Research Article

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Comparative Study Between *Quercus Infectoria* Galls Extract and Glimepiride On Pancreas and Some Blood Parameters in Diabetic Rats

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Abstract: Diabetes mellitus (DM) is a metabolic disease involving improperly high blood glucose level, hyperlipidemia and hypoinsulinemia. The aim of current study is to assess and compare the influence of *Quercus infectoria* galls (*QI*g) extract and glimepiride on pancreatic β-cells secretory function and serum lipid profile in diabetic rats. In the present study, thirty six male rats were used and divided into six groups (n=6), including negative control, STZ induced diabetic control, treated diabetic rats with glimepiride (200mg/Kg Bw), and treated diabetic rats with different concentrations of *QI*g extract, 200 mg, 400 mg and 800 mg/Kg Bw for 42 days, respectively. At the end of the experiment, all rats were fasted overnight, blood samples were collected for measuring fasting blood glucose, insulin hormone and lipid profile. All rats were then scarified by using chloroform in order to take part of the pancreatic tissue. The results showed a significant decrease in blood glucose, total cholesterol, triglycerides, low density lipoprotein, very low-density lipoprotein, HbA1c% with obvious elevation in serum insulin hormone and high-density lipoprotein in diabetic rats treated with *QI*g extract and glimepiride compared with diabetic untreated group. In addition, high atherogenic index in diabetic rats was significantly reduced by different concentrations of *QI*g extract and glimepiride. Histopathological results revealed that there was a significant and dose-dependent morphological restoration in the pancreatic structure damages especially at high dose of the extract. These findings provide a new insight into the role of *QI*g methanolic extract in maintaining normal blood glucose and adequate pancreatic insulin hormone secretion in diabetic rats, amelioration of dyslipidemia and associated cardiovascular alterations with subsequent improvement in pancreatic structural damages in diabetic rats.

Keywords: ***Quercus infectoria*** **galls, Insulin, HbA1c, atherogenic index, pancreas**

Introduction

Diabetes mellitus (DM) is a systemic metabolic disease characterized by abnormal elevation in blood glucose level beyond normal range (hyperglycemia) as well as hyperlipidemia and hypoinsulinemia. This disease is caused by decrease in both insulin secretion and insulin action (1, 2). Hyperglycemia is the predominant cause of diabetic complications (3). It is associated with alterations in glucose, lipid metabolism, modification in liver enzyme levels, development of vascular diseases such as neuropathy, nephropathy, cardiovascular and cerebrovascular disease (4). There are two most common types of diabetic mellitus including (i) type 1 diabetes (T1D) which is characterized by autoimmune destruction of pancreatic beta cells (β-cell), leading to a completely diminished production of insulin (5) , (ii) type 2 diabetes (T2D) which is associated with impaired response to insulin and β-cell dysfunction causing hyperglycemia attributable to disturbances in carbohydrate, and lipid metabolism (6). It has been described that pancreatic ß-cells and insulin secretion are affected in both major types of DM (7). Recently, medicinal plants are involved in the treatment of various diseases including diabetes. The antidiabetic effects of most medicinal plants are associated with the presence of active chemical ingredient including carotenoids, flavonoids, terpenoids, alkaloids and glycosides (8). Moreover, the anti-hyperglycemic effects of medicinal plants are often due to their ability to improve the performance of pancreatic tissue which is done by increasing insulin secretions or reducing the intestinal absorption of glucose (9). The hypoglycemic effect of some herbal extracts has been confirmed in human and animal models of diabetes. Many studies are underway to find new effective agents that can increase or preserve islet β-cell mass and function, providing a plant to lower the burden of morbidity from DM and its complications (10). Insulin and oral hypoglycemic agents are the most widely used drugs for lowering blood sugar in diabetic patients, but these drugs also have various side effects such as hypoglycemia, weight gain, lactic acidosis, and some organ damage (11). Most recent study has found that *QI*g extracts contain various chemical constituents such as tannins, flavonoids, saponins, anthraquinones, triterpenes, gallic acid, syringic acid, ellagic acid and submarines that play a vital role in regulating blood glucose levels (12). In addition, *QI* galls that grown in the Mediterranean area, especially in Greece, Syria, Iraq, and Asia Minor (13) possess a wide range of medicinal properties such as astringent, anti-inflammatory, antiviral, antidiabetic, antibacterial, antiulcerogenic and gastro-protective activities (14, 15). Furthermore, it has been found that glimepiride play a vital role as antidiabetic activity in regulating insulin hormone secretion, blood glucose level and some liver enzymes activities in experimental diabetic rat model (16).

However, little is known about the role of *QI*g extract in regulating pancreatic β-cell secretory function and improving hyperglycemia, dyslipidemia and protection against cardiovascular diseases (CVD) in diabetes. In addition, the information concerning to the influence of *QI*g extract and glimepiride on the histological changes in the pancreas of diabetic rats are rather limited and not clearly understood. Thus, our study aimed to assess and compare the influence of *QI*g extract and glimepiride on pancreatic β-cell secretory function, hyperglycemia, dyslipidemia and histological alteration in STZ-induced diabetic rats.

Material and Methods

Preparation of *QI* galls methanolic extract : During July of 2018, the plant had been collected in bulk from mountains around Sulaimani province and authenticated by Kurdistan Botanical Foundation as *Quercus infectoria* galls. Plant sample was then washed with distilled water to remove adhering dirt and dust. The samples were dried in the shade, away from direct sunlight exposure, under room temperature and humidity conditions. The dried aerial parts were ground into a fine powder using pistol, mortar and electrical blender. It was then stored in a closed airtight container at 4°C for further use. Fifty gram of the grinded aerial part of the fine powder was soaked in 600ml of solvent (80% methanol (w/v). The mixture was incubated in the shaking incubator at 200 rpm and 25°C for 48-72 h. Then, it was filtered using filter paper (110mm) several times to get rid of solid part completely. The filtered yellow solution was put in the rotary evaporator at 5 rpm and 35 degrees to remove the methanol. The aqueous residue was kept in dark container at $^{-}20°C$. The aqueous residue was later lyophilized by running freeze dryer for 48 hours to get the powder. For running of freeze drier machine, the solution was put in -80°C freezer and liquid nitrogen was used for transporting of frozen samples.

Determination of LD50 of *QI*g extract :

Determination of the 50% of lethal dose (LD50) of the in- vivo studied compound was detected in the rats according to the "up-and-down" procedure by Dixon, (1990). All animals were orally administered with different doses of the *QI*g extract after conducting series of test levels. With equal spacing between doses, a series of trails were carried out using this method: increased dose following a negative response and decreased dose following a positive response. Testing continued until chosen "nominal" sample size was reached. LD50 were determined after reading final result (response-dead (X) or non-response alive (O).The following equation was then applied LD50 = XF + Kd. The estimate of LD50 is XF + Kd, where (XF) is the final test level and (K) is the interval between dose levels, where (d) is the tabulated value (17). In the present experiment, 10 animals of white rats 12-15 weeks' old were used. Graded doses of extract to each animal in series of concentrations (1200, 1400, 1600, 1800, 2000 ,2200 , 2400,2200,2400,2200 mg /Kg Bw in 1 ml of normal saline) were administered and chosen with equal spacing (concentrations between doses were 200 mg / Kg Bw). Mortality was recorded after 24 h in such a way that each animal treated with one dose and after 24 h if the animal lives, it was recorded as O and then increased the dose. While, X recorded for the animal death and then decreased the dose according for the result of the animal the code which formed as being (OOOX) and according for Dixon value was get (see Table 1 in results). The LD50 was determined according to the formula employed by Dixon (1980).

LD50 =Xf+ Kd

LD50 = 2200 + 0.741) x 200

LD50 = 2348 mg / kg b.w

Experimental Design

Experiments were performed on thirty six male Wistar rats, weighing 200–285 g and aging 10-15 weeks. All animals were housed under safe laboratory conditions in a temperature-controlled room (22–24◦C) and kept on a 12 h light/dark cycle in animal laboratory house at college of Veterinary medicine/Veterinary teaching hospital-University of Sulaimani. All rats had access to food and water ad *libitium*. The animals were provided by the Experimental Animal Center of biology, University of Sulaimani, Kurdistan, Iraq. Experimental animals were randomly divided into six groups (n=6), including negative control (C): rats were given normal saline using oral gavage tube (group 1), diabetic control (DC) without treatment (group 2), treated diabetic rats with glimepiride (200mg/ Kg Bw, group 3), and treated diabetic rats with different concentrations of *Q. Infectoria* galls (*QI*g) extract, 200 mg, 400 mg and 800 mg/Kg Bw, group 4,5 and 6 respectively. The examination period was continuous for 42 days.

Induction of diabetes

Diabetes mellitus was induced by a single intraperitoneal (IP) injection of freshly prepared Streptozotocin (Sigma-Aldrich, Saint Louis, MO) at a dose of 55 mg/kg b.w. dissolved in normal saline (28). After 72 h of STZ injection, an overnight fast, blood was taken from the tail vein of the rats. Accu-Chek monitoring used for checking the changing in the blood glucose level, rats with blood glucose higher than 250 mg/dl were selected for the diabetic groups and involved in the examination. In the first day after 6h of STZ injection, rats were developed a hypoglycemia due to the insulin release from destroyed beta cells. Injection of STZ lead to intensive hypoglycemia and this may cause death to many animals. To avoid this, drinking water containing 10% dextrose were given to rats directly after I.P injection of STZ. To take care about rats, blood glucose was measured after 42 days of the experiment from tail puncture.

Blood collection and analysis

At the end of the treatment period, all rats were fasted overnight, weighed, blood samples were collected from caudal vena cava in non-heparinized blood tube for determination of insulin hormone level and glycated hemoglobin (HbA1c). Lipid profile tests (cholesterol, triglyceride, HDL, LDL and VLDL) were done using the standard kit and measured using an auto-analyzer in blood sample contained anticoagulant.

Atherogenic index of plasma, which is a mathematical relationship between TG and HDL-C, was used as an additional index to assess cardiovascular (CV) risk factors. Atherogenic index of plasma (AIP) and percentage protection against CVD were calculated by using the following formula (19).

Atherogenic index of plasma (AIP): log $\frac{Triglyceride}{HDL-C}$

Protection against CVD (%): $\frac{(AIP) of\ diabetic\ control - AIP\ of\ diabetic\ treated\ group}{(AIP) of\ diabetic\ control\ group}$

Histopathological Studies

Histopathological analysis was performed at 42^{nd} day of the experiment. Tissue sections were then taken from pancreas of rats. Pancreas was removed, washed by normal saline and fixed with neutral buffered formalin solution (10%). It was dehydrated and infiltrated by paraffin liquid and embedded in molten paraffin liquid at 60ºC. After cooling down at 20ºC, solidified paraffin blocks were cut into 3-5µm sections, put on the slides, deparaffinized in xylene for 35 min, rehydrated, washed in water, stained with hematoxylin and eosin (H&E) stain. Finally, the slides were examined and evaluated using microscope (20).

Statistical analysis

All the data were expressed as mean ± SEM (Standard Error). Statistical analysis was performed by SPSS version 24 one-way ANOVA followed by post hoc =Duncan multiple range test. Differences between groups were considered significant at $p<0.05$ level.

Results

LD50 of *QI*g extract

Results of the study demonstrated that the LD50 of QIg extract in the rat produced no death or signs of toxicity till the dose of 2000 mg/Kg Bw (Table 1).

Effects of *QI*g extract on blood glucose levels

In order to assess the role of the *QI*g extract in the regulation of the blood glucose level, diabetic rats were treated with different concentrations of *QI*g extract. Results showed that induced diabetic rats revealed a marked elevation of blood glucose level ($p<0.05$) following 42 days of experiment compared with negative control rats. Interestingly, treatment of diabetic rats with different concentration of *QI*g extract showed a significant decrease in blood glucose level, in a dose dependent manner compared with diabetic control rats, with 800 mg/kg of *QI*g extract produced almost the same result as synthetic anti-diabetic drug (Glimepiride 200 mg/kg) by 42 days of treatment (Table 2).

Table 1: Result of LD50 given *QI*g extract by oral route in rat according to the Up and down method.

Treatment	Animal used	Dose Range	Difference between doses	Results after 24 hours	LD50 Mg/ Kg Bw
QI galls extract	10	1200-2400 mg/kg Bw	200	Ooooxoxo	2348 mg/ Kg Bw

Table 2: Effect of different concentrations of *QI*g extract and glimepiride on blood glucose level of diabetic rats. (Mean±SE), n=6.

Groups	Fasting Blood Glucose level (mg/dl)
Control negative	110.60 ± 5.11 [a]
STZ-induced Diabetic group	569.40 ± 20.47 [b]
Diabetic group+Glimepiride (200 mg/ Kg Bw)	398.22 ± 21.64 [c]
Diabetic group+200 mg/Kg Bw of *QI*g extract	516.12 ± 38.85 [b]
Diabetic group+400 mg/ Kg Bw of *QI*g extract	470.20± 43.95 [c]
Diabetic group+800 mg/ Kg Bw of *QI*g extract	461.41± 16.00 [c]

Different superscript letters denote significant difference within the column at $p < 0.05$.

Effect of *QI*g extract on serum insulin and HbA1c

The results showed that there was a significant decrease ($P<0.05$) in serum insulin hormone and a significant increase in serum HbA1c% in non-treated diabetic rats compared with control group. However, treated diabetic group with *QI*g extract revealed a significant increase in serum insulin hormone level and significant decrease in serum HbA1c%, in a dose dependent manner, compared with diabetic group, especially with high dose (800 mg/kg b.w) of *QI*g extract that produced same effect of glimepiride to restore the insulin hormone level and serum HbA1c% back to normal level compared to negative control (Table 3).

Table 3: Effect of *QI*g extract on the serum insulin level and HbA1c of all groups after 42 days of experiment. (Mean±SE), n=6.

Groups	Insulin (MU/ml)	HbA1c (%)
Control negative	0.20±0 [b]	3.40±0 [a]
STZ-induced Diabetic group	0.10±0 [a]	7.94±0.33 [c]
Diabetic group+Glimepiride (200 mg/ Kg Bw)	0.20±0 [b]	3.40±0 [a]
Diabetic group+200 mg/ Kg Bw of *QI*g extract	0.16±0.02 [b]	6.64±0.24 [b]
Diabetic group+400 mg/ Kg Bw of *QI*g extract	0.18±0.02 [b]	6.52±0.23 [b]
Diabetic group+800 mg/ Kg Bw of *QI*g extract	0.20±0 [b]	3.40±0 [a]

Different superscript letters denote significant difference within column at $p < 0.05$.

Effect of *QI*g extract on lipid profiles

As illustrated in table (4), results showed that STZ-induced diabetes caused a significant increase ($p<0.05$) in serum levels of cholesterol, triglyceride, LDL and VLDL with significant decrease ($p<0.05$) in serum level of HDL in diabetic control group compared with negative control group. While the animal treated on *QI*g extract revealed a significant decrease in serum level of cholesterol, triglyceride, LDL, VLDL and marked elevation ($p<0.05$) of serum level of HDL in treated diabetic group compared with non-treated diabetic group almost similar to the effect of synthetic antidiabetic drug glimepiride.

Table 4: Effect of different concentrations of *QI*g extract and glimepiride on the lipid profile in diabetic rats. (Mean±SE), n=6.

Groups	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Control negative	57.80 ± 70.17^{a}	99.61 ± 7.90^{a}	43.41 ± 1.70^{c}	9.83 ± 0.58^{a}	19.92 ± 1.58^{a}
STZ-induced Diabetic group	87.41 ± 21.37^{b}	253.60 ± 4.24^{c}	23.40 ± 1.00^{a}	17.81 ± 0.37^{c}	50.72 ± 2.41^{c}
Diabetic group+Glimepiride (200 mg/ Kg Bw)	56.40 ± 10.00^{a}	110.60 ± 24.64^{a}	31.00 ± 4.90^{ab}	12.40 ± 1.69^{ab}	21.72 ± 4.90^{a}
Diabetic group+200 mg/ Kg Bw of *QI*g extract	61.81 ± 5.70^{a}	220.80 ± 25.29^{b}	28.63 ± 1.51^{ab}	15.23 ± 0.66^{bc}	44.16 ± 5.00^{bc}
Diabetic group+400 mg/ Kg Bw of *QI*g extract	54.23 ± 2.20^{a}	198.83 ± 5.402^{b}	30.59 ± 0.55^{ab}	14.80 ± 1.15^{bc}	39.76 ± 4.68^{bc}
Diabetic group+800 mg/ Kg Bw of *QI*g extract	58.00 ± 2.51^{a}	177.00 ± 23.73^{b}	30.62 ± 1.15^{ab}	14.24 ± 1.46^{bc}	35.40 ± 4.74^{b}

Different superscript letters denote significant difference within column at $p < 0.05$.

Effect of *QI*g extract on atherogenic index

The current study revealed that atherogenic index (AIP) of diabetic group were significantly increased (p<0.05) compared with negative control group. Nonetheless, treatment of diabetic rats with glimepiride caused a significant decline (P<0.05) in the AI back to the normal level compared with negative control group. Similarly, in the other treatments, different concentrations of *QI*g extract caused a significant decrease (P<0.05) in the AI in a dose dependent manner after 42 days of treatment compared with diabetic non-treated rats. In addition, glimepiride produced high and significant protection against cardiovascular diseases in diabetic rats compared with different concentrations of *QI*g extracts. There was no significant difference ($P>0.05$) among different concentration of *QI*g extract in protection of diabetic rats against CVD (Table 5).

Table 5: Effect of different concentrations of *QI*g extract and glimepiride on atherogenic index in diabetic rats. (Mean ± SE), n= 6.

Groups	Atherogenic index	Protection against CVD (%)
Control negative	0.35 ± 0.04^{a}	Not treated
STZ-induced Diabetic group	1.03 ± 0.03^{c}	Not treated
Diabetic group+Glimepiride (200 mg/ Kg Bw)	0.35 ± 0.56^{a}	65.76 ± 5.52^{b}
Diabetic group+200 mg/ Kg Bw of *QI*g extract	0.86 ± 0.06^{b}	16.52 ± 5.84^{a}
Diabetic group+400 mg/ Kg Bw of *QI*g extract	0.77 ± 0.48^{b}	24.8 ± 4.73^{a}
Diabetic group+800 mg/ Kg Bw of *QI*g extract	0.74 ± 0.69^{b}	27.55 ± 6.61^{a}

Different superscript letters denote significant difference within column at $p < 0.05$.

Histopathological findings of pancreas

The histological examination for pancreas, at the end of experiment, in negative control group showed normal pancreatic histoarchitecture with uniformly arranged pancreatic acini and normal islet cells (Figure 1). Nevertheless, histological sections from rat pancreas in diabetic group showed extensive fatty infiltration, distributed diffusely within the pancreatic acinar cells together with degeneration and necrosis of islet cells compared to negative control group (Figure 2). Both the 800mg/Kg Bw of *QI*g extract and glimepiride produced same effect in regeneration of pancreatic lesions both in the acinar epithelium and Islets of Langerhans compared with control and diabetic groups (Table 6). Moreover, there was no fatty infiltration of pancreatic acini with obvious increasing and regeneration of

Islets of Langerhans in diabetic rats treated with glimepiride compared with diabetic groups (Figure 3). Histopathological examinations of pancreas in rats treated with 200mg/Kg Bw *QI*g extract showed fatty infiltration of pancreatic acinar cells with moderate islets cells, pancreatic acini epithelium degeneration and necrosis (Figure 4). Histopathological examinations of pancreas in rats treated with 400mg/Kg Bw *QI*g extract showed moderate vacuolar degeneration in pancreatic acinar epithelium together with mild necrosis of Islets of Langerhans (Figure 5). Interestingly, there was significant morphological regeneration in the pancreatic islets of Langerhans's evident by typically arranged cellular content together with complete improvement in the morphology of pancreatic acini in diabetic rats treated with 800mg/Kg Bw *QI*g extract for 42 days compared with diabetic group (Figure 6).

Table 6: Scoring of pancreatic lesions in the different experimental groups. Scoring for the pancreatic lesions were classified as (-) no change, (+) mild change, (++) moderate change, and (+++) severe change according to (21), in different experimental groups.

lesions	Control negative	STZ-induced Diabetic group	Diabetic group+Glimepiride (200 mg/kg)	*Diabetic group+200 mg/kg of QIg extract*	*Diabetic group+400 mg/kg of QIg extract*	*Diabetic group+800 mg/kg of QIg extract*
Acinar epithelium						
Fatty infiltration	-	+++	-	+++	++	-
Vacuolar swelling	-	++	+	++	++	+
Necrosis	-	+++	-	++	+	+
Islets of Langerhans						
Cellular degeneration	-	+++	++	++	++	++
Necrosis	-	+++	+	++	+	+

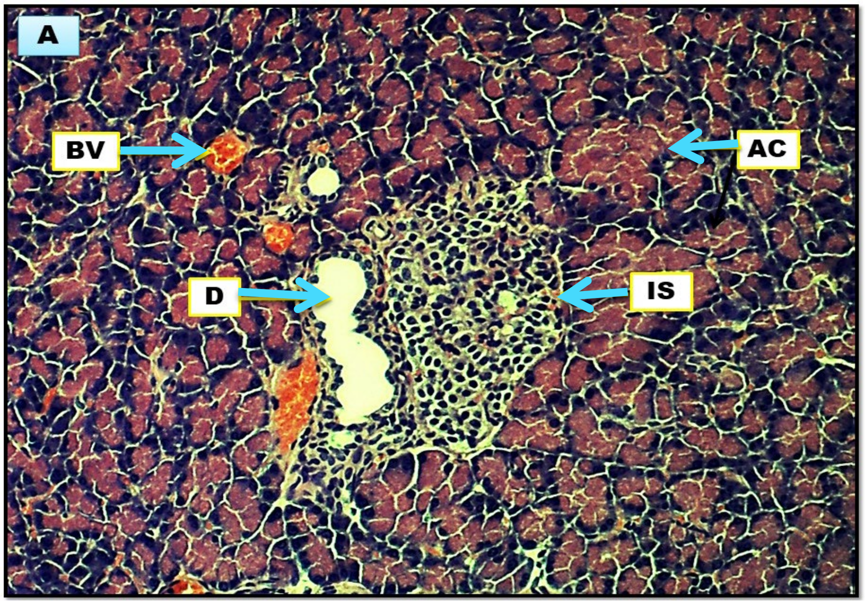

Figure 1: Photomicrograph of Pancreas from negative control group (A), showing normal morphological appearance of pancreatic acini (AC), along with typical organization of Islet of Langerhans (IS), evident by standard cellular arrangement. Congestion of blood vessels (BV) together with presence of exocrine ductal (D) in the given section. (H&E stain 100X).

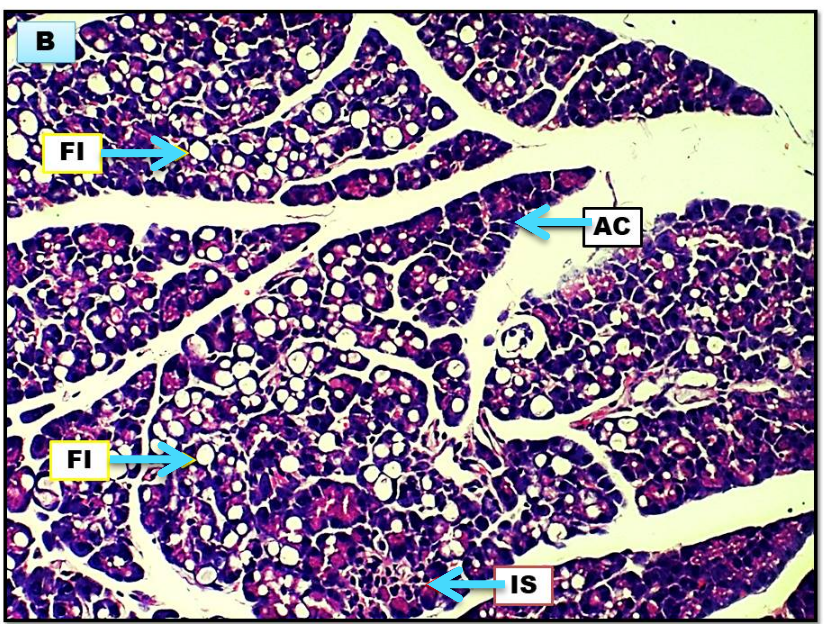

Figure 2: Photomicrograph of Pancreas from diabetic group, showing extensive fatty infiltration (FI), distributed diffusely within the pancreatic acinar cells (AC) of pancreatic lobules. In addition, the section reveals significant atrophy, vacuolation and necrosis of an Islet of Langerhans associated with pyknotic nuclei (IS) (H&E stain 100X).

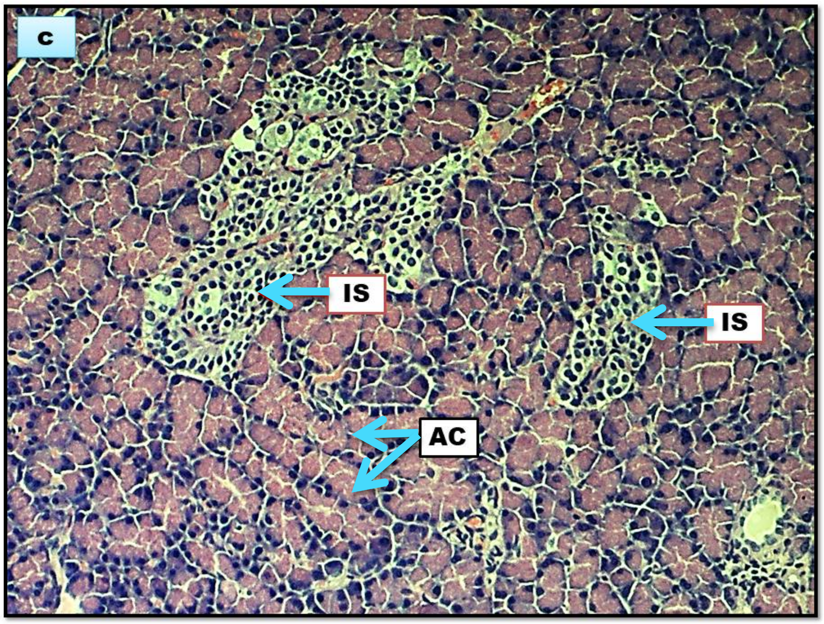

Figure 3: Photomicrograph of pancreas in diabetic treated group with Glimepiride 200mg/kg revealing significant morphological improvement of the pancreatic Islets of Langerhans (IS) evident by typically arranged cellular content together with complete improvement in the morphology of pancreatic acini (AC). (H&E stain 100X).

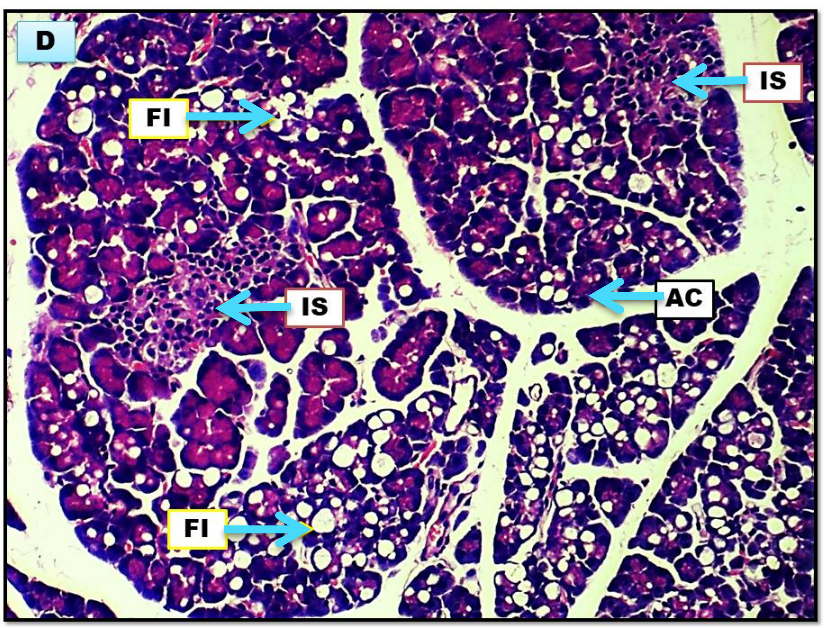

Figure 4: Photomicrograph of pancreas from treated group, with 200mg/kg (*QI*g) extract, showing moderate fatty infiltration (FI), distributed throughout the pancreatic acini (AC) in the given section. As well as, the section discloses pancreatic Islets (IS) manifested by increase cellular content with moderate cellular degeneration. (H&E stain100X).

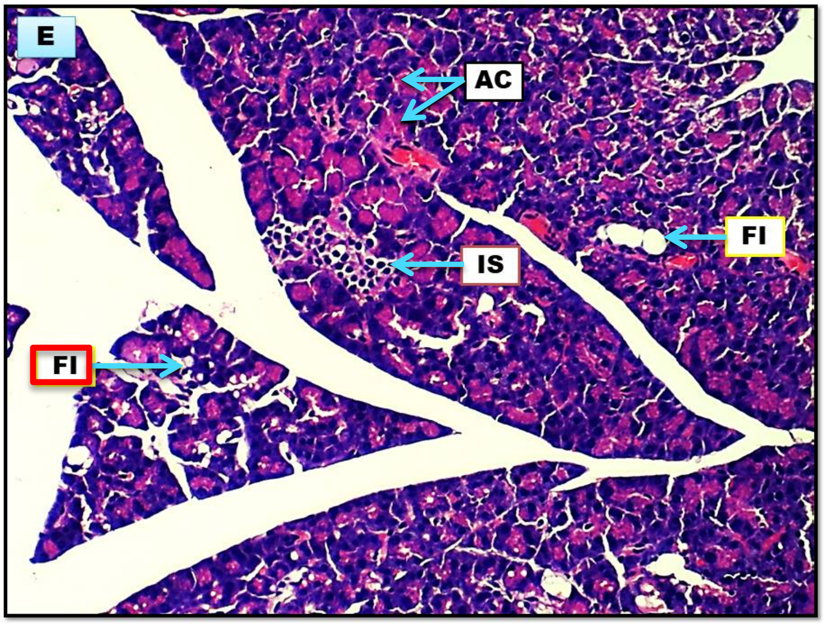

Figure 5: Photomicrograph of Pancreas from treated group, with 400mg/kg *QI*g extract revealing significant reduction of fatty infiltration (FI), evident morphologically by almost normal appearing pancreatic acini (AC). The section shows pancreatic islets of Langerhans (IS) with some degenerative changes. (H&E stain 100X).

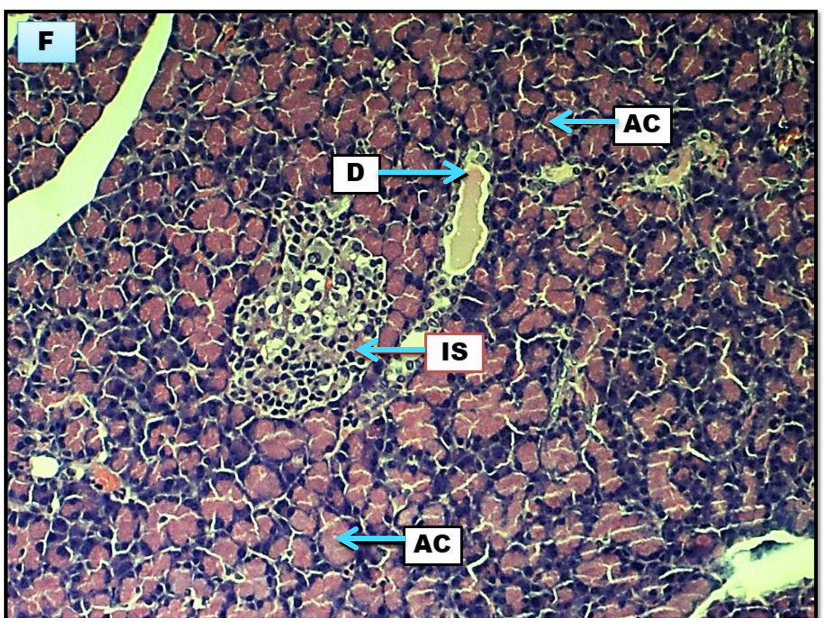

Figure 6: Photomicrograph of pancreas from treated group, with 800mg/kg *QI*g extract, demonstrates significant regenerative changes in the morphology of pancreatic acini (AC) together with the pancreatic islets of Langerhans (IS). The slide showing section through an exocrine ductal system (D). (H&E stain 100x).

Discussion

The LD50 test of *QI*g extract in the rat showed no death or signs of toxicity till the dose of 2000 mg/kg, suggesting that the *QI*g extract was well tolerated and the experimental doses were quite safe (22). It has been demonstrated that excess blood glucose level reacts non-enzymatically with hemoglobin to form glycosylated hemoglobin (HbA1C) during diabetes. As a result, the rate of glycosylation is proportional to the concentration of blood glucose (23). The value of glycosylated hemoglobin (HbA1c) has been used as an index of estimation average blood glucose and long-term glycemic status for monitoring the blood glucose control in diabetic patients (24). The increased glycated hemoglobin is associated with loss of β-cell function and has been implicated in the complications of diabetes mellitus (25). The results of the current study demonstrated that, similar to glimepiride, administration of different concentration of *QI*g extract for 42 days were effectively reduced the diabetic induced hyperglycemia and high value of HbA1c%, potentially through enhancing pancreatic β-cell secretory function. Consistence with this, it has been shown that antidiabetic activity of glimepiride is via maintaining pancreatic β-cell secretory function in experimentally induced diabetic rats (16). In addition, the flavonoids and saponins contents in *QI*g extract play a crucial role in maintaining normal blood glucose level through stimulation of pancreatic β-cells to secrete insulin hormone or abolishing intestinal glucose absorption (26, 27). On the other hand, the hypoglycemic effect of *QI*g extract might be due to alkaloid and saponin contents of *QI*g extract on stimulating glucose transporter (GLUT4) expression on the plasma membrane and thus enhancing cellular glucose uptake via insulin dependent mechanism (28).

Interestingly, treating diabetic rats with different concentration of the *QI*g extract for 42 days revealed an insulinotropic effect of the extracts by producing significant increase in serum insulin hormone level, in a dose dependent manner, comparing to non-treated diabetic rats, most obviously with 800mg/kg Bw *QI*g extract that mimicked the effect of glimepiride in restoration of insulin secretion back to normal level compared with normal group. One possible explanation might be via effects of *QI*g extract on inhibiting HMG-CoA reductase activity, potentially through flavonoid dependent mechanism (29), since reduced insulin hormone secretion in diabetes might be associated with the increased activity of HMG-CoA reductase to produce TG and blood cholesterol (30). This finding is in agreement with previous study that inadequate insulin hormone secretion in diabetes is associated with increased synthesis of cholesterol via increased hydroxyl-methyl-glutaryl-CoA (HMG-CoA) reductase activity, which is responsible for cholesterol biosynthesis (29). Furthermore, STZ-induced diabetes caused a significant increase in serum cholesterol, triglycerides, LDL, VLDL with marked decrease in serum HDL consistent with previous finding that showed hyperglycemia in diabetic condition is usually accompanied by an increase in plasma level of cholesterol, triglycerides, LDL, VLDL and decreases in HDL potentially through mobilization and releasing of free fatty acid from peripheral tissue by activation of hormone-sensitive lipase during insulin deficiency (31). Conversely, results of the present study showed that application of various concentrations of *QI*g extract for 42 days resulted in a significant improvement in serum lipid profile via dose dependent manner comparing to diabetic non-treated group. One potential explanation for this might be due to the influence of active ingredients of *QI*g extract, including flavonoids, saponin, tannin, and alkaloids since these active ingredients play an important role in regulating blood cholesterol level in diabetic condition (26, 32) especially saponins through hypolipidemic effects via suppression of luminal cholesterol absorption and stimulation of biliary cholesterol excretion (33). In addition, increment of serum HDL level in treated diabetic rats by various *QI*g extract is crucial for improvement of the anti-atherogenic capability of the therapy as HDL-cholesterol plays a vital role in transporting of cholesterol from peripheral tissues to the liver for metabolism and employs part of its anti-atherogenic activity through neutralizing LDL oxidation (34). Interestingly, treatment of diabetic rats with different concentrations of the *QI*g extract showed a dose depend decrease in atherogenic index and marked increase in the percentage of protection against cardiovascular disease, which was more obvious with the high dose (800mg/Kg Bw) of the *QI*g extract, potentially via its active constituents. Consistent with this, previous study has been found that extracts of those medicinal plants containing phytoconstituents such as alkaloids, flavonoids, saponins, tannins, can markedly reduce the atherogenic indexes in diabetic rats (35). This potentially suggests that *QI*g extract plays an essential role in preventing atherogenesis in diabetic condition and thus might potentially works as cardioprotective via reducing the relative risk of coronary heart diseases (CHD) encouraged by diabetes mellitus (36). Furthermore, there were a wide range of histological abnormalities of pancreas in diabetic group exhibited by extensive fatty infiltration distributed diffusely within the pancreatic acinar cells compared with normal control group, potentially through complication of diabetic induced hyperglycemia, which enhances various metabolic changes in cell signaling pathways leading to inflammation, pro-inflammatory cytokine production, ROS generation, oxidative stress, and subsequently cell apoptosis (37). The present study revealed that STZ-induced diabetes produced destroying of pancreatic β-cells, even at a single dose of 55 mg/kg of body weight, with sever distributed fatty infiltration, potentially through disturbance in fatty acid composition of pancreatic islets (38). Consistence with this, previous study has demonstrated that single dose of STZ injection in rats may produce pancreatic β-cell destruction and reduction of insulin hormone secretion (39). Treatment of diabetic rats with different concentration of *QI*g extract caused an obvious restorating effects on the morphological alteration of pancreatic tissue and β-cells regeneration, potentially through the flavonoid content and antioxidant activity of the of the *QI*g extract to alleviate the diabetic-induced oxidative stress on pancreatic tissue cells. This is consistent with previous results who demonstrated that the majority of the natural bioactive compounds, including flavonoids, and its antioxidant activity are responsible for pancreatic β-cell regeneration and enhancement of β-cell function as well as with the most recent study that revealed the potent antioxidant activity of *QI*g extract (40). Likewise, glimepiride caused a significant morphological regeneration of the pancreatic islets of Langerhans and pancreatic acini, potentially via stimulating insulin secretory pathways through activation of cell signaling pathway-dependent mechanism that play a crucial role in restoration of pancreatic β-cells structures and function (41) since the surviving cells of pancreas, stable (Quiescent) cells are able to proliferate and regenerate to replace the destructive pancreatic β-cells (42).

Conclusion

The current study revealed that various concentrations of *QI*g extract exert antihyperglycemic and insulinotropic effect in a dose dependent manner similar to that of antidiabetic agent, glimepiride 200mg/kg along with restorations of histological morphology of pancreatic tissue back to normal in diabetic rats. In addition, different concentrations of *QI*g extract play an important role in reducing atherogenic index and increasing of protection against cardiovascular diseases through marked improvement in blood lipid profile alterations.

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Conflict of Interest

The authors report no conflicts of interest.

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دراسة مقارنة بين مستخلص ثمرة البلوط الصبغي *Quercus Infectoria* ودواء جليميبيريد Glimepiride على البنكرياس وبعض مؤشرات الدم في الجرذان المصابة بداء السكري

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الخلاصة

داء السكري هو مرض استقلابي يشمل ارتفاع مستوى السكر في الدم بشكل مفرط ويشمل ايضا فرط في شحميات الدم ونقص الأنسولين. الهدف من الدراسة الحالية هو تقييم ومقارنة تأثير مستخلص ثمرة البلوط ودواء جليمبيريد على الوظيفة الإفرازية لخلايا بيتا البنكرياسية ومؤشر الدهون في دم الفئران المصابة بداء السكري.تم في هذه الدراسة استخدام ستة وثلاثين من ذكور الجرذان حيث قسمت إلى ست مجموعات (ن = ٦) ،شملت مجموعة الكونترول السالبة الغير المعالجة ,مجموعة داء السكري المستحدث **(**STZ) ومجموعة الجرذان المصابة بالسكري والمعالجة بتراكيز مختلفة من مستخلص الجليميبيريد (٢٠٠مجم / كجم من وزن الجسم) ومستخلص ثمرة البلوط الصبغي بتراكيز ٢٠٠ ملجم,٤٠٠ملجم و٨٠٠ ملجم /لكل كغم من وزن الجسم ولمدة ٤٢ يومًا على التوالي.وفي نهاية التجربة تم جمع عينات الدم من كل الفئران الصائمة طوال الليل وذلك لقياس مستوى كلوكوز الدم والانسولين ومؤشر الدهون , . ثم تم خدش جميع الفئران باستخدام الكلوروفورم من أجل الحصول على جزء من أنسجة البنكرياس. حيث أظهرت النتائج انخفاضًا ملحوظًا في جلوكوز الدم ، والكوليسترول الكلي ، والدهون الثلاثية ، والبروتين الدهني منخفض الكثافة ، HbA1c% للغاية، و مع ارتفاع واضح في هرمون الأنسولين في الدم والبروتين الدهني عالي الكثافة في الفئران المصابة بداء السكري والمعالجة بمستخلص *Ql*g والجليمبيريد مقارنة بالمجموعة المصابة بداء السكري والمجموعة غير معالجة. بالإضافة إلى ذلك ، لوحظ انخفاض كبير لمؤشر تصلب الشرايين في الفئران المصابة بداء السكري والمعالجة بتركيزات مختلفة من مستخلص *Ql*g و glimepiride.حيث أظهرت المقاطع النسيجية أن هناك ترميما شكليا واضحا اعتمد على جرعات المستخلص ساعدت في أعادة ترميم بنية البنكرياس خاصة عند الجرعات العالية من هذا المستخلص. توفر هذه النتائج نظرة فطنة وجديدة لدور مستخلص *Ql*g الميثانولي في الحفاظ على مستوى الجلوكوز الطبيعي في الدم وإفراز هرمون الأنسولين الكافي في بنكرياس الفئران المصابة بداء السكري ، وتحسين شحوم الدم والتغيرات القلبية الوعائية المرتبطة بها ,مع التحسن اللاحق في البنية الهيكلية المتضررة للبنكرياس في الفئران المصابة بداء السكري.

الكلمات الأساسية: مستخلص ثمرة البلوط الصبغي (*Ql*g) ، الأنسولين ، HbA1c ، مؤشر تصلب الشرايين ، البنكرياس.