

EVALUATION OF THE PROPHYLACTIC EFFECT OF POMEGRANATE PEEL EXTRACT AND PUMPKIN SEED OIL ON THE PERIODONTIUM OF RATS RECEIVING METHOTREXATE: HISTOLOGIC AND IMMUNOHISTOCHEMICAL STUDY

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ABSTRACT

Objectives: One antimetabolite that is frequently used to treat cancer is methotrexate (MTX). Its frequent usage has been linked to negative skeletal effects such decreased bone production and bone loss. This research's objective was to assess the preventive role of pomegranate peel extract and pumpkin seed oil on the histological and immunohistochemical changes of periodontium of rats receiving methotrexate (MTX).

Material and Methods: Thirty-two male albino rats were randomly distributed into four groups.: Group I; normal, Group II; received MTX, Group III and Group IV; received MTX + pomegranate peel extract (PPE) and MTX +pumpkin seed oil (PSO) respectively. Mandibles were viewed under a light microscope for histological analysis using hematoxylin and eosin, specific Masson stain, and immunohistochemical evaluation using the TRAP labelling index that was calculated and statistically assessed.

Results: group II showed partial resorption for lining of alveolar bone with dissociation of PDL fibers that devoid of fibroblast with more improvement in the histological result in PSO group compared PPE group. Regarding the immunohistochemical results, PSO group displayed a significant decrease in TRAP labelling index compared to other groups.

Conclusion: both PPE and PSO could be used as a prophylactic dietary supplement against the adverse effect of MTX on the periodontium although PSO showed more promising effect.

KEY WORDS: Methotrexate, Pomegranate Peel Extract, Pumpkin Seed Oil, periodontium.

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INTRODUCTION

Chemotherapy has been shown to have detrimental effects on bone, affecting bone remodelling and bone mass, in both human and animal investigations [1,2,3,4]. Anti-metabolite methotrexate (MTX) is a commonly used chemotherapeutic medication. It inhibits the conversion of folic acid to tetrahydrofolic acid, which is necessary for DNA synthesis and cell replication, by competing with it for the folate binding site of the enzyme dihydrofolate reductase (DHFR) [5,6,7]. It has been demonstrated that MTX causes osteopenia, fractures, and bone discomfort in children with leukaemia [8,9]. Previous studies using rat models have demonstrated that MTX decreases trabecular bone volume, which is associated with increased adipogenesis, enhanced osteoclastogenesis, and decreased osteogenesis potential within the bone marrow, and thus a lower osteoblast number but a higher osteoclast density on the bone surface as well as a higher adipocyte density in the bone marrow [1,2,10,11,12]. Because high-dose MTX can decrease osteoblast volume without reducing number, it may be hazardous for these cells for a brief period of time. Additionally, MTX may have a negative impact on osteoid thickness [13]. Both therapeutic and toxic effects of MTX have emerged as a result of the changes made by MTX on many metabolic pathways, including DNA synthesis [14]. In addition, MTX treatment is known to cause bone marrow toxicity [15].

There has been a lot of debate, regarding the possible function of natural substances in protecting against the harmful side effects of chemotherapy and enhancing its effectiveness. Indeed, research has shown that polyphenolic compounds—more especially, flavonoids such as genistein, daidzein, oleuropein, and hydroxytyrosol—have an effect on osteoporosis [16], inhibit the differentiation of osteoclasts [17], and stimulate the creation of osteoblasts [18].

Pomegranate peel extract (PPE), which contains hydrolyzable tannins, flavonoids (catechins, anthocyanins, and other complex flavonoids), and other

bioactive compounds, has been advocated as a rich source of nutrients. Phenolic acids (hydroxycinnamic and hydroxybenzoic acids), pedunculagin, punicalin, and punicalagin, all of which have been demonstrated to have positive effects on health [19,20]. Pomegranate byproducts also contain protein, minerals (calcium, phosphorus, magnesium, potassium, and sodium), and fatty acids (mostly the punicic, linoleic, and oleic acids present in the seeds) [21]. These compounds vary in their chemical nature and play a significant role as antioxidant [22], anti-inflammatory [23], anticarcinogenic [20], antiatherosclerotic [24] antifungal, antibacterial and antiviral drugs in a few human studies, animal models [20] and on cell lines [25].

Also, pumpkin contains highly active molecules called carotenoids, which include lutein, zeaxanthin, and α -carotene. These components improve bone mineral density, minimise fracture risk, and hinder osteoporosis from progressing [26]. Additionally, pumpkin is simple to use in an osmotic dehydration procedure that permits the enrichment of its tissues with calcium salts [27]. Pumpkin that has been enhanced with nutrients includes inulin, which raises the bioavailability of calcium, as well as other components that might strengthen bones, such as lutein, β -cryptoxanthin, and [28,29,30]; as well as a pigment that prevents bone resorption and lowers oxidative stress [31,32].

In this context This study's objective was to figure out the impact of MTX on the condition of the periodontium and assess the protective effects of PPE and PSO against periodontal ligaments (PDL) and alveolar bone resorption that MTX-induced in albino rats.

MATERIALS AND METHODS

Pomegranate Peel Extract

PPE was prepared at laboratory of Chemistry Department, Faculty of Science, Suez Canal University. The pomegranate fruits (*Punica*

granatum L., post-Ghermez variety, 5-64-WS) were purchased from local market and properly cleansed and gently peeled. The peels were divided into small pieces and dried in an air circulation tray dryer at 60 °C for 48 hours. The powdered peels were then ground in a powerful kitchen grinder and sieved using, ASTM No. 10 (1.651 mm). Centrifugation (5,000 rpm for 10 min at 5 °C) and filter sterilization (0.45 m) were used to produce the clear extract from the powder (10 gram), which was extracted at room temperature for 1 hour with 25 ml of boiling distilled water ^[33].

Pumpkin seed oil

This experiment used pumpkin seed oil obtained from <https://www.imtenan.com/Egypt>. Oil was produced from Cucurbita maxima and Cucurbita stilbo seeds using natural cold-pressing methods. The stock oil had a concentration of 1g/mL and was diluted to 1.5 mg/mL with Corn oil as Vehicle.

Sample size calculation:

The sample size for this study was calculated according to **Charan and Biswas, 2013** ^[34] used the following equation:

$$N = \frac{(Z_{\alpha})^2 * (SD)}{(d)^2}$$

N = Total sample size, Z_{α} = Is Standard normal variate and its equal 1.96 at $P < 0.05$, SD = Standard deviation of variable, d = Absolute error or precision

$$\text{Total sample size } N = \frac{(1.9)^2 \times (5.77)^2}{(2)^2} = 31.97 \text{ (32 sample)}$$

Animals

Thirty-two adult male Wistar rats weighing 200 ± 15 gram, 6-8 weeks of ages were used in the investigation. With authorization from the Faculty of Dentistry at Suez Canal University. During the study, the animals were housed in a metal cages and kept in a temperature-controlled environment

of 23°C degrees Celsius, with 12 hours' light-dark cycles and a relative humidity of 40%, as well as free access to food and drink. All procedures of the research were carried out in accordance with WHO-2011 guidelines, and the current study has received approval by the Faculty of Dentistry's Research Ethics Committee (Research Ethics Committee number 701/2023).

Study design

The animals were given identification numbers and at random, they were allocated into 4 separate groups. The randomization was performed using a computer-generated protocol. Group I (negative control group): comprised of 8 normal rats. Group II (MTX group): (Positive control group) consisted of 8 rats, the rats were received methotrexate (MTX) 10mg/kg intramuscularly for 3days, beginning from the 10th day ^[35]. Group III (PPE + MTX group): comprised of 8 rats, the rats were received 500mg/ml/kg/day pomegranate peel extract (PPE) oral administration through a gastric tube once daily for 21days and methotrexate (MTX) 10mg/kg intramuscularly for 3days, starting on the day 10 ^[35]. Group IV (PSO + MTX group): comprised of 8 rats, the rats were received pumpkin seed oil 1.5 ml/kg/day by oral administration through a gastric tube once daily for 21days and methotrexate (MTX) 10mg/kg intramuscularly for 3days, starting on the day 10 ^[36].

Histological observation

At day 22, after completing the experimental procedures, all rats were euthanized by ketamine anesthesia (80mg/kg, i.p.). Then the mandible was dissected and the samples were taken from individual groups. Samples were fixed in 10% Neutral Buffered Formalin (NBF) then decalcified in 10% EDTA solution for two months. The specimens will be then washed properly under running water, dehydrated by transferring through ascending grades of alcohol, then transferred to xylene to

clear the specimens from alcohol and then cut into 4-6 micrometre slices in a mesio-distal directions, which were subsequently fixed in paraffin and prepared^[37] for staining with Hematoxylin and eosin stain (H&E) and with special stain; Masson's trichrome for histological examination, and Immunohistochemical localization: The influence of treatments on osteoclastogenesis were detected by Tartrate resistant acid phosphatase (TRAP) staining for immunohistochemical examination where sections of the mandibles measuring four microns thick were put on optiplus positively charged slides for staining. All sections were examined by light microscope (Olympus model: BX60F5 – Olympus optical company, Japan) with different magnifications at Clinical Pathology department, faculty of Medicine, Cairo University. All the examinations were carried out by blinded examiners.

Statistical analysis

The statistical tests were used to collect, compute, tabulate, and statistically evaluate all of the data. To check the validity of the samples' normal distribution, Shapiro-Wilk normality testing was applied. The formula for calculating descriptive statistics was Mean Standard Deviation (SD). The four groups were compared using one-way ANOVAs. Pairwise comparisons were made using Tukey's post hoc analyses.

P value ≤ 0.05 is considered statistically significant. The SPSS software for Windows version 26.0 (Statistical Package for Social Science, Armonk, NY: IBM Corp) was used for all analysis, and a P-value of 0.05 was used to indicate significance.

RESULTS

Under a light microscope, the positive control group II demonstrated pathological characteristics distinct from negative control group I. (fig. 1) and represented by reduction in thickness at some parts of the surface epithelium and ridges, few clear cells were found near basal layers of epithelium which are less defined. In lamina propria, collagen destruction was distinct in the connective tissue fibers. Loss of osteoblastic layer lining both the alveolar bone and bone marrow and partial loss of dental septum with disorientation and aggregation of collagen fibers in PDL adding no evidence for the presence of fibroblast in focal areas. Lot of inflammatory cells among bone marrow and multiple of osteoclasts based in howships lacune along irregular alveolar bone and scattered red corpuscles were evidenced (fig.2). Group III rats treated with PPE at 500 mg/kg showed no adverse effects against this dose given as a prophylactic agent against MTX. showed some signs of improvement as incomplete reconstruction of collagen fibers within the lamina propria with few scattered fibroblasts and less clear cells. Lining

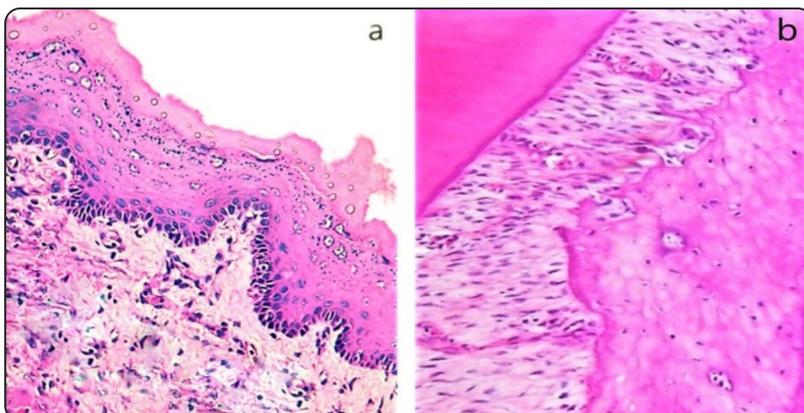


Fig. (1) A photomicrograph of control group I; (a) revealing normal histological features of the gingiva for the surface epithelium of the keratinized stratified squamous type with intact collagen fibers in the lamina propria (H&E, orig magnific. 400). (b) showing normal orientation of PDL with presence of fibroblast and normal lining of alveolar bone (H&E, orig. magnific. 200).

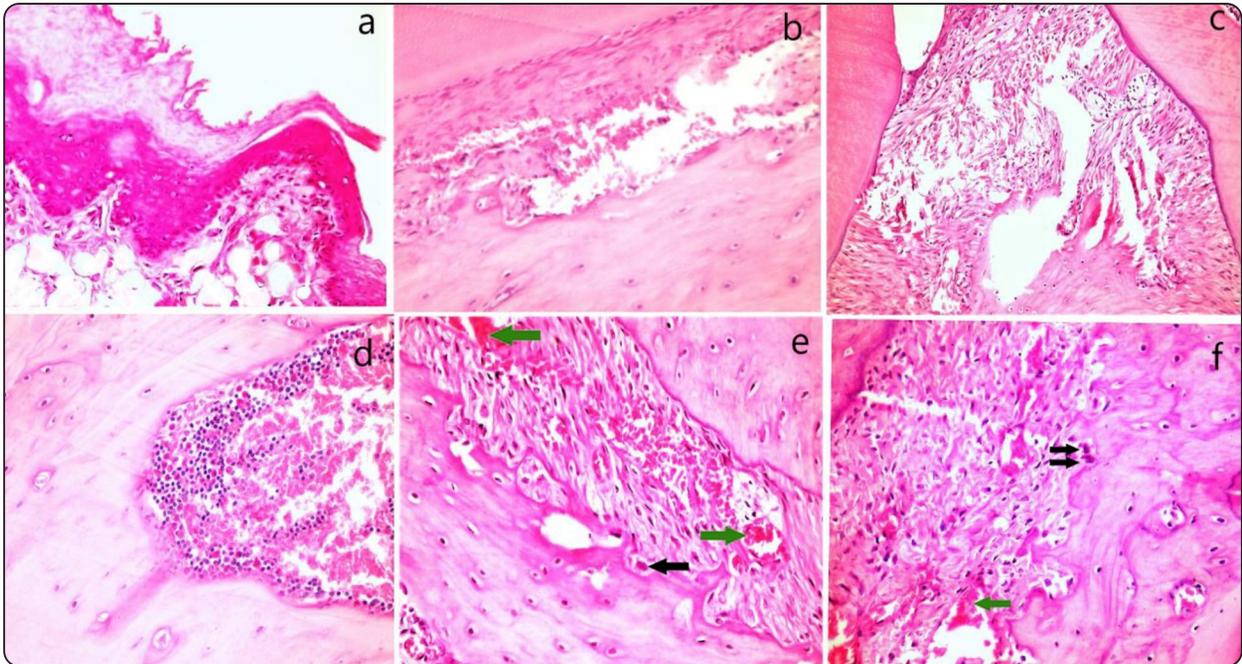


Fig. (2) A photomicrograph of group II MTX; (a) displaying gingiva with few clear cells scattered with loss of epithelial ridge and Lamina propria connective tissue dissociation were detected (H&E. orig. magnific. 400). (b,c) partial resorption for lining of alveolar bone with dissociation of PDL fibers that devoid of fibroblast (HX&E. orig. magnific 200). (d) cellular infiltration in bone marrow in spongiosa (HX&E. orig. magnific 400). (e,f) osteoclasts were detected in their howships lacune (black arrows). Notice the existence of red blood cells (green arrows) (HX&E. orig. magnific. 400).

of alveolar bone has been partially restored as well as PDL collagen fibers inspite of the inflammatory cell infiltration and the presence of diffused red blood cells and no osteoclasts were detected (fig. 3). Group IV, which received PSO treatment where rats showed no adverse effect from the administered dose, displayed normal epithelium thickness and pattern, as well as well-defined collagen fibers in the lamina propria and almost no connective tissue dissociation. Collagen fibers were reconstructed and restored their orientation at PDL region. Absence of irregularities along the alveolar bone with no sight of osteoclasts. Bleeding was reduced and observed in focal areas spotted by red blood cells (fig.4).

Special stain ; Masson trichrome:

Low intensity of staining for Masson trichrome of collagen fibers at the lamina propria of the gingiva and PDL tissue for group II was obtained while more intensity of staining of the treated groups III

and IV to Masson's trichrome stain was detected. Staining intensity was semiquantitatively scored on the base of the following categories: 0, absent; 1, weak; 2, moderate; and 3, intense. (fig.5)

Immunohistochemical results:

The group II animals given MTX injections displayed a highly significant TRAP labelling index which may be due to a possible increase in the osteoclastic activity of the alveolar bone. Compared to the control group, Rats in groups III and IV had higher TRAP labeling index. Staining intensity was semiquantitatively scored on the base of the following categories: 0, absent; 1, weak; 2, moderate; and 3, intense. (fig.6)

Statistic results

All the thirty-two animals were included in the statistical analysis and data were presented as the mean \pm standard deviation (SD) in the table

and graph. The results in table 1, presented a considerable significant difference for the TRAP between the studied groups using One Way ANOVA ($F= 63.72, P<0.0001$). Pair wise comparison displayed highly significant difference between GI with GII and GIII, GII with GIII, while there is no significant between GI with GIV. The highest mean value was recorded in GII (163.48 ± 10.17) followed by GIII (125.27 ± 2.02) and GIV (101.69 ± 3.41) while GI had the lowest value (89.82 ± 7.01). While

studied groups for the Masson trichrome revealed that there is clear significant difference between the groups ($F= 76.63, P<0.0001$). significant difference between GI with GII, GIII and GIV also, GII with GIII and GIV was detected by Pair wise comparison, while there is no significant difference between GIII with GIV. The high mean value was recorded in GI (204.07 ± 7.85) followed by GIV (175.42 ± 5.43) and GIII (170.19 ± 7.01) while GII recorded the lowest value (123.93 ± 5.67) (fig, 7)

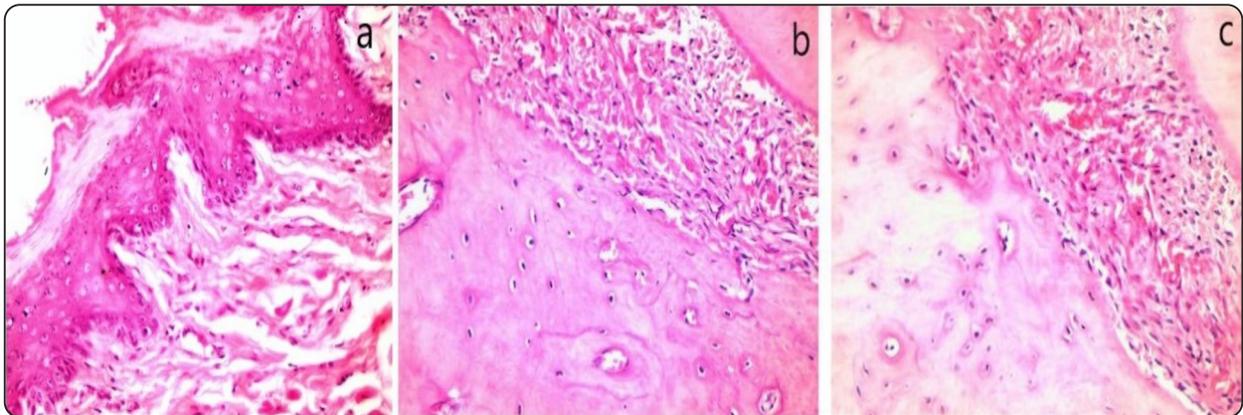


Fig. (3) A photomicrograph of group III PPE+MTX; (a) showing increase in epithelial ridge of gingiva with partial regeneration of collagen fibers at lamina propria (HX&E. orig. magnfic. 400), (b,c) showing PDL with more collagen fibers but less organized and less resorbed sites along alveolar bone (HX&E. orig. magnfic. 400).

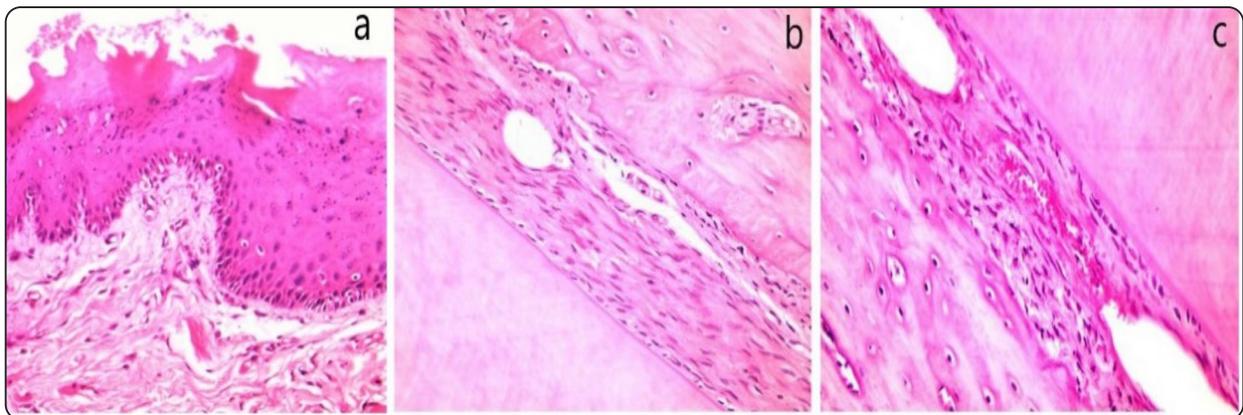


Fig. (4) A photomicrograph of group IV PSO+MTX; (a) revealing fewer clear cells, nearly normal-looking epithelium, and lamina propria. (HX&E. orig. magnfic. 400). (b,c) showing well organized collagen fibers and fibroblast with almost normal lining of alveolar bone. Notice the reduction in red blood cells (HX&E. orig. magnfic. 400).

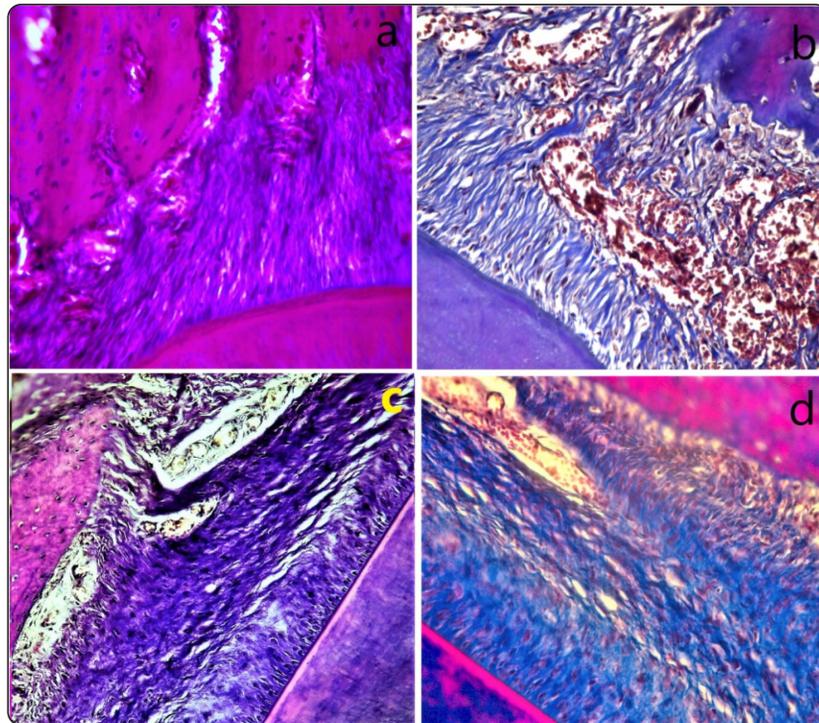


Fig. (5) (a) A photomicrograph of normal PDL showing highly positive staining of collagen bundles in group I to Masson's trichrome stain. (b) showing low intensity in group II while (c) showing moderately positive staining with partial unfilled with collagen bundles in group III and (d) showing highly positive staining in group IV (Masson's trichrome orig. magnific. 400)

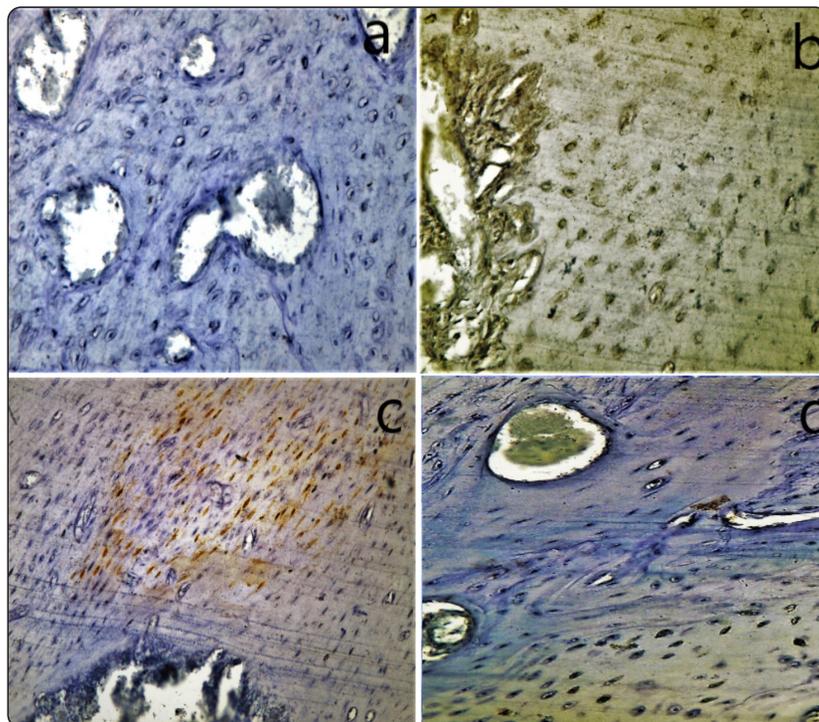


Fig. (6) (a) A photomicrograph of group I alveolar bone demonstrating negative staining reactivity to TRAP (b) showing highly positive staining reactivity in group II while (c) showing moderately positive staining reactivity in group III while (d) in group IV showed moderate to high positively reactivity to TRAP (TRAP. orig. mag. 400)

TABLE (1) Masson trichrome and TRAP labeling index of the different groups.

	Mean± SD			
	Masson trichrome	TRAP		
GI	204.07±7.85 ^a	98.82 ±7.01 ^c		
GII	123.93±5.67 ^c	163.48± 10.17 ^a		
GIII	170.19±7.01 ^b	125.27 ±2.02 ^b		
GIV	175.42±5.43 ^b	101.69 ±3.41 ^c		
F	76.63	63.72		
P value	<0.0001**	<0.0001**		
Pair wise comparison using Tukey`s post hoc				
	Mean difference	P value	Mean difference	P value
GI vs GII	80.14	<0.0001**	-64.66	<0.001**
GI vs GIII	33.88	0.003**	-26.46	0.009**
GI vs GIV	28.65	0.012**	-2.88	0.940
GII vs GIII	-46.3	<0.0001**	38.21	0.001**
GII vs GIV	-51.5	<0.0001**	61.79	<0.001**
GIII vs GIV	-5.2	0.845	23.58	0.033**

** , and different letters means significant difference at $P<0.05$ SD= standard deviation

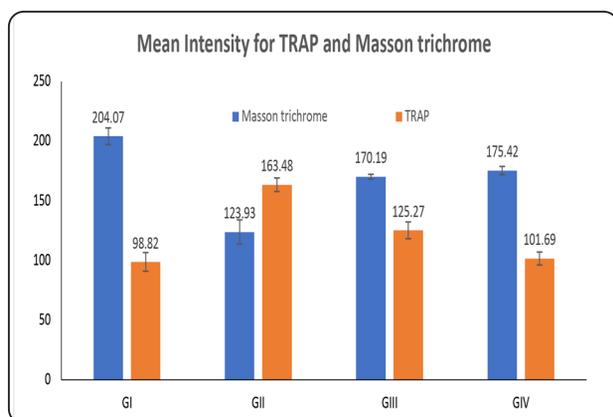


Fig. (7) An evaluation chart illustrates the staining reactivity for osteoclasts and collagen fibers of the different groups to TRAP and Masson respectively.

DISCUSSION

There have been reports that MTX has detrimental effects on bone. The term “MTX osteopathy” was first used to describe a clinical syndrome in children receiving low dose MTX for long-term maintenance therapy after being diagnosed with acute lymphoblastic leukaemia. The syndrome was characterized by stress fractures of the lower extremities, diffuse bone pain, and osteoporosis^[18,38]. Therefore, cancer patients are

increasingly resorting to alternative therapies, such as natural products (nutraceuticals), in search of additional safe and non-toxic treatments to preserve bone during chemotherapy in order to improve bone health and quality of life^[39]. Accordingly, the present study designed to evaluate the protective effect of Pomegranate Peel Extract and Pumpkin Seed Oil On The Periodontium Of Rats Receiving Methotrexate.

The present study showed significant increase of the inflammatory cells after MTX injection, and the thickness of the epithelium decreased with observed hemorrhage in PDL that agrees with other studies showed significant harm in the MTX-treated group, showing infiltration, a considerable increase in mononuclear inflammatory cells, epithelial destruction, and multiple hemorrhagic zones with mucus exudates^[40,41]. Reduced proliferative potential of the epithelium caused by methotrexate is the cause of decreased epithelial thickness. Dihydrofolate reductase (DHFR) is inhibited by MTX, an anti-folate medication that restricts the production of nucleotides^[42]. Consequently, the formation of purines and pyrimidines is hindered, thereby impeding DNA synthesis, a critical process

for the cell cycle^[43]. Resulting in ulceration, inflammation, and direct degradation of epithelial cells^[44].

It is thought that the increased inflammatory cytokines caused by MTX could activate eNOS, endothelial-induced nitric oxide synthase, which would produce NO and combine with peroxynitrite to form superoxide radicals^[45]. This potent oxidant can either directly damage cells by nitrating tyrosine, causing irreversible protein dysfunction, or indirectly by promoting the creation of other reactive chemicals with cell-toxic properties. Thus, extravascular haemorrhage occurred in the MTX group^[46].

Regarding the alveolar bone, MTX group showed irregular alveolar bone and partial loss of dental septum with loss of osteoblastic layer lining both the alveolar bone and bone marrow and multiple of osteoclasts based in howships lacune which confirmed by the immunohistochemical results. The histological examination in present study for MTX group also came in agreement with several studies studies in rat models have revealed that severe MTX chemotherapy reduced trabecular bone volume and osteoblast counts^[1, 3, 10].

The Wnt/catenin pathway has been shown to control bone formation by inducing osteoblastogenesis and inhibiting the apoptosis of the osteoblast^[47, 48]. Notch signalling is recognised to be primarily inhibitory in osteogenesis. It is widely established that the crosstalk between canonical Wnt signalling and Notch signalling pathways regulates osteogenesis and skeletal homeostasis^[49, 50]. Yaser et al., 2021^[51] reported that MTX treatment stimulates Notch2 signalling pathway in osteoblasts and reduces Wnt signalling in osteoblasts, confirming the inhibitory effect of MTX on Wnt/catenin pathway, which was improved to some extent when cells were treated with MTX and antiNotch2 antibody.

In the current study Group III treated with as a prophylactic agent against MTX showed some signs of improvement as incomplete reconstruction

of collagen fibers within the lamina propria and the alveolar bone has been patially restored and no osteoclasts were detected that was in line with the findings of the immunohistochemical localization of TRAP in group III. By enhancing ALP activity and calcium nodule formation, PGPE greatly boosted osteoblastogenesis. Furthermore, serum from PGPE animals was found to be capable of inhibiting RANKL-induced osteoclast development by down-regulating the expression of particular osteoclast markers (calmodulin, CCR2, calcitonin receptor, cathepsin K, and MMP-9)^[52]. that comes in compatible with our findings regarding the lining of alveolar bone that has been patially restored and no osteoclasts were detected.

Group III treated with PPE against MTX showed improvement as incomplete reconstruction of collagen fibers within the lamina propria with few scattered fibroblasts that came close to the study of Hayouni et al. (2011)^[53] that have shown that the pomegranate peel 5% methanolic extract-based ointment boosted collagen, DNA, and total protein content, as well as wound contraction rate. it was documented that PPE gel can expedite fibroblast infiltration, collagen regeneration, vascularization, and epithelialization by increasing no production and the expression levels of TGF-1, VEGF, and EGF in wound tissue^[54]. Furthermore, it has been demonstrated that PPE promotes wound healing by boosting FN1 gene expression and extracellular matrix components such as GAGs and collagen levels, and so can be used as a therapeutic agent for wound healing^[55].

Our research demonstrated that PSO increased fibroblast and epithelial proliferative capacity and decreased osteoclastic activity. This was confirmed in this investigation by the histology and immunohistochemical findings that is consistent with Bardaa et al.'s study, which demonstrated that the proliferative phase of the cut wound, which includes the development of granulation tissue, angiogenesis, fibroblast migration, and collagen synthesis, began on day 3 and was characterised by high collagen and fibroblast density and a

low number of macrophages in the pumpkin oil group^[56]. Pumpkin active constituents, tocopherols, fatty acids, and phytosterols, combine synergistically to generate this effect. These bioactive components contribute to the oil's ability to regenerate by providing connective tissue matrix, reinstalling PDL fibres, and minimizing resorbing sites along the alveolar bone. They also aid in the migration of fibroblasts. Through its antioxidant characteristics, the oil's vitamin E content supports in this healing process by preventing cell degeneration and stimulating DNA production^[57]. Furthermore, it is regarded as a potent peroxy radical scavenger, as it prevents free radicals from harming biological cell membranes. It prevents oxidative DNA damage and counteracts the increased production of reactive oxygen species^[58]. A previous study indicated that PSO, a great source of minerals, vitamins, and antioxidants, created a protective impact through its anti-inflammatory and antioxidant capabilities^[59].

The hydrolysable tannins procyanidins and others have a high affinity for proline, which is a major component of collagen (10% by residue for type 1 collagen)^[60, 61]. Polyphenols have various degrees of affinity for collagen^[62]. Stronger cross-links formed by polyphenol binding to collagen appear to form covalent and hydrogen connections, as well as boost collagen's hydrophobicity and resistance to collagenase^[63, 64]. Which approach our results obtained by Masson trichrome stain that showed high positive reaction of collagen bundles (175.42) with significant difference from group II (-51.5) that approached to normal intensity and orientation to group I (28.65) ($p < 0.05$).

In addition, group IV received the PSO seems to reduce the bleeding and extra vasation of red corpuscles from the blood vessels. As a result, the haemostatic action of the tested oil that was revealed in this investigation may offer a justification for its therapeutic effects. In fact, fibrin, once stabilised, is essential for the initial stages of skin healing. It facilitates the chemotactic effect that attracts fibroblasts and encourages these cells to produce collagen^[63].

According to a study on PSO, polyphenolic compounds stay in soft tissues longer than they do in the blood^[64], and after 1-6 hours of consumption, rats and mice's intestines and livers have shown signs of polyphenol metabolites^[64]. Studies in vitro and fewer investigations in vivo on rats indicate potential for polyphenol-mediated stimulation and inhibition of osteoblasts and osteoclasts, respectively^[65]. In accordance with an in vitro study, polyphenols may prevent bone loss by blocking the pathways which trigger RANKL to cause osteoclast differentiation, whether due to estrogenic activity, various gene expression patterns that results in osteoblast differentiation, or by proactively scavenging ROS^[66]. Rats fed blueberry-rich diets showed a slower rate of bone resorption by reducing RANKL expression from marrow stromal cells, which is more directly related to osteogenesis^[67] that confirm our results regarding localization of TRAP as an indication for the reduction of osteoclastic activity in group IV (101.69) showing a significant difference from group II (61.79) ($p < 0.05$).

Some limitations of this work is that systemic inflammatory biomarkers were not investigated, which could assist analyze the probable explanation of how MTX accelerated alveolar bone loss in rats. This is an animal research with a small number of animals. The pathogenic mechanism in rats may be different from that in humans. As a result, human interpretation and translation must be done with caution. However, the evidence obtained in this animal model is important for progressing in the formulation of human clinical results or eventually becoming compatible with human outcomes.

CONCLUSION

Considering the findings of the present study, PSO reduces the periodontal destruction induced by MTX through decreasing inflammatory activity, osteoclast activity, and fibroblast death. This was demonstrated by the histopathological analysis, which revealed significant periodontal protection with substantially fewer inflammatory

cells, osteoclasts, and haemorrhage in the MTX and PSO treated group corresponding to the immunohistochemical findings, PSO treated group was also demonstrated to have an antiapoptotic impact by lowering the expression of caspase 3 in rats given MTX^[42]. The general dental practitioners in particular should be more aware of a potential connection between MTX therapy, delayed healing, and the possibility of bone fracture given that MTX is a commonly prescribed medication in cancer and RA protocols in the absence of anti-resorptive and anti-angiogenic supplements as a prophylactic agent against the adverse side effects that have been experimentally proven to be associated with MTX drugs.

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Competing interests

The authors declare no competing interests.

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