Research Article

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Effect of *Moringa oleifera* on serum YKL-40 level: *In vivo* rat periodontitis model

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Abstract: Periodontitis is a chronic inflammatory disease that destroys the bones and soft tissues that support the tooth as a result of inflammatory reactions. YKL-40 is an inflammatory marker associated with inflammation and is also associated with periodontal diseases. *Moringa oleifera* (MO) is a plant rich in high nutritional values, minerals, vitamins, and other essential phytochemicals. The aim of this study was to investigate the effect of MO administration on serum YKL-40 levels in an experimental periodontitis model. In the study, a total number of 24 female Wistar albino rats, which were 4–5 months old with a body weight of 275 ± 25 g, were used. Animals were divided into three groups. 1st group: Control (n = 8), 2nd group: periodontitis group (PG) (n = 8), 3rd group: Periodontitis group (PG + MO) with an additional 200 mg/kg/4 weeks MO (n = 8). In the PG and PG + MO were formed experimental periodontitis model. Compared to the PG, the decrease in the interleukin-6 (IL-6) and YKL-40 values in the PG + MO ($p < 0.05$, $p < 0.05$) was found to be significant in terms of statistical evaluation. As a result, MO decreased YKL-40 levels in the experimental periodontitis model. Although further research is needed, drugs containing MO can be used in the treatment of periodontal diseases.

Keywords: experimental periodontitis, YKL-40, *Moringa oleifera*, Chitinase 3-like protein 1, *Moringa* tree

1 Introduction

Periodontitis is a chronic inflammatory disease that is initiated by microbial infections and results in the destruction of the bone and soft tissues that support the tooth as a result of inflammatory reactions [1]. Periodontitis is considered the main cause of tooth loss [2]. It is the inflammation in the periodontium characterized by the deterioration of the balance between the biofilm attached to the tooth surface and the host defense mechanism over time. At the end of a series of inflammatory processes in periodontitis, periodontal tissues lose their physiological properties. As a result, findings such as periodontal pocket, bleeding in the examination of the gingival groove with the periodontal probe, gingival recession, and loss of attachment in the ongoing process are seen. With the continuation of this physiopathological condition, migration, mobility, and tooth loss are observed in the teeth [3].

Moringa oleifera (MO) is a tree that grows in tropical and subtropical regions of the world. In terms of high nutritional value, every part of the tree is precious. The leaves of MO are rich in minerals, vitamins, and other essential phytochemicals. Studies on its use as an antioxidant, anticancer, anti-inflammatory, antidiabetic, and antimicrobial agent in therapeutic use are continuing [4,5]. In experimental studies with MO, it was observed that there was a significant decrease in gene expression of pro-inflammatory cytokines tumor necrosis factor alpha (TNF-α), IL-6, and interleukin 1 beta (IL-1β) [6].

YKL-40 (Chitinase 3-like protein 1, CH3L1) is an inflammatory marker associated with both acute and chronic inflammation [7,8]. YKL-40 is secreted from neutrophils and macrophages activated by arthritic chondrocytes in acute or chronic inflammation [9]. It has been shown that YKL-40 is also produced by vascular smooth muscle and endothelial cells [10] and embryonic and fetal cells [11]. In addition, YKL-40 is a growth factor for connective tissue cells (fibroblasts, chondrocytes, and human synovial cells) and initiates a signaling cascade that leads to increased cell proliferation in these cells [12]. Conversely, YKL-40 has a role in angiogenesis by stimulating adhesion, migration,

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and reorganization of vascular endothelial cells [12,13]. It has been reported that YKL-40 is highly multifunctional and may play a central role primarily in homeostasis-related pathological conditions [14]. In addition to all these, it has been reported that the amount of serum YKL-40 increases in periodontitis and with the progression of periodontitis [1,15].

The aim of this study was to investigate the effect of MO administration on serum YKL-40 in experimental periodontitis model.

2 Materials and methods

The study was approved by NEU Experimental Animals Local Ethics Committee (2021-048). Then the study was performed at KONÜDAM, within the same university. The shelter and care of the animals were secured in the same center in accordance with the regulations on experimental animals.

In the study, a total of 24 female Wistar albino rats, which were 4–5 months old with a body weight of 275 ± 25 g, were used. The animals were housed as five rats in each cage and, maintained under standard conditions of temperature (22°C) and humidity (50%), in an air-conditioned room (12 full changes of air per 1 hour), with regular 12 hours light/dark cycle. Animals were divided into three groups. 1st group: Control (n = 8), 2nd group: periodontitis group (PG; n = 8), 3rd group: Periodontitis group (PG + MO) with additional 200 mg/kg/4 weeks MO (n = 8).

Experimental periodontitis was induced via ligation method [16,17]. Experimental animals were set under combined anesthesia with ketamine-HCL (75 mg/kg) and xylazine (10 mg/kg) intraperitoneally. And then, silk ligatures (4-0) (Dogsan Pharmaceuticals Industry, Istanbul, Turkey) were firmly placed bilaterally into the subgingival location of the mandibular first molar teeth. For ligature placement, a slight separation in the interproximal area between molars with the use of a periodontal probe (Hu-Friedy, Chicago, Illinois) was performed [18,19].

The ligatures were evaluated every other day, gently displaced apically into the gingival sulcus to provide a subgingival position, and repositioned if necessary to maintain the ligature into the subgingival position, thus alveolar bone destruction and gingival recession was observed at 14 days. All ligation procedures were performed by an experienced operator (F.U.Y).

Preparation of MO extract: 50 grams of dried MO leaves were ground at a frequency of 50 hertz for a total of 10 min at intervals (IKA T 65 Digital Ultra-Turrax). 250 ml of methanol was added to it and left to stir for 48 h at room temperature at 500 rpm under a magnetic stirrer (HeidolpH MR Hei-Tec). In order to obtain methanol extract, it was centrifuged at 4,500 rpm for 5 min and the precipitate was discarded, and the supernatant was separated into a drying oven (Core NF 1200R). In order to remove the methanol in the supernatant parts and to obtain the powdered *Moringa* extract, methanol was first evaporated under the heater at 70°C for 4 h, then it was kept in an oven at 40°C for 2 days and dried completely (Nüve FN 120). The resulting dry powder was mixed for 5 min in order to reach the same size.

Before administrating the prepared MO dry powder, it was dissolved in drinking water and the mixture was homogenized. MO applications were administered by oral gavage, at the same time every day, at the indicated doses for 4 weeks. The groups that did not receive MO were given the same amount of drinking water by oral gavage to experience the same stress.

Collection of serum samples: On the last day of the study, 24 rats were bled by cardiac puncture after ketamine–xylazine (50 mg/kg to 10 g/kg) (Ketalar, Pfizer, Turkey – Rompun, Bayer, Turkey) anesthesia. Then, sacrification was carried out. Blood taken from the heart was transferred to biochemistry tubes with gel and clot activator. Then, as is described, it was performed [20].

The levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, and creatinine were measured using commercially available kits based on routine methods on Architect C8000 System (Abbott Laboratories, Abbott Park, Illinois, USA) [21]. Rat YKL-40/CHI3L1 (Cat No: E0899Ra) ELISA kit for YKL-40 levels, Rat IL-10 (Cat No: E0108Ra) ELISA kit for IL-10 levels, Rat IL-6 (Cat No: E0135Ra) ELISA kit for IL-6 levels were analyzed according to the manufacturer's instructions (Bioassay Technology Laboratory Rel Assay Diagnostics).

2.1 Statistical analysis of data

All data were analyzed using Statistical Package for the Social Sciences (SPSSv16.0; SPSS, Chicago, IL, USA) statistical software. The conformity of the data to the normal distribution was made using the Shapiro–Wilk test. Statistical comparisons were made with the test of variance (One Way ANOVA), including correction for Bonferroni. The results are given in the table as mean value + standard error of mean (SEM). Values less than $p < 0.05$ were considered statistically significant.

3 Results

There was no significant difference between the groups in terms of statistical evaluation of ALT, AST, urea, and creatinine serum levels, which are liver and kidney function tests. The increase in IL-6 and YKL-40 values in the PG compared to the control group ($p < 0.05$, $p < 0.001$) was found to be significant. The decrease in the IL-6 and YKL-40 values and the increase in IL-10 values in the PG + MO, compared to the PG ($p < 0.05$, $p < 0.05$, $p < 0.05$), were found to be significant in terms of statistical evaluation. More detailed results are given in Figures 1–3.

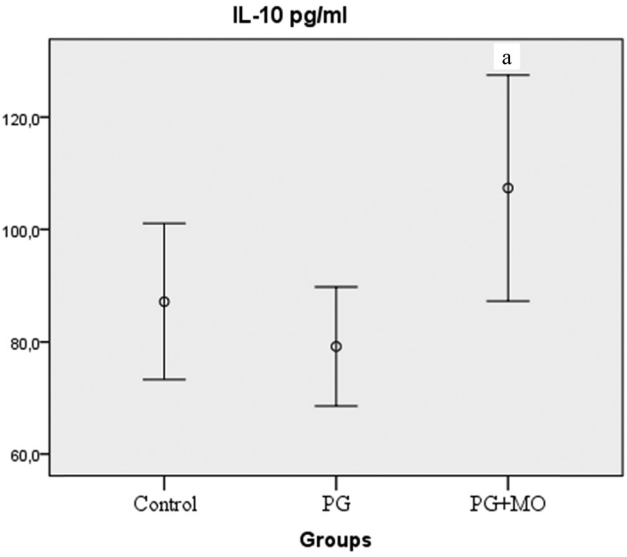


Figure 2: IL-10 values in the groups. The groups were compared using ANOVA between groups values. PG: Periodontitis group, PG + MO: Periodontitis + 200 mg/kg *Moringa oleifera* group. Different superscripts indicate significant difference (lowercase letters: control, PG; number: $p < 0.05$, $p < 0.008$, $p < 0.001$).

4 Discussion

In this study, the effect of MO administration on periodontal inflammation on YKL-40, IL-6, and IL-10 was evaluated by creating an experimental periodontitis model in rats. First of all, an experimental periodontitis model was created on rats selected as experimental animals. In this model, experimental periodontitis was induced by ligature ligation to the first molar teeth for 14 days. Although experimental periodontitis models have been created in different studies in the literature, in different time periods, Kuhr et al. have attached ligatures to the molars of rats and evaluated alveolar bone loss morphometrically in the sections they took from jaw samples on 1st, 15th, 30th, and 60th days after sacrification. They stated that the bone loss on the 30th and 60th days continued in the form of a slight increase [16]. In the light of this information, the experimental periodontitis model in our study was created over a period of 2 weeks.

Periodontal disease encompasses multifactorial disease involving bacterial biofilm and formation of the

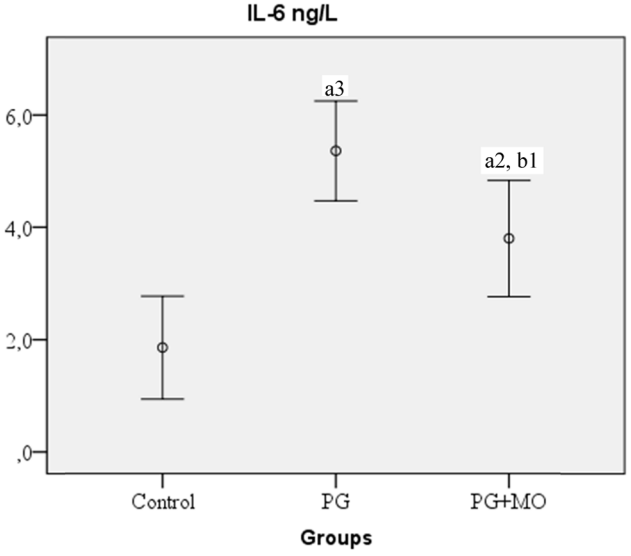


Figure 1: IL-6 values in the groups. Between groups* compared to; (control: a), (PG: b), [(1: $p < 0.05$), (2: $p < 0.008$), (3: $p < 0.001$)] values. *: According to ANOVA. PG: Periodontitis group, PG + MO: Periodontitis + 200 mg/kg *Moringa oleifera* group.

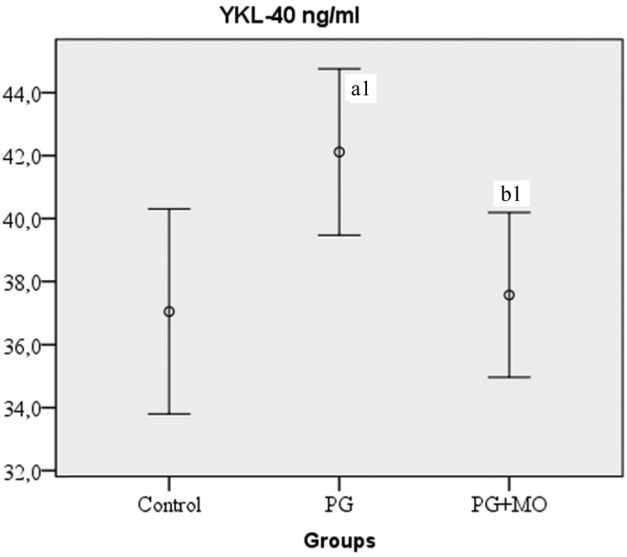


Figure 3: YKL-40 values in the groups. The groups were compared using ANOVA between groups values. PG: Periodontitis group, PG + MO: Periodontitis + 200 mg/kg *Moringa oleifera* group. Different superscripts indicate significant difference (lowercase letters: control, PG; number: $p < 0.05$).

inflammatory response. Bacterial biofilm has been shown to be the primary etiological factor in the initiation of gingivitis and destruction of periodontal tissues. Although a pathogen is required for the formation of periodontal disease, the progression of periodontal disease is dependent on the host response to pathogenic bacteria colonizing the tooth surface. Many bacterial products can affect the host's response. These products initiate a local host response that includes generation of prostaglandins and cytokines, release of inflammatory cells, activation of osteoclasts, and production of lytic enzymes and reactive oxygen species. A better understanding of the pathogenesis of periodontal disease is very important for the treatment of the disease. Therefore, innovations are being researched in the treatment and follow-up of periodontal diseases. Animal studies are frequently used to elucidate this pathogenesis and to develop treatment methods [22].

Leira Y et al. (2020) in their study they created a periodontitis model in rats, followed IL-6 and IL-10 levels for 14 and 21 days. Researchers reported in their studies that IL-10 levels decreased significantly over time, IL-6 and TNFα levels increased significantly, periodontitis occurred at the end of 14 days, and this was accompanied by alveolar bone loss [23]. Keles et al. (2014) reported that YKL-40 and IL-6 levels in gingival crevicular fluids and serum were higher than in healthy subjects ($p < 0.01$), and YKL-40 and IL-6 levels were also higher in patients with periodontitis than in patients with gingivitis ($p < 0.01$) [1]. The same researchers, in another follow-up study, reported that YKL-40 and IL-6 levels were significantly decreased in the saliva and gingival crevicular fluids of patients with chronic periodontitis (non-surgical) after treatment ($p < 0.001$, $p < 0.001$). They reported that monitoring the level of YKL-40 in body fluids before and after periodontal treatment may be necessary to evaluate the current state of periodontitis [15]. In a similar study, patients with gingivitis and patients with periodontitis were compared and they reported that serum YKL-40 levels were significantly higher in patients with periodontitis ($p \leq 0.001$). They found a significant increase in serum YKL-40 concentration with the clinical parameter directly related to pocket depth and then they reported that YKL-40 was associated with the severity of periodontitis [24].

Leaves of MO are extremely nutritious and rich in amino acids, vitamins, minerals, and natural antioxidants [25,26]. Therefore, MO has been studied in many diseases. However, there are a few studies in the literature about its use in the treatment of periodontitis in the last two years [27]. Wang et al. reported that MO suppressed inflammation by increasing the expression of p38α/MAPK14-OPG/RANKL in an experimental periodontitis model [19]. Again, Duartre et al. compared the use of miswak and the use of MO toothpaste in patients with moderate gingivitis. As a result, they reported that toothpaste with MO can help improve oral hygiene [28].

In our study, a dose of 200 mg/kg MO was administered because it was seen that when MO was given at this dose in the experimental periodontitis model in our previous preliminary study with MO, it was seen to reduce inflammation. In that study, 100, 200, and 400 mg/kg MO doses were administered. There was no significant difference between the groups in the levels of TNF α, IL-1β, malondialdehyde, superoxide dismutase, and glutathione peroxidase between the group in which we administered 100 mg/kg MO and the PG. Therefore, we interpreted this dose as not showing a protective effect. There was a significant difference in these biochemical parameters between the 400 mg/kg MO group and the PG group. But we observed in a few animals in the 400 mg/kg MO group that there was a 1.5–2-fold increase in ALT and AST levels. In the group administered with 200 mg/kg MO, there was a significant difference both in terms of biochemical parameters and no significant difference was observed in liver and kidney function tests. Therefore, we preferred 200 mg/kg MO dose in this study. Our research results are consistent with the studies mentioned above, and YKL-40 levels, which are known to increase with periodontitis, decreased as a result of MO administration [1,15]. We think that this decrease occurs as a result of suppression of inflammation.

Since MO has anti-inflammatory, antibacterial, and antioxidant properties, we thought that it would contribute to these anti-inflammatory properties in the group administered with MO. Therefore, the anti-inflammatory cytokine IL-10 values were included in the study. Thus, it was aimed to evaluate the change in anti-inflammatory properties between groups [4,5,29]. Accordingly, IL-6 levels decreased significantly in the PG + MO (1.86 ± 0.39) compared to the PG (5.36 ± 0.38), whereas IL-10 values increased significantly in the PG + MO (107.4 ± 8.51) compared to the PG (79.2 ± 4.48). However, the fact that IL-10 levels did not decrease significantly in PG compared to the control group may be due to individual differences (Figure 2). In addition, we think that the significant increase in IL-10 levels in the PG + MO group compared to the PG group is due to the known antioxidant properties of MO [25,26]. In other words, we think that MO suppressed inflammation by contributing to the antioxidant mechanism of animals in PG + MO.

There is no difference between the groups in terms of liver and kidney function tests (Table 1). In other words, it can be said that the administration of the MO dose used

Table 1: Comparison of liver and kidney function tests and IL-6, IL-10, and YKL-40 values in study groups (mean value ± SEM)

Parameters	Control	PG	PG + MO
ALT (U/l)	63.5 ± 2.4	65.5 ± 5.4	63.9 ± 2.5
AST (U/l)	103 ± 2.9	102.6 ± 5.4	101.9 ± 4.9
Urea (mg/dl)	30.4 ± 0.7	29.5 ± 0.9	28.1 ± 0.8
Creatinine (mg/dl)	0.55 ± 0.01	0.54 ± 0.01	0.52 ± 0.01
IL-6 (ng/l)	1.86 ± 0.39	5.36 ± 0.38 [a3]	3.80 ± 0.44 [a2,b1]
IL-10 (pg/ml)	87.2 ± 5.87	79.2 ± 4.48	107.4 ± 8.51 [b1]
YKL-40 (ng/ml)	37.1 ± 1.38	42.1 ± 0.12 [a1]	37.58 ± 0.11 [b1]

The groups were compared using ANOVA between groups values. PG: Periodontitis group, PG + MO: Periodontitis + 200 mg/kg *Moringa oleifera* group. Different superscripts indicate significant difference (lowercase letters: control, PG; number: $p < 0.05$, $p < 0.008$, $p < 0.001$).

in the experiment for 4 weeks was not toxic in clinical and biochemical evaluation and does not have any side effects. These findings in our study are consistent with other studies in the literature evaluating ALT, AST [30,31], urea, and creatine [32,33] levels.

Limitations of our study are that serum YKL-40 levels were not measured before periodontitis occurred in all groups at the start of the study. If it had been measured, YKL-40 would have been evaluated both within and between groups.

It is the first study in which the level of YKL-40 was determined among the studies in which the experimental periodontitis model was created by applying MO. In addition, since YKL-40 values increase in different physiopathological conditions, it is a more randomized study than human studies, since pathological conditions in which other YKL-40 levels are affected, except periodontitis, are postponed. In this respect, it sheds light on clinical studies.

As a result, MO suppressed YKL-40 levels in the experimental periodontitis model. Although further research is needed, drugs containing MO can be used in the treatment of periodontal diseases. In addition, toothpastes containing different amounts of MO can be produced and further studies can be conducted to investigate the protection of these toothpastes against periodontal diseases.

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Conflict of interest: The authors declare no conflict of interest.

Ethical approval: The conducted research is not related to either human or animal use.

Data availability statement: Derived data supporting the findings of this study are available from the corresponding author on request.

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