AUSTRALIAN JOURNAL OF BASIC AND APPLIED SCIENCES

ISSN:1991-8178 EISSN: 2309-8414
Journal home page: www.ajbasweb.com

Effects of Açaí and Ginger in Senile Rats with Experimental Periodontitis. Histological and Biochemical Study

[1]Azza S. Koura, [2]Heba A. Shawky, [3]Nevien M. Ahmed

[1]*Associate Professor of Oral Biology, Faculty of Dentistry, Alexandria University, Egypt.*
[2]*Lecturer of Oral Medicine and Periodontology, Faculty of Dentistry, Pharos University in Alexandria, Egypt.*
[3]*Lecturer of Biochemistry,Faculty of Dentistry, Pharos University in Alexandria, Egypt.*

Address For Correspondence:
Heba A. Shawky,Lecturer of Oral Medicine and Periodontology, Faculty of Dentistry, Pharos University in Alexandria, Egypt.
E-mail: shawky_heba@yahoo.com

ARTICLE INFO

Article history:
Received 26 July 2016
Accepted 21 September 2016
Published 30 September 2016

Keywords:
Açai, aging, ginger, inflammatory mediators, oxidative stress, periodontitis.

ABSTRACT

Background: Aging process is associated with an increased prevalence of periodontal disease. Different herbal supplements possess antimicrobial, anti-inflammatory and antioxidative properties for controlling the process of aging as well as for treatment of periodontal diseases. The positive effects of ginger have been established in different studies. Likewise, açai berry has been proven to owe numerous health benefits. **Objective:** The current study was performed to investigate the protective effect of açai and ginger supplementation on senile rats with induced periodontitis. **Materials and Methods:** The study was performed on 40 Male albino Wistar rats with advanced age (24 months) where periodontitis was induced in 30 rats. The rat population wasdivided into four groups: control group, periodontitis group, ginger treated and açai treated groups. Histological examination and biochemical procedures for analyzing inflammatory mediators and oxidative stress were performed after 30 days of treatment.**Results:** Periodontitis resulted in a significant increase in inflammatory mediators as well as lipid peroxidation (MDA) with a significant decrease in antioxidant enzymes. Administration of ginger or acai was associated with significant reduction in levels of inflammatory mediators and MDA and a significant increase in antioxidant enzymes. Histological examination proved that ginger exhibited good response regarding the cellularity and PDL fibers organization.Açai showed moderate degree of mineralization of alveolar bone, variable sizes of bone marrow spaces and numerous osteocytes. **Conclusion:** Our data demonstrated protective effect of both ginger and açai berry concerning the oxidative stress and inflammatory mediators. Histological observations showed better progress towards normal tissue in açai group than those of ginger group.Hence,açaí may be considered as a promising supplement in periodontal therapy as well as an anti-aging compound.

INTRODUCTION

Periodontitis is a multifactorial infectious disease caused by the interaction between specific invasive oral pathogens that colonize dental plaque biofilms on tooth surface and host immune response(Ji *et al*., 2015; Amro *et al*., 2016). Several risk factors may affect the rate of disease progression though, they are not considered as a causative factor of the disease. Risk factors can either by modifiable which include environmental or behavioral risk factors (e.g. smoking, poor nutrition, alcohol consumption, diabetes and stress) or non-modifiable risk factors as host response and genetic factors(Van Dyke and Dave, 2005). Aging is usually accompanied with increased prevalence of periodontal diseases. With increasing age, thinning and diminished keratinization of the gingival epithelium have been detected. A decrease in cellularity of the gingival connective tissue and an increase in the amount of intercellular substance was also denoted. Besides, the rate of collagen synthesis and

ToCite ThisArticle: Azza S. Koura, Heba A. Shawky, Nevien M. Ahmed., Effects of Açaí and Ginger in Senile Rats with Experimental Periodontitis. Histological and Biochemical Study. ***Aust. J. Basic & Appl. Sci.,*** *10(14): 10-19, 2016*

oxygen consumption was shown to be reduced by age(Andreescu *et al*., 2013). Regarding the periodontal ligament, the principle fibers become thickened showing wavy and broad bundles with a reduction in cell density and organic matrix production(Benatti *et al*., 2008). Also, in advanced age the cementum increases in width and the process of cementum formation becomes predominantly acellular(Foster *et al*., 2007). The most noticeable changes are seen in the alveolar bone. Senile atrophy, loss of bone mineral density, reduction in vascularity and in number of bone trabeculea, thinning of cortical plates, thickening of collagen fibers and a decrease in the bone formation have all been noted with advanced age (Benatti *et al*., 2006; Misawa-Kageyama *et al*., 2007; Leong *et al*., 2012). The amount of periodontal destruction during periodontitis increases by age. This can be attributed to the longer periods of exposure to different destructive process as plaque biofilm, chronic mechanical trauma or even iatrogenic damage (Renvert *et al*., 2013). Previous studies(Ebersole *et al*., 2008; Liang *et al*., 2010) demonstrated a greater inflammatory response in older age group subjects. The increased response was correlated to the differences in T and B cells as well as the levels of cytokines (Gomez *et al*., 2008; Pawelec and Muller, 2013; Malikabood *et al*., 2016).

Both inflammation and oxidative stress are increased with advanced age. There is evidence that inflammation may cause an increase in the production of free radicals leading to enhanced oxidative stress(George, 2014). Also, the severity of periodontal disease is affected by age. Periodontitis in the elderly subjects is associated with increased systemic inflammatory markers as C-reactive protein, interleukin-6, and tumor necrosis factor-alpha (Bretz *et al*., 2005). In addition, (Chapple and Matthews, 2007) found a large evidence for the destruction of periodontal tissue by reactive oxygen species (ROS).

Different botanicals were used in treatment of periodontitis and as anti-aging agents.Ginger (Zingiberofficinale Roscoe) belongs to the family Zingiberaceae. It originated in South-East Asia and has been used in traditional botanical medicine(Nicoll and Henein, 2009). Ginger constitutes of volatile and non-volatile oils. Volatiles oils include sesquiterpene and monoterpenoid hydrocarbons while non-volatiles include gingerols, shogaols, paradols, and zingerone(Jolad *et al*., 2004). Ginger constituents possess various pharmacological effects as anti-platelets, anti-tumor and anti-hepatotoxic (Lantz *et al*., 2007; El-Sharaky *et al*., 2009).Accordingly,(Dugasani *et al*., 2010)demonstrated that [6]-gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol revealed substantial scavenging activities against superoxide radical and hydroxyl radical and that the free radical scavenging activity could be enhanced with increasing concentration of these Gingerol compounds. Owing to its potent antioxidant activity, (Mahmoud and Hegazy, 2016)concluded that ginger appeared to be a promising anti-aging agent. In addition, (Park *et al*., 2008) demonstrated that the ginger extracts displayed antibacterial activities against virulent types of periodontal pathogens as *Porphyromonasgingivalis* and *Prevotellaintermedia* and that [10]-gingerol and [12]-gingerol effectively inhibited the growth of these bacteria.

Açaiberry (*Euterpeoleracea*) is a fruit found in the Amazon River area in South America. It contains high levels of phytochemicals, particularly, anthocyanins, proanthocyanidins and other flavonoids(Schauss *et al*., 2006). Açai pulp possessed antioxidant, anti-inflammatory and anti-carcinogenic properties(Poulose *et al*., 2012; Stoner *et al*., 2010; Sun *et al*., 2010).The antioxidant capacity of açaí was found to be excellent against peroxyl radicals and good against peroxynitrite (Lichtenthäler *et al*., 2005). Thus, (Laslo *et al*., 2013) determined that açai supplementation was effective in promoting healthy aging in oxidative stressed animals by reducing the protein levels of genes involved in oxidative stress response and cellular growth.

The aim of this study is to investigate the effects of acai and ginger supplementation in treatment ofperiodontitis induced in senile rats.

MATERIALS AND METHODS

This study was carried out on 40 Male albino Wistar rats aging 24 months, their body weight ranged between 300 – 350 gm. Animals were obtained from the animal house of Medical Research Institute, Alexandria University. During the study the animals were kept in polypropylene cages, 10 rats eachwith ad libitum access to water and normal diet. The roomtemperature was about 22-24°C and the animals wereexposed to 12:12 hours light dark cycles.

Induction of periodontitis:

30 rats were anesthetized with an intra-peritoneal injection of ketamine and xylazine (90 and 15 mg/kg, respectively). A sterile silk ligature (4/0) was placed around the cervices of mandibular molars in both sides. The ligatures were knotted on the vestibular side(Brito *et al*., 2013). These ligatures acted as a nidus for plaque accumulation to initiateperiodontal disease. After 14 days, the ligatures were removed and the whole rat population was randomly assigned into four groups:

Group I (control group):
10 normal rats

Group II (periodontitis group):
10 rats with ligature induced periodontitis

Group III (ginger group):
10 rats with ligature induced periodontitis receiving one mL of ginger (100mg/kg body weight) (Habib *et al*., 2008) daily via oro-gastric tube for 30 days.

Group IV (açaí group):
10 rats with ligature induced periodontitis receiving one mL of açaí (100mg/kg body weight)(Zapata-Sudo *et al*., 2014) daily via oro-gastric tube for 30 days.

Histological Examination:
The animals were sacrificedunder ether anesthesia;mandibles specimens were collected, cut into two halves,fixed in 10% bufferedformalin and demineralized in 5% nitric acid. Followingthese treatments, the specimens were dehydrated and embedded inparaffin.Thesections were stained with hematoxylin and eosin (H&E) and withTrichrometo be evaluated by light microscopy(Bashkar, 1990).

Biochemical Analysis:
Blood samples were collected and gingival tissues from the rats were minced and homogenized in appropriate buffer and then centrifuged according to the instructions of the biochemical assay. The supernatant was stored at -80°C till further analysis of various measurements.

Cytokines assays:
The levels of Interleukin-6 (IL-6), Interleukin-1 beta (IL-1β), Interleukin-10 (IL-10), and Tumor necrosis factor-alpha (TNF-α) in the gingival samples were determined by specific enzyme-linked immunosorbent assay (ELISA) techniques according to the manufacturer's instructions. All of the samples and standards were assayed in duplicate. Serum C-reactive protein (CRP) concentration was measured by ELISA Kit. All ELISA kits were purchased from (eBioscience, USA).

Oxidative stress assays:
Malondialdehyde (MDA) resulting from lipid peroxidation was quantified in the tissue homogenates after their reaction with thiobarbituric acid in acid medium, according to the method described by (Bird and Draper, 1984).

Superoxide dismutase (SOD) activities were assayed in the tissue homogenates by the method of (Misra and Fridovich, 1972)at 480 nm for 4 min on a Hitachi U-2000 spectrophotometer. Catalase (CAT) activity was estimated by using the methodof (Aebi, 1984). Glutathione (GSH) levels in gingival tissue were measured as a marker for antioxidant activity, GSH method is based on the reduction of 5,5dithiobis(2-nitrobenzoic acid) (DTNB) with GSH to produce a yellow compound. The reduced chromogen is directly proportional to GSH concentration and its absorbance can be measured at 405 nm by using a commercial kit (Biodiagnostic, Egypt).

All of the enzyme activities were expressed in µmol/mg or U/mg of protein and the tissue protein was estimated according to the method of (Lowry *et al*., 1951).

Statistical analysis:
All the statisticaldata were processed using statisticalprogram SPSSversion 20.0. Values of the measured parameters were expressed as meanvalue ± SD and the difference between the studied groups was determined using F –test.Significance was considered at p values <0.05.

Results:
Inflammatory mediators:
Our results showed that levels of CRP, IL-6, IL-1B, IL-10 and TNF-α were all significantly elevated ($p<0.001$) in the periodontitis group compared with the control group. In contrast, treatment with ginger or açaí significantly decreasedCRP, IL-6, IL-1B, IL-10 and TNF-α compared with the periodontitis group. The reduction in all inflammatory cytokines was more pronounced in acai group compared with ginger group with significant difference concerning IL-1B, IL-10 and TNF-α (Table 1).

Oxidative stress:

A significant elevation in MDA level was observed in periodontitis group witha significant decrease in Catalase, SOD and GSH levels in the same group compared with the control group. Ginger and açaí groups showed significant improvement concerning both MDA and antioxidant enzymes. Comparing the effect of ginger and açaí, the level of MDA in acai group was significantly lower than that of ginger group. Regarding the antioxidant enzymes, higher levels were detected in acai group with significant difference in GSHlevels(Table 2).

Table 1:Comparison between all studied groups according to inflammatory mediators.

	Control (n = 10)	Periodontitis (n = 10)	Ginger (n = 10)	Açaí (n = 10)	p
CRP (mg/dL)	16.40 ± 2.66	29.96[a] ± 3.38	22.44[ab] ± 4.83	19.36[b] ± 2.57	<0.001*
IL-6 (pg/mL)	1.62 ± 0.27	5.79[a] ± 0.76	3.08[ab] ± 0.41	2.61[ab] ± 0.34	<0.001*
IL-1B (pg/mL)	21.93 ± 1.73	84.88[a] ± 5.60	54.65[ab] ± 11.07	42.29[abc] ± 7.21	<0.001*
IL-10 pg/mg protein	4.66 ± 0.56	14.66[a] ± 0.63	10.23[ab] ± 0.72	9.17[abc] ± 0.83	<0.001*
TNF-αpg/mg protein	13.29 ± 0.72	31.71[a] ± 2.62	22.38[ab] ± 2.21	18.51[abc] ± 3.14	<0.001*

Normally distributed data was expressed in mean ± SD and was compared using F test (ANOVA)
*: Statistically significant at $p \leq 0.05$
a: Significant with control, b: Significant with periodontitis, c: Significant with ginger

Table 2:Comparison between all studied groups according to oxidative stress

	Control (n = 10)	Periodontitis (n = 10)	Ginger (n = 10)	Açaí (n = 10)	p
MDA (nmoL/g tissue)	21.14 ± 0.92	72.95[a] ± 8.09	39.82[ab] ± 7.31	29.53[abc] ± 3.78	<0.001*
Catalase (µmoL/mg/protein/min)	641.5 ± 32.70	479.0[a] ± 27.05	523.6[ab] ± 29.97	554.2[ab] ± 37.44	<0.001*
SOD U/mg protein/min	13.89 ± 0.84	9.53[a] ± 0.60	10.96[ab] ± 1.21	11.88[ab] ± 0.83	<0.001*
GSH (µmoL/g tissue)	102.1 ± 8.77	62.48[a] ± 8.61	77.04[ab] ± 11.40	90.90[bc] ± 9.72	<0.001*

Normally distributed data was expressed in mean ± SD and was compared using F test (ANOVA)
*: Statistically significant at $p \leq 0.05$
a: Significant with control, b: Significant with periodontitis, c: Significant with ginger

Histological Finding:

Histological examination of sections stained with H&E and Trichromerevealed:

Group I (control group):

The alveolar bone of control group showed smooth boundary facing periodontal ligament (PDL). Also bone marrow spaces were variable in size (Fig. 1). Thick PDL fibers withminimalcellularityandnormal arrangement were observed. The alveolar bone was well mineralized (Fig. 2).

Group II (Periodontitis group):

The apical part of the root showed hypercementosis with disorganized PDL fibers. Some areas of the root surface appeared degenerated. Several reversal lines were observed in the alveolar bony socket (Fig. 3). Osteoclast cells were seen in theirhowshipslacunae,the PDL fibers were degenerated in certain areas and root cementum appeared with resorbed areas (Fig. 4).

Group III (Ginger group):

PDL fibers appeared moderately reorganized. Immature thin alveolar bonetrabeculae with wide marrow spaces were observed. Minute areas of root resorption were also seen (Fig. 5). The apical root region revealed hypercementosis with reorganized PDL fibers. New alveolar bone trabeculae with resting lines and numerous osteocytes were observed (Fig. 6).

Group IV (Açaí group):

The newly formed interdental bone appeared with numerous osteocytes in their lacunae and osteoblasts were seen as a continuous layer lining bonetrabeculae. The PDL fibers were well organized (Fig.7).Several resting lines were seen in the newly formed alveolar bone with prominent haversian system formation. Continuous layer of cementoblastswas seenon root surface (Fig.8). The alveolar bone showed smooth boundary facing PDL (Fig. 9). Also, it appeared with proper degree of mineralization together with marrow spaces of normal cellularity (Fig. 10).

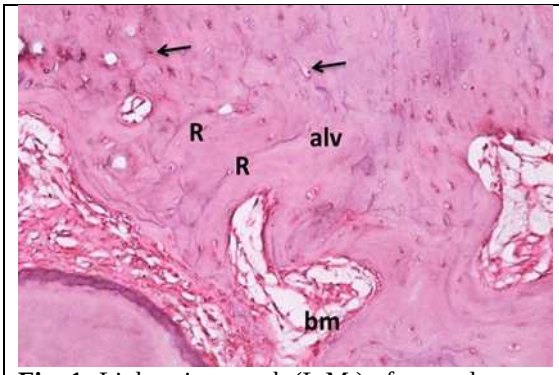

Fig. 1: Light micrograph (L.M.) of control group showing well-formed alveolar bone (**alv**) with relative smooth boundary facing the PDL, numerous ostoecytes (arrows) and several resting lines are seen (**R**). Moderately distributed Bone marrow spaces (**bm**) are observed (H&E) x 200.

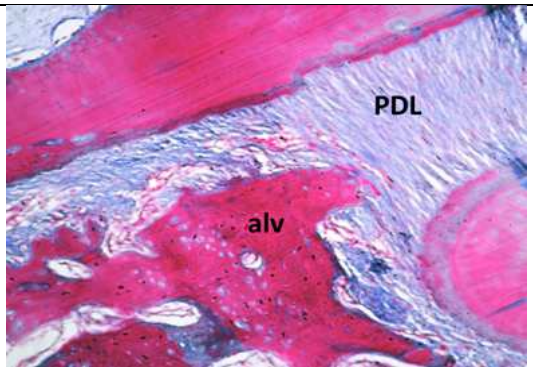

Fig. 2: L.M. of control group showing thick PDL fibers with minimal cellularity. Note well mineralized alveolar bony socket (**alv**) (Trichrome) x 200.

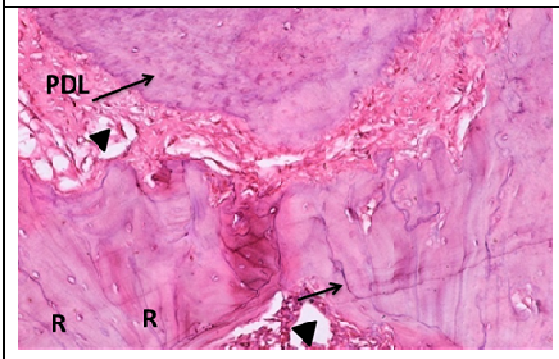

Fig. 3: L.M. of periodontitis group showing heypercementosis at the apical part of the root (arrows). Disorganized PDL fibers with areas of degeneration (arrows heads). The alveolar bony socket reveals several reversal lines (**R**) (H&E) x 400.

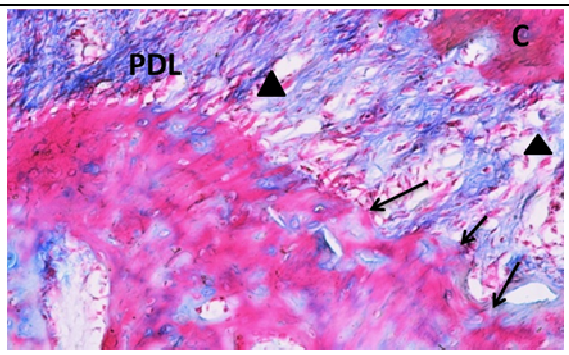

Fig. 4: L.M. of periodontitis group showing alveolar bony socket with osteoclast cells in howship's lacunae (arrows) with degenerated areas in PDL (arrows head). The root cementum appears with resorbed areas (**C**) (Trichrome) x 400.

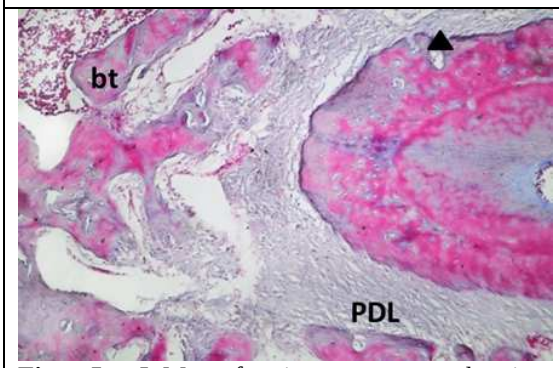

Fig. 5: L.M. of ginger group showing moderately reorganized PDL fibers. Thin immature alveolar bone trabeculae (**bt**) and wide marrow spaces. Minute areas of root resorption could be seen (arrow head) (Trichrome) x 200.

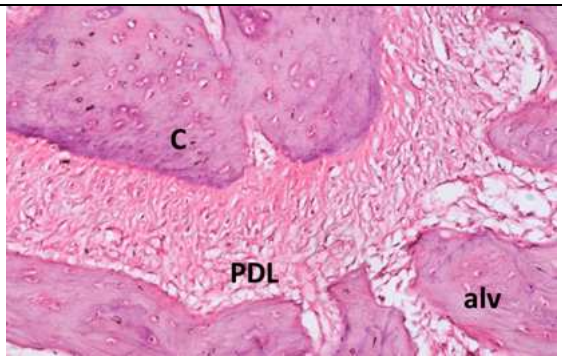

Fig. 6:L.M. of ginger group showing hypercementosis (**C**) at the apical region with reorganized PDL fibers. Newly formed alveolar bone with resting and reversal lines with numerous osteocytes (H&E) x 400.

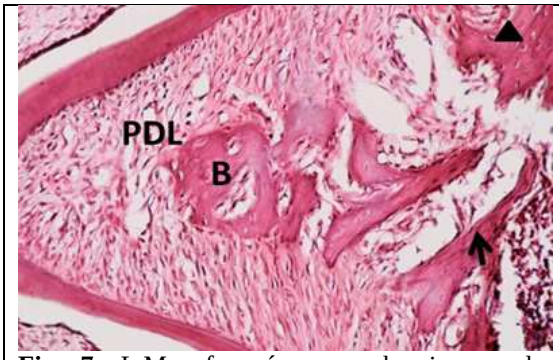

Fig. 7: L.M. of açaí group showing newly formed interdental bone (**B**) with osteoblasts lining bony trabeculae (arrow). Numerous osteocytes could be seen in their lacunae (arrow head). The PDL fibers are well organized (H&E) x 200.

Fig. 8: L.M. of açaí group showing reorganized apical fibers of PDL. New alveolar bone formation with several resting lines (**R**). Haversian system is seen in the alveolar bony socket (arrows) (H&E) x 400.

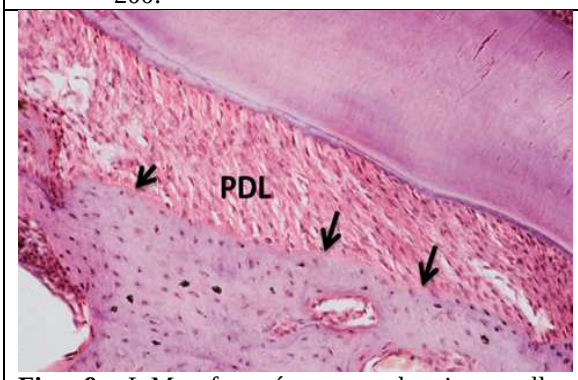

Fig. 9: L.M of açaí group showing well reorganized PDL fibers with high cellularity. Well-formed alveolar bone with relative smooth boundary facing PDL is seen (arrows) (H&E) x 200.

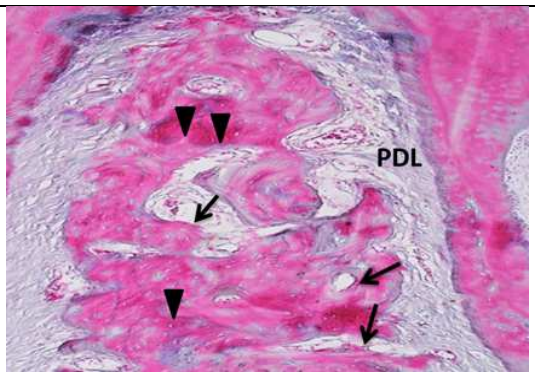

Fig. 10: L.M. of açaí group showing inter-radicular bone with proper degree of mineralization. Variable sizes of bone marrow spaces with normal cellularity can be observed (arrows). Resting lines (arrows head) can be seen. PDL fibers are well organized (Trichrome) x 200.

Discussion:

Aging is usually associated with alteration in the periodontitum. These histopathologic and clinical periodontal changes should be distinguished from pathological condition(Huttner *et al*., 2009). It was suggested that with increasing age, periodontal inflammation tends to develop at a higher rate in response to the prolonged period of plaque accumulation, and also that wound healing occurs at a slower rate (Farias Gomes *et al*., 2010).

The current study was performed on senile rats aging 24 months. Results revealed a significant increase in the levels of all examined inflammatory mediators (CRP, IL-6, IL-1β, IL-10 and TNF-α) in periodontitis group compared withcontrol group. These results were in convenience with those of (Liang *et al*., 2010) who reported that old mice displayed significant increase in periodontal bone loss, accompanied by elevated expression of pro-inflammatory cytokines (IL-1β, TNF-α, and IL-17A). In accordance, various studies(Persson *et al*., 2005; Salzberg *et al*., 2006; Gomes-Filho *et al*., 2011) have demonstrated the positive association between periodontitis and high serum CRP levels and between CRP and advanced age groups(Woloshin and Schwartz, 2005). CRP is a pattern recognition molecule; an extremely sensitive non-specific acute-phase marker for inflammation, and is regulated by cytokines like IL- 6, IL-1βand TNF-α(Ebersole and Cappelli, 2000).

In the present study we compared the effects of ginger and açaí on senile rats with experimental periodontitis. Results showed that both ginger and açaí caused a significant decrease in all examined inflammatory mediators. These results agree with (Mahluji *et al*., 2013)who demonstrated that ginger offers a high anti-inflammatory activity through its inhibition to IL-6, TNF-α and CRP. Also, our results are in accordance with (Qu *et al*., 2014) who proved that acai effectively inhibited the expression of TNF-α and IL-6. In our study, the decrease in levels of inflammatory mediators was more prevalent in the açaí group compared to the ginger group with a significant difference regarding IL-1B, IL-10 and TNF-α. These results pointed to the superior effect of açaí over ginger. Moreover, (Xie *et al*., 2012) reported that açaí exhibit a strong anti-

inflammatory activity by potent inhibition of nuclear factor-kappa B *(*NF-κB)activation and MAPK pathway which in turn results in inhibition of the expression of various types of pro-inflammatory cytokines.

Concerning oxidative stress, in the current study, the level of MDA was significantly higher in periodontitis group than in control group. While antioxidant enzyme (catalase, SOD and GSH) showed a significant decrease in periodontitis group which emphasized that periodontitis elevated the oxidative stress. These findings agree with previous studies (Akalin *et al*., 2007; Wei *et al*., 2010; Masi *et al*., 2011)that showed an increase in levels of oxidative stress in serum, saliva, or gingival crevicular fluid in patients with periodontitis compared with control group.

On the other hand,both ginger and acai showed a significant decrease in MDA and an increase in antioxidant enzymes which agrees with(Rotimi *et al*., 2015)who suggested that ginger treatment exerts a therapeutic protective effect in rats bypromoting the antioxidant defense systems. Accordingly, (Young *et al*, 2005)suggested that the effective role of ginger was due to 6-gingerol, tannins, and other polyphenolic compounds.Comparing the action of açaí and ginger, the present results showed that açaí treatment decreased MDA and increased antioxidant enzymes more than ginger. Consequently, (Alexander *et al*., 2006) reported that açaí exhibited high antioxidant capacity in vitro, especially for superoxide ($O_2^{\cdot-}$) and peroxyl scavenging. Moreover, (Schreckinger *et al*., 2010) reported that theaçaí berry contain high levels of antioxidants as vitamins (A and E) and numerous polyphenols such as anthocyanins, procyanidin oligomers, vanillic acid, syringic acid, para-hydroxybenzoic acid (*p*-hydroxybenzoic acid), protocatechuic acid and ferulic acid.

The rat periodontal model was used in our study as various studies found that there is a lot of similarity between rats and humans in the development and resolution of inflammation as well as the gingival wound healing(Oz and Puleo, 2011; Jacob and Nath, 2013). In this study periodontitis group showed disturbance in PDL fibers arrangement and areas of bone resorption along the surface of the alveolar bone trabeculae with inflammatory cell infiltration. Histological examination revealed that both ginger and açaí treatment groups exhibited a nearly smooth boundary surface of alveolar bone facing reorganized PDL fibers with increased osteoblastic activity in spite of the rats senility.

Ginger group showed good response regarding the organization of PDL fibers with increased cellularity. These findings agree with (Ezzat and Fares, 2014) who concluded that systemic administration of ginger resulted in improvement in the histopathology of gingiva and PDL compared with the periodontitis group. The efficacy of ginger in treatment of periodontal disease was demonstrated in different studies(Park *et al*., 2008; Chatterjee *et al*., 2011). On the other hand, rats treated with açaí showed larger bone masses with proper degree of mineralization and variable sizes of bone marrow spaces with numerous osteocytes. Haversian systems and resting lines were obviously seen. PDL fibers were well organized. These observations showed better progress towards normal tissuein the acai than in the ginger group.

Conclusion:

From the current results we concluded that both ginger and açaí were effective in treatment of periodontitis. Acai group exhibited better progress towards normal tissue along with improved biochemical results. Thus,açaí may be considered as a promising supplement in periodontal therapy as well as an anti-aging compound. Future studies concerning the impact of açaí on periodontal disease are recommended since according to the authors' knowledge, no studies have been implemented on that point.

REFERENCES

Aebi, H., 1984. Catalase in vitro. Methods in Enzymology, 105:121-126.

Akalin, F.A., E. Baltacio-glu, A. Alver and E. Karabulut, 2007. Lipid peroxidation levels and total oxidant status in serum, saliva and gingival crevicular fluid in patients with chronic periodontitis. Journal of Clinical Periodontology, 34(7):558-565.

Alexander, G.S., W. Xianli, B. Prior, H. Dejian and O. John, 2006. Antioxidant capacity and other bioactivities of the freeze-dried Amazonian palm berry, euterpeoleraceae mart. (acai). Journal of Agricultural and Food Chemistry, 54(22): 8604-8610.

Amro, S., H. Othman and A. Koura, 2016.Scanning Electron Microscopy and Energy-Dispersive X-ray Analysis of Root Cementum in Patients with Rapidly Progressive Periodontitis.Australian Journal of Basic and Applied Sciences, 10(10): 16-26.

Andreescu, C.F., L.L. Mihai, M. Răescu, M.J. Tuculină, C.N. Cumpătă and D.L. Ghergic, 2013. Age influence on periodontal tissues: a histological study. Romanian journal of morphology and embryology, 54(3 Suppl):811-815.

Bashkar, S.N., 1990. Orban's Oral Histology and Embryology: 11th Ed. Baltimore, Boston, Chikago, London, Piladephia, Sydney, Toronto: Mosby st. Louis, pp: 339-341, 349-350, 365, 470-473.

Benatti, B.B., J.B. Neto, M.Z. Casati, E.A. Sallum, A.W. Sallumand F.H. Nociti, 2006. Periodontal healing may be affected by aging: a histologic study in rats. Journal of Periodontal Research, 41(4):329-333.

Benatti, B.B., K.G. Silvério, M.Z. Casati, E.A. Salloum and F.H. Nociti, 2008.Influence of aging on biological properties of periodontal ligament cells. Connective tissue research, 49(6):401-408.

Bird, R.P. and A.H. Draper, 1984.Comparative studies on different methods of malondyaldehyde determination. Methods in Enzymology, 90:105-10.

Bretz, W.A., R.J. Weyant, P.M. Corby, D. Ren, L. Weissfeld, S.B. Kritchevsky, T. Harris, M. Kurella, S. Satterfield, M. Visser and A.B. Newman, 2005. Systemic inflammatory markers, periodontal diseases, and periodontal infections in an elderly population. Journal of the American Geriatrics Society, 53(9):1532-1537.

Brito, LC., S. Dalbó, T.M. Striechen, L.C. Brito, S. DalBó, T.M. Striechen, J.M. Farias, L.R. Olchanheski, R.T. Mendes, J.C. Vellosa, G.M. Fávero, R. Sordi, J. Assreuy, F.A. Santos and D. Fernan, 2013. Experimental periodontitis promotes transient vascular inflammation and endothelial dysfunction. Archives of Oral Biology, 58(9):1187-1198.

Chapple, I.L. and J.B. Matthews, 2007.The role of reactive oxygen and antioxidant species in periodontal tissue destruction. Periodontology, 43:160-232.

Chatterjee, A., H. Bhattacharya and A. Kandwal, 2011.Probiotics in periodontal health and disease. Indian Society of Periodontology, 15(1): 23-28.

Dugasani, S., M.R. Pichika, V.D. Nadarajah, M.K. Balijepalli, S. Tandra and J.N. Korlakunta, 2010. Comparative antioxidant and anti-inflammatory effects of [6]-gingerol, [8]-gingerol, [10]-gingerol and [6]-shogaol. Journal of Ethnopharmacology, 127(2):515-520.

Ebersole, J.L. and D. Cappelli, 2000.Acute-phase reactants in infections and inflammatory diseases.Periodontology, 23:19-49.

Ebersole, J.L., M.J. Steffen, J. Gonzalez-Martinez and M.J. Novak,2008.Age and oral disease effects on systemic inflammatory and immune parameters in nonhuman primates. Clinical and Vaccine Immunology, 15(7):1067-1075.

El-Sharaky, A.S., M.A. Newairy, S.M. Kamel and S.M. Eweda, 2009. Protective effect of ginger extractagainstbromobenzene-induced hepatotoxicityin male rats. Food and Chemical Toxicology, 47(7): 1584-1590.

Ezzat, B. and A.E. Fares, 2014. The effect of orally administered curcumin and /or ginger on escherichia coli induced periodontitis in rats (histopathological, immunohistochemical, and stereomicroscopic study). Egyptian Dental Journal, 60(2): 1465-1477.

Farias Gomes, S.G., C.B. Meloto, W. Custodio and C.M. Rizzatti-Barbosa, 2010.Aging and the periodontium. The Brazilian Journal of Oral Sciences, 9(1):1-6.

Foster, B.L., T.E. Popowics, H.K. Fong and M.J. Somerman, 2007. Advances in defining regulators of cementum development and periodontal regeneration. Current Topics in Developmental Biology, 78:47-126.

George, H.,2014. Aging and its Impact on Innate Immunity and Inflammation: Implications for Periodontitis. The Journal of Oral Biosciences, 56(1): 30-37.

Gomes-Filho, I.S., J.M. Freitas Coelho, S.S. da Cruz and J.S. Passos, C.O. Teixeira de Freitas, N.S. AragãoFarias, R. Amorim da Silva, M.N. Silva Pereira, T.L. Lima, M.L. Barreto, 2011. Chronic periodontitis and C-reactive protein levels. Journal of Periodontology, 82(7):969-978.

Gomez, C.R., V. Nomellini, D.E. Faunce and E.J. Kovacs,2008. Innate immunity and aging. ExperimentalGerontology, 43(8):718-728.

Habib, S.H., S. Makpol, N.A. Abdul-Hamid, S. Das, W.Z. Ngah and Y.A. Yusof, 2008. Ginger Extract (ZingiberOfficinale) has Anti-Cancer and Anti-Inflammatory Effects on Ethionine-Induced Hepatoma Rats. Clinics, 63(6):807-813.

Huttner, E.A., D.C. Machado, R.B. de Oliveira, A.G. Antunes and E. Hebling, 2009.Effects of human aging on periodontal tissues. Special Care in Dentistry, 29(4):149-155.

Jacob, S.P. and S. Nath, 2013. Rat gingival model for testing drugs influencing inflammation. International e-Journal of Science, Medicine & Education, 7(2): 8-16.

Ji, S., Y.S. Choi and Y. Choi, 2015.Bacterial invasion and persistence: critical events in the pathogenesis of periodontitis? Journal of Periodontal Research, 50(5): 570-585.

Jolad, S.D., R.C. Lantz, A.M. Solyom, G.J. Chen, R.B. Bates and B.N. Timmermann, 2004. Fresh organically grown ginger (Zingiberofficinale): Composition and effects on LPS-induced PGE2 production. Phytochemistry Journal, 65(13):1937-1954.

Lantz, R.C., G.J. Chen, M. Sarihan, A.M. Solyom, S.D. Jolad and B.N. Timmermann, 2007.The effect of extracts from ginger rhizome on inflammatory mediator production.Phytomedicine, 14(2-3): 123-128.

Laslo, M., X. Sun, C.T. Hsiao, W.W. Wu, R.F. Shen and S. Zou, 2013. A botanical containing freeze dried açai pulp promotes healthy aging and reduces oxidative damage in sod1 knockdown flies. American Aging Association, 35(4):1117-11132.

Leong, N.L., J.M. Hurng, S.I. Djomehri, S.A. Gansky, M.I. Ryder and S.P. Ho, 2012. Age-related adaptation of bone-PDL-tooth complex: Rattus-Norvegicus as a model system. PLoS One, 7(4):e35980.

Liang, S., K.B. Hosur, H. Domonand, G. Hajishengallis, 2010. Periodontal inflammation and bone loss in aged mice. Journal of periodontalresearch,45(4):574-578.

Lichtenthäler, R., R.B. Rodrigues, J.G. Maia, M. Papagiannopoulos, H. Fabricius and F. Marx, 2005.Total oxidant scavenging capacities of Euterpeoleracea Mart. (Açaí) fruits. International Journal of Food Sciences and Nutrition, 56(1):53-64.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951.Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry, 193(1): 265-275.

Mahluji, S., A. Ostadrahimi, M. Mobasseri, V. EbrahimzadeAttari and L. Payahoo, 2013. Anti-Inflammatory Effects of *ZingiberOfficinale* in Type 2 Diabetic Patients. Advanced Pharmaceutical Bulletin,3(2): 273-276.

Mahmoud, Y.I. and H.G. Hegazy, 2016. Ginger and alpha lipoic acid ameliorate age-related ultra-structural changes in rat liver. Biotechnic&Histochemistry, 91(2):86-95.

Malikabood, F., R.J. Alwarid and L.J. Witwit, 2016.Estimation of Some Immunological Parameters amongChronic Periodontitis Patients with Cardiovascular Disease.Australian Journal of Basic and Applied Sciences,10(10): 27-30.

Masi, S., K.D. Salpea, K. Li, M. Parkar, L. Nibali, N. Donos, K. Patel, S. Taddei, J.E. Deanfield, F. D'Aiuto and S.E. Humphries, 2011. Oxidative stress, chronic inflammation, and telomere length in patients with periodontitis. Free Radical Biology and Medicine, 50(6):730-735.

Misawa-Kageyama, Y., T. Kageyama, K. Moriyama. S. Kurihara, H. Yagasaki, T. Deguchi, H. Ozawa and N. Sahara, 2007.Histomorphometric study on the effects of age on orthodontic tooth movement and alveolar bone turnover in rats. European Journal Of Oral Sciences,115(2):124-130.

Misra, H.P. and I. Fridovich, 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase.The Journal of Biological Chemistry, 247:3170-3175.

Nicoll, R. and M.Y. Henein, 2009.Ginger (Zingiberofficinale Roscoe): A hot remedy for cardiovascular disease? The International Journal of Cardiology, 131(3):408-409.

Oz, H.S. and D.A. Puleo, 2011.Animal models for periodontal disease. Journal of Biomedicine and Biotechnology, pp: 754857.

Park, M., J. Bae and D.S. Lee, 2008. Antibacterial activity of [10]-gingerol and [12]-gingerol isolated from ginger rhizome against periodontal bacteria. Phytotherapy Research, 22(11):1446-1449.

Pawelec, G. and L. Muller, 2013.Introduction to Ageing of the Adaptive Immune System. In Immunosenescence, Ed. Bosch, J.A. New York: Springer, pp: 17-33.

Persson, G.R., T. Pettersson, O. Ohlsson and S. Renvert, 2005. High-sensitivity serum C-reactive protein levels in subjects with or without myocardial infarction or periodontitis.Journal of Clinical Periodontology, 32 (3):219-224.

Poulose, S.M., D.R. Fisher, J. Larson, D.F. Bielinski, A.M. Rimando, A.N. Carey, A.G. Schauss and B. Shukitt-Hale, 2012. Anthocyanin-rich acai (Euterpeoleracea Mart.) fruit pulp fractions attenuate inflammatory stress signaling in mouse brain BV-2 microglial cells. The Journal of Agricultural and Food Chemistry, 60(4):1084-1093.

Qu, S.S., J.J. Zhang, Y.X. Li, Y. Zheng, Y.L. Zhu and L.Y. Wang, 2014. Protective effect of Acai berries on chronic alcoholic hepatic injury in rats and their effect on inflammatory cytokines. ZhongguoZhong Yao ZaZhi, 39(24):4869- 4872.

Renvert, S., R.E. Persson and G.R. Persson, 2013. Tooth loss and periodontitis in older individuals: results from the Swedish National Study on Aging and Care. Journal of Periodontology, 84(8):1134-1144.

Rotimi, S., I. Adelani, A. Okafor, W. Iyanda-Joel and O. Rotimi, 2015.Effects of Ginger Juice Aflatoxin-Induced Oxidative Stress in Rats. The Federation of American Societies for Experimental Biology, 29(1):621-625.

Salzberg, T.N., B.T. Overstreet, J.D. Rogers, J.V. Califano, A.M. Best and H.A. Schenkein, 2006. C-reactive protein levels in patients with aggressive periodontitis. Journal of Periodontology, 77 (6):933-939.

Schauss, A.G., X. Wu, R.L. Prior, B. Ou, D. Patel, D. Huang, J.P. Kababick,2006. Phytochemical and nutrient composition of the freeze-dried amazonian palm berry, Euterpeoleraceae Mart. (acai). The Journal of Agricultural and Food Chemistry, 54(22):8598-8603.

Schreckinger, M.E., J. Lotton, M.A. Lila and E.G. de-Mejia, 2010. Berries from South America: a comprehensive review on chemistry, health potential, and commercialization. Journal of Medicinal Food, 13(2):233-246.

Stoner, G.D., L.S. Wang, C. Seguin, C. Rocha, K. Stoner, S. Chiu and A.D. Kinghorn, 2010.Multiple berry types prevent N-nitrosomethylbenzylamine-induced esophageal cancer in rats. Pharmaceutical Research, 27(6):1138-1145.

Sun, X., J. Seeberger, T. Alberico, C. Wang, C.T. Wheeler, A.G. Schauss and S. Zou, 2010. Açai palm fruit (Euterpeoleracea Mart.) pulp improves survival of flies on a high fat diet. Experimental Gerontology, 45(3):243-251.

Van Dyke, T.E. and S. Dave, 2005.Risk Factors for Periodontitis. Journal of the international academy of Periodontology, 7(1):3-7.

Wei, D., X.L. Zhang, Y.Z. Wang, C.X. Yang and G. Chen, 2010. Lipid peroxidation levels, total oxidant status and super oxide dismutase in serum, saliva and gingival crevicular fluid in chronic periodontitis patients before and after periodontal therapy. Australian Dental Journal, 55(1):70-78.

Woloshin, S. and L.M. Schwartz. 2005. Distribution of C-reactive protein values in the United States. The New England Journal of Medicine, 352(15):1611-1613.

Xie, C., J. Kang, Z. Li, A.G. Schauss, T.M. Badger, S. Nagarajan, T. Wu and X. Wu, 2012. The açaí flavonoid velutin is a potent anti-inflammatory agent: blockade of LPS-mediated TNF-α and IL-6 production through inhibiting NF-κB activation and MAPK pathway.Journal of Nutritional Biochemistry,23(9):1184-1191.

Young, H.Y., Y.L. Luo, H.Y. Cheng, W.C. Hsieh, J.C. Liao and W.H. Peng, 2005.Analgesic and anti-inflammatory activities of [6]-gingerol. Journal of Ethnopharmacology, 96(1-2):207-210.

Zapata-Sudo, G., J.S. da Silva, S.L. Pereira, P.J. Souza de Moura and R.T. Sudo, 2014.Oral treatment with *Euterpeoleracea* Mart. (açaí) extract improves cardiac dysfunction and exercise intolerance in rats subjected to myocardial infarction. BMC Complementary and Alternative Medicine,14: 227-232.