Evaluation of Hypoglycemic Effect of Qurs-e-Ziabetus Khas in Alloxan Monohydrate Induced Diabetic Albino Rabbits

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Abstract

This study was carried out to evaluate the use of *Qurs-e-Ziabetus Khas* in hyper-glycaemia clinically. The anti-diabetic effect of *Qurs-e-Ziabetus Khas* was studied in adult healthy albino rabbits of either sex weighing 1.5-2 Kg at the doses of 400, 800 and 1200 mg/kg orally in the form of suspension. The animals were randomly divided into diabetic control, diabetic treated and standard groups, and each group consisting of 6 rabbits. Initially animals were made diabetic by injecting alloxan monohydrate at the dose of 150mg/Kg intravenously. The blood samples were obtained from the marginal ear veins at 0 h (initial), and after the test drug administration at 2, 3 and 6th hour. Blood glucose was estimated by the End Point O-Toluidine method. The maximum reduction of blood glucose occurred after 3rd hour of test drug administration at the dose of 1200 mg/kg orally as compared to animals of control group. The test drug was also compared with a standard drug glibenclamide in a dose of 1.5 mg/kg. The obtained data were analyzed by one way ANOVA with post hoc't' test. The findings indicate that the *Qurs-e-Ziabetus Khas* has significant effect in non insulin dependent diabetes mellitus and scientifically validated the claims of Unani physicians that this drug possesses antidiabetic effect.

Key words: Qurs-e-Ziabetus Khas, Hyper-glycaemia, Alloxan monohydrate, Antidiabetic

Introduction

Diabetes is the world's largest endocrine disease with deranged carbohydrate, lipid and protein metabolism. It is distinguished by irrelevant hyperglycemia produced by insufficiency of insulin at the cellular level (Sidhu, *et al*., 2014). It is reported that diabetic patient are increasing every year globally (Samyal *et al*., 2014), and inferring that more than 400 million people of the worldwide will be effected from hyperglycemia by 2030 (Samyal *et al*., 2014). The occurrence rate of diabetes in India is 1-5% (Sen *et al*., 2016). Statistical projection suggests that the number of diabetic patients will rise from 15 million in the year 1995 to 57 million in 2025, making India the country with the highest number of diabetics in the world (Shalam *et al*., 2006). It is a major public health problem in the developed as well as developing countries. It is ranked seventh among the leading causes of death, and third when all its fatal complications are taken into account. Large-vessel atherosclerosis is the most common cause of death in diabetes (Trivedi *et al*., 2004).

The phytoconstituents such as flavonoids and polyphenol components have an ability to enhance glucose transport and metabolism in muscle and/or to stimulate

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insulin secretion and play a chief role to delay digestion and absorption of carbohydrates lowering the postprandial glucose levels by inhibiting á-glucosidase and áamylase enzyme (Das *et al*., 2016 and Mukesh *et al*., 2013)

There is an increasing demand of antihyperglycemic natural products by patients, due to obvious side effects associated with the use of mainstream medicine such as insulin and oral hypoglycemic agents (Zhang *et al*., 2007 and Badole *et al*., 2006).

In the interest of patients there is a need for more effective, durable, safer and cost effective anti-diabetic agents. Therefore, the World Health Organization (WHO) has recommended the evaluation of plants effectiveness and suggested to use of herbal medicines where the conventional treatment of diabetes is not satisfactory (Samyal *et al*., 2014). This has lead to increasing demand of research on natural products with antidiabetic activity with minimal or no side effects (Singh *et al*., 2007). More than 400 traditional plants have been recorded to possess antidiabetic activity, but few of these are scientifically validated for their efficacy (Barhate and Kulkarni, 2007). Many herbal products, including several metals and minerals have been described for the cure of diabetes mellitus in ancient literature of Unani System of Medicine. Herbal preparations alone or in combination with oral hypoglycemic agents sometimes produce a good therapeutic response in some resistant cases where modern medicines alone fail (Ghosh *et al*., 2006).

Qurs-e-Ziabetus Khas (QZK) is a well known Unani Pharmacopoeial preparation mainly based on different medicinal plants used to treat diabetes since ancient times (Anonymous, 2006). But to the best of our knowledge this formulation is not evaluated scientifically for its antidiabetic activity. Therefore, this study was carried out to assess and scientifically validate the use of *Qurs-e-Ziabetus Khas* in hyper-glycaemia. The ingredients of QZK are Tabasheer (calcinated pith of *Bambusa arundinacea* Retz.), Satt-e-Gilo (dried extract of the stem of *Tinospora cordifolia* Miers.), Maghz-e-Khasta-e-Jamun (seed kernel of *Eugenia jambolana* Lam.), Gurmar Buti (*Gymnema sylvestre* Schult.), Kushta-e-Zumurrud (calx of Emerald), Kushta-e-Baiza-e-Murgh (calx of Egg shell) and Loab-e-Aspghol (Seeds mucilage of *Plantago ovata* Forsk) (Anonymous, 2006).

The anti-diabetic effect of *Qurs-e-Ziabetus Khas* was studied in adult healthy albino rabbits of either sex weighing 1.5-2 Kg at the doses of 400, 800 and 1200 mg/kg orally in the form of suspension. The animals were made diabetic using alloxan monohydrate (150 mg/kg; i.v.) and glibenclamide was used as a reference drug (1.5 mg/kg; p.o.).

Material and Methods

The study was conducted in the department of Ilmul Advia, A.K. Tibbiya College, AMU, Aligarh, during 2009-2010.

Preparation of Qurs-e-Ziabetus Khas

The ingredients of QZK are shown in table 1 (Anonymous, 2006).

Table 1: Ingredients of Qurs-e-Ziabetus Khas

S. No.	Ingredients	Weight (g)	Scientific Name
1.	Tabasheer	25	*Bambusa arundinacea*
2.	Satt-e-Gilo	25	*Tinospora cordifolia*
3.	Maghz-e-Khasta-e-Jamun	50	*Eugenia jambolana*
4.	Gurmar Buti	50	Gymnema sylvestre
5.	Kushta-e-Zumurrud	10	Calx of Emerald
6.	Kushta-e-Baiza-e-Murgh	10	Calx of Egg shell
7.	Loab-e-Aspghol	Q.S.	*Plantago ovata*

The QZK was prepared according to the following steps:-

Step I Procurement, authentication and identification of ingredients

Step II Processing of raw materials

Step III Preparation of Tablet (Qurs)

Step I: The raw materials were purchased from the local market of Aligarh and their identity, purity and quality were checked in the pharmacognosy section of the Department of Ilmul Advia, Faculty of Unani Medicine, AMU, Aligarh, and found at par with the standards of Unani and Ayurvedic Pharmacopoeia of India (Anonymous, 1986, 1992, 1997, 1999, 2001, 2007 and Vohora, 2008).

Step II: All the ingredients of QZK except Satt-e-Gilo and Loab-e-Aspghol were powdered in an electric grinder and passed through sieve number 80 to obtain fine powder. The fine powder alongwith Satt-e-Gilo was mixed properly and then excipient Loab-e-Aspghol was added and finally dough was made. Further this wet mass was made in to granules by passing through 12 mesh sieve and dried at room temperature (Anonymous, 1968; Anonymous, 1970).

Step III: Tablets were prepared of 500mg each according to the method described in the Pharmacopoeia of India (Anonymous, 1970), by automatic tablet making machine in Dawakhana Tibbiya College, AMU, Aligarh.

Animal maintenance

Experiments were carried out in healthy adult albino rabbits of either sex weighing 1.5-2 kg. The animals were kept in animal house of the Department of Ilmul Advia, Faculty of Unani Medicine, A.M.U., Aligarh, under hygienic and standard laboratory conditions at uniform temperature. All the animals were fed, Standard animal diet and water-ad-libitum.

Drugs and chemicals

Alloxan monohydrate was used for inducing diabetes at the dose of 150 mg/kg intravenously in healthy albino rabbits (Baily and Baily, 1943; Pincus *et al*., 1954; Bander *et al*., 1969). Glibenclamide (Daonil) was used as standard referent drug which was administered in a dose of 1.5 mg/kg orally to the animals.

Preparation of Test Drug Material

Fresh suspension of powdered drug was prepared in distilled water with 2% gum acacia powder (S.d. Fine Chemical Ltd.), which was administered orally in the animals with the help of feeding canula after shaking the suspension well. The dose for the albino rabbit was calculated by extrapolating the human dose of test drug by conversion factor of 12 for rabbit (Nair and Jacob, 2016). Hence the three different doses selected for the study of hypoglycaemic activity of test drug i.e. 400, 800 and 1200 mg/kg.

Hypoglycaemic activity of Qurs-e-Ziabetus Khas

The hypogycaemic effect of QZK was assessed on healthy albino rabbits. For study rabbits were divided into 5 groups and each group consisting of 6 albino rabbits. Group I served as control group and distilled water was given in the dose of 5 ml/kg orally. Group II is standard group and treated with referent drug Glibenclamide (Daonil) in the dose of 1.5 mg/kg orally. Group III, IV and V were test drug treated group and administered with the oral doses of QZK in 400, 800 and 1200 mg/kg respectively.

All the animals were fasted for 24h before alloxan injection. Diabetes was observed in the rabbits (fasting Blood Glucose Levels ranged from 200-250 mg/100ml) within 24h after injection of alloxan. The effect of the oral administration of QZK was observed in Group III, IV and V for 6h after drug administration. The blood samples of all the groups were taken from the marginal ear veins at 0h (before treatment), then at 2, 3 and 6h after the treatment. Serum was separated and serum glucose level was estimated by the End Point O-Toluidine Method, (Mukherjee, 1997; Hultman, 1959). The rabbits were fed water only during experiments.

Procedure

Bloods were collected in to test tubes. Serums were separated from blood by centrifuging the blood at 5000 rpm for 15 minutes in centrifuging machine. Then three test tubes were labelled as B for blank, S for standard and T for test (unknown) and the following reagents were pipetted into them as shown in table 2.

Table 2: Mixing of reagents in different test tubes

S. No.	Reagents	B	S	T
1.	Glucose reagents	5.0 ml	5.0 ml	5.0 ml
2.	Distilled water	100 µl	–	–
3.	Glucose standard	–	100 µl	–
4.	Specimen (serum)	–	–	100 µl

The contents in test tubes were mixed by lateral shaking of each test tube separately. All test tubes were put in to vigorously boiling water bath at 1000C for exactly 9 minutes, and then test tubes were removed quickly and cooled to room temperature by placing them in cold water for 3 minutes. The contents of tubes were transferred to cuvette and ABSORBANCE (O.D) of all tubes were measured against blank adjusted to 630 ± 20 nm on the filter wheel using Lab System Analyzer.

Calculation

The concentration of glucose in serum of unknown sample was calculated by the following formula:-

$$\frac{R.T}{R.S} x\,100 = mg/dl$$

Where, R.T = optical density of unknown sample

R.S = optical density of standard solution

Statistical Analysis

All the data were analyzed by one way-Analysis of variance (ANOVA) with post hoc 't' test.

Results

Control group: This group doesn't get any medication except distilled water. Therefore, there is no significant difference in serum glucose level before and after the treatment. The results are shown in table 3.

Standard group: In standard group the glucose level in serum reduced significantly after 3h ($P<0.001$) and continued for 6h when compared with control group. After the 6^{th} of the treatment, glucose level slightly increased. The results are presented in table 3.

Test drug treated groups: QZK in the doses of 400 and 800mg/kg doesn't show any significant reduction in serum glucose level in any animal at any time interval in comparison to control group. While the animals of group treated with the dose of 1200mg/kg of QZK shows significant difference in serum glucose level after 3h ($P<0.05$) of test drug administration, when compared to control group. However

there is an insignificant rise in serum glucose level after 6h of treatment. The results are depicted in table 3.

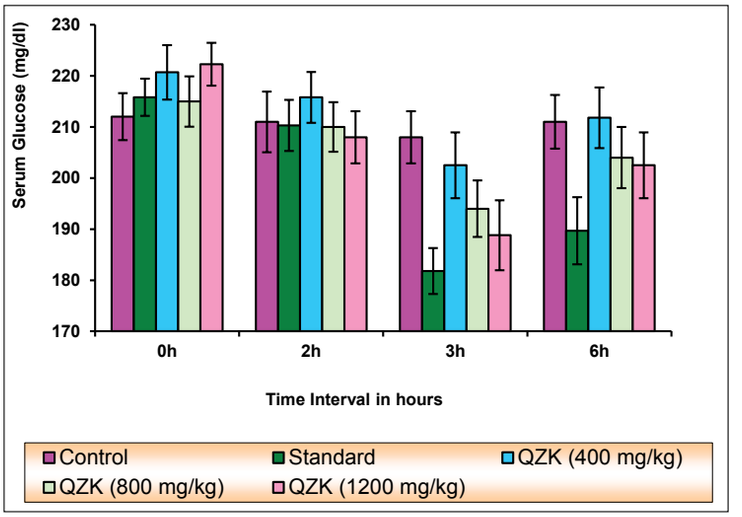
The comparison of the efficacy of test drug groups QZK in all the given doses with standard and control group has also been depicted in figure 1.

Table 3: Serum glucose level of different group before and after the treatment

Group	Initial (Mean ± SE)	Serum Glucose Levels (mg/dl) (Mean ± SE)		
	0h	2h	3h	6h
Control	212 ± 4.6	211 ± 5.95	208 ± 5.10	211 ± 5.26
Standard	215.8 ± 3.64	210.3 ± 5.02	181.8 ± 4.48***	189.7±6.57**
QZK (400 mg/kg)	220.7 ± 5.34	215.8 ± 4.97	202.5 ± 6.44	211.8 ± 5.95
QZK (800 mg/kg)	215 ± 4.93	210 ± 4.85	194 ± 5.55	204 ± 6.00
QZK (1200 mg/kg)	222.3 ± 4.20	208 ± 5.10	188.8 ± 6.85*	202.5 ± 6.44

(Tabulated values are mean ± SE; n=6; * P<0.05; ** P<0.01; *** P<0.001)

Fig. 1: Comparision of Effect of QZK in different doses (400, 800 and 1200 mg/kg) with Glibenclamide (1.5 mg/kg) and control on Serum Glucose Levels in Diabetic Rabbits



Discussion

Diabetes is a group of Syndromes characterized by hyperglycemia, due to altered metabolism of lipids, carbohydrates and proteins in result of complete or relative insufficiency of insulin secretion or insulin action (Satyanarayana *et al.*, 2007). Consumption of calorie-rich diet, obesity and sedentary lifestyle led to a tremendous increase in the number of diabetics' worldwide (Asulander *et al.*, 2002).

Presently two main groups of substances are recognized as oral hypoglycemic agents. They are certain sulphonamide derivatives (Sulphonylureas) and

guanidine derivatives (Biguanides). They are being used by 30% of all diabetics, but produce some undesirable effects such as vertigo, headache, gastric and hepatic disorders, poor renal functions, vit. B_{12} deficiency and cardiac disorders etc. (Goodman and Gilman, 1992; Laurence *et al*., 1997).

However, hyperglycemia can be treated quickly with allopathic drugs but at the same time they may cause various side effects like hypoglycemia, peripheral neuropathy and gastrointestinal disturbances etc. To avoid these side effects both patient and researchers are more eager to get and to investigate the new alternate method to cure the hyperglycemic condition. So, herbal medicines are gaining importance because of least adverse effects. In India plants were used for health care since 5000 years. In Indian system of medicine, crude drugs are obtained from plants and there are about 8000 herbal remedies for human ailments, besides the numerous plants used in folk medicine by tribal and rural people (Chikaraddy and Maniyar, 2017).

Hence this study has been undertaken to evaluate the hypoglycemic activity of Qurs Ziabitus Khas. All the ingredients of QZK viz. Satt-e-Gilo, Maghz-e-Khasta-e-Jamun, Gurmar Buti, Kushta-e-Zumurrud, Kushta-e-Baiza-e-Murgh and Loab-e-Aspghol are reported to have antidiabetic activity (Modak *et al*., 2007, Dymock *et al*., 1891, Rustenbeck, 2007, Kabiruddin, 1967, Said, 1997 and Ceranic *et al*., 2006) except Tabasheer (calcinated pith of *Bambusa arundinacea*) but the ethanolic extract of leaves of Bambusa arundinacea have been reported to possess hypoglycemic activity in alloxan induced diabetic rabbits (Gupta *et al*., 2004).

Study reveals that the test drug in the multiple doses of 400, 800 and 1200 mg/kg reduced the blood glucose level in alloxan induced diabetic rabbits from the beginning of 2nd hour. While the maximum reduction of blood glucose level seen after 3rd hour. Further the significant effect was produced by the dose of 1200 mg/kg ($P<0.05$) at 3rd hour against control group. The possible mechanism of action of the test drug may be like that of glibenclamide. The test drug may acts by stimulating the β-cells of the Pancreas which stimulate the secretion of insulin and thereby reduce the blood glucose level.

Conclusion

The test drug (QZK) appears to be quite safe, comprehensive, as it will produce the hypoglycaemic effect and can be used effectively for the treatment of diabetes.

The results of this study suggest that the test drug may be effective in non insulin dependent diabetes mellitus (NIDDM) at all the doses while having maximum effect at1200 mg/kg. The study also validates the description of Unani literature as Qurs-e-Ziabetus Khas has been described to possess antidiabetic activity. This study also substantiates the use of this drug as antidiabetic agent in clinical

practice by Unani physicians. Further to say that there should be more studies conducted on QZK to ascertain its exact mechanism of action and the role of its phytochemical constituents for anti-hyperglycemic activity.

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