Original Article

Effect of High Fat Diet on the Rat Submandibular Salivary Glands with the Possible Protection by Gomisin A: Light and Scanning Electron Microscopic Study

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ABSTRACT

Introduction: Obesity is an epidemic health problem leads to adverse effects on different body organs especially salivary glands. Gomisin A is a diben¬zocyclooctadiene lignan derived from Schisandra Chinensis fruits. It possesses anti-oxidation, anti-inflammatory and anti-obesity actions.

Aim of the Work: This study was conducted to evaluate the effect of high fat diet on the rat submandibular salivary glands with the possible protection by gomisin A, using histological, immunohistochemical and scanning electron microscopic study.

Materials and Methods: For 6 weeks' duration, 50 adult male albino rats (150-200 gm) were used divided into 5 groups (10 rats each); Group 1: Control; Group 2: High fat diet (HFD): rats received high fat diet; Group 3: High fat diet (HFD)+ dimethyl sulfoxide (DMSO): rats received DMSO 1 hr. before HFD; Group 4: High fat diet (HFD) + Gomisin A low dose (GLD): rats received gomisin A in a low Dose 5 mg/kg/day 1 hr. before HFD; and Group 5: high fat diet (HFD) + Gomicin A high dose (GHD): rats received a high dose of gomisin A (20 mg/kg/day) 1 hr. before HFD, doses taken orally by intragastric tube.

Results: High fat diet group showed significantly increased gland weight. H&E sections showed disturbed architecture, congested blood vessels, inflammatory cellular infiltrations, acinar cells with cytoplasmic vacuoles, and darkly stained nuclei, beside vacuolated ducts. Also, significantly decreased proliferating cell nuclear antigen (PCNA) reaction, significantly decreased beta cell lymphoma-2 (Bcl-2) reaction, and duct cells with lost membranous expression for E-cadherin appeared. Additionally, the scanning electron microscope showed distended lobules while shrinkage of others, apparently increased acini with accumulated secretion in the secretory granules and apparently dilated ducts. These degenerative changes were attenuated by gomicin A at dose dependent manner.

Conclusion: High fat diet induced structural changes in the rat submandibular salivary glands, and gomisin A could protect against these changes.

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Key Words: Gomisin A; Light; high fat diet; scanning electron microscope; submandibular salivary glands.

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INTRODUCTION

Obesity is an epidemic health problem that has reached pandemic proportions recently. It could affect the quality of life because of its associated complications like diabetes mellitus, sterility, asthma and cancer in association with cardiovascular, renal and hepatic disorders[1,2]. The consumption of food with high energy fat could lead to many negative effects like adipose tissue accumulation with the risk of obesity. Also, the natural products such as phytochemicals that are present in fruits and vegetables have been reported to be vital modulators of the obesity-associated risks[3,4].

Higher caloric intake of carbohydrates and lipids causes an increase in insulin resistance, that can be defined as reduced sensitivity of tissues to insulin hormone[5,6]. Many researches indicates that lipids are more specifically involved in the onset of insulin resistance in the peripheral tissues. It results from an imbalance between increased free fatty acids cellular supply and their inadequate utilization[7,8]. Nowadays, insulin resistance has a great attention from scientists and clinicians as a serious health problem as a main cause of diabetes mellitus[9].

Obesity could lead to adverse effects on different body organs especially salivary glands (SGs)[10]. SGs are responsible for the secretion of different enzymes, and growth factors needed for the biological balance of the oral cavity as well as for its protection[11]. To date, the studies relating obesity to the salivary gland structural alterations are insufficient; although its relation to several oral pathologies such as periodontitis, xerostomia and tooth caries[12,13].

Gomisin A (GA) is a major dibenzocyclooctadiene lignan derived from Schisandra Chinensis fruits[14]. The Schisandra Chinensis fruits has been widely used for traditional medicinal purposes in China, Japan, Korea, and Russia. It possesses many interesting biological effects such as anti-obesity[15], anti-oxidant[16], anti-inflammatory[17], antiviral[18], anti-aging[19,20]. Moreover, it was reported to have a hepato as well as cardio-protective effects[21].

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Based on the above mentioned data, this study was conducted to evaluate the effect of high fat diet on the submandibular salivary glands with the possible protection by gomisin A, using histological, immunohistochemical and scanning electron microscopic study.

MATERIALS AND METHODS

Materials

1. High fat diet was prepared through the addition of 20% animal fat + 1% cholesterol to the animal standard diet. It was prepared every two days, and kept at 4◦C until used. It should be left at the room temperature 1 hr. before use.
2. Cholesterol (Sigma Company, Egypt): A white powder in plastic bottles each containing 100 grams.
3. Gomisin A (Sigma, Egypt): A white to beige powder. The stock concentration of gomisin A was prepared in 20 mg/mL dimethyl sulfoxide (DMSO).

Animals

50 adult male albino rats (150-200 gm) were used. They were obtained from the animal house, Tanta University, Egypt. Animals were kept at 22°C, with a relative humidity of 60%. Also, animals were kept in clean properly ventilated cages with the same environmental conditions. They were allowed free access to a balanced diet and water ad libitum.

The present research was carried out in accordance with the guidelines for the care and use of the experimental animals in Tanta University, Faculty of Medicine, Egypt. Additionally, the approved code of the Local Ethics Committee of the Faculty for the research is (34820/8/21).

The experimental Groups

1. **Group 1:** (Control group): included 10 rats that were further subdivided into subgroup a: 5 rats received no treatments and subgroup b: 5 rats received dimethyl sulfoxide (DMSO) (solvent of Gomisin) in a dose equivalent to its corresponding doses for 6 weeks.
2. **Group 2:** High fat diet group (HFD): 10 rats received high fat diet orally through an intragastric tube for 6 weeks.
3. **Group 3:** High fat diet + dimethyl sulfoxide group (HFD+DMSO): 10 rats received DMSO 1 hr. before HFD orally through an intragastric tube for 6 weeks.
4. **Group 4:** High fat diet + Gomisin A low dose group (HFD+ GLD): 10 rats received gomisin A in a low Dose 5 mg/kg/day 1 hr. before HFD orally through an intragastric tube for 6 weeks.
5. **Group 5:** High fat diet + Gomicin A high dose group (HFD+ GHD): 10 rats received a high Dose of gomisin A (20 mg/kg/day) 1 hr. before HFD orally through an intragastric tube for 6 weeks.

The doses were adjusted according to Yun and Jung, (2017)[22] and Yao *et al*., (2011)[23].

By the end of the experiment, rats were anesthetized by an intraperitoneal injection of pentobarbital (60 mg/kg) then, the submandibular salivary glands (SMGs) were dissected out, washed with saline. Finally, one part was taken and processed for light microscopic study (histological and immunohistochemical) while the other was processed for scanning electron microscope.

Processing for light microscopic study

Specimens from the SMGs were fixed in 10% formalin buffered saline then, dehydration, clearance and finally embedding in hard paraffin. Then, through a rotatory microtome (Leica, US); 5 µm sections were obtained[24].

Hematoxylin & Eosin (H&E)

Sections were deparaffinized, hydrated then, stained with hematoxylin for 10 minutes followed by counterstaining in 1% Eosin solution. At last, sections were dehydrated, cleared and mounted. Nuclei stained blue with hematoxylin, while eosin-stained the cytoplasm as well as connective tissue fibers with pink coloration[24].

Immunohistochemistry for PCNA (Proliferating cell nuclear antigen), Bcl-2 (Beta cell lymphoma-2) and E-cadherin (Epithelial- cadherin)[24-27]:

Paraffin sections of 5 µm thickness were obtained and situated on positive slides then deparaffinized, and rehydrated. 0.3% hydrogen peroxide/methanol were used to block the endogenous hydrogen peroxidase activity. Then, 10% normal goat serum was added followed by incubation overnight at 4°C in a humid chamber with mouse monoclonal anti- PCNA (1:75) (Santa Cruz Biotechnology, USA), anti-Bcl2 (1:50) (Santa Cruz Biotechnology, USA) and anti-E-cadherin antibodies (1:100) (Santa Cruz Biotechnology, USA). Sections then were incubated with the biotinylated goat anti-rabbit secondary antibody (1:100, Vector Labs, Peterborough, United Kingdom) 30 minutes at room temperature followed by the addition of 3, 3′-diaminobenzidine chromogen. Later, counterstaining with Mayer's hematoxylin and examination under a light microscope (Olympus, Japan). The positive immunohistochemical results for PCNA was the nuclear brownish staining while a brown coloration in the cytoplasm of the acinar and ductal epithelial cells for positive Bcl-2 immunoreaction, while E-cadherin showed positive membranous reactions. For negative controls; sections were routinely processed with the omission of the primary antibodies. On the other hand, the positive control for PCNA was small intestine while that of the Bcl-2 was tonsils and mammary epithelium for E-cadherin.

Scanning electron microscopic processing

Tissues were fixed at 0.25% glutaraldehyde and 0.25% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2, for about 15 minutes at room temperature. Then, post-fixed in 1.25% potassium ferrocyanide and 1% osmium tetroxide for 2 hours. followed by embedding in agarose, sectioned and macerated with 0.1% osmium tetroxide for 44± 60 hour at room temperature in a rotating agitator. Later, dehydration, drying and coating with platinum. Finally, examination by JEOL scanning electron microscope, The Electron Microscopic Unit, Faculty of Medicine, Tanta University, Egypt[28].

Morphometric studies

Measurement of the submandibular salivary glands' weight

The submandibular salivary glands of the different experimental groups were extracted and purified from the visible blood rudiments and fat, then were weighted.

Mean number of positive PCNA nuclei

Five non-overlapped sections were randomly selected from five selected slides of each experimental group. Then, the mean number of PCNA positive nuclei was evaluated using an Olympus, Japan light microscope with a magnification of x 400.

Area percentage (%) of positive Bcl-2 immunohistochemical reactions

Bcl-2 positive cells were examined by Leica analyzer computer system (Leica, japan). Bcl-2 positive immunostaining was measured as area % in a standard measuring frame in five fields of each experimental group at a magnification of x 400 by a light microscope. Then the mean values as well as the standard deviation were recorded per each specimen.

Statistical analysis

Data were expressed as means ± standard deviation (SD) through using Minitab Statistical Software program for Windows (version 16.1, Minitab Inc., State College, USA). Student t-test (student two-sample test) was performed after evaluation of F-test (Fisher-test) to compare the HFD, with the control group. While the one-way ANOA test (analysis of variance test) was used to compare between the HFD+DMSO; HFD+GLD; and HFD+GHD groups with the HFD group. When *P* (probability value) is less than 0.05; it is considered as significant.

RESULTS

Submandibular salivary glands' weight

As regards SMGs' weight of the different experimental groups; it showed significant increase in the groups of HFD when compared to the control group. While showed significant decrease in the groups of HFD+GLD and HFD+GHD as compared to the HFD group, while non-significant difference of the HFD+DMSO group compared to the HFD group (Histogram 1).

H&E results

The normal histological structure of the submandibular salivary gland was seen in H&E stained sections. It was consisted of lobules separated by connective tissue septa containing blood vessels and excretory ducts (Figure 1 A1). The serous acini were seen spherical in shape with basophilic pyramidal shaped cells having rounded basally situated nuclei. Considering the glandular ducts; there were intercalated ducts (IDs) seen lined by short cuboidal cells with centrally located rounded nuclei, granular convoluted tubules (GCTs) lined by tall columnar cells with eosinophilic cytoplasm and rounded basally situated nuclei, and striated ducts (SDs) lined by columnar cells with centrally located rounded nuclei and basal striations (Figure 1 A2).

HFD group exposed disturbed glandular architecture with multiple degenerative changes (Figure 2 B1). These were in the form of congested apparently dilated blood vessels. Besides, acinar cells with many cytoplasmic vacuoles and some darkly stained nuclei. Considering the glandular ducts; they showed vacuolated cytoplasm, and darkly stained nuclei (Figure 2 B2). Moreover, inflammatory cellular infiltrations were observed (Figure 2 B3). On the other hand, HFD+DMSO group showed congested apparently dilated blood vessels, acinar cells with many cytoplasmic vacuoles and some darkly stained nuclei with IDs; GCTs and SDs with vacuolated cytoplasm, and darkly stained nuclei (Figure 2 C).

HFD+GLD group showed moderate improvement with blood vessel congestion and some serous acini, GCTs and SDs with vacuolations (Figure 3 D). As regards the HFD+GHD group, it showed a nearly normal histological glandular structure with nearly normal serous acini, IDs, GCTs and SDs (Figure 3 E).

PCNA immunohistochemical results

Showed negative PCNA immunohistochemical reaction of the serous acini (Figure 4 A).

The control group showed positive immunohistochemical reaction of most nuclei of the acinar cells for PCNA, while only some nuclei of the IDs, GCTs and SDs cells (Figure 4 B). On the other hand, the HFD and the HFD+DMSO groups revealed few serous acinar cells as well as IDs, GCTS, and SDs cells with positive PCNA immunohistochemical reaction (Figures 4 C,D respectively). By observation of the HFD+GLD group; it showed some nuclei of the acinar cells as well as few of the glandular ducts with positive PCNA reaction (Figure 4 E). While, the HFD+GHD group revealed nearly normal immunohistochemical reaction with positive PCNA expression of most nuclei of the acinar cells, and some nuclei of that lined the IDs, GCTS, and SDs (Figure 4 F).

As regards the mean number of PCNA positive nuclei of the different experimental groups; there was a significant decrease of the HFD group when compared to the control

group. Whereas, a significant increase was observed in the groups of HFD+GLD and HFD+GHD compared to the HFD group. While, the HFD+DMSO group showed a non-significant difference when compared to the HFD group (Histogram 2).

Bcl-2 immunohistochemical results

Showed negative Bcl-2 cytoplasmic reaction of the serous acini and glandular ducts (Figure 5 A).

Immunohistochemical results for Bcl-2 of the control group showed strong positive cytoplasmic reaction of most of the GCTs and SDs cells. While, the IDs revealed negative reaction besides moderate reaction of few of the serous acinar cells (Figure 5 B). On the other hand, the HFD & HFD+DMSO groups expressed mild Bcl-2 cytoplasmic reaction of both GCTs and SD cells while negative cytoplasmic reactions of both IDs and the serous acinar cells (Figures 5 C,D respectively). Considering, the HFD+GLD group; it showed GCTS and SD cells with moderate positive Bcl-2 cytoplasmic reaction and negative for the serous acinar cells (Figure 5 E). Finally, the HFD+GHD group revealed a nearly normal image with strong positive cytoplasmic reaction of most of the GCTs, and SDs cells, negative reaction of the IDs and moderate reaction of few of the serous acinar cells (Figure 5 F).

Considering the area % of Bcl-2 immunohistochemical reaction of the different experimental groups; there was a significant decrease considering the groups of the HFD group when compared to the control group. Whereas, a significant increase was observed in the groups; HFD+GLD and HFD+GHD in comparison to the HFD group. Meanwhile, the HFD+DMSO group showed a non-significant difference as compared to the HFD group (Histogram 3).

E-Cadherin immunohistochemical results

Showed a negative E-Cadherin expression of the serous acini and glandular ducts (Figure 6 A).

Control group revealed strong positive membranous reaction for E-cadherin of the serous acinar as well as the duct cells (Figure 6 B). On the other hand, lost membranous expression of serous acinar, ID, GCTs and SD cells for E-cadherin of both HFD & HFD+DMSO groups was observed (Figures 6 C,D respectively). Moreover, the HFD+GLD group showed positive reaction for the E-cadherin which was more cytoplasmic than membranous in some GCTs (Figure 6 E). While, HFD+GHD group revealed a picture similar to the control with positive membranous reaction for E-cadherin of the serous acinar, IDs, GCTs and SDs (Figure 6 F).

Scanning electron microscopic (SEM) results

The control group showed multiple gland lobules separated by interlobular spaces which contained collagen fibrils as well as blood vessels (Figure 7 A). The acini contained many secretory granules of variable sizes and shapes that was surrounded by multiple blood capillaries (Figure 7 B). Moreover, the duct formations were seen at the areas in-between the secretory granules of the acinar cells (Figure 7 C). Additionally, the cut surface of each lobule revealed openings of the secretory ducts besides multiple secretory acini (Figure 7 D).

The HFD group showed distension of some lobules while shrinkage of the others (Figure 8 A). Considering the acini; they were apparently increased in size with distention and accumulated secretion in the secretory granules (Figure 8 B). In addition, apparently dilated ducts were observed at the cut section of the lobules (Figure 8 C).

The HFD+DMSO group showed distension of some lobules while shrinkage of the others (Figure 9 A). Considering the acini; they were apparently increased in size with distention and accumulated secretion in the secretory granules (Figure 9 B). In addition, apparently dilated ducts were observed at the cut section of the lobules (Figure 9 C).

The HFD+GLD group revealed some distended lobules between nearly normal others (Figure 10 A). Also, there were some distended secretory acini by the accumulated secretions while others were nearly normal in size (Figure 10 B). Additionally, some ducts appeared apparently dilated while the others were normal in appearance (Figure 10 C).

As for the HFD+GHD group; it showed a SEM appearance like the control group with multiple lobules separated by interlobular spaces (Figure 11 A). Also, there were acini with many secretory granules of variable sizes and shapes surrounded by blood capillaries (Figure 11 B). Moreover, the cut surface with gland openings of the secretory ducts besides multiple secretory acini (Figure 11 C).

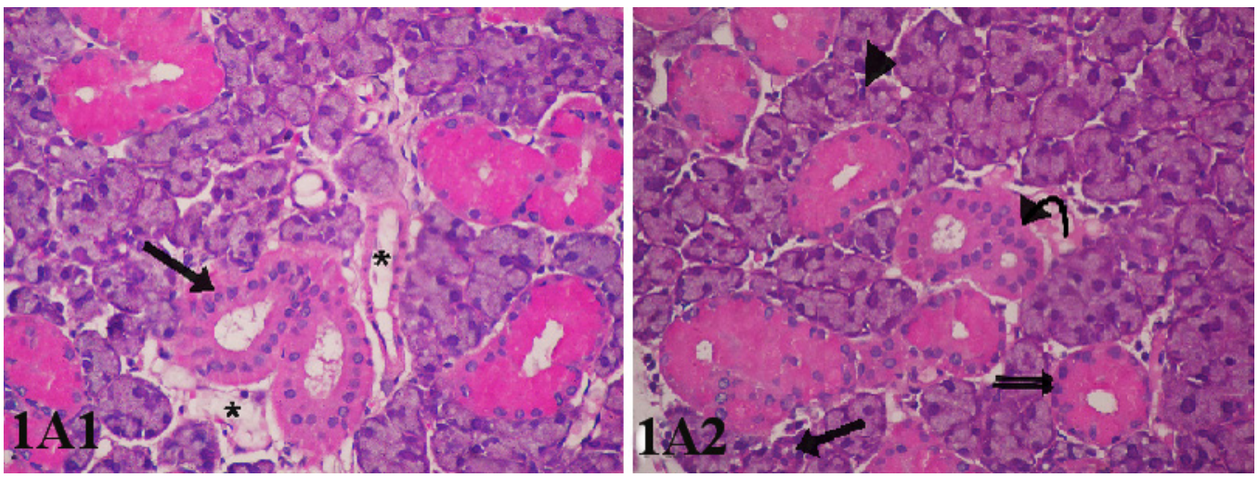


Fig. 1: A1) Control: gland lobules separated by connective tissue septa containing blood vessels (*) and excretory ducts (→). A2) Control: spherical serous acini with basophilic pyramidal shaped cells and rounded basally situated nuclei (►), intercalated ducts (IDs) lined by short cuboidal cells with centrally located rounded nuclei (→), granular convoluted tubules (GCTs) lined by tall columnar cells with eosinophilic cytoplasm and rounded basally situated nuclei (double arrow), and striated ducts (SDs) lined by columnar cells with centrally located rounded nuclei and basal striations (curved arrow) (H&E x400).

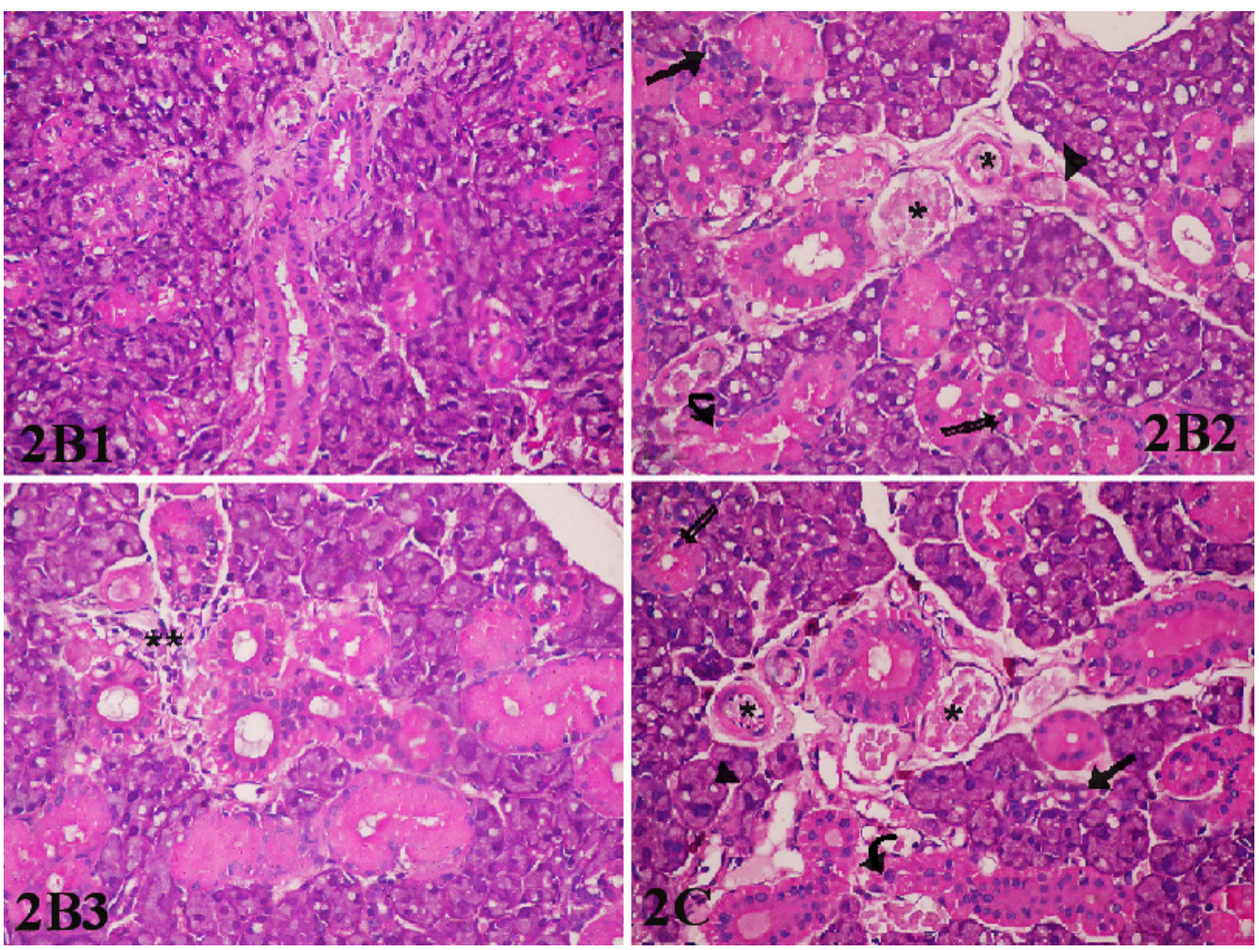


Fig. 2: B1) HFD group: disturbed glandular architecture with multiple degenerative changes (H&E x 200). B2) HFD group: congested apparently dilated blood vessels (*), serous acinar cells with many cytoplasmic vacuoles and some darkly stained nuclei (►), IDs (→); GCTs (curved arrow), and SDs with vacuolated cytoplasm, and darkly stained nuclei (double arrow) (H&E x400). B3) HFD group: inflammatory cellular infiltrations (**) (H&E x400). C) HFD+DMSO group: congested apparently dilated blood vessels (*), acinar cells with many cytoplasmic vacuoles and some darkly stained nuclei (►), IDs (→); GCTs (double arrow) and SDs (curved arrow) with vacuolated cytoplasm, and darkly stained nuclei (H&E x400).

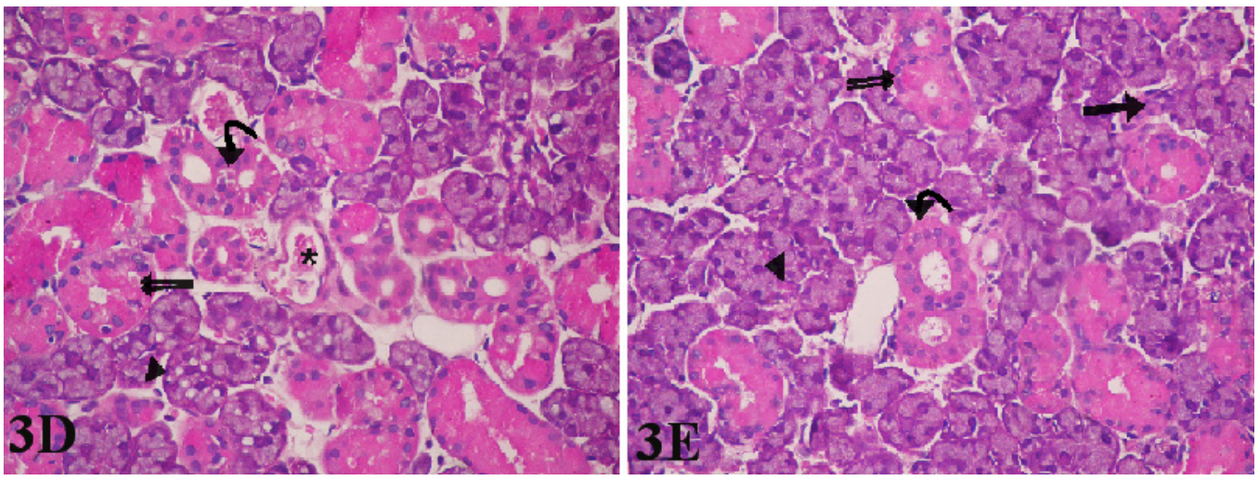


Fig. 3: D) HFD+GLD group: moderate improvement with blood vessel congestion (*) and some serous acini (►), GCTs (double arrow) and SDs with vacuolations (curved arrow). E) HFD+GHD group: nearly normal serous acini (►), IDs (→); GCTs (double arrow) and SDs (curved arrow) (H&E x400).

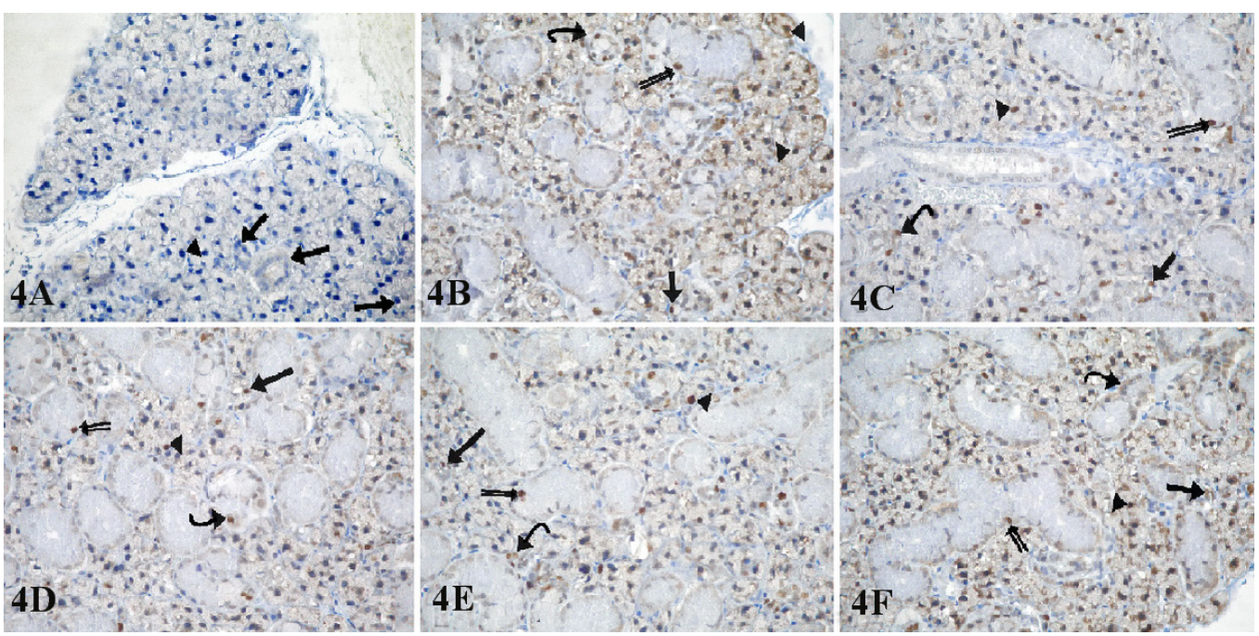


Fig. 4: A) Negative PCNA immunohistochemical reaction of the serous acini (►) and glandular ducts (→). B) Control: positive PCNA expression of most nuclei of the acinar cells (►) and some nuclei of that lined the IDs (→), GCTs (double arrow) and SDs (curved arrow). C) HFD group: few serous acinar cells (►), IDs (→), GCTS (double arrow), and SDs cells (curved arrow) with positive PCNA immunohistochemical reaction. D) HFD+DMSO group: few serous acinar cells (►), IDs (→), GCTS (double arrow), and SDs cells (curved arrow) with positive PCNA immunohistochemical reaction. E) HFD+GLD group: some nuclei of the acinar cells (►) and few of IDs (→), GCTS (double arrow), and SDs (curved arrow) with positive PCNA reaction. F) HFD+GHD group: positive PCNA expression of most nuclei of the acinar cells (►) and some nuclei of that lined the IDs (→), GCTS (double arrow), and SDs (curved arrow) (PCNA immunohistochemical reaction x 400).

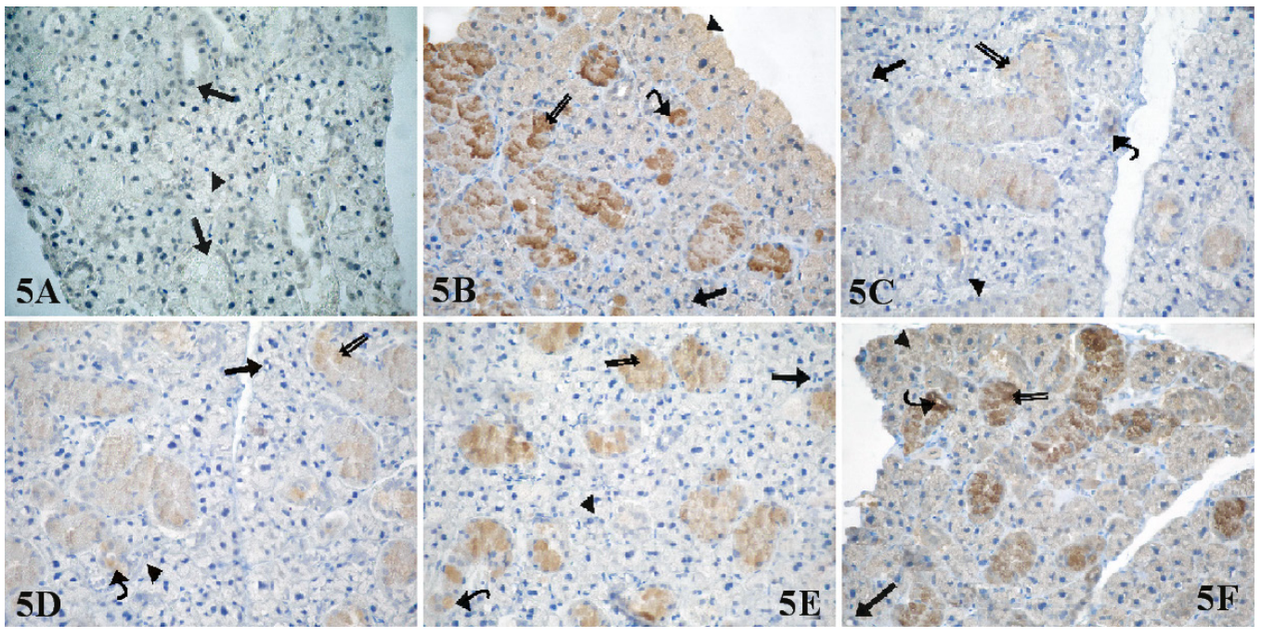


Fig. 5: A) Negative Bcl-2 cytoplasmic reaction of the serous acini (►) and glandular ducts (→). B) Control: strong positive cytoplasmic reaction of most of the GCTs (double arrow), and SDs cells (curved arrow), negative reaction of the IDs (→) and moderate reaction of few of the serous acinar cells (►). C) HFD group: mild Bcl-2 cytoplasmic reaction of both GCTs (double arrow), and SDs cells (curved arrow), negative reaction of the IDs (→) and the serous acinar cells (►). D) HFD+DMSO group: mild Bcl-2 cytoplasmic reaction of both GCTs (double arrow), and SDs cells (curved arrow), negative reaction of the IDs (→) and the serous acinar cells (►). E) HFD+GLD group: GCTS (double arrow), and SDs cells (curved arrow) with moderate positive Bcl-2 cytoplasmic reaction and negative for the ID (→) and serous acinar cells (►). F) HFD+GHD group: strong positive Bcl-2 cytoplasmic reaction of most of the GCTs (double arrow), and SDs cells (curved arrow), negative reaction of the IDs (→) and moderate reaction of few of the serous acinar cells (►). (Bcl-2 immunohistochemical reaction x 400).

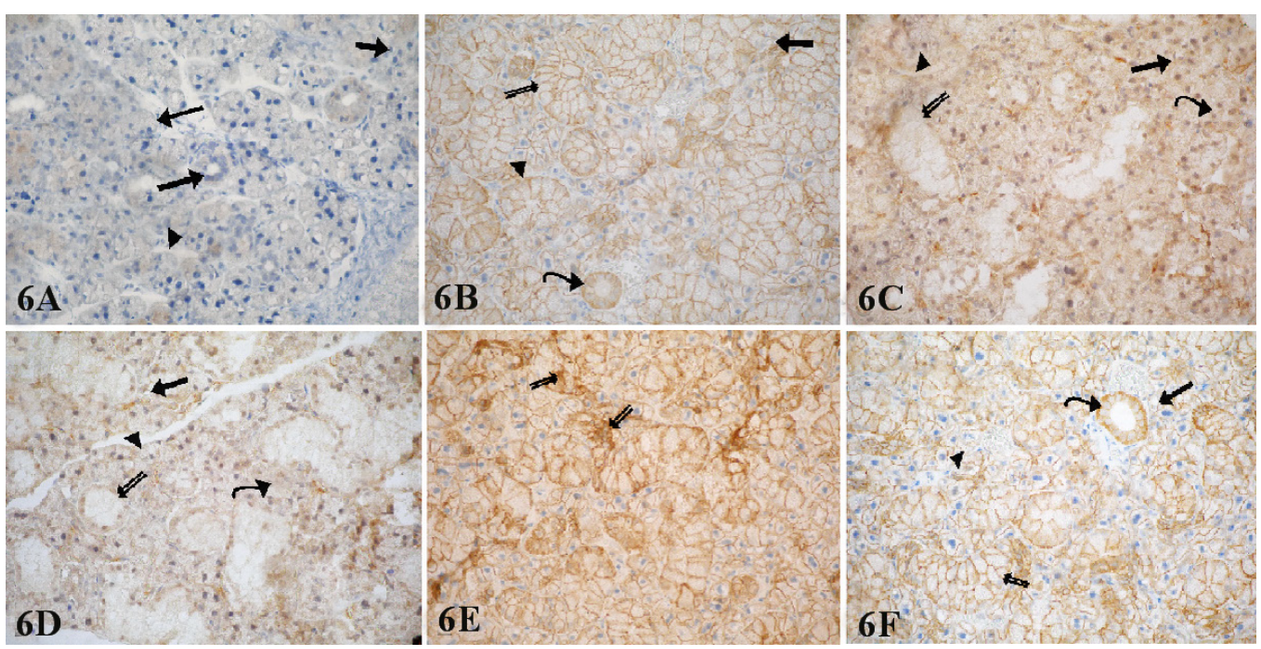


Fig. 6: A) Negative E-Cadherin expression of the serous acini (►) and glandular ducts (→). B) Control group: strong positive membranous reaction for E-cadherin of the serous acinar (►), IDs (→), GCTs (double arrow) and SDs (curved arrow). C) HFD group: lost membranous expression of serous acinar (►), IDs (→), GCTs (double arrow) and SDs (curved arrow). D) HFD+DMSO group: lost membranous expression of serous acinar (►), IDs (→), GCTs (double arrow) and SDs (curved arrow). E) HFD+GLD group: positive reaction for the E-cadherin which was more cytoplasmic than membranous in some GCTs (double arrow). F) HFD+GHD group: strong positive membranous reaction for E-cadherin of the serous acinar (►), IDs (→), GCTs (double arrow) and SDs (curved arrow). (E-Cadherin immunohistochemical reaction X 400).

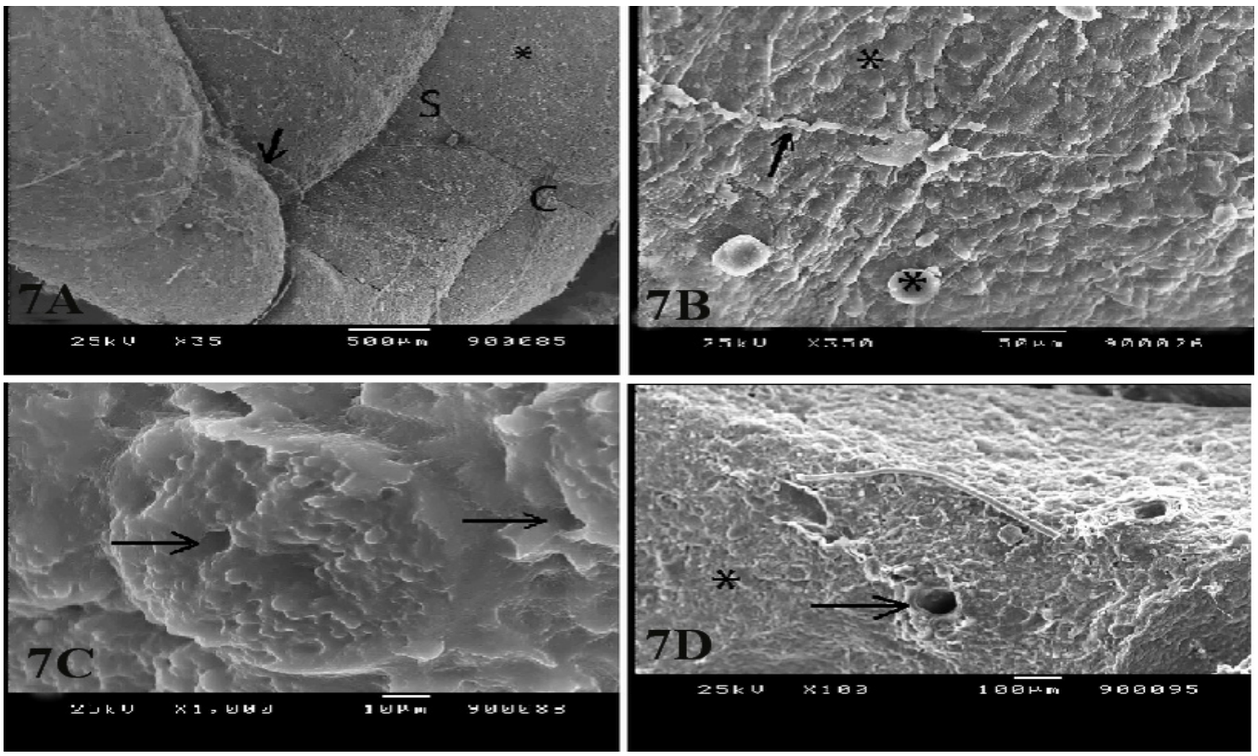


Fig. 7: Scanning electron microscopic results of the control group. A) multiple gland lobules (*) separated by interlobular spaces (S) contained collagen fibrils (C) and blood vessels (→). B) The acini contained many secretory granules of variable sizes and shapes (*) surrounded by multiple blood capillaries (→). C) duct formations (→) at the areas in-between the secretory granules of the acinar cells. D) the cut surface of each lobule revealed openings of the secretory ducts (→) besides multiple secretory acini (*).

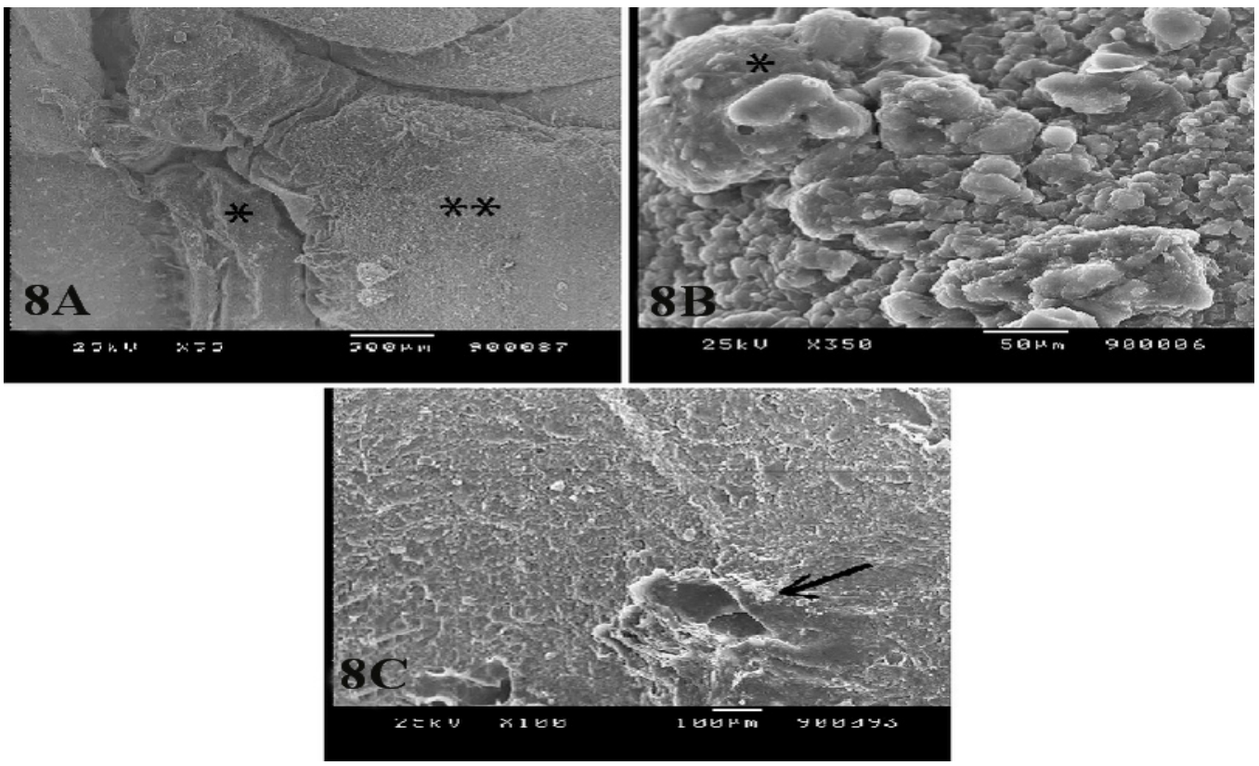


Fig. 8: Scanning electron microscopic results of the HFD group. A) distension of some lobules (**) while shrinkage of the others (*). B) secretory acini that apparently increased in size with distention and accumulated secretion in the secretory granules (*). C) apparently dilated ducts at the cut section of the lobules (→).

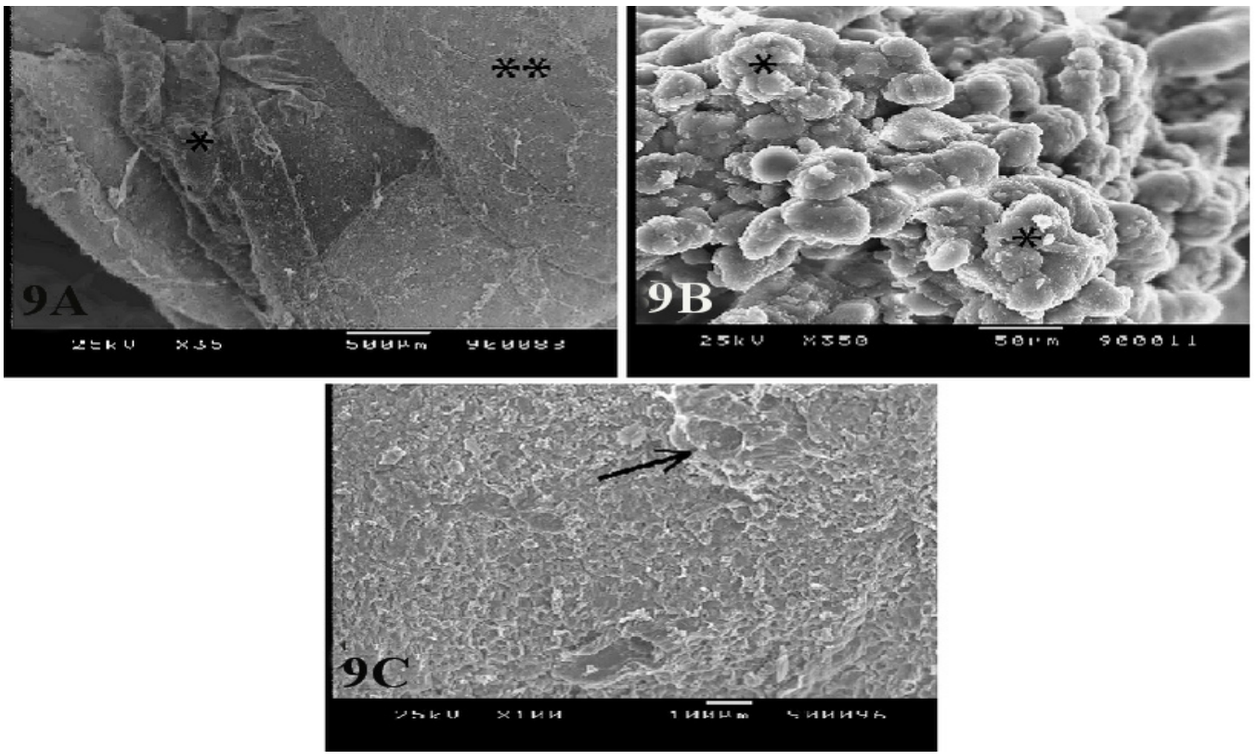


Fig. 9: Scanning electron microscopic results of the HFD+DMSO group A) distension of some lobules (**) while shrinkage of the others (*). B) the acini were apparently increased in size with distention and accumulated secretion in the secretory granules (*). C) apparently dilated ducts at the cut section of the lobules (→).

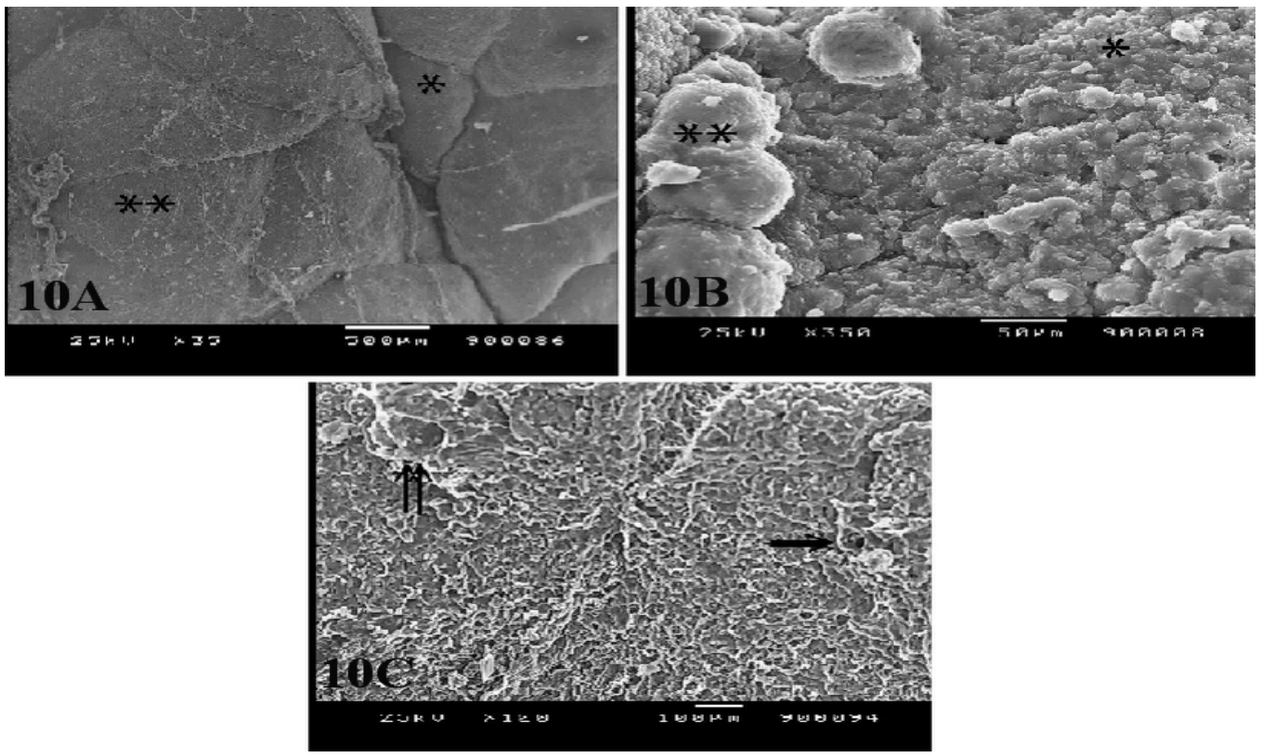


Fig. 10: Scanning electron microscopic results of the HFD+GLD group A) some distended lobules (**) between nearly normal others (*). B) some distended secretory acini by the accumulated secretions (**) while others were nearly normal in size (*). C) some ducts appeared apparently dilated (→) while the others were normal in appearance (double arrow).

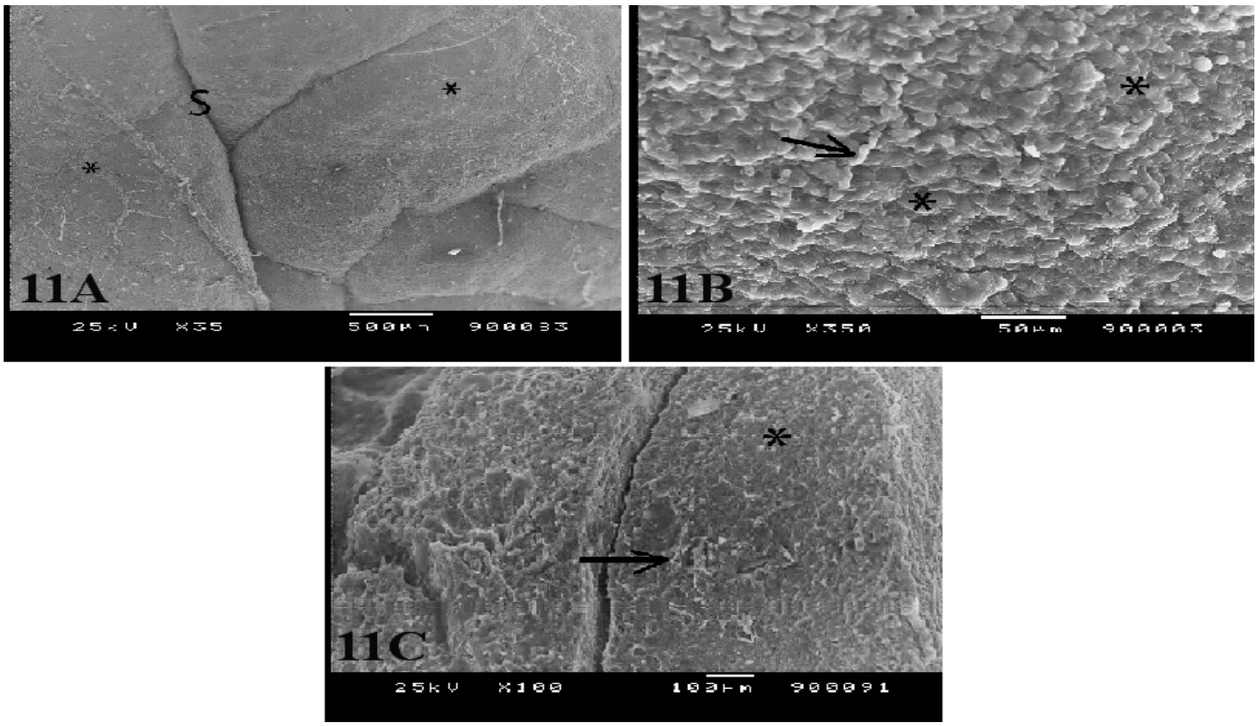
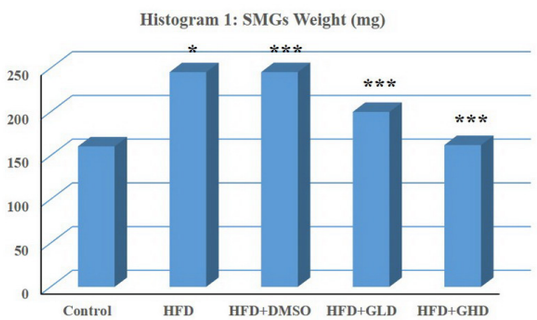
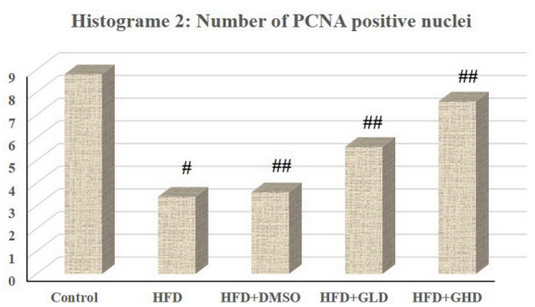


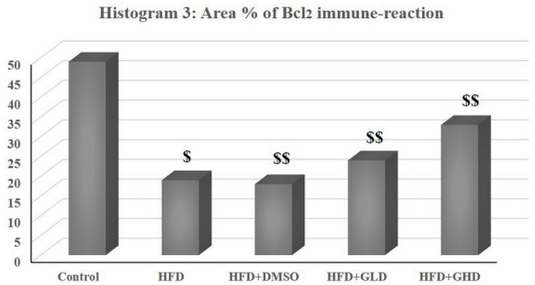
Fig. 11: Scanning electron microscopic results of the HFD+GHD group A) multiple lobules (*) separated by interlobular spaces (S). B) The acini with many secretory granules of variable sizes and shapes (*) surrounded by blood capillaries (→). C) lobules cut surface with the openings of the secretory ducts (→) besides multiple secretory acini (*).



Histogram 1: Weight of the SMGs of the different experimental groups. Data expressed as means ± SD. * HFD (high fat diet) group compared to the control group; *** HFD +DMSO (high fat diet+ dimethyl sulfoxide), HFD+GLD (high fat diet+ gomisin low dose), and HFD+GHD (high fat diet+ gomisin high dose) compared to the HFD group.



Histogram 2: Number of PCNA positive nuclei of the different experimental groups. Data expressed as means ± SD. # HFD (high fat diet) group compared to the control group; ## HFD +DMSO (high fat diet+ dimethyl sulfoxide), HFD+GLD (high fat diet+ gomisin low dose), and HFD+GHD (high fat diet+ gomisin high dose) compared to the HFD group.



Histogram 3: The area % of Bcl-2 immunohistochemical reaction of the different experimental groups. Data expressed as means ± SD. $ HFD (high fat diet) group compared to the control group; $$ HFD +DMSO (high fat diet+ dimethyl sulfoxide), HFD+GLD (high fat diet+ gomisin low dose), and HFD+GHD (high fat diet+ gomisin high dose) compared to the HFD group

DISCUSSION

Consuming high fat diet leads to obesity in humans and animals[29]. Obesity is an alarming medical problem and a risk factor for development of many diseases such as diabetes mellitus, cardiovascular diseases and some types of cancer[1].

The HFD animal model is one of the most reliable used models as it simulates the most common route of obesity in humans as well as its associated metabolic effects such as insulin resistance[30]. The studies that have reported the possible effects of excess lipid deposition on the structure and function of salivary glands are insufficient, and so far the relation between obesity and xerostomia is being debated among researchers. Natural herbal products have been reported to be vital modulators of the obesity related risks[31]. Therefore, in the present study, we examined the effect of a high fat diet (HFD) on the rat SMGs with the evaluation of the possible protective role of gomisin A.

In this study, light microscopic examination of the HFD group exposed disturbed glandular architecture with multiple degenerative changes affecting the acinar cells as well as the glandular ducts as both of them revealed vacuolated cytoplasm and darkly stained nuclei. Similar results were recorded by other researchers[32]. In addition, other clinical studies have reported a higher incidence of xerostomia, dental caries and bad oral hygiene in obese patients than in normal weight ones, denoting obesity related salivary glands hypofunction[10].

The intra-cytoplasmic vacuolations observed in this study were suggested to be of lipid nature as they were dissolved during samples fixation and processing for H&E staining leaving empty vacuoles. These cytoplasmic vacuoles might be attributed to fatty degenerative changes within the glandular acinar cells, as it was reported that excess lipid is temporarily preserved as droplets in the cytoplasm of salivary glandular secretory cells altering the lipid fractions of salivary glands causing their hypofunction[7]. Similar results were recorded by some investigators who reported massive accumulation of lipid droplets in the salivary glandular cells of diabetic rats[33]. In this regard, some authors reported high prevalence of xerostomia in diabetic patients mostly due to salivary gland impaired function[34].

It is well evidenced that excess lipids are stored in adipocytes and used as a source of energy during fasting. However, in HFD, the lipids accumulate in other tissues such as skeletal muscle, liver, kidney, heart and pancreas causing their dysfunction[35].

In recent years, it was evidenced that the imbalance between reactive oxygen species (ROS) levels and anti-oxidants might have an essential role in the development of various salivary gland disorders[36]. Besides, it has been stated that the excess fatty acid induced by HFD disrupts the SMGs anti-oxidative systems, leading to DNA, protein and lipid oxidation, and increases the ROS production that results in oxidative stress with subsequent cellular damage[36].

In this study, HFD provoked inflammatory signs that were in the form of congested dilated blood vessels with inflammatory cellular infiltrations. These findings were similar to that reported in other studies. It might be explained to be an inflammatory response to deliver more blood to the areas of degeneration. A growing evidence suggests a strong association between HFD induced obesity and inflammation as the excess fatty acids increased insulin resistance causing fat droplets and glucose storage in salivary glands, causing their inflam¬mation[37].

Moreover, diffusion of oxygen and nutrients to parenchymal cells might be restricted by these inflammatory changes decreasing parenchymal cells survival and functions.[38]. Moreover, SEM results showed distended acini with accumulated secretion in their secretory granules, and this might cause the observed significant increase in SMG weight of HFD group compared to control. Additionally, dilatation of glandular ducts was observed in the HFD group, and this could be attributed to accumulation of salivary secretion and failure of its excretion due to glandular injury and dysfunction provoked by lipid accumulation as discussed before[39].

Apoptosis plays critical roles in a variety of physiological processes in life. The defects in the apoptotic process results in various diseases. The apoptosis involves many families of proteins. Bcl-2 proteins are one of the families that has an anti-apoptotic activity[40]. In the current study, immunohistochemistry for Bcl-2 was performed to assess the degree of cellular apoptosis. The results showed that HFD provoked apoptotic changes in the SMG acinar and ductal cells as manifested by decreased expression of anti-apoptotic Bcl-2 protein. This was similar to other studies which demonstrated the saturated fatty acids associated pro-inflammatory and apoptotic responses in salivary glands of obese-insulin resistant rats, as well as in human salivary gland epithelial cells of diabetic patients[41]. The apoptotic cell death was also reported in diabetic wound healing as well as diabetic cardiomyopathy[42].

E-cadherin is a transmembrane glycoprotein that is expressed on the cell surface of most epithelial tissues at the level of adherent intercellular junctions. It is a cell adhesion molecule that plays an essential role in maintaining epithelial integrity, cellular polarity as well as cellular differentiation[43]. In this study, HFD significantly decreased membranous expression of E-cadherin in acinar and ductal cells, and thus altered the integrity of SMG epithelial cells. In other studies, the integrity of intercellular tight junctions was reported to be disrupted in SMG of diabetic rat models, and these injured junctions might be responsible for hyposalivation[44].

The immunohistochemical results of the present study revealed decreased glandular cell proliferation in HFD as evidenced by the significant decrease in the mean number of PCNA positive nuclei of the HFD group compared to

control group. Similar results were recorded in a previous study that examined the effect of maternal diabetes on the offspring SMG structure. It reported minimal reaction of PCNA in the SMG of offspring of diabetic mothers, which might be attributed to the increased cell apoptosis[45]

On the other side, our result showed that treatment with gomisin A dose dependently minimized the HFD-induced SMG structural changes in rats. This was manifested by the significant improvement of the SMG weight as well as improvement of the SMG histological, immunohistochemical and SEM changes caused by HFD. This could be attributed to the antioxidant capacity of Gomisin A, as many studies have suggested that the antioxidant compounds from Schisandra chinensis including gomisin A, gomisin J, schisandrin B, schisandrin C reduce oxidative stress caused by excessive ROS production[16].

In addition, recent studies declared that in early stages of adipogenesis, gomisin N; a lignin from Schisandra chinensis; was able to inhibit intracellular lipid accumulation by inhibiting cell proliferation and cell cycle progression, and it was able to attenuate hepatic steatosis in HFD-induced obesity in mice[46]

The present study showed that gomisin A administration decreased the inflammatory cellular infiltration. The anti-inflammatory effect of gomisin A has been well documented in many in in-*vitro* and in-*vivo* studies that contributed the gomisin A anti-inflammatory activity to its effect on the inducible nitric oxide (iNOS) and cyclooxygenase-2(COX-2) pathway. Others reported that, gomisin A anti-inflammatory effect could be due to its attenuating the production of inflammatory genes such as TNF-α, IL-1β, and IL-6[47].

The present study illustrated that gomisin A was beneficial in attenuating the degree of apoptotic cell death of SMGs. This could be mediated through increasing the anti-apoptotic activity of Bcl-2 as observed from the immunohistochemical results of the current work. The anti-apoptotic activity of gomisin A was also recorded by other investigators to be through suppression of active caspase-3[48].

It was stated that the complex relation between cell death and cell proliferation of SMGs is a key feature in maintenance of their normal structure and function. So, with this concept, the current results revealed that gomisin A administration preserved to some extent the SMGs proliferative cells as illustrated in the immunohistochemical results of this study. Which showed a significant increase in the mean number of PCNA positive nuclei of gomisin A treated HFD groups compared to the HFD group, indicating a normal cellular proliferation that maintains the integrity of SMG epithelial cells. Recent studies reported that gomisin A might act as an anti-proliferative agent by arresting the cell cycle[49].

Altogether, our results clearly revealed that HFD induced SMG structural changes in rats, and gomisin A administration could be beneficial in attenuating these changes. This might be through its anti-oxidant and anti-inflammatory and anti-apoptotic actions in addition to its proliferative effects. So, it was suggested that gomisin A has a role as a natural therapeutic agent for the protection of obesity-associated salivary glands complications.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

تأثير النظام الغذائي الغني بالدهون على الغدد اللعابية تحت الفك السفلي في الجرذان مع إمكانية الحماية بواسطة جوميسين أ : دراسة ضوئية ومجهرية إلكترونية ماسحه

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المقدمة: السمنة هى مشكلة صحية وبائية يمكن أن تؤدي إلى آثار سلبية على أعضاء الجسم المختلفة وخاصة الغدد اللعابية. جوميسين أ هو قشوردابينزوسيكلوأوكتادين رئيسي مشتق من ثمار شيزاندرا تشينينسيس التى لديها تأثيرات مضادة للأكسدة و للإلتهابات وللسمنة.

الهدف: أجريت هذه الدراسة لتقييم تأثير النظام الغذائي الغني بالدهون على الغدد اللعابية تحت الفك السفلي للجرذان مع إمكانية الحماية بواسطة جوميسين أ بإستخدام دراسة هستولوجية ،هستوكيميائية مناعية ومجهرية إلكترونية ماسحه.

المواد وطرق البحث: لمدة ٦ أسابيع ، تم إستخدام ٥٠ من الجرذان البيضاء البالغة ١٥٠-٢٠٠ جم مقسمة إلى ٥ مجموعات (١٠ جرذان لكل مجموعة) ؛ المجموعة ١: (المجموعة الضابطة) ؛ المجموعة ٢: مجموعة النظام الغذائي عالي الدهون: تناولت نظام غذائي عالي الدهون. المجموعة ٣: مجموعة نظام غذائي عالي الدهون + DMSO: تناولت DMSO قبل النظام الغذائي عالي الدهون بساعة واحدة؛ المجموعة ٤: مجموعة النظام الغذائي عالي الدهون + جوميسين أ بجرعة منخفضة: تناولت جرعة منخفضة من جوميسين أ (٥ مجم / كجم / يوم(قبل النظام الغذائي عالي الدهون بساعة واحدة ؛ والمجموعة ٥: نظام غذائي عالي الدهون + جوميسين أ بجرعة عالية: تلقت جرعة عالية من جوميسين أ بجرعة عالية (٢٠مجم / كجم / يوم) قبل النظام الغذائي عالي الدهون بساعة واحدة. تم أخذ الجرعات عن طريق الفم بواسطة أنبوبة داخل المعدة.

النتائج: أظهرت مجموعة النظام الغذائي عالي الدهون زيادة ذات دلالة إحصائية في وزن الغدد ، كما أظهرت السكاشن المصبوغة بالهيماتوكسيلين والإيوسين إضطراب في بنية الغدد ، إحتقان الأوعية الدموية ، تسلل خلوي إلتهابي ، العديد من الفجوات السيتوبلازمية فى خلايا العنيبات وبعض الأنوية ذات البقع الداكنة بالإضافة إلى القنوات الغدية المفرغة. كما ظهر أيضا إنخفاض كبير في تفاعل PCNA ، وإنخفاض ذو دلالة إحصائية في تفاعل Bcl-٢ ، وفقدان التعبير الغشائي E-cadherin لخلايا العنيبات ، GCTs ، ID و SD .بالإضافة إلى ذلك ، أظهرت دراسة المسح الإلكتروني المجهرية إنتفاخ بعض الفصيصات مع إنكماش للبعض الآخر، مما أدى إلى زيادة ظاهرية فى العنيبات مع الإنتفاخ والإفراز المتراكم في الحبيبات الإفرازية والتمدد الظاهرى للقنوات. وقد تم تحسين هذه التغييرات التنكسية بواسطة جوميسين أ بطريقة معتمدة على الجرعة.

الإستنتاج: تسبب النظام الغذائي عالي الدهون فى إحداث تغييرات تركيبية في الغدد اللعابية تحت الفك السفلي للجرذان، هذا وقد تمكن جوميسين أ من الحماية ضد هذه التغييرات.