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ORAL OR INJECTABLE ALOE VERA? APPROACHES FOR TREATING GINGIVITIS ASSOCIATED WITH LIGATURE INDUCED PERIODONTITIS IN WISTAR RATS

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ABSTRACT

Periodontitis is a set of inflammatory diseases affecting the periodontium, and the tissues that surround and support the teeth. Mouthwash solutions are mainly used for their antiseptic properties. They currently include synthetic agents, or essential oils. Many natural extracts may also be used, these associate both antiseptic effects and action on host response, due to their antioxidant, immunoregulatory, and analgesic, buffering, or healing properties. The best known are avocado oil, manuka oil, propolis oil, grapefruit seed extract, pycnogenol, Aloe Vera, Q10 coenzyme, green tea, and megamin.

AIM: The aim of this study is to assess and compare between the Oral and systemic administration of Aloe Vera on gingivitis Associated with induced periodontits in male Wistar rat.

METHODS: Forty adult male Wistar rats with an average weight 150-250 g, 6–7 weeks old were will be assigned to the ligature, and divided randomly into four groups with 10 rats in each group: The first group (control) were daily intraperitoneally injected with saline (5mg/kg). The rats of the second group were given Aloe Vera extract intraperitoneally (300mg/kg) starting one day before ligature and continuing for one month. The rats in the third group were given saline (5mg/kg) orally, while rats in the fourth group were given Aloe Vera extract (300mg/kg) by oral gavage starting one day before ligature and continuing for one month. At the end of 30 days for all groups, all animals were sacrificed, the gingiva was dissected, processed, and a set of sections were stained with haematoxylin and eosin for detection of any morphological changes. The other two sets of sections were labeled for localization of PCNA and caspase-3. The epithelial thickness was measured, and the data were calculated, analyzed and computed to compare the two routes of administration on the induced periodontitis.

RESULTS: There was a high significant difference between all groups in their epithelial thickness measurements and high significant difference between the oral and control groups. The immunohistochemistry revealed that there was an overall high significant difference in the PCNA and caspase-3 area% between the studied groups. In addition there has been a high significant increase the PCNA reaction in the oral and intraperitoneal groups versus the control group, while in caspase-3 immunostaining there has been a statistically significant increase in the control group versus both the oral and the peritoneal groups.

Key words: Aloe Vera, Periodontitis, Gingivitis, immunohistochemistry.

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INTRODUCTION

Periodontitis is the inflammation of the gingiva, periodontal ligament and the supporting alveolar bone. It involves progressive loss of the alveolar bone around the teeth, and if left untreated, can lead to loosening and subsequent loss of teeth. Periodontitis is caused by microorganisms that adhere to, and grow on the tooth surfaces, along with an overly aggressive immune response against these organisms (Savage et al 2009).

In some people, gingivitis progresses to periodontitis with the destruction of gingival fibers, the gum tissues separate from the tooth and deepened sulcus, (periodontal pocket). Subgingival microorganism colonizes the periodontal pockets and cause further inflammation in the gum tissues and progressive bone loss. (Lalla et al 2007)

In the 1999 Armitage had classified periodontal diseases and conditions into seven major categories of which the last six are termed destructive periodontal disease because they are essentially irreversible. The seven categories are as follows: Gingivitis, Chronic periodontitis, Aggressive periodontitis, Periodontitis (as a manifestation of systemic disease), necrotizing ulcerative gingivitis/ periodontitis, abscesses of the periodontium and combined periodontic-endodontic lesions.

Inflammation of the periodontium may result from many causes (bacteria, trauma,..). However, most forms of gingivitis and periodontitis result from the accumulation of tooth adherent microorganisms. Prominent risk factors for development of chronic periodontitis include the presence of specific sub gingival bacteria, tobacco use, diabetes, age and male gender (Grossi et al 1994, 1995).

The non-specific plaque hypothesis estimated that there may be over 400 distinct species that can be found in the dental plaque, and when extensive efforts were made to classify the cultivable isolates, many were found to be previously undescribed species. In fact, most cultivable species were present in such low proportions that it was difficult to identify any single species as being uniquely associated with periodontal disease (Moore et al 1994).

Removal of microbic plaque and calculus is necessary to establish periodontal health. The first step in the treatment of periodontitis involves non-surgical cleaning below gum line with a procedure called scaling and debridement. (Beirne et al 2005)

Multiple clinical studies have shown that non-surgical scaling and root planning is usually successful if the periodontal pockets are shallower than 4-5 mm (Stambaugh et al 1981, Waerhaug 1978), if non-surgical therapy is found to have been unsuccessful in managing signs of disease activity, periodontal surgery may be needed to stop progressive bone loss and regenerate lost bone where possible. There are many surgical approaches used in treatment of advanced periodontitis, including open flap debridement, osseous surgery, as well as guided tissue regeneration and bone grafting (Kaldahl et al 1996, Hirschfiejd et al 1978).

Most alternative “at-home” gum desease treatments involve injecting anti-microbial solution, such as hydrogen peroxide, into periodontal pockets via slender applicators or oral irrigators. This process disrupts anaerobic microorganism colonies and is effective at reducing infections and inflammation when used daily (Cutler et al 2000).

Periodontitis can be treated in a noninvasive manner by means of periostat (subantimicrobial dose of doxycycline), an FDA- approved, orally-administered drug that has been shown to reduce bone loss. Its mechanism of action in part involves inhibition of Matrix metalloproteinases (such as collagenase), which degrade the extracellular matrix under inflammatory conditions. This ultimately can lead to reduction of alveolar bone-loss in patients with periodontal disease, as well as patients without periodontitis (Greenstein 1999).

The inability of the normal adult population to perform adequate tooth brushing has led to the search for chemotherapeutic agents in order to im-

prove plaque control (Nogueira et al 2000). Chemicals such as triclosan and chlorhexidine, have been used as mouth rinses or added to dentifrice to avoid plaque formation and development of gingivitis (Moran et al 2001).

The demand for using natural products in the prevention and treatment of oral conditions has increased recently and could be of benefit to low-socioeconomic level urban and rural communities (Botelho et al 2007).

Among the various currently available herbal agents, Aloe Vera is a plant commonly found in deserts (which is stem less, drought-resisting succulent of lily family). Its foliage, extract and resin contains over 70 biologically active compounds and has been claimed to have antimicrobial, anti-inflammatory and healing properties and are indicated to hepatic and stomach diseases (Davis et al 1992, Shelton 1991). Aloe Vera plays an important role in many medical properties as a traditional medicine in treatment of many pathological disease and conditions such as Insomnia, Burns, Ulcerative colitis, Psoriasis, wound healing as it is rich in minerals (magnesium, calcium, chromium, copper, iron, etc), enzymes, amino acids, vitamins as antioxidants, including vitamin E, vitamin C, flavenoids, carotenoids and tannins (Marshall 1990, Surjushe et al 2008, Miladi 2008).

The antimicrobial effect of a dentifrice containing Aloe Vera has been demonstrated in an in vitro study, in which this phytotherapic agent inhibited the growth of diverse oral microorganisms, such as streptococcal mutans (S mutans), streptococcal sangius (S sangius), Actinomyces viscosus (A viscosus) and candida albicans (C albicans) (Lee et al 2004). The only study evaluating the clinical effects of Aloe Vera showed a significant reduction of gingivitis and plaque accumulation after use of a mouth rinse containing this natural product (De Oliveira et al 2008).

To the present date, there is no reported controlled trial evaluating the efficacy of Aloe Vera in the control of gingivitis and periodontitis. Therefore, purpose of the present study was to assess and compare between the oral and systemic Aloe Vera administration in the control of gingivitis associated with periodontitis.

MATERIAL & METHODS

Preparation of the formulation

Leaves of Aloe Vera were collected, left to rest in distilled water for 8 hours to eliminate aloine, and then cut into pieces. Afterwards, pulp fragments were liquefied, sieved, filtered with negative pressure to obtain the juice, and freeze-dried. They were then sterilized by gamma ray and stored at 4˚C in Eppendorf tubes. The extract was re-suspended in distilled water for use.

Animal model:

Forty adult male Wistar rats with an average weight 150-250 g, 6–7 weeks old were obtained from Veterinary Research institute, Cairo University, Egypt. The animals were housed in controlled environment (temperature 25 ± 2° and 12 hr dark/light cycles) and fed with standard pellets diet, tap water adlibitum. They were kept in individual cages in a controlled room (temperature 20–25°C; humidity 70–80%). The animal room was under standard conditions. All experiments were conducted in the animal house in faculty of Oral and Dental Medicine, Cairo University, Egypt according to the recommendations of the ethics committee on animal's experimentation of the school.

Experimental procedure:

Induction of Periodontitis

The rats were assigned to the ligature. All procedures of periodontal disease induction were performed under general anesthesia by intramuscular injection of a combination of 0.1ml ketamine hydrochloride (50 mg/ ml) and 0.05 ml

xylaine hydrochloride (2 g/100 ml) for each 100 g body weight. After anesthesia, sterile 4/0 silk ligature was placed around the bilateral mandibular first molars (Villalobos et al 2001).

Administration of Aloe Vera Gel extract

The forty animals were divided randomly into four groups with 10 rats in each group: The rats in the first group (control) were daily intraperitoneally injected with saline (5mg/kg). The rats of the second group were given Aloe Vera extract intraperitoneally (300mg/kg) starting one day before ligature and continuing for one month (Smenoff et al 2008). The rats in the third group were given saline (5mg/kg) orally, while rats in the fourth group were given Aloe Vera extract (300mg/kg) by oral gavage starting one day before ligature and continuing for one month (Madan et al 2008). At the end of 30 days for all groups, all animals were sacrificed with cervical translocation, and the gingiva was dissected using a scalpel. All the gingival specimens were then washed and fixed in 4% buffered formalin.

After fixation, the specimens were dehydrated, cleared and embedded in paraffin. Sections of 4-5μm thickness were collected on positively charged microscope slides and stained with haematoxylin and eosin for detection of any morphological changes (epithelial, vascular, and collagenous) that might have been induced by gingivitis and periodontitis. Other two sets of sections were labeled for localization of PCNA and caspase-3.

Histomorphometric analysis

Histomorphometric analysis was performed in haematoxylin and eosin sections for measuring the epithelial thickness and in immunolabelled sections for measuring the area % for caspase-3 and PCNA immunostaining. The data were obtained using Leica Owen 500 image analyzer computer system. The fields were randomly selected and the image analyzer was calibrated automatically to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer unit.

Statistical analysis

The data obtained from both the histomorphometrical and radiographic analysis were statistically described in terms of range, mean value ± standard deviation (±SD), median. Kruskal-Wallis analysis of variance (ANOVA) test was used to make comparison between the studied groups with Conover-Inman test for independent samples as post hoc multiple 2-group comparisons. A probability value (p value) less than 0.05 was considered statistically significant.

RESULTS

Sue to similarity between the two control groups and to avoid repetition, the two control groups were displayed as a single group. The groups were as follow: control, intraperitoneal and oral groups.

Heamatoxylin and Eosin stain

The control and intraperitoneal groups showed increase in their epithelial thickness, while the oral group revealed a decrease in its epithelial thickness. The gingival epithelial ridges from the control and intraperitoneal groups were flattened and had tortuous appearance, on the other hand the oral group showed normal epithelial configuration. Intra-cellular edema had been observed in the basal and parabasal cells of the control and intraperitoneal groups. Also, the surface keratin layer in the control group demonstrated shedding, while increase keratin thickness was recorded in the intraperitoneal group and re-epithelization of the keratin was noticed in the oral group (Fig 1).

The lamina propria of the control group demonstrated strong inflammatory cell infiltration, dilated and conjugated blood vessels with extravasation of RBCs, and irregular arrangement as well as degeneration of the collagen fibers. On the other hand, the intraperitoneal group revealed mild to moderate increase in the number of inflammatory cells in both papillary and reticular layers with mild increased number of arterioles, and dilated lymph vessels. As

for the oral group, there was decrease in the inflammatory cells number with increase fibroblastic proliferation, normal collagen fibers distribution and multiple small blood vessels were demonstrated as well (Fig 2).

Immunoperoxidase staining

Proliferating cell nuclear antigen (PCNA):

In the control group there was a mild reaction to PCNA in 60% of cases while 40% showed negative reaction, while in the intraprtitoneal group moderate reaction was revealed in both epithelium and lamina propria. On the other hand, the oral group demonstrated strong reaction of basal epithelial cells and the blood vessels endothelial cells. This group also revealed an increased fibroblastic proliferation (Fig 3).

Caspase-3:

In the control group, strong reaction was observed in both epithelium and connective tissue and in endothelial cells of the blood vessels. In the intraperitoneal group, moderate reaction was detected in the whole epithelium, inflammatory cells as well as fibroblasts in the connective tissue stroma. On the other hand, the oral group showed mild to negative reaction in both epithelium and lamina propria (Fig4).

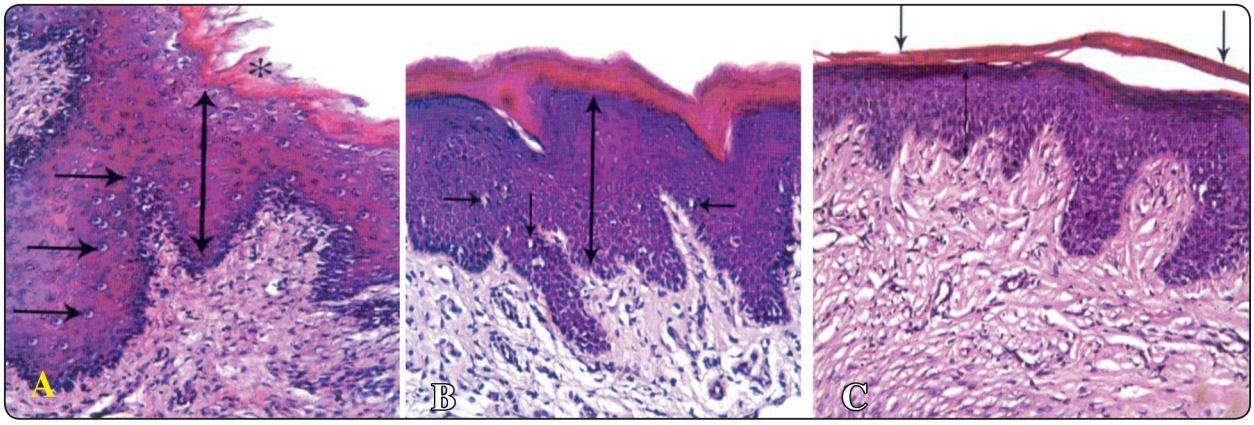

Fig. (1) Light micrograph plate of the gingiva showing: increase in the epithelial thickness and tortuous epithelial ridges with intracellular edema in both control (a) and intraperitoneal (b) groups, while the oral group (c) shows decrease epithelial thickness with normal configuration. (Hx&E. stain. Orig. Mag.200)

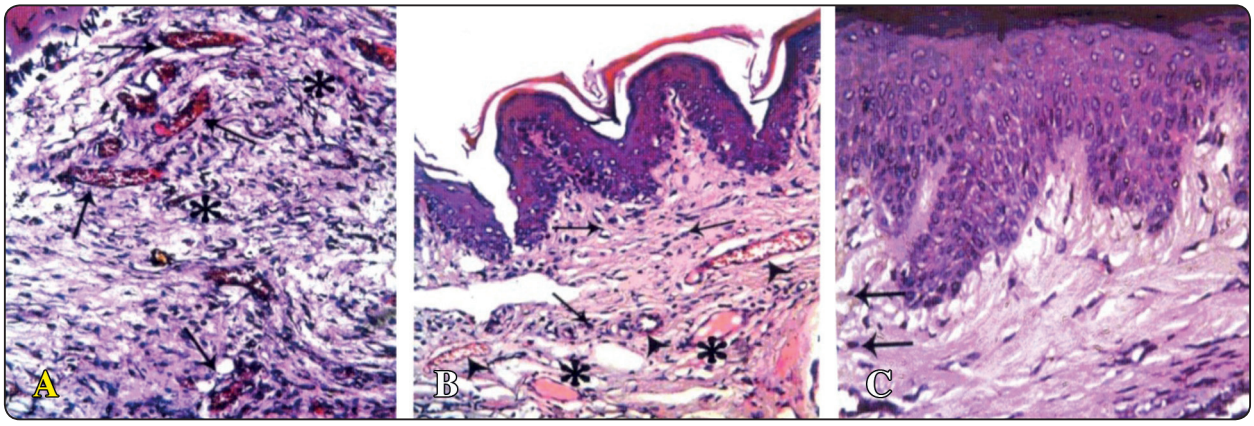

Fig. (2) Light micrograph plate of the gingiva showing strong inflammatory cells infiltration in control group with irregular collagen configuration and conjugated bvs (a), mild inflammatory cells with increased dilated bvs in the intraperitoneal group (b), while the oral group (c) shows decrease inflammatory cells with small bvs and normal collagen configuration. (Hx&E. stain. Orig. Mag.200)

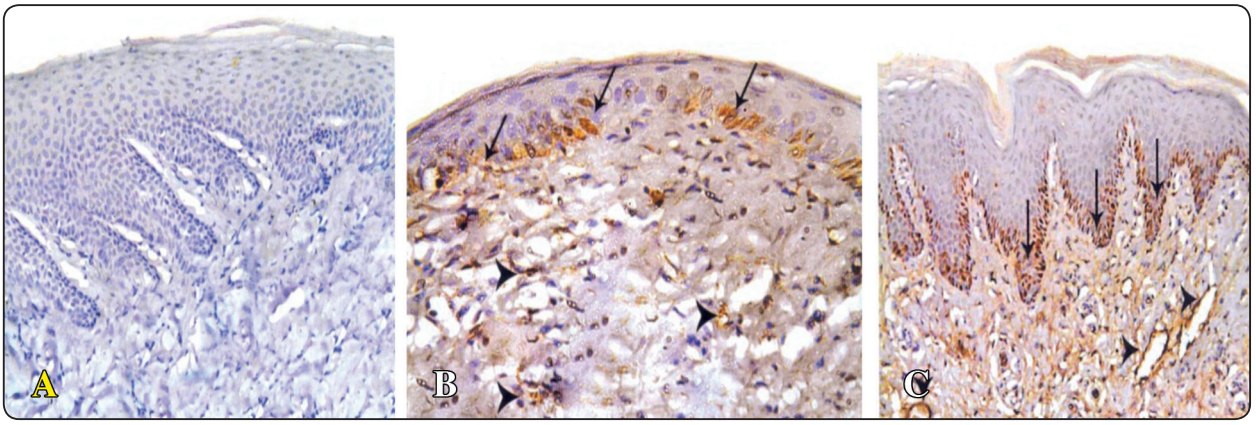

Fig. (3) Light micrograph plate of the gingiva showing negative PCNA reaction in control group (a), moderate reaction in the intraperitoneal group (b), and strong reaction in the oral group (c). (DAB, Orig. Mag.200)

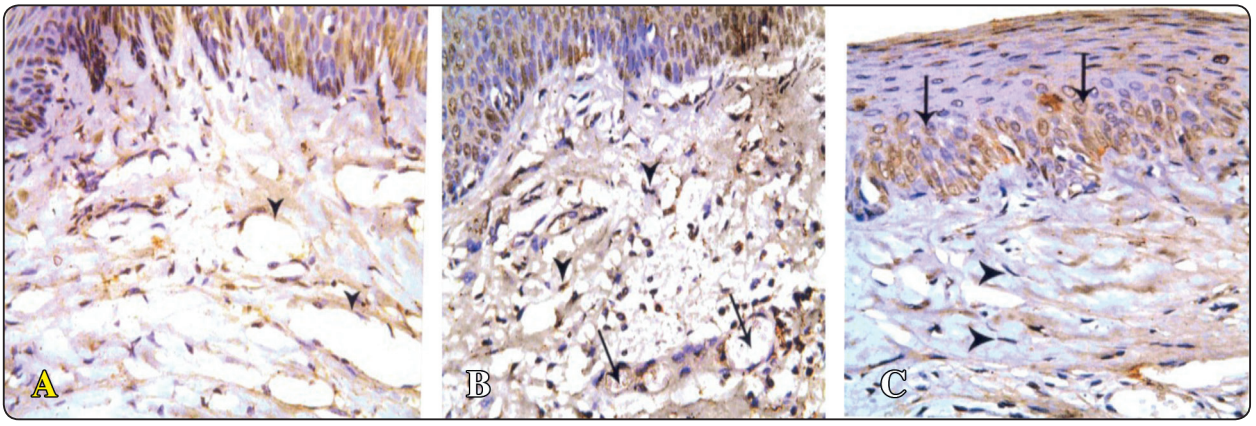

Fig. (4) Light micrograph plate of the gingiva showing strong caspase-3 reaction in control group (a), moderate reaction in the intraperitoneal group (b), and mild reaction in the oral group (c). (DAB, Orig. Mag.400)

Histomorphometric analysis

Epithelial Thickness:

The histomorphometric analysis revealed that the mean epithelial thickness was increased in the intraperitoneal and control groups compared to the oral group as shown in table (1) and Fig (5). There was a statistical significant difference between all groups in their epithelial thickness measurements (P=0.0163), there was a high statistical significant difference in the epithelial thickness between the oral and the control groups (P=0.0065). On the other hand, there was a non significant difference between the control and intraperitoneal groups as well as between the oral and intraperitoneal groups (P=0.1822 and 0.0738 respectively).

PCNA (area %):

It was found that there was an overall high significant difference in the PCNA area % immunostaining between the three studied groups (P=<0.0001). In addition, there has been a high significant increase in the PCNA reaction in the oral and intraperitoneal groups versus the control group (P=<0.0001). A non significant difference in the PCNA immunoreactions between the oral and intraperitoneal groups has been recorded (P=0.0586) table (2) and Fig(6).

Caspase-3 (area %):

An overall high significant difference in the caspase-3 immunostaining area % was found between the three studied groups (P=<0.0001). There has been a statistically significant increase in the caspase-3 reaction in the control group versus both the oral and the intraperitoneal groups (P=<0.0001). Furthermore, a statistically significant increase in the caspase-3 immunoreaction in the intraperitoneal group in comparison to the oral group was also present (P=<0.0186) table (3) and Fig (7).

TABLE (1) Mean values & standard deviation of the epithelial thickness in the studied groups

Variables	**Control Group**	**Oral Group**	**Intraperitoneal**
Number of samples	10	10	10
Mean	182.217	118.589 [a]	148.690
Standard deviation	61.678	21.900	45.098
P value	0.0163*		

statistically significant difference** **[a] statistically significant with the control group

TABLE (2) Mean values & standard deviation of the PCNA area percentage in the studied groups

Variables	**Control Group**	**Oral Group**	**Intraperitoneal**
Number of samples	10	10	10
Mean	32.309	74.002 [a]	64.958[a]
Standard deviation	11.479	12.742	6.190
P value	<0.0001*		

statistically significant difference** **[a] statistically significant with the control group

TABLE (3) Mean values & standard deviation of thecaspase-3 area % in the three studied groups

Variables	**Control Group**	**Oral Group**	**Intraperitoneal**
Number of samples	10	10	10
Mean	69.452	27.315 [a]	39.593[b]
Standard deviation	11.951	13.356	6.830
P value	<0.0001*		

statistically significant difference** **[a] statistically significant with the control group

[b] statistically significant with the control group and the oral group

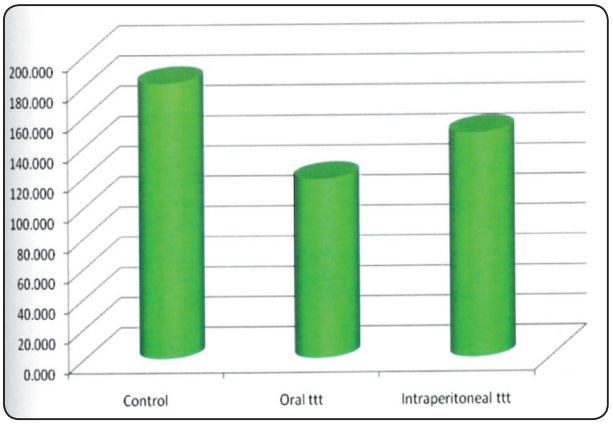

Fig. (7) Mean caspase-3 area % between the three studied groups

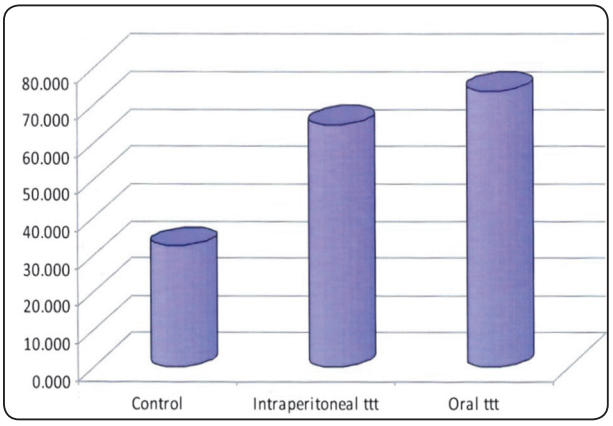

Fig. (6) Mean PCNA area % between the three studied groups

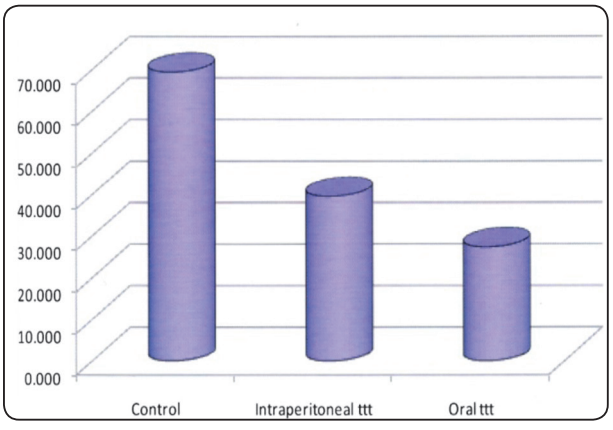

Fig. (5) Mean epithelial thickness between the three studied groups

DISCUSSION

Periodontal disease is the most prevalent chronic inflammatory condition of the soft and hard supporting tissues of the teeth, and is a major cause of tooth loss in adults (Dongari et al 1998). It comprises a variety of pathological conditions of the tooth supporting structures characterized by the presence of bacterial challenge eliciting a potentially destructive host response (Lang et al 1996).

Treatment of periodontitis using natural products is a great challenge. Aloe Vera is a natural compound which has been used as a therapeutic product by human for hundreds of years (Reyonolds et al 1999). In the present study, it had been used in treating gingivitis associated with ligature induced periodontitis by two different routes of administration: orally and intraperitoneally.

In the present study, the control group (periodontitis group) showed increased epithelial thickness and increased number of inflammatory cells in the lamina propria. Also, intracellular edema of basal and parabasal cells had been demonstrated.

Previous studies have attributed that the host-mediated response in periodontal disease involves the activation of the innate immunity, specifically by upregulation of pro-inflammatory cytokines from monocytes/polymorphonuclear leukocytes, and down regulation of growth factors from macrophages (Offenbacher et al 1986). The host mediated response in periodontal disease is also characterized by the production of inflammatory mediators (Offenbacher et al 1984).

Treatment using Aloe Vera in this study revealed marked decrease in the inflammatory reaction in the oral group in comparison with that in the control group. In the current research, increased inflammation in the control group compared to the oral group was demonstrated by hyperplasia in epithelium while the oral group showed normal epithelial thickness. The gingival of the control

group showed strong inflammatory cell infiltration in the lamina propria, the intraperitoneal group also revealed mild to moderate increase in the number of inflammatory cells in both papillary and reticular layers. The oral group on the other hand, showed decrease in the number of inflammatory cells.

Various authors have demonstrated the anti-inflammatory effect of Aloe Vera to be associated with inhibition of cyclooxygenase activity, which prevents the synthesis of prostaglandins that are the fundamental chemical mediators in inflammatory processes (Davies et al 1984). Moreover, Davis et al 1994 demonstrated the effectiveness of Aloe Vera in wound treatment reduction of inflammation due to the action of mannose-6-phosphatase, a major sugar present in Aloe Vera.

The identification of the connective tissue metabolites in the gingival crevicular fluid resulting from the degradation of periodontal tissues which provides evidence for a role for Reactive Oxygen Species (ROS) in tissue destruction associated with inflammatory periodontal diseases (Battino et al 1999). ROS are generated predominantly by polymorohonuclear leukocytes during an inflammatory response and are regarded as being highly destructive in nature; they are active in increased apoptosis in deepest layer of sulcular pocket (Jarnbring etv al 2002).

The antioxidant activity of Aloe Vera has been previously reported by Rajasekharan etal 2005, who reported that some compounds located in the leaf cortex were responsible for most of the Aloe Vera antioxidant activity and this was attributed to its protection against lipid peroxidation (Chandan et al 2007).

In the ongoing study, the use of the Aloe Vera with both routes increased the number of samall blood vessels infiltrating the lamina propria. Concurrent with our results, Aloe Vera contains *B*-sitosterol compound which has shown to enhance angiogensis in chick embryo chorioallantoic membrane assay (Moon et al 1999).

Our histological results revealed that the number of fibroblasts was significantly increased in the oral group compared to the control and intraperitoneal groups. This finding is consistent with previous studies that showed Aloe Vera gel crude extract or its components have the ability to stimulate proliferation of normal and diabetic skin fibroblasts in both in vivo and in vitro studies (Boudreau et al 2006, Jettanacgeawcheawchankit et al 2009).

The immunohistochemical results of the present study have revealed the greatest PCNA area percentage in the oral group which was statistically significant with that in the control group, indicating the presence of proliferative fibroblasts and endothelial cells, which were incorporated in epithelial regeneration. This could be attributed to the assumption that the glycoprotein fractions of Aloe Vera together with bradykinase activity and other stimulants of cell proliferation were identified in the non-dialized fraction of the Aloe Vera (Yagi et al 2003).

On the other hand, caspase-3 results of the current work revealed the highest caspase-3 area percentage in the control group, followed by the intraperitoneal group, while the least was in the oral group with a statistically high significant difference between the three groups.

In many cell types such as macrophages, neurons and thmocytes; nitric oxide (NO) is a form of ROS, activates apoptosis (Kim et al 2001). Induction of apoptosis may be also seen as a response to oxidative DNA damage, which occurred by ROS, and especially by nitric oxide. Both in experimental models and human samples of periodontal diseases, an increase in local NO production has been reported (Kendall et al 2001). The induction of NO synthesis expression may also inhibit fibroblast proliferation and induce apoptosis, contributing to the imbalance of tissue destruction with tissue repair that is characteristic of gingivitis and periodontitis.

An established event in most apoptotic cells is the generation of ROS in the cytosol, which directs the cell and its neighboring cells towards the path of death (Chipuk et al 2005). However, with Aloe Vera leaf treatment, no ROS generation was evident (Dutta et al 2007).

CONCLUSION

The histological, immunohistochemical and morphometric results of the present study have proved the efficacy of Aloe Vera in treating gingivitis. However, the oral administration proved to be more beneficial rather than intraperitoneal route.

REFERENCES

1. Armitage G C (1999): Development of a classification system for periodontal diseases and conditions. Annals of Periodontolgy, 4: 1-6.
2. Battino M, Bullon P, Wilson M, Newman H (1999): Oxidative injury and inflammatory periodontal diseases: the challenge of anti-oxidants to free radicals and reactive oxygen species. Critical Reviews in Oral Biology and Medicine, 10 (4): 458-476.
3. Beirne PV, Forgie A, Clarkson JE, Worthington HV (2005): Recall intervals for oral health in primary care patients. Cochrane Database for Systematic Reviews, 18 (2): CD004346.
4. Botelho MA, Nogueira NAP, Bastos GM, Fonseca SGC, Lemos TLG, Matos FJA (2007): Antimicrobial activity of the essential oil from Lippia Sidoides, carvacrol and thymol against oral pathogens. Brazillian Journal of Medical and Biological Research, 40 (3): 349-356.
5. Boudreau MD, Beland FA (2006): An evaluation of the biological and toxicological properties of Aloe barbadensis (Miller), Aloe Vera. Journal of Environmental Science and Health- Part C: Environmental Carcinogenesis & Ecotoxicology Reviews, 24: 103-154.
6. Chandan BK, Saxena AK, Shukla S, Sharma N, Gupta DK, Suri KA (2007): Hepatoprotective potential of Aloe barbadensis against carbon tetrachloride induced hepatotoxicity. Journal of Ethnopharmacology, 111 (3): 560-566.
7. Chipuk JE, Green DR (2005): Do inducers of apoptosis trigger caspase-indeprndent cell death? Nature Reviews, Molecular Cell Biology, 6: 268-275.
8. Cutler CW, Stanford TW, Abraham C (2000): Clinical benefits of oral irrigation for periodontitis are related to reduction of pro-inflammatory cytokine levels and plaque. Journal of Clinical Periodontology, 27: 134-143.
9. Davis RH, Stewart GJ, Bregman PJ (1992): Aloe vera and the inflamedsynovial pouch model. J Am Podiatr Med Assoc, 82: 140±8.
10. Davis RH, Donato JJ, Hartman GM, Haas RC (1994): Antiinflammatory and wound healing activity of a growth substance in Aloe Vera. Journal of American Podiatric Medical Association, 84 (2): 77-81.
11. Davies P, Bailey PJ, Goldenberg MM, Ford-Hutchinson AW (1984): The role of arachidonic acid oxygenation products in pain and inflammation. Annual Review of Immunology, 2: 335-357.
12. De Oliveira SM, Torres TC, Pereira SL, Mota OM, Carlos MX (2008): Effect of a dentifrice containing Aloe Vera on plaque and gingivitis control. A double-blind clinical study in humans. Journal of Applied Oral Science, 16 (4): 293-6.
13. Dongari AI, Ebersole JL (1998): Increased presence of interleukin-6 and IL-8 secreting fibroblast subpopulations in adult periodontitis. Journal of Periodontology, 69:899-910.
14. Dutta A, Bandyopadhyay S, Mandal C, Chatterjee M (2007): Aloe Vera leaf exudates induces a caspase independent cell death in Leishmania donovani promastigotes. Journal of Medical Microbiology, 56: 629-636.
15. Greenstein G (1999): The role of periostat in the management of adult periodontitis. A critical Assessment, 20 (7): 664-668.
16. Grossi SG, Zambon JJ, Ho AW (1994): Assessment of risk for periodontal disease. I. Risk indicators for attachment loss. Journal of Periodontology, 65: 260-267.
17. Grossi SG, Genco RJ, Machtei EE (1995): Assessment of risk for periodontal disease. II Risk indicators for alveolar bone loss. Journal of Periodontology, 66: 23-29.
18. Hirschfeid L, Wasserman B (1978): Along-term survey of tooth loss in 600 treated periodontal patients. J Periodontol, 49: 225-37.
19. Jarnbring F, Somogyi E, Dalton J, Gustafsson A, Klinge B (2002): Quantitative assessment of apoptotic and proliferative gingival keratinocytes in oral and sulcular epithelium in patients with gingivitis and periodontitis. Journal of clinical Periodontology, 29 (12): 1065-1071.
20. Jettanacheawcheawchankit S, Sasithanasate S, Sangvanich P (2009): Acemannan stimulates gingival fibroblast proliferation; expression of keratinocyte growth factor-1,

vascular endothelial growth factor, and type I collagen; and wound healing. Journal of pharmacological science, 109: 525-531.

21. Kaldahl WB, Kalkwarf WL, Patil KD, Molvar MP, Dyer JK (1996):.Long-term evaluation of periodontal therapy: Incidence of sites breaking down. J Periodontal, 67: 103-8.
22. Kendall HK, Marshall RI, Bartold PM (2001): Nitric oxide and tissue destruction. Oral Diseases, 7: 2-10.
23. Kim PK, Zamora R, Petrosko P, Billiar TR (2001): The regulatory role of nitric oxide in apoptosis. International Immunopharmacology, 1: 1421-1441.
24. Lalla E, Cheng B, Lal S, Kaplan S (2007): Diabetes mellitus promotes periodontal destruction in children. Journal of clinical Periodontology, 34: 294-298.
25. Lang NP, Tonetti MS (1996): Periodontal diagnosis in treated periodontitis. Why, When and How to use clinical parameters. Journal of Clinical Periodontology, 23: 240-250.
26. Lee SS, Zhang W, Li Y (2004): The antimicrobial potential of 14 natural herbal dentifrices: results of an in vitro diffusion method study. Journal of American Dental Association, 135 (5): 1133-1141.
27. Madan J, Sharma Ak, Inamdar N, Rao HS, Singh R (2008): Immunomodulatory properties of Aloe Vera in mice. Int J Green Pharm, 2: 152-4.
28. Marshall JM (1990): Aloe Vera gel: What is the evidence? Pharmaceutical Journal, 24: 360-362.
29. Miladi S, Damak M (2008): In Vitro Antioxidant Activities of Aloe Vera Leaf Skin Extract. Journal de la Societe Chimique de Tunisie, 10: 101-109.
30. Moran J, Addy M, Newcombe RG, Marlow I (2001): A study to assess the plaque inhibitory action of newly formulated triclosan toothpaste. Journal of Clinical Periodontology, 28 (1): 86-89.
31. Moon EJ, Lee Y, Lee OH (1999): A novel angiogenic factor derived from Aloe Vera gel: B-sitosterol, a plant sterol. Angiogenesis, 3: 117-123.
32. Moore WEC, Moore LH (1994): The bacteria of periodontal diseases. Journal of Periodontology, 5: 66-77.
33. Nogueira-Filho GR, Toledo S, Cury JA (2000): Effect bof 3 dentifrices containing triclosan and various additives: an experimental gingivitis study. Journal of Clinical Periodontology, 27 (7): 494-498.
34. Offenbacher S, Odle BM, Gray RC, Van Dyke TE (1984): Crevicular fluid prostaglandin E levels as a measure of the periodontal disease status of adult and juvenile periodontitis patients. Journal of Periodontal Research, 19 (1): 10-13.
35. Offenbacher S, Odle BM, Van Dyke TE (1986): The use of crevicular fluid prostaglandin E2 levels as a predictor of periodontal attachment loss. Journal of Periodontal Research, 21 (2): 101-112
36. Rajasekeran S, Sivagnanam K, Subramanian (2005) : Antioxidant effect of Aloe vera gel extract in streptozotocin-induced diabetes in rats. Pharmacological reports, 57: 90-96.
37. Reyonolds T, Dweek AC (1999): Aloe Vera leaf gel: A review update. Journal of Ethnopharmacology, 68: 30-37.
38. Savage A, Eaton K A, Moles D R, Needleman L (2009): systematic review of definitions of periodontitis and methods that have been used to identify this disease. Journal of Clinical Periodontology, 36: 458-467.
39. Shelton M (1991): Aloe Vera, its chemical and therapeutic properties. International Journal of Dermatology, 30: 679-683.
40. Semenoff TA, Semenoff-Segundo A, Bosco AF, Nagata MJ, Garcia VG, Biasoli ER (2008): Histometric analysis of ligature-induced periodontiotis in rats: A comparison of histological section planes. J Ap-pl Oral Sci., 16 (4): 251-256.
41. Stambaugh RV, Dragoo M, Smith DM, Carasali L (1981): The limits of subgingival scaling. Int J Periodontics Restorative Dent, 1 (5): 30-41.
42. Surjushe A, Vasani R, Saple DG (2008): Aloe Vera: A short review. Indian Journal of Dermatology, 53 (4): 163-166.
43. Villalobos OJ, Salazar CR, Sanchez GR (2001): Efecto de un enjuague bucal compuesto de Aloe Vera en la placa bacteriana e inflamacion gingival. Acta Odontologica Venzolana, 39 (2): 16-24.
44. Waerhaug J (1978): Healing of the dento-epithelial junction following subgingival plaque control, As observed in human biopsy material. J Periodontal, 49: 1-8.
45. Yagi A, Takeo S (2003): Antiinflammatory constituents, aloesin and aloemannan in Aloe species and effects of tanshinon VI in saliva miltiorrhiza on heart. Journal of Pharmaceutical Society of Japan, 123 (7): 517-532.