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Effects of QZ-16 on blood glucose and lipids in Streptozotocin induced diabetic rats

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SUMMARY

The present study was designed to investigate the hypoglycaemic and hypolipidaemic activities of Qurs-e-Ziabetus 16 (QZ-16) in Streptozotocin (STZ) induced diabetic rats. QZ-16, a polypharmaceutical herbomineral formulation developed on the principles of Unani medicine is used for non-insulin dependent diabetes mellitus (NIDDM). The elevated levels of fasting blood glucose, serum levels of cholesterol, triglycerides and urea observed in rats treated with STZ (55 mg/kg body wt.) were significantly reduced by the treatment of QZ-16 (240 mg/kg, p.o.) and gliclazide (30 mg/kg, p.o.). The reduced HDL cholesterol levels were also increased by the QZ-16 and gliclazide treatments in the STZ induced diabetic rats. These data show that QZ-16 has hypoglycaemic, hypolipidaemic properties in STZ induced diabetic rats.

Key words: Qurs-e-Ziabetus 16; Unani medicine; Hypoglycaemia; Hypolipidaemia; Streptozotocin

INTRODUCTION

QZ-16 is a polypharmaceutical herbomineral formulation containing five herbal ingredients namely *Azadirachta indica* A. Juss (Family: Meliaceae) (Leaves), *Gossypium herbaceum* Linn. (Family: Malvaceae) (Seeds), *Gymnema sylvestre* R.Br. (Family Asclepiadaceae) (Leaves), *Rosa damascena* Mill (Family: Rosaceae) (Petals), *Syzigium cumini* (Linn) Skeels. (Family: myrtaceae) (seeds) along with two Kushtas, Kushta Hartal (Preparation: 25 g of Hartal is ground in 125 g of extract of *Calotropis gigantea* (Linn) R. Br. Cakes of the mass are then wrapped in the leaves of *Ricinus communis* linn and then heated for three hours after putting in the ash of leaves of *Ficus religiosa* Linn. After cooling it to room temp Kushta Hartal is obtained) (Rafiquddin, 1980) and Kushta Baiza-e-Murgh (Preparation: 100 g of post-e-baiza-e-murgh (shell of hen's egg) is soaked in sufficient quantity of Aab-e-lemu (lemon extract) in a porcelain pot till dried. It is then kept in earthen discs sealed with a process of Gil-e-hikmat & subjected to a fire of 70× 70×70 cm pit. The process in repeated twice) (National Formulary, 1983).This formulation is used by Unani physicians in the management of NIDDM (Vohora, 1983).

A preliminary study with QZ-16 in our laboratory showed the reduction of blood glucose and food intake and enhanced oral glucose tolerance in neonatally STZ induced diabetes in rats (Najmi *et al.*, 1999).

The present investigation is designed to present an experimental evidence that treatment with QZ-

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16 can result in reduction in the degree of hyperlipidaemia along with the reduction in the blood glucose in experimental diabetes.

MATERIALS AND METHODS

Drugs and chemicals

QZ-16, was provided by Hamdard (wakf) Labs. Ghaziabad, India. STZ was purchased from Sigma, USA. Gliclazide was purchased from Serdia, Mumbai, India. All the biochemicals and chemicals used were of analytical grade.

Animals

Albino rats of Wistar strain weighing 100 - 150 g were used for the study. Animals were supplied by central Animal House facility of Hamdard University and kept under standard laboratory conditions in 12 h light/dark cycle at 25 ± 2°C. Animals were supplied with pellet diet (Lipton, India) and water ad libitum.

Experimental protocol

Fasting blood glucose levels of all the animals were determined and then divided into two groups. In group A, 10 animals were administered citrate buffer of pH 4.5 by tail vein. The animals in group B were administered STZ 55 mg/kg i.v. after dissolving in 0.1 M citrate buffer pH 4.5 after an overnight fast (Cam *et al.*,1993). After 5 days period the fasting blood glucose level was determined for all the animals and 5 rats were randomly selected from group A showing normal glucose levels and were included in group I. From group B rats showing fasting blood glucose levels more than 250 mg/dl (Patel *et al.*, 1997) were selected and randomly divided into three groups II, III and IV each containing 6 rats (Ignacimuthu *et al.*, 1998).

Group I (Vehicle treated) served as normal control and received normal saline twice daily. Animals in group II (STZ- treated) served as toxic control and received normal saline 1 ml/kg p.o twice daily. Rats in group III (STZ treated) received QZ-16 (240 mg/kg p.o) twice daily. Rats in group IV (STZ treated) received gliclazide (30 mg/kg p.o) twice daily.

Treatment was continued for a period of 30 days and on 31st day the blood was collected from retro orbital plexus under light ether anesthesia for estimation of various biochemicals. The blood samples were allowed to clot for 30 - 40 min. serum was separated by centrifugation at 37°C and was used for biochemical estimation.

Biochemical estimations

Biochemical parameters blood glucose (Brahm and Trinder., 1972), serum total cholesterol (Warnick *et al.*, 1985), serum triglycerides (Fossati *et al.*, 1982), HDL Cholesterol (Demacher *et al.*, 1980; Warnick *et al.*, 1985), serum urea (Teitz, 1976) were analyzed according to the reported procedures.

Statistical analysis

Results are expressed as mean ± SEM. The significance of difference was assessed by using students '*t*' test and ANOVA. F ratio was also calculated. *P* values < 0.05 were regarded as significant.

RESULTS

A significant increase in the fasting blood glucose levels was observed in the STZ treated animals (group II, III and IV) as compared with citrate buffer treated animals (group I). Treatment of STZ challenged animals (group II, III & IV) by QZ-16 (240 mg/kg p.o) and gliclazide (30 mg/kg p.o) twice daily for 30 days respectively brought about significant reduction in the fasting blood glucose levels as compared with the fasting blood glucose levels in these animals before treatment with QZ-16 and gliclazide and also with STZ challenged animals treated with normal saline (1 ml/kg p.o) twice daily for 30 days (group II) (Table 1).

Levels of serum cholesterol, triglycerides and urea increased by STZ treatment were decreased significantly by the treatment of rats with QZ-16

Table 1. Effect of QZ-16 and gliclazide on fasting blood glucose levels in control and streptozotocin induced diabetic rat

	Blood glucose conc. mg/dl	
Group	Before Treatment	After Treatment
I	107.72 ± 2.71	112.01 ± 2.88^{ns}
II	322.11 ± 12.10	332.44 ± 13.42^{ns}
III	328.25 ± 14.64	184.35 ± 16.40**
IV	332.89 ± 14.37	179.11 ± 14.22**

Values represents ± SEM (group I, n = 5, group II, n = 6). After treatment values are compared with before treatment values by paired '*t*' test. $^{**}P < 0.01$, ^{ns}= not significant.

(240 mg/kg p.o) and gliclazide (30 mg/kg p.o) twice daily for 30 days, Moreover the significant decrease in the HDL cholesterol observed in animals treated with STZ (group II) as compared with normal control group (group I) was also increased by QZ -16 and gliclazide treatments (Table 2).

DISCUSSION

STZ injection in rats causes hyperglycaemia, hypercholesterolemia, hypertriglyceridemia, increased serum urea conc. etc. (Silva *et al.*, 2001; Umrani *et al.*, 2002). Glucose lowering effect of QZ-16 may possibly be due to the presence of *Azadirachta indica*, *Gossypium herbaceum*, *Gymnema sylvestre, Syzigium cumini*, and Kushta Hartal (Rizvi and Rizvi, 1987; AttaurRehman and Zaman, 1989; Handa *et al.*, 1989; Bopanna *et al.*, 1997; Sugihara *et al.*, 2000). Decoction of leaves of *Azadirachta indica* has been shown to possess antidiabetic activity. Nimbidin a constitument of this plant significantly delays the peak rise in blood sugar after glucose administration (Bopanna *et al.*, 1997). The leaves extract (500 mg/kg B. wt.) and seed oil (5 ml/kg b. wt.) of *Azadirachta indica* have been reported to decrease glycaemia in alloxan diabetic rabbits (Khosla *et al.*, 2000). *Gymnema sylvestre* contains gymnemic acid which acts by increasing insulin release from β-cells of langerhan's (Shanmugasundaram *et al.*, 1981; Persaud *et al.*, 1999), and glycogen synthetase (Shanmugasundaram *et al.*, 1983). Hexokinase catalyses the entry of glucose in to alternative metabolic pathways and glycogen synthetase is responsible for control of glycogen metabolism. Besides this it can abolish the sensations of sweet and bitter taste (Bailey and Caroline, 1989; Ye *et al.*, 2001). It also causes suppression of glucose absorption by inhibiting glucose uptake in the intestine (Shimizu *et al.*, 1997). In a clinical study leaf extract of *Gymnema sylvestre* was found to produce significant reduction in blood glucose, glycosylated hemoglobin and glycosylated plasma proteins (Baskaran *et al.*, 1990). Epinephrin induced hyperglycaemia was also antagonized by *Gymnema sylvestre* (Gupta and Variyar, 1961). Seeds of *Syzigium cumini* contains glycoside jumboline which prevents the conversion of starch into sugar (Nadkarni, 1979). Oral administration of aqueous extract (2.5 ml/kg B. wt.) of the seeds has been shown to significantly reduce the blood glucose and increase total hemoglobin content (Prince *et al.*, 1998).

The aqueous extract (2.5 ml/kg B. wt.) of

Table 2. Effect of QZ-16 and gliclazide on serum lipid and urea levels in control and streptozotocin induced diabetic rats (mg/dl)

Group	Triglycerides	Cholesterol	HDL Cholesterol	Urea
I	76.54 ± 4.04	77.41 ± 1.53	43.64 ± 0.75	35.96 ± 1.52
II	173.66 ± 11.61**	129.49 ± 3.83***	28.47 ± 1.78**	97.99 ± 1.40***
III	57.28 ± 7.94**	75.44 ± 5.96**	37.18 ± 2.07*	50.63 ± 2.86***
IV	80.36 ± 9.90**	85.54 ± 3.56**	38.39 ± 3.02*	41.55 ± 4.26***
F-ratio	34.99$^{\#}$	39.68$^{\#}$	9.81$^{\#}$	103.25$^{\#}$

Values represent ± SEM, (group I, n = 5, group II, n = 6). Group II is compared with group I and groups III & IV are compared with group II. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{\#}P < 0.005$

Syzigium cumini results in the decreased free radical formation in the tissues studied (Prince *et al.*, 1998). *Azadirachta indica* (Arivazhagan *et al.*, 2000) and *Rosa damascena* (Bolwell *et al.*, 1998) have also been shown to possess antioxidant property. It has been reported that in diabetes, plasma lipid peroxide levels are increased, it has also been shown that increased plasma lipid peroxidations are accompanied by the depression of glutathione-dependent antioxidant defense mechanism of the body (Uzel *et al.*, 1987; Baydas *et al.*, 2002). Several other investigators have also reported that increased oxidative stress plays an important role in the pathogenesis of diabetes mellitus (Maxwell *et al.*, 1997; Desco *et al.*, 2002; Zobali *et al.*, 2002). Hence it can be hypothesized that antioxidant property of *Azadirachta indica, Rosa damascena* and *Syzigium cumini* may also contribute in the overall improvement in diabetic state of the rats in the present study.

Kushta Hartal has been shown to produce hypoglycaemia significantly and reduce other diabetic symptoms in a clinical study (Rizvi and Rizvi, 1987). Although Kushta Baiza Murgh is used in diabetes in unani medicine but no clinical or experimental reports are available about its efficiency (Vohora, 1990).

Abnormalities of fat metabolism are also accelerated in diabetes mellitus. The manifestations of disordered lipid metabolism are so prominent that diabetes has been called more a disease of lipid than that of carbohydrate metabolism (Ganong, 1991). Elevated level of serum total cholesterol, and triglycerides were found in the STZ diabetic rats (Umrani *et al.*, 2002), these are increased in diabetic patients also (Zargar *et al.*, 1997). Rise in cholesterol may be due to increase in plasma concentration of VLDL and LDL which may be due to increased hepatic production of VLDL and decreased removal of VLDL and LDL from circulation (Ganong, 1991), because of decreased activity of insulin dependent lipoproteins lipase (Stephen *et al.*, 1996).

Azadirachta indica (Bopanna *et al.*, 1997; Khosla *et al.*, 2000) and *Gymnema sylvestre* (Murshed *et al.*, 1996; Bhandari *et al.*, 1998; Shigematsu *et al.*, 2001) which are constituents of QZ-16, have been reported to possess hypolipidaemic and hypoglycaemic properties.

QZ-16 and gliclazide treatment significantly reduced the serum conc. of triglycerides, total cholesterol, which might be due to the improvement of the glucose tolerance (Najmi *et al.*, 1999). The elevated levels of HDL cholesterol concentration observed in QZ-16 and gliclazide treatment rats might be due to the influence of the these drugs on carbohydrate and related metabolism.

STZ is known to produce nephrotoxicity by increasing the generation of free radicals (Mukhopadhyay, 1995; Degenhardt *et al.*, 2002) in the renal cells. The oxidative stress might be responsible for elevated urea level. Sustained hyperglycaemia also produces impairment of renal function (Pickup and Milan, 1990; Umrani *et al.*, 2002). Since QZ-16 contains antioxidant and hypoglycaemic principles. The reduction in serum urea might be due to the antioxidant and hypoglycaemic mechanisms.

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