رقم المقالة	الترقيم	عدد الصفحات	ص الألوان	صور 1 لون	صور 4 لون	ملاحظات
31		13	5		9	

THE EFFECT OF ORALLY ADMINISTERED CURCUMIN AND /OR GINGER ON ESCHERICHIA COLI INDUCED PERIODONTITIS IN RATS (HISTOPATHOLOGICAL, IMMUNOHISTOCHEMICAL, AND STEREOMICROSCOPIC STUDY)

Bassant A. Ezzat * and Amira E. Fares **

ABSTRACT

Objective: The aim of the present study was to determine whether Curcumin and /or Ginger can inhibit the inflammatory responses and prevent the alveolar bone loss in experimentally induced periodontitis in rats.

Design: Fifty adult male Wistar rats with an average weight of 150-200 g were randomized into five groups, control, E. coli induced periodontitis, curcumin treated, ginger treated and curcumin-ginger treated groups. After two weeks, the rats were sacrificed. The gingiva and periodontal ligaments were examined histologically, immunohistochemically and histomorphometrically. Bone loss was evaluated by stereometric analysis.

Results: severe destruction with areas of hydropic degeneration was observed in the gingival epithelium of group II as well as loss of normal arrangement of the PDL and appearance of resorption lacunae in both cementum and alveolar bone surfaces. Minimal recovery was observed in curcumin treated group and a valuable recovery were seen in both ginger treated and curcumin-ginger treated groups. MMP-1 immunostaining was increased in all experimental groups as compared to control (ANOVA) test revealed a statistically significant difference among all groups. Tukey's post-hoc revealed that the difference between group I and groups II and III was significant. However, no significant difference was seen between group I and groups IV and V. Furthermore, there was no significant difference between the three treatment groups III, IV and V. Stereomicroscopic results revealed bone resorption in all experimental groups. The greatest mean value was in-group II.

Conclusions: systemic administration of curcumin was not reliable in improving the condition of periodontitis. However, preferable results were revealed with ginger and combined mixture of curcumin and ginger administration.

Key words: E. coli**,** curcumin, ginger, periodontitis, MMP-1

*Assistant professor, Oral Biology, Faculty of Oral and Dental Medicine, Cairo University.
**Lecturer, Oral Biology , Faculty of oral and Dental Medicine, Cairo University.

INTRODUCTION

Periodontal disease is the leading cause of tooth loss in adults. Periodontitis is initiated by tooth-associated microbial biofilms triggering an altered host response leading to soft tissue inflammation and alveolar bone loss [1]. Periodontal infections are not only an important impact factor to human oral health but also it implicated in a variety of other diseases, such as cardiovascular disease, stroke, and aspiration pneumonia [2]. A number of studies have indicated that the main pathogen of periodontal disease is the bacterial plaque accumulation including Porphyromonasgingivalis and Escherichia coli [3-5]. The bacterial pathogen is known to play a critical role in the initiation of inflammatory processes in periodontal tissues and host immune response by releasing several inflammatory mediators such as proinflammatory cytokines, prostaglandin E_2, reactive oxygen species and matrix metalloproteinase. These harmful factors result in loss of the supporting periodontal tissues including the collagen of periodontal ligament, cementum and alveolar bone [6,7]. Periodontal therapy is aimed at achieving the complete regeneration of these structures [8].

Currently, the fundamental treatment of periodontitis is aimed to reduce the pathogenic microbiota by instrumental debridement [9]. However, mechanical instrumentation fails to completely eliminate the pathogenic bacteria that have invaded into soft tissue and anatomically inaccessible areas such as the furcation area and root depression. Owing to these unavoidable limitations, development of potential therapeutic drugs with an ability to regulate the host immune and bacteria-mediated inflammatory interactions is a valuable approach for prevention and treatment of periodontal diseases [10]. Recently, many natural products have been reported to improve the pathological symptoms of periodontitis [11,12], suggesting that natural herbs may be potential drugs for periodontal therapy.

Curcumin (diferuloylmethane) is a spice used extensively in Southeast Asia for centuries. This yellow pigment is extracted from the rhizomes of the plant *Curcuma longa*, and is recognized for its medicinal properties including anti-inflammatory, anti-oxidant, anti-proliferative, anti-angiogenic, and anti-tumor activities [13-17]. The anti-carcinogenic properties of curcumin have been demonstrated in animal models and human studies [18,19]. Curcumin's anti-neoplastic activity, along with its low molecular weight and apparent lack of toxicity (use of up to 8 g/day), makes it an ideal foundation for the development of new, synthetic chemotherapeutic agents [20]. Interestingly, curcumin was able to block ulceration by induction of collagenization and angiogenesis in gastric tissues via upregulation of MMP-2, VEGF, and transforming growth factor (TGF)-β at protein and messenger ribonucleic acid (mRNA) levels [21].

On the other hand, Ginger belongs to the family Zingiberaceae. It originated in South-East Asia and used in many countries as a spice and condiment to add flavor to food. [22]. Ginger has staring potential for treating a number of ailments including degenerative disorders (arthritis and rheumatism), digestive health (indigestion, constipation and ulcer), cardiovascular disorders (atherosclerosis and hypertension), diabetes mellitus, and cancer[23-27]. It also has anti-inflammatory and anti-oxidative properties for controlling the process of aging [28,29]. Furthermore, it has antimicrobial potential as well which can help in treating infectious diseases [30].

Based on the anti-inflammatory effect of curcumin and ginger and the anti- microbial effect of ginger, the present study was conducted to determine whether curcumin and /or ginger can inhibit the inflammatory responses and prevent the alveolar bone loss in experimentallyinduced periodontitis in rats.

MATERIALS AND METHODS:

Experimental procedure

Fifty adult male Wistar rats with an average weight of 150-200 g were used in this study. They were randomly assigned to five groups, 10 rats each. The rats of all groups were lightly anaesthetized with surgical doses of 3% sodium pentobarbitone (35 $mg \cdot kg^{-1}$). Group I (control group) was injected with 0.1 ml of 9% saline into gingival crevice of mandibular 1st molars of both sides every other day for two weeks. Experimental periodontal disease was induced in rats of other experimental groups (II & III, IV & V) by injection of 0.1 ml of E. coli [31] bacterial suspension, which was diluted to obtain approximately 10^{10} cells/ml into the gingival crevice of mandibular 1stmolars of both sides every other day for two weeks. In addition, concomitant curcumin (suspended in 0.5% w/v sodium carboxymethyl-cellulose solution) was given to the rats of group III by the intragastric route daily at a dose of 100 mg/kg for 2 weeks [32]. Ginger was given to rats of group IV by the intragastric route daily at a dose of 200 mg/kg for 2 weeks [33]. Both curcumin and ginger were given to rats of group V by the intragastric route daily at doses of 100 mg/kg and 200 mg/kg respectively for 2 weeks [32-33].

The animals were sacrificed following the last E. coli infection dose by ketamine over dose. The mandibles were dissected. The right side of each mandible was used for light microscopic examination, immunohistochemical and histomorphometric analysis, forming a total of 50 bone segments (10 specimens from each group). The degree of alveolar bone loss was evaluated by stereometric analysis in the other 50 bone segments (10 specimens from each group) of the left sides of the mandibles.

Light microscopic examination

Specimens were immediately fixed in 10% neutral formalin for 48 h, washed and soaked in 10% Ethylenediaminetetraacetic acid (EDTA) for decalcification for 4 weeks, and then rinsed in distilled water. Specimens were dehydrated in ascending grades of alcohol and embedded in paraffin. From each mandible, 40 to 50 mesio-distal serial sections of 5 μm thickness were cut from the predilection site: the lingual cortical plate that supports the incisors and the alveolar bone apical to the first molar. The sections were subjected to haematoxylin and eosin stain according to the conventional method. Histopathologic examination was performed using light microscopy.

Immunohistochemistry (IHC)

Sections of 5 μm were placed on positive charged slides. The sections were deparaffinized with xylene, and rehydrated in a series of ethanols. Endogenous peroxidase was blocked by 3% hydrogen peroxide in methanol for 30m. For epitope retrieval, slides were heated in a microwave oven at 92°C for 20m in a PBS buffer. They were then incubated overnight at 4°C with the primary antibodies anticollagen I, MMP1 at 1:250 (Santa Cruz, CA, USA). Sections were subsequently incubated for 1h with biotinylated goat anti-rabbit antibody IgG and then for 30m with Streptavidin-HRP peroxidase (Santa Cruz, CA, USA). Color reaction product was visualized by using diaminobenzidine-(DAB-) H_2O_2 as substrate for peroxidase. All sections were counterstained with hematoxylin, dehydrated, and covered. Incubations with phosphate-buffered saline containing 1% bovine serum albumin were used as negative controls. The negative control was obtained by omitting the primary antibody from the protocol outlined above.

The data of immunohistochemical reaction were obtained using Leica Qwin 500 image analyzer computer system (England). The image analyzer consisted of a coloured video camera, coloured monitor, hard disc of IBM personal computer connected to the microscope, and controlled by Leica Qwin 500 software. The area percentage occupied by MMP-1 was measured using an

objective lens of magnification 40 x, i.e. of a total magnification of 400. Ten fields were measured for each specimen. After grey calibration, the image was transformed into grey delineated image to choose areas exhibiting positive reactivity with accumulation of all grades of reactivity (minimum, median and maximum). Areas of positive reaction were then masked by a blue binary color. The area percentage was calculated in relation to a standard frame of area 118476.6 μm^2. Mean value were obtained for each specimen.

Stereomicroscopic analysis

The mandibles of left side were excised and defleshed after immersion in 8% sodium hypochlorite for 4 hours. The specimens were washed in running water and dried with compressed air. To outline the cemento-enamel junction (CEJ), 1% methylene blue (Sigma-Aldrich®, Saint Louis, USA) was applied to the specimens for 1 minute and then washed in running water. The specimens were fixed in wax with their occlusal plane kept parallel to the ground. The external surface of the mandible was examined using sharp image digital 130 x USB microscope camera Leica DFC 290. Images were transferred to the computer using computer software (digital viewer) and saved as TIF format with the aid of paint program (accessories, windows XP). A millimetric ruler was photographed together with all specimens. Alveolar bone loss was determined on the buccal surface of the mandibular first molars by distance of the CEJ from the alveolar bone crest (ABC) using a calibrated dissecting microscope (magnification, ×30). Measurements were made along the axis of each root in three regions of the first molar (three roots). The total alveolar bone loss was obtained by taking the sum of the linear recordings from the buccal tooth surface of the roots and dividing by three. (Liu, 2008)[34]

Statistical analyses

The data obtained from stereomicroscopic and immunohistochemical analyses were statistically described in terms of mean ± standard deviation (± SD). Comparison between the study groups was done using one way analysis of variance (ANOVA) test with Tukey's post-hoc for multiple 2-group comparisons. A probability value (*p* value) less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs Microsoft Excel 2007 (Microsoft Corporation, NY, USA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

RESULTS

Histopathological Results

The control group (GI)

The gingiva of the Wistar albino rats exhibited typical keratinized stratified squamous epithelium with basal, prickle, granular and keratinous layers, the epithelial ridges were long, slender and numerous, a few number of intraepithelial lymphocytes was observed. The lamina propria was composed of well-formed collagen fibers, small sized blood vessels and few inflammatory cells (Fig. 1 a). The periodontal ligaments (PDL) of the rats of the same group were organized with small interstitial tissue spaces extending along the entire surface of PDL close to the alveolar bone surface. A thin layer of cementoid tissue and cementoblasts was found covering the root surface (Fig.1 b).

The periodontitis group (G II)

Severe destruction in both epithelium and lamina propria was observed in the gingiva of group II. The epithelium showed reduction in its thickness with hyperorthokeratosis. The epithelial cells showed hydropic degeneration with appearance of large amount of vacuoles of different sizes. The epithelial ridges were narrower and more irregular than that appeared in the control group. The prickle cell layer revealed severe paranuclear haloing and hyalinization of some of its cells. The basal cell layer

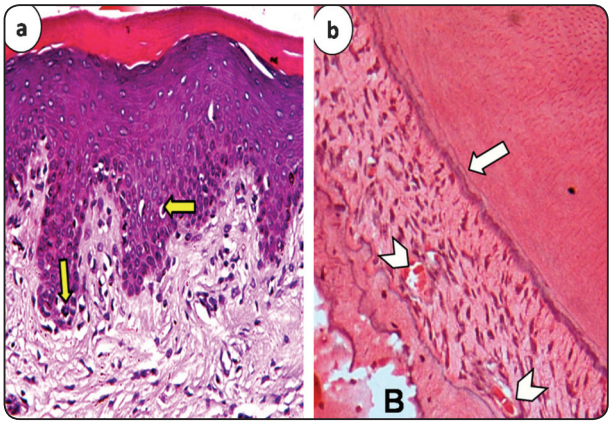

Fig. (1): a- Aphotomicrograph of the gingiva of the control group showing normal histological structure of both epithelium and lamina propria with long slender epithelial ridges, few intraepithelial lymphocytes (arrows) .b- A photomicrograph of the PDL of the control group showing well organized PDL fibers and fibroblasts, small interstitial tissue spaces (arrow heads) were close to the alveolar bone surface (B) and a thin layer of cementoid tissue covering the root surface (arrow) (H&E X 200).

showed loss of its palisade arrangement. Loss of the basement membrane continuity has been revealed. The lamina propria showed dissociation of the collagen and muscle fibers, multiple multinucleated foreign body giant cells, dilated blood vessels and increased inflammatory cell infiltration,(Fig.2a& b).

Loss of normal arrangement of the PDL fibers and fibroblasts, increases in the size of interstitial tissue spaces with marked dilatation of its blood vessels were observed. Resorption lacunae and multinucleated osteoclasts were observed in both cementum and alveolar bone surfaces (Fig. 2c&d).

The Curcumin group (G III)

The gingiva of group III revealed a minimum degree of recovery of the epithelium when compared to that of group II, the vacuolization was more localized in the upper layers of the epithelium and the basement membrane was almost straight. The lamina propria showed dissociated collagen and muscle fibers (Fig. 3a). Compared to that revealed in GII, the PDL showed nearly the same

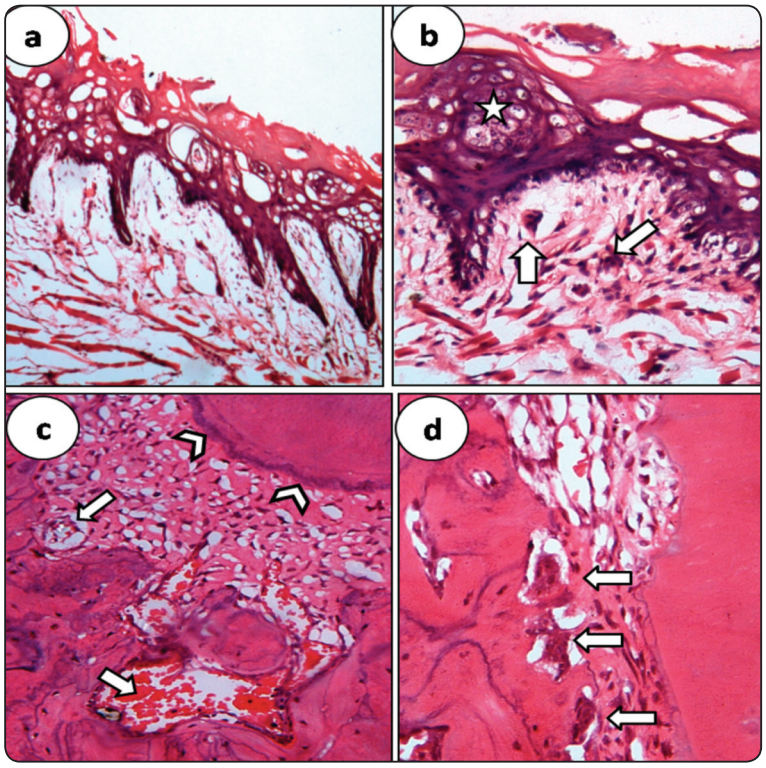

Fig. (2): A photomicrograph of the gingiva of group II showing: (a) Vacuoles of variable sizes in the epithelium, few irregular epithelial ridges , dissociation of collagen fibers as well as the underlying muscles. (b) Hydropic degeneration within the epithelium (star) and multinucleated foreign body giant cells in the underlying lamina properia (arrows). (H&E X 200,400). photomicrograph of the PDL of group II showing: (c) disorganized PDL and fibroblasts, large sized interstitial tissue spaces (arrows)with dilated blood vessels and an irregular layer of cementoid tissue (arrow heads) covering the root surface. (d) A photomicrograph showing resorption of both cemntum and alveolar bone surfaces with appearance of multiple osteoclasts in their lacunae (arrows) (H&E X 200,400).

disorganization with resorption of both cementum and alveolar bone surfaces (Fig.3d).

The Ginger group (G IV)

The gingiva of group IV revealed mild acanthosis of the epithelium with smaller and fewer vacuoles than that observed in both groups II & III. the epithelial ridges were short and broad. the collagen fibers and the underlying muscles were improved (Fig. 3b).

Re-organization of the PDL and fibroblasts was seen at the cemental side with smooth cementum surface, while at the bone side, disorganized PDL was still observed with multibleresorption lacunae and dilated blood vessels (Fig. 3e).

The Curcumin- Ginger group (G V)

The gingiva of group V showed nearly normal structure with broader and fewer epithelial ridges. A mild degree of dissociation appeared in the lamina propria and the underlying muscles (Fig. 3c).

The architecture and arrangement of the PDL of group V were similar to that observed in control group except for mild dilatation of blood vessels within the interstitial tissue spaces (Fig.3 f).

Immunohistochemical Results

We evaluated the effect of E-coli induced periodontitis and three possible natural treatments on the degree of extracellular matrix proteins degradation using MMP-1 IHC.

The control group: (G I)

The gingiva of the control group I exhibited mild to moderate reaction in the basal and prickle cell layers of the epithelium and underlying lamina propria. (Fig. 4a). The PDL of the same group exhibited very mild MMP-1 immunostaining localized mainly in the interstitial tissue spaces (Fig.4 c).

The periodontitis group: (G II)

The gingiva of group II showed strong reaction of MMP-1in the basal cell layer of the epithelium

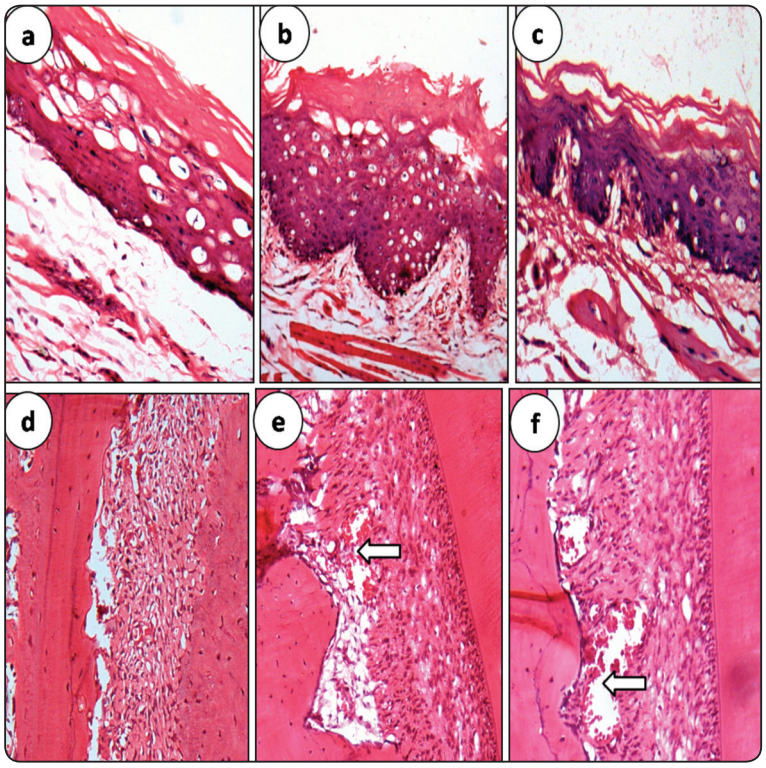

Fig. (3): A photomicrograph of the gingiva of group III showing: (a) Vacuolization localized in the upper layers of epithelium, the basement membrane is almost straight . the lamina propria showing dissociated collagen and muscle fibers. (b) The gingiva of group IV showing mild acanthosis with smaller and fewer vacuoles than that observed in both groups II & III and more organized collagen and muscle fibers. (c) The gingiva of group V showing nearly normal structure of the gingiva with broader and fewer epithelial ridges and mild dissociation of the keratinous layer (H&E X 200). (d) A photomicrograph of PDL of group III showing disorganized PDL and fibroblasts and resorption of both cementum and alveolar bone surfaces. (e) PDL of group IV exhibited reorganization at the cemental side with smooth cementum surface. At alveolar bone surface, the PDL revealed disorganization, howships lacunae and dilated blood vessels (arrow) (f) The PDL of group V showing nearly the same features as seen in group IV except for mild dilated blood vessels (arrow) (H&E X 200).

and a moderate reaction in the remaining epithelial layers as well as the underlying lamina propria (Fig. 4b). The PDL of the same group II showed strong MMP-1 immunostaining along the entire width of periodontal ligaments (Fig. 4d).

The Curcumin group (G III)

The gingiva exhibited strong MMP-1 immunostaining in the basal and suprabasal layers of the epithelium and moderate reaction in the underlying lamina propria (Fig. 5 a). In addition, the PDL of group III revealed strong reaction to MMP-1 localized the interstitial tissue spaces and discrete areas of PDL (Fig. 5 d).

The Ginger group (G IV)

The gingiva showed mild reaction to MMP-1 in the keratinized layer of epithelium and moderate immunostaining in the underlying lamina propria (Fig. 5 b). The PDL of group IV revealed mild reaction in the PDL region adjacent to the alveolar bone and moderate reaction of the interstitial tissue spaces (Fig. 5 e).

The Curcumin-Ginger group (G V)

Group V exhibited mild MMP-1 immunostaining in the basal and prickle cell layers of gingival epithelium and in the underlying lamina propria (Fig. 5 c).The PDL of the same group showed mild MMP-1 immunostaining in the interstitial tissue spaces (Fig. 5 f).

The area percentage occupied by MMP-1 of the control and experimental groups is summarized by means and standard deviation in Table 1.The greatest mean value of area % of MMP-1 immunoexpression was in G2 (6.27±1.31), while the lowest mean value was in G1 (3.25±1.21). One way analysis of variance (ANOVA) test revealed that the difference between the different groups was statistically significant (p=0.011). Tukey's post-hoc revealed that the difference between group I and groups II and III was significant were p= .0053 and p=0.029 respectively. However, no significant difference was seen between group I and groups IV and V were p= 0.360 and p=0.258 respectively. Furthermore, there was no significant difference between the three treatment groups III, IV and V.

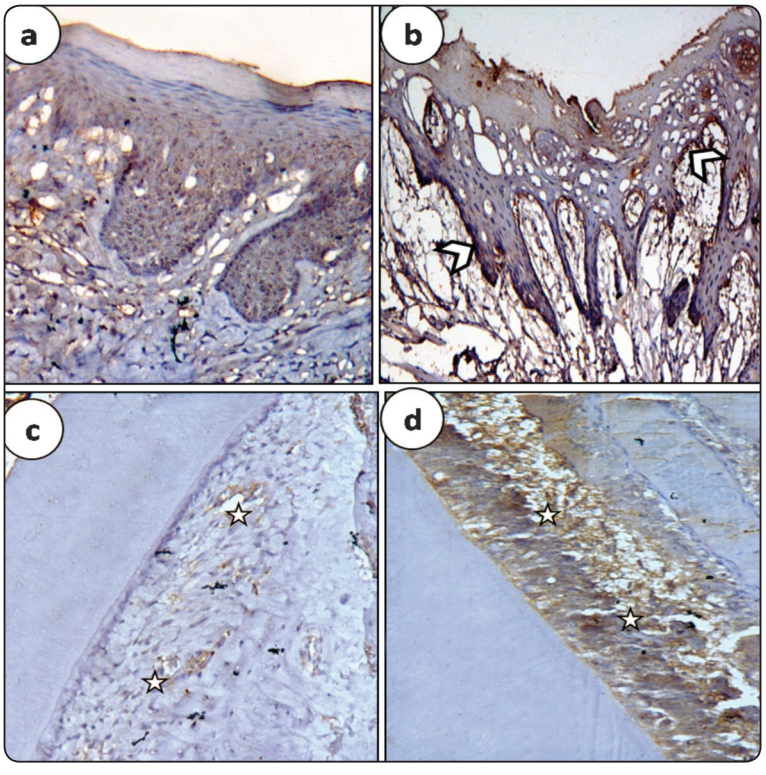

Fig. (4): a- A photomicrograph of the gingiva of the control group showing mild to moderate immunoreactivity to MMP-1 in the basal and prickle cell layers of the epithelium and underlying the lamina propria. b- The gingiva of group II showing reaction of MMP-1in the basal cell layer of the epithelium (arrow heads) and a moderate reaction in the remaining epithelial layers and the underlying lamina properia .c- A photomicrograph of the PDL of the control group showing very mild MMP-1 immunostaining localized in the interstitial tissue spaces(astrisks). d- The periodontal ligaments of group II exhibiting strong MMP-1 immunostaining along the entire width of periodontal ligaments (asterisks) (MMP-1 X 200).

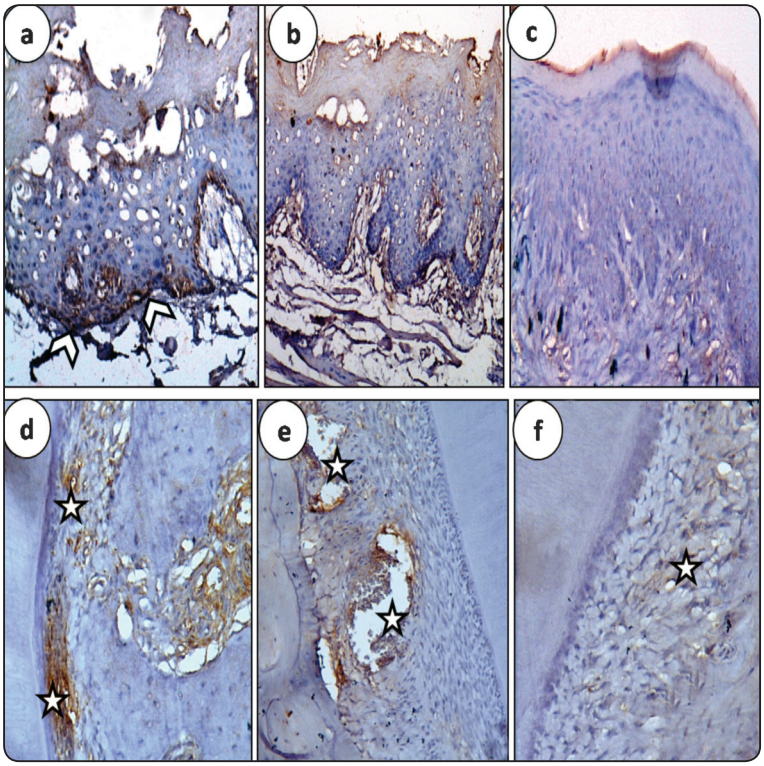

Fig. (5): (a)- A photomicrograph of the gingiva of group III showing strong MMP-1 immunostaining in the basal and suprabasal layers (arrow heads) of the epithelium and moderate reaction in the underlying lamina propria. (b)- The gingiva of group IV showing mild reaction to MMP-1in the keratinized layer of epithelium and moderate reaction in the underlying lamina propria. (c)- The gingival epithelium of group V showing mild MMP-1 immunostaining in the basal and prickle cell layers and in the underlying lamina propria. (d)-A photomicrograph of the PDL of group III showing strong reaction of MMP-1 immunostaining localized in the interstitial tissue spaces (astrisks). (e) -The PDL of group IV revealed mild reaction of MMP-1 in the region adjacent to alveolar bone surface and moderate reaction in the interstitial tissue spaces (sterisks). (f)- Group V exhibiting mild MMP-1 immunostaining in the interstitial tissue spaces(asterisks) (MMP-1 X 200).

TABLE (1) Mean values and Standard Deviation of the area percentage of MMP-1 activity in the gingiva and PDL of the control and experimental groups

	G1	G2	G3	G4	G5
Number of samples	10	10	10	10	10
Mean	3.25^{a}	6.27^{b}	$5.67^{b,c}$	$4.09^{a,c}$	$4.07^{a,c}$
SD	1.21	1.31	1.65	1.51	0.90
Maximum	5.33	8.11	7.51	6.34	5.09
Minimum	2.19	5.06	3.91	2.14	2.95
F value	4.360				
P value	0.011*				

SD=Standard Deviation, *statistically significant

Tukey's post hoc test: means with different letters are significantly different

Streomicroscopic results

Evident bone resorption was observed 2 weeks after inoculation with E. Coli. The greatest mean value was in group II (1.165±0.348), while the lowest mean value was in group I (0.416±0.039). One way analysis of variance (ANOVA) test revealed that the difference between the five groups was statistically significant ($p<0.0001$), (Table 2). Tukey's post-hoc revealed that the difference between group I and groups IV & V was not statistically significant($p=0.0555$,$p=0.1908$) respectively. However, group I significantly differed from group II and group III where ($p= 0.0014$, $p= 0.0007$) respectively. Moreover, the difference between group II and group III was statistically significant ($p=0.0457$). (Table 2)

TABLE (2) Mean values and Standard Deviation of alveolar bone loss obtained by linear measurement of exposed roots of control and experimental groups

	G1	G2	G3	G4	G5
Number of samples	10	10	10	10	10
Mean	0.416[a]	1.165[b]	0.768[c]	0.55[a]	0.487[a]
SD	0.039	0.348	0.141	0.128	0.104
Maximum	0.461	1.555	0.941	0.73	0.634
Minimum	0.365	0.71	0.596	0.442	0.403
F value	13.508				
P value	<0.0001***				

SD= Standard Deviation, ***extremely statistically significant

Tukey's post hoc test: means with different letters are significantly different

DISCUSSION

Individuals with periodontal disease have increased risk of tooth loss, particularly in cases associated with loss of alveolar bone and PDL. 35 The primary etiological agent of this inflammatory disease is a polymicrobial complex, predominantly Gram negative anaerobic or facultative bacteria within the sub-gingival biofilm. These bacterial species initiate the production of various cytokines. These cytokines produce reactive oxygen species (ROS) superoxide via the respiratory burst mechanism as the part of the defence response to infection. ROS have deleterious effects on tissue cells when produced in excess. [36]

In this study, periodontal disease was induced in rats by injections of 0.1 ml suspension of gram-ve facultative anaerobic bacteria E.Coli into the gingival crevice of mandibular first molar every other day for 2 weeks, whereas the control group was injected with 0.1 ml of 9% saline. Severe destruction was demonstrated in the periodontium. This destruction was evaluated histopathologically, immunohistochemically, histomorphometrically and streomicroscopically.

Rats are often used in models of experimental periodontitis because periodontal anatomy in the molar region shares some similarities with that of humans. Furthermore, rats are easy to handle and can be obtained with different genomes and microbial status. [37]

In the present research, destruction was observed in the epithelium and lamina propria of the gingiva of group II represented in hydropic degeneration within the epithelium, few irregular epithelial ridges, dissociated collagen fibers and multiple multinucleated giant cells. The same destruction was observed in PDL with disorganized collagen fibers and fibroblasts and presence of multiple resorption lacunae in both cementum and bone surfaces.

Previous studies were induced experimental periodontitis by repeated injection of E. Coli endotoxin. They attributed the periodontal tissue destruction to the upregulation of proinflammatory cytokines such as Prostaglandin E2 and leukotriens. [38,39] Moreover, marked increase in protein carbonyl levels (biomarker for oxidative damage) in gingival cervicular fluid reflect oxidative tissue damage occurred in periodontitis. [40]

The usage of curcumin in the herein study via oral route in group III showed a minimum degree of recovery compared to periodontitis group II. Various preclinical cell culture and animal studies

suggested that curcumin is highly pleiotropic molecule because of its pharmacological properties including anti-inflammatory41, antimicrobial42 and wound healing activities. [43]

It was also observed that topical application of curcumin have shown to improve significantly wound healing in view of a rapid wound contraction , a greater content and a better arrangement of collagen.[43]

United States of Food and Drug Administration has declared curcumin as "generally regarded as safe". In spite of its efficacy and safety, curcumin has not yet been approved as a therapeutic agent. The major drawback of curcumin is poor oral bioavailability which is related mainly to its poor aqueous solubility, intestinal metabolism, rapid hepatic metabolism and rapid systemic clearance. [44]

In the present study, ginger administration in group IV exhibited improvement in the histopathology of gingiva and PDL compared to the periodontitis group II.

Ginger is known for its strong free-radical reducing efficacy. It is mediated mostly by its phenolic constituents.[46] It recorded improvement of the antioxidant parameters and decreased production of free radicals derivatives in rats subjected to renal injury by toxic carbon tetrachloride. [47]

Ginger was also able to reduce inflammatory pain and chronic swelling of osteoarthritis or rheumatoid arthritis 48. The mechanism of the anti-inflammatory action of ginger was first confirmed to be due to dual inhibition of COX and 5-lipoxygenase, enzymes essential for arachidonate metabolism [49], and then extended to down-regulation of the induction of inflammatory genes. The latter effect means that ginger can modulate pathophysiological pathways activated in chronic inflammation. [50]

It was reported that combination of curcumin and ginger extract functions to improve wound-healing in corticosteroid-damaged skin. The two agents act through complementary mechanisms. A major contribution of the ginger extract, is to increase vascularity and blood flow in the repairing tissue, while the major effect of curcumin is on matrix remodeling.[51]

Interestingly, the immunohistochemical and histomorphometrical data of the ongoing study were corroborated with histopathological results. Comparing the area percentage occupied by MMP-1 in 5 studied groups revealed an increase in the MMP-1 immunostaining in all experimental groups II,III,IV and V. The increase was statistically significant ($p= 0.011$). Moreover, significant difference was also observed between control group I and experimental groups II and IV ($p=0.0053$, $p=0.029$) respectively. Furthermore, no significant difference was existed between the three treatment groups III, IV and V.

Fibroblasts are the major cell type in gingiva and PDL.They play a key role in maintenance and remodeling of extra cellular matrix (ECM) by producing various structural components, such as collagen, elastin, glycoprotein and glycosaminoglycans. In addition, they also synthesize and secrete various members of MMPs proteolytic enzymes that play a major role in the degradation of collagens and proteoglycans in periodontal tissues.[52] The activity of MMPs is largely regulated by several naturally occurring inhibitors like tissue inhibitors of metalloproteinases (TIMPs).[53] Overall, the expression and regulation of MMPs and TIMPs in fibroblasts are crucial for maintenance of tissue homeostasis and periodontal health.

Disturbance of this balance is critically implicated in the destruction of periodontal tissues.[54] In normal conditions, MMPs are involved in the remodeling and turnover of periodontal tissues under the strict control of TIMPs. Increased production of MMPs 1–3 are observed in periodontitis in response to

periodontopathic bacteria that results in excessive connective tissue breakdown.[54]

In the human skin culture system, the ginger extract raises the level of matrix metalloproteinase-1 (MMP-1) – known to be associated with vascular growth into collagen [55] while curcumin has a strong positive effect on collagen synthesis and suppresses the increase in MMP-1 induced by ginger.(56) These data provide a basis for suggestion that the two agents have complementary effects when added together.

The stereomicroscopic results of the ongoing research provided good support for the histological and immunohistochemical findings. They were reliable in tracing the amount of alveolar bone loss. The alveolar bone loss was detected in all experimental groups. Significant loss of alveolar bone was observed in groups II and III when compared to control group I (p=0.0014 and p= 0.0007) respectively. However, the bone loss was insignificant in groups IV and V when compared to the control group.

Recent reports proved that curcumin and ginger were able to reduce bone loss by reducing osteoclastogenesis and impaired RANKL signaling, suggesting their potentials as therapeutic agents for osteoporosis. [57-59]

The overall results of this study clearly demonstrated that systemic administration of curcumin was not reliable in improving the condition of periodontitis. However, combined mixture of curcumin and ginger revealed more promising results.

Improvement of the bioavailability of curcumin is a challenge. So, it is recommended to perform several studies and compare between different curcumin formulations and /or alternative drug delivery system in order to optimize the curcumin bioavailability.

REFERENCES

1- Savage A, Eaton KA, Moles DR, Needleman I. A systemic reviewof definitions of periodontitis and methods that have been used to identify this disease. J Clin Periodontol 2009; 36:458-467.

2- Scannapieco FA, Dasanayake AP, and Chhun N, "Does periodontal therapy reduce the risk for systemic diseases?" Dental Clinics of North America 2010; 54(1):163–181.

3- Ramseier CA, Kinney JS, Herr AE, Braun T, Sugai JV, Shelburne CA, Rayburn LA, Tran HM, Singh AK, Giannobile WVIdentification of pathogen and host-response-markers correlated with periodontaldisease. J Periodontol. 2009 ;80(3):436-46.

4- Cortelli JR, Cortelli SC, Jordan S, Haraszthy VI, and Zambon J. "Prevalence of periodontal pathogens in Brazilians with aggressive or chronic periodontitis," J Clin Periodontol 2005;32 (8):860–866.

5- Amel Y, Bouziane D, Leila M and Ahmed B. Microbiological Study of Periodontitis in the West of Algeria. World Journal of Medical Science 2010; 5 (1): 07-12.

6- Deo V and Bhongade ML. "Pathogenesis of periodontitis: role of cytokines in host response," Dentistry Today 2010; 29(9): 60–66.

7- Herath TD, Wang Y, Seneviratne CJ, Darveau RP, Wang CY, Jin L. The expression and regulation of matrix metalloproteinase-3 is critically modulated by Porphyromonas gingivalis lipopolysaccharide with heterogeneous lipid A structures in human gingival fibroblasts. BMC Microbiol 2013;13:73.

8. Lin N.H., Gronthos S., Mark B.P. Stem cells and future periodontal regeneration. Periodontology 2009; 51: 239-251.

9- Apatzidou DA, Kinane DF. Nonsurgical mechanical treatment strategies for periodontal disease. Dent Clin North Am 2010;54(1):1-12.

10- Huang RY, Lu SH, Su KW, Chen JK, Fang WH, Liao WN, Chen SY, Shieh YS.Diacerein: a potentialtherapeuticdrug for periodontaldisease. Med Hypotheses 2012; 79(2): 165-7.

11- Sastravaha G, Gassmann G, Sangtherapitikul P, and Grimm WD. "Adjunctive periodontal treatment with Centella asiatica and Punica granatum extracts in supportive periodontal therapy," J Int Acad Periodontol 2005;7(3)70–79.

12- Pimentel SP, Barrella GE, Casarin RC, et al., "Protective effect of topical Cordia verbenacea in a rat periodontitis

model: immune-inflammatory, antibacterial and morphometric assays," BMC Complement Altern Med 2012;12: 224.

13- Anand P, Sundaram C, Jhurani S, Kunnumakkara AB, Aggarwal BB. Curcumin and cancer: an "old-age" disease with an "age-old" solution. Cancer Lett 2008;6(1):133–164.

14- Ammon HP, Wahl MA. Pharmacology of Curcuma longa. Planta Med 1991;6(1):1–7.

15- Weir NM, Selvendiran K, Kutala VK, Tong L, Vishwanath S, Rajaram M, Tridandapani S, Anant S, Kuppusamy P. Curcumin induces G2/M arrest and apoptosis in cisplatin-resistant human ovarian cancer cells by modulating Akt and p38 MAPK. Cancer Biol Ther 2007;6(2):178–184.

16- Ghosh SS, Massey HD, Krieg R, Fazelbhoy ZA, Ghosh S, Sica DA, Fakhry I, Gehr TW. Curcumin ameliorates renal failure in 5/6 nephrectomized rats: role of inflammation. Am J Physiol Renal Physiol 2009;6(5):1146-1157

17- Anand P, Thomas SG, Kunnumakkara AB, Sundaram C, Harikumar KB, Sung B, Tharakan ST, Misra K, Priyadarsini IK, Rajasekharan KN. Biological activities of curcumin and its analogues (Congeners) made by man and Mother Nature. Biochem Pharmacol 2008;6(11):1590–1611

18- Ono M, Higuchi T, Takeshima M, Chen C, Nakano S.Differential Anti-tumor Activities of Curcumin against Ras- and Src-activated Human Adenocarcinoma Cells. Biochem Biophys Res Commun 2013 May 29 [Epub ahead of print]

19- Tan Q, Wu J, Li Y, Mei H, Zhao C, Zhang J. A supermolecular curcumin for enhanced antiproliferative and proapoptotic activities: molecular characteristics, computer modeling and in vivo pharmacokinetics. Nanotechnology 2013 ;24(3):035102.

20- Sampaio FJ. Anti-neoplastic activity of curcumin in PCa. Int Braz J Urol 2009;6(3):254–255.

21- Sharma AV, Ganguly K, Paul S, Maulik N, Swarnakar S. Curcumin heals indomethacin-induced gastric ulceration by stimulation of angiogenesis and restitution of collagenfibers via VEGF and MMP-2 mediated signaling. Antioxid Redox Signal 2012;16(4):351-62.

22- Mashhadi NS, Ghiasvand R, Askari G, Hariri M, Darvishi L, Mofid MR. Anti-oxidative and anti-inflammatory effects of ginger in health and physical activity: review of current evidence. Int J Prev Med 2013;4(Suppl 1):S36-42.

23- Akhtar N and Haqqi TM . Current nutraceuticals in the management of osteoarthritis: a review Ther Adv Musculoskelet Dis 2012 June; 4(3): 181–207.

24- Haniadka R, Saldanha E, Sunita V, Palatty PL, Fayad R, Baliga MS. A review of the gastroprotective effects of ginger (Zingiber officinale Roscoe). Food Funct 2013 Jun 28;4(6):845-55.

25- Nicoll R, Henein MY. Ginger (Zingiber officinale Roscoe): A hot remedy for cardiovascular disease? Int J Cardiol 2009;131:408–9.[PubMed]

26-Li Y, Tran VH, Duke CC, Roufogalis BD. Preventive and Protective Properties of Zingiber officinale (Ginger) in Diabetes Mellitus, Diabetic Complications, and Associated Lipid and Other Metabolic Disorders: A Brief Review. Evid Based Complement Alternat Med 2012;2012:516870.

27- Brahmbhatt M, Gundala SR, Asif G, Shamsi SA, Aneja R.Ginger phytochemicals exhibit synergy to inhibit prostate cancer cell proliferation. Nutr Cancer 2013 Feb;65(2):263-72.

28- Van Breemen RB, Tao Y, Li W. Cyclooxygenase-2 inhibitors in ginger (Zingiber officinale) Fitoterapia 2011;82:38–43.

29- Krim M, Messaadia A, Maidi I, Aouacheri O, Saka S.Protective effect of ginger against toxicity induced by chromate in rats. Ann Biol Clin (Paris) 2013;71(2):165-73.

30- Thongson C, Davidson PM, Mahakarnchanakul W, Vibulsresth P. Antimicrobial effect of Thai spices against Listeria monocytogenes and Salmonella typhimurium DT104. J Food Prot 2005;68(10):2054-8.

31- Alma Linggar Jonarta AL, Asmara W, Astuti I and Tandelilin R.Systemic IL-1β and TNF-α Productions of E. coliLipopolysaccharide-Induced Periodontitis Model on Rats. The Indonesian J Dent Res 2010, Volume 1, No.1

32- Guimarães MR, Coimbra LS, de Aquino SG, Spolidorio LC, Kirkwood KL, Rossa C Jr.Potent anti-inflammatory effects of systemically-administered curcumin modulates periodontal disease in vivo. J Periodontal Res 2011; 46(2): 269–279.

33- Nasri H, Nematbakhsh M, Ghobadi S, Ansari R, Shahinfard N, Rafieian-Kopaei M.Preventive and curative effects of ginger extract against histopathologic changes of gentamicin-induced tubular toxicity in rats. Int J Prev Med 2013;4(3):316-21.

34- Liu R, Desta T, Raptis M, Darveau RP, Graves DT. P. gingivalis and E. coli lipopolysaccharides exhibit different systemic but similar local induction of inflammatory markers. J Periodontol 2008;79(7):1241-7.

35- Trombetta-Esilva J, Yu H, Arias DN, Rossa C Jr, Kirkwood KL, Bradshaw AD. LPS induces greater bone and PDL loss in SPARC-null mice. J Dent Res 2011;90(4):477-82.

36- Dahiya P, Kamal R, Gupta R, Bhardwaj R, Chaudhary K, KaurS. Reactive oxygen species in periodontitis. J Indian Soc Periodontol 2013;17(4):411-416.

37- Oz HS, Puleo DA. Animal models for periodontal disease. J Biomed Biotechnol 2011;2011:754857.

38- Gürkan A, Emingil G, Nizam N, Doğanavşargil B, Sezak M, Kütükçüler N et al. Therapeutic efficacy of vasoactive intestinal peptide in escherichia coli lipopolysaccharide-induced experimental periodontitis in rats. J Periodontol 2009 ;80(10):1655-64.

39- Vardar S, Buduneli E, Baylas H, Berdeli AH, Buduneli N, AtillaG.Individual and Combined Effects of Selective Cyclooxygenase-2 Inhibitor and Omega-3 Fatty Acid on Endotoxin-Induced Periodontitis in Rats. J Periodontol 2005 ;76(1):99-106.

40- Pradeep AR, Ramchandraprasad MV, Bajaj P, Rao NS, Agarwal E. Protein carbonyl: An oxidative stress marker in gingival crevicular fluid in healthy, gingivitis, and chronic periodontitis subjects. Contemp Clin Dent 2013;4(1):27-31.

41- Strasser E-M, Wessner B, Manhart N, Roth E. The relationship between the anti-inflammatory effects of curcumin and cellular glutathione content in myelomonocytic cells. Biochemical Pharmacology 2005; 70(4): 552–559

42- Han S, Yang Y. Antimicrobial activity of wool fabric treated with curcumin. Dyes and Pigments 2005;64(2):157–161.

43- Dai M, Zheng X, Xu X, Ye Kong X, Yi Li X, Guo G, et al. Chitosan-Alginate Sponge: Preparation and Application in Curcumin Delivery for Dermal Wound Healing in Rat. J Biomed Biotechnol 2009; 2009: 595126.

44- Rajasekaran SA.Therapeutic potential of curcumin in gastrointestinal diseases. World J Gastrointest Pathophysiol 2011;2(1):1–14.

45- Račková L, Cupáková M, Tažký A, Mičová J, Kolek E, Ko□t'álová D. Redox properties of ginger extracts: Perspectives of use of ZingiberofficinaleRosc. asantidiabetic agent. InterdiscipToxicol 2013;6(1):26-33.

46- Hamed MA, Ali SA, El-Rigal NS.Therapeutic potential of ginger against renal injury induced by carbon tetrachloride in rats. Scientific World Journal 2012;2012:840421.

47- Bliddal H, Rosetzsky A, Schlichting P, Weidner MS, Andersen LA, Ibfelt HH, et al. A randomized, placebo-controlled, cross-over study of ginger extracts and ibuprofen in osteoarthritis. Osteoarthritis Cartilage 2000;8:9–12.

48- Kiuchi F, Iwakami S, Shibuya M, Hanaoka F, Sankawa U. Inhibition of prostaglandin and leukotriene biosynthesis by gingerols and diarylheptanoids. Chem Pharm Bull (Tokyo) 1992;40:387–391.

49- Lee HY, Park SH, Lee M, Kim HJ, Ryu SY, Kim ND et al.Dehydro-[10]-gingerdione from ginger inhibits IKKβ activity for NF-ϰB activation and suppresses NF-ϰB-regulated expression of inflammatory genes. Br J Pharmacol 2012;167(1):128-40.

50- Bhagavathula N, Warner RL, DaSilva M, McClintock SD, Barron A, Aslam MN et al. Combination of curcumin and ginger extract improves abrasion wound healing in corticosteroid-damaged hairless rat skin. Wound Repair Regen 2009; 17(3): 360.

51- Hosokawa I, Hosokawa Y, Ozaki K, Yumoto H, Nakae H, Matsuo T. Proinflammatory effects of muramyldipeptide on human gingival fibroblasts. J Periodontal Res 2010;13:193–199.

52- Brew K, Dinakarpandian D, Nagase H. Tissue inhibitors of metalloproteinases: evolution, structure and function. Biochim Biophys Acta 2000;13:267–283.

53- Herath TD, Wang Y, Seneviratne CJ, Darveau RP, Wang CY, Jin L. The expression and regulation of matrix metalloproteinase-3 is critically modulated by Porphyromonasgingivalis lipopolysaccharide with heterogeneous lipid A structures in human gingival fibroblasts. BMC Microbiol 2013; 30:13-73.

54. Varani J, Perone P, Warner RL, Dame MK, Kang S, Fisher GJ, Voorhees JJ. Vascular tube formation on matrix metalloproteinase-1 - damaged collagen. Brit J Cancer 2008;98:1646–1652.

55- Onodera S, Kaneda K, Mizue Y, Koyama Y, Fujinaga M, Nishihira J.Macrophage migration inhibitory factor up-regulates expression of matrix metalloproteinases in synovial fibroblasts of rheumatoid arthritis. J Biol Chem 2000 ; 7;275(1):444-50.

56- Kim WK, Ke K, Sul OJ, Kim HJ, Kim SH, Lee MH et al. Curcumin protects against ovariectomy-induced bone loss and decreases osteoclastogenesis. J Cell Biochem 2011;112(11):3159-66.

57- Zhou T, Chen D, Li Q, Sun X, Song Y, Wang C.Curcumin inhibits inflammatory response and bone loss during experimental periodontitis in rats. Acta Odontol Scand 2013 Mar;71(2):349-56.

58- Sung B, Murakami A, Oyajobi BO, Aggarwal BB.Zerumbone abolishes RANKL-induced NF-kappaB activation, inhibits osteoclastogenesis, and suppresses human breast cancer-induced bone loss in athymic nude mice. Cancer Res 2009 Feb 15;69(4):1477-84.