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Institute of Integrative Omics and Applied Biotechnology Journal 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 Integrative Omics and Applied Biotechnology (IIOAB) Journal



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# ARTICLE MOLECULAR DIAGNOSIS OF HUMAN PAPILLOMAVIRUS (HPV) GENOTYPES IN TEHRAN CITY

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#### ABSTRACT



**Background** and **objective:** The human papilloma virus is known as one of the cause's anogenital cancers, including cervical cancer there is a strong link between presences of different types of human papilloma viruses to the development of genital lesions. HPV16 and HPV18 were defined as most important etiologic agents for cervical dysplasia and carcinoma. The aim of present research was to find genital HPV genome in patients with cervical cancer and control group and to investigate incidence of HPV16, HPV18, HPV31, HPV33, HPV45 and HPV51 types among patients admitted to medical centers. **Materials and Methods:** Fifty samples of women cervical paraffin-embedded tissue, HPV positive from pathology department of Imam Khomeini (RA) were collected. For this purpose, DNA was extracted and purified using a DNA Extraction Kit (Cinnagen, Tehran, Iran] from 50 patients with cervical cancer attending the Imam Khomaini Hospital (Tehran, Iran) and amplified by PCR. The nucleotide sequences were analyzed using the BLAST program (http://www.ncbi.nlm.gov/ BLAST). **Results:** The molecular and pathological investigation of samples showed that %71 of cases were HPV-positive. Out of them, %41 of samples was determined HPV 16, %28 HPV18, %10 HPV31, %5 HPV33, %7 HPV45 and %9 HPV51. **Conclusion:** As a matter of fact, the virus is not related to significantly detectable symptoms, and also the extreme prevalence of HPV in Iran, early detection of HPV can be prohibit preventing cancer development from precancer lesions. Therefore, using molecular techniques like PCR may contribute effectively to identifying HPV cases to diagnose in a proper time.

# INTRODUCTION

KEY WORDS Papiloma virus; cervical cancer; cancer diagnosis

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The human papilloma virus is known as one of the cause's anogenital cancers, including cervical cancer [1]. Which approximately 30-40 percent of them are due to contamination of mucous, especially in the anogenital areas [2] lack of ability to viral culture, makes the initial identification of the virus problem. And up to now more than 100 different types of human papillomavirus have been identified with the help of molecular techniques [1]. PCR technique is the ability to replicate virus DNA using primers specific for the particular type of virus as one of the most sensitive methods to detect HPV in genital tissues are considered. This method along with cytological methods can increase the resolution and accuracy in investigating clinical patients. Papilloma viruses have circular double-stranded DNA genomes with sizes close to 8 kb. Due to their small size, their molecular biology is very complex [5]. Human papilloma virus genome consists of three initial proteins E1-E8, L1-L2 protein secondary and non-coding control region and the LCR or URR [6 and 7, 8, 9, 10, 11, 12]. Cervical cancer is the first epidemiological study was conducted by Rigoni-Stern in 1842 showed that the incidence of cervical cancer is more common in married women [13]. The HPV genotypes in terms of cancer risk can be grouped as high-risk (HR) or low-risk (LR): LR HPV includes types 6, 11, 40, 42, 43, and 44, whereas HR HPV includes types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66.

On the basis of all married women 20-65 years after first entering the screening Pap test every year once they are done [14 and 15]. HPV is the most common disease and showed that the incidence of cervical cancer is more common in married women [13]. HPV is the sexually transmitted disease (STD) acquired from the oncoviruses comes to the types 6 and 11 account not only the anogenital region but also in the respiratory tract, lungs, mucous membranes, skin, head and neck and mouth and the injury, including warts anogenital that an infectious transmitted disease in both sexes by sexuality [16]. Types 16 and 18 caused serious problem for women including cancers of the cervix, vulva, anus, vagina, etc. [17]. Anogenital infection in the form of warts. Condylomata acuminata, and mucosal lesions of the cervix (CIN) appears [18] for cervical lesions CIN1, CIN2, CIN3 or classified CIS [19, 20]. The lesion is usually diagnosed as CIN1 [21]. There lesion in the middle and upper thirds as CIN2, CIN3 is detected, indicating the progress of cervical lesions and cervical cancer [22]. Cervical cancer occurs most often between the ages of 30 to 55 years but has recently been observed in young women. Cervical cancers in developing countries, including Iran, are more common than in developed countries [23]. By early detection of the HPV virus can be prevent many cancers. All diagnostic methods has advantages and disadvantages, but new molecular methods of detection and genotyping of HPV have a higher sensitivity and specificity (28). PCR amplification of the target DNA using different today and Hybrid Capture HPV virus detection is done accurately [24, 20]. The objectives of this study were to investigate molecular diagnostics HPV genotypes (HPV) causes' cervical cancer in Tehran city.



# MATERIALS AND METHODS

#### Tissue samples

A total of fifty cervical paraffin-embedded (FFPE) tissue samples from 50 different women who were diagnosed as HPV positive were selected for DNA extraction and human papillomavirus (HPV) genotyping and were retrieved from the archives pathology department of Imam Khomeini hospital (Tehran, Iran), from October 2014 to February 2016.

#### Tissue retrieval and DNA extraction

For all 50 samples, paraffin-embedded blocks were examined for adequate tissue volume, defined roughly as an area at least twice that of a 1 mm micro-punch. Only one block per case was used, and within that block, one contiguous area of tumor was selected for analysis. One-millimeter disposable sterile micro-punches were used to punch tissue cores from marked paraffin embedded blocks. Between 3 and 7 1-mm cores per block were obtained. Tissue cores were first de paraffinized and proteinase K-digested according to the manufacturer's instructions. DNA was extracted using a commercially available DNA extraction Rima Pure FFPE kit (Recover all Total Nucleic Acid Extraction Kit for formalin fixed, paraffin-embedded Tissues, Life Technologies, Grand Island, NY) that uses a spin column-based extraction technique to maximize nucleic acid purity. Final DNA concentrations were determined using a Nano-drop 1000 spectrophotometer. Samples were included for PCR amplification and sequencing if the DNA concentration was greater than 6 ng/ $\mu$ L in the 50  $\mu$ L DNA extraction volume.

#### PCR and Sanger sequencing

system.

At least 200 ng of DNA was PCR amplified using forward and reverse primers that targeted HPV types 16, 18, 31,33,45,51 Primer sequences were as mentioned I [Table 1]. The PCR reactions were carried out in a reaction volume of 25  $\mu$ l containing genomic DNA 30-40 ng, 1 mol/l dNTP 2  $\mu$ l, 25 mM MgCl2 1.5  $\mu$ l, 10x PCR Buffer 2.5  $\mu$ l, 10 pM primer 0.5  $\mu$ l each, 1 U Taq polymerase (CinnaGen) 0.4  $\mu$ l, and DDH20. The reactions were carried out in the following thermo cycler conditions: denaturation at 94 °C for 5 min, 36 cycles of 94 °C for 30 s, 63 °C for 30 s, and 72 °C for 30 s, and final elongation step at 72 °C for 10 min. PCR products were visualized by gel electrophoresis and staining with ethidium bromide in a digital camera

The presence of HPV different genotypes (HPV16, HPV18, HPV31, HPV33, HPV45 and HPV51) in patients were determined by using following primers sequence as mentioned in [Table 1].

Туре	Primer	Sequences	Length [bp]	Annealing Temperature	Reference
HPV-16	F	ACCCAGTATAGCTGACAGT	252	48 °C	Raji, 2011 [32]
	R	CTCGTTTATAATGTCTACACA			
HPV-18	F	ATAGCAATTTTGATTTGTC		44 °C	Raji, 2011 [32]
	R	AAACTCATTCCAAAATATG			
HPV-31	F	CACAACATTTGATTTGTCCC	351	54/3 °C	Raji, 2011 [32]
	R	CTCGTTTATAATGTCTACACA			
HPV-33	F	ATGCACAACTTGCAGATTC	449	57 °C	Raji, 2011 [32]
	R	AAACTCATTCCAAAATATG			
HPV-45	F	GCTACAGCTGTTATTACGCAG	455	60 °C	Weyn, 2007 [33]
	R	GCAATTGTGCAGGTTTAC			
HPV-51	F	CCTAAAACCTCAACGCGTGCTGCT	452	52 °C	Weyn, 2007 [33]
	R	TTGTTGTGCATTGCCATTTGC			

Table1: Primers used of the detection of HPV genotypes 16, 18, and 31,33,45,51

After extracting DNA and insert samples in agars gel 5/1% and electrophoresis presence of HPV in the sample was determined. Next the samples with HPV-DNA typing kits for DNA Technology were selected and prepared by the reaction mixture to separate in micro tubes to which 10 ml and 5 ml sample buffer and the same amount as the reaction mixture or positive control or added based on the order of 45 cycles with temperatures kit (Denaturation 94 °C, 64 °C primer binding temperature of 70 °C Extension) PCR reactions done and in the end product by 2% agarose gel stained with Ethidium Bromide was observed and interpreted

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according to the kit manual. Symptoms of HPV type 33 bp 449 to get the band is. A band types 16 and 31 and 51 bp 642 signs are present. Size bp 285 bp 291 bands and HPV types 18 and 45 are respectively. Then the final PCR products were sequenced by Macrogen Company in South Korea.

#### RESULTS

A total of fifty cervical paraffin-embedded (FFPE) tissue samples from 50 different women who were diagnosed as HPV positive were collected for DNA extraction and human papillomavirus (HPV) genotyping and were retrieved from the archives pathology department of Imam Khomeini hospital (Tehran, Iran), DNA was extracted and purified using a DNA Extraction Kit (Cinnagen, Tehran, Iran) from 50 patients with cervical cancer attending the Imam Khomaini Hospital (Tehran, Iran). The quality of DNA extraction method using electrophoresis in agarose gels and stained with ethidium bromide were studied. The mean age of the study population was 50 years and their age range 25 to 76 years old. The maximum number of patients at the age of 36 to 55 age category with the abundance of % 53.2 [Fig. 1].

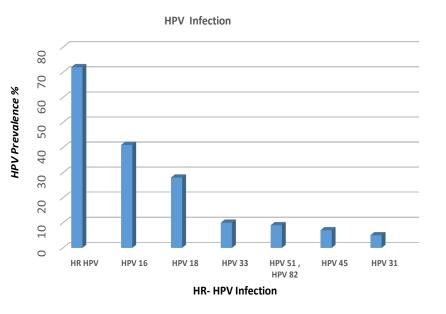


Fig. 1: Distribution as percentage of the most prevalent HPV genotypes in the selected population.

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Results of the molecular and pathological investigation of samples showed that 36 women (%71) were positive for HPV. For HR HPV infection, HPV 16 (41%) was the most common type, followed by HPV 18 (28%), HPV 31 (10%), HPV 51 and HPV 82 (9%), HPV 45 (7%) and HPV33 (5%) [Fig. 2].

	2	3	4	5	6	7
						. i. ji
500		512			-	-
400		415	-	-		
300						
200						
100						

Fig. 2: Agarose gel electrophoresis of human papillomavirus (HPV) polymerase chain reaction amplification products HPV 16 and HPV 18.

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Nucleic acid sequences of the different types of HPV were compared with those available in the GenBank database using NCBI/BLAST to search for related sequences.

Results of homology comparison in in the GenBank database using NCBI/BLAST shows that HPV 16 were 89% homologous with HPV16 from Germany; (Gene Bank ACCESSION Number: AJ313181) and 85% homologous with HPV16 from South Korea; (Gene Bank ACCESSION Number: KT428600) [Table 2]. HPV 18

were 93% homologous with HPV16 from Brazil; (Gene Bank ACCESSION Number: KP965187.1) and 93% homologous with HPV18 from Brazil; (Gene Bank ACCESSION Number: KP965186.1) [Table 2]. HPV 31 were 89% homologous with HPV31 from Italy; (Gene Bank ACCESSION Number: JN041177.1) and HPV 33 were 92% homologous with HPV33 from Mongolia; (Gene Bank ACCESSION Number: KC862076.1) [Table 2] [Fig. 3]. HPV 45 were 94% homologous with HPV45 from USA; (Gene Bank ACCESSION Number: KC470254.1), HPV 51 were 98% homologous with HPV51 from USA; (Gene Bank ACCESSION Number: U45917.1) and HPV 51 were 98% homologous with HPV82 from USA; (Gene Bank ACCESSION Number: KF436795.1) [Table 2].

 Table 2: Homology of the Nucleic Acid Sequences of the different types of HPV in the GenBank
 Database (NCBI)

HPV Types	Homology [%]	Accession No.	Country
HPV16	89%	AJ313181.1	Germany
HPV16	85%	KT428600.1	South Korea
HPV18	93%	KP965187.1	Brazil
HPV18	93%	KP965186.1	Brazil
HPV31	89%	JN041177.1	Italy
HPV31	88%	JN041176.1	Italy
HPV33	92%	KC862076.1	Mongolia
HPV33	92%	KC862075.1	Georgia
HPV33	92%	KC862074.1	Vietnam
HPV45	94%	KC470254.1	USA
HPV45	94%	EF202160.1	USA
HPV51	98%	U45917.1	USA
HPV51	97%	KF436874.1	USA
HPV82	82%	KF436795.1	USA
HPV82	82%	KF436794.1	USA

Human papillomavirus type 31 isolate 11\_TV31 L1 gene, partial cds

viruses | 4 leaves

Human papillomavirus type 31 isolate 4941ENG minor capsid protein L2 (L2) and major capsid protein L1 (L1) genes, complete cds

Human papillomavirus type 31 isolate QV14043, complete genome

viruses | 5 leaves

Human papillomavirus type 31 isolate 7611ENG minor capsid protein L2 (L2) and major capsid protein L1 (L1) genes, complete cds

viruses | 53 leaves

Human papillomavirus type 31 isolate 5001ENG minor capsid protein L2 (L2) and major capsid protein L1 (L1) genes, complete cds

Human papillomavirus type 31 variant L1 and L2 capsid protein genes, complete cds

Human papillomavirus isolate 06JAN\_PHL\_MY010\_09 L1 capsid protein (L1) gene, partial cds

0	vinuses   4 leaves
-0	Human papillomavirus type 31 isolate 16_TV31 L1 gene, partial cds
	viruses   8 leaves
	♥◀viruses   4 leaves
	Human papillomavirus type 31 isolate 20_TV31 L1 gene, partial cds
	- ● • viruses   4 leaves
	Human papillomavirus isolate PT105-05 L1 protein gene, partial cds
	HPV31_23F
	Human papillomavirus type 31 isolate 31_TV31 L1 gene, partial cds

Fig. 3: Phylogenetic Tree of Human Papillomavirus type 31.

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#### DISCUSSION

The genotype-specific prevalence of HPV in Tehran city women, is essential for achieving further progress in cervical cancer prevention. According to our knowledge, this is the first validation set to comprehensively describe prevalence of HPV infection of Tehran city women. The overall prevalence of HPV infection was %71 (n= 36). The results of this study indicate a much higher prevalence of HPV than the overall prevalence worldwide [34]. Age-specific HPV prevalence estimates were highest in women younger than 34 years and prevalence decreased in the 35-44 year-group [35]. Age-specific HPV prevalence were highest in Tehran City women with 36 years old. For HR HPV infection, HPV 16 (41%) was the most common type, followed by HPV 18 (28%), HPV 31 (10%), HPV 51 and HPV 82 (9%), HPV 45 (7%) and HPV33 (5%). Differences in the prevalence of HPV genotypes might be related to the geographical location factors and biological interactions between different HPV types and Immunity system of host [36]. This is the first prevalence report of HPV 82 in Iran. Results of Bruni et al., [37] indicated that among the women with type-specific HPV data, the 5 most common types worldwide were HPV-16 (3.2%), HPV-18 (1.4%), HPV-52 (0.9%), HPV-31 (0.8%), and HPV-58 (0.7%). Identification of HPV infection is the central causal agent of cervical neoplasia has created new research fronts in primary and secondary prevention of this disease. Sequence similarity searching to identify homologous sequences is one of the first, and most useful informative, steps in any analysis of newly determined sequences. Homologous sequences have similar structures, and frequently, they have similar functions as well. Results of homology comparison in the GenBank database using NCBI/BLAST shows that HPV 16 were 89% homologous with HPV16 from Germany: Gene Bank ACCESSION Number: AJ313181 [38]. HPV 18 were 93% homologous with HPV16 from Brazil; Gene Bank ACCESSION Number: KP965187.1 [39]. HPV 31 were 89% homologous with HPV31 from Italy; Gene Bank ACCESSION Number: JN041177.1 [40]. HPV 33 were 92% homologous with HPV33 from Mongolia; Gene Bank ACCESSION Number; KC862076.1 [41]. HPV 45 were 94% homologous with HPV45 from USA; Gene Bank ACCESSION Number: KC470254.1 [41], HPV 51 were 98% homologous with HPV51 from USA; Gene Bank ACCESSION Number: U45917.1 [42]. Many studies have shown that some types of high-risk HPV types are known to play a major role in creating this type of cervical cancer so that more than 99 % of cervical cancers have been identified in the world [43].

#### CONCLUSION

This finding adds knowledge to molecular diagnosis of human papillomavirus (HPV) genotypes information in Tehran city, HPV epidemiological investigation, and addresses further studies aimed to consider public health for identifying groups at high-risk (HR) for cervical cancer. The relatively ease and economic accessibility of the PCR technique can potentially have an impact in HPV screening in Iran.

CONFLICT OF INTEREST There is no conflict of interest.

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# TAMPER PROTECTION FOR DYNAMIC SERVICE LEVEL AGREEMENT IN INTELLIGENT AGENT BASED MOBILE CLOUD FRAMEWORK

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# ABSTRACT



In utility computing, customer requests varying services from software to infrastructure as and when needed. As a result, there are many mobile cloud providers provide this services on demand basis as per current market needs. To ensure compliance and security, these providers establish a contract popularly known as Service Level Agreement (SLA) which states the services and management aspects. Such SLA is usually managed by third party entities and in some case by intelligent autonomic agents. But owing to the growing needs and demands of customers, dynamic SLA was offered by the service providers. A dynamic SLA is a redefined SLA whenever new services are demanded by the customer after SLA negotiation. Consequently these dynamic SLA need tamper protection from unauthorized users to prevent unnecessary changes which may lead to SLA violation consequently losing the trust of customers. This paper proposes a data integrity protection for the dynamic SLA using visual cryptography and semi-homomorphic encryption in intelligent agent based service level architecture. As a result the autonomous intelligent agents, customers and service providers can modify the SLA without decrypting the encrypted SLA and Sitekey authentication thereby achieving tamper protection. Furthermore this also ensures the multiparty involvement in dynamic SLA management in intelligent agent based service level architecture.

# INTRODUCTION

#### KEY WORDS

Service Level Agreement (SLA); tamper protection; dynamic SLA; data integrity; homomorphic encryption

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\*Corresponding Author Email: erodearvind@gmail.com Tel.: +91-9944323386 Mobile cloud computing has become an integral part of both small and large organizations day to day business life which in turn resulted in its explosive growth. According to a recent survey conducted by Forbes, nearly 75 percent of the business users are using mobile cloud platform in one way or another. To achieve compliance and trust between mobile cloud customers and service providers, a contractual agreement was established popularly known as Service Level Agreement (SLA).A SLA is a statement of obligations and expectations between a customer and service provider stating the services, target, restrictions, optional services and penalties and scope of availability. SLA management usually consists of three phases namely creation, operation and removal phase. In creation phase, service provider is discovered and SLA is defined and agreement is established between the service provider and customer.

During operation phase, SLA is monitored for service satisfaction and violation and in case of SLA violation, termination of SLA is carried out. If SLA is violated, then penalties are enforced during removal phase. A Static approach to SLA management is followed in almost all scenarios of mobile cloud computing. But static SLA often tends to wastage of resources of both user and provider.

In recent years, services provided by mobile cloud providers are inter dependent on each other i.e. some services relies on completion of the previous services task and resources are also reallocated based on completion of the previous services task. Hence there is increasing need for modifying SLA after creation phase continuing through operation phase. This gave rise to a solution that is dynamic SLA.A dynamic SLA is a renegotiated SLA which contains new terms and services after a proper SLA negotiation. These dynamic SLA thereby make an intelligent decision in making use of the resources effortlessly and efficiently.

Often this SLA management is entrusted with third party entities. Their main work is to monitor whether the Service Level Objectives stated in the SLA are met or violated and reported properly to the customer. But often there are issues of trust. To overcome this advantage, use of autonomous intelligent agent to manage SLA was proposed here.

But in this intelligent agent based architecture, SLA terms are modified and updated both by customers and service providers as needed giving rising to the question of protection against tampering. Henceforth in this paper, we propose a security framework model which follows homomorphic encryption to encrypt the SLA. Then the encrypted SLA is modified without decrypting the SLA while modifying the terms and only after successful renegotiations the modified terms are enforced in the original encrypted SLA after verifying the authenticity, authorization and access permission of the modifier making it more secure. Subsequently data protection of dynamic SLA is also achieved.

This paper is organized as follows. Section 2 deals with related works researched for this proposal and section 3 describes the modified SLA management for dynamic SLA with looping. Section 4 explains in brief about the homomorphic encryption and their mechanism is explained with a scenario for proper understanding. Then we propose the use of modified Dynamic SLA in IAIS architecture with tamper protection using homomorphic encryption in section 5. In section 5.5 and 5.6, we will discuss about the



performance evaluation and security analysis of or proposed methodology. Finally we conclude in Section 6.

# RELATED WORKS

The inclusion of SLA management in utility or cloud computing was supported by GRID and telecommunication systems for the purpose of standardizing the process between cloud consumers and cloud service providers [1].Inter cloud, a form of mobile cloud also followed the cloud framework for SLA managements which has gained importance due to Semi-Markov Decision Process (SMDP).[2]

SLA management is important for cloud service providers as they act as a trust material between cloud consumer and cloud service provider. Ruben Trapero et al [3] proposed Security Service Level Agreement secSLA which adopted various security control frameworks such as CCM [4] and RATAX [5]. The various phases of secSLA was implemented in SPECS [6]. The implemented SPECS project aimed at developing an framework for security SLA which supported the entire lifecycle of secSLA.

Although the various proposed framework for ensuring security have addresses many issues, there is an issue of trust between mobile CSP and users. To prevent this we are proposing the dynamic SLA management which encompasses seven phases which will be discussed below.

To ensure authentication and confidentiality in this framework, we proposed the use of Visual cryptography technique [7] to create a SITEKEY which will be explained in the consecutive sections. Also to ensure the tamper protection of SLA document we make use of semi-Homomorphic encryption [8] technique and propose a novel semi-homomorphic algorithm which was discussed in section 5.4

# DYNAMIC SLA MANAGEMENT

As discusses previously, Service Level Agreement is a critical component which ensures correct services are rendered and correct QoS parameters are established to meet the Service Level Objective. To enforce dynamic SLA management by the intelligent agents, we propose a change in the SLA management phases where we include a renegotiation phase in addition to the already existing three phases namely creation, operation, renegotiation and removal phases which is depicted in the [Fig. 1].

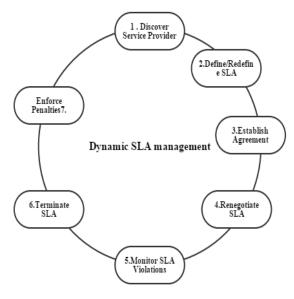


Fig. 1: Dynamic SLA management

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The method for dynamically modifying the SLA involves the following steps

- Discover Service provider based on the need of service by referring preferred service provider
   Define/Redefine SLA
  - 3. Mutual Agreement on the terms of SLA is established.
  - 4. Renegotiate SLA terms to include new services and go to step 2
  - 5. SLA violation is monitored
  - 6. SLA is terminated
  - 7. Penalties are enforced

Step 1, 2 and 3 involves the creation phase with step 4 forming the renegotiation phase and step 5 and 6 constitutes the operation phase and finally step 7 encompass the removal phase of modified dynamic SLA



management. The main difference between the static SLA management and dynamic SLA management is the inclusion of renegotiation phase.

# HOMOMORPHIC ENCRYPTION

Homomorphic encryption is a special kind of encryption which allows computation to be performed on the encrypted data which yields the same result as the computation performed on the original data. In simple words, it allows the multi users of an encrypted entity to modify the entity without disrupting the original entity. This scheme was first proposed by Gentry in [9]. As an application, let's say for banking, a user details will be stored in an encrypted form in an untrusted server and if the user wants to query and make minor modification to the details without the knowledge of decryption key, the homomorphic encryption is the suitable mechanism. This method was first developed from RSA which has a multiplicative homomorphic encryption but it was known then as privacy homomorphism. This was later developed into fully homomorphic encryption, partial homomorphic encryption and semi homomorphic encryption with applications varying from multi-party computation, secret sharing, electronic voting etc.

To support our proposal, we will be using semi homomorphic encryption scheme which supports multiparty computation. Because in our intelligent agent based SLA management, both the service provides and customers will modify the SLA terms after renegotiation. We will be making use of semi homomorphic encryption .We make use of a relaxed homomorphic encryption scheme which can recover the plaintext as long as the computed function cannot increase the size of the input too much.

# PROPOSED FRAMEWORK

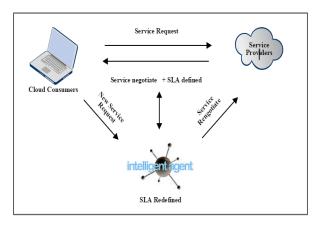
This paper proposes use of dynamic SLA in Intelligent Agent based architecture as an extension to the scheme proposed in [10, 11]. This proposed framework employs intelligent autonomous dynamic SLA management with tamper protection for SLA. Instead of entrusting a third party entity with SLA management, this framework makes use of an self-deployed intelligent agent for the following purposes namely SLA definition and agreement, SLA renegotiation, Redefining SLA and Encrypting SLA using semi homomorphic encryption, SLA violation, termination and Penalty enforcement.

#### SLA Definition and agreement

Intelligent agent is initialized in this phase before SLA negotiation begins. Then, the intelligent agent selects a suitable service provider from a possible list of service providers. Next steps "Define SLA" and "Agreement Establishing between Service Provide and Consumer" are dependent on each other. Then SLA terms and components are defined and commitment protocol, preferred query language are established. After negotiation on both sides, a mutual agreement is established.

#### **SLA Renegotiation**

To achieve efficient resource allocation of both users and service provider, we are using dynamic SLA which allows new service request to be added and SLA to be modified after renegotiation between two parties and SLA terms are redefined as needed which is shown in the [Fig. 2].



#### Fig. 2: Dynamic Intelligent agent based dynamic SLA management

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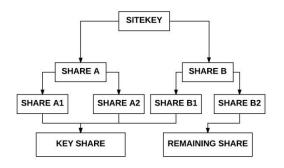
#### **SLA Redefinition**

Since we need to modify the SLA to encompass the new service request, care should be taken to prevent unnecessary modification to the SLA components and values.

Tamper Protection for Dynamic SLA



As previously mentioned, to protect against unnecessary and unauthorized modification of SLA, we propose a new tamper resistance SLA management which will be explained in the following section. Let us explain the concept with the following scenario. Suppose user A uses his/her smart card to make a transaction with user B and the smart card is tamper resistance, then the both user A and B cant non-repudiate the transaction which occurred between them. In the scenario above, tamper resistance is left to the third party in most cases whose trustworthiness cannot be verified who in turn causes the problems of integrity between User A and User B. To overcome such difficulties, we in our mobile cloud framework propose a tamper resistance SLA renegotiation. Both the cloud consumer and cloud service provider must share a SITEKEY which is generated using Visual cryptography and they must submit their Sitekey shares which when overlaid together must ensure the authenticity of both cloud consumer and cloud service provider as explained in the following [Fig. 3].



#### Fig. 3: Creation of site key authentication

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In the first level of security, two image shares are generated from the snapshot image by the cloud consumer and service provider namely Share A and Share B. In the next level, the Share A is encrypted again to generate two shares namely Share A1 and Share B2. Likewise, the Share B is encrypted again to generate two shares namely Share B1 and Share B2. Randomly, any three shares form (Share A1/A2/B1/B2) is combined to form the key image share and remaining share is kept aside as an image share. Key image share is shared with the cloud consumer over a secure communication channel. Then the remaining share is stored in the cloud service provider secure database. The above process is explained in the Fig.3. During SLA renegotiation phase, cloud consumer enters their username or customer id along with their image share to authenticate them to the cloud service provider. Then cloud service provider retrieves their share for the corresponding cloud consumer from their database using customer id.

Then the cloud consumer's key image share and cloud service provider's remaining image share retrieved from their database are stacked over one another to retrieve the original snapshot image or Sitekey. Then the SLA renegotiation takes place between the cloud service provider and Cloud consumer and the modified SLA is stored in the semi homomorphic encrypted method [11] to ensure additional security to tamper resistance dynamic SLA management between the cloud consumer and cloud service provider as explained in the [Fig. 4].

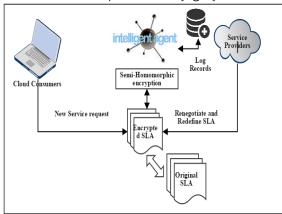


Fig. 4: Tamper Protection for SLA using semi homomorphic encryption

Algorithm for Semi-homomorphic encryption and Decryption

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Step 2: Choose 4 bit random integer X' Compute R0 = AB and R1 = AC + XX'

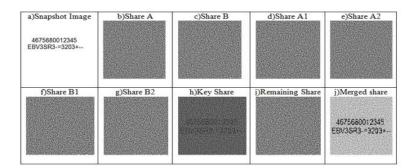
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Step 3 : Accept Number N from user by converting the SLA document into integers using ASCII encoding Step 4: R2 = [T1 R1] mod R0 Encryption Cipher Text C = [N + T2 R2] mod R0 (T1, T2 are a 4-bit random integer) Step 5: Decryption N = (C mod A) mod X

# **RESULTS AND PERFORMANCE EVALUATION**

Although achieving security is the main target of this proposal, the proposed system is simulated using Java and the following scenario was created wherein which the cloud consumers and cloud service provider used their Sitekey image to authenticate each other in turn using the following methodology. According to the proposed methodology, we create a snapshot image as in Fig 5(a). Later, we embedded the secret message in the snapshot image using the above proposed steganography method. Then we generated two image shares namely image share A and B using (2, 2) VCS as in [Fig. 5(b) and (c)] respectively. Furthermore, we generated two image shares namely image shares namely image share A1 and A2 using (2, 2) VCS from Share A as in [Fig. 5(d)] and [Fig. 5(e)] respectively. Likewise, we generated two more image shares namely image share B1 and B2 using (2, 2) VCS from Share B as in [Fig. 5(h)] and [Fig. (i)] respectively. Upon overlaying those image shares, we received the merged shares as in [Fig. 5(j)].



#### Fig. 5: Experimental results

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To include further Tamper protection, we propose the use of semi-homomorphic encryption for storing the SLA by implementing the proposed framework in AWS cloud. The process of implementing the Java module in AWS cloud was explained using the following [Fig. 6].

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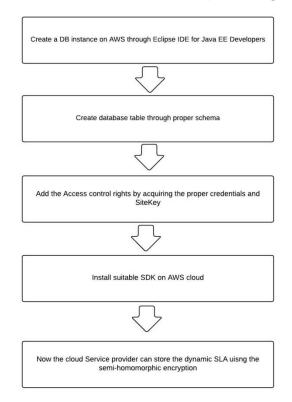


Fig. 6: Dynamic SLA encryption using semi Homomorphic encryption

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# SECURITY ANALYSIS

In this section, we are analyzing the security of our proposed solution against some security attacks to know their resisting quality, advantages, disadvantages and method extension.

#### Secret sharing

The proposed solution is implemented using hierarchical visual secret sharing scheme where the cloud consumer's key share and cloud service provider's share are both required to retrieve the secret image. The key share is kept by the cloud consumer and the second share is stored the Cloud service provider's database in a secure manner.

#### Man-in-the-middle attack

Since only one share is sent across secure communication channel, the man-in-the-middle attack will not succeed in obtaining the access. Adversely, if the share is intercepted by the intruders and the share is duplicated to generate fake share. If the intruders provide the fake share in the payment website which when stacked together with cloud service providers share may retrieve the original image but the cloud service provider will detect anomalies since the fake secret message decoded will not match the cloud consumer entered secret message stored in the cloud service providers secure database suing semi homomorphic encryption.

#### Security image attack

Security image attack is a special type of attack against SiteKey where the image and phrase column will be replaced with a maintenance message in the fake website which will look legitimate. This can be avoided only through proper awareness among users about this attack.

#### CONCLUSION

In this paper, we have overcome the difficulties of trustworthiness and integrity by the use of Visual cryptography schemes. Furthermore, it prevents the identity theft by malicious insiders and phishing attack by malicious outsides by the combined use of visual cryptography and semi-homomorphic encryption technique. It also limits the information shared between the cloud service provider and the cloud consumer. It provides protection for high profile users by the use of SiteKey authentication from spear phishing attacks and the modified SLA management for dynamic SLA with looping enables tamper protection against the dynamic SLA management.

#### CONFLICT OF INTEREST There is no conflict of interest.

#### **ACKNOWLEDGEMENTS** None

FINANCIAL DISCLOSURE None

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# ARTICLE ECHOCARDIOGRAPHIC EVALUATION OF PATIENTS WITH PERSISTENT ST SEGMENT ELEVATION AFTER SUCCESSFUL PRIMARY ANGIOPLASTY

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# ABSTRACT



**Introduction**: Percutaneous intervention[PCI]is an accepted method of reperfusion in patients with acute ST segment elevation myocardial infarction [STEMI]; resulting in better left ventricle function and increase in life expectancy. However, if PCI fail to completely recover ventricular function, mortality and left ventricular failure may increase. Establishing coronary blood flow in angiography is not always indicating proper cardiac circulation and cardiac events may occur later in life. There are many factors related to patient's outcomes after primary PCI. ST segment resolution is one this factors which is noninvasive and indicates reperfusion. However, the relation between ST segment changes and echocardiographic findings is not widely studied. The aim of present study is evaluation of electrocardiography [ECG] and echocardiography finding in post STEMI patients undergoing PCI. **Material and methods**: one hundred and seventy patients who had STEMI and underwent successful PCI were chosen by convenience sampling and enrolled in this study. After considering exclusion criteria, every patient had 12 lead ECG 60min, 90min, 120min, 1st day and 2 month after PCI. Also, a transthoracic echocardiography [TTE] was performed after PCI, and 2 more TTE were performed day after and 2 months after PCI. ST segment resolution was evaluated in every ECG and the results were compared with TTE findings. **Results**: Mean±SD of time duration between onset of symptoms and calling EMS [OSCE], door to balloon time [DTB], time duration between first medical staff visit and angioplasty[FMVA] were 103.4±56.63 min, 35.58±4.43 min and 60.58±4.43 min respectively. **Conclusion**: In-hospital and pre-hospital delays are two prominent findings in our study which were related to inappropriate ST segment resolution after successful PCI in STEMI patients. Also, patients with Anteroseptal and anteroseptolateral MI will show lesser ST segment resolution

#### INTRODUCTION

#### KEY WORDS Myocardial infarction; angioplasty; echocardiography

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\*Corresponding Author Email: panjalizb931@mums.ac.ir Tel.: +989155683036 Fax: +985138544504 increase from 1990 to 2013 despite of decrease in deaths attributed to epidemiologic changes[1]. By recent development diagnosing and managing cardiovascular disease, mortality rate from these disease is reducing in some regions such assome European countries which have reported median annual reduction of case fatality rate around 5%[2]. Myocardial infarction [MI] is a sever manifestation of cardiovascular disease. The incidence rate of MI in Iran is reported to be 73.3 per 100000 which is higher than some Arab countries in middle east [3]. Acute MI is divided into 2 main categories according to ST segment appearance on electrocardiography [ECG] as ST elevation MI [STEMI] and non ST elevation MI [NSTEMI]. The end feature of MI is myocyte necrosis and will lead to ventricular function disruption[4]. In patients with STEMI, complete total occlusion is usually seen and reperfusion in needed to be established with 60 to 90min[4]. Early results of successful angiography shows improved left ventricular function and increased patient's survival. While there are some non-invasive markers suggested for assessing reperfusion, these results are compared mostly with angiography results [5, 6]. Angiographic assessment is based on thrombolysis in myocardial infarction [TIMI] in infarct related artery. While primary angioplasty mostly lead to early and complete reperfusion; however, reopening of coronary vessels doesn't indicated successful reperfusion [5-7]. An angiographic snapshot of blood flow through the infracted myocardium will not guarantee later intact coronary microvasculature [8]. There are many imaging techniques available for detecting myocardial perfusion defects such as cardiac magnetic resonance imaging [CMR] and Echocardiography[9, 10]. However, there are less expensive and widely used techniques for determining reperfusion injury such as ECG is available which requires more attention of researchers[11]. Myocardial perfusion defects, which are predictable from ECG, are significantly different from angiographic findings of TIMI 3 in an infarct related artery. This fact challenges the concept that TIMI grade 3 flow is a sufficient indicator of reperfusion. Approximately one third of acute MI patients shows impaired micro vascular reperfusion after successful recanalization[11]. Persistent ST segment elevation shortly after successful recanalization is thought to be related with sustained trans-mural injury[11]. ST segment monitoring after primary PCI is a simple and validated method for assessing adequate myocardial perfusion. The resolution is correlated with restoration of blood flow and myocyte function. As well as determining reperfusion, ST resolution is related to mortality rate of patients[12]. Also, ST segment resolution is thought to be related with ejection fraction but not widely studied. Recently, Hallen et al. reported that left ventricular ejection fraction is only related to sum ST segment deviation resolution in 90min after PCI. [13]. ST segment resolution is related to cardiac ejection fraction in different times. Present research tried to find any

Cardiovascular diseases are major cause of mortality word wide. The mortality rate has showed 40.8%



relation between ST segment changes after successful PCI of STEMI patients and their echocardiographic features as an indicator of cardiac global function. The aim of this study is Echocardiographic evaluation of patients with persistent ST segment elevation after successful primary angioplasty.

# MATERIAL AND METHODS

Present case control study was approved by Mashhad University of Medical Sciences research and ethic committee and conducted on patients who were referred to cardiology department of Ghaem hospital in Mashhad, Iran. Patients with the impression of STEMI were chosen by convenience sampling. Those who had cardiac pain for more than 12 hours before admission, had severe renal insufficiency, their angioplasty results were other than TIMI 3, had cardiopulmonary resuscitation before angioplasty, had history of open heart surgery or angioplasty, previous history of MI, heart failure and severe concomitant valvular disease were excluded from this study. After performing a transthoracic echocardiography by a cardiologist, every patient underwent PCI and infarct related artery flow was evaluated.Response to PCI was evaluated by ECG of 60min, 90min, 120min, one day and 2 months after PCI.No response was considered as depression lower than 30%. Also, participants underwent transthoracic echocardiography before hospital discharge and 2 months after.

Characteristics of participants will be presented using descriptive statistics including measures of central tendency, frequency and distribution in the form of appropriate tables and figures. Then, cardiac function and ejection fraction will be presented as continuous variable using mean and standard deviation. The Chi-square test will be used for the statistical analysis for categorical variables. The student t-test will be used for continuous variables in case of normal distribution while the Mann-Whitney test will be used for comparison of non-normally distributed variables.

# RESULTS

One hundred and seventy patients [mean±SD:49.96±3.16 years- ranging between 35 to 65 years old] who have successfully underwent angiography were enrolled in present study. Most of the patients were male [52.4%]. Mean±SD of low density lipoprotein [LDL], cholesterol, triglyceride and high density lipoprotein [HDL] level were 121.14±18.53 mg/dl, 196.4±20.01 mg/dl, 190.06±18.58mg/dl and 37.26±6.99 mg/dl respectively. Other descriptive statistics of discrete demographic variables are summarized in [Fig. 1]. Mean±SD of time duration between onset of symptoms and calling EMS [OSCE], door to balloon time [DTB], time duration between first medical staff visit and angioplasty [FMVA] were 114.4±56.63 min, 35.58±4.43 min and 60.58±4.43 min respectively. Mean ejection fraction and systolic volumes were showed an increasing pattern during the study period [Table 1].Delayed referral in different stages were significantly related to ejection fraction and systolic volume which is summarized in [Table 2]. Hypertensive patients had lower systolic volume [P=0.000] and patients with hypercholesterolemia had higher systolic volume at the time of admission and at the end of first day [P=0.024]. Also, Inferior and inferoRV posterior MI had significantly higher ejection fractions at the end of first day and second month [p=0.000 for both].Meaningful relations between ST segment resolution indifferent stages and echocardiographic findings are summarized in [Table 3].

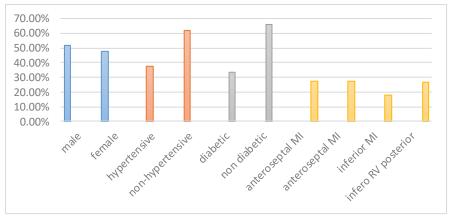


Fig. 1: Descriptive statistics of discrete demographic variables [MI: myocardial infarction, RV: right ventricle].



#### Table 1: Patient's descriptive features

Age	49.92	±	0.242
HDL	37.34	±	0.53
LDL	119.38	±	1.46
TG	190.45	±	1.44
cholesterol	194.81	±	1.51
SYMtoEMS	133.15	±	4.02
DtoDtime	36.15	±	0.35
FMCtoDtime	61.15	±	0.35

Table 2: Patient's echocardiographic features

		ESV	EF
age	Pearson Correlation	029	018
	p-value	.590	.745
HTN	Pearson Correlation	.037	.089
	p-value	.499	.101
High LDL	Pearson Correlation	.037	.026
	p-value	.498	.634
High TG	Pearson Correlation	032	060
	p-value	.553	.272
hypercholesteolemy	Pearson Correlation	.026	.012
	p-value	.627	.827
Time from symptom	Pearson Correlation	156**	313
onset to callEMS	p-value	.004	.000
Door to device time	Pearson Correlation	071	060
	p-value	.194	.270
First medical contact	Pearson Correlation	071	060
to device time	p-value	.194	.270

# Table 3: Meaningful relation between ST segment resolution and echocardiographic variables

					p-value <sup>*</sup>				p-value <sup>*</sup>
Sex	Man	9.79	±	.206	1.000	8.33	±	.202	0.803
	Woman	9.89	±	.145		8.65	±	.194	
DM	Yes	9.68	±	.155	0.875	7.78	±	.253	0.002

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	NI	0.00		470		0.04		101	
	No	9.92	±	.176		8.84	±	.164	
HTN	Yes	9.73	±	.27	1.000	8.19	±	.232	0.74
	No	9.91	±	.123		8.67	±	.175	
MI	Anteroseptal	9.94	±	.205	0.989	6.51	±	.212	<0.001
	Anteroseptolateral	9.92	±	.194		8.30	±	.253	
	Inferior	9.49	±	.477		10.38	±	.298	
	Inferorvposterior	9.89	±	.208		9.43	±	.201	

Ejection fraction [EF], systolic volume [SV], transthoracic echocardiography [TTE].

# DISCUSSION

Successful PCI in most cases is not the end point of complete reperfusion. While there are other imaging techniques introduced for assessing reperfusion, other noninvasive and widely available monitoring techniques such as 12 lead ECG is considered to be helpful. ST segment resolution after successful PCI is an indicator of cardiac function in future which takes effects from other modalities such as possible delays in establishing reperfusion and previous medical condition.

Over past few years, still there is approximately a 2 hours duration between initiation STEMI symptoms and seeking medical care. This duration is shorter for patients who are directly transferred to hospital by emergency medical services[EMS][5]. This delayed referral is mostly due to patient's previous bias from a heart attack. Most of patients think that heart attack is dramatically characterized by sever crushing left sided chest pain[5]. Considering the pain a self-limited or not serious, attempting for self-treatment and not knowing the importance of immediate action and calling EMS are other possible reasons for late referral[5]. According to the latest American heart association guideline for management of patients with STEMI, door to balloon time lesser than 90min and door to needle time lesser than 30min is recommended for centers with capability of performing PCI[5]. Also, first-medical-contact-to-balloon optimal time is reported to be less than 90min[14]. The multicenter study by Kassaian et al. was the first study on management of patients with ACS syndrome in our country who followed their patients for one year [15]. Composition of Iranian patients with ACS according to their ACS type is most likely similar to developed European countries and different from developing countries of Africa and Middle East [15]. According to their study, Iranian physicians are treating ACS patients highly in adherence with guideline recommendation for in-hospital management but underuse dual antiplatelet therapy at discharge [15]. Iranian patients with STEMI are more likely to be presented to hospital lately. However, in hospital reperfusion therapy of these patients is quite appropriate [15]. In our study the mean time between initiation of symptoms and calling EMS was lesser than Kassaian et al. study. Also, the mean door needle and door to balloon time was 45.6 and 82.9 min respectively in Kassaian et al. study which is greater than our study [15]. Uncertainty of diagnosis, presence of other medical life threatening conditions or delays in filling and understanding the informed contests are possible factors increasing in hospital delays [5]. While reducing in-hospital delays seem difficult, educating the patients and their families will be the first and most important step in reducing the pre-hospital delay. By introducing all possible signs of heart attack, even if patients didn't take their symptoms seriously, their family members will encourage them for seeking prompt medical care.

There are various techniques reported for evaluation of cardiac reperfusion and remodeling. Farag et al. study evaluated cardiac remodeling after PCI by using echocardiography in 232 patients with STEMI. They defined cardiac remodeling as 20% increase in left ventricle end diastolic volume after six months. Symptoms to balloon time and symptoms to door time were both significant predictor of left ventricle remodeling. This findings highlights the lack of awareness about myocardial infarction symptoms and the necessity of in-time referral[16].

CMR is new imaging technique which will provide important information such as infarct size, micro vascular obstruction, myocardial salvage index and left ventricle ejection fraction which are all incremental prognostic value in patients with STEMI [10]. Nguyen et al. found that infarct scar site is an important prognostic factor for diastolic remodeling after STEMI. They evaluated their patients by serial



transthoracic echocardiography and cardiac magnetic resonance imaging [CMR] at median of 4 and 55 days after STEMI. Larger infarct scar site following STEMI results in adverse diastolic remodeling and emphasis a larger group of at risk patients. Their finding revealed an important link between myocardial damage and serially evaluated diastolic function [17].

Lenz et al. introduced real time myocardial perfusion echocardiography as a noninvasive bedside technique for evaluation of major adverse cardiac events in patients with STEMI [9]. They compared CMR infarct mass and real time myocardial perfusion echocardiography in 27 patients after early reperfusion. Myocardial blood flow and infarct mass after adjusting risk factors were independent predictors of major cardiac events [9].

Despite of CMR and real time myocardial perfusion echocardiography, 12 lead ECG is still an inexpensive and easily applicable tool for assessing cardiac condition after PCI. ST segments changes are also used for evaluation of myocardial reperfusion. These changes can be considered as a reliable non-invasive marker for cardiac reperfusion [6, 7]. Sanati et al. reported that time from onset of symptoms to initiation of catheterization is significantly related to magnitude of ST segment resolution[18]. Delay in performing PCI will result in higher rate of reperfusion failure and lead to adverse clinical outcome. Their median of presentation time were 3,5 and 5.5 hours for patients with complete, partial and no resolution respectively. They also concluded that involvement of left anterior descending artery is associated with poorer myocardial reperfusion after successful PCI. Also in our study, Anteroseptal and anteroseptolateral MI showed poorer ST segment resolution. More degree of ST resolution will be achieved if right coronary artery is treated as culprit artery[18]. They explained this finding according the ability of right ventricle in handling ischemia[18]. Jiecheng et al. study evaluated long term effect of early and subsequent no-reflow phenomenon after PCI in 420 patients with STEMI. They reported that in early no-reflow patients despite of severe baseline angiographic and clinical characteristics, poorer long term prognosis, lower left ventricular ejection fraction and larger left ventricular end diastolic diameter is prominent[19].

# CONCLUSION

In-hospital and pre-hospital delays are two prominent findings in our study which were related to inappropriate ST segment resolution and lower ejection fraction after successful primary PCI in STEMI patients. Also, patients with Anteroseptal and anteroseptolateral MI will show lesser ST segment resolution.

CONFLICT OF INTEREST There is no conflict of interest.

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None FINANCIAL DISCLOSURE None

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# ARTICLE VISION BASED GESTURE RECOGNITION: A COMPREHENSIVE **STUDY**

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# ABSTRACT



Computer vision involves identifying the behavior, interpreting background scene and understanding the scenario much similar to the manner in which human brain thinks and reacts. This intelligence in tuning the computer to understand objects under study and their behavior is highly essential across various domains such as security, health care, medical science, educational systems, etc., . As part of this work, a comprehensive study of various works done towards hand and face based gesture recognition has been listed and the concepts and technical aspects used behind have been compared. Also, an architecture for designing a vision based intelligence system which has the intelligence to understand the situation or scene and react to the same is proposed.

# INTRODUCTION

# **KEY WORDS**

Gesture Recognition; Hand Gesture; Face Gestures; Computer Vision; GCAIS

An intelligence system essentially tracks the entire activities which include actions, behaviors, interactions and happenings. But, the potential of this system can be maximized by inducing some intelligence and training the system to be context aware and capable of making decisions and in turn perform a sequence of alerting mechanisms. This process of making the system intelligent is easier said than implementable. Multiple steps are involved in acquiring this intelligence which starts with preprocessing of images to capture the scene or scenario at different levels of abstraction, detecting the objects of interest and tracking the same in the given scene or sequence, training the system to understand multiple contexts and able to apply the same in real time and make educated decisions (predictions) and perform corresponding actions. Detection and tracking of objects involves studying the motion or movement of the object. The features of motion of object such as movement pattern, trajectory, inter-object distance etc., [1] are highly significant in the study of motion of object. In general, any intelligent system requires subsystems to analyze interactions, activities, context understanding and detecting objects as shown in [Fig.1].

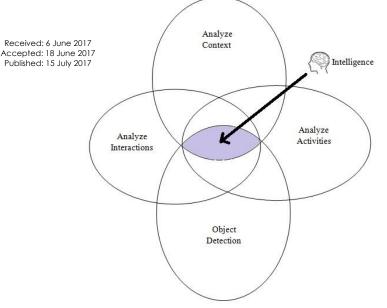


Fig.1: Subsystems to acquire intelligence.

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It can be seen that complete intelligence is acquired when all the four subsystems go hand in hand. Developing an intelligent surveillance system is application specific and requires multiple templates that represent the object's behavior. These predefined templates are in other words termed as priori. However, not all characteristics of the object can be determined using the priori information and proper training data for the system plays a vital role in determining the accuracy of intelligence acquired.

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# RECENT WORK DONE TOWARDS VISION BASED GESTURE RECOGNITION

The spectrum of vision based applications include analysis of complex scientific data which is used across multiple domains such as medical science, military applications, surveillance, health care, educational developments etc,. [Table 1] shows some of the recent work performed towards vision based gesture recognition based on face and hand gestures.

Table 1: Recent work related to vision based gesture recognition

Reference	Work done	Feature extractor	Classifier used	Database
number [2]	Human motion classification using 2D stick-model matching regression coefficients	Polynomial Regression Data Fit	Bayes, Lazy, Function, Meta, Misc, Rules and Trees classifiers	Own Database
[3]	An Architecture for Personality- Based, Nonverbal Behavior in Affective Virtual Humanoid Character	3D Hybrid model which considers non-verbal behavior	Rule Based	Own Database
[4]	Gaze patterns during scene processing in typical adults and adults with autism spectrum disorders	Statistical methods	Rule Based	Medical Database
[5]	Walking behavior change detector for a "smart" walker	Gait Analysis	Binary ANN cascade classifiers	Own Database
[6]	Framework for Traffic Personnel Gesture Recognition	Cumulative Block Intensity Vector (CBIV) of n- frame cumulative difference	Support Vector Machine (SVM), Decision Tree and Random Forests (RF)	Own Database
[7]	Gait-Based Emotion Detection of Children with Autism Spectrum Disorders: A Preliminary Investigation Flexible human action recognition in depth video	Gait Analysis	Rule Based	Own Database
[8]	sequences using masked joint trajectories	3D Hybrid models	(SVM), Hidden Markov Model (HMV)	Own Database
[9]	Detecting & interpreting self- manipulating hand movements for student's affect prediction	Sobel-operated local binary pattern (SLBP)	Three-layered Bayesian network (BN)	Own Database
[10]	Framework of Single-Frame Face Superresolution Across Head Pose, Facial Expression, and Illumination Variations	face hallucination, Face	Pattern Matching	CAS-PEAL-R1 Face Database and CMU PIE database
[11]	Real-World and Rapid Face Recognition Toward Pose and xpression Variations via Feature Library Matrix	3D face reconstruction, feature library matrix	Support Vector Machines	Face Recognition Technology (FERET), Carnegie Mellon University- Pose, Illumination, and Expression (CMU-PIE), and Labeled Faces in the Wild (LFW) face databases
[]	Recognition of Face Expressions using Local			
[12]	Principal Texture Pattern	LPTP, LBP	Support Vector Machines	CK and JAFFE
[13]	Hand Body Language Gesture Recognition Based on Signals From Specialized Glove and Machine Learning Algorithms	Glove Based	Probabilistic neural network, Support Vector Machine, and k-nearest neighbors algorithm	Real time
[14]	Nonparametric Feature Matching Based Conditional Random Fields for Gesture Recognition from Multi-Modal Video	Probabilistic Model	Structured Support Vector Machines, Naïve Bayes	MSRC-12 Dataset
[15]	Direction Estimation for Pedestrian Monitoring System in Smart Cities: An HMM Based Approach	2D camera model	Hidden Markov Model	CASIA Dataset A, CASIA Dataset B and NITR Conscious Walk Dataset (Own Dataset)
[13]	Vision-based position computation from in-vehicle video log images for road sign inventory	3D based Analytical Model of Road Sign Positioning (AM- RSP) using first order approximation	Mean and deviation of 3D position errors of road sign are computed to present both the position measurements and	



uncertainties

#### TAXONOMY OF GESTURES

The taxonomy of gestures can be drawn based on various considerations such human computer interaction based gesturing, gesture mappings, physical characteristics etc. [Fig.2] shows the taxonomy based on Human Computer Interaction based gesture classification.

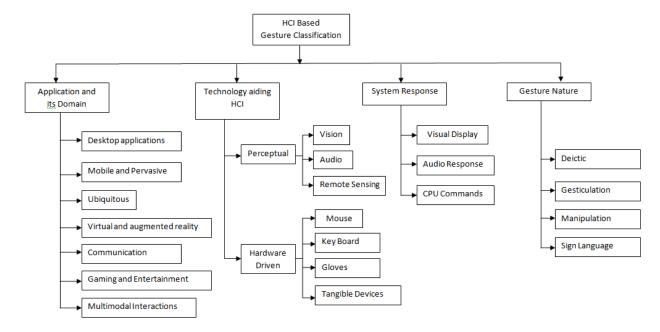
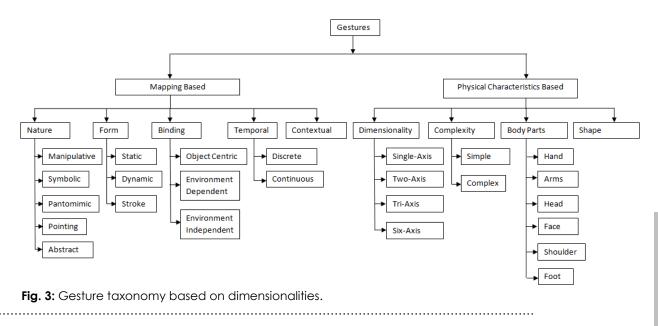


Fig. 2: Gesture taxonomy based on human computer interaction.

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Gesture taxonomy can also be classified based on the dimensions under consideration. These include various dimensional aspects which map the gestures to the nature of activity performed, whether the form is static or dynamic, whether the contextual parameters are taken into consideration and whether the data available is discrete or continuous in nature and so on. [Fig.3] shows the classification of gestures based on the dimensional aspects.

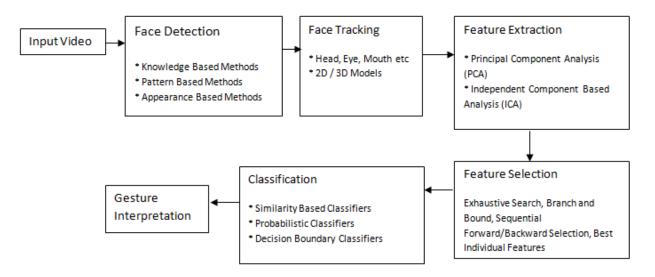


# FACE GESTURE RECOGNITION TECHNIQUES

Face gesture recognition requires the face to be detected first and then the detected face is tracked. This is followed by extracting and selecting the salient features that will be required for determining the facial COMPUTER SCIENCE



gestures.[ Fig. 4] shows the sequential flow of these steps along with the algorithms used across each step.



#### Fig. 4: Phases involved in facial gesture interpretation along with algorithms.

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Based on the requirement and the necessity of the application, the respective algorithms are chosen across each phase. [Table 2] lists the various algorithms used across each phase of gesture interpretation along with its salient features.

Module	Algorithm	Sub-types if any	Significance
	Knowledge Based Methods		Rule based Ruled-based methods that encode our knowledge of human faces
	Feature Invariant Methods		Algorithms that try to find invariant features of a face despite it's angle or position.
Face Detection	Template Matching Methods		These algorithms compare input images with stored patterns of faces or features.
	Appearance Based Methods (SVM, Naïve Bayes, Nueral Network Based etc)		A template matching method whose pattern database is learnt from a set of training images
	Individual Feature Tracking (Head, eye, mouth etc,.)		Certain features tracked specifically
Face Tracking	2D / 3D Models		2D models track face and output is shown in 2D image space. In 3D models pose and orientation variations are also considered
	Principal Component Analysis (PCA)		Eigenvector-based, linear map
	Kernel PCA		Eigenvector-based , non-linear map, uses kernel methods
	Weighted PCA Linear Discriminant		PCA using weighted coefficients Eigenvector-based, supervised
	Analysis (LDA)		linear map
	Semi-supervised Discriminant Analysis (SDA)		Semi-supervised adaptation of LDA
Feature Extraction	Independent Component Analysis (ICA)		Linear map, separates non- Gaussian distributed features
	Neural Network based methods		Diverse neural networks using PCA, etc
	Multidimensional Scaling (MDS)		Nonlinear map, sample size limited, noise sensitive.
	Self-organizing map (SOM)		Nonlinear, based on a grid of neurons in the feature space
	Active Shape Models (ASM)		Statistical method, searches boundaries
	Active Appearance Models		Evolution of ASM, uses shape and

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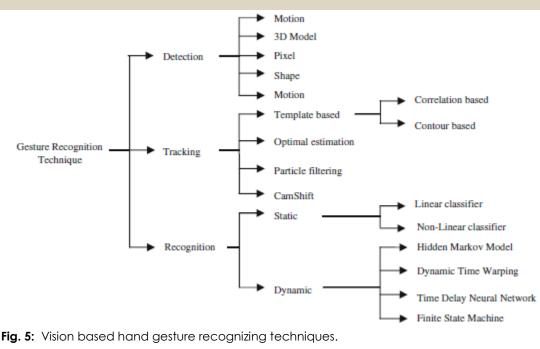


		[	1
	(AAM)		texture
	Gabor wavelet transforms		Biologically motivated, linear filter
	Discrete Cosine Transform (DCT)		Linear function, Fourier-related transform, usually used 2D-DCT
	MMSD, SMSD		Methods using maximum scatter difference criterion.
	Exhaustive search		Evaluate all possible subsets of features. Optimal, but too complex.
	Branch and bound		Can be optimal. Complexity of max O(2^n).
Feature Selection	Best individual features		Evaluate and select features individually. Simple but noit very effective.
	Sequential Forward Selection (SFS)		Evaluate growing feature sets (starts with best feature). Retained features cannot be discarded.
	Sequential Backward Selection (SBS)		Evaluate shrinking feature sets (starts with all the features). Deleted features cannot be revaluated.
		Template Matching	Assign sample to most similar template. Templates must be normalized
		Nearest Mean	Assign pattern to nearest class mean
	Similarity Based Classifiers	K-NN	Like 1-NN, but assign to the majority of k nearest patterns.
		Vector Quantization Methods	Assign pattern to nearest centroid. There are various learning methods
		Self Organizing Maps	Assign pattern to nearest node, then update nodes pulling them closer to input pattern
Classifiers	Probabilistic Classifiers	Bayesian	Assign pattern to the class with the highest estimated posterior probability.
		Logistic Classifier	Predicts probability using logistic curve method
		Fisher Linear Discriminant (FLD)	Linear classifier. Can use MSE optimization
		Binary Decision Tree	Nodes are features. Can use FLD. Could need pruning
	Decision Boundary Classifiers	Perceptron	Iterative optimization of a classifier (e.g. FLD)
		Radial Basis Network	Optimization of a Multi-layer perceptron. One layer at least uses Gaussian transfer functions.
		Support Vector Machines	Maximizes margin between two classes.

# HAND GESTURE RECOGNITION TECHNIQUES

Hand gesture recognition techniques involve detecting the hand movements, tracking the same and then recognize the respective gestures. As suggested by Rautaray S.S. et.al., [17] [Fig. 5] shows the taxonomy for various hand gesture recognizing techniques.

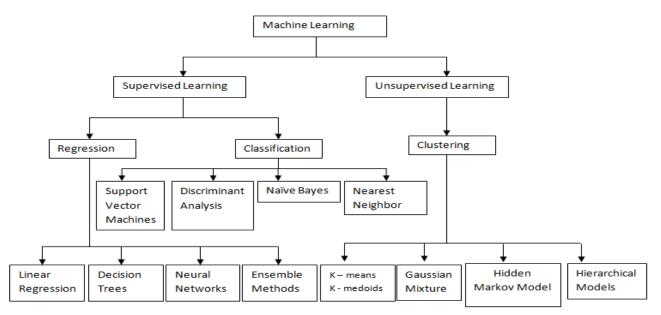




# TAXONOMY OF MACHINE LEARNING TECHNIQUES FOR GESTURE

#### RECOGNITION

Machine learning involves studying how to automatically learn to make accurate predictions based on the past observations. [Fig.6] shows the taxonomy of machine learning techniques.



#### Fig. 6: Machine learning techniques for gesture recognition

..... In general machine learning techniques can be broadly classified into Supervised and Unsupervised learning. Supervised learning involves developing a predictive model based on both input given to the system and output obtained. Unsupervised learning involves discovering an internal representation from the input data alone. These machine learning techniques are selected according to the nature of the input and the system requirements. [Table 3] lists the merits and demerits of various machine learning techniques.

Table 3: Merits and demerits of different machine learning techniques

Machine learning technique	Advantages	Disadvantages
	Simple	Applicable for simple
	Consistent Result	applications only
1R (1 Rule)	Handles Missing Data	Susceptible to over fitting

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		More assumptions
		Slow results due to redundant
Naïve Bayes	Missing values neglected	attributes
		Complex when compared with
Bayes Network classifier	Less Assumptions	Naïve Bayes
	Easily Understandable	Mismatch exists between
	Works well with continuous numeric	assumed and actual
Statistical Distributions	attributes	distributions
Perception	Simple and can handle multiple functions	Can handle linear functions only
		Difficulty in explaining
		classification rules
		Requires large input for effective
Back Propagation	Handles non-linear functions	learning / training
		An attribute with few values can
		reduce the result accuracy
Divide and Oceanies	Description of the second state of the second state	Replicated sub-tree problem
Divide and Conquer	Provides visual representation for rules	occurs
		Difficult to depict exact structure
	Lindler both linear and you linear date	to user
Constin Algorithms	Handles both linear and non-linear data	Can sometimes be slow to arrive
Genetic Algorithms	Seeks global maximization	at a good result
		Determining appropriate weights for attributes is difficult
	Adding now instances increase learning	
Instance Based	Adding new instances increase learning	Time consuming for large data
Installe Daseu	Simple to implement	sets

# ARCHITECTURE OF GESTURE BASED CONTEXT AWARE INTELLIGENT SYSTEM (GCAIS)

Based on the knowledge acquired by understanding the taxonomies of the various means for studying face and hand gestures, an architecture for gesture based context aware intelligent system is proposed as shown in [Fig 7]. The input to this gesture-based context aware intelligent system may be a video sequence or a live video stream. From the input video, the face and hand portions are detected by the system. This is followed by a series of pre-processing activities that include normalization, thresholding, image resizing, cropping and noise removal. After this, the refined contents are analyzed. The facial reactions are analyzed and the gesture is studied. Similarly the hand movements are analyzed and the hand gestures are identified. Using these gestures, the computational intelligence is acquired by applying various options like feature matching, machine learning techniques, pattern recognition concepts etc.

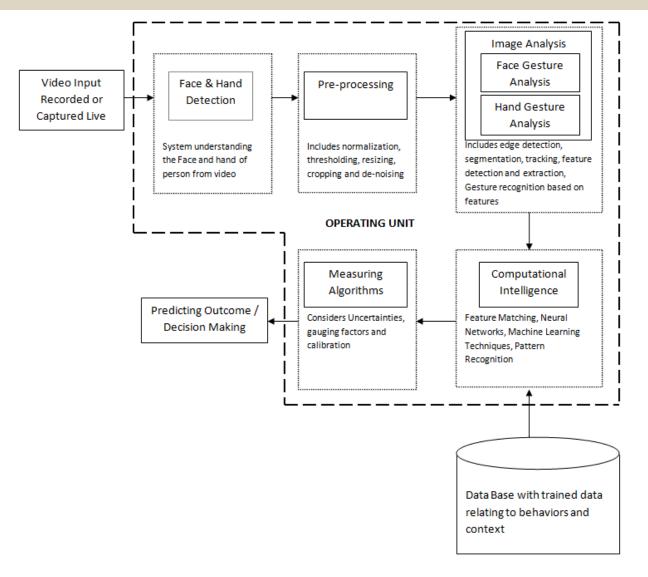
While computing intelligence, the analyzed gestures are compared with the trained data related to gestures which is available in the database. Similarly, the context in video is compared against the contextual patterns available in the database. The relevancy of gestures is mapped to the context to study the contextual behavior. The computed intelligence is subjected to various measuring algorithms which takes in order to consideration various uncertainties, calibration and gauging factors. Based on all these aspects the outcome is predicted or a decision making is performed. It can be seen from the above architecture that acquiring computational intelligence through various machine learning algorithms plays a significant role in determining the prediction or decision making.

#### RESEARCH CHALLENGES TOWARDS GESTURE RECOGNITION

According to T.Fang et al. [18], the major challenges faced in Facial Emotion Recognition are Database Challenges, Algorithm Improvement and availability of standardized protocols. In addition, as stated by Zeng et al. [19] another important challenge for the system is to be context aware while making gesture interpretations. This is again an unexplored area in Gesture recognition. Also, the limitations in the availability of hardware components like sensors make it difficult to acquire proper data to prepare well trained datasets [20]. Another key challenge to gesture interpretation is that a same expression may be displayed at different intensity levels [21][22]. It is highly essential for a Gesture based intelligence system to address these challenges while interpreting these gestures.

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#### Fig. 7: Architecture of GCAIS

# CONCLUSION AND FUTURE WORK

A comprehensive survey of the various techniques, methods and algorithms involved in computer vision based face and hand gesture recognition was carried out and the respective taxonomies were studied. This included study of various techniques involved in face and hand gesture recognition. Also, architecture for a context aware gesture based intelligence system was proposed. Future work will involve implementing the GCAIS architecture using desirable techniques and quantize the results using suitable metrics.

#### CONFLICT OF INTEREST

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None

#### ACKNOWLEDGEMENTS

None

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ARTICLE



# **QOS-AWARE CLOUD SERVICE SELECTION IN DYNAMIC** PERVASIVE ENVIRONMENTS

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# ABSTRACT



Most of the web service selection methods don't account for conflicting objectives. These work by combining the objective function into a single objective function by scalarization. When the value of weights is, not given and fixed weights are used, this can lead to problems. In this paper, modified reinforcement learning algorithms are proposed which are used to derive Single policy and multiple policy solutions respectively and applies the concept of multi-agent Reinforcement learning and value iteration respectively in the original algorithm which uses fixed weights, our algorithm modifies the weights in order to achieve output. Our proposal algorithm dynamically updates the weight for the objectives functions in the value function vector to achieve accuracy especially for large no. of states (value function approximation). Our modified algorithm uses the combination of multi objectives reinforcement learning with value function approximation to achieve better results. We are formulating the problem as a multi objectives partially observable Markov Decision Model problem description. In pervasive environment, such technique can prove to be useful for selecting web services. Examples of such services are E- Governance applications.

# INTRODUCTION

**KEY WORDS** cloud computing, web service selection,reinforcement learning

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the form of ontology of the domain. Selecting web services out of a set of web services in order to create a composite web services is a complex task. When selecting web services from the set, certain QoS parameters have to be considered,

A large number of services are being offered on mobile today like E Governance application. A large amount of study has been undertaken to examine web service composition in dynamic pervasive

environments l.e. which are aware of the environment and change their behavior accordingly (content

aware).Web service composition engine takes in data which mostly consists of content sensitive data in

QoS-aware web service selection has been widely researched in areas of service oriented Architecture (SOA) and service oriented computing (SOC)[1]. All the existing approaches deals with evaluating objective functions of QoS parameters by combining them into a single objective function.

More-over, they give solutions which are not optimal. We propose two hybrid algorithms which formulate the web service selection as a multi-objective optimization problem .This involves simultaneous optimization of multiple objectives [1].

We have used 5 parameters which are mentioned in the problem description some of them are conflicting objectives like availability and response which are both positive when one has to be maximum and one has to be minimum in response to wrong inputs.

Availability= Q availability [29]

this is known as QoS aware service composition.

Response time =  $[100*(exp(-r^2)*exp(-r+0.1r))/(1+exp(-r+0.1r))]$  [29]

In single policy algorithm, weight is assigned by us whose purpose is to create a bias between preference of services and it is updated to maximize the LMS (Least mean square error) between them.

The problem of selecting web services has been studied in great detail. Various Techniques have been applied to selection of web services such as mixed integer Programming which formulates the problem as a multi-objective mixed integer programming problem [1]. In mixed integer linear programming, all variables do not belong to integer group but only a few.

Another formulation of the web service composition problem is the knapsack problem which is multiple choice and multi-dimensional (MMKP). In MMKP,

Weight= (w1,w2,w3,...,wd) Capacity = (c1,c2,c3,...,cd) [26] The aim is to maximize the ratio of sum of value of items in the knapsack to the sum of weights in such dimension of the knapsack. [26]. Another formulation uses an immune binary PSO (IDIPSO) is proposed.

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IDIPSO uses immune concepts and methods with PSO to solve the web service composition problem. [5] The web service composition problem is formulated as multi-objective ant colony optimization and is solved using multi objective ant colony optimization. [6]

Petri net based algebra is also used to model web service composition process related work. [27]This algebra is expressive enough to capture the semantics of complex web service composition. [27] Another formulation of web service composition uses the LIAC algorithm to construct the service path [14]. Web service composition has applications in pervasive computing.

#### **Related Work**

Wang et al [1] proposed a method to select a web service from a set of web service using mixed integer programming. Confora et al [1] proposed a method to select a web service from a set of web service using GA (genetic algorithm). Kiu and Lin [1] proposed a method to select a web service from a set of web service by formulating it as a knapsack problem which is both multiple choice and multi- dimensional.

The problem of selecting a web service from a set of web service can be solved using non-linear techniques as proposed by Grossmann [3]. In (Ran 2003) the main idea is to incorporate the QoS awareness into the UDDI registries as to facilitate QoS aware service discovery.

IDIPSO uses immune concepts and methods with PSO to solve the web service composition problem .[5] The problem of selecting web service from a set of web service can be solved by using multi-objective ant colony optimization.[5] The problem of selecting web service from a set of web services can be solved using elitist MOEA ,Zitzler's and Thielem's SPFA [6], Knowles and corne's Pareto-archived PAES [7] and Rudolph elitist GA [8]. The problem of selecting a web service from a set of web service can be solved by using Petri nets as proposed by Hamadi and Benatallah [9].

The problem can also be solved using Maxmilien and Singh's [10] approach which is based on learning based on historical information but without explanation.[11] and PICO as proposed by kalasapur et al [11] which solves the problem using graph theory and the LIAC algorithm as proposed by Raman and Katz [12].

Various middleware has been proposed such as spider net by Gu et al to solve the above problem. [15] the problem of selecting web services from a set of web services is a pervasive involvement has been studied by Sonia, Nicolas, Valerie [13].Application of the above problem to business process has been proposed by F. Koshkina And M. Von Bruegel [16].In software agent oriented approach solve the above problem has been proposed by Za aria maonnar, sorayukocicedic and hamdii vahyavi [17].

#### Objective function for QoS parameters for web services

We are using Response time, availability, throughput, successability, reliability as our objectives.

Response Time- Response time is the overall time required to complete a server request. [29]

Q (Response time) =  $[100*(exp(-r^2)*exp(-r+0.1r))/(1+exp(-r+0.1r))]$  [29]

Availability - It is a factor which measures whether the server is up after selection [29]

Throughput - It is referred as total number of completed transactions by a web service over a time period

Successability – It is defined as the extent to which web services yield successful results over request messages.

Reliability - It is ability of a web service to perform well over a given time span [29].

Most of the web service selection methods don't account for conflicting objectives. These work by combining the objective function into a single objective function by scalarization. Eg- when there are conflicting inputs to a web service i.e. parameters conflicts, the availability is high which is to be maximized and the response time is also high which is to be minimized.

#### Markov decision process

We formulate the problem as an MOMDP-WSC (Multi Objective Markov Decision Process Web Service Composition). This is applied to multi objective optimization in partially observable environment. MDPs has applications in optimization problems and reinforcement learning and dynamic programming. MDPs is a random process .An MDPs consists of transition diagram between states and a reward function R(s, s') after moving from one state to another. [28]

An MOMDP-WSC is defined as a 6-tuple MOMDP-WSC-[(s), Si, St, A, P, R]

S- Set of states agents can be in



Si- state from which agents starts
St – set of terminals
A-Set of action (web services) that can be executed by the agent (web services composition engine)
P is the probability of going from one state to another by choosing an action.

Output of MOMDP-WSC is an optimal decision policy

π: S → A

Each policy of MOMDP-WSC can define a single workflow, and therefore, the task of our service composition model is to identify the set of Pareto optimal policies that gives the best trade-offs among multiple QoS criteria. [1]

Multi objective reinforcement learning for service composition [1]

The goal of MORL is to require the set of Pareto- Optimal Policies in MOMDP model [1]

Moreover,  $[V(s) = (V_1^{\pi} (s), V_2^{\pi}(s), ..., V_m^{\pi}(s)]$ 

Is the value vector of scale s under policy  $\boldsymbol{\pi}$  and it is defined by

 $\begin{array}{lll} E_{\pi} & - \mbox{ Mean} \\ S_{\pi} & - \mbox{ State} \\ r_{\pi} & - \mbox{ Reward vector} \\ r & - \mbox{ Discount rate parameter} \end{array}$ 

We also define the Q - learning vector

 $Q_{\pi}$  (s,a) = E  $_{\pi}$  { $\sum_{n=0}^{n} \{\sum_{k=0}^{n} a_{k} = 0 \}$  [1]

The MORL consists of two classes of algorithms, single policy algorithm and multiple policy algorithm. Single policy finds the single state to action mapping of an MDP formulation using multi agent reinforcement learning. The multiple policy algorithms produce many solutions in form of policies of the MDP formulation. [1]

#### Value function approximation [2]

Value Function Approximation deals with approximation of value function using another function, we approximate the weight of value function using value function approximation which helps us to obtain an accurate solution for large number of actions.

The aim is to estimate the value function with function approximation

$$v^{(s,w)} \stackrel{\sim}{\sim} v_{\pi}$$
 (s)  
or q^{(s,a,w)}  $\stackrel{\sim}{\sim}$  q  $_{\pi}$  (s,a)

And to generalise from seen states to unseen states [2]

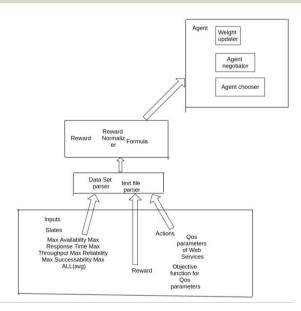
Update the weights of the value vectors dynamically (with the following formula)

 $V(S, W) = X(S)^{T}W = E\sum_{nJ=1}X_{J}(S)w_{J}[2]$ 

Objective function for W:-  $J(w) = E \pi[(v_{\pi}(s) - x(S)^{T}w)^{2}] [2]$ update rule is particularly simple-DERIVATIVE  $V^{(s,w)} = x(S)$   $\Delta w = \alpha(v \pi(S) - v^{(s,w)})(x(S)) [2]$ update = step size x prediction error x feature value. We apply the above value approximation to the single policy and multiple policy algorithm.

Architecture diagram





#### Fig. 1: Modified single policy reinforcement learning algorithm.

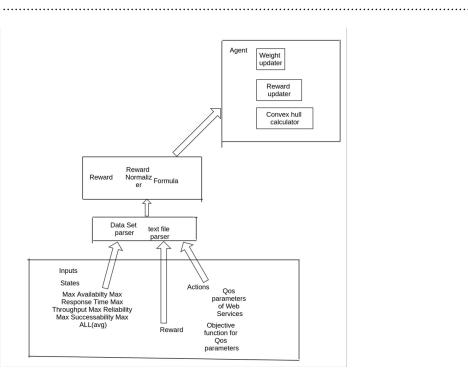


Fig. 2: Modified multiple policy reinforcement learning algorithm.

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# METHODOLOGY

#### Modified single policy multi objective service composition

In the first algorithm, each QoS objective is implemented as a separate Q learning agent [1] at every state & each agents selects its own such service (agent's performance) that optimizes its own QoS objectives. The relative gain is calculated obtained by agents in choosing its own web service to the reward obtained by choosing the service recommended by other agent. The agents negotiates among themselves to reach on optimum decision policy.

Our modified algorithm states that we assign a performance to the objective function by multiplying with its weights. The optimum decision policy is calculated by choosing its action with maximum state action value (reward). The weights are updated dynamically by using value function approximation in order to minimize its least square error between its optimum decision value function and the current value function (current



iteration) which helps to iteratively achieve the optimal decision policy in a shorter time and provide approximately accurate to a large no. of actions. (Fig 1)

First, we randomly choose an agent which selects an optimal action based on epsilon –greedy policy. The remaining agents optimizes the remaining objective function assigned to them by calculating the relative reward gained by choosing their choice to optimize the objective function assigned to them or following the choice of the randomly chosen agent (single policy algorithm).

```
Modified single policy algorithm
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The algorithm consists of the following steps

```
1. Agent negotiation (discussed above)
2. Weight updation
Function single_policy_algorithm() [1]
Choose a starting states from set of states
k=random integer between 1 to the no of actions
Re_k=0//maximum reward so far
Re_i=0 // maximum reward at each iteration
deltaW=0 //change in weight
gamma=0.01 //discount factor for reinforcement learning equation
epsilon=0.01 //error limit
ak=index of service with maximum re ward of agent k 's objective function
repeat
for all agents I except k do
t=maximum of agent_i 's objective function
t2=W[agent_i]*(max-R_[s,ak,agent_i]) #agent negotiation relative reward //obtained when agent_i
choose his choice rather than following the choice of randomly //chosen agent
deltaW= alpha * (Re_k-t2)*(t2/W[agent_i]) //change in weight
W[agent_i]=W[agent_i]+(deltaW) #weight updation
if t2 > max3: //choosing max Re_i
{
  max3=t2
Re_i=t2
ifRe_i>Re_k : #in general case we keep Re_i for all agents except k
{// if obtained reward is greater than maximum reward
         Re k=Re i
k=agent_i #argmax(R_agent_i([s,a,agent_i]))
}
if delta < abs(Re_i-t):
                           //convergence condition
delta= abs(Re_i-t)
if (delta < epsilon * (1 - gamma) / (gamma)) :
break
maxiter=maxiter+1
print ("Iteration No", maxiter)
if maxiter == 15:
break
If maxiter == 15:
break
}
```

### Multiple policy multi-objective service composition

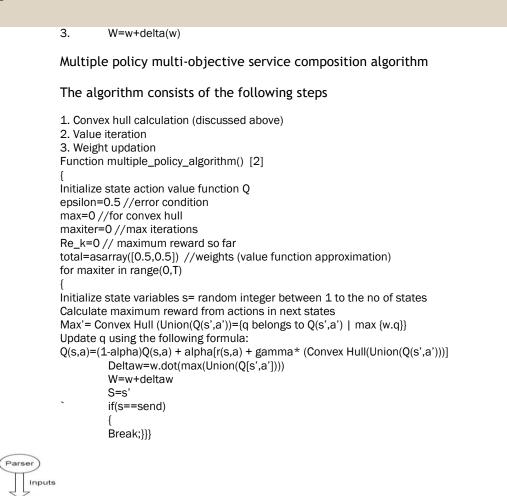
The multiple policy algorithm uses an exact method to solve the MDP formulation of the problem known as value iteration is use to solve the problem, this is combined with dynamic programming which updates the state value action vector by calculating the convex hull in 2 dimensions.

In multiple policy algorithm, we use value iteration and dynamic programming to calculate the convex hull in 2 dimensions. We then choose the action which is optimal i.e., which has maximum value when we dot product of the reward with weight .we update the state action value function according to value iteration and weights according to value approximation. We have used availability and response time as parameters as they are conflicting objective because when we supply wrong inputs to web service the availability is positive and response time is negative.(Fig 2)

We compute the convex hull in 2 dimensions. There two objectives using the following equations  $\rightarrow$ 

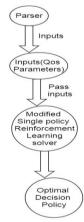
- Convex Hull(Union(Q(s',a'))={q belongs to Q(s',a') | max {w.q}}
- Q(s,a)=(1-alpha)Q(s,a) + alpha[r(s,a) + gamma\* (Convex Hull(Union(Q(s',a')))]







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Inputs(Qos Parameters

> Pass inputs

Modified ultiple policy einforcemen Learning solver

> Optimal Decision Policy

Fig. 4: Flow diagrams of single policy reinforcement learning algorithm.

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Data Set (Table 1) is parsed using Data Set Parser. Inputs in form of vectors are passed to the modified single policy reinforcement learning solver and modified multiple policy reinforcement learning solver. From both the algorithms, we get the optimal decision policy (Fig. 3 and 4)

### EXPERIMENT RESULTS AND ANALYSIS

We have considered the following dataset

Table 1: Sample QWS dataset with QOS parameters

Name of web service	Availability	Response Time	Throughput	Successability	Reliability
MAPP Matching	302.75	89	7	90	73
MSSOAP INTEROP	482	85	16	95	73
STRIKE IRON	3321	89	1.39	96	73
HOLIDAY WEB SERVICE	126	98	12	100	67
GALEX	107	87	1.89	95	73
INTEROP	107	80	1.7	81	67
EMBOSS4	255	98	1.29	99	67
EMBL-EBI	136	76	2.79	76	60
SERVICEOBJECTS.COM	102	91	15.300	97	67
LEAD TOOLS	93	96	13.5	99	67

Single policy algorithm

Algorithm trace out

Agent negotiation and weight updation and accumulated reward

1. Agent negotiation relative reward obtained when agent\_i choose his choice rather than following the choice of randomly chosen agent 2. Reward updation 3. Weight updation 4. Choosing action with max reward Index of web services with Maximum value of objective function: 2 delta W -3.50398068269 Re\_i: 3.94629541198 Index of web services with Maximum value of objective function: 2 delta W 0.0 Re i: 0.0 Index of web services with Maximum value of objective function: 2 delta W 0.0 Re i: 0.0 Index of web services with Maximum value of objective function: 2 delta W 0.0 Re\_i: 0.0 Index of web services with Maximum value of objective function: 2 delta W 0.0 Re\_i: 0.0 Index of web services with Maximum value of objective function: 2 delta W 0.0 Re\_i: 0.0 Index of web services with Maximum value of objective function: 2 delta W 0.0 Re\_i: 0.0 Re\_k: 3.94629541198 Iteration No 1 Accumulated Reward: 3.94629541198 and so on till convergence condition or till max iterations.

### Multiple policy algorithm

The algorithm consists of the following steps:

- 1. Epsilon-greedy strategy:
- 2. Convex hull calculation
- Value iteration
- 4. Weight updation



#### Algorithm trace out

Modified multiple policy algorithm with value function approximation Maximum value of objective function: [[0.010160994923391649, 12.239028778368278, 0.72789115734524901, 1.0, 0.53846153846153844]] Index of web services with Maximum value of objective function: [3] Accumulated Reward: 162.072528122

Iteration No 1

Maximumvalueofobjectivefunction:[[0.010160994923391649,12.239028778368278,0.72789115734524901,1.0,0.53846153846153844],[0.004398966982931054,38.41560588468586,0.02721089075305546,0.2083333333333333334,0.5384615384615384615384],[0.013426147004615304,50.0,0.10204081599553161,0.0,0.0]]Index of web services with Maximum value of objective function:[3, 5, 7]Accumulated Reward:541.358115854

Iteration No 2

Maximumvalueofobjectivefunction:[[0.010160994923391649,12.239028778368278,0.72789115734524901,1.0,0.53846153846153844],[0.004398966982931054,38.41560588468586,0.02721089075305546,0.208333333333333334,0.53846153846153846],[0.013426147004615304,50.0,0.10204081599553161,0.0,0.0],[0.004398966982931054,38.41560588468586,0.02721089075305546,0.20833333333333334,0.5384615384615384615384],[0.013426147004615304,50.0,0.10204081599553161,0.0,0.0]]Index of web services with Maximum value of objective function:[3, 5, 7, 5, 7]Accumulated Reward:1200.85210952

Iteration No 3

Maximum value of objective function: [[0.010160994923391649, 12.239028778368278, 0.72789115734524901, 1.0, 0.53846153846153844], [0.004398966982931054, 38.41560588468586, 0.02721089075305546, 0.208333333333333334, 0.5384615384615384], [0.013426147004615304, 50.0, 0.10204081599553161, 0.0, 0.0], [0.004398966982931054, 38.41560588468586, 0.02721089075305546, 0.20833333333333334, 0.5384615384615384], [0.013426147004615304, 50.0, 0.10204081599553161, 0.0, 0.0], [0.004398966982931054, 38.41560588468586, 0.02721089075305546, 0.2083333333333334, 0.5384615384615384], [0.013426147004615304, 50.0, 0.10204081599553161, 0.0, 0.0], [0.004398966982931054, 38.41560588468586, 0.02721089075305546, 0.2083333333333334, 0.5384615384615384], [0.013426147004615304, 50.0, 0.10204081599553161, 0.0, 0.0]] Index of web services with Maximum value of objective function: [3, 5, 7, 5, 7, 5, 7] Accumulated Reward: 1807.09099934

Iteration No 4

Maximumvalueofobjectivefunction:[[0.010160994923391649,12.239028778368278,0.72789115734524901,1.0,0.538461538461538441,[0.004398966982931054,38.41560588468586,0.02721089075305546,0.20833333333333334,0.5384615384615384615384],[0.013426147004615304,50.0,0.10204081599553161,0.0,0.0],[0.013426147004615304,50.0,0.10204081599553161,0.0,0.0],[0.013426147004615304,50.0,0.10204081599553161,0.0,0.0],[0.013426147004615304,50.0,0.10204081599553161,0.0,0.0],[0.013426147004615304,50.0,0.10204081599553161,0.0,0.0],[0.013426147004615304,50.0,0.10204081599553161,0.0,0.0],[0.013426147004615304,50.0,0.10204081599553161,0.0,0.0],[0.013426147004615304,50.0,0.10204081599553161,0.0,0.0],[0.013426147004615304,50.0,0.10204081599553161,0.0,0.0],

Index of web services with Maximum value of objective function: [3, 5, 7, 5, 7, 5, 7, 7]

Accumulated Reward: 2468.55487898

Parameter	Meaning	Value
Alpha	learning rate	0.1
Gamma	discount factor	0.01

The experiments results were to examine the ability of the hybrid algorithm. results are as follows:

1-Singly policy reinforcement learning algorithm 2-improved algorithm



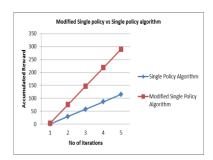
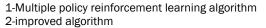


Fig. 5: Modified single policy vs single policy algorithm.

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As you can see in the above chart, the accumulated reward for modified single policy algorithm is greater than the accumulated reward for original algorithm. This is true for each iteration, thus the quality of solutions obtained by Modified single policy algorithm is greater than single policy algorithm.(Fig 5)

Thus the modified algorithm yield better results than original algorithm



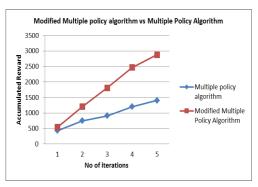


Fig. 6: Multiple policy algorithm vs modified multiple policy algorithms.

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As you see in the above chart the accumulated reward for modified multiple policy algorithm is greater than the accumulated reward algorithm for original multiple policy algorithm. This is true for each iteration, thus the quality of solutions obtained by Modified multiple policy algorithm is greater than multiple policy algorithm.(Fig 6)

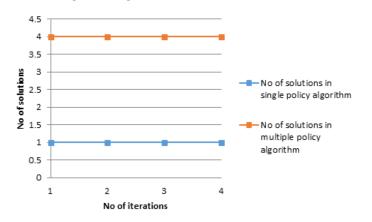


Fig. 7: No of solutions in single policy algorithm an multiple policy algorithms.

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As you can see from the above chart, the no of solutions obtained in multiple policy algorithms is greater than no of solutions obtained in single policy algorithm. Thus, multiple policy algorithms is better than single policy algorithm. Thus, we can obtain multiple solutions to the same problem in multiple policy algorithms as compared to single policy algorithm.(Fig 7)

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### CONCLUSION

We propose modified reinforcement learning algorithm, the result have shown that the modified reinforcement learning algorithm is more effective than the original algorithm for selecting a web services in a pervasive environments. The future work is to further modify learning algorithm to achieve better results. The effectiveness of the proposed reinforcement learning algorithm is shown in the results.

The scenario addressed by single policy algorithm is that we have partially observable environment and we want to find the optimal state of action mapping of the MDP formulation of the problem .This is single in number in single policy algorithm .The scenario addressed by multiple policy algorithm is that we want to find multiple solutions to the MDP formulation of the problem. The future work involves optimization of the value function approximation technique to yield better results.

### CONFLICT OF INTEREST

The authors have no competing conflicts of interest.

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#### AUTHOR CONTRIBUTION

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ARTICLE



## WATER SCARCITY IN EGYPT: CAUSES AND CONSEQUENCES

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### ABSTRACT



Water quality is now a major concern for all countries around the world, it depends on the location of the source and the state environmental protection in a given area. Therefore, the quality and the nature of water may be determined by physical and chemical characters. So, water resources are the critical factor affecting production, services, and sustainable development in Egypt. Egypt is facing four major constraints with respect to its water resources: (I) A fixed water supply and rocketing population growth. (II) Difficulties in the country's relationship with the Nile Basin states. (III) Independence of South Sudan declared in July 2011. (IV) Climate change and its hidden future. These four factors pose a number of questions related to the availability of water and the amount of supply that will be allocated for different consumptive and non-consumptive activities and development programs. Egypt also faces the dilemma of pollution of water resources by industrial wastewater, agricultural drainage water and municipal wastewater which may cause many risks to the health of citizens and threaten the safety of the water situation in Egypt.

### INTRODUCTION

KEY WORDS Water resources, Scarcity, Pollution, Climate change

Received: 27 February 2017 Accepted: 24 April 2017 Published: 1 Aug 2017 Since the 1970's, water uses in Egypt have exceeded the available resources. Accordingly, the government has provided additional resources by recycling drainage and wastewater, trapping water losses, and with water use rationalization practices [1]. However, at present, there are significant challenges to water resources management in Egypt. Beginning with a single source of water the Nile, uncertainties in climate, developments upstream, and population growths have characterized efforts to anticipate potential future water constraints. The Minister of Water Resources and Irrigation has stated that the challenges facing the water sector in Egypt are enormous and require the mobilization of all resources and the management of these resources in an integrated manner. This is especially true as the amount of available water resources is fixed, meanwhile water demands continue to grow in the years ahead due to population growth, increased food demand, and expansion and modernization of the industrial base, and improved standards of living [2].

In fact, Egypt's water problem cuts two ways: on the one hand a scarcity of fresh water to drink and irrigate crops, on the other, an overabundance of salt water, already rendering farmlands barren, and threatening to drown the low-lying areas along the Mediterranean coast. Coastal erosion, a consequence of both the Aswan High Dam and global warming-related sea rises, is readily observable in Egypt [3]. All the lakes suffer the additional burden of fertilizer run-off from surrounding farmland, also the fishing communities have disappeared, and it is also threatened by diminished water quality [3]. As the acceleration of the productivity is the main objective of Egyptian economy, however, this is reached sometimes at the expense of sustainability. The challenge the policy-maker faces is how the development can reduce poverty, but to be made compatible with the maintenance of the natural resources [4].

According to United Nations projections, the population of Egypt will grow from 62.3 million in 1995 to 95.6 million by 2026 and will likely reach 114.8 million before it stabilizes in the year 2065. In spite of Egypt using most of its share of the Nile water for irrigation, at present it imports more than half of its food grains. Increasing demand for food in the future will certainly bring further pressure on the scarce water supply [5]. However, it is found that in Egypt, despite considerable planning capacities, many water policy outcomes are influenced by developments beyond the control of the water ministry [6].

Water resources and supply systems in Egypt

Water resources in Egypt are limited to the following resources:

Nile water

\*Corresponding Author Email: withyou\_2010@yahoo.com Tel.: +201015119474 Egypt is dependent on Nile River as the major source of its water supply for all economic and service activities. Nile River is considered as one of the most important rivers in the world; it is the life artery of Egypt. Throughout the known Egyptian history, Nile River had dominating influences on the economy, culture, public health, social life and political aspects. Nile River basin covers an area of about 3,000,000 square km, through ten African countries: Burundi, Democratic Republic of Congo, Egypt, Eritrea, Ethiopia, Kenya, Rwanda, Sudan, Tanzania and Uganda. The supply of Nile water is allocated by the Nile Water Agreement, signed with the Sudan in 1959 prior to the construction of Aswan High Dam; Egypt's quota is fixed at 55.5 billion m<sup>3</sup>/year. This quota constitutes about 90% of the country's water budget; the remaining 10 % represents minor quantities of renewable and fossil groundwater, and a few showers of rainfall [7].



Aswan High Dam (AHD) is the major regulatory facility on the river. It began operation in 1968, ensuring Egypt's control over its share of water, and guiding its full utilization. Downstream from AHD, the Nile water is diverted from the main stream into an intensive network of canals through several types of control structures, providing water for agricultural and other uses [8, 9 and 10]. Being the most downstream country on the Nile, Egypt is affected by climate change impacts, not only within its borders, but also within the whole basin; which it shares with 9 other countries. Economic developments in upstream countries and measures they might take to adapt to climate change are likely to put more pressure on water resources in Egypt. Therefore, it is of prime importance for Egypt, amongst other Nile countries, to assess the hydrological impacts of climate change on the Nile. From the other hand, several studies showed that Nile River is very sensitive to temperature and precipitation changes mainly because of its low runoff/rainfall ratio (4%) [11 and 12].

There are two branches of Nile River; Rosetta and Damietta. Rosetta is the western branch of about 239 km long and variable widths of 450-1000 m. It is characterized by variable depths with an average range of 12-20 m. The water level in Rosetta Branch is controlled by two barrages; Delta Barrage in the south and Idfina Barrage at about 197 km to the north. On the other hand, the Damietta Branch is about 230 km long with variable widths of 300 and 500 m. This branch is characterized by variable depths with an average of about 8 m. The Nile water quality is characterized by high nutrients concentrations. Nile River from Aswan to El-Kanater Barrage receives wastewater discharge from 124 point sources, of which 67 are agricultural drains and the remainders are industrial sources [13].

#### Rainfall

Rainfall is limited to the coastal strip running parallel to the Mediterranean Sea and occurs only in winter season. The amount is small ranging between 80 to 280 mm/year and provides an overall volume of 1.3 billion m<sup>3</sup>/year. Rain is also erratic with respect to space and time and mainly utilized for agricultural purposes. Rainfall amount cannot be considered a reliable source of water due to high spatial and temporal variability [7 and 12].

### Groundwater

Although there is less groundwater than Nile water, the existence of aquifers in the desert, where no Nile water can be conveyed, makes this resource extremely precious. Desert aquifers are mostly deep and nonrenewable, meaning that they are mined and the cost of exploitation is high. The average pumping rate from these aquifers at the present time is about 1.5 to 1.85 billion m<sup>3</sup>/year although the actual potential is 3.5 billion m<sup>3</sup>/year. The second source of groundwater is the Nile aquifer, which lies below cultivated land in the valley and Delta. The reservoir is fed by surplus irrigation water and seepage from the Nile and its branches. Obviously, water in the reservoir is shallow and renewable and the quality is not as good as the deep groundwater in the desert. The third, smaller groundwater reservoir is the coastal aquifer, which is fed by rain falling on the coastal strip, forming lenses of freshwater, which sit above the saline groundwater and run parallel to the shoreline [7].

#### Non-conventional water sources

Given the continuous need to increase water resources and bridge the gap between supply and demand; Egypt has a long history of water reuse [7]. While conventional (renewable) resources are extremely scarce, non-conventional water sources provide highly valued supplies. This water is generally used for agriculture, landscaping, and industrial uses through specialized processes [12], these sources include:

#### Treated domestic sewage

Treated domestic sewage is being reused for irrigation with or without blending with fresh water. The increasing demands for domestic water will increase the total amount of sewage available for reuse. It is estimated that the total quantity of reused treated wastewater in Egypt is about 0.3 BCM in 2013 [12].

#### Agricultural drainage water

The amount of water that returns to drains from irrigated lands is relatively high (about 25 to 30%). The total amount of reused water is estimated to be 13 BCM in 2013. The reuse practices increase the overall efficiency of the system as comparable to the efficiency of modern irrigation systems [7]. Reuse of non-conventional water sources such as agricultural drainage water and treated sewage water cannot be added to Egypt's fresh water resources. In fact, using these sources is a recycling process of the previously used Nile fresh water in such a way that improves the overall efficiency of the water distribution system, however, appropriate strategies are needed for managing soil, water, and crops when these resources are used for irrigation [7 and 12].

### Desalination

Desalination of seawater in Egypt has been given low priority as a water resource because the cost of treatment is high compared with other sources. Desalination is actually practiced in the Red Sea coastal area to supply tourism villages and resorts with adequate domestic water supply where the economic



value of the water is high enough to cover the treatment costs. It may be crucial to use such resource in the future if the growth of the demand for water exceeds all other available water resources. However, its use will depend on technological development in this field [12].

#### Pollution

Pollution is considered as one of the most serious problems that faces human societies in the whole world especially in the developing countries. Though produced by man himself and his activities, it has deleterious effects on human's environments and resources, so pollution and its effects are considered as one of man's greatest crimes against himself. It is well known that all water resources in Egypt depending on surface water which could be exposed to many pollutants and contaminants; natural contamination arising from leaching, and normal geological weathering processes in the surrounding watersheds, Domestic and industrial wastewater may be disposed directly into surface water and percolate into ground water and agricultural activities are also a contributor to water pollution. Within limits, materials like organics and microorganisms can be degraded by the water's natural-purification process, or by flowing through wetlands such as the Delta. In contrast, inorganic pollutants are not affected by natural purification processes; their concentrations may only be reduced by dilution. Toxic compounds arising from industrial discharges (heavy metals, herbicides and pesticides, plus inert suspended or dissolved solids) destroy the natural biological activity in the water. Oxygen balance is also affected due to organic or inorganic substances, oils and detergents that hinder oxygen transfer across the air-water interface [14]. Aquatic habitats, especially the freshwater ecosystems, are more subjected to pollution than other environments, because of water use in industrial processes as well as discharge of effluents from industry and urban developments. Most aquatic ecosystems can cope with a certain degree of pollution, but severe pollution is reflected in a change in the fauna and flora of the community, which suffer such pollution. The sources of contaminants can be grouped into three categories: industrial activities, municipal wastewater, and agricultural drainage water [15].

#### Industrial activities

Although industrialization is considered the cornerstone of the development strategies due to its significant contribution to the economic growth and hence human welfare, however, in most developing countries it led to serious environmental degradation. Chemicals, food, metal products, and textiles are the most prominent branches in Egypt. The worst industrial waste liquids are those heavily laden with organic or heavy metals or with corrosive, toxic or microbial loaded substances; such waters endanger public health through the direct use as well as through feeding with fish that live in the polluted streams. Some groups of chemicals, such as carcinogenics, mutagenics and neurotoxins, are even unaffected by the usual methods of water treatment. The threats imposed by chemical discharges comprise contamination of drinking water supplies, phyto- and aquatic toxicity, destruction of agriculture as well as fisheries, bioaccumulation, and biotransformation. As it might be expected, the mid-stream conditions of the Nile are still, on an average, at a fairly clean level owing to dilution and degradation of the pollutants discharged. The riverbanks, however, are much more polluted. Inefficient production in some industries (e.g., oil and soap) generates waste that contains raw material as well as products, a costly burden to the national economy and the consumer [16 and 17]. Evidently, efficient production causes lesser pollution. Cleaner production is defined by UNEP's Industry and Environment Program Activity Center as "the continuous application of an integrated preventive environmental strategy to processes and products to reduce risks to humans and the environment". Obviously, cleaner production is the unique answer for the industrial pollution in Egypt [16].

#### Municipal wastewater

Egypt had 372 municipal wastewater treatment plants in 2012, treating an average of 10.1 million cubic meters per day [18]. In the rural areas accommodating about half of the population, 95% of the people have no access to sewer systems or wastewater treatment facilities. The "septic tank" is the most common disposal facility where excreta and a limited amount of sludge water can be collected for biological digestion. The digested excreta leach into the soil surrounding the tank and hence subject shallow groundwater to pollution [16]. Furthermore, secondary treatment cannot be satisfactory in emphasizing the quality of wastewater for reuse or in preventing further pollution with pathogenic bacteria and other microorganisms [19]. Admittedly, the unused drainage water led into waterways transfers its pollution burden to the surface water which might be contaminated with pollutants from domestic sources. Typhoid, paratyphoid, infectious hepatitis, and infant diarrhea are some endemic diseases indicating deterioration of water quality in Egypt. Despite the assiduous endeavours for public awareness through the media, the prevalence of Bilharzia substantiates the lack of rural sanitation against the traditional contamination of surface waters with human wastes, i.e., urine and faeces [16].

#### Agricultural drainage Water

The increased nutritional needs due to the high population growth in Egypt imposed major challenges to the agricultural policy over decades. the rate of growth in agricultural production increased since the construction of Aswan High Dam, many programs of land reclamation were implemented; transformation of basin irrigation to permanent irrigation make agriculture consumes more than 85% of Egypt's share of



Nile water annually. Although the country lost part of its fertile land to urbanization, this has been balanced by expansion of agricultural areas [12]. The intensive use of chemical fertilizers and insecticides was essential for the exploitation of the available land. A part of the used fertilizers is usually drained into the surface and groundwater systems along the Nile. The use of these sources for drinking water supply is at risk due to the presence of nitrogen and phosphorus salts [20]. The heavy implementation of pesticides, as in the case of cotton crop, poses serious environmental risk. Some pesticide residues were found in canals and drains, however, their concentration was far below the guidelines set by WHO [21]. In the downstream direction the water quality gradually deteriorates due to the poorly treated wastewater discharges from both domestic and industrial activities and uncontrolled mixing with water from polluted drains. Therefore, they contain high levels of various pollutants, such as faecal bacteria, heavy metals, pesticides and insecticides. Some drains should be considered as open sewage system that smell is very bad due to the production of hydrosulfide [16].

#### Water scarcity

Average per capita fresh water availability in Egypt is on a steady decline from about 1,893 cubic meters per year in 1959 to about 900 cubic meters in 2000, to 700 cubic meters in 2012. According to government, population in Egypt will likely reach 98.7 million in 2025. According to the Ministry of Water Resources and Irrigation, Egypt will need 20 percent more water by 2020, Egypt already uses 127% of its water resources; meaning that Egypt imports 27% of its water used through imported food and other products; and by 2020 Egypt could be using 147%. United Nations now says Egypt could be water scarce by 2025. Assuming that Egyptians' population carries on growing, The land reclamation projects in deserts and the fact that Egypt is already importing more than 50% of the cereals it consumes, Egypt cannot meet its food demand by relying on Nile water for irrigation [22]. Adding to Egypt's precarious water situation, the evaporation from the surface of the long Lake Nasser apparently exceeds the earlier estimated amount. Egypt is already utilizing most of the flow of the Nile and it plans to use even more. Additionally, Egypt has built several irrigation projects such as the Isna Barrage, Nag Hammadi Barrage, and Asyut Barrage, which will certainly be affected by decreased flow from Aswan [5]. It is forecasted that in 2025 the population of Egypt will increase to about 95 million, leading to a decrease in per capita water availability per year assuming that total water availability remains constant. Moreover, developments in Sudan, Ethiopia or other riparian countries could reduce water availability to Egypt [23]. Potential risks around water availability include the huge population density and deteriorated water pipe network which causes a huge water loss that reach the values of 34-35% which is equal to around 791 million m3/year; which if saved can provide fresh potable water to additional 11 million inhabitants. This combination of water scarcity and pollution of the available water resources could be one of the worst resource crises Egypt faces. Industrial facilities are source of industrial waste water, which is considered one of the major causes of water pollution in Egypt and therefore, these facilities must be inspected and subjected to Egyptian laws; which preserve a certain balance between economic gain and preservation of water resources from pollution.

#### Water demands

Rapidly growing demand for water in Egypt in response to an increasing population led to higher crop intensities and horizontal expansion. The logical sequence in water resource management is to examine the present demand of water for all purposes, to determine how the demand in various sectors will be affected by growth then to project what the water demand will be in the short and in the long term future [24]. Various demands for freshwater are exerting excessive pressure on the available water supply as follows:

**Agriculture:** The agricultural sector is the highest freshwater consumer as cropping patterns are a crucial factor in water resources management, especially under the free-market policy. The prediction of future water requirements depends upon the best estimation of cropping patterns. The Ministry of Water Resources and Irrigation (MWRI) in Collaboration with the Ministry of Agriculture & Land Reclamation have planned an ambitious program for reclamation [24]. However, irrigation mainly consumes the bulk of the available water supplies. Despite losses of agricultural land to urbanization, the cropped area statistics indicate a very modest increase during the last years due to the increase in cropping intensity, complying with the national plan to achieve food security and the distribution of crop areas then has not change significantly during the last decade [2 and 24].

**Municipal water demands:** Estimation of the municipal water use depends on population growth rates, the consumption in liter/capita/day, and distribution system losses.

**Industrial water demands:** industrial facilities demands of water should be regulated in a certain way preserving a balance between economic gain and resources preservation.

**Navigation and Hydropower Water Use:** From February to September, water released from the High Aswan Dam (HAD) for irrigation, municipal, and industrial purposes is sufficient to maintain the required navigational draft in the Nile.



**Outflows to Sea:** Water enters the system at the High Aswan Dam and flows to the sea as freshwater through the Rosetta and Damietta branches, and as drainage water through the main drains [24].

### Challenges of water quality and climate change

Due to the progressive increase of population and the continuous expansion of urbanized areas, pollution dilemma has increased too. One of the greatest water related challenges facing Egypt is the pollution of its surface and ground water resources from agricultural, domestic and industrial sources. Although monitoring water quality of the Nile system started as early as the 1980s, the complexity of water quality management still required the development of other mechanisms including policies, institutional and governance arrangements, infrastructure for monitoring and analytic laboratories, awareness and skilled human resources. The cost of environmental degradation due to water quality deterioration is relatively high with serious health and quality of life consequences; it was also found that the level of bacteria is higher than what is permitted for both municipal and irrigation purposes. The closed water system of the country makes it more vulnerable to quality deterioration in a northward direction [25]. Control of the groundwater withdrawals, especially in the Northern oases and Siwa oasis, is necessary in order to prevent the deterioration of groundwater quality [26]. Contamination arises from both point and diffuse sources. Inadequate treatment of industrial and domestic wastewater and the progressive increase of the population and industrial activities have created significant pollution dilemma with serious health effects [24]. The severity of water quality problems in Egypt varies among different water bodies depending on the flow use pattern, the population density, the extent of industrialization, the availability of sanitation systems as well as the social and economic conditions [2].

The poor quality of drinking water is of great importance since the sources of raw water for many areas have become increasingly polluted. Therefore, it requires more sophisticated treatment to produce drinking water of adequately quality. In addition, water treatments units are not always functioning properly as a result of lacking maintenance and proper operation. Even when water treatment is satisfactory, drinking water is sometimes contaminated in leaking distribution network, which are infiltrated for example by sewage [2]. In addition, despite of the flourishing fish production in Egypt, only 17 species remain as of 1995 out of 47 species which used to be available in 1948 [2]. In fact, MWRI cannot solve the pollution problem alone, but requires a combination of all the efforts of all people and community groups and also needs to collaborate with different stakeholders including the polluting sectors such as industries, agriculture, and municipal water users. However, water quality control is not generally a top priority in the respective ministries, and their departments dealing with issue of water quality may lack full internal support [6]. At present, industrial facilities managers, engineers and workers in Egypt do not have the sufficient knowledge and know-how enough to deal with the industrial pollution. Neither their academic, nor their on-the-job training have provided them with knowledge and experience necessary for identifying and dealing with environmental problems [25].

In an initiative to contain water pollution without confronting the business interests of the industries, the Egyptian Environmental Affairs Agency EEAA supports efforts to improve the capacity of industrial plants for wastewater treatment. However, the Ministry of State for the Environment itself is also considered a still relatively weak actor in the water sector by many observers. In summary, decisions regarding water quality control are very much subject to bargaining over stakeholder interests, both within the government and between the government and water users [6]. The anticipated environmental deterioration that may take place in the northern lakes should be closely monitored and evaluated [26]. As for the challenge of the climate change, in recent years, a large part of the scientific community has made efforts analyzing the impact of projected climate change on water resources and proposing adaptation strategies. In this context, a number of studies analyzed the effects of climate change on the hydrology of the River Nile Basin (RNB), the world's longest river. In fact, the RNB could be vulnerable to water stress under climate change because of the limited water availability and the increasing demand for water from different sectors [23]. Furthermore, climate change is likely to affect water availability to Egypt, although the direction of change is uncertain. Some studies foresee a decline of up to 70 percent in Nile water availability, while other studies project an increase in Nile water levels by 25 percent [27].

### Population and Its distribution in Egypt

Egypt's resident population surpassed 83 million in 2003, according to the Central Agency for Public Mobilization and Statistics [28], making it the most populous country in the Arab World and the third largest in Africa. A recent report published by the Information and Decision Support Center (IDSC) [40]; places population growth rates at close to 2% per year. Egypt's population growth rate remains high compared to growth rates of less than 1% in Europe [29]. Egypt's surface area is almost entirely desert. Since the climate is hyper-arid, the presence of Nile water concentrates the population within the confines of the valley and delta. A commonly quoted number is 96% of the population living on 4 % of the land [30]. Rural population densities as high as 1,600 people per km2 exert profound pressure on natural resources of land and water. The rapid urbanization and urban encroachment around the metropolitan areas of Cairo and Alexandria, as well as around the cities in the delta, is reducing available agricultural land [31]. According to the United Nation's Food and Agriculture Organization (FAO), since 1997, Egypt's rural population as a percentage of the country's total population has been relatively stable, declining by only 1.7%, and now makes up almost 56% of the population [32]. It is worth noting that, despite the relatively



stable percentage of rural population, the percentage of the labor force in agriculture has declined to only 24% of the total national labor force [32].

### Water resources and water security in Egypt

Without significant rainfall, Egypt is classified as a hyper-arid country, and its agricultural production is almost wholly dependent on irrigation [30]. Freshwater resources are limited to the Nile River and groundwater stored in shallow and deep aquifers. National dependence on freshwater withdrawal from the Nile is estimated at between 95% and 98% [16 and 33]. Desert groundwater withdrawals, accounting to a few billion m3 yearly, are mainly drawn from the deep Nubian Sandstone aquifer supplying the Western Desert, which is depleting at an increasing rate. Egypt's set share of the annual Nile flow is 55.5 billion m3 [1 and 34]. The primary function of the Aswan High Dam is to provide Egypt with water security, protecting it from the unpredictability of the annual flow. About 85% of Nile water is devoted to the agricultural sector, and releases and quantities are timed according to the national agricultural cycle. The country fell below the World Bank's water scarcity threshold of 1,000 m<sup>3</sup> of renewable water available per capita per year in 1997 [30]. By 2007, this figure sunk below the 700 m<sup>3</sup> international water poverty limit [35]. Economic growth in Egypt also threatens the quantity and quality of water resources, inflating the existing issue of contaminated shallow groundwater from industrial chemicals, and excessive fertilizer and pesticides use [36]. Egyptian farmers still overwhelmingly practice flood irrigation, which results in evaporative loss and over-irrigation, causing soil damage and rises in groundwater tables [37]. In 2005, only 6% of Egypt's cultivated area was equipped with modern pressurized irrigation systems [1]. Inadequate agricultural drainage coupled with the Nile Delta's particularly flat slope contributes to salinization of soil and water resources. Local demand for water is also increasing, both through horizontal growth from desert land reclamation projects and vertical growth in terms of production intensification. Policy changes that give more freedom to farmers in cropping pattern choices, while stimulating yield increases have led to an overall increase in water demand [37]. El-Agha et al., (2011) [35] and Radwan (1998) [38] reported a mismatch between irrigation demand and supply at the level of main canals in the Nile Delta, and Radwan (1997) [39] blamed bureaucratic, overly centralized government administration and management for inefficiencies in irrigation water supply and distribution [35 and 39].

### Industrial pollution in Egypt

Assessing the industrial landscape of Egypt, wastewater from various industrial processes could regularly contain pollutants such as asbestos, lead, mercury, cadmium, arsenic, sulfur, oils and petrochemicals. All of these are very hard or even impossible to separate from the water causing permanent damage once dumped in it. Furthermore, radioactive materials can also be found in wastewater from ore processing such as uranium, thorium, iodine, cesium and radon and cause pollution to both surface and underground water. To date however, industrial polluters have continued to pollute unabated, despite laws which expressly state they cannot pollute above certain levels. What we now see is uncontrolled polluted wastewater from leather tanning and dyeing processes, sugar distillation factories, chemical producing factories, building materials industry - including cement, the food canning industry, paper and wood pulp processing factories and the electrical industry (amongst many others). Despite the argument that a level of deregulation is required to bring in investors to rebuild the economy in Egypt, it should not come at the cost of the lives of those supposedly benefitting from the economic improvement. Egyptians suffering from long-term illnesses and in need of medical care owing to kidney failure, cancer or the Hepatitis C Virus (10-20% of the population). Worryingly high and increasing rates of renal diseases and renal failure: roughly 30% of which are caused by Schistosomiasis. The highest rates of Schistosomiasis contributing to renal failure are in Lower and Upper Egypt: the areas with least access to safe drinking water and sufficient sewage treatment infrastructure. Public Health researchers based in El Minia identified Drinking unsafe water and exposure to pesticides as the cause of renal diseases for an estimated 72% of patients. The dilemma manifests in case of rural villages along the Nile which are not connected to mains water and without sufficient means to buy bottled water, drinking the untreated and polluted river water. Industrial pollution is causing many risks for Egyptian fishermen who losing their livelihoods because the fish can no longer survive in the water, on the other hand making sources of food to be polluted: fish, fruit and vegetables grown using polluted water and soil. The added cost to farmers whose land remains permanently polluted and unusable whilst the groundwater and water sources are also polluted, finally, loss of tourism due to lack of safe access to clean water and unsightly surroundings. In 2008, the EEAA recorded that roughly 102 industrial plants are discharging their waste water either directly into the Nile or through the municipal system. The waste produced from these industries contains some of the most hazardous detergents, heavy metals, and pesticides of all. Such industrial contaminants dumped in the Nile have reached levels of almost 4.5 tons per year. And the percentage of industrial organic pollutants thrown in the water is roughly 270 tons per day. The only published inventories of industrial facilities violating the regulations are 5 years or more old, therefore there is no longer an up to date dataset of the facilities in question sufficient to operate good monitoring and enforcement activities as required.

### CONCLUSION

Egypt, as a developing country is facing a crisis in water resources sustainability and management. The water shortage dilemma is not related only to increasing demands, but rather also to poor infrastructure



and management practices. It has limited access to funding, and infrastructure requires urgent upgrading. The water sector in Egypt is facing many challenges including water scarcity and deterioration of water quality due to population increase and lack of financial resources. The fragmentation of water management and lack of awareness about water challenges are also clear challenging problems. Water scarcity is the most serious dilemma facing Egypt at the present time in addition to the problems caused by pollution of water resources by various forms of contaminants loaded with sewage, agricultural drainage water and industrial wastewater which in fact posing continuous challenges facing the government and the institutions responsible for the preservation of water resources in Egypt and make it very necessary and imperative to maintain water resources by all means including rationalize the consumption, reduce the waste and strict laws governing the process of industrial wastewater pumping on water resources , as well as improve the quality of sewage treatment to reduce pollution in addition to the search for alternative sources of water , such as desalination of sea water.

### CONFLICT OF INTEREST

There is no conflict of interest.

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# ARTICLE AMINO ACID PROFILE OF SOME NEW VARIETIES OF LEGUMINOUS SEEDS

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### ABSTRACT



**Background:** Legumes play an important role in the traditional diets of many regions throughout the world. They are low in fat and are excellent sources of protein, dietary fibre, and a variety of micronutrients and phytochemicals. Utilization of legume seeds in daily diet not only will be a good option to overcome problem of protein-caloric-malnutrition among the people of low income group and also it supports the vegetarian community in Indian prospects. Availability of the essential amino acids under legume seeds enhances nutritional benefits. The quality of a seed depends on the total amount of amino acids present in protein. The present investigation is an attempt to determine amino acid composition in selected hybrid varieties of legume seeds viz Glycine max (NRC-37), Vigna radiata (LGG-460), Phaseolus mungo (LBG-20), Cicer arietinum (JG-130) and Lens esculenta (JL-3). The HPLC technique was applied to ascertain nutritional quality for seeds. All the varieties were found rich source of amino acids and presenting high nutritive value.

### INTRODUCTION

KEY WORDS Amino acid; HPLC technique; Leguminous seeds; Protein

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\*Corresponding Author Email: chem.249@gmail.com Tel.: +91-7208118781 Legumes are cultivated for their seeds from many years. These seeds were used for food consumption and for industrial oil production. Legumes include beans, lentils, lupins, peas, peanuts and other podded plants that are used as food [1]. Legumes are a significant source of protein, lipids, dietary fibres, carbohydrates and dietary minerals. Like other plant based foods, pulses contain no cholesterol and little fat or sodium [2]. Legumes have played an important role in the diets of many regions in the world. It is difficult to imagine the cuisines of Asia, India, South America, the Middle East, and Mexico without picturing soybeans, lentils, black beans, green beans, chickpeas, and pinto beans, respectively [3,4].

It has long been established that protein is an essential nutrient that needs to be obtained from diets. Protein is an indispensable requirement for growth and maintenance of any living creature. However, the ultimate source of protein is its amino acid composition. A protein molecule is a long chain of amino acids linked with peptide bonds (i.e., an amino acid linked to another amino acid). Dietary protein is digested and absorbed into amino acids. These amino acids play central roles both as building blocks of the body's proteins and as intermediates in metabolism, controlling virtually all cellular processes and reactions in living cells [5, 6]. Amino acids contribute significantly to the health of the nervous system, muscular structure, hormone production, vital organs, and cellular structure. More important, many physiological processes relating to exercise require amino acids for energy, recovery, muscle hypertrophy, and strength gain [7].

Proteins obtained from different food stuffs; supports diet and consider as a main ingredient for healthy life. The rich sources of amino acids are meat, fish, dairy products, and vegetables such as legumes and grains. Legumes have been shown to be rich in dietary protein. However, the utility of protein does not depend on its quantity but is known to be affected by (i) essential amino-acid composition, (ii) amino-acid imbalance, (iii) biological availability of essential amino acid, (iv) digestibility and (v) interference due to anti-nutritional factors [8]. Scientists and nutritionist were concerned about enough quantity of amino acids in diet must be an important factor in order to maintain health. Availability of the essential amino acids under legume seeds enhances nutritional benefits. The present investigation is an attempt to determine amino acid composition in hybrid varieties of *Glycine max* (NRC-37), *Vigna radiata* (LGG-460), *Phaseolus mungo* (LBG-20), *Cicer arietinum* (JG-130) and *Lens esculenta* (JL-3) by HPLC technique.

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### MATERIALS AND METHODS

#### Collection of samples

New hybrid varieties of Leguminous seed of *Glycine max* (NRC-37), *Vigna radiata* (LGG-460), *Phaseolus mungo* (LBG-20), *Cicer arietinum* (JG-130) and *Lens esculenta* (JL-3) under investigation were collected from Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur (MP). These seed varieties were high yielding, taken short cultivation time and have resistance for diseases. The study was conducted in the Soybean Processing & Utilization Centre, Central Institute of Agricultural Engineering Bhopal, (M.P.) India.

### Amino acid determination method

#### Instrumentation

Amino acids were determined by high performance liquid chromatography LC-10A (Shimadzu Corporation, Kyoto, Japan) according to the method of Cserhati et.al. [9] and Kerese et.al. [10]. Like an amino acid analyser, this system uses a column packed with the styrene-divinylbenzene copolymer with sulphonic group, i.e. a strongly acidic cation exchange resin (Shim-pack Amino-Na or Amino-Li), for separation. Amino acid injected and separated by means of a binary gradient eluting method using two liquids of standard solution and then, fed to detection unit. When arginine (the final peak) elute, the column was automatically cleaned and equilibrated to be ready for the next analysis. Ophthaladehyde (OPA) was used as the derivatizing reagent for detection through a reaction process. In the presence of thiol (SH) compound, OPA react rapidly with compounds with primary amino (-NH2) group into a strongly fluorescent substance. This post column derivatization and fluorescence detection method enables selective detection of each amino acid at high sensitivity.

### Sample preparation by hydrolysis

Alkali hydrolysis: Tryptophan destroyed during acidic conditions therefore alkaline hydrolysis is used for determination of tryptophan, which is stable under basic conditions. For alkaline hydrolysis 500 mg finely ground samples were hydrolyzed by adding 4.83g barium hydroxide and 5ml of boiling water. The mixture was evacuated and then heated at 120°C for 8 hours. After hydrolysis, the pH was adjusted to 3 with HCl, and diluted to 25ml with HPLC grade distilled water. 1ml of sample was vacuum dried using flash evaporator and finally dissolved in citrate buffer (0.1M; pH 2.20).

Acid hydrolysis: Acid hydrolysis was carried out with 6N HCl at 110°C to 20-22 hrs in evacuated and sealed tubes. The hydrolysate was filtered and diluted to 250ml. 1ml of sample was vacuum evaporated at 40°C until dryness. The content was dissolved in citrate buffer (0.1M; pH 2.20). 20µl of this derivatized were injected directly into the HPLC.

### Chemicals and buffer solutions

- i) Buffer Solution (Citrate buffer 0.1M; pH 2.20)
- ii) 10% Brij-35<sup>™</sup> solution (Sigma USA)
- iii) Reaction solution A [Sodium hypochlorite solution (NaClO solution)]
- iv) Reaction solution B (OPA solution –Sigma USA)
- v) Sample diluents: (0.2N Na<sup>+</sup> (sodium citrate) pH 2.20)
- vi) Standard Amino acid Solution (Sigma USA)
- vii) HPLC Grade water (Millipore)
- viii) Mobile phase A, B & C

Mobile phase A: 58.8 gm of sodium citrate containing 0.2N sodium (pH 3.20), 210 ml 99.5% ethanol and 50ml (60%) Perchloric acid makes up the volume up to 3 litres with HPLC grad water.

Mobile phase B: 58.8g of sodium citrate containing 0.6N sodium (pH 10), 12.4 g Boric acid and 30 ml 4N NaOH solution make up the volume up to 1 litre with HPLC grad water.

Mobile phase C: For washing only: 4g NaOH diluted up to 500ml with HPLC grad water.



#### Chromatographic conditions

Shim-pack VP-ODS (150mm X 2.0mm) i.d. 5-µm (Equivalent to Shimadzu P/N 228-34937-94) column, protected by C-8 guard, was used for amino acid analysis at column temperature 35 °C. Mobile phase was pumped through the column at a flow rate of 0.3ml/minute and the injection volume was 20 µl. Detection was accomplished by Shimadzu Fluoresce detector which was operated using an excitation wavelength of 350 nm and emission wavelength of 450 nm. The chromatographic run time was kept 90 minutes and resolution of amino acid derivatives was routinely accomplished by using binary gradient system.

### **RESULTS AND DISCUSSION**

The nutritional quality of protein depends upon the total amount of amino acids present in it, relative proportion of the constituent amino acid and the degree to which the animal can liberated and utilize the amino acids from the protein i.e. amino acid availability. The amino acids compositions of different varieties of leguminous seeds viz *Glycine max* variety NRC-37, *Vigna radiata* variety LGG-460, *Phaseolus mungo* variety LBG-20, *Cicer arietinum* variety JG-130, *Lens esculenta* variety JL-3 are mentioned in tabular form in the [Table 1] and chromatograms are represented in [Fig. 1] to [Fig. 5]

Table 1: Amino acid composition of new variety of leguminous seeds (g/100g seed sample)

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Amino Acids	Glycine max NRC-37	Vigna radiata LGG-460	Phaseolus mungo LBG-20	Cicer arietinum JG-130	Lens esculenta JL-3
Aspartic acid	0.902	0.821	0.322	0.483	0.063
Threonine*	2.033	0.373	0.901	0.599	1.283
Serine	0.100	0.044	0.167	1.220	1.004
Glutamic acid	-	0.011	-	-	0.073
Proline	0.092	0.016	0.230	0.045	0.317
Glycine	4.002	0.077	0.045	0.554	1.663
Alanine	0.324	0.023	0.200	-	2.201
Cystine	0.691	0.028	0.311	0.232	0.301
Valine*	2.202	1.192	1.188	0.844	1.244
Methionine*	0.561	0.035	0.350	0.088	0.203
Isoleucine*	2.100	1.310	1.298	0.848	1.082
Leucine*	3.332	1.882	1.905	1.600	1.901
Tyrosine	1.435	0.400	0.342	0.493	0.784
Phenylalanine*	2.072	1.401	1.190	0.974	1.072
Histidine*	0.662	0.662	0.662	0.438	0.662
Tryptophan*	0.442	0.659	0.192	1.322	0.066
Lysine*	1.253	1.744	1.522	1.266	1.760
Arginine	0.456	2.001	1.829	1.566	2.110

<sup>\*</sup>Essential Amino Acids

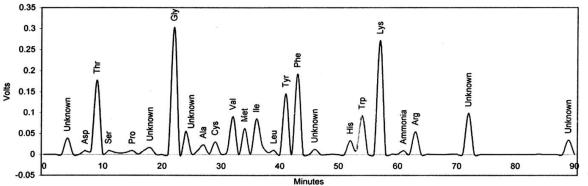


Fig. 1: Chromatogram of amino acid composition of Glycine max variety NRC-37.

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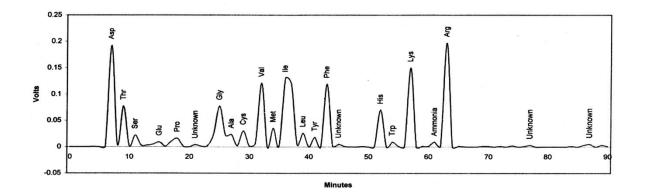
From the perusal of the data of [Table 1] and [Fig. 1] it appears that in the seed proteins of *Glycine max* variety NRC-37, the percentage of Glycine was found maximum (4.002g/100g). Glycine has several important roles within the body. It is essential for the production of many different acids, including nucleic acids, bile acids, creatine phosphate and porphyrins. This amino acid is closely associated with the central nervous system and the digestive system. Glycine helps with the

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breakdown of fat by regulating the concentration of bile acids. Glycine is also required for the biosynthesis of heme. Heme is a key component of haemoglobin. Haemoglobin is essential in the maintenance of red blood cell integrity and optimal oxygen carrying capacity. Glycine is used prostatic for treating schizophrenia, stroke, benign hyperplasia (BPH). and some rare inherited metabolic disorders. It is also used to protect kidneys from the harmful side effects of certain drugs used after organ transplantation as well as the liver from harmful effects of alcohol [11, 12]. However, other amino acids found in increasing order were proline, serine, alanine, tryptophane, arginine, methionine, histidine, cystein, aspartic acid, lysine, tyrosine, threonine, phenylalanine, isoleucine, valine, and leucine. These results also found good agreement with other varieties legume and oil seed [17, 18, 19].



### Fig. 2: Chromatogram of amino acid composition of Vigna radiata variety LGG-460

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Results of [Table 1] and [Fig. 2] shows that the seed proteins of *Vigna radiata* variety LGG-460 was found to contain highest amount of arginine (2.001g/100g). Body uses the amino acid arginine to make nitric oxide. Nitric oxide helps lower blood pressure by relaxing muscles in the blood vessels. It's produced in heart muscles, where it regulates contractions. It may also prevent atherosclerosis by inhibiting the development of plaque in the arteries. Nitric oxide is the active ingredient in nitroglycerin, a medication used to relieve angina, or chest pain caused by coronary heart disease [13, 14]. However, other amino acids also found in the decreasing order were leucine, lysine, phenylalanine, isoleucine, valine, aspartic acid, histidine, tryptophane, tyrosine, threonine, glycine, serine, methionine, cystein, alanine, proline and glutamic acid. These results also found close proximity with other varieties legume seeds [20, 21].

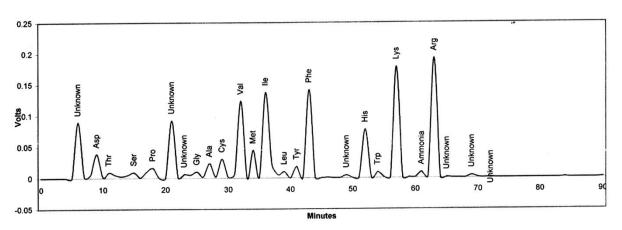


Fig. 3: Chromatogram of amino acid composition of Phaseolus mungo variety LBG-20.

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Data present in [Table 1] and [Fig. 3], it appears that the seed proteins of *Phaseolus mungo* variety LBG-20 were found to contain highest amount of leucine (1.905g /100g). Leucine is the branchedchain amino acids that enhance energy, increase endurance, and aid in muscle tissue recovery and repair. It also lowers elevated blood sugar levels and increases growth hormone production. It is one of three essential amino acids that increase muscle mass and help muscles recover after exercise. It also regulates blood sugar and supplies the body with energy. These



functions make it invaluable when the body is stressed. Leucine is used clinically to help the body heal, and it also affects brain function. It is concluded that the role of leucine In vivo is to provide a signal that amino acids are available, which in combination with the signal of energy availability from insulin, stimulates muscle protein synthesis [15]. However other amino acids in the decreasing order were arginine, lysine, leucine, phenylalanine, valine, threonine, histidine, methionine, tyrosine, aspartic acid, cystein, proline, alanine, tryptophan, serine and glycine. These results also found good accordance with the reported values [21, 22, 23].

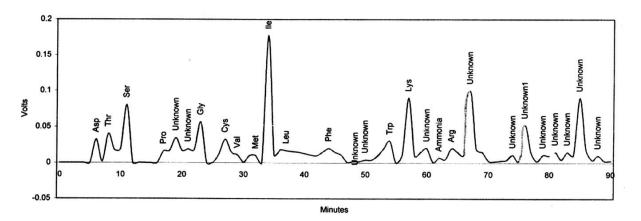


Fig. 4: Chromatogram of amino acid composition of Cicer arientinum variety JG-130. 

From the data given in [Table 1] and [Fig. 4] it appears that the seed proteins of Cicer arietinum (JG-130) was found to contain highest amount of leucine (1.600g/100g) followed by arginine (1.566g/100g). The role of leucine and arginine are discussed in previous varieties of Phaseolus mungo and Vigna radiata respectively [13, 14, 15]. However, other amino acids in decreasing order were tryptophan, lysine, serine, phenylalanine, isoleucine, valine, threonine, glycine, tyrosine, aspartic

acid, histidine, cystein, methionine and proline. These results also found good agreement with other varieties of legumes [20, 24, 25]. 0.25 0.2 0.15 ₹ 0.1 0.05

Minutes

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Fig.5: Chromatogram of amino acid composition of Lens esculenta variety JL-3.

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Results of [Table 1] and [Fig. 5] represent that Lens esculenta variety JL-3 was found to contain highest amount of alanine (2.201g/100g). Alanine is one of the simplest amino acids which involved in the energy-producing breakdown of glucose. Alanine plays a key role in glucose-alanine cycle between tissues and liver. In conditions of sudden anaerobic energy need, when muscle proteins are broken down for energy, alanine acts as a carrier molecule to take the nitrogen-containing amino group to the liver to be changed to the less toxic urea, thus preventing build-up of toxic products in the muscle cells when extra energy is needed [16, 17]. However, other amino acids in the decreasing order were arginine, leucine, lysine, glycine, threonine, valine, isoleucine, phenylalanine, serine, tyrosine, histidine, proline, cystein, methionine, tryptophan, glutamic acid, and aspartic acid. Several investigators have also been studied the amino acid composition in some leguminous seeds [22, 23, 24].

0

-0.05

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### CONCLUSION

From the above analysed data it conclude that the varieties of legume seeds taken under this study were found to rich source of some essential (leucine, isoleucine, valine, phenylalanine, lysine & threonine) and non-essential amino acids (glycine, arginine, cystine, tyrosine & aspartic acid). These seeds may support the essential amino acid requirement of human diet except S-containing amino acids. In order to complete this deficiency leguminous seeds were supplemented with other vegetables, cereals and dairy products. Utilization of legume seeds in daily diet not only will be a good option to overcome the problem of protein-caloric-malnutrition among the people of low income group but also it supports the vegetarian community in Indian prospects.

#### CONFLICT OF INTEREST

There is no conflict of interest.

#### **ACKNOWLEDGEMENTS**

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FINANCIAL DISCLOSURE None

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# ARTICLE A HEURISTIC APPROACH FOR GRAPH BASED MACHINE TRANSLATION

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### ABSTRACT

**Background:** Due to the drastic increase in electronic gadgets and widespread use of the computers two-third of the world population is using one or another type of software's. So this insists software industry develop software's based on linguistic approach. This makes machine translation as one of the most important key research topics in the current era. Many automatic machine translation methodologies exist like linear String matching, Pre context matching and much more. But every system has one or more exceptions that may yield some wrong outputs on some selected occasions. So as a tiny step towards this, **Methods:** this paper deals with the machine translation technique using the graph based approach where for the given phrases in English as respective Hindi phrases are identified. This technique explicitly uses the matrix space translation method for word identification which is catalyzed by the process of similarity index of Jaccard distance. The when the English phrases are given to the system as input, the output is Hindi phrase sentences. **Conclusions:** So, The proposed system using graph based approach having an advantage of poly-directional traversing ability to identify larger semantics between the two vocabularies. This is an added advantage to increase the performance of the system to give best results.

### INTRODUCTION

#### **KEY WORDS**

Sub graph matching; Correlation; Pruning; Similarity index; Pattern identification.

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\*Corresponding Author Email: priyankamalviya1006@gm ail.com Tel.: +91 9175935295 Machines producing the translation from natural language to other without human assistance is termed as Machine Translation [1]. Computational linguistics is Major Domain research domain facilitating text to speech based translation from one language to other. A major challenge is design and development of complete automated Machine Translation System with limited language modeling and other limitations. A Large training dataset of the parallel corpus has been required to achieve reasonable translation. Currently, multilingual machine translation is necessitated to overcome language gap. [2]Target language lexicons with syntactical modeling are basically done in deriving translated text. Future more semantics have been considered inaccurate translation. In commonly observed that target language might have SOV(Sentence Object Verb) pattern whereas source might be in SVO(Sentence Verb Object) pattern. Modeling of this divergence has been done using rules [1]. In context to India 18 constitutional languages with 10 scripts exists and a large number of sub-local languages have been derived. Commonly Adopted Approach is Rule-based Statistical and Hybrid Translation.

**Rule-based Translation:** Translation system build on language protocol has been termed as a rule based system. Rule-based Translation is based on word to lexicon translation, Expert crafted rules have been stored in a database [3],[16],[28].

**Statistical translation:** Phrase to phrase Translation [30] is termed as statistical translation overlooking language format. Fluency has been achieved with statistical translation; as such most industry products like Google translator are based on statistical translation.

**Graph database:** Graph database seems to be the promising solution to model large and huge data. Currently, social networking and routing data are been modeled using graph database [4],[30]. **Correlation:** In most data mining application confidence is a major factor affecting the relevance of output. Correlation is a mathematical value that assists in computing strong relationship with two features. [30] This research work is been organized in six sections as shown below Section I Introduction Section II Related Work, Section III proposed Methodology Section IV results and Discussion Section V Conclusion and Future Scope

### RELATED WORK

To achieve optimal translation results, pattern based Ambiguity is to be modeled. Similarity and dissimilarity measures assist in evaluating pattern based similarity. Solving Classification and clustering problem [7].

System [8] methods focuses on "language divergence" challenge in translation. Interlingua and transfer based Approach for English to Hindi Translation. Work under Research [9] presents Angla Hindi an enhanced version of ANGLABHARATI system. Core Methodology is rule based Interlingua translation. Proposed System uplifts performance with example and statistical analysis.

System [3] has innovated simplified Methodology based on Integration twin techniques firstly ordering English sentences To Hindi Syntax, secondly applying Hindi Suffix. The system has been developed based



smaller dataset. System methods [10] have been delivered at IIT Delhi and address pattern ambiguity in English to Hindi translations. Word net has been found to be the solution to eliminate pattern divergence. The [11] system has based on the monolingual corpus. Corpus has been developed consisting of 44 million sentences and 787 million tokens. Corpus-based approach enhances statistical translation. The corpus is freely accessible. Scope remains to clean and make better corpus dataset.

System [12] addresses a new challenge in translation. Code-mixing frequently encountered today, is mixing of two languages and using them. In the case of English and Hindi, Hinglish is the outcome. System address to identify foreign clauses in translation and perform the pure translation are used in several reports. System have specialized analyzers for Hindi-English summing unknown words. Overall perspective on Indian language has been addressed [13]. Large divergence remains in Indian languages, official reports have been built in local language building language gap. System [14] is designed on joint channel model with the alternative hypothesis. NEW 2009 dataset has been used to train system. The accuracy of 0.47 and F-score of 0.86 has been observed. Limitation observed is for nonstandard runs system performance falls. The scope of work is the enhancement of ranking algorithm token set modification. Matra fully automated system for English to Hindi translation has been described by [15]. The methodology of Matra is robust parsing, Incremental progress has been observed with enhancement in linguistic capabilities. System [16] introduces English to Hindi (EHMT) Translation system. The methodology is analyzing sentence structure of input English and generating Hindi Output based on recursive Tree writing algorithm. Word-Sense Ambiguity is majorly observed Challenge in language translation. [17] This system addresses word sense ambiguity and proposes a framework to eliminate Ambiguity.

These methodology adopted is the rule based correction with statistical input. Ambiguity has been handled effectively with word net implementation. In this System work author [18] marks that language divergence is the major challenge in English to Hindi Translation. Example based Translation system commonly face this challenge. Proposed framework identifies Divergence. Level based Translation system has been implemented by [13]. In the actual scenario no fully automated system exists for any language translation. Challenge observed in the context of translation and audience view.

Statistical translation corrective methodology has been adopted [19]. Effective corrections are done on statistical translation result in better output. A source parser and rule learner model are been presented. The author summarizes the overall status of Machine translation systems [20]. Standalone system, webbased software has been developed in the form of plug-ins for translation. Elaborated survey has been presented on every Indian translation system recognized.

System [21] addresses English to Marathi translation, Adopted methodology is Hybrid Translation. Proposed System implements Statistical translation and then applies rule-based translation for effective translation output. Hybrid Methodology [22] is adapted in English to Marathi translation produces better output. Observations marked by this method are rule based System are costlier and lack fluency. On another hand statistical system lack accuracy but have higher fluency.

Proposed statistical based translation correction with rule based enhances the system. This system [23, 24] available online feature graph based search and effectiveness of graph model. Graph model has been used in the scenario where large data exists to be modeled like social networks, routing paths. Graph based approach assist in mining patterns between objects. Comparative analysis of graph-based search presents Neo4j (Graph database to be better than RDBMS data schema. Graph data model is the scope of data mining and surely would assist large data handling effectively.

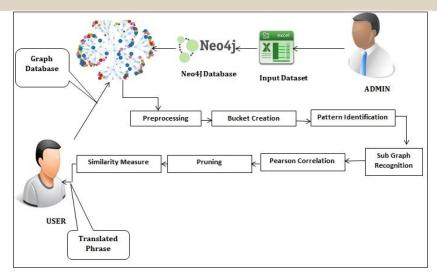
This system [26] has been designed on Moses and deep learning framework. System work [27] focuses on combining rule-based and statistical-based approaches. Each methodology [27], [29], [30] has got own benefits. The fusing of two techniques has been done to achieve the better system.

This system [25] first attempts to find research problem in machine translation. Proposed methodology is statistical machine translation based on graph based Approach. Above article is an extension [20] with complete research evaluation and algorithmic strategy. Graph-based Approach is ultimately the best approach only first of kind innovation presented by above system methods.

### MATERIALS AND METHODS

The proposed system of machine translation from English to Hindi phrases can be detailed in the above overview [Fig. 1]. The Methodologies of this approach is deeply discussed in the below-mentioned steps.





### Fig. 1: System model.

#### A. Step 1:

The proposed system is based on the graph based approach for identification of the proper phrases for the Hindi language for the given phrase in English as input.

As the very initial step of the system we need to train the proposed model with different words in English and their respective alternatives in the Hindi language. So the system needs to feed by this database to train itself by the creation of hyper-graph for this process. A hyper-graph is crafted based on the identification of proper nodes which are unique in their category. This identification of unique elements will be repeated to all categories like English words, Hindi translated words, phrase adjectives, etc.

Once the unique nodes are identified, then these nodes are bounded by the respective edges which indicate the relationship between the two nodes in the form of closest semantics.

Then by using these nodes and edges a hyper-graph is created where common features found clustered on traversing with inner edge formation in between the tiny clusters. This graph is stored in most advanced graph database like neo4j.which will be used further when query phrases are given to the system as input.

#### ALGORITHM 1: Hyper graph Creation

//Input: Data collection Set  $S = {s_i, h_i, s_m}$ //Output: Hyper Graph G (si,hi,sm) Where Si - English Words (node) hi - Hindi words(node) sm- Semantic Step 0: Start Step 1: Get the Set S Step 2: FOR i=0 to Size of S Step 3: Separate s<sub>i</sub>, h<sub>i</sub> into List L<sub>s</sub>, L<sub>h</sub> Step 4: END FOR Step 5: Get unique elements form Ls and Lh Step 6: N<sub>s</sub>=Size of L<sub>s</sub>( Number of nodes for English words) Step 7: Nh=Size of Lh( Number of nodes for Hindi words) Step 8: FOR i=0 to Size of Ns Step 9: FOR j=0 to Size of N<sub>h</sub> Step 10: Identify the relational Edges E using sm Step 11: Form Graph G Step 12: END FOR Step 13: END FOR Step 14: return G Step 15: Stop

B. Step2

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Here in this step user will feed the phrases in the English language which are preprocessing to remove any special symbols and then these words are tokenized in a vector for the further processing.

<sup>.....</sup> 



#### C. Step 3:

Here in this step, the internally hidden words from a word are identified by using matrix space translation process. Where all the word combinations are evaluated by combining with the next character of the word. By doing this system derives other existing words from the given words. This can be easily denoted with the following model.

If the user is given the word called "Going" then our system identifies all its combination words like go, goi, goin, going. Then these four combinations are checked in the dictionary for their existence in English language and then compute a relationship between the words. Like in the considered example system identifies "go" and "going" as the two words from the given word "going" and identifies "present continuous "as the relationship between the two words. This can be represented by the algorithm 2.

#### ALGORITHM 2: MATRIX SPACE TRANSLATION

//Input: Data collection Set **W** = {wi} //Output: Matrix space Set **M** 

Step 0: Start Step 1: Get the Set W Step 2: FOR i=0 to Size of W Step 3: get S<sub>i</sub> of Wi Step 4: FOR j=0 to length of si Step 5: sb<sub>i</sub>=substring (s<sub>i</sub>, 2 $\rightarrow$ j) Step 6: Add sb<sub>i</sub> to M Step 7: END FOR Step 8: END FOR Step 9: return M Step 10: Stop

### D. Step 4:

After the step of matrix space translation with derived words from this is used to create multiple patterns of resulted language words. These patterns are derived with the well-organized combination of words in the entire possible manner. This pattern creation is achieved by using power set generation techniques which yield true combination of words which are represented in the form of vector.

*E.* Step 5:

This is the part where already created graph database has been queried to get the best possible subgraphs for the created patterns. And these subgraphs are analyzed for their correlation with the Hindi words based on the semantics. While performing this correlation, two lists have been created for both the language words for all the possible subgraphs lists to measure the correlation between them using Pearson correlation, which can be represented using equation 1

$$r = \frac{\sum xy - \frac{\sum x \sum y}{n}}{\sqrt{(\chi^2 - \frac{\sum x^2}{n})}\sqrt{(y^2 - \frac{\sum y^2}{n})}}$$

Where

x is the entities of query phrase y is the entities of resulted phrase n is the size List

On evaluating this correlation value will be yielded by r in between 0 and 1. So any value which is nearer to 1 always indicates better correlation.

So the system prunes the values which are less than mid probability, i.e. 0.5 and then all other remained phrases are collected in a list for further investigation of similarity measure.

F. Step 6:

This is the last step of the proposed methodology were finally extracted phrases of Hindi language are measured for the similarity index using Jaccard distance. All the characters of extracted phrase words are exchanged with the proper ASCII values of Hindi language to display the results title.



### **RESULTS AND DISCUSSION**

To deploy the proposed model system uses Java technology with Net-beans as IDE and neo4j as the graph database. The system is put under the hammer for vigorous tests to evaluate its performance by conducting several tests as discussed below.

### Mean Reciprocal Ratio (MRR)

After translation of the phrases from English to Hindi system is to rank the answers in between 0 to 6 as per the satisfaction. For all the ranks from 0 to 6 Reciprocal ratios are assigned (RR) **as 1/1, 1/2, 1/3, 1/4, 1/5, 0.** 

For example if ranked the system as 2 for a result, then reciprocal ratios will 0.5. If it is 3 then reciprocal ratios will 0.33. For any value that is 6 then RR is 0.

The mean reciprocal rank (MRR) is the average score over all yielded answers.

$$MRR = \frac{\sum_{i=1}^{N} \frac{1}{Rank_{\underline{i}}}}{N}$$

Where,

 $Rank_i$  is the rank of the first correct occurrence in the top five ranks for yielded results N is the number of tested results.

The system performed an experiment to evaluate the rank retrieval using the MRR, and the results are summarized as shown in [Table 1].

Table 1. WIRK for unlerent Runs				
No of Phrase	MRR			
10	0.98			
20	0.88			
30	0.79			
40	0.89			
50	0.88			

On plotting graph for the values tabled in [Table 1], observe that proposed model yields average MRR of 0.822 that is 82.2 % and this shows the good sign of any translation system in heuristic approach.

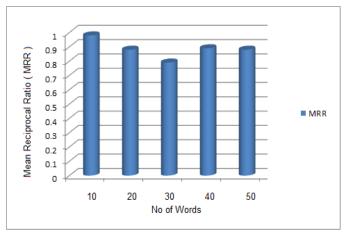


Fig. 2: Performance evaluation through.

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### Performance evaluation

Headings, The performance of the system are measured using most common and effective features like precision and recall. Precision is used to evaluate the relative preciseness of the system which always exposes the fact of Positive-ness of the system. Whereas Recall is using to measure the relevance of the system and it shows the sensitivity of the system through which it has been evaluated.

Precision can be stated as ratios of relevant phrases are translated to the sum of Relevant and irrelevant phrases are translated.

Which can be more deeply explain with the below equation

$$P(x) = (R/(R + IR))*100$$



#### Where,

P(x): Precision Function R: Number of Relevant phrases is translated IR: Number of Irrelevant Phrases is translated

Recall can be stated as ratios of Relevant phrases are translated to the sum of relevant phrases are translated and relevant phrases are not translated. Which can be more deeply explain with the below equation

$$R(x) = (R/(R+!R))*100$$

Where,

P(x): Recall Function R: Number of Relevant phrases is translated ! R: Number of Relevant Phrases is not translated

System conducted the experiment based on precision and recall parameter and gathered information is tabulated in the below [Table 2].

		Table 2: Precision and Recall Performance			
No. of Phrases	R	!R	IR	Precision	Recall
10	10	0	0	100	100
20	16	1	3	84.21053	94.11765
30	25	0	5	83.33333	100
40	38	1	1	97.4359	97.4359
50	47	1	2	95.91837	97.91667

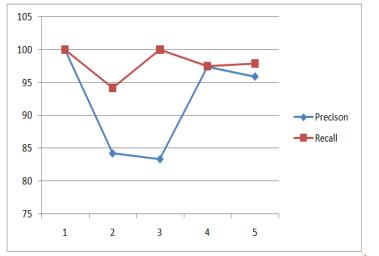


Fig. 3: Performance comparisons of precision and recall evaluation. .....

> By observing the above plot, observe that both precision and recall are achieved approximately 90% of performance accuracy which is the good sign of the proposed system using graph-based approach for machine translation process.

### CONCLUSION

This Research work addresses English to Hindi translation problem. System accepts English phrases generating Hindi translation on higher accuracy and fluency. The quality of translation depends on size of input of existing system, lack word ambiguity and lack rule knowledge. Search space for translating is large in existing system, which are major challenges addressed by our research work. Most of the rule based machine translation systems are having larger probability of yielding non semantic results. This is all due to missing of very much needed semantic relationship between the two vocabularies. So graph based approach of the proposed system is having an advantage of poly-directional traversing ability to identify larger semantics between the two vocabularies. This is an added advantage to increase performance of



the system to its best. And this can be analyzed in the prior section with linear rule based system of machine translation. The analysis of the system performance can be done by the Precision and recall method which observe that both precision and recall are achieved approximately 90% of performance accuracy which is the good sign of the proposed system using graph based approach for machine translation process.

Graph based machine translation system can be enhance in the future by adding large and very large phrase conversion techniques using huge databases in distributed systems and cloud computing paradigm. The work can be extended to include multilingual corpus of different languages in the source-target pair. The target and source languages can be increased from present one language. The system can also be put in the web-based portal to translate content of one web page in English to Hindi. A mobile application can also be developed in which message containing English text is sent to the client in Hindi language. The translated text can be reordered and processed to overcome grammatical mistakes which will be part of post-processing. This will improve score of human evaluation. What has been achieved by above work:-

- Accuracy and fluency in balance way is been achieved which existing system lack.
- Graph based approach enhances search process.
- First of kind work to model Machine translation in graph scenario.
- As system data increases precision enhances.

# CONFLICT OF INTEREST

#### FINANCIAL DISCLOSORE None

#### ACKNOWLEDGEMENTS

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# ARTICLE PROTECTIVE ROLE OF LETTUCE (Lactuca sativa L.) ON ETHANOL ALCOHOL CONSUMPTION IN MALE REPRODUCTIVE SWISS MICE

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### ABSTRACT



The present study aimed to evaluate the protective effects of Lettuce (Lactuca sativa L.) against the toxicity caused by ethanol consumption in reproductive system of mice. The histopathological changes in testis and epididymis antioxidant enzymes activities of catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MAD) and glutathione peroxidase in testis-homogenized tissue were investigated. Twenty mice were categories into four groups, each with five mice. Control group: received normal saline (0.25ml), second group received lettuce leaf extract (150 mg/kg body weight), third ethanol group received 50% ethanol, and the fourth is Lettuce-Ethanol group received ethanol 50% + lettuce extract (150 mg/kg body weight). Treatment was carry out for 20 days. The results indicated various histological changes in testicular and epididymis tissue induced by ethanol consumption with significant decreased in antioxidant activity levels and increased in lipid peroxidation compared to control group and co-administration with lettuce compensated the damage effects significantly. We concluded that co-treatment mice with lettuce prevent the adverse effect of ethanol toxicity in reproductive male system which proposed the highly antioxidant properties.

### INTRODUCTION

**KEY WORDS** 

Lactucasativa L. Testicular antioxidant activity, Testes histopathological changes.

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Green leafy vegetables (fresh vegetables) and fruits are important components of a healthy diet [1]. They have dietetic, nutritive value and some may also have the medicinal value [2]. They are providing important vitamins, minerals, and phyto-nutrients [3]. Phyto-nutrients can act as antioxidants, which help to prevent chronic diseases like cancer and cardiovascular diseases [4]. Also many epidemiological studies demonstrated the relationship between dietary habits and disease risk. Because of these potential benefits, many healthy programs around the world have been promoting the consumption of fresh vegetables to prevent diseases [5]. Now days the use of alternative medicine in particular herbal therapies has been propagated among people, because of its economic and no/low side effects [6] Consumption of medicinal plants have the concerned due to the essential antioxidant probable of the phytochemicals that diminish the free-radical and protecting against induced oxidative damage. One of the most common vegetable that consumed as salad in the world is Lettuce (Lactuca sativa L.) and that belongs to a member of the composite. Lettuce exhibits healthy properties mainly due to the presence of antioxidant compounds (vitamins C and E, carotenoids, polyphenols) alongside significant fibre content and useful amounts of certain minerals [7]. Therefore it used for treatment of a variety of disorders such as insomnia, neurosis, dry coughs, rheumatic pain [8], and anxiety [9].

The reproductive system, like other body's systems, is affect by many exo and/or endo factors that may have positive or negative effects; for this, many studies are trying to find alternatives like plants to eliminate the effects of negative factors and as a treatment for some cases because of its low cost and the possibility of consumption daily. Alcohol like many factors showed negative effects on many body organs especially the reproductive organs. The essential component of alcoholic beverages is ethanol, the substance responsible for chemical addiction and for a chronic, progressive disease, the alcoholism. Ethanol acts as a toxic component to vigorous organs, acting harmfully on different tissues [10 - 12]. In human, high alcohol consumption is associated with serious damage of spermatogenesis [13]. That reduces sexuality on fertilization. Accordingly, in this study we focused on lettuce as a protective factor and its ability in reducing the negative effects of chronic alcohol consumption.

### MATERIALS AND METHODS

Lettuce leave extraction: Air-dried young Lettuce leaves were ground to powder and the extract prepared using Soxhlet extraction method, ethanol was used as a solvent at a ratio of 1:10 w/v. the extract was evaporated and reconstituted to 10 ml with distilled water and kept at 4 C until used.

Animals: Fifteen mice were housed in animal laboratory of biological department / college of education of pure sciences, Ibn Al-Haitham / University of Baghdad under controlled environmental conditions (12L:12D light cycles; 24C°± temperature). Water and food were given ad libitum.

Experimental design: Animals were divided into three groups, 5 each as follows:

- Control group: received normal slain 1-2-
  - Lettuce group: received leave lettuce extract (150 mg/kg)
- 3-Ethanol group: received 50% ethanol orally for 20 days
- 4-Lettuce-Ethanol group: received ethanol for 20 days + Lettuce extract (150 mg/kg body weight).



**Prepare tissue for histological studies:** The right testis fixed in 10% formaldehyde, followed by dehydration with gradual series of alcohol (30 – 100%) and cleared in two changes of xylene, then embedded in paraffin wax for sections. Hematoxylin (Harrison) - Eosin stain was used and sections visualise under light microscope [14].

**Tissues preparations for biochemical studies:** Animals were cervical decapitation, after dissected testis, the left testis washed with saline solution and homogenize with KH<sub>2</sub>PO<sub>4</sub> buffer (100 mM) with EDTA (1 mM, pH 7.5). The homogenize tissue was centrifuged at 13000 g for 20 min at 4 C and supernatant kept for the following biochemical assays.

**Determination of lipid peroxidation:** Lipid peroxidation was determined according to Guidet and Shah [15]. briefly; 1 ml supernatant of homogenise testes tissue mixed with 1 ml of trichloroacetic acid

17.5 % and 1ml of 0.6% thiobarbituric acid. The mixture was incubated in water bath at  $100^{\circ}$  for 15 min, after cooling 1ml of 70% TCA was add and left to stand at room temperature for 20 min. samples mixture then centrifuged at 2000 rpm for 15 minutes, and the absorbance was measured at 532 nm.

**Determination of catalase activity:** CAT enzyme activity was determined followed a method of Aebi [16]. Briefly, 100  $\mu$ L of supernatant homogenized tissue was added to 1.9 mL of 50 mM phosphate buffer (pH 7.0). Then 1.0 mL of freshly prepared 30 mM H<sub>2</sub>O<sub>2</sub> was added to start the reaction. The rate of change was measured at 240 nm.

**Determination of glutathione peroxidase activity:** Activity of GSH-Pxs was determined according to Godin et al. [17]. The assay mixture contained of phosphate buffer (50 mM, pH 7.0), glutathione (50 mM), 0.1 mL of 30 units/mL glutathione reductase, 0.1 mL of EDTA, 0.1 mL of NADPH (2 mM) and 0.2 mL of samples. The reaction was started by the addition of 0.2 mL of 0.25 mM H<sub>2</sub>O<sub>2</sub>. The rate of change of absorbance was recorded at 340 nm spectrophotometrically for 3 min.

**Determination of superoxide dismutase activity:** SOD was determined according to Beyer and Fridovich [18]. Briefly, The assay mixture contained of phosphate buffer (50 mM) with EDTA (0.1 mM, pH 7.8); 0.03 g/mL, L-methionine; 1.41 mg/mL nitroblue tetrazolium chloride (NBT•2HCl); 1% Triton X-100® and 0.4 mL of the samples. The reaction was started by adding 10 µL riboflavin. The mixtures then put in an aluminum box with fluorescent lamps of 20 W for 10 min, and the absorbance was read at 560 nm before and after lighting.

#### Statistical analysis

Statistical analysis was performed using Minitab 16. All results are presented as mean  $\pm$  SEM. Significance was accepted at P  $\leq$  0.05. The antioxidant treatment data was analysed by one-way analysis of variance (ANOVA) followed by Fisher's least Significant Difference (LSD) test.

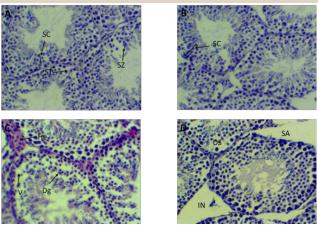
### RESULTS

**Changes in enzymes activity induced by ethanol and lettuce extract:** Ethanol treatment significantly decreased the activities of CAT, SOD, glutathione peroxidase and increased in MAD levels as shown in [Table 1] Treatment with lettuce extract significantly improved were the enzyme activities increased whereas MAD level decreased significantly in the tissue homogenate as compared to ethanol treated group.

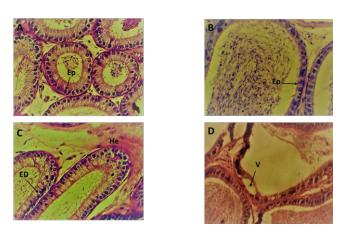
**Testicular histology:** The light microscopy results showed normal and intact spermatogenesis epithelium in the control and lettuce treated groups [Figure 1 A and B]. However, different histo pathological alterations were noticed in group treated with ethanol compared to control group. Detected injuries in the seminiferous tubules showed disruption, exfoliation of germinal cells and detachment of different stages of spermatocytes into the lumen [Figure 1 C]. In the other hand, atrophy and degeneration of spermatocytes lining the seminiferous epithelium appeared to be exhausted with appearance of vacuolated tubules. Some of Leydig cells showed nuclear destruction, blood vessels of the interstitial tissue appeared larger and congested. Lettuce + ethanol treated group showed improved in seminiferous tubules structure involving normal spermatogenesis cells along with the interstitial tissue [Figure 1 D].

**Epididymis:** Epididymis epithelium of control groups as well as lettuce treated group exhibited the typical ciliated epithelial cells (columnar cells in the head and cuboidal cells in the tail) based on basement membrane and enclosed by smooth muscles with connective tissue [Figure 2 A and B]. Epididymis epithelium of ethanol treaded groups showed declined in the epithelial cell height, detachment of epithelial cells from basement membrane and decreased in sperms numbers with occurs of vacuolated epithelial cells and some necrotic cells. Enlargement of interstitial spaces between the tubules also found [Figure 2 C and D], where the epididymis sections of Lettuce + ethanol treated group revealed ameliorated epithelial architecture.





**Fig. 1:** Crosse section of testes mice showing (A) Control group with normal spermatocytes cells (SC) and sperm (SZ) with Sertoli cell (ST). (B) Testes of mice treated with (150 mg/kg) lettuce extract showing that most seminiferous tubules appeared intact (SC). (C) Testes of mice treated with 50 % of ethanol showing interstitial hemorrhage (He), degeneration (Dg) of some spermatocytes, large vacuole in spermatocytes cytoplasm (V). (D) Testes mice treated with 50% of ethanol + lettuce showing moderated lesion including: disruption and exfoliation in some tubules (DS), seminiferous tubule atrophy (SA), large interstitial space (IN). Stained with H&E, sectioned 400 X.



**Fig. 2:** Cross section of epididymis in mice of (A) Head of control and group treated with lettuce leave extract showing normal columnar epithelium. (B) Tail of control and group treated with lettuce extract showing standard cuboidal cell lining in basement membrane. (C) Head of mice treated with 50% ethanol showing detachment of epithelium, enlargement of interstitial space and hemorrhage. (D) Tail of mice treated with 50% ethanol showing decreased epithelial cell high with appearance of vacuole. Stained with H&E, sectioned 400X.

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Table 1: Antioxidant enzymes activity (CAT), (SOD), (MAD) and glutathione peroxidase in homogenised testes tissue of albino mice treated with ethanol extracts of Lactuca sativa L

Groups	Dose Mg/kg	SOD U/ml	CAT U/min	MAD μmol/ml	PXs µM/ml		
		Treatment for 20 days					
control	Normal saline	A, C 78.9±0.3	A,C44.1±0.3	A, D41.4±1.1	B 27.16±1.5		
ethanol	50%	B, C 56.1±0.4	C28.4±0.3	D57.7±1.2	B18.28±1.4		
Lettuce	150 mg/kg	C73.2±0.2	C 39.0±0.4	D 45.5±1.7	B 26.50±1.6		
Ethanol +	50% + 150	A, B 67.1±0.4	A36.7±0.6	A51.0±1.5	B 25.04±1.6		
lettuce	mg/kg						

A: significance from control group at P  $\leq$  0.05, B: significance from ethanol group at P  $\leq$  0.01, C: significance from ethanol group at P  $\leq$  0.001, D: significance from ethanol group at P  $\leq$  0.05.

### DISCUSSION

Alcohol consumption has been identified as contributor to chronic tissue injury. This study was carried out to investigate the protective role of Lettuce leave extract in testicular tissue and as well as the antioxidant



level in mice administrated ethanol. Results observed in the present study suggested that ethanol induced significant decrease in CAT, SOD, and GS-Pxs activity levels, along with increase in MDA level in testis. Furthermore, study results have been shown that ethanol is capable of affecting seminiferous epithelium and altering spermatogenesis. This results were consistent with previous studies which have been determined the effects of ethanol on different animal tissues through metabolic activation to highly reactive substances such as free radicals. Maneesh et al., [19] suggested that histopathological changes observed in rat testes treated with ethanol was due to increase the oxidative stress, as a consequence of free radicals levels increased and a declined in antioxidant defense. Moreover, the other suggested mechanism of the antioxidant activity decreased in testes induced by chronic alcohol feeding, may be due to the enhanced of lipid peroxidation [20]. Histopathological damages in our results is in concert with a study by [21] which suggested that alcohol has a direct and in toxic effect from the decreased in seminiferous tubular function due to negative feedback of fertility hormones; and indirect effect through the HPG axis. The increased in MAD level in this study supported the deleterious effect of alcohol in spermatocytes; spermatozoa are particularly susceptible to oxidative stress-induced damage because their plasma membrane contain large quantities of polyunsaturated fatty acid and their cytoplasm contains low concentration of scavenging enzymes [22]. It has been reported that both acute and chronic consumption of alcohol causes histo-testicular damage, which obstructs spermatogenesis with, decreased in sperm count because of prolonged oxidative stress [23]. On the other hand, mitochondrial function can be impaired by alcohol, thus, promotion of spermatocytes cell death (apoptosis and necrosis) indicated as a result [24].

The interested results in this study was the significant enhanced of lettuce treatment in both histological and antioxidant activities and reduced the lipid peroxidation, this results proposed the free radical scavenging role of the lettuce extract. Oxidative damage can arise as consequence of oxidative stress caused by significant production of reactive oxygen species (ROS), or diminished antioxidant defense mechanisms [25]. Testis is susceptible to oxidative damage than other organs because of its low antioxidant capability and occurrence of polyunsaturated fatty acids in its cell membrane, which are easy targets to oxidative damage by free radicals. Thus, the need for useful green therapeutic agents for enhancing male fertility has been required [26]. Bioactive phytochemicals founds in plants such as saponins, alkaloids and flavonoids have been determined to be valuable therapies for various diseases. For example, flavonoids have been found to be a valuable spectrum of biological activities such as, antiinflammatory, anti-microbial, anti-proliferative and antioxidant agent [27]. Hefnawy and Ramadan [28] suggested that lettuce extract capable of reduced lipid peroxidation of ethanol induced oxidative damage in liver and testes in rats due to the presence of flavonoids and saponins in the extract. Additionally, the antibictrial and antiviral activities of lettuce methanol extract due to highest total phenolic contents [29]. Moreover, the protective properties of hydro-alcohol extract of Lactuca sativa against DNA and protein oxidation with highly value of scavenging activity has been demonstrated [30].

### CONCLUSION

Taken together, the results of this study provided new evidence in some potential mechanisms of the effect evince protective role of ethanol extracts of Lactuca sativa L to reduce alcohol histopathological impact on male fertility.

CONFLICT OF INTEREST There is no conflict of interest.

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