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Dear Esteemed Readers, Authors, and Colleagues,

I hope this letter finds you in good health and high spirits. It is my distinct pleasure to address you as the Editor-in-Chief of Integrative Omics and Applied Biotechnology (IIOAB) Journal, a multidisciplinary scientific journal that has always placed a profound emphasis on nurturing the involvement of young scientists and championing the significance of an interdisciplinary approach.

At Integrative Omics and Applied Biotechnology (IIOAB) Journal, we firmly believe in the transformative power of science and innovation, and we recognize that it is the vigor and enthusiasm of young minds that often drive the most groundbreaking discoveries. We actively encourage students, early-career researchers, and scientists to submit their work and engage in meaningful discourse within the pages of our journal. We take pride in providing a platform for these emerging researchers to share their novel ideas and findings with the broader scientific community.

In today's rapidly evolving scientific landscape, it is increasingly evident that the challenges we face require a collaborative and interdisciplinary approach. The most complex problems demand a diverse set of perspectives and expertise. Integrative Omics and Applied Biotechnology (IIOAB) Journal has consistently promoted and celebrated this multidisciplinary ethos. We believe that by crossing traditional disciplinary boundaries, we can unlock new avenues for discovery, innovation, and progress. This philosophy has been at the heart of our journal's mission, and we remain dedicated to publishing research that exemplifies the power of interdisciplinary collaboration.

Our journal continues to serve as a hub for knowledge exchange, providing a platform for researchers from various fields to come together and share their insights, experiences, and research outcomes. The collaborative spirit within our community is truly inspiring, and I am immensely proud of the role that IIOAB journal plays in fostering such partnerships.

As we move forward, I encourage each and every one of you to continue supporting our mission. Whether you are a seasoned researcher, a young scientist embarking on your career, or a reader with a thirst for knowledge, your involvement in our journal is invaluable. By working together and embracing interdisciplinary perspectives, we can address the most pressing challenges facing humanity, from climate change and public health to technological advancements and social issues.

I would like to extend my gratitude to our authors, reviewers, editorial board members, and readers for their unwavering support. Your dedication is what makes IIOAB Journal the thriving scientific community it is today. Together, we will continue to explore the frontiers of knowledge and pioneer new approaches to solving the world's most complex problems.

Thank you for being a part of our journey, and for your commitment to advancing science through the pages of IIOAB Journal.

Yours sincerely,

Vasco Azevedo

Vasco Azevedo, Editor-in-Chief
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ARTICLE

CHARACTERIZATION OF AN EXTRACELLULAR CHITOSANASE FROM THE SOIL BACTERIUM *BACILLUS CEREUS* CH12

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ABSTRACT

An extracellular chitosanase producing bacterium was isolated from soil samples in Mysuru, Karnataka, India and identified as Bacillus cereus CH12 based on morphological, biochemical and 16S rRNA gene sequence. Presence of the chitosanase gene was detected by partial gene sequencing. The chitosanase showed an activity of 11.16 U/ml after three days of incubation with 90% deacetylated chitosan. The enzyme was purified by gel filtration and its molecular weight was 30 kDa. Optimum temperature of the enzyme was 40 ^{o}C and optimum pH was 6.0. The enzyme was stable at a pH range of 5.0-8.0 and up to a temperature of 50 ^{o}C. The enzyme showed high specificity towards carboxymethyl cellulose (CM-cellulose). Analysis of the hydrolytic product of the enzyme showed the presence of chitooligosaccharides of a degree of polymerization of 3-5. Bacillus cereus CH12 chitosanase can be useful in the degradation of cellulose and chitosan containing biomass and in the production of chitooligosaccharides.

KEY WORDS
Bacillus cereus; Chitosan; Chitosanase; Extracellular; Chitooligosaccharides

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INTRODUCTION

Chitosan is a D-glucosamine polymer made of N-acetyl-D-glucosamine linked by β-1,4-glycosidic bonds [1, 2]. Chitosan is a deacetylated derivative of chitin [1, 3]. Chitosan is manufactured in industries by chemical deacetylation of chitin and biologically by the deacetylation of chitin through a chitin deacetylase enzyme. Fungi like *Absidia, Mucor* and *Rhizopus* are known to have chitosan in the cell walls of their mycelia and sporangiophores. It is also present in the exoskeletons of the insects and predominantly in crustacean shells [1, 4]. Chitosan and the derivatives of chitosan have various applications - they are being used as permeability control agents, adhesives and as flocculating and chelating agents. They have also emerged as potential biomaterial for food, pharmaceutical, textile industry and in wastewater treatment [1, 5]. Chitin and chitosan have been receiving lot of importance in the recent times due to their derivatives - the chitooligosaccharides. Chitooligosaccharides have several beneficial properties [4, 5]. Chitooligosaccharides have low viscosity, they have shorter chains and most importantly, they are water soluble [6]. Chitosan can be converted to chitooligosaccharides either by chemical or enzymatic methods. However, chemical methods of chitosanase production have several shortcomings - production of large amounts of glucosamine, which is the monomer of chitin, low yields of chitooligosaccharides, cost effectiveness and problems of environmental pollution. Therefore, biological methods of chitooligosaccharide production have attracted more attention and are highly advantageous [7]. Chitosanases (EC 3.2.1.132) are enzymes that hydrolyze chitosan (1, 3, 8). Chitosanases have been classified under family 8, 46, 75 and 80 respectively based on their amino acid sequence similarities. Among these families, the glycoside hydrolase family 8 (GH-8) has the highest number of bifunctional enzymes with cellulolytic and chitosanolytic activities. The GH-8 family also contains cellulase, xylanase, lichenase and others [9, 10]. Chitosanases are used in the production of biologically functional chitooligosaccharides and in the control of fungal pathogens [11, 12]. Chitooligosaccharides produced enzymatically are a better alternative to chemical methods of degradation, which use acids for hydrolysis resulting in lower yields [13]. Use of chitosanases for the enzymatic degradation of the vast biomass of chitosan available in nature has become advantageous over chemical methods due to its environmental compatibility, reproducibility and low cost [14]. A chitosanase producing bacterium *Bacillus cereus* CH12 was isolated, the enzyme chitosanase was purified and its biochemical characteristics were studied. This may serve as a potential enzyme for the degradation of biomass and in the production of useful chitooligosaccharides.

MATERIALS AND METHODS

Materials

Chitosan of 75% degree of deacetylation, Glucosamine hydrochloride and chitin (Himedia laboratories, Mumbai, India), 90% degree of deacetylation chitosan, chitosan lactate (Everest Biotech, Bengaluru, Karnataka, India) and Sephadex G-75 (Sigma) were used. Other chemicals were of analytical grade (SRL, Mumbai India and Himedia Laboratories, Mumbai India). Pre-stained protein marker (Fermentas) was used to determine the molecular weight of the protein. The soil for screening was obtained from garden soil samples at Mysuru, Karnataka, India.

Screening of chitosanase producing bacteria from soil

Soil samples were serially diluted and plated on to a chitosanase screening medium as described by Johnsen et al., 2010 [15]. The medium (1 litre) was prepared by adding 1.8 g of 90% deacetylated chitosan to 200 ml of distilled water and sterilizing it by autoclaving. At room temperature, 18 ml of sterile 1 molar HCl was added in order to bring the chitosan to solution. The chitosan solution was then dissolved by stirring for two hours. The dissolved chitosan was poured into 700 ml of an autoclaved, warm medium at a slow rate, while stirring vigorously so that the small colloidal particles were well maintained. This medium had the following composition; KH_2PO_4 – 9 g, K_2HPO_4 – 6 g, Tryptone – 8 g, Yeast extract – 4 g and Agar – 15 g. Vogel's trace elements (2 ml) were added to the medium. The composition of Vogel's trace elements is as follows – Citric acid H_2O – 5 g, $ZnSO_47H_2O$ – 5 g, $Fe(NH_4)_2(SO_4)_26H_2O$ – 1 g, $CuSO_45H_2O$ – 0.25 g, $MnSO_4H_2O$ – 0.05 g, H_3BO_4 – 0.05 g, $NaMoO_42H_2O$ – 0.05 g per 100 ml. Finally, 2 ml of sterilized $MgSO_46H_2O$ (203 g/l) was added. The final volume of the medium was adjusted to 1 litre. The bacterial isolates were incubated on the chitosanase detection medium for a period of 3-4 days. An isolate named CH12, showing significant chitosanase activity and a clear prominent halo around the bacterial growth was selected for further study. The isolate CH12 was identified taxonomically according to the Bergey's Manual of Systematic Bacteriology [16]. The identity of the organism was confirmed by Polymerase chain reaction (PCR) and the partial amplification of the 16S rRNA gene. The following primers were used for amplification: 27F- 5'- GAGTTTGATCCTGGCTCA-3', 1492R- 5'-TACGGCTACCTTGTTACGACTT-3' [17]. The sequence was amplified by Sanger Dideoxy method and the amplified sequence was compared with published sequences through an NCBI BLAST search. Chitosanase gene was identified by partial amplification of the gene by PCR. The primers were designed on the basis of the chitosanase gene of the bacterium *Bacillus cereus* ATCC 14579 (Accession number: NC_004722) obtained from a search in the NCBI database. The following PCR conditions were used – 95 ^{0}C for 15 minutes, 30 cycles at 95 ^{0}C for 30 seconds, 48 ^{0}C for 30 seconds, 72 ^{0}C for 1 minute and a final extension at 72 ^{0}C for 7 minutes. The primers had the following sequences: Forward primer: 5'-CTTCAGAAGGTCAAGGGTATGG-3', reverse primer: 5'-CCAATCAGATGGTCTCGTATCAA-3'.

Assay for chitosanase activity

Chitosanase was assayed by modifications of methods described by El – Sherbiny, 2011 [18] and Sinha et al., 2012 [12]. Chitosanase was assayed by measuring the reducing sugars produced using 0.18% of 90% deacetylated chitosan as a substrate. The reaction mixture consisted of 200 µl of enzyme solution, 500 µl of the substrate and 800 µl of sodium acetate buffer (pH 6.0). The mixture was incubated at 37 ^{0}C for 1 hour. The reaction was terminated by heating the mixture in a boiling water bath (100 ^{0}C) for 2 minutes. The supernatant was separated by centrifugation and the reducing sugars in the supernatant were measured by a method described by Imoto and Yagishita 1971 [19] using glucosamine hydrochloride as a calibration standard. One unit of chitosanase was defined as the amount of enzyme that liberated 1µmol of D-glucosamine per minute under the above mentioned conditions.

Purification of chitosanase

The isolate CH12 was cultured on the chitosanase detection medium (1 litre) except for agar. The culture was incubated for a period of 3-4 days. After incubation, the cell supernatant was separated by centrifugation at 8,000 rpm at 4 ^{0}C for 15 minutes. The resulting supernatant (950 ml) was subjected to ammonium sulphate precipitation. Ammonium sulphate (662 g/l) was added to the supernatant (950 ml) in order to achieve 90% saturation. This mixture was stored overnight at 4 ^{0}C and the resulting precipitate was centrifuged at 12,000 rpm at 4 ^{0}C for 15 minutes. The precipitate was pooled, and an appropriate amount of sodium acetate buffer (pH 6.0) was added in order to dissolve the precipitate. This resulting crude enzyme solution was concentrated by dialysis against the same buffer (Sodium acetate, pH 6.0) at 4 ^{0}C for a period of 48-72 hours. The dialyzed crude enzyme was then loaded on to a Sephadex G-75 gel filtration column (60x2 cm). 4 ml fractions were collected at a flow rate of 22 ml/hour. Protein profile was examined at 280 nm. Protein concentration of the enzyme was determined by Lowry's method [20] using Bovine Serum Albumin as a standard.

Determination of molecular weight of chitosanase

The molecular weight of the purified chitosanase enzyme was determined using 12% Sodium dodecyl sulphate – polyacrylamide gel electrophoresis (SDS-PAGE) [21] and stained with Coomassie Brilliant Blue R-250. Pre-stained protein marker was used for identification of the molecular weight of the protein.

Effect of pH, temperature, metal ions and substrates on enzyme activity

Enzyme activity for the purified enzyme was tested for its behavior with respect to different parameters like temperature, pH, effect of metal ions and specificity to substrates [3, 13, 22]. To determine the optimum pH, the enzyme and substrate were incubated in buffers of different pH values. The buffers used were 100 mM solutions of McIlvaine buffer (pH 3.0, pH 4.0), sodium acetate buffer (pH 5.0), potassium phosphate buffer (pH 6.0, 7.0, 8.0) and bicarbonate-carbonate buffer (pH 9-10). For the determination of pH stability, the enzyme solution was pre-incubated at 4 ^{0}C for 1 hour in the buffers as described above and the residual activity was measured. For the determination of optimum temperature, a mixture of enzyme,

substrate and buffer was incubated at various temperature ranges from 10-80 ^{0}C at pH 6.0. Temperature stability was determined by checking the residual activity after pre-incubation of the enzyme for 1 hour at pH 6.0 and various temperatures as described above without the substrate. Metal ion effect was determined by pre-incubating the enzyme with 10 mM metal ions like Mn^{2+}, Ca^{2+}, Mg^{2+}, Mo^{2+}, Ba^{2+}, Zn^{2+}, Cu^{2+}, Hg^{2+} and Fe^{2+}. Substrate specificity was tested by selecting substrates of chitosan and cellulosic nature. Chitosan of different degrees of deacetylation (75%, 90%), chitosan lactate, colloidal chitin, CM-cellulose, insoluble cellulose and xylan were used to analyze the substrate specificity.

Analysis of the hydrolytic products of chitosanase

An enzymatic hydrolysate was prepared from the enzyme solution as described by Wang et al., 2012 [14]. For the production of chitooligosaccharides, 90% deacetylated chitosan was used as a substrate (0.18% w/v, 50 mM sodium acetate buffer pH 6.0). 1ml of enzyme was incubated with 1ml of substrate at 40 ^{0}C for 2 hours. The enzymatic hydrolysate was collected after incubation and this solution was concentrated to 1/5 of its original volume in a vacuum concentrator. This was followed by the addition of methanol solution (90% concentration, v/v) until yellow agglomerates were formed in solution. The resulting solution was concentrated in a vacuum concentrator and was collected after drying completely. This sample was subjected to Matrix - assisted laser desorption/ ionization time of flight (MALDI-TOF) mass spectral analysis (UltrafleXtreme MALDI TOF/TOF (Bruker Daltonics)).

RESULTS

Screening of chitosanase producing bacteria from soil

The bacterial isolates were screened for chitosanase production and one isolate CH12 was selected [Fig. 1]. The strain CH12 was identified by its biochemical and molecular characteristics. The organism was Gram positive, aerobic, rod shaped and motile. The bacterium was positive for Malonate, Voges-Proskaeur, Nitrate, β- haemolysis and catalase tests. It was negative for Citrate, O-Nitrophenyl-β-D-galactopyranoside (ONPG) and arginine. It was positive for the hydrolysis of sucrose, glucose and trehalose and negative for mannitol and arabinose. The organism could grow up to a temperature of 45 ^{0}C, could not grow in acidic pH but tolerated an alkaline pH up to 10.0. According to sequencing of the 16S rRNA gene, the organism showed close similarity (99%) to *Bacillus cereus*. Therefore, based on the morphological, biochemical characters and the results of the 16S rRNA gene sequencing, CH12 was identified as *Bacillus cereus* and named as *Bacillus cereus* CH12. The partial 16S rRNA gene sequence was deposited in GenBank with the accession number KR818841. The partial chitosanase gene was sequenced and deposited to Genbank with the accession number KX375814. The sequence showed high homology (99%) to *Bacillus* chitosanases.

Fig. 1: Culture plate of *Bacillus cereus* CH12 showing clear zone of chitosan hydrolysis around the bacterial growth after 3 days of incubation.

Assay for chitosanase activity

Bacillus cereus CH12 was grown on chitosanase detection media of various chitosan concentrations. A medium with 0.18% of 90% deacetylated colloidal chitosan [15] was found to be most suitable for chitosanase production in this study. Maximum chitosanase activity was seen when cultures were grown at 37 ^{0}C for three days. At the end of the incubation period, a maximum activity of 11.16 U/ml of chitosanase was seen. According to previous reports of chitosanase activities among *Bacillus* species, chitosanase from *Bacillus circulans* WL-12 showed an activity of 1.2 U/ml after two days of cultivation [8], wild strain of *Bacillus* sp. TS showed an activity of 5 U/ml [7]. *Bacillus megaterium* P1 and *Bacillus cereus* D11 and showed activities of 1 U/ml and 4.85 U/ml respectively [2, 22]. Activity of chitosanase of *Bacillus cereus* CH12 (11.16 U/ml) was higher than many chitosanases produced by wild strains of *Bacillus* species so far when grown in a chitosan medium.

Purification of chitosanase

Culture supernatant (950ml) of *Bacillus cereus* CH12 was purified by precipitation with 90% ammonium sulphate, dialysis and Sephadex G-75 gel filtration chromatography. The gel filtration yielded 60 fractions of 4ml each. The fractions were assayed for chitosanase activity and the protein profile was monitored by checking the absorbance at 280 nm. The active fractions were pooled and concentrated by a vacuum concentrator. After the final purification step, 16.2 mg of protein and 5.9 fold purity was obtained. Percentage yield was 18.1.

Determination of molecular weight of chitosanase

Purity of the protein was analysed by SDS-PAGE and a single band of 30 kDa was obtained [Fig. 2].

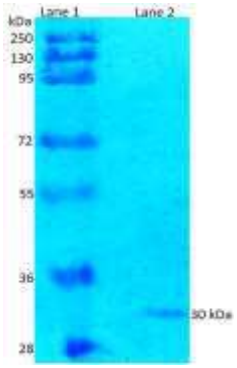

Fig. 2: SDS-PAGE of the purified protein. Lane 1: Molecular marker, Lane 2: Purified enzyme

Effect of pH, temperature, metal ions and substrates on enzyme activity

Optimum pH of the enzyme was 6.0. The activity was low in the pH below 5.0 and above 7.0. The enzyme was stable at pH 5.0-8.0 when incubated in various buffers at 4 ^{0}C as described earlier. The pH stability of chitosanases from *Bacillus* species ranged from 4-10 [Fig. 3A]. The enzyme had an optimal temperature of 40 ^{0}C, was stable up to a temperature of 50 ^{0}C and all its activity was intact at this temperature. When heated to higher temperatures for one hour, the enzyme retained 66% of its activity at 60 ^{0}C. There was a gradual decline of activity at higher temperatures and almost no activity was seen above 60 ^{0}C [Fig. 3B]. In order to determine the effect of various metal ions, CH12 chitosanase was incubated with different metal ions [table 1] at 10 mM concentration. The enzyme was completely inhibited by Hg^{2+} and Mn^{2+}. The activity was reduced by 67% when incubated with Zn^{2+} and Fe^{2+} ions when compared to the control. However, the enzyme was completely inhibited by Mn^{2+} ions. This result was different when compared to other chitosanases. Chitosanase inhibition by Mn^{2+} ions is an unusual characteristic among chitosanases and most microbial chitosanases are not inhibited by Mn^{2+} ions [5]. A similar inhibition by Mn^{2+} was reported in chitosanase of *Serratia marcescens* TKU011 [23]. Chitosanase from *Bacillus* sp. Strain KCTC 0377BP showed 69% inhibition in enzyme activity in the presence of Mn^{2+} ions [5]. Analysis of the substrate specificity of the purified enzyme was done with different substrates [Table 2] ranging from colloidal chitosan of different degrees of deacetylation, chitin to cellulosic substrates and chitosan lactate. The activity was highest in 90% deacetylated chitosan. The enzyme also retained 91% of activity on CM-cellulose. The enzyme showed little activity on xylan, insoluble cellulose and colloidal chitin.

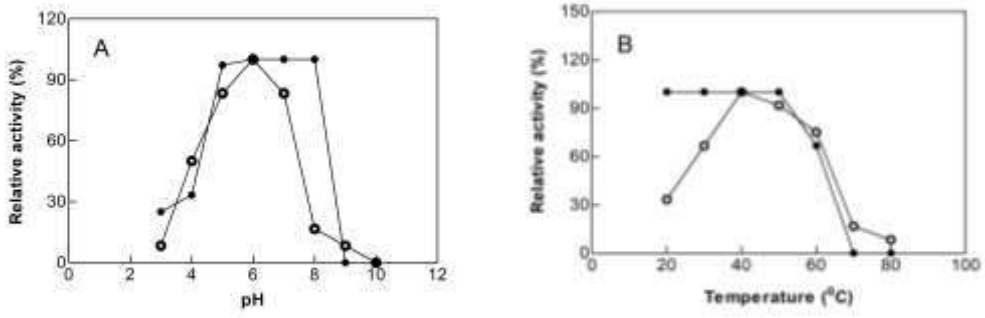

Fig. 3 [A]: Effect of pH (open circles) and pH stability (closed circles) of *Bacillus cereus* CH12 chitosanase. **B:** Effect of temperature (open circles), temperature stability (closed circles). 100% activity refers to the temperature and pH where the activity and stability are highest

Table-1: Effect of metal ions on chitosanase from *Bacillus cereus* CH12. 100% activity implies - incubation of the enzyme without metal ions.

Metal ion	Relative activity (%)
None	100
Mn^{2+}	0
Ca^{2+}	83
Mg^{2+}	83
Mo^{2+}	67
Ba^{2+}	75
Zn^{2+}	92
Cu^{2+}	33
Hg^{2+}	0
Fe^{2+}	33

Table-2: Substrate specificity of *Bacillus cereus* CH12 chitosanase. Activity values were relative to the maximum activity seen in 90% deacetylated chitosan.

Substrate	Relative activity (%)
Colloidal chitosan (90% deacetylated)	100
Colloidal chitosan (75% deacetylated)	83
Chitosan lactate	67
Colloidal chitin	0
CM-cellulose	91
Insoluble cellulose	47
Xylan (from oat spelts)	0

Analysis of hydrolytic products

The hydrolytic products of *Bacillus cereus* CH12 chitosanase were subjected to MALDI-TOF mass spectrometric analysis in the positive mode. The oligosaccharides present in the precipitate were obtained as sodium adducts [M+Na]. From the MALDI-TOF analysis, chitooligosaccharides of a degree of polymerization (DP) of 3 to 5 were identified. They had the *m/z* (mass to charge ratio) values of 524.3, 551.2, 647.7, 727.3, 749.3 and 888.7. The ionic compositions of the chitooligosaccharides were $(GlcN)_3$, $(GlcN)_4$, GlcNAc-$(GlcN)_3$, (GlcN)-$(GlcNAc)_3$, GlcNAc-$(GlcN)_4$. The results are similar to ionic compositions previously reported [24-27].

CONCLUSION

A bacterium with chitosan hydrolyzing property was isolated and identified as *Bacillus cereus* CH12. The presence of the chitosanase gene was identified in the bacterium by partial sequencing. When this sequence was translated into an amino acid sequence and subjected to a protein BLAST search, it showed homology to those sequences, which had the GH-8 signature motif - "ATDGDLDIAYSLLLAHKQW" [28]. GH-8 is a family with chitosanase, cellulase, lichenase, xylanase and other enzymes. The chitosanase enzyme also retained 91% of its initial activity on CM-cellulose. High activity on CM-cellulose is a characteristic of GH-8 enzymes [22]. An important feature of an enzyme that can be used in biodegradation is the production of oligosaccharides as a product of enzyme hydrolysis. Analysis of the hydrolytic product of *Bacillus cereus* CH12 chitosanase showed the presence of oligosaccharides of a degree of polymerization from 3-5. Absence of monosaccharides specifies that the enzyme may have an endo-splitting nature. However, this has to be confirmed with further studies. *Bacillus cereus* CH12 could be cultured up to a maximum temperature of 45 ^{0}C and the maximum chitosanase activity was at 40 ^{0}C. Activity of chitosanase of *Bacillus cereus* CH12 (11.16 U/ml) was higher than many chitosanases produced by wild strains of *Bacillus* species reported in literature so far when grown in a chitosan medium. In conclusion, chitosanase of *Bacillus cereus* CH12 had a high activity on chitosan, high relative activity on CM-cellulose and could produce chitooligosaccharides. This makes the organism very suitable for utilization in biodegradation and production of chitooligosaccharides.

CONFLICT OF INTEREST
There is no conflict of interest

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None

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No financial support was received for this study

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ARTICLE

EFFECT OF IRANIAN CRACK ON TESTOSTERONE AND GONADOTROPIN LEVELS IN ADDICTED MEN

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ABSTRACT

Background: *Substance abuse is associated with a wide range of side effects such as hormonal and reproductive disorders. Iranian Crack is a new form of narcotic substance that widely used in Iran during last decade. The aim of this study was determination of the effects of Iranian Crack on serum testosterone and gonadotropin levels in addicted men.* ***Methods:*** *In this case-control study, participants were screened for eligibility, and then, serum levels of testosterone and gonadotropin (LH and FSH) hormones in 54 Iranian Crack addicts men were compared with 45 healthy subjects. Hormone levels in serum were measured by ELISA technique.* ***Results:*** *Results indicated that serum FSH levels in addict men was significantly lower than healthy subjects (p = 0.03). Serum LH and Testosterone levels in case group had not significant difference with control group.* ***Conclusions:*** *According to our results, chronic use of Iranian Crack lead to a reduction in FSH levels, and this reduction may impair the reproductive function in addicted men.*

KEY WORDS
Iranian Crack, Addiction, Testosterone, FSH, LH

INTRODUCTION

Substance abuse is a major problem among the various societies throughout the world [1]. Opioids have been used for a long time for the treatment of acute and chronic pain [2]. Opioids induced a lot of side effects such as addiction, hypogonadism, immune suppression, osteoporosis and hyperalgesia [3]. Also, opioids act on endocrine system that may lead to further serious adverse effects [4].

The gonadotropins (FSH, LH) are secreted from pituitary gland and acts via hypothalamus-pituitary-gonadal axis, which stimulates gonadal endocrine function and gametogenesis in males [5]. Inhibition of this axis will lead to reduction of semen quality, sperm count, impairment of erection and finally infertility [6]. Several studies have evaluated the relation between drug use and Hypothalamic-Pituitary-Gonadal axis hormones, it has been demonstrated that opioid abuse may result in hypogonadism and infertility by decreasing release of gonadotropin releasing hormone (GnRH) [7], LH, FSH and testosterone hormones [8-10]. In addition has effect on sperm motility [11] and morphology [12].

There is a long history of opioid abuse in Iran [13]. In recent years, Iranian Crack consumption in addicts has increased. Iranian Crack is a new form of narcotic substance that has widely used in Iran in the past years [14]. Farhoudian A *et al*. showed that Iranian Crack contains heroin, acetaminophen, caffeine, morphine, codeine, thebaine and acetylcodeine, so they concluded that the Iranian Crack is heroin-based and hence is quite different from common Crack Cocaine found in the Western countries [15].

Despite many studies on the effects of opioids on different body systems, the effects of long term Iranian Crack consumption on endocrine system especially on sex hormones is not determined. Therefore, the aim of this study was determination of effects of Iranian Crack use on serum testosterone and gonadotropins in addicted men.

MATERIALS AND METHODS

Study subjects

In this case-control study, 84 addicted men were screened for eligibility (cases, group 1). They had Iranian Crack dependency and referred to Bahari Digar and Ofogh Bidari Addiction Treatment Centers at Zahedan city of Iran (2016). At the same time, 60 healthy men (control, group 2) among volunteer blood donors were screened. Healthy subjects were enrolled using local advertisements for a study of reproductive hormones. The study was explained to the participants, and written informed consent was collected from all the subjects. In addition, the ethics committee of Iranshahr University of Medical Sciences has approved this study (No. IR.IRSHUMS.REC.1394.4).

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Inclusion/exclusion criteria

Participants were entered in the study if they were between 20 and 50 years old. Participants had no history of known medical or surgical condition that could influence their fertility. Healthy subjects had no self-reported drug use history, and among them, who that had similar conditions (such as education, cigarette smoking, social and cultural features) with addicted men were selected as the control group.
The exclusion criteria which determined by interview and examination, consisted of having a history of any genitourinary surgery, epididymo-orchitis, cryptorchidism, varicocele, sexually transmitted disease, daily alcohol consumption, cancer, diabetes, serious medical illnesses that required pharmacological treatment, and any neurological or psychiatric disease. Also, polyconsumers were excluded from the study. Finally, 54 addicted men and 45 healthy subjects that met study criteria were recruited into the study.

Measures

All participants were examined by a physician before inclusion in the study. Personal interviews were done with all participants to obtain relevant clinical data: age, weight, height, educational years, marital status, history of alcohol consumption, infertility status, history of any medical problem and treatment, history of smoking and history of drug use. Then, additional information from Iranian Crack-dependent subjects (duration of drug use, average daily dose, and route of drug administration) were also noted in the questionnaire.
In this study, laboratory tests included serum levels of testosterone, LH and FSH.

Determination of testosterone and gonadotropin concentrations in serum

Serum testosterone, LH and FSH levels were examined for all subjects. Examination for Iranian Crack group was performed at baseline (before starting to treatment programs in the addiction treatment centers). Five milliliters of venous blood was collected (between 8 and 10 AM) then allowed to clot at room temperature, and the blood was centrifuged at 3500 rpm for 10 min instantly. Serum was obtained, and then stored at -80°C until use. Serum levels of testosterone, LH and FSH were measured by ELISA technique.

Statistical analysis

Statistical analysis was done using software SPSS-18. Descriptive statistics (mean, standard deviation) were calculated for age, weight, height, educational years, marital status, smoking status, duration of drug use, average daily dose and serum levels of hormones. Data analysis performed by Chi-Square and Independent sample *t*-test. $P \leq 0.05$ was considered significant.

RESULTS

Demographic characteristics of participants

Demographic characteristics of Iranian Crack addicts (group1) and healthy subjects (group 2) are summarized in [Table 1]. Fifty-four Iranian Crack addicts and forty-five healthy subjects were recruited. Weight in addicted group was significantly lower than healthy subjects ($p = 0.001$). There was no significant difference in any other variables between cases and healthy subjects. Duration of Iranian Crack use ranged from 1 year to 20 years, with an average duration of 4.64 ± 3.48 years. dose of Iranian Crack use per day ranged from 0.5 to 6.0 g/day, with the average dose of 1.05 ±0.56 g/day. In all addict men, route of drug administration was smoking.

Testosterone and gonadotropin (LH and FSH) levels of Iranian Crack addicts and healthy subjects

[Table 2] shows serum testosterone, LH and FSH levels of participants. Mean serum FSH levels of Iranian Crack addicts were significantly lower than healthy subjects ($p = 0.03$).Testosterone levels were also lower in Iranian Crack addicts, although the difference was not statistically significant. Serum levels for LH were similar in the two groups.

Table 1: Demographic characteristics of the participants

Characteristics	Cases (n=54)	Controls (n=45)	P value*
Age (years)	31.24±0.96	32.44±1.27	0.44
Weight (kg)	67.03±1.45	74.46±1.7	**0.001**
Height (cm)	171.04±0.96	172.73±1.79	0.38
Education (years)	7.16 ± 0.56	7.13 ± 0.69	0.97
Marital status, no (%)			
Married	32(59.25)	26(57.77)	0.75
Never married	21(38.9)	17(37.77)	
Divorced, separated or		2(4.46)	

widowed	1(1.58)		
Smoking status, no (%) Consumption Non consumption	 35(64.81) 19(35.19)	 24(53.3) 21(46.7)	0.24
Duration of dependency (year)	4.64 ± 3.48	-	NA

Note: Bold value indicates p < 0.05. Key: NA, not applicable.
*Derived from the Chi-Square test or Independent sample *t*-test.

Table 2: Testosterone, LH and FSH levels of participants

Hormones	Cases (n=54)	Controls (n=45)	P value*
T (ng/ml)	6.2±0.43	6.6±0.39	0.5
FSH (mIU/ml)	3.25±0.26	4.32±0.42	**0.03**
LH (mIU/ml)	3.18± 0.3	2.74±0.3	0.32

Note: Bold value indicates p < 0.05. Key: T, testosterone; FSH, follicle stimulating hormone; LH, luteinizing hormone. *Derived from Independent sample *t*-test.

DISCUSSION

To our knowledge, for the first time, this study was performed to determine the effect of Iranian Crack consumption on testosterone and gonadotropin levels in addicted men. Iranian Crack is a new form of narcotic substance that widely used in Iran during past years [14]. Iranian Crack is heroin-based substance, but it has complex composition that contains heroin, acetaminophen, caffeine, morphine, codeine, thebaine and acetylcodeine [15]. There is no study about Iranian Crack effects on body systems and hence, it seems that conducting of studies in this field could be essential.

In our study, men in group 1 showed significant decrease in FSH level when compared to the control group. But, serum testosterone and LH levels in case group had not significant difference with control group. The results of this study are not in agreement with the opioid studies, because previous studies have shown that chronic opioid administration decreases testosterone, LH, whereas FSH is not affected [8, 16-19]. These contradictory results can due to different composition of Iranian Crack. Although it is opioid-based, but it has complex and different composition.

Vuong and his colleagues (2010) concluded that in both animals and humans, opioids can not significantly alter FSH levels [16]. However, our results indicate that Iranian Crack can change serum FSH levels in human. This reduction may be due to a subsequent of the direct effect of Iranian Crack on the pituitary or may be due to effect on hypothalamus.

Some studies have suspected that opioids inhibit testosterone and LH secretion through effect on the hypothalamic-pituitary axis [18, 20-21]. Besides, opioids can directly decrease testosterone secretion by their effect on the testes [22]. But in our study, testosterone and LH did not change significantly in the case group when compared to the controls. These results can due to little effect of Iranian Crack on testosterone and LH secretion.

CONCLUSION

The findings of this study showed that Iranian Crack addiction decrease FSH levels in addicted men. Hence, it can have a negative effect on reproductive system.

CONFLICT OF INTEREST
Authors declare no conflict of interest.

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IIOAB JOURNAL

SHORT COMMUNICATION

A NEW SUSTAINABLE APPROACH FOR LACCASE PRODUCTION AND BIOREMEDIATION

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ABSTRACT

Background: *Fungal laccase is a ligninolytic enzyme with great biotechnological interests into bioremediation, but the optimization need of their production conditions on an industrial scale difficult its commercial viability. Thus, a promising alternative is the utilization of agroindustrial wastes and here we propose açaí (Euterpe sp.) wastes as an alternative substrate from Amazon rainforest.* ***Methods:*** *We cultivated the fungus in solid-substrate fermentation method using açaí wastes (bagasse and seed) as substrate. We performed a synthetic dyes decolorization activity assay using Reactive Black 5, Reactive Blue 4, AB129, Acid Red 1 and RBBR as substrates.* ***Results:*** *We report that white rot fungus grows well in the solid substrate and the maximum laccase activity was in 21st day of cultivation. Laccase was able to degrade all synthetic dyes tested, with better activity on Acid Blue 129 and RBBR.* ***Conclusions:*** *Our work demonstrate that the white rot fungus cultivation in açaí wastes provides a significant laccase activity and also produces an enzymatic complex able to degrade synthetic dyes, what provides useful insights into the development of industrial laccase production and the sustainable commercial exploitation of agroindustrial waste products from Amazon rainforest.*

KEY WORDS
Ligninolytic enzymes, bioremediation, synthetic dyes, white rot fungi, Euterpe

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INTRODUCTION

Laccase is a ligninolytic enzyme that have attracted great attention due to their potential to recovery environments contaminated by chemical compounds coming from industrial processes and which are inappropriately disposed, such as synthetic dyes mainly related to textile and paper industries [1-3]. In nature laccase can be produced by a wide range of conditions and by a diverse array of bacteria, plants and fungi [4]. The white rot fungi are efficient ligninolytic enzymes producers and for this reason have been the subject of numerous scientific works, which have tried to manipulate their enzyme production for commercial exploitation [5-6].

At present, the key limitation impeding to large scale production of fungal ligninolytic enzymes, including laccase, is the availability of commercially viable fungal culturing conditions [6]. One of the most promising alternative is the utilization of agricultural residues as substrates for optimizing production of these enzymes [7]. The fruit of the palm *Euterpe* sp., which grows widely in the Amazon region and is called "*açaí*" in Portuguese, is an important component of the Amazon regional economy [8]. Commercial processing of açaí generates an abundance of biological waste products (including seeds and fibres), which are not commercially exploited and are often inappropriately discarded in the environment. This wasted biomass is a rich source of protein and carbohydrates, like other agricultural residues such as orange [9] and banana peels [10]. For this reason, açaí wastes may represent a natural substrate with great potential for the production of ligninolytic enzymes such as laccase, which can have numerous applications such as bioremediation.

The great potential of laccase for environmental decontamination is related to their ability to oxidize phenolic compounds, such as synthetic dyes, which can be potent soil and water pollutants, resulting in several changes in physical and biological environmental factors, also with risks to human health [11] Following laccase-degradation of phenolic compounds, contaminated environments are able to recover to their natural states and for this reason laccase is often viewed as a potentially important biological tool for environmental bioremediation. In this context synthetic dyes Acid Blue 129, Acid Red 1, RBBR, Reactive Blue 4 and Reactive Black 5 have been used as experimental models to investigate the ability of laccase to degrade phenolic compounds [12].

The aim of our work was to investigate the potential of açaí commercial-processing waste-products for laccase production by a white rot fungus and the ability of these enzymes to degrade synthetic dyes.

MATERIALS AND METHODS

Microorganism

We collected a basiodiomycetes fungus from a basidiocarp growing on decomposing wood waste, in the campus of Universidade Federal do Amazonas (UFAM). The microorganism was cultivated in Potato-Dextrose-Agar (PDA) medium (pH 5.0), incubated at 27°C until the experiment.

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Cultivation in agroindustrial waste and enzymatic extract obtainment

We cultivated the fungus in solid-substrate fermentation method using açaí (*Euterpe* sp.) wastes (bagasse and seed) as substrate. Our experiments were divided in two treatments with different percentages of açaí wastes mixed with oat bran, in a total of 10g with 60% of humidity, as following: treatment 1 (90% of açaí wastes and 10% of oat bran) and treatment 2 (70% of açaí wastes and 30% of oat bran). We carried the experiment during 35 days at 27ºC and every seven days we collected three samples of each treatment. We added acetate buffer (1M; pH 5.0) in each sample and filtered to obtain the enzymatic extract.

Laccase activity assay

We registered the laccase activity by spectrophotometry using 2,2'-azinobis- (3-etilbenzotiazoline-6-sulfonate) (ABTS) as substrate. We prepared a reaction mixture (1ml) with 0.1ml of ABTS, 0.4ml enzyme extract and 0.5ml of acetate buffer (0.1M; pH 5.0). We registered the absorbance at 420nm in 30s intervals, during 5min. We considered one unit of enzyme activity as the amount of enzyme capable of oxidizing 1µmol of ABTS per minute (U/L).

Synthetic dyes decolorization assay

We measured the decolorization enzymatic ability in the same way as described by [12]. We used as substrate Reactive Black 5 (9.15 mg.l^{-1}), Reactive Blue 4 (35mg.l-1), AB129 (83.3 mg.l^{-1}), Acid Red 1 (10mg.l^{-1}) and RBBR (50mg.l^{-1}). We registered the absorbance during 10min: Reactive Black 5 (597nm), Reactive Blue 4 (595nm), AB 129 (629nm), Acid Red 1 (506nm) and RBBR (592nm). We considered a decolorization activity unit as capable of catalyzing a reduction of 0.01 in absorbance per minute (U/L).

RESULTS AND DISCUSSION

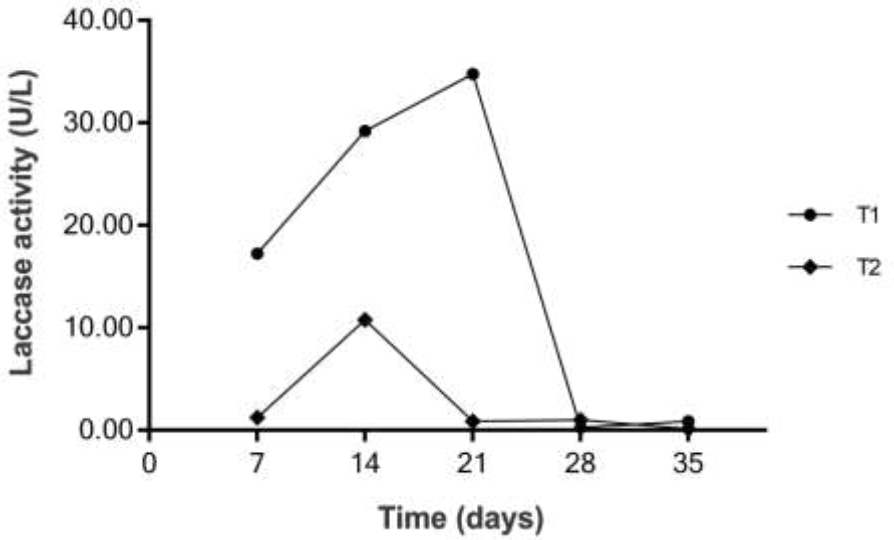

Fig. 1: Laccase activity (U/L) during 35 days of cultivation in açaí (*Euterpe* sp.) wastes. This represent the results of enzymatic assays with the treatment 1 (T1) and treatment 2 (T2) extracts using ABTS as substrate.

The white rot fungus successfully colonized all the solid substrate until the 7th day of experiment in both treatments, but the highest laccase activity was registered between the 14th (treatment 2, ~10.80 U/L) and 21st (treatment 1, ~34.77 U/L) days. After 28th day of experiment laccase activity declined substantially, being recorded ~1.26 U/L in treatment 1 and ~0.14 U/L in treatment 2. Humidity, temperature and the supply of carbon and nitrogen directly influence gene expression of enzymes in fungi [13]. The açaí wastes are a rich nitrogen, amino acids and carbohydrates source, what probably stimulates the mycelial growth and thus the laccase production to obtain these nutrients from the substrate. Similar results were found for different white rot fungi cultivated in other agroindustrial wastes as orange peels [9], banana peels [10] and sawdust [14], which corroborates the idea that agroindustrial wastes are viable alternatives for the fungal ligninolytic complex production.

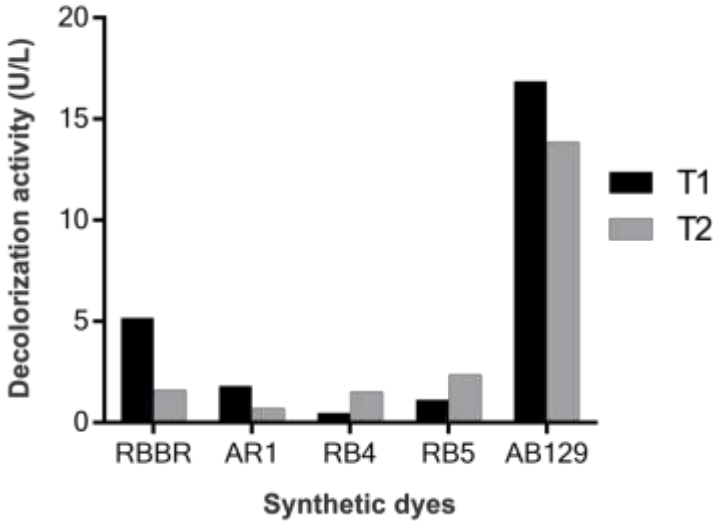

Fig. 2: The decolorization assay of synthetic dyes by the fungal enzymatic activity obtained in the treatment 1 (T1) and treatment 2 (T2).

We performed the decolorization assay using the enzymatic extract obtained on the 21st (treatment 1) and 14th (treatment 2) days of experiment, when the higher laccase activity was seen (Fig. 1). We registered the highest decolorization activity of RBBR (~5.17 U/L), Acid Red 1 (~1.80 U/L) and AB 129 (~16.87 U/L) in the treatment 1 (Fig. 2), which also showed the higher laccase activity (Fig. 1). However we observed the opposite results for Reactive Blue 4 (~1.53 U/L) and Reactive Black 5 (~2.37 U/L), for which decolorization activity was higher in the treatment 2 (Fig. 2). We suggest that other ligninolytic enzymes may have acted together laccase in a mediator-involved dye decolorization mechanism in both treatments, which was also suggested by [15], and they were more efficient in degrading Reactive Blue 4 and Reactive Black 5 than other synthetic dyes. However, it is known that laccase can act alone in the degradation of synthetic dyes, as [12] observed in biochemical assays with laccase produced by *Trametes trogii*, where the laccase inhibition stopped the synthetic dyes decolorization completely. Thus our data corroborate the key role of laccase in the degradation of synthetic dyes.

CONCLUSION

Our results demonstrate that the açaí wastes as a solid fermentation substrate apparently offer a favorable nutrients source for white rot fungus cultivation and provide an expressive laccase activity. There was also a production of an enzymatic complex capable to degrade synthetic dyes, which can cause several ecological problems and can persist in the environment for many years.

As far as we concerned this is the first report of açaí wastes as substrate for white rot fungi cultivation to produce ligninolytic enzymes. Our work provides potentially useful insights into the development of industrial laccase production and the sustainable commercial exploitation of agroindustrial waste products from Amazon rainforest.

CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

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FINANCIAL DISCLOSURE

None

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ARTICLE

IDENTIFYING SITES FOR PROMOTING ECOTOURISM IN PHULWARI-KI-NAL WILDLIFE SANCTUARY (PWLS), SOUTHERN ARAVALLI HILLS OF INDIA

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ABSTRACT

Background: *Promoting eco-tourism in Wildlife Sanctuaries without compromising conservation can provide an opportunity for interested people to be in the midst of biodiversity and rare biota. The study was carried out in Phulwari-ki-nal Wildlife Sanctuary of Rajasthan with an objective to identify potential sites for promoting ecotourism. Plant wealth of the region was enumerated using vegetation samples and information obtained from vegetation samples like richness, diversity, density of all life forms were brought into GIS platform. Normalization and scaling techniques was adopted and weightage was given according to floral components to generate floral diversity map. Based on thematic maps plant diversity hotspot, tourism spots, path for nature walk and habitats for threatened plants and orchids distributions were identified to promote the Ecotourism in PLWS.*

KEY WORDS
Aravalli, Phulwari-ki-nal, Ecotourism, thematic map, GIS

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INTRODUCTION

Phulwari-ki-Nal one of the ecological fragile area, forming the catchments of Wakal (Sabarmati) river is the lifeline of this sanctuary [1]. Presence of diverse micro and macro habitats is said to support considerable number of medicinally important plants and rich flora and fauna [2]. The southernmost sanctuary of Udaipur district and Rajasthan state, which is contiguous with the Polo forests of Vijaynagar Range of North Gujarat Region, forms a bridge for the wild animals to cross between the two forests. It is situated in 24° 00' - 24°10'N latitude and 73° 10' - 73° 20' E longitude [3]. The climate of the area is sub-tropical, characterized by three distinct seasons, Summer (March – June), Rainy season (July – September) and Winter (October – February). Rains, generally starts from mid June and continue till September. The forest of PWLS falls into the II Dry Tropical Forest as per classification [4]. This is further sub-classified into 5B - Northern Tropical Dry Deciduous Forest and C2 – Northern Dry mixed Deciduous Forest. The river and stream courses being rich in moisture provide special microhabitats, which encourage tall evergreen trees with dense undergrowth. The natural regeneration of all species is generally profuse and abundant [3].

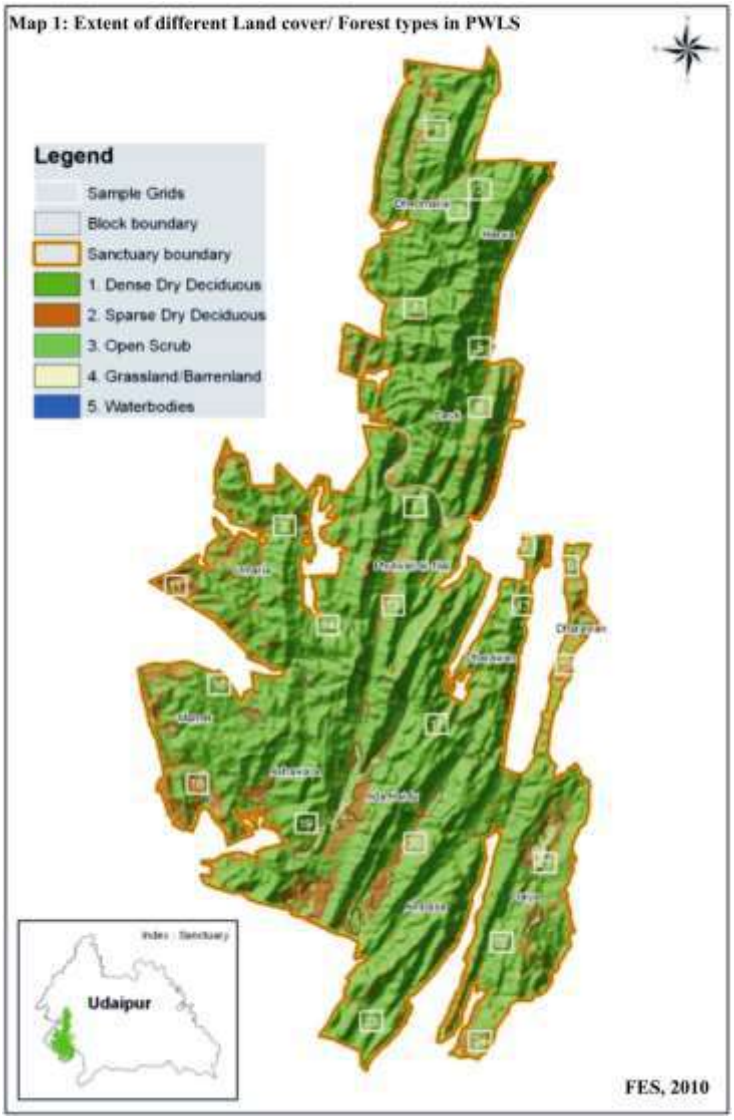
A number of taxa of angiosperms are present in addition to that it is a home for orchids, tuberous plants, climbers and lianas. Based on published information a total 346 species belonging to 20 life forms [Table 1] and had a five species of Orchids [2, 5, 6, 7, 8, 9]. Crustose lichens are also present on rock and tree trunks that increase the floral diversity values of PWLS [2]. There are several deep pools along the riverbed Sabarmati, which are ideal home for crocodiles and other water dependent fauna. This forest is bestowed with patches of *Madhuca latifolia* groves, some extending to even c. 20 ha and forms an ideal habitat for the threatened Indian Flying Squirrel [2]. This sanctuary with its dense forest has rendered protection to threatened and conservation significant floral species viz. *Anogeissus serecea*, *Chlorophytum borivilianum*, *Commiphora wightii*, *Gloriosa superba*, *Streculia urens*, *Tecomella undulata*, and faunal species like Indian Balloon Frog, Mugger or Marsh Crocodile, Alexandrine Parakeet, Grey Jungle Fowl, Aravalli Red Spurfowl, Flying squirrel, Sloth Bear, Leopard, Ratel and Pangolin [2]. The proposed study is "to examine the floral components and promote the ecotourism using geo-spatial technology that will attract the more tourists and also offer economical benefits to the villagers present in and around of PWLS.

MATERIALS AND METHODS

The approach followed is an integrated method that accounts ecological, social, cultural factors of plants resources and biological factors influences the diversity of plants. This was carried out in three steps. (i) The mapping of forest and other related parameters. (ii) Field survey both rapid and intensive and group specific methods to collate information on floral biodiversity. (iii) The collected data processed in GIS flat form. Interpolated data helps in developing floral biodiversity map with Low, Moderate, High and Critical Areas of PWLS.

Mapping the Land use and Forests

This process was started by procuring the topo maps of PAs with the boundaries for the respective forest department, digitized using GIS software and over laid on the LISS IV P6 satellite data of November-December 2006, after geo-referencing boundaries and important places. Then the landscapes falling within the boundaries were classified into different land use categories, with details on broad forest types [10]. Following ground truthing method, the vegetation map was finalized [Map 1].



Map 1: Extent of different land cover/ forest types in PWLS.

Field Data Collection

The entire area (vegetation map) of PWLS was divided into 1 km x 1 km grids. Based on the extent of vegetation types, grids were identified, it was sampled to assess the floral diversity and its components. The selection of grids based on approachability, accessibility, spatially distributed covering different altitude categories and distributed entire landscapes of respective vegetation type [Map 1].

The sampling was being done along a transect running in the diagonal axis of the grid, which extend to about 1.4km. In the case of sacred groves, reserved forest and community forest depending upon their extent of area, transects were used along with perambulation to record the floral components. Along this transect, taxa specific techniques was being adopted. At every 200 m intervals plots are being used to quantify vegetation [11, 12]. The plots are of varying size with 15m radius plots for trees, 5 m radius for shrubs, climbers and recruitment (gbh < 20cm and height >50cm) class of tree species and five plots of 1m x 1m for herbs, grass and regeneration class of tree and shrub (< 50cm height) species, smaller plots nested within the larger plot. For all trees (>20cm gbh at 1.3 m height) information on gbh, height, cutting, lopping signs if any and phenology at the time of sampling are being recorded. For shrubs, the species, cutting, lopping, browsing signs were being documented along with their phenology. In the case of climbers, liana, orchids the species, host plant species, height reached were documented. The species, numbers and percent cover (line-intercept method) was noted for the herbs and the grass species [13]. The numbers were being documented for the recruitment and regeneration class also. Surveys were conducted in all the three seasons (summer, winter & monsoon). In addition to the quantification, listing of plants species as part of inventorization of each selected grids was also being done.

The threatened floral species were also searched along the entire length of transect within a width of 10 – 15m (belt-transect). Along these transects whenever a targeted species was located a species specific plot (vary in size according to the plant form) was used to enumerate and record the abundance, phenology, regeneration, associated species, macro & micro habitat parameters (habitats, terrain, slope, substrate, soil type, soil moisture, ground cover, canopy cover and other related environmental information) and site specific threats to the threatened plants.

The data thus collected were analyzed in grid wise, information like species richness, diversity and density of trees, its regeneration and recruitment, shrubs, climbers, herbs, grass, threatened species and % ground cover were derived into map. All these factors were brought into GIS domain and prepared floral diversity map of PWLS.

Mapping of Floral diversity & Analysis

All these non spatial and spatial data (research components) were brought into GIS domain as separate layer to contribute maps on floral diversity. This analyzed data in terms of each column as variables were attached to the respective grid and used to generate the Arcinfo vector coverage file. Further the grid centers were extracted from Arcinfo coverage file using toolbox of ArcGIS as centroid along with the analyzed data. This point layer of centroids were used to perform the simple ordinary Kriging in Spatial Analyst to generate interpolated continuous raster layer as Arcinfo grids, such Kriging calculates the interpolated values for that particular parameter where the sampling grids was lacking within the extent of PWLS. With this approach floral diversity maps were prepared and ranked in the form of Low, Moderate, High and Critical Areas. Such continuous raster layers were reclassified into four categorical layers or classes as Low, Moderate, High and Very High. Further, distribution of threatened and endemic species, orchid with help of GPS reading that was taken at the time of data collection, were also added to floral diversity map to identify the sites for promoting eco-tourism in PWLS.

RESULTS

Species Richness of PWLS

The secondary information collected on the flora of PWLS, it was apparent that in total 346 species. As part of inventory, a total 515 species of plants was recorded belonged to 297 genera and 89 families. So the PWLS is a home for 616 species belongs to 340 genera and 92 families [3]. These species were represented from 21 life forms or habits [Table 1].

Table 1: Overall Status of Plants Species in Phulwari Ki Nal Wildlife Sanctuary

S. No	Habit/Life Forms	Primary Information			Secondary Information			Overall species in Phulwari Ki Nal WLS		
		F	G	S	F	G	S	F	G	S
1	Aquatic Herb	1	2	2	1	1	1	1	2	2
2	Climber	5	9	12	6	10	12	7	12	15
3	Climbing Herb	1	3	4	1	2	2	1	3	4
4	Climbing Shrub	1	1	1	0	0	0	1	1	1
5	Grass	1	30	53	1	27	35	1	38	69
6	Herb	49	122	201	32	75	112	52	135	239
7	Orchid	1	5	6	1	5	5	1	7	9
8	Parasite	2	3	4	2	3	3	2	3	5
9	Prostrate Herb	2	3	3	1	1	1	2	3	3
10	Scandent Shrub	2	2	3	2	2	2	2	2	3
11	Sedge	1	4	10	1	1	2	1	4	10
12	Shrub	17	28	32	13	22	24	19	35	40
13	Small Tree	17	20	22	16	16	18	19	22	25
14	Straggling Shrub	8	9	11	6	6	6	9	10	12
15	Tree	33	55	81	32	57	79	33	61	94
16	Twiner	5	6	11	6	8	10	6	8	13
17	Twining Herb	4	10	15	3	6	9	5	11	18
18	Twining Shrub	4	6	6	3	4	4	5	8	8

19	Under Shrub	12	32	32	8	17	19	12	28	40
20	Woody Climber	3	3	4	1	1	1	3	3	4
21	Woody Twiner	2	2	2	1	1	1	2	2	2
	Total	**89**	**297**	**515**	**75**	**235**	**346**	**92**	**340**	**616**

Diversity of Plant species in PWLS

Quantification of plants species was performed through grid based method. On the whole 280 plant species were recorded from 67 families and 192 genera. Mature tree showed richness of 52 species with the diversity of 2.55 and density of 234/ha. Regeneration showed 43 species with the diversity of 2.66 and density of 14068/ha. The regeneration ranges from 1000/ha to 42286/ha. 47 species were recorded from recruitment stage, with the diversity of 2.26 and density was 1695/ha (it ranges from 249/ha to 2659/ha). Shrub with a richness of 51 species and diversity showed 2.93, density was 740 shrubs/ha, climbers were 27 species, diversity 2.54 and density 259/ha. Herb form showed 109 species, diversity 2.55 and density was 17/sqm with the ground cover of 21%, finally grass were 28 species with the diversity of 2.12, density was 7/sqm and ground cover was 7 % [Table 2].

Table 2: Floristic Diversity of Phulwari Ki Nal Wildlife Sanctuary

Overall	Mature Tree	Regeneration	Recruitment	Shrub	Climber	Herb	Grass	Total
Family	29	26	26	23	12	31	2	67
Genus	41	38	39	45	21	76	19	192
Species	52	43	47	51	27	109	28	280
Density	234	14068	1695	740	259	17	7	0
Abundance	2432	1034	5006	2186	814	12190	5042	28704
Diversity	2.55	2.66	2.26	2.93	2.54	2.55	2.12	3.82
% Cover	0	0	0	0	0	21	7	28

The mapping of the floral diversity showed that the vegetation assemblage was moderate all over the sanctuary with high diversity found in two distinct patches, one in the central part and other larger patch located in the lower half at the central portion extending to the western boundary of the study area [Map 2]. Very high floral diversity was also recorded at two locations but in comparatively smaller patches. One located at the northern boundary of the Phulwari-ki-Nal forest block and another at the northeastern boundary of Ambasa and northwestern boundary of Daiya forest blocks [Map 2]. The block wise diversity revealed that Dharavan, Daiya, and Adahaldu forest blocks had high to moderate floral diversity, while Phulwari-ki-Nal, Ambasa and Ashawara blocks had moderate to high diversity. Devali, Harwa, Umaria and Mamer forest blocks were found with moderate diversity. Dhedmariya block had moderate to low diversity [Map 2].

Distribution of Threatened Plants and Orchids

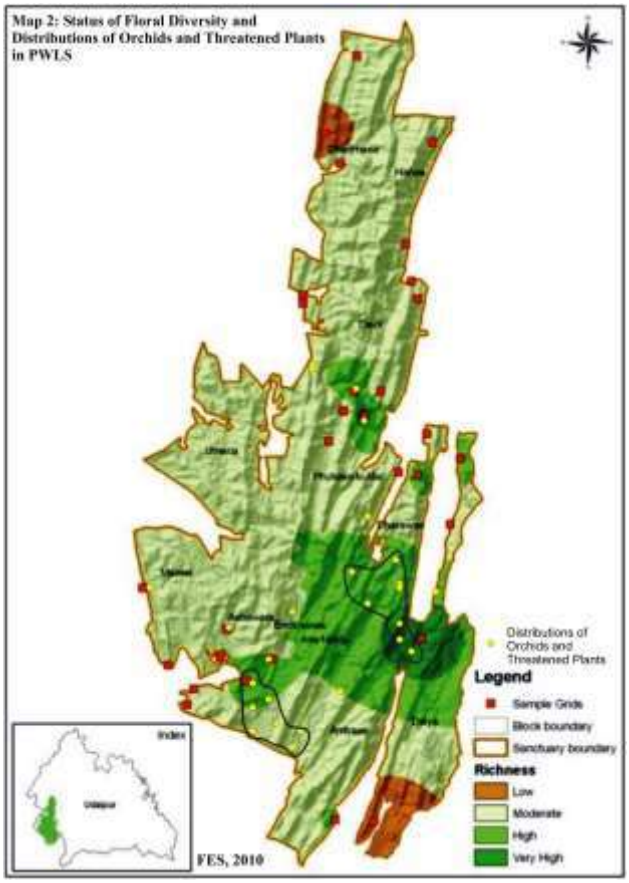
The presence of 25 threatened plants species belongs to 25 genera and 18 families were recorded from PWLS. The threat status revealed that four were endangered, 12 vulnerable, two near threatened, one intermediate and six with status as unknown [Table 3]. The PWLS is a home of 9 orchid species, based on available information 4 aerial and 5 ground orchids were recorded from this sanctuary [Table 4].

The mapping of the richness of the orchids and threatened species being found in central portion and below, majorly two groups, one is located in south west (Ambasa, Ashawara blocks) and other in south east (Dharavan, Ada Haldu and Ambasa blocks) part of PWLS. Few scattered locations in blocks of Phulwari-Ki-Nal, Mamer, Ashawara and Devli were also recorded.

Table 3: Status of Threatened plants species in Phulwari Ki Nal Wildlife Sanctuary

S. No	Scientific Name	Status	Total
1	Alysicarpus tetragonolobus Edgew.	?	49
2	Ampelocissus latifolia (Roxb.) Planch.	?	45
3	Anogeissus sericea Brandis var. nummularia King ex Duthie	I	12
4	Barleria acanthoids Vahl	VU	9
5	Blepharis linariaefolia Pers.	VU	3
6	Boswellia serrata Roxb. ex Cocls.	EN	23
7	Butea monosperma var lutusa (Witt.) Maheshwari.	?	2
8	Celastrus paniculata Willd.	VU	27

9	Costus speciosus (Koenig ex Retz.) Sm.	VU	6
10	Feronia limonia (L.) Swingle	VU	2
11	Gloriosa superba L.	VU	47
12	Habenaria furcifera Lindl.	?	8
13	Manilkara hexandra (Roxb.) Dub.	EN	3
14	Moringa concanensis Nimmo ex Dalz. & Gibs.	VU	4
15	Nervilia aragoana Gaud.	?	168
16	Ocimum gratissium L.	VU	4
17	Oroxylum indicum (L.) Vent.	EN	5
18	Ougeinia oogeinsis (Roxb.) Hochr.	EN	1
19	Peristylus stocksii (Hk. F) Kranz.	?	68
20	Pueraria tuberosa (Roxb. ex Willd.) DC.	VU	5
21	Sarcostemma viminale (L.) R. Br. subsp. viminale	VU	1
22	Schrebera swieteinoides Roxb.	VU	10
23	Soymida ferbrifuga (Roxb.) A. Juss.	NT	9
24	Sterculia urnes Roxb.	VU	3
25	Terminalia arjuna (Roxb. ex DC.) Waight & Arn.	NT	9
	Total Abundance		**523**
	Species Richness		**25**



Map 2: Status of Floral Diversity, Distribution of Orchids and Threatened Plants in PWLS.

Tourism Hot spots

Important areas for conservation through ecotourism were selected from the blocks, which has high numbers of threatened plants and orchids. The selected sites [Map 3] for promoting ecotourism activities are Ambasa-Ashawara (high to moderate diversity) and Dharavan-AdaHaldu-Ambasa (high to very high diversity of floral components). Apart from identified sites, the tourism activity can be promoted in floral diversity rich areas of Map 2 (northern boundary of the Phulwari-ki-Nal forest block, northeastern boundary of Ambasa and northwestern boundary of Daiya forest blocks). Further, the area marked under the low diversity and moderate diversity of vegetation can be improved through conservation measures involving eco-tourist. Tourist will inspire with the ambiance of natural forest cover and values, showing as a model for improving the degraded lands through active participations.

Map 3: Sites for Promoting Ecotourism in PWLS.

Table 4: Status of Orchid species in Phulwari Ki Nal Wildlife Sanctuary

Sl. No	Scientific Name	Pri. Info.	Sec. Info.	Orchid in PWLS
1	Acampe praemorsa (Roxb.) Blatt. & McCann.		*	*
2	Aerides crispum Lindl.	49		*
3	Aerides maculosum Lindl.	*		*
4	Eulophia ochreata Lindl.		*	*
5	Habenaria furcifera Lindl.+	8		*
6	Habenaria longicorniculata Grah.		*	*
7	Nervilia aragoana Gaud.+	168	*	*
8	Peristylus stocksii (Hk. F) Kranz.+	68		*
9	Vanda tessellata (Roxb.) Hook. ex G. Don	*	*	*

+ Threatened Species

CONCLUSION

Promoting tourism is a basic principle of wildlife conservation. Phulwari-ki-nal Wildlife Sanctuary has a good scope of tourism since it has rich floral diversity along with multifarious tribal culture. This sanctuary is a natural home 616 species that includes 9 orchids and 25 locally and globally endangered plant species. But this sanctuary not familiar for the ecotourism activities, very few people visits this sanctuary. Apart from conventional practices, the science and technology approaches will help the forest department do a systematic plan for ecotourism activities. i.e developing tourism facilities, nature trails, identifying more watching point, camping site, publicity, brochure and signage in this sanctuary. Also these kinds of study very useful to have a regular monitoring of biodiversity components and developing a long term conservation plan for PWLS.

CONFLICT OF INTEREST

None

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None

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ARTICLE

COMPARING THE EFFECTS OF REMIFENTANIL, ALFENTANIL, SUFENTANIL, AND FENTANYL ON THE INCIDENCE OF EPIGASTRIC PAIN AFTER ANESTHESIA WITH LARYNGEAL MASK AIRWAY (LMA) IN CATARACT SURGERY

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ABSTRACT

Introduction: *One of the complications of general anesthesia and anesthetic drugs, especially opioids is abdominal pain. The aim of this study is to examine the effects of remifentanil, alfentanil, sufentanil, and fentanyl on the incidence of epigastric pain after anesthesia with LMA in cataract surgery.* ***Materials and Methods:*** *In this analytical cross-sectional study, 104 patients in each group of fentanyl, alfentanil, sufentanil, and remifentanil who underwent cataract surgery and general anesthesia with LMA in operating room of Motahari Hospital, Jahrom were studied. Pearson correlation test was used to determine the relationship between the occurrence of epigastric pain and opioid.* ***Results:*** *In fentanyl group, out of 104 patients, 20 patients (19.2%) had mild abdominal pain, 6 patients (5.8%) moderate abdominal pain, and four patients (3.8%) had severe abdominal pain. In alfentanil group, 11 patients (10.6%) had mild abdominal pain and six patients (5.8%) had moderate abdominal pain. In sufentanil group, 15 patients (14.4%) had mild abdominal pain, five patients (4.8%) had moderate abdominal pain, and 4 (3.8%) had severe abdominal pain. In remifentanil group, 20 patients (19.2%) had mild abdominal pain and 15 patients (14.4%) had moderate abdominal pain. The incidence of abdominal pain has a significant relationship with opioid (P=0.009).* ***Conclusion:*** *According to the present study, it was revealed that the incidence of abdominal pain in remifentanil group is more than the others and in alfentanil group is less than the other groups, which indicates opioid acute tolerance in association with remifentanil.*

KEY WORDS
cataract, epigastric pain, laryngeal mask airway

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INTRODUCTION

Any opacity in the lens of the eye is called cataract [1]. Among the major known causes of cataract age, genetic factors, inflammation, blow, and so on can be noted [2]. Ninety percent of all cataracts are related to age. [3] In various studies, it is found that the prevalence of cataract in women is more than in men and most patients are older than 65 [4]. Sudden movements or attempts to cough while eyeball is open can lead to protrusion of eye contents and permanent damage to the eye. For these reasons, when general anesthesia is chosen for cataract surgery, it is necessary to maintain sufficient depth of anesthesia [5]. The greatest responsibility of the anesthesiologists is to provide enough breathing for patient, and the most vital factor in this regard is airway protection [5]. Placing LMA is a non-invasive alternative approach appropriate to replace endotracheal intubation and in short-term surgeries and in difficult intubation, it is an acceptable method that due to lack of need for laryngoscopy does not have the strong adverse consequences related to it [6 -8]. Establishing hemodynamic stability with LMA shows that, it can easily be used in people with cardiovascular and respiratory diseases [9]. Placing LMA during anesthesia induction prevents the sharp rise in blood pressure and tachycardia during induction of anesthesia [10]. Therefore, a simple way to keep the airways that brings about effective breathing and oxygenation is LMA [11]. Fentanyl analgesic, with 80 times more analgesic effect as morphine was introduced to medicine as intravenous anesthetic in the 1960s. Its main use is as pre-medication and sedation before anesthesia in the operating room. Today, fentanyl is widely used for anesthesia and for relieving pain. Its effect mechanism is μ opioid receptor agonist and its side effects are decreased diastolic blood pressure and blood-oxygen saturation decrease, nausea, and vomiting [12]. Sufentanil is used as the main or supplemental anesthesia drug due to its power to weaken the central nervous system. This drug is 7 times more powerful than fentanyl, and its return from anesthesia is more rapid than fentanyl [13]. Alfentanil is a preserving drug in anesthesia and pain control after surgery. Its effect is short-term and is a narcotic painkiller used as supplementary medicine as well as for induction of anesthesia. Through intrathecal and dural injection, this medicine is used to create analgesia after surgery [14]. Remifentanil has more rapid effect onset and shorter duration of effect, so that in comparison with fentanyl and the similar drugs, it has shorter half-life [about 5 minutes]. Moreover, like alfentanil, it reaches its peak quickly. Remifentanil power is slightly less than fentanyl. Remifentanil has a significant role in modern anesthesia and is a safety drug for continuous infusion. Its most common use is administration along with propofol in total intravenous anesthesia (TIVA). In addition, it is useful as a single dose when a short-term analgesia is needed [13]. In the study by Jalalian Taghadomi et al. who had studied the role of remifentanil in the development of abdominal pain after cataract surgery, the results showed that in remifentanil group, 20 patients (40%) complained of severe abdominal pain during recovery and in the propofol group, only three subjects (6%) complained of blurred abdominal pain around the abdomen. In remifentanil group, in 14 patients with

abdominal pain, intravenous injection of hyoscine was effective, while it had no effect on the propofol group. According to the mentioned study, abdominal pain is a rare complication, but according to clinical observations of the researcher in the operating room, it seems that the prevalence of abdominal pain (in the epigastric region) in operating rooms of Jahrom hospitals is more than the amount listed in other studies. The objective of this study is to find the prevalence of this complication and to study the related factors to reduce this complication. Therefore, studying the prevalence of epigastric pain after anesthesia with LMA with remifentanil, alfentanil, sufentanil, and fentanyl drugs after cataract surgery appears necessary.

METHODS

The present study was analytical cross-sectional. Sampling method was convenience. The sample in this study was 104 patients, studied in fentanyl, alfentanil, sufentanil, and remifentanil groups, 416 patients were studied. Inclusion criteria included all patients of 50 to 75 years of age group that undergo cataract surgery with phacoemulisification device and general anesthesia with opioid that fall into classification Group ASA I (American Society of Anesthesiologists) (without sickness and healthy) and ASA II (with mild systemic disease). Exclusion criteria included a history of chronic abdominal pain, ischemic heart disease, gastroesophageal reflux, pulmonary aspiration, gastrectomy, diabetes, history of extensive abdominal surgery, gastric and duodenal ulcer history, and any change in homodynamic condition of the patient that requires injection of other drugs. Before implementing the project, the researcher received the approval of project and licensing from research committee, permission from the Ethics Committee, and coordinated with operation room officials, and Motahari Hospital administration. Patients, undergoing eye surgery (cataract) for whose anesthesia opioid is used, after recovery and gaining full consciousness are examined and asked about the presence or absence of epigastric pain (as yes or no). Previously, the patients are also asked question about the history of stomach pain, so as during the examination after consciousness not to mistake the abdominal pain because of a history of stomach problems with cramps caused due to the use of opioid. The patients are also asked questions about the novelty or old pain. It should be noted that in all the patients studied to induce anesthesia, sodium thiopental (3-4 mg/kg) and atracuriom (0.5 mg/kg), and for the maintenance of anesthesia, propofol (50-150 µg/kg/min) with an infusion pump were used. Moreover, alfentanil (25µg / kg), sufentanil (0.25µg / kg), fentanyl (2-4µg / kg), and remifentanil (1-2µg / kg) were used. The results of the information contained in the questionnaire were statistically analyzed using SPSS version 22 and Pearson Chi-Square test.

RESULTS

In this study, 416 patients who underwent cataract surgery under general anesthesia with LMA entered the study. They were 53.1% male and 46.9% female. Among them, 23.6% were at age 55-50 years, 19.0% at age 60-56 years, 18.8% at age 61-65 years, 16.1% at age 66-70 years, and 22.6% were aged 75-71 years. The incidence of epigastric pain after anesthesia with LMA in cataract surgery was 25.5%. Ninety eight point three percent of patients had duration of 30-39 minutes of mask use, and 1.7 percent had 40-50 minutes if mask use. Among the patients, 18.5% were in the weight range of 60-69 kg, 54.3% were 70-79 kg, 21.9 percent were 80-89 kg, and 5.3% were in 100-90 kg weight range. The patients were divided into four groups of 104: remifentanil, alfentanil, sufentanil, and fentanyl.

Table 1: The incidence of epigastric pain according to the drug

Opioids	Epigastriac pain			
	No pain	Mild	Average	Severe
Fentanyl	74	20	6	4
	71.2%	19.2%	5.8%	3.8%
Alfentanil	87	11	6	0
	83.7%	10.6%	5.8%	0.0%
Sufentanil	80	15	5	4
	76.9%	14.4%	4.8%	3.8%
Remifentanil	69	20	15	0
	66.3%	19.2%	14.4%	0.0%
Total	310	66	32	8
	74.5%	15.9%	7.7%	1.9%

Table 2: Comparison of the effect of remifentanil, alfentanil, sufentanil, and fentanyl on the incidence of epigastric pain using Chi-square test

		Epigastric pain		Pearson Chi-Square	p-value
		no	Yes	9.166	0.027
		n (%)	n (%)		
opioid	fentanyl(N=104)	74(71.2)	30(28.8)		
	alfentanil(N=104)	87(83.7)	17(16.3)		
	sufentanil(N=104)	80(76.9)	24(23.1)		
	remifentanil(N=104)	69(66.3)	35(33.7)		

Chi-square test results show that there is a significant difference between remifentanil, alfentanil, sufentanil, and fentanyl on the incidence of epigastric pain after anesthesia with LMA in cataract surgery (p-value = 0.027). Remifentanil and alfentanil had the highest and lowest incidence of pain in the epigastric region respectively. The incidence of epigastric pain in patients using fentanyl has been 28.8%, in alfentanil 16.3%, in sufentanil 23.1%, and in emifentanil 33.7%.

In the fentanyl group, 20 patients had (19.2%) mild epigastric pain, 6 patients (5.8%) moderate epigastric pain, and four patients (3.8%) had severe epigastric pain. In alfentanil group, 11 patients (10.6%) had mild epigastric pain, and six patients (5.8%) had moderate epigastric pain. In sufentanil group, 15 patients (14.4%) had mild epigastric pain, five patients (4.8%) had moderate epigastric pain, and four patients (3.8%) had severe epigastric pain. In remifentanil group, 20 patients (19.2%) had mild epigastric pain, and 15 patients (14.4%) had moderate epigastric pain.

Table 3: Incidence of epigastric pain in terms of surgery time

Epigastric pain		Time (minutes)	
		30-39	40-50
No pain		307	3
		99.0%	1.0%
Mild		64	2
		97.0%	3.0%
Average		32	0
		100.0%	0.0%
Severe		6	2
		75.0%	25.0%
Total		409	7
		98.3%	1.7%

Chi-square test results show that the incidence of abdominal pain has a significant relationship with operation time (P-value=0.000)

Table 4: Incidence of epigastric pain based on gender

Epigastric pain		Gender	
		Men	Women
No pain		177	133
		57.1%	42.9%
Mild		26	40
		39.4%	60.6%
Average		13	19
		40.6%	59.4%
Severe		5	3
		62.5%	37.5%
Total		221	195
		53.1%	46.9%

Chi-square test results show that epigastric pain has no significant relationship with gender (P-value=0.138). Chi-square test results show that epigastric pain has a significant relationship with patients' age (P-value =0.003).

DISCUSSION

The aim of this study was to examine the effects of remifentanil, alfentanil, sufentanil, and fentanyl on the incidence of epigastric pain after anesthesia with LMA in cataract surgery. The main finding in this study was that the incidence of abdominal pain has a relationship with opioid (P-value=0.27), so that the incidence of epigastric pain in remifentanil group (33.6%) is more than in all groups and is the lowest in alfentanil (16.3 %). Few studies have examined the effect of remifentanil, alfentanil, sufentanil, and fentanyl on the incidence of epigastric pain after surgery. Among few studies conducted, the study by Jahanbakhsh et al. (2007) at Khatam Alanbya Hospital, Mashahd, which evaluated 100 patients, is consistent with results obtained from our study [14]. They found that, 40 percent of patients who received remifentanil had abdominal pain during recovery. In our study, 34.6% of patients in the remifentanil group complained of abdominal pain while in the fentanyl group 28.8%, in alfentanil 16.3%, and in sufentanil group 23.1% complained of abdominal pain. Our results are in line with the results of this study [14].

In the research by Guignard B, Bossard AE, Coste C et al. (2010) on 50 patients undergoing major abdominal surgery, it was found that in remifentanil group, the required morphine for pain relief after surgery was twice the patients in desflurane group. In our study, the highest incidence of abdominal pain was in the remifentanil group [15]. However, in the study by Gustorff B, Hanlik G, Hoerauf KH et al. (2012) on 20 patients, which was conducted in remifentanil and control groups, there was no significant difference in pain perception threshold after 180 minutes infusion that is in contrast with the result of result of our study [16]. In research by Schraag S, Checketts MR, Kenny GN (2009) on 51 patients receiving alfentanil after cardiac surgery, and 30 patients receiving remifentanil after orthopedic surgery, no significant difference was observed in pain.

This result was inconsistent with the results of our study as well [17]. In the study by Derrode, N, et al. (2009) on 50 patients who underwent laparoscopic ventral surgery, the consumption of morphine in remifentanil was more than sufentanil group in the first 2 hours after surgery, which is in line with our research [18,19]. Another result of our study was that the incidence of abdominal pain is correlated with operation time (P=0.000). It was also observed that abdominal pain has no significant relationship with gender (P-value=0.138). The rate of incidence of abdominal pain in female patients was more than male. Another result of our study was that the incidence of abdominal pain is correlated with the patient's weight (P-value =0.035). It was also observed that the incidence of abdominal pain is correlated with patient age (P-value=0.003). However, no other studies have been conducted in this area to examine the results of our study further, and with this small number of studies, there is more need for such research.

CONCLUSION

According to the present study, it was revealed that the incidence of abdominal pain in remifentanil group is more than the others and in alfentanil group is less than the other groups, which indicates opioid acute tolerance in association with remifentanil.

SUGESSIONS

In general, given much controversy about the effect of the opioid, especially remifentanil on postoperative epigastric pain, it is recommended that in future studies broader, more comprehensive, and more studies be conducted on the role of opioids, especially remifentanil, in this regard. Due to the high rate of epigastric pain in remifentanil, it is better to use other opioid with longer effect in surgeries.

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CONFLICT OF INTEREST

None

FINANCIAL DISCLOSURE

None

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ARTICLE

IDENTIFYING PROCESS IMPROVEMENT OPPORTUNITIES IN GYNECOLOGY CLINIC BY VALUE STREAM MAPPING

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ABSTRACT

Introduction: *Value stream mapping is one of the most important and useful tools of lean thinking to determine non-value adding activities and improve service delivery process. The present study aimed to identify improvement opportunities in visit process in the gynecology clinic using value stream mapping.* ***Methods:*** *This was a descriptive case study. In this study, flowchart of outpatient visit was drawn through observation and confirmed by semi-structured individual interviews with those involved in this process. Current value stream map was drawn and discussed in a focus group discussion. Various types of wastes, root causes and elimination strategies were evaluated. Then, future value stream map was drawn using Edraw.* ***Result:*** *Only 20% of current value stream map of outpatient visit in the gynecology clinic consisted of value adding activities. The patients spent between 79% and 85.2% of their time on waiting depending on type of the patients. The number of value adding steps increased from 20% to 33% while the number of non-value adding steps reduced from 73.3 to 33% in future value stream map.* ***Conclusion:*** *most wastes in outpatient visit process in the genecology clinic can be detected and compensated by minimal costs. This requires scientific tools like lean thinking [e.g. value stream mapping] for process improvement.*

KEY WORDS
value stream mapping, lean, process improvement, clinic, outpatient

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INTRODUCTION

Increasing costs of global health care systems is one of the main concerns of managers and decision makers in the health system [1]. Iranian health system as other health systems is facing the challenge of increased costs. Accordingly, health care cost has increased 71 times during the last twenty years [2]. Despite various investments, health system faces such challenges as poor quality of services and healthcare, medical errors, long waiting time, low levels of patient satisfaction and wastes in health care services [3]. A significant portion of health care processes (between 30% and 60%) added no value tothe patients [4].

In this regard, a variety of quality improvement methods are developed to address the inadequacies reported in healthcare services. Lean thinking is one of these methods, which has emerged from manufacturing industry. Lean thinking is a management strategy universally applicable across all organizations because it deals with work processes. Lean is a set of theories and practices, which create maximum value for the patient by reducing wastes and waiting times [5]. Lean management can be summarized as five principles of Specify value from patient perspective, identifying value stream, Make the process and value flow, creating a pull system and a Pursue perfection [6]. The most important and most widely used lean tool is value stream mapping [VSM], which is referred to as a bridge between lean concepts and methods [7]. VSM is a visual representation of the flow of materials, information and people from the beginning to the end of a process. This includes all activities in that process categorized in three categories of value adding [VA] activities, non-value adding [NVA] activities and essential non-value adding activities [8, 9]. Value adding activities directly meet customer needs [10]. Essential non-value adding activities add no value to the customers but are currently inevitable due to technical knowledge and available assets [10]. In addition, any activity that spends time, space or resources but does not create any value for the customer is called non-value adding activity [10]. VSM visualizes the whole process in the system and delineates wastes and sources of wastes. This is an effective tool for improvement in communication process [9]. This tool helps managers to identify priorities for process improvement. VSM presents a visual display that enables the stakeholders (physicians, senior managers and accreditation assessors) to understand the given process easier and more comprehensively. There are two types of value stream map: a) current value stream map (CVSM) that displays current state of material and information flow in the system; b) future value stream map (FVSM) that shows ideal state of the system [11].

Outpatient department (OPD) is the first one who is in contact with the hospital and is one of the most important aspects of the health system. Nowadays, a major part of diagnosis and even treatment is performed on an outpatient basis with great advances in medical technology. This has increased the size, variety and severity of outpatients [12]. OPD deal with various problems such as over crowding and delays that lead to dissatisfaction of the patient [13]. OPD department is less considered than other wards and departments in a hospital despite growing importance of this department in the hospitals. Most OPD departments are not prepared to meet probable challenges. Various studies should be conducted in the field of personal and organizational changes in OPD departments [14]. Long waiting time and complexity of services in the studied hospital increased the sensitivity of making decision on promoting quality of this

process. The present study aimed to determine improvement opportunities in visit process in a genecology clinic in a large public teaching hospital in Tehran using VSM.

MATERIALS AND METHODS

This was a case study performed in a genecology clinic in teaching hospital in Tehran in 2015. Visit process at the genecology clinic was evaluated in this study with participation of personnel. A sample of 30 individuals were selected using convenience sampling method to determine patient flow and measure waiting time in the clinic. The patients were selected based on Jimerson's recommendations. He believed that selection of 30 samples gives reliable results for evaluation of process engineering [15].

Data was collected through observation, interviews, documentation review and focus group discussions at multiple steps. First, flowchart of visit process at the gynecology clinic was drawn through observation in. The scholar attended the clinic and accompanied the patient from the beginning to the end of the process [from admission to discharge from the gynecology clinic]. Accuracy of the flowcharts was confirmed through semi-structured individual interviews with the participants. Patient waiting time and cycle time of each activity was measured using a chronometer. The collected data was logged into collection forms, which were a combination of the forms used in the studies conducted by Rodrigurez and Casey [16, 17]. Accordingly, a draft of value stream map was prepared. The CVSM was evaluated in a focus group discussion where VA activities, NVA activities and barriers to process improvement were identified. Improvement strategies were offered after identifying root causes and evaluation of wastes. The value stream map was drawn at the final step using Edraw version 7.6.

Data validity or credibility was confirmed through reflecting on participant review. Data reliability was confirmed through peer review. The study design was confirmed in the Research Ethics Committee in Shahid Beheshti University of Medical Sciences with Approved Act No 66000474 on 11/2/2015.

RESULTS

The findings showed a typical patient would wait 143.3 minutes when visiting the genecology clinic for the first time to receive medical services, which lasted for 28.7 minutes according to the value stream map.

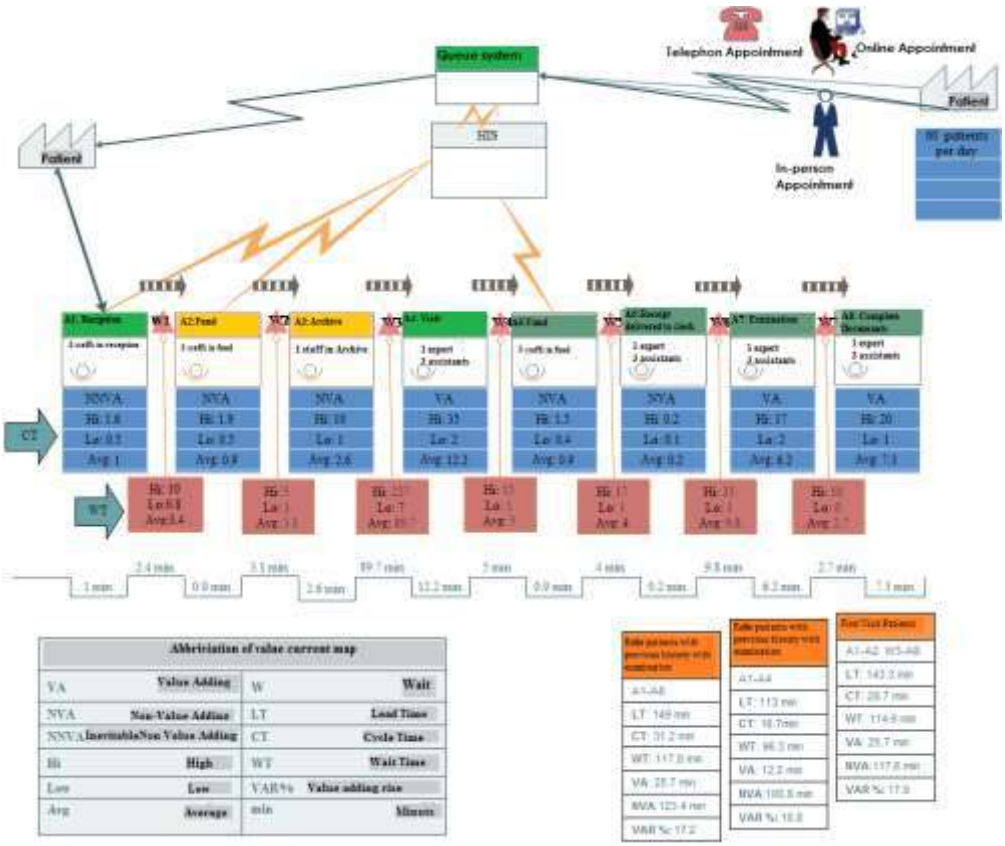

Fig. 1: Current value stream map for outpatient visit in the genecology clinic

In other words, 80% (114.6 minutes0 of the patient time is spent on waiting on the system and only 20% (28.7 minutes) of the patient time was spent on receiving medical care by the personnel on average.

Waiting time for follow-up patients t who were both visited and examined was calculated as 79% (117.8 minutes) on average. Waiting time for the patients who were only visited and not examined was calculated as 85.2% (96.3 minutes). VA activities time for these patients were respectively 17.9% (25.7 min), 17.2% (25.7min) and 10.8% (12.2 min). Then, CVSM was drawn [Fig. 1]. This map was studied during a focus group discussion and available wastes were identified from a lean perspective. Effective strategies were proposed to eliminate or reduce these wastes.

There was no continuous flow in visit process due to waiting time between various activities. In addition, visit process in the genecology clinic followed a push system in which the patient should wait between different activities. The patients were driven from one activity to another one throughout the value stream regardless of readiness of personnel to offer health care services. The push system results in accumulation and waiting of the patients between different activities.

The FVSM [Fig. 2] aimed to draw continuous patient flow of the outpatient visit. If impossible, the activities were linked to each other through a pull system. Work cells was the first step to avoid push system in order to reorganize the activities and eliminate waiting time between different activities. A continuous patient flow is developed by eliminating unnecessary waiting times, increasing NVA activities and reorganizing the activities.

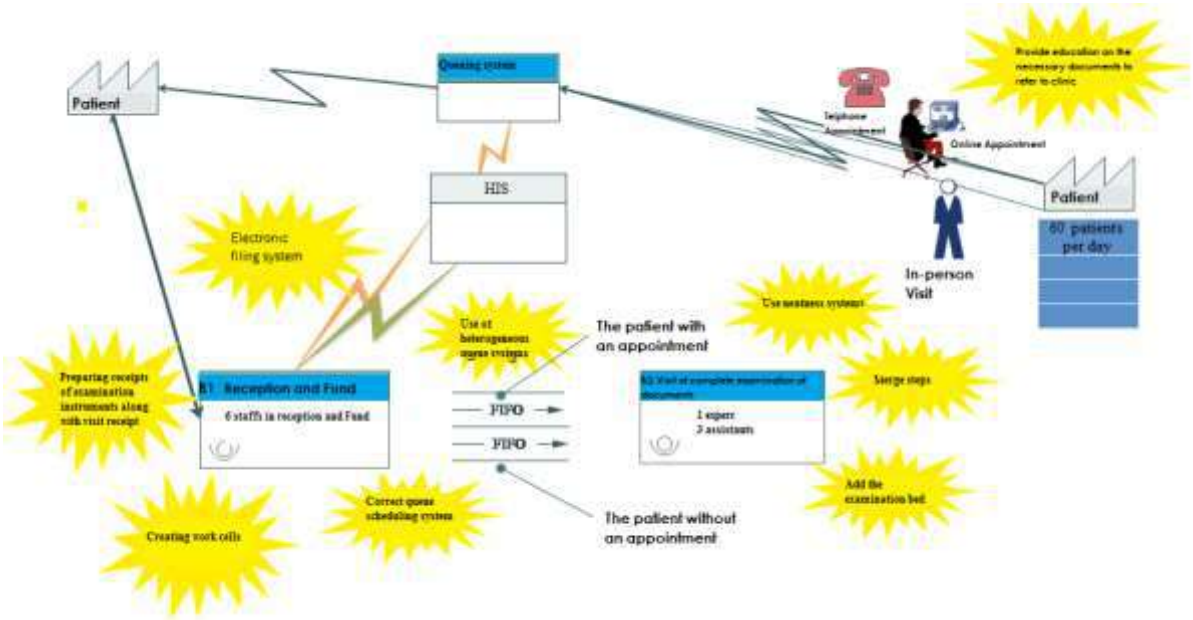

Fig. 2: Future value stream map of outpatient visit in the gynecology clinic

Electronic filing system was used to not only eliminate the archive step and the waiting time before this step but also eliminate the wastes caused by additional movements, correction and transfer. Delays identified in the CVSM were caused by additional movements and transfer, which were eliminated using 5s as well as identification and control of required papers and items at the beginning of the shift. These issues were eliminated in the FVSM.

The highest waste in visit process in the genecology clinic was detected in waiting time prior to physician visit, which was accounted for 60% of total Cycle time on average. This issue was resolved in the FVSM using heterogeneous queuing system and modification of queue scheduling system.

The FVSM consisted of two activities after reorganizing the activities and developing work cells. These two activities were attached to each other using FIFO lines in the proposed map to avoid a push system and create a pull system. FIFO lines are suitable to attach movements in visit process in order to implement a pull system. This is because the patients are stable in visit process in contrast to emergency cases. Thereby, the process is organized using FIFO lines, which are scheduled using appointment system.

A mixed-registration-type appointment system was proposed in this study. Thus, 50% of available time slots were assigned to scheduled patients in a heterogeneous and alternative manner. Other appointments were assigned to walk-ins patients. In previous appointment system, walk-ins were visited after the patients with prearranged appointments. Thereby, they waited shorter time to be visited. Thus, two FIFO lines were defined for the clinic secretary. The first line belonged to pre-scheduled patients and the second line belonged to walk-ins patients. Therefore, the patients were called from the first line at first. If no patient answered, the second line would be called.

When maximum number of patients [predefined by the system] was detected in FIFO lines or at least one patient had spent the highest possible time on waiting [predefined by the system], a reactive plan for reallocation of resources would be implemented. The residents were asked to help to maintain a continuous flow in the visit process due to low number of patients in the clinic for prenatal care and prevention and treatment of cancer and plenty of free time of the residents in this clinic. Therefore, the secretary at genecology clinic would refer the patients to the clinic for prenatal care and prevention and treatment of cancer as soon as the number of waiting patients or waiting time reached the maximum threshold. As a result, patient waiting time is controlled and predicted by FIFO lines. However, effective management of FIFO lines requires careful planning and willingness of the employees to help each other to maintain a continuous movement in the visit process. In other words, the residents would be drifted from one activity to another one, which necessitates a strong relationship between the personnel and attitude change toward effective cooperation in the process.

According to the above-mentioned materials, the FVSM follows a push system that allows continuous flow of the patient. Seven lean wastes were identified in the FVSM and effective strategies were proposed to eliminate or reduce these wastes. A review and comparison between the current and future maps revealed that complexity of the process has significantly decreased. The FVSM contains only two work cells and FIFO lines. Eight activities and seven waiting times in the CVSM were reduced to two activities and one waiting time in the future value steam map. The number of VA steps has increased from 20% to 33% while the number of NVA steps has decreased from 73.3% to 33%.

DISCUSSION

The findings showed that the time of VA activities has increased from 10.6 to 17.9 depending on type pf the patients. The s spent from 79% to 85.2% of their time on waiting at the genecology clinic. These findings are consistent with findings of those studies representing huge waste of time and high share of NVA activities in the field of health. The findings of a study in primary care in the UK showed that 67 processes are available in main services for healthcare in which 65% are in vain and can be eliminated in the FVSM [18]. Abu-Hamdet al. showed that 78% Drug round process refers to NVA activities on average in Ireland [19]. Analysis of value stream map for purchasing endovascular stents showed that only two processes from total 13 processes were VA activities and only 1.92% of the time was spent on purchasing stents as a VA activity and only 15.4% of human resources performed VA activities [20]. A study in the Netherlands showed that only 13.3% of esophageal cancer treatment activities are value adding [21]. The present study and the above-mentioned studies showed inefficiency of patient flow in many health care facilities and highlighted the necessity to identify wastes and take measure to improve this process. In other words, many efforts of staff and other rare and valuable resources are dedicated to NVA activities. In this system, the patients are involved with long processes, waiting times and huge wastes. Therefore, the patient as the main customer is not satisfied despite efforts of employees and resource allocation. Thus, the necessity for process improvement methods such as lean is highlighted more than ever.

Long waiting times at the clinics in public hospitals are main obstacles to service delivery in the OPD department, which was addressed in previous studies. AeenParast showed that waiting time to be visited by a physician was 87.4 minutes at orthopedic clinics [22]. Another study at clinicsin Shariati Hospital in Tehran showed that average patient waiting time was 121 minutes [23]. All these findings are consistent with findings of this study and show that examination room is the bottleneck of services delivery to outpatients. Long waiting time is the most important factor for dissatisfaction of patients in the OPD. Previous studies suggested that reduced waiting time is effective in overall satisfaction with outpatient services [24]. According to the above issues, attention of senior managers, periodic monitoring, determining root causes of waiting times and bottlenecks are necessary to improve the process of providing outpatient services.

The findings showed that such factors as inefficiency of appointment system , presence of large numbers of walk-ins in early hours of the shifts and lack of timely presence of physicians at the clinic lead to long waiting time. Hong believed that root causes of many problems in the OPD are inefficient appointment system and schedule of service delivery. These findings are consistent with findings of the present study [24].

Various studies have been conducted in the field of reduced waiting time where scholars have used two methods.

1- Changes in distribution of the patients in the system through appointment system [, 25].
2- Changes in clinic schedules and hours of human resource activities [26].

Since this study was conducted in a teaching hospital and residents were involved in education and healthcare activities at the beginning of the day, it was not possible to change working hours of the residents in an effective manner to improve visit process. In such circumstances, it is essential to schedule arrival of the patients at the clinic in order to not only reduce patient waiting time, but also increase human resource and equipment productivity.. An efficient appointment is one of the most important priorities in improving service delivery [27]. It seems that using an efficient appointment system and regulation of services delivery can reduce waiting times and increase satisfaction of the patients and the personnel.

The results showed that the delays caused by Transportation and Motion (e.g. the physician goes to another room to get examination instruments, the physician or the personnel go from one room to another one to search for necessary papers and equipment) can be eliminated using 5s. Searching for necessary equipment and forms were causes of waste in a urology clinic in a University Hospital in Canada, which were resolved using the 5s [28]. Mahabadi also used this method in a studyto reduce patient presence in the system [29]. Khatibi also stated that one of the five most important causes of interruptions in operations was unavailability of equipment [30]. The results of this study are consistent with findings of the present study, which showed that 5s is an effective strategy for improvement in clinic workspace, decrease in time of implementation of tasks, faster access to necessary equipment and tools.

The present study had several limitations. First, identification of all wastes in the visit process is a time-consuming and challenging task, which requires a culture based on lean thinking at all levels of the organization to identify a thorough list of these factors. Second, only improvement opportunities were identified in the visit process. It is expected to achieve significant results in case that an effective intervention was applied to the case study.

CONCLUSION

The findings of this study showed that most of the proposed strategies could be implemented solely by modifying the existing processes without using either new human resources or expensive technologies. Hopefully, these strategies would reduce waiting time of the patients visiting the clinic and consequently might reduce average time of patient visit process. Education and institutionalization of lean management [particularly VSM] can be an effective step to facilitate implementation of healthcare reform plan, particularly the service package designed to improve the quality of visit process in outpatient clinics.

CONFLICT OF INTEREST
There is no conflict of interest.

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ARTICLE

MOLECULAR STUDY OF CUTANEOUS LEISHMANIASIS HUMAN RESERVOIRS AND INFECTIONS IN BASTAK

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ABSTRACT

Introduction: *Leishmaniasis is one the six most important diseases in tropical areas. Study and research on various aspects of it is recommended and supported by the World Health Organization. Broom tail rodents (Rodentia: Muridae: Gerbillinae) are the most important reservoir hosts of parasite causes wet cutaneous leishmaniasis (or Zoonotic Cutaneous Leishmaniasis (ZCL) disease) means leishmania major. Different species of rodents from different regions of Iran play role in maintenance of the parasite. The purpose of this research is molecular study of cutaneous leishmaniasis human reservoirs and infections in Bastak County, Hormozgan Province, Iran.* ***Materials and Methods:*** *Live traps were used to catch rodents. The rodents were anesthetized with chloroform in the field and four slides were prepared from earlobe of each animal by sanding method and their morphometric characteristics were completely measured and recorded. Rodent abdomen was opened with blade and the liver and the spleen of each sample were kept in a numbered container containing 70% ethanol. The prepared slides were examined with microscopic (Giemsa staining) and molecular methods. Finally, using Nested-PCR method, they were evaluated using species-specific primers (LIN R4, LIN 17, LIN 19).* ***Results:*** *A total of 108 rodents from five species of three different genera were collected. Meriones persicus species with 26.9% was the predominant species. A contamination in T. indica species and two cases of contamination in two samples of female M. hurrianae were observed. L. major species was also identified.* ***Conclusions:*** *Broom tail rodents are abundance and have relatively high diversity in Bastak County. They live in proximity to human dwellings. Tatera indica and Merioneshurrianae species are introduced as the disease reservoir hosts in this area and use of molecular and PCR methods provides the possibility of faster diagnosis of parasite species.*

KEY WORDS
Cutaneous leishmaniasis, Nested-PCR, Tatera indica, Meriones hurrianae, L. major

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INTRODUCTION

Leishmaniasis (Cutaneous leishmaniasis (CL)) has long been known in Iran. In Iranian ancient books, including Canon of Medicine compiled by Avicenna (Ibn Sina), a wound called “khyroniyeh” was mentioned. Its treatment was difficult and lasted a long time and was resistant to different medications. By referring to signs and its symptoms mentioned about the wound, it is thought to be lesion of leishmaniasis [1]. Cutaneous leishmaniasis is a chronic disease of skin and named differently in various areas (e.g., it is known to Salak in Shiraz and Lakehye Sal in Mashhad). The disease is especially observed in warm seasons and in tropical regions. It is caused by a protozoan of genus *leishmania* and *Trypanosomatidae* family. It can be transferred between human and some animals, including rodents and dogs and it can also transfer by a sandfly (*Phlebtomus*) from a patient to others. Leishmaniasis arises at first as a small papule and then becomes larger (nodule), and finally converts to wound. It is possible that the wounds recover spontaneously in a few weeks or even several years [2].

Leishmaniasis is one of the most common parasitic diseases in Iran that is called urban (dry) and rural (wet) Leishmaniasis. *Leishmaniatropica* and *L. major* species are agents of urban (dry) leishmaniasis and rural (wet) leishmaniasis, respectively [3].

The presence of parasite can be identified by microscopic observation of slides stained with Giemsa. However, its type is not recognizable [4]. Molecular techniques are widespread in recent years to detect leishmaniasis. One of these techniques is determination of parasite type by PCR method. This method can be done even with small number of parasites in a sample [5]. Leishmaniasis is not so problematic in comparison with other diseases. However, the wounds caused by it due to various reasons may leave some traces as improper and disfiguring scars. Identification of parasite species is effective in adoption of the disease prevention and control programs. Its classification to different species and strains is very difficult due to several reasons and similarity in appearance of parasite species. Epidemiological and clinical evidence are not alone effective in differentiating between various species [7]. Since the first step in planning for control and fighting a disease is determination of exact specification of pathogens, molecular studies that in addition to detection of genetic diversity among species and within species, diagnose the genus of *Leishmania*, are of great importance [7, 8].

So, study and investigation of cutaneous leishmaniasis molecular reservoirs and human infections is necessary to control the parasite. Since the evaluation process of the disease is greater in warm climates, Bastak County with a warm climate is a suitable area to evaluate the subjects.

MATERIALS AND METHODS

To determine reservoirs of cutaneous leishmaniasis and parasites cause the disease in the patient, rodents’ hunting was performed in spring, autumn and winter of 2014 in selected villages of Bastak County (the city of Bastak location; longitude: 54 degrees and 23 minutes; Latitude: 27 degrees and 14 minutes; height 2120 m above sea level), which were selected from different climatic regions of the County. Once in a month and each time 10 Sherman live traps were installed at sun sets around active

nests and they were collected in morning of the next day. Date palm, cucumber and walnuts were used as bait.

After collecting the traps containing rodent, they were placed in a plastic bag and were anesthetized with chloroform-soaked cotton. When the researchers became sure of the animal death, its abdomen was opened using a sterile surgical blade and the liver and spleen were placed in a numbered container containing 70% ethanol. After clearing the abdomens, rodent bodies in containers containing 10% formalin were sent for taxidermy and identification to Zoology Laboratory of Shiraz University. Rodents' identification was conducted in Zoology Laboratory of Natural History Museum of Shiraz University and by the help of Iranian rodent diagnostic key [1]. To confirm the suspicious samples, they were sent to Rodent Research Center of Mashhad University.

Two slides were prepared from Serosity of the ear and the tail of each animal using sanding method. The prepared slides from the ears and the tails of the rodents were examined using microscopic method and Giemsa staining for observation of amastigotes. In case of observation of Leishman body, DNA extraction process began for the positive slides using Motazedian et al. method [9]. DNA extraction from the liver and the spleen was also carried out using Azizi et al. method [10-12]. Nested-PCR-based approach was used for proliferation of variable region of Leishmania kinetoplast DNA (k DNA) minicircle. LIN R4, LIN 17 and LIN 19 were used in this approach as a forward primer in both stages, reverse primer of the first stage and reverse primer of the second stage, respectively.
-LIN R4: 5'- GGG GTT GGT GTA AAA TAGGG-3'.
-LIN 17: 5'- TTT GAA CGG GAT TTC TG-3'.
-LIN 19: 5'- CAG AAC GCC CCT ACC CG-3'.
This technique was designed in 2000 by Aransay et al. and it was used by the author in numerous studies for diagnosis of Leishmania parasite in carriers' body and Leishmania reservoirs [13].
The reference strains of the parasite were used as follows to set up PCR reaction: the strains
Infantum: MCAN/IR/96/Lon49Le.
Le. Tropica: MHOM/IR/89/ARD2
Major: MHOM/IR/54/LV39Le.
The above strains were used for comparison. These strains were taken from Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences. Negative control, which was the extracted DNA from the liver of healthy rodents, was used for all reactions to ensure of the accuracy of performance. Thermal profile at the first stage was as follows: after a preliminary heat at 94˚C for 5 min (Initial Denaturation), proliferation continued for 30 seconds at 94˚C (Denaturation), 30 seconds at 52˚C (Annealing) and 1 minute at 72˚C (Extension) and finally, 10 minutes at 72˚C (Final Extension) (the first stage was repeated in 30 cycles). The second phase of thermal profile was also similar to the first stage, but without preliminary heat and the heat related to Annealing stage was 58˚C. The second thermal profile was repeated 33 cycles.

After proliferation of DNA, 5 microliter of PCR product mixed with loading buffer was electrophoresed for 40 min on 1.5% agarose gel containing ethidium bromide. By detection in UV Transilluminator device, the obtained bonds from the samples were compared with each other and studied. They were also compared with the obtained bonds from the standard strains and were recognized.
The results were analyzed using SPSS statistics 19 and Post Hoc test, Anova, paired t-test and chi-square tests.

RESULTS

108 rodents of 5 different species were caught in this study. Rodents' hunting was done completely out of rural places and borders and as a result, no house mice (Mus Musculus) commonly hunted in the studies for determination of rodent fauna, were caught. Species, frequency and percentage of rodent hunting in the studied areas of Bastak County are listed in [Table 1] and [Table 2]. Frequency percentage of species in the city of Kukherd showed that the highest frequencies with 57.1% and 42.9% was related to Meriones hurrianae and Gerbillus nanus species, respectively [Table 1]). Frequency percentage of species in Gachuyeh Village showed that the highest frequencies with 56.3%, 37.5% and 6.3% were related to Meriones hurrianae and Gerbillus nanus and Meriones lybicus species, respectively [Table 1]. Frequency percentage of species in Hanguyeh Village showed that the highest frequencies with 55%, 40% and 5% were related to Meriones hurrianae and Gerbillus nanus and Meriones persicus species, respectively [Table 1]. Frequency percentage of species in the city of Bastak showed that the highest frequency with 100% was related to Tatera indica species [Table 1].

Frequency percentage of species in Guwdah Village showed that the highest frequencies with 50%, 27.8%, 11.1% and 11.1% were related to Meriones persicus, Tatera indica, Meriones hurrianae and Meriones lybicus species, respectively [Table 2]. Frequency percentage of species in Deh Tall Village showed that the highest frequencies with 36.4%, 36.4% and 27.3% were related to Meriones persicus, Tatera indica and Meriones lybicus species, respectively [Table 2]. Frequency percentage of species in Dehang Village showed that the highest frequencies with 47.8% and 52.2% were related to Meriones persicus and Tatera indica species, respectively [Table 2].

Table 1: Frequency and percentage of rodent hunting in the villages studied in Bastak County

Location / Species	Kukherd Frequency	Kukherd %	Gachuyeh Frequency	Gachuyeh %	Hanguyeh Frequency	Hanguyeh %	Bastak Frequency	Bastak %
Meriones persicus	0	0%	0	0%	1	0.9%	0	0%
Tatera indica	0	0%	0	0%	0	0%	2	1.9%
Meriones hurrianae	4	3.7%	9	8.3 %	11	10.2%	0	0%
Meriones lybicus	0	0%	1	0.9 %	0	0%	0	0%
Gerbillus nanus	3	2.8%	6	5.6 %	8	7.4%	0	0%
Total	7	6.5%	16	14.8 %	20	18.5%	2	1.9%

Table 2: Frequency and percentage of rodent hunting in the villages studied in Bastak County

Location / Species	Guwdah Frequency	Guwdah %	Deh Tall Frequency	Deh Tall %	Dehang Frequency	Dehang %	Total Frequency	Total %
Meriones persicus	9	8.3%	8	7.4 %	9	8.3%	8	7.4%
Tatera indica	5	4.6%	8	7.4 %	5	4.6%	8	7.4%
Meriones hurrianae	2	1.9%	0	0%	2	1.9%	0	0%
Meriones lybicus	2	1.9%	6	5.6 %	2	1.9%	6	5.6%
Gerbillus nanus	0	0%	0	0%	0	0%	0	0%
Total	18	16.7%	22	20.4 %	18	16.7%	22	20.4%

Using Fisher's Exact Test, it was found that there was a significant relationship at level of 1% between studied location and species (P-Value=0.000).

Sampling process using microscopic method was done from all hunted rodents to recognize Leishmania contamination. They were also studied in terms of DNA detection of Leishmania parasite in their liver and spleen. The results are separately specified in Table 3. Using Fisher's Exact Test, it was found that there was no significant relationship between contamination and species (P-Value=0.707). The approved contamination in all samples was related to female Tatera indica and female Meriones hurrianae species [Fig. 1].

Table 3: Results related to identification of Leishmania reservoirs

Contamination / Species	Microscopic Frequency	Microscopic %	PCR Frequency	PCR %	Non Frequency	Non %	Total Frequency	Total %
Meriones persicus	0	0%	0	0%	29	26.9%	29	26.9%
Tatera indica	1(♀)	0.9%	1(♀)	0.9%	25	23.1%	27	25.0%
Meriones hurrianae	1(♀)	0.9%	2(♀)	1.9%	23	21.3%	26	24.1%
Meriones lybicus	0	0%	0	0%	9	8.3%	9	8.3%
Gerbillus nanus	0	0%	0	0%	17	15.7%	17	15.7%
Total	2	1.9%	3	2.8%	103	95.4%	108	100.0%

Fig. 1: Amastigote of Leishmaniasis major in prepared sample from Meriones hurricane species, Bastak County.

The results of Nested-PCR: determined genes by LIN R17, LIN R4 and LIN 19 primers on agarose gel using Nested-PCR are shown in[Fig. 2].

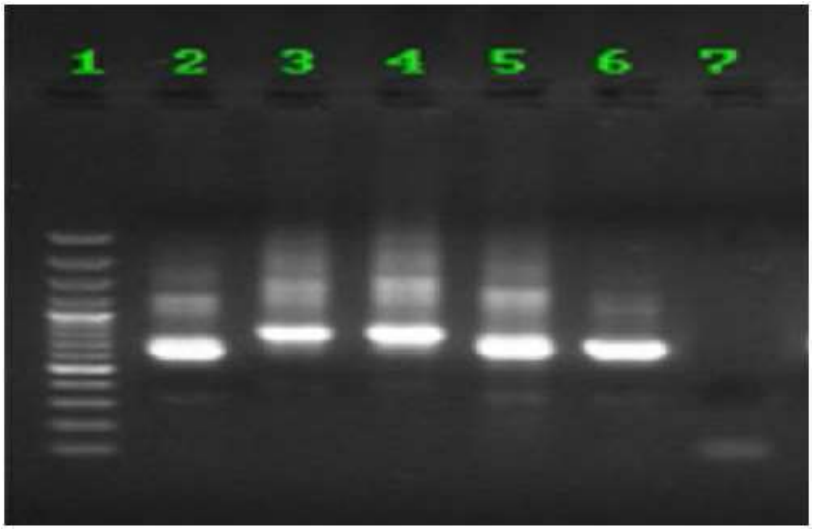

Fig. 2: The results of electrophoresis of Nested-PCR products related to rodent samples of Bastak County, Hormozgan Province, Iran, with LIN R4, LIN R17 and LIN 19 primers in 1.5% agarose gel; size marker (column 1); L. major standard strain (column 2); Leishmania tropica standard strain (column 3); Leishmania infantum standard strain (column 4); a Meriones hurrianae sample (column 5); a Tatera indica sample (column 6); negative control (column7).

DISCUSSION

Five species of rodents (Gerbillinae subfamily) were identified as broom tail rodents in this study in Bastak County. The highest frequencies among the collected samples were related to Meriones persicus (26.9%), Tatera indica (25%), Meriones hurrianae (24.1%), Meriones lybicus (8.3%) and Gerbillus nanus (15.7%), respectively. Since the sampling was done in the burden of rural areas, no house mice (Mus Musculus) commonly reported in the studies for determination of rodent fauna, were caught.

So far, a few studies have been done related to broom tail rodents in Hormozgan Province. In the study conducted by Solymani et al. only Rhombo-mys opimus species were collected from Kahorestan region in Bandar Abbas [14]. The remarkable point in the present study was that Rhombo-mys opimus species, the main reservoir host of the disease in most central areas and north eastern regions of Iran, did not collected. Although animal fauna may be different in various geographical conditions, the presence of high similarity of climatic conditions in Bastak and Kahorestan areas makes necessary revision and repeat of a similar study in Kahorestan area.

Hanafi et al. reported two species of Meriones persicus and Tatera indica in Haji Abad County. These two was also the dominant species in the current study. But no Leishmania contamination was observed by the researchers in the collected samples [15].
Leishmania contamination was observed with microscopic and molecular methods in the two rodent species of Meriones persicus and Tatera indica. Only one case of contamination to L. major was observed in Tatera indica species with both identification methods. One case of contamination in one female sample and two cases of contamination in two female samples were proved in Meriones hurrianae specie, using microscopic and specific PCR methods, respectively. It is worth noting that the microscopic method was only performed by investigation of prepared slides from rodents' ears. However, the molecular method was taken both on positive skin smears and the liver and spleen samples. So, the molecular method was able to identify positive the two slides prepared from ears which had been diagnosed negative by microscopic method. This reveals more sensitivity of the molecular method in detection of parasite.

Indian gerbil (Tatera indica) infected to L. major has been reported in several studies, especially in the west and the southwest regions of Iran. The species is reported by Javadian et al. as the main reservoir host of wet Leishmaniasis in Ilam and Khozestan Provinces [16]. Nesokia indica and Meriones libycus species are considered as the second reservoir hosts of the disease. The species were reported by Askari et al. as Leishmania reservoir in Kherameh region, Fars Province [17]. Contamination to the similar

species was also detected using microscopic and molecular methods in Larestan County, Fars Province located in vicinity of Hormozgan Province. This was reported by Mehrabani et al. [18].

Indian desert jird species, Meriones hurrianae, is considered as the main rural reservoir host in the southeast of Iran, where Tatera indica acts usually as the second reservoir [19]. The infection of this species to L. major was identified by Mohebali et al. using microscopic and RAPD-PCR methods and iso-enzymic analysis in the southeastern of Iran (e.g. the south of Baluchestan, and Dashtyari regions, Konarak and Chabahar) [20]. The contamination to this species has been limited in Iran to the southeastern areas and this contamination was also reported in the west of Hormozgan province in the present study. Meriones hurrianae species infected by L. major was also reported in Rajasthan Province in India [21].

Based on the extensive studies of researches, the contamination of Gerbillus nanus to L. major has not been mentioned in any scientific sources. For the first time and using microscopic and molecular methods, Mehrabani et al. reported two rodent samples of Gerbillus spp. genus infected to L. major in Larestan region, Fars Province. They did not report the rodent species. However, based on the size of the samples (two cases of contaminations) which were big, the two samples were not certainly from G. nanus species [18]. Of course, some cases of contamination to Gerbillus species, including Gerbillus pyramidum and Gerbillus dasyurus to L. major have been reported in some countries of Middle East, including Egypt, Jordan and Palestine. However, Gerbillus nanus was found in the present study but did not infect to L. major, parasite [22, 23].

Two species of rodents' contaminations mentioned above were observed with both the microscopic and specific PCR methods. The molecular method was more successful in detection of contaminations, so that 2 and 3 cases of contaminations were identified using microscopic and molecular methods, respectively. Since parasite DNA is searched in PCR method and its sensitivity and specificity is high, can be expected that its' diagnostic power to be higher than the microscopic method which is highly dependent on skill and recognition ability of microscopist. This issue has been highlighted by studies of other researchers [17, 24].

CONCLUSION

Based on the above findings, leishmaniasis in this emerging focus was of rural or wet type and was caused by *L. major*. Two *Meriones hurrianae* and *Tatera indica* species of rodent were introduced as the disease reservoir hosts. So, considering the type of primers and used sequence in this study to differentiate species of cutaneous leishmaniasis pathogens in the city of Bastak, finding a practical method with high sensitivity and performance is essential to enhance accuracy and to provide possibility of early diagnosis.

CONFLICT OF INTEREST
There is no conflict of interest.

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None

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ARTICLE

INVESTIGATING THE EFFECT OF REGULAR AEROBIC ACTIVITY ON YOUNG FEMALES HEMATOLOGY

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ABSTRACT

Introduction: *Physical activity, as a complementary element of a healthy lifestyle, has numerous advantages. It suggests the healthy impacts of exercise and the correlation between physical activity with appropriate frequency, intensity, time, and health. Therefore, the aim of this study was to determine the effect of eight weeks of aerobic exercise on serum levels of red blood cells, hemoglobin, hematocrit, and platelet in young females.* ***Methods:*** *This research is a quasi-experimental study. In this study, 30 females studying at the Jahrom University of Medical Sciences were participated voluntarily. They were assigned in two groups experimental group (n=15) with an mean age 21.93 ± 2.34, height 157.93 ± 4.57 , weight 60.00 ± 8.09 and BMI 24.10 ± 3.20 and control group (n=15) with mean age 22.33 ± 2.69, height 168.60 ± 10.54, weight 66.60 ± 13.15 and BMI 23.33 ± 3.53. The experimental group program includes eight weeks of running, three times a week with 60 to 65 percent of maximum heart rate for 45 minutes and with observing the principle of overload until the eighth week of 75 to 80 percent of maximum heart rate. To measure blood factors (red blood cells, hemoglobin, hematocrit, and platelet), blood samples were taken from participants in two stages (72 hours before and eight weeks after executive program). In order to analyze the data, independent t test was used.* ***Results:*** *The results showed that comparing hematologic factors in the experimental group increased red blood cells (p<0.009), hemoglobin (p<0.0001), hematocrit (p<0.0001), and platelet (p<0.001) significantly compared to the control group.* ***Conclusion:*** *Considering the results of this research, it can be concluded that regular exercises can have positive effects on red blood cells, hemoglobin, hematocrit, and platelet of people, especially the young females.*

KEY WORDS
aerobic activity, red blood cells, hemoglobin, hematocrit, platelet, young females

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INTRODUCTION

Exercise, as one of the physical activity options, can be effective not only at championship dimension, but also in maintaining the body's health. However, its implementation and intensity should be so that its side effects to be reduced in terms of health [1]. In studies conducted in this regard, it has been stated that blood tissue plays an important role in exercise activities due to transporting blood to cells, disposal of waste material, the sustainability of the body fluids, hormones, being involved in regulating hemostasis in blood glucose, and hormonal adaptations during exercise activities. Conflicting studies have been conducted so far to investigate the impact of these factors on exercise and physical activities [2]. Considering the incorrect form of exercise and its impact on blood changes of athletes, it has been stated that changes in red blood cells depend on intensity and type of activity performed and the readiness of individuals, so that in high-contact exercises like Kung Fu and the activities carried out with high intensity, a significant decrease was found in the number of red blood cells due to the increased vascular hemolysis [3]. Reduction in blood indices level can decrease oxygen transport to active tissues. As a result, oxygen required for aerobic metabolism of muscle cells decreases. Accordingly, the active muscles supply their needed energy from anaerobic pathways and their dependency to immediate fuels [glycogen and creatine phosphate] increases. This mechanism leads to the accumulation of lactic acid, increased perception of fatigue, and depletion of immediate energy stores, which ultimately creates fatigue and decreased performancein athletes [4]. Therefore, exercise without considering its correct form, can leave harmful effects on body and effort in performing correct form of exercise can increase the efficiency of the body, especially the blood factors of athletes. Some studies have shown that the number of circulating red blood cells can increase as result of release of stored red cells in the spleen induced by physical activity [5]. Research has shown that exercise causes increased red blood cells, so that concentration of red blood cells can increase by 25% during the exercise [6].

Exercise hematology has taken great steps in the past 30 years and it has been emerged as a specialized sub-branch of science [7, 8]. Physical activity that increases physical power causes a wave of change in the body, including peripheral blood erythrocytes system. It was also reported that inactivity reduces plasma volume and total volume of red blood cells, leading to reduced circulating blood volume and reduced performance of body [9] and the number of red blood cells in active tissues increases while performing physical activity. This phenomenon is created due to increased blood flow associated with an increase in the number of red blood cells and slight increase in hemoglobin and hematocrit immediately after the exercise [10].

In addition, it has been stated that increased red bloods leads to increased blood concentration, increased capability in carrying oxygen from blood, leading to increased performance and efficiency during physical activity. On the other hand, physical work capacity and the maximum oxygen consumption in humans depend on transporting oxygen to tissues involved in activity. In addition, blood performance is determined under the influence of factors like Circulating blood volume, oxygen-carrying capacity of blood by hemoglobin by [7].

As mentioned, these changes in some cases may cause a risk to health and athletic performance can be reduced. Increased blood viscosity impairs oxygen delivery to the muscles and body tissues and it causes

resistance against capillary blood circulation, fatigue, and reduced ability in athletic performance [11]. Studies on exercise activities effects on hematological variables continue, so that researchers have found contradictory results on the impact of physical activities on these variables. Despite significant developments in the various areas of hematology and exercise, the long-terms effect of aerobic exercises on blood factors has not been clearly stated and there are many conflicts in this regard. As women and girls are always exposed to anemia and decreased blood elements for a variety of causes such as menstruation and pregnancy at different stages of life, the investigation of impact of aerobic activities on blood elements, especially in females, seems to be necessary. Therefore, with regard to what was said above and importance of the issue, the aim of this study was to investigate the effect of aerobic training on hematological changes, especially in young females.

MATERIALS AND METHODS

This research is quasi-experimental study and it is applied in terms of objective. The study population consisted of 30 female students of Jahrom University of Medical Sciences [25-18 years old] who participated voluntarily in the study. A sample of 30 subjects was assigned in two groups of experimental [aerobic exercise] and control groups, each containing 15 subjects. After selecting subjects, they were informed of research topic, objective, method, as well as applications and potential risks of the study.
Pre-test session was hold one day before exercises to assess weight, height, and body composition. Height and weight were measured by wall stadiometer and digital scale, respectively. For weight measurement, the subjects stood beside wall without shoes and socks, so that back of them was stuck to wall and their head position was toward forward. Then, their weight was recorded in kilograms. To measure the weight, subjects stood on the scale and their weight was recorded in kilograms. Then, subjects were divided into two experimental and control groups.

The experimental group exercise program included eight weeks running, three times a week with 60 to 65 percent of maximum heart rate for 45 minutes at the beginning by observing overload principle, reached to 75 to 80 percent of maximum heart rate until eighth week. Exercises started with 15-minute warm-up including stretching and jogging started and ended with 15 minutes of cooling down the stretching. The control group received no regular exercise during the study and they continued their normal activities before the study. Blood samples were taken 72 hours before and 72 hours after the last exercise session to determine the pre-test and post-test**.**

Before blood sampling, subjects took a break for 20 minutes. Then, venous blood sampling was performed from the subjects' right hand in a sitting position to assess red blood cells, hemoglobin, hematocrit, and platelet. In this study, descriptive statistics was used to describe the collected data and inferential statistical methods were used to test the hypotheses. In the descriptive statistics section, indicators of mean and standard deviation were used. Then, independent t-test was used to intergroup means comparing. SPSS 18 software was used to calculate the statistical results of study. Significance level was also $p<0.5$.

Findings

Demographic characteristics of research participants included age, height, weight and body mass index [BMI] shown in [Table 1]. The mean age in the control group and experimental group was 22.33 ± 2.69 and 21.93 ± 2.34 years, respectively that significant difference was not found in this regard. The mean height in the experimental group and the control group was 168.60 and 157.93 cm, respectively.
In addition, mean weight in the control group and experimental group was 66.6 and 60.0 respectively, which significant difference was not found between them in this regard, and body mass index in the experimental group and control group was 24.1 Kg per m^2 and 232.33 Kg per m^2, respectively.

Table 1: Descriptive variables of subjects studied

Variable group	nfeatures mean and SD		
Experimental group	15	Age [years]	34.2±21.93
		Height [cm]	54.4±157.93
		Weight [kg]	09.8±60.00
		Body mass index [Kg per m^2]	20.3±24.10
Control group	15	Age [years]	69.2±33.22
		Height [cm]	54.10±168.60
		Weight [kg]	15.13±66.60
		Body mass index [Kg per m^2]	53.3±23.33

Descriptive statistics of variables of hemoglobin and red blood cells in the experimental group and the control group are shown in [Table 2]. The results show that the control and experimental groups was significantly different in pre-test in terms of descriptive statistics. The concentration of red blood cells, hemoglobin, hematocrit, and platelets in the experimental group in pre-test stage was close to the control group. In the post-test experimental group, the increase in red blood cells, hemoglobin, hematocrit and platelet was observed compared to pre-test groups, indicating the effect of exercise carried out in this

study of the investigated factors. However, in the post-test control group compared to pre-test group, significant changes were not observed in red blood cells, hemoglobin, hematocrit, and platelet.

Table 2: Descriptive statistics of subjects studied

platelet	Hematocrit	Hemoglobin	Red Blood Cell	variable stages	
66.7±178.33	91.1±40.40	69.0±12.92	45.0±69.4	Pre-test	Experimental
91.66±252.93	56.2±90.44	34.1±15.42	31.0±074.5	Post-test	
27.7±177.40	11.2±40.16	91.0±70.12	41.0±57.4	Pre-test	Control
72.7±26.177	04.2±40.20	75.0±83.12	45.0±53.4	Post-test	

To compare the variables of study in the pre-test stage, independent t-test was used for subjects in both experimental and control groups. [Table 3] shows that the homogeneity assumption of the subjects is confirmed in pre-test considering non-significance of research variables in two control and experimental groups.

Table 3: Compares of research variables in the pretest stage for subjects in both control and experimental groups

statistic Variable		Mean difference	SD	T value	Significance
Experimental and control groups	Red Blood Cell	0.054	0.16	0.334	0.741
	Hemoglobin	0.23	0.29	0.790	0.436
	Hematocrit	0.074	0.74	0.100	0.921
	Platelets	1.21	2.73	0.443	0.661

To compare Red blood cell between experimental group and control in post-test stage, independent t-test was used. [Table 4] shows the results of this test. Independent t-test results showed that in the post-test stage, there is significant difference between control and experimental groups in terms of the effect of aerobic exercise on red blood cells.

Table 4: Results of independent t-test [variable of red blood cells in the two groups]

Statistic Group		mean±SD	Mean difference±SD	n	Levin test for equality of variances F coefficient	Significance	T value	df	Significance
Red Blood Cell	Experimental	5.074±0.31	0.544±0.15	15	.0118	.0733	.0018	28	.0009
	Control	4.53±0.45		15					

To compare hemoglobin between experimental group and control in post-test stage, independent t-test was used. [Table 5] shows the results of this test. Independent t-test results showed significant difference between control and experimental groups in terms of the effect of aerobic exercise on hemoglobin in the post-test stage.

Table 5: Results of independent t-test [hemoglobin variable in the two groups]

Statistic Group		mean±SD	Mean difference± SD	n	Levin test for equality of variances Coefficient F	Significance	T value	df	Significance
Hemoglobin	Experimental	15.42±1.34	2.59±0.45	15	4.306	0.047	5.386	28	0.0001
	Control	12.83±0.75		15					

Independent t-test results in [Table 6] showed significant difference between control and experimental groups in terms of the effect of aerobic exercise on hematocrit in the post-test stage.

Table 6: Results of independent t-test [hematocrit variable in the two groups in post-test stage]

Statistic / Group		mean±SD mean±SD	Mean difference±SD	n	Levin test for equality of variances: Coefficient F	Levin test for equality of variances: Significance	T value	df	Significance
Hematocrit	Experimental	44.90±2.56	4.7±0.99	15	0.411	0.527	4.139	28	0.0001
	Control	40.20±2.04		15					

As Levin test did not confirm the homogeneity of variances significant, we reported modified t-test results. Independent t-test results in [Table 7] showed that significant difference between the experimental group and control group in terms of the effect of aerobic exercise effect on platelets variable in post-test stage.

Table 7: the results of independent t-test [platelet variable in two studied groups in post-test stage]

Statistic Group		mean±SD mean±SD	Mean difference±SD	n	Levin test for equality of variances: Coefficient F	Levin test for equality of variances: Significance	T value	df	Significance
Platelet	Experimental	252.93±66.91	75.67±18.10	15	22.55	0.0001	4.2	15.487	0.001
	Control	177.26±7.72		15					

DISCUSSION

Serum levels of red blood cells in young females changed significantly after eight weeks of aerobic activity. According to results of this study, red blood cells in experimental groups increased significantly compared to control group and this difference was significant [p<0.009].

Results of this study were in line with results of study conducted by Ramazanpoor et al [2001] who examined the effect of aerobic exercise on hemoglobin, red blood cells, hematocrit, iron, serum ferritin, and transferrin in young females. They showed a significant increase in the red blood cells in subjects [12]. Ramazani et al [2012] examined the effect of eight weeks of resistance periodic and continuous exercise on some hematologic parameters in non-athlete males. The results showed that the levels of red blood cells, hemoglobin, hematocrit, increased significantly in three groups [13].

Marjani et al [2009] examined the effect of one session of physical activity on some blood elements of athletes and the results showed that the level of red blood cells was significantly increased at the end of exercise [14]. Additionally, the results of this study were in line with results of Mousavi Zadeh et al. [2009] who examined the effect of aerobic exercise on hematological indices in female students and they found significant reduction in hematologic indices of young females [15]. Dehghan and Pouya [2012] examined the effects of 8 weeks of exercise on blood factors of young females and they found no significant differences in red blood cells, haemoglobin, and haematocrit [16] that it was not consistent with the results of our study. Lack of consistency between current study and presented studies can be justified by intensity and duration of readiness exercise of subjects, difference in type of exercise presented, exercise pressure, and base level of blood levels.

Physical activities, especially aerobic activities, increase blood circulation and increased need of muscles to oxygen, so that oxygen consumption in the muscles is 100 times than resting time [17]. Additionally, the primary driver for the production of erythropoietin is the amount of oxygen available to meet the metabolic needs of the body tissues that it is one of the causes for increased need of body tissues to oxygen of aerobic physical activity [18]. In this regard, the occurrence of hypoxia conditions during exercise and adaptations resulting from exercise cause an increase this hormone from the kidneys and liver in small quantities.

Therefore, regular exercises increased releasing of red blood cells through hormone aritropoitin [19, 20]. Hence, the results of this research will be justified that the regular exercises have led to increased hormone aritropoitin, increased blood flow, and finally increased red blood cells in young females. It is in line with the previous studies conducted in this regard. Serum hemoglobin values of young females changed significantly after eight weeks of aerobic activity. According to the results of the research, hemoglobin value increased in the experimental group compared to control group, that this difference was statistically significant [P<0.0001]. The results of this investigation were in line with results of a study conducted by Ramazani et al [2012], who examined the effect of eight weeks of resistance periodic and continuous exercise on some hematologic parameters in non-athletic males. The results showed that the levels of red blood cells, hemoglobin, and hematocrit increased significantly in three groups [13]. Gray [1993] studied the effect of aerobic exercise on blood hemoglobin and the results showed that aerobic

exercise increased significantly the hemoglobin concentration after exercise [21]. Marjani et al [2009] examined the impact of one session of physical activity on some blood elements of athletes and hemoglobin level increased at the end of exercise [14, that it was in line with result of our study. Ghanbari Niaki et al [2006] examined the effects of three-day non-consecutive running on hematology variables and observed a significant decrease in hemoglobin levels, that it was inconsistent with this study. One reason of consistency of this study with presented studies could be participation of young subjects [20]. Lack of consistency of the present study with presented studies could be reasons of differences in size and duration and frequency of exercise program. The need to increased oxygen during exercise can provoke hematopoietic cells to produce more oxygen carriers [12]. Since 92 percent of the oxygen in the blood is carried by hemoglobin, there is high correlation between oxygen-carrying capacity and range of hemoglobin density [14]. In addition, as hemoglobin contains 33.5% of internal compounds of red blood cells, it is obvious that an increase in red blood cells is followed by increased hemoglobin [22]. According to the results of this research, it can be stated that there is a positive and linear relationship between increased hemoglobin and increased red blood cells. Hence, the need for more oxygen provokes oxygen carriers [hemoglobin, and subsequently red blood cells] to supply body oxygen as result of physical activities. Therefore, the results of this research indicated that exercises increased the values of hemoglobin in the subjects.

Serum levels of hematocrit young females changed significantly after eight weeks of aerobic exercise. According to the results of this study, levels of hematocrit in the experimental group increased compared with the control group and this difference was statistically significant [$p<0.0001$].

The results of research are in line with results of study conducted by Ramezani et al [2012] on the effect of eight weeks of resistance periodic and continuous exercise on some hematologic parameters in non-athletic males. The results showed that the levels of red blood cells, hemoglobin, and hematocrit increased significantly in three groups [13]. Several factors can be responsible for changes in hematocrit during sports activities, including the change and displacement of fluids, reduced water, and the release of red blood cells from the spleen [15]. Additionally, changes in fluids and dehydration and decreased plasma volume can lead to increased hematocrit [6]. Given that hematocrit is the percentage of red blood cells, then it is possible that hematocrit percentage increase due to increased red blood cell [14]. The results of this study suggest that the increase in red blood cells and hemoglobin can be a major cause of

increased hematocrit in the subjects, but plasma and reduced blood viscosity during physical activities should not be ignored in this regard. However, as lack of plasma volume control was limitation of this study, increase in percentage of red blood cells can increase the level of hematocrit in subjects.
Serum levels of platelets in young females after eight weeks of aerobic activity increased significantly. According to results of this study, platelet values increased in the experimental group compared with the control group, and this difference was significant [$p<0.001$]. The results of this research are in line with results of study conducted by Marefati et al [2012] who compared the effect of aerobic exercise on blood platelets of young females. The results showed that aerobic exercise caused significant increase in blood platelet of subjects [23].

Platelet increase mechanism could be due to increased blood return from the vascular bed spleen, bone marrow and the accumulation of pulmonary artery blood flow within the muscles involved [2], because it has been reported that injection of epinephrine causes strong vasoconstriction of spleen, where about one-third of platelets are stored. This mechanism can explain the reason for increase in the amount of circulating platelets in the exercise [24]. According to results of this study, exercise in this research caused an increase in the level of blood platelets of young females by creating spleen contraction due to increased levels of epinephrine.

CONCLUSION

The results of the investigation showed that the regular exercises caused an increase in blood elements studied in young females by increasing the hormone erythropoietin, and increased blood flow. Therefore, the use of regular exercises and activities is recommended by observing scientific principles in order to increase the factors of red blood cell, hemoglobin, hematocrit, and platelet in people. Therefore, according to the results of this research, it is recommended for educational institutions to take measures to reduce the health problems of the students and to reduce blood problems of this class of society by planning the efficiency of the exercises in the academic area of students.

CONFLICT OF INTEREST
To the best of our knowledge, no conflict of interest exists.
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IIOAB JOURNAL

ARTICLE

DETECTION OF CANCER STEM CELLS MARKER IN PROSTATE CANCER USING A NANOMECHANICAL MEMBRANE-TYPE SENSOR

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ABSTRACT

In this study, we have used arrays of self-sensing piezoresistive nanomechanical membrane (NMM) to detect CD133 antigen, a protein surface marker associated with stem cells in prostate cancer. The sensing principle is based on the surface stress changes induced by antigen–antibody interaction on the NMM surfaces. NMM consists of a membrane suspended by four piezoresistive sensing components. The isotropic surface stress on the membrane results in a uniaxial stress in each sensing component, which efficiently improves the sensitivity. According to the experiments, it was revealed that NMMs have surface stress sensitivities in the order of 1.5 (mJ/m). This matter allows them to detect CD133 concentrations as low as 300 pg/ml or 150 pM. This indicates the fact that the self-sensing NMM approach is beneficial for detecting disease markers. Moreover, the performance of the NMM was compared with other detection methods and the results indicated a better performance for NMM.

KEY WORDS
Piezoresistive nanomechanical membrane; Cancer stem cell; CD133; Surface stress

INTRODUCTION

IMicro and nanofabrication technologies are new alternatives for fabricating high sensitive mechanical devices for many applications including clinical diagnosis, food quality control, and environmental monitoring [2, 3, 11]. The central element in many traditional mechanical biosensors is a small cantilever that is sensitive to the biomolecule of interest. It is possible to operate micro-cantilever sensors in two different modes, i.e. cantilever bending (surface stress method) and resonance response variation (microbalance method). In the first mode, static mode, the induced surface stress that is due to the presence of the adsorbates results in a deflection in the cantilever [26], while in the second mode, dynamic mode, the adsorbates change the resonance frequency of a cantilever due to mass loading [12].

A sensitive readout system is crucial for monitoring the deflection of cantilevers. For this reason several read-out methods have been presented. The most extended readout methods for biosensing are optical, and piezoresistive ones. The optical method is simple to implement and shows a linear response with sub-angstrom resolution, also is currently the most sensitive method. This method is employed for detecting the cantilever deflection in most studies [12, 20-22]. Nevertheless, the optical detection mechanism presents some disadvantages for example, bulky, time-consuming laser alignment on each cantilever, low applicability for large one- or two-dimensional arrays, and the difficulty of performing measurements in opaque liquids, such as blood, may hinder the potential application of this method for actual applications. The piezoresistive sensing method is known as a good alternative for the optical detection in biosensing application. The benefit of this method is that the principle works well in both liquid and gas phase and large arrays can be realized and read-out. Also, the technique is applicable for static as well as dynamic measurements [1, 9, 18, 19]. Although piezoresistive cantilevers have proven to be highly beneficial detection methods, without effective mechanical amplification schemes, their sensitivity is far below that of optical methods. In order to overcome this problem, several researches have focused on applying structural modification, such as making a through hole [18], patterning the cantilever surface [16], or variation of geometrical parameters (e.g., length, width, and overall shapes) [10, 17, 27]. Although all these methods have proven to improve the sensitivity of piezoresistive cantilevers for surface stress sensing, they have still not yielded significant stress amplification to make piezoresistive detection comparable to the optical approach, which this can be due to the fact that all these approaches rely on suppressing one of the isotropic stress components. Analytical consideration of strain amplification schemes for sensing applications based on the strategies of the constriction and double lever geometries [8, 27] has resulted in the introduction of NMMs, which have shown a considerable improvement in amplifying piezoresistive detection signals. Yoshikawa *et al, [28]* have experimentally evaluated a prototype nanomechanical membrane and the results have illustrated a significant sensitivity for piezoresistive cantilevers. In comparison with the standard piezoresistive cantilever, this study demonstrated a factor of more than 20 times higher sensitivity than that obtained with a standard piezoresistive cantilever. Presently, prostate cancer is considered as the most prevalent form of cancer in men. Recently, increasing evidence for a hierarchically organized cancer stem cell (CSC) model emerged for different tumours entities, including prostate cancer. CSCs are defined by several characteristics including self-renewal, pluripotency and tumorigenicity and are thought to be responsible for tumour recurrence, metastasis and cancer related death. A common strategy for CSC identification is flow-cytometry using assumed specific CSC surface markers, e.g., CD44 or CD166. However,

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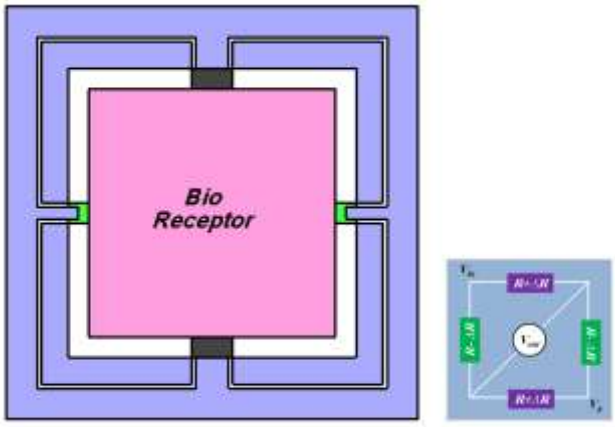
many of the surface proteins used to identify CSCs are also expressed on physiological stem cells and/or progenitor cells. Human CD133 antigen, also known as AC133, was recently identified as a CSCs marker in prostate cancer [13, 24, 28].In this work, a signal transduction biosensor has been used as a novel electrical detection for identifying CD133 marker. A direct nano-mechanical response of micro-fabricated self-sensing NMM was used to detect the surface stress changes of antigen–antibody specific binding. After injecting the CD133 target, as model biocontents, the piezoresistive responses were carefully analyzed and the feasibility of the piezoresistive membranes for biosensing were discussed in terms of device performance measures such as sensitivity, accuracy, and specificity. At the end, the results were compared with a standard cantilever.

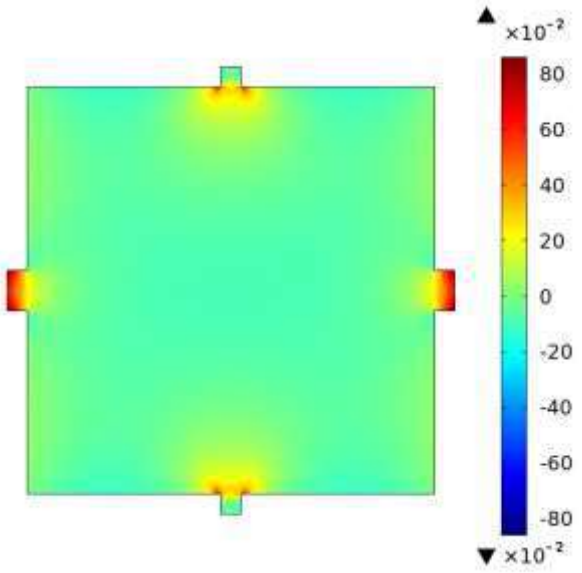
There are very few studies regarding the wearing and laundering of lab coats in hospitals and medical practice. This study highlights the role of lab coats acting as vector for transmitting health care infections to the patients and the common areas where contamination occurs.

Theoretical background

Molecular adsorptions on a surface do not only add mass, but also can induce surface tension or surface stress [15]. As the molecules bind, surface stress is developed — owing to electrostatic repulsion or attraction, steric interactions, hydration and entropic effects — and this can induce deflection in the mechanical element. In the piezoresistive micro/nanomechanical sensors the electrical resistivity of a piezoresistive film varies with the applied surface stress. The resistance of the silicon piezoresistor is a function of stress and the orientation of the piezoresistors. The relation between resistivity and stress can be expressed as [31]:



(a)



(b)

Fig. 1: (a) A schematic of the NMM sensor with piezoresistive sensing component (b) distribution of ΔR/R on the surface of NMM with a dimension of 400 µm 400 µm 2 µmwhen a compressive surface stress of -1.0 N/m applied uniformly calculated by finite element analyses (FEA) using COMSOL Multiphysics 4.2.

$$[\frac{\Delta R}{R_0}] = \{\pi\}[\sigma] \quad (1)$$

where R_0 is the isotropic resistivity of the unstressed crystal, σ_i is the stress components, and the terms π_{ij} the component of the piezoresistance tensor. According to equation (1), for plain stress (i.e., $\sigma_z = 0$), relative resistance change can be described as follows:

$$\frac{\Delta R}{R_0} \approx \frac{\pi_{44}}{2}(\sigma_x - \sigma_y) \quad (2)$$

From equation (2), it is clear that ($\Delta R/R_0$) is completely dependent on σ_x and σ_y values. In cantilevers sensors, surface stress induces an isotropic stress, and the piezoresistive signal is nearly zero except at the clamped end where the isotropic symmetry is broken. Thus, the sensor sensitivity efficiently reduces in comparison with cantilevers when a point force is applied at the free end. According to this problem NMM approach was presented by Yoshikawa et al, [28].

A simple illustration of the final NMM sensor with piezoresistive sensing component can be observed in [Fig.1a]. Owing to equation (2), isotropic surface stress leads to zero piezoresistive signal, but in the NMM structure the isotropic deformation effectively converts into a concentrated force at the connection between the membrane and the piezoresistive sensing component. [Fig. 1b] shows ($\Delta R/R_0$) distribution for NMM with a dimension of 400 µm x 400 µm x 2 µm, when a compressive surface stress of -1.0 N/m is applied uniformly on the NMM. COMSOL Multiphysics 4.2 finite element software was used for extracting ($\Delta R/R_0$) distribution. The number of elements for modeling the sensor was about 25000, which gave sufficient resolution for the present simulation.

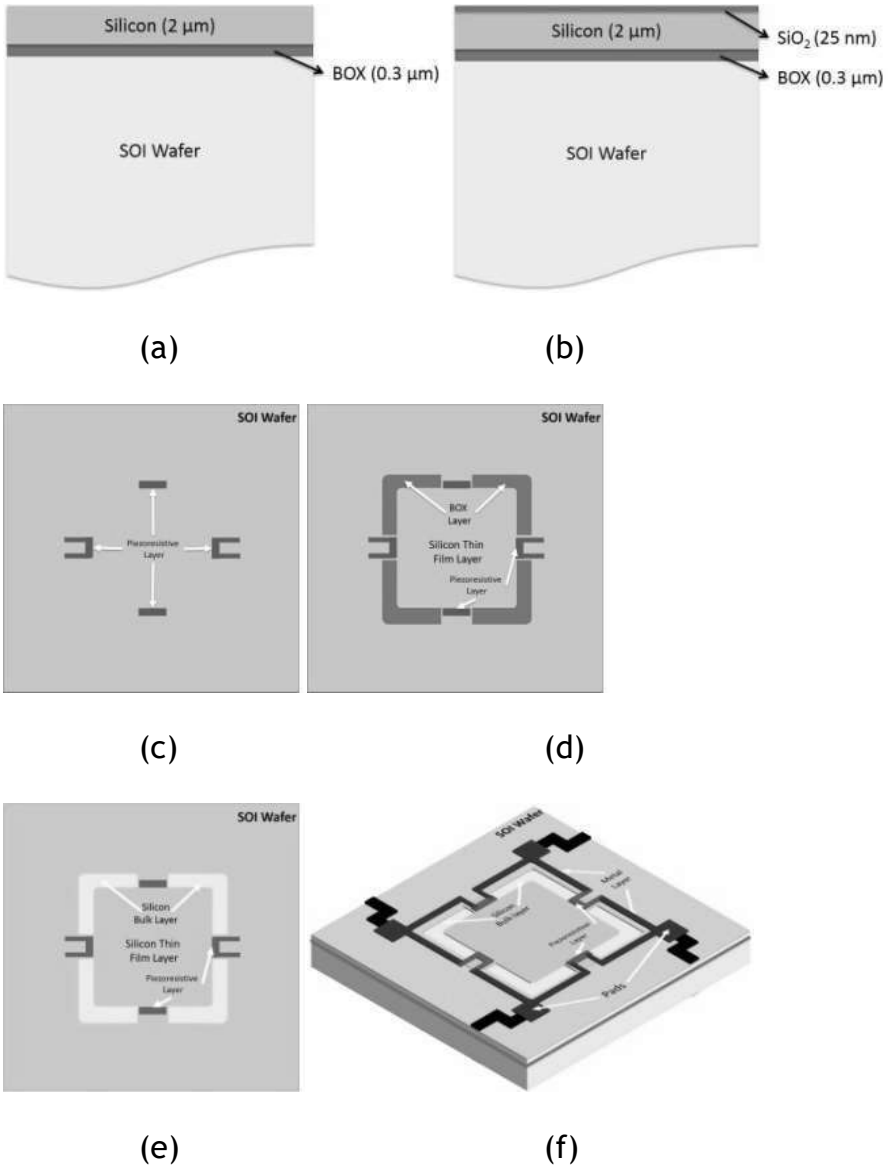
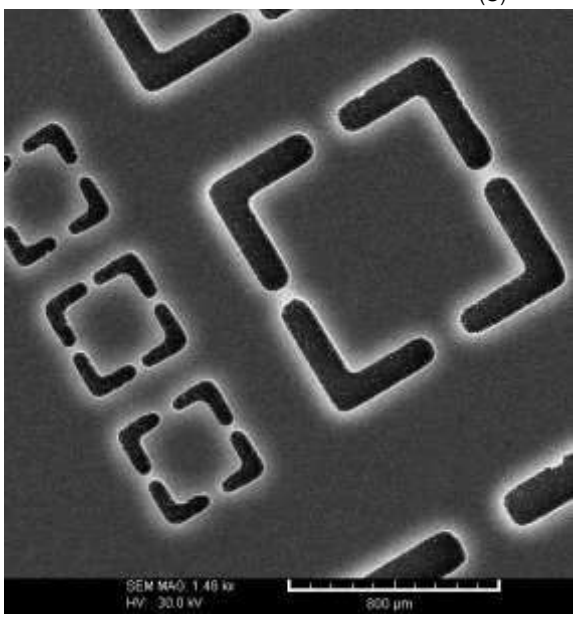


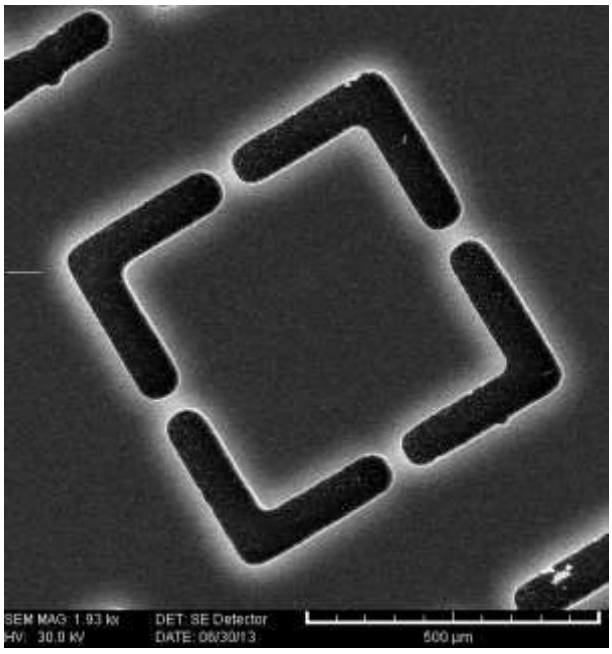
Fig. 2: Fabrication sequence of NMM.

The membrane-type geometry allows us to place a full Wheatstone bridge on the chip, when all four resistors are practically equal and the relative resistance changes are small, the total output signal V_{out} can be approximated by:

$$V_{out} = \frac{V_{in}}{4}\left(\frac{\Delta R_1}{R_1} - \frac{\Delta R_2}{R_2} + \frac{\Delta R_3}{R_3} - \frac{\Delta R_4}{R_4}\right) \quad (3)$$



(a)



(b)

Fig. 3: Scanning electron micrograph (SEM) of (a) NMM array chip with two different dimension of 800 µm x 800 µm x 2 µm and 400 µm x 400 µm x 2 µm which fabricated in a same array (b) high magnification micrograph of 400 µm x 400 µm x 2 µm NMM.

According to equations (1-3), the average values of relative resistance change in the NMM has a higher value in comparison with the standard cantilever (about 43 times) [28].

The intrinsic noise level for the modified piezoresistor can be estimated by Johnson (thermal) and Hooge (1/f) noise equations [4, 5, 23]. The total intrinsic noise for NMM is reported as 0.01- 0.5 µV [7], which is still lower than the experimental noises (2.0~2.5 µV), mainly caused by the electrical circuit.

MATERIALS AND METHODS

Fabrication of NMM sensor

We used Silicon on Insulator (SOI) wafers with a 2 µm device layer and a 0.3 µm buried oxide (BOX) layer as the substrate material [Fig. 2a]. Then a 25 nm silicon dioxide layer was grown by a thermal oxidation to electrically insulate the device layer from the subsequent metal layers [Fig. 2b]. The first lithographic process to define the first metal layer for electrode and sensor platform for subsequent liftoff process has been accomplished. After patterning, the photoresist, chrome (10 nm) and gold (50 nm) layers were deposited by e-beam evaporator and patterned by a liftoff process with the previously patterned photoresist [Fig. 2c]. The patterned metal layer from previous step and the patterned layer of photoresist, from the second photolithographic process were used to define the areas to be etched to define the sensor structure. The exposed device layer was etched completely by RIE to define the sensor structure. Then, a third photolithographic step for the second liftoff process, followed by the deposition of a 30-nm chrome layer and a 150-nm gold layer for wire-bonding pads. After the liftoff, a release window was photolithographically defined by the fourth lithographic process [Fig. 2d] and the exposed BOX was etched by RIE leaving the Si substrate exposed [Fig. 2e]. Then the wafer was diced into individual chips. Through the release window, the exposed Si substrate was etched by vapor phase etching using xenon difluoride (XeF2) to release the sensor structure. After XeF2 etching, the photoresist and the BOX were removed by BHF etching and solvent cleaning. The die was cleaned with oxygen plasma and then a 100-nm thick silicon dioxide layer was deposited with plasma enhanced chemical vapor deposition (PECVD) for insulation. Chrome (20 nm) and gold (50 nm) layers were deposited using an e-beam evaporator for an immobilization layer for protein–protein interaction. The PECVD oxide on the bonding pads was selectively etched for wire-bonding. Then each die was attached to a custom made printed circuit board (PCB) and was wire-bonded. [Fig. 3] presents the final picture of NMMsin different sizes fabricated in the same array using a Scanning electron micrograph (SEM).

CD133 antibody immobilization process

A fresh piranha solution (a 4:1 ratio of H_2SO_4 (98.08%) and H_2O_2 (34.01%)) was used to wash and clean the membranes, in order to remove experimental contamination of the Au surface. After 1 min, the membranes were taken out of the solution and were rinsed using deionized water. To complete the cleaning process, the rinsed membranes were dried using a stream of N_2 gas. For 2 h at room temperature in darkness a 0.1 M deoxygenated cysteamine (Sigma, 95%) aqueous solution was used to functionalize the devices. Then, NMMs were washed with deionized water and soaked in water for 12 h to remove the physically adsorbed cysteamine. Moreover, for creating a covalent cross-linker molecule between the amine groups on the NMM surface and antibodies, chips were soaked in a 5% solution of gluteraldehyde (Sigma, 50%) in borate buffer for 2 hours. Following this and all subsequent steps, device chips were washed twice, each washing step was for two minutes, in purified DI water on an orbital shaker operating at 95 RPM. It should be mentioned that fresh water was used between washes. The reason of using water instead of buffer for washing was to prevent the abundant formation of buffer salt crystals on the surface of devices which make the sensors effectively useless.

Next, one hour incubation was used to immobilize the monoclonal anti-CD133 (Fitzgerald Industries International Inc., Concord, MA, USA), affinity-purified, with a concentration of 50 mg/mL on the surface. By immersing the NMM in 50 mM solution of glycine for 30 minutes unreacted gluteraldehyde was then quenched. In addition, dissolved bovine serum albumin (BSA, Sigma, St. Louis, MO, USA) in phosphate buffered saline (PBS) with 10 mg/ml concentration was used to prevent non-specific binding. For this purpose, the membranes were immersed in this solution for 1 h at room temperature. Then, they were rinsed with PBS (pH 7.4) containing polyoxyethyethylenesorbitan monolaurate (Tween 20, St. Louis, MO, USA) and finally washing was performed by only using PBS solution.

Electrical measurements

For the electrical measurement of sensor, internal dc-bias Wheatstone bridge was used. A bridge supply voltage of 1.5V was applied using a dc power supply (Agilent, E3631A), and the sensor output voltage was measured by a multimeter (keithley, 2010 7-1/2). Moreover, a faraday cage was adopted for noise reduction. The above components were used to measure the piezoreisitive response of the NMM in a liquid environment.

RESULTS AND DISCUSSION

In order to reach results with high reliability, the surfaces of the membranes were stabilized by treating them with a PBS buffer. The PBS buffer was directed with a typical flow rate of 0.4 – 0.5 ml/hour, for 1 h, to the NMM sensor arrays using a flexible PDMS polymer microfluidic channel sealed to the device chip.

As a general trend, at the point of initial injection of the PBS buffer the induced voltage of the NMM increased rapidly and steadily decreased with time, which in this case the induced voltage of the NMM reached dynamic equilibrium after 10 min. For the bio-assay, CD133 antigens were injected into each liquid chamber, including the stabilized membrane. The liquid temperature was precisely controlled and external noise sources were excluded using a shield box. In order to estimate the nonspecific adsorption on the NMM surface, the concentration of BSA in all solutions was stabilized at 0.1 mg/ml.

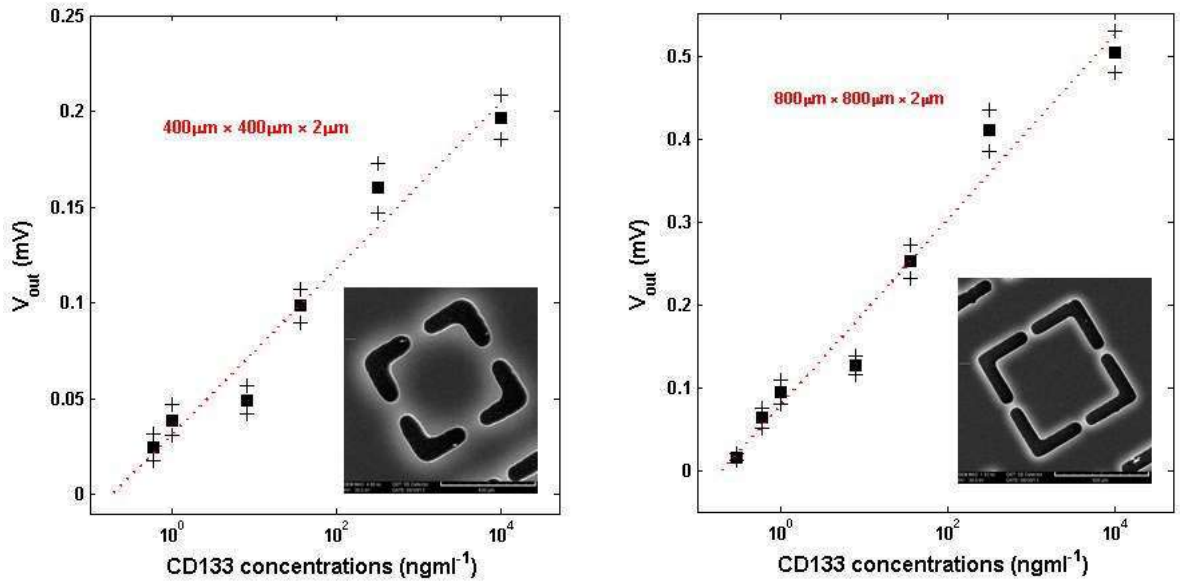


Fig. 4: Steady-state output signals (V_{out}) as a function of CD133 concentrations for two different NMM geometries. Every data point on this plot represents an average of output signals obtained in multiple experiments done with different NMM, whereas the range of output signals obtained from these experiments is shown as the error bar.

[Fig. 4] shows the steady-state output signals (V_{out}) as a function of CD133 concentration in a BSA background for different dimensions of NMM. By using a 400 µm x 400 µm x 2 µm NMM, the lowest CD133 concentration that we could clearly detect above noise was 0.6 ng/ml. However, when a 800 µm x 800 µm x 2 µm NMM was used, CD133 concentration as low as 0.3 ng/ml was detectable. The experimental results presented a range of linearity of 0.3 ng/ mL to 10 µg/mL and 0.6 ng/mL to 10 µg/mL for 800 µm x 800 µm x 2 µm and 400 µm x 400 µm x 2 µm NMM, respectively. The minimum detectable surface stress for each sensor can be obtain when the output signals are equal to the noise values. By using the experimental results, 1.5 and 2.5 mJ/m were respectively the minimum surface stress sensitivities for the 800 µm x 800 µm x 2 µm and 400 µm x 400 µm x 2 µm NMM. In order to check the sensitivity of the present NMM -based biosensor, the results have been compared with other label free biosensing technologys. In Table 1, the minimum detection limits (LOD) of NMMs with different dimensions have been compared with a standard cantilever (MCL) [4-14], surface-plasmon resonance (SPR) [6], quartz crystal monitor (QCM) [30], and electrochemical [29] sensors. In most cases the NMM -based biosensor has the lowest LOD. Results indicat that NMM has comparable sensitivity with the optical read-out methods, moreover its sensitivity is significantely higher than a standard piezoresistive cantilever. This table quite well reflects the potential of the NMM -based biosensor in the pharmaceutical and medical diagnosis fields.

Table 1: The minimum limit of detection (LOD) of biomarker by different biosensors

[a] 400µm x 400µm x 2µm.

Category	Detection conditions	LOD
NMM	0.1 mg/ml BSA	0.6 ng/ml [a]
	0.1 mg/ml BSA	0.3 ng/ml [b]
MCL [30]with reference cantilever, piezoresistive detection	0.1 mg/ml BSA	10 ng/ml
MCL [4] no reference cantilever, optical detection	1.0 mg/ml HSA	0.2 ng/ml
SPR [25] Direct immunoassay	0.3 mg/ml BSA	300 ng/ml
QCM [28] Direct assay based on yeast cells strategy	serum	5 ng/ml
Electrochemical [14] Amperometric Sandwich immunoassay	phosphate buffer	0.25 ng/ml

[b] 800µm x 800µm x 2µm.

CONCLUSION

We have reported a novel signal transduction biosensor for detecting CD133, using a unique micro-fabricated self-sensing array of NMM sensors. Unlike cantilever sensors, which are based on optical readout systems, the NMM integrated piezoresistive readout sensors facilitate the detection of compact devices in even non-transparent environments. In comparison with traditional piezoresistive based cantilever sensors [9-16-20], our unique NMM design significantly improves sensor sensitivity that allows us to detect CD133 concentrations as low as 300 pg mL^{-1}, or 150 pM.

CONFLICT OF INTEREST
There is no conflict of interest.

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None

FINANCIAL DISCLOSURE
None

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ARTICLE

LIGHT AND ELECTRON MICROSCOPIC INVESTIGATION OF SEMELIL (ANGIPARS™) ON THERAPEUTIC PROCESS AFTER CHRONIC MYOCARDIAL INFARCTION IN RABBIT

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ABSTRACT

One of the main therapeutic intentions of modern cardiology is to contrive strategies aimed at decreasing myocardial necrosis and improving cardiac healing following myocardial infarction (MI). This investigation was contrived to study the protective effects of Semelil (ANGIPARS™), a native herbal medicine, on MI in the rabbit model. In this experimental study, Twenty New Zealand white rabbits were utilized. Rabbits were allocated to equal groups: control plus vehicle; sham plus vehicle; ischemia plus vehicle; Semelil 10 mg/kg, respectively. MI was created by the complete closure of Left Anterior Descending Coronary Artery (LADC). The animals were treated with Semelil 10 mg/kg daily for 14 days. Electrophysiological, Biochemical, histological and ultrastructural studies were used for detecting protective effects of Semelil. Based on our data, Semelil ameliorated the electrocardiogram (ECG) pattern. Besides, treatment with Semelil improved Creatine Kinase, creating kinase isoenzyme and Lactate dehydrogenase levels comparing to the ischemia group. Morphological data showed that Semelil could protect cardiomyocytes against myocardial infarction insults. The results demonstrate that Semelil may have protective effects against ischemic damages induced by LADC obstruction in male rabbits due to its anti-inflammatory and antioxidant properties.

KEY WORDS
Myocardial infarction; Mitochondria; Semelil; necrosis; Rabbits

INTRODUCTION

Cardiovascular disease, prevalently MI, is the prominent cause of mortality worldwide [1]. The decreases in the myocardial bloodstream because of the coronary artery obstruction can significantly conciliation the energy metabolism. The myocardial tissue is aerobic and its metabolism is strictly dependent on oxygen accessibility, which is approved by plenty of myoglobin and mitochondria in the cardio myocytes [2]. Moreover, the high-energy need of cardio myocytes for its contracture is met almost solely by mitochondrial oxidative phosphorylation [3]. These changes lead to the high sensitiveness of myocardial cells to oxygen shortage. The decrease of heart oxygen supply because of coronary occlusion leads to the reduction of oxygen provision to the mitochondria to uphold oxidative phosphorylation and cardiac ischemia ultimately [4]. Cardiac remodeling subsequent either ischemia or MI primarily develops to recompense for deteriorating cardiac function with the indication of hypertrophy, inflammation, cardio myocyte apoptosis and necrosis. Primarily these changes are advantageous but eventually transition to diminishing cardiac function and eventuate to heart deficiency [5]. The inflammatory responses stimulated by MI play a major impress in the pathogenesis of myocardial ischemia. These inflammatory reactions are found to intensify myocardial damage and remodeling after MI [6, 7], which eventuate to healing and wound formation [8, 9]. Medical trials comparing different myocardial advocating strategies and anti-inflammatory medicines are strongly needed. Clinically, there is noticeably intensifying release of heart damage enzyme markers (creatine kinase, MB-creatine kinase, LDH, et al.) in the serum level of patients with MI injury [10]. In this investigation, we mainly studied on an extract herbal and focused on the intricate mechanisms of their beneficial effects on ischemic heart diseases, especially on myocardial ischemic. *Melilotus Officinalis* has been produced as a component of a native drug by trade nomination of Semelil (ANGIPARS™). In vivo studies in mice, rabbit and dogs and in vitro studies in several based cell lines have approved its safety [11-13]. Prior examinations have revealed advantageous results of Semelil such as improvement of blood flow, the decrease of inflammation, the improvement of immune system and lymphedema [14-17]. Effects of clinical studies on Semelil revealed its safety and usefulness in human diabetic wound [18]. This extract herbal has been revealed to have the strong antioxidant concoction such as 7 hydroxycoumarin and flavonoids [15, 19]. Despite the clinical procedure of Semelil in Iran, fewer attentions have been paid to the importance of its management on the cardiovascular system. Recently, joukar showed the pretreatment influence of Semelil on isoproterenol-induced cardiac damage for two days can help to keep the heart contractility and consequently blood pressure balance in myocardial damage conditions [20]. In this investigation, we studied Post-treatment influence of Semelil on permanent closure of LADC for two sequential weeks based on electrophysiological, biochemical, histological and ultra-structural indices.

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MATERIALS AND METHODS

The animal preparation

In this experimental study, the examination was concurred by the animal Care Committee of Tehran heart Researches Center (EC/THRC/696) ordaining on the care and handling of laboratory animals. We used Twenty-five adult male New Zealand white rabbits (weight range 2–3.2 kg; Pasture Institute, Tehran, Iran). The rabbits were kept alone in metal cages and organized in a room with the stable temperature (24±2°C) and stable lighting cycle that contain 12 hours light-dark. The rabbits were allocated to equal groups: control plus vehicle (Ethanol 86 %, n=5); sham plus vehicle (n=5); ischemia plus vehicle (n=5); Semelil 10 mg/kg (thirty minutes after LADC obstruction was injected intraperitoneally for two weeks continually, n=5).

Induction of regional myocardial ischemia

The rabbits under deep anesthesia with ketamine and xylazine (100 mg/kg, 10 mg/kg i.m) were intubated and well-ventilated with a combination of 02 and N20. The body temperature of the rabbits was maintained by using a thermostatically warming plate at 37°C throughout surgery [21]. During the operation, an electrocardiogram (ECG) with six-lead was recorded. The chest was exposed through the fifth intercostal space, and then the pericardium was cut. The root regional of LADC was permanently ligated with silk suture. A successful setup of permanent coronary obstruction was concluded by the occurrence of superficial cyanosis of regional myocardial and typical ST segment elevation. None LADC ligation was managed in the sham operation group.

Drug solution preparation

The Semelil (ANGIPARS™) was prepared by Rose Pharmed Co. (Tehran, Iran) that contained the ethanol extract of *Melilotus Officinalis* was mixed with different content of selenium, sodium phosphoglycerol, urea, and fructose. The Semelil was diluted in normal saline for using.

Monitoring of the ECG

We screened each New Zealand white rabbit with the ECG in 1st and 30th days after the project under anesthesia by intraperitoneal injection of 50 mg/kg and xylazine 10 mg/kg in all groups. The hair was shaved for the location of electrodes. Electrodes were connected to each leg for limb leads. Six electrodes were connected to chest leads. The presentation of successful coronary obstructions was confirmed by observing the increase of ST-segment elevation and alterations in the QRS wave on the ECG tapes.

Biochemical analysis

The sampling and storage of blood and the evaluation of hemostatic factors have been depicted in detail elsewhere [22]. Blood was taken from the vein of ear rabbits in 1st and 30th days. The blood samples were gathered in sterile tubes and centrifuged at 2500 rpm for 10 minutes. The serums were separated and gathered for biochemical evaluations. Serum was utilized for the test of biochemical factors such as Creatine Kinase (CK) creatine kinase isoenzyme in heart (CK-MB) and Lactate dehydrogenase (LDH) were studied (Roche kit, Germany). The results were declared as U/L for CK, CK-MB, and LDH.

Light microscopic analysis

Rabbits were euthanized with sodium pentabarbitone (100 mg/kg) administrated intraperitoneally. The hearts were removed and cut into five transverse sections from apex to base. The samples were then flushed with normal saline and fixed in 10% buffered formalin for 96 h. The specimens were processed for light microscopy study according to the standard method [23]. Morphologic characteristics of cell necrosis demonstrate cell swelling, disturbance of cell membrane, hyper-eosinophilia and karyolysis [24]. Necrotic cells were enumerated in five different fields applying light microscopy.

Electron microscopic

Ultra structural indices of Cardio cytes were studied in different groups. Left ventricle was cut into small sections (1mm3) and fixed in 2.5% glutaraldehyde in a phosphate buffer (PH 7.4 for 2 h at 4 °C). Then samples were fixed in 1% osmium tetroxide (1 h at 4 °C). After frequent washing with buffer, Samples were dehydrated in ethanol sequence and embedded in Epon 812 (TAAB Laboratories Equipment Ltd, UK). Ultrathin sections were sliced and stained using uranyl acetate and lead citrate [25] and evaluated by transmission electron microscope (Philips, EM 208, Netherlands).

Statistical analysis

The results were evaluated by SPSS, version 21.0 and were presented as means ± SEM. All numerical information in text, tables and figures were studied by one-way ANOVA and the Bonferroni post-hoc test (P<0.05).

RESULTS

Effects of Semelil 10 mg/kg on electrocardiogram

Control group displayed a standard electrocardiograph pattern. Induction of ischemia caused significant changes in ECG differ from the control group at 1st after LADC ligation as presented in [Table 1]. Semelil 10 mg/kg failed to amend ECG disturbances at 30th after LADC ligation [Table 1].

Table 1: Electrocardiography results in different groups

Groups	Normal	ST flat	T inversion	ST depression	ST elevation	Q wave
1 days after LADC ligation						
Control vehicle	6	0	0	0	0	0
Sham vehicle	6	0	0	0	0	0
Ischemia vehicle	0	1	1	6	3	1
Semelil 10 mg/kg	0	1	1	6	3	1
30 days after LADC ligation						
Control vehicle	6	0	0	0	0	0
Sham vehicle	6	0	0	0	0	0
Ischemia vehicle	0	1	1	6	3	1
Semelil 10 mg/kg	0	0	1	1	2	1

Biochemical parameters

The effects of Semelil 10 mg/kg on serum marker enzymes as LDH, CK, and CK-MB are shown in [Table 2]. LDH decreased significantly (P<0.01) in Semelil 10 mg/kg group compared with the ischemic group at 30th after LADC ligation. CK and CK-MB enzyme significantly decreased (P<0.001) in Semelil 10 mg/kg group compared with the ischemic group at 30th after LADC ligation [Table 2].

Table 2: Hepatic and cardiac biomarkers results in different groups

Time point and parameter	Control vehicle	Sham vehicle	Ischemia vehicle	Semelil 10 mg/kg
1 days after LADC ligation				
CK (U/L)	1413±42	1605±27.9	8392±244.8	8250±162.5
CKMB (U/L)	16.9±0.5	19.2±0.63	100.7±2.9	99±3.6
LDH (U/L)	76±5.7	106±11.8	3930±133.3	2451±288.3
30 days after LADC ligation				
CK (U/L)	1156±23	1389±11.8	7755±323.9	2771±288.3*
CKMB (U/L)	13.8±0.28	16.5±0.41	93.06±3.8	33.2±1.9*
LDH (U/L)	84±5.3	86±8.8	2852±208	375±38.3*
Values are means ± SD. CK: Creatine Kinase; CK-MB: creatine kinase isoenzyme in heart; LDH: Lactate dehydrogenase; * P < 0.001 compared to control group.				

Changes in myocardial structure

Normal morphology of the myocardiocytes was observed with no indication of microscopic modifications in the control plus vehicle and sham plus vehicle groups [Fig. 1 A and B]. In the ischemic group was observed disturbance myocardial fibers, pyknotic nucleus and scar formation [Fig. 1 C and C1].

In Semelil 10 mg/kg group was observed nearly typical cardiac cells with the minimal injury [Fig. 1 D]. Myocardial fibers were organized in neat rows compared to the ischemic group [Fig. 1 D1]. There was a significant difference (P<0.05) of the Necrotic cells in the treatment group compared with ischemic groups [Fig. 2 and Table 3].

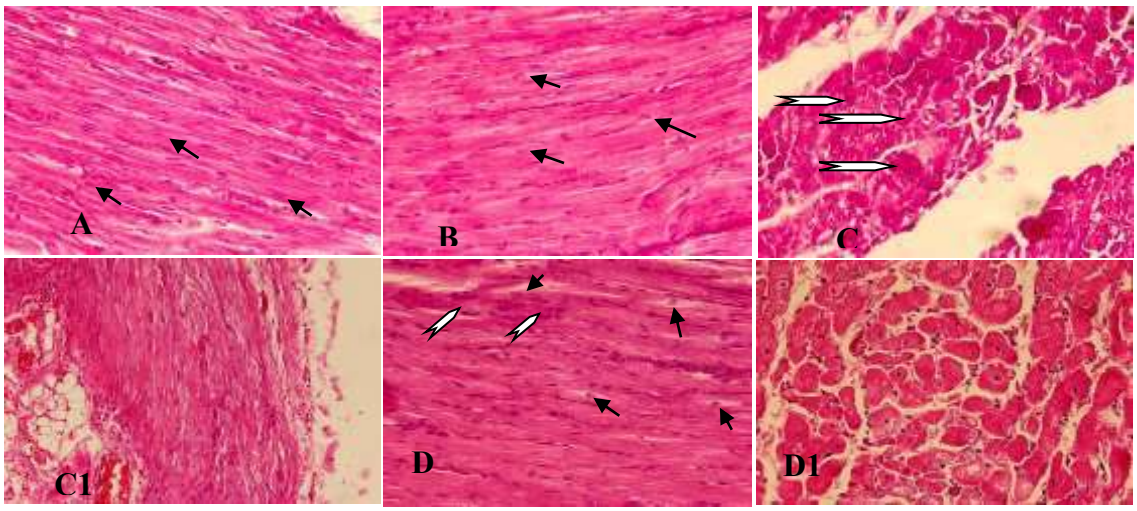

Fig: 1. Hematoxylin & eosin staining of the pathological structure by light microscopy at 30th after LADC ligation: (A) control (B) sham plus vehicle; muscle fibers and the nuclei form are normal; Arrows show the surviving myocardium. Ischemic group (C and C1); disturbance myocardial fibers, pyknotic nucleus (C) and scar formation (C1); chevrons show the Necrotic cells. Semelil 10 mg/kg group (D and D1); nearly typical cardiac cells (D) and myocardial fibers were organized in neat rows (D1); Chevrons show the cells death and arrows show the surviving myocardial (magnification x400).

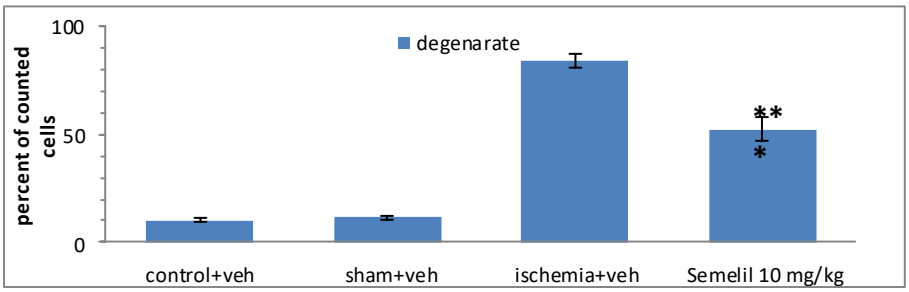

Fig: 2. The Effect of Semelil 10 mg/kg at 30th after LADC ligation in the heart rabbit; Results are expressed as mean ± S.E.M and data were studied by One-way ANOVA followed by the Bonferroni post-hoc test. ***Significantly different from control.

Table 3: Effect of Semelil 10mg/kg on Permanent obstruction of LADC Model in the heart rabbit

GROUP	(ALL)*	(DC)**	(DC/ALL×100)***
Control vehicle	521±7.81	53.6±2.85	10.28±0.65
Sham vehicle	528±9.30	60±4.19	11.36±0.87
Ischemic vehicle	339±10.65	285.8±13.03	84.30±3.33
***Semelil* 10 mg/kg**	543.4±10.88	284.2±27.28	52.30±5.47#

Five groups (n=5) were counted.
*Average numbers of all the myocytes
**Average numbers of the degenerated cells (DC)
***Percent average of the cells death (DC/ All×100)
#P<0.05 for *Semelil* 10 mg/kg

Changes in myocardial ultrastructure

Ultrathin Images in the control, sham plus vehicle groups demonstrate regular organization of the myofibrils [Fig 3 B2] and the mitochondria with plentiful regular cristae in between [Fig. 3 A2]. Striations are apparent

among the well organized myofibrils with H line [Fig. 3 A1]. The Z-line [Fig. 3 A] and intercalated disk [Fig. 3 B] are also seen. The ischemic injury led to lysis of the myofibrils [Fig. 4 C1 and C2] and massive fragmentation. Mitochondria [Fig. 4 C2] display either complete loss or vacuolization of the cristae [Fig. 4 C2]. Disruption and expansion of Z-lines are also seen. Semelil 10 mg/kg group displays organized myofibrillar [Fig. 4 D1] with mitochondria [Fig. 4 D2] in between. The mitochondria maintain a normal organization. Focal areas of myofibrillar loss [Fig. 4 D1] are seen.

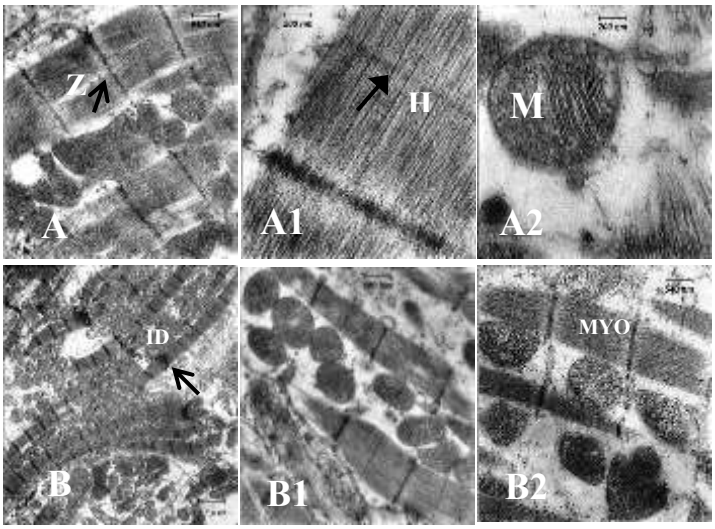

Fig. 3: Electron micrograph of the left ventricular myocardium of the control (a, a1and a2; scale bar 200 nm and 640nm) and sham vehicle (b, b1 and b2; scale bar 2.5µm and 640nm) groups at 30th after ladc ligation; displaying z line (a); striations are apparent among the well-organized myofilaments with h line (a1); mitochondria (m) (a2) with plentiful regular cristae in between and intercalated disk (id) (b); regular organization of the myofibrils (myo) (b2).

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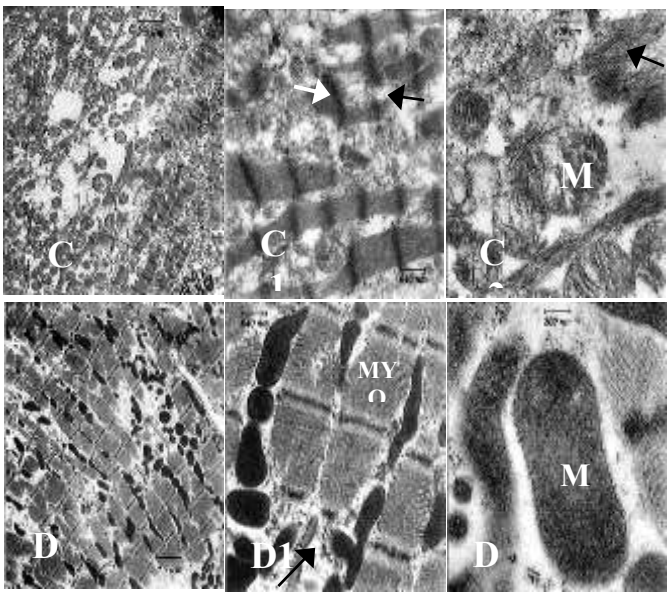

Fig. 4: Electron micrograph of the left ventricular myocardium of the ischemic group at 30th after ladc ligation (c, c1and c2; scale bar 2.5µm, 400nm, and 640nm) displays lysis of the myofibrils (c1 and c2-black arrows) and massive fragmentation. mitochondria (m) (c2) display either complete loss or vacuolization of the cristae (c2). disruption and expansion of z lines (white arrows) is also seen. semelil 10mg/kg group at 30th after ladc ligation (d, d1and d2; scale bar 2.5µm, 200nm and 640nm) displays organized myofibrils (myo) (d1) with mitochondria (m) (d2) in between. the mitochondria maintain a normal organization. focal areas of myofibrilar loss (d1-black arrow.

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DISCUSSION

MI is related to an inflammatory response, which is a prerequisite for therapeutic and scar formation [9]. In addition, ischemia leads to activation of nitric oxide synthesis (NOs) then produces nitric oxide (NO) and several enzymes as LDH, CK, CK-MB from the myocardium is secreted and can be created in peripheral blood almost two hours later [26]. These combinations cause to disturbed mitochondrial, lake of ATP and eventually death of myocardial cells [27]. Electrocardiogram (ECG) is regarded the most prominent clinical device for the diagnosis of some types of MI, specifically for the revelation of ST-segment elevation myocardial infarction (STEMI). In this study, we provided the indications for the cardio protective effects of the Semelil 10 mg/kg and its main constituent on numerous parameters of plasma level of myocardial enzymes and cardiac structure and ultrastructure in the chronic MI model. Semelil 10 mg/kg management significantly ameliorated the ECG pattern that indicating its protective influences on cardiac function. In this examination, treatment with Semelil led to the decrease in the plasma level of enzymes such as LDH, CK, and CK-MB. Structural investigations of this group demonstrated that Semelil 10 mg/kg prevented cell degeneration, myocardial necrosis, and nuclei shrinkage. Ultra structural studies of the treatment group revealed that Semelil 10 mg/kg improved neat organized in myocardial fibers with mitochondria. Semelil is a novel drug product including herbal extract with known useful effects specifically on diabetic foot [18]. Some of its constituents are *Melilotus Officinalis* extract, fructose, urea and selenium as prepared by the manufacturer. *Melilotus Officinalis* extract can decline activity of circulating phagocytes and has antioxidants, anti-inflammatory and anti-edematous influences [15, 28]. Selenium is a prominent potent anti-inflammatory and antioxidant medicine that may have cardio protective results [29]. Furthermore, experimental evidence has demonstrated that fructose can prevent the inflammatory response and improve the ischemic injury [30]. Also, the previous examination has revealed that urea could improve cardiac blood flow, oxygenation and have antioxidant and cardio protective effects [31]. The exact protective mechanism of Semelil on MI is not known yet. This examination was just concentrated on the common effect of Semelil on myocardial survival in rabbit heart and not its mechanism. Further investigations focusing on microcirculation of pre & post occlusion LADC and inflammatory factors are needed to find the correlated mechanisms.

CONCLUSION

This study revealed that herbal extract decreased the plasma level of myocardial enzymes, myocardial degeneration and necrosis. Furthermore, this herbal drug prevents disrupted mitochondria, sarcolemma breakage. These protection mechanisms may be associated to antioxidants, anti-inflammatory and anti-edematous influences.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

ACKNOWLEDGEMENTS

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FINANCIAL DISCLOSURE

The authors report no financial interests or potential conflicts of interest

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ARTICLE

INVESTIGATING OF REMOVAL ACTIVITY OF SYNTHESIZED ZINC TITANIUM OXIDE NANOPARTICLES ON THE WASTEWATER TREATMENT

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ABSTRACT

In this study, ZnTiO3 nanoparticles were successfully prepared by sol-gel method. For preparation of these nanoparticles, zinc acetate and tetra-n-butyl orthotitanate were used as the sources of zinc and titanium, respectively. Stearic acid was used as the complexing agent. Infrared (FT-IR) spectroscopy, X-ray diffraction (XRD), and scanning electron microscopy (SEM) were used for characterization, phase detection, and determination of the size and morphology of the particles, respectively. The size of the synthesized zinc titanium oxide nanoparticles was obtained approximately between 37-56 nm.To investigate the efficiency of the synthesized nanoparticles in removal of pollutants, malachite green was used as the model pollutant. To obtain the optimum condition, the effects of concentration, pH, time, and the amount of ZnTiO3 adsorbent were investigated. Studies showed that ZnTiO3 nanoparticles demonstrated good efficiency in removal of malachite green.

KEY WORDS
Nanoparticle, removal, ZnTiO3, sol-gel, malachite green

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INTRODUCTION

Healthy water which is free of toxic chemicals and pathogens is necessary for human health and it is considered a crucial material in major industries such as electronics, pharmaceuticals, and food industries. Due to expansion of drought, increase of population, intensification and improvement of health regulations, and increase of water consumption, supplying healthy water for the world is becoming challenging [1].

Though 2/3 of the surface of the earth is covered with water, nowadays there is water scarcity in human societies. With the increase of the world population, increase of drinking water consumption on one hand and contamination of a remarkable portion of drinking water on the other hand, will accelerate the decrease of drinkable water sources. According to the United Nations predictions, 48 countries (i.e. 32% of the world's population) will face water shortage in 2025 [2].

Underground waters finally find their ways into the rivers and seas. Therefore, contamination of underground waters can be a serious bio-environmental hazard. Dye pollutants are relatively much more hazardous compared to other pollutants. In fact, the color of these pollutants is a factor which can be used for detection of dangerous pollutants. Textile waste water is known as one of the biggest sources of pollutants which leave bio-environmentally destructive impacts. The dye material present in these waste waters is potential hazards for underground waters and inhabitants of these ecosystems. Water scientists and engineers are working toward increasing water quality according to the strict regulations and standards as water consumption is increasing. There are different methods to remove this substance from the aqueous solutions including adsorbent materials [3-6]. Beak et al. investigated MG removal from the aqueous solutions by degreasing coffee beans [7]. Rais Ahmad and coworkers studied the adsorption of MG on the Ginger wastes (TGW) by batch and column methods. The effect of various factors were also studied including initial dye concentration, contact time, pH and temperature [8]. The progresses of engineering sciences at the nano scale have provided unprecedented opportunities for development of acceptable processes for cost effective and bio-environmentally compatible water purification. To purify textile waste water and remove dyes from it various methods can be utilized. One of the most cost effective and common methods is application of adsorbents capable of trapping the dyes present in these waste waters. These adsorbents have different types which can be categorized in different groups according to their adsorption capacity, yield, and their efficiency in removal of these dyes [9-12]. Titanium dioxide nanoparticles have relatively wider application in removal of bio-environmental pollutants [9]. Many researchers have taken advantage of using adsorbent for removal of pollutants [13-18]. Novelty of this research is the application of zinc titanium oxide nanoparticles as the adsorbent in removal of pollutants, for the first time. For preparation of these nanoparticles, sol-gel method was used due to simplicity and controllability of the morphology of the nanoparticles.

MATERIALS AND METHODS

The Instruments and material

Scanning electron microscopy (SEM) was used for determination of particle size. FT-IR (Perkin Elmer) and XRD instruments were used for investigation of structural properties of the nanoparticles. A dual-beam spectrophotometer (Perkin Elmer UV/Vis 25) was used for measurement of the sample absorbance. Furthermore, a pH meter (Mettler Toledo) was used for pH measurement. Zinc acetate, tetra-n-butyl

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orthotitanate, and stearic acid were purchased from Merck Company of Germany. Deionized water was used during the experiment for preparation of the solutions.

Zinc titanium oxide nanoparticles preparation method

In this study, a type of wet chemistry synthetic procedure, stearic acid gel was used for preparation of pure zinc titanium oxide nanoparticles. In addition, this synthetic procedure is easily controlled and is an appropriate method. Sol-gel method demonstrates interesting results such as low temperature, homogeneity and higher purity. At first, zinc acetate (1 mol) and tetra-n-butyl orthotitanate (1 mol) were added to 2 moles of melted stearic acid at 73 ˚C and stirred. Then, it was put in an electric oven and heated up to 400 ˚C to dry up. It was kept at the same temperature (400 ˚C) for another 1 h to remove the stearic acid impurities. Finally, the temperature was raised up to 850 ˚C with a rate of 5˚/min to complete the calcination process. The zinc titanium oxide nanoparticles were ready for FT-IR, XRD and SEM analysis.

Procedure

In this research, some zinc titanium oxide adsorbent was added to the solutions of malachite green with different concentrations at ambient temperature. Then, at different time intervals, free concentrations of the solutions were recorded by a dual-beam spectrophotometer. The optimum factors such as pH, contact time, concentration of MG and the amount of adsorbent were determined from diagrams according to the above factors.

RESULTS AND DISCUSSION

Characterization and investigation of zinc titanium oxide nanoparticles

Characterization by infrared spectroscopy

[Fig. 1] illustrates the infrared spectrum of the zinc titanium oxide nanoparticles. In the FT-IR spectrum, the bands at 548.76 cm^{-1} and 836.81 cm^{-1} refer to the formation of $ZnTiO_3$. Furthermore, the absorption band at 3434.06 cm^{-1} in the spectrum, is attributed to the stretching vibrations of –OH groups of water.

Fig. 1: FT-IR spectrum of zinc titanium oxide nanoparticles.

Investigation of the phase and morphology of zinc titanium oxide nanoparticles by X-ray diffraction method

[Fig. 2] demonstrates the XRD pattern for zinc titanium oxide nanoparticles. According to the results, the formed nanoparticles are of cubic type which is in complete agreement with single XRD data. It should be noted that, there are very little amounts of titanium dioxide in the products which can be easily ignored.

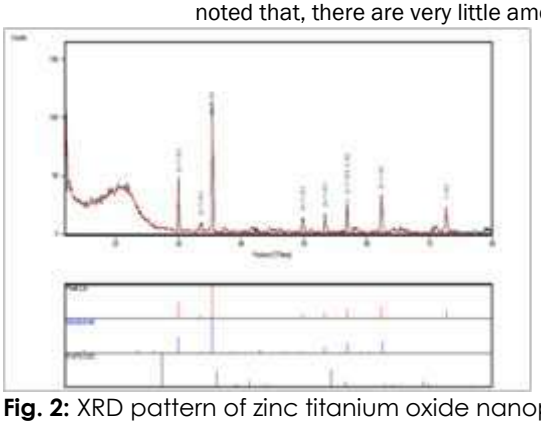

Fig. 2: XRD pattern of zinc titanium oxide nanoparticles.

Investigation of scanning electron microscopy spectrum

[Fig. 3] shows the scanning electron microscopy spectrum of the zinc titanium oxide nanoparticles. As shown in this fig., the average particle size obtained is around 41 nm.

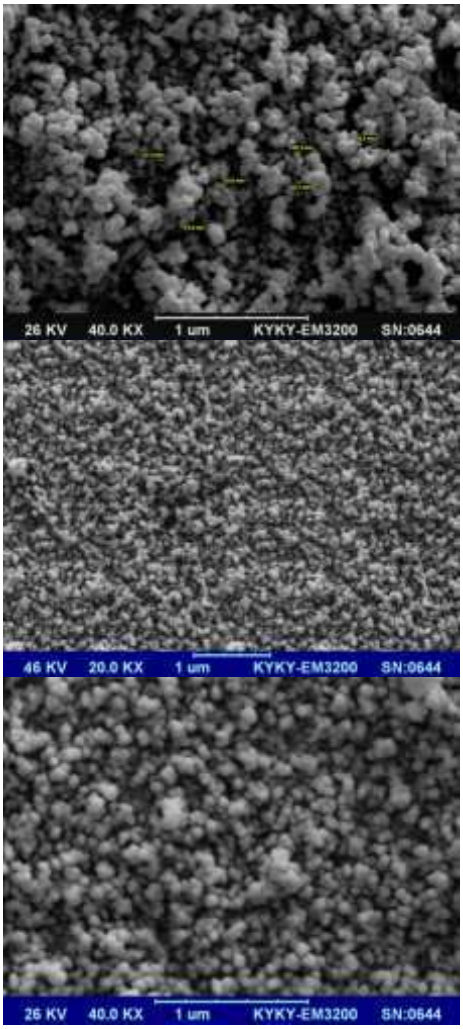

Fig. 3: SEM images of zinc titanium oxide nanoparticles.

Investigation of the efficiency of zinc titanium oxide adsorbent in removal of malachite green pollutant

Plotting the calibration curve for malachite green adsorption versus its different concentrations

For measuring the concentration of MG, its absorption properties in UV-Vis region were used. The absorption spectrum was plotted by a dual-beam spectrophotometer within the range of 350-800 nm for 1-10 ppm samples of malachite green to determine the maximum wavelength. As shown in [Fig. 4], the maximum absorption for malachite green is at 625 nm. Hence, this wavelength was used to measure the concentration of malachite green.

Plotting the calibration curve is essential for determination of the concentrations of the samples whose colors have been removed by absorbent. Thus, different concentrations of the malachite green were prepared and their absorptions were recorded and presented at 625 nm. The calibration curve in [Fig. 5] is plotted on the basis of the data presented in [Table 1].

Table 1: Concentration changes and absorptions

Concentration of malachite green (mg/L)	Absorption
1	0.201
2	0.601
3	0.9754
4	1.4183
5	1.9118

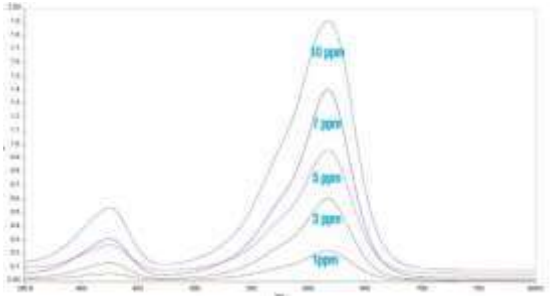

Fig. 4: UV-Vis spectrum of Malachite green.

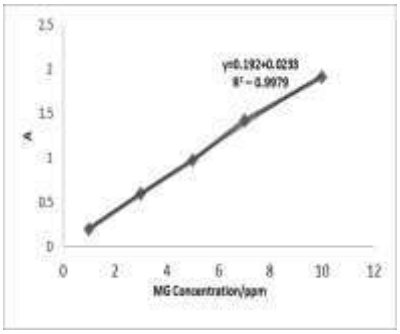

Fig. 5: Calibration curve of malachite green.

Investigation of the removal of malachite green in different pH values

pH is one of the most important factors which can increase the adsorption capacity of the adsorbent considering the point of zero charge potential, pH_{PZC}. To obtain the pH_{PZC}, 1 g of the adsorbent was added to eight beakers and their pH_1 values were adjusted between 2-9 using 0.1 M solutions of NaOH and HCl. Then, their pH_2 values were recorded after 24 h using a pH meter. Finally, ΔpH was plotted against pH_1 and the point where this curve crossed pH_1 diagram was considered as the pH_{PZC} or point of zero charge pH. As demonstrated in Fig. 6 the point of zero charge pH corresponds to the adsorbent 6 in which pH the adsorbent is electrically neutral. Because malachite green is a cationic dye, negative charges must surround the adsorbent. Thus, at pH>6 negative charges surround the adsorbent and the removal rate increases. Table 2 shows pH_1, pH_2, and ΔpH variations according to pH_1, where pH_1 is the hydrogen potential in the first day and pH_2 is the hydrogen potential after 24 h. As shown in table 3, 0.1 g of the zinc titanium oxide nanoparticles was added to 50 mL of 10 mg/L of malachite green and shaken for 30 minutes on a shaker. It was then filtered using a syringe and the absorbance of the solution was red and its concentration was calculated using the calibration curve and then placed in formula (1) to calculate the removal percentage where, C_0 is the initial concentration (mg/L) and C is the final concentration (mg/L) of malachite green and the diagram of removal percentage (R%) is plotted according to pH [Fig. 7]. As observed in [Fig. 7], the maximum amount of removal occurs at pH=8. Therefore, we will use pH=8 for optimization of other parameters.

$$\%Removal = \frac{(C_0 - C)}{C_0} \times 100 \qquad (1)$$

Table 2: pH_1, pH_2, and ΔpH variations according to pH_1

pH 1	pH2
2	-0.8
3	-2.4
4	-3.7
5	-4.9
6	-6
7	-7.2
8	-8.6
9	-10

pH 1	ΔpH	
2	1.2	
3	0.6	
4	0.3	
5	0.1	
6	0	PH_{PZC}=6
7	-0.2	
8	-0.6	
9	-1	

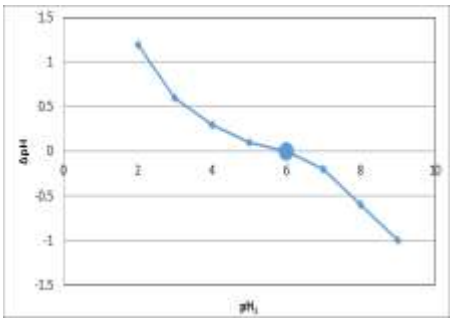

Fig. 6: Diagram of ΔpH variations according to pH_1.

Table 3: The effect of pH on the removal percentage

concentration=10 ppm	Volume=50	Time=30	Dosage=0.1
%Removal	A_{30}	A_0	PH
25.38	1.321	1.771	6
51.74	0.727	1.508	7
69.46	0.437	1.432	8
63.92	0.463	1.284	9
		%Removal	PH
		25.38	6
		51.74	7
		69.46	8
		63.92	9

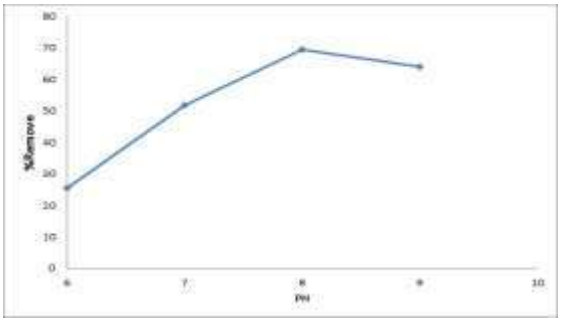

Fig. 7: Diagram of removal percentage of zinc titanium oxide nanoparticles versus pH.

Investigation of removal of malachite green on different dosage of zinc titanium oxide nanoparticles

Different amounts of the zinc titanium oxide nanoparticles were added to 50 mL of 10 mg/L solutions of malachite green with pH adjusted to 8 and were shaken for 30 minutes on a shaker. Then, they were filtered by insulin syringes and the absorbance values of the solutions were recorded. The concentrations were calculated using the calibration curve and placed in the formula (1) to calculate the removal percentage. As [Fig. 8] indicates, the removal percentage increases with the increase of the amount of the

adsorbent. This means that following increasing the amount of the adsorbent, the available sites for adsorption and results in improvement of dye removal increase. If the amount of the adsorbent is more than the optimum amount, the condensation and covering the molecular sites of the adsorbent results in a reduction in adsorption rate. According to the [Fig. 8], it is observed that the malachite green removal percentage increases with the increase of the adsorbent dosage until reaching a maximum at a dose of 0.7 g and then remains constant. Thus, the optimum dose of the nanoparticles was chosen as 0.7 g.

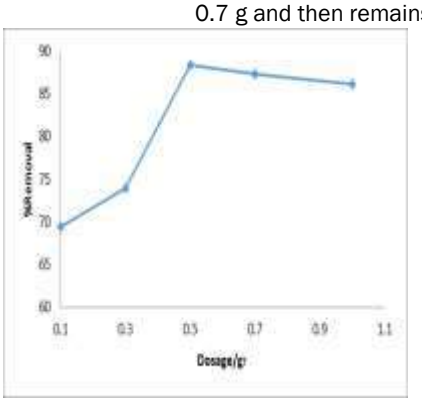

Fig. 8: Removal percentage of malachite green in different dosage of zinc titanium oxide nanoparticles.

Investigation of malachite green removal percentage based on different contact times

0.5 g of the zinc titanium oxide nanoparticles was added to 50 mL of 10 mg/L solution of malachite green with the pH adjusted to 8 and was shaken on a shaker. The absorbance of the solution was measured by a dual-beam spectrophotometer in 20 minutes' intervals after filtration by an insulin syringe. The concentrations were calculated from the calibration curve equation. The removal percentage was calculated by formula (1). [Fig. 9] indicates that the removal percentage reached a constant value after 60 minutes. Therefore, 60 minutes is considered as the optimum contact time for removal of the pollutant malachite green dye from aqueous media.

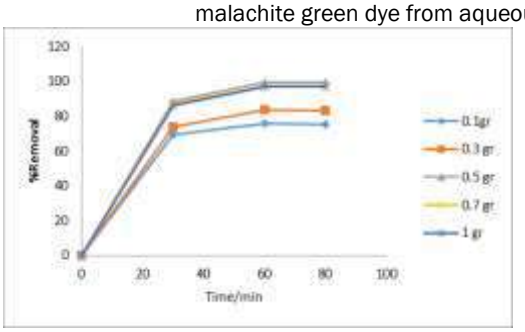

Fig. 9: Removal of malachite green on different contact time.

Investigation of the malachite green removal percentage with different concentration at optimum conditions

Zinc titanium oxide nanoparticles (0.5 g) were added to a 50-mL solution of malachite green with concentrations varying from 1 to 10 mg/L with the pH adjusted to 8. The solutions were shaken for 60 minutes on a shaker and then filtered and the absorbance values were measured by a dual-beam spectrophotometer. The concentrations were calculated using the calibration curve equation and the removal percentages were calculated by formula (1). [Fig. 10] demonstrated that the removal percentage of the solution with lower concentration is higher than the rest.

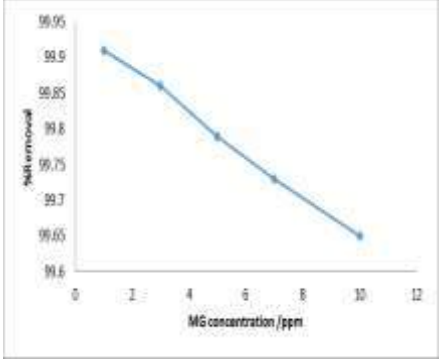

Fig. 10: MG removal percentage using the zinc titanium oxide nanoparticles in different concentrations of MG.

DISCUSSION

Camel was secured in sternal recumbence and fistula was repaired under xylazine sedation. Xylazine @ 0.3 mg/kg body weight administered intramuscularly and local infiltration of 2% Lignocaine was made. Partially masticated feed material was recovered from the buccal fistula along with pocket by help of allies forceps and pocket was emptied. The fistula was debrided. One soft circular leather piece of size slightly greater than diameter of fistula was placed on inner oral mucosal opening along with thread which was come out through buccal fistula opening [Fig. 2]. The wound edge was freshened with B.P. blade to improve vascularity. Buccal fistula was repaired with catgut no. 2 and skin was sutured with silk thread. Another rectangular hard leather piece of size slightly greater than diameter of fistula was placed on outer skin opening of fistula and knot was secured on the outer hard leather piece [Fig. 3].

Table 1: Prediction time of parallel machines and prediction accuracy

Machine	Prediction time	Prediction accuracy
P1	0.29 sec	98%
P2	0.32 sec	98%
P3	0.29 sec	100%
P4	0.31 sec	96%

The wound edge was freshened with B.P. blade to improve vascularity. Buccal fistula was repaired with catgut no. 2 and skin was sutured with silk thread. Another rectangular hard leather piece of size slightly greater than diameter of fistula was placed on outer skin opening of fistula and knot was secured on the outer hard leather piece [Table 1].

CONCLUSION

This research demonstrated that zinc titanium oxide nanoparticles were synthesized successfully by sol-gel method. Infrared spectroscopy (FT-IR), X-ray diffraction (XRD), and scanning electron microscopy (SEM) were used for characterization, phase detection and determination of the size and morphology of the particles. According to the results, the prepared nanoparticles were of cubic type which was in complete agreement with single crystal XRD data. The size of the synthesized zinc titanium oxide nanoparticles was between 37-56 nm.

Malachite green was used as a model pollutant to investigate the efficiency of the synthesized nanoparticles in removal of pollutants. To obtain the optimum condition the impacts of the concentration of the pollutant, pH, time and the amount of ZnTiO3 were studied. The studies showed that ZnTiO3 nanoparticles demonstrate good efficiency in removal of malachite green.

Hence, according to these results, zinc titanium oxide nanoparticles can be applied as an available adsorbent with high removal percentage.

CONFLICT OF INTEREST
There is no conflict of interest.

ACKNOWLEDGEMENTS
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FINANCIAL DISCLOSURE
None

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ARTICLE

METAGENOME OF INDIAN ONE RUPEE COIN REVEALS PLETHORA OF MICROBIOTA

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ABSTRACT

Background: *Currency coins are widely exchanged inanimate object in the world and thus are potential source of pathogenic microorganisms. Most of the earlier studies focused upon culture dependent screening of currency coins and proved less fruitful.* ***Methods:*** *We studied an Indian One Rupee Coin through metagenomic approach. Metagenomic DNA was isolated and Illumina Mi-seq PCR and sequencing was carried out. Further, denoising, chimera checking, SFF file generation, quality file generation, sequence clustering, taxonomic identification and data analysis were performed.* ***Results:*** *Among the trimmed kingdom, bacteria ranked first (99.81%) and the rest were no hit. Among trimmed phylum and trimmed class, actinobacteria was abundant (90.52% and 90.45%) respectively. Among the trimmed order and family, propionibacteriales and Propionibacteriacea were found to be copious (88.67% and 88.53%) respectively. Among genus, Propionibacterium was found to be abundant (88.53%).* ***Conclusions:*** *Possible pathogenic microorganisms found at species included: Corynebacterium accolens, Corynebacterium kroppenstedtii, Propionibacterium granulosum, Staphylococcus aureus, Finegoldia magna, Listeria monocytogenes and Staphylococcus epidermidis and thus coins are potential source of pathogenic microorganisms.*

KEY WORDS
Coin, Metagenome, Currency, India

INTRODUCTION

Currency coin is a potential source of pathogenic microorganisms. Currency coins are widely exchanged inert object in the world. Most of the studies payed attention upon culture dependent selection of currency coins and proved less productive. To our best knowledge, this scientific communication of metagenomic study of Indian currency coin revealed a plethora of potential pathogenic bacteria that might play a significant role in disease spread and possible antibiotic resistance spread.

MATERIALS AND METHODS

Sample collection and metagenomic DNA isolation

An Indian One Rupee coin was obtained. Metagenomic DNA was isolated from an Indian One Rupee coin by adding 0.25 ml of saline sample to powermag bead plate and further powermag bead solution, lysis solution and RNase A was added. The powermag bead plate was placed in the 96 well plate shaker and later centrifuged. The supernatant was transferred to a clear powermag 1ml collection plate. By using inhibitor removal technology, powermag IRT solution was added and incubated at 4˚C and later centrifuged. 450 µl of supernatant was transferred to a new powermag collection plate and again centrifuged. 450 µl of supernatant was transferred to a kingfisher deep well 96 plate and the metagenomic DNA was isolated with the technical support of Rocio Navarro Garcia and J. Delton Hanson in Research and Testing Laboratory, USA (Research and Testing Laboratory, 4321 Marsha Sharp FWY, Door #2, Lubbock, Texas 79407, USA).

Sequencing of isolated metagenomic DNA

Illumina Mi-seq was performed [1] with the technical support of above mentioned personnel in Research and Testing Laboratory, USA. Illumina Mi-seq PCR technique is an accurate and widely used technique [1]. PCR was performed in a two step process according to Users' manual. The forward and reverse primers namely TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG and GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG were used for first round of PCR. PCR was performed according to Users' manual with the technical support of already mentioned personnel in Research and Testing Laboratory, USA in 25 µl reactions with 1 µl template and 1 ul 5 uM primer on ABI verity thermo cyclers (Applied Biosytems, California, USA) using Qiagen Hot Star Taq master mix (QiagenInc, California, USA) under the following conditions: 95 ˚C for 5 minutes, 25 cycles of 94 ˚C for 30 S, 54 ˚C for 40 S, 72 ˚C for 1 minute, one cycle of 72 ˚C for 10 minutes and 4 ˚C hold. The forward and reverse primers namely AATGATACGGCGACCACCGAGATCTACAC (i5 index)- TCGTCGGCAGCGTC and CAAGCAGAAGACGGCATACGAGA (i7 index)- GTCTCGTGGGCTCGG were used for second round of PCR according to Users' manual. PCR was performed according to Users' manual as before with the technical support of personnel in Research and Testing Laboratory, USA. PCR products were visualized in e-gels according to Users' manual (Life Technologies, New York, USA), pooled in equal molar and all the pooled products were size selected into 2 rounds according to Users' manual using AgencourtAmpure XP (BeckmanCoulter, Indiana, USA) with the technical support of personnel in Research and Testing Laboratory, USA. A 0.7 ratio for both rounds size chosen pool was then quantified using a Quibit 2.0 fluorometer (Life Technologies) according to Users' manual and loaded on an illumina Mi-Seq (Illumina, , California, USA) 2 x 300 flow cell at 10 pM and PCR products were sequenced according to Users' manual with the technical support of personnel in Research and Testing Laboratory, USA.

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Bioinformatics analysis

After completion of sequencing, FASTQ data was segregated as paired and single end. Paired end FASTQ data was merged and was send to converter along with single end FASTQ data. FASTA/ Qual file was generated and denoising [2] , chimera detection [3] was performed. Diversity analysis consisted of SFF file generation [4], quality file generation [5], sequence clustering [6], taxonomic identification [7] using OTU selection (default) / dereplication and data analysis using RDP and USEARCH (default) [8,9,10].

RESULTS AND DISCUSSION

KRONA depiction [Fig. 1] indicated among trimmed kingdom, bacteria ranked first (99.81%) and the rest were no hit [Table 1]. Among trimmed phylum and trimmed class, actinobacteria was abundant (90.52% and 90.45%) respectively. Among trimmed order and family, propionibacteriales and Propionibacteriacea were found to be copious (88.67% and 88.53%) respectively.

Among genus, Propionibacterium was found to be abundant (88.53%). Apart from actinobacteria, firmicutes (6.13%) dominated the trimmed taxa percentage [Table 2]. Bacilli was found to be second position (5.17%) among trimmed class [Table 3]. A slightly significant percentage of bacillales (3.82%), lactobacillales (1.35%) and corynebactetriales (1.30%) were present apart from leading propionibactetriales (88.67%) [Table 4]. Apart from leading genus Propionibacterium (88.53%), other prominent genus included Corynebacterium (1.06%), Staphylococcus (3.45%), Streptococcus (1.00%) [Table 6]. Prominent species found included *Pr*opionibactetrium acnes (88.2%), *Staphylococcus epidermidis* (3.37%) [Table 7].

Among the species found, many of them have been earlier reported to cause various infections: *Actinomyces* sp. (Oral- cervico facial disease) [11], *Corynebacterium accolens* (Pelvic osteomyelitis) [12], *Corynebacterium kroppenstedtii* (Inflammatory breast disease) [13], *Streptomyces* sp. (Mycetoma) [14], *Propionibacterium granulosum* (Septicemia) [15], *Bacteroides* sp. (Root canal infection) [16], *Porphyromonas* sp. (Infection in anatomic cells) [17], *Staphylococcus aureus* (Skin infection, respiratory infection) [18], *Peptostreptococcus* sp. (Brain, liver, abcesses, soft tissue infection) [19], *Veilionella* sp. (Endocarditis) [20], *Finegoldia magna* (Prosthetic infection) [21], *Methylobacterium* sp. (general infection) [22], *Listeria monocytogenes* (Meningitis, sepsis) [23], *Wolbachia* (Infection in ovary) [24] and *Staphylococcus epidermidis* (Infection in heart valves and joints) [25].

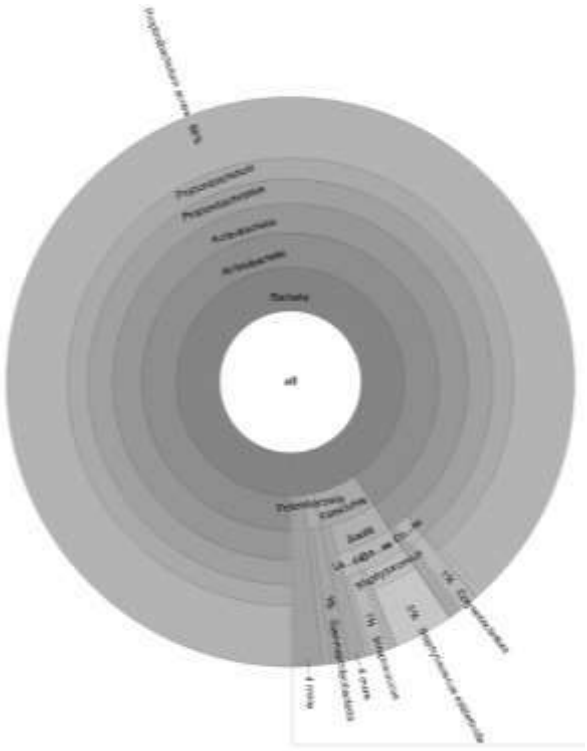

Fig. 1: Krona hierarchical pie chart of One Rupee coin metagenome Among trimmed kingdom, bacteria ranked first (99.81%) and the rest were no hit. Among trimmed phylum and trimmed class, actinobacteria was abundant (90.52% and 90.45%) respectively. Among genus, Propionibacterium was found to be abundant (88.53%).

Table 1: Kingdom percentage

Kingdom	Trimmed Taxa Percentage
Bacteria	99.81
No hit	0.18

Table 2: Phylum percentage

Phylum	Trimmed Taxa Percentage
Actinobacteria	90.52
Bacteroidetes	0.65
Cyanobacteria	0.15
Firmicutes	6.13
Proteobacteria	1.52
Unclassified	0.07
No hit	0.18
Unknown	0.74

Table 3: Class percentage

Class	Trimmed Taxa Percentage
Actinobacteria	90.45
Rubrobacteria	0.01
Bacteroidia	0.35
Flavobacteria	0.03
Sphingobacteria	0.20
Bacilli	5.17
Clostridia	0.54
Negativicutes	0.11
Tissierellia	0.05
Alphaproteobacteria	0.14
Betaproteobacteria	0.36
Gammaproteobacteria	1.00
Unclassified	0.07
No Hit	0.18

Table 4: Order percentage

Order	Trimmed Taxa Percentage
Actinomycetales	0.01
Corynebacteriales	1.30
Geodermatophilales	0.03
Micrococcales	0.15
Propionibacteriales	88.67
Streptomycetales	0.07
Rubrobacterales	0.01
Bacteroidales	0.35
Flavobacteriales	0.03
Sphingobacteriales	0.20
Bacillales	3.82
Lactobacillales	1.35
Clostridiales	0.50
Selenomonadales	0.11
Tissierellales	0.05
Rhizobiales	0.07
Rhodobacterales	0.03
Rickettsiales	0.03
Burkholderiales	0.21
Neisseriales	0.09
Enterobacteriales	0.56
Oceanospirillales	0.01
Pseudomonadales	0.28
Xanthomonadales	0.13
No Hit	0.18

Table 5: Family percentage

Family	Trimmed Taxa Percentage
Actinomycetaceae	0.01
Corynebacteriaceae	1.06
Mycobacteriaceae	0.23
Geodermatophilaceae	0.03
Microbacteriaceae	0.14
Micrococcaceae	0.01
Nocardioidaceae	0.14
Propionibacteriaceae	88.53
Streptomycetaceae	0.07
Rubrobacteraceae	0.01
Bacteroidaceae	0.10
Porphyromonadaceae	0.01
Prevotellaceae	0.23
Chitinophagaceae	0.04
Sphingobacteriaceae	0.16
Bacillaceae	0.27
Listeriaceae	0.09
Staphylococcaceae	3.45
Lactobacillaceae	0.31
Streptococcaceae	1.00
Flavobacteriaceae	0.03
Lachnospiraceae	0.02
Peptostreptococcaceae	0.07
Veillonellaceae	0.11
Peptoniphilaceae	0.05
Methylobacteriaceae	0.05
Xanthobacteraceae	0.02
Rhodobacteraceae	0.03
Anaplasmataceae	0.03
Burkholderiaceae	0.14
Enterobacteriaceae	0.56
Oceanospirillaceae	0.01
Moraxellaceae	0.18
Pseudomonadaceae	0.10
Xanthomonadaceae	0.13
Unknown	0.74
No Hit	0.18

Table 6: Genus percentage

Genus	Trimmed Taxa Percentage
Actinomyces	0.01
Corynebacterium	1.06
Mycobacterium	0.23
Blastococcus	0.03
Microbacterium	0.14
Arthrobacter	0.01
Nocardioides	0.14
Propionibacterium	88.53
Streptomyces	0.07
Rubrobacter	0.01
Bacteroides	0.10
Porphyromonas	0.01
Prevotella	0.23
Segetibacter	0.04
Pedobacter	0.16
Anoxybacillus	0.27
Listeria	0.09
Staphylococcus	3.45
Lactobacillus	0.31
Streptococcus	1.00
Oribacterium	0.02
Peptostreptococcus	0.07

Veillonella	0.11
Finegoldia	0.05
Methylobacterium	0.05
Xanthobacter	0.02
Rubellimicrobium	0.03
Wolbachia	0.03
Burkholderia	0.14
Tepidimonas	0.07
Buchnera	0.06
Escherichia	0.36
Marinomonas	0.01
Acinetobacter	0.08
Psychrobacter	0.09
Pseudomonas	0.10
Stenotrophomonas	0.13
Unclassified	0.07
Unknown	0.74
No Hit	0.18

Table 7: Species percentage

Species	Trimmed Taxa Percentage
Corynebacterium accolens	0.02
Corynebacterium kroppenstedtii	0.50
Corynebacterium sp.	0.17
Microbacterium oleivorans	0.03
Microbacterium phyllosphaerae	0.10
Arthrobacter sp.	0.01
Nocardioides sp.	0.14
Propionibacterium acnes	88.27
Propionibacterium granulosum	0.19
Propionibacterium sp.	0.06
Streptomyces sp.	0.03
Rubrobacter sp.	0.01
Bacteroides sp.	0.10
Porphyromonas sp.	0.01
Segetibacter sp.	0.04
Pedobacter duraquae	0.16
Anoxybacillus sp.	0.27
Listeria monocytogenes	0.09
Staphylococcus aureus	0.07
Staphylococcus epidermidis	3.37
Lactobacillus jensenii	0.31
Streptococcus sp.	0.70
Peptostreptococcus sp.	0.07
Veillonella sp.	0.11
Finegoldia magna	0.05
Methylobacterium sp.	0.01
Rubellimicrobium sp.	0.03
Buchnera aphidicola	0.06
Acinetobacter sp.	0.08
Psychrobacter sp.	0.09
Pseudomonas sp.	0.10
Stenotrophomonas sp.	0.13
No Hit	0.18

Table 8: Possible pathogenic bacteria

Bacteria	Reported disease	Trimmed Taxa Percentage	Reference
Actinomyces	Oral- cervico facial disease	0.01	11
Corynebacterium accolens	Pelvic osteomyelitis	0.02	12
Corynebacterium kroppenstedii	Inflammatory breast disease	0.50	13
Streptomyces sp.	Mycetoma	0.03	14
Propionibacterium granulosum	Septicemia	0.19	15
Bacteroides sp.	Root canal	0.10	16

	infection		
Porphyromonas sp.	Infection in anatomic cells	0.01	17
Staphylococcus aureus	Skin infection, respiratory infection	0.07	18
Peptostreptococcus sp.	Brain, liver, abcesses, soft tissue infection	0.07	19
Veilionella sp.	Endocarditis	0.11	20
Finegoldia magna	Prosthetic infection	0.05	21
Methylobacterium sp.	General infection	0.015	22
Listeria monocytogenes	Meningitis, sepsis	0.09	23
Wolbachia	Infection in ovary	0.03	24
Staphylococcus epidermidis	Infection in heart valves and joints	3.37	25

CONCLUSION

Possible pathogenic microorganisms found at species included: *Corynebacterium accolens, Corynebacterium kroppenstedii, Propionibacterium granulosum, Staphylococcus aureus, Finegoldia magna, Listeria monocytogenes* and *Staphylococcus epidermidis*.

CONFLICT OF INTEREST
There is no conflict of interest.

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AUTHOR CONTRIBUTION
All authors have contributed equally to this work.

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ROLE OF INTRA-GASTRIC TRANEXAMIC ACID IN MANAGEMENT OF ACUTE UPPER GASTROINTESTINAL BLEEDING

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ABSTRACT

Background and Aims: *There are controversies about the safety and haemostatic efficacy of systemic Tranexamic Acid (TA) in patients with Upper Gastrointestinal Bleeding (UGIB). The goal of this study was to determine the efficacy of a single dose of TA, administered topically, on bleedings from benign peptic lesions.* ***Methods:*** *We assessed the effects of intra-gastric administration of TA on 131 patients presenting with hematemesis, melena, or both in a prospective double-blind randomized placebo-controlled trial. TA was administered at a dose of 1 gram diluted in 250 cc of saline solution via nasogastric tube. Our primary outcome parameter was the amount of blood needed for transfusion (units of packed cells).* ***Results:*** *UGIB-related mortality rate was seen to be lower in TA treated group, but the difference did not reach the level of significance (p=0.150). Transfusion requirements were significantly higher in patients not receiving TA (p<0.001). The number of re-bleeding episodes was 4 (6%) in TA group and 12 (18.8%) in placebo group (p=0.033). There was also a significant difference between the 2 groups in the number of emergency endoscopies; Six (9%) in TA group vs. 14 (21.9%) in the placebo group (p=0.040).* ***Conclusions:*** *We believe that intra-gastric administration of TA is safe, cost effective, well tolerated and can be performed easily. Further investigation is needed to evaluate the efficacy and safety of this new method for management of acute UGIB .Since we had some limitations in our sample size, additional evidence is needed before any treatment recommendations can be made.*

KEY WORDS
Upper Gastrointestinal Bleeding, TranexamicAcid, Peptic Ulcer, Antifibrinolytic Drug, Topical

INTRODUCTION

Despite advances in diagnosis and treatment of upper gastrointestinal bleeding (UGIB), the mortality and morbidity rates are still high [1, 2]. Bleeding peptic ulcers are responsible for about half of all upper gastrointestinal hemorrhages [3, 4].

Tranexamic acid (trans-4-aminomethylcyclohexane-1-carboxylic acid; TA), a potent anti-fibrinolytic agent, has been found to bind to lysine binding sites of plasmin and plasminogen [5]. It saturates the lysine binding sites of human plasminogen, displacing plasminogen from the fibrin surface, which leads to inhibition of fibrinolysis [6]. Systemic TA appears to be effective in diminishing blood loss in various pathological conditions such as menorrhagia [7, 8], gastric and duodenal ulcers [9, 10], orthopaedic surgery [11, 12], intraoperative and postoperative bleeding in cardiac surgery [13, 14], bleeding from traumatic injuries [15].

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There are some reports about complications of systemic TA administration. It increased the risk of thromoembolic events [16, 17], and seizure [18, 19] in susceptible patients. However, topical TA has been successfully used to control bleeding in urologic, gynaecologic, oral, otolaryngeal surgeries [20-22] and total knee arthroplasty [23]. Topical application of TA in patients undergoing primary coronary artery bypass grafting led to a significant reduction in postoperative blood loss without adding extra risk to the patient too [24]. Also, topical TA significantly reduced mean blood losses after minor oral surgeries in patients receiving oral anticoagulants without discontinuation of anticoagulation regimen [25].

To our knowledge, there is no report of a controlled trial of topical intra-gastric anti-fibrinolytic therapy in UGIB. The goal of the current study was to determine the efficacy of a single dose of TA, administered topically, to control bleedings from benign peptic lesions.

MATERIALS AND METHODS

Study design

We assessed the effects of intra-gastric administration of TA on acute UGIB in a prospective double-blind randomized placebo-controlled trial in 131 patients with hematemesis, melena, or both by a random number sequence. After eligible subjects received detailed explanations about the protocol, informed consent is obtained. This study is conducted in concordance with the principles of the Declaration of Helsinki. Approval for the conduct of this study was obtained from the Research Ethics Committee and Institutional Review Board of Tehran University of Medical Sciences (Ethic code: 90/d/2781/130). This trial has also been registered at International Clinical Trials Registry Platform (registration number: IRCT201201148721N1at http://www.who.int/ictrp).

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Setting and selection of participants

This study was conducted at Hazrat Rasool General Hospital. All patients with an initial clinical diagnosis of UGIB were primarily recruited. Endoscopic examination was performed in all recruited patients within 24 hours of presentation and consequently, any patient without a demonstrable benign gastric or duodenal lesion was excluded from the study.Patients would not be eligible for inclusion in this study if they were pregnant or lactating women, patients having a gastrointestinal malignancy, patients having a history of thromboembolism, myocardial infarction, ischemic cerebrovascular accident, end stage renal disease, allergy to TA, ongoing anticoagulation therapy, congenital or acquired coagulopathy, or patients being reluctant to enroll in this study. A total of 272 patients entered the trial and 141 were excluded, leaving 64 placebo treated and 67 TA treated patients for analysis [Fig. 1]. The most common reason for exclusion was having no gastric or duodenal source of bleeding identified at endoscopy.

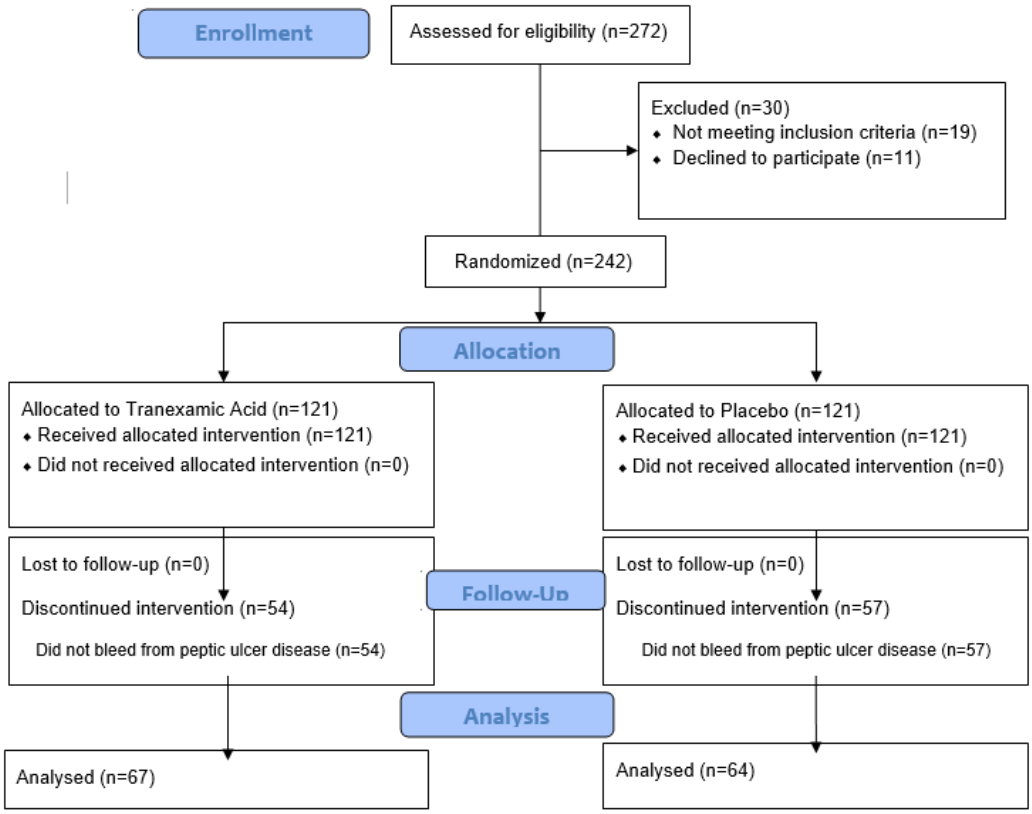

Fig. 1: The overall patient selection process

Outcomes

Our primary outcome parameter was the amount of blood needed for transfusion (units of packed RBCs); secondary outcome parameters included rebleeding events, need for surgical intervention, postoperative 30-day mortality rates, and occurrence of deep vein thrombosis (DVT).

Intervention

Participants were randomized to receive either TA or placebo in addition to the usual conventional treatment. All patients underwent resuscitation with crystalloids, insertion of nasogastric tube and gastric lavage with saline and intravenous pantoprazole. Also patients with UGIB presenting to ED were managed in consultation with department of gastroenterology. TA (Tranexip: Caspian Tamin Co., Rasht, Iran) was administered at a dose of 1 gram diluted in 250 ml of saline solution via nasogastric tube started in the first 30 minutes of patients' arrival at the ED. TA or placebo (physiologic saline) for infusion was prepared by the institution's pharmacy in two 250cc bags indistinguishable in shape and color identified only by random numbers, with the constituents unknown to the administering emergency resident. The placebo group received the same treatment protocol as the TA group except for the infusion of normal saline solution not containing TA. The randomization codes were known only to the pharmacist in charge and revealed only at the end of the study. Rebleeding was defined as bleeding after a silent period of more than six hours, or hypotension (<100 mm Hg systolic blood pressure) associated with a drop in the hemoglobin concentration of 2 g/dl or more and/or endoscopic evidence of fresh rebleeding. Although a hemoglobin level less than7 g/dl was the starting point for packed RBC transfusion, some other factors

also play roles, such as a clinical assessment of shock, hematocrit level, amount of blood loss, age, presence or absence of symptoms, underlying cardiopulmonary status and clinical judgment of ED attending physician. The number of PRBC units used for blood transfusion for each patient was counted up to the time of discharge from hospital: One unit of PRBC was equal to 250ml of this product. In-hospital mortality was recorded and all patients were followed for 4 weeks. Basic data, laboratory study, and endoscopy findings were obtained and recorded. A Doppler ultrasound study of lower limb veins was performed anytime there was a clinical suspicion for DVT (swelling and/or tenderness of calf muscles and/or unexplained postoperative hypoxemia). Rockall score was calculated for each participant at the beginning of treatment and after the endoscopy [26]. Surgical intervention was considered when intervention by endoscopic techniques failed or was contra-indicated.

Data analysis

The statistical analyses were performed by Statistical Package for Social Sciences (SPSS) for Windows 16.0 (SPSS Inc., Chicago, IL, USA). To test the normality of the distribution of the continuous variables, the Kolmogorov-Smirnov test was performed. Descriptive statistics are given by means and standard deviations for normally distributed data. Categorical data are subsumed by absolute and relative frequencies. In analytical statistics, Nominal or ordinal variables were compared between groups by chi-square test and Fisher's exact test, depending on the expected cell counts of the corresponding crosstabs. In addition, unpaired Student *t* test was used when the variables fulfilled the presumption of normal distribution (only age), whereas the Mann-Whitney *U* test was used when the variables were not normally distributed. The Spearman rank order correlation was used to test correlations between transfusion requirements and age. The results of the two-sided tests were considered significant if the p value was less than 0.05. As there were no previously published data, we used the data of previous studies evaluating the effects of intravenous or oral form of TA to estimate the sample size. The null hypothesis was defined as there is no statistically meaningful difference in amount of blood needed for transfusion between those who either did or did not receive TA, and the alternative hypothesis was that the amount of blood transfusion of those who received TA was less than that of those not treated with TA. The Altman's nomogram was used to calculate the sample size; for a power being equal to 90%, the sample size would be 130. We included 272 patients initially because our estimate for the proportion of episodes caused by peptic ulcers was approximately 50% of all UGIB events.

RESULTS

A total of 272 patients with UGIB were admitted to our ED during the study period, of which 131 (48.16%) were diagnosed to bleed from peptic ulcers. Sixty-seven patients were allocated to TA and 64 to placebo. The average age of participants was 64.25 years (± 13.92 years). The treatment group and the placebo group were well comparable regarding gender, age, *Helicobacter pylori* infection, endoscopy findings, Rockall score, and laboratory values; no statistically significant difference was found [Table 1].

Table 1: Presentation of findings on admission and post intervention findings in the participants Data are presented as incidence (%), mean ± standard deviation. PRBC: Packed Red Blood Cell

Variables	All Participants (n=131) Number of patients (%) or mean±*SD*	TA Group (n=67) Number of patients (%) or mean±*SD*	Placebo Group (n=64) Number of patients (%) or mean±*SD*	*P* value
Findings on Admission				
Age (years)	64.25±13.92	63.83±13.60	64.70±14.34	P=0.723
Male Gender	82(62.6%)	41(61.2%)	41(64.1%)	P=0.857
Rockall Score	3.48±1.57	3.52±1.60	3.43±1.55	P=0.759
H.pylori	91 (69.46)	45 (67.16)	46 (71.8575)	P=0.078
Post Intervention Findings				
Mortality	13 (9.9%)	4 (6%)	9 (14.1%)	P=0.150
Rebleeding	16 (12.2%)	4 (6%)	12 (18.8%)	P=0.033
Emergency Endoscopy	20 (15.3%)	6 (9%)	14 (21.9%)	P=0.040
Units of PRBC	2.32±1.47	1.77±1.08	2.90±1.61	P<0.001

Mortality– There were 13(9.92%) cases expired (30-day mortality) in our study population; 4 in TA group

(5.97%) and 9 in placebo group (14.06%). UGIB-related mortality was reduced in TA treated patients but the difference did not reach the level of significance. (p=0.150)
During the study no emergency surgery for UGIB was done in our hospital. Thromboembolic complications (arterial or venous thrombosis) were seen in neither of the groups within 30 days. No other side effects were observed during treatment with intra-gastric TA.

Transfusion- Transfusion requirements (as units of PRBC) were higher in patients not receiving TA. Patients in TA group received 1.77 (SD=1.08) units averagely but the average amount of packed RBCs received by placebo group was 2.9 (SD=1.61) units. This difference was significant statistically. (p<0.001)

Rebleeding- During the follow-up period (4 weeks after initiation of the treatment), the rebleeding frequencies differed between the two groups significantly. The rebleeding episodes were 4 (6%) in TA group and 12 (18.8%) in placebo group. (OR=3.635; 95% CI= 1.106 - 11.943; p=0.033)

Emergency Endoscopy- Significant difference was found in the rate of emergency endoscopies between the two groups. There were 6 (9%) emergency endoscopies in patients received TA whereas patients treated with placebo underwent 14 (21.9%) emergency endoscopies. (OR=2.847; 95% CI 1.019 - 7.949; p=0.040). In addition, patients' age were significantly correlated with transfusion requirements. (Spearman correlation coefficient: 0.711, $P = 0<0.001$)

DISCUSSION

The dosage used for topical administration of TA is much lower and effects are shorter than that used in oral or intravenous routes [9, 27]. The results of this study indicate that topical intra-gastric TA may be as an aid to conventional treatment and illustrates a novel application for TA in patients bleeding from benign lesions in stomach or duodenum. Although we did not measure the plasma level of TA to determine the degree of its systemic absorption, the prompt response of the patients to direct administration suggests that the beneficial effects probably originates from topical influence of TA.

Blood products can expose the patient to the risks of blood-borne diseases, graft-versus-host reactions, and acute hemolytic reactions, all of which increase patient mortality [28, 29]. Therefore, reducing blood loss and consequent transfusions are important priorities in acute UGIB. In our study, the use of intra-gastric TA reduced the risk of exposure to allogenic blood products.

Systemic TA in UGIB: The effect of TA is uncertain in the treatment of acute UGIB [30, 31]. Gluud et al performed a systematic review and evaluated seven double-blind randomized trials. They concluded that no significant differences were found on bleeding, surgery, transfusion requirements and also mortality between TA and placebo group. They stated that systemic TA could not be recommended for UGIB [31].

Topical TA: TA has been used topically in different pathological situations. Topical application of TA in patients undergoing coronary artery bypass operations efficiently has reduced postoperative bleeding [24, 32, 33]. De Bonis and colleagues did not detected TA in any of the blood samples blindly collected from 24 patients underwent coronary artery surgery to verify whether any systemic absorption of the drug occurred [32]. Wong and colleagues assessed the efficacy and safety of the topical application of TA on postoperative blood loss in patients undergoing primary unilateral total knee arthroplasty. They found that topical application of TA directly in the surgical wound reduced postoperative bleeding by 20-25% compared with placebo, with no clinically important increase in complications being identified in the treatment groups [23]. Some studies showed minor oral surgery, such as single tooth extraction or implant placement in patients on anticoagulant agents can be performed safely without any modification of the ongoing anticoagulant therapy when local haemostatic measures such as TA mouthwashes or gauzes saturated with TA were applied, thus minimizing costs and reducing discomfort for patients [34-36]. Results of another study indicated that the mean intra-operative bleeding rate in TA group was significantly lower than that of placebo group in endoscopic sinus surgery [37]. Topical TA has also been successfully used to control bleeding in parotid surgery [38], hemophiliacs undergoing oral surgery [39] and lung surgery [40].

Mechanisms: Fibrinolytic activity in the upper gastrointestinal tract may be a factor contributing to hemorrhage that often complicates gastroduodenal ulcers and erosions [41]. Fibrinolytic activity has been described in the margins of peptic ulcers [42]. Fibrin is readily degraded by gastric juice and duodenal juice. Gastric juice is particularly potent and a haemostatic fibrin clot in the stomach must be regarded as a potential substrate for its action [41].

TA has a plausible mode of action in UGIB. It is a plasminogen inhibitor, and plasminogen activators have been found in the gastric and duodenal mucosa. Cox et al reported that plasminogen activator and free plasmin were present in gastric venous blood obtained at operation in a higher proportion of patients with peptic ulcer than in a control group [43]. In addition, TA has been shown to inhibit the fibrinolytic action of pepsin [41]. This effect is independent of changes in PH, which may have particular relevance for patients who are critically ill and whose gastric contents may remain acidic despite treatment with histamine H2 receptor antagonists [44]. On the other hand, gastric juice from patients with bleeding ulcers was found to have a plasmin-like fibrinolytic activity [45]. Antifibrinolytic treatment and an increase in gastric PH are

possibly the necessary combination for successful haemostatic treatment [42]. The fibrinolytic activation was more pronounced in the wound than in the general circulation. TA inhibited fibrinolysis in blood from the wounds, but had no effect on fibrinolysis in plasma from peripheral venous circulation. The diminished postoperative blood loss in patients treated with TA is probably induced by decreased fibrinolysis in the wound. TA, by reducing local fibrinolysis, decreases plasminogen consumption and thus maintains a higher plasminogen level in the wounds [46].

The principle for the potential effectiveness of TA includes the following activities: local inhibition of fibrinolytic activity in bleeding lesions, inhibition of fibrinolytic activity of pepsin [41, 43], stabilization of haemostatic clots [47], and specific systemic improvement of haemostatic impairment [48].

Limitation: We did not measure the plasma level of TA in patients. The number of the patients was relatively small and the inclusion criteria were strictly tight.

CONCLUSION

We believe that intra-gastric use of TA is safe, cost effective, well tolerated and can be performed easily. The present study suggests that TA may reduce transfusion requirements and rebleeding events. Obviously, further investigation is needed to evaluate the efficacy and safety of this new method for management of acute UGIB and because of limitations in the sample size, additional evidence is needed before any treatment recommendations can be made. Further studies must be carried out to clarify whether this topical effect could also be seen in patients with malignant lesions.

CONFLICT OF INTEREST
There is no conflict of interest.

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ARTICLE

THE EFFECTIVENESS OF ACCEPTANCE AND COMMITMENT THERAPY ON PSYCHOLOGICAL WELL-BEING IN WOMEN WITH MS

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ABSTRACT

Introduction: *Acceptance and commitment therapy (ACT) is a third generation behavior therapy in treatment of mood and anxiety disorders. The purpose of this study was to investigate the effectiveness of acceptance and commitment therapy on psychological well-being in women with MS.* ***Method:*** *In this study which was a Quasi-Experimental with pre-test, post-test and control group, the sample group was selected by available sampling method from patients referred to the Kermanshah MS association. Therefore, 30 female subjects were chosen randomly and assigned to two control & experimental groups (each with 15 subjects). To assess the severity of psychological well-being, psychological well-being short form Reef was used respectively in pre-test. The experimental group experienced the treatment based on acceptance and commitment therapy in eight; two hours sessions and the control group did not receive any treatment. These questionnaires again conducted on both group in post-test.* ***Results:*** *The results showed that psychological well-being scores of experimental group significantly increased and in one-month fallow up did not significantly differ. So, treatment based on the acceptance and commitment therapy caused significant changes in the treatment of psychological well-being in women with MS.* ***Conclusion:*** *According to the findings, acceptance and commitment therapy is efficacious on increase of psychological well-being of multiple sclerosis patients. So it can be applied as useful method of intervention for improving psychological symptoms in patients with Multiple Sclerosis.*

KEY WORDS
Acceptance and Commitment Therapy, Psychological Well-being, MS

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INTRODUCTION

Multiple sclerosis, also known as MS, is an autoimmune, inflammatory, chronic and progressive disease that is characterized by demyelinating neurological damages in the white matter of the brain, spinal cord, and optic nerves [28]. It is both one of the most common neurological diseases among human beings and the most debilitating illness among the youth [13]. In 2011, the National Multiple Sclerosis Society announced that more than 2.1 million people were suffering from this disease worldwide (Moss-Morris& et al, 2012). Moreover, MS is relatively common in Iran, where its incidence, in spite of the lower statistics for Asians (3-5 cases per 100,000), is around 15-30 cases per 100,000 people [26].According to the Iranian Multiple Sclerosis Society, there are approximately 40,000 patients in the country, of which 9,000 cases have been registered, and this number still keeps growing [1].

This disease, like any other autoimmune illnesses, is more prevalent among women, and its incidence is twice as high among females as males [10]. The risk of developing MS is higher in the 20-40 age range, and its diagnosis is based on MRI studies [6]. In addition, the most vulnerable age for MS is the 20-40 age range at which women of childbearing age are with the most familial and social responsibilities [7].As a result, the disease can damage the productive forces of society, and everyone is affected by this illness. Furthermore, the empirical literature on MS patients point towards the high levels of depression, distress, anxiety, poor mental health, low quality of life (QOL), and problems with social roles and relations [18].In addition to being the result of the direct effects of inflammation and destruction of the nerve sheath, these psychological symptoms may ensue from disabilities and psychosocial issues resulting from a chronic debilitating illness with unknown etiology and unpredictable re lap secourses [14].Therefore, since the research shows that the psychological factors are often better predictors of the differences in coping with the disease compared to other factors such as neurological disabilities, severity of symptoms and the duration of the disease, etc. [18], taking the psychological components of the disease into account and taking interventions in this regard can play crucial roles in one's adaptation to one's physical condition.

Due to the vital roles that the psychological well-being plays in various mental-social and even physical aspects of one's life, numerous studies have been conducted about well-being and its components. Some scholars consider the psychological well-being the equivalent of happiness and emotional interaction with others [24]. Additionally, based on Ryff and Keyes' pattern of psychological well-being, this construct comprises the six components of purpose in life, positive relations, personal growth, self-acceptance, autonomy, and environmental mastery. From this perspective, the health index is not defined as 'lacking the disease,' so that one's well-being rather than sickness is emphasized[25].The debilitating nature of multiple sclerosis, affecting one's personal, social, occupational, physical and mental life, is important from the viewpoints of both the patient due to the serious concerns about the disease and specialists and researchers who are still overwhelmed by the theoretical and practical ambiguities and failures regarding understanding this disease, especially its etiology, prevention, prognosis and treatment. Hence, the identification of programs in the form of training interventions towards improving the psychological well-

being of these patients seems essential. So, the present study aimed to investigate the effectiveness of acceptance and commitment therapy (ACT) on the psychological well-being of women with MS.

MATERIALS AND METHODS

The present study also followed a pretest-posttest design with experimental and control groups, and the statistical population comprised all MS-stricken women residing in Kermanshah, Iran. Moreover, the simple random sampling was employed to select the MS-stricken subjects [15] in each of the experimental and control groups) from the registered members of Kermanshah MS Society. It is worth noting that the number of subjects in the present study partially corresponded to the appropriate number of subjects suggested in the clinical literature, ranging from seven to ten members and in some other cases between ten and fifteen members. As for sample selection, the inclusion criteria included the diagnosis of multiple sclerosis, being female, and having middle school education and above. Also, the exclusion criteria were diagnosis of physical illnesses, other mental disorders, and receiving psychological treatment. Not to mention, this research was recorded with the registration code of IRCT2016020625433N2 in the clinical trial center.

After sampling, the subjects were randomly assigned to two experimental and control groups, and then the pretest was performed in both groups. After that, the experimental group was collectively provided with the independent variable, i.e., acceptance and commitment therapy (ACT), in eight 90-minute sessions once a week. As for the control group, no intervention was offered. Upon completion of the sessions, the posttest took place. The treatment plan given to the experimental group is briefly shown in [Table 1].

The ACT protocol, which was based on an unpublished manual used in a previous study [29] focused on changing expectations from elimination of pain to living as well as possible with chronic pain. Discussions and experiential exercises were used to demonstrate the futility of control-oriented strategies such as thought suppression and attempts to eliminate pain, distress, and other negative experiences. Mindfulness strategies were taught in order to develop the skill of allowing negative experiences such as muscle tension or discomfort, negative thoughts, and emotional distress to pass through consciousness without requiring the expenditure of energy or psychological resources to control or alter them [16]. Participants were also encouraged to identify their personal values and set and pursue short- and long-term goals consistent with those values in order to achieve improved quality of life and functioning.

Table 1: Session outlines for acceptance and commitment therapy (ACT)

Session	ACT
1	The limits of control (short and long-term costs and benefits; finger traps), focus on experience (body scan)
2	Values (what you care about, how you want to live your life)
3	Cognitive defusion (observing thoughts without trying to evaluate or change them)
4	Mindfulness (being in the moment, raisin exercise)
5	Committed action (“road map” connecting values, goals, actions, obstacles, and strategies)
6	Review and continued action in support of values
7	Review and continued action in support of values
8	Moving forward

Ryff's Psychological Well-being scale (PWB): This scale was developed by Ryff in 1980. The original scale contained 120 questions, but in further studies done afterwards, shorter forms of the scale were proposed with 84, 54, and 18 questions. In the present study, the 18-item scale was utilized with six-point Likert Scaling (ranging from strongly disagree to strongly agree). In addition, the validity and reliability of this scale has been reported in numerous preceding studies. In a study conducted by Dierendonck (2005), the internal consistency of the subscales of the psychological well-being scale (PWB) was appropriate, and their Cronbach's alpha was between 0.77 and 0.90. The correlations of the psychological well-being scale (PWB) with life satisfaction scale, happiness inventory and Rosenberg self-esteem scale (RSES) were 0.47, 0.58, and 0.46, respectively. In a study performed by Zanjani Tabasi (2004), the reported internal consistency for the entire psychological well-being scale (PWB) was 0.94, and between 0.63 and 0.89 for the subtests. Moreover, in the present study, the correlation coefficient for the entire test through test-retest was 0.76, and between 0.67 and 0.73 for the subtests ($p<0.001$). In addition, the Cronbach's alpha was 0.83 in the present study [11].

To analyze the collected data and given the research questions, the univariate ANCOVA was employed in addition to descriptive statistics. As for data analysis, the SPSS-23 was employed.

RESULTS

Totally 30 women with the age range of 18-55 years were selected from the intended population and were included in the research. About 55% were in the age range of 18-28 years, 30% were in the age range 29-39, and 15% were 40 years and older. As for education, 42.54% of them had high school diploma or lower levels and 28.64% had technical education after high school diploma, Collegiate 28.82%.

[Table 2] shows mean and standard deviation for scores of psychological well-being test in the studied groups in pretest-posttest and follow-up stages.

Table 2: Mean and standard deviation of psychological well-being scores

Groups	Pretest		Posttest		Follow-up	
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
Commitment and Acceptance Therapy	73/87	11/76	85/20	13/89	84/07	7/32
Control	74/80	10/99	72/93	5/36	73/07	6/10

As seen in [Table 2], mean in the experimental group (Commitment and Acceptance Therapy) increased from 73.87 at pre-test stage to 85.20 at post-test stage. But no significant change was observed in the control group in pretest and posttest stages. Considering the difference observed in the mean of the study groups, average psychological well-being in experimental group indicates the effectiveness of the aforesaid procedure.

The Leven's test was used to assess the equality of variances in psychological well-being scores. The results of the Leven's test are provided in [Table 3].

Table 3: Results of the Leven's test to examine the equality of variances in psychological well-being scores

Variable	F	df_1	df_2	Sig
Psychological well-being	2/9	1	28	0/07

In order to evaluate the presumptions of the analysis of covariance (ANCOVA), firstly the homogeneity of slopes of pretests and posttest scores were calculated. Multivariate ANCOVA was used to compare experimental and control groups with respect to psychological well-being scores. The results showed that the tests were significant ($P<0.01$). This means that there was a significant difference at least between two groups. The results are shown in [Table 4].

Table 4: Results obtained from multivariate analysis of covariance on mean scores of posttest of variables in two groups

	value	F	Hypothesis df	Error df	Sig	Square Eta
Pillai's trace	0/80	11/34	14	2	0.001	0/80
Wilks lambda	0/20	11/34	14	2	0.001	0/80
Hoteling's trace	4	11/34	14	2	0.001	0/80
Roy's largest rot	4	11/34	7	2	0.001	0/80

ANCOVA was conducted to find out the difference observed. Considering the calculated effect size, 80% of total variances of experimental and control groups was the result of effectiveness of the independent variable. Moreover, statistical power of the test was 0.80 which means that the test was able to reject the null hypothesis with a power of 80%. [Table 4] only states that in one of the areas there is a significant difference between experimental and control groups. Multivariate analysis of covariance (MANCOVA) was used to distinguish which area was significantly different. The results are shown in [Table 5].

Table 5: Results obtained from multivariate analysis of covariance (MANCOVA): mean scores of posttest of psychological well-being dimensions in the experimental and control groups

Sources Change	Mean Square	Degrees of freedom	F	Sig	Square Eta
Independence	69.08	1	27.31	0.001	0.55
Dominance over the environment	13.14	1	7.42	0.012	0.25
Self-development	66.40	1	25.89	0.001	0.54
Positive relation with people	20.72	1	5.62	0.027	0.20
Being targeted in life	21.44	1	9.58	0.005	0.30
Self-acceptance	102.74	1	15.96	0.001	0.42
Total psychological well-being	1033.87	1	15.42	0.001	0.41

Results of [Table 5] show that there was a significant difference in psychological well-being dimensions including independence, dominance over the environment, self-development, Positive relation with people, being targeted in life, and self-acceptance between the two study groups. Overall psychological well-being with F=15.42 was significant (P= 0.001). Considering the Eta square of 0.41, it can be said that 41% of the changes in the dependent variable was due to effectiveness of the independent variable.

DISCUSSION AND CONCLUSION

The present study aimed to investigate the effectiveness of acceptance and commitment therapy (ACT) on the psychological well-being of women with MS. As the results of the present study demonstrated, the acceptance and commitment therapy (ACT) significantly enhanced the psychological well-being of patients with multiple sclerosis in the experimental group compared to the control group. This result was consistent with the results of a study conducted by [22] in which it was reported that the acceptance and commitment therapy (ACT) influenced the treatment of anxiety and depression of women suffering from MS. In addition, this finding of the present study was concurrent with the results of a study conducted by Izadi et al. (2013) [8], in which it was reported that the acceptance and commitment therapy (ACT) could lessen obsession, depression, and anxiety in patients suffering from MS. In acceptance and commitment therapy (ACT), patients are trained in mindfulness each session. By the same token, Brown & Ryan (2003) concluded that mindfulness and presence of mind increase one's wellbeing [3]. In line with the results of the present study, Arch and Craske (2005) [2] concluded that the participants that concentrated for 15 minutes experienced mental clarity and reduced physical stress compared to those lacking such concentration [5]. Given that it has been proven that psychological stress can activate multiple sclerosis, thus, not only does the commitment and acceptance therapy increase the psychological well-being, it also reduces stress in patients suffering from multiple sclerosis, as a result of which the recurrence rate of this disease is lessened in these patients. Therefore, it seems that the psychological interventions, including the acceptance and commitment therapy, can play efficacious roles in increasing the psychological well-being and overall mental health of patients suffering from multiple sclerosis.

In this therapy, the practices of behavioral commitment along with diffusion and acceptance techniques as well as detailed discussions about one's values and goals and the need for specification of values all led to an increase in the psychological well-being of women suffering from MS. Further, in this treatment, the main goal of placing greater emphasis on one's inclination towards inner thoughts was to assist one to experience one's worrisome thoughts just as a thought, to become aware of the inefficient nature of one's current programs, and to handle what was of importance in one's life in line with one's values rather than responding to those thoughts. Here, through replacing ego as background, clients could smoothly experience one's unpleasant inner events in the present and were able to restrain oneself from nasty reactions, thoughts, and memories. In fact, it aimed to affirm one's psychological flexibility. As the results of the statistical analysis showed, this approach led to a major rise in the psychological flexibility of this group of patients. In practice, the central processes of ACT teach one how to desist from worrisome deterrent thoughts, how to conceptualize such thoughts, how to affirm self–observation, how to accept rather than control the internal events, and how to clarify one's values. In this therapy, one learns how to accept rather than desist from one's feelings. It also raises awareness about one's thoughts and thinking process and links them towards the realization of goal-oriented activities. In short, AC Taims to teach one to experience rather than obstruct one's thoughts and feelings, and one is asked to do one's utmost towards the realization of goals and values [22].

One of the limitations of the present study was the limited number of the sample under study because the male patients were not included in the study. Accordingly, it is recommended that larger groups that includes men be investigated in future studies towards greater validity of this therapy with greater

confidence. In addition, it is suggested that this method of treatment be compared to other methods to determine how they are different from each other.

CONFLICT OF INTEREST

There is no conflict of interest.

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FINANCIAL DISCLOSURE

None

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ARTICLE

THE FACTORS RELATED TO THE SEVERITY OF TRAFFIC ACCIDENT VICTIMS OF MOTOR BIKERS AT EMERGENCY DEPARTMENT (IGD) OF GENERAL HOSPITAL

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ABSTRACT

Background: Traffic accidents are road traffic incident in which at least caused by a vehicle which caused injury, damage or loss to the owner or the victim. The purpose of this study was to determine the correlation between identifying factors with the severity of traffic accident victims of motor bikers at emergency Unit (IGD) of General Hospital in Kendari. Research method was analytical research with cross sectional approach. This research was conducted in the district general hospitals kendari and sample size was 443 people. Univariate and bivariate analysis used to determine the frequency and the relationship between the variables were analyzed through statistical test. Bivariate analysis using chi-square test *Chi square (Test of Independence)* with a significant level (α = 0,05). The results showed that there was no correlation between the use of helmets with the severity of traffic accident victims motorcycles (ρValue=0.054), there was no correlation between the use of mobile phones with the severity of traffic accident victims motorcycles (ρValue=1.000). There was a relationship between vehicle speed with the severity of victims of traffic accidents of motorcyclists (ρValue=0.003). Overall conclusion, the use of helmets and cell phones are not related, meanwhile the vehicle speed related to the severity of traffic accident victims in patients.

KEY WORDS
Traffic Accidents, Helmet Use, Cell Phone Use, and Speed

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INTRODUCTION

One of the main causes of death is injuries. WHO projections, in 2005 the injury was ranked as the fourth leading cause of death in all ages around the world, and the third on the global burden. But population growth and prosperity have become one cause of the increase in road traffic accidents. Sometimes traffic accidents (kalimat ini dihapus saja: on these roads) can lead to injuries or material losses even to eliminate human lives [1].

In 2013 the number of traffic accident occurrences in Indonesia was 101 037 and the number of victims was 159 677 people. the victim died as many as 25 157 people with CFR 15.75%, seriously injured 29 544 people with the proportion 18:50%, and minor injuries 104.978 people with the proportion of 65.74%. In Southeast Sulawesi in 2015 there were 821 occurrences of traffic accidents, the number of victims of accidents was 1,295 people, victim died as many as 166 people with 20 CFR, 21%, 277 seriously injured people to the proportion of 24.53% and slightly injured 852 people with the proportion of 75.46%. In the city of Kendari in 2014 there were 316 occurrences of traffic accidents. The number of victims of accidents was as many as 425 people. The victim died 54 at 17:08 CFR%, 122 seriously injured people to the proportion of 32.88% and slightly injured 249 people with the proportion of 67.11%. Then in 2015 decreased, there were 296 occurrences of traffic accidents. The number of victims of accidents was 362 people. The victim died as many as 33% of people with CFR 11:14, 97 seriously injured people to the proportion of 29.48% and slightly injured 232 people with the proportion of 70.51% [2].

In 2013 the number of traffic accident victims was 679 people. The victim died with CFR 1:42 10%, 77 seriously injured people to the proportion of 11:50% and 592 lightly injured people to the proportion of 88.49%. In 2014 the number of traffic accident victims increased by 757 people. The victim died was 13 people with CFR 1.71%, serious injuries increased by 61 people with the proportion of 8.19% and slightly injured 683 people with the proportion of 91.80%. Then there was an increase in 2015 with 895 occurrences of traffic accidents. The victim died as many as 10% of people with CFR 1:11, 85 seriously injured people to the proportion of 9.60% and slightly injured 800 people with the proportion of 89.38% [3].

MATERIALS AND METHODS

Research design

A quantitative research with cross sectional survey design. This study aimed to explain the influence between variables. Research that had been carried out was designed to analyze the relationship between the use of helmets, use of cellular phone, and vehicle speed with the severity of the patient's traffic accident motorcycle users in the emergency department (IGD).

Research sites

The experiment was conducted on patients of traffic accident victim of motorcyclists in intensive care units darudat (IGD) General Hospital of Kendari city 2016.

Population and sample

The population was all motor bikers accident patients who were in the emergency unit of Regional General Hospital of Kendari amounted to 4.405 people. A simple random sampling method was used to obtain a sample of 443 people.

Data Collection

The primary data obtained directly from respondents using a questionnaire (questionnaire), which has been tested for the validity and reliability. Secondary data were obtained from the General Hospital of Kendari city and previous researches related to the incidence of traffic accidents (hapus saja: in patients).

Processing and analysis of data

Processing and analysis of data using a computer. data processing begun with editing, coding, entry and tabulation. After that, the data were analyzed with SPSS 16. Data analysis was divided into two, namely univariate and bivariate analysis. Univariate analysis was used to determine the frequency distribution and independent variables associated. The bivariate analysis using Chi-square test statistic (hapus aja:is) (Test of Independence) with a significant level ($\alpha = 0.05$) was developed to analyze the relationship between independent variables and the dependent variable.

RESULTS

The results of the **univariate** analysis of factors causes of traffic accidents and the severity of traffic casualties motorcyclists can be seen in [Table I].

Table I: Results of univariate analysis and factors contributing to the severity of traffic accidents motorcycle users

Variable	Dimension	Frequency (n)	Percentage (%)
The severity of the accident victim	Minor injuries Serious wound	211 232	47.8 53.5
The use of helmets	Standardized Non standardized	119 324	26.7 73.3
Use of Cell Phones	Use Don't Use	266 177	60.0 40.0
Vehicle speed	Moderate Fast	152 290	34.4 65.6

The results of the analysis of [Table I] show that the motorcycle rider as much as 53.5% suffered serious injuries. The use of unstandardized helmet was accounted for 73.3%, using a mobile phone while driving was 60% and the very fast driving speed was accounted for 65.5%.

The results of the bivariate analysis of factors causes of traffic accidents (helmet usage, mobile phone usage and vehicle speed) with the severity of the victim's user traffic of motorcycles can be seen in the following Table II, III and IV.

Relationship between helmet usage and severity of victims of motorcycle rider traffic accidents in general hospital

Table 2: Analysis of the relationship between helmet usage and the severity of motorbike accident patients

The use of helmets	The severity of the accident victim				Amount		P Value	phi Ø
	Minor Injuries		Serious Wound					
	n	%	n	%	n	%		
Standardized Non standardized	99 121	66,7 40,9	49 175	33,3 59,1	148 296	100 100	0,054	0,228
Total	212	47,8	231	52,2	443	100		

The results in [Table 2] study show that there was no association between the use of helmets with the severity of traffic accident victims of motorcycle users in the emergency room (ER) Regional General Hospital of Kendari city. This is indicated by p_{value} of 0.054 is greater than $\alpha = 0.005$. Therefore, the use of helmets does not affect the severity of victims of motorcycle riders traffic accidents, and their relationship with phi Ø = 0.228.

Relationship between cell phone usage and severity of victims of motorcycle rider traffic accidents in general hospital

The use of mobile phones is described as the cell phone usage while driving. test results show no association between mobile phone use and the severity of traffic accident victims of motorcycle users in the emergency unit of Regional General Hospital of Kendari.

Table 3: Analysis of the relationship between cell phone use and severity of traffic accident victims of motorcycle users

Use of Cell Phones	The severity of the accident victim				Amount		P Value	phi Ø
	Minor Injuries		Serious Wound					
	n	%	n	%	n	%		
Use	112	48,1	110	51,9	222	100	1,000	0,009
Do not Use	100	47,2	121	52,8	221	100		
Total	212	47,8	231	52,2	443	100		

The results in [Table 3] study show there was no association between mobile phone use with the severity of traffic accident victims of motorcycle users in the emergency room (ER) Regional General Hospital of Kendari city. This is indicated by p value of 1,000 is greater than $\alpha = 0.005$. Means the use of cell phones does not affect the severity of victims of traffic accidents motorcycle riders, and the absence of a very weak relationship with phi Ø of 0.009.

Relationship between riding speed and severity of victims of motorcycle rider traffic accidents in general hospital

Riding speed is depicted as how much the speed of vehicles driven by motorcyclists in driving. the results showed there is no relationship between the speed and the severity of traffic accident victims of motorcycle users in the emergency unit of Regional General Hospital of Kendari.

Table 4: The analysis of relationship between vehicle speed and severity of traffic accident victims of motorcycle users

Speed	The severity of the accident victim				Amount		P Value	phi Ø
	minor injuries		serious wound					
	n	%	n	%	n	%		
Moderate Fast	129 93	71,0 35,6	53 168	29,0 64,4	182 261	100 100	0,003	0,337
Total	212	47,8	231	52,2	443	100		

The results in [Table 4] study show that there is a relationship between vehicle speed and the severity of traffic accident victims of motorcycle users in the emergency room (ER) Regional General Hospital of Kendari city. This is indicated by pvalue 0.003 is greater than $\alpha=0.005$. means the vehicle speed influences the severity of victims of traffic accidents motorcycle riders, and the strength of the relationship with phi Ø of 0.228.

DISCUSION

Relationships between helmet use and severity of traffic accident victims of motorcycle riders in the emergency unit of general hospital

The results showed that there was no association between the use of helmets with the severity of the accident victim in patients IGD Regional General Hospital. other studies show that helmet use was not statistically sufficient evidence of an effect on the severity, but the effect on the level of awareness of the driver involved in the accident [4].

Severity is influenced by the level of consciousness, thus indirectly affects the use of helmet . compliance with the level of traffic trauma in a traffic accident motorcycle driver has been studied [5]. Accidents involving motorcycle riders or passengers can lead to serious injuries, even death. This is due to the lack of protection of motor bikers. When compared to cars, motorcycles do not have the instruments absorbers, seat belt in order to withstand the impact. The wound in the head is the biggest part of the severe and fatal accidents suffered by motorcyclists. Type of head damage such as cracking on the cranium. The location of the wound on the forehead, back or side of the head, especially in the right position to deal with traffic from the front. Excellence Motorcycles have a smaller size makes it easy to oncoming motorists move in traffic. however, this can make the motorcycle riders involved in accidents that can easily cause serious injury to the rider.

Further impact of head injury is that it can cause disturbances in the brain, central nervous system and spinal cord top. Motorcyclists also could have a concussion, injury to the foot even death. To protect motorists from the prevalent traffic accidents, the government also requires the use of SNI helmets (Indonesian National Standard) to anticipate worse possibility.

The results showed that most respondents did not use a helmet because of the close distance factor. With regard that the higher underestimation of the severity of the motorbike accident victims.

In the implementation of helmet standards program in Kendari, Kendari City Traffic Polrestabes has prioritized the application of proper coordination with the total police force of Kendari which Polrestabes and the entire police station in the city of Kendari. Police force can optimize the lowest part of the implementation of helmet standards program throughout the city of Kendari. Coordination between Satlantas Polrestabes the entire police station in the city of Kendari was done to achieve standardized policy implementation program in accordance with Regulation Legislation Number 22 Year 2009 regarding Traffic and Road Transportation, which requires all road users, especially users of two-wheelers to wear helmets in accordance with SNI standard.

Implementation of standard helmet program provides enormous benefits. The benefits are felt by the public, among others, can improve the security and safety when driving on the highway and can reduce the high risk of a fatal accident for the rider. Motorcyclists urged to always use a helmet in accordance with applicable regulations.

Achievement of the objectives of the implementation of standardized helmet program is not maximal yet, because in the reality, this experiences various obstacles, such as suburban communities still do not understand the purpose of the implementation of the policy. Many violations and traffic accidents happened in rural areas in Kendari.

Implementation process consists of the improvement of facilities and infrastructure provided by Polrestabes Kendari to support the process of socialization. Facilities and infrastructure are provided such as the installation of banners, distribution of brochures on how to use a helmet (directly to the motorcycle rider), as well as the delivery of information through the mass media. Implementation of the program's implementation standard helmet is necessary to support the activities that are more effective in the socialization process. So, that people can understand the purpose of the policy, and make the program sustainable.

Associated with the implementation of policies, actors implementing the policy play primary responsibility to implement policies to the target group which is a whole community of Kendari. An active policy implementers must provide and serve. Expected that people can understand clearly and have the benefit after following the policy.

Facilities and infrastructures are supporting tools in the implementation of the policy. About the completeness and the quality and quantity must be considered in accordance with the needs of the policy program. If it is provided well, at least make it easier for executive actors in achieving its policy objectives.

Implementation of standard helmet in Kendari by the Traffic Polrestabes Kendari city has been in communication with providing information to the public through the mass media and radio, distribution of free brochures or pamphlets to motorists, the socialization directly to a number of areas, and provide information on the rules and cross. Particularly regulations that require wearing a SNI standard helmet.

Indicators clarity of communication is also important to determine the success of public policy implementation. The effective implementation happens if the decision makers already know what they will do. Understanding of what should be done can be run smoothly when communication goes well in addition to policy communication must be precise, clear and consistent. Clarity of communication includes transmitting information between Polrestabes with Traffic, as well as between Satlantas with the community.

In practice, there are still many people who do not understand the intent of the government to implement the obligation to use helmets that comply with safety standard. Thus, the number of traffic violations will rise higher and higher. Compliance with the driver of the provisions for the use of a standard helmet, generally no more than an attempt to escape from the police. While the philosophical value of these provisions is not a driver of self-awareness to the ISO-standard helmet use.

The implementation of this standard helmet has not run smoothly because many people do not understand about the purpose of the program. Society as a target group in the implementation of standard helmet program, believes that this program only benefited a group of parties, namely helmet employers and of course the police will do a lot of speeding tickets.
Basically, people as the target groups need to support this policy. In the implementation, it is not running optimally, so there are still many people do not understand the purpose of this program implementation. In addition, the relatively high penalties for the perpetrators of the offense of not using helmets that comply with safety standards, making many people, especially the motorcycle feel too heavy financial penalties

are applied. Expected in the next execution, the police have to work harder, so that people can understand the purpose of the program is the implementation of ISO-standard helmet. Public awareness needs to be fostered and developed through development patterns more effectively and intensively.

Relationships between cell phone use and severity of traffic accident victims of motorcycle riders in the emergency unit of general hospital

The results of analysis of this study showed that there was no correlation between the cellular phone usage and severity of traffic accident victim of motorcycle users. The results of this study are different from previous research that states that there is an association of cell phone use as a factor in motorcycle accidents and biological use of mobile phones as a risk factor for the incidence of motorcycle accidents [6]. Other studies have also suggested that there is a relationship of cell phone use with the incidents resulted in casualties on the severity of the rider [7].

Motorists using mobile phones while driving, are 4 times more likely to develop a risk of collision compared to riders who do not use cell phones. collisions are most common in riders due to cell phone use. Therefore, for motorists who use mobile phones have risk of traffic accidents by 4.3 times compared to riders who do not use cell phones. While the study finding suggests the use of mobile phones while driving causes fortunate consequences in the event of a collision on the highway. Driving while using a cell phone (talking, texting, use the facilities of the game) is an activity that endanger the safety of the riders themselves and other road users. Communicating using a mobile phone while driving will distract the driver in running his vehicle so that conditions like this opportunity for the RTA on the highway [8].

The majority of motorists who do not use cell phones while driving preferred not to use cell phones while driving because they fear the lack of focus, causing an accident while driving. they also said that should not use mobile phones while driving because it can endanger themselves and others.

Most motorists are aware of the dangers of cell phone use while driving. due in large cities congested road they do not pay attention to the dangers of cell phone use, while in the small town streets were deserted of vehicles as well as better awareness for not using a cell phone while driving.

Relationships between Riding Speed and severity of traffic accident victims of motorcycle riders in the emergency unit of general hospital

Results of analysis showed an association of mobile phone usage with the severity of traffic accidents motorcycle users. This is consistent with previous studies which stated that the speed has a significant relationship with victim severity of traffic accidents [9]. IGD research results in Fatmawati, states that high speed has a relationship with the severity of motorcycle accident victims[10].
Incidence of traffic accidents in urban mainly is caused by a high rate of speed riders which was more than 50 km / h. riding at high speed increases opportunity to accidents by 2:54 times (OR = 2.54) compared with the low-speed riders, (95% CI = 1623.98) [11]. zone speed of 20 km / h is an effective measure in reducing injuries and deaths from traffic accidents [12].

Acceleration of the vehicle by the riders affected by a number of factors, factors related to the driver (age, gender), factors related to roads and vehicles (the state of roads, the quality, the road surface, vehicle power, and maximum speed), whereas environmental factors and the road is the composition and density of the road, speed prevailing weather conditions.

Motorists in running vehicles should pay attention to speed signs as well as to other conditions such as rainy weather, vehicle density and other factors that do not allow the vehicle to be driven at high speed. Riders sometimes forget to pay attention to signs of speed and other circumstances. In fact, at high speed, the rider will be difficult to control the vehicle if it is willing to stop the vehicle suddenly and therefore will lead to traffic accidents.

The risk for the occurrence of death and injury increases with increase in driving speed. Vehicle speed of 20 meters per hour has a 5% risk of causing death while the speed of 85 meters per hour increased the risk of death to 85%. Motorcycle accident due to high speed when driving the vehicle in general suffered a severe injury due to clash intense and powerful that allows the victim thrown away from the spot of the incident.

The physical condition of the road as well as a seamless reveal no significant difference in the proportion of injury severity. Smooth road conditions encourage motorcyclists to accelerate the speed while the condition of the road motorcyclists is usually less cautious in driving the vehicle though at a low speed.

CONCLUSION

There was no association of helmet use and Cell Phones use with the severity of traffic accident victims patient in the emergency unit of general hospital Kendari. While the speed related to the severity of traffic accident victims in the emergency unit. enactment of the rules to wear international standard helmets, do

not use cell phones while driving, and keep the vehicle speed motorcycle.

CONFLICT OF INTEREST

The author states do NOT have competing interests The Subscription with Jobs

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ARTICLE

EFFECTS OF DIFFERENT NITROGEN AND SOLUPOTASSE FERTILIZER RATE ON YIELD AND YIELD COMPONENTS OF POTATO

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ABSTRACT

The effect of different nitrogen and Solupotasse fertilizer levels on yield and tubers quality, germination percentage and rate after storage was investigated in a split plot experiment based on a RCBD with three replications, in 2014. The main plot and subplot were four N fertilizer levels (0, 125,250 and 375 kg N/ha) and four K fertilizer levels (0, 2,4 and 6 kg K/ha) respectively. The results showed that the main effect of N and K fertilizer were significant on mean tuber number and fresh tuber yield at P>1% and germination percentage at 5% probability level. The interaction effect was significant on mean tuber number, and fresh tuber yield at P>1% and starch percentage at P>5%. It showed that the application of 375 kg N/ha had a significant effect on measured traits and ranked in the superior group. Also application of 0 kg/ha solupotasse was ranked in the superior group. For tuber number per plant, application of N fertilizer 375 kg/ha and K fertilizer 4 kg/ha, in terms of fresh tuber yield, application of N fertilizer 375 kg/ha and K fertilizer 0 kg/ha and for starch percentage, application of N fertilizer 0 kg/ha and K fertilizer 4 kg/ha ranked in the superior group.

KEY WORDS
Germination, Storage, Starch, Tuber number.

INTRODUCTION

Potato is one of the main tubers and nutritious crops, which also is very important due to nutritive value and economical. This crop with high performance in unit level is containing abundant carbohydrate and also biological value of protein is high [Mohammadi, 2000].

Studies have shown that utilization of microelements cause development of performance and quality of crops . Enrichment of agriculture crops for increasing of healthy level of society production of seeds with high growth rate and sprout rate is very beneficial [Mohammadi, 2000]. Potassium acts as an osmoticum in plants and is important for the translocation of sugars and synthesis of starches in potatoes [Harris, 1978, Kunkel and Thornton, 1971]. Fertilizer K applications are often required for optimum potato yields because of a relatively high K requirement compared with other irrigated crops. Fertilizer K-source is known to affect tuber specific gravity (SG). [Dubetz and Bole 1975] observed no K effect on yield, number of tubers, or weight of tubers at various N rates, but K decreased SG.

Fertilizer N applications are generally needed since the mineralization of soil organic N does not satisfy the N requirements of the potato plant [Mcdole et al, 1991]. Belanger et al.[2000] found that application of appropriate amounts of nitrogen [80 kg/ha] resulted in more favorable effects than higher rates. Waddell et al. [1999] and Saeidi et al. [2009a , 2009b] reported that application of nitrogen, led to increase in tuber yield than control. This rate has been obtained 34.3% by Marguerite et al. [2006].

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Nutrient management is a controllable input that potato growers utilize to ensure high tuber yields and quality. Both N and K fertilization are often required for maximum production. Optimum recommendations can only be made if the specific effects of K-sources and their interaction with N rates are known. So the objective of nutritional quality and yield of potato tuber cultivar Agria, by applying nitrogen and solupotasse fertilizer levels and determine the best fertilizer rates to get the highest yield and quality of potato tuber along with the lowest environmental accumulation.

MATERIALS AND METHODS

The study area is located Jolge Rokh, Khorasan Razavi , Iran with longitude of 30°17' and latitude of 56°57' in 2013 – 2014. Annual average temperature in research area was 15.6° C and average rainfall was 260 mm and also it was 985 m higher than sea level.

A split-plot field design with four replication in a randomised block design was employed. Main plot provided four levels of nitrogen fertilization [0, 125, 250, 375 kg per ha] . Each Main plot was subdivided into two levels of solupotasse fertilization [0, 2, 4, 6 kg per ha] . Solupotasse solution was sprayed in three stages. Phosphate application remained constant for each level of solupotasse.

Whole amount of phosphate and third of the urea at the time of preparing seed bed was used. Seed cultivation with density 12.5 plants per m2 at May, was done, plants distance on rows were 20 cm. Rows were spaced 40 cm and plots contained 5 rows each 3 meters. Control of weed was done through mechanical and in two times manually. Three plants selected randomly, then plants were transferred to the laboratory. This experiment measured properties such as number of leafs, number of branch, plant height, number and weight of tubers per plant, average weight of tubers per plant, tubers yield per unit,

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starch percentage and leaf chlorophyll content . In order to calculate starch percent of tuber, Mccriddy et al. [1950] method was used. Leaf chlorophyll content measured using chlorophyll meter device [model: SPAD-502] were calculated.

After harvesting five tubers from each plot randomly selected and stored in refrigerator at 7 °C for 90 days. Following the expiration of 90 days, samples were taken for experimental analysis. After reconditioning, for 10 days germination percentage and germination rate measured.

Results were analyzed by SAS software, mean comparisons were done via Duncan's multiple range test and graphs were drawn by Excel software.

Table 1 : Soil characteristics examined at the depth of 0 – 30 cm

Texture	pH	Ec ds/m	TNV %	O.C %	Sand %	Silt %	Clay %	P (mg/kg)	K (mg/kg)
Sandy Silt	7.9	1.78	17.6	0.56	41	35	24	12.0	213

RESULTS AND DISCUSSION

Mean leaf number/plant

Analysis of variance [Table 2] showed that the main effect of N fertilizer was significant on mean leaf number at 1% probability level. The main effect of K fertilizer and N fertilizer × k fertilizer on leaf number was not significant. Also, means comparison table [Table 3] showed that all four K fertilize levels had no significant differences in their leaf number and were ranked in same group. Among N fertilizer levels, application of 375 kg N/ha gave rise to the greatest leaf number. The results were not in agreement with Vos [1995] who reported that the effect of different N fertilizer level was significant on Branch number and declined with increase in nitrogen supply.

Table 2: Analysis of variance of effect of N and K fertilizer levels on different traits of potato

Source of variance	df	Mean leaf number	Mean branch number	Mean plant height	Mean tuber number	Mean tuber weight	Fresh tuber yield	Dry matter percentage	Starch percentage	Leaf chlorophyll content	Germination percentage	Germination rate
Replication	2	1.31	2.27	3.06	0.89	2515525	13.77*	0.49	3.55	26.02	681.58**	1.08
N Fertilizer	3	48.55	16.74*	449.38**	295.8**	3415566.30	2305.38**	2.47*	1.64	189.16	217.52	34.05*
R*N	6	0.78	3.24	1.36	14.72	6180258.80	2.15	0.34	1.97	135.23	54.08	3.80
K Fertilizer	3	1.05	068	0.16	5.55*	10837.13	29.55**	0.36	0.30	63.01	146.90*	47.77
N*K	9	0.53	1.66	1.37	7.16**	92456.10	13.64**	0.39	0.99*	79.09	46.65	38.87
R*N*K	24	0.5	2.77	0.81	1.65	68759.11	0.56	0.27	0.33	34.67	37.48	27.68

* and ** show significance at 5% and 1% probability level.

Table 3: Means comparison of effect of different N and K fertilizer levels on different traits of potato

Source of variations	Men leaf number / plant	Mean branch number / plant	Mean plant height (cm)	Mean tuber number / plant	Mean tuber weight / plant	Fresh tuber yield (t/ha)	Dry matter percentage (%)	Starch percentage (%)	Leaf chlorophyll content	Germination percentage	Germination rate
N Fertilizer levels											
0 kg/ha	11 b	5.75 a	50.75 d	17.08 b	2827.91 a	19.91 d	22.69 a	18.83 a	40.83 a	41.25 a	24.41 a
125 kg/ha	10.83 b	5.91 a	53.66 c	17.25 ab	2928.25 a	26 c	22.65 ab	18.04 a	45.35 a	37.16 a	28 a
250 kg/ha	12 b	7.91 a	60.5 b	24 a	2897.08 a	41.83 b	22.45 ab	18.19 a	45.15 a	42.5 a	26.33 a
375 kg/ha	15.16 a	7.8 a	64.08 a	27 a	3211.25 a	49.91 a	21.71 b	18.07 a	49.93 a	47.5 a	27.91 a
K fertilizer level											
0 kg/ha	11.91 a	6.83 a	57.16 a	20.83 a	2943.5 a	36.41 a	22.64 a	18.09 a	46.87 a	43.08 a	29.33 a

2 kg/ha	12.5 a	6.83 a	57.41 a	21.83 a	2980.91 a	34.41 b	22.27 a	18.09 a	43.46 a	45.41 a	27 a
4 kg/ha	12.08 a	7.16 a	57.25 a	22 a	2938.83 a	32.58 c	22.29 a	18.09 a	44.53 a	42.75 a	25.5 a
6 kg/ha	12.5 a	6.58 a	57.16 a	20.66 a	3001.25 a	34.25 b	22.32 a	18.09 a	46.39 a	37.16 a	24.83333 a

Mean branch number/plant

Analysis of variance [Table 2] showed that the main effect of N fertilizer was significant on mean branch number at 5% probability level. The main effect of K fertilizer and N fertilizer × k fertilizer on branch number was not significant. Also, means comparison table [Table 3] showed that all four K fertilize levels had no significant differences in their branch number and were ranked in same group. Among N fertilizer levels , application of 375 kg N/ha gave rise to the greatest leaf number. The results were in agreement with taghdiri and sepehri [2010] who reported that the effect of different N fertilizer level was significant on leaf number.

Mean plant height

Analysis of variance [Table 2] showed that the main effect of N fertilizer was significant on mean plant height at 1% probability level. The main effect of K fertilizer and N fertilizer × k fertilizer on mean plant height was not significant. Also, means comparison table [Table 3] showed that all four K fertilize levels had no significant differences in their mean plant height and were ranked in same group. Among N fertilizer levels, application of 375 kg N/ha gave rise to the greatest mean plant height. The results were in agreement with Jafari and Heidari [2014] who reported that the effect of different N fertilizer level was significant on plant height.

Mean tuber number/plant

The main effect of N fertilizer levels on mean tuber number/plant was significant at 1% probability level [Table 2]. The main effect of K fertilizer was significant at 5% probability level [Table 1]. Also N fertilizer × K fertilizer interaction was significant at 1% probability level [Table 1]. Also, means comparison table [Table 3] showed that among different N fertilizer levels, application of 125 kg N/ha was lower than the other three levels and other levels was ranked in the superior group. But all four K levels had no significant differences in their mean tuber number/plant and were ranked in same group. In N fertilizer ×K fertilizer interactions, application of 375 kg N/ha was better than the other levels at K level of 4 kg/ha and were ranked in superior group a. This is in agreement with foregoing researches [Aghighi et al, 2011;Saeedi, 2007; Koochaki, 2006, Bansal,2011].

Mean tuber weight/plant

Analysis of variance [Table 2] showed that the main effect of N fertilizer, K fertilizer and N fertilizer × K fertilizer on mean tuber weight per plant was not significant. Also, means comparison table [Table 3] showed that all fertilizer levels had no significant differences in their mean tuber number/plant and were ranked in same group. The results were not in agreement with Jafari and Heidari [2014], Zelalem[2009] who reported that the effect of different N fertilizer level was significant on tuber weight.

Fresh tuber yield

Analysis of variance [Table 2] showed that the main effect of N fertilizer , K fertilizer and N fertilizer × K fertilizer interaction was significant on fresh tuber yield at 1% probability level. Among N fertilizer levels, the fertilizer level of 375 kg had the strongest effect on fresh tuber yield and produced the highest yield. Also among K fertilizer levels, the fertilizer level of 0 kg had the strongest effect on fresh tuber yield and produced the highest yield. Among the interactions too, N fertilizer level of 375 kg/ha at K fertilizer level of 100 kg /ha ranked in the superior group and no-fertilizer level ranked in the inferior group. The increase in the application of N fertilizer up to a certain level increases the potato yield, but since then, it has no effect on the increase in yield [Westerman et al., 1985]. Jindong et al. [2006] stated that if the amount of applied fertilizer is greater than field capacity, the excessive fertilizer leaches to underground waters, which is harmful to ecosystems. Therefore, the recommendation regarding fertilizer type and level for a crop and field must be based upon genuine and delicate experiments. But This research was in agreement with Lie et al. [2003] who reported that high levels of fertilizer maximizes the net efficiency by neutralizing the adverse effects of soil quality on yield.

Dry matter percentage

Analysis of variance [Table 2] showed that the main effect of N fertilizer was significant on dry matter percentage at 5% probability level. The main effect of K fertilizer and N fertilizer × k fertilizer on dry matter percentage was not significant. Among N fertilizer levels, 0 kg/ha had the greatest effects on dry matter content and ranked in the superior group. The results were not in agreement with Moosavi et al. [2001] who reported that the effect of different N fertilizer level was insignificant on dry matter content. Krijthe [1982] reported that the excessive level of available N fertilizer stimulates reformation of tubers and may

Lead to the lengthening of tuber formation period and the difference in tubers maturity which in turn, leads to the difference in tubers dry matter content.

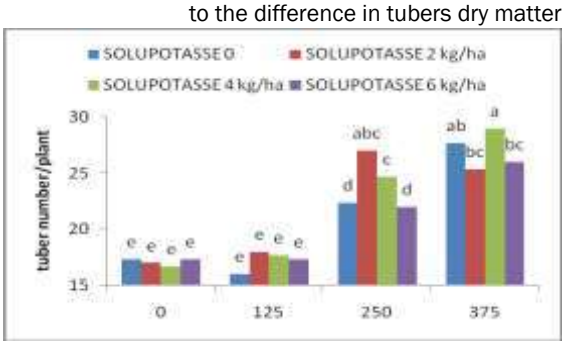

Fig. 1: Interaction between different N fertilizer levels and different K fertilizer on tuber number/plant.

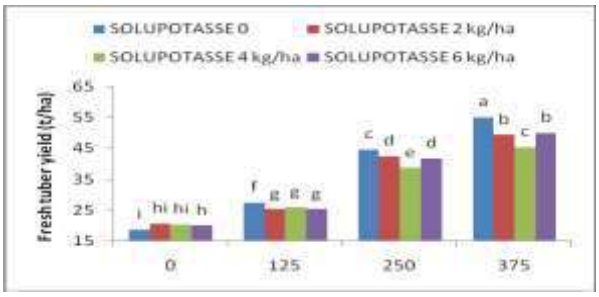

Fig. 2: Interaction between different N fertilizer levels and different K fertilizer on fresh tuber yield.

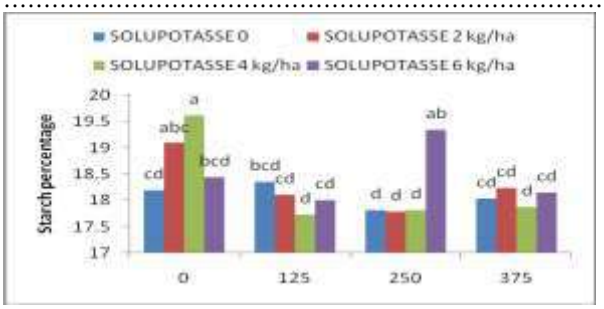

Fig. 3: Interaction between different N fertilizer levels and different K fertilizer on starch percentage.

Starch percentage

The main effect of N fertilizer and K fertilizer on starch percentage was not significant , But the interaction between them was significant on starch percentage at 5% probability level. According to means comparison table N fertilizer level of 0 kg/ha at K fertilizer level 6 kg/ha had the strongest effect on starch content and ranked in the superior group. Since starch forms 60-80% of dry matter, there was a special correlation between starch content and tuber dry matter. Starch is the main compound of potato tuber, making 3/4 of dry matter and depends mostly on cultivar. It plays an important role in the quality of products and is an important factor affecting potato cooking quality [Jafarian, 2000].

Leaf chlorophyll content

Analysis of variance [Table 2] showed that the main effect of N fertilizer, K fertilizer and N fertilizer × K fertilizer on leaf chlorophyll content was not significant. Also, means comparison table [Table 3] showed that all fertilizer levels had no significant differences in their mean leaf chlorophyll content and were ranked in same group. The results were not in agreement with Arshadi et al. [2012] who reported that the effect of different N fertilizer level was significant on leaf chlorophyll content

Germination percentage

Analysis of variance [Table 2] showed that the main effects of K fertilizer was significant on germination percentage at 5% probability level. The main effect of N fertilizer and N fertilizer × k fertilizer on germination percentage was not significant. Among K fertilizer levels, 0 kg/ha had the greatest effects on germination percentage.

Germination rate

Analysis of variance [Table 2] showed that the main effects of N fertilizer was significant on germination rate at 5% probability level. The main effect of K fertilizer and N fertilizer × k fertilizer on germination rate was not significant. Among N fertilizer levels, 125 kg/ha had the greatest effects on germination rate.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

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FINANCIAL DISCLOSURE

None

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ARTICLE

ASSESSMENT OF THR EFFECT OF BISPECTRAL INDEX (BIS) MONITORING ON AWARENESS DURING ANESTHESIA IN PATIENTS CANDIDATES FOR NON EMERGENCY CESAREAN SECTION

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ABSTRACT

Background: *Awareness during general anesthesia is potentially an unpleasant experience among patients undergoing surgical procedures. Despite the loss of consciousness induced by anesthesia, patients may experience pain during surgical processes due to their sensory perceptions and improper pain management. Anesthesiologists can estimate the level of patients' unconsciousness, based on their clinical experience and skills, as well as such as changes in patients' clinical signs (e.g., blood pressure, heart rate, sweating and tears in the eyes). However, since this sign is neither accessible nor reliable at all times, bispectral index (BIS) monitoring is required during the general anesthesia, particularly in the cesarean section. Methods: This double-blinded, randomized, clinical trial was performed on 214 women (> 15 years of age) with (American Society of Anesthesiologists) ASA class I and II, undergoing cesarean section at Taleghani Hospital of Arak, Iran. The participants undergoing general anesthesia were randomly divided into intervention (with BIS monitoring) and control groups (without BIS monitoring). The level of subjects' awareness during anesthesia was determined by interviews (using specific structured questions) within 24 hours after the surgery and 3-6 days following the procedure at the post-anesthesia care unit.* ***Results:*** *Awareness during anesthesia was reported in 8 out of 107 cases (7.4%) in the control group (awareness score ≥ 2). However, this event was observed in none of the participants (0%) in the intervention group. Based on Kruskal-Wallis test results, level of awareness during anesthesia in the control group was higher than the intervention group (P<0.001).* ***Conclusion:*** *Based on the finding, level of awareness during anesthesia was dramatically lower in subjects with BIS monitoring, compared to those without BIS monitoring (traditional anesthetic induction).*

KEY WORDS
Awareness, BIS monitoring, Non-emergency Cesarean Section

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INTRODUCTION

Awareness during general anesthesia is a potential and major concern among patients and anesthesiologists during surgical processes. As estimated, over 50% of patients are concerned about this unpleasant event. Awareness during anesthesia can cause various side-effects such as neuroticism, anxiety and irritability among patients. Despite the loss of consciousness induced by anesthesia, patients may experience pain during surgery due to their sensory perceptions and improper pain management [1, 2].

Commonly, anesthesiologists prescribe a dose of analgesic medications for the patients, based on their medical experience and expertise. They can estimate the level of patients' unconsciousness by evaluating their clinical signs (e.g., blood pressure, heart rate, sweating, tears in the eyes and body movements). Since evaluation of the majority of these signs is neither accessible nor reliable at all times, bispectral index (BIS) monitoring during general anesthesia, particularly in abdominal surgeries, is essential [3].

BIS is a statistical index, derived from electroencephalographic parameters, which categorizes the level of patients' consciousness as follows: burst suppression: 0-30, deep hypnosis: 30-40, general anesthesia: 40-65, sedation: 65-85 and awake: 85-100.

Although intraoperative awareness with explicit recall of sensory perceptions during surgery is a rare event, it can lead to post-traumatic stress disorder (PTSD) among patients with such an experience [1, 2, 4]. Initially, monitoring devices for evaluating the depth of anesthesia were introduced in the developed countries, especially in the United States. In these countries, use of such systems has been recommended by medical authorities, considering the high reported rate of awareness during surgery, deep anesthesia and uncontrolled use of analgesics. Use of these devices is increasing in other countries, although the available technologies are quite restricted, given the complexity of anesthetic induction.

According to published approved data, BIS monitoring, as the main introduced technology in this area, is applied in approximately 73% of most prominent hospitals and 53% of operating rooms in the United States. Today, use of this technology has been reported in almost 160 countries around the world. Factors such as high cost, unfamiliarity, unawareness of BIS advantages and low quality of care provision have led to the slow development of this technology in developing countries [5-7].

Use of BIS monitoring will be necessary in near future without any doubts. In addition to enhancing the quality of care provision via proper anesthesia management, this technology could prevent patients' awareness during surgery, reduce the risk of drug poisoning/overuse and distinct anesthetic-induced sleep from loss of consciousness (i.e., coma) [4]. In fact, as previously mentioned, these problems are quite common in Iranian hospitals [1].

Considering the risk of post-traumatic stress disorder (PTSD) in patients under anesthesia and the rarity of information on the complexity of this issue [2], the level of patients' awareness during general anesthesia and the effects of BIS monitoring need further studies [8]. Given the specific circumstances required for the administration of some analgesic medications, this study aimed to determine the level of awareness among 214 women (> 15 years of age) with ASA class I and II, undergoing abdominal surgery at Taleghani Hospital of Arak, Iran.

MATERIALS AND METHODS

This double-blinded, randomized, clinical trial was performed on 214 patients, candidates for cesarean (aged 15-45 years) with ASA class I and II, referring to Taleghani Hospital of Arak, Iran. The study samples were randomly divided into groups A and B. The sample size was calculated at 107 cases per group. Unlike group B (control group), BIS monitoring was employed in group A (intervention group). The number of cases was equal in the two groups.

These patients are randomized with sample randomized sample size.

$$n = \frac{\left[Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right]^2 [P_1(1-P_1) + P_2(1-P_1)]}{(P_1 - P_2)^2}$$

$$n = \frac{(1.93 + 2.33)^2[0.2(1-0.2) + 0.1(1-0/1)]}{(0.2-0.1)^2}$$

N= 107

$$Z_{1-\frac{\alpha}{2}} - 1.96$$

$$Z_{1-\beta} = 2.33$$
$$P_1 = 0.2$$
$$P_2 = 0.1$$

The inclusion criteria were as follows: 1: Being a candidate for non-emergency cesarean section; 2: gestational age of 37-42 weeks; 3: Lack of any systemic disorders; 4: ASA class I or II; 5: age range of between 15-45 years-old; 6: No chronic drug abuse; 7: no prior history of heart, liver or kidney disorders; 8: maximum surgery duration of 60 min; and 9: undergoing surgery by one single surgeon.

On the other hand, the exclusion criteria were as follows: 1: intubation for more than 35 sec (since problematic intubation is one of the causes of intraoperative awareness); 2: preeclampsia or chronic hypertension; 3: morbid obesity (BMI>35 kg/m2); 4: ASA class > II; 5: systemic or mental disorders; and 6: duration of surgery > 90 min.

In this study, the subjects were blind to the group they were assigned to. Also, considering the double-blind study, awareness during anesthesia was not evaluated by an anesthesiologist. Instead, trainees were instructed to assess the level of awareness during anesthesia in educational classes. The assigned classes were held by an anesthesiologist and the project manager before implementing the intervention.

In this study, all subjects underwent general anesthesia by thiopental (2-4 mg/kg) and succinylcholine (1-2 mg/kg). Afterwards, the subjects were mechanically ventilated and received 50% 02, 50% N20 and 1% isoflurane under anesthesia and. if required, muscle relaxants (0.2-0.5 mg/kg of atracurium) were used, as well. After delivery, 50-150 µg of fentanyl was prescribed for the patient.

In the intervention group, BIS monitoring was performed every 15 min during anesthetic induction alternatively, laryngoscopy, intubation, surgical incision, extubation and the end of the procedure. In the intervention group, in case of increased blood pressure or heart rate or BIS > 60 was reported, use of narcotics, anesthetic gases and medications was improved to increase the depth of anesthesia. In the control group, in case of increased blood pressure or heart rate, tears in the eyes or limb movements, the mentioned medications were prescribed.

At 12 and 24 hours after the surgery, a questionnaire on the level of awareness during anesthesia was completed. Additionally, the level of subjects' awareness during anesthesia was measured via interviews and the designed questionnaire. All of the questionnaire and interviews data were kept secret. In order to analyze the obtained findings, statistical tests including Kurskal-Wallis test, Chi-square, parametric tests and ANOVA were performed, using SPSS version 16.

RESULTS

Awareness during anesthesia was reported in 8 out of 107 cases (7.4%) in the control group (awareness score ≥ 2). However, awareness during anesthesia was observed in none of the participants in the

intervention group (0%). Based on Kruskal-Wallis test results, level of awareness during anesthesia in the control group was higher than the intervention group (P<0.001) [Fig. 1].

The mean score of awareness in the control group was 1.64 in 8 subjects experiencing awareness during anesthesia. The mean blood pressure during anesthesia was 8.1 in the intervention group and 9.5 in the control group. Based on Kruskal-Wallis test results, the mean blood pressure was significantly lower in the intervention group, compared to the control group (P<0.01) [Fig. 2].

The mean heart rate was estimated at 94.6 bpm in the intervention group and 102.1 bpm in the control group. Based on Kruskal-Wallis test results, there was a significant difference between the two groups, and the mean heart rate in the intervention group was lower than the control group (P<0.01) The mean heart rate was 94.6±3.4 in the intervention group and 102.1±4.5 in the control group. There was a significant difference between the two groups and the mean heart rate was lower in the intervention group, compared to the control group (P<0.01) [Fig.3].

Moreover, according to Kruskal-Wallis test results, there was no significant difference between the two groups in terms of oxygen saturation (SpO2); in fact, the mean SpO2 was almost equal in the two groups (96%) (P<0.05).

Additionally, according to Kruskal-Wallis test results, age and duration of surgery were not significantly different between the two groups (P>0.05). The mean age of the participants was 27.3±2.4 years, which was almost similar in the two groups (P>0.05).

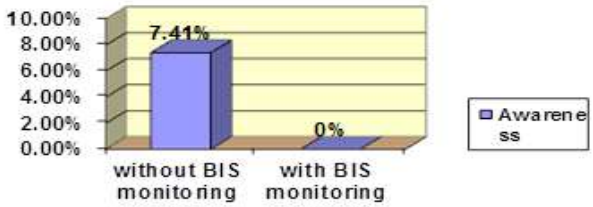

Fig. 1: The frequency distribution of awareness during anesthesia in subjects with and without BIS monitoring.

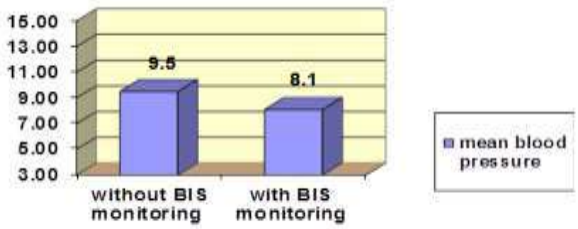

Fig. 2: The mean blood pressure in subjects with and without BIS monitoring.

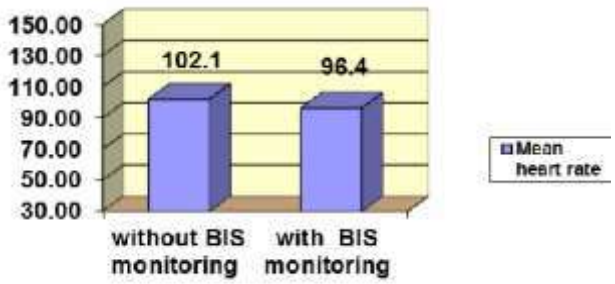

Fig. 3: The mean heart rate in subjects with and without BIS monitoring.

Based on the findings, the mean minimum alveolar concentration (MAC) of isoflurane was 0.5±0.1 in the intervention group and 1±0.2 in the control group (P≤0.01); there was a significant difference between the two groups and MAC of isoflurane was lower in the intervention group, compared to the control group [Table 1].

Table 1: The mean minimum alveolar concentration (MAC) of isoflurane in the intervention and control groups

Groups	Mean	SD	P-value
Intervention group	0.5	0.1	≤0.01
Control group	1	0.2	

DISCUSSION

Characterization of the difference between the incidence and level of awareness during anesthesia in patients with and without BIS monitoring could cause enhancement in the condition of patients who experienced awareness during anesthesia. This event is dependent on the condition of patients and use of anesthetics. Awareness during anesthesia can cause various problems such as neuroticism, anxiety, irritability, depression and even suicide in some cases. Despite the loss of consciousness induced by anesthesia, patients may experience pain during surgical procedures due to their sensory perceptions and improper pain management. Anesthesiologists can estimate the level of patients' unconsciousness, based on their clinical experience and expertise, such as changes in patients' clinical signs (e.g., blood pressure, heart rate, sweating and tears in the eyes).

Since the majority of these signs are not reliable at all times, use of bispectral index (BIS) during general anesthesia, particularly in abdominal surgeries, is required. It seems that BIS monitoring could lead to a substantial reduction in patients' awareness during anesthesia [8].

The risk of awareness during anesthesia is higher in some surgeries such as open heart, cesarean and trauma surgeries. In such surgeries, anesthesiologists cautiously prescribe analgesics in accordance with patients' specific conditions; these prescriptions may increase the risk of awareness during surgery in some cases [8-11].

In this study, awareness during anesthesia was assessed in patients, who were candidates for non-emergency cesarean. Based on our findings, awareness during anesthesia was reported in 8 out of 107 cases (7.4%) without BIS monitoring (awareness score ≥ 2). On the other hand, awareness during anesthesia was observed in none of the participants with BIS monitoring.

In the present study, based on Kruskal-Wallis test's results, level of awareness during anesthesia in patients without BIS monitoring was higher than those with BIS monitoring (P<0.001). Similar results have been reported in previous studies. In a study by Ekman et al. (2004) in Scandinavia on the effect of BIS monitoring on awareness during anesthesia, a 77% decline in awareness during anesthesia was reported [10].

Additionally, in a study by Avidan et al. (2008) on awareness during anesthesia and BIS monitoring, it was reported that this technology does not provide routine standard anesthesia. However, the incidence of awareness during anesthesia would decline if BIS value did not exceed 60 [12].

Moreover, Azemati et al. (2004) conducted a study in Iran in order to compare the incidence of awareness during anesthesia in 151 women undergoing cesarean section and anesthesia via propofol, thiopental and halothane. The results showed that awareness during anesthesia may cause some side-effects such as neurosis, anxiety and irritability, which can manifest in form of dreaming or complete recall of intraoperative events. Awareness during anesthesia was more common in some surgeries such as cardiac and cesarean [13]. This study was indicative of the relatively high incidence of awareness during anesthesia in cesarean section.

Additionally, Khalili et al. (2007) conducted a study at Isfahan University of Medical Sciences to compare the depth of anesthesia, based on BIS value in 114 cases, undergoing intravenous and inhalation anesthesia. As the findings indicated, clinical symptoms, which are commonly applied to evaluate the depth of anesthesia, are insufficient and inadequate. The depth of anesthesia could be correctly set by BIS monitoring; also, the required dose of anesthetics could be reduced in some cases via BIS monitoring [14].

In the present study, the mean MAC of isoflurane during surgery was 0.5±0.1 in cases with BIS monitoring and 1±0.2 in patients without BIS monitoring. As the results indicated, a significant difference was detected between the two groups and MAC of isoflurane was lower in the BIS monitoring group (P≥ 0.01). In other words, use of anesthetic gas in patients benefiting from BIS monitoring was lower than those with no BIS monitoring.

In a previous study accordance with the present research, the dose of required anesthetics was lower in patients with BIS monitoring, compared to those without BIS monitoring (traditional method) [14]. The results of the majority of these studies are in consistence with the present findings. According to literature review, BIS monitoring plays a basic role in decreasing awareness during anesthesia [9, 14, 15].

Application of BIS monitoring during general anesthesia in surgeries, particularly open heart surgery and cesarean, is effective in accurate administration of anesthetics. Accordingly, the accurate use of drugs can lead to a significant decline in the rate of intraoperative awareness (via accurate and timely administration of sufficient anesthetic doses), prevent the overdose of anesthetics and reduce the use of such drugs; it also can lead to a reduction in the costs and problems induced by anesthetics.

Further research is required to compare the depth of anesthesia in different anesthetic techniques and to evaluate the effect of BIS monitoring on reduced awareness during anesthesia in other surgeries such as open heart surgery.

CONCLUSION

In conclusion, this study showed that BIS monitoring could be effective in reducing awareness during anesthesia among pregnant women undergoing non-emergency cesarean.

CONFLICT OF INTEREST

The authors declare no competing interests in relation to the work.

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ARTICLE

COMPARISON OF KETOROLAC, APOTEL AND THEIR COMBINATIONS FOR PAIN CONTROL IN ACUTE CHOLECYSTITIS

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ABSTRACT

Background: *Pain is a common problem between patients and delays returning to daily life. So the aim of this study is to compare ketorolac, apotel and their combination in management of acute cholecystitis pain.* ***Methods:*** *In this clinical trial study, 90 patients with acute cholecystitis candidate for cholecystectomy surgery selected for study based on inclusion and exclusion criteria and were randomly divided into three equal groups. The first group received ketorolac 30 mg, while the second group received apotel 2 gr, and the third group received a combination of both medications. Patients' pain was evaluated with visual analog scale in 5 steps.* ***Results:*** *The results of this study showed that simultaneous administration of ketorolac 30 mg and apotel 2 gr is more analgesic than any of them alone.* ***Conclusions:*** *Ketorolac and paracetamol are two analgesics used commonly in clinic. The present study demonstrated that simultaneous administration of these medications in a same time will significantly manage the pre-operative pain of cholecystitis.*

KEY WORDS
Cholecystitis, Ketorolac, Pain, Paracetamol

INTRODUCTION

Gallstones are common problem of the digestive system that afflict 10% of the people in the world. As many as 80% of the people with gallstones show no signs. One to three present of the patients with marked gallstones with its symptoms such as acute Cholecystitis [1]. Acute Cholecystitis is diagnosed based upon clinical symptoms and signs in patients with peritonitis localized in the upper, right-hand quadrant of the abdomen. Obstruction of the Cystic duct as a result of stone will cause in distension of the gallbladder, inflammation and edema of gallbladder wall [1, 2, 3]. Acute Cholecystitis begins with a biliary colic attack, but the pain doesn't subside and it may last for several days. Patients referred to hospital with acute cholecystitis require reception of intravenous liquids, anti-biotic and analgesia and the final treatment for them is Cholecystectomy. To prevent the pain caused by the acute inflammation of gallbladder, analgesics are used such as non-steroid narcotics and anti-inflammatories. As various many studies indicate, morphine results in high sphincter Oddi pressure, thus it should never be prescribed in patients with Biliary colic [1, 4, 5]. The potential problem of using narcotics in patients suffering fromwith Biliary colic or Cholecystitis is its interference with HIDA scan which is the definitive method used to diagnose acute Cholecystitis [2]. Non-steroid anti-inflammatory medicines don't result in contraction of Oddi sphincter and show no interference with HIDA scan. By harnessing Prostaglandins, non-steroid anti-inflammatories prevent the progress of acute gallbladder inflammation and the resulting contraction complications in the initial phases of acute cholecystitis. One of these non-steroid, anti-inflammatory medicines that its effect on reducing the biliary colic pain has been approved is Ketorolac. The main advantage of using Ketorolac is its analgesic effect without reducing the performance of the central nervous system which is typical of narcotics. Ease of prescription, immediate commencement of the effect and its durability make Ketorolac a good choice to reduce pain in the emergency ward of a hospital [3, 5, 6]. Apotel (acetaminophen) also reduces production of prostaglandins. The analgesic properties of apotel can be justified by harnessing nitric oxide synthase enzyme and the analgesic mechanism which depends on supra-spinal serotonin [4, 5, 7, 8]. Finally, the present research aims to compare the effect of ketorolac and apotel and their mixture on controlling acute pre-operation cholecystitis pain.

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MATERIALS AND METHODS

For this double-blind, randomized clinical trial. All patients referring to Vali Asr and Amir Al-Momenin hospitals of Arak, Iran with the possible symptoms of acute cholecystitis (fever, pain in the upper, right-hand quadrant of the abdomen, leukocytosis, etc.) who had undergone sonography, were selected for the research based on the exclusion and inclusion criteria after taking their written informed consent. All patients underwent standard, anti-biotic (Ceftriaxone and Metronidazole) and hydration treatment. The pain scale of the patients was measured using VAS criteria which is essentially a 10 cm ruler expanding from 0 to 10. In this ruler, zero indicates no pain, while 10 indicates intolerable pain. The patients were asked to mark their pain on this ruler and the distance between the mark made by the patient and point zero shows patient's pain. Then patients were divided into 3 groups based on the block model. The patients in first group who had received 30 mg ketorolac solved in 100 cc normal saline for 30 minutes. Patients in the second group received 2 mg apotel solved in 100 cc normal saline for 30 minutes (mixed group) received 30 mg ketorolac and apotel 2 g solved in 100 cc normal saline. The pain scale of the patients was measured 0.5, 1, 2, and 6 hours before operation based upon visual analog scale (VAS). To measure the inflammation factor in 0 and 6 time, LFT was also measured. To observe the rule of double-blindness, the medicines were given by an assistant and the pain level was measured by plan executor who had no information concerning the groups. Those patients who had VAS level of 10 one hour after

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prescription of medicine or placebo were excluded and underwent routine treatment using Pethidine. Then, necessary tests were used to analyze the tests in SPSS software version 20. The medicines used were produced by Exir Iran Co. (ketorolac) and Uni Farma Co. (Apotel). The data was entered in SPSS, and Chi square and one-sided variance analysis tests were utilized to compare the data of each group. The P-value level below 0.05 was considered to be significant. The sample size was calculated using the following formula. Considering the type of research, randomized clinical trial method was used for sampling. The patients with acute cholecystitis were selected and divided into three groups using the following formula:

$$N = \frac{\left(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta}\right)^2 (S_1^2 + S_2^2)}{(\mu_1 - \mu_2)}$$

Inclusion criteria: patients diagnosed with acute cholecystitis, aging 18 to -65- years -old.
Exclusion criteria: patient's declined to take participate in the research, aging younger than 18 and older than 65 years old

- Pregnancy or breast feeding, clinical symptoms indicating sepsis or other inflammatory diseases
- Lack of cooperation on the side of patient to determine level of pain, no toleration of pain, sensitivity to non-steroid anti-inflammatories and acetaminophen, renal or liver failure, history of digestive system bleeding
- Coagulation disorders, symptoms indicating neuropathy, patients with gangrenous cholecystitis
- Patients diagnosed with generalized Peritonitis

RESULTS

As many as 90 patients with acute cholecystitis were studied in this clinical trial. There were 15 male and 15 female patients in ketorolac group 14 female and 16 male in apotel group and 13 female and 17 male in the group that received both the apotel and ketorolac. The chi square test showed no significant difference between the three groups in terms of gender (P = 0.875). The following average ages were observed: 38.23 ± 8.13 years in ketorolac group, 34 ± 5.98 years in apotel group and 36.06 ± 7.73 in the mixed group. One way ANOVA results showed no statistically significant difference between the three groups in terms of their age (P = 0.089). Patients' pain levels were measured 0.5, 1, 2, and 6 hours immediately following diagnosis and before the operation based on VAS. The following values for VAS before receiving medicines in each group: 7.33 ± 1.15 in ketorolac group, 7.1 ± 66.26 in apotel group, and 7.43 ± 1.07 in the mixed group. One way ANOVA results found no statistically significant difference between groups in terms of pain levels before receiving the medicines (P = 0.528). The average pain score 30 minutes after receiving the medicine in groups receiving ketorolac, apotel, and both medicines was 2.76±0.43, 3±0.83, and 2.73±0.44 respectively. One way ANOVA results found no statistically significant difference between groups in terms of pain levels 30 minutes after receiving the medicines (P = 0.177). The average pain score 1 hour after receiving the medicine in groups receiving ketorolac, apotel, and both medicines was 3.23±0.62, 3.0±13.34, and 2.96±0.41 respectively. One way ANOVA results found no statistically significant difference between groups in terms of pain levels 1 hour after receiving the medicines (P = 0.097). The average pain score 2 hours after receiving the medicine in groups receiving ketorolac, apotel, and both medicines was 4.83±0.59, 4.0±46.62, and 4.33±0.99 respectively. One way ANOVA results found a statistically significant difference between groups in terms of pain levels 2 hours after receiving the medicines (P = 0.035). The average pain score 6 hours after receiving the medicine in groups receiving ketorolac, apotel, and both medicines was 6.83±0.87, 6.6±1.13, and 5.4±0.89 respectively. One way ANOVA results found a statistically significant difference between groups in terms of pain levels 6 hours after receiving the medicines (P = 0.0001) [Fig. 1]. Liver enzymes of the patients were measured at the beginning and 6 hours after receiving the medicine and no significant difference was observed in their levels (P = 0.45).

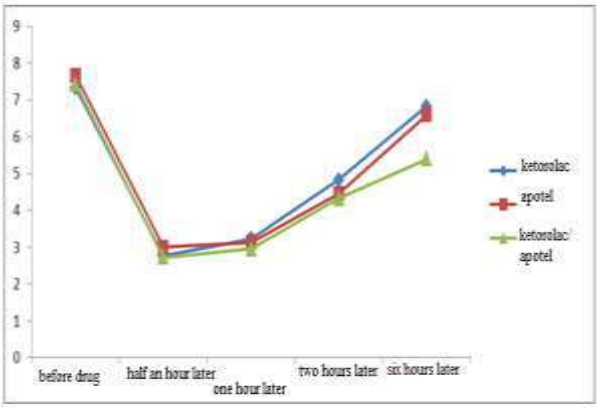

Fig. 1: Average pain scores before and after receiving the drug.

DISCUSSION

As the results of the present research, the simultaneous prescription of ketorolac (30 mg) and apotel (2 g) has a much stronger analgesic effect than prescribe them individually for cholecystitis pain. Pethidine is regularly used now to reduce the pain in patients with acute cholecystitis. Considering the current sanctions and shortage of this medicine observed in hospitals, it is necessary to find alternative medicines for these patients. According to the results of this research, a mixture of ketorolac and Paracetamol can be a good choice to reduce the pain in patients with acute cholecystitis. In a review research conducted by Hilsted et al., it was shown that mixing Paracetamol and non-steroid anti-inflammatory medicines was very useful to reduce acute pains. As many as eight clinical trials on this issue have been published over the recent years [9,10]. Four of these researches have confirmed that mixing one NSAID with paracetamol has a much better analgesic influence in compare of using the, individually. In their researches, Romanstad et al. added that Propacetamol to ketorolac and measured their analgesic effects by tolerating painful pressure [11]. It showed that 30 mg ketorolac resulted in high levels of pain toleration which were not more than the basic levels of pain toleration. However, addition of two gr Propacetamol (equal to 1 gr apotel) to 30 mg ketorolac could significantly enhance pain toleration [11]. This result supports the hypothesis of mixing Paracetamol with an NSAID to reduce acute pains which is confirm the results of present study. Pain is an unpleasant feeling and an emotional experience accompanied by real or possible damages caused to tissues or it is justified by such damages [3, 5, 12]. Multimodal analgesia includes mixing different sets of painkillers to amplify the effect and complications of medicines. Mixing paracetamol and non-steroid anti-inflammatory drugs (NSAIDs) is largely used in clinics [13, 14]. The hypothetical cause of this issue is the site and different performance of these two medicines (central nervous system vs. peripheral nervous system, serotoninergic system vs. synthesis of prostaglandins) [13, 15, 16]. It has been shown that the inhibitory effect of paracetamol on synthesis of prostaglandins can be observed in peripheral tissues as well. As a result, apotel can have significant peripheral effects only if it is prescribed along with an NSAID besides its central effects [10, 15, 17, 18]. Various materials such as modified biliary fats, prostanoids and cytokines can act as mediators of damage during cholecystitis [1, 12]. Prostaglandins can play a major role as mediators of acute inflammatory procedures through various effects such as hyperemia, edema, and contractile dysfunction [15, 16]. In this research, we used ketorolac which is an inhibitor of prostaglandin synthesis to reduce inflammation. Using NSAIDs to reduce acute cholecystitis pain in various researches has resulted in positive effects. Parkman et al studied the effects of Indomethacin and placebo on inflammation and contraction of gallbladder. In this research, the researchers realized that inflammation and contractile dysfunction of gallbladder had vanished after common bile duct ligation within 6 and 24 hours after operation [15]. In another research, Olsen et al compared the effects of ketorolac and butorphanol in treating the biliary colic pain. The results of our research point to this fact that both drugs help reduce the pain in the patients with biliary colic, thus they may be used in the emergency service unit of hospitals [3]. The results of our research also confirmed the analgesic effect of ketorolac in acute cholecystitis. Patients reported no severe complication in our research. However, in the research conducted by Olsen, 26% of the patients who had received ketorolac were exhibiting symptoms of nausea and vomiting [3]. This research was conducted on patients with biliary colic, while the current research studied the pain levels of patients with acute cholecystitis before cholecystectomy.

CONCLUSION

Ketorolac and apotel are two analgesic medicines used separately to reduce pain in different patients. According to the results of this research, simultaneous application and use of these medicines in a same time can effectively reduce the pain caused by acute cholecystitis in the period before and after operation.

CONFLICT OF INTEREST
The authors declare no competing interests in relation to the work.

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There is no financial disclosure.

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