

Raisins Preserve Thyroid Gland Function and Structure in an Animal Model of Hypercholesterolemia

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Abstract

Background: Statins are among the first line of pharmacological treatment of lipid disorders and lowering serum cholesterol, but they have many side effects. **Aim:** The study aim was to evaluate the role of raisins in protecting the thyroid function and structure in a rat model of hypercholesterolemia, through biochemical and histopathological investigation. **Materials and Methods:** Thirty male rats were randomly divided into three groups ($n = 10$ each) of albino rats included the control, high cholesterol diet (HCD)-fed for 13 weeks and HCD plus Raisins were included in this study. Blood levels of glucose, insulin, cholesterol, lipids, thyroid-stimulating hormone (TSH), T3, T4, oxidants/anti-oxidants were assessed. Thyroid gland was processed and examined histopathologically using light and electron microscopy. **Results:** Feeding HCD resulted in hypercholesterolemia in rats after 13 weeks as evidence by lipid profile. Ingestion of raisins along with HCD resulted in a significant ($P < 0.001$) decrease in the levels of insulin, blood glucose, thyroxine (T4) and malondialdehyde (MDA), while the levels of TSH, T3 and total anti-oxidant capacity significantly ($P < 0.001$) elevated. Raisins histologically alleviated the HCD-induced structural changes in the thyroid glands that included degenerated mitochondria and increased lipid droplets in the cytoplasm. **Conclusions:** Simultaneous administration of raisins along with HCD, administrated for a short time, could modulate the negative effect on thyroid gland structure and function.

Keywords: Anti-oxidants, high fat, hypercholesterolemia, raisins, structure, thyroid hormones, ultrastructure

INTRODUCTION

Dyslipidemia is a common health problem linked to high morbidity and mortality. It is an important risk factor for coronary artery disease and stroke because of atherosclerotic plaque deposition in the coronary arteries. Moreover, it increases the risk for thromboembolism and kidney affection that may progress to glomerulosclerosis or tubulointerstitial injury. Dyslipidemia downregulates the activity of lipoprotein lipase enzyme, present in the endothelium, fatty tissue and muscles) and hepatic lipase resulting in the decrease of lipoprotein clearance and increase of lipoprotein synthesis.^[1,2]

In dyslipidemia, the levels of low-density lipoprotein (LDL) cholesterol and triglycerides (TG) are high, while the level of high-density lipoprotein (HDL) cholesterol is low.^[3] By the age of 50 years, men with untreated familial hypercholesterolemia

are at a 50% risk for coronary diseases while the risk is about 30% in untreated females by the age of 60 years.^[4]

In Egypt, cardiovascular deaths are very common and represent about 15% of the cardiovascular deaths in the Middle East and North Africa. Moreover, dyslipidemia has a high prevalence that may reach up to 71% in Egyptian females.^[5]

Thyroid hormone plays a regulating role in lipid synthesis, metabolism, and mobilization. Among the characteristic features of hypothyroidism are elevated levels of serum cholesterol, LDL cholesterol (LDLC), apolipoprotein B (apo B), and lipoprotein (a).^[6]

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High-fat diet and endocrinal malfunction account for metabolic syndrome and insulin resistance.^[7] There are many categories of drugs that can lower cholesterol levels. Statins are considered the first line for controlling dyslipidemia.^[8,9] Statins can decrease levels of LDL through inhibiting the activity of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which resulting in decreased liver cholesterol and increased LDL receptors in the liver, which increases LDL clearance.^[10] Studies showed that statin use may induce diabetes mellitus, peripheral neuropathy, depression, Parkinson's disease, cognitive impairments, dysfunctional breathing, interstitial pneumonitis, hearing loss, early premature cataracts, herpes zoster, bladder wall instability, interstitial cystitis, impotency, and cancer.^[11]

Ezetimibe is a dyslipidemic agent used to treat people with hyperlipidemia. Ezetimibe is an inhibitor of intestinal cholesterol absorption and is indicated in reducing total cholesterol, LDL, apo B, and non-HDL. However, ezetimibe may rarely cause acute autoimmune hepatitis or severe cholestatic hepatitis.^[12,13] Bile acid sequestrants, for example, colestipol, and colesevelam, can manage hypercholesterolemia via binding with intestinal bile acids and interfering with their reabsorption. Therefore, a compensatory increase in bile acid synthesis takes place in the liver through the conversion of cholesterol into bile acids. When the level of hepatic cholesterol is decreased, hepatic LDL receptors are up-regulated, causing increased LDL clearance.^[14] However, Bile acid sequestrants may affect the gastrointestinal tract and is associated with constipation that might be severe. Moreover, patients usually have abdominal discomfort, bloating, and aggravation of hemorrhoids. Hence, a large number of patients discontinue bile acid sequestrants therapy.^[10] On the other hand, many studies showed that there are natural compounds can be effective in lowering serum cholesterol.^[15]

Raisins, the dried grapes of different cultivars of *Vitis vinifera*, are widely consumed all over the world.^[16] Raisins contain a large amount of phenolic acids, polyphenols, and flavonoids which are powerful antioxidants. They chelate metals, inhibit cellular proliferation, modulate enzymatic activity and signal transduction pathways.^[17] Intake of raisins with high-fat diet reduced mesangial expansion, glomerular capillary congestion, and fibrosis of the kidney.^[18] Moreover, raisins had a cardio-protective effect and were able to preserve the function and structure of cardiac muscle in hypercholesterolemic rat model through decreased blood vessels affection, cellular infiltration and cardiomyocytes' degeneration. In addition, raisins reduced immunoexpression of alpha-smooth muscle actin and hence decreased fibrosis.^[19] In hypercholesterolemic rats, ingestion of red grape juice could alleviate the high cholesterol diet (HCD)-induced structural changes in the thyroid gland, decrease both thyroid hormones and blood glucose levels, improve the lipid profile and increase anti-oxidants levels.^[20]

No studies have assessed the efficacy of raisins in alleviating the impact of hypercholesterolemia on the thyroid gland.

Hence, this study aimed to evaluate the protective role of raisins on the thyroid function and structure in the hypercholesterolemic rat model, through biochemical and histopathological examination.

MATERIALS AND METHODS

Animal experiments

Thirty male albino rats, with weights (225–285 g), were purchased from King Fahd Medical Research Centre, King Abdulaziz University (KAU), Jeddah, Saudi Arabia. They were kept under standardized and hygienic conditions “12 h cycles of day and light, at a room temperature of 27°C ± 1°C, 55% humidity” and were fed the commercial diet and water supplemented with *ad libitum* for 2 weeks and left to acclimatize. Ethical approval for this study was obtained from “the biomedical research ethics committee at the Faculty of Medicine, KAU.”

Rats were equally assigned, at random, into three groups. Group 1 (control), received saline through a nasogastric tube, group 2 (HCD) were fed HCD for 13 weeks, group 3 (HCD + R) were fed HCD along with raisin homogenate for 13 weeks through a nasogastric tube for 13 weeks.^[21] The HCD was formed of 4% cholesterol and 1% cholic acid added to 95% rat chow.^[22]

Preparation of raisins homogenate

Raisins were purchased from nut store and were verified by a specialist in Botany at Faculty of Sciences, KAU. Raisins homogenate in water was prepared in the Laboratory of Nutrition at the Faculty of Science, KAU, using a sterilized blender. Raisins chemical composition was mentioned before.^[19]

Biochemical assays

Blood was collected by capillary glass pipettes from the retro-orbital sinus of the rats after being fast overnight at the 1st, 6th and 13th weeks. Biochemical assays include insulin, blood glucose levels and lipid profile included cholesterol, TG, HDL and LDL levels were assessed. Blood glucose level was assessed using Accu-Chek Active monitoring device (Roche Diagnostics, Germany).^[23] Insulin was measured using Insulin-Ak ELISA (DPC, USA).^[24] Lipid profile was assessed using lipid assay kits (Randox Laboratories Ltd., UK).^[25]

Histopathological investigations

After completing 13 weeks, rats were anesthetized, decapitated and the thyroid glands were dissected then processed into paraffin blocks that were sectioned and stained with haematoxylin and eosin and toluidine blue.^[26]

Small parts of thyroid tissues (1 mm) were fixed with glutaraldehyde (4%), and 500–800 Å Ultrathin sections were prepared then stained with uranyl acetate and lead citrate.^[27] The ultrastructural examination was performed using transmission electron microscope (TEM) “JEM-100 C × 11; Jeol, Assuit, Egypt at TEM Unit of Assiut University, Egypt.”

Statistical analysis

Data of the study were analyzed using the (SPSS, version

16; SPSS Inc., Chicago, Illinois, USA). Analysis of variance (ANOVA) (f-test), followed by a Least significant difference *post hoc* test was utilized for comparing the parametric data for significance, while Kruskal-Wallis ANOVA, followed by a *post hoc* test (based on the Dunn procedure) was used for nonparametric data. *P* value was considered to be significant when <0.05.

RESULTS

Lipid profile

Administration of HCD for 13 weeks significantly increased ($P < 0.001$) cholesterol, TG, and LDL levels and significantly reduced ($P < 0.001$) HDL level compared to the control group. On the other hand, the levels of cholesterol, TG and LDL showed a significant reduction ($P < 0.001$) whereas, HDL showed significant ($P < 0.001$) increase in the group received HCD + raisins compared to those received HCD alone [Figure 1].

Blood glucose and insulin

Blood glucose and insulin levels showed a significant increase ($P < 0.001$) in the group received HCD for 13 weeks compared to the control group. Administration of raisins along with HCD

significantly ($P < 0.001$) reduced the blood glucose and insulin levels compared to the group received HCD alone [Figure 2].

Thyroid functions

The level of thyroid-stimulating hormone (TSH) significantly increased ($P < 0.001$) after administrating HCD for 13 weeks either alone or along with raisins compared to the control group. In addition, T4 significantly ($P < 0.001$) increased where T3 significantly decreased in the HCD group compared to the control. On the other hand, T4 significantly ($P = 0.004$) and T3 level significantly decreased ($P < 0.001$) in the group received HCD plus raisins [Figure 3].

Oxidants/antioxidants level

It was noticed that the level of malondialdehyde (MDA) significantly ($P < 0.001$) increased in the group received HCD alone compared to the control, whereas it significantly decreased ($P = 0.003$) in the group received HCD along with raisins. In contrast to that, total anti-oxidant capacity (TAOC) significantly decreased ($P = 0.002$) in the group received HCD compared to the control whereas it significantly ($P = 0.001$) elevated in the group received HCD plus raisins [Figure 2].

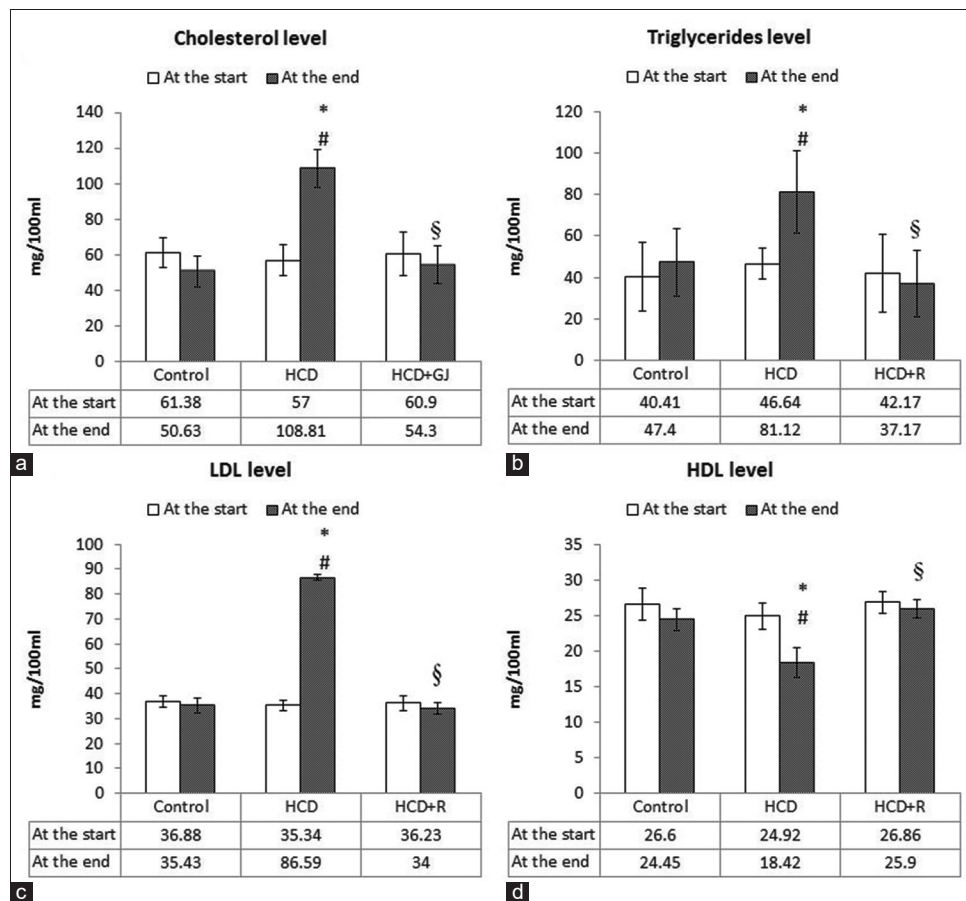


Figure 1: Effect of raisins on the cholesterol (a), Triglycerides (b), low-density lipoprotein (c) and high-density lipoprotein (d). Results are presented as mean and Standard deviation * significant compared to the level at the start of the experiment. # Significance compared to the control. \$ Significance compared to high cholesterol diet group. HCD: High cholesterol diet, R: Raisins, TSH: Thyroid stimulating hormone, T3: Triiodothyronine, T4: Free thyroxine, MDA: Malondialdehyde, TAOC: Total anti-oxidant capacity

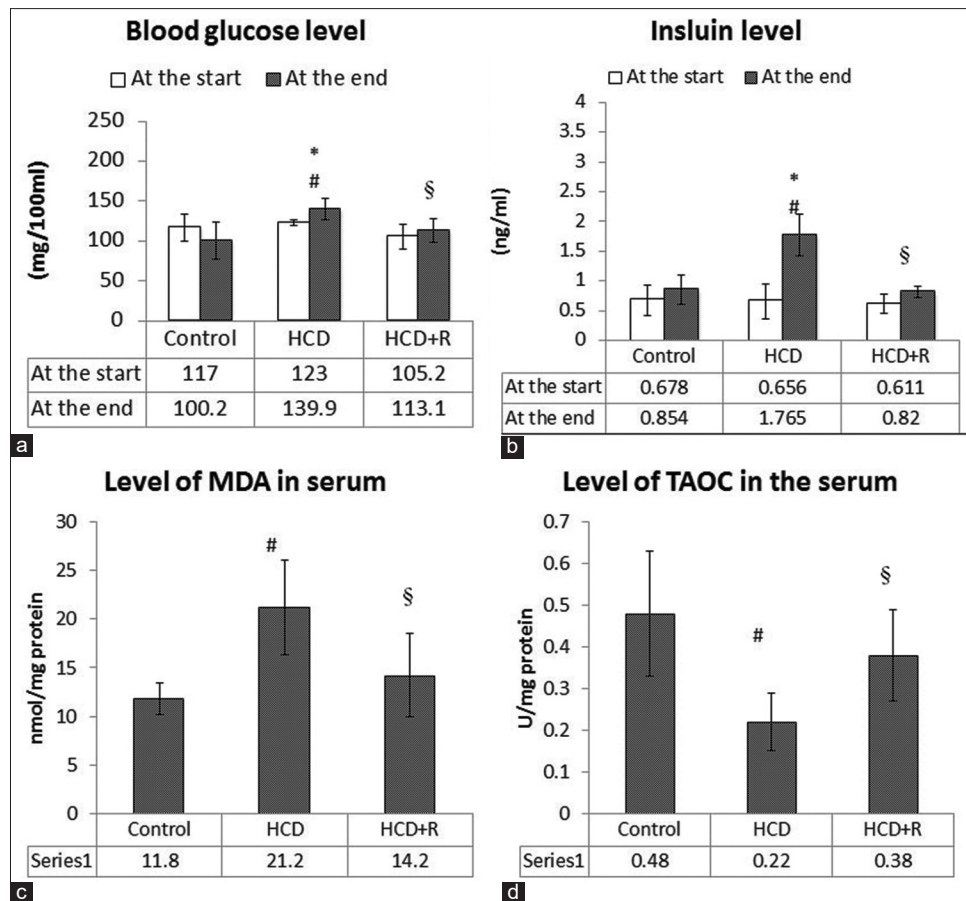


Figure 2: Effect of raisin on the blood glucose (a), insulin (b), malondialdehyde (c) and total antioxidants capacity (d) levels. Results are presented as mean and standard deviation *Significant compared to the level at the start of the experiment. #Significance compared to the control. \$ Significance compared to high cholesterol diet group. HCD: High cholesterol diet, R: Raisins, MDA: Malondialdehyde, TAOC: Total antioxidants capacity

Histopathological results

When the thyroid gland of the control group was examined using the light microscope, it was observed that it had intact structure. It was formed of intact thyroid follicles filled with homogenous colloid and lined with cubical cell layer with rounded vesicular nuclei (follicular cells) as well as large parafollicular cells that had rounded vesicular nuclei and pale cytoplasm [Figure 4]. On the other hand, thyroid gland of the HCD group showed many irregular follicles contained vacuolated colloid and lined with follicular cells with vacuolated cytoplasm and pyknotic nuclei. Thyroid gland of HCD group treated with raisins showed more or less intact follicles apart from few follicles that showed vacuolated follicular cells with dark nuclei as well as vacuolated colloid [Figure 4].

The ultrastructure of the thyroid gland was examined using the TEM. It was noticed that the follicular cells of control rats showed intact euchromatic nuclei and cell organelles included lysosomes, mitochondria, endoplasmic reticulum and secretory granules [Figure 5]. On the other hand, some follicular cells, in the thyroid gland of the HCD group, were degenerated and had vacuolated mitochondria as well as areas of the lost matrix. Although the nuclei of some cells appeared

euchromatic, they possessed heterochromatin and showed increased lysosomes and many lipid droplets. The cellular structure of thyroid follicular cells of the HCD group treated with raisins exhibited more or less normal structure apart from some cytoplasmic vacuoles and few lipid droplets that were observed in few cells. The limited number of the degenerated cell was also noticed [Figure 5].

The ultrastructure of the parafollicular cells was also examined. It was found that the parafollicular cell of control rat appeared intact with the euchromatic nucleus, intact mitochondria, lysosomes and membrane-bound cytoplasmic granules. Contrary to that, many parafollicular cell of the thyroid gland from rats received HCD showed signs of cellular degeneration in the form of shrunken nuclei, swollen mitochondria, dilated endoplasmic reticulum, and disrupted membrane-bound cytoplasmic granules. All these signs were rarely encountered in the parafollicular cells of thyroid glands of the group treated with raisins while receiving HCD [Figure 6].

DISCUSSION

Many previous studies were conducted to assess the effect of thyroid gland dysfunction on the lipid metabolism and cholesterol level in blood, while few studies, had assessed the

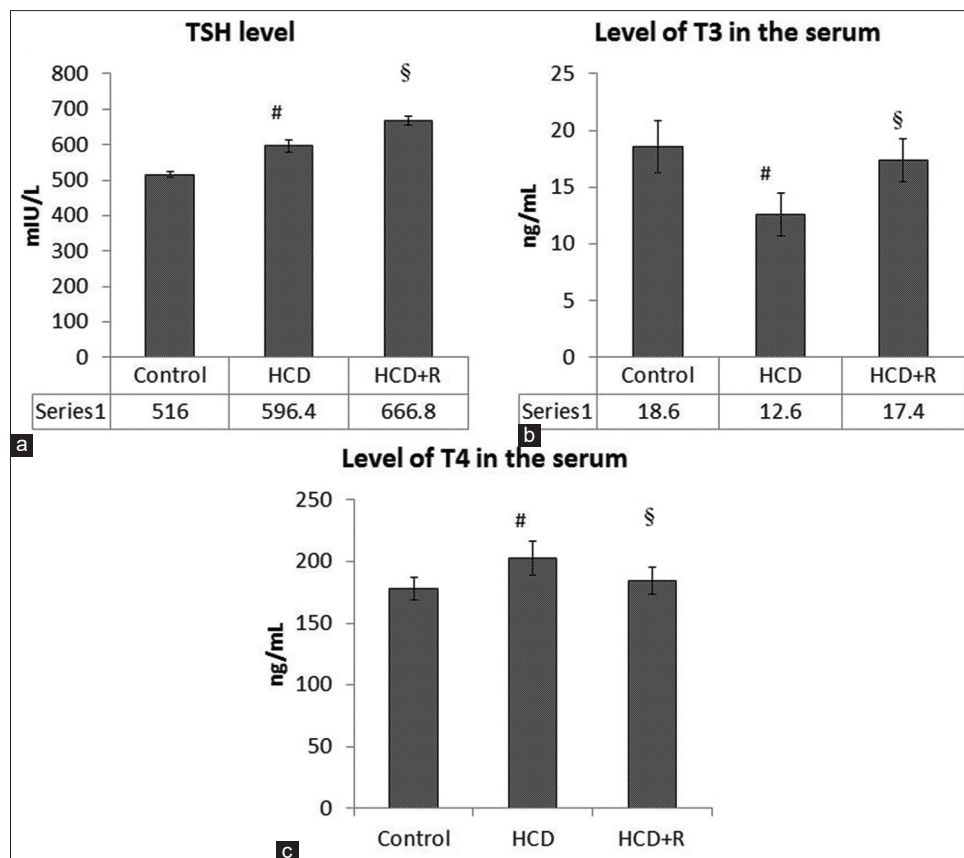


Figure 3: Effect of raisin on the thyroid-stimulating hormone (a), T3 (b) and T4 (c) levels. Results are presented as mean and standard deviation *significant compared to the level at the start of the experiment. # Significance compared to the control. § Significance compared to high cholesterol diet group. HCD: High cholesterol diet, R: Raisins, TSH: Thyroid-stimulating hormone, T3: Triiodothyronine, T4: Free thyroxine

effect of HCD on the thyroid gland. Therefore, this current study was performed to investigate the impact of HCD on the structure and function of the thyroid gland. Hypothyroidism is usually associated with hypercholesterolemia due to decreased activity of the LDL receptor which can uptake LDL. A reduced level of T3 can affect control of sterol regulatory element-binding protein 2, which modulates the activity of HMG-CoA reductase and cholesterol biosynthesis. A natural thyroid hormone derivative-3,5-diiodothyronine, was linked in lipid catabolism and lipogenesis, via a different pathway other than that of T3. Moreover, T2 could repress the transcription factor carbohydrate-response element-binding protein.^[28]

Thyroid hormones stimulate both cholesteryl ester transfer protein, and lipoprotein lipase affecting metabolism of HDL and TG-rich lipoproteins respectively. It was documented that thyroid gland dysfunction affected lipids resulting in hypercholesterolemia, hypertriglyceridemia and reduced HDL levels.^[29]

The current study demonstrated that HCD was significantly increased both TSH and T4 while T3 was significantly reduced in comparison to the control group. Bansal and Jaswal^[30] also documented the same results which could be due to the decreased activity as well as mRNA expression of 5'-deiodinase (5'-DI) enzyme with HCD. Basically, 5'-DI

transforms T4 to T3, and therefore lesser the expression of this enzyme lesser will be T3 production.

In comparison to the HCD group, this work documented a significant increase in both TSH and T3 with the combined administration of raisins and HCD while T4 was significantly reduced. Raisins are rich in fibers and polyphenols such as resveratrol and rutin.^[31,32] Resveratrol could influence the metabolism and actions of thyroid hormones at tissues causing a significant increase in T3, T4 and TSH levels.^[33] Moreover, resveratrol has many beneficial health properties such as its anti-oxidant, anti-inflammatory, chemopreventive actions, enhancement of iodide trapping and stimulation of TSH secretion.^[34] However, rutin can result in a slight decrease of serum T4 and T3 without influencing TSH. It also stimulates the expression of both the TSH receptor and sodium-iodide symporter; therefore, thyroid iodide uptake was increased.^[35] Villanueva *et al.* reported that T3 reduced the oxidative damage through the enhancement of expression of glutathione and the antioxidant enzyme catalase.^[36]

At the end of this work, the HCD-administered group showed significant elevation of blood cholesterol, TG, LDL, glucose and insulin levels while HDL significantly decreased. Previous studies reported the same results.^[37,38] Increased levels of TG can cause insulin resistance as it induces subclinical

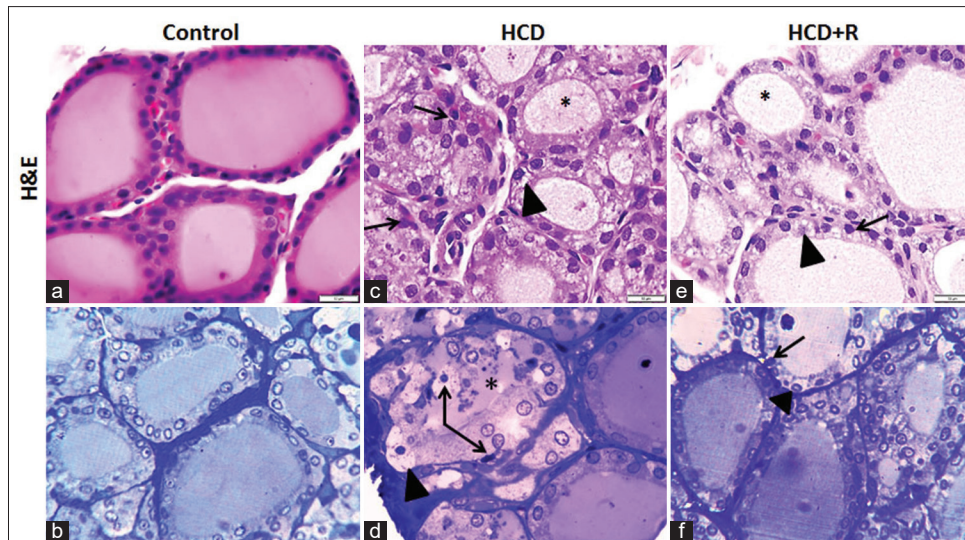


Figure 4: Thyroid gland of control (a and b), hypercholesterolemic (c and d) and hypercholesterolemic rat treated with raisins (e and f). Note, the thyroid follicles of the control rat appear intact while those of hypercholesterolemic rat showing irregular follicles with little and vacuolated colloid (*) and some follicular cells have vacuolated cytoplasm (arrow head) and pyknotic nuclei (arrow). Thyroid gland of hypercholesterolemic rat treated with raisins showing more or less intact follicular shape and epithelial lining apart from few follicles that show vacuolated follicular cells (arrowhead) with dark nuclei (arrow) as well as vacuolated colloid (a, c, e stained with H and E and b, d, f stained with Toluidine blue $\times 400$)

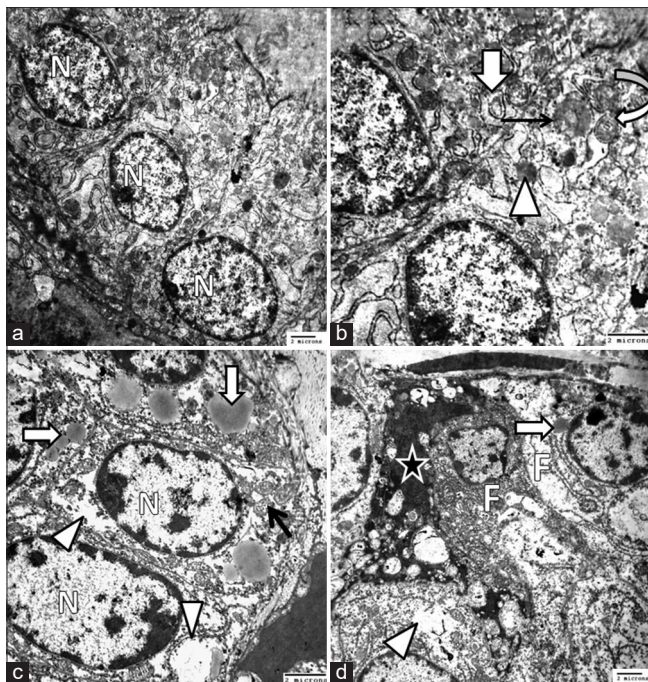


Figure 5: Thyroid gland of control rat (a and b) shows lipid droplets (thick arrow). Thyroid gland of hypercholesterolemic rat treated with raisins (d) shows follicular cells with euchromatic nuclei (N), lysosomes (arrow head), mitochondria (curved arrow), secretory granules (thin arrow) and endoplasmic reticulum (thick arrow). Thyroid gland of hypercholesterolemic rat (c) showing some follicular cells with euchromatic nuclei (N) and increased heterochromatin, areas of the lost matrix (asterisk), vacuolated mitochondria (thin arrow), increased lysosomes and more or less intact follicular cells (F) apart from the presence of cytoplasmic vacuoles (arrow head). Notice the presence of one degenerated cell (star) and few lipid droplets (Thick arrow) (transmission electron microscope, a and d $\times 5800$, b and c $\times 7200$)

inflammation and disruption of the cascade linking insulin receptors with glucose transporters.^[39] On the other hand, intake of raisins with HCD; in this study, markedly reduced the blood glucose, insulin, cholesterol, TG, and LDL levels, whereas HDL significantly elevated. These findings were similar to that of Puglisi, *et al.*^[40] and Esfahani, *et al.*^[41] Raisins contain a significant amount of polyphenols and dietary fibers that can potentially decrease both plasma TG and LDLC by reducing apo E and inhibition of microsomal triglyceride transfer protein, respectively, as shown with lyophilized grape powder supplementation.^[40,42]

The decrease of both glucose and insulin levels in HCD and raisins administered group of this work was in agreement with the previous study of Esfahani *et al.*^[41] who documented that fructose constitutes about 50% of carbohydrate content of raisins and has a low glycemic index. Fructose intake elevates the concentration of “fructose-1-phosphate” in the liver which competes with “fructose-6-phosphate” for binding to glucokinase regulatory protein. This leads to the release of glucokinase which increases glucose uptake and thus decreases postprandial glucose level.^[43,44] The decreased insulin level in HCD and raisins administered group may be attributed to high fructose content of raisins which does not stimulate insulin secretion.^[45,46]

The HCD group of the present study showed that MDA was significantly increased while TAOC was significantly reduced. This finding was supported by Fernández *et al.* who reported that changes in thyroid hormones were associated with an enhanced generation of reactive oxygen species (ROS) in different tissues.^[47] This result was in agreement with other studies revealed that thyroid oxidative stress is presented as

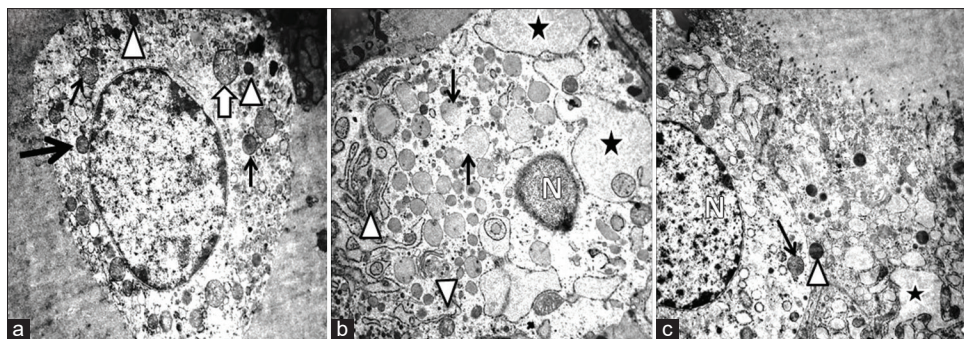


Figure 6: Thyroid gland of control rat (a) show a parafollicular cell with euchromatic nucleus, intact mitochondria (thick arrows), lysosomes (arrow heads) and membrane-bound cytoplasmic granules (thin arrows). Parafollicular cell from thyroid gland of hypercholesterolemic rat (b) shows shrunken nuclei, degenerated mitochondria (arrow head), dilated endoplasmic reticulum (star) and disrupted membrane-bound cytoplasmic granules (thin arrows). Parafollicular cell from thyroid gland of hypercholesterolemic rat treated with raisins (c) shows euchromatic nucleus (N), intact mitochondria (arrows) and lysosomes (arrow head), few dilated endoplasmic reticulum (star), and disrupted membrane-bound cytoplasmic granules (thin arrows). (transmission electron microscope, a, b, and d $\times 7200$, c $\times 5800$)

a marked elevation in ROS and MDA “a biomarker of lipid peroxidation” and a significant decrease in TAOC.^[48,49]

In the present work, electron microscopic examination revealed degeneration of both follicular and parafollicular cells and their organelles such as mitochondria. Moreover, accumulation of lipid droplets was observed. The same results are reported by Ayoub *et al.*^[20] These degenerative changes may be due to oxidative stress. Lipids are considered targets for free radicals which resulted in overproduction of ROS causing damage of cell components, and mitochondrial degeneration.^[33,50,51] Consumption of HCD markedly elevate lipid load in the cells or might result in an excessive increase of ROS, causing lipid peroxidation and organ damage through the effect of ROS on biological membranes.^[52]

Intake of raisins antagonizes the degenerative effect of hypercholesterolemia. Raisins are characterized by 27 phenolic compounds from four phenolic families, “i.e., flavan-3-ols, phenolic acids, flavonols, and anthocyanins.” Different types of raisins have variable phenolic and anthocyanins content. There are robust associations between antioxidative capability and both phenolic and flavonol content. White and red raisins have the highest antioxidant capacity and total phenolic content. It was recommended to intake these two cultivars due to their high phenolic content and antioxidant activity.^[53] Moreover, phenolic compounds in grapes have significant anti-inflammatory effects through the inhibition of pro-inflammatory factors release.^[54,55]

CONCLUSIONS

High-fat diet induced degenerative changes of thyroid gland while intake of raisins improved these changes and modulated the levels of thyroid hormones. It is recommended to test the efficacy of raisins in improving the thyroid function in patients with hypercholesterolemia together with thyroid problem.

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Conflicts of interest

There are no conflicts of interest.

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