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

Abdulaziz Omar Alshwerf¹, Laila El Sayed Amin², Fatma Ibrahim³ and Jilan Youssef⁴.
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.

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

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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.

Key words:- Moringa Oleifera(MO), E.coli, Periodontitis(PD), IL-6, Leptin(Ob).
Leptin formed mainly by adipocytes and secreted into systemic circulation, performs regulatory functions including consuming, storage of energy and bone metabolism. 

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. 

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.

Moringa leaves and flowers are used as a significant source of vitamins (A, B and C) and minerals (Calcium, iron, Phosphours and Magnesium). 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.

**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.
- **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.

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:**

**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        | 0.56±0.31    | 0.64±0.48           | 0.56±0.35           |

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**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.  

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.  

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.  

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. 

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. Choi et al., 2014 who reported the production of IL-6 that were induced by prevotella intermedia LPS. 

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. IL-6 stimulates gingival fibroblasts to produce collagenolytic enzymes, resulting in periodontal tissue destruction.

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.

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 Lucaciuc 2015, Çalışır et al., 2016 comes in agreement 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.

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.

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).

Swathi et al., 2016 studied the effects of MOE on PD pathogens like Aggregatobacter 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.

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. 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.

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