Original Article

The Possible Therapeutic Role of Ginger Extract in the Effect of Chronic Aluminum Toxicity on Rat Periodontium (Histological and Immunohistochemical Study)

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ABSTRACT

Background: Chronic aluminum intoxication enhanced the risk for different body tissues in human and animals. Ginger administration may minimize the harmful effects of metal ions toxicity.

Objective: This study has attempted to assess and compare the effects of chronic aluminum intoxication and concomitant ginger treatment on different structures of rat periodontium.

Methodology: 21 adult male albino rats were divided into three equal groups: For three months; groupI received sterile 0.9% saline/day orally, groups II and III received (20mg/kg/day) aluminum chloride orally in drinking water; then, only groupIII received (150mg/kg/day) ginger oil extract orally for 4 weeks. Some of dissected mandibular halves were used for bone mineral density (BMD) measure. Other halves were decalcified and processed for H&E and immunohistochemical (using anti-CD68+ and anti-osteopontin antibodies) examination.

Results: Histopathologically, groupII showed apparently decreased periodontal fibers density, wide degenerative areas and marked inflammatory infiltrates. Likewise, CD68+ was significantly expressed in giant cells in periodontal ligament (PDL) and at resorption surfaces of cementum and bone in groupII. These changes were improved in groupIII with a significant decrease of CD68+ positive cells in PDL. Cementum and bone of groupII presented significant destructive changes that were mostly restored in groupIII. Comparing to groupI, osteopontin expression in groupII significantly increased in acellular cementum(AC)>cellular cementum(CC)>bone> PDL. In comparison, this reactivity was significantly increased in bone>CC>AC in groupIII while osteopontin expression in PDL was mild but with significant increase comparing to groupI. BMD of alveolar bone was highest in groupI>groupIII>groupII with a significant differences.

Conclusion: We concluded that detrimental alterations associated with chronic Al toxicity were markedly detected in PDL>bone>CC>AC that could result in serious clinical outcomes. Ginger extract greatly ameliorated these changes in bone>CC>AC>PDL confirming its strong curative potency against aluminum associated oxidative damage thus it is indicated for use in further new therapeutic approaches.

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Key Words: Aluminium, CD68+, ginger, osteopontin, periodontium.

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INTRODUCTION

Aluminum (Al) is omnipresent in the environment; it is liberated either naturally or from different anthropogenic sources. It is widely used and found in food products, water purification, medicines, antiperspirants, cosmetics, toothpastes and industry. Most relevant routes of exposure to Al are through mouth and nose along with the trans or percutaneous absorption[1,2]. The soluble Al is potentially toxic and more bioavailable than the insoluble one[3]. Aluminum could be toxic to humans, animals and plants[4]. Different Al forms are environmental xenobiotics provoking neurotoxicity, cytotoxicity, hepatotoxicity and nephrotoxicity[5]. Symptoms of Al toxicity such as muscle weakness and spontaneous bone fractures could be difficult to be correlated to Al toxicity since they are frequently nonspecific[6].

Periodontitis, as a chronic destructive disease, is characterized by inflammation of dental the supporting tissues giving rise to periodontal tissue injury along with alveolar bone loss[7]. It was documented that both tooth loss and mobility were manifested secondary to aluminum toxicity and became a significant clinical problem[8]. Periodontal regeneration which is a very complex healing process necessitates a coordinating reaction of the periodontal soft and hard tissue cells[9].

Ginger is a well-known dietary adjuvant contributing to food taste and flavor. It is one of the top antioxidant food and believed to contain a number of main constituents include volatile oil, phenol, proteolytic enzymes, vitamin B6, vitamin C, calcium, magnesium, phosphorus, potassium and linoleic acid[10]. It was documented to regulate the progression of different oxidative stress-related diseases. Ginger has been

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stated to have antiemetic, antioxidant, antithrombotic, anti-inflammatory, anti-hepatotoxic, and immunomodulatory effects in addition to inhibition of carcinogenesis[5].

Since aluminum can be toxic and the influence of its toxicity on dental tissues is still poorly investigated. We evaluated and compared, in the present study, the effect of chronic aluminum toxicity on the dentoalveolar complex in rat model as well as the ensuing therapeutic potentiality of ginger extract oil.

MATERIALS AND METHODS

2.1. Ethical Clearence

This study was approved and performed in accordance with the regulations of the Research Ethics Committee (FDASU-REC) of the Faculty of Dentistry, Ain Shams University, Egypt .

2.2. Animals

Twenty one adult male albino rats (200-220 gm body weight and aged 5-6 months) were housed under controlled temperature, humidity and dark-light cycle. This was done under supervision of specialized veterinarian since their housing till getting rid of sacrificed bodies. The rats were kept under good ventilation and adequate stable diet consisting of fresh vegetables, dried bread and tap water throughout the experimental period (4 months).

2.3. Experimental Design

After one week acclimatization period, the animals were randomly divided into three groups (seven rats each) as follows:

Group I (Control): The rats were treated daily with sterile 0.9% saline and were given by oral gavages[10,11].

Chronic aluminum toxicity was induced in group II and III.

Group II (AL toxicity group): The rats were treated daily with 20 mg/kg BW aluminum chloride (AlCl3) (Sigma, St. Louis, MO, USA) dissolved in drinking water and were given by oral gavages for 3 months[10,11].

Group III (Ginger treated group): After AlCl3 administration for 3 months[10,11], the animals were treated with a daily dose of 150 mg/kg BW of ginger oil extract (Sigma, St. Louis, MO, USA) by oral gavages for 4 weeks[10].

By the end of experimental periods (4 months), animals of each group were sacrificed by intracardiac administration of anesthetic overdose (sodium thiopental 80 mg/kg BW[12]). The lower jaws were dissected free and split into two halves using surgical scissors[13]. Getting rid of sacrificed rats bodies were done according to the ethical committee rules in the incinerator of Ain Shams University Hospital. The specimens were rinsed in a water beaker and placed in capsules carrying codes for each rat to be prepared and processed.

First half: was processed for routine histological H&E staining and immunohistochemical staining[13]. Second half: was kept undecalcified in formalin to measure the bone mineral density (BMD) using dual-energy X-ray absorptiometry (DEXA)[7].

2.4. Histopathological Examination

Jaw specimens were immediately fixed in 10% formaldhyde solution for 72 hours. Afterward, the decalcification of the specimens was done with 10% ethylene diamine tetra-acetic acid (EDTA) solution for approximately 5 weeks at 4 °C. Dehydration in increasing concentrations of alcohol was performed with a subsequent alcohol clearance by xylene. Then, infiltration and embedding of specimens in paraffin were carried out. Mounting of 5µm thick sections were done on regular glass slides. Sections were stained by Hematoxyline and Eosin (H&E) for routine histological examination[13] by a research light microscope (Olympus® BX 60, Tokyo, Japan).

2.5. Immunohistochemical Examination

Immunolabeling for detection of osteopontin (OPN) and (cluster of differentiation 68) CD68+, using osteopontin (rabbit polyclonal antibody) and anti CD68+ (mouse monoclonal antibody) respectively, were carried out. Immunohistochemically, the tissues sections were stained by biotin-streptavidin method. Serial paraffin-embedded sections (4-5µm) mounted on positively charged slides were deparaffinized, rehydrated and antigen retrieved. All sections were placed in phosphate-buffered saline at pH 7.4, treated with protein block for 5 min to avoid false staining. Afterwards, incubation for 30 min with primary antibody of CD68+ as well as the incubation for 1 hour with primary antibody of polyclonal osteopontin was done. The sections were exposed for 30 minutes to secondary antibody, washed in PBS and incubated for 5 min with DAB (diamino-benzidine) for visualization then washed. Next, the counter-staining of the slides with hematoxylin was performed and last, after drying, the slides were coverslipped. Assessment using a light microscope (Olympus® BX 60, Tokyo, Japan) was achieved[9,14].

2.6. Measurement of Bone Mineral Density (BMD)

Bone mineral density was measured by Dual X-ray absorptiometry (DEXA) at an area of 1.6 x1.6 mm^2 in the mandibular alveolar bone exactly beneath the mesial root of the first molar. This measurement was achieved using GE Lunar Prodigy machine. Seven specimens were selected to run the morphometric measures[7,15].

2.7. Histomorphometric and Statistical Analysis

Analysis of the immunohistochemical results of the studied groups was assessed using digital camera and software (Leica Qwin 500) of Leica microscope. The pixels created with the image analysis program by the image analyzer changed to real micrometer units[16]. The obtained data from histomorphometric analysis and bone mineral density were analyzed using Statistical Package for Social Science software computer program version 23 (SPSS, Inc., Chicago, IL, USA). Data were illustrated in mean and standard deviation. One way Anal¬ysis of variance

(ANOVA) and Tukey were used for comparing data. P value less than 0.05 was considered sta¬tistically significant[7].

RESULTS

3.1. Histopathological Results

Group I (Control group): H&E stained sections of the control group exhibited normal architecture and stainability of periodontium included gingiva, periodontal ligament, alveolar bone and cementum. Principal fiber bundles of periodontal ligament (PDL) exhibited interstitial tissue spaces and properly oriented normal fibroblasts along the wavy collagen fibers. The thin acellular cementum (AC) as well as the slightly eosinophilic cellular cementum (CC) normally covered the root. The surface of both cementum types was bordered by cementoblasts interposed between PDL fiber bundles. Likewise, spiderlike oval cementocytes were entrapped in CC (Figures 1a, 1b and 1c). The normally architectured alveolar bone presented well-organized trabeculae of woven and lamellar bone containing osteocytes. Plump osteoblasts lined the marrow spaces and alveolar bone trabeculae. Moreover, presence of cementoid and osteoid layers on bone and cementum surfaces respectively was obviously revealed (Figures 1b and 1c).

Group II (AL toxicity group): Three months after AlCl3 administration, disorganization of extracellular matrix and periodontal collagen fibers was detected in addition to the apparent decrease of collagen fibers density comparing to control group. Besides the wide degenerative areas, partly or complete loss of fibers attachment to bone and cementum were also seen along with areas of fibers hyalinization as well as fibrosis. Dilated and congested blood vessels were persistent finding in this group. Fewer disoriented irregularly shaped fibroblasts with pyknotic nuclei were also noticed in addition to the accentuated inflammatory and giant cells infiltrates in gingiva and PDL. These infiltrates were associated with the destructive changes of cementum and alveolar bone. The irregularity in cementum surface and in the apparent thickening of both AC and CC together with the observed giant cells at the surface periphery in addition to the reversal cement lines revealed cementum resorption. Apparently widened cementocytic lacunae of CC as well as increased amount of cementoid in both cementum types were also displayed. Apparent variation in the stainability of cementum and bone was detected as well (Figures 1d, 1e and 1f). Noticeable degenerative changes of alveolar bone were manifested by trabecular alterations in thickness and architecture besides the increase in the thickness of osteoid seam. Apparent decrease of bone cells (osteocytes, osteoblasts, osteoclasts) and randomly distributed osteocytes with degenerative signs were also elucidated. Areas of fatty degeneration and immense inflammatory cell infiltration within the widened marrow spaces were seen. Bone resorption, multinucleated osteoclasts in Howship's lacunae and reversal lines were also evident (Figures 1d and 1e).

Group III (Ginger treated group): Comparing to group II, cementum, PDL and alveolar bone mostly showed features resembling those of the control group. In addition, the apparent increase of the PDL fibroblasts as well as fiber bundles in density, thickness and organization with apparently increased fibroblasts were displayed. Apparently, fewer degenerative areas with fewer inflammatory cell infiltrates could be detected. Yet, some periodontal areas exhibited disorganized extracellular matrix and fibers with few dilated blood vessels. AC and CC seemed more regular but with hypercementosis and marked cementoid layer in CC. Noticeably increased deposition of bone matrix, less widened marrow spaces contained few inflammatory cells and apparently thicker bony plates with less alveolar bone resorption were distinguished. Apparently normal osteocytes were detected in this group. Apparently increased areas of bone remodeling along with numerous reversal lines separating old bone from the newly formed bone, as well as filling cones, were also demonstrated (Figures 1e and 1 f).

3.2. Immunohistochemical and Statistical Results

3.2.1. Immunohistochemical and Statistical Results for CD68+

Group I (Control group): In normal dentoalveolar complex, CD68+ expression was almost negative in AC and CC as well as in PDL (Figures 2a, 2b and Figure 6).

Group II (AL toxicity group): Comparing to control group, a significant (*P value* <0.001) strong immunopositivity to CD68+ was elucidated in mononuclear and multinuclear giant cells which appeared compact in shape. The evident cells in the PDL tissue and in the vicinity of blood vessels may be marked as monocytes and macrophages. Those detected at resorption sites adjacent to hyalinized areas of PD fibers on both AC and CC surfaces and cutting cones could be marked as odontoclasts whereas osteoclasts were observed on the alveolar bone surfaces and cutting cones. These results were consistent with the occurrence of apparent moderate to severe root and bone resorption (Figures 2c, 2d and Figure 6).

Group III (Ginger treated group): This group displayed a significant decrease (*P value* <0.001) of CD68+ positive cells in the dentoalveolar complex comparing to group II. But a significant increase (*P value*= 0.009) was still presented in this group compared to control group. The illustrated positive cells at cementum (AC and CC) surface and/or at bone surface were seen at the sites of tissue apposition and repair. This was consistent with the attenuated root and bone resorption (Figures 2d, 2e and Figure 6).

3.2.2. Immunohistochemical and Statistical Results for Osteopontin (OPN)

Group I (Control group): In normal dentoalveolar complex, the OPN expression was weak to mild in PDL and very weak in the fully calcified CC and bone. OPN expression was apparently moderate in AC and mild in scarce cementoblasts surrounding AC and CC along with few cementocytes in CC. Scarce OPN positive osteoblasts were also elucidated in alveolar bone with absence and/or negative reaction of cement lines in addition to the mildly reacted bone matrix (Figures 4a, 4b, 5 & 6) and (Tables I & II).

Group II (AL toxicity group): Comparing to control group, a significantly increased immunopositivity to OPN was distinguished in this group (Figure 5 and Table I); AC > CC > alveolar bone > PDL with insignificant differences between CC and bone as well as between bone and PDL (Figure 6 and Table II). Moderate immunoexpression of OPN was localized in PDL mostly near cementum and alveolar bone surfaces. Very weak expression was also detected in the fully mineralized CC and bone matrices similar to the control group. Significantly increased OPN staining was perceived in AC as well as in the outer layers of CC and alveolar bone. Intensely stained new cementoblasts and osteoblasts were also noticed near the root and alveolar bone surfaces. Zones of superficial resorption exhibited strong OPN positivity in numerous odontoclasts and osteoclasts along the irregular cementum and bone surfaces respectively, as well as at the resorptive surface-clast cells interface. A mild to moderate OPN reactivity was also displayed in few reversal cement lines in cementum and alveolar bone separating the old mineralized tissues from the newly formed un/mineralized matrices (Figures 4c, 4d and 4e).

Group III (Ginger treated group): This group comparing to group II displayed a statistically significant increase in OPN expression (Figure 5 and Table I); in alveolar bone > CC > AC with significant differences (Figure 6 and Table II). Yet, the PDL presented a low significant decrease in OPN reactivity in comparison with group II as it showed only a mild expression near the cementum and bone surfaces but with a significant increase when compared to the control group (Figures 4f, 4g, 5 and Table I). In contrast to the other PD tissues, PDL significantly showed the least expression to OPN in this group (Figure 6 and Table II). OPN immunoreactivity in the old fully calcified CC and bone was very weak. Secondary to the superficial root and bone resorption detected in group II, a strong OPN expression in the external layers of root cementum and alveolar bone was illustrated in this group. Increased intensity of OPN reactivity was displayed in new cementoblasts, AC and in the apparently thickened newly formed cementum (AC and CC with cementoid). The strong the OPN positivity was also noticed in new osteoblasts, few osteoclasts at the oppositional bone surfaces and in the apparently thickened areas of the newly formed bone with osteoid. In addition, a potent remodeling process was demonstrated by numerous thickened reversal cement lines in cementum and much more in bone exhibiting a strong OPN staining (Figures 4f, 4g).

3.3. Bone mineral density and statistical Results

An example of DEXA output scan to the medial aspect of rat hemimandible was presented the included the region of interest just beneath the roots of first molar in (Figure 7). For such a region, the bone mineral density was measured. Data obtained from the assessed bone mineral density in the studied groups were summarized by mean and standard deviation (SD) values. Group I showed the highest mean values of BMD with significant differences comparing to group II (*P value* <0.001) and to group III (*P value* = 0.016). Likewise, the least mean values of BMD presented in group II with significant differences (*P value* <0.001) comparing to group III and to group I as aforementioned (Figure 8).

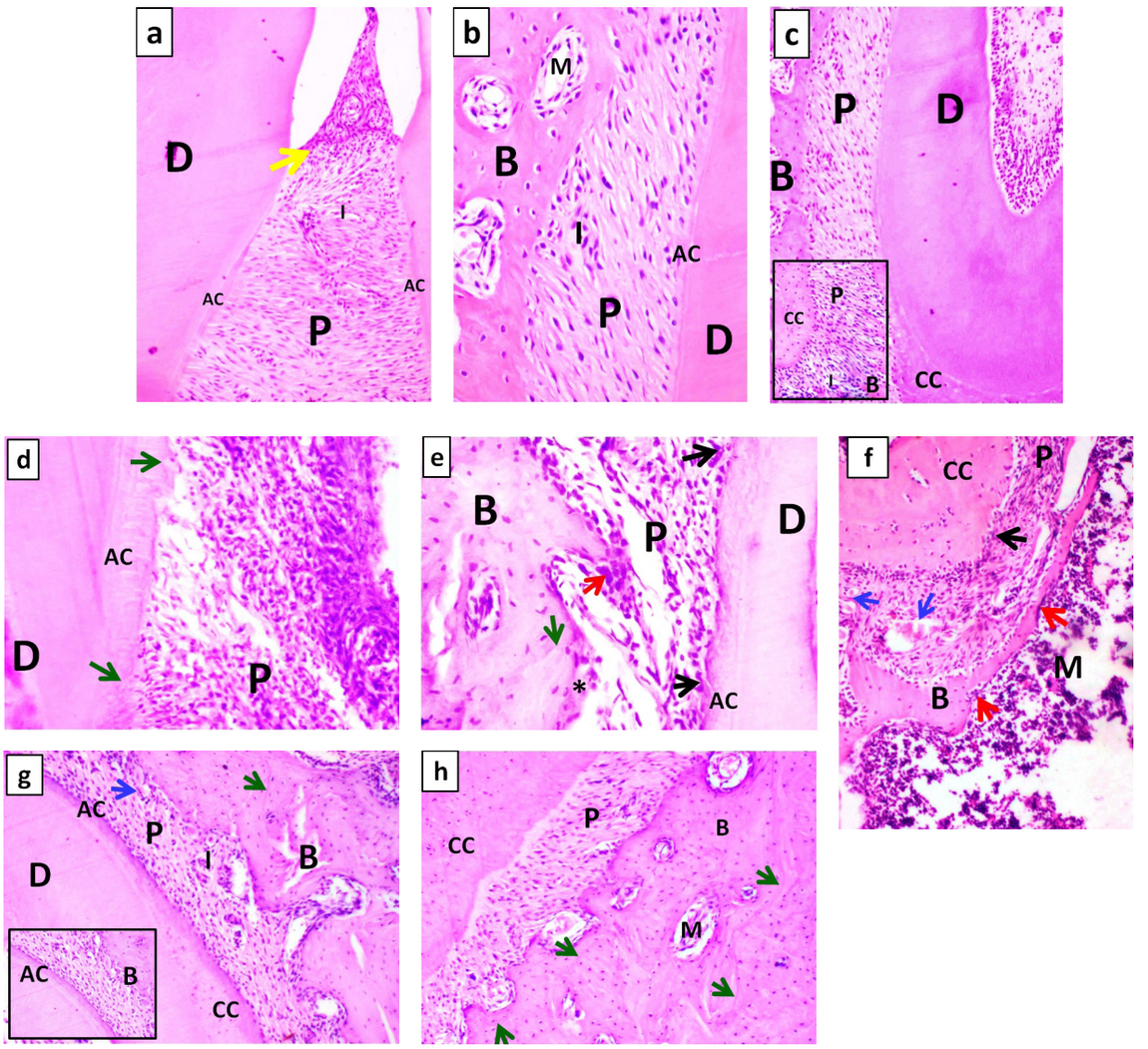

Fig. 1: Photomicrographs of rat dentoalveolar complex; (Control) groupI showing (a) regular AC, normal gingival and PDL fibers (H&E X 100), (b) normal architecture of alveolar bone (H&E X 200), (c) normal slightly oesinophilic CC and adequate cementoid and osteoid layers (H&E X 100, Inset: X 200). GroupII showing (d) disorganization and alterations of PD fibers, wide degenerative areas with inflammatory infiltrates, irregular apparently thickened AC with reversal lines (H&E X 200), (e) giant cells at surface periphery of cementum and alveolar bone besides reversal lines in bone, increased cementoid and osteoid seams (H&E X 200), (f) apparently widened cementocytes lacunae in apparently thickened CC, trabecular bone alterations (H&E X 100). Group III showing (g) apparently fewer degenerative areas and inflammatory infiltrates in PDL, almost regular cementum, marked bone deposition (H&E X 100, Inset: X200). (h) Denser PD fibers, increased bone remodeling with numerous reversal lines and filling cones (H&E X 100). Dentin (D), periodontal ligament (P), acellular cementum (AC), interstitial tissue space (I), bundle bone (B), marrow spaces (M), cellular cementum(CC) , gingival interdental papilla (yellow arrow), reversal lines (green arrows), giant cells on cementum surface (black arrows), giant cells on bone surface (red arrows), osteoid seam (asterisk), blood vessels (blue arrows).

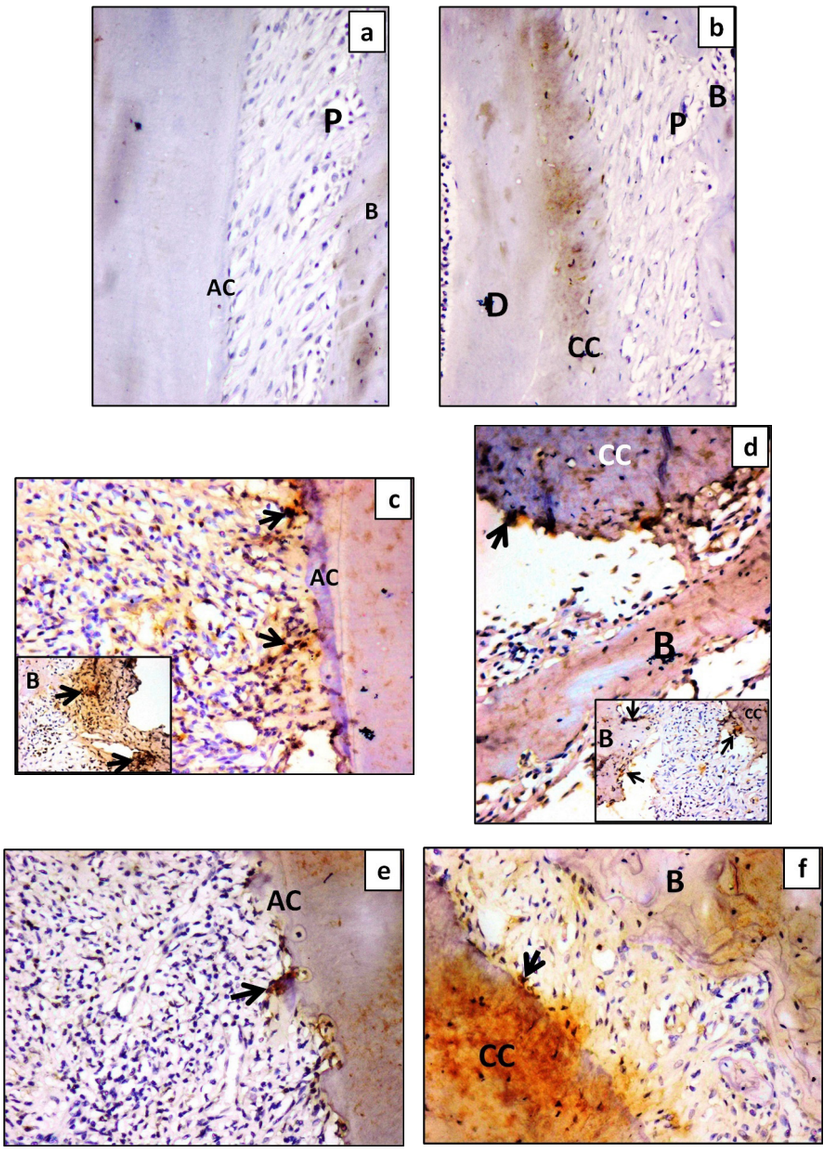

Fig. 2: Photomicrographs of rat dentoalveolar complex showing the immunohistochemical reactivity to anti-CD68+, C-ve group (I) showing (a) negative immunoreactivity in PDL, AC, (b) in CC and alveolar bone. Group (II) showing (c) strong reaction of mononuclear and multinuclear giant cells with a compact form in PD tissue, in the vicinity of blood vessels, at resorption sites of AC, (d) of CC and at alveolar bone surface. Group (III) showing (e) apparently decreased CD68+ positive cells in PDL, few positive cells at sites of apposition on AC surface, (f) on CC and alveolar bone surface. (Original magnification, X200, Insets: X 200). Dentin (D), periodontal ligament (P), acellular cementum (AC), cellular cementum (CC) , alveolar bone (B), CD68+ positive cells (black arrows)

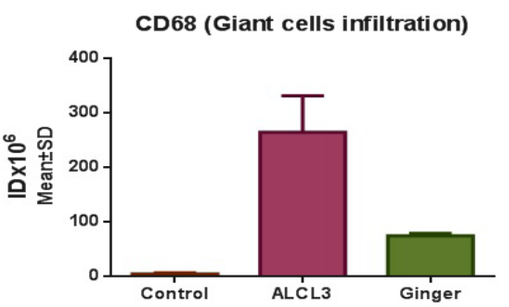

Fig. 3: Bar charts showing mean ±SD of CD68+ immunoreactions (Integrated density x10^6) in PDL among all studied groups

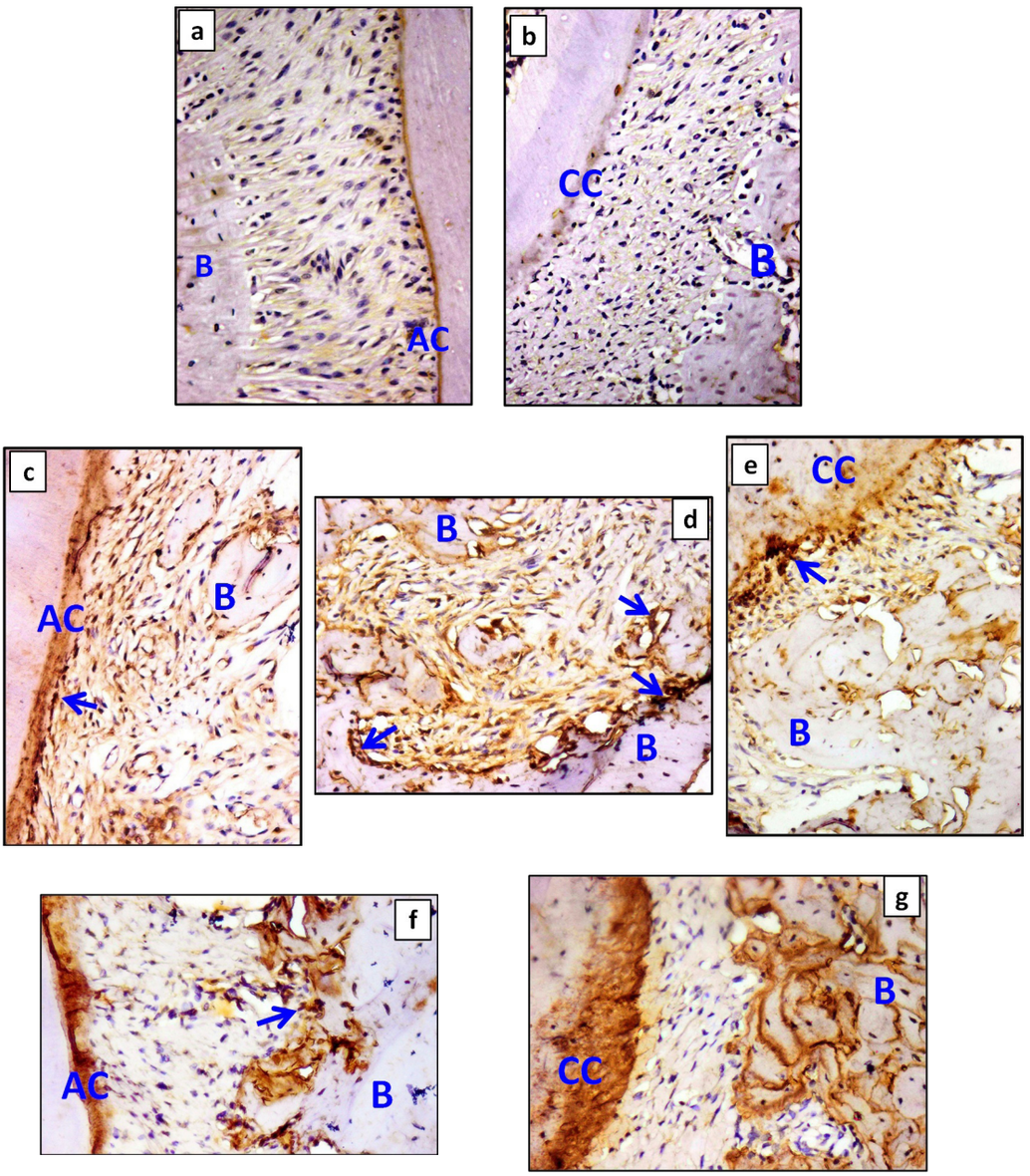

Fig. 4: Photomicrographs of rat dentoalveolar complex showing the immunohistochemical reactivity to anti-osteopontin, C-ve group (I) showing (a) faint OPN reaction in PDL, scarce cells in cementum and bone besides the fully mineralized alveolar bone but apparently moderate in AC, (b) weak OPN positivity in few cementocytes of CC. Group (II) showing (c) apparently increased reactivity in AC, new cementoblasts , odontoclasts, resorptive surfaces, reversal lines as well as in PD tissue near cementum surface (d) and near bone surface besides the strongly reacted new osteoblasts, osteoclasts, resorption surfaces and reversal lines, (e) obviously reacted odontoclasts and resorptive surfaces of CC along with reversal lines. Group (III) showing (f) apparent strong reactivity in AC, thickened increased reversal lines, thickened areas of newly formed bone, osteoblasts and few osteoclasts at apposition surfaces, (g) mild reaction in PDL near cementum and bone surfaces, strong expression in outer newly formed CC and in active remodeled bone areas with numerous thickened reversal lines (Original magnification, X200). Acellular cementum (AC), cellular cementum (CC) , alveolar bone (B), giant cell at resorptive surface interface (blue arrows).

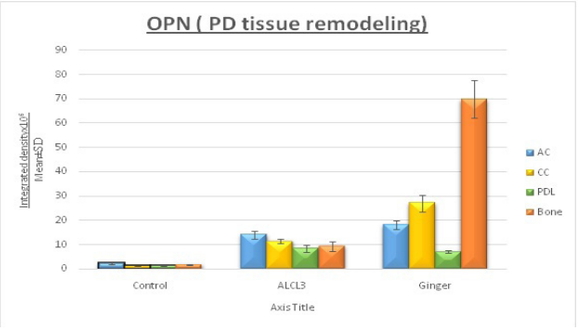

Fig. 5: Bar charts showing mean ±SD of OPN immunoreactions (Integrated density x10^6) in PDL, AC, CC & alveolar bone among all studied groups

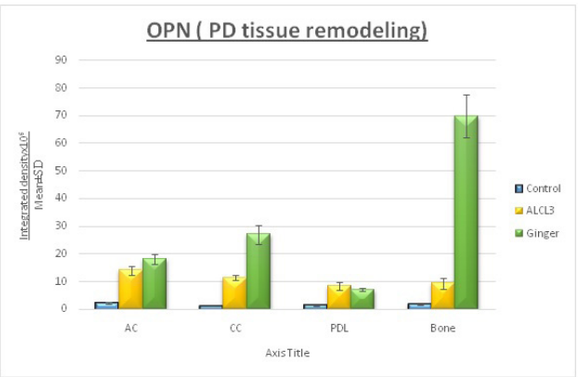

Fig. 6: Bar charts showing mean ±SD of OPN immunoreactions (Integrated density x10^6) in PDL, AC, CC and alveolar bone in each studied group

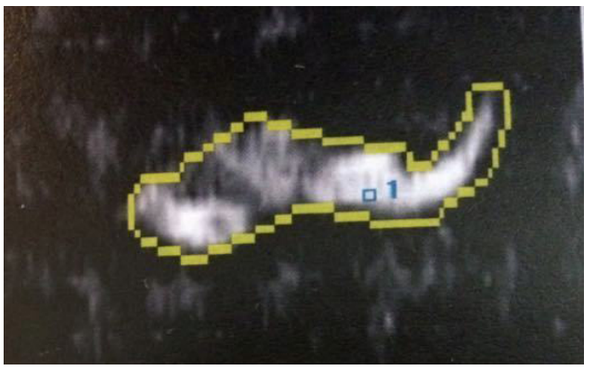

Fig. 7: DEXA scan of medial aspect of rat hemimandible showing the region of interest for which bone mineral density was measured

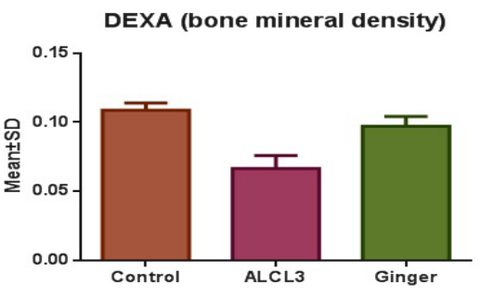

Fig. 8: Bar charts showing mean ±SD of BMD among all studied groups

Table I: Showing OPN Immunoreactions (Integrated density x10^6) among the studied groups

	Control	ALCL3	Ginger
AC	2.231±0.28	14.11±1.61	18.08±1.79
Posthoc		P1=<0.001*	P2= <0.001* P3=<0.001*
CC	1.13±0.11	11.39±0.89	27.12±3.39
Posthoc		P1=<0.001*	P2= <0.001* P3= <0.001*
PDL	1.36±0.21	8.49±1.40	7.10±0.68
Posthoc		P1=<0.001*	P2= <0.001* P3= 0.026*
Bone	1.81±0.18	9.41±1.99	69.83±7.73
Posthoc		P1=0.016*	P2= <0.001* P3= <0.001*

Data expressed either as mean±SD
SD: standard deviation P:Probability *:significance <0.05
Test used: One way ANOVA followed by post-hoc Tukey
P1: significance between Control Group & ALCL3 Group
P2: significance between Control Group & Ginger Group
P3: significance between ALCL3 Group & Ginger Group

Table II: Showing OPN Immunoreactions (Integrated density x10^6) in PDL, AC, CC and alveolar bone in each studied group

	AC	CC	PDL	Bone	P
Control	2.231±0.28	1.13±0.11	1.36±0.21	1.81±0.18	<0.001*
Post-hoc		P1=<0.001*	P1=<0.001* P2= 0.2	P1=0.004* P2=<0.001* P3=0.003*	
ALCL3	14.11±1.61	11.39±0.89	8.49±1.40	9.41±1.99	<0.001*
Post-hoc		P1=0.013*	P1=<0.001* P2= 0.008*	P1=<0.001* P2=0.097 P3=0.67	
Ginger	18.08±1.79	27.12±3.39	7.10±0.68	69.83±7.73	<0.001*
Post-hoc		P1=0.012*	P1=<0.001* P2=<0.001*	P1=<0.001* P2=<0.001* P3=<0.001*	

Data expressed either as mean±SD
SD:standard deviation P:Probability *:significance <0.05
Test used: One way ANOVA followed by post-hoc Tukey
P1: significance vs ACC
P2: significance vs CC
P3: significance vs PDL

DISCUSSION

Recent toxicology studies were conducted on the largely accumulated aluminum salts in earth because of their toxic implications. Al induced toxicity was reported to inhibit normal development and growth in rats and to cause intensified histological changes[1,17]. In the present study, Wister albino rats were administered a daily oral dose (20 mg/kg body weight) of aluminum chloride (AlCl3) in drinking water for three months to simulate the chronic Al mediated toxic conditions in humans. Ginger is one of the botanicals which gained popularity among physicians to be used in the treatment of different diseases since it is rich in valuable bioactive constituents[10]. Several reports[1,9] studied the curative effect of ginger in restoring Al-associated

alterations in some oral tissues. Yet, up to our knowledge no detailed histological studies had been performed to investigate the effects of both chronic Al toxicity and subsequent ginger remedy on different structures of rat periodontium.

In the present work, H&E stained sections of group II (Al toxicity) exhibited significant degenerative changes of periodontium compared to control group. Apparent decrease of collagen fibers density of this group was observed in addition to fibers hyalinization, wide degenerative areas and loss of fibers attachment. Besides the accentuated inflammatory infiltrates, fewer fibroblasts were also illustrated with pyknotic nuclei. Similar detrimental alterations of connective tissue were attributed to Al-associated functional alterations of fibroblasts[1]. Injury to fibroblasts and other periodontal cells was provoked by the cytotoxic effects of metal ions via the overproduction of reactive oxygen species leading to a slower cellular turnover rate, lessened cell viability as well as proliferation and to the altered capability of repair[18,19]. The resultant changes of PDL and supporting tissues triggered the loss of fibers attachment, tooth mobility and loss. This was mainly afforded through the activity of clasts cells and matrix metalloproteinases in addition to the intense inflammatory infiltrates including macrophages mediated by cytokines and chemokines that were mostly released by the inflammatory cells themselves[18]. The authors added that residual Al deposits were observed in different tissues as well as in macrophages[1]. In harmony with our finding of the increased dilated congested blood vessels in Al toxicity group, some Al intoxication rat studies illustrated a significant elevation of serum cholesterol and triglycerides assignable to hepatic impairment as well as pancreatic beta cell dysfunction and thus hyperglycemia. These changes caused lipoprotein lipase hypoactivity in blood vessels which in turn elevated the cholesterol levels[5,10].

Comparing to group II, the ginger treated group (group III)exhibited an apparent increase of collagen fibers density more or less resembling those of the control group. Amelioration of all aforementioned deleterious PDL changes of group II was distinguished in this group. In concurrence, Halawa *et al*.[1] declared that ginger via its constituents including flavonoids acts as a potent anti-inflammatory agent through the direct inhibition of leukotriene and prostaglandins. It is significantly used to remedy the injurious effects of Al toxicity on different tissues through its chelating potentiality for heavy metals. Minimal or no MMPs stimulation was detected with no destruction of collagen fibers[1] proving that alterations of PD fibroblasts were renovated and thus promoted the high turnover rate and rapid remodeling of PDL[18]. Furthermore, Osama *et al*[10] found that ginger improved cholesterol levels by increasing the hepatic cholesterol hydroxylase activity and cholesterol excretion from the body as well as by its hypoglycemic effect through stimulation of insulin secretion and thus enhanced lipoprotein lipase activity[10].

Akin to our results, previous chronic AL intoxicated rat studies demonstrated marked infiltration of macrophages in connective tissues[1]. Giant phagocytic cells generally regulate and play an important role in inflammation[20]. Thus, the current study focused on the CD68+ expression and distribution of the giant phagocytic cells within rat periodontium among the studied groups. CD68+, a glycoprotein was utilized as a general marker for bone marrow-derived giant cells such as macrophages, their monocytes precursors, odontoclasts and osteoclasts[14,21]. In this work, CD68+ positive cells were scarcely detected in the PDL of the control group under normal conditions. This could be attributed to that the CD68+ antigen was localized on the membranes of the phagolysosomal apparatus of phagocytic cells and thus CD68+ expression was correlated with the phagocytic activity[21,22]. For that reason, group II sections in comparison presented a statistically significant strong CD68+ expression of by mononuclear and multinuclear giant cells in the PDL, in the vicinity of blood vessels, and at the resorption sites on cementum and alveolar bone surfaces adjacent to the hyalinized PD fibers. Analogous to the histological and CD68+ expression findings of group II in this work, some authors observed that CD68+ positive clasts cells moved along surface during bone and cementum resorption forming scalloped cutting cones[9,23]. This distribution of the CD68+ cell infiltrates confirmed their roles in regulating inflammation as well as their involvement in cementum and bone resorption besides collagen destruction. The potent aluminum pro-oxidant activity elicited oxidative deterioration, mitochondrial damage and lysosomal increase[1,5,22]. Subsequent activation of NF-κB, release of proinflammatory cytokines as tumour necrosis factor-α by macrophages and receptor activator of nuclear factor kappa-b ligand (RANKL) by osteoblasts/ cementoblasts together with the Al-associated disrupted balance between formative and resorptive activities of bone and cementum cells as well as the generated superoxides at the resorptive surfaces interface were all involved in the regulation of inflammatory clastogenesis[24]. Wei *et al*.[25] clarified that chronically Al treated rats exhibited considerable production of soft tissue-based bone together with immature collagen matrix which in turn stimulated and enhanced bone resorption via the increase of osteoclastic differentiation[25]. On the other hand, a significant decrease of the CD68+ positive PD cells in the ginger treated group of this study was elucidated. In confirmation, it was stated that the therapeutic activity of ginger against oxidative stress is mediated by the polyphenolic antioxidant activity, free radicals scavenging action and by protection of lipids in the cellular membranes from oxidation in Al intoxicated rats[1,10]. Furthermore, ginger selectively suppresses macrophage activation and function along with the proinflammatory mediators' release[26].

The routine histological sections of group II (Al toxicity) exhibited significant destructive changes of cementum and alveolar bone compared to control group. Comparable changes of cementum and bone were also illustrated secondary to chronic periodontal inflammatory disease in other reports[13].

In agreement with our results, Sivakumar *et al*.[27] recorded reduced biochemical constituents as well as changes of bone and cementum surface architecture consequent to aluminum toxicity[8,27]. The detected irregularity in cementum thickness and surface was possibly due to the interruption of the continually cemental deposition which was triggered by the inflammatory periodontal disease[13]. Moreover, we attributed the observed dissimilar stainability in both cellular cementum (CC) and alveolar bone in this group to the changed calcium balance. Aluminum may interfere with both intestinal calcium absorption and hard tissues mineralization causing reduced calcium retention and lower strength. Al has a high affinity for phosphates in the lipid bilayer leading to loss of membrane integrity and cell signaling[5]. The structural alterations of the cellular membranes inhibited many enzymes such as alkaline phosphatase (membrane-bound enzyme) elaborated by the mineralized tissues forming cells[10]. Because of the direct effect of Al intoxication, the phosphate transport to the mineralization front through the activity of alkaline phosphatase was strongly inhibited owing to Al deposition at the mineralization front and cement lines thus strongly reduced crystal growth and caused accumulation of organic matrix. Together with the Al-associated inhibited differentiation of mineralized tissue forming cells, these changes could interfere with mineralized tissue remodeling. Furthermore, the indirect effect might occur due to aluminum accumulation in the parathyroid glands inhibiting both parathyroid hormone-calcium as well as vitamin D-calcium axes[2,25]. Analogous to our observations on the microarchitectural deterioration of cementum and bone, some authors considered chronic periodontitis as a type of bone and cementum resorptive disease that could be associated with connective tissue degradation, loss of fiber attachment, vascular angiopathy and atherosclerosis[24,28]. Comparing to group II, cementum and alveolar bone of the ginger treated group mostly showed improved features simulating those of the control group. We ascribed this improvement to the abovementioned role of ginger in the inhibition of the inflammatory process as well as in the increase of the anabolic activity in cementum and bone. Either new bone or new cementum formation is the key event of PD regeneration by attaching the newly forming PD collagen fibers to the altered cementum and alveolar bone surfaces[9]. In addition, the observed cellular hypercementosis in this group was considered as an adaptive response of periodontal tissues to injury[29]. In parallel with other researches[23], the noticed filling cones in the alveolar bone of ginger group in this work were observed in association with central capillaries besides osteoblasts which deposited the new bone in concentric lamellae[23]. In consensus with these results, it was believed that flavonoids and polyphenols, which are greatly found in the ginger oil extract used in our study, have a potent anabolic effect on induction, increased expression as well as release of bone sialoprotein and osteopontin (OPN) in periodontium. These proteins are crucial non-collagenous matrix proteins implicated in hydroxyapatite crystals nucleation[30-32].

To support the histological findings of our study, immunohistochemial localization of OPN in rat periodontium was carried out. Osteopontin, a glycoprotein nucleates hydroxyapatite crystals and regulates crystal growth thus influencing the mineralization extent. OPN is secreted at different stages of cementum and bone formation as well as remodeling. It is accumulated and expressed in both tissues with similar distribution[9,31,32]. Comparing to the herein control group, the Al toxicity group showed a statistically significant increase in the OPN immunopositivity so that it was highly expressed in acellular cementum (AC) > cellular cementum (CC) > alveolar bone > PDL with insignificant differences between CC and bone as well as between bone and PDL. The highest significant increase of OPN reactivity in AC comparing to the other PD tissues in group II followed by control group was explained by Thomas and Thomas[31]. The authors found that OPN deposition was markedly concentrated in the interfibrillar spaces that are large in normal AC since it is mostly formed of extrinsic fibers[31]. Whereas the further increased expression in the AC of group II could be attributed to the newly forming repair tissues as a response Al-associated resorptive changes. At the same time, we ascribed the much significant increase of OPN expression in the AC of group III to the marked cementum remodeling triggered by the anabolic effect of ginger flavonoids[30] but this reaction was significantly lesser in both bone and CC of this group.

In the current study, the significant strong OPN expression in CC and alveolar bone of group II comparing to control group was observed in the superficial resorption; (in odontoclasts, osteoclasts and at resorptive surfaces interface); as well as in the outer newly forming layers, new formative cells and few reversal lines. Accordingly, it was affirmed that OPN has a role in the superficial resorption as it was expressed in the formed lamina limitans on the mineralized tissues by the positive cementoblasts and osteoblasts to accelerate the adhesion of the strongly OPN reacted clasts cells contributing to their activation and thus tissue resorption[7,31]. Then, the resorptive surfaces and cutting cones enclosed the precursors of the formative cells for repair which deposited a thin organic coating composed largely of OPN that served as a cohesive mineralized layer between new and old bone and/or cementum[9,23]. This strong expression of OPN along the cementum and bone surfaces was followed by condensation of connective tissue matrix[9]. In inflammation, the soluble mediators like cytokines and growth factors secreted by inflammatory cells and from degraded tissues contributed to periodontium regeneration. They seemed to be rather higher in cementum and alveolar bone with low concentration in soft PD tissues. Yet, the presence of these mediators in one periodontal element could influence the other tissues. But the role of these mediators was assumed to be minor due to the relative large amounts of the inflammatory products in the local environment[33]. Moreover, cementum was illustrated to better promote migration and adhesion of the preferential progenitor cells and thus the formation of new cementum and bone via its specific proteins and growth factors than the

other periodontal tissues in injury[33,34].

The ginger treated group of this study presented a statistically significant OPN expression comparing to group II; in alveolar bone > CC > AC with significant differences. This strong OPN reactivity emerged in the apparently thickened newly formed alveolar bone and CC as well as in the new formative cells along with few osteoclasts at the appositional bone surfaces. In confirmation of these findings, it was declared that following chronic inflammation, the distribution and /or amount of OPN during both repair and remodeling processes were altered in PDL as well as in matrices and surfaces of the adjacent mineralized tissues. The strong expression of OPN along the surfaces serves as an attractant to progenitor cells of bone and cementum and is also expressed during cellular differentiation. In addition, OPN accumulates in the newly formed mineralized tissues and matrices[7,9,31]as it is strongly bound to hydroxyapatite crystals[9,23] thus new cementum and bone formation proceeds revealing the significant role of OPN in bone and cementum repair[7,9,31]. Similar to other reports[7,31], the increase of the thickened and intensely reacted reversal lines to OPN in the ginger group comparing to group II was distinguished in the remodeled areas much more in bone and lesser in cementum consequent to the post clastic cells activity. In concurrence to these results, Salmon *et al*.[35] affirmed that although the various similarities in matrix composition and morphology in both bone and cementum, particularly that cementoblasts were considered merely positional osteoblasts, but still differ in numerous significant respects. Cementum, unlike bone, is non-innervated, avascular plus its growth by apposition without considerable remodeling or turnover[35]. In addition, despite the occurrence of the cementocytic interconnecting network in cellular cementum, the high vascularity along with the osteocytic network in bone as well as the abovementioned release of soluble mediators would enhance fluid and mediatorsexchange in bone more than cementum. So, adaptive cellular activities were stimulated on bone surfaces maintaining the mineralized bone matrix metabolically active[29,36]. Therefore, repair and regeneration were predictable and more rapid in alveolar bone but unpredictable and intricate with low quality in cementum. Furthermore, it was suggested that each matrix includes unique proteins that might give insight to their physiologic dissimilarities. Hence, the response of both alveolar bone and cementum to therapeutic interventions in disease is most probably quite different[35]. Periodontal regeneration study of Liu *et al*.[34] reported the simultaneous regeneration of cementum, bone and PDL though the osteogenic process possibly preceded cementum repair and PD fiber differentiation.

Comparing to the weak/ mild expression of OPN in the PDL of both control group and group III with significant differences in the current study, the PDL of group II exhibited a significantly increased moderate reactivity to OPN. In agreement with this finding, it was found that OPN was localized and upregulated in the PDL of the diseased root surfaces adjacent to resorption areas and acted as a macrophage chemotactic factor. It was supposed that fibroblasts, osteoblasts and cementoblasts secrete OPN in response to injury to induce clastic cells migration to resorption sites[31,37,38]. Likewise, RANKL secreted by fibroblasts and osteoblasts (or cementoblasts) is essential for clast cells maturation and it was suggested to be involved in the intracellular production of OPN in clast cells by several pathways[37]. Furthermore, OPN was also expressed in the newly formed PDL cells, mesenchymal stem cells and in the interfibrillar spaces particularly close to the surfaces during cementum repair and bone regeneration. Therefore, preserved PDL is essential for the formation and regeneration of adjacent mineralizing tissues as it is rich in pro-mineralization factors and serves as a source for the precursors of PD cells. Moreover, OPN shows a protective role during cementum and bone formation by acting as a regulator/ inhibitor of mineralization and thus manages PDL width and prevents ankylosis[31,32,39]. Consistent with the herein OPN results, it was clarified that OPN plays an important role in regulation mineralization of periodontium as well as formation and resorption of both bone and cementum[31,37]. Its significance in mineralization arises with cellular cementum, and at the bone-PDL border. It also influences PDL tissue properties but does not directly control AC apposition[32].

Since Al intoxication rat studies declared that the deleterious effects of Al primarily targeted bone and inhibited bone matrix formation and mineralization[17]. To enhance the above-mentioned histological and immunohistochemical results of alveolar bone, we evaluated the effect of Al intoxication as well as the consecutive ginger administration on the mineral density of alveolar bone (BMD) using dual-energy X-ray absorptiometry in the mandibular first molar region as a standard method. DEXA-BMD is not a volumetric measure, it indicates for the degree of mineral concentration and bone loss in the cortical and cancellous bones of small animals[40]. Analysis of DEXA-BMD results elucidated statistically significant differences among the three studied groups with the highest BMD found in the control group whilst the lowest was recorded in group II (Al toxicity). The reduced BMD detected in the Al group specimens complemented the osteopontin results of the resorbing bone as well as the routine histological results which illustrated the enlarged marrow spaces as an indirect measure of bone loss along with osteopenia[41]. Onoyama *et al*.[42] asserted that bone strength is regulated by both bone density and quality. One of the factors that determine bone quality is the micro-architecture that was significantly deteriorated in the Al group according to the aforementioned histological results. Conforming to other authors[42], the earlier mentioned Al-associated microangiopathy and atherosclerosis contributed to the reduced cell function provoking the decrease in bone mass and quality. These findings indicated the reduction of alveolar bone strength in the Al group of this study. On the other hand, the significant restoration of BMD in the ginger group of the current work was mediated by the beneficial effect of ginger in its ability to downregulate oxidative distress, inflammation

and apoptosis. Ginger polyphenols could also prevent bone loss as well as the decrease of bone density and strength in addition to its potentiality to increase osteoblastic activity and thus enhance bone mineralization[10,43].

From all previous findings, we concluded that chronic Al exposure was associated with detrimental alterations of histology, CD68+ and osteopontin expression along with bone strength in the dentoalveolar complex of Albino rats. This deterioration was highly detected in PDL>bone>CC>AC that could result in serious clinical outcomes. Concomitant administration of ginger extract greatly ameliorated these changes in bone>CC>AC>PDL confirming the strong protective and curative potency of ginger against the oxidative damage induced by aluminum thus indicated the use of ginger in further new therapeutic approaches.

DATA AVAILABILITY

"All data underlying the results are available as part of the article and no additional source data are required."

ABBREVIATIONS

(Al): Aluminum; **(AlCl3):** Aluminum chloride; **(PDL):** Periodontal ligament; **(AC):** Acellular cementum, **(CC):** Cellular cementum; **(H&E):** Hematoxyline and Eosin; **(CD68+):** Cluster of differentiation 68 ; (OPN): Osteopontin; **(DEXA):** Dual-energy X-ray absorptiometry, **(BMD):** Bone mineral density

CONFLICTS OF INTEREST

There are no conflicts of interest

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الملخص العربى

الدور العلاجي المحتمل لمستخلص الزنجبيل فى تاثير سُمية الالومنيوم المزمنة علي دواعم اسنان الجرذ (دراسة نسيجية ومناعية)

فاطمة عادل سعد
مدرس بيولوجيا الفم- جامعة المستقبل في مصر

نبذة مختصرة: تسمم الألومنيوم المزمن عزَّزَ من الخطرعلي أنسجة الجسم المختلفة في الانسان والحيوانات. قد يقلل تناول الزنجبيل من الآثار الضارة لسمية أيونات المعادن .

الهدف من التجربة: حاولت هذه الدراسة تقييم ومقارنة آثار تسمم الألمنيوم المزمن وعلاج الزنجبيل المصاحب علي المكونات المختلفة لدواعم اسنان الجرذ.

التجربة: تم تقسيم 21 من الفئران البيضاء الذكور البالغين إلى ثلاثة مجموعات متساوية: المجموعة الاولي الظابطة : تلقت محلول ملحي 0.9 ٪ يوميا عن طريق الفم ، تلقت المجموعة الثانية و الثالثة كلوريد الألومنيوم 20 مجم / كجم عن طريق الفم في ماء الشرب يوميًا لمدة ثلاثة أشهر ؛ ثم بعد ذلك تلقت المجموعة الثالثة فقط مستخلص زيت الزنجبيل يوميًا 150 مجم / كجم عن طريق الفم لمدة 4 أسابيع. ثم تم فصل الفك السفلي، واستخدام بعض أنصاف الفك السفلى لقياس كثافة المعادن في العظام. بينما البعض الاخر تم إزالة الكِلْس منهم ومعالجتهم للفحص الهستولوجي الروتيني وللفحص المناعي باستخدام الأجسام المضادة لـكلاً من عنقود تمايز68 و الأُستيوبونتين .

النتائج : أظهرت نتائج الفحص الهستوباثولوجي لعينات المجموعة الثانية علي ما يبدو انخفاض كثافة الألياف بالرباط اللثوي، مناطق تنكسية واسعة وتسلل ملحوظ للخلايا الالتهابية. وبالمثل ، وجدت زيادة كبيرة في التفاعل المناعي لعنقود التمايز 68 في الخلايا العملاقة بالرباط اللثوي وعلي أسطح ارتشاف سطح الجذر والعظم السنخي في المجموعة الثانية. وقد وجد تحسن في هذه التغيرات في المجموعة الثالثة مع انخفاض كبير في الخلايا الإيجابية لعنقود التمايز68 في الرباط اللثوي . بينما أظهر الملاط والعظم السنخي بالمجموعة الثانية تغيرات متدهورة كبيرة مقارنةً بالمجموعة الاولي والتي تحسنت لدرجة كبيرة في المجموعة الثالثة. وبالمثل، قد زاد التفاعل المناعي للأُستيوبونتين في المجموعة الثانية بشكل ملحوظ مقارنة بالمجموعة الاولي في الملاط اللا خلوي (الأكثر) مصحوبا بالملاط الخلوي ثم العظم السنخي وأخيرا الرباط اللثوي (الاقل). وأيضاً بالمقارنة زاد هذا التفاعل زيادة كبيرة في المجموعة الثالثة بالعظم السنخي (الأكثر) مصحوبا بالملاط الخلوي واخيرا بالملاط اللاخلوي(الاقل)، بينما في هذه المجموعة التفاعل المناعي للأستيوبونتين في الرباط اللثوي كان ضعيف ولكن بزيادة ملحوظة مقارنةً بالمجموعة الاولي. وبالنسبة لكثافة المعادن في العظم السنخي كان أعلى بكثير في المجموعة الاولي ثم بالثالثة والاقل بالمجموعة الثانية وبفوارق ملحوظة .

الاستنتاج: ومما سبق تم إستنتاج أن التغيرات الضارة المرتبطة بالسمية المزمنة لـلأمونيوم قد وجدت بشكل ملحوظ في الرباط اللثوي (الاكثر) ثم بالعظم السنخي يليه الملاط الخلوي واقلهم الملاط اللا خلوي و يمكن أن يؤدي ذلك إلى نتائج مَّرَضية كبيرة. وقد حَسَّن مستخلص الزنجبيل بشكل كبير هذه التغيرات في ثم العظم السنخي (الأكثر) ثم الملاط الخلوي يليه الملاط الخلوي واقل تحسن بالرباط اللثوي مما يؤكد قوته العلاجية القوية ضد الأكسدة المرتبطة بالألومونيوم ، وذلك يدل علي امكانية استخدامه في طرق علاجية جديدة أخرى.