EFFECT OF NIGELLA STIVA AND LIPIDUM SATIVUM SEEDS ON PERIODONTAL LIGAMENTS AND ALVEOLAR BONE STATUS OF HYPERCHOLESTEROLEMIC RATS: HISTOPATHOLOGIC AND IMMUNOHISTOCHEMICAL STUDIES

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ABSTRACT

Background: Plant bioactive chemicals have a wide range of pharmacological properties that are thought to be advantageous in the treatment and prevention of a variety of diseases, including hypercholesterolemia. The aim of the present study was to evaluate the effect of Nigella Sativa (NS) and Lipidum Sativum (LS) seeds on periodontal ligament (PL) status and alveolar bone (AB) of hypercholesterolemia (HC) rats.

Materials and methods: 40 rats were randomly divided into four main equal groups and classified as follows: group I: negative control. Group II: HC rats. Group III: HC rats treated with NS seed. Group IV: HC rats treated with LS seed. Rats were euthanized and the jaws of rats were detached for evaluation of PL and AB and processed for histologic and immunohistochemical evaluation of vascular endothelial growth factor (VEGF) and tumor necrosis factor-α (TNF-α).

Results: rats fed with hypercholesteremic diet showed a significant increase (P≤0.001) in total serum cholesterol compared to normal and both treated groups that reflect on the histopathologic condition of PL and alveolar bone showed massive degeneration of collagen fibers and alveolar bone in the HC group. While both treated groups showed more improvement in the condition of PL and alveolar bone. The immunohistochemical results showed a statistically significant decrease (P≤0.001) in VEGF and an increase in (TNF-α) in HC group compared to both treated groups.

Conclusion: Both NS and LS were effective in decreasing total serum cholesterol and could be used as an adjunct to the standard anti hypercholesteremic drugs.

KEYWORDS: hypercholesterolemia, Nigella Stiva, Lipidum Sativum, periodontal ligaments, alveolar bone, VEGF, TNF-α
INTRODUCTION

Hypercholesterolemia is a form of hyperlipidemia that cause atherosclerosis, and chronic inflammation,\(^1\) and aggravates the loss of alveolar bone induced by periodontal disease.\(^2\) It is diagnosed by the increase in the serum or plasma level of very-low-density lipoprotein cholesterol (VLDL-C), low-density lipoprotein cholesterol (LDL-C), and total cholesterol (TC).\(^3,4\)

A review of the literature points to a bidirectional relationship between hyperlipidemia and periodontitis. One of the explanations for this link is that dyslipidemia and periodontal disorders share comparable inflammatory mechanisms.\(^5\) Lipids can modify macrophage gene expression to create activated macrophage, which raises the levels of proinflammatory cytokines like TNF and interleukin-1(\(\text{IL-1}\)) as well as important polypeptide growth factors like platelet-derived growth factor and transforming growth factor-\(\text{I}\).\(^6,7\) Furthermore, regardless of the mechanism of induction, serum lipids can boost polymorphonuclear neutrophil (PMN) production while impairing wound healing by blocking macrophage synthesis of critical polypeptide growth factors.\(^8\)

The deposition of cholesterol in many cells, such as endothelial cells, has been linked to decreased antioxidant responses and increased formation of reactive oxygen species (ROS). Excess ROS buildup can cause inflammation, cell death, and metabolic disturbances. As a result, substances with antioxidant and anti-inflammatory capabilities, as well as the ability to reduce hypercholesterolemia and other hyperlipidemias, can help to avoid the metabolic changes caused by excessive lipid levels.\(^9\)

Medicinal herbs are an important source of bioactive chemicals that can have anti-hyperlipidemic actions affected by oxidative stress and inflammation suppression.\(^10\) Extensive research on Nigella Sativa (NS) or black cumin has been conducted, and a wide range of its pharmacological actions have been investigated, including anti-diabetic, anticancer, immunomodulator, analgesic, antimicrobial, anti-inflammatory, spasmylytic, bronchodilator, hepatoprotective, renal protective, gastro-protective, antioxidant properties, and it has been studied for its lipid-modifying abilities.\(^10,11&12\)

Garden cress, commonly known as Lepidium Sativum (LS), is a Brassicaceae family member prevalent in Egypt and West Asia. It has a significant antioxidant activity owing to the presence of phenolic chemicals.\(^13\) LS seed extracts have previously been shown to produce hypoglycemic effects,\(^14\) it possesses antihyperlipidemic and antihypercholesterolemic properties,\(^15\) as well as kidney and liver protection.\(^15\)

In light of the above information, the current study was designed to assess the impact of Nigella Stiva and Lepidium Sativum on the periodontal ligament and alveolar bone of rats with hypercholesteremia and immunohistochemical expression of VEGF and TNF-\(\alpha\) in the PL and AB.

MATERIAL AND METHODS

Sample size calculation:

The sample size was determined using an effect size of 0.65 and alpha (\(\alpha\)) and beta (\(\beta\)) level of 0.05, i.e., power = 85 percent; the minimum sample size (\(n\)) calculation was a total of 40 samples for four groups.

Animals and ethics:

A total of 40 healthy adult male albino rats with an average body weight of 200 ± 20 gm were obtained from the Laboratory House of College of Veterinary Medicine and Animal Resources, Suez Canal University, Egypt. Before starting the experiment, rats were acclimated for 10 days. All animals were fed with a basal laboratory diet and supplied drinking tap water ad libitum. The typical diet comprised of corn starch (497g/kg), casein (200g/kg), mineral mixture (100g/kg), sucrose (100g/kg), cellulose (30g/kg), corn oil (50g/kg), DL-methionine (3g/kg) and vitamin mixture (20g/kg).\(^17\) The laboratory animals

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were put up in standard raised wire bottom cages in order to maintain hygienic conditions, five rats per cage, housed in air-conditioned rooms at 21-23°C and 60-65% of relative humidity, and maintained on a 12 h light/12 h dark cycle.

The rats were maintained in accordance with the USA National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. The present study has been approved by the Research Ethical Committee faculty of Dentistry Suez Canal University (serial number 257/2020).

Induction of hypercholesterolemia:

Cholesterol and cholic acid were obtained from EL-Gomhoria Co., Egypt as a pure powder. 4% cholesterol and 0.5% cholic acid (hypercholesteremic diet, HCD) were added to the basal diet for 8 weeks. Bile salt mixture (2.5 gr) is necessary for intestinal absorption of cholesterol. 

The plants material:

N. Sativa (NS) and Lepidium sativum L. seeds (LS) were obtained from Agricultural Research Center. Garden cress seeds were left to dry in a hot air (40–60°C) and were crushed in a miller before the dietary supplementation.

Experimental design:

Forty rats were randomly divided according to dietary intake into four main equal groups, 10 rats each, placed in individual cages and classified as follows:

**Group I:** (negative control) 10 rats were fed an ordinary diet only during the entire experimental period of 8 weeks.

The remaining rats were fed a basic diet with 2% cholesterol and 0.5% cholic acids for 4 weeks to induce HC then divided into 3 groups and remained fed on HCD during the experimental period (8 weeks).

**Group II:** 10 rats were fed with HCD.

**Group III:** 10 rats were fed with HCD admixed with 7.5 g/kg body weight/day crushed NS seed.

**Group IV:** 10 rats were fed with HCD mixed with 6 g/kg body weight/day crushed LS seeds.

At the end of the experiment, the rats were euthanized (8 weeks). A heart puncture was used to collect 5 mL blood samples. The obtained blood samples were centrifuged for 10 minutes at 3000 rpm to extract serum for total serum cholesterol assessment.

Histopathologic Analysis:

The jaws of rats were detached for PL and AB evaluation. For bone decalcification, the specimens were assembled and immersed in 10% formalin after 24-48 hours they were inserted in 10% EDTA (pH 7.4) and every week for 3-5 weeks, the solution was replaced. The specimens were flushed with (phosphate-buffered saline) PBS and then immersed in 70%, 80%, 96% ethanol (90 minutes for each one), two absolute ethanol immersions (60 minutes each), two xylol immersions (90 minutes each) and two liquid paraffin immersions at 60°C (120 minutes each). Finally, sections of 5 μm thickness were prepared using a microtome and mounted on adhesive-coated glass slides. Sections were stained with hematoxylin-eosin for histological examination and Streptavidin-biotin immunohistochemical method for VEGF and TNF-α identification.

Immunohistochemical Analyses:

The expressions of VEGF and TNF-α were demonstrated using commercial indirect immunoperoxidase streptavidin/biotin (ThermoScientific, CA, USA) kits. In the oven at 60°C, the paraffin sections were incubated for 30 min, then deparaffinized in xylol, and rehydrated in alcohol series and distilled water. The sections were incubated in 0.1% hydrogen peroxide solution for 10 min at room temperature to eliminate endogenous peroxidase activity. Sections were then washed with TBS and boiled...
with Citrate Buffer (pH 6.0) solution for 20 min for retrieval of the antigen, Ultra V Block solution was applied for 10 min to complete the protein blocking phase. The primary antibody (VEGF and TNF-α) for the test antigen was inserted in the sections without washing protein blockage serum and incubated in a humidity chamber at room temperature for 60 minutes. The sections have been stained with chromogen AEC for a color reaction following application of biotin-labeled antimouse, rat, rabbit polyclonal secondary anti-mouse (Novacastra, catalog No; RE7103) for 15 min and streptavidin-peroxidase enzymes for 15 min. The sections were treated with Mayer’s hematoxylin for 1 minute for contrasting staining and sealed with a water-based mounting medium. Immunohistochemical slides were investigated, and microphotographs were captured under the light microscope of the Olympus BX50 with a DP70 digital camera connected. Then, the optical density of (VEGF and TNF-α) positive cells and the intensity of the immunostaining were assessed using the J Image analyzer computerized system.

**Histopathogical results (Hematoxylin & eosin)**

**Control group (group I):**

Light microscopic examination of the periodontal ligament (PDL) and alveolar process of the control group shows that the PDL is formed of cells and intercellular substance, the latter is formed of well-oriented fibers mostly collagen and ground substance in addition to the blood vessels, lymphatics, and nerves. The gingival and interdental (transeptal fibers) group of fibers are present in the cervical region, the alveodental group of fibers are extending from the cementum to the alveolar bone and divides into the alveolar crest, horizontal, oblique, apical, and interradicular groups. The fibroblast cells had basophilic cytoplasm and nuclei that usually appeared elongated and had the direction of adjacent collagen fibers.

The attachment epithelium is usually thin and consists of few layers of nonkeratinized stratified squamous epithelium. The epithelial cells are resting on smooth basement membrane with no evidence of epithelial ridges. The subepithelial connective tissue (lamina propria) was formed of cells and intercellular substance was formed of collagen fibers embedded in the ground substance. The cells are mainly fibroblasts, progenitor cells, and defensive cells. Sometimes, inflammatory cells could be detected in the lamina propria. The alveolar process reveals its compact bone consists of Haversian canals surrounded by concentric lamellae of bone. Well-developed osteocytes were present within their lacunae. Marrow cavities cannot be neglected (Figures 1; A,B, C).

**Group II animals (Hypercholesterolemic group):**

The periodontal ligament obtained from hypercholesterolemic rats showed marked degeneration, dissociation, and loss of orientation of the collagen fibers and their frequent detachment either from the cementum, bone, or both. There was apparent reduction in the number of fibroblasts which revealed marked cytoplasmic vacuolization and loss of normal arrangement. Cholesterol clefts were demonstrated between the collagen fibers together with marked fibroblastic fatty infiltration and degeneration. Areas of fatty infiltration totally replace the periodontal ligament. Cells of the attachment epithelium revealed signs of degeneration in focal areas manifested as hydropic degeneration and fatty infiltration. A tendency for apical migration with pocket formation was seen. Pathological folding of the basement membrane was also encountered. The subjacent lamina propria revealed areas of cellular and fibrous degeneration and increase in chronic inflammatory cells infiltration. Blood vessels presented a marked increase in the thickness of their walls. Massive osteoclastic alveolar bone resorption resulted in marked rarefaction of bone trabeculae, multinucleated osteoclasts were identified within their Howship’s lacunae, in addition to extreme widening of the marrow cavities associated with extensive fatty and massive inflammatory cell
infiltration were the most observed effect and common feature. Cementum showed surface irregularity cannot be neglected (Figure 1; D, E, F).

**Group III and group IV animals (Rats fed with HCD admixed with crushed NS and LS respectively):**

The examined periodontal ligament and alveolar process of group III and group IV rats fed with HCD admixed with crushed NS and LS seeds respectively showed an improvement in the condition of the periodontal fibers and the cells although areas of degeneration of fibers and cells were still existing, dissociation of the collagen fibers was limited to focal areas. Dentogingival junction showed improvement of the surface epithelium and lamina propria. A decrease in the tendency of bone resorption was observed clearly and the widening of the marrow cavities of the alveolar bone was limited compared to those of the hypercholesterolemic group. Osteoclastic activity get decreased leaving a lot of empty Howship’s lacunae, decreased reversal lines indicating a turnover rate close to the control (Fig. 1, G- L)

![Photomicrograph of the specimens taken from all groups.](image-url)

**Fig. (1)** A Photomicrograph of the specimens taken from all groups. A,B,C. showing the PDL bundles of a control specimen, gingival G, Transeptal T, alveolar crest C, horizontal H, oblique O, apical A. The dento-gingival junction showing the attachment epithelium and subjacent lamina propria as well as the alveolar bone were seen (Hx & E., Mag. 40, 100, 200). D, E, F) showing massive degeneration, destruction of the collagen fibers D. Cholesterol clefts, fatty infiltration. Massive osteoclastic alveolar bone resorption, bone rarefaction, multinucleated osteoclasts were identified within their Howship’s lacunae, extreme widening of the marrow cavities with extensive fatty and massive inflammatory cell infiltration stars. Apical migration arrow and fatty degeneration of the attachment epithelium. G-L) showing group III and group IV rats revealed improvement in the condition of the periodontal fibers, cells, dentogingival junction and alveolar bone. Areas of degeneration D still existing. (Hx & E., Mag. 200).
Immunohistochemical results:

Immunohistochemical localization of vascular endothelial growth factor

The new blood vessels formation can be detected by the immunohistochemical expression VEGF. Sections of the periodontal ligament, alveolar bone and dentogingival junction of the negative control group displayed moderate to strong staining reaction to VEGF mono-colonal antibody (Fig. 2; A, B). Those sections of HC rats revealed a remarkable reduction in the staining reaction compared to the control group (Fig. 2; C, D). While those specimens of rats fed with HCD admixed with crushed NS and LS seeds respectively showed their examined tissues showed strong staining reactivity. (Fig. 2; E-H).

Immuno-histochemical localization of TNF-α

TNF-α expression (brown staining) in normal control tissue shows no to weak expression localized to the periodontal ligament, alveolar bone, the epithelium, and lamina propria of the dentogingival junction (Fig. 3; A, B). While rats fed with HC diet showed massive expression of TNF-α all around the above-mentioned tissues, particularly in the areas of severe inflammation, tissue degeneration and destruction (Fig. 3; C, D). Samples of rats fed with HCD admixed with crushed NS and LS seeds respectively showed nearly completely withdraw expression of TNF-α in the above-mentioned tissues (Fig. 3; E-H).

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Fig. (2) A photomicrograph of the periodontal ligament and bone incubated with mouse monoclonal antibody of VEGF. A, B) Group I: negative control group showed moderate to strong immunostaining reactivity of the periodontal ligament, and alveolar bone. C, D) Group II: (HC) showed mild to moderate immunostaining reactivity. E, F) Group III: (HC+NS) showed strong immunostaining reactivity. G, H) Group IV: (HC+LS) showed strong immunostaining reactivity (Orig. Mag. 200).
**Statistical Analyses**

Data were expressed as means ± standard deviation (SD). The data of total serum cholesterol and immunohistochemical scores were analyzed using SPSS windows program version 15 (SPSS Institute, Inc., Chicago, IL, USA). The One-way Analysis of Variance (ANOVA) was used to analyze the data. A post hoc test was carried out to identify whether there is any significant difference between the individual groups. P-value less than 0.05 was considered to be significant.

**Total serum cholesterol:**

The total serum cholesterol level showed a statistically significant increase (P< 0.01) in group II compared to the normal group and groups III and IV. (Table 1)

**TABLE (1) Comparison of means and standard deviation of total serum cholesterol between the groups at the end of the studies**

<table>
<thead>
<tr>
<th>Biochemical parameter</th>
<th>Group I (control)</th>
<th>Group II (HC)</th>
<th>Group III (HC+NS)</th>
<th>Group IV (HC+LS)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>50.93±4.28</td>
<td>261.42±8.52</td>
<td>74.65±3.37</td>
<td>76.09±3.89</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Different letters mean significant difference at P<0.05

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Fig. (3) A photomicrograph of the periodontal ligament and bone incubated with mouse monoclonal antibody of TNF-α. A,B) Group I: (negative control) showed mild immunostaining reactivity. (C,D) Group II: (HC) showed strong immunostaining reactivity. E,F) Group III: (HC+NS) showed moderate immunostaining reactivity. G, H) Group IV: (HC+LS) showed moderate immunostaining reactivity (Orig. Mag. 200).
VEGF:

The expression of VEGF showed a clear significant difference between the studied groups for the using one-way ANOVA (F= 43.45, P< 0.001). The pair-wise comparison showed a significant difference between each group except NS treated group vs LS treated group. The high value was recorded in group NS treat (106.23±7.15) followed by group LS treat (100.47±4.02) while HC group was the lowest one (72.75±6.48). (Table 2)

**TABLE (2)** Descriptive statistics and results of One way ANOVA test for comparison between means of positive cells expressed VEGF in the four groups

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>F-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>92.50b</td>
<td>9.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HC</td>
<td>72.75c</td>
<td>6.48</td>
<td>43.45</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>NS treat</td>
<td>106.23a</td>
<td>7.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LS treat</td>
<td>100.47a</td>
<td>4.02</td>
<td></td>
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</tbody>
</table>

Multiple Comparisons using Tukey post-hoc

<table>
<thead>
<tr>
<th>Pair wise</th>
<th>Mean difference</th>
<th>95% Confidence Interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Vs HC</td>
<td>19.75</td>
<td>11.30-28.19</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Normal Vs NS treat</td>
<td>-13.73</td>
<td>-22.17-5.28</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Normal Vs LS treat</td>
<td>-7.97</td>
<td>-16.41-0.47</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>HC Vs NS treat</td>
<td>-33.48</td>
<td>-41.92-25.03</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>HC Vs LS treat</td>
<td>-27.72</td>
<td>-36.16-19.27</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>NS treat Vs LS treat</td>
<td>5.76</td>
<td>-2.68-14.02</td>
<td>0.273</td>
</tr>
</tbody>
</table>

**TNF-α**

The results of immunohistochemical expression of TNF-α (pro-inflammatory cytokine) showed a clear significant difference between the studied groups for the TNF-α using One-way ANOVA (F= 266.621, P< 0.001). The pair-wise comparison showed a significant difference between each group except NS treat vs LS treat. The high value was recorded in HC group (156.11±11.34) followed by group LS treat (81.99±7.80) while the control group was the lowest one (50.90 ±8.84). (Table 3)

**TABLE (3)** Descriptive statistics and results of One way ANOVA test for comparison between means of positive cells expressed TNF-α in the four groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
<th>F-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>50.90a</td>
<td>8.84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hypercol</td>
<td>156.11a</td>
<td>11.34</td>
<td>266.621</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>NS treat</td>
<td>78.95b</td>
<td>6.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LS treat</td>
<td>81.99b</td>
<td>7.80</td>
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</table>

Multiple Comparisons using Tukey post-hoc

<table>
<thead>
<tr>
<th>Pair wise</th>
<th>Mean difference</th>
<th>95% Confidence Interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Vs Hypercol</td>
<td>-105.21</td>
<td>-115.70-94.72</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Normal Vs NS treat</td>
<td>-28.05</td>
<td>-38.54-17.56</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>Normal Vs LS treat</td>
<td>-31.09</td>
<td>-41.58-20.60</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>HC Vs NS treat</td>
<td>77.16</td>
<td>66.67-87.65</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>HC Vs LS treat</td>
<td>74.12</td>
<td>63.63-84.61</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>NS treat Vs LS treat</td>
<td>-3.04</td>
<td>-13.53-7.45</td>
<td>0.860</td>
</tr>
</tbody>
</table>

*The test used: ANOVAs test (F) ns; means no significance at \( P<0.05 \)

**DISCUSSION**

Given the link between hypercholesterolemia, higher fasting plasma total cholesterol levels, and periodontal disease, \( 20-23 \) the present study was designed to evaluate the effect of dietary intake of NS and LS on the periodontal ligaments and alveolar bone of hypercholesteremic rats and immunohistochemical analysis of the expression of VEGF and TNF-α in the PL and AB.
In the current study, the biochemical results reported that high dietary cholesterol for eight weeks caused a considerable rise in total serum cholesterol in HC group compared to the control (P< 0.01). Whilst the total serum cholesterol statistically significantly (P< 0.01) decreased in NS and LS treated groups compared to HC group. Our result coincides with previous studies. 12, 24 , 25,26

In view of the present investigation, it was obvious that the hypercholesteremia in group II resulted in marked structural degenerative changes within the periodontal ligament, dentogingival junction and alveolar bone manifested as massive fatty infiltration and degeneration. In addition, vascular changes demonstrated as thrombotic lesions and atherosclerosis were encountered. Moreover, our investigation presented fatty infiltration of the periodontal ligament, dentogingival junction that led to massive degeneration and destruction of collagen fibers.

In line with these observations, María et al., 27 and Macri et al., 28, documented that a high-cholesterol diet was linked to a reduction in periodontal bone support. Previous research has shown that an excess of saturated fat and cholesterol in the diet leads to hyperlipidemia, this causes a pro-inflammatory state by increasing oxidative stress, dysfunction of the endothelial cells, and a decrease in vascular nitric oxide bioavailability, resulting in morphological changes in the periodontal microcirculation. 29-31 Additionally, a high-cholesterol diet may boost the proliferative activity of the cells within the junctional epithelium and cause alveolar bone loss in rats.32 Also, Elisa et al., 33 proved that animals with hypercholesterolemia were more likely to acquire the severe induced experimental periodontal disease than those fed a conventional diet.

Vasculogenesis and angiogenesis promoted by the release of VEGF, a signal protein.34 VEGF is a component of the system that restores oxygen delivery to tissues when blood circulation is insufficient.35 Our immunohistochemical results revealed decreased expression of VEGF in the HC sections compared to their controls. In response to localized tissue ischemia, HC inhibits angiogenesis and collateral vessel development, potentially by reducing tissue nitrous oxide (NO) bioactivity. 36 Also, O’Reilly et al., 37 documented that antiangiogenic protein endostatin was highly expressed in HC animals. Endostatin may not only reduce angiogenesis but also endothelial cell migration and proliferation, additionally promote apoptosis.

It was documented that hypercholesterolemia is a syndrome that causes a significant rise in the production of cytokines, interleukins (IL-1, IL-1, IL-6, and IL-17), and (TNF-α) by monocytes/polymorphonuclear leukocytes (PMNs) furthermore a decrease in the synthesis of growth factors by macrophages. Such a condition may enhance the development of periodontal disease. 38,39 This is consistent with our immunohistochemical analysis which revealed marked expression of TNF-α in group II animals that received HCD for two months. In our opinion that HC could indirectly increase the circulation of inflammatory molecules that may cause and augment the pathological changes observed in the present investigation.

The strong expression of TNF-α in the PL and AB of HC rats was consistent with the histopathologic findings of the present studies that revealed massive alveolar bone resorption leading to marked rarefaction of bone trabeculae. TNFα appears to stimulate and increase osteoclast differentiation mediated by receptor activator of NF-kB (RANK) signaling so, enhancing bone resorption. TNF-α increases the availability of receptor activation via activator of NF-kB by promoting the expression of (RANK) on hematopoietic precursor monocyte/macrophage. 40 Overall, this cytokine can provide an environment abundant with osteoclastic development and activity factors, as well as promote their precursors to migrate to the site of bone destruction.41 The findings of the present study were inline with the study
of Mandal et al.,\textsuperscript{42} who confirmed that hypercholesterolemia activates osteoclasts which contribute to alveolar bone loss via boosting RANKL expression in osteoblasts and lowering osteoprotegerin (OPG) expression in osteoblasts.

In the present investigation, hypercholesteremic rats fed with NS (group III) and LS (group IV) showed a statistically significant decrease (P≥0.001) in total serum cholesterol compared to group II. While there was no statistically significant difference between groups (III & IV).

These findings were coordinated with the study of Shafiq et al.,\textsuperscript{43} who reported that NS markedly affects the lipid profile through its ability to decrease the serum cholesterol LDL and triglycerides levels in animals and humans. The presence of thymoquinone (TQ), which is the primary component of seed essential oil, has already been linked to the majority of its bioactivities.\textsuperscript{44-46} TQ has been shown to lower oxidative stress and enhance the lipid profile, which in turn improves high blood cholesterol levels and inhibits plaque formation.\textsuperscript{47} Also, Al Nageeb et al.\textsuperscript{48} reported that the hypocholesterolemic impact of N. Sativa seed and oil might be attributable to the total dietary fiber, insoluble dietary fiber, and soluble dietary fiber content of the seed.

The hypocholesterolemic effects of LS displayed in the present study come in accordance with previous research in rats on a high cholesterol diet found that LS seed powder reduced LDL-c and VLDL-c while increasing HDL-c in the serum of rats fed a high fat and cholesterol diet. The reduction of cholesterol production might explain LS’s hypocholesterolemic impact.\textsuperscript{17,49} This is accomplished by inhibiting HMG-CoA reductase, the rate-limiting enzyme responsible for the initial step in cholesterol production. It was further found that the hypocholesterolemic impact of LS may be attributable to its high antioxidant content (vitamin C, E, carotenoids, polyphenols, and flavonoids). With just a single electron transfer, these chemicals may scavenge free radicals, superoxide, and hydroxyl radicals.\textsuperscript{50,51}

To the best of our knowledge, no previous studies compare the effect of NS and LS on the periodontium of hypercholesterolemic rats

The histopathologic finding of the periodontal ligament and alveolar process of NS and LS treated groups which showed an improvement in the condition of the periodontal fibers and the cells as well as the dentogingival junction. Decrease in the tendency of bone resorption was observed clearly and the widening of the marrow cavities of the alveolar bone was limited compared to those of the hypercholesterolemic group.

The immunohistochemical findings in group III and IV were consistent with the histopathologic findings where there was a strong expression of VEGF in both groups which was statistically significant (Ps≤0.001) for HC group these findings may be attributed to their anti-hypercholesterolemic effect that may improve the endothelial dysfunction induced by hypercholesterolemia so enhance angiogenesis.

These findings coincide with the study of Starke et al.,\textsuperscript{51} who reported that Nigella sativa is thought to have an angiogenic impact via increasing VEGF receptor-2 dependent endothelial cell proliferation and migration.

Also, when compared to the HC group, both treated groups showed a significant reduction in TNF-α expression, which coincides with the studies done by Chehl et al.,\textsuperscript{52} and Raish et al.,\textsuperscript{53} revealed that TNF-α and IL-6 mRNA were considerably reduced by NS seed extract, TQ and LS ethanolic extract. This is consistent with the results of the current investigation.

The anti-inflammatory properties of TQ of NS have been demonstrated experimentally.\textsuperscript{54} It lowers nitric oxide (NO) levels by suppressing iNOS mRNA transcription by macrophages and suppressing pro-inflammatory cytokines such as IL-1b, IL-6, TNF-α, interferon-c, and prostaglandin while increasing anti-inflammatory IL-10.\textsuperscript{55,56} Regarding LS, the presence of glycoside, alkaloids, tannin
(Phenolic substance), flavonoids, and amino acids such as glutamine, cysteine, and glycine has been discovered in LS seeds. Its constituents are intermediates in the synthesis of the endogenous antioxidant glutathione, tannin, and flavonoids, all of which have antioxidant properties. Free radicals are scavenged, which inhibits the oxidative processes that cause degenerative disease. 57,58,59

CONCLUSION

According to the findings of this investigation, HC may increase the risk of periodontal disease. Not only by increasing the likelihood of an ischemic event in tissues but also by worsening inflammatory and tissue damage via increased production of the pro-inflammatory cytokine, TNF-α, as well as reduced VEGF expression. Crushed NS and LS seeds supplementation improved the state of the damaged periodontal ligament fibers and cells, dentogingival junction, and alveolar bone, coupled with TNF-α withdrawal and enhanced VEGF production. Both NS and LS have the potential to be employed as adjunctive therapies for hypercholesterolemia. The present research will be useful in attracting greater interest in medicinal plants in the future by identifying as well as generating unique clinical uses and new medication formulations.

REFERENCES


