Original Article

Evaluation of Thymus Vulgaris (Thyme) Role in the Protection and Treatment of the Parotid Gland of Triton WR-1339 Induced Hyperlipidemia in Adult Male Albino Rats

Noha M. Issa[1], Dalia A. Noya[2] and Rasha M. Salama[1]

[1]Department of Anatomy and Embryology, [2]Department of Histology and Cell Biology, Faculty of Medicine, Menoufia University, Egypt

ABSTRACT

Background: Hyperlipidemia is a common problem. It is a well-known aggravating factor in the pathogenesis of many diseases so that, this study was planned to study the structural changes of parotid gland in Triton WR-1339-induced hyperlipidemia rat model and to investigate the (protecting and treating) effect of Thymus vulgaris extract (Thyme) as a natural product.

Materials and Methods: Fifty male albino rats were randomly divided into five groups. Group I (Control G), Group II (Thyme G): rats received Thyme (500 mg/kg body weight) for ten days, Group III (Hyperlipidemia G): rats received a single intraperitoneal dose of Triton WR1339. Group IV (Protected G): rats received Thyme for ten days and the induction of hyperlipidemia occurred on the seventh day of the experiment. Group V (Treated G): rats received Thyme 24 hours after induction and throughout the experiment. Three days after induction of hyperlipidemia, blood samples were collected to measure serum total cholesterol & triglycerides. The parotid gland was processed for histological, histochemical and immmunohistochemical studies. Morphometrical and statistical analysis were done.

Results: The hyperlipidemia rat model induced by Triton WR1339 developed acute inflammation of parotid gland leading to obvious histological findings and significant increase in the immunohistochemical markers of inflammation and fibrosis. These findings were ameliorated by thyme as a protecting agent and to a significant lesser extent as a treatment.

Concolusion: Thyme can be used as a protective agent against the parotid gland lesions caused by hyperlipidemia through its anti-inflammatory, antioxidant and hypolipidemic properties.

Received: 25 September 2020, **Accepted:** 04 November 2020

Key Words: Hyperlipidemia, parotid gland, thyme.

Corresponding Author: Dalia A. Noya, MD, Department of Histology and Cell Biology, Faculty of Medicine, Menoufia University, Egypt, **Tel.**: +20 1068464307 , **E-mail:** dalia_noya@yahoo.com

ISSN: 1110-0559, Vol. 44, No.3

INTRODUCTION

Hyperlipidemia is a state of increased plasma lipids over the normal range[1]. Triton WR1339, a non-ionic agent causing actively surface detergency leads to an elevation of triglycerides and total cholesterol plasma concentration. Triton WR-1339 could induce hyperlipidemic rat model to evaluate potential hypolipidemic drugs is accepted globally[2]. Hyperlipidemia may attribute to the disease directly and indirectly through (inflammation and impaired immune response)[3], also abnormal cell proliferation and apoptosis are various biological effects in response to accumulation of lipids[4]. Apoptosis induced by hyperlipidemia is the corner stone to develop salivary glands (mainly parotid gland) dysfunction[5,6].

Secretion of saliva is the most important role of parotid gland, which has a pivotal role to maintain the oral homeostasis[7,8]. In addition, its secretion is a part gastrointestinal tract function. However, many factors as aging, inflammation, medication and autoimmune diseases may lead to parotid gland dysfunction[9]. Salivary secretion is controlled by water channel proteins on the secretory cells, called aquaporin-5 (AQP5)[10]. Normally, nitric oxide is produced in the parotid gland for benefit action through a reaction catalyzed by inducible nitric oxide synthase (i-NOS)[10]. If nitric oxide is overproduced, it leads to the destruction of parotid gland's cells[11].

Recently, herbal extracts which are culturally acceptable, economically feasible and have antioxidants activities could replace many drugs in various therapy regimens[12]. Thymus vulgaris (Thyme) is a medicinal plant belongs to the Lamiaceae family[13]. It is a pleasant smelling herb in the Mediterranean region[14]. The main ingredients of thyme are phenols, including flavonoids, thymol, carvacrol, eugenol, luteolin and saponins, these polyphenols are responsible for its antioxidant and free radical scavenging activities[15,16]. Thymus vulgaris extract was described as an immunomodulator, anti-inflammatory agent[17], vasorelaxant, hypolipidaemic and hypoglycaemic[18,19].

Thus in the current study, we investigated the effects of hyperlipidemia on the parotid gland and the influence of thyme on it to measure the potential hypolipidemic effects of this natural compound.

 DOI: 10.21608/ejh.2020.44047.1361

MATERIALS AND METHODS

Chemicals

Triton WR-1339 was delivered from Sigma–Aldrich (St. Louis, MO, USA). Triton WR-1339 was dissolved in saline solution (pH 7.4).

Thymus Vulgaris extract (the trade name is thyme; super concentrated liquid herbal extract presented as a bottle containing 330 mg thymus vulgaris/ml) and all markers were purchased from local distributer (Sigma chemical) Cairo, Egypt.

Hyperlipidemia modeling

The hyperlipideamia was achieved by a single intraperitoneal injection of Triton WR1339 (400 mg/kg body weight) dissolved in 0.9% saline (pH 7.4) to overnight fasted albino rats based on[20]. After one hour of tritonization, the animals were given feed ad libitum; Hyperlipidemia was confirmed 72 hours after triton injection by determining the level of serum total cholesterol and triglyceride.

Experimental design

All experimental protocols were approved by the Ethics Committee of the Medical Research Institute, Alexandria University, Egypt. Fifty adult male albino rats weighting 200 - 210 gm obtained from Medical Research Institute, Alexandria University, Egypt. Firstly, the animals were left for 72 hours for acclimatization, at temperature of (23±2 ºC) with a 12 hours light / dark cycle. All rats have free access to food, tap water and standard laboratory chow. The rats were randomly divided into 5 groups of 10 in each:

Group I (Control G): it included ten rats, 5 rats remained without treatment throughout the experiment and 5 rats received a single intraperitoneal dose of 1 ml 0.9% saline (pH 7.4) on the seventh day of the experiment.

Group II (Thyme G): it included ten rats received thyme in an oral daily dose of 500 mg/kg body weight[21] (0.3 ml of thyme/rat) for ten days.

Group III (Hyperlipidemia G): it included ten rats received a single intraperitoneal dose of Triton WR1339 (400 mg/kg body weight) dissolved in 1.0 ml 0.9% saline (pH 7.4) on the seventh day of the experiment.

Group IV (Protected G): it included ten rats received thyme in an oral daily dose of 500 mg/kg body weight (0.3 ml of thyme/rat) for ten days; it was started on the first day of the experiment and for ten days, the induction of hyperlipidaemia was done by a single intraperitoneal dose of Triton WR1339 (400 mg/kg body weight) dissolved in 1.0 ml 0.9% saline (pH 7.4) on the seventh day of the experiment.

Group V (Treated G): it included ten rats received thyme throughout the experiment in an oral daily dose of 500 mg/kg body weight (0.3 ml of thyme/rat); it was started 24 hours after induction of hyperlipidaemia by a single intraperitoneal dose of Triton WR1339 (400 mg/kg body weight) dissolved in 1.0 ml 0.9% saline (pH 7.4) on the first day of the experiment.

Three days after induction of hyperlipidemia (10 days from the start of experiment), all animals were weighted and then sacrificed after being anesthetized by an intraperitoneal injection of 40 mg/kg pentobarbital sodium[22]. The blood samples were collected from the rat's tails to measure the levels of serum total cholesterol and triglyceride.

Biochemical analysis

Blood samples collected before scarification of the animals under anesthesia were centrifuged at 1500 × g for 15 min to separate serum. Serum total cholesterol and triglycerides were determined using commercial kits (Labtest Diagnostica, MG, Brazil) through enzymatic colorimetric methods, the results were expressed as mg/dl. This was done in central lab, Faculty of Medicine, Menoufia University.

Histological analysis

A skin incision was done ventral to the external ear,the parotid glands were dissected carefully, weighed and put in 10% formalin solution then processed routinely by embedding in paraffin; finally, the parotid glands were sectioned into 5 µm sections for histological (Hx.&E. and Mallory's trichrome stains), histochemical (Periodic acid schiff (PAS) stain) and Immunohistochemical stains with anti-bcl-2 antibody (ab196495), anti-α SMA antibody (ab32575) and anti-TNF-α antibody (ab66579) by avidin-biotin complex method. Positive control for Bcl 2 and TNF – α was lymphoid tissues and for α SMA was muscle tissue.

Morphometric analysis

Image J software (Maryland, USA) used to evaluate the different parameters in ten fields (non – overlapping, x100 and x400) for each specimen from five rats per experimental group. The area percentage of Mallory's trichrome stain, PAS reaction together with immunohistochemical stains for the number of positive cells stained with α SMA, area percentage of Bcl2 and area percentage of TNF α. This was done in the Anatomy Department, Faculty of Medicine, Menoufia University, Egypt.

Statistical analysis

By using SPSS program version 20.0, those quantitative data were assessed and expressed as mean and standard deviation. Mann Whitney U test was used to test the difference between each two independent groups. The statistically significant *P value* was considered lower than (0.05).

RESULTS

Biochemical, morphometric, and Statistical results

Body and parotid gland weight

Regarding the final weight of the body and the parotid gland, the rats of hyperlipidemia group (III) showed significant increase (*p value* < 0.05) of the both parameters as compared to the control group (I), while both parameters in protected group (IV) were significantly decreased (*p value* < 0.05) as compared to the hyperlipidemia group (III) (Histogram 1) .

Biochemical results

Regarding the serum triglyceride and cholesterol, the rats of hyperlipidemia group (III) showed significant increase (*p value* < 0.05) of the both parameters as compared to the control group (I), while the both parameters in protected (IV) group were significantly decreased (*p value* < 0.05) (Histogram 2).

The surface area percentage of Mallory's trichrome stain

The surface area percentage of Mallory's trichrome stain in the parotid connective tissue spetae, around ducts and blood vessels is significantly increased (*p value* < 0.05) in hyperlipidemia group (III) as compared to the control (group I). Protected (group IV) showed significant decrease (*p value*< 0.05) in surface area percentage of the Mallory's trichrome stain around ducts and acini as compared to hyperlipidemia (group III) (Histogram 3).

The surface area percentage of PAS stain

Hyperlipidemia group (III) showed significant decrease (*p value* < 0.05) of the mean PAS stained area percentage as compared to control group (I). In protected group (IV) there was significant increase (*p value* < 0.05) of the mean PAS stained area percent as compared to hyperlipidemia group (III) (Histogram 3).

The number of α SMA stained cells

The hyperlipidemia group (III) showed significant increase (*p value* < 0.05) in the mean number of cells that show positive cytoplasmic immunoreactivity for α SMA compared to control (group I). Protected group (IV) showed significant decrease (*p value* < 0.05) in the mean number of cells with cytoplasmic immunoreactivity for α SMA as compared to hyperlipidemia group (III) (Histogram 4).

The surface area percentage in BcL2 immunostain

Significant decrease (*p value* < 0.05) in the mean surface area percentage of cytoplasmic immunoreactivity for Bcl2 in the parotid gland sections of hyperlipidemia group (III) was recognized as compared to control group (I). Parotid gland sections of Protected group (IV) stained with Bcl2 showed significant Increased (*p value* < 0.05) in the mean surface area percentage of positive cytoplasmic immunoreaction for Bcl2 as compared to hyperlipidemia group (III) (Histogram 5).

The surface area percentage in TNF- α immunostain

Parotid gland sections of hyperlipidemia group (III) showed significant increase (*p value* < 0.05) of cytoplasmic immunoreaction for TNF- α mean surface area percentage in the acini and ducts as compared to control group (I). Protected group (IV) revealed significant decrease (*p value* < 0.05) of cytoplasmic immunoreactivity surface area percentage for TNF-α as compared to hyperlipidemia group (III) (Histogram 5).

Histological results

Regarding group II (Thyme G), it showed add similar histological, histochemical, and immunohistochemical picture as instead of highlighted words as group I (control G).

Hematoxylin and Eosine (Hx. & E.) stain

The parotid gland of control group (I) showed closely packed serous acini that were lined by pyramidal cells with basal rounded nuclei, enclosing narrow lumen. Striated ducts located between serous acini were lined by cuboidal cells with rounded vesicular nuclei (Figures 1a, 2e). The parotid gland of hyperlipidemia group (III) showed disturbed acinar architecture, distorted acini with variable sized intracellular vacuoles were detected. Acinar cells showed irregular displaced pykontic nuclei. Apparently thickened connective tissue septae contained ducts, blood vessels and inflammatory cells. The ducts appeared dilated and irregular with retained secretion in their lumens. Their lining cells contained intracytoplasmic vacuoles and pyknotic nuclei. Some ducts showed different degrees of degeneration leaving homogenous acidophilic areas. Blood vessels were dilated and congested with visible red blood corpuscles inside. Intense monocellular inflammatory infiltrations were detected in the connective tissue septum with scattered epitheliod cells in between (Figures 1b,2f).

Parotid gland sections of protected group (IV) stained with Hx.&E. showed more or less normal histological features of parotid gland as serous acini appeared with regular outlines; their lining pyramidal cells showed basal rounded nuclei. Very few affected acini with intracytoplasmic vacuoles were present among plenty of healthy ones. Striated ducts appeared with regular lumen and were lined by cubical cells with rounded vesicular nuclei (Figures 1c,2g).

Sections of the parotid gland of treated group (V) stained with Hx.& E. showed many irregular degenerated acini with intracytoplasmic vacuoles among few normal acini. Some acinar cells showed pyknotic nuclei, while other showed shadows of krayolytic nuclei. Some of the cell lining of ducts showed shadows of degenerated nuclei, normal ducts were also seen (Figures 1d,2h).

Mallory`s Trichrome stain

Sections stained with Mallory`s trichrome showed

minimal amounts of collagen fibers around ducts and blood vessels, and scanty collagen fibers around acini in the control group (I) (Figure 3a). Hyperlipidemia group (III) showed apparent increase in the collagen fibers deposited in the connective tissue spetae and around ducts and blood vessels (Figure 3b).Protected group (IV) showed minimal to moderate amounts of collagen fibers around ducts and acini (Figure 3c). While treated group (V) showed apparent increase in the amounts of collagen fibers around ducts and blood vessels in the connective tissue septum as compared to control group (Figure 3d).

Histochemical results

Periodic Acid Scheif (PAS)

The Control group (I) sections stained with PAS showed serous acini especially their basement membranes strongly stained with PAS reaction as deep magnata color (Figure 4a). While a weak PAS reaction was detected in the acini and their basement membranes of hyperlipidemia group (III). Also ductal cell basement membranes showed faint PAS +ve reaction (Figure 4b).Protected group (IV) showed strong +ve PAS reaction in the acini and in the basement membranes surrounding the acini and ducts (Figure 4c). Treated group (V) showed moderate PAS reaction in the acini and ducts basement membranes (Figure 4d).

Immunohistochemical results

1- Alpha smooth muscle actin (α SMA) immunostain

The parotid gland sections of Control group (I) showed minimal cytoplasmic immunoreactivity for α -SMA in myoepithelial cells around acini (Figure 5a). Hyperlipidemia group (III) showed apparent increase in the positivity of the α -SMA cytoplasmic immunoreactivity in the myoepithelial cells surrounding the parotid acini compared to control parotid gland (Figure 5b). Protected group (IV) showed minimal cytoplasmic immunoreactivity for α SMA in the myoepithelial cells surrounding the acini and ducts (Figure 5c).Treated group (V) showed increase in the cytoplasmic immunopositivty for α SMA in the myoepithelial cells surrounding the acini and ducts (Figure 5d). This was confirmed by the morphometric and statistical results.

2- Bcl2 immunostain

The parotid gland sections stained with Bcl2 revealed the control group (I) with strong positive (+ve) cytoplasmic immunoreactivity reaction in the cells of acini and ducts (Figure 6a). Hyperlipidemia group (III) showed weak cytoplasmic immunoreactivity for Bcl2 (Figure 6b). The protected group (IV) showed strong positive cytoplasmic immunoreaction for Bcl2 (Figure 6c). While the treated group (V) showed minimal cytoplasmic immunopositivty for Bcl2 (Figure 6d).

3- Tumor necrosis factor alpha (TNF-α) immunostain

The parotid gland sections stained with TNF- α showed minimal cytoplasmic immunoreactivity for TNF- α in control group (I) (Figure 7a). The hyperlipidemia group (III) showed strong cytoplasmic immunoreaction for TNF- α in the acini and ducts (Figure 7b). The protected group (IV) showed minimal cytoplasmic immunoreactivity for TNF- α (Figure 7c). The treated group (V) showed moderate cytoplasmic immunoreactivity in the cells of the parotid glands (Figure 7d).

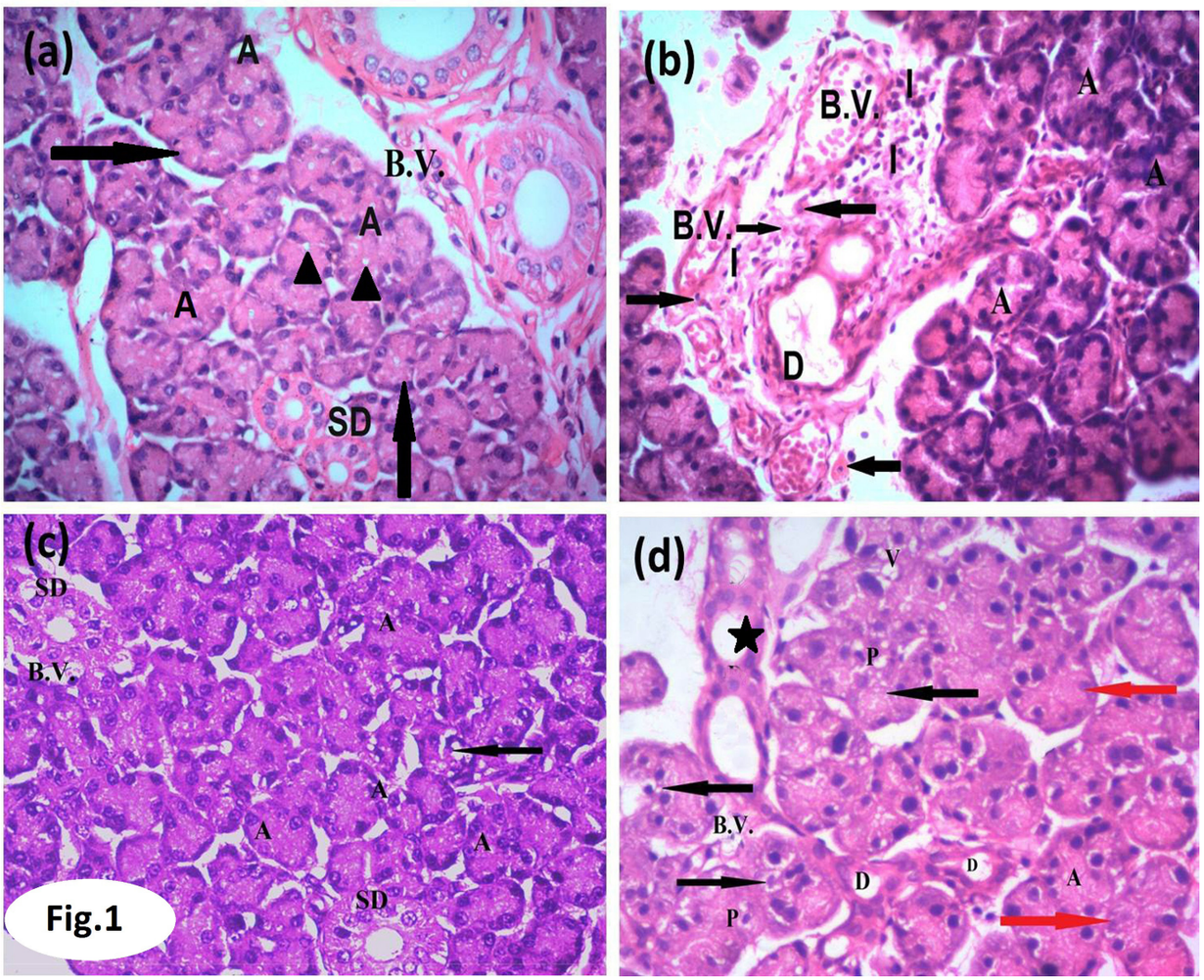


Fig. 1: A micrograph of parotid gland sections stained with Hx& E: (a) Control group (I) showing normal acini (A) lined by pyramidal cells with basal rounded nuclei (→), enclosing narrow lumen (►) in between striated ducts (SD) are present. (b) Hyperlipidemia group (III) showing distorted acini (A). apparently thickened connective tissue septae contains ducts with dilated irregular lumen with retained secretion (D), dilated congested blood vessels (B.V), intense inflammatory cells infiltrates (I). Notice the scattered epitheliod cells (→). (c) Protected group (IV) showing normal acini (A) with few scattered affected ones (→). Striated ducts (SD) with regular lumen with normal blood vessels (B.V) are also seen. (d) Treated group (V) showing irregular degenerated acini (→) with intracytoplasmic vacuoles (V). Some acinar cells showing pyknotic nuclei (P) while others showing shadows of karyolytic nuclei (red →). Normal blood vessels (B.V) with both degenerated (D) and healthy ducts (*) are seen (x200).

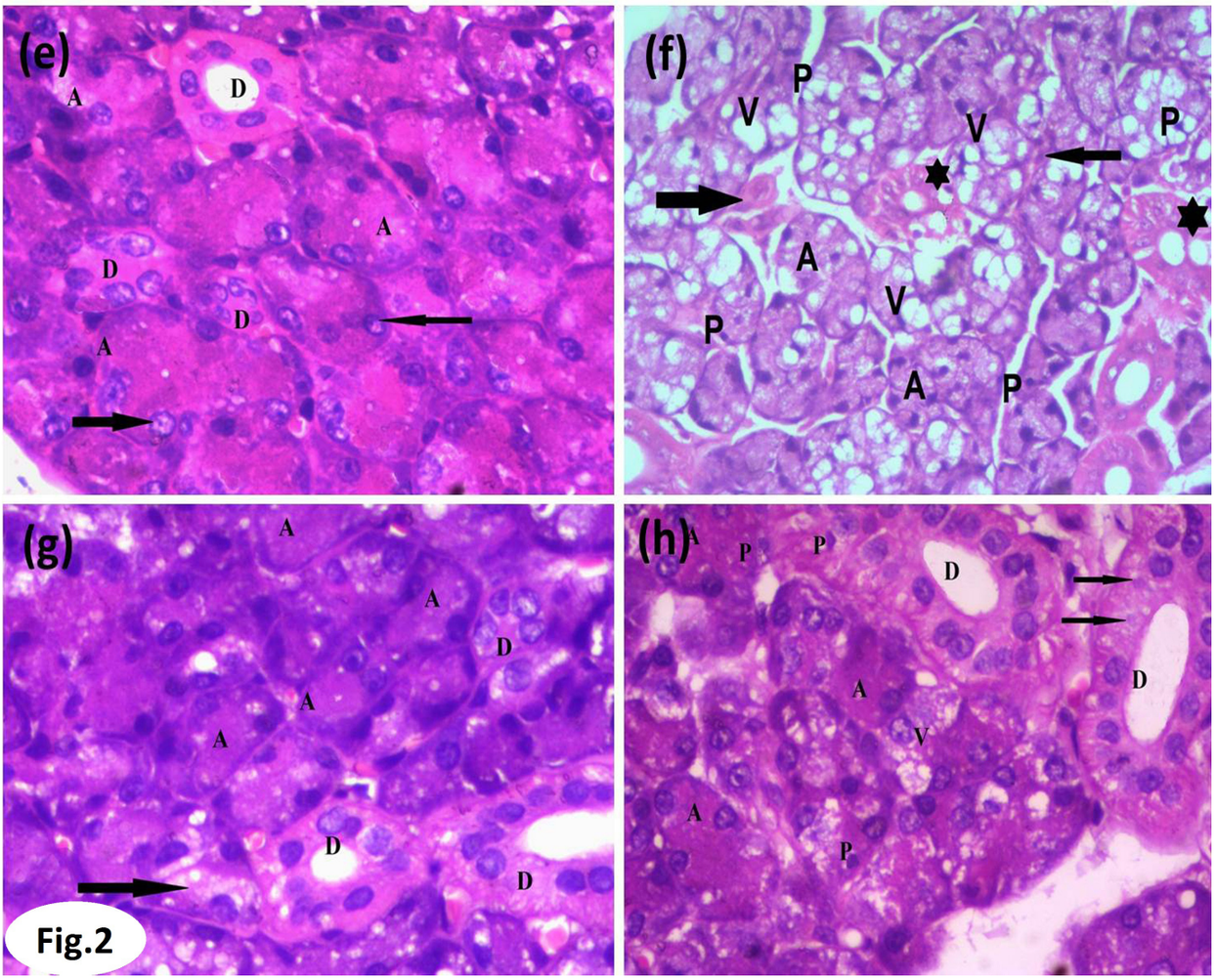


Fig. 2: A micrograph of parotid gland sections stained with Hx& E: (e) Control group (I) showing pyramidal cells with basal rounded vesicular nuclei (→) lining the acini (A) and duct (D). (f) Hyperlipidimia group (III) showing distorted acini (A) with variable sized intracellular vacuoles (V). Acinar cells showing irregular, displaced and pyknotic nuclei (P). Some ducts showing different degrees of degeneration leaving homogenous acidophilic areas (→), another ducts showing vacuoles in their lining cells (*). (g) Protected group (IV) showing acini (A) with regular contour with very few acini with intracytoplasmic vacuoles are noticed (→). Ducts appeared normal (D). (h) Treated group (V) showing many irregular acini with vacuolated cytoplasm (V) and pykontic nuclei (P) among few normal acini (A). Some of the cells lining ducts showing shadows of karyolytic nuclei (→) (x400).

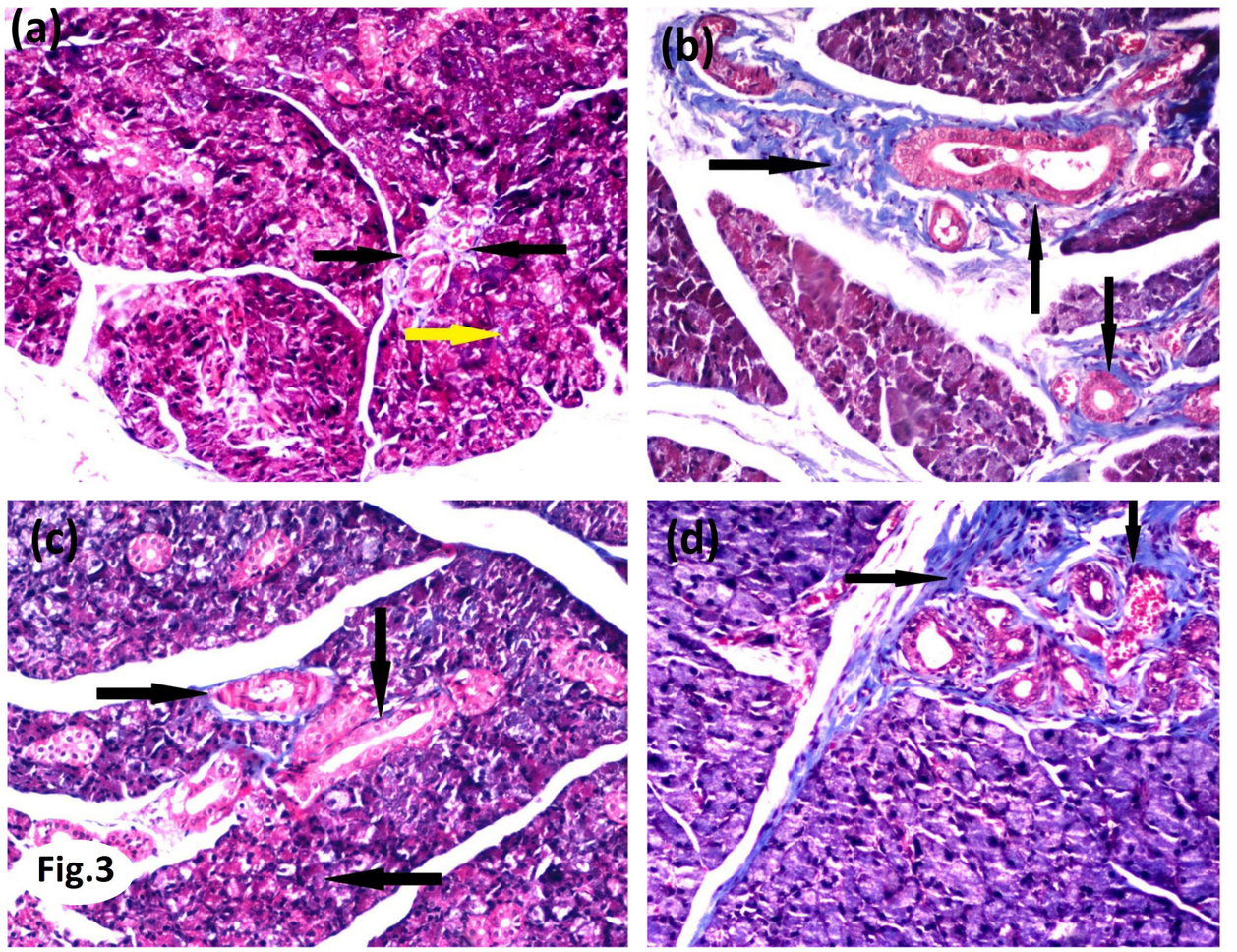


Fig. 3: A micrograph of parotid gland sections stained with Mallory`s trichrome:(a) Control group (I) showing minimal amounts of collagen fibers around ducts and blood vessels (black arrow), and scanty collagen fibers around acini (yellow arrow). (b) Hyperlipidemia group (III) showing apparent increase in the collagen fibers deposited in the connective tissue spetae and around ducts and blood vessels (→). (c) Protected group (IV) showing minimal to moderate amounts of collagen fibers around ducts and acini (→). (d) Treated group (V) showing apparent increase in the amounts of collagen fibers around ducts and blood vessels in the connective tissue septum (→) (x 100).

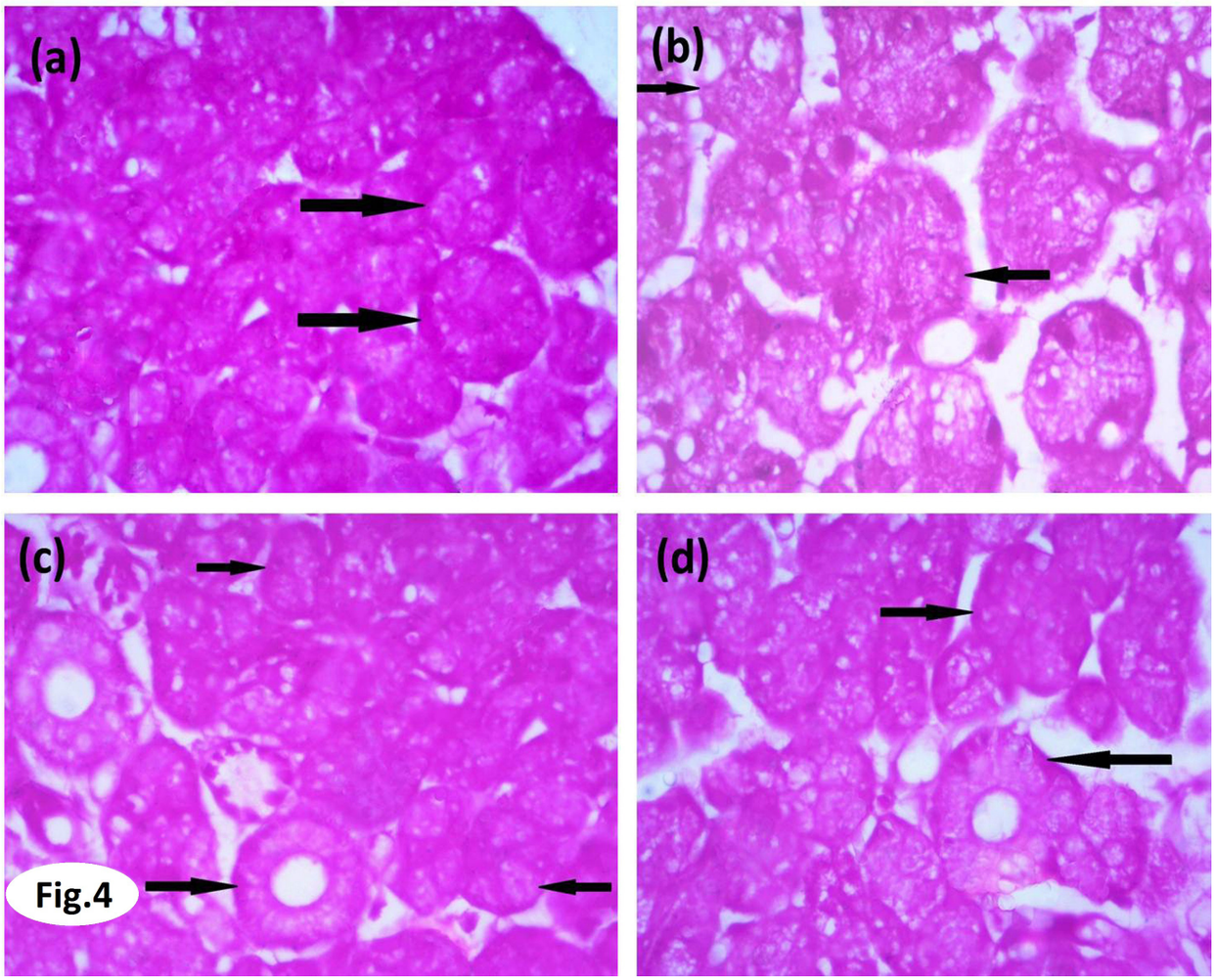


Fig. 4: A micrograph of parotid gland sections stained with PAS: (a) Control group (I) showing serous acini especially their basement membranes strongly stained with PAS reaction as deep magnata color (→). (b) Hyperlipidemia group (III) showing weak PAS reaction in the acini and their basement membranes, also ductal cell basement membranes showed faint PAS +ve reaction (→). (c) Protected group (IV) Showing strong +ve PAS reaction in the acini and in the basement membranes surrounding the acini and ducts (→). (d) Treated group (V) showing moderate PAS staining in the basement membranes surrounding the acini and ducts (→) (x 400).

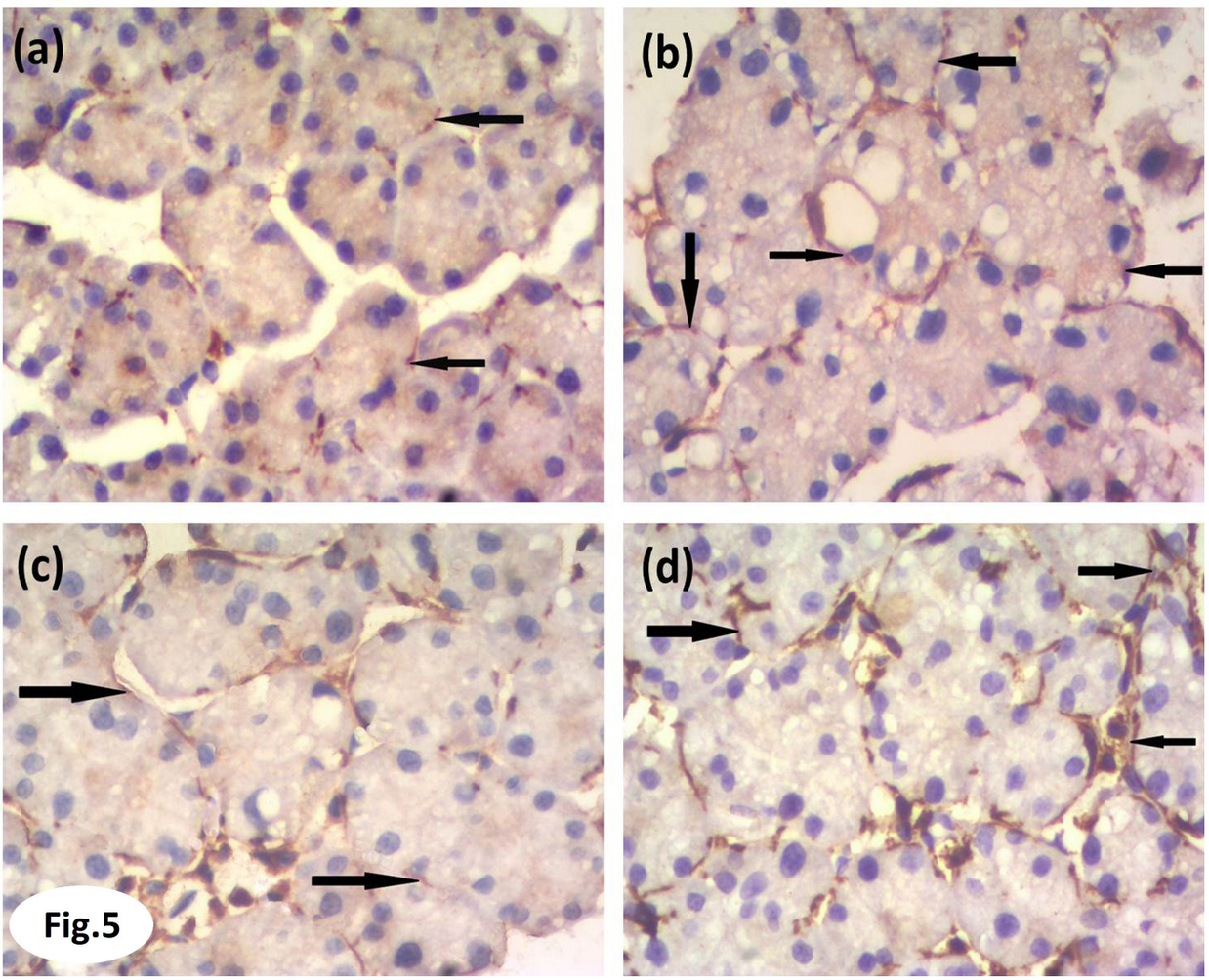


Fig. 5: A micrograph of parotid gland sections stained with α SMA: (a) Control group (I) showing minimal cytoplasmic immunoreactivity for α SMA surrounding the acini (→). (b) Hyperlipidemia group (III) showing apparent increase in the positivity of the α SMA cytoplasmic immunoreactivity surrounding the parotid acini compared to control parotid gland (→). (c) Protected group (IV) showing minimal cytoplasmic immunoreactivity for α SMA surrounding the acini and ducts (→). (d) Treated group (V) showing increase in the cytoplasmic immunopositivty for α SMA surrounding the acini and ducts (→) (x 400).

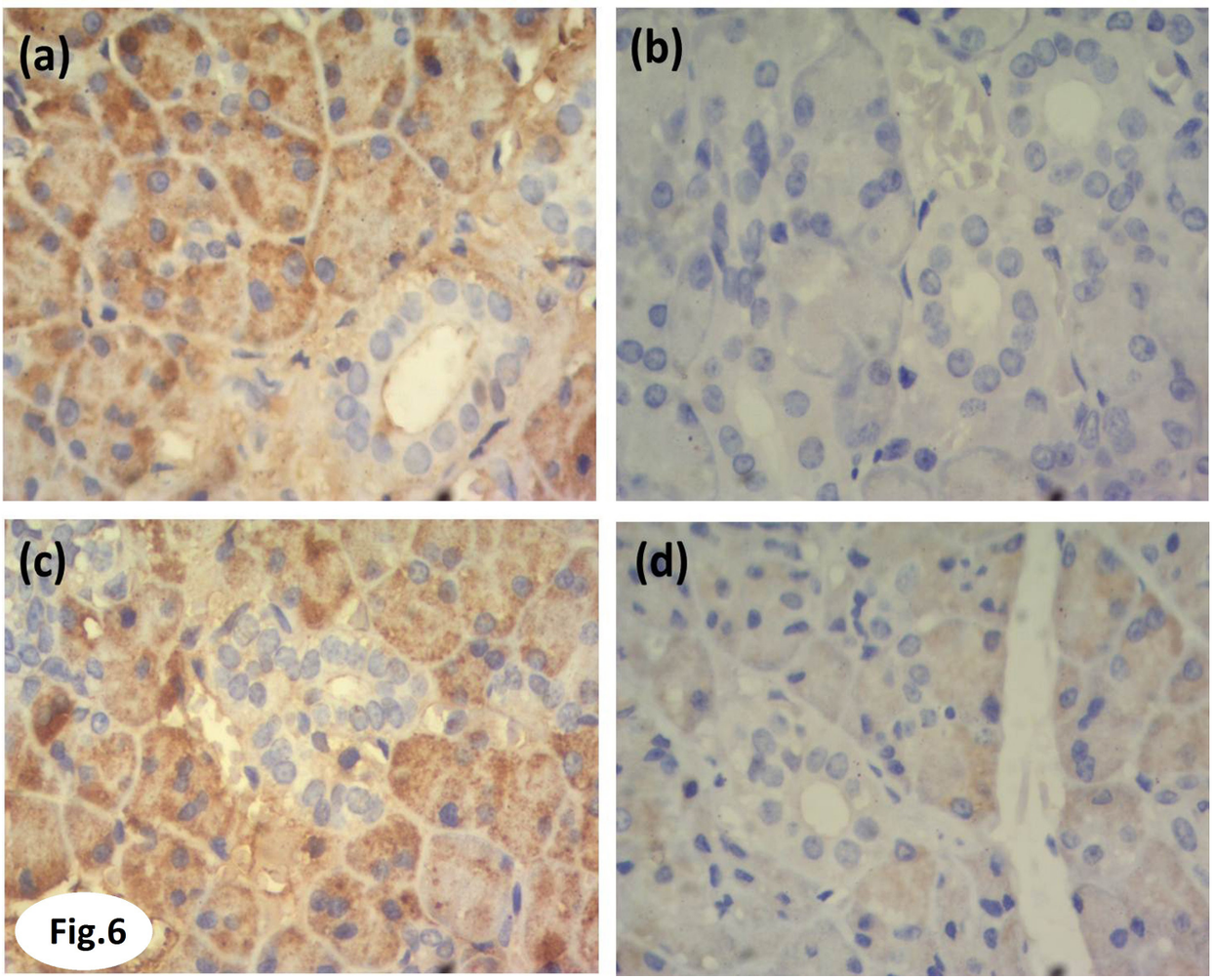


Fig. 6: A micrograph of parotid gland sections stained with Bcl2: (a) Control group (I) showing strong positive (+ve) cytoplasmic immunoreactivity reaction in the cells of acini and ducts. (b) Hyperlipedemia group (III) showing weak cytoplasmic immunoreactivity for Bcl2. (c) Protected group (IV) showing strong positive cytoplasmic immunoreaction for Bcl2. (d) Treated group (V) showing minimal cytoplasmic immunopositivty for Bcl2 (x 400).

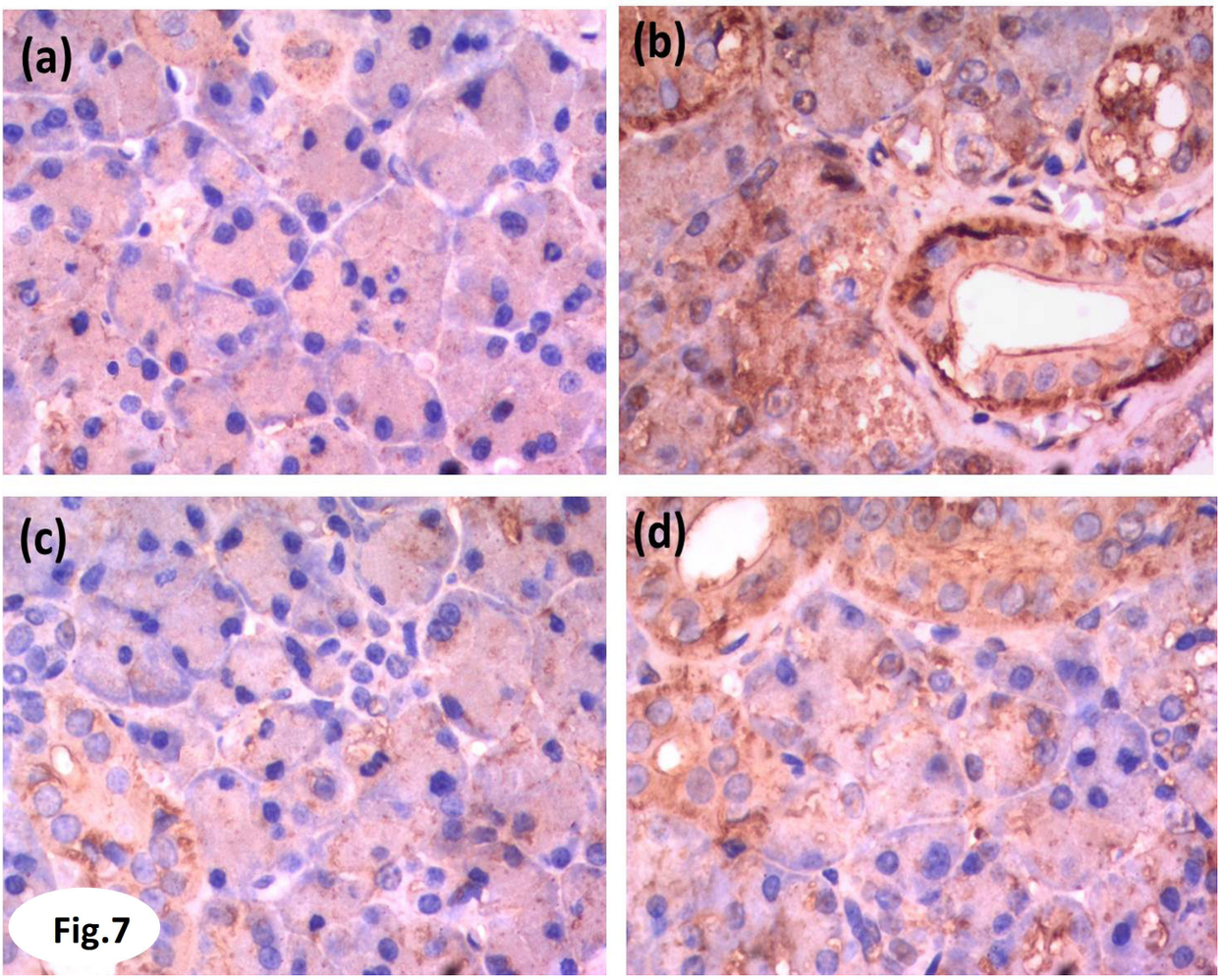
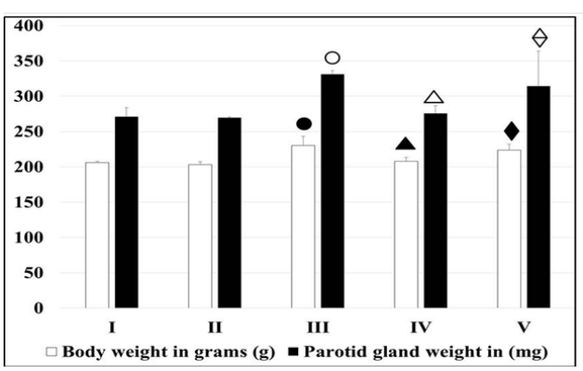
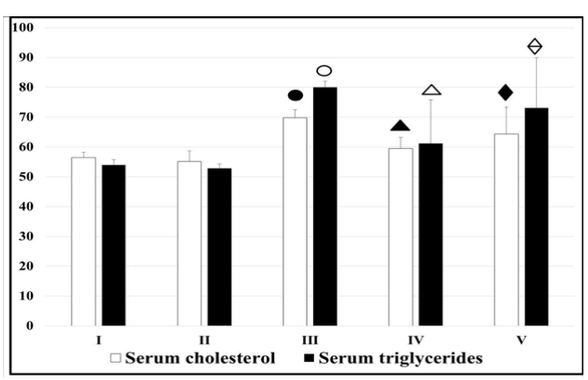


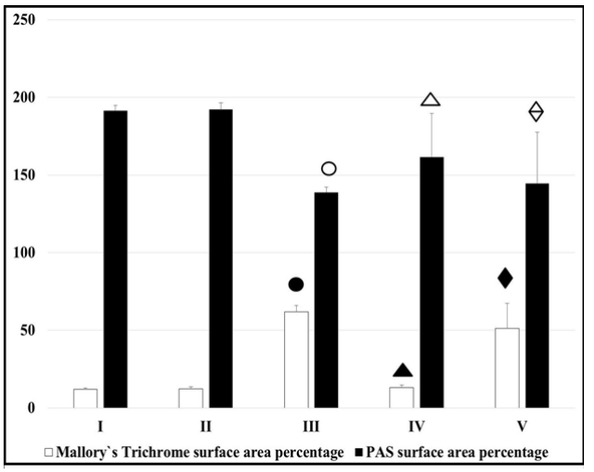
Fig. 7: A micrograph of parotid gland sections stained with TNF- α: (a) Control group (I) showing minimal cytoplasmic immunoreactivity for TNF- α. (b) Hyperlipidemia group (III) showing strong cytoplasmic immunoreaction for TNF- α in the acini and ducts. (c) Protected group (IV) showing minimal cytoplasmic immunoreactivity for TNF- α. (d) Treated group (V) showing moderate cytoplasmic immunoreactivity in the cells of the parotid glands (x 400).



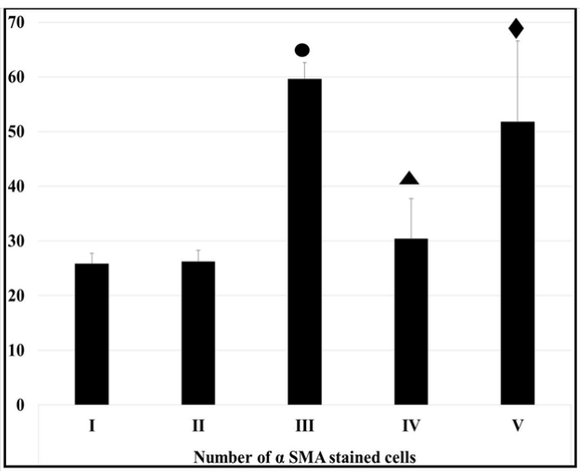
Histogram 1: The mean of body weight (g) and parotid gland weight (mg)



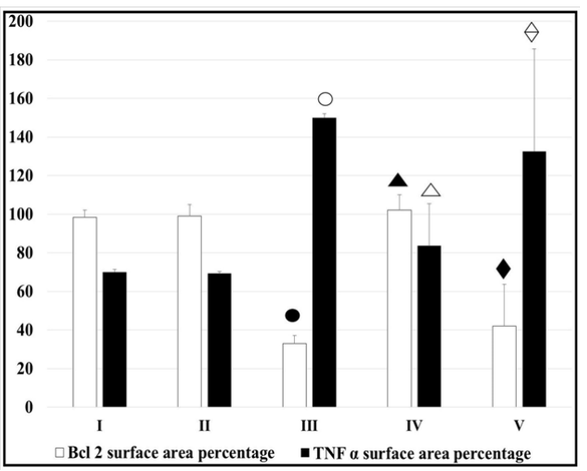
Histogram 2: The mean of serum total cholesterol and triglycerides (mg/dl)



Histogram 3: The means of surface area percentage of Mallory`s Trichrome and PAS stains.



Histogram 4: The mean number of α SMA stained cells.



Histogram 5: Bcl2 and TNF α mean surface area percentage.

DISCUSSION

Hyperlipidemia (high level of serum triglycerides and cholesterol) is a critical condition associated with oxidative damage and dysfunction of many organs including the salivary glands[23-25]. Lipid lowering drugs have been implicated in serious side effects as found by some researchers[26]. This study was planned to evaluate the effect of thyme (natural lipid lowering agent) in the protection and treatment of the parotid of hyperlipidemia rat model. In the present study, the body and parotid weight of rats of hyperlipidemia group was significantly increased as compared to control group. Moreover, their serum cholesterol and triglycerides were significantly increased as compared to that of control group. Also the parotid gland of hyperlipidemia group showed disturbed architecture with wide interlobular and intralobular spaces. The acini appeared distorted with variable sized intracellular vacuoles. These histological changes might be owed to hydropic degeneration in the parotid followed by intracellular lipid infiltration as recognized by the study done by some scientists[27]. All these findings ensured the efficiency of Triton WR 1339 as a hyperlipidemic agent through the inactivation of lipoprotein lipase enzyme leading to lipid accumulation in the blood followed by tissue infiltration as explained by the work of some scientists[2]. Also the parotid gland of hyperlipidemic rats showed pyknosis (apoptosis) of acinar and ductal nuclei, this is in agreement with findings recorded in previous study[28] that reported that the high fat diet triggered the apoptosis of the rat parotid gland and explained it by the oxidative stress. The result of the current study emphasized on this by significant decrease in the expression of Bcl2 (the antiapoptotic immunohistochemical marker) in the parotid of hyperlipidemic rats as compared to that of control group. The hyperlipidemia activates (nicotinamide adenine dinucleotide phosphate (NADPH) and xanthine) oxidase enzymes generating reactive oxygen species (ROS) and inhibits the scavenging enzymes as superoxide dismutase causing the oxidative stress[29]. Hyperlipidemia is associated with lipid peroxidation and cell injury [26]. This explained the parotid duct degeneration of hyperlipidemic rats with appearance of homogenous acidophilic areas with ductal dilatation and retention of its secretions. Also the microscopic examination of the parotid gland of hyperlipidemic rats showed dilatation and congestion of the blood vessels with intense inflammatory cellular infiltrations appeared in the connective tissue septum with scattered epitheliod cells in between. Vessels dilatation was a compensatory event to reverse the tissue degeneration as a part of the inflammatory process together with inflammatory cells infiltration[28,30]. This explanation is in agreement with the significant increase in the expression of the TNF α (inflammatory immunohistochemical marker) in the parotid gland of hyperlipidemia group as compared to the control group. TNF α is a proinflammatory mediator that is released in response to the oxidative stress

and regulate the inflammatory cascade together with endothelial dysfunction[31]. In the present study, significant increase in number of α SMA immunopositive cells in the parotid gland of hyperlipidemic group as compared to the control group was detected. The α SMA stained cells resemble the smooth muscle cells in their capability to contract and the fibroblast cells in their capability to initiate the fibrosis[32]. A previous study[28] correlated the increased α SMA immunopositive cells numbers in the parotid gland of hyperlipidemic rats to overcome the parotid dysfunction induced by the hyperlipidemia. Also these cells could produce the collagen type I & III initiating the fibrosis around the acini and between the lobules interfering with its function[33]. So the collagen deposition in the connective tissue septae and around blood vessels and ducts increased the Mallory's trichrome stain surface area in the parotid gland of hyperlipidemic rats. All these findings denoted the occurrence of fibrosis in the parotid gland of hyperlipidemic rats which is in agreement with previous experimental work[28]. There was a significant decrease in the mean area percent stained with PAS in the parotid gland of the hyperlipidemic group as compared to control group. This may be due to decreased ability of the gland to synthesize zymogen granules with diminished secretory activity of amylase exerted by degenerated acini leaving empty secretory granules that react weakly with PAS stain[34].

On administration of thyme for rats of protected group, their parotid gland showed nearly normal arrangement of many healthy acini, ducts and blood vessels with few affected acini. However, many irregular acini with pyknotic nuclei appeared in the parotid sections of treated group. The parotid sections of protected group showed significant up - regulation of PAS reaction, down – regulation of Mallory's trichrome stain as compared to that of hyperlipidemia group. Also the expression of TNF α and α SMA was significantly decreased; however, the Bcl2 expression was significantly increased as compared to the hyperlipidemia group. But the treated group showed significant difference from the protected group regarding all the statistical parameters. These findings were explained by the hypolipidemic, antioxidant and anti-inflammatory activities of thyme as reported by some scientists[35]. Thyme is a very potent ROS scavenger reducing the oxidative stress and lipid peroxidation. This is in agreement with the findings reported by some authors[36] who mentioned that thyme improved the mitochondrial biogenesis and the antioxidant capacity denoting the improved tissue oxygenation. Some scientists[37] explained the redox activity of thyme by its rich content of phenolic and flavonoid compounds as catechin, coumarin, cinnamic acid, rutin, ferulic acid and quercetin. While the hypolipidaemic effect of thyme was owed to its ingredient of thymol and carvacrol that could attenuate the activity of the cholesterol-synthesizing enzyme; Hydroxymethyl-glutaryl Coenzyme A reductase thus reducing the cholesterol level in blood. However, only the carvacrol ingredient of thyme could lower the triglycerides in the blood as reported by some scientist[38].

CONCLUSION

Thyme is a natural hypolipidemic, antioxidant and anti-inflammatory agent that could protect and to a significant lesser extent treat the parotid gland of hyperlipidemia rats model that were induced by Triton WR 1339.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Karin, A.; Brita, K.; Susanne, F.; Helena, P.; Margareta, O. and Bjorn Z. A (2008): two-year clinical lifestyle intervention program for weight loss in obesity. Food Nutr Res.; 52.
2. Bertges, LC; Mourão, CA; Souza, JB and Cardoso, VAC (2011): Hyperlipidemia induced by Triton WR1339 (Tyloxapol) in Wistar rats. Rev Bras Cien Med Saúde. ; 1(1):32–34.
3. Shamshiev, AT; Ampenberger, F.; Ernst, B.; Rohrer, L. Marsland, BJ and Kopf M.(2007): Dyslipidemia inhibits Toll-like receptor-induced activation of CD8 α-negative dendritic cells and protective Th1 type immunity. Journal of Experimental Medicine.; 204(2):441-452.
4. Cominacini, L.; Pasini, AF; Garbin, U. and Davoli, ML.(2000): Oxidized low density lipoprotein (ox-LDL) binding to ox-LDL receptor-1 in endothelial cells induces the activation of NF-kappa B through an increased production of intracellular reactive oxygen species. J Biol Chem.; 275(17):12633-12638.
5. Choi, JS; Park, IS; Kim, SK; Lim, JY and Kim, YM.(2013): Analysis of age-related changes in the functional morphologies of salivary glands in mice. Archives of Oral Biology.;58(11):1635-1642.
6. Choi, JS; Park, IS; Kim, SK; Lim, JY and Kim YM. (2013): Morphometric and functional changes of salivary gland dysfunction after radioactive iodine ablation in a murine model. Thyroid.;23(11):1445-1451.
7. Nederfors, T. (2000): Xerostomia and hyposalivation. Adv Dent Res.;14:48-56.
8. Proctor, GB and Carpenter, GH. (2007): Regulation of salivary gland function by autonomic nerves. Auton Neurosci. ;133(1):3-18.
9. Matsuzaki, T; Suzuki, T; Koyama, H; Tanaka, S and Takata, K. (1999): Aquaporin-5 (AQP5), a water channel protein, in the rat salivary and lacrimal glands: Immunolocalization and effect of secretory stimulation. Cell Tissue Res.;295(3):513–21.
10. Konttinen, YT; Platts, LA; Tuominen, S; Eklund, KK; Santavirta, N; Törnwall, J; Sorsa, T; Hukkanen, M and Polak, JM (1997): Role of nitric oxide in Sjögren's syndrome. Arthritis Rheum.;40(5):875-83.

11. Lomniczi, A; Suburo, AM; Elverdin, JC; Mastronardi, CA; Diaz, S; Rettori, V and McCann, SM.(1998): Role of nitric oxide in salivary secretion. Neuroimmunomodulation.; 5(5):226-33.
12. Abiy, Y; Sten, T and Trease, G. (2005): Antimicrobial flavonoids from the stem bark of Erythrina burtii. Fitoterapia.;76(5):496-499.
13. Dogu-Baykut, E; Gunes, G and Decker, EA.(2014): Impact of shortwave ultraviolet (UV-C) radiation on the antioxidant activity of thyme (Thymus vulgaris L.). Food Chemistry.; 157:167-173.
14. Domaracky, M; Rehak, P; Juhas, S and Koppel, J.(2007): Effects of selected plant essential oils on the growth and development of mouse pre-implantation embryos in *vivo*. Physiol Res.;56(1):97-104.
15. Amarowicz, R; Zegarska, Z; Rafałowsi, R; Pegg, RB; Karamac, M and Kosin, A. (2008): Antioxidant activity and free radical-scavenging capacity of ethanolic extracts of thyme, oregano, and marjoram. European Journal of Lipid Science and Technology.;111(11):1-7.
16. Dorman, HGD and Deans SG. (2000): Antimicrobial agents from plants: antibacterial activity of plant volatile oils. J Appl Microbiol.;88(2):308-316.
17. Ocaña, A and Reglero, G. (2012): Effects of thyme extract oils (from Thymus vulgaris, Thymus zygis, and Thymus hyemalis) on cytokine production and gene expression of ox LDL-Stimulated THP-1-Macrophages. J Obes. ;(2):104706.
18. El Nekeety, AA; Mohamed, SR; Hathout, AS; Hassan, NS and Aly, SE. (2011): Antioxidant properties of Thymus vulgaris oil against aflatoxin- induce oxidative stress in male rats. Toxicon.;57(7-8):984-991.
19. Vigo, E; Cepeda, A; Gualillo, O and Perez-Fernandez, R. (2004): In-*vitro* anti-inflammatory effect of Eucalyptus globulus and Thymus vulgaris: nitric oxide inhibition in J774A.1 murine macrophages. J Pharm Pharmacol.;56(2):257-263.
20. Oh, PS; Lee, SJ and Lim, KT.(2006): Hypolipidemic and antioxidative effects of the plant glycoprotein (36 kDa) from Rhus verniciflua stokes fruit in Triton WR-1339-induced hyperlipidemic mice. Biosci Biotechnol Biochem.; 70(2):447-456.
21. Noor, H. Swayeh; Ahmed, R. Abu-Raghif; Ban J. Qasim and Hayder, B. Sahib (2014): The protective effects of the Thymus Vulgaris Aqueous Extract against induced hepatic toxicity in rabbits. Int. J. Pharm. Sci. Rev. Res.,29(2):187-193.
22. Kalleny, N.K. and Soliman, N.B.(2011): light and electron microscopic study on the effect of topically applied hyaluronic acid on experimentally induced corneal alkali burn in albino rats. Egyptian journal of histology.;34: 829-848.
23. AboGazia,M.(2013):Preclinicalimmunohistochemical biomarkers for testicular changes in hyperlipidaemic rats and the role of ocimum gratissimum. Egyptian journal of histology.; 36(4):751-762.
24. Izumi, M; Hida, A; Takagi, Y; Kawabe, Y; Eguchi, KT and Nakamura, T.(2000): MR imaging of the salivary glands in sicca syndrome: Comparison of lipid profiles and imaging in patients with hyperlipidemia and patients with Sjögren's syndrome. Am J Roentgenol.;175(3):829-834.
25. Lukach, L; Maly, A; Zini, A and Aframian, DJ. (2013):Morphometrical study of minor salivary gland in xerostomic patients with altered lipid metabolism. Oral Diseases. ;20(7):714-719.
26. Mohamed, AS; Ibrahim, WM; Zaki, NI; Ali, SB and Soliman, AM. (2019): Effectiveness of Coelatura aegyptiaca Extract Combination with Atorvastatin on Experimentally Induced Hyperlipidemia in Rats. Evidence-Based Complementary and Alternative Medicine. ;9 pages.
27. Daskala, ID and Tesseromatis, CC.(2011): Morphological Changes of Parotid Gland in ExperimentalHyperlipidemia.IntJDent.;2011:928386.
28. Altayeb, ZM.(2017): Possible Protective Role of Green Tea Extract on Male Rat Parotid Gland in High Fat Diet Induced Obesity (Histological Study). JMH.;2(1):69-80.
29. Hopps, E; Noto, D; Caimi, G and Averna, MR. (2010): A novel component of the metabolic syndrome: the oxidative stress. Nutr Metab Cardiovasc Dis.; 20(1):72-77.
30. Ogaly, AH; Khalaf, AA; Ibrahim, MA; Galal, MK and Abd-Elsalam, RM.(2015): Influence of green tea extract on oxidative damage and apoptosis induced by deltamethrin in rat brain. Neurotoxicology and Teratology.;50:23-31.
31. Dela Paz, NG; Simeonidis, S; Leo, C; Rose, DW and Collins, T.(2007): Regulation of NF-κB-dependent gene expression by the POU domain transcription factor Oct-1. J Biol Chem. ;282(11):8424–8434.
32. Todd, NW; Luzina, IG and Atamas, SP. (2012): Molecular and cellular mechanisms of pulmonary fibrosis. Fibrogenesis & Tissue Repair.; 5(1):11.
33. Sun, KH; Chang, Y; Reed, NI and Sheppard, D.(2016): α-Smooth muscle actin is an inconsistent marker of fibroblasts responsible for force-dependent TGFβ activation or collagen production across multiple models of organ fibrosis. Am J Physiol Lung Cell Mol Physiol. ;310(9):L824-36.
34. Elghamrawy, T. (2015): The effect of liquid diet on the parotid gland and the protective role of L-carnitin: Immunohistochemical and ultrastructural study.Folia morphologica;74(1) :42-9.

35. Ramchouna, M; Harnafib, H; Alemc, C; Bücheled, B; Simmetd, T; Rouise, M and Atmania, F. (2012): Hypolipidemic and antioxidant effect of polyphenol-rich extracts from Moroccan thyme varieties. Clinical Nutrition ESPEN.;7(3):E119–E124.

36. Khani, M; Motamedi, P; Dehkhoda, MR; Nikukheslat, SD and Karimi, P. (2017): Effect of thyme extract supplementation on lipid peroxidation, antioxidant capacity, PGC-1α content and endurance exercise performance in rats. J Int Soc Sports Nutr.;14:11.

37. Wisam, SU; Nahla, TK and Tariq, NM.(2018): Antioxidant Activities of Thyme Extracts. Pakistan Journal of Nutrition. ;17(1):46-50.

38. Khaksar, V; Krimpen, MV; Hashemipour, H and Pilevar, M.(2012): Effects of thyme essential oil on performance, some blood parameters and ileal microflora of Japanese quail. The Journal of Poultry Science. ;49(2):106-110.

الملخص العربى

تقييم دور الزعتر في وقاية و علاج الغدة النكفية في ذكور الجرذان البيضاء البالغة المصابة بارتفاع دهون الدم المستحث بعقار Triton WR-1339

نهى محيي عيسى[١]، داليا عبد الرازق نويه[٢]، رشا ممدوح سلامة[١]

[١]قسم التشريح والجنة، [٢]قسم الأنسجة والخلايا - كلية الطب - جامعة المنوفية

الخلفية والهدف من البحث: ارتفاع دهون الدم مشكلة شائعة. وهي عامل اساسي في التسبب في العديد من الامراض .كذلك استخدام مثبطات الدهون يسبب العديد من المشكلات الصحية. لذلك تم عمل هذه الدراسة لمعرفة التغيرات النسيجية في الغدة النكفية في الجرذان التي تعاني من ارتفاع دهون الدم المستحث بعقار Triton WR-١٣٣٩ وللتحقق من التاثير الوقائي والعلاجي للزعتر كمنتج طبيعي

المواد والطرق المستخدمة: تم تقسيم خمسين من ذكور الجرذان البيضاء بشكل عشوائي إلى خمس مجموعات. المجموعة الأولى (الضابطة) ، المجموعة الثانية : تلقت الجرذان الزعتر (٥٠٠ مجم / كجم من وزن الجسم) لمدة عشرة أيام ، المجموعة الثالثة: تلقت الجرذان جرعة واحدة داخل الغشاء البريتوني مقدارها ١,٠ مل من Triton WR1339. المجموعة الرابعة : تلقت الجرذان الزعتر لمدة عشرة أيام و تم استحثاث زيادة في دهون الدم في اليوم السابع من التجربة. المجموعة الخامسة: تلقت الجرذان الزعتر بعد ٢٤ ساعة من استحثاث لزيادة دهون الدم وطوال التجربة. بعد ثلاثة أيام من استحداث ارتفاع دهون الدم ، تم جمع عينات الدم لقياس الكوليسترول الكلي والدهون الثلاثية في الدم. تمت معالجة الغدة النكفية للدراسات النسيجية والنسجوكميائية والكيميا مناعية. وتم إجراء التحليل المورفومتري والإحصائي.

النتائج: الجرذان المصابة بارتفاع دهون الدم المستحث بعقار Triton WR1339 طورت التهابًا حادًا في الغدة النكفية مما أدى إلى نتائج نسيجية واضحة وزيادة ملحوظة في العلامات الكيميائية و المناعية للالتهاب والتليف. تم تحسين هذه النتائج عن طريق الزعتر كعامل وقاية وبدرجة أقل كعلاج.

الاستنتاج: يمكن استخدام الزعتر كعامل وقائي ضد مشاكل الغدة النكفية الناتجة عن زيادة دهون الدم من خلال خصائصه المضادة للالتهابات ومضادات الأكسدة.