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Impact of *Quercus infectoria* Galls Extract on Thyroid Gland and Testicular Functions in Diabetic Rats

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A B S T R A C T

Diabetes mellitus has been reported to be accompanied by thyroid and testicular dysfunctions. The objective of this study was to investigate the effect of *Quercus infectoria* galls (QIg) extract on the thyroid gland and testicular functions in diabetic rats. Sixteen rats were randomly divided into four equal groups, consisting of normal control, diabetic untreated control, diabetic treated with oral administration of 500 mg/kg BW and 1000 mg/kg BW, respectively for 15 days. Serum blood glucose, thyroid stimulating hormone (TSH), triiodothyronine (T3), thyroxine (T4), testosterone (T), and luteinizing hormone (LH) were assessed. At the end of the experimental period, the rats were euthanized for histopathological analysis of thyroid gland and testis. Furthermore, immunohistochemistry was used to assess the expression of thyroid transcription factor-1 (TTF-1) in the thyroid gland of rats. The significant increase in serum blood glucose level in diabetic rats (DC) was markedly decreased by treatment with QIg extract (500 mg and 100 mg/kg BW) almost to the normal level. The reduced thyroid hormones, both the T3 and T4 were significantly recovered after 15 days of treatment with QIg extract (500 mg and 100 mg/kg BW). Whereas serum concentration of testosterone was significantly reduced in diabetic rats with QIg extract (500 mg and 100 mg/kg BW) treatment. Histopathological analysis of diabetic rats showed a wide range of morphological alterations in thyroid gland and testicular structures, which were almost completely, restored back to normal by treatment of rats with QIg extract. Furthermore, results showed overexpression of TTF-1 in the thyroid gland of diabetic rats, which was recovered back to normal expression after 15 days of treatment with QIg extract. These findings may provide new insights into the potential role of QIg extract as a promising therapeutic agent against diabetic complications in thyroid gland and testicular functions.

Keywords: diabetes, thyroid gland, testis, TTF-1, *Quercus infectoria* galls extract

INTRODUCTION

The impairment of metabolic regulatory mechanism that associated with diabetes mellitus leads to secondary pathophysiological alterations in a wide range of body organs (1), most commonly thyroid gland dysfunction in human and animals (2, 3). Diabetes mellitus is a serious and chronic metabolic disorder is caused by absence or insufficient amount of secreted insulin (4), and characterized by hyperglycemia, polyuria, and polyphagia (5). Defects in the molecular mechanisms that tightly regulate the synthesis and secretion of insulin hormone can lead to disturbance in the metabolic process and development of diabetes mellitus (6). Type 2 diabetes

mellitus founds in 90–95% of all the diabetic cases, results either from pancreatic beta cells dysfunction and inadequate amount of insulin secretion or desensitization of insulin receptors that blocks glucose entry into the cell (7).

It has been suggested that there are synergistic effects between insulin and thyroid hormone to regulate the glucose and lipid metabolic pathways (8) as well as direct influence of thyroid hormone on testicular cell activity through thyroid receptor mediated mechanism in testicular cells (9). In addition, it has been shown that thyroid hormones play a vital role in modify the effects of other hormones (10). Thyroid hormones secretion is regulated by anterior pituitary gland through releasing of thyroid stimulating hormone (STH), which in turn, enhances the follicular epithelial cells of thyroid gland to secrete T3 and T4 (11).

A clinical study showed that there is a relationship between a low level of thyroid hormones and diabetes mellitus, including insulin resistance, which is the major cause of reduced glucose metabolism in type 2 diabetes mellitus (12). Furthermore, it has been shown that diabetes mellitus and thyroid diseases are the two most common endocrine metabolic disorders in the general population (13) and tend to coexist in patients (14). Moreover, it has been demonstrated that there is a marked increase in the prevalence of thyroid disease in diabetic patients (3) that can have a major impact on glucose homeostasis (14). In addition, thyroid hormones have a counter-regulatory impact on the hyperglycemia, and it is well known that hyperthyroidism worsens metabolic control in diabetic patients (15). Moreover, it has been demonstrated that thyroid hormones play a vital role in regulating carbohydrate metabolism and pancreatic beta cells function since thyroid hormones directly control the insulin secretion by pancreatic beta cells. Low level of thyroid hormones produces an obvious decrease in glucose-dependent insulin secretion by pancreatic beta cell while hyperthyroidism leads to an increase in pancreatic beta cell response to glucose as a consequence of an increase in pancreatic beta cell mass (16, 17). Furthermore, diabetes mellitus induces a wide range of functional and structural complications in testis, including testicular dysfunction accompanied by reduced spermatogenesis, oligospermia, low sperm motility, reduced seminal fluid volume and testosterone hormone, leading to male infertility (18).

It has shown that medicinal plants, such as *Allium cepa* L., bulbus; *Azadirachta indica* A. Juss., folium; *Momordica charantia* L., fructus; *Panax ginseng*; and *Rehmannia glutinosa* are an important source of phytochemical compounds, which can be used in treating of various diseases, including diabetes mellitus (19) due to the presence of active phytochemical ingredients (20, 21). This is more likely due to the ability of phytochemical ingredients in the medicinal plants to improve the performance of pancreatic tissue function via ameliorating the number of pancreatic beta- cells (22). In addition, phytochemical ingredients augments glucose uptake by translocation of glucose transporter protein (GLUT) to the cell membrane of skeletal muscles and adipocytes (23), which plays a vital role in regulating entire body glucose homeostasis.

Furthermore, it has been demonstrated that the extract of *Quercus infectoria* galls (QIg) is contained medicinal active constituents of flavonoids, alkaloid, saponins, and tannins (24), which are responsible for its antidiabetic activity and play a crucial role in regulating glycaemia (25). These studies have provided valuable indications that QIg extract is able to modulate blood glucose levels. In addition, previous studies have linked the function of thyroid hormone with the development and function of pancreatic islet cells (26). Moreover, thyroid hormone plays a crucial role in testicular cell proliferation and differentiation in rats and other mammals (27, 28). In addition, a study by Korejo et al. (29) has indicated that diabetes is always accompanied by dysfunction in the thyroid gland, which leads to inhibition of testicular and epididymal development as well as male infertility. Furthermore, diabetes mellitus induces alteration in circulatory and testicular levels of sex hormones, including testosterone, LH, and follicular stimulating hormones (FSH) in human and animals (30). Likewise, diabetes mellitus produces male infertility through elevation of reactive oxygen species (ROS) parallel with decline in cellular antioxidant activity, in the testes and epididymis (31). In addition, diabetes mellitus directly influences the hypothalamic-pituitary-gonadal axis in human by reducing the testosterone hormone level (32). However, to the best of our knowledge, it is yet unknown the role of QIg extract in regulating thyroid gland and testicular activity in diabetic rats. Thus, this study was aimed to explore the influence of QI galls extract on thyroid gland and testicular activity in diabetic rats.

MATERIALS AND METHODS

Plant Material and Extract Preparation

The local galls of *Q. infectoria* in Sulaimani province were identified and authenticated by Kurdistan Botanical Foundation. Voucher sample was preserved in research center at the College of Veterinary Medicine, University of Sulaimani, for further reference. The galls of *Q. infectoria* were air-dried at room temperature (28±2°C) and then grinded into fine powder (33).

The dried powdered material (50 g) was mixed with 80% methanol, and then incubated at 25°C for 2-3 days in the shaking incubator (Labtek, Australia). Then, the extract was evaporated by rotary evaporator (Labtek, Australia), and then the aqueous residue was lyophilized for 2 days to get the dried powder of QIg extract as described by (34). Then the dried extract was kept in sealed container at -20°C for future use.

Experimental Animals and Treatments

Sixteen apparently healthy male Wistar rats, body weight approximately 200 g, were purchased from animal house, College of Veterinary Medicine, University of Sulaimani, Veterinary Teaching Hospital, Kurdistan region, Iraq and used in this study. They were housed, in an ordinary cage and hygienically kept with freely accessed to diet and drinking water and were maintained on a 12 h light/dark cycle with sufficient ventilation. This study was authorized by the committee of the animal welfare, University of Sulaimani, College of Veterinary Medicine. Animals were kept under controlled conditions two weeks prior to experiment for acclimatization and received standard diet and water *ad libitum*.

Diabetes mellitus was induced in 12 rats by intraperitoneal injection of a single freshly prepared streptozotocin (STZ) (55 mg/kg BW, Sigma-Aldrich). On day 3 after STZ injection, rats were fasted for overnight, and blood glucose concentration was measured. Rats with fasting blood glucose levels over 300 mg/dL were considered as diabetic and included in the study (Figure 1) (35, 36). Confirmed diabetic rats were divided randomly into four groups with 4 rats each (n=4) as follows: the first group (C) served as negative control, received distilled water orally via gastric gavage needle, 0.1 mL/100 g BW/day for the entire study period; the second group (DC) referred to as diabetic control, received distilled water orally via intragastric tube, 0.1 mL/100 g BW day for the entire study period; the third (DQIg-500) and fourth (DQIg-1000) groups were diabetes treatment groups which received oral QIg extract at dose rates of 500 mg/kg BW/day and 1000 mg/kg BW/day, respectively, over a period of 15 days post-induction of diabetes.

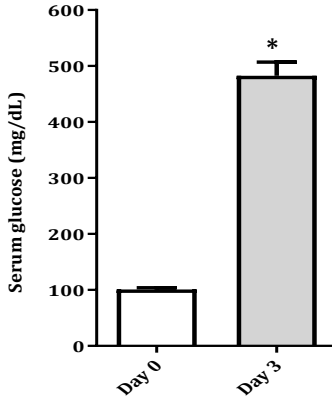

Figure 1. Serum glucose level in all rats at day zero and day 3 before treatment. Data are shown as mean ±SEM, n=16. **P*<0.05 compared to control.

At the end of the experimental period, the rats were fasted overnight with free access to drinking water. Blood samples were immediately collected directly from heart puncture. Then serum was collected by transferring of the whole blood sample into sterile microtubes for centrifugation at 3000 × g for 5 min and isolated serum was used for measuring the concentration of blood glucose, TSH, T3, T4, LH and testosterone (36). Analysis of all hormones was performed by using MINI VIDAS® compact multiparametric immunoanalyzer (BIOMÉRIEUX S.A., France.

Histopathological and Immunohistochemistry Study

At the end of the experiment, the rats were anesthetized by intraperitoneal injection of a mixture of ketamine 30 mg kg/BW and xylazine 20 mg kg/BW (Alfasan, Netherlands) at a dose of 0.01 mL/g BW, then euthanized by cervical dislocation. The thyroid glands and right testis of each rat in each group were quickly removed and fixed in a 10% neutral buffered formalin (DAKO, Germany). After fixation, samples were processed for paraffin embedding, and then two sections of 4 µm thick were taken and stained with hematoxylin and eosin stain and examined under light microscopy (Leica, Germany).

The second tissue section of paraffin block from thyroid glands was mounted on a positively charged slide and processed to apply the immunohistochemistry staining technique according to the producer's instructions that supplied with the TTF-1 monoclonal antibody kit (Bio SB, USA). A total staining score was obtained by multiplying the positive reactivity extent and level of staining intensity making a range of 0-12.

Statistical Analysis

All results were presented as mean±SEM. number of the animals in each group n=4. For data presentation and statistical analysis, GraphPad Prism 5 software (GraphPad Software, Inc., CA, USA) was used for statistical analysis and one-way ANOVA (with Tukey's multiple comparison post hoc test). $P \leq 0.05$ were considered statistically significant.

RESULTS

A series of experiments were performed to assess the effect of two different doses of QIg extract, 500 mg, and 1000 mg/kg BW for 15 days, on the regulation of blood sugar in hyperglycemic rats. Results showed that high serum glucose level was significantly reduced by treating of diabetic rats both with the 500 mg and 1000 mg doses of QIg extract compared to control diabetic group (Figure 2). This suggests that QIg extract played an important role in the regulation of blood sugar level in diabetic rats.

Since the pituitary TSH responsible for enhancing the process of thyroid hormones (T3 and T4) biosynthesis and secretion (37), the influence of diabetes mellitus and QIg extract was assessed on the activity of pituitary gland. The activity of pituitary gland to secret TSH was not affected by alteration of pancreatic beta cell function in diabetic group compared to control group ($P>0.05$). Results showed that, similar to the lack of effect of diabetes on TSH secretion, treatment of diabetic rats with different doses of QIg extract had no significant effect ($P>0.05$) on serum TSH

concentration compared to both the diabetic control and control group (Figure 3).

Untreated diabetic rats exhibited a significant decrease (P<0.05) in both the T3 and T4 hormones secretion by thyroid gland compared to control group. Interestingly, treatment of rats with QIg extract, 1000 mg/kg BW, caused a significant increase (P<0.05) both in the T3 and T4 hormone secretion by thyroid gland compared to diabetic group, almost to the same level as in healthy control group. There was slight, but not significant, increase (P>0.05) in T3 and T4 hormone secretion by thyroid gland in diabetic rats treated by 500 mg/kg BW of QIg extract compared to diabetic control group (Figures 4A, B).

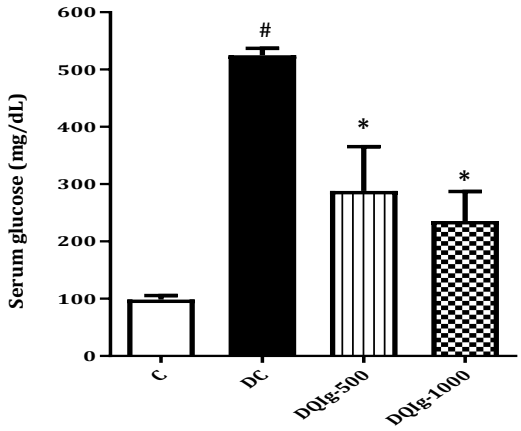

Figure 2. Effects of *Quercus infectoria* galls (QIg) extract on serum glucose concentration in diabetic male Wistar rats. Data are shown as mean±SEM, n=4. C, negative control; DC, diabetic control; DQIg-500, diabetic rats treated with 500 mg/kg BW/day of QIg extract; DQIg-1000, diabetic rats treated with 1000 mg/kg BW/day of QIg extract. #P<0.05 compared to NC, *P≤0.05 compared to DC

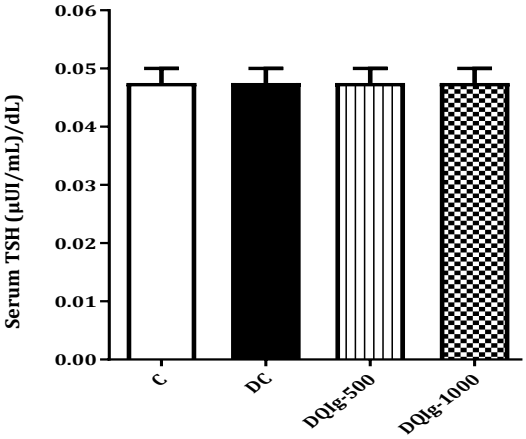

Figure 3. Effects of *Quercus infectoria* galls (QIg) extract on serum thyroid stimulating hormone (TSH) concentration in diabetic male Wistar rats. Data are shown as mean±SEM, n=4. C, negative control; DC, diabetic control; DQIg-500, diabetic rats treated with 500 mg/kg BW/day of QIg extract; DQIg-1000, diabetic rats treated with 1000 mg/kg BW/day of QIg extract

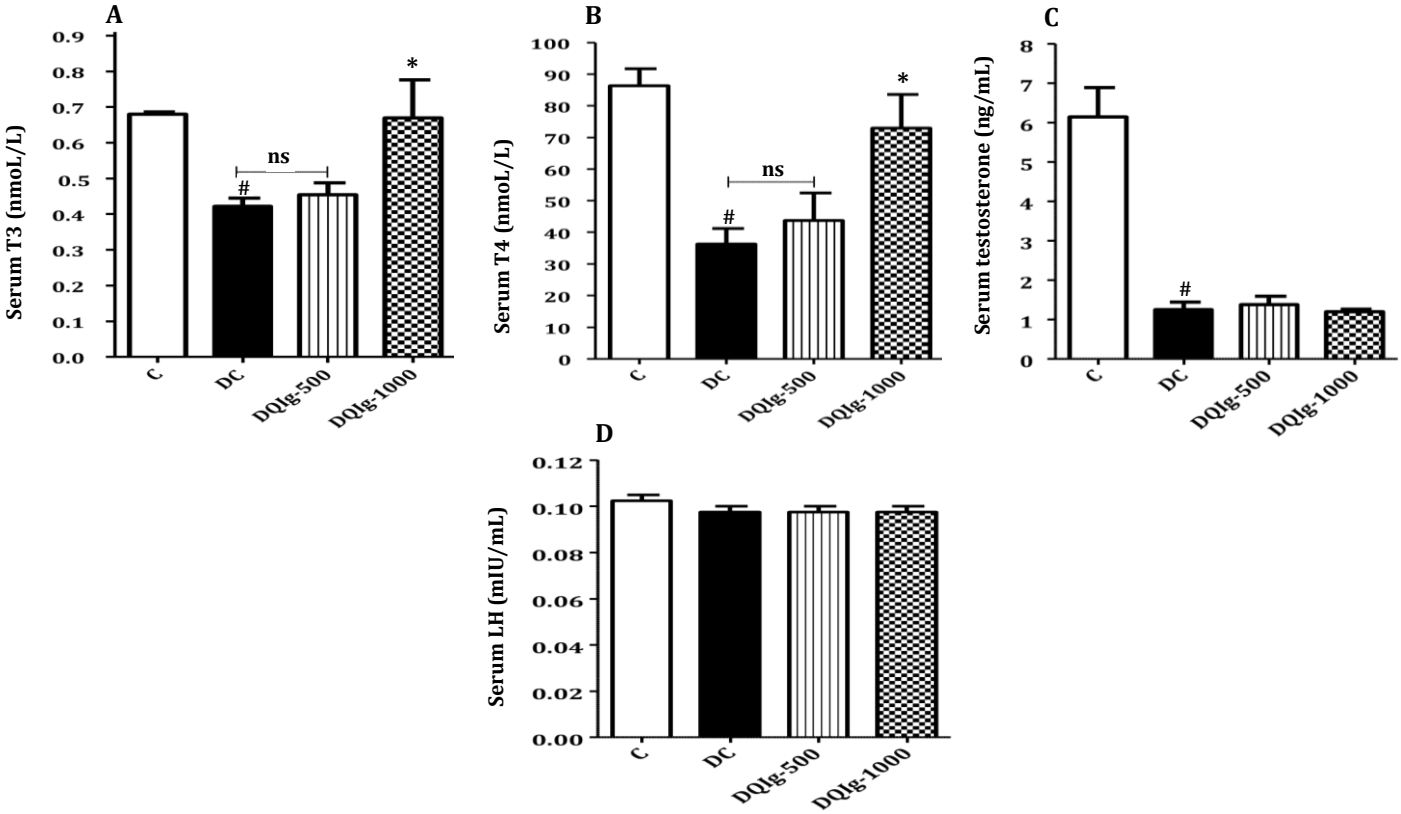

Figure 4. Effects of *Quercus infectoria* galls (QIg) extract on **A)** serum triiodothyronine (T3) and **B)** thyroxine (T4), **C)** serum testosterone and **D)** luteinizing hormone (LH) in diabetic male Wistar rats. Data are shown as mean±SEM, n=4. C, negative control; DC, diabetic control; DQIg-500, diabetic rats treated with 500 mg/kg BW/day of QIg extract; DQIg-1000, diabetic rats treated with 1000 mg/kg BW/day of QIg extract. #P<0.05 compared to C, *P<0.05 compared to DC, ns=P>0.05

Testosterone hormone secretion by testicular cells was significantly reduced (P<0.05) in DC group compared to control rats. Reduction in testosterone hormone secretion was not recovered after 15 days of treating diabetic rats

with different doses of QIg extract compared to diabetic control group (Figure 4C). Results showed that LH secretion by pituitary gland did not alter (P>0.05) by experimentally induced diabetes, nor did it by treatment of diabetic rats with different doses of QIg extract (Figure 4D).

Histopathological Study

The histopathological assessment of testicular sections in control group after 15 days were illustrated in Figures 6a-c. Comparing to control group, the microscopic examination of seminiferous tubules in DC group revealed epithelial lining distortions, abnormal cellular attachment in the germ lineage, mild degeneration of germ cell epithelium with severe spermatogenic arrest, decrease in the number of spermatozoa, and reduction in round spermatids, as well as marked interstitial degeneration (Figures 6d-f).

Histopathological results in treated diabetic group with oral administration of QIg extract showed obvious restoration of seminiferous tubule abnormalities and enhanced the mitotic activity of spermatogenic cells and Leydig cells compared to DC group (Figures 7d-f).

The influence of QIg extract was more potent in rats treated by 1000 mg/kg BW since the microscopic examination characterized with well-organized and nearly normal histological structures of seminiferous tubules with germinal cells, as well as maintaining their associations and cellularity with the intact interstitial cells (Figures 7a-f).

The microscopic examination of tissue sections from rat thyroid glands of control group revealed normal variable-sized and well-organized thyroid follicles, and excessive amounts of colloid was seen inside of thyroid follicles (Figures 8a, b). However, histopathological examination of thyroid glands in diabetic rats showed fewer follicles in the resting (inactive) stage in comparison to the control healthy group. The follicular cells were flat simple squamous (reduction in the height of the follicular cells) within the cavity, as well as focal necrosis of follicular and parafollicular cells that distorted the thyroid gland's structures (Figures 8c, d).

Treatment of diabetic rats with QIg extract produced ameliorative effect on the histoarchitecture of the thyroid gland compared to DC group. The results revealed mild degeneration of thyroid follicular and parafollicular cells, as well as a mild reduction in the thickness or height of follicular cells in DQIg-500 treated group (Figures 9a, b). While microscopic assessment of thyroid section from DQIg-1000 group showed more obvious regenerating and had normal histoarchitecture, as well as normal and intact cellularity (Figures 9c, d).

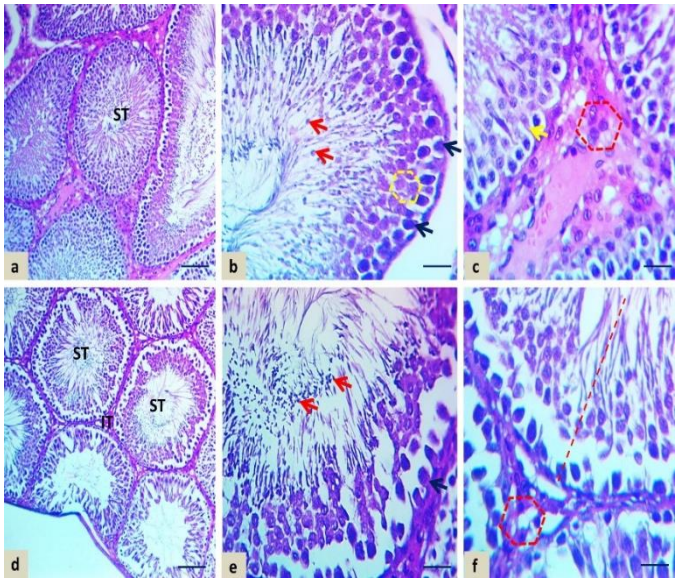

Figure 6. Microscopic photographs of seminiferous tubules sections of the right testis of male Wister rats in negative control (**a-c**) and diabetic control (**d-f**). **a**, Normal spermatogenic arrangement and cellularity (ST) (H&E, 200×); **b**, well-organized interstitial space (IT) with intact spermatogenic cells including spermatogonia (black arrows), primary spermatocytes (yellow polygonal) and round spermatids (red arrows) (H&E, 400×); **c**, intact Sertoli cells (yellow arrows) and Leydig cells (red polygonal) (H&E, 400×). **d,** Dilated seminiferous tubules (ST) and mild degenerated interstitial space (IT) (H&E, 200×); **e**, moderate-marked degeneration of spermatid as designated by red arrow (H&E, 400×); **f**, hypospermatogenesis and vacuolization in spermatogenic cells (red dash line) with degenerated Leydig cells (red polygonal) (H&E, 400×)

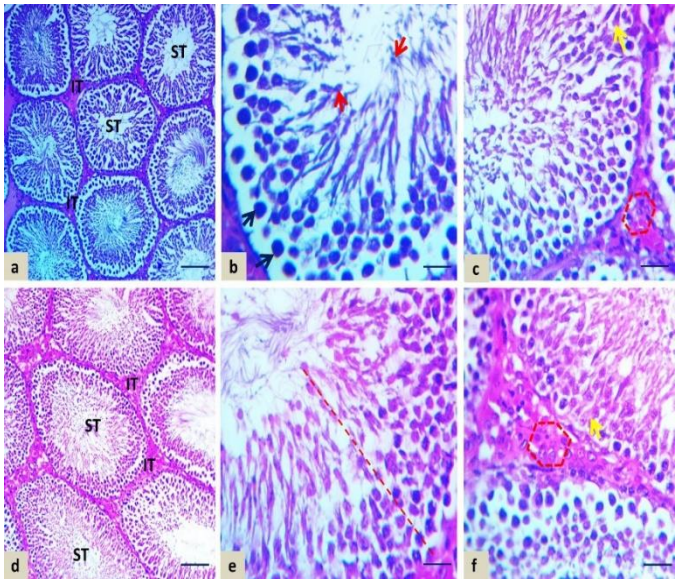

Figure 7. Microscopic photographs of seminiferous tubules sections of the right testis of diabetic male Wister rats treated with 500 mg/kg BW (**a-c**) and 1000 mg/kg BW (**d-f**) of *Quercus infectoria* galls (QIg) extract. **a,** Mild dilation of the seminiferous tubule (ST) and degenerated interstitial space (IT) (H&E, 200×); **b,** mild degenerated spermatid (red arrow) (H&E, 400×); **c**, mild degenerated Sertoli cells (yellow arrow) and Leydig cells (red polygonal) (H&E, 400×). **d**, Normal organized seminiferous tubule (ST) and interstitial space (IT) (H&E, 200×); **e**, Intact spermatogenic cells (red dash line) (H&E, 400×); **f,** intact Sertoli cells (yellow arrow) and Leydig cells (red polygonal) (H&E, 400×)

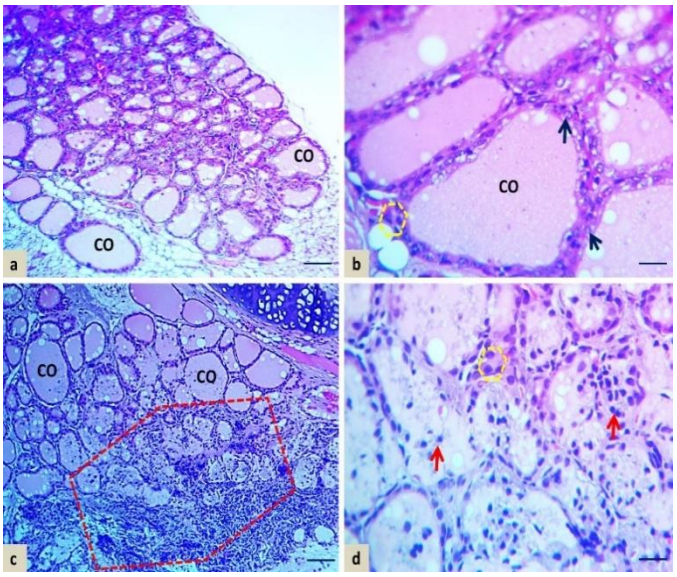

Figure 8. Microscopic photographs of thyroid gland sections of male Wister rats in negative control (**a** and **b**) and diabetic control (**c** and **d**). **a,** Well organized thyroid follicles that contain a large amount of colloid (CO) (H&E, 100×); **b**, normal height of follicular cells (black arrow) and intact parathyroid cells (yellow polygonal) (H&E, 400×). **c**, distorted thyroid gland (red polygonal) with degeneration of follicular cells and reduction of colloid (CO), inflammatory cells infiltration (H&E, 100×); **d**, focal necrosis of thyroid follicular cells (red arrows) and parafollicular cells (yellow polygonal) (H&E, 400×)

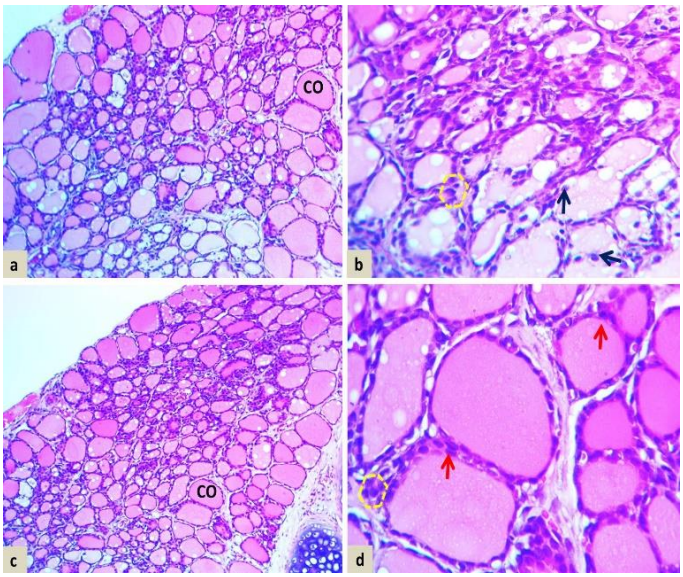

Figure 9. Microscopic photographs of seminiferous tubules sections in the diabetic male Wister rats treated with 500 mg/kg BW (**a** and **b**) and 1000 mg/kg BW (**c** and **d**) of *Quercus infectoria* galls (QIg) extract. **a**, Mild degeneration of follicular cells (black arrows) that contain a large amount of colloid (CO) (H&E, 100×); **b**, mild vacuolization of follicular cells (black arrows) and parafollicular cells (yellow polygonal) (H&E, 400×). **c**, Well organized thyroid follicles with normal colloidal content (CO) (H&E, 100×); **d**, normal height of follicular cells (red arrow) and intact parathyroid cells (yellow polygonal) (H&E, 400×)

Immunochemistry Study

Study of immunohistochemical staining of tissue sections from the thyroid glands revealed a wide range of scores of nuclear TTF-1 expressions in the epithelial cells of thyroid follicle in different groups of rats. Results clearly showed that there is a minimal expression of TTF-1 (sum score 1) in thyroid follicular cells of rats in the healthy control group. However, there was a diffuse and strong expression of nuclear TTF-1, (sum score 12), in rat thyroid follicle epithelial cells of diabetic control group (Figure 10). This suggests that diabetes play a negative role in the regulation of TTF-1 expression and thyroid cellular differentiation.

Interestingly, treatment of diabetic rats with different doses of QIg extract improved the nuclear TTF-1 expression, almost to the same level as in control healthy group, especially in treated group with 1000 mg/kg/BW of QIg extract since there was a focal but weak expression, (sum score 2), of nuclear TTF-1 in the thyrocytes. In addition, treatment of diabetic rats with 500 mg/kg/BW of QIg extract showed reduction in the TTF-1 expression by producing a diffuse but moderate intensity expression, (sum score 6), of TTF-1 in the thyrocytes (Figure 11). This indicates that QIg extract would potentially eliminate the stimulatory influence of diabetes on the overexpression of nuclear TTF-1 in the thyrocytes.

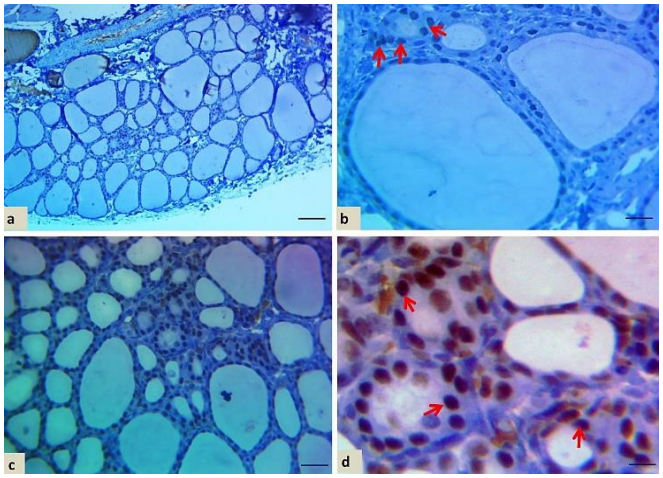

Figure 10. Immunohistochemical brownish nuclear staining of TTF-1 expression in thyroid gland of control and diabetic rats (**a** and **b**): Minimal, weak expression (sum score 1) in rat of negative control group, (100× and 400×), (**c** and **d**): Diffuse, strong expression (sum score 12) in positive control group, (200× and 400×)

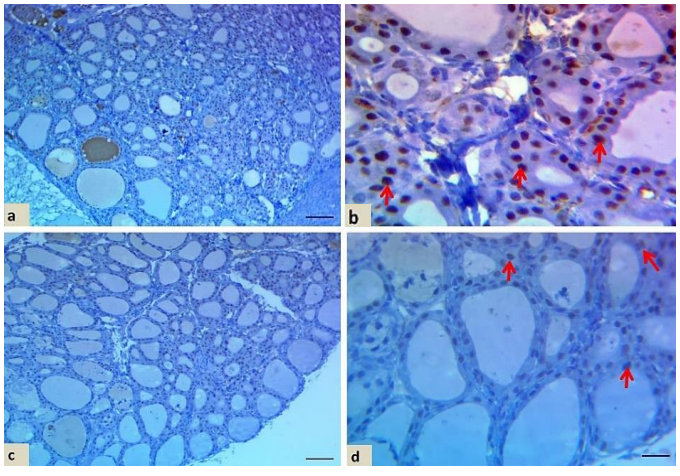

Figure 11. Immunohistochemical brownish nuclear staining of TTF-1 expression in thyroid gland of diabetic rats treated with 500 mg/kg BW and 1000 mg/kg BW of QIg extracts for 15 days (**a** and **b**): Diffuse, moderate expression (sum score 6) in 500 mg/kg/BW extracted QIg group, (100× and 400×), (**c** and **d**): Focal-weak expression (2) in group in 1000 mg/kg/BW extracted QIg group, (100× and 400×)

DISCUSSION

Current results have shown that QIg extract markedly reduced the elevated blood sugar level in diabetic rats at the end of 15 days of treatment, compared to normal control group indicating that QIg extract play a crucial role as antihyperglycemic effect in diabetic rats. This potentially due to the presence of various active ingredients, such as alkaloids and flavonoids that play an important role in the regulation and decreasing of blood sugar level (24) through dependent mechanism of maintaining pancreatic β-cells performance to increase insulin secretion or decreasing intestinal glucose absorption (38).

It has been demonstrated that the function of thyroid gland is impaired in type 2 diabetes (39), while the serum level of TSH was not changed (40). Consistence with this, results of the current study revealed that high blood glucose level in diabetic rats caused a significant decrease in thyroid hormones level, both for serum T3 and T4 while did not change the TSH level. This could be potentially by the impact of ketoacidosis resulted from diabetes, which can decrease both serum T3 and T4 levels but remaining the TSH levels normal (41). Treatment of diabetic rats with different doses of QIg extract for 15 days enhanced thyroid gland functions by increasing both the serum T3 and T4hormone secretion; it could be that QIg extract play a role as antioxidant substance and free radical scavenging activities to prevent intracellular accumulation of oxidative substances. On the other hand, a significant decrease in testosterone hormone secretion in DC group might be due to low level of thyroid hormones in diabetic rats because thyroid hormones directly control the testicular Leydig cells differentiation and testosterone formation through thyroid hormone receptors-dependent mechanism in testicular cells (9). In addition, diabetes mellitus causes testicular dysfunction through inducing degeneration and apoptosis of testicular cells (42). However, reduced serum testosterone level did not recover in treated diabetic rats after 15 days of treatment with different doses of QIg extract. One possible explanation could be the contemporaneous influence of both the diabetes and hypothyroidism on the reduction of serum testosterone concentrations (29), which might need longer duration of treatment with QIg extract to restore testicular function. However, neither diabetes nor QIg extract treatment had any effect on the LH secretion could be explained that the activity of pituitary gland was not changed by day 15 of streptozotocin-induced diabetes mellitus in rats (40).

Furthermore, rats in diabetic group exhibited various histological abnormalities of thyroid gland compared to control group. This might be due to diabetic complication resulted from hyperglycemia, which stimulates the alteration of various metabolic signaling pathways leading to inflammation, pro-inflammatory cytokine production, ROS generation, oxidative stress, and consequently cellular death (43). Treatment of diabetic rats with different doses of QIg extract restored the normal histoarchitecture of thyroid glands. One explanation could be that QIg extract possess a strong antioxidant activity that play a vital role in chelating metal ions, scavenging intracellular free radicals and anti-inflammatory abilities (44). In addition, the current study indicates that combined diabetes and hypothyroidism can stop rat spermatogenesis, these results were consistence with (29). Treatment of diabetic rats with QIg extracts improved testicular dysfunction by restoration of seminiferous tubule deformities and may be simulation of activity of spermatogenic cells. A possible mechanism for this might be that QIg extract markedly reduced blood glucose level, which abolishes the inhibitory effects of hyperglycemia on tight junction proteins (45) of blood-testis barrier (BTB) (29), and thereby BTB support Sertoli cells to regulate luminal environment of a seminiferous tubule by inhibiting the permeability of cytotoxic agents (46). Furthermore, it has been shown that TTF-1 is limited to the thyroid follicular epithelial cells and play a critical role in thyroid gland epithelial cells differentiation (48), and development of thyroid glands (48), thus controlling the mechanism of cellular differentiation in thyroid gland (49). Immunochemistry results of this study revealed overexpression of TTF-1 (sum score 12) in thyroid gland of diabetic group, compared to minimal expression in normal group, which potentially influences the rate of cellular proliferation and tumorigenic potential of thyrocytes (49). This could be caused by the deleterious effects of diabetes induced hyperglycemia that alters metabolic balance and significantly enhances cell cycle and cellular proliferation (50, 51). However, treatment of diabetic rats with different doses of QIg extract recovered the TTF1 expression in thyrocytes. This could be through the role of QIg extract in regulating the TTF-I expression. These results are consistence with (52). QIg extract could play a vital role in abolishing of cell proliferation, cell cycle arrest, and stimulation of apoptosis, and thereby autophagy through down regulation of intracellular signaling pathway in a wide range of human cancer cell lines (52).

The present work provided further insights into the use of QIg extract as potential promising future therapeutic agent against diabetic complications, through regulation of thyroid hormones secretion as well as testicular function accompanied with improvement in histopathological alterations of thyroid gland and testes in diabetic rats. Furthermore, QIg extract plays a significant role in regulating cell cycle and cellular proliferation, potentially through regulatory dependent mechanism of TTF-1 expression in thyrocytes. Future experiments would be of interest to assess the exact molecular regulatory mechanism of QIg extract on thyrocytes and testicular cells functions in diabetes.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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تأثير مستخلص ثمرة البلوط الصبغي *Quercus infectoria* على وظائف الغدة الدرقية والخصى في الجرذان المصابة بداء السكري

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الخلاصة

أعتبر داء السكري من الأمراض المصاحبة لحدوث خلل في وظائف الغدة الدرقية والخصى. تم إعداد هذه الدراسة على الفئران المصابة بمرض السكري لفحص تأثير مستخلص ثمرة البلوط الصبغي على كل من وظائف الغدة الدرقية والخصى. وقسمت بشكل عشوائي ستّة عشرة من الفئران على أربع مجاميع تتكون كل من، مجوعة سيطرة غير مصابة وثلاث مجاميع اخرى مصابة بداء السكري وكانت واحدة منها غير معالجة بينما المجموعتين الاخريين معالجة فمويا بجرع ٥٠٠ ملغم لكل كغم من وزن الجسم و١٠٠٠ ملغم لكل كغم من وزن الجسم من مستخلص نبات البلوط على التوالي ولمدة خمسة عشرة يوما. حيث تم تقيم مستويات كل من الكلوكوز في مصل الدم اضافة الى كل من الهرمونات المحفز للغدة الدرقي، ثلاثي اليودوثيرونين (الثايروكسين، التيستيسترون) والهورمون اللوتيني . وفي نهاية التجربة تم التضحية بالفئران بشكل رحيم وذلك من اجل اجراء التحليل التشريحي المرضي للغدة الدرقية والخصى. اضافة الى استخدام الكيمياء النسيجية المناعية لتقيم التعبير المناعي لعامل نسخ الغدة الدرقية الاول في الغدد الدرقية للفئران. وقد لوحظ انخفاض كبير في مستوى السكر في دم الفئران المصابة بداء السكري التي عولجت بجرعة ٥٠٠ ملغم/ كغم من وزن الجسم من بمستخلص نبات البلوط وعودته الى المستوى الطبيعي تقريبا. وبشكل ملحوظ عادت هورمونات الغدة الدرقية بعد خمسة عشرة يوما من العلاج بمستخلص نبات البلوط وبجرعة ٥٠٠ ملغم لكل كغم من وزن الجسم. في حين انخفض تركيز هرمون التستوستيرون في الدم بشكل كبير في كل مجاميع التجربة. حيث أظهر التحليل التشريحي المرضي للفئران المصابة بداء السكري مجموعة واسعة من التغيرات الشكلية في الغدة الدرقية وهياكل الخصية، والتي تم استعادتها بالكامل تقريبًا عن طريق علاج الفئران بمستخلص نبات البلوط. علاوة على ذلك أظهرت النتائج إفراطًا في التعبيرعن العامل نسخ الغدة الدرقية الاول في الفئران المصابة بداء السكري، والذي تم استعادته مرة أخرى إلى التعبير الطبيعي بعد خمسة عشرة يومًا من العلاج بالمستخلص النباتي. وفرت هذه النتائج رؤى جديدة حول دور مستخلص نبات البلوط كعامل علاجي واعد ضد مضاعفات مرض السكري في وظائف الغدة الدرقية والخصية.

الكلمات المفتاحية: مرض السكر، الغدة الدرقية، الخصية، عامل نسخ الغدة الدرقية-١، مستخلص ثمرة البلوط الصبغي، *Quercus infectoria*