Original Article

Effects of Ocimum Sanctum (Tulsi) on Blood Glucose level of Alloxan induced Hyperglycemic Rats

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Abstract

Blood Glucose lowering properties of Ocimum Sanctum (OS), a renowned medicinal species, were assessed by measuring the levels of blood glucose in the plasma of the hyperglycemic Long Evans rats. The feeding of 1% powder of OS with normal diet for 21 days to hyperglycemic rats significantly reduced serum blood glucose level ($p < 0.01$). The present study suggests that the blood glucose lowering effects of OS probably serve as a new potential natural product for the treatment of hyperglycemia.

Keywords: *Diabetes mellitus, Ocimum Sanctum, Blood glucose.*

Introduction

Diabetes Mellitus is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin. Lack of insulin affects the metabolism of carbohydrate, protein and fat and can cause a significant disturbance of water and electrolyte homeostasis. Death may result from acute metabolic derangement[1]. As the disease progress tissue or vascular damage ensues leading to severe diabetic retinopathy, nephropathy, neuropathy, cardiovascular complication and ulceration. Thus Diabetes covers a wide range of heterogeneous disease. In type 2 diabetes mellitus, there is impaired pancreatic beta cell function due to oxidative stress leading to "relative" insulin deficiency together with resistance to the action of insulin in the liver and muscle. Insulin resistance leads to elevated insulin secretion in order to maintain normal blood glucose levels. However, in susceptible individuals the pancreatic beta cells are unable to sustain the increased demand for insulin and slowly progressive insulin deficiency develops[2]. A currently favored hypothesis is oxidative stress, though a single unifying mechanism of super oxide production, is the common pathogenic factor leading to insulin resistance, beta cell dysfunction, impaired glucose tolerance (IGT) and ultimately to Type 2 diabetes[3,4]. Ocimum Sanctum has specific aromatic odor because of the presence of essential or volatile oil, mainly concentrated in the leaf[5]. This aromatic volatile oil mainly contains phenols, terpenes and aldehydes[6,7,8]. Tulsi has also important constituent of Eugenol (1hyrdoxy-2 methoxy-4 allylbenzene). Eugenol is an active phenolic compound (volatile oil) of O.Sanctum. Dry tulsi leaf powder when fed with alloxan induced diabetic rats, eugenol has been shown to efficiently inhibit lipid peroxidation[9]. Lipid per oxidation is a marker of cellular oxidative damage initiated by reactive oxygen species. It was reported that diabetics are highly sensitive to oxidative stress. These compounds may be contributed to the fact that the phenolic compound of O.Sanctum extract acts as free radical scavengers which reduces fasting blood sugar level significantly[9].

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Materials and Methods

The study was carried out in the laboratory of the Department of Pharmacology and Therapeutics of Sir Salimullah Medical College, Dhaka in collaboration with Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka during the period from January 2010 to December, 2010. 30 Long rats weighing between 150-200 gms were used as experimental animals. They were kept in cages in an animal house at 23 ± 2^{o} c under 12 hour light-dark cycle.

The rats were divided into 5 groups as follows:

(i) Group A: Rats fed on basal (laboratory) diet for 21 days with no O.S added. This group served as a control for normally fed rats.

(ii) Group B: Rats fed on basal diet plus 1% powder O.S for 21 days.

(iii) Group C: Rats fed on basal diet plus 1% powder with no O.S for 21 days. This group served as a diabetic control group.

(iv) Group D: Alloxan induced rats fed on basal diet for 21 days.

(v) Group E: Alloxan induced rats fed on basal diet plus 1% O.S for 21 days (4th day of the Experiment)

Experimental protocol: Total 30 adult male rats were taken 6 (six) in each group. The experiment was divided into 2 (two) parts:

Experiment I: Rats of group A and B were tested to demonstrate the effect of O.Sanctum on serum blood glucose level of normal diet fed rats.

Experiment II: This part of experiment was designed to demonstrate the effect of O.Sanctum on serum blood glucose level of alloxan induced hyperglycemic rats including group -D & group -E.

Sacrifice of Rats and Collection of Samples

The rats were fasted for 18 hours before collection of blood samples. Sacrifice under ketamine anesthesia after completion of treatment. Blood was collected in test tubes. These were kept in a slanting position till clotting of blood had occurred. Serum was separated from the clot after centrifugation in a centrifuge machine. The serum was collected in small test tubes and kept at 0^{0} c. The samples were stored until analysis of the serum for blood glucose.

O.sanctum Collection: The leaf of Tulsi was collected from BCSIR campus. Leaves were washed in tap water and then left to dry at room temperature for 4-5 days. The dried leaves were crushed to make powder and stored in clean sterile glass container. The dried leaf powder was then added to make up 1% in the diet.

Statistical Analysis: Results were expressed as the mean ±S.D (n=6). All parameters for inter group differences were analyzed by one -way ANOVA. The correlations were evaluated by Simple Regression Analysis.

Result

In Experiment I the effect of O.Sanctum on serum glucose in normal adult male rats was observed and it was found that serum glucose level was slightly changed in O.Sanctum fed rat (Group B) compared to those in the control Group A, but the changes was not statistically significant as shown in Table-I & Figure 1

Table-I: Comparisons of FBG level between day 1 for Group A & day 22 for Group B (A∞B). (N=6)

Test Day	Mean ± SE FBG level (mmol/l)		
1	Group A	5.08 ± 0.14	NS at
22	Group B	5 ± 0.05	p > 0.10 level

SE=Standard Error
N.S=Not significant at p< 0.10

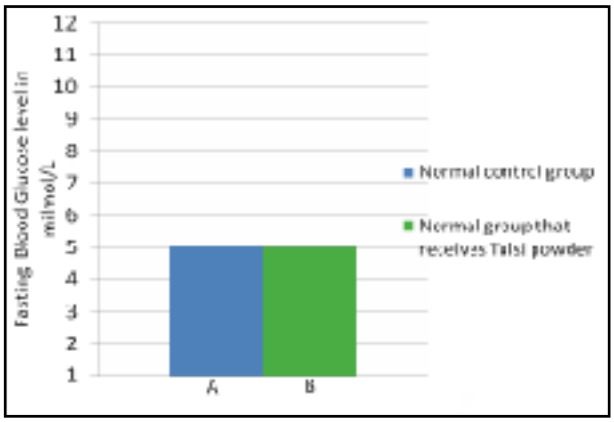

Figure 1: Bar diagram showing the FBG level (mmol/l) on day 1 for Group A & on day 22 for Group B.

In Experiment II the effect of O.Sanctum on the diabetic rats (Group C) was observed and it was found that there

was a significant ($p < 0.01$) increase in serum glucose level compared to those of the normal diet fed rats (Group A) as shown in Table-II & Figure 2

Table-II: Comparisons of FBG level between day 1 for Group A & day 4 for Group C (A∞C). (N=6)

Test Day	Mean ± SE FBG level (mmol/l)		
1	Group A	5.08 ± 0.14	S* Significant at
4	Group C	11.16 ± 0.68	p < 0.01 level

S.E=Standard Error
***Significant at p< 0.01**

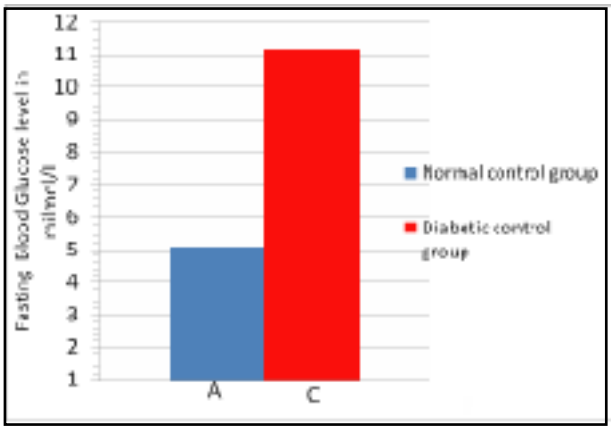

Figure 2: Bar diagram showing the FBG level (mmol/l) on day 1 for Group A & on day 4 for Group C.

In Experiment II the effect of O.Sanctum on the diabetic rats (Group D) was observed and it was found that there was a significant ($p< 0.001$) increase in serum glucose level compared to those of the normal diet fed rats (Group A) as shown in Table-III & Figure 3

Table-III: Comparisons of F.B.G. level between day 1 for Group A & day 22 for Group D (A∞ D). (N=6)

Test Day	Mean ± SE FBG level (mmol/l)		
1	Group A	5.08 ± 0.14	S* Significant at
22	Group D	12.4 ± 0.42	p < 0.001 level

S.E=Standard Error
*Significant at p< 0.001

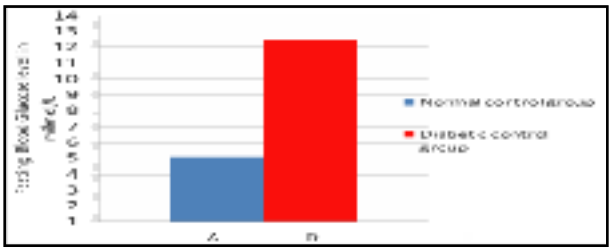

Figure 3: Bar diagram showing the FBG level (mmol/l) on day 1 for Group A & on day 22 for Group D.

Results of blood glucose levels of Group E when compared to Group D showed that there was significantly decreased ($p< 0.01$) as shown in Table-IV & Figure 4 . There was significantly decreased S.glucose level in group D when compared to group E.

Table-IV: Comparisons of F.B.G. level between day 22 for Group D & day 25 for Group E (D∞E). (N=6)

Test Day	Mean ± SE FBG level (mmol/l)		
22	Group D	12.4± 0.42	S* Significant at
25	Group E	10.53 ± 0.19	p < 0.01 level

S.E=Standard Error
***Significant at p< 0.01**

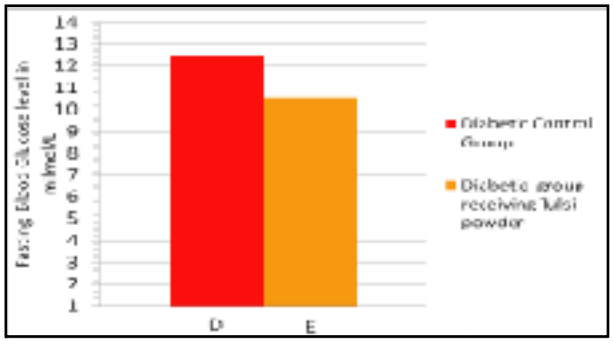

Figure 4: Bar diagram showing the FBG level mmol/l on day 22 for Group A & on day 21(4th to25th) for Group D.

Discussion

The present study provides evidence that the feeding of 1% powder of O.Sanctum (Tulsi) to rats significantly ameliorates the plasma glucose level only in experimentally induced hyperglycemic rats but not in normoglycemic rats, suggesting that Tulsi feeding does

not affect glucose metabolism at the basal levels requisite for normal homeostatic functions of the body. The present research work has been undertaken based upon the above mentioned expectations. The study was carried out to evaluate the effect of O. Sanctum leaf powder on blood glucose level in normal and experimentally induced diabetic rats. O. Sanctum leaf powder 1% in diet was given orally for duration of 21 consecutive days in both normal and alloxan induced diabetic rats.

The dose and route of administration of alloxan was selected as previous observation. The dose of O. Sanctum leaf powder used in this study was selected as dose used[9].The project was divided into two parts: Experiment-1 and Experiment-2. In Experiment-1 the effect of O. Sanctum leaf powder was observed on normal adult Evan rats. Two groups of adult male rats were taken with 6 rats in each group. One group was kept as the control group A which received laboratory diet and the other group B was treated with O. Sanctum leaf powder 1% in diet given orally.

Eugenol has been shown to efficiently inhibit lipid peroxidation. Lipid peroxidation is a marker of cellular oxidative damage initiated by reactive oxygen species. It was reported that diabetics are highly sensitive to oxidative stress. Eugenol (1 hydroxy-2 methoxy- 4 allylbenzene) the active compound present in O.Sanctum. These compounds may be attributed to the fact that the phenolic compound of O.Sanctum extract act as free radical scavengers[10].

In Experiment -2, the effect of O .Sanctum leaf powder was observed in alloxan induced diabetic rats[11]. Eighteen (18) rats were divided into 3 groups, 6 rats allocated per group. Group- C rats was administered 100 mg/kg bw alloxan intraperitoneally and received only lab diet and d /w for 4 days & Group- D for 22 days and served as diabetic control group. Group E was treated with 1% of O. Sanctum leaf powder in diet for 22 days after alloxan induction. The observation suggested that the Mean ± SE FBG level (mmol/l) of Group C on day 4 was 11.16 ± 0.68. The Mean ±SE FBG level (mmol/l) of Group A on day1 was 5.08 ± 0.14. The increase in blood glucose level was highly significant p< 0.01. It was concluded that alloxan is a potent hyperglycemic agent in rats. In this experiment administration of a single dose of alloxan 100 mg/kg bw intraperitoneally in rats caused an increase blood glucose level. The Mean ± SE FBG level (mmol/l) of Group D on day 22 was 12.4 ± 0.42 .The Mean ± SE FBG level (mmol/l) of Group A on day 1 was 5.08 ± 0.15. The increase in blood glucose level was highly significant at p<.001. It was concluded that alloxan is a potent hyperglycemic agent in rats. In this experiment administration of a single dose of alloxan 100 mg/kg bw intraperitoneally in rats caused an increase blood glucose level. The Mean ± SE FBG level (mmol/l) of Group D on day 22 was 12.4 ± 0.42 . The Mean ± SE FBG level (mmol/l) of Group A on day 1 was 5.08 ± 0.15. The increase in blood glucose level was highly significant at p< 0.001. It was conducted that alloxan is a potent hyperglycemic agent in rats. The Mean ± SE FBG level (mmol/l) of Group E who received post treatment with O. Sanctum leaf powder for 25 days after alloxan induction on day 25 was 10.53 ± 0.19. The Mean ± SE FBG level (mmol/l) of Group D who served as diabetic model rat on day 22 was 12.4 ±0.42. The difference in corresponding blood glucose level was statistically significant at p< 0.01. So it was concluded that O.Sanctum leaf powder significantly reduces blood glucose level in alloxan induced diabetic rat. Some researchers like shweta Gupta et al, A. Sarkar & V. Vats et al[12,13,14,15] suggested that, significant decrease in blood glucose level observed in the alloxan induced hyperglycemic rats[11]. Some investigators like in V.RAI, U. IYER, Jyoti Sethi et al, N.A Zeggwagh et al.& Thamolwan, Songsak observed positive influence of tulsi powder in alloxan induced hyperglycemic rats[16,17,18,19]. These results also comply with the present study. In the present study, it was not possible to isolate active ingredients contained in O.Sanctum as this was not within the scope of the present work. Evidence from this study confirms that O. Sanctum leaf powder has hypoglycemic action in experimentally induced diabetic rats. It is now well established that hyperglycemia is almost everywhere a well-known consequence of the aging process. Moreover hypertension, heart failure, CAD, CVD &MI may follow consequently if hyperglycemia is not treated. Therefore, treatment of hyperglycemia is urgent.

Conclusion

From this study, we can be concluded that 1% powder of O.Sanctum exhibits significantly hypoglycemic effect in hyperglycemic adult male rats. It is as well a good source of nutrition that may also act as a prophylactic agent against hyperglycemia related complications. Before stabilizing O.Sanctum as a therapeutically effective hypoglycemic agent, further studies should be carried out to determine the active

principle responsible for hypoglycemic effect and its cellular mechanism of action.

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