Possible Protective Role of Green Tea Extract on Male Rat Parotid Gland in High Fat Diet Induced Obesity (Histological Study)

Original Article

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ABSTRACT

Background: Obesity is a major health problem that affects up to 30% of the adult population all over the world. It is a well-known aggravating factor in the pathology of many organs. The investigation of the anti-obesity properties of food components is a popular field of research and one of these natural agents of interest is green tea extract (GTE).
The present study aimed to assess the possible protective role of GTE on the parotid gland of male rat in high-fat diet (HFD) induced obesity histologically and immunohistochemically.
Materials and Methods: 40 adult male rats were divided equally into 4 groups.
Group I: rats were divided equally into two subgroups.
Group II: rats received of GTE 200 mg/kg body weight / day for12 weeks.
Group III: rats were fed HFD for 12 weeks.
Group IV: rats received GTE in conjunction with HFD 200 mg/kg body weight /day for 12 weeks. Parotid sections were subjected to histological, histochemical, immunohistochemical and morphometric studies. Serological, weight and waist circumference assessment were performed.
Results: group III: was comparable to group I Showed disarrangement of the acinar cells, cytoplasmic vacuoles, congested and dilated blood vessels. Marked fibrosis and cellular infiltration were observed. An increase in the immune reaction for α-SMA and moderately increase in Ki-67.
Group IV: Showed a reduction in all changes.
Conclusion: GTE play a role in the protection from HFD induced toxic changes on the parotid of rat.

Key Words: α-SMA, Ki-67, GTE, HFD, parotid.

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INTRODUCTION

Obesity has become a big health problem, as a growing proportion of the population worldwide is overweight and very likely to become obese[1]. Obesity affects about 500 million adults and 40–50 million children globally. More than that disturbing is rapid growth spread of clinically serious obesity with serious common illness manifestations[2].

Obesity has been established as a risk factor for many diseases including cardiovascular disease (CVD), Type 2 diabetes mellitus (T2DM), hypertension, renal disease, and neurologic dysfunction. Furthermore, obesity has been causally linked to a variety of cancers either as a risk factor or as a negative factor for prognosis[3].

About 80% of individuals with type II diabetes are classified as overweight or obese and 30% of obese children under the age of 12 years display insulin resistance[4].Scientists and media have put HFD as an important factor of obesity[5].

Most anti-obesity pharmacological approaches were found to have adverse effects and few of them were proved to be safe. That is why the investigation of the anti-obesity properties of food components have become a popular field of research to discover new natural safer agents. Green tea (GT) is among natural agents that have anti-obesity beneficial effects[6]. In addition, GT is one of the most commonly consumed drinks in the world. There has been an increase in the interest to discuss its different properties being a potent antioxidant, anti-inflammatory, antiproliferative, anticarcinogenic, anti -cariogenicand antimicrobial[7]. Exposure to GT can take place in several forms: consumed as dilute beverages or concentrated supplements or applied topically[8].

Salivary gland secretions are rich in fluids, ions, and proteins important for oral health and integrity of the teeth[9]. The saliva also produces antimicrobial substances such as immunoglobulins, lysozyme, lactoferrin, and salivary peroxidase. The latter can interfere with oral bacteria metabolism producing acid and preventing multiplication or killing them directly[10].The salivary gland parenchyma is a site for sterol biosynthesis, like liver, as it was proven biochemically[11].

The salivary glands were proved to lose function regularly with significant reduction of saliva production after exposure to HFD[12].

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Myoepithelial cells (MECs), are positive for alpha smooth muscle actin (α-SMA)[13]. Ki-67 expression is a member to detect the cell mitotic activity[14]. Antigen Ki-67 is a nuclear protein that is associated and necessary for cellular proliferation[15].

Aim of the work: The present study aimed to assess the possible protective role of GTE on the parotid gland of male rat in HFD induced obesity histologically and immunohistochemically.

MATERIALS AND METHODS

Used drug:

Green tea extract (GTE) was obtained from: Arab Company for Pharmaceuticals & Medicinal Plants (MEPACO-MEDIFOOD) – Egypt. Each tablet contains 300 mg.

Animals and diets :

The study included 40 healthy adult male albino rats 3–4 months old, range of weight was between190200-gmsobtained from the Animal House of Moshtohor Faculty of Veterinary Med¬icine, Benha University and acclimatized to the laboratory conditions. They were housed in plastic cages (five animals/cage) in a temperature-controlled environment (2124°-C), humidity (60 ± 10%)under a 1212/ h light/dark cycle and were given free access to food and water ad libitum. The guidelines for the ethical care and treatment of animals, given by the Local Ethical Committee of the Faculty of Medicine, Benha University, were strictly followed.

After acclimatization, rats were randomly divided into 4 groups Each of the following groups included 10 rats.:

Group I (Control group): 10 rats were all fed on standard rat show and they were subdivided into two subgroups each containing five animals.

Subgroup IA: kept as a negative control, they were left without intervention for 12 weeks.

Subgroup IB: kept as a positive control each animal was given 0.5 ml of distilled water (the vehicle of GTE) 2 times /day by a gastric tube 5 days/week for 12 weeks.

Group II(GTE group): rats were all fed on standard rat chow and were given GTE at a dose of 200 mg/kg bodyweight[16] at two divided doses/day[17].each dissolved in 0.5ml distilled water by gastric tube in conjunctions with standard rat chow for 5 days /week for 12 weeks[16].

GTE was prepared freshly every day, in the form of 0.5 ml distilled water containing 100 mg /kg GTE.

Group III:(HFD group)Rats were allowed a free access to HFD 5days/week for 12 weeks.[4]

Group IV: (GTE and HFD group) Rats received GTE orally at the same dose by the same route as in GII in conjunction with HFD for the same period.

The composition of diet according to authors[18] was as follows:

Balanced diet for feeding rats of GroupI and GroupII: 10g protein, 10g fat, 74.4 g carbohydrates, 3.5 g mineral, 1 g vitamin, 0.1g methionine and 1 g fiber.

HFD for induction of obesity feeding rats of Group III and Group IV: 10 g protein, 30g fat, 54.4 g carbohydrates, 3.5 g mineral, 1 g vitamin, 0.1g methionine and 1 g fiber. These forms of diet were prepared at the Feeding Department of the Faculty of Veterinary Medicine, Benha University.

General observations

During the experimental period, general appearance and the amount of food consumed, were checked daily.

At the initial time and at the end of the experiment, body weight and waist circumferences of all rats were recorded[2].

Biochemical analysis: After the end of the experimental period, the rats in all groups were fasted for 16 hours, then were sacrificed under anesthesia[19] The blood collected from the left ventricle of each rat was placed in sterile, clean centrifuge tubes and the serum was separated for measuring the levels of total cholesterol(TC) and triglyceride(TG) using a radioimmunoassay kit (Biochemicals, Costa Mesa, CA, USA).

Histological study:

The right parotid salivary glands were dissected of all rats and were fixed in neutral buffered 10% formalin, dehydrated, embedded in paraffin, cut into 5-µm sections, stained with H&E to verify histological structure, Mallory's trichrome stain to assess collagen fibers content.

Histochemical study Periodic acid Schiff (PAS) staining was used to detect the presence of glycoprotein at the basement membrane[20].

Immunohistochemical study using alpha smooth muscle actin (α-SMA)to identify the MECs[13]and Ki-67 immune staining was used to detect the cellular proliferation.[21].

Immunostaining for α-SMA :

The sections were blocked with 1.5% normal goat serum in PBS and were then incubated for 45 min at room temperature with 6 ml prediluted primary (1ry) mouse monoclonal anti-α-SMA antibody (Ab) (ab5694) (Dako Corporation, Glostrup, Denmark) stored at +4°C. Sections were subsequently incubated with a second-stage biotinylated antibody (biotin-conjugated goat anti-rabbit IgG, 1:200, 1 h, at room temperature). After rinsing in PBS, the reaction products were visualized by immersing the section into the chromogen diaminobenzidine. Finally, the sections were counterstained with Mayer's hematoxylin, dehydrated, and mounted. The positive tissue control was a specimen of human tonsil. α-SMA positive cells showed brown cytoplasmic reaction. The negative control was processed in the same way, but omitting the step of 1ry Ab[13].

Immunostaining for Ki-67:Antigen retrieval was performed with citrate buffer (pH 6) at 97ºC for 20 min. The slides were incubated 1 hour at room temperature with 6 ml diluted (1:100)anti- Ki-67 mouse monoclonal Ab (ki67/MKI67 -Sigma Aldrich (Germany). Sections then were washed 3 times in PBS5 minutes for each and incubated for further 30 minutes with biotinylated goat anti-rabbit 2ry Ab diluted 1:1000, followed by washing for 10 minutes. After rinsing in PBS, the reaction products were visualized by immersing the section into the chromogen diaminobenzidine. Finally, the sections were counterstained with Mayer`s hematoxylin, dehydrated, and mounted. Positive results for the Ki-67 immune reaction were indicated by brown staining of the nucleus. The positive control for Ki67 was a specimen of human tonsil. Negative control was performed by omitting the step of 1ry Ab application[21].

Morphometric study:

Using Leica Quin 500LTD (Cambridge, UK) computer assisted image analysis system, the count of vesicular nuclei was performed in H&E stained sections, that of α-SMA +ve MECs and that of Ki-67 positive nuclei were performed using interactive measurements menu. In addition, the area % of collagen fibers and PAS positive staining of basement membrane was measured using binary mode. The measurements were done in 10 high power fields.

Statistical analysis**[22]**: Statistical evaluation of the morphometric results was performed by using SPSS software (version 20; SPSS Inc., Chicago, Illinois, USA). Values were expressed as means ± SD and compared using one way analysis of variance (ANOVA). For hormonal results, differences were assessed using Student's t-test. Values of $P < 0.05$ were considered statistically significant.

RESULTS:

No mortality occurred throughout the experimental period.

Histological results

No significant differences in the microscopical pictures of rat's parotid glands sections of both control groups were seen.

Group I (the control group)

H&E examination: Sections of the parotid gland of the rat in control group revealed the normal architecture. It is formed of stroma which consists of the connective tissue capsule and the septa extending from capsule and dividing the glands into lobules. The parenchyma between the septa consists of groups of pure serous acini were lined by pyramidal cells having rounded central vesicular nuclei and basal basophilic cytoplasm. The duct portion consists of striated, interlobular and intra-lobular ducts were easily identified. There was capillary network in between the acini and in a connective tissue septum(Figures 1&2)

Mallory's trichrome stained sections showed blue multiple collagen fibers in the capsule and fewer fibers in the septa between the lobules (Figure.3).

PAS stain: showed obvious positive (a magenta color) PAS reaction in the basement membrane of the acini (Figure. 4).

Immunoreaction to α-SMA immune reaction showed brown cytoplasm in multiple MECs at the periphery of the acini and intra-lobular ducts. Positive reaction is also seen in the lining of blood vessels (Figure. 5).

Immunoreaction to Ki-67 showing positive immune reaction in few nuclei of acinar cells and the cells lining an intra-lobular duct (Figure. 6).

Group II: light microscopic examination including the H&E, Mallory's trichrome PAS stain immunohistochemical α-SMA and KI-67 comparable to the control group showed no significant differences.

Group III (HFD group) revealed widened connective tissue septa between some lobules and homogenous acidophilic material in others and disorganized acini in some areas could be observed. Some acinar cells showed dark nuclei and others showed cytoplasmic vacuoles. Inter lobular septa contain dense connective tissue fibers with cellular infiltration between the lobules and around a dilated duct (D). Two dilated and congested blood vessels are seen. loss of the architecture of multiple lobules(Figures 7,8,9).

Mallory's trichrome stained sections clarified dense collagen fibers in the connective tissue septa surrounding some lobules. Dense intra lobular connective tissue noticed (Figure 10).

PAS stained tissue showed obvious positive PAS reaction in the basement membrane of some acini and absent in others (Figure.11)

Immunohistochemical examination showed: an apparent increased α-SMA immunoreaction in MECs (red arrows) at the periphery of the acini , interlobular ducts and in the lining of blood vessels(Figure12).

Ki- 67 immuno reactivity in some nuclei of the lining cells of acini and some cells lining an intralobular duct (Figure. 13).

Group IV: (HFD and GTE group)showed the capsule, septa between the lobules. An interlobular duct in a connective tissue septum and intra-lobular ducts in between the acini apparently normal serous acini, intra-lobular duct, interlobular ducts and non-congested blood vessel in a slightly thickened connective tissue septum containing homogeneous material(Figure 14&15).

Mallory's trichrome stained sections clarified the moderate amount of collagen fibers appeared in between the lobes and lobules and around ducts and blood vessels (Figure 16).

PAS stain: obvious positive PAS reaction in the basement membrane of the acini (Figure 17).

Immunoreaction to α-SMA Sections showed α-SMA immunoreaction in multiple MECs at the periphery of the acini and intra-lobular ducts (Figure 18).

Immunoreaction to Ki-67 immunoreaction in few nuclei of acinar cells (Figure 19).

Morphometric and statistical result:

The difference between the results of the studied parameters in subgroups IA, IB, of the control group were statistically insignificant (P >0.05). Thus, we chose subgroup IA (negative control) as the control groupin the statistical analysis compared between group I, group II, group III and group IV. Statistical analysis of the results showed a significant increase (P <0.05) in the mean body weight(Table1,Histogram1) and waist circumferences (Table2,Histogram2) in GIII compared than group II, IV or control group. A significant increase in Mean number of vesicular nuclei (P <0.05) in Group I ,II and GIV compared with GIII (Table3,Histogram3). A significant increase (P <0.05) in the mean area % of collagen fibers stained by Mallory's trichrome, but a significant decrease (P <0.05) in the mean area % of PAS staining in group III compared with Groups I ,II and IV(Table4,Histogram4). A significant increase (P <0.05) in the Mean number of positive α-SMA in cytoplasm of MECs and mean number of positive Ki-67 nuclei expression in group III compared with control group, group II and group IV(Table5,Histogram5). A significant increase (P <0.05) in the values of total serum cholesterol and triglyceride in group III compared with groups I ,II and IV (Table6, Histogram6).

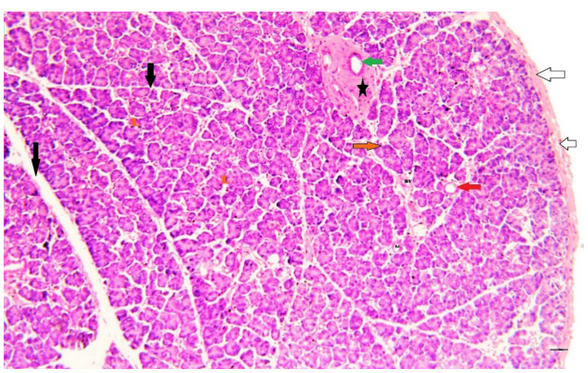


Fig. 1: A photomicrograph of a section in the parotid gland of a control rat showing the capsule(white arrow), the septa (black arrow) between the lobules (L), groups of acini (yellow arrow). Note an interlobular duct (green arrow) in a connective tissue septum (star) and intralobular ducts in between the acini (red arrow) (H & E x 100).

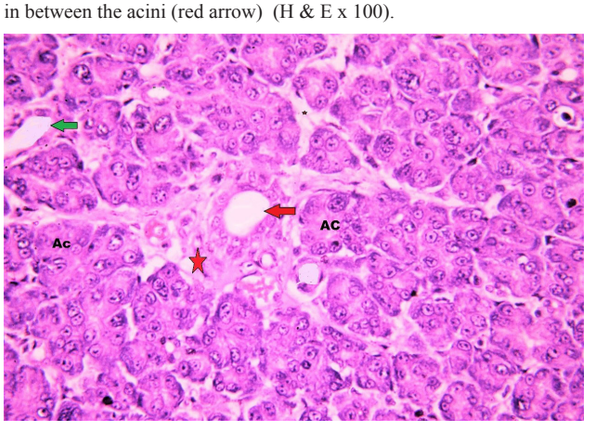


Fig. 2. A photomicrograph of a section in the parotid gland of a control rat showing pure serous acini (Ac), the lining cells show basophilic cytoplasm and central rounded vesicular nuclei. Note interlobular septa (star), interlobular duct (red arrow) and intra lobular duct (green arrow) (H&E, × 400).

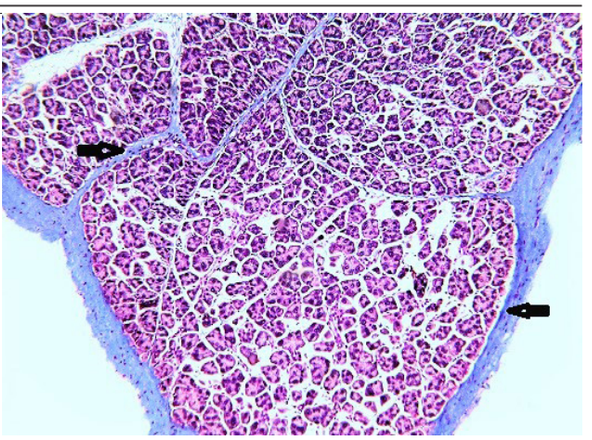


Fig. 3. A photomicrograph of a section in the parotid gland of a control rat showing multiple collagen fibers (arrow) in the capsule and fewerfibers(arrow) in the septa between the lobules (Mallory's trichrome × 100) .

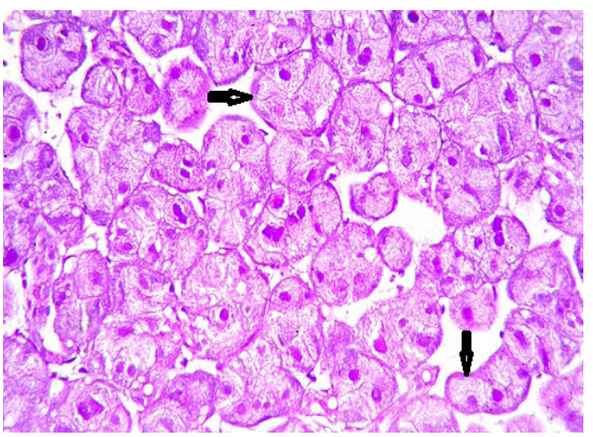


Fig. 4: A photomicrograph of a section in the parotid gland of a control rat showing obvious positive PAS reaction in the basement membrane of the acini (arrow) (PAS ×400).

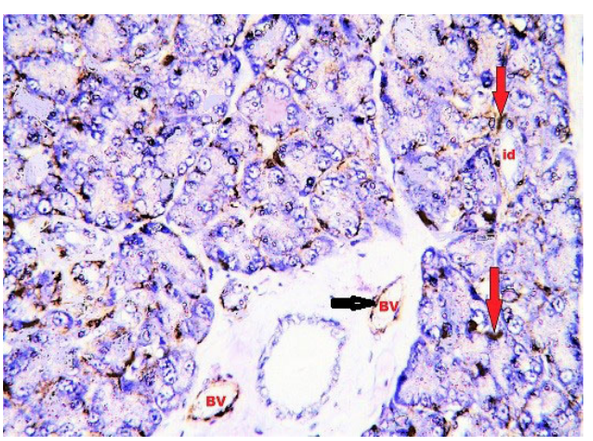


Fig. 5: A photomicrograph of a section in the parotid gland of a control rat showing α-SMA immunoreaction in multiple MECs at the periphery of the acini (red arrows) and intralobular ducts (id). Positive reaction (black arrow) is also seen in the lining of two blood vessels (BV) (α-SMA × 400)

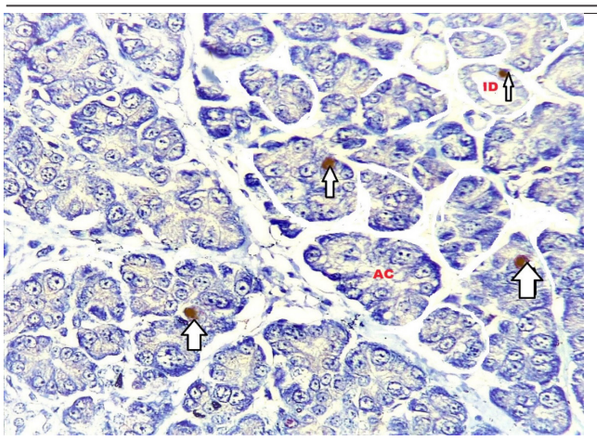


Fig. 6: A photomicrograph of a section in the parotid gland of a control rat showing +ve Ki-67 immunoreaction in few nuclei of acinar cells and the cells lining an intralobular duct (arrows) (Ki-67 x 400).

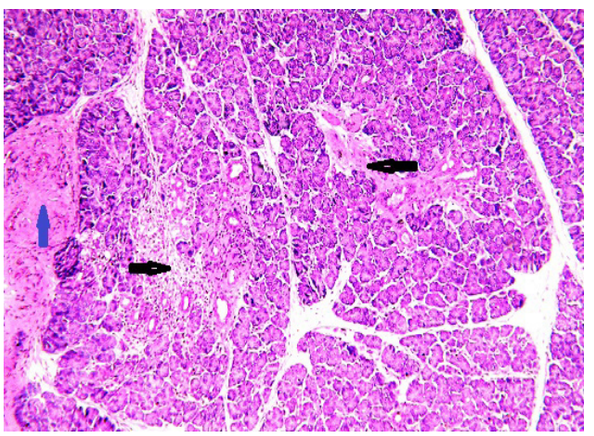


Fig. 7: A photomicrograph of a section in the parotid gland of a rat in Group III (HFD) showing widened connective tissue septa (arrow) between some lobules and homogenous acidophilic material (blue arrow) in one of them(H&E × 100).

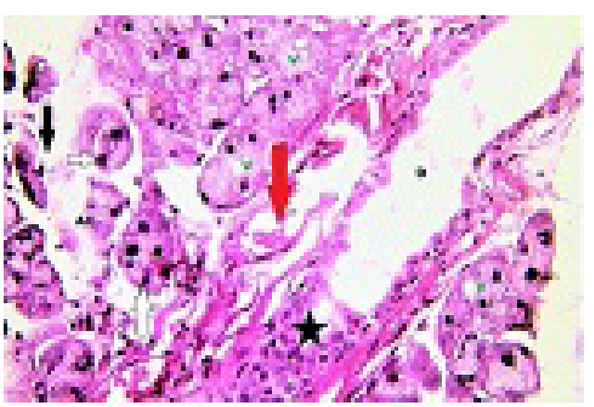


Fig. 8: A photomicrograph of a section in the parotid gland of a rat in Group III showing a disorganized acinus (AC). Some acinar cells showed dark nuclei (white arrows) and others showed cytoplasmic vacuoles (v).Inter lobular septa contain dense connective tissue fibers with cellular infiltration (star) between the lobules and around a dilated duct (D). Two dilated and congested blood vessels are seen (red arrow) (H&E, × 400).

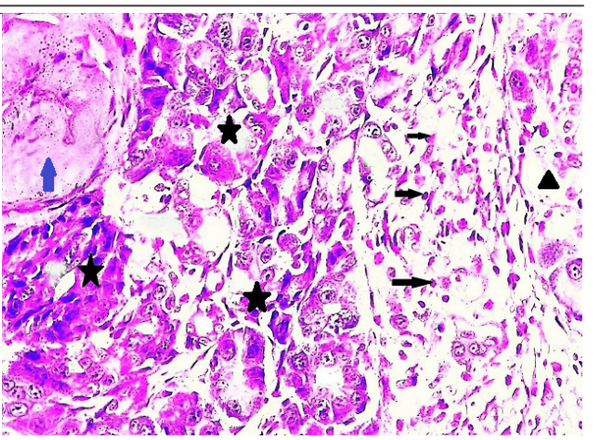


Fig. 9. A photomicrograph of a section in the parotid gland of a rat in Group III showingloss of the architecture of multiple lobules (stars). Obvious cellular infiltration (arrows) is noticed in a connective tissue septum with wide separation of the fibers (arrowhead).Note accumulated homogenous acidophilic material in another septum (blue arrow) (H&E, × 400).

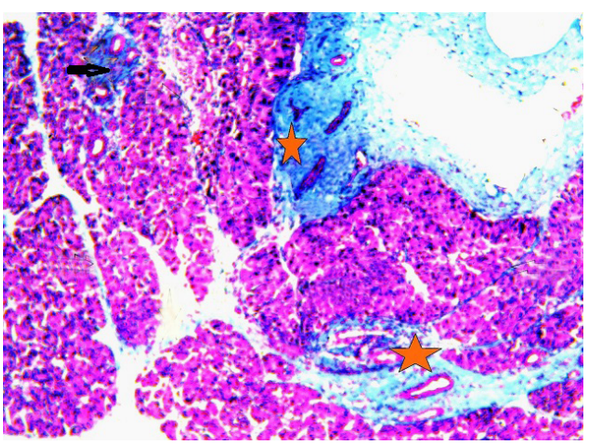


Fig. 10. A photomicrograph of a section in the parotid gland of a rat in Group III showing dense collagen fibers in the connective tissue septa surrounding some lobules (star). Notice dense intra lobular connective tissue (arrow) (Mallory's trichrome ×100).

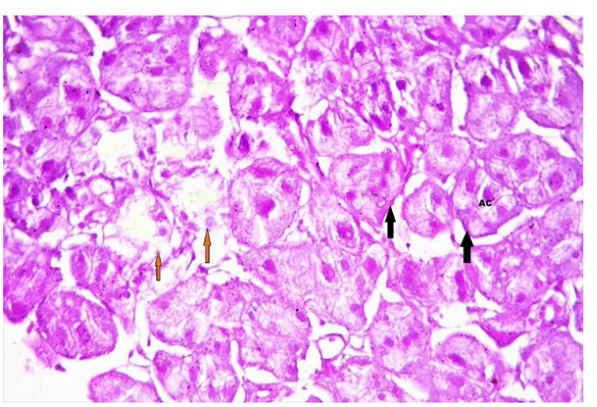


Fig. 11. A photomicrograph of a section in the parotid gland of a rat in Group III showingobvious positive PAS reaction in the basement membrane of some acini (black arrows) and absent in others (yellow arrows) (PAS ×400)

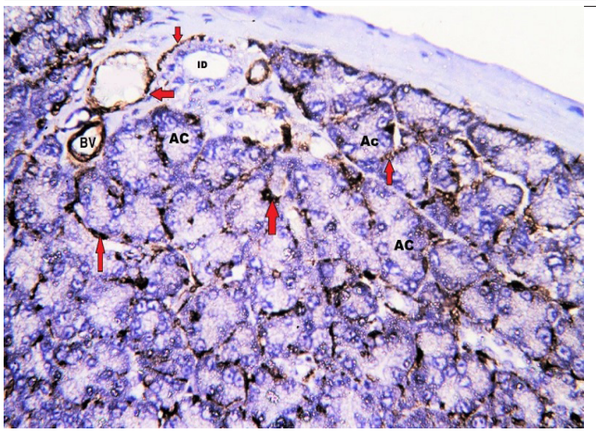


Fig. 12. A photomicrograph of a section in the parotid gland of a rat in Group III showing an apparent increased α-SMA immunoreaction in MECs (red arrows) at the periphery of the acini (AC), interlobular ducts (ID) and in the lining of blood vessels (BV) (α-SMA × 400).

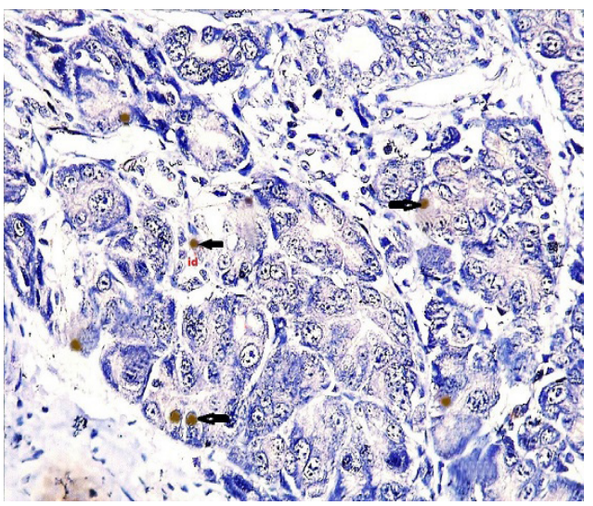


Fig. 13: A photomicrograph of a section in the parotid gland of a rat in Group III showing Ki-67 immunoreaction (arrow) in some nuclei of the lining cells of acini (AC) and some cells lining anintralobular duct (id) (Ki-67 X 400).

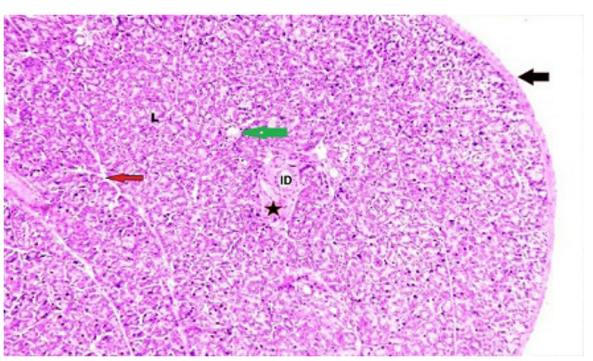


Fig. 14: A photomicrograph of a section in the parotid gland of a rat in group IV (GTE+HFD) showing the capsule (arrow), septa (red arrow) between the lobules (L). Note an interlobular duct (ID) in a connective tissue septum (star) and intralobular ducts in between the acini (green arrow) (H & E x 100).

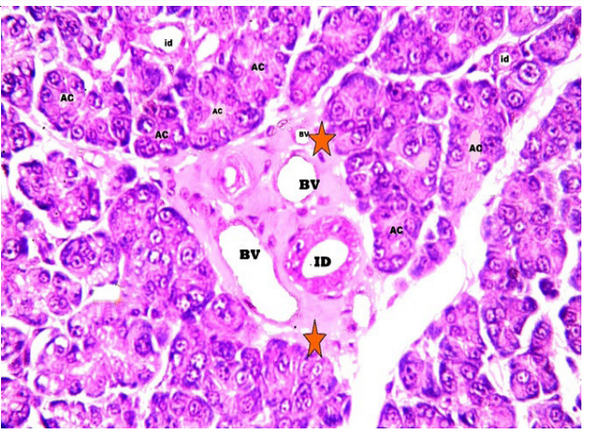


Fig. 15: A photomicrograph of a section in the parotid gland of a rat in Group IV showingapparently normal serous acini (AC), intra-lobular duct(id), interlobular ducts (ID) and noncongestedblood vessel(BV) in a slightly thickened connective tissue septum containing homogeneous material (bluearrow)(H&E X400)

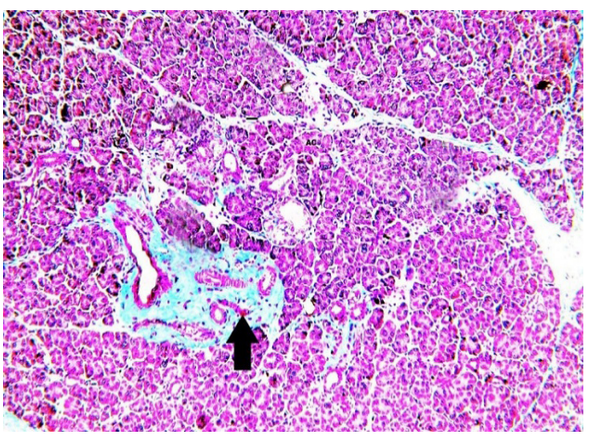


Fig. 16: A photomicrograph of a section in the parotid gland of a rat in Group IV showing a dense collagen fibers (black arrow) in a connective tissue septum (Mallory's trichrome, x100).

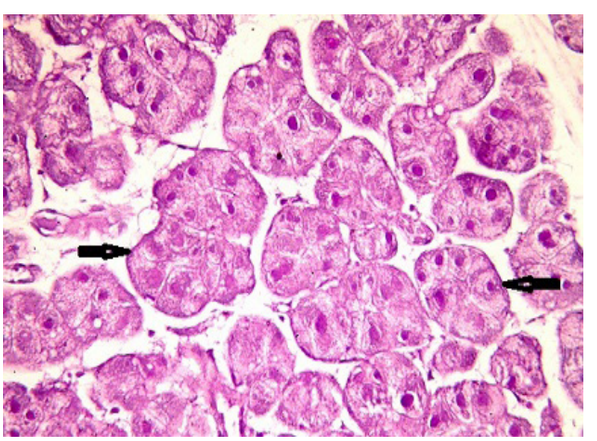


Fig. 17: A photomicrograph of a section in the parotid gland of a rat in group IV showing obvious positive PAS reaction in the basement membrane of the acini (arrow) (PAS ×400).

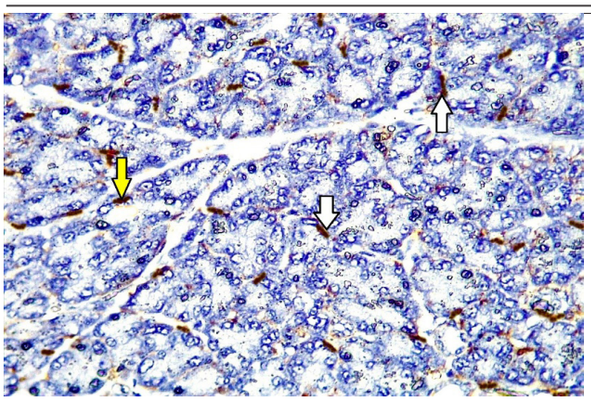


Fig. 18: A photomicrograph of a section in the parotid gland of a rat in Group IV showing α-SMA immunoreaction in multiple MECs at the periphery of the acini (white arrows) and intralobular ducts (yellow arrow) (α-SMA× 400).

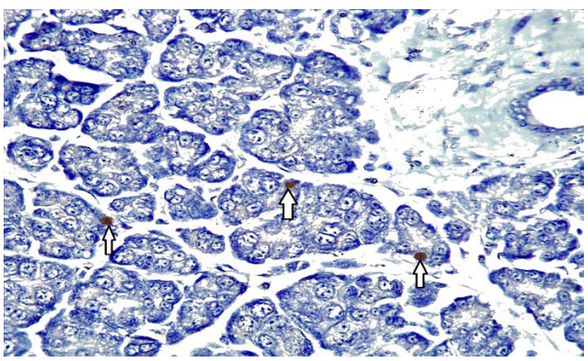


Fig. 19: A photomicrograph of a section in theparotid gland of a rat in Group IV showingKi- 67 immunoreaction in few nuclei (white arrows) of acinar cells (Ki-67 ×400).

Table 1. The mean body weight (g±SD) in the studied groups

	GroupI (control)	GroupII (GTE)	GroupIII (HFD)	GroupIV (GTE+HFD)
Body weight	192.3±9.6	190.9±9.5	251.9 ± 11.7*	199.9 ± 8.9

*significantP <0.05

Histogram 1: Mean values of body weight in the studied groups

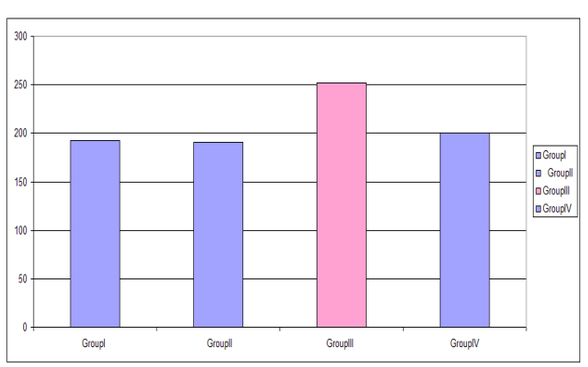


Table 2. The mean waist circumference (cm±SD) in the studied groups

	GroupI (control)	GroupII (GTE)	GroupIII (HFD)	GroupIV (GTE+HFD)
Waist circumference	13.5 ±1.5	13.1±1.2	24.6 ± 1.9*	14.9 ± 1.4

*significantP <0.05

Histogram 2: Mean values of waist circumference in the studied groups

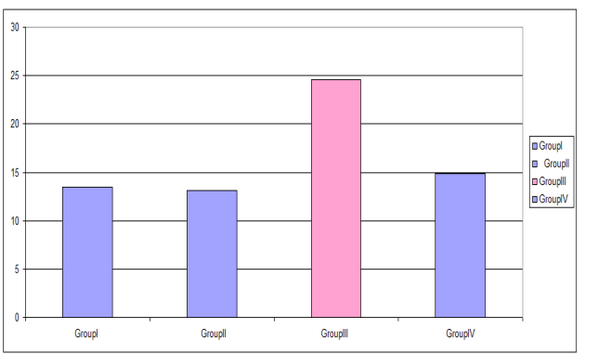


Table 3. The mean count of vesicular nuclei in the studied groups

	GroupI (control)	GroupII (GTE)	GroupIII (HFD)	GroupIV (GTE+HFD)
Count of vesicular nuclei	9.1 ± 1.3	8.6 ± 1.2	4.1 ± 0.4*	8.8± 1.1

*significantP <0.05

Histogram 3: Mean number of vesicular nuclei in the studied groups

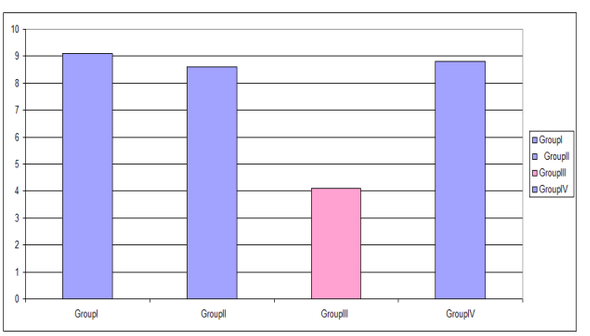
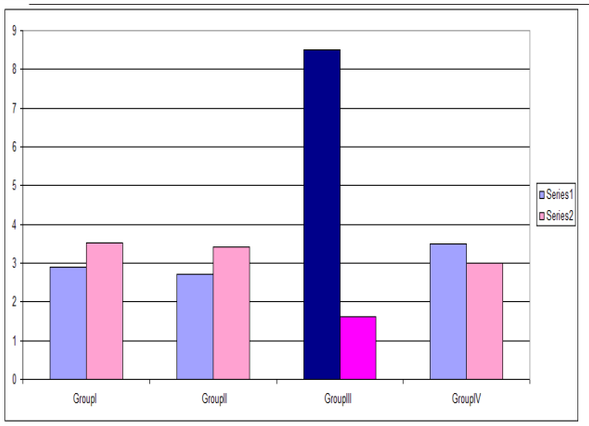


Table 4. The mean area% of collagen fibers and PAS +ve staining in the studied groups

	GroupI (control)	GroupII (GTE)	GroupIII (HFD)	GroupIV (GTE+HFD)
Collagen fibers	2.9± 0.4	2.7± 0.3	8.5±1.1*	3.5±0.6
PAS +ve staining	3.51± 0.2	3.43± 0.3	1. 61± 0.2*	3.01±0.3

*significantP <0.05



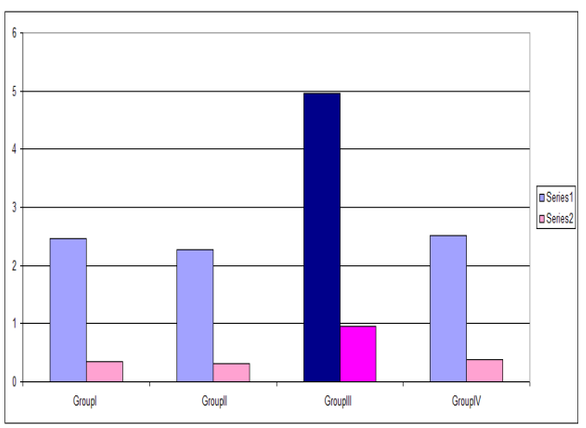
Series 1: collagen fibers Series 2: PAS +ve staining

Table5. The mean number of α-SMA +ve MECs and Ki67 + ve nuclei in the studied groups

	GroupI (control)	GroupII (GTE)	GroupIII (HFD)	GroupIV (GTE+HFD)
α-SMA+ve MECs	2.47± 0.2	2.27± 0.3	4.96 ± 0.7*	2.52±0.2
Ki-67 +ve nuclei	0.35 ±0.04	0.31 ±0.04	0.95±0.20*	0.38±0.05

*significantP <0.05

Histogram 5: Mean number of α-SMA +ve MECs and Ki67 +ve nuclei in the studied groups



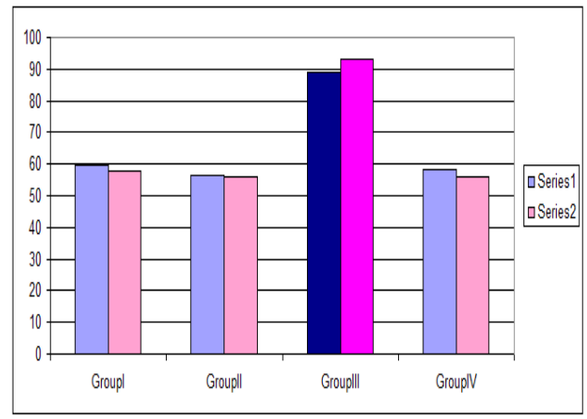
Series 1: α-SMA +ve MECs Series 2: Ki67 +ve nuclei

Table (6) Mean values of serum cholesterol and serum triglycerides (mg/dl ± SD) in the studied groups

	GroupI (control)	GroupII (GTE)	GroupIII (HFD)	GroupIV (GTE+HFD)
Serum cholesterol	59.5±2.45	56.5±2.13	88.8 ± 3.67*	58.2 ±1.35
Serum triglycerides	57.9 ± 3.5	55.8 ± 3.2	93.2 ± 4.5*	55.9 ± 2.5

*significantP <0.05

Histogram 6: Mean values of serum cholesterol and triglycerides in the studied groups



Series 1: serum cholesterolSeries 2: serum triglycerides

DISCUSSION

Obesity is one of the most widespreadunexpectedly medical problems. It is the good document that obese patients are associated with serious morbidities, of which a high incidence of type 2 diabetes, hyperlipidemia, hypercholesterolemia, and cardiovascular, liver, and renal disease[23].

Epidemiological studies have revealed a strong relationship between HFD intake and obesity. Excess caloric intake and lack of energy expenditure are generally accepted to disrupt hepatic lipid metabolism and induce obesity and insulin resistance[24&25]. Rats and are assumed an appropriate model for studying dietary obesity for human. Salivary glands and saliva are the important maintenance setting of oral health and play a role in carbohydrate metabolism. The type of diet influences the amount and nature of secretion[26]. This is the reason for the selection of parotid gland of rat to evaluate the histological alterations as observed in adult albino rats subjected to HFD. In this study rats of group III showed, a highly significant increase in mean body weight and waist circumference compared to control group that observably Specific places toward central or abdominal obesity. Authors said, the exposure to HFD for long periods results in a positive energy balance and obesity in experimental models such as rats which considered a model for human obesity. This reflected visceral rather than subcutaneous allocation of fat tissue[27]. Otherwise, no significant increase in rats Group II (fed GTE)or rats in group IV(fed GTE with HFD), but were preserved nearly as a control. These findings were explained by GTE has thermogenic properties and promotes fat oxidation this lead to control of body composition. Also, epigallocatechin-3-gallate (EGCG) is the most abundant catechin in green tea with several potential health effects, including anti-obesity and weight maintenance properties[28].The GTE is unlike the other sympathomimetic drugs, whose use as anti-

obesity thermogenic agents are limited by their adverse cardiovascular effects, and inappropriate for obese individuals with hypertension and other cardiovascular complications[29]. This is the reason for the selection of GTE for this respect.

In the present study, HFD altered the histological structure of parotid glands. Abnormal architecture, fibrosis, mononuclear infiltration, dilatation of blood vessels with hemorrhage, and dark-stained nuclei, numerous intracellular vacuoles in acinar cells were observed. These findings were in accordance with those were found that prolonged HFD intake lead to oxidative stress this was harmful to pancreas and can induce pancreatic abnormalities. Many studies have suggested that pancreas and parotid gland presented morphological and functional similarities[2& 30]. In this study, the connective tissue stroma of the parotid glands of rats in group III showed dense in interlobular and moderate intra lobular fibrosis, characterized by a significant increase mean area % of collagen fibers . These findings were in accordance with authors[29] who mentioned that HFD cause hypercholesterolemia. The latter was considered a risk factor for hepatic fibrosis. It was hypothesized that hypercholesterolemia modulates several interlinked pathways that promote deposition and degradation of extracellular matrix. Also, It might be a response of fibroblast simulating factor that activate fibroblast to increase the synthesis of collagen fibers. Other studies found that oxidation products such as lipid peroxidation products can stimulate α-collagen expression and collagen synthesis[2,37,38]. Some authors considered, fibrosis was a defensive and protective tissue response to limit the toxic effect of hypercholesterolemia[13].Therefore, it could be suggested that interstitial fibrosis was a key factor in the dis-arrangement and outline deformities of some acini in the parotid gland. Otherwise the accumulation of lipid droplets in-between the secretory portions also exerts pressure on their margins so that they appeared have irregular margins.

This study showed cellular infiltration and congested blood vessels at some areas in parotid sections. These findings explained with other studies, these findings might be a part of inflammatory response to bring more blood to the areas of fibrosis or degeneration. Also they said that epidemiologic evidence suggests an important link between obesity and inflammation[31]. Increased fat consumption was associated with gradual imbalance of homeostasis and alterations of capillaries in the parotid. HFD were also reported to cause atherosclerosis and hypertension. Atherosclerotic vessels were fragile, and easy to produce hemorrhage within the glands[32]. These inflammatory changes may restrict diffusion of nutrients and oxygen to parenchymal cells and adversely affect the function and survival of parenchymal cells[15].

There were suggestions that, green tea catechins affect lipid metabolism by different methods and prevent the appearance of atherosclerotic plaque. GTE intake decreases the absorption of triglycerides and cholesterol. Green tea consumption decreased total plasma cholesterol and triglycerides[32]. The latter explain the presence of a significant increase of values of total cholesterol and triglycerides in this study in rats fed HFD but normal in control group and rats were given GTE or rats were given GTE with HFD (group II and IV).In the present study, parotid sections in rat fed with HFD had adversely affected its histological structure in the form of numerous intracellular vacuoles. These vacuoles might be attributed to degenerative changes within the acinar cells (fatty degeneration). In the routinely processed H&E sections, the lipid droplets were dissolved during fixation and processing of the tissues, leaving empty vacuoles. Similar results were observed in this study. These results were in accordance with other studies as long term HFD increased lipid accumulation within the kidneys, liver, and pancreas.Rather than, an imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Catechins protect against these problems by contributing, along with antioxidant vitamins (i.e., vitamins C and E) and enzymes[33].

In this study, the parotid gland rat in group III which took GTE with HFD showed similar to control group. The latter explained by The GTE is rich in antioxidant polyphenolic flavonoids, carotenoids, tocopherols (vitamin E derivatives) and vitamin C. and minerals act as co-factors in antioxidant enzymes: zinc, selenium and manganese[12&13]. Dilatation of the ducts could be attributed to accumulation of the salivary secretion and failure of excretion due to glandular injury and dysfunction, as lipid accumulation promotes gland dysfunction as previously explained[34].

IN this study showed a significant decrease in mean area percent of PAS positive material others in parotid gland of rat group III compared to control group as a result of depletion of basement membrane at some injured areas in response to toxic effects of HFD as explained before. Preserved this topic in group II and group IV nearly as control group. The latter was the role of GTE in cell protection from breakthrough inflammation. These findings were in agreement with other studies[35].

In this study, the MECs could be demonstrated by immunohistochemistry for α-SMA that has been considered as a specific marker expressed by the smooth muscle cells early during their differentiation. By quantitative and morphometric analysis, a highly significant increase in α-SMA immunoreactivity around acini and intra lobular ducts were observed in parotid sections of rat fed HFD. The MECs were observed to undergo morphological and proliferative changes during both atrophy and regeneration of acinar cells with subsequent increase in their size and number[18]. In stressful conditions, recovery of gland function it seems that require increasing the secretory capacity of the surviving cells. This need more MECs to squeeze the accumulated secretion[17].

In this study,increase in Ki-67 immuno- reaction in parotid of rat fed on HFD but appeared nearly as

acontrol in GIII in which rat took GTE plus HFD. This explained by the previous studies thatepigallocatechin gallate (EGCG) in green tea could normalize abnormal cell proliferation of the salivary gland. Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species[21].

The administration of green tea extract that protects salivary gland from apoptosis by increasing significantly the anti-apoptotic activity, it can be used as a criterion and can be used as, a novel approach to decrease the cytotoxicity of HFD on parotid gland[34].

The complex relation between cell proliferation, differentiation, and apoptosis is a cardinal feature in maintenance of the normal structure and function of the parotid gland. Rearrangement of tissue is an important feature of the recovery from injury[11,12].

A recognized indicator of cell mitotic activity is Ki-67, a moderate increase in Ki-67 expression is indicative of the parotid gland has the ability to resist degeneration cell mitotic activity and proliferation. Ki-67 monoclonal antibodies detect a nuclear antigen expressed exclusively at the level of cells in the proliferation phase(phases G1, S, G2 and mitoses), but not in the G0 phase. There for,Ki-67 antibodies allow for the immunohistochemical determination of the tissue growth fraction[21].

The human parotid gland is well characterized intra-lobular adipose tissues, whereas the a dipocytes are not prominent in the rodent parotid gland this explain, the fat cells could not be detected in all examined sections of parotid glands of rats even group III which fed HFD for 12 weeks in this study[2].

CONCLUSION

Supplementation with GTE at a concentration of 200mg/kg/day are useful as anti-obesity agents and can be used to prevent the impact histological changes of obesity on the parotid gland. Other studies are needed to exclude the possible adverse effect of high dose of GTE on different body organs. Also, further studies are required to document the effect of lipid deposition on glandular function, which could affect the oral health. Reducing body weight is essential for decreasing the risk of developing cardiovascular disease and other health problems.

ACKNOWLEDGEMENTS

Conflicts of interest

There are no conflicts of interest.

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الملخص العربى

تأثير مستخرجات الشاي الأخضرعلى الغدة النكافية لدكور الجرذان البالغة مع استحداث السمنة دراسة هستولوجية

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المقدمة: أصبحت السمنة من أهم وأخطر المشكلات الصحية التي يعاني منها نسبة كبيرة من الأشخاص في مختلف أنحاء العالم . وقد أصبحت هده النسبة في تزايد مستمر مما يزيد من خطورتها.
يعتبر الشاي الأخضر من أوسع المشروبات المستخدمة في العالم وقد ظهرت خصائصه الطبية بوضوح . ويعتقد أن المادة الفعالة فيه هي متعدد الفينول هي المسؤلة عن تحسن الصحة وتخفيف أثر السمنة.

الهدف من البحث: استهدفت هده الدراسة معرفة وتقييم التأثير الحمائي لمستخلص الشاي الأخضر هستولوجيا على الغدة النكفية بعد استحداث السمنة تجريبيا .

المواد وطرق البحث: استخدم في هده الدراسة أربعون جرذًا بالغا وقسمت إلى أربعة مجموعات بالتساوي المجموعة الأولى الضابطة والمجموعة الثانية أعطيت مستخلص الشاي الأخضر لمدة 12 اسبوعا والمجموعة الثالثة المتأثرة أعطيت غداء عالي الدهون لمدة 12 اسبوعا والمجموعة الرابعة أعطيت مستخلص الشاي الأخضر عن طريق أنبوب الفم وقد تغدت على غداء عالي الدهون لمدة 12 اسبوعا . دبحت المجموعات بعد انتهاء المدة وأخدت الغدد النكفية وتم تحضيرها للدراسة بالمجهر الضوئي

النتائج: أوضحت النتائج وجود زيادة ذات مغدى في أوزان الجرزان وكدلك تغيرات شكلية وخلوية في الحويصلات والقنوات للغدد النكفية في المجموعات المتأثرة مثل وجود فجوات سيتوبلازمية وأنوية داكنة ووجد العديد من الألياف وخلايا النسيج الضام المتسللة فى الحواجزبين الفصيصات وحول القنوات والأوعية الدموية عند مقارنتها بالمجموعة الضابطة ووجد تحسن ملحوظ في المجموعة الرابعة التي أعطيت مستخلص الشاي الأخضر مع الغداء عالي الدهون

الخلاصة: أن مستخلص الشاي الأخضر له تأثير فعال في حماية الغدة النكفية من التغيرات الهستولوجية التي طرأت عليها مثل التليف واحتقان الأوعية الدموية والفجوات السيتوبلازمية إثر تناول نظام غدائي عالي الدهون .