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

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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 was divided 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 açai 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, açai 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 be modifyable 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...
The ligatures were knotted on the vestibular side to initiate periodontal disease. After 14 days, the ligatures were removed and the whole rat population was randomly assigned into four groups: of periodontitis induced in senile rats.

Induction of periodontitis:

University. During the study the animals were kept in polypropylene cages, 10 rats each with ad libitum access to water and normal diet. The room temperature was about 22-24°C and the animals were exposed to 12:12 hours light dark cycles.

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. Ginger constitutes of volatile and non-volatile oils. Volatiles oils include sesquiterpene and monoterpenoid hydrocarbons while non-volatiles include gingerols, shogaols, paradols, and zingerone. Ginger constituents possess various pharmacological effects as anti-platelets, anti-tumor and anti-hepatotoxic. Ginger constituents possess various pharmacological effects as anti-platelets, anti-tumor and anti-hepatotoxic. Accordingly, 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, demonstrated that ginger appeared to be a promising anti-aging agent. In addition, 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. The aim of this study is to investigate the effects of acai and ginger supplementation in treatment of periodontitis 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 each with ad libitum access to water and normal diet. The room temperature was about 22-24°C and the animals were exposed 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. These ligatures acted as a nidus for plaque accumulation to initiate periodontal 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 sacrificed under ether anesthesia; mandibles specimens were collected, cut into two halves, fixed in 10% buffered formalin and demineralized in 5% nitric acid. Following these treatments, the specimens were dehydrated and embedded in paraffin. These sections were stained with hematoxylin and eosin (H&E) and with Trichrometo 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 method of (Aebi, 1984). Glutathione (GSH) levels in gingival tissue were measured as a marker for antioxidant activity, GSH method is based on the reduction of 5,5-dithiobis(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 statistical data were processed using statistical program SPSSversion 20.0. Values of the measured parameters were expressed as mean value ± 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 decreased CRP, IL-6, IL-1B, IL-10 and TNF-α compared with the periodontitis group. The reduction in all inflammatory cytokines was more pronounced in açaí 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 with a 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 açaí group was significantly lower than that of ginger group. Regarding the antioxidant enzymes, higher levels were detected in açaí group with significant difference in GSH levels (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 ± 3.38                     | 22.44 ± 4.83                    | 19.36 ± 2.57                    | <0.001*  |
| IL-6 (pg/mL)                    | 1.62 ± 0.27                      | 5.79 ± 0.76                      | 3.08 ± 0.41                     | 2.61 ± 0.34                     | <0.001*  |
| IL-1B (pg/mL)                   | 21.93 ± 1.73                     | 84.88 ± 5.60                     | 54.65 ± 11.07                   | 42.29 ± 7.21                    | <0.001*  |
| IL-10 pg/mg protein             | 4.66 ± 0.56                      | 14.66 ± 0.63                     | 10.23 ± 0.72                    | 9.17 ± 0.83                     | <0.001*  |
| TNF-α pg/mg protein             | 13.29 ± 0.72                     | 31.71 ± 2.62                     | 22.38 ± 2.21                    | 18.51 ± 3.14                    | <0.001*  |

Normally distributed data was expressed in mean ± SD and was compared using F test (ANOVA)

*: Statistically significant at p ≤ 0.05

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

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 10)</th>
<th>Periodontitis (n = 10)</th>
<th>Ginger (n = 10)</th>
<th>Açaí (n = 10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/L tissue)</td>
<td>21.14 ± 0.92</td>
<td>72.95 ± 8.09</td>
<td>39.82 ± 7.31</td>
<td>29.53 ± 3.78</td>
<td>&lt;0.001*</td>
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<tr>
<td>Catalase (μmol/mg/protein/min)</td>
<td>641.5 ± 32.70</td>
<td>479.0 ± 27.05</td>
<td>523.6 ± 29.97</td>
<td>554.2 ± 37.44</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>SOD U/mg protein/min</td>
<td>13.89 ± 0.84</td>
<td>9.53 ± 0.60</td>
<td>10.96 ± 1.21</td>
<td>11.88 ± 0.83</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>GSH (μmol/L tissue)</td>
<td>102.1 ± 8.77</td>
<td>62.48 ± 8.61</td>
<td>77.04 ± 11.40</td>
<td>90.90 ± 9.72</td>
<td>&lt;0.001*</td>
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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 with minimal cellularity and normal 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 their howships lacunae, 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 bon trabeculae 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 bonetabeculae. 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 cementoblasts was seen on 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 osteocytes (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 hypercementosis 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.
In our study, the decrease in levels of inflammatory mediators was more prevalent in the ginger group compared with control group. These results were in coincidence 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-1β, and 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 açaí 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-1β, 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-

**Discussion:**

Aging is usually associated with alteration in the periodontium. 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 with control group. These results were in coincidence 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-1β, and TNF-α (Ebersole and Cappelli, 2000).

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inflammatory activity by potent inhibition of nuclear factor-kappa B (NF-kB) 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 açaí 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 by promoting 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$_2^-$) and peroxyl scavenging. Moreover, Schreckinger et al., 2010 reported that the açaí 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 tissue in the açaí 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**


