Journal Homepage: - www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI: 10.21474/IJAR01/5337
DOI URL: http://dx.doi.org/10.21474/IJAR01/5337

RESEARCH ARTICLE

PROTECTIVE EFFECT OF MORINGA OLEIFERA EXTRACT ON EXPERIMENTALLY LPS-INDUCED PERIODONTITIS.

Abdulaziz Omar Alshwerf[1], Laila El Sayed Amin[2], Fatma Ibrahim[3] and Jilan Youssef[4].

1. BDS, Faculty of Dentistry, Tripoli University, Libya.
2. Lecturer of Oral Biology, Faculty of Dentistry, Mansoura University, Egypt.
3. Professor of Oral Biology, Faculty of Dentistry, Mansoura University, Egypt.
4. Professor of Oral Medicine, Periodontology and Oral Radiology, Faculty of Dentistry, Mansoura University, Egypt.

Manuscript Info

Manuscript History

Received: 05 July 2017
Final Accepted: 07 August 2017
Published: September 2017

Key words:-
Moringa Oleifera(MO), E.coli, Periodontitis(PD), IL-6, Leptin(Ob).

Abstract

Periodontitis (PD) are an inflammatory disorder in which tissue damage occurs through complex interactions between periodontal pathogens and components of the host mechanisms. Natural agents having antimicrobial and anti-inflammatory activities might be able to control the inflammatory diseases such as PD. This study designed to investigate the effect of Moringa Oleifera Extract (MOE) on healing of induced PD. Experimental periodontal disease was induced in rats by injecting LPS in the gingival tissues on the distobuccal aspect of lower first molars (30 ug LPS, 3 times/week for 2 weeks). MOE was administered to rats daily via oral gavage by dose 300 mg/kg once daily. The inflammatory status was evaluated by descriptive analysis of Ob and IL-6 serum level on H&E-stained sections. MOE Group B showed significant decrease in periodontal inflammation. In conclusions: MOE potently inhibits innate immune responses associated with periodontal disease, suggesting a therapeutic potential in this chronic inflammatory condition.

Introduction:-

Periodontal disease denotes a group of oral inflammatory infections. This infectious disease severity varies from minor and reversible gingiva inflammation (gingivitis) to chronic damage of CT.[1] This process causes separation of the gum tissues from the tooth, producing a periodontal pocket and bone loss, which causing tooth loosening. [2]

Lipopolysaccharide (LPS) is a main compound present in the membrane of Gram negative bacteria. It has the ability to initiate immune responses by stimulating cells that reside in the periodontal tissues, leading to releasing of large numbers of inflammatory mediators including interleukins, chemokines and adhesion molecules. [3]

After recognition and presentation of microbes to the appropriate cells, cytokines of the innate response, including tumor necrosis factor alpha (TNF- α), interleukin-1beta (IL-1β) and interleukin-6 (IL-6), are the first to appear in the periodontal disease pathogenesis pathways. [4] IL-1β and IL-6 are signature innate cytokines and have been characteristically associated with inflammatory cell migration and osteoclastogenesis. [5]

Corresponding Author:- Abdulaziz Omar Alshwerf.
Address:- BDS, Faculty of Dentistry, Tripoli University, Libya.

Leptin formed mainly by adipocytes and secreted into systemic circulation, performs regulatory functions including consuming, storage of energy and bone metabolism. [6]

There is a close relationship between the high incidence of oral diseases and microorganisms and because of growing antibiotic bacterial resistance, toxic and harmful side effects associate with the use of some common antibacterial agents; there is a need for alternative treatment options and therapies that are effective and safe and affordable such as herbal therapies. [7] Phytochemicals are, in the strictest sense of the world, chemicals produced by plants, which may have an impact on health. Moringa Oleifera contains several phytochemicals, some of which are of special interest because of their medicinal properties. [8]

Moringa leaves and flowers are used as a significant source of vitamins (A, B and C) and minerals (Calcium, iron, Phosphours and Magnesium). [9] Leaves and stems of MO are well-known to have a huge quantity of their calcium in their calcium oxalate crystals. It contain, more vitamin A than carrot, more calcium than milk, more iron than spinach, more vitamin c than oranges and more potassium than in banana. [10]

Materials and Methods:-

Thirty-six healthy male albino rats ranging in weight from (150-200gm) were used in the study, housed in Nile Center for Experimental Research, Mansoura City, Egypt. According to the ethical committee of (Mansoura University- Dentistry). Rats were kept in suitable circumstances, such as, temperature, humidity.

The animals were divided into three main groups (12 rats for each):
Group A (Control group): rats were left without any intervention
Group B (LPS group): rats were injected by 30µg of LIP E.Coli in the lower gingiva at the distobuccal aspect of the first molar, three times a week for 10 days of induction of PD. [13]

Group C (LPS and MO group): This group manipulated as a group B. Additionally, it was received MO by dose 300 mg/kg once daily along the period of study . [14]

Then, serum level of IL-6 was being measured in all groups for assurance of PD induction in groups (B and C).

The MO leaves were purchased from herbal store, Mansoura, Egypt. The extraction, purification, and extract preparation were carried out in Liver Research Lab, Department of Pharmacognosy, Faculty of Pharmacy, Mansoura University, Egypt.

Periodontitis induction:-
General anesthesia was induced with intraperitoneal injections of ketamine hydrochloride (50 mg/ Kg) and xylazine hydrochloride (5 mg/ Kg), then were fixed on his back. 30 µg of E.Coli LPS (Strain 055:B5 – Sigma Chem Co.,St. Louis, MO, USA) was dissolved in phosphate buffered saline (PBS), then injected bilaterally and the needle held in place for several seconds post injection in order to ensure that LPS was not lost by the needle track

Blood sampling collection and cytokine measurement

Blood samples were withdrawn from the eye of all rats at 1st day and other blood samples were collected from cardiac at 15th days into heparin-coated micro-capillaries. The samples were centrifuged for 15 min. The serum was carefully harvested in dry clean Wasserman tubes using a Pasteur pipette and kept frozen until examination at -20 oC. Then determine concentration of IL-6 and Leptin in serum by ELISA kit.

Animals Sacrification: twelve animals from each group after two weeks were exposed to halothane over dose. Then the mandibles were split into two halves for decalcification and were prepared for histological examination.

Results: 1- Blood analysis results:
Table (1): Comparison of leptin level change in the studied groups at baseline , 2 weeks and after one month of the study showed a statistically significant difference in group B & C.

	Studied groups	Baseline n=12	Two weeks after n=6	one month after n=6
Leptin	Group A	0.56±0.31	0.64±0.48	0.56±0.35

	Significance		t=0. 08 p=0.94	t=0.907 p=0.406
	Group B	0.64±0.39	1.15±0.53	0.87±0.47
	Significance		t=2.58 p=0.049*	t=3.77 p=0.009*
	Group C	0.76±0.47	0.39 ± 0.17	0.08±0.03
	Significance		t=2.68 p=0.043*	t=2.59 p=0.049*

Table (2): Comparison of IL-6 level change in the studied groups at baseline, 2weeks and after one month of the study showed only statistically significant difference was found in group C at two weeks

		Baseline n=12	Two weeks after n=6	one month after n=6
IL- 6	Group A	40.75±20.43	21.27±4.9	17.58±1.88
	Significance		t=2.159 p=0.083	t=9.597 p=0.001*
	Group B	47.17±21.71	51.903±26.9	46.38±17.09
	Significance		t=2.38 p=0.063	t=1.016 p=0.356
	Group C	39.49±10.39	14.92±0	11.91±4.5.
	Significance		t=5.39 p=0.003*	t=6.347 p=0.001*

Light microscopic results:-

1-Haematoxyline and Eosin stain (H & E):

Group A (control group): After 2 weeks the sections showed normal histological structure of the periodontium with gingiva consisted of typical keratinized stratified squamous epithelium with basal, prickle, granular and keratinized layer, the lamina properia composed of well formed collagen fibers and small blood vessels Figs (1), 1.

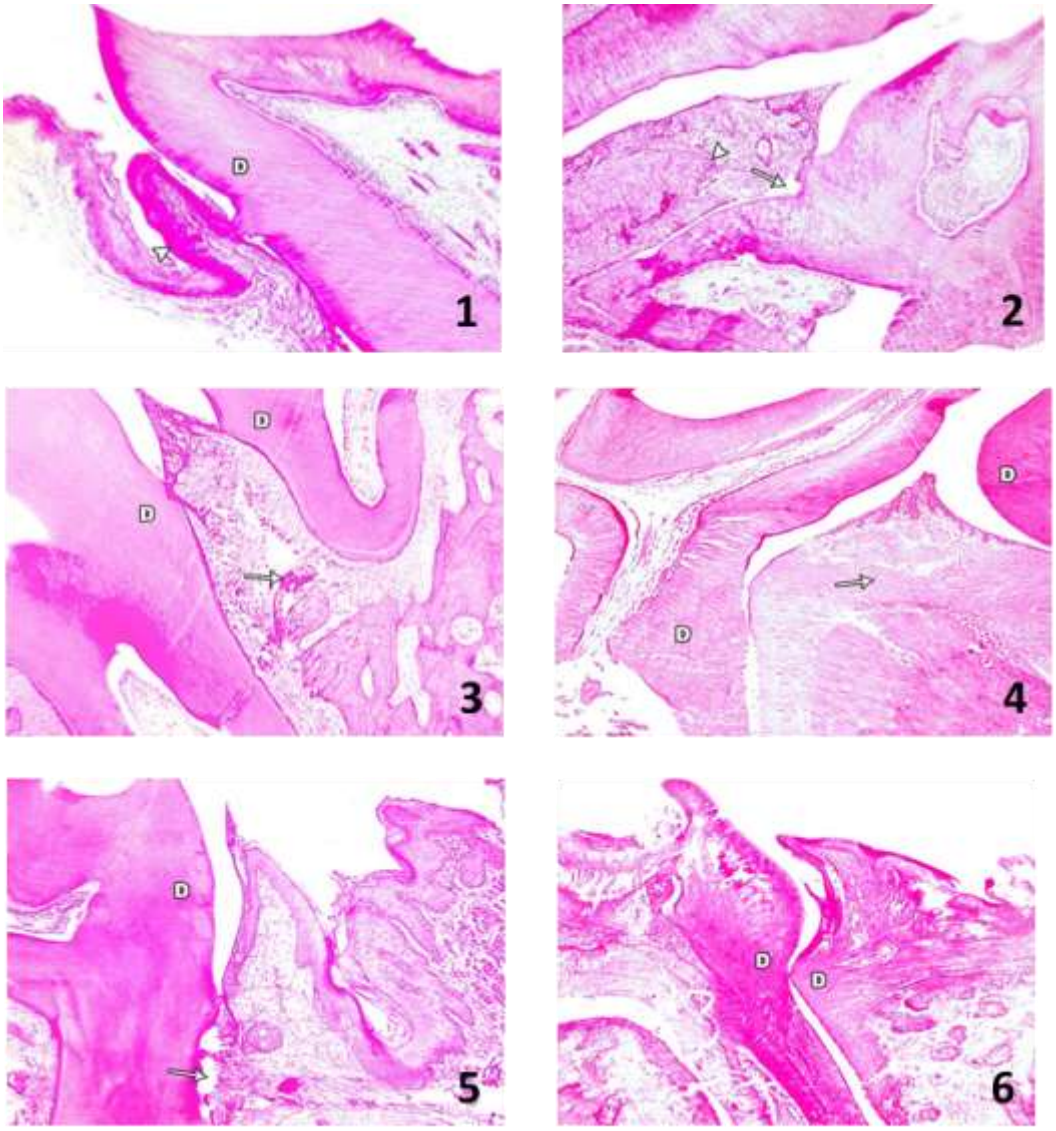
Group B (LIP group): After 2 weeks the sections showed external resorption of the cementum in the cervical area with the irregular orientation of (PDL) and sever destruction of the gingiva, the epithelium showed hydropic degeneration with appearance of large amounts of vacuoles and infiltration of the inflammatory cells in the lamina properia Figs (1), 2.

Group C (LIP and MO treated group): After 2 weeks the gingiva appeared with loss of its regular structure and inflammatory cell accumulation and irregular arrangement of (PDL) fibers Figs (1), 3.

Group A (control group): After 4 weeks the sections showed well organized gingival epithelial layers and regular arrangement of the periodontal ligament fibers with a small number of blood vessels Figs (1), 4.

Group B (LIP group): After 4 weeks the specimens showed irregular areas of external root resorption and with the destruction of periodontal ligament fibers and large number of inflammatory cells and bone resorption in some specimens Figs (1), 5.

Group C (LIP and MO treated group): After 4 weeks the periodontium showed regeneration with rearrangement of periodontal ligament fibers and normal structure of the gingiva and moderate vasculature with bone deposition in alveolar bone crest Figs (1), 6.



Figs (1): (H&E stain x100) photomicrograph of (1) control group showing the normal gingival epithelium (arrowhead), (D) indicates the dentin. (2) group B showing external resorption of the cementum of the cervical area (arrow) with disorganized PDL fibers and alveolar bone crest resorption (arrow head). (3) showing the almost normal architecture of the gingival epithelial layers, PDL fibers and normal alveolar bone crest (arrow), (D) indicates the dentin. (4) Photomicrograph of control group (after 4 weeks) well organized gingival epithelial layers and periodontal ligament (PDL) fibers (arrow), (D) indicates the dentin (H & E X 100). (5)Photomicrograph of group B (after 4 weeks) showing large areas of resorption in the cementum covering the root surface (arrow), (D) indicates the dentin. (H & E X 100). (6)Photomicrograph of group C (after 4 weeks) showing the normal architecture of the periodontium, (D) indicates the dentin. (H & E X 100).

Discussion:-

The plant has numerous medicinal applications and is used as a traditional medicine for the treatment of various illnesses. [11] Moringa leaves contain a rich source of minerals and proteins with eight essential amino acids. Amino acids are important, especially for infants who unable to make enough protein for their growth requirements. [12]

The results of the present study showed no statistically significant at baseline Ob levels between study groups in serum while the comparison of Ob (after 2 weeks) between study groups was highly statistically significant difference in groups B&C. These results conducted by Johnson and Shari 2001 observed that Ob levels were highest

in the healthy gingiva, which decreased in PD. This variation was attributed to the enhanced microvasculature found in PD that caused the removal of leptin from gingival tissue and an increase in the serum Ob level. [13]

Also Karthikeyan and Pradeep 2007 who carried out a study suggested that greater the periodontal destruction, the lesser is the gingival crevicular fluid Ob concentration and greater in the serum. [14]

In the present study, we found a slightly increase in serum of IL-6 in group B as compared to group A & group C at baseline. In addition, serum of IL-6 there was showing significantly higher after 2 weeks in group B than in group C. The increased levels of IL-6 found in group B explained by inflammatory reactions to bacterial. These results confirmed by Monea et al., 2014 that reported that IL-6 levels elevated in the serum of chronic periodontitis compared to periodontal healthy control subjects. [15] Choi et al., 2014 who reported the production of IL-6 that were induced by prevotella intermedia LPS . [16]

The increased levels of IL-6 found in study groups explained by Noh et al., 2013 they explained the IL-6 is expressed in a variety of situations involving host immune responses and inflammatory reactions to bacterial LPS. [17] IL-6 stimulates gingival fibroblasts to produce collagenolytic enzymes, resulting in periodontal tissue destruction. [18]

In the group C the level of IL-6 in serum, revealed a significant reduction after treatment with MO in comparison to its level at baseline. These results were in accordance with Kardesler et al., 2010 and Kocak et al. 2016 they observed that periodontal treatment can decrease circulating inflammatory mediators such as IL-6 due to inflammation control. [19-20]

In the current study, histopathological results for PD group (group B) after 2 weeks showed external resorption of the cementum in the cervical area with the sever destruction of the gingiva, the epithelium showed sign of PD hydropic degeneration with appearance of large amounts of new vasculature and infiltration of the inflammatory cells in the lamina properia. Ionel and Lucaciu 2015, Çalışır et al., 2016 comes in aggrement with our result, they found that PD in rats after 4 weeks revealed pronounced inflammation of PDL and advanced resorption of the alveolar bone crest. [21-22]

While after 4 weeks, the same group showed irregular areas of external root resorption with the destruction of periodontal ligament fibers and massive inflammation. hasan and palmer 2016 aggremented with our results. They found that advanced periodontitis after 4 weeks revealed collagen and bone loss, reparative fibrotic response which become more evident with time and dense infiltration of inflammatory cells. [23]

The histopathological results after treatment with MO (group C) showed the gingiva appeared with almost regular structure with moderate vasculature, inflammatory cell reduction and nearly regular arrangement of (PDL) fibers and no alveolar bone crest resorption. Dike and Luteino 2015, studied the effect of aqueous extract of MO seed on hematological parameters and the spleen in male albino rats. They reported that the administration of the MOE showed normal histological features of lymphoid nodules (white pulp) embedded in the matrix (red pulp). [24]

Swathi et al., 2016 studied the effects of MOE on PD pathogens like Aggregatebacter actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi) and Fusobacterium nucleatum (Fn) through subgingival plaque samples were collected from patients with chronic periodontitis, cultivated, and incubated anaerobically as per the standard procedure. The subcultured strains of Aa, Pg, Pi and Fn are tested with the prepared extracts of MO. Their results showed data supporting the use of the MO as a natural antimicrobial agent in periodontal therapy. [25]

MO leaves act as a good source of natural antioxidant due to the presence of various types of antioxidant compounds such as ascorbic acid, flavonoids, phenolics particular essential amino acids such as methionine, cystine, tryptophan and lysine and carotenoids. [29] In consistent with our results, Nagashree et al., 2011 reported that MOE (200 mg/kg bw/day) contains substances that act as an antioxidant and prevent the damage produced by arsenite (significantly) decrease in the blood cell counts and Hb of albino rats. [26]

Therefore the identification of the antibacterial effects of these herbal extracts opened a new avenue to futuristic concepts and potential applications in periodontal therapy.

Reference:-

1. How KY, Song KP and Chan KG. Porphyromonas gingivalis: An Overview of Periodontopathic Pathogen below the Gum Line. Front Microbiol. 2016; 7: 53.
2. Pihlstrom BL, Michalowicz BS and Johnson NW. Periodontal diseases. Lancet 2005; 366: 1809–1820.
3. Gorąca A, Huk-Kolega H, Kleniewska P, Piechota-Polańczyk A and Skibska B. Effects of lipoic acid on spleen oxidative stress after LPS administration. Pharmacol Rep 2013; 65: 179–18.
4. Garlet GP. Destructive and protective roles of cytokines in periodontitis: a re-appraisal from host defense and tissue destruction viewpoints. J Dent Res 2010; 89: 1349– 1363.
5. Fonseca JE, Santos MJ, Canhao H and Choy E. Interleukin-6 as a key player in systemic inflammation and joint destruction. Autoimmun Rev 2009: 8: 538–542.
6. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L and Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature. 1994; 372: 425–432.
7. Palombo EA. Traditional medicinal plant extracts and natural products with activity against oral bacteria: potential application in the prevention and treatment of oral diseases. Evid-Based Compl. AlT. 2011; 2011: 15.
8. Bose CK. Possible role of Moringa Oleifera Lam. Root in epithelial ovarian cancer. MED Gen MED. 2007; 9: 26.
9. Khalafalla MM, Abdellatef E, Dafalla MM, Nassrallah AA, Aboul-Enein KM, Lightfoot DA, et al., Active principle from Moringa oleifera Lamleaves effective against two leukemias and a hepatocarcinoma. Afr. J. Biotechnol. 2010; 9: 8467-8471.
10. Singh N and Gilca M. Herbal Medicine: Science embraces tradition: A new insight into ancient Ayurveda. Lambert Academic Publishing AGandCo KG Dudweiler Landstr 99,66123. Saarbrucken, Germany: ISBN 978-3-8383-2145-5.
11. Mukunzi D, Nsor-Atindana J, Zhang XM, Gahungu A, Karangwa E and Mukamurezi G. Comparison of volatile profile of Moringa oleifera leaves from Rwanda and China using HS-SPME. Pak J Nutr 2011; 10: 602-608.
12. Tiloke C, Phulukdaree A and Chuturgoon AA. The antiproliferative effect of Moringa oleifera crude aqueous leaf extract on cancerous human alveolar epithelial cells. BMC complementary and alternative medicine. 2013; 13: 226
13. Johnson RB and Serio FG. Leptin within healthy and diseased human gingiva. J Periodontol. 2001; 72: 1254–1257.
14. Karthikeyan BV and Pradeep AR. Gingival crevicular fluid and serum leptin :their relationship to periodontal health and disease. J Clin Periodontal 2007; 34: 467-472.
15. Monea A, Gruber R, Elod N , Bereşescu G , Moldovan C and Monea M. Saliva and serum of TNF-α and IL- 6 in a sample of Romanian adult subjects with type 2 diabetes mellitus and periodontal disease. *European Scientific Journal* , 2014 ; 10: 1857 -7881.
16. Choi EY, Jin JY, Choi JI, Choi IS and Kim SJ. DHA suppresses Prevotella intermedia lipopolysaccharide-induced production of proinflammatory mediators in murine macrophages. *Br J Nutr*, 2014; 111: 1221–1230.
17. Noh MK, Jung M, Kim SH, Lee SR, Park KH, Kim DH, et al., Assessment of IL-6, IL-8 and TNF-α levels in the gingival tissue of patients with periodontitis. *Expermental And Therapeutic Medicine*, 2013; 6: 847-851.
18. Takashiba S, Naruishi K and Murayama Y. Perspective of cytokine regulation for periodontal treatment: fibroblast biology. *Journal of periodontology* , 2003; 74: 103-110.
19. Kardesler L, Buduneli N, Cetinkalp S & Kinane D F ."Adipokines and Inflammatory Mediators after Initial Periodontal Treatment in Patients with Type 2 Diabetes and Chronic Periodontitis," Journal of Periodontology, 2010; 81: 24-33.
20. Kocak E, Sağlam, Kayış SA, Dündar N, Kebapçılar L, Loos BG, et al., Nonsurgical periodontal therapy with/without diode laser modulates metabolic control of type 2 diabetics with periodontitis: a randomized clinical trial, 2016; 31: 343-353.
21. Ionel A, Lucaciu O, Moga M, Buhatel D, Ilea A, Tabaran F, et al., Periodontal disease induced in Wistar rats - experimental study, Human & Veterinary Medicine OPEN ACCESS International Journal of the Bioflux Society. 2015; 7: 90-95.
22. Çalışır M, Akpınar A, Poyraz O, Göze F and Çınar Z. Histopathological and morphometric investigation of the effects of systemically administered humic acid on alveolar bone loss in ligature-induced periodontitis in rats. Journal of Periodontal Research 2016; 51: 499-507.
23. **Hasan A, Palmer RM. A** clinical guide to periodontology: Pathology of periodontal disease. BDJ. 2016; 216:457-61.
24. Dike EC, Mohammed A and Satya P. Effect of aquous extract of Moringa Oleifera seed on heamatological parameters and the spleen in male albini rats. Journal of dental and medical sciences. 2015; 14: 35-41.

25. Swathi K, Savita AM, Pallavi Nanaiah. K, Abhilash N, Vaijinathrao SS and Abdul H. Antimicrobial effects of phyllanthus emblica extract and Moringa Oleifera Extract on specific periodontopathogens - an in vitro study, International Jornal of Analytical, pharmaceutical and biomedical sciences. 2016; 5: 2278-0246.
26. Nagasheree R, Latha R and Karthikeyan V. Effect of leaves of Moringa Oleifera on biochemical and physiological parameters in rats. Journal of natural remedies . 2011; 11: 1.