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Dear Esteemed Readers, Authors, and Colleagues,

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

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

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

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

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

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

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



Yours sincerely,

*Vasco Azevedo*

**Vasco Azevedo**, Editor-in-Chief  
Integrative Omics and Applied Biotechnology  
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# AMELIORATIVE ROLE OF ANTIOXIDANT MICRONUTRIENTS: SELENIUM, VITAMINS C AND E ON OXIDATIVE STRESS AND WOUND HEALING IN TYPE 2 DIABETIC PATIENTS WITH FOOT ULCER IN IBADAN

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## ABSTRACT

Wound healing is a complicated process that requires several steps. The involvement of hyperglycaemia and oxidative stress (OS) in healing process has been reported. This study investigated the effects of selenium, vitamins C and E supplementation on OS indices such as lipid peroxides (LPO) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) in wound healing in diabetic patients with foot ulcer. The study comprised fifty non-diabetics (Group A) and fifty diabetics (Group B) between 40 and 60 years. Group B were divided into supplemented (1) and non-supplemented (2) subgroups. Patients in B1 were given 1000mg vitamin C + 400 mg vitamin E + 100 µg selenium for 16 weeks. Subgroup B2 received no supplementations. Wound healing process was assessed in Group B using 'ABDEFS' tools of evaluating chronic ulcers. Blood samples of 10 ml were collected. 8-OHdG was determined by ELISA; LPO, TAS, SOD and GPx were measured spectrophotometrically. Data were analysed statistically using Wilcoxon tests at  $p \leq 0.05$ . Before supplementation, increases of 79.37 and 54.91% respectively in LPO and 8-OHdG levels with decrease of 53.52% in TAS were observed in Group B compared with Group A. After supplementation, reductions of 22.50 and 22.48% respectively in LPO and 8-OHdG with 10.77% increase in TAS were found in subgroup B 1 compared with subgroup B2. Decreased 'ABDEFS'-score of 22.91% was observed in subgroup B1 compared with subgroup B2 thus revealing better healing. Antioxidant micronutrient supplementation demonstrated ameliorative effect on oxidative stress and wound healing in Type 2 diabetes with foot ulcer.

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### KEY WORDS

Diabetic foot ulcer; Oxidative stress; Antioxidant micronutrients; Type 2 diabetes mellitus

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## [I] INTRODUCTION

Diabetes mellitus (DM) is a growing non-communicable disease worldwide. It is a metabolic disorder characterized by chronic hyperglycaemia with disturbances in carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both [1]. Among the associated morbidities of DM is impaired/delayed wound healing. Healing is a normal physiological process that proceeds through a series of co-ordinated cellular and cytokine-mediated events, culminating in the restoration of functional integrity of tissues [2]. Healing is delayed for several reasons under certain circumstances such as diabetes, starvation, ageing and in immunocompromised situations [2]. The effects of diabetes on healing are diverse, multifactorial, complex and inter-related. Diabetes mellitus, to some extent, affects almost all stages of wound healing [3]. Free radicals and their scavenging systems are known to have a very important role in healing of normal and/or delayed healing of

wounds [4]. The magnitude of free radical generation and the antioxidant defence systems are also known to be altered in diabetic condition [2]. Hence, this present study aimed at investigating the possible ameliorative effect of antioxidant micronutrients on oxidative stress and wound healing in Type 2 diabetes mellitus with foot ulcer.

## [II] MATERIALS AND METHODS

### 2.1. Patient Selection

Fifty Type 2 diabetic subjects with Wagner's Grade 2 foot ulcer (i.e. ulcer without abscess or osteomyelitis) attending the Medical Out-Patient (MOP) Unit and patients admitted in the Medical Wards (MW) of University College Hospital, Ibadan, and Adeoyo Hospital, Ring Road, Ibadan, Nigeria were recruited into the study as test group (Group B). This test group comprises male (54%) and non-pregnant/lactating female (46%) between the ages of 40 and 60 years (52.04±8.65). Fifty non-

diabetic healthy subjects matched for age and sex were recruited as control group (Group A) after signing the informed consent form.

The diabetic subjects were on diet restriction and/or oral hypoglycaemic agents. They were divided into two groups of twenty-five each as follows: Supplemented Type 2 diabetes mellitus with diabetic foot ulcer (Group B1) and Non-supplemented Type 2 diabetes mellitus with diabetic foot ulcer (Group B2). Group B1 were given 1000 mg ascorbic acid (Ascomed, Kunimed Pharmchem Limited, Lagos), 400 mg  $\alpha$ -tocopherol ( $\alpha$ -tocopherol acetate, Korea United Pharm. Int'l Inc.) and 100  $\mu$ g Se (Selenium ACE, Wassen International Limited, UK), dissolved in 100 ml of vanilla flavoured milky beverage while Group B2 received 100 ml of vanilla flavoured milky beverage only for 16 weeks (The amount of vitamins C and E in Selenium ACE has been incorporated in the daily dosages. Vanilla flavour was used to improve the taste of mixture). Patients were not given placebo because of their diabetic state. Both the supplemented and non-supplemented subjects were given the same medical management in terms of glucose control and wound dressing. In order to evaluate their compliance, they were asked to come back every fortnight to receive the next antioxidant supplements until the end of study.

This study was approved by the Joint Ethical Committee of the University of Ibadan and the University College Hospital Institutional Review Committee (UI/UCH IRC) (approval number UI/IRC/03/0096).

## 2.2. Blood Sample Collection

Ten millilitres aliquots of venous blood were drawn after a ten-hour overnight fast and collected into heparinised and EDTA sample tubes. The blood samples were collected at the beginning (0 week) of the study, 8 and 16 weeks after daily supplementation. All samples were centrifuged at 3000 rpm for 10 minutes. Plasma and haemolysate were stored at  $-80^{\circ}\text{C}$  until the day of analysis and vitamin C samples were analysed within 3 hours of collection. Antioxidant enzymes (SOD and GPx) were measured in heparinised whole blood, total antioxidant status (TAS) was measured in heparinised plasma, while Se, vitamin C, Vitamin E and oxidative status parameters (LPO, 8-OHdG) were determined in EDTA plasma.

## 2.3. Biochemical Analyses

Lipid peroxide concentrations were measured spectrophotometrically at 560 nm using the ferrous oxidation with xylenol orange (FOX VERSION

II) assay according to the method of Nourooz-Zadeh et al. [5]. This method is based on the principle of rapid peroxide-mediated oxidation of  $\text{Fe}^{2+}$  to  $\text{Fe}^{3+}$  under acidic conditions. Plasma levels of 8-OHdG were measured at 450nm on a microplate plate reader using a commercial kit from the Japan Institute for the Control of Aging (Fukuoro, Japan). The method is based on a competitive in-vitro enzyme-linked immunosorbent assay for quantitative measurement of this DNA metabolite in tissue, serum and plasma [6]. Erythrocyte SOD activity was determined by the method of Arthur and Boyne [7] using Randox kit (Randox Laboratories, UK). This method uses xanthine and xanthine oxidase (XOD) to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. Superoxide dismutase activity was then measured by the degree of inhibition of this reaction spectrophotometrically at 505 nm. The determination of erythrocyte GPx activity was based on modified method of Paglia and Valentine [8] using Randox kit (Randox Laboratories, UK). This method involves the oxidation of glutathione (GSH) by cumene hydroperoxide catalysed by GPx. In the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to  $\text{NADP}^{+}$  and the decrease in absorbance is then measured spectrophotometrically at 340nm. Total antioxidant status (TAS) in heparinised plasma was determined by the method of Miller et al. [9] using Randox kit (Randox Laboratories Limited, U.K). In this method, ABTS(R) (2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]) was incubated with metmyoglobin, a peroxidase, and  $\text{H}_2\text{O}_2$  to produce the radical cation  $\text{ABTS(R)}^{\cdot+}$ . This reaction has a relatively stable blue-green colour which was measured at wavelength of 600 nm. Antioxidant in the added sample caused suppression of this colour production to a degree which is proportional to their concentration. Vitamin C (ascorbic acid) was determined spectrophotometrically at wavelength of 700 nm in plasma by the acid phosphotungstate method of Aye Kyaw [10]. Vitamin E ( $\alpha$ -tocopherol) in plasma was extracted using xylene, and its level was assayed spectrophotometrically at 520 nm according to the method of Baker and Frank [11]. Selenium in plasma was determined by the method of Pleban et al. [12].

## 2.4. Clinical assessment of ulcer

The 'ABDEFS' tool of evaluating chronic ulcers [13] was used in clinical assessment of wound healing progress. This system consists of scoring the ulcer based on certain features that begin with the first few English alphabets (excluding letter "c" but including letter "s") as follows:

A.	Aetiology:	1. Local, e.g. trauma, infection 2. Controlled systemic disease 3. Systemic disease, uncontrolled 4. Malignancy
B.	Base:	1. Soft, mobile 2. Hard fixed
D.	Discharge:	1. Slight to moderate 2. Copious, purulent, etc
E.	Edge:	1. Flat, shelving, punched out 2. Undermined, raised
F.	Floor:	1. Predominantly granulation 2. Predominantly sloughy
S.	Size:	1. Less than or equal to 2.5 cm in one dimension 2. Greater than 2.5 cm in one dimension.

Points corresponding to appropriate description were allocated to each feature on the ulcer. The minimum possible score of 6 denotes the best healing ulcers while the maximum score of 14 denotes the worst healing ulcers. The 'ABDEFS' assessing tool was selected because the six different aspects of 'ABDEFS' scoring system were various characteristics normally utilized in the description of an ulcer. This tool was easy to measure and no special training was required.

## 2.5. Statistical analysis

All data were presented as Mean  $\pm$  Standard Deviation (SD) for 50 subjects in each group at baseline and 25 subjects in each subgroup after supplementation. The Statistical Package for Social Sciences (SPSS) (version 16) was used for statistical evaluation. Significance of differences was determined using Wilcoxon Test. Percentage (%)

increase or decrease was used to interpret the differences between supplemented and non-supplemented subgroups. Statistical significance was set at  $p \leq 0.05$ .

### [III] RESULTS

At the beginning of the study, significantly higher values were observed in fasting plasma glucose ( $13.01 \pm 3.50$  vs  $5.09 \pm 0.53$  mmol/L), glycated haemoglobin A1c ( $8.51 \pm 2.0$  vs  $4.08 \pm 0.75$  %), body mass index ( $26.59 \pm 2.44$  vs  $22.93 \pm 1.69$  kg/m<sup>2</sup>), lipid peroxides ( $56.34 \pm 10.04$  vs  $31.41 \pm 15.96$   $\mu$ mol/L) and 8-hydroxyl-2'-deoxyguanosine ( $49.37 \pm 7.78$  vs  $31.87 \pm 11.58$  ng/ml ( $p < 0.001$ ) when patients with diabetic foot ulcer (Group B) were compared with non-diabetic subjects (Group A). The data also revealed lower activity of superoxide dismutase ( $4169.15 \pm 635.78$  vs  $4408.61 \pm 1630.42$  U/g Hb;  $p > 0.05$ ) and higher activity of glutathione peroxidase ( $1281.64 \pm 240.36$  vs  $1055.49 \pm 308.79$  U/g Hb;  $p < 0.001$ ) in Group B compared with Group A. In addition, decreases were observed in total antioxidant status ( $0.66 \pm 0.13$  vs  $1.42 \pm 0.17$  mmol/L), vitamin C ( $0.003 \pm 0.002$  vs  $0.01 \pm 0.003$  mmol/L) and vitamin E ( $0.05 \pm 0.02$  vs  $0.06 \pm 0.005$  mmol/L) and selenium ( $0.46 \pm 0.12$  vs  $0.81 \pm 0.27$   $\mu$ mol/L) ( $p < 0.001$ ) when Group B patients were compared with Group A subjects.

However, supplementation with combined Se, vitamins C and E at 8 and 16 weeks resulted in decreases of 6.86, 7.33, and 6.05% at 8 weeks and of 9.07, 11.97 and 10.44% at 16 weeks respectively in BMI, FPG and HbA1c of the supplemented (B1) subgroup. Reductions of 4.57 and 6.95% in BMI, of 4.58 and 10.04% in FPG and 4.77 and 7.10% in HbA1c were observed at 8 and 16 weeks respectively in non-supplemented (B2) subgroup. Lipid peroxide and 8-OHdG levels were observed to decrease in subgroup B1 with percentage decreases of 12.51 and 10.43% ( $p > 0.05$ ) respectively at 8 weeks and of 22.50 and 22.48% ( $p < 0.01$ ) respectively at 16 weeks, but increased in the non-supplemented group (B2) by 8.45 and 18.73% in LPO and by 15.30 and 30.0% in 8-OHdG at 8 and 16 weeks respectively. Total antioxidant status increased by 6.15% ( $p > 0.05$ ) and 10.77% ( $p < 0.01$ ) respectively at 8 and 16 weeks of supplementation in the supplemented group, while decreases of 4.48 and 8.96% respectively were recorded at the same period of supplementation in the non-supplemented group. The 'ABDEFS' tool was observed to decrease by 11.01 and 22.91% respectively at 8 and 16 weeks compared with baseline ( $8.08 \pm 1.38$ ,  $7.0 \pm 1.26$  vs  $9.08 \pm 1.61$ ) in the supplemented subgroup while increase of 6.87 and 15.02% was found in the non-supplemented subgroup ( $9.96 \pm 1.27$ ,  $10.72 \pm 1.24$  vs  $9.32 \pm 1.44$ ), at the same period of supplementation [Table-1].

**Table 1. Effect of 8 and 16 weeks of supplementation with Se, vitamins C and E on Oxidative stress indices in supplemented diabetic foot ulcer patients (Group B<sub>1</sub>) and non-supplemented diabetic foot ulcer patients (Group B<sub>2</sub>)**

90Parameters (n=25)	Group B <sub>1</sub> (0 wk) (Mean±S.D)	Group B <sub>1</sub> (8 wk) (Mean±S.D)	Group B <sub>1</sub> (16 wk) (Mean±S.D)	<sup>1</sup> Wilcoxon (p-value)	<sup>2</sup> Wilcoxon (p-value)	Group B <sub>2</sub> (0 wk) (Mean±S.D)	Group B <sub>2</sub> (8 wk) (Mean±S.D)	Group B <sub>2</sub> (16 wk) (Mean±S.D)	<sup>3</sup> Wilcoxon (p-value)	<sup>4</sup> Wilcoxon (p-value)
BMI (kg/m <sup>2</sup> )	26.68±2.72	24.85±2.90	24.26±2.84	2.839* (0.005)	3.377* (0.001)	26.49±2.17	25.28±1.76	24.65±1.77	1.722 <sup>ns</sup> (0.085)	2.489 <sup>†</sup> (0.013)
FPG (mmol/L)	12.92±3.57	11.98±3.36	11.38±3.40	2.059 <sup>†</sup> (0.040)	2.570 <sup>†</sup> (0.010)	13.10±3.50	12.50±3.52	11.78±3.63	0.605 <sup>ns</sup> (0.545)	1.372 <sup>ns</sup> (0.170)
HbA1c (%)	8.43±1.94	7.92±1.77	7.55±1.67	1.790 <sup>ns</sup> (0.073)	2.287 <sup>†</sup> (0.022)	8.59±2.10	8.18±1.90	7.98±1.86	0.600 <sup>ns</sup> (0.549)	0.727 <sup>ns</sup> (0.467)
LPO ( $\mu$ mol/L)	62.28±7.36	54.49±8.06	48.27±10.15	0.740 <sup>ns</sup> (0.459)	2.600 (0.009)	50.41±8.85	54.67±13.24	59.85±13.45	0.283 <sup>ns</sup> (0.778)	1.009 <sup>ns</sup> (0.313)
8-OHdG (ng/ml)	53.51±5.23	47.93±8.38	41.48±9.82	0.969 <sup>ns</sup> (0.333)	2.812 (0.005)	45.23±7.78	52.15±9.98	58.80±11.91	1.400 <sup>ns</sup> (0.162)	2.812 (0.005)
SOD (U/g Hb)	4123.99±634.43	4128.62±632.42	4239.29±669.45	0.040 <sup>ns</sup> (0.968)	0.578 <sup>ns</sup> (0.563)	4214.33±644.93	4009.28±513.51	3789.33±469.20	1.413 <sup>ns</sup> (0.158)	2.516 <sup>†</sup> (0.012)
GPx (U/g Hb)	1325.28±282.90	2307.26±2559.90	2853.99±3434.48	1.413 <sup>ns</sup> (0.158)	2.516 <sup>†</sup> (0.012)	1237.99±184.26	3245.38±3349.87	3159.66±3329.29	2.193 <sup>†</sup> (0.028)	2.408 <sup>†</sup> (0.016)
TAS (mmol/L)	0.65±0.10	0.69±0.09	0.72±0.08	1.292 <sup>ns</sup> (0.196)	2.705 (0.007)	0.67±0.15	0.64±0.13	0.61±0.14	0.772 <sup>ns</sup> (0.440)	1.575 <sup>ns</sup> (0.115)
Vitamin C (mmol/L)	0.003±0.002	0.01±0.005	0.01±0.005	3.296 (0.001)	3.956 (0.000)	0.003±0.002	0.003±0.002	0.003±0.003	0.229 <sup>ns</sup> (0.819)	0.121 <sup>ns</sup> (0.904)
Vitamin E (mmol/L)	0.05±0.02	0.06±0.02	0.07±0.02	2.759 (0.006)	3.297 (0.001)	0.05±0.02	0.05±0.02	0.04±0.02	0.071 <sup>ns</sup> (0.943)	1.529 <sup>ns</sup> (0.126)
Se ( $\mu$ mol/L)	0.47±0.12	0.53±0.12	0.58±0.12	1.884 <sup>ns</sup> (0.060)	2.852 (0.004)	0.51±0.13	0.46±0.14	0.43±0.14	1.257 <sup>ns</sup> (0.209)	1.924 <sup>ns</sup> (0.054)

ns – Not significant ( $p > 0.05$ ); \*Significant at  $p < 0.001$ ; <sup>†</sup>Significant at  $p < 0.05$ ; <sup>†</sup>8 weeks values were compared with baseline values (0 week); <sup>†</sup>16 weeks values were compared with baseline values (0 week)

### [IV] DISCUSSION

Wound healing in diabetic patients is usually poor and slow. It is a complicated process that involves several steps and any alteration at any step may further slow down an already slow process. Factors affecting healing could either be 'general'

resulting from lack of vitamin C, protein deficiency, ageing and other diseases such as diabetes, jaundice, uraemia, and Cushing's disease; or it may be 'local' for example sepsis, impaired blood supply (ischaemia) and presence of dead or damaged tissues [2]. The possibility of altered free radical scavenging systems as a major cause for delayed healing cannot



be ignored, since free radicals and antioxidant defence systems play an important role in many disorders including diabetes and its complications [2]. Though poorly controlled metabolic disorders such as diabetes mellitus are associated with poor wound healing [14], the specific role of oxidative stress has received little attention. Our data indicate that optimum antioxidant status can significantly enhance healing of wounds.

At the beginning of this study, indices of diabetes were found to be higher in Type 2 diabetes mellitus with diabetic foot ulcer compared with non-diabetic control subjects. Elevated FPG has been shown to generate oxidative stress (OS) through several mechanisms [15]. An increase in FPG has been hypothesized to induce increase generation of lipid peroxide (LPO), a marker of lipid peroxidation, leading into excessive production of HO<sup>•</sup> which then attack the C-8 position of deoxyguanosine and the subsequent loss of a hydrogen atom from the intermediate to form 8-hydroxyl-2'-deoxyguanosine (8-OHdG), a marker of DNA damage. Therefore, increase in FPG results in elevated levels of LPO and subsequently increase production of 8-OHdG. This hypothesis was indeed confirmed by the result obtained in this study as LPO and 8-OHdG levels were observed to increase in Group B compared with Group A. These increases in LPO and 8-OHdG levels in diabetic foot ulcer patients compared with non-diabetic control subjects resulted in lowering SOD activity and increasing GPx activity. These findings agree with the studies of Merzouk and Coll. [16] and Gupta and Chari [17]. The decrease in SOD activity may be due to the over production of peroxides which then resulted in increasing GPx activity as a compensatory mechanism to remove the excess peroxides generated from the dismutation process thus preventing further tissue damage in these patients.

Total antioxidant status (TAS), a useful indicator of risk from diseases associated with free radical activity, and which may indicate the need for antioxidant therapy was observed to be lower in diabetic foot ulcer patients compared with non-diabetic subjects. This finding in TAS of diabetic foot ulcer patients agree with the previous studies conducted in Type 1 and Type 2 diabetic patients [18] where significant decreases were reported. Significant decreases were similarly observed in non-enzymatic antioxidant micronutrients. These decreases in the antioxidant micronutrients are in accordance with the findings of Gupta & Chari [17] and Karatas et al. [19], they reported lower levels of vitamin C. Merzouk et al. [16] reported decreases in vitamin E levels while Kljai and Runje [20] and Kornhauser et al. [21], reported lower levels of selenium in diabetic subjects compared with non diabetic subjects. The low vitamin C level in diabetic foot ulcer patients may be due to its consumption in scavenging free radicals and/or its involvement in regenerating vitamin E from the generated  $\alpha$ -tocopheroxyl radicals as well as its participation in collagen synthesis in these patients. The hyperactivity of GPx, a seleno-enzyme [22], indicated above in diabetic foot ulcer patients may justify the reduction of selenium. This micronutrient may be increasingly consumed in the biosynthesis of glutathione peroxidase enzyme in these patients.

Following 16 weeks of supplementation with combined Se, vitamins C and E coupled with diet restriction and oral hypoglycaemic agent administration, decreases in BMI, FPG and HbA1c were found in the supplemented subgroup. Similarly, in the non-supplemented subgroup, reductions in the diabetes indices were observed at the same period of supplementation. These reductions in diabetes indices in both subgroups B1 and B2 indicated good management of blood glucose in these patients. Although, good management of blood glucose was demonstrated in both subgroups as revealed by the reductions in diabetes indices, LPO and 8-OHdG levels were only observed to decrease in the supplemented group (B1) but increased in the non-supplemented group (B2). The TAS was observed to increase in the supplemented diabetic foot ulcer patients, as a result of the reduction in the oxidative stress indices while in the non-supplemented group a non-significant decrease was recorded. This findings suggested that supplementation with combined Se, vitamins C and E was able to reduce LPO and 8-OHdG levels thus improving the total antioxidant status while absence of supplementation exacerbate the antioxidant defence status.

Wound healing process was measured by the 'ABDEFS' assessing tool in diabetic foot ulcer group. This tool revealed improvement in wound healing in the supplemented group compared with the non-supplemented group. Vitamin C has been reported to be involved in two stages of wound healing. These are the inflammatory stage, in which vitamin C enhances neutrophil migration and lymphocyte transformation, and the proliferative stage where it is necessary for collagen synthesis [23]. Supplementation of diabetic ulcer with vitamin C therefore ensures the availability of this vitamin in these two phases thus speeding up the healing as depicted by the 'ABDEFS' result.

## [V] CONCLUSION

Good glycaemic control, as indicated by reduction in levels of BMI, FPG and HbA1c in both supplemented and non-supplemented diabetic foot ulcer patients, could not improve oxidative stress and wound healing on its own. Reduction in oxidative stress indices coupled with better healing of wound was achieved by good glycaemic control and administration of antioxidant micronutrients. These findings signify the ameliorative role of the antioxidant micronutrients supplementation on oxidative stress and subsequently on wound healing in Type 2 diabetics with foot ulcer.

## FINANCIAL DISCLOSURE

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## CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.



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## REFERENCES

- [1] American Diabetes Association. [2004] Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 27(1): 5–10.
- [2] Rasik AM, Shukla A. [2000] Antioxidant status in delayed healing type of wounds. *Int J Exp Pathol* 81: 257–263.
- [3] Greenhalgh DG. [2003] Wound healing and diabetes mellitus. *Clin Plast Surg* 30(1): 37–45.
- [4] McDaniel DH, Ash K, Lord J, Newman J, Zukowski M. [1998] Accelerated laser resurfacing wound healing using a triad of topical antioxidants. *Dermatol Surg* 24: 661–664.
- [5] Nourooz-Zadeh J, Tajaddini Sarmadi J, McCarthy S, Betteridge DJ, Wolff SP. [1995] Elevated levels of authentic plasma hydroperoxides in NIDDM. *Diabetes* 44: 1054–1058.
- [6] Toyokuni S, Iwasa Y, Kondo S, Tanaka T, Ochi, H. 1999. Intracellular distribution of 8-hydroxy-2'-deoxyguanosine. An immunocytochemical study. *J Histochem Cytochem* 47(6): 833–836.
- [7] Arthur JR, Boyne R. [1985] Superoxide dismutase and glutathione peroxidase activities in neutrophils from selenium deficient and copper deficient cattle. *Life Sci* 36: 1569–1575.
- [8] Paglia DE, Valentine WN. [1967] Glutathione peroxidase determination. *J Lab Clin Med* 70: 158.
- [9] Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Milner A. [1993] Determination of plasma total antioxidant status. *Clin Sci* 84: 407–412.
- [10] Aye K. [1978] A simple colorimetric method for ascorbic acid determination in blood plasma. *Clin Chim Acta* 86: 153–157.
- [11] Baker H, Frank O. [1968] Determination of Vitamin E. *Clinical Vitaminology, Wiley, New York* 1: 72.
- [12] Pleban PA, Munyani A, Beachum J. [1982] Determination of selenium concentration and glutathione peroxidase activity in plasma and erythrocytes. *Clin Chem* 28: 311–316.
- [13] Oluwatosin OM, Alawale O, Olabanji K, Tijani M. [1998] ABDEFS' scoring system: a new method of evaluating chronic ulcers. *Afr J Med Sci* 28: 153–154.
- [14] Falanga V. [2000] Classification for wound bed preparation and stimulation of chronic wounds. *Wound Repair Regen* 8(5): 347–352.
- [15] Piconi L, Quagliaro L, Ceriello A. [2003] Oxidative Stress in Diabetes. *Clin Chem Lab Med* 41(9):1144–1149.
- [16] Merzouk S, Hichami A, Madani S, Merzouk H, Berroughet AY. [2003] Antioxidant status and levels of different vitamins determined by high performance liquid chromatography in diabetic subjects with multiple complications. *Gen Physiol Biophys* 22: 15–27.
- [17] Gupta MM, and Chari S. [2005] Lipid peroxidation and antioxidant status in patients with diabetic retinopathy. *Indian J Physiol Pharmacol* 49(2):187–192.
- [18] Akinosun OM, Bolajoko EB. [2007] Total antioxidant status in type 2 diabetic patients: experienced at University College Hospital (UCH) Ibadan, Nigeria. *Nigerian J Clin Pract* 10(2): 126–129.
- [19] Karatas F, Halifeoghi I, Karatepe M, Konar V, Canatan H, Colak R. [2006] Evaluation of changes in levels of serum selenium, malondialdehyde and antioxidant vitamins (A, E, C) in diabetic patients. *Arastirma* 20(6): 391–395. retrieved on February 20, 2008 from <http://www.fusabil.org>.
- [20] Kljai K, Runje R. [2001] Selenium and glycogen levels in diabetic patients. *Biol Trace Elem Res* 83: 223–229.
- [21] Kornhauser C, Garcia-Ramirez JR, Wrobel K, Perez-Luque E, Garray-Sevilla M, Wrobel, K. [2008]. Serum Selenium and Glutathione Peroxidase concentration in type 2 diabetes mellitus patients. *Primary Diabetes Care (PCD 64; Number of pages 5)*. Retrieved on April 4, 2008 from <http://www.intl.elsevierhealth.com/journals/pcd/>. Available @ [www.sciencedirect.com](http://www.sciencedirect.com)
- [22] Abul HT, Mathew TC, Abul F, Al-Sayer H, Dashti HM. [2002] Antioxidant enzyme level in the testes of cirrhotic rats. *Nutrition* 18(1): 56–59.
- [23] MacKay D, Miller AL. [2003] Nutritional Support for Wound Healing. *Altern Med Rev* 8(4): 359–377.

## COMPARATIVE INVESTIGATION OF COLD PRESSED ESSENTIAL OILS FROM PEEL OF DIFFERENT MANDARIN VARIETIES

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### ABSTRACT

The object of this study was to investigate the composition of different varieties of cold pressed mandarin peel oils by using Gas Chromatography-Mass Spectrometry. Results obtained from this study showed the major volatile compounds counting for >90% of total volatile flavour compounds. Identification and quantification of the volatiles indicated the most abundant compounds as monoterpene hydrocarbons (limonene, sabinene,  $\beta$ -pinene,  $\gamma$ -terpinene, myrcene,  $\alpha$ -pinene and  $\alpha$ -terpinolene), following by esters (methyl antranilate, neryl acetate and geranyl acetate), alcohols (linalool) and aldehyde compounds (decanal,  $\alpha$ -sinensal,  $\beta$ -sinsnal and peril aldehyde). Principle component analysis (PCA) was employed to discriminate among the cold pressed mandarin oils based on their varieties and to evaluate their sensorial attributes. Results from PCA analysis showed classification of the samples of red mandarin oil from South Africa and unknown region in the same grope which was separated from yellow and sweet "Murcot" mandarin oil.

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#### KEY WORDS

Diabetic Cold pressed mandarin oil, esters, alcohols, GC-MS, alkanes, principle component analysis (PCA).

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### [1] INTRODUCTION

Cold pressed citrus oils represent a large portion of the world market for flavour ingredients, food industry, and aromatherapy as well as perfume industry [1]. An expanding market for mandarin flavoured drinks uses the oils of lemon, orange, mandarin, grapefruit and bitter orange [2]. The flavour of citrus oils is usually defined as refreshing, green, citrus and it is extremely important for the flavouring of food, beverages, confectionary, cookies and dessert. From this point of view, the main role of mandarin oils is covering the "off flavor" in lemon and orange drinks.

Chemical composition of the cold-pressed citrus includes different volatiles, such as terpenes and oxygenated compounds, and nonvolatiles, such as waxes and pigments [3-10]. Terpene content should be minimal in order to protect the oils from oxidation. The most dominant monoterpene limonene does not contribute to the overall flavor of mandarin oil and its high concentration (usually over 90 %) is responsible for oil instability as well as unpleasant "off" flavor. Furthermore, most of the volatile compounds can provide antifungal activity of oils [11-14]. Except gas chromatography, other analytical techniques were applied for examination of volatile compounds of citrus peel oils [15, 16].

The chemical composition of the citrus oils is usually affected to the geographical region and micro-ecological environment. In the work of Vercera et al., the chemical composition of two varieties of Nova and Satsuma peel mandarin oils from Uruguay was object of study [17]. Effect of geographical origin to the volatiles of different lemon peel oils was object of study in the work of Kostadinović et al. [18].

The flavor and the taste typical for "sweet mandarin", as in other citrus, is provided by oxygenated terpenes such as alcohols, esters and aldehydes. These volatile compounds can be "the key" for the overall flavor of mandarin oil even in the concentration less than 1%. Cold pressed mandarin oil usually is used as a blend with other citrus oils as orange and lemon or as a mask for unpleasant flavor in the food, aromatherapy and perfume industry [19, 20].

The first objective of the present study was investigation of the effect of variety on the composition of different cold pressed mandarin oils.

The second objective of the study was classification of the four samples of cold pressed mandarin oils regarding their volatile composition. Results from the percentage of the compounds

were submitted to Principal Component Analysis (PCA) in order to find relationship between abundance of the volatiles and variety of the mandarin from which the peel oil was cold-pressed.

## [II] MATERIALS AND METHODS

### 2.1. Samples of cold pressed mandarin oils

The four commercial samples of cold pressed mandarin oils were obtained from Aromaceuticals (Texas, United States).

### 2.2. Apparatus

The flavour compounds of three varieties cold pressed mandarin oils (red, yellow and sweet "Murcot") were analyzed using a Hewlett-Packard 6890 GC equipped with a Mass Spectrometer (MS) and an HP-5 cross-linked fused-silica capillary column (i.d. = 0.25 mm, length = 30 m, film thickness = 0.25  $\mu$ m) supplied by Agilent inc. (PA, USA). In qualitative and quantitative analyses, 0.2  $\mu$ l of cold pressed mandarin oil was injected in the split mode (1:100). Oven temperature was programmed at 40°C isothermally for 5 min, then ramped to 180°C at 4°C/min and then increased to 260°C at 20°C/min and held for 10 min at the final temperature. Helium was used as the carrier gas with a flow-rate of 5.3 (ml/min). Injector and detector temperatures were set at 270 and 300°C, respectively. The volatile flavour compounds were initially detected and confirmed using a Hewlett-Packard 6890N GC system (Wilmington, DE) and the NIST library version 2.0. The components of the essential oil were identified by comparison of their linear retention indices, determined in relation to a homologous series of n-alkanes (C8-C32), with those from pure standards or reported in literature. Comparison of fragmentation patterns in the mass spectra with those stored on databases and MS data of our collection was also performed.

### 2.3. Statistical analysis

On Principal component analysis (PCA) was performed to gain an overview of how the samples were correlated to each other with regard to equilibrium volatile headspace concentration. Correlation matrix was applied in multivariate analysis with Minitab software release 14 so that the data was autoscaled by variable to give the same weight to all components

## [III] RESULT AND DISCUSSION

The volatile fraction composition of cold pressed essential oils from the peel of different species of mandarin was studied using GC-MS. The obtained TIC chromatogram is presented in [Figure-1](#). More than seventy components were identified and quantified using mass spectra and linear retention indices presented in [Supplementary Table-1](#). The monoterpene limonene was the most abundant component even though not in a quantity expected for fresh mandarin oil. Relatively lower quantity of limonene suggested other extraction procedures apart from cold pressing for protecting the oils from oxidation [21].  $\alpha$ -pinene and  $\alpha$ -tujene were the most abundant compounds after limonene responsible for the flavor of the mandarin oil.  $\alpha$ -copaene, trans- $\alpha$ -bergamotene and  $\beta$ -farnesene were the most important sesquiterpenes responsible for the sweet taste of the "murcot" mandarin oil [22]. Octanal, nonanal, decanal from aliphatic aldehydes and geranial and  $\alpha$ -sinensal from the class of

monoterpene aldehydes were dominant in all samples of mandarin oils. Methyl antranilate was the main ester which contributes the most to the overall aroma of mandarin oil. Linalool was the major alcohol responsible for the sweet and floral smell of the oil. Significant quantity of flavones was detected around 250°C during chromatographic analysis. Polyphenolic compounds 4',5,6,7,8,-pentamethoxy flavone (tangeretin), 3,3',4',4,5,5',7,8-heptamethoxy flavone and 3',4',5,6,7,8-hexamethoxy flavone (nobiletin) in the role of natural antioxidants protected the oils from oxidation.

Regarding the total quantities of chemical classes of the volatile compounds sesquiterpenes, alcohols and aldehydes were the most dominant of volatiles in all samples of the oils [[Figure-2](#)]. Red mandarin oil from South Africa had the highest quantities of esters and oxides which can be indication of oxidized oil. Acids and ketones were the least abundant compounds which contribute to the chemical composition of mandarin oils. Oils pressed from the peel of yellow mandarin and red mandarin from South Africa contained higher quantities of flavones which acts as natural antioxidants and protect the oils from oxidation. Nobiletin (3',4',5,6,7,8-hexamethoxy flavone) was the most abundant flavone in examined samples of mandarin oils.

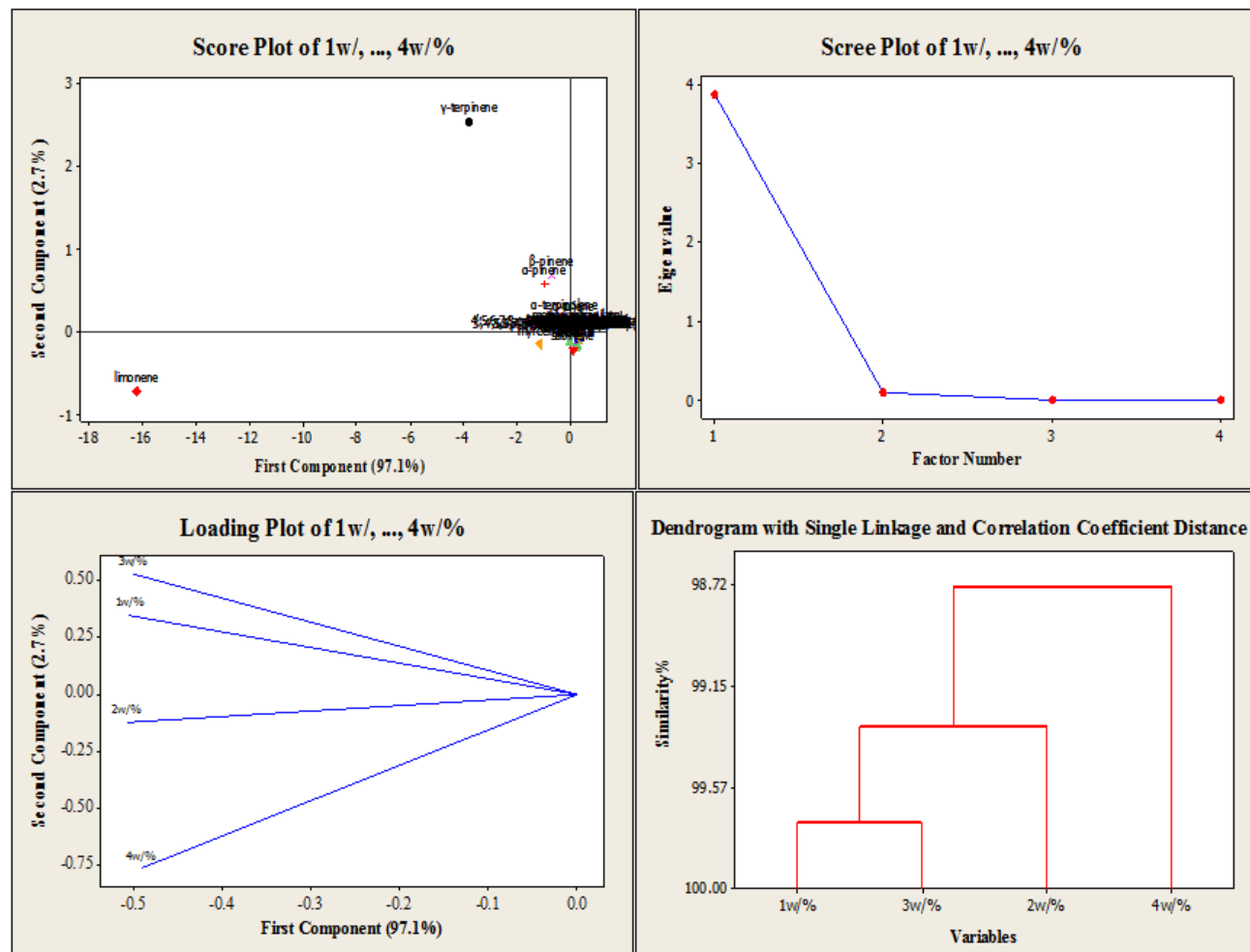
However, the quantitative information summarized in [Supplementary Table-1](#) and [Figure-1](#) was not enough to evaluate the similarities and differences between the cold pressed mandarin oils considered in this study.

A multivariate pattern recognition approach should be more effective in recognizing differences among the samples analyzed. The results showed the comparison between the major chemical classes of volatile chemical compounds of three varieties of cold pressed mandarin oils [[Supplementary Table-1](#)]. Apart from monoterpenes as the most abundant group of volatiles, three varieties of cold pressed mandarin oil had the highest quantities of sesquiterpenes, alcohols and aldehydes. Esters and oxides were present in lower quantities and the least abundant were acids, ketones and flavones. Sesquiterpenes are a major group of flavour compounds in citrus oils in term of quantity.

PCA score plots were used to determine whether for different cold pressed mandarin oils could be grouped into different classes [[Figure-3](#)]. To focus on the differences among the cold pressed mandarin oils and target volatile compounds, cluster observation and cluster variable dendrograms were constructed using the nearest neighbour [[Figure-3](#)]. The results indicated that first two principal components explained 97.1% and 2.7% of the total variability, respectively. Despite first two principal components, which showed 99.8% of total variation, the remaining principal components didn't account for any variability and were probably unimportant.







**Fig. 3. PCA scatter plots and cluster dendrograms discriminating cold pressed mandarin oils and their volatile flavour compositions into different classes.**

Except for some monoterpene hydrocarbons namely  $\alpha$ -pinene,  $\beta$ -pinene, limonene and  $\gamma$ -terpinene, the other target volatile compounds could be classified in one group in PC1, because the coefficients of these volatile compounds were the same negative sign located in PC 1 [Figure– 3]. In most cases, the differentiation or closeness between the volatile flavour compounds directed in negative side of PC 1 was dependent on their chemical classes. The second principal component (PC 2) accounted for very small proportion (2.7%) of the total variability. As shown in Figure– 3, most of target volatile flavour compounds identified in cold pressed lemon oils namely alcohol (i.e. linalool, terpinen-4-ol,  $\alpha$ -terpineol and geraniol), ester compounds (i.e. citronellyl acetate, neryl acetate and geranyl acetate) and aldehyde compounds (i.e. octanal decanal, citronellal, nonanal, neral and geraniol) were classified with the same negative sign in PC 2; while  $\alpha$ -pinene,  $\beta$ -pinene and  $\gamma$ -

terpinene were placed in the positive side of PC 2 [Figure– 3]. Limonene and  $\gamma$ -terpinene were separated from the other volatiles because their percentage was significantly higher in comparison to other volatile compounds.  $\alpha$ -pinene and  $\beta$ -pinene were closer to other compounds on PCA score plot because their abundance were lower than the abundance of the limonene and  $\gamma$ -terpinene. PCA score plot significantly differentiated the cold pressed mandarin oils from each other [Figure– 3]. As it is notable from the loading plot, samples 1 and 3 were closer in comparison to the samples 2 and 4.

Finally, their similarity was confirmed in the dendrogram with single linkage and correlation coefficient distance. Samples of cold pressed mandarin oil from South Africa and red mandarin oil from unknown region were classified as the most similar. If we compare the percentage of the volatiles for samples 1 and 3 [Supplementary Table-1] we can see that those two samples

had the most similar composition. Because all the samples have been processed with the same technology at the same season, quantitative differences in the essential oil compositions might be due to the genetic origin which belongs to the different variety.

## [V] CONCLUSION

In the present study, high resolution gas chromatography (HRGC) equipped with mass spectrometer (MS) was employed for the qualitative and quantitative analyses of volatile flavour compounds of different varieties of cold pressed mandarin oils. Multivariate analysis techniques aided in the interpretation of chemical data obtained for volatile components of mandarin oils. Differences among individual components provided less useful information, mainly because there is often a wide variation in the volatile fraction composition from different samples of the same oils; since multivariate analysis involves variability of several or all of the compounds; it seems that the discrimination results were less affected by such variation. Although the same technology was applied to process the studied cold pressed mandarin oils, quantitative differences in the essential oil composition could be attributed to the different varieties. The samples of red mandarin oil from South Africa and red mandarin oils from unidentified region were grouped in the same cluster and clearly separated from the yellow and sweet “Murcot” variety of mandarin oil.

## FINANCIAL DISCLOSURE

This work is not supported by any financial assistance.

## CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

## REFERENCES

- [1] GA Burdock. [1995] Fenaroli's handbook of flavor ingredients, CRC Press Inc, Boca Raton, Ann Arbor, London and Tokyo.
- [2] Price S. [1993] The aromatherapy workbook. Thorsons, UK.
- [3] ML Lota, D de Rocca Serra, F Tomi, J Casanova. [2000] Chemical variability of peel and leaf essential oils of mandarins from Citrus reticulata Blanco, *Biochemical Systematics and Ecology* 28(1): 61–67
- [4] ML. Lota, D de Rocca Serra, F Tomi, J Casanova. [2000] Chemical variability of peel and leaf essential oils of 15 species of mandarins, *Biochemical Systematics and Ecology* 29(1): 77–104.
- [5] D Sciarrone, L Schipilliti, C Ragonese, P Quinto Tranchida, P Dugo, et al. [2010] Thorough evaluation of the validity of conventional enantio-gas chromatography in the analysis of volatile chiral compounds in mandarin essential oil: A comparative investigation with multidimensional gas chromatography, *Journal of Chromatography A* 1217(7):1101–1105
- [6] S Moufida, B Marzouk. [2003] Biochemical characterization of blood orange, sweet orange, lemon, bergamot and bitter orange, *Phytochemistry* 62(8):1283–1289.
- [7] C Blanco Tirado, EE Stashenko, MY Combariza, JR. Martinez. [1995] Comparative study of Colombian citrus oils by high-resolution gas chromatography and gas chromatography-mass spectrometry, *Journal of Chromatography A* 697(1-2): 501–513,
- [8] L Espina, M Somolinos, S Lorán, P Conchello, D García, R Pagán. [2011] Chemical composition of commercial citrus fruit essential oils and evaluation of their antimicrobial activity acting alone or in combined processes, *Food Control* 22(6): 896–902,
- [9] B Campisi, G Dugo, A Cotroneo, L Favretto. [1995] Chemometric analysis and extraction processes of mandarin essential oils, *Analytica Chimica Acta* 312(2): 199–205.
- [10] L Danielski, G Brunner, C Schwänke, C Zetzl, H Hense, J PM. [2008] Donoso, Deterpenation of mandarin (Citrus reticulata) peel oils by means of countercurrent multistage extraction and adsorption/desorption with supercritical CO<sub>2</sub>, *The Journal of Supercritical Fluids* 44(3): 315–324.
- [11] M Viuda-Martos, Y Ruiz-Navajas, J Fernández-López, J Pérez-Álvarez. [2008] Antifungal activity of lemon (Citrus lemon L.), mandarin (Citrus reticulata L.), grapefruit (Citrus paradisi L.) and orange (Citrus sinensis L.) essential oils, *Food Control* 19(12): 1130–1138.
- [12] Hugo Merle, Montserrat Morón, M.Amparo Blázquez, Herminio Boira. [2004] Taxonomical contribution of essential oils in mandarins cultivars, *Biochemical Systematics and Ecology* 32(5): 491–497.
- [13] Karim Hosni, Nesrine Zahed, Raouf Chrif, Ines Abid, Wafa Medfei. [2010] Composition of peel essential oils from four selected Tunisian Citrus species: Evidence for the genotypic influence, *Food Chemistry* 123(4): 1098–1104.
- [14] M Chutia, P Deka Bhuyan, MG Pathak, TC Sarma, P Boruah [2009] Antifungal activity and chemical composition of Citrus reticulata Blanco essential oil against phytopathogens from North East India, *LWT - Food Science and Technology* 42(3):777–780,.
- [15] B Steuer, H Schulz, E Läger. [2001] Classification and analysis of citrus oils by NIR spectroscopy, *Food Chemistry* 72(1):113–117.
- [16] H Merle, M Morón, M Blázquez, H Boira. [2004] Taxonomical contribution of essential oils in mandarin cultivars, *Biochemical Systematics and Ecology* 32(5): 491–497.
- [17] A Verzera, A Trozzi, A Cotroneo, Daniel Lorenzo, E. Dellacassa. [2000] Uruguayan Essential Oil. 12. Composition of Nova and Satsuma Mandarin Oils, *Journal of Agriculture and Food Chemistry* 48 (7): 2903–2909.
- [18] S Kostadinović, M Stefova, D Nikolova, D Nedelcheva, N Martinez. [2010] Multivariate analysis discrimination of various cold-pressed lemon oils from different geographical regions, *Journal of Food, Agriculture and Environment* 8(2): 132–136.
- [19] VG Cooksley. [1996] Aromatherapy: A lifetime guide to healing with essential oils, Prentice-Hall Inc, New Jersey, USA.
- [20] MA Ferhat, BY Meklati, F Chemat. [2007] Comparison of different isolation methods of essential oil from Citrus fruits: cold pressing, hydrodistillation and microwave ‘dry’ distillation, *Flavor and Fragrance Journal* 22:494–504.
- [21] F Shahidi. [2005] Bailey's industrial oil and fat products, Wiley, New York.
- [22] RJ Braddock. [1999] Handbook of citrus by-products and processing technology, Wiley, New York.



SUPPLEMENTARY TABLE (As supplied by author)

Supplementary Table: 1. Volatile composition of cold pressed peel oils from red (1), yellow (2), red from South America (3) and sweet "Murcot" (4) mandarin expressed as percentage (%).

No.	Components	*LRI	1w/%	2w/%	3w/%	4w/%	Aroma descriptor
<b>Hydrocarbons</b>							
<i>Monoterpenes</i>							
1.	$\alpha$ -tujene	926	1.23	0.15	1.46	0.02	-
2.	$\alpha$ -pinene	932	4.61	2.09	7.20	2.78	sweet
3.	camphene	947	0.06	0.04	0.20	0.03	camphoraceous
4.	sabinene	973	0.50	1.86	0.00	1.70	-
5.	$\beta$ -pinene	976	5.17	2.30	5.27	0.00	wet soil
6.	myrcene	992	5.91	4.52	3.13	7.15	warm
7.	$\alpha$ -terpinene	1024	0.43	0.18	0.26	0.13	lemony
8.	$\alpha$ -phelandrene	1005	0.00	0.00	0.11	0.00	-
9.	$\delta$ -3-carene	1010	0.00	0.28	0.10	0.00	-
10.	limonene	1033	46.91	56.60	46.71	74.29	fresh
11.	<i>trans</i> -iso-limonene	1073	0.05	0.00	0.00	0.01	-
12.	<i>trans</i> - $\beta$ -ocimene	1054	0.36	0.41	0.11	0.12	-
13.	$\gamma$ -terpinene	1063	17.31	12.59	19.97	0.17	lime-like
14.	$\alpha$ -terpinolene	1090	2.92	2.82	1.13	0.19	citrus
15.	$\delta$ -4-carene	1352	0.04	0.00	0.00	0.00	-
	<b>Total terpenes</b>		85.50	83.84	85.65	86.59	
<i>Sesquiterpene</i>							
16.	$\delta$ -elemene	1559	0.03	0.03	0.00	0.00	-
17.	$\alpha$ -copaene	1377	0.17	0.19	0.07	0.24	-
18.	$\beta$ -cubebene	1392	0.14	0.15	0.04	0.21	-
19.	$\beta$ -elemene	1393	0.12	0.12	0.04	0.10	fruity
20.	<i>trans</i> - $\alpha$ -bergamotene	1438	0.24	0.00	0.01	0.00	-
21.	<i>trans</i> - $\beta$ -caryophyllene	1421	0.32	0.18	0.35	0.10	fresh
22.	$\alpha$ -humulene	1455	0.06	0.05	0.06	0.04	woody
23.	$\beta$ -farnesene	1458	0.07	0.10	0.02	0.26	fruity
24.	$\alpha$ -amorphene	1478	0.02	0.02	0.01	0.00	-
25.	germancren-D	1482	0.12	0.14	0.00	0.13	
26.	aromadendrene	1489	0.02	0.00	0.00	0.00	
27.	byclogermacrene	1498	0.00	0.03	0.00	0.04	

28.	valencene	1494	0.08	0.13	0.00	0.08	woody
29.	$\alpha$ -selinene	1498	0.15	0.00	0.13	0.00	-
30.	$\alpha$ -farnecene	1509	0.00	0.00	0.30	0.21	-
31.	$\alpha$ -murolene	1502	0.03	0.00	0.00	0.05	
32.	<i>trans</i> - $\alpha$ -bisabolene	1545	0.04	0.08	0.00	0.00	dry
33.	$\beta$ -bisabolene	1887	0.78	0.65	0.00	0.00	-
34.	$\delta$ -cadinene	1525	0.18	0.21	0.06	0.28	-
35.	germancren-B	1559	0.03	0.00	0.00	0.00	-
	<b>Total sesquiterpenes</b>		<b>2.60</b>	<b>2.08</b>	<b>1.09</b>	<b>1.74</b>	
<b>Alcohols</b>							
36.	<i>p</i> -menta- <i>trans</i> -2,8-dien-1-ol	1124	0.09	0.09	0.11	0.10	-
37.	<i>cis</i> -sabinene hydrate	1070	0.05	0.03	0.04	0.02	-
38.	terpinene-4-ol	1179	0.18	0.08	0.14	0.00	woody
39.	$\alpha$ -terpineol	1193	0.43	0.21	0.87	0.32	floral
40.	linalool	1103	1.23	1.72	0.60	1.90	sweet
41.	<i>cis</i> -carveol	1224	0.09	0.12	0.31	0.08	-
42.	<i>trans</i> -carveol	1205	0.00	0.00	0.03	0.00	-
43.	thymol	1296	0.08	0.00	0.11	0.00	-
44.	elemol	1552	0.06	0.05	0.00	0.09	-
45.	nerolidol	1566	0.02	0.03	0.00	0.01	-
46.	spatulanol	1581	0.01	0.02	0.01	0.01	-
	<b>Total alcohols</b>		<b>2.24</b>	<b>2.35</b>	<b>2.22</b>	<b>2.53</b>	
<b>Aldehydes</b>							
<i>Aliphatic</i>							
47.	octanal	1004	0.00	0.36	0.00	1.32	-
48.	nonanal	1108	0.07	0.05	0.00	0.38	floral
49.	decanal	1207	0.39	0.43	0.07	1.28	marine
50.	tridecanal	1510	0.03	0.00	0.00	0.00	-
51.	tetradecanal	1614	0.00	0.00	0.00	0.29	-
52.	(E,E)-2,4-decadienal	1319	0.02	0.02	0.00	0.02	-
<i>Monoterpenic</i>							
53.	neral	1243	0.19	0.16	0.00	0.09	lemony
54.	citronellal	1155	0.12	0.12	0.02	0.50	powerful floral
55.	geranial	1272	0.25	0.26	0.00	0.16	flowery, fruity
56.	undecanal	1308	0.00	0.00	0.00	0.12	-
57.	peryl aldehyde	1276	0.17	0.14	0.00	0.00	-
58.	$\beta$ -sinensal	1700	0.19	0.00	0.00	0.00	-
59.	$\alpha$ -sinensal	1757	1.09	0.79	0.16	0.00	-
	<b>Total aldehydes</b>		<b>2.52</b>	<b>2.33</b>	<b>0.25</b>	<b>4.16</b>	

Esters							
60.	octyl acetate	1214	0.00	0.00	0.00	0.05	-
61.	methyl antranilate	1343	0.24	0.35	1.03	0.00	-
62.	citronelyl acetate	1355	0.05	0.05	0.08	0.13	rosy
63.	neryl acetate	1367	0.23	0.27	0.02	0.20	very sweet
64.	geranyl acetate	1385	0.11	0.08	0.04	0.04	dry
<b>Total esters</b>			<b>0.63</b>	<b>0.75</b>	<b>1.17</b>	<b>0.42</b>	
Oxides							
65.	cis-limonene oxide	1135	0.10	0.07	0.44	0.09	citrus like
66.	trans-limonene oxide	1139	0.07	0.09	0.61	0.07	citrus like
67.	cariophyllene oxide	1586	0.01	0.02	0.44	0.02	woody
<b>Total oxides</b>			<b>0.08</b>	<b>0.18</b>	<b>1.49</b>	<b>0.18</b>	
Acids							
68.	Octanoic acide	1184	0.05	0.03	0.04	0.00	-
69.	Decanoic acide	1375	0.03	0.02	0.04	0.00	-
70.	Hexadecanoic acid	1876	0.09	0.00	0.04	0.01	-
<b>Total acids</b>			<b>0.17</b>	<b>0.05</b>	<b>0.12</b>	<b>0.01</b>	
Ketones							
71.	L-carvone	1246	0.15	0.19	0.10	0.17	-
72.	piperiton	1257	0.01	0.00	0.00	0.00	-
73.	nutcatone	1808	0.01	0.02	0.00	0.00	grapefruit
<b>Total ketones</b>			<b>0.17</b>	<b>0.21</b>	<b>0.10</b>	<b>0.17</b>	
Flavones							
74.	4',5,6,7,8-pentamethoxy flavon (tangeretine)	-	0.17	0.09	0.25	0.00	-
75.	3,3',4',5,5',7,8-heptamethoxy flavon	-	0.00	0.00	0.18	0.18	-
76.	3',4',5,6,7,8-hexamethoxy flavon (nobiletin)	-	0.00	0.44	0.11	0.24	-
<b>Total flavones</b>			<b>0.17</b>	<b>0.53</b>	<b>0.53</b>	<b>0.42</b>	

\* Linear retention index obtained by GC-MS with HP-5 column.



## SOME PHYSICAL PROPERTIES OF RICE SEED (ORIZA SATIVA)

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### ABSTRACT

Physical and mechanical properties of rice are necessary for the design of equipment to handle, transport, process and store the crop. These properties were evaluated as a function of moisture content of grain. The objective of this work was to determine the physical and mechanical properties of rice. The grain was tested for bulk density, true density, sphericity, porosity, angle of internal friction and coefficient of friction with various materials at 12% moisture content (dry basis, db). The average length, width, thickness and the average thousand grain weight of the rye grains were, 7.43mm, 2.75mm, 2.53mm and 26.91 g. The static coefficient of friction 0.4835, 0.4061, and 0.3670 for wood, galvanized iron and glass surfaces respectively. The higher friction coefficient values were observed on wood surface and the lowest on steel surface.

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#### KEY WORDS

Rice; Angle of repose; coefficient of friction; Physical properties

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### [I] INTRODUCTION

Rice (*Oryza sativa* L.) is the basic food for two-thirds of the world population and is the most important cereal crop cultivated in the world [1, 2]. The physical and mechanical properties of rice, which are important in the design and selection of storage structures and storage and processing equipment, depend on grain moisture content. Therefore, the determination and consideration of properties such as bulk density, true density, angle of internal friction and static coefficient of friction of grain has an important role [3, 4, 5]. The principal axial dimensions of rye seeds are useful in selecting sieve separators and in calculating power during the rye milling process.

Knowing the grain's bulk density, true density and porosity can be useful in sizing grain hoppers and storage facilities: they can affect the rate of heat and mass transfer of moisture during the aeration and drying processes. A grain bed with low porosity will have greater resistance to water-vapor escape during the drying process, which may lead to the need for higher power to drive the aeration fans. Cereal-grain kernel densities have been of interest in breakage susceptibility and hardness studies [6]. The static coefficient of friction is used to determine the angle at which chutes must be positioned to achieve consistent flow of materials through the chute. Such information is useful in sizing motor requirements for grain transportation and handling [7]. Other researchers have determined the properties of different types of grains and seeds: canola and wheat [8] lentils; [9]; sunflower seeds [10]; black pepper [11]; pigeon peas [12]; cotton [13]; millet [14]; popcorn [15]; caper seeds [16]; pistachio nuts [5]; and barley [17].

Many studies have reported on the physical, chemical and surface properties of wheat husks, rye husks and soft wood and their polypropylene composites [18]; rheological properties of dough and sensorial quality of bread made from a whole meal rye wheat blend with the addition of gluten [19]; and rheological properties of rye mash prepared by pressure less liberation of starch in mashing and fermentation [20]. But there has been no study to date on the physical properties of rice seeds.

This study investigated the some physical and mechanical properties of the variety of rice typically cultivated in Iran. The parameters measured were bulk density, true density, angle of internal friction and static coefficient of friction.

### [II] MATERIALS AND METHODS

#### 2.1. Sample Preparation

Rice seeds were collected in October 2010 from a farm in north province of Iran. The variety (Sadri) used in the study is the prevalent variety in the studied region. This work was carried out in the Laboratory of Physical Properties and packaging in Department of Food Science and Technology in University of Tehran, Karaj, Iran. The seeds were cleaned in an air-screen cleaner to remove all foreign matter such as dust, dirt, stones, chaff and immature and broken seeds. The seeds' initial moisture content was determined by the oven method [21].

The samples were placed into polyethylene bags and sealed. The sealed samples were kept in a curing room for two days to enable the moisture to distribute uniformly throughout the grains. After the grains reached equilibrium moisture, each sample was placed in a desiccator. Before

each test, the required quantity of samples was taken out of the desiccator and allowed to warm to room temperature.

For grain moisture content considered, 50 grains were selected at random from the chamber, dried down to the desired moisture content, and the length, width and thickness were measured in three mutually perpendicular directions using a micrometer gauge reading to 0.001 mm. Several investigators [22, 23, 24, 25, 26, 27] have measured these dimensions for other grains and seeds in a similar manner to determine size and shape properties. The sphericity was calculated using (1) and (4), the volume using Eq. (5) and the surface area using (6) and (7). Grain mass was measured with a sensitive electronic balance of 0.001 gm sensitivity.

After the determination of the dimensions, all other measurements which followed were replicated five times at the moisture content considered and the averages were calculated. The grain or true density was determined using the toluene displacement method [26]. The bulk density was determined with a weight per hectoliter tester which was calibrated in kg per hectoliter [27, 28, 29, 30]. This may also be done using the air comparison pycnometer [31]. The porosity was then calculated using Eq. (8).

According to Mohsenin (1970), the degree of sphericity,  $\phi$  can be expressed as follows [25]:

$$(1) \phi = \frac{(LWT)^{1/3}}{L}$$

Where L is the grain length, W the grain width and T is the grain thickness.

$$(2) D_g = (LWT)^{1/3}$$

$$(3) \phi = \frac{D_g}{L}$$

The geometric mean diameter,  $D_g$  is given by (Sreenarayanan, Subramanian, & Visvanathan, 1985; Sharma, Dubey, & Teckchandani, 1985); Jain and Bal (1997) have also stated that the sphericity,  $\phi$  seed volume, V and grain surface area, S may be given by [28, 30, 32]:

$$(4) \phi = \left[ \frac{B(2L - B)}{L^2} \right]^{1/3}$$

$$(5) V = \frac{\pi B^2 L^2}{6(2L - B)}$$

$$(6) S = \frac{\pi B L^2}{2L - B} \text{ where } B = (WT)^{0.5}$$

The surface area, S was also found by McCabe, Smith, and Harriott (1986) [33] to be given by:

$$(7) S = \pi D_g^2$$

The bulk density was determined by dividing this weight by the 20 cc volume. This method was repeated five times; the averaged bulk density was thus determined. True density – defined as the ratio of the volume of particles – was determined using the water displacement method to the known volume of the sample.

According to Mohsenin (1970) [25] and Thompson and Isaacs (1967) [31], the porosity ( $\epsilon$ ) is given by:

$$(8) \epsilon = \frac{[(\rho_g - \rho_b)100]}{\rho_g}$$

Where  $\rho_b$  is the bulk density and  $\rho_g$  is the true or grain density

The static coefficients of friction were determined with respect to three surfaces: wood, glass and galvanized iron. A hollow metal cylinder [Figure-1] of diameter 75mm and depth 50mm and open at both ends was filled with the seeds at the desired moisture content. It was then placed on an adjustable tilting surface such that the metal cylinder. The surface was tilted gradually until the filled cylinder just started to slide down [34].

The static angle of repose is the angle with the horizontal at which the material will stand when piled. This was determined by using an apparatus [Figure-2] consisting of a plywood box 140 x 160 x 35 mm and two plates: fixed and adjustable. The box was filled with the sample, and the adjustable plate was inclined gradually, allowing the seeds to follow and assume a natural slope [21]. Finally, the data were analyzed statistically and figures were plotted using Excel software (2003).

All the experiments were replicated three times, unless stated otherwise, and the average values calculated. All the data was statistically analyzed for various parameters of study at different moisture contents using the SPSS statistical program. Duncan's multiple comparison was used to determine the difference existing at a 1% level of significance.

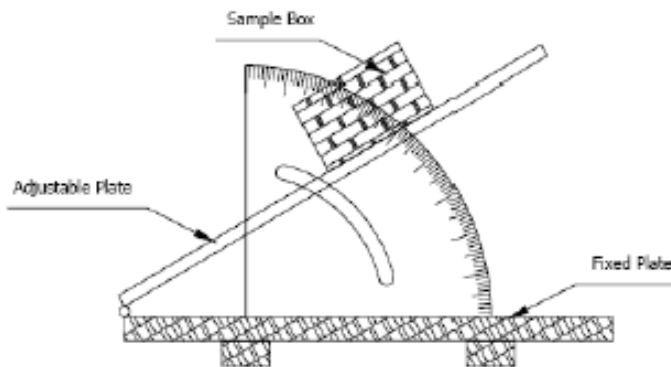


Fig: 1.



Fig: 2.

Fig: 1. Apparatus to determine emptying Angle of Repose. Fig: 2. Apparatus to Determine Coefficient of Static Friction

### [III] RESULTS AND DISCUSSION

The average values of the physical properties, grain specific gravity, bulk density and porosity for the studied factors are shown in [Table-1]. The mean dimensions of about 40 samples at a moisture content of 12% (Wb) were: length 7.43mm, width 2.75mm and thickness 2.53mm. A summary of the physical properties of rice is shown in [Table-1]. Seeds graded uniformly, according to size, provide uniform germination and usually give increased harvesting yield. Effective grading according to width, through sieves with round holes, occurs when the particles lie along the axis perpendicular to the surface of the sieve.

For this, the sieve must be vibrating vertically. When the length of the particle is no more than twice the width, the grading is satisfactory even on sieves which vibrate horizontally [35]. Considering the latter fact, for grading the tested cultivars, grading based on horizontal vibration can be performed. The equivalent diameters for Sadri cultivar were 3.48.

**Table: 1.** Some physical properties of Sadri variety

Property	Variety (Sadri)
	Mean± SD
Length <sup>ns</sup> (mm)	7.43±1.02
Width <sup>a</sup> (mm)	2.53±0.33
Thickness <sup>ns</sup> (mm)	2.75±0.68
Equivalent diameter <sup>b</sup> (mm)	3.48±0.42
Sphericity <sup>ns</sup> (%)	4.352±0.31
Thousand weight of grains <sup>ns</sup>	28±3.41
Porosity (%)	46±1.08
Bulk density (kg m <sup>-3</sup> )	541±3.02
True density (kg m <sup>-3</sup> )	1108.98±35.70
Angle of repose <sup>ns</sup> (deg.)	34±0.28

**Table: 2.** Static Coefficient of friction of Sadri variety against different surface

Property	Variety (Sadri)
	Mean± SD
Length <sup>ns</sup> (mm)	7.43±1.02
Width <sup>a</sup> (mm)	2.53±0.33
Thickness <sup>ns</sup> (mm)	2.75±0.68
Equivalent diameter <sup>b</sup> (mm)	3.48±0.42
Sphericity <sup>ns</sup> (%)	4.352±0.31
Thousand weight of grains <sup>ns</sup>	28±3.41
Porosity (%)	46±1.08
Bulk density (kg m <sup>-3</sup> )	541±3.02
True density (kg m <sup>-3</sup> )	1108.98±35.70
Angle of repose <sup>ns</sup> (deg.)	34±0.28

### [IV] CONCLUSION

This investigation into the properties of rye grains gives rise to a number of conclusions. This paper concludes with information on engineering properties of Sadri variety which may be useful for designing much of the equipment used for rice processing. The static coefficient of friction was highest for wood, followed by galvanized iron and glass. The static coefficient of friction and angle of repose is necessary to design conveying machine and hoppers used in planter machines. When seeds are ground in mills, the rupture force must be known in order to achieve desirable properties without unnecessary expenditure of energy.

### FINANCIAL DISCLOSURE

This work is not supported by any financial assistance.

### CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

### REFERENCES

- [1] Zhout Z, Robards K, Heliwell S, Blanchard C. [2002] Ageing of stored rice: changes in chemical and physical attributes, *Journal of Cereal Science* 35, pp. 65–78.
- [2] Trop Rice International Rice Research Institute [1998–2004] Main Milling Practices (pp.199). Available from [http://www.knowledgebank.irri.org/troprice/Main\\_Milling\\_Practices.htm](http://www.knowledgebank.irri.org/troprice/Main_Milling_Practices.htm).
- [3] Mohsenin N. [1980] Physical properties of plant and animal materials, New York: Gordon and Breach.
- [4] Molenda M, Montross MD, Horabik J, Ross IJ. [2002] Mechanical properties of corn and soybean meal. *Transactions of the ASAE* 45(6): 1929–1936.
- [5] Kashaninejad M, Mortazavi A, Safekordi A, Tabil L G. [2006] Some Physical Properties of Pistachio (*Pistacia vera* L.) nut and its kernel. *Journal of Food Engineering* 72 (1): 30–38.
- [6] Chang C S. [1988] Porosity and density of grain kernels. *Cereal Chemistry* 65(1): 13–5
- [7] Ghasemi Varnamkhasia M, Moblia H, Jafaria A, Keyhania A R, Heidari Soltanabadib M, Rafieea S, Kheiralipoura K. [2008] Some physical properties of rough rice (*Oryza Sativa* L.) grain. *Journal of Cereal Science* 47 (3):496–501.
- [8] Bargale P C, Irudayaraj J, Marquis B. [1995] Studies on rheological behavior of canola and wheat. *Journal of Agricultural Engineering Research* 61: 267–274.
- [9] Çarman K. [1996] Some physical properties of lentil seeds. *Journal of Agricultural Engineering Research* 63: 87–92.
- [10] Gupta RK, Das S K. [1997] Physical properties of sunflower seeds. *Journal of Agricultural Engineering Research* 66 (1): 1–8.
- [11] Murthy CT, Bhattacharya S. [1998] Moisture dependant physical and uniaxial compression properties of black pepper, *Journal of Food Engineering* 37 pp. 193–205.
- [12] Baryeh EA, Mangobe BK. [2002] some physical properties of QP-38 variety pigeon pea. *Journal of Food Engineering* 56: 59–65.

- [13] Ozarslan C. [2002] Physical properties of cotton seed. *Bio-systems Engineering* 83: 169–174.
- [14] Baryeh EA. [2002] Physical properties of millet. *Journal of Food Engineering* 51: 39–46. 8-Baryeh, E.A. and Mangobe, B.K. 2002. Some physical properties of QP-38 variety pigeon pea. *Journal of Food Engineering* 56: 59–65.
- [15] Karababa E. [2006] Physical properties of popcorn kernels. *Journal of Food Engineering* 72 (1): 100–107.
- [16] Dursun E, Dursun I. [2005] Some physical properties of caper seeds. *Biosystems Engineering* 92(2): 237–245.
- [17] Öztürk T, Esen B. [2008] Physical and mechanical properties of barley. *Agricultura tropica et subtropica*. 41(3):117-121.
- [18] Bledzki A K, Mamun AA, Volk J. [2010] Physical, chemical and surface properties of wheat husk, rye husk and soft wood and their polypropylene composites. *Composites part A-applied science and manufacturing* 41(4):480–88.
- [19] Saiz A I, Iurlina M O, Borla O P, Fritz R. [2007] Rheological properties of dough and sensorial quality of bread made from a whole meal rye-wheat blend with the addition of gluten. *Italian journal of food science* 19(4):439–48.
- [20] Czuprynski B, Kotarska K, Kosowski G, Wolska M. [2003] Rheological properties of rye mash prepared by the method of pressure less liberation of starch in mashing and fermentation. *Polish Journal of Food and Nutrition Sciences* 12(2): 19–6.
- [21] Tabatabaeifar A. [2003] Moisture-dependent physical properties of wheat, *International Agrophysics* 17, pp. 207–211. View Record in Scopus | Cited By in Scopus (16)
- [22] Shepherd H, Bhardwaj R K. [1986] Moisture dependent physical properties of pigeon pea. *Journal of Agricultural Engineering Research* 35, pp. 227–234.
- [23] Dutta SK, Nema VK, Bhardwaj RK. [1988] Physical properties of gram. *Journal of Agricultural Engineering Research* 39:pp. 259–268.
- [24] Joshi DC, Das SK, Mukherji RK. [1993] Physical properties of pumpkin seeds. *Journal of Agricultural Engineering Research* 54: pp. 219–229.
- [25] Mohsenin NN. [1970] Physical properties of plant and animal material, Gordon and Breach, New York.
- [26] Singh KK, Goswami TK. [1996] Physical properties of cumin seed. *Journal of Agricultural Engineering Research* 64:pp. 93–98.
- [27] Deshpande SD, Ojha TP. [1993] Physical properties of soybean. *Journal of Agricultural Engineering Research* 56:pp. 89–98.
- [28] Sharma S K, Dubey K, Teckchandani CK. [1985] Engineering properties of black gram, soybean and green gram. *Proceedings of Indian Society of Agricultural Engineers* 3:pp. 181–185.
- [29] Suthar SH, Das SK. [1996] some physical properties of karingda seeds. *Journal of Agricultural Engineering Research* 65:pp. 15–22.
- [30] Jain RK, Bal S. [1997] Properties of pearl millrt, *Journal of Agricultural Engineering Research* 66:pp. 85–91.
- [31] Thompson R A, Isaacs G W. [1967] Porosity determination of grains and seeds with air comparison pycnometer. *Transactions of ASAE* 10: pp. 693–696.
- [32] Sreenarayanan VV, Subramanian V, Visvanathan R. [1985] Physical and thermal properties of soyabean. *Proceedings of Indian Society of Agricultural Engineers* 3:pp. 161–169.
- [33] McCabe WL, Smith JC, Harriott P. [1986] Unit operations of chemical engineering, McGraw-Hill, New York.
- [34] Razavi S, Milani E. [2006] some physical properties of the watermelon seeds, *African Journal of Agricultural Research* 13: pp. 65–69.
- [35] Klenin NI, Popov IF, Sakun VA. [1986] Agricultural Machines, Theory of Operation, Computation of Controlling Parameters and the Condition of Operation. Reka Printers Private Limited, New Dehli.



# MULTIDISCIPLINARY MANAGEMENT OF A PATIENT WITH PAPER PIN BITING HABIT: A CASE STUDY

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## ABSTRACT

Habits can, and all too frequently do, cause aesthetic or functional problems in the mouth. For this reason, destructive habits need to be diagnosed and corrected as early as possible. Many patients are unaware that even holding pins or needles in mouth can cause severe dental problems. This clinical report demonstrates successful multidisciplinary approach to a full mouth rehabilitation of a 50-year-old patient with paper pin biting habit whose dentition had been esthetically and functionally compromised because of dentition wear, and reduced vertical dimension. The objective of complete mouth rehabilitation was to reconstruct, restore, and maintain health of the entire oral mechanism. Anterior bite plane was used after the decision of increasing vertical dimension, constructed using anatomical landmark, facial and physiologic measurement. The treatment entailed using posts and cores, metal-ceramic restorations, and a fixed partial denture. This case report shows that a satisfactory clinical result of severely worn dentition can be achieved by multidisciplinary approach.

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### KEY WORDS

Anterior bite plane; habits; rehabilitation; tooth wear.

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## [I] INTRODUCTION

Habitual use of the teeth for purposes other than chewing food is known as a "factitious habit" (or a "parafunctional habit"). Examples include biting fingernails, trimming fishing line, chewing ice or popcorn kernels, paper pin biting and resting the front teeth on sewing needles, bobby pins or nails. Placing various types of pins, needles, or even bobby pins in mouth is not an uncommon habit, particularly among people who knit and sew or have a stressful office job [1]. These habits can cause chipping or cracking of the teeth, which can further lead to severe attrition of teeth and loss of vertical dimension of occlusion (bite collapse).

Bite collapse can result in damage to the jaw joints; severe pain or dysfunction in the jaw joints (temporomandibular dysfunction, or TMD). A critical aspect for successful treatment is to determine the occlusal vertical dimension (OVD) and the inter-occlusal rest space (IRS) [2]. Articulated study casts and diagnostic wax-up can provide important information which is helpful for the evaluation of treatment options [3]. A systematic approach for managing tooth wear can lead to a predictable and favourable prognosis [4].

This clinical report demonstrates successful multidisciplinary approach to a full mouth rehabilitation of a patient with paper pin biting habit whose dentition had been esthetically and functionally compromised. Written consent was obtained from the patient(s) or their relative(s) for publication of study".

## [II] CASE HISTORY

A 50 year old man, moderately built with good general health reported to the department of Conservative dentistry and Endodontics, Dr HSJ Institute of Dental Sciences and Hospital, Chandigarh, with a chief complaint of difficulty in chewing and poor aesthetics because of worn out teeth. Patient's medical and family history was non contributory. His personal history revealed that he was biting on paper pins for past 15yrs.

Intra oral examination revealed a generalized loss of dental substance that was greater in the maxillary and mandibular anteriors [Figure-1A-C]. Maxillary left canine was worn to the gingival level. Maxillary right first molar and mandibular left first molar were absent. Maxillary right second premolar was palatally placed along with disto-palatal rotation of maxillary right first premolar [Figure-1B]. Multiple teeth were carious. Dentofacial analysis demonstrated a visually shortened facial height for the lower third of the face and enlarged interocclusal space, implying loss of vertical dimension of occlusion.

Radiographic examination included full mouth IOPA and OPG showing features of generalized attrition and pulp exposure [Figure-2]. Diagnostic maxillary and mandibular casts were prepared. On the basis of clinical and radiographic examination, full mouth rehabilitation was planned to restore the function, esthetics, speech and comfort of the patient. The patient was

informed of the diagnosis and the treatment planning. His consent was taken before the start of the procedure.



Fig: 1. A) Frontal, B) Maxillary, and C) Mandibular view of severely attrited teeth



Fig: 2. Pre-operative OPG

### [III] TREATMENT

Root canal treatment of maxillary and mandibular anteriors and premolars was started. During the treatment, root of maxillary left central incisor got fractured and root of mandibular right lateral incisor was found to be calcified. Therefore, they were planned for extraction. Carious teeth were restored. Sidewise orthodontic treatment for the derotation of maxillary right first premolar was planned. Maxillary right first premolar was mesio-lingually and maxillary right first molar was disto-lingually present due to the presence of maxillary right second premolar between the two teeth. Therefore, it was decided to

extract maxillary right second premolar and to derotate the two malaligned teeth. The first molar was banded with a preformed band (0.005" X 0.180") having triple buccal tube (0.022" slot Roth) welded on the buccal side and a weldable lingual button on the lingual aspect of the molar band. The first premolar was also banded with a self fabricated band from premolar band material (0.004" X 0.150") and a weldable lingual button was welded onto it on the lingual side [Figure-3A]. A closed coil NiTi spring ( 9mm length) was then attached to the two lingual buttons with the help of a stainless steel ligature wire (0.009" diameter) to provide reciprocal force ( disto-lingual on the premolar and mesio-lingual on the molar) to bring about the correction of the rotations. The corrections took about 2 months

after which both the bands on the premolar and the molar teeth were removed.

Initially this case was overwhelming, as there were so many factors necessary to achieve a successful treatment outcome. After mounting and studying the casts, it was obvious that the patient's vertical dimension had to be increased to a proper, comfortable position, which has been called the physiologic neuromuscular position. For this the patient's casts were mounted on a semi-adjustable articulator (Hanau TM Modular Articulator; Whip Mix Corp., Louisville, USA) using a face-bow record and an interocclusal record that was made with the aid of bite wax material.

The new VDO was set by 5 mm increase in the incisal guidance pin of the articulator, utilizing which an anterior bite plane was made.

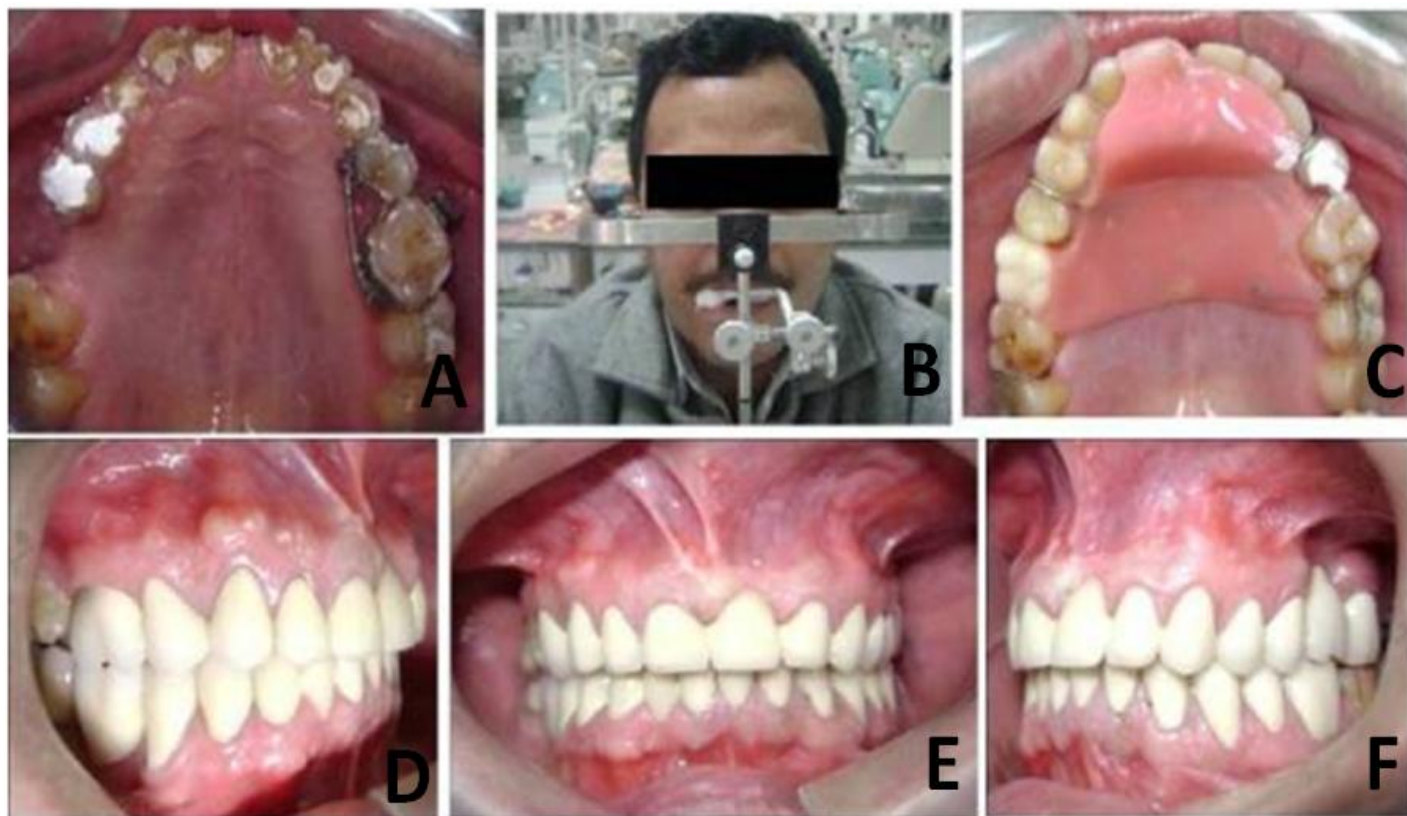
Anterior bite plane is a modified Hawley's appliance with a flat ledge of acrylic behind the upper anteriors [Figure-3C]. When the patient bites, the mandibular incisors contact the bite plane thus disoccluding the posteriors which are then free to erupt<sup>5</sup>. Along with the plate two acrylic teeth were also added to temporarily replace missing molars and maxillary left central

incisor. He was advised to wear the splint throughout the day and night.

The adaptation of patient to the increased VDO of 2mm was evaluated during 1-month trial period. No muscle tenderness and temporomandibular discomfort was found. Next increment of 3mm was further added for another 2 months.

Composite build-ups of maxillary right central incisor, lateral incisor, canine and maxillary left lateral incisor, canine first and second premolar were done. Prefabricated metal posts were placed in mandibular left central and lateral incisor and mandibular right central incisor and composite build-ups were done.

The crown and bridge work was divided into 4 stages. Firstly the maxillary anteriors followed by mandibular posteriors, then mandibular anteriors and finally the maxillary posterior teeth [Figure-3D-F]. Postoperative OPG is shown in Figure-4. During the prosthetic work, intentional root canal treatment of maxillary left second molar was planned. The patient was followed up for one year on a regular three month recall appointment schedule and he was satisfied with the results.



**Fig: 3.** A) Maxillary molar and premolar are banded for derotation, B) Face bow transfer to record in vertical dimension, C) Anterior bite plane occlusal view, D) After treatment intraoral view, right lateral view, E) Frontal view, and F) Left lateral view



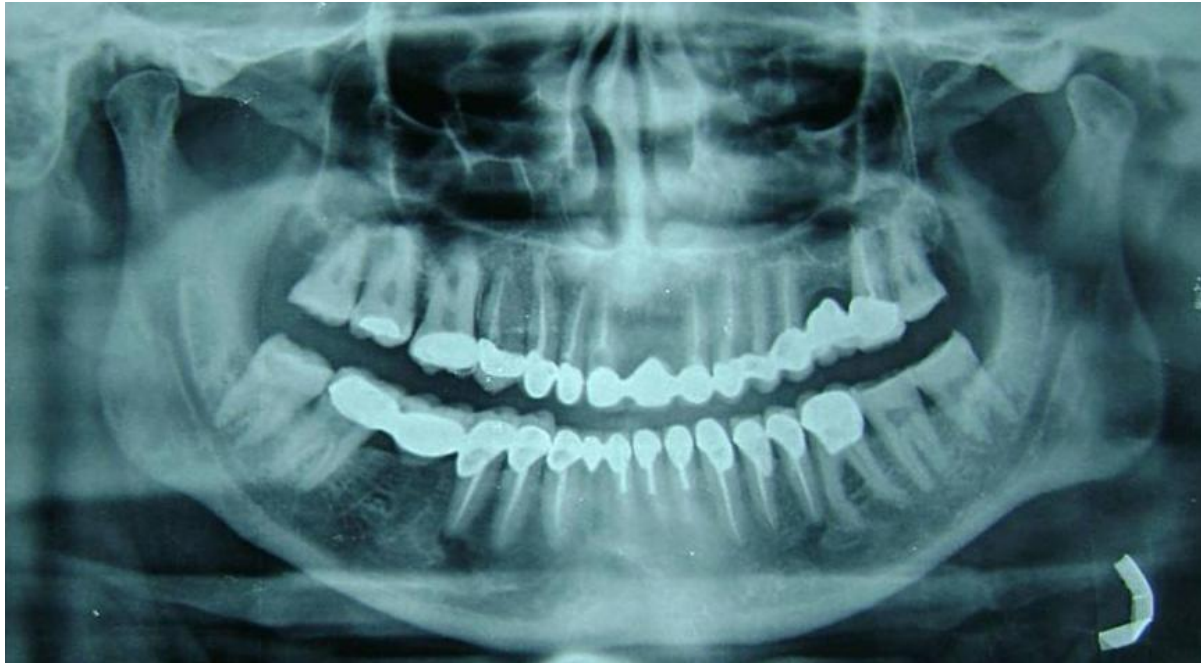


Fig: 4. Post-operative OPG

#### [IV] DISCUSSION

A habit can be defined as the tendency towards an act that has become a repeated performance, relatively fixed, consistent and easy to perform by an individual. Habits are acquired as a result of repetition [6]. In the initial stages there is a conscious effort to perform the act. Later the act becomes less conscious and if repeated often enough may enter the realms of unconsciousness. Certain habits either temporarily or permanently are harmful to teeth and supporting structures. Thus, patient may require full mouth rehabilitation after a certain time period.

The objective of complete mouth rehabilitation must be the reconstruction, restoration, and maintenance of the health of the entire oral mechanism [7].

Depending on the degree of tooth wear, full-mouth rehabilitation of worn dentitions can be accomplished by conservative composite resin restorations, resin bonded ceramic crowns and post-and-core and cast restorations [8-10]. The use of composite resins for severely worn dentitions is a relatively cost-effective option when patients cannot afford an expensive restorative rehabilitation, but a successful treatment is linked to patient-related factors, such as oral hygiene and feeding habits [11], treatment plan-related factors, including occlusal adjustment, bruxism control and GERD treatment [12], in addition to the technique and materials to be employed [13]. Nonetheless, the composite resin restoration could not be used for the patient in this case as the remaining tooth structures were too small to have sufficient retention of composite. Therefore, the conventional treatment modality that includes splint, careful monitoring, and definitive prosthesis, was chosen.

The increase of VDO was determined not by standardized aesthetic golden proportion of anterior teeth but by patient's physiologic factor like interocclusal rest space and speech. Anterior bite plane was used as a means to raise the vertical dimension of occlusion (VDO) for 3 months. Basic function of a splint is referred to as muscle deprogrammer and it helps the condyle in returning to their centric relation position [14]. The patient was carefully monitored for 3 months to evaluate the adaptation to the removable occlusal splints.

In this case fixed partial dentures were preferred over implants because of financial reasons.

#### [V] CONCLUSIONS

Treatment of patients with destroyed dentition is very difficult clinical procedure and challenging for dental profession. The use of diagnostic elements and a preoperative treatment plan allows the clinician to identify areas of concern, allows the desired protocol for restoration. The normal healthy function of the masticating apparatus is the ultimate aim of full mouth rehabilitation.

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#### CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.



## REFERENCES

- [1] Goldstein RE, Curtis JW, Farley BA. Oral habits. Available at: [www.scribde.com](http://www.scribde.com).
- [2] Doan PD, Goldstein GR. [2007] The use of a diagnostic matrix in the management of the severely worn dentition. *Journal of Prosthodontics* 16:277–281.
- [3] Song MY, Park JM, Park EJ. [2010] Full mouth rehabilitation of the patient with severely worn dentition: a case report. *Journal of Advanced Prosthodontics* 2: 106–110.
- [4] Mahboub F, Fard EM, Geramipناه F, Hajimiragha H. Prosthodontic.[2009] Rehabilitation of a Bruxer Patient with Severely Worn Dentition: A Clinical Case Report. *Journal of Dental Research, Dental Clinics, Dental Prospects* 3(1):28–31.
- [5] Bhalajhi SI. Management of deep bite In: Bhalajhi SI (2ed). Orthodontics, the art and science. Arya publishing house, 2000:414–415.
- [6] Funk and Wagnalls. [1952] New Practical Standard Dictionary.
- [7] Kazis H, Kazis AJ. [1960] Complete mouth rehabilitation through fixed partial denture prosthodontics. *Journal of Prosthetic Dentistry* 10:296–303.
- [8] Barron RP, Carmichael RP, Marcon MA, Sandor GK. [2003] Dental erosion in gastroesophageal reflux disease. *Journal of the Canadian Dental Association* 69(2):84–89.
- [9] Little JW. [2002] Eating disorders: dental implications. *Oral Surgery, Oral Medicine, Oral Pathology Oral Radiology, and Endodontology* 93(2):138–143.
- [10] Smales RJ, Berekally TL. [2007] Long-term survival of direct and indirect restorations placed for the treatment of advanced tooth wear. *European Journal of Prosthodontics and Restorative Dentistry* 15(1):2.
- [11] Gandara BK, Truelove EL. [1999] Diagnosis and management of dental erosion. *Journal of Contemporary Dental Practice* 1(1):16–23.
- [12] Parker MW. [1993] The significance of occlusion in restorative dentistry. *Dental Clinics of North America* 37(3):341–351.
- [13] Roeters JJ. [2001] Extended indications for directly bonded composite restorations: a clinician's view. *Journal of Adhesive Dentistry* 3(1):81–87.
- [14] Okeson JP. [2003] Occlusal Appliance Therapy. In: Management of Temporomandibular Disorders and Occlusion, 5<sup>th</sup> Ed Mosby Company, St. Louis, Missouri, p. 509–512.

# ORGANIZATION AND MANAGEMENT OF DRUGS FOR PEACEKEEPING HOSPITAL IN AFRICA: EXPERIENCE FROM CHINA

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## ABSTRACT

Maintaining international peace and security is an important purpose of peacekeeping operations. Now United Nations (UN) are playing an important role in abating regional conflicts preventing or reducing the bloody conflict and the peaceful settlement of international disputes. China has consistently supported and actively participated in the UN peacekeeping operations, making a positive contribution to world peace. South Sudan became independent in 9 July 2011, and we participated in the first peacekeeping operation to South Sudan in January 2012. Now we would like to share experience of participation in drug support of level II hospital in South Sudan, and we hope this article will work for the follow-up pre-deployment medical detachment of the UN peacekeeping operations and the implementation of their medical task.

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### KEY WORDS

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## [I] REPORT

### 1.1. Acquired Preparation of drugs

All drugs of level II hospital are prepared from home country. Before deployment on UN operations, self-sustained requirements should be transported by airlift and sealift to South Sudan. Emergency drugs should be airlifted, because it often takes over 3 months for sea shipment to arrive. Preparation of drugs should begin as early as possible. It is better to begin with the pre-deployment organizing and training, because after the training, there would be little time left, we have no chance to deal with emergency. For example, if some drugs purchased are nearing their expiration date, they would be of no use after their arrival by sea. On the other hand, packing lists in two languages should be accomplished after packing; it is also difficult to complete this in limit time.

### 1.2. Transport and storage of drugs

Most of drugs are transported through sealift because of the weight limit of airlift. However, sealift takes a long time and air humidity is high, we should take necessary precaution for the drugs. For example, glass packaging should be avoided for large liquid solutions, to prevent breakages. When packing, some buffering should be prepared in case the drugs are squeezed. In addition, the box should be strong, durable and sealed. Some drugs, such as aerosol containing propellants can not be transported by air, while other drugs that need to be kept cold should be airborne. In peacekeeping mission area, the

environmental temperature can reach up to 40°C. Storage condition can not meet the requirements of many drugs. Because of the adverse conditions of transportation and storage, we should check the drug quality carefully when dispensing prescriptions. For example, we cannot dispense leaking eye drops or if a vitamin C injection turns yellow, it should be rejected.

### 1.3. Checking and disposal of expired drugs

Because the medical contingent bring with them many drugs, at some point, some drug do expire. In addition, we have to store some emergency drugs. Gradually, many drugs expire. So we check the expiration date of each drug every month and pick out outdated drugs in time. It is also necessary to check the expiration date when dispensing prescriptions. In order to avoid unnecessary wastage, we donate the excess drugs to a local public hospital in proper time. Humanitarian aids to the civilians should be performed under the united standard and planning of UN agencies. The health education and medical skill training are more emphasized than the donations of medical materials. It is important for the sector to build the outer medical support system.

### 1.4. Preparing return and evacuation

Pharmacist should make drug raising plan and complete inventory count before evacuation for a smooth handover.

However, epidemiologic studies have documented a range of psychiatric disorders among personnel serving in the military such as, general anxiety disorder, panic disorder, and major depression [1]. Peacekeepers are widely homesick, or anxious at that time. Carelessness in working may lead to adverse consequences. For example, if the pharmacist can not take notice of the shortage of antipyretics, or there are plenty of these drugs which are about to expire, then the following detachment can not supply antipyretics in time and future medical work would be seriously influenced by the lack of these drugs. So the pharmacist should have a high sense of responsibility, working hard and carefully.

## [II] CONCLUSION

Within the deployment of the UN peacekeeping mission in south Sudan and the specific medical conditions of Chinese peacekeeping medical detachment, we summarized some useful experience. We think it will provide useful reference for the follow-up pre-deployment medical detachment of the UN peacekeeping operations.

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## CONFLICT OF INTERESTS

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## REFERENCES

- [1] Sanyal C, Asbridge M, Kisely S, et al. [2011] Prevalence and determinants of antidepressant use among Canadian Forces members experiencing major depressive episodes. *J Popul Ther Clin Pharmacol* 18: e54-e64.

## CONGENITAL ANOMALIES

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### ABSTRACT

*Congenital anomalies are present at birth and manifest in different parts of the body. But certain anomalies are esthetically compromised which affect the patient psychologically. Here we report four different cases with congenital anomalies. All these anomalies had additional orofacial manifestations. Case reports with review of literature are discussed.*

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### [I] INTRODUCTION

Congenital anomalies do occur because of a variety of etiologies. It is the prime duty of a clinician to diagnose the patients with congenital anomalies promptly so that proper care could be rendered to the patients. They vary in their clinical presentations. Approximately 2% of infants are born with extrinsically caused deformations that usually arise during late fetal life from intrauterine constraint. Approximately 30% of deformed infants have two or more deformations. Deformed infants tend to show catch-up growth toward their genetic potential during the first few postnatal months after release from the intrauterine constraining environment. Congenital deformations of the head and neck are common, and most resolve spontaneously within the first few days of postnatal life. When they do not, further evaluation may be necessary to plan therapeutic interventions that may prevent long-term consequences [1]. This article would present a series of case reports manifesting with various congenital anomalies.

### [II] CASE REPORT

#### 2.1. Case Report-1

A male patient 27 years of age on examination of left side preauricular area there was a sinus opening with no discharge and the patient revealed its presence since childhood. On examination of hands there was syndactyly of 2nd and 3rd finger

with single palmar crease. Verbal consent was taken from the patient for the photographs [Figure-1].

The opening of the preauricular sinus has also been reported along the posteriosuperior margin of the helix, the tragus or the lobule [2]. The majority of patients with preauricular sinus are asymptomatic [3, 4]. A thorough history of head and neck examination is mandatory in all cases, seeking evidence of associated anomalies. Where it is an isolated, asymptomatic finding no treatment is required.

Syndactyly is a shared feature of more than 28 syndromes, including Poland, Apert, and Holt-Oram syndromes. Syndactyly is a failure of differentiation in which the fingers fail to separate into individual appendages. This separation usually occurs during the sixth and eighth weeks of embryologic development [5].

A simian crease is a single line that runs across the palm of the hand. People normally have three creases in their palms. A single transverse palmar crease formed by fusion of the proximal and distal palmar creases a common but not pathognomonic feature of Down syndrome; also found in 1% of the normal population. The simian crease is the most medically researched marker found on the hands because it is the most noticeable. A very high percentage of Down syndrome children have this marker, however, this doesn't mean everyone with this marker has Down [6].





Fig-1: Syndactyly of 2<sup>nd</sup> and 3<sup>rd</sup> finger with single palmar crease

## 2.2. Case Report-2

A 13 years old male patient on examination had broad nasal bridge, hypoplastic ala nasi, short first finger, clinodactyly of fifth finger, genu valgum, metatarsus adductus. Intraorally he had bifid tongue in the anterior region which continued posteriorly as a fissure in the midline. He also had ankyloglossia along with malocclusion. Verbal consent was taken from the patient for the photographs [Figure-2].

“Clinodactyly” or radio-ulnar deviation of the digits is a common congenital anomaly of the hand with a reported

incidence of 19.5% in a non-Caucasian population. It is reported in about 1% of normal newborns and 10% of abnormal newborns. The most commonly affected digit is the little finger; the proximal phalanx of the thumb is the second most common site of occurrence. An inherited disorder with a dominant trait, the condition is usually bilateral. It usually results from an angulated short tubular bone - the “delta” phalanx - the middle phalanx commonly as the middle phalanx is the last bone in the hand to ossify [7].



Fig-2: Short first finger, clinodactyly of fifth finger, bifid tongue, ankyloglossia

Ankyloglossia, commonly known as tongue tie, is a congenital oral anomaly which may decrease mobility of the tongue tip and is caused by an unusually short, thick lingual frenulum, a membrane connecting the underside of the tongue to the floor of the mouth. Ankyloglossia varies in degree of severity from mild cases characterized by mucous membrane bands to complete ankyloglossia whereby the tongue is tethered to the floor of the mouth [8, 9].

### 2.3. Case Report-3

A female patient 7 years of age on examination had triangular shaped forehead, negative canthal tilt, microphthalmia. No other abnormalities were observed. Verbal consent was taken from the patient for the photographs [Figure-3].

Metopic synostosis is a relatively rare form of nonsyndromic synostosis. The incidence of metopic synostosis was reported to be 0.3 per thousand 1,000 live births. Under normal conditions, metopic suture remains patent until 2-3 years of age and so frontal bone development continues. However, premature closure of the metopic suture results in trigonocephaly. In

trigonocephaly, a 'keel-shaped' deformity of the forehead is present and this is characterized by a prominent midline ridge, bilateral frontotemporal constriction with compensatory biparietal expansion, supraorbital and lateral orbital retrusion and hypotelorism. Besides that, interorbital distance decreases, orbita is rotated posterolaterally and radiological findings show superomedial harlequin deformity [10]. Trigonocephaly appears as part of the phenotype of some well known syndromes, such as opitz trigonocephaly syndrome, craniotelencephalic dysplasia, Say Meyer syndrome, VSR syndrome, and in autosomal dominant trigonocephaly without associated anomalies [11].

The major causes for this disorder are genetic but environmental factors have also been implicated such as exposure to radiation, chemicals, or viruses. Microphthalmia in newborns is sometimes associated with fetal alcohol syndrome or infections during pregnancy, particularly herpes simplex virus, rubella and cytomegalovirus, but the evidence is inconclusive. Genetic causes of microphthalmia include chromosomal abnormalities Patau syndrome, Triploid Syndrome, and Wolf-Hirschhorn Syndrome or monogenetic Mendelian disorders [12].



Fig-3: Triangular shaped forehead, negative canthal tilt, microphthalmia

### 2.4. Case Report-4

A female patient 16 year of age came to our department for regular check up. She gave history of consanguineous marriage and frequent fracture of bones on any severity of trauma. On examination she had bluish discoloration of sclera of eyes. On forehead she had scar resembling cigarette foil appearance. Her oral examination revealed no abnormality except flourosis. Radiological investigation revealed normal thickness of enamel and dentin. So a provisional diagnosis of osteogenesis imperfect was given. Verbal consent was taken from the patient for the photographs [Figure-4].

Osteogenesis imperfecta (OI) is a heterogeneous group of genetic disorders that affect connective tissue integrity [13].

Tissues in which the principal matrix protein is type I collagen (mainly bone, dentin, sclerae, and ligaments) can be affected. The resultant abnormalities include blue sclera, rigidity of the osseous tissue, hearing loss, dentinogenesis imperfecta (DI), growth deficiency, laxity of the joints, and any combination of these characteristics [14]. The incidence of OI in infancy is about 1 per 20,000-30,000 in an Australian study [15].

There are extreme phenotypic variations within the OI



population. Four types of OI including mild, perinatal lethal, progressive deforming, and moderately severe were classified according to clinical, genetic, and radiographic criteria [16].

Each of the 4 types of OI is further subdivided on the basis of the absence or presence of D [17].



**Fig-4: Bluish discolouration of sclera of eyes, flourosis**

### [III] CONCLUSION

A thorough knowledge about the developmental anomalies is essential so as to diagnose the ailments of the patients and

intervene at an earlier stage. Hence, as a futuristic approach; proper genetic counseling is essential to prevent such anomalies in the progeny of the affected family.

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### CONFLICT OF INTERESTS

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### REFERENCES

- [1] Gorlin RJ, Cohen MM, Hennekam. [2001] Syndromes of Head and Neck. Oxford University Press, New York.
- [2] Chami RG, Apesos J. [1989] Treatment of asymptomatic preauricular sinuses: challenging conventional wisdom, *Ann Plast Surg*, 23: 406–411.
- [3] O'Mara W, Guarisco L. [1999] Management of the preauricular sinus, *J La State Med Soc.*, 151(9):447–450.
- [4] Lau JT. [1983] Towards better delineation and complete excision of preauricular sinus, *Aust N Z J Surg*, 53: 267–269.
- [5] E Gene Deune. [2012] Syndactyly. <http://emedicine.medscape.com/article/1244420-overview> [assessed 12/07/2012].
- [6] Malla T, Malla KK, Gauchan E, Brijesh S, Sharma R. [2010] Incidence of Simian Crease in Normal Nepalese Children. *Kathmandu University Medical Journal* 8(3):410–414.
- [7] Ravishanker R, Bath AS. [2004] Distraction - A Minimally Invasive Technique for Treating Camptodactyly and Clinodactyly. *MJAFI*, 60(3): 227–230.
- [8] Messner AH, Lalakea ML. [2002] "The effect of ankyloglossia on speech in children". *Otolaryngology--head and neck surgery: official journal of American Academy of Otolaryngology-Head and Neck Surgery* 127 (6): 539–545.
- [9] Horton CE, Crawford HH, Adamson JE, Ashbell TS. [1969] "Tongue-tie". *The Cleft palate journal* 6: 8–23.
- [10] Akan M, Avci G, Silav G, Aköz T, Elmaci I. [2011] Surgical Treatment of Trigenocephaly. *J Neurol Sci [Turk]* 28:042–050.
- [11] Zanini, Silvio A, Neto, Eliseu Paglioli, Viterbo, Fausto, da Costa, Antonio Richieri, Tershakowec M. [1992] *Trigenocephaly* 3(2):85–89.
- [12] Microphthalmia. [2012] <http://en.wikipedia.org/wiki/Microphthalmia> [assessed 12/07/2012]
- [13] Niyibizi C, Smith P, Mi Z, Robbins P, Evans C. [2000] Potential of gene therapy for treating osteogenesis imperfecta. *Clin Orthop Relat res* (379 suppl), S126–133.
- [14] Marini JC. [1988] Osteogenesis imperfecta: comprehensive management. *Adv Pediatr* 35:391–426.
- [15] Sillence DO. [1981] Osteogenesis imperfecta: an expanding panorama of variants. *Clin Orthop Rel Res* 159:11–25.
- [16] Sillence DO, Senn A, Danks DM. [1979] Genetic heterogeneity in osteogenesis imperfecta. *Am J Med Genet* 16: 101–116.
- [17] Sillence DO. [1988] Osteogenesis imperfecta nosology and genetics. *Ann NY Acad Sci* 543:1–15.