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Protective Effect of Galactomannan Extracted from Iraqi *Lycium Barbarum* L. Fruits Against Alloxan-Induced Diabetes in Rats

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Abstract: *Lycium barbarum* L. (*Solanaceae*) is widely used in Iraqi Ayurvedic medicine for the treatment of diabetes mellitus. The present investigation was done to evaluate the effects of polysaccharide (galactomannan) from *Lycium barbarum* L. Fruits (GLBF) on serum blood glucose, serum lipid profile and lipid peroxidation and antioxidant defence system in liver and kidney of alloxan-induced diabetic rats. GLBF was found to be non-toxic at 1000 mg kg^{-1}, as no deaths or hazardous signs were recorded during treatment or the observation period (24 and 72 h) in either control or treated groups of mice. In GLBF (500 mg kg^{-1}), the onset was 4 h, the peak effect was 6 h but the effect waned at 24 h. In the chronic study, repeated administration (once a day for 21 days) of the glibenclamide and GLBF caused a significant reduction in the serum glucose level as compared to the diabetic control group. GLBF (500 mg kg^{-1}) treatment prevented a decrease in the body weight of the diabetic rats. Moreover, the results revealed that GLBF (500 mg kg^{-1}) treatment for 21 days significantly ($p<0.01$) reduced the levels of lipid profile, lipid peroxidation, improving kidney and liver functions, enhanced insulin level and increased the levels of enzymatic and non-enzymatic antioxidants. The findings in this study suggest that polysaccharide (galactomannan) from *Lycium barbarum* L. Fruits (GLBF) possess good pharmacological activities, which might be helpful in controls the blood glucose level, improves body weight, lipid metabolism and prevents diabetic complications associated with lipid peroxidation and also maintains the antioxidant enzymatic and non-enzymatic in experimental diabetic rats. The extract seems promising for the development of a phytomedicine for diabetes mellitus.

Keywords: *Lycium Barbarum*, Hypoglycemic, Hypolipidemic, Antioxidant, Alloxan, Galactomannan

Introduction

Diabetes mellitus, an endocrine disorder is associated with depleted insulin secretions with altered carbohydrate, lipid and protein metabolism. Complications such as renal failure, coronary artery, cerebral-vascular disease, neurological complications, blindness, limb amputation, long term damage, dysfunctions, failure of various organs and eventually premature death are associated with chronic hyperglycemia (Lanjhiyana *et al*., 2011). There is a reservoir of basic information that suggests the involvement of oxidative stress in the pathogenesis of diabetes mellitus. It is now recognized that sustained hyperglycemia in diabetic patient, causes protein glycation and generates free radicals through auto-oxidation and polyol pathways (Ramakrishna and Jailkhani, 2008; Sharma *et al*., 2003). High levels of free radicals with concurrent decline of antioxidant defense mechanism may lead to damage of cellular organelles and enzymes (Ottaviano *et al*., 2008). This can culminate in increased lipid peroxidation and development of insulin resistance, which may consequently promote the development of complications of diabetes mellitus (Demozay *et al*., 2008). The use of oral antidiabetics is limited due to their adverse side effects including hematological, cutaneous and gastrointestinal reactions, hypoglycaemic coma and disturbances of liver and

kidney functions. In addition, they are not suitable for use during pregnancy (Alarcon-Aguilara *et al*., 1998). Medicinal plants continue to provide valuable therapeutic agents, in both modern medicine and in traditional system. The doubts about the efficacy and safety of the oral hypoglycemic agents have prompted a search for safer and more effective drugs in the treatment of diabetes (Reaven *et al*., 1983). In spite of the fact that insulin has become one of the most important therapeutic agents known to medicine, researchers have been making efforts to find insulin substitutes from synthetic or plant sources for the treatment of diabetes. Many herbs have remained as an alternative to conventional therapy especially in poor areas where insulin is not readily available (Sanchez *et al*., 1994). Since time immemorial, patients with non-insulin requiring diabetes have been treated orally in folk medicine with a variety of plant extracts. In Iraq, a number of plants are mentioned in ancient literature (Ayurveda) for the cure of diabetic conditions. *Lycium barbarum* L. (*Solanaceae*) is widely distributed in Iraq. This plant is well known in Arabic and English system as 'Ausaj, Osaj, Sarim' and 'Box Thorn, Matrimony-Vine', respectively (Chakravarty, 1976). Information gathered from some herbalists (in Basra governorate, Iraq) that the plant is useful to reduce blood sugar, blood cholesterol, rheumatism, heart and liver ailments, infant convulsions and also to aid digestion and to preventing and treating cancer, hepatitis, hypo-immunity function. In this study, the effect of oral dietary supplementation of GLBF in the prevention and/or treatment of alloxan induced diabetes in rat model was investigated. The study also investigated the potential role of antioxidant activity of this material in protecting from diabetes. The findings from this study may add to the overall value of the medicinal potential of the plant.

Material and Methods

Plant Materials and Chemicals/Reagents

Lycium barbarum L. fruits, used in this study, were collected on April 2013 from Abu-Al-Khaseeb region (Southern of Basrah), which is the well-known production area of *Lycium barbarum* L. in Basrah/Iraq. The plant was botanically authenticated and voucher specimens 3943 were deposited in the Herbarium of Basrah (Iraq, Basrah, College of Science, University of Basrah). All the chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO), Riedal de Haën (Germany), Fluka (Switzerland), Randox (United Kingdom) and solvents were from E. Merck (Darmstadt, Germany). All of the reagents were prepared in deionized distilled water to eliminate the contamination of metal ions.

Experimental Animals

Albino rat (150-220 g) of both sex were used for the present study. Healthy albino mice of either sex (20-30 g) were used for study sub-acute (short term) toxicity of polysaccharides (galactomannan) extract (GLBF). The animals, with no prior drug treatment, were housed in polypropylene cages (five in cage) under a 12 h light/12h dark cycle in a controlled temperature room (25±2°C). All the animals were acclimatized to the laboratory conditions for a week before use. They had free access to food and water. All studies were carried out by using five groups of six animals (3 males and 3 females).

Extraction of Galactomannan

Extraction of the water-soluble polysaccharides (galactomannan) from fruits of *Lycium barbarum* L. was done using the procedure of (Kooiman, 1971), with minor modification. Fruits were dried at 60°C and ground to fine powder. The ground powder samples were refluxed to remove lipids with chloroform: methanol solvent (2:1) (v/v). After filtering, the residues (100 g) were air-dried, suspended in 500 mL distilled water for 3 d at 0°C and were homogenized using a blender. The viscous mass was further stirred using a magnetic stirrer overnight at room temperature and centrifuged at 9000×g at 0°C. The clear supernatant liquid was separated from the residue and an equal amount of 95% ethanol was added to the supernatant liquid with continuous stirring. The resulting white precipitate was allowed to settle and separated by decantation. The precipitate was washed with ethanol and freeze dried. To purify the gum, the dried gum was redissolved in distilled water with continuous stirring until completely dissolved. Fehling's solution was added to the gum solution resulting in the formation of light blue precipitate. The precipitate was separated by decantation, washed and suspended with distilled water and 2 M HCl solution. The resulting mixture was stirred and an equal amount of 95% ethanol was added to regenerate the gum. The galactomannan extract was washed again with ethanol and freeze dried. The dried *L. Barbarum* Polysaccharides (LBP) obtained were stored in a refrigerator till further use.

Toxicity Evaluation in Mice

To study sub-acute (short term) toxicity, four groups of 9 weeks Swiss albino mice of both sexes (8) ranging in weight from 4-45 g were used and were orally fed with GLBF in increasing dose levels of 100, 500,1000 mg kg^{-1} body weight. The animals were observed after 30 min and 2 h. The following profiles were observed (Turner, 1965). Behavioural profile: Alertness, restlessness, irritability and fearfulness. Neurological profile: Spontaneous activities, reactivity, touch response, pain response and gait. Autonomic profile: Defecation and urination.

Induction of Diabetes Mellitus

The animals were fasted for 12 h with free access to water prior to the induction of diabetes which was carried out by single intraperitoneal injection of alloxan

monohydrate dissolved in sterile cold normal saline at a dose of 150 mg kg^{-1} body weight (El-Demerdash *et al*., 2005). Since alloxan is capable of producing fatal hypoglycemia as a result of massive pancreatic release of insulin, the rats were treated with 20% glucose solution intraperitoneally after 6 h. They were kept for the next 24 h on 5% glucose solution bottles in their cages to prevent hypoglycemia (Dhanapani *et al*., 2002). Four days after alloxan injection, the blood samples were freshly withdrawn from tail-tips of the experimental rats by the retro orbital plexus technique using capillary glass tubes. Serum was separated in an electric centrifuge at 3000×g for 10 min and glucose levels was measured using the glucose oxidase peroxidase (GOD-GPD) method (Trinder, 1969) using a commercial kit (Span Diagnostics, Stuart India) and only those rats with fasting blood serum glucose greater than 200 mg dL^{-1} will be included in the study (Dhanapani *et al*., 2002).

Experimental Design

Overnight fasted rats were divided into five groups (n = 6) as follows:

Group I: Served as normal healthy control received only normal saline, 5 mL kg^{-1} body weight (p.o)
Group II: Served as diabetic control received only normal saline, 5 mL kg^{-1} body weight (p.o)
Group III: Diabetic rats served as standard received 10 mg kg^{-1} body weight of Glibenclamide (p.o)
Group IV: Diabetic rats received 250 mg kg^{-1} body weight of GLBF (p.o)
Group V: Diabetic rats received 500 mg kg^{-1} body weight of GLBF (p.o)

Effect of GLBF on Serum Glucose Level in Normal and Diabetic Rats (Acute Study)

The procedure described by (Badole *et al*., 2006) was adopted for this study. Animals were fasted for 12 h but were allowed access to water. Initial blood samples were obtained for determination of basal glucose levels from tail-tips using capillary tubes. Blood samples were then taken at 2, 4, 6 and 24 h after administration of control, standard or different doses of GLBF.

Effect of GLBF on Serum Level in Normal and Diabetic Rats (Chronic Study)

A published method by (Singh *et al*., 2010) was adopted, with slight modification. This study was involved administration of control, standard or different doses of GLBF for a period of 21 days. Serum glucose levels were estimated on days 0, 7, 14 and 21. Mean changes in serum glucose were calculated.

Effect of GLBF on Body Weight (g) in Normal and Diabetic Rats

This test was assessed according to the method described by (Badole *et al*., 2006), with minor modification. During the study period of 21 days the rats were weighed daily and their body weights were recorded. From this data, mean changes in body weight and SEM were calculated and tabulated.

Biochemical Investigations

At the end of the experiment, all the rats were sacrificed by decapitation under light ether anesthesia after fasting for 12 h. Blood samples were collected directly from heart and serum was separated by centrifugation for 5 min and was kept at -20°C for the biochemical assay of creatinine, urea, Alkaline Phosphatase (ALP), Total Cholesterol (TC), Triglycerides (TG) and High Density Lipoprotein (HDL) and total proteins levels (Kumar *et al*., 2010). The LDL-cholesterol level was calculated by using the formula: LDLc= Total cholesterol-(HDLc + (Triglyceride/5)), where (Triglyceride/5) = VLDL-cholesterol (Freidewald 1972). Serum alanine transaminase (ALT) and Aspartate Transaminase (AST) were measured by using NADH oxidase reaction (Neely *et al*., 1985). Serum insulin levels were determined using insulin ELISA kit (Kratzsch *et al*., 1990). The organs namely liver and kidney were excised, immediately washed with cold saline. The tissues were weighed and 10% tissue homogenate was prepared with 0.025m Tris-Hcl buffer, pH 7.5. After centrifugation at 10,000×g for 10 min, the resulting supernatant was used for the estimation of enzymatic and non-enzymatic antioxidants such as Superoxide Dismutase (SOD), (Sajeeth *et al*., 2011) catalase (Lee *et al*., 1999), Glutathione Peroxidase (GPx) (Garfinkel *et al*., 1992) and glutathione (GSH) (Saneviratane *et al*., 2009). Lipid peroxidation was estimated by thiobarbituric acid reaction in both liver and kidney (Ohkawa *et al*., 1979).

Statistical Analysis

The data were expressed as mean values ± SEM and tested with analysis of variance followed by Dunnett's t-test. P-values <0.05, 0.01 were considered to be statistically significant.

Results

Toxicity Evaluation in Mice

No death or hazardous signs were recorded during treatment or the observation period (24 and 72 h) in either control or treated groups of mice with GLBF (100, 500 and 1000 mg kg^{-1}). Therefore, 500 mg kg^{-1} was chosen as the maximum dose for further experimentation.

Effect of GLBF on Serum Glucose Level in Normal and Diabetic Rats (Acute Study)

Table 1 showed that the blood glucose levels were significantly decreased at time 2, 4 and 6 h after

administrated of a single dose of GLBF (250 and 500 mg kg^{-1}) with glibenclamide (10 mg kg^{-1}). The reduction in serum glucose from basal value (before) at 6 h after administration of glibenclamide and GLBF (250 and 500 mg kg^{-1}) were 180.27, 122.53 and 171.33 mg dL^{-1}, respectively. The onset of the antihyperglyceim effect of glibenclamide and GLBF (500 mg kg^{-1}) were at 2 h and that of the GLBF (250 mg kg^{-1}) was at 4 h; the peak effect was at 6 h but the effect waned at 24 h. The significant reduction in serum glucose from basal value (before) at 24 h was 100.74 mg dL^{-1}.

Effect of GLBF on Serum Glucose Level in Normal and Diabetic Rats (Chronic Study)

The perusal of Table 2 concerning antidiabetic activity in alloxan induced diabetic rats showed that the test extract (GLBF) in both dose levels, reduces the blood glucose significantly ($p<0.01$) starting from 7 day to the end of 21 day of the study in a dose dependent manner, while the standard drug, glibenclamide showed similar effect during the course of the experiment. However the blood glucose level at the end of 21 day was 149.42 and 126.46 mg dL^{-1} of the groups treated with the doses of GLBF 250 and 500 mg kg^{-1} respectively, while standard drug showed 117.34 mg dL^{-1} at the same time. On the other hand, same Table 2 reflects that reducing insulin level in diabetic rats was also significantly improved by treatment of GLBF.

Effect of GLBF on Body Weight (g) in Normal and Diabetic Rats

In the body weight measurement, normal vehicle control animals were found to be gained in their body weight but diabetic rats showed a significant reduction in the body weight, which reversed significantly ($p<0.05$) by GLBF treated groups (250 and 500 mg kg^{-1}) and glibenclamide (10 mg kg^{-1}) treated group during 21 days treatment, as shown in Table 3.

Effect of GLBF on Lipid Profile in Normal and Diabetic Rats

In diabetic rats the administration of GLBF exhibited a very highly significant hypolipidemic effect ($p<0.01$) as shown in Table 4. When compared to control (healthy rats), serum Total Cholesterol (TC), Triglycerides (TG), LDL and VLDL levels were increased and HDL-c decreased clearly in diabetic rats. The standard drugs as well as GLBF (250 and 500 mg kg^{-1}) plant extracts used in the experimental study significantly decreased ($p<0.01$) the levels of serum cholesterol, triglycerides, LDL levels and serum VLDL, whereas HDL cholesterol level was improved in both standard and test drug, after 21 days treatment.

Effect of the GLBF on Serum Biochemical Parameters in Normal and Diabetic Rats

The effect of GLBF on liver and kidney functions are represented in the Table 5. The levels of liver function marker enzymes ALT, AST and ALP were significantly elevated in alloxan-induced diabetes. The rats treated with GLBF (250 and 500 mg kg^{-1}) showed significant ($p<0.01$) reduction in the elevated levels of these enzymes in a dose dependent manner. On the other hand, kidney function markers like creatinine and urea were elevated in the alloxan-induced diabetic rats when compared with the normal rats. GLBF reduced both the levels in dose dependent manner. Also, the lowered level of total protein in diabetic rats was significantly ($p<0.01$) elevated, after 21 days treatment (Table 5).

Effect of the GLBF on Lipid Peroxidation and Antioxidants Activities in Liver and Kidney of Normal and Diabetic Rats

Table 6 and 7 shows the activities of the antioxidants, enzymatic CAT, SOD, GPx and non-enzymatic GSH and lipid peroxidation in liver and kidney of normal and diabetic rats. The above mentioned antioxidants showed a significant decrease ($p<0.01$), but the lipid peroxidation level was significantly increased ($p<0.01$), in both liver and kidney of diabetic rats when compared with normal rats. The standard drugs as well as GLBF (250 and 500 mg kg^{-1}) treated rats proved a significant increase ($p<0.01$) in the levels of CAT, SOD, GPx and GSH when compared with diabetic rats, whereas lipid peroxidation showed a significant decrease.

Table 1. Effect of GLBF on serum glucose level in normal and diabetic rats (acute study)

		Blood glucose levels (mg/dL)				
Groups	Dose (mg/kg)	0 h	2 h	4 h	6 h	24 h
Group I	Healthy control	113.71±3.41	112.28±2.34	113.29±4.31	115.42±4.70	114.14±3.26
Group II	Diabetic control	317.37±5.32	322. 12±2.23	325.83±4.51	329.85±2.43	332.56±2.18
Group III	Standard	314.52±3.61	247.31±4.84*	196.79±4.16**	134.25±3.43**	203.32±3.75*
Group IV	250	313.85±1.14	271.23±3.51	227.42±2.05*	191.32±1.35**	235.51±2.45*
Group V	500	314.64±4.15	254.14±2.36*	203.37±4.21**	143.31±2.42**	214.35±3.47*

N = 6, values are mean ±SEM, *$p<0.05$, **$p<0.01$, as compared to control group

Table 2. Effect of GLBF on serum level in normal and diabetic rats (chronic study)

		Blood glucose levels (mg/dL)				
Groups	Dose (mg/kg)	Day 0	Day 7	Day 14	Day 21	Serum insulin (U/dL)
Group I	Healthy control	114.37±5.32	114.46±3.26	113.31±4.25	112.91±3.31	4.28±1.07
Group II	Diabetic control	314.24±2.37	351.47±2.86	393.31±4.53	426.12±3.12	1.19±0.09
Group III	Standard	315.32±2.47	214.37±2.91	146.42±2.51**	117.34±3.12**	3.29±1.76**
Group IV	250	319.69±2.73	250.41±2.63	196.57±3.14*	149.42±2.34*	2.18±1.22*
Group V	500	311.73±3.41	231.27±1.93	159.46±4.63**	126.46±4.21**	2.79±1.98**

N = 6, values are mean ±SEM, *p<0.05, ** p<0.01, as compared to control group

Table 3. Effect of GLBF on body weight (g) in normal and diabetic rats

		Body weight (g)			
Groups	Dose (mg/kg)	Day 0	Day 7	Day 14	Day 21
Group I	Healthy control	206.31±2.43	212.34±2.61	215.47±3.07	219.26±2.52
Group II	Diabetic control	209.24±1.72	204.17±1.87	196.23±1.39	190.19±1.87
Group III	Standard	206.71±2.64	207.31±2.03*	209.34±1.94*	211.41±1.61**
Group IV	250	208.32±1.09	206.43±1.74*	207.13±2.07*	208.76±1.64*
Group V	500	207.42±1.46	206.93±1.14*	208.17±1.93*	209.37±1.73*

N = 6, values are mean ±SEM, *p<0.05, **p<0.01, as compared to control group

Table 4. Effect of the GLBF on lipid profile in normal and diabetic rats

		Serum lipid profile levels (mg/dL)				
Groups	Dose (mg/kg)	Total cholesterol	HDL-Cholesterol	Triglycerides	LDL	VLDL
Group I	Healthy control	99.64±3.51	34.29±3.12	87.45±3.23	47.6±3.44	17.39±1.47
Group II	Diabetic control	177.56±2.41	23.15±2.09	172.39±2.41	119.34±2.07	34.37±2.13
Group III	Standard	104.51±3.34**	40.34±3.13**	92.17±2.93**	45.41±3.08**	18.23±2.14**
Group IV	250	128.51±1.24*	29.27±1.71*	122.24±1.43*	74.55±1.13*	24.34±1.26*
Group V	500	112.69±2.52**	34.21±1.97**	102.63±1.36**	56.16±2.45**	20.53±1.66**

N = 6, values are mean ±SEM, *p<0.05, **p<0.01, as compared to control group

Table 5. Effect of the GLBF on serum biochemical parameters in normal and diabetic rats

		Serum biochemical parameters					
Groups	Dose (mg/kg)	Total protein (g/dL)	Urea (g/dL)	Creatinine (g/dl)	AST (U/L)	ALT (U/L)	ALP (U/L)
Group I	Healthy control	6.43±1.12	32.47±2.49	0.83±0.07	45.31±1.25	52.94±2.31	135.21±1.56
Group II	Diabetic control	4.39±1.67	57.21±2.82	1.18±0.06	107.12±1.34	105.41±2.11	196.78±1.83
Group III	Standard	6.37±1.13**	35.18±2.28**	0.86±0.03**	48.09±1.71**	52.54±2.34**	136.35±1.26**
Group IV	250	5.28±0.98*	44.76±2.54*	1.09±0.07*	75.11±1.21*	64.23±1.97*	155.69±1.73*
Group V	500	6.23±1.24**	36.53±1.48**	0.91±0.04**	52.07±1.45**	53.03±2.08**	139.94±1.09**

N = 6, values are mean ±SEM, *p<0.05, **p<0.01, as compared to control group

Table 6. Effect of the GLBF on lipid peroxidation and antioxidants activities in liver of normal and diabetic rats

Groups	Dose (mg/kg)	LPO (nm MDA formed/ mg tissue)	CAT (µm H_2O_2 consumed/min /mg protein)	SOD (U /mg protein)	GPx (µg GSH utilized/min /mg protein)	GSH (µg GSH consued/min /mg protein)	LPO (nm MDA formed /mg tissue)
Group I	Healthy control	31.86±1.28	0.924±0.04	1.376±0.02	0.574±0.04	0.935±0.05	31.86±1.28
Group II	Diabetic control	64.37±1.42	0.513±0.03	0.922±0.04	0.353±0.07	0.585±0.03	64.37±1.42
Group III	Standard	31.06±1.74**	0.914±0.02**	1.311±0.03**	0.534±0.08**	0.959±0.02**	31.06±1.74**
Group IV	250	47.63±1.88*	0.747±0.05*	1.162±0.04*	0.417±0.03*	0.626±0.05*	47.63±1.88*
Group V	500	35.27±1.96**	0.829±0.03**	1.252±0.05**	0.468±0.04**	0.862±0.07**	35.27±1.96**

N = 6, values are mean ±SEM, *p<0.05, **p<0.01, as compared to control group

Table 7. Effect of the GLBF on lipid peroxidation and antioxidants activities in kidney of normal and diabetic rats

Groups	Dose (mg/kg)	LPO (nm MDA formed/ mg tissue)	CAT (µm H_2O_2 consumed/min /mg protein)	SOD (U /mg protein)	GPx (µg GSH utilized/min /mg protein)	GSH (µg GSH consued/min /mg protein)	LPO (nm MDA formed /mg tissue)
Group I	Healthy control	26.41±0.15	0.968±0.07	0.766±0.08	0.768±0.05	0.536±0.03	26.41±0.15
Group II	Diabetic control	48.35±0.31	0.643±0.06	0.442±0.04	0.450±0.06	0.346±0.09	48.35±0.31
Group III	Standard	26.32±0.27**	0.915±0.04**	0.759±0.03**	0.759±0.09**	0.510±0.06**	26.32±0.27**
Group IV	250	34.09±0.41*	0.723±0.03*	0.534±0.02*	0.567±0.04*	0.423±0.02*	34.09±0.41*
Group V	500	29.98±0.35**	0.819±0.02**	0.757±0.03**	0.669±0.08**	0.487±0.05**	29.98±0.35**

N = 6, values are mean ±SEM, *p<0.05, **p<0.01, as compared to control group

Discussion

As is the case with other diseases, medicinal plants have been used since ancient times to treat and manage diabetes mellitus in traditional medical systems of many cultures throughout the world (Sathya Meonah *et al*., 2012). The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts like the Vedas and Bible have been traced to the occurrence of natural products with medicinal properties (Ajay Kumar *et al*., 2010). Currently, medicinal plants continue to play an important role in the management of diabetes mellitus, especially in development countries, where many people don't have access to conventional antidiabetic therapies (Blade *et al*., 2006). The currently available drug regimens for management of diabetes mellitus have certain drawbacks and therefore there is a need to find safer and more effective antidiabetic drugs (Duraipanadiyan *et al*., 2006). Alloxan causes diabetes through its ability to destroy the insulin-producing beta cells of the pancreas (Sharma and Kumar, 2011). *In vitro* studies have shown that alloxan is selectively toxic to pancreatic beta cells, leading to the induction of cell necrosis (Jorns *et al*., 1997). The cytotoxic action of alloxan is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration, leading to a rapid destruction of beta cells (Szkudelski, 2001). According to earlier studies, plant extracts causes antihyperglycemic effect by promoting regeneration of β-cells or by protecting these cells from destruction, by restricting glucose load as well as by promoting unrestricted endogenous insulin action. Antihyperglycemic effect may also be caused by the effect of plant extract on β-cells to release insulin or activate the insulin receptors to absorb the blood sugar and stimulate the peripheral glucose consumption (Jadhav *et al*., 2009). Present study was undertaken to evaluate the hypoglycemic activity of GLBF in normal and alloxan induced-diabetic rats. The results of both acute and chronic study showed that alloxan-induced diabetic rats presented obvious hyperglycemic symptoms, but GLBF produces a significant antihyperglycemic effect when oral administration to alloxan-diabetic rats (dosage of 500 mg kg^{-1} is more effective than that of 250 mg kg^{-1}). The comparable effect of the GLBF extract with the reference drug glibenclamide may suggest similar mode of action. Glibenclamide is a potent, second-generation, oral sulfonylurea antidiabetic agent used as an adjunct to diet in order to lower blood glucose levels in patients with diabetes mellitus. The hypoglycaemic action of glibenclamide is due to the stimulation of pancreatic islet cells, which results in an increase in insulin secretion. The effects of sulfonylurea are initiated by binding to and blocking on the ATP-sensitive K^+ channel, which has been cloned. The drugs thus resemble physiological secretagogues (e.g., glucose, leucine), which also lower the conductance of this channel. Reduced K^+ conductance causes membrane depolarization and an influx of Ca^{2+} through the voltage sensitive Ca^{2+} channel. Prolonged administration of glibenclamide also produces extra pancreatic effects that contribute to its hypoglycaemic activity (Shah *et al*., 2006). This let us to consider that GLBF may work by the same mode of action of glibenclamide drug or may act by stimulating utilization of glucose by peripheral tissue or the involvement of hepatic factors like activation of glycogen synthetase or release of somatomedin having insulin like activity or inhibition of glucagon release (Suganya *et al*., 2012). However, the mechanism of this extract (GLBF) used has not been clearly defined. Further experiments are needed to determine its mechanism of action. Alloxan causes a massive reduction in insulin release by the destruction of β-cells of the islets of langerhans, thereby inducing hyperglycaemia (Grover *et al*., 2000). Insulin deficiency leads to various metabolic alterations in the animals viz increased blood glucose, increased cholesterol, increased levels of alkaline phosphate and transaminases (Sharma *et al*., 2010). The results of the present study indicate that GLBF reduces the glucose level in animals made diabetic with alloxan. Alloxan has been shown to induce free radical production and cause tissue injury. The pancreas is especially susceptible to the action of alloxan induced free radical damage. In the present investigation, repeated administration of GLBF for 21 days has increased the insulin level. Therefore, the antihyperglycemic activity of GLBF was associated with an increase in plasma insulin levels, suggesting that the

activity could be due to insulinogenic activity of the extract. The increased levels of insulin in diabetic treated rats in this study, indicate that GLBF stimulates insulin secretion from the remnant β cells or/and from regenerated β cells. Similar effect, i.e., insulinogenic activity with the treatment of some medicinal plants was shown by (Karunanayake *et al*., 1984; Cakici *et al*., 1994). Further investigation is expected to characterize the active hypoglycemic principle. Induction of diabetes with alloxan is associated with the characteristic loss of body weight which is due to increased muscle wasting and due to loss of tissue protein. When diabetic rats were treated with GLBF or glibenclamide, the weight losses was recovered and shows an increase in the body weight. The capability of GLBF to protect body weight loss seems to be related to its ability to reduce oxidative damage, its protective effect in controlling muscle wasting i.e., reversal of gluconeogenesis and may also be due to the improvement in insulin secretion and glycemic control (Shirwaikar *et al*., 2006). Lipid plays an important role in the pathogenesis of complications involved with diabetes mellitus. The elevated levels of serum cholesterol and triglycerides with reduced level of serum HDL cholesterol in diabetic condition, poses to be a rises of factor for developing microvascular complication leading to atherosclerosis and further leads to cardiovascular diseases like coronary heart disease. The abnormal high concentration of serum lipid in diabetic mainly due to increased mobilization of free fatty acids from peripheral fat depots, since insulin inhibits the hormone sensitive lipase, insulin deficiency or insulin resistance may be responsible for dislipidimia (Daisy *et al*., 2008). Present study showed that alloxan induced diabetic untreated rats showed significantly increased serum lipid profiles except HDL when compared with the control rats. However, treatments with GLBF or glibenclamide were significantly improvement in the lipid profile when compared to the diabetic untreated rats. Similarly the high density lipoprotein which was reduced in the diabetic untreated rats was significantly increased in the groups administered the GLBF extract. The diabetes-induced hyperlipidemia might be due to excess mobilization of fat from the adipose tissue because of underutilization of glucose. The result of our study is in accord with the findings of other researchers who reported that Many plants extracts have potential therapeutic value in combating artherosclerosis which is one of the major complications of diabetes by lowering serum lipids particularly total cholesterol, triglyceride and low density lipoprotein level (Luka and Tijjani, 2013). This effect not only due to better glycemic control but could also been due to inhibition of the pathway of cholesterol synthesis and increased HDL/LDL ratio may be due to the activation of LDL receptors in hepatocyte, which is responsible for taken up LDL into the liver and reduce the serum LDL level (Singh *et al*., 2010). Liver and kidney damages were the prominent side effect in severe diabetic patients as well as in severe alloxan induced diabetes. The observed marked increases in the activities of aminotransferases (AST and ALT) and Alkaline Phosphatase (ALP) in the diabetic untreated rats are indications that the liver resulted in cytotoxic injury when compared with the control. Larcan *et al.* (1979) reported that the necrotized property can be occurring in liver of diabetic patients. Therefore, the increment of the activities of AST, ALT and ALP in serum may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream (Navarro *et al*., 1993), which gives an indication on the hepatotoxic effect of alloxan. Conversely, a marked decrease in the activities of these marker enzymes was observed in the GLBF extract treated diabetic rats when compared to the diabetic untreated rats which imply a decrease in the rate and magnitude of tissue cell injury and it is also in accord with the observed protective effect of plant extract (GLBF) against alloxan-induced diabetes in rats. Measurement of the activities of "marker" enzymes or biomarkers in body fluids can be used in assessing the degree of assault and the toxicity of a chemical compound on organs/tissues (Mafulul *et al*., 2013). These results are in agreement with those obtained by Ohaeri (2001). On the other hand, renal disease is one of the most common and severe complications of diabetes. Several tests are commonly used in clinical practice to screen for renal disease, monitor the progression of known disease and monitor the effects of potentially renal toxic drugs. The most common tests include the serum total proteins, urea and creatinine. In the present study, a significant reduction in the level of serum insulin and elevation in the levels of serum creatinine and urea indicate impaired renal function of untreated-diabetic animals when compared with control group. Insulin is a physiological factor, which plays an important role in the maintenance of protein balance, since it not only stimulates the uptake of amino acids and protein synthesis, but also inhibits protein degradation (Pathak and Dhawan, 1988). The extract GLBF was showed significantly increased the total protein and lowered the serum urea and creatinine levels by enhancing the renal function that is generally impaired in diabetic rats. This result is going with a previous study (Daisy *et al*., 2007).

Like many chronic diseases, diabetes is widely believed to increase oxidative stress. A possible mechanism for oxidative stress in diabetes is autooxidation of sugar and unsaturated lipid that leads to the production of free radicals, which damage the macromolecules (Suganya *et al*., 2012). Hypoinsulinemia in diabetes increases the activity of the

enzyme fatty acyl coenzyme A oxidase which initiates β-oxidation of fatty acids, resulting in lipid peroxidation (Memişoğullari and Bakan, 2004). The involvement of oxidative stress in the causation of diabetes mellitus was well documented and the importance of antioxidants in the duration of diabetes mellitus was also reported. The major organs involved in diabetic complication are liver and kidney. The current study showed a significant elevation of plasma malondialdehyde (an indicator of lipid peroxidation) contents in liver and kidney of diabetic rats. The GLBF significantly reduced the lipid peroxidation product levels in diabetic rats, confirming that GLBF is potent antioxidants. In diabetes an increase in oxidative stress arises due to compromise in natural antioxidant mechanisms and an increase in oxygen free radical production (El-Demerdash *et al*., 2005). Any compound-natural or synthetic-with antioxidant activity might totally or partly alleviate the oxidative damage (Sepici-Dincel *et al*., 2007). SOD and CAT are the two major scavenging enzymes that remove radicals *in vivo*. In the present study, SOD and CAT activities were significantly decreased in the liver and kidney of diabetic rats. A decrease in the activity of these antioxidants can lead to an excess availability of superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), which in turn generate hydroxyl radicals ($^{\bullet}OH$), resulting in initiation and propagation of lipid peroxides. SOD can catalyze dismutation of $O_2^{\bullet-}$ into H_2O_2, which is then deactivated to H_2O by CAT or GPx (Pari and Latha, 2004; Kumuhekar and Katyane, 1992). Normally, SOD works in parallel with selenium dependent GPx, which plays an important role in the reduction of H_2O_2 in the presence of reduced glutathione forming oxidized glutathione (GSSG) and thus, it protects cell proteins and cell membranes against oxidative stress. GPx has a key role in enzymatic defense systems and reduces organic peroxides into their corresponding alcohols. In our study, GPx activity was significantly decreased in the liver and kidney of diabetic rats. The significant decrease in GPx activity could suggest inactivation by ROS, which are increased in diabetic rats. The decrease may also be due to the decreased availability of its substrate, GSH, which has been shown to be depleted during diabetes (Ugochukwu *et al*., 2004; Ardestani and Yazdanparast, 2006). GSH is important in the circumvention of cellular oxidative stress, detoxification of electrophiles and maintenance of intracellular thiol redox state. GSH is reported to be essential for recycling of other antioxidants like antioxidant vitamins (Ardestani and Yazdanparast, 2006) and acts as a substrate for GPx and Glutathione-S-Transferace (GST) that are involved in preventing the deleterious effect of oxygen radicals (Sajeeth *et al*., 2011). In our study, liver and kidney of diabetic rats exhibited a decreased level of GSH which might be due to increased utilization for scavenging free radicals and increased consumption by GPx and GST. Treatment with GLBF and glibenclamide reversed GSH level could be due to decreased utilization of GSH to reduce the lipid peroxidation and also ameliorated SOD, CAT and GPx activities in liver and kidney of diabetic rats. These results suggest that GLBF has free radical scavenging activity, which may exert a beneficial effect against pathological changes caused by ROS. In addition, the over expression of these antioxidant enzymes in diabetic rats treated with GLBF implies that this potential oxidant defense is reactivated by the active principles of GLBF with a resulting increase in the capacity of detoxification through enhanced scavenging of oxy radicals.

Conclusion

Finally, it is concluded that the consumption of GLBF caused a significant reduction in glucose level of alloxan diabetic rats. The result of the present study showed that GLBF brings back the blood glucose and body weight to normal in diabetic rats. In addition, this agent was capable of improving serum insulin, hyperlipidaemia and the impaired liver and kidney functions. Moreover, the treatment of rats with GLBF for 21 days was effective in controlling the hyperglycemia, hyperlipidaemia and the oxidative damage. This investigation is helpful for understanding mechanism of action of *Lycium barbarum* L. fruits and its active ingredients and also reveals the potential of *Lycium barbarum* L. for use as a natural oral agent with hypoglycemic, hypolipidemic and strong antioxidant effects.

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Ethics

Author confirms that this manuscript has not been published elsewhere and is not under consideration by another journal. Also, the manuscript represents honest work and the author has approved the manuscript and agrees with the order in which his name appears on the title page.

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