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The possible protective effects of virgin olive oil and Nigella Sativa seeds on the biochemical and histopathological changes in pancreas of hyperlipidemic rats

The effects of olive oil and Nigella Sativa seeds on the changes in pancreas of hyperlipidemic rats

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Abstract

Background: Hyperlipidemia is a risk factor for the development and progression of atherosclerosis and is linked to various diseases. This study was done to evaluate the possible protective effects of virgin olive oil and Nigella sativa seeds on the biochemical and histopathological changes which occurred in the pancreas of the rats. The study lasts 8 weeks and included 24 albino rats that were divided into four groups (6 rats each); group I, control group, fed with normal standard diet, group II fed with high fat diet (HFD), group III fed with HFD and virgin Olive oil, group IV fed with HFD and Nigella sativa seeds powder.

Materials and Methods: After finished the experiment, blood samples collected and assessed for the lipid profile, fasting blood sugar, pancreatic amylase and insulin levels. Then, the rats were sacrificed and the pancreata were extracted and slices of them were processed for histological examination using hematoxylin stain and Masson's Trichrome stain. Small fragments from the tail of the pancreata were extracted and processed for electron microscopic
examination. The statistical analysis of the data using the appropriate statistical tests was also conducted.

**Results:** In the present study, the serum lipid profile in hyperlipidemic rats are ameliorated in rats fed on high fat diet with virgin olive oil or Nigella sativa seeds powder as a significant decrease in total cholesterol, LDL-cholesterol and triglycerides. Moreover, while Nigella sativa decreases HDL, virgin olive oil increases significantly HDL. Also a significant decrease in the serum levels of blood glucose and amylase and a significant increase in insulin levels are present in these groups. The histological and ultrastructural results revealed regeneration of the exocrine and endocrine parts of the pancreatic tissues from the hyperlipidemic rats fed with virgin olive oil or Nigella sativa seeds.

**Conclusions and recommendations:** From this study, the biochemical results were paralleled to the histological and ultrastructural results, so it could be concluded that virgin olive oil and Nigella sativa seeds had anti-hyperlipidemic and hypoglycemic effects and they could protect the pancreas from hyperlipidemia-induced injury and the daily consumption of virgin olive oil and Nigella sativa seeds in the diets is highly recommended.

**Key Words:** Pancreas, Virgin olive oil, Nigella sativa seeds, Hyperlipidemia, Pancreatitis

**Introduction**

Nowadays, with the excited lifestyle, people less frequently eat fresh fruits and green vegetables that enforces their antioxidant and immunity status. Moreover, consuming high fat diet and insufficient physical activity are factors contributing to the development of hyperlipidemia, which is a major risk factor for the development of atherosclerosis and is highly related to many diseases (1).

**Virgin olive oil** is an efficient food with several nourishing elements like monounsaturated fatty acids. Also, it is a great source of phytochemicals, containing polyphenolic compounds (2, 3). It is healthier to increase the consumption of Monounsaturated Fatty Acids (MUFA) replacing the Polyunsaturated Fatty Acids (PUFA), hence it lowers the risk of atherosclerosis as it reduces the circulating lipoprotein’s sensitivity to peroxidation (4).
Virgin olive oil is an essential ingredient of the Mediterranean diet and is appreciated worldwide because of its nutritional benefits in metabolic diseases, including type 2 diabetes. It augment the key risk factors for cardiovascular disease, for instance the blood pressure, the glucose metabolism, the lipoprotein and antithrombotic profiles (4, 5). Clinical studies provided evidence that consumption of olive oil might lower the risk of heart disease as lowered blood cholesterol levels and reduced LDL cholesterol oxidation (6).

These outcomes are attributed beside the monounsaturated fatty acids (MUFA) to the minor compounds of virgin olive oil as hydrocarbons, polyphenols, tocopherols, sterols, triterpenoids and other nutrients. Most of these components have certified antioxidant properties and hypolipidemic and anti-inflammatory effects (7, 8). Furthermore, diet enriched with MUFA prevents central body fat distribution and decreases postprandial adiponectin expression induced by a carbohydrate-rich diet in insulin-resistant individuals (9).

Nigella sativa seed (N. sativa) is a plant cultured in many countries in the Mediterranean region and Asia, it is known as “Sannouj, Habbat el Baraka or Black seed”. Nigella sativa “black seeds” has long been used as a nice flavor added to variety of Persian cuisine such as salads, pastries and bread, pickle and sauces (9). Organic composition of Nigella sativa seeds includes oil, carbohydrate, fibers, proteins and saponin. The fixed oil chemical compositions of Nigella sativa are linoleic acid, oleic acid, arachidic acid, eicosadienoic acid, palmitic acid, stearic acid and myristic acid (10). The major phenolic compounds of N. sativa seeds are p-cymene (37.3%), thymoquinone (13.7%), carvacrol (11.77%), carvone (0.9%), and thymol (0.33%) (11).

The active constituent of N. sativa, is principally thymoquinone (TQ), has potential therapeutic properties; it exhibited anti-inflammatory effects on several inflammatory disorders including encephalomyelitis, colitis, edema and arthritis through suppression of prostaglandins and leukotrienes as inflammatory mediators (12). Nigella sativa seeds have also antihistaminic and antidiabetic activities are used traditionally for treatment of asthma, cough, bronchitis, headache, rheumatism, fever, and influenza (13).
Nigella sativa seeds are well-known also for their potent antioxidative effects. Some studies demonstrated that N. sativa seeds might preserve significantly the spatial cognitive in rats challenged with chronic cerebral hypoperfusion (14, 15).

Since virgin olive oil and Nigella sativa seeds are commonly used in the diets of Mediterranean countries and Asia and there is little data about plants which has both hypoglycemic and hypolipidemic effects, Therefore, the aim of this study is to evaluate the possible protective effects of virgin olive oil and Nigella sativa seeds on the biochemical and histopathological changes which may occur in the pancreas of the rats which fed with high fat diets.

Ethical approval

This study was conducted according to guidelines and protocols approved by the ethical committee for animal care and use in King Fahd Medical Research Centre (KFMRC), KAU, Jeddah, Saudi Arabia, which are in accordance with the guidelines of the Canadian Council on Animal Care.

Material:

Virgin olive oil and Nigella sativa seeds were purchased from the local traditional market, Jeddah, KSA.

Experimental animals

The albino rats weighing (180 - 230 g) were obtained from the Animal Experimental Unit of King Fahd Medical Research Center, King Abdul Aziz University, Jeddah, KSA. The rats were housed in well- aerated cages in an animal room and maintained in a temperature- controlled room (24°C ± 1°C) with a 12 h light/12 h dark cycle, 55% ± 10% humidity. They were fed with normal standard diet and water ad libitum.

Induction of hyperlipidemia:
The albino rats fed with high fat diet (HFD) in the form of butter, in a dose of 20 gm/100 gm (20%) of diet (16) for 8 weeks. After 3 weeks, blood samples were taken for lipid profile analysis to ensure the occurrence of hyperlipidemia.

**Experimental design:**

A total of 24 rats were used in the study for 8 weeks. The albino rats were divided into 4 groups (n=6), **Group 1**, control group, which fed with normal standard diet and used as reference. **Group II**, hyperlipidemic (non-treated ) group, fed with HFD in a dose of 20 gm/100 gm (20%) of diet (16). **Group III**, fed with HFD and Virgin Olive oil. The animals received 1.5 mg/kg of Virgin olive oil every day by intragastric intubation (17). **Group IV**, fed with HFD and Nigella sativa seeds powder. The seeds were powdered and dissolved in freshly prepared water and the animals received 300mg/kg everyday by intragastric intubation (18).

**Methods:**

1. **Blood analysis:**


2. **Technique for histological study:**
The rats were anaesthetized lightly by diethyl ether inhalation and the abdominal cavity was incised at the midline. The pancreata were removed and specimen were fixed by immersion in formaldehyde 10% for three days. The specimen were dehydrated in ascending grades of ethyl alcohol and cleared in benzene. The specimens were soaked for 3 changes in paraffin and were finally embedded in paraffin wax. A microtome was used to cut the paraffin blocks into serial transverse sections at thickness of 6-8um. The sections were attached to an albumenized glass slides. They were stained with **Hematoxylin and Eosin stain** to study the general structure and **Masson’s trichrome stain** to demonstrate the collagen fibres in the pancreas (19). Slides were photographed using a light microscope (BX51, Olympus, Tokyo, Japan) fitted with an Olympus digital camera, Anatomy Department, Faculty of Medicine, KAU, KSA).

### 3. Technique for transmission electron microscopic study:

Small fragments from the tail of the pancreata were extracted. Samples were fixed by immersion in 5% phosphated buffered glutaraldehyde (PH 7.3). The tissue pieces were post-fixed in 1% buffered osmium tetroxide. Then, specimens were dehydrated, cleared and embedded in Epon 812 (20). Semithin sections (1mm thick) were stained with 1% **toluidine blue** in borax for light microscopy at the Regional Mycology and Biotechnology center, Al-Azhar University, Cairo, Egypt. Selected areas were trimmed down for ultrathin sectioning. Ultrathin sections (60a nm) were cut, doubly stained with **uranyl acetate and lead citrate** (21) and examined using a transmission electron microscope (JEOL 1010 EX II, Japan) at the electron microscopic unit, Ain Shams University, Cairo, Egypt.

### 4. Statistical analysis:

The data were statistically analyzed using SPSS statistical software, version 19.0 (SPSS Inc., Chicago, IL, USA) for Windows. The results were presented as means ± SDs. The differences between the continuous data were analyzed using one-way ANOVA. Data was presented as means ± standard deviations (SD). Differences were considered statistically significant when P<0.05.

### RESULTS:

**Biochemical Results**:

I. Lipid profile concentration:
Table (1) showed that serum levels of triglyceride, cholesterol and LDL in G2 fed on HDL were significantly higher than those of G1 (P < 0.001). The rats in G3 (fed on HFD and virgin olive oil) and G4 (fed on HFD and Nigella sativa seeds) showed a significant decrease (P < 0.001) in the serum levels of triglyceride, cholesterol and LDL as compared with G2.

**II. Fasting blood glucose (FBG) concentration:**
Table (1) showed that fasting blood glucose (FBG) concentration in serum of G2 was increased significantly (P < 0.001) as compared with control G1. The rats in G3 and G4 showed a significant decrease (P < 0.001) in FBG as compared with G2.

**III. Serum pancreatic amylase:**
Table (1) showed the mean serum amylase level in rats in G2 increased significantly as compared with the control rats (P < 0.001). In G3 and G4 decreased significantly (P < 0.001) as compared with G2.

**IV. Serum insulin concentration:**
Table (1) showed the mean serum insulin level in G2 decreased significantly as compared with the control rats (P < 0.001). In G3 and G4 increased significantly (P < 0.001), as compared with G2.

Table (1). Effects of Virgin olive oil and N. sativa seeds supplementation on blood lipid profile, glucose, pancreatic amylase and insulin in rats.

<table>
<thead>
<tr>
<th></th>
<th>G1 (control)</th>
<th>G2 (HFD)</th>
<th>G3 (HFD + Olive oil)</th>
<th>G4 (HFD + N. sativa seeds)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Triglyceride</strong></td>
<td>72.5 ± 5.3 ***</td>
<td>107.69 ± 1.4 ***</td>
<td>77.69 ± 1.39***</td>
<td>60.69 ± 1.4 ***</td>
</tr>
<tr>
<td><strong>Cholesterol</strong></td>
<td>84.4 ± 3.1 ***</td>
<td>212.53 ± 2.7 ***</td>
<td>103.53 ± 3***</td>
<td>100.53 ± 2.62 ***</td>
</tr>
<tr>
<td><strong>LDL-C</strong></td>
<td>47.6 ± 0.6 ***</td>
<td>151.87 ± 2***</td>
<td>51.87 ± 2.42***</td>
<td>64.87 ± 2.4***</td>
</tr>
<tr>
<td><strong>HDL-C</strong></td>
<td>29.4 ± 0.5 ***</td>
<td>40.01 ± 0.2 ***</td>
<td>37.01 ± 0.19***</td>
<td>25.01 ± 0.19***</td>
</tr>
</tbody>
</table>
Data are expressed as mean±/ standard deviation. 1P: Significance versus G1 (Control group) The number of animals was 6 for each group, All values are expressed as means ± SE; Significantly different from untreated hyperlipidemic rats (* p < 0.05, ** p < 0.01 and *** p < 0.001); Significantly different from control.

<table>
<thead>
<tr>
<th>Glucose (mg/dl)</th>
<th>89.7 ± 2.2</th>
<th>197.84 ± 2 ***</th>
<th>144.84 ± 1.9 ***</th>
<th>123.84 ± 1.85***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic amylase (U/L)</td>
<td>85.7 ± 2***</td>
<td>153.72 ± 2***</td>
<td>119.72 ± 2.8 ***</td>
<td>77.72 ± 2.17***</td>
</tr>
<tr>
<td>Insulin (mc IU/mL)</td>
<td>3.76 ± 0.1***</td>
<td>1.23 ± 0.2 ***</td>
<td>3.42 ± 0.2 ***</td>
<td>3.63 ± 0.5 ***</td>
</tr>
</tbody>
</table>

Histological results:

Microscopic examination of Hx &E stained sections from the pancreata of control group 1 rats revealed a normal pancreatic architecture which is organized into lobes and lobules (Fig. 1a). The pancreas was formed of exocrine and endocrine parts. The acini represented the exocrine part and islets of Langerhans represented the endocrine part.

The acini appeared having different sizes and shapes and most of them formed of a single row of pyramidal cells which have dark basophilic nuclei and apical acidophilic zymogen granules (Fig.2a). The islets of Langerhan's appeared as noncapsulated pale stained rounded or oval areas with regular outlines inside the pancreatic lobules. They composed of mainly red B-cells and few peripheral dark A-cells. B- cells are formed most of the density of the islet and occupying the central and peripheral regions of the islet (Fig.3a).

Sections from the pancreata of group 2 rats (fed on HFD) showed a marked thickening and congestion of intralobular blood vessels with widening of intralobular ducts (Fig.1b). The cytoplasm of the pancreatic acini contained many vacuoles and most of their nuclei showed a marked degree of degeneration as they were small, dark and pyknotic (Fig.2b). Most of islets of Langerhans lost their regular outlines with a marked reduction in their sizes and numbers. Most of B- cells appeared degranulated with small degenerated nuclei (Fig.3b). Sections from
the pancreata of group 3 rats (fed on HFD and virgin olive oil) showed that the pancreas nearly restored its normal structure (Fig.1c). The pancreatic acini were a moderately improved in shape and most of them had no vacuolation (Fig.2c). Most of nuclei and zymogen granules appeared nearly similar to those of control. The islet of Langerhans appeared somewhat similar to control group and most of beta-cells in the central and peripheral regions of the islet with normal density (Fig.3c).

Sections from the pancreata of group 4 (rats fed on HFD and Nigella sativa seeds) showing that it is nearly similar to control group (Fig.1d). The pancreatic acini were markedly improved in shape with no vacuolation (Fig.2d). The nuclei and zymogen granules appeared nearly similar to those of control. The islet of Langerhans appeared nearly similar to control group with nearly regular outlines. The beta-cells appeared with normal density in the central & peripheral regions of the islet (Fig.3d).

In sections stained with Masson's trichrome from the pancreata of control group 1, a normal distribution of collagen fibres as appeared as fine collagen fibres in the interlobular septa and around the acini. The collagen fibres are surrounded the interlobular vessels and ducts. Mild collagenous fibrous content appeared incompletely surrounding the islets of Langerhans (Fig.4a). In sections from the pancreata of group 2 fed on HFD, thick collagenous fibres appeared surrounding the dilated interlobular vessels and ducts. Large areas of hemorrhage was appeared (Fig.4b). In sections from the pancreata of group 3 fed on HFD and virgin olive oil and from group 4 fed on HFD and Nigella sativa seeds, the distribution of collagenous fibrous content (Fig.4c and 4d) were improved and decreased in thickness as compared with those of group 2.

Electron microscopic results:

1. Exocrine part:

Electron microscopic examination of ultrathin sections from the pancreatic exocrine cells of control rats of group 1 showed that the apical cytoplasm contains numerous zymogen granules with variable sizes and shapes (Fig.5a). The basal cytoplasm contained quantities of ribosome-attached rough endoplasmic reticulum( RER). Some condensing vacuoles containing secretory
material of low density were seen in the vicinity of the Golgi apparatus, and a few lysosome-like bodies were detected in the apical cytoplasm of the cell. Mitochondria appeared as oval and rod-bodies in both the apical and basal parts of the cell. The nuclei were smoothly rounded in general and occupied the basal portion of the acinus. (Fig.6a).

Electron microscopic examination of ultrathin sections from the pancreatic exocrine cells of rats of group 2 fed with HFD revealed a marked vascular congestion with partial destruction of the blood vessel's walls. The zymogen granules were markedly decreased in number and densities. The cytoplasm of the acinar cells was full with fat droplets and phagocytic vacuoles (secondary lysosomes). The rod-shaped mitochondria appeared swollen (Fig.5b). The rough endoplasmic reticulum appeared swollen, it took the shape of large spherical to irregular vesicular profiles containing fibrillar materials. The nuclei appeared pyknotic with irregular nuclear membranes (Fig.6b). Electron microscopic examination of ultrathin sections from the pancreatic exocrine cells of rats of group 3 fed with HFD and virgin olive oil revealed that the zymogen granules were increased in number and density (Fig.5c). Most of Mitochondria and RER restored their shapes. Most of nuclei appeared with normal shapes with regular nuclear membranes. The chromatin matrix restored its normal arrangement and shape. Few fat droplets were present (Fig.6c).

Electron microscopic examination of ultrathin sections from the pancreatic exocrine cells of rats of group 4 fed with HFD and Nigella sativa seeds revealed that the zymogen granules were markedly increased in number and density (Fig.5d) and became similar to those of control group (Fig. 5a). Most of Mitochondria and RER restored their shapes. The nuclei appeared with normal shapes with regular nuclear membranes (Fig.6d).

2. Endocrine part:

Electron microscopic examination of ultrathin sections from the islets of Langerhans of control rats of group 1 (Figs.7a &8a) revealed that the islet cells were characterized essentially by the morphological features of their endocrine granules. Granules of B-cells appeared rounded in shape variable in size and contained homogenous cores, which were separated from the surrounding membrane by wide electron-lucent halos. Most of these granules had dark electron dense cores. The nuclei of B-cells were irregular in outline and contained both hetero and
euchromatin. Heterochromatin was mostly attached to the nuclear envelope. The A-cells were present in peripheral position beside the acinar cells. Their characteristic granules were more numerous and electron dense than the B-granules and were surrounded by narrower halos and closely applied limiting membranes. The nuclei were regular in outline with small chromatin clumps.

Electron microscopic examination of ultrathin sections from the islet of Langerhans of rats of group 2 fed on HFD revealed that the B-cells showed multiple signs of degeneration as marked degranulation of the B-cells and A-cells with degeneration of their nuclei and mitochondria and rough endoplasmic reticulum appeared swollen (Figs. 7b&8b). A marked vacuolation of the cytoplasm was also present.

Electron microscopic examination of ultrathin sections from the islet of Langerhans of rats of group 3 fed on HFD and virgin olive oil revealed that most of B-cells and A-cells showing marked signs of improvement as restoring the density of the granules and the nuclei became regenerated with normal shape and normal distribution of chromatin matrix (Figs. 7c&8c). Most of mitochondria and RER appeared as those of the control group. Few cytoplasmic vacuoles were present.

Electron microscopic examination of ultrathin sections from the islet of Langerhans of rats of group 4 fed on HFD and Nigella sativa seeds revealed that B- and A-cells are nearly similar to the control cells (Figs. 7d&8d).

**Discussion**

**Blood Analysis**

In the present study, the fasting blood sugar and mean serum amylase levels are increased significantly in G2 rats fed on HFD as compared with the control rats and the serum insulin levels are decreased significantly in G2 rats. These results suggesting that hyperlipidemia predisposes to pancreatitis and are in agreement with the results of other studies (22, 23).

In the present study, the serum lipid profile in hyperlipidemic rats are favorably modified in G3 fed on virgin olive oil and G4 fed on Nigella sativa seeds powder as a significant decrease in total cholesterol, LDL-cholesterol and triglycerides. Moreover, while Nigella sativa decreases
HDL, virgin olive oil increases significantly HDL. Also a significant decrease in the serum levels of blood glucose and amylase and a significant increase in insulin levels are present in these groups. These results suggesting the improvement of the hyperlipidemic pancreatitis.

These results are in agreement with many authors (24-26), they mentioned that the bioactivity of olive tree by product extracts might be related to antioxidant and phenolic composites like oleuropein, hydroxytyrosol, oleuropein aglycone, and tyrosyl. They had shown that oleuropein is related to improved glucose metabolism. The hypoglycemic and antioxidant effects of oleuropein had been reported in alloxan diabetic rabbits. In streptozotocin (STZ) induced diabetic rats, olive leaf extract has decreased serum concentrations of lipids, glucose, uric acid, creatinine, and liver enzymes.

These results are in accordance with many authors (27, 28) who mentioned that the Nigella sativa oil has an antilipidemic action and this action might due to their ability to stimulate insulin secretion from a stimulatory effect on β cell function. The treatment of hypercholesterolemic rabbits with Nigella sativa seeds powder or oil showed hypocholesterolemic and antiatherogenic cardioprotective properties (29, 30).

The consumption of olive oil might reduce the risk factors of heart disease, as lowered blood cholesterol levels and lowered LDL cholesterol oxidation and that it might also possibly influence inflammatory, thrombotic, hypertensive and vasodilator mechanisms (4, 6).

The protective antioxidant effects of olive oil and Nigella sativa oil was explained (31). The mechanism of olive oil might be due to the presence of MUFA, oleic acid, vitamin E omega 3, and oleuropein which were responsible for its antioxidant effect. The mechanism of nigella sativa in decreasing lipid profile (TC, TG, LDL-C and VLDL-C) due to the presence of monounsaturated fat and phenols. Nigella sativa oil rich with conjugated linoleic acid, thioquinon and nigellon dithymoquinone) that was providing the protective antioxidant effect (31). A diet supplemented with virgin olive oil can modify pancreatic cell function as assessed by Ca2 immobilization and amylase release evoked by secretagogues in rat pancreatic acinar cells (32).

In this study microscopic examination of pancreatic tissues of the rats fed with HFD for 8 weeks showed marked affection of its exocrine and endocrine parts, as marked collagenous thickening of interlobular and intralobular ducts were detected with congestion of dilated intralobular blood
vessels. The cytoplasm of the pancreatic acini contained many vacuoles and most of their nuclei showed a marked degree of degeneration as they were small, dark and pyknotic. Most of islets of Langerhans lost their regular outlines with an marked reduction in their sizes and numbers. Most of B-cells appeared degranulated with small degenerated nuclei. Large areas of hemorrhage with a marked vacuolation appeared in the islets.

These results are in agreement with many studies (23, 33, 34), which mentioned the role of lipids in progressive pancreatic diseases.

In this study, microscopic examination of pancreatic tissues of the rats fed with HFD with either olive oil or with Nigella sativa seeds for 8 weeks showed a marked improvement of pancreatic tissue especially with Nigella sativa group. The collagenous fibres distribution around the ducts, vessels and islets were decreased as compared with hyperlipidemic group and appeared nearly similar to those of control group. The pancreatic acini were a moderately improved in shape and most of them had no vacuolation. Most of nuclei and zymogen granules appeared nearly similar to those of control. The islet of Langerhans appeared somewhat similar to control group with normal density of B-cells in the central and peripheral regions of the islet.

In this study, electron microscopic examination of the pancreas of hyperlipidemic rats showed a marked degeneration of both exocrine and endocrine parts. The exocrine part showed a marked vascular congestion, the zymogen granules were markedly decreased in number and densities, the cytoplasm of the acinar cells was full with fat droplets and phagocytic vacuoles, the rough endoplasmic reticulum RER appeared swollen, it took the shape of large spherical to irregular vesicular profiles containing fibrillar materials. The nuclei appeared pyknotic or with irregular outlines. The endocrine part showed that the B-cells and A-cells had multiple signs of degeneration as marked degranulation of the B-cells with degeneration of their nuclei. Marked vacuolation of the cytoplasm was present.

The hyperlipidemia induced by high-fat feeding affected B-cells functions as decreasing the insulin secretion (34). Qingqiang, et al. (2014) reported case studies of correlation between high blood lipid levels and chronic pancreatitis (35).

In this study, electron microscopic examination of the pancreas of rats feed on HFD with virgin olive oil or with Nigella sativa seeds powders showed a marked improvement of exocrine and
endocrine parts of the pancreas. The exocrine part showed the zymogen granules were increased in number and density especially with nigella sativa group. Most of nuclei appeared with normal shapes with regular nuclear membranes. The chromatin matrix restored its normal arrangement and shape. Few fat droplets were present. The endocrine part revealed that most of B-cells and A-cells showing marked signs of improvement as restore the density of the granules and the nuclei became regenerated with normal shape and normal distribution of chromatin matrix.

These results were in agreement with Natalicchio, et al. 2018, who confirmed the positive effects of the main polyphenols contained in extravirgin olive oil on beta-cell function and survival suggesting that extravirgin enriched with these compounds might improve insulin secretion and promoted glycemic control in type 2 diabetic patients (36).

Many studies confirmed the ability of Nigella sativa to preserve pancreatic beta-cell integrity, to induce lipid peroxidation, and to increase antioxidant defense system activity (37). However, this latter suggestion may be contradictory as lipid peroxidation is the primary marker of oxidative stress. The effects of Nigella sativa on oxidative stress and cell damage in streptozotocin-induced diabetic rats. They suggested that Nigella sativa treatment exerted a therapeutic protective effect in diabetes by decreasing oxidative stress and preserving pancreatic cell integrity (38, 39).

**Conclusions**

From this study, the biochemical results were paralleled to the histological and ultrastructural results, it could be concluded that virgin olive oil and Nigella sativa seeds had antihyperlipidemic and hypoglycemic effects and they could protect the pancreas from hyperlipidemia-induced injury and the daily consumption of virgin olive oil and Nigella sativa seeds in the diets is highly recommended.

**Conflicts of interest**: The authors have declared that no conflicts of interest exist.

**Acknowledgement:**
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REFERENCES


Legends of figures:

Fig. (1): Photomicrographs of transverse sections from the rat pancreas of :

a. G1(control) showing the normal pancreatic structure. The exocrine acini(c), endocrine islets of Langerhan's (I), intralobular blood vessels (V) and ducts (d).

b. G2(Fed with HFD) Showing a marked thickening and congestion of intralobular blood vessels (V) with widening of intralobular ducts (d).

c. G3(Fed with HFD & virgin olive oil) showing that the pancreas nearly restored its normal structure.

d. G4(Fed with HFD & Nigella sativa seeds) showing that it is nearly similar to control group. H & E scale=100μm.

Fig.(2): Photomicrographs of transverse sections from the rat pancreas of:

a. G1(control) showing the acini (C) have different sizes and shapes. Each acinus is formed of a single row of pyramidal cells which have dark basophilic nuclei(n) and apical acidophilic zymogen granules(Z).

b. G2(Fed with HFD) Showing that the acinar cytoplasm (C) containing many vacuoles (O). Most of the nuclei (n) showing a marked degree of degeneration.

c. G3(Fed with HFD & virgin olive oil) showing the pancreatic acini (C) are a moderately improved in shape and most of them had no vacuolation.
d. **G 4** (Fed with HFD & Nigella sativa seeds) showing the pancreatic acini (C) are markedly improved in shape with no vacuolation. *Hx & E scale bar = 10μm*

**Fig. (3): Photomicrographs of transverse sections from the rat pancreas of:**

a. **G1** (control) showing a large islet of Langerhan’s. It is composed of mainly red B-cells (B) and few peripheral dark A-cells (A).

b. **G2** (Fed with HFD) Showing that most of B-cells (B) in islet of Langerhans are degranulated with small degenerated nuclei.

c. **G3** (Fed with HFD & virgin olive oil) showing that most of B-cells (B) in the islet with normal density.

d. **G4** (Fed with HFD & Nigella sativa seeds) showing normal density of B-cells (B) as compared with control. *Hx & E scale bar = 10μm*

**Fig. (4): Photomicrographs of transverse sections from the rat pancreas of:**

a. **G1** (control) showing fine collagenous fibres (g) around the interlobular vessels and ducts. Fine collagenous fibres appeared also inside and around the islet of Langerhans (I).

b. **G2** (Fed with HFD) Showing thick collagenous fibres (g) around a dilated destructed interlobular vessel (V) containing a large area of hemorrhage.

c. **G3** (Fed with HFD & virgin olive oil) showing a moderate distribution of collagenous fibrous (g) content around the interlobular vessels (V), ducts and islets (I).

d. **G4** (Fed with HFD & Nigella sativa seeds) showing a moderate distribution of collagenous fibrous content (g) around the interlobular vessels (V), ducts and islets (I).

*Masson’s Trichrome Stain Scale Bar = 50μm*
Fig. (5): Electron micrographs of ultrathin sections from pancreatic exocrine cells of:

a. **G1**(control) showed numerous dark zymogen granules (Z) with variable sizes and shapes in the apical cytoplasm.

b. **G2**(Fed with HFD) showed the zymogen granules (Z) are markedly decreased in number and densities, a marked vascular congestion in a destructed blood vessel wall’s (V) and phagocytic vacuoles in the cytoplasm (0).

c. **G3**(Fed with HFD & virgin olive oil) showed the zymogen granules (Z) are increased in number and density and nearly similar to the control.

d. **G4**(Fed with HFD & Nigella sativa seeds) showed that the zymogen granules (Z) are markedly increased in number and density similar to control. *Double stain Scale Bar = 2 \mu m X 1000*

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Fig.(6): Electron micrographs of ultrathin sections from pancreatic exocrine cells of:

a. **G1**(control) showed the rough endoplasmic reticulum (RER), mitochondria (m). Mitochondria appeared as oval and rod-bodies in both the apical and basal parts of the cell. Showing the rounded nuclei (n) with regular nuclear membranes.

b. **G2**(Fed with HFD) showed the swollen rough endoplasmic reticulum (RER). Showing the nuclei (n) are pyknotic or with irregular outlines. Showing also the mitochondria (m) appeared swollen.

c. **G3**(Fed with HFD & virgin olive oil) showed most of mitochondria (m) and rough endoplasmic reticulum (RER) restored their shapes. Most of nuclei (n) appeared with normal shapes with regular nuclear membranes.

d. **G4**(Fed with HFD & Nigella sativa seeds) showed most of mitochondria (m) and rough endoplasmic reticulum (RER) restored their shapes. The nuclei (n) appeared with normal shapes with regular nuclear membranes. *Double stain Scale Bar = 2\mu m X 2000*

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Fig.(7): Electron micrographs of ultrathin section from an islet of Langerhans of:
a. **G1** (control) showed the B–cells (B) and A-cells (A) with their specific endocrine granules. The nuclei of B-cells contain both hetero and euchromatin. The nuclei of A-cells contain small chromatin clumps.

b. **G2** (Fed with HFD) showing marked degranulation of the B-cells and A-cells with degeneration of their nuclei. Showing also a marked vacuolation of the cytoplasm(0), swollen mitochondria (m) and rough endoplasmic reticulum(RER).

c. **G3** (Fed with HFD & virgin olive oil) showing most of B-cells(B) and A-cells(A) restoring the density of the granules and the nuclei are regenerated with normal shape and normal distribution of chromatin matrix. Most of mitochondria and RER appeared as those of the control group. Few cytoplasmic vacuoles are present.

d. **G4** (Fed with HFD & Nigella sativa seeds) showed that B-cells (B) and A-cells (A) are nearly similar to control. *Double stain Scale Bar = 2μm X 1000*

**Fig.(8): Electron micrographs of ultrathin section from an islet of Langerhans of:**

a. **G1** (control) showing the granules of beta-cells(B) are rounded in shape, contained dark electron dense cores and surrounded by wide electron-lucent halos. Showing the granules of A-cells (A) are more numerous and electron dense than the B-granules and are surrounded by narrower halos.

b. **G2** (Fed with HFD) showing marked degranulation of the B-cells(B) with degeneration of their nuclei. Showing also a marked vacuolation of the cytoplasm(0).

c. **G3** (Fed with HFD & virgin olive oil) showing most of B-cells(B) and A-cells(A) restoring the density of the granules and the nuclei are regenerated with normal shape and normal distribution of chromatin matrix. Few cytoplasmic vacuoles are present.

d. **G4** (Fed with HFD & Nigella sativa seeds) showed that B-cells(B) and A-cells (A) are nearly similar to control. *Double stain Scale Bar = 2μm X 2000*