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Research Article
Antidiabetic Efficacy of Methanolic Crude Extract of *Quercus dilatata* Fruit: A Randomized Control Trial

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Abstract

Background and Objective: *Quercus dilatata* is traditionally used for the treatment of various human diseases. The present study was conducted for the determination of *in vivo* anti-diabetic activity and diabetes complications preventive effect of the *Quercus dilatata*. **Methodology:** The methanol extract of *Quercus dilatata* (200 and 400 mg kg^{-1}; orally) was administered in alloxan induced (150 mg kg^{-1}; intraperitoneally) diabetic rats. Glibenclamide (10 mg kg^{-1} day^{-1} orally) was used as a standard drug. Diabetic rats boosted the levels of blood glucose, serum lipids (triglycerides, cholesterol, low density lipoprotein and serum high density lipoprotein levels), liver enzymes (serum alanine transaminase (ALT), alkaline phosphatase (ALP) and total bilirubin), serum urea, creatinine and total protein. **Results:** After 21 days of treatment, the plant extract at a dose of 200 and 400 mg kg^{-1} day^{-1} significantly reduced the elevated blood glucose level in diabetic rats by 114.975 and 111.520%, respectively. Extracts of *Quercus dilatata* (both doses) proved to have anti-diabetic activity by decreasing the elevated level of plasma blood glucose, with subsequent increase in body weight. Extracts of *Quercus dilatata* (both doses) also proved to have lipids, hepatic and renal protective activity by reducing the elevated level of enzymes and other biochemical markers. Extract at a dose of 200 mg kg^{-1} revealed highly significant ($p<0.05$)results as compared to 400 mg kg^{-1} dose. **Conclusion:** On the basis of the results it is concluded that *Quercus dilatata* extract can be used to treat diabetes, kidney and liver disorders and serum lipids related abnormality.

Key words: *Quercus dilatata*, diabetes, liver enzymes, serum lipids, urea creatinine

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Modern diabetic medicines offer a variety of effective treatment options but can also have several side effects[1]. Pharmacological and clinical trials of medicinal plants have shown anti-diabetic effects and repair of β-cells of islets of Langerhans[2]. A wide and diverse range of plants have been reported in the literature to prevent and treat diabetes. The plant extracts have several phytochemicals including flavonoids, terpenoids, saponins, tannin, cardiac glycoside, coumarins, anthraquinone and phlobatannin, these affect various metabolic cascades, which directly or indirectly affect the level of glucose in the human body[3]. These have produced potent hypoglycemic, anti-hyperglycemic and glucose suppressive activities. The effects achieved by either increase in serum insulin level or increase in the production of insulin from pancreatic β-cells, inhibit glucose absorption in the gut, stimulate glycogenesis in liver or increase glucose utilization by the body[4-6]. These compounds also exhibit their antioxidant, hypolipidemic, anti-cataract activities, restored enzymatic functions, repair and regeneration of pancreatic islets and alleviation of liver and renal damage[7]. The aim of present pharmacological study was the screening of the *Quercus dilatata* plant extract by using alloxan induced diabetic rats.

MATERIALS AND METHODS

Extraction: The authenticated plant of *Quercus dilatata* was collected from vicinity of District Bannu, KPK (Pakistan). A specimen voucher was submitted in the herbarium of Botany Department (MS-12). Plant material was shade dried at room temperature (32°C) and the dried plant material was grounded into a moderately coarse powder using domestic electric grinder. The powdered plant material was soaked in 70% methanol for 10 days. The suspension was then filtered to obtain the extract. The extract was then evaporated using the rotary evaporator at 40°C until it was completely dried. A solid methanol extract of *Quercus dilatata* was obtained and stored for further investigations.

Animals: Healthy albino rats of either sex were selected for the study. The study was carried in accordance with the rules and regulations laid by the Institutional Animal Ethics Committee (IAEC number 2314). The animals were housed under standard laboratory conditions of light and dark cycles at room temperature of 25°C with free access to food and water. The basal body weights to the nearest gram were noted. Rats were starved 12 h prior to the study. The extracts of *Quercus dilatata* leaves were evaluated in five groups of 5 animals each.

Acute toxicity test: Healthy albino rats of either sex were taken as 3 groups of 6 rats each. Animals were fasted overnight but there was free access to water prior to experiment. One group was taken as normal group and other two as experimental groups. The extract of *Quercus dilatata* at dose levels of 1 and 2 g kg^{-1} b. wt., was administered once to each experimental group. The volume of administered dose was 1 mL. The rats were observed for 24 h no mortality was recorded[8].

Alloxan induced albino rat model for diabetes: Diabetes was induced by administering alloxan (150 mg kg^{-1} b. wt., intraperitoneally) in normal saline in a volume of 1 mL kg^{-1}. After two days of alloxan injection, animals showing the fasting blood glucose level more than 200 mg dL^{-1} were considered as diabetic[9-10]. After that diabetic induced animals were used for experimental purposes.

Experimental groups: Healthy rats were randomly allotted into 5 groups of five animal (n = 5) each.

Group 1: Served as normal control and received water

Group 2: Served as diabetic control and received water

Group 3: Diabetic animals treated orally with glibenclamide at a dose of 10 mg kg^{-1} day^{-1}

Group 4: Diabetic animals treated orally with extract of *Quercus dilatata* at a dose of 200 mg kg^{-1} day^{-1}

Group 5: Diabetic animals treated orally with extract of *Quercus dilatata* at a dose of 400 mg kg^{-1} day^{-1}

The drug treatment was carried out every day at morning with the help of 16 gauge ball tipped feeding tube[11]. Blood sugar was determined at 0, 7th, 14th and 21st day of drug treatment. On the same day body weight was determined. After 21 days of drug treatment serum lipids, kidney profile, liver function tests, total bilirubin and total proteins were evaluated. Blood samples were withdrawn by end tail vein cutting method from animals and blood glucose level was determined by using one touch electronic glucometer ACU check[12].

RESULTS

Acute toxicity test: Acute toxicity studies revealed that the extract of *Quercus dilatata* was safe up to 2000 mg kg^{-1} of b. w.t. and approximate LD_{50} is more than 2000 mg kg^{-1}. Any toxic reactions or lethality or moribund state was observed to the end of the study period. This is not surprising as *Quercus dilatata* is used as a vegetable locally and as a medicine traditionally.

Effect of *Quercus dilatata* extract on physiological parameters: The body weight changes showed that there is significant increase in the body weight of the extract treated groups in comparison to the diabetic group over the period of 21 days. The decrease in the body weight was shown by diabetic group but the extract treated groups showed significant ($p<0.05$) increase in the body weight as shown in Table 1.

Effect of *Quercus dilatata* extract on blood glucose levels of normoglycemic rats: The effect of extract on blood glucose levels of normoglycemic rats revealed the *Quercus dilatata* extract (200 mg kg^{-1}) showed significant ($p<0.01$) decrease in the blood glucose levels of non-diabetic rats as shown in Table 2; thus showing the hypoglycemic nature of the extract of this plant.

Effect of *Quercus dilatata* extract on glucose levels: As shown in Table 2, the induction of diabetes has caused initial increase in the blood glucose levels of all the diabetic groups. The diabetic control group showed increase throughout the study period in comparison with normal control group. However, the extract treated groups showed significant ($p<0.05$) decrease in the blood glucose levels as compared with the diabetic control. The effect was more pronounced in *Quercus dilatata* (200 mg kg^{-1}) group 4 followed by *Quercus dilatata* (400 mg kg^{-1}) group 5 and standard (10 mg kg^{-1}) group, with the percentage variations as shown in Table 2.

Effect of *Quercus dilatata* extract against alloxan hepatotoxicity in rat liver: Effect of alloxan in experimental animals were not limited to the pancreas but induces injuries in other organs as well. The results obtained with alloxan induced hepatic injuries and recovery effects of *Quercus dilatata* and glibenclamide are given as follow in Table 3.

Effect of *Quercus dilatata* extract on liver function test in rat: Levels of ALP and ALT and total bilirubin in serum are highly susceptible to toxic chemicals and oxidative stress, used as biochemical markers to evaluate the hepatic injury. The preventive effects of *Quercus dilatata* on the activity of liver serum marker enzymes are shown in Table 3. Alloxan treatment significantly increased the activity of liver serum marker enzymes which were attenuated significantly ($p<0.05$) by orally administration of extract of *Quercus dilatata* near to control group dose dependently. Similarly 10 mg kg^{-1} b. wt., of glibenclamide treatment significantly ($p<0.001$) erased the effect of alloxan intoxication and serum level of these enzymes returned to control group.

Table 1: Effect of *Quercus dilatata* extract on body weight of diabetic rat

	Body weight (g)				
Groups	0th day	7th day	14th day	21st day	Variation (%)
Normal	222.5±6.5	223.5±0.565	224±0.353	224±0.353	0.50
Diabetic control	210±4.7	205.0±3.53	205±3.53	200±7.07	16.66
Glibenclamide (10 mg kg^{-1})	224±4.8	225.0±0.7**	227±2.12**	230±4.24**	7.00
Quercus dilatata (200 mg kg^{-1})	193±5.0	195.0±1.41**	200±4.94**	215±15.53**	98.91
Quercus dilatata (400 mg kg^{-1})	219±4.5	236.5±12.37**	250±21.92**	261±29.19**	108.00

Data is represented as Mean±SD, **Indicate significance at $p<0.05$ (Dunnett's-test), Normal was compared with the diabetic control, diabetic control were compared with the standard and extract treated groups

Table 2: Effect of *Quercus dilatata* extract on blood glucose levels of diabetic rat

	Blood glucose level (mg dL^{-1})			
Groups	0th day	7th day	14th day	21st day
Normal	91.0	92±0.7	94±2.12	94.5±2.47
Diabetic control	270.5	271±4.5	277±4.9	279.0±6.01
Glibenclamide (10 mg kg^{-1})	495.0	175±6.2**	171±2.1**	96.0±2.1**
Quercus dilatata (200 mg kg^{-1})	380.5	232±5.0**	116±1.2**	109.0±9.9**
Quercus dilatata (400 mg kg^{-1})	269.0	219±3.3**	105±1.6**	98.0±10.9**

Data is represented as Mean±SD, **Indicate significance at $p<0.05$ (Dunnett's-test), Normal was compared with the diabetic control, diabetic control were compared with the standard and extract treated groups

Table 3: Effects of extract of *Quercus dilatata* on liver function test

Treatments	ALT (μL)	Total bilirubin (mg dL^{-1})	ALP (μL^{-1})
Control	141.3±3.1	1.0±0.02	235.5±6.0
Diabetic control	242.5±5.5	1.4±0.01	198.9±5.3
Glibenclamide (10 mg kg^{-1})	219.7±3.3	0.7±0.1	274.1±3.7
Quercus dilatata (200 mg kg^{-1})	219.1±7.6	0.7±0.02	167.6±5.0
Quercus dilatata (400 mg kg^{-1})	135.6±6.7**	0.6±0.01**	246.3±4.1**

Data is represented as Mean±SD, **Indicate significance at p<0.05 (Dunnett's-test), Normal was compared with the diabetic control, diabetic control were compared with the standard and extract treated groups, ALT: Alamin transaminase, ALP: Alkaline phosphates

Table 4: Effect of *Quercus dilatata* extract on serum level of triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol, VLDL cholesterol and CPK in rat

Groups	TG (mg dL^{-1})	Cholesterol (mg dL^{-1})	HDL (mg dL^{-1})	LDL (mg dL^{-1})	VLDL (mg dL^{-1})	CPK (mg dL^{-1})
Control	92.3±6.2	84.3±5.1	40.4±3.2	26.3±0.1	18.4±3.1	170.1±5.1
Diabetic	168.5±4.7	93.6±3.8	24.1±6.0	36.5±3.5	33.6±2.6	449.4±5.0
Glibenclamide (10 mg kg^{-1})	110.2±5.0**	79.1±4.7**	30.6±7.2**	27.8±2.6**	22.2±1.7**	254.2±7.3**
Quercus dilatata (200 mg kg^{-1})	91.7±3.8**	82.5±5.6**	40.7±5.5**	27.3±1.6**	22.7±2.3**	240.7±3.4**
Quercus dilatata (400 mg kg^{-1})	116.2±4.8**	89.8±3.2**	56.3±3.3**	20.9±2.1**	23.1±1.1**	238.3±4.1**

Data is represented as Mean±SD, **Indicate significance at p<0.05 (Dunnett's-test), Normal was compared with the diabetic control, diabetic control were compared with the standard and extract treated groups, Tg: Triglycerides, HDL: High density lipoprotein, LDL: Low density lipoprotein, CPK: Creatine phosphokinase, VLDL: Very low density Lipoprotein

Table 5: Effect of *Quercus dilatata* extract on serum urea and creatinine

Treatments	Urea (mg dL^{-1})	Creatinine (mg dL^{-1})
Control	40.2±5.7	0.6±0.1
Diabetic control	58.5±5.0	1.9±0.3
Glibenclamide (10 mg kg^{-1})	35.3±3.9**	0.6±0.1**
Quercus dilatata (200 mg kg^{-1})	47.8±4.7**	0.6±0.2**
Quercus dilatata (400 mg kg^{-1})	43.6±6.1**	0.5±0.09**

Data is represented of Mean±SD values, **Indicate significance at p<0.05 (Dunnett's-test), Normal was compared with the diabetic control, diabetic control were compared with the standard and extract treated groups

Effect of *Quercus dilatata* extract on serum total cholesterol, triglyceride, LDL-cholesterol and HDL-cholesterol: Free radicals especially hepatotoxin reacts with polyunsaturated fatty acids which causes lipid peroxidation and effect lipid profile. Serum level of total cholesterol, triglyceride LDL-cholesterol and HDL-cholesterol of the present study are summarized in Table 4. The diabetic rats showed elevated level of lipid parameters like total cholesterol, triglyceride LDL-cholesterol and HDL-cholesterol. These parameters were significantly restored by extract (200 and 400 mg mL^{-1} b. wt.) near to control group. More significant (p<0.05) protective observations were found by treatment with the higher dose (400 mg mL^{-1} b.wt.) of the extract of *Quercus dilatata*.

Effect of *Quercus dilatata* extract on serum urea and creatinine in rats: Protective effect of *Quercus dilatata* extract versus diabetic rat, changes in serum urea and creatinine as well as in non-treated control rats are shown in Table 5. Diabetic rats showed increased serum level of kidney profile; serum urea, creatinine and decreased level of serum total protein as compared to the control group. Serum level of urea and creatinine were significantly (p<0.05) returned towards the normal level by post treatment of *Quercus dilatata* extract (200 and 400 mg mL^{-1} b.wt.) as compared to control group. Extract (both doses) significantly reversed the serum level of above parameters. Significant (p<0.05) returned were also recorded by extract treatment of non-diabetic group.

DISCUSSION

Despite the fact that diabetes has high prevalence, morbidity and mortality globally, it is regarded as non-curable but controllable disease[13]. Many natural active compounds have been isolated from plants of different species. These active principles are complex carbohydrates, alkaloids, flavonoids, saponins, amino acids, steroids, peptides, terpenoids and others. These compounds have been shown to produce potent hypoglycemic, anti-hyperglycemic and glucose suppressive activities[4]. These compounds may also exhibit antioxidant, hypolipidemic and anticataract activities and restore enzymatic functions, repair and regeneration of pancreatic islets and the alleviation of liver and renal damage[7]. Methanol extract of *Quercus dilatata* (200 and 400 mg kg^{-1} b.wt.) showed significant (p<0.05) effect on the serum glucose levels of rats (Table 2). The anti-diabetic effect of the plant in diabetic rats was studied during 21 days treatment. The difference observed between the initial and final fasting serum glucose levels of extract treated diabetic rats revealed anti-diabetic effect of *Quercus dilatata* throughout the period of study. The effect of the extracts was compared to that of reference standard.

An increase in the ALT and ALP activities was recorded in diabetic rats in comparison with non-diabetic rats, indicating an altered liver function in diabetic condition (Table 3). In diabetic animals a change in the serum enzymes is directly related to changes in the metabolism in which these enzymes are involved. The increased levels of transaminases which are active in the absence of insulin because of increased availability of amino acids in diabetes[14-15] are responsible for the increased gluconeogenesis and ketogenesis observed in diabetes. In the present study, the *Quercus dilatata* extract significantly ($p<0.05$) decreased total bilirubin, ALT and ALP enzyme activities in alloxan induced diabetic rats (Table 3). Hence, the improvements noticed in the level of these enzymes were as a consequence of an improvement in the carbohydrate, fat and protein metabolism. The restoration of bilirubin and ALT levels after treatment also indicated a revival of insulin secretion. Elevation of ALP has been reported in diabetic rats. This increase in ALP was significantly ($p<0.05$) reversed by the extract of *Quercus dilatata.*

The status of kidney function may be provided by analysis of blood[16]. During normal condition the serum level of urea and creatinine remains at normal unless there is pathogenesis. The high levels of urea and creatinine indicate the kidney injuries induced through chemical treatment[17-18]. Diabetic group showed significantly increased in blood urea and creatinine showing renal injuries[19]. Protein high level in urine showed the nephrotoxicity. The data of this study showed that plant extract of *Quercus dilatata* significantly ($p<0.05$) restored urea and creatinine and increased the level of proteins in blood (Table 5).

Moreover, increased glucose level in diabetic rats was associated with a high serum concentration of total cholesterol and triglycerides as present in the normal diabetic conditions[20]. However, extracts of *Quercus dilatata* at a dose level of 400 and 200 mg kg^{-1} reversed the diabetes induced hyperlipidemia compared to the diabetic control group (Table 4). In extract treated rats, there was a reduction in the levels of total cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol showing the hypolipidemic effect of *Quercus dilatata* plant. The hypolipidemic effect may be due to inhibition of fatty acid synthesis. In normal metabolism insulin activates the enzyme lipoprotein lipase and hydrolysis triglycerides and the deficiency in insulin results in inactivation of these enzymes thereby causing hypertriglyceridemia[21]. The significant reduction of serum lipid levels in diabetic rats after treatment with extract of *Quercus dilatata* may be directly attributed to improvements in insulin level. Extract of *Quercus dilatata* appear to be attractive material for further studies leading to possible drug development for diabetes.

CONCLUSION

It is concluded extract of *Quercus dilatata* can be used to treat diabetes and to control abnormal levels of lipids, renal biochemical and liver enzymes which can cause many diseases.

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