ORIGINAL ARTICLE

Antidiabetic Effect of Kernals of *Balanites roxburghii* *i*n Normal and Alloxan-induced Diabetic Rats

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ABSTRACT

The antidiabetic activity of *Balanites roxburghii* was carried out in normal and alloxan-induced diabetic rats. Oral administration of methanolic extract of kernals (0.1 and 0.3g/kg body weight) significantly lowered the blood glucose levels. The activity can be attributed to reducing the intestinal absorption of glucose. The activity reported was dose-dependent.

Keywords: *Balanites roxburghii, Diabetes, Rat*

Diabetes mellitus is a chronic metabolic disorder, caused by insulin deficiency, often combined with insulin resistance. Presently, synthetic oral hypoglycemic agents, insulin products and some herbal medicines are being in practice to control the disorder and there is an increased demand to use natural products with antidiabetic activity due to the side effects associated with the use of insulin and oral hypoglycemic agents [1,2]. *Balanites roxburghii,* a medicinal herb, belongs to family Balanitaceae, found in drier parts of India and Myanmar. It has been reported to contain alkaloids, saponins, flavonoids, phenols, steroids and carbohydrates. Its relative species, *B.aegyptica* was used in Sudanese folk medicine for treatment of jaundice [3]. In Egyptian folk medicine, the fruits are commonly used as an oral antidiabetic drug [4]. An aqueous extract of the mesocarp of the fruits of *B.aegyptiaca* was reported to exhibit a prominent antidiabetic activity in streptozotocin (STZ)-induced diabetic mice [5] and alloxan-induced diabetic rats [6]. So far no work has been reported on the hypoglycemic and antihyperglycemic activity *of B.roxburghii* kernals. Hence, the present study was aimed to determine these activities.

MATERIALS AND METHODS

The plant was authenticated by Prof. Raju S. Vastavaya, Taxonamist, Department of Botany, Kakatiya University, Warangal. Fresh fruits (Voucher number: PLB-049, deposited in: Herbarium, director: Prof. Raju S. V.) from the plant were collected and kernals were separated. Dried kernals were converted to coarse powder, macerated with methanol (30:70) and filtered. The filtrate was air dried giving an extract (MBR, yield: 15% w/w). The extract was suspended in gum acacia in water as a suspending agent for the purpose of oral administration. Male Wister rats (180-220g) were used. They were kept at $25 \pm 2°C$ in a 12h light dark cycle and fed the standard pellet rat diet and water ad libitum. Institutional Animal Ethics Committee, constituted under the guidelines of CPCSEA, Ministry of Environment, Govt. of India, New Delhi, approved all the animal experimental protocols. The MBR was administered orally in doses of 0.1, 0.3, 1 and 2g/kg body weight to groups of rats (n = 6) and percentage mortality was noted beginning at 24 h up to a period of 7 days.

Table 1. Hypoglycemic effect of MBR in normal rats

Treatment group	Dose g/kg (p.o)	Blood glucose levels (mg/dL) Pre-treatment/Post-treatment (h)						
		0	2	4	6	8	12	24
Control (Group-I)	-	70.07 ± 10.64	68.88 ± 8.10	68.07 ± 4.91	69.88 ± 5.52	70.69 ± 6.34	69.79 ± 7.69	72.08 ± 8.38
Glimeperide (Group-II)	0.01	72.60 ± 5.03	47.71 ± 8.76 (30.26)[***]	48.71 ± 7.08 (32.79)[***]	52.50 ± 10.36 (27.78)[*]	54.05 ± 8.52 (25.30)[*]	56.40 ± 9.90 (17.32)[*]	71.45 ± 8.15
MBR (Group-III)	0.1	76.83 ± 4.34	77.38 ± 7.40	76.85 ± 4.21	65.37 ± 9.14 (15.06)	54.15 ± 7.57 (29.28)[**]	70.46 ± 8.34 (7.99)	76.95 ± 8.45
MBR (Group-IV)	0.3	73.20 ± 6.85	74.56 ± 7.06	71.40 ± 8.88 (2.27)	54.40 ± 8.83 (25.44)[*]	50.08 ± 10.20 (31.83)[**]	62.64 ± 7.15 (13.65)	72.31 ± 5.35

All values are expressed as mean ± SEM of six rats in each group. Values given in the parenthesis are percent blood glucose reduction as determined in comparison with levels at time = 0, in corresponding group. Statistically significant *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ as compared to control respective time.

Table 2. Oral Glucose Tolerance Test of MBR in normal rats

Treatment group	Dose g/kg (p.o)	Blood glucose levels (mg/dL) Pre treatment/Post treatment (h)					
		0 min	30 min	60 min	90 min	120 min	240 min
Control (Group-I)	-	73.08 ± 7.75	126.73 ± 7.66	128.70 ± 6.95	104.09 ± 8.05	81.71 ± 7.66	77.75 ± 3.47
Glimeperide (Group-II)	0.01	72.37 ± 7.24	89.04 ± 5.62 [***]	82.80 ± 5.36 [**]	68.16 ± 6.97 [*]	54.07 ± 6.85	52.47 ± 3.82
MBR (Group-III)	0.1	73.12 ± 9.51	118.23 ± 10.58	121.61 ± 10.82	91.19 ± 6.41 [**]	80.78 ± 11.82	67.55 ± 10.93
MBR (Group-IV)	0.3	76.72 ± 8.80	117.42 ± 6.63	113.37 ± 6.17 [*]	80.33 ± 4.66 [***]	74.35 ± 6.03	63.19 ± 10.90

All values are expressed as mean ± SEM of six rats in each group. Statistically significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ as compared to control respective time.

Pharmacological activity
Effect on normal rats

Rats were divided in four groups of six rats each. The blood glucose concentration of fasted rats of all groups was determined at zero time as: Group I (control rats); group II (MBR 0.1g/kg); group III (MBR 0.3g/kg), group IV (Glimeperide 0.01g/kg). All groups were treated orally. Serum glucose concentration of rats of all groups was determined at 2, 4, 6, 8, 12 and 24 h later.

Effect on oral glucose tolerance test (OGTT)

Rats were divided in four groups of six rats each. Initial glycemia was determined in fasted rats of all groups as Group I (control rats); group II (MBR 0.1g/kg); group III (MBR 0.3g/kg), group IV (Glimeperide 0.01g/kg). All groups were treated followed by glucose (50% w/v glucose in distilled water) administration at a dose of 2g/kg orally. Blood glucose levels were determined at 30, 60, 90, 120, 180 and 240 min.

Effect on alloxan-induced diabetic rats

Diabetes was induced by single intra-peritoneal administration of alloxan monohydrate in rats (0.125 g/kg). The blood samples were collected on 15th day and blood glucose levels were estimated. Rats having blood glucose levels above 300 mg/dL were selected for further experiments and divided in four groups of six

rats each. The blood glucose concentration of glucose was determined at zero time. All groups were treated orally. Serum glucose concentration of rats of all groups was determined at 2, 4, 6, 8, 12 and 24 h later.

Collection of blood and determination of blood glucose

Blood samples were collected from retro-orbital plexus [7] and the glucose content was determined using glucose-oxidase method [8].

Effect on glucose uptake by isolated rat hemidiaphram

Animals were fasted for overnight. They were killed by decapitation and diaphragms were taken out quickly avoiding trauma and divided into two halves. The hemidiaphragms were then rinsed in cold Tyrode solution (without glucose) to remove any blood clots and were placed in a small conical flasks containing 2 ml tyrode solution with 2 g/L glucose and incubated for 30 min at 37°C in an atmosphere of 95% O_2 - 5% CO_2 with shaking at 140 cycles per min. Four sets of experiments were performed [9].

All the groups contained three animals each. The animals were killed by decapitation and diaphragms were exposed. Group I received tyrode solution with glucose (2 g/L) only and served as control. Group II received tyrode solution with glucose (2 g/L) + Insulin (0.25 IU/mL). Group III received tyrode solution with

Table 3. Effect of MBR in Alloxan-induced diabetic rats

Treatment group	Dose g/kg (p.o)	Blood glucose levels (mg/dl) Pre treatment/Post treatment (h)						
		0 h	2 h	4 h	6 h	8 h	12 h	24 h
Control (Group-I)	-	338.42 ± 17.96	333.43 ± 14.58	336.35 ± 16.72	338.91 ± 15.44	336.70 ± 12.28	340.19 ± 18.87	342.71 ± 14.73
Glimeperide (Group-II)	0.01	352.75 ± 14.00	224.15 ± 9.64 (36.35)***	235.01 ± 15.72 (33.28)***	267.69 ± 14.39 (23.98)***	282.37 ± 15.82 (19.80)***	301.18 ± 10.74 (14.49)***	340.49 ± 7.43
MBR (Group-III)	0.1	333.93 ± 15.13	334.95 ± 13.77	333.11 ± 11.94 (0.60)	313.92 ± 10.05 (5.91)*	289.72 ± 7.34 (13.11)***	326.86 ± 5.51 (2.01)	333.52 ± 18.43
MBR (Group-IV)	0.3	345.17 ± 13.04	346.26 ± 16.92	329.68 ± 14.98 (4.38)	277.06 ± 14.29 (19.65)***	247.61 ± 17.30 (27.47)***	280.97 ± 14.08 (18.54)***	330.00 ± 12.88

All values are expressed as mean ± SEM of six rats in each group. Values given in the parenthesis are percent blood glucose reduction as determined in comparison with levels at time = 0, in corresponding group. Statistically significant, $*p < 0.05$, $***p < 0.001$ as compared to control respective time.

Table 4. Effect of MBR on glucose uptake by isolated rat hemidiaphram

Treatment group	Incubation medium	Glucose uptake (mg/g per 30 min)
I	Tyrode solution with glucose (2 g/L) Control group	4.06 ± 1.13
II	Tyrode solution with glucose (2 g/L) + Insulin (0.25 IU/mL)	8.90 ± 4.01***
III	Tyrode solution with glucose (2 g/L) + MBR (0.025 g/mL)	4.78 ± 2.21
IV	Tyrode solution with glucose (2 g/L) + Insulin (0.25 IU/mL) + MBR (0.025 g/mL)	7.58 ± 4.49***

Results are expressed as mean ± SEM from three observations. $***p < 0.001$ vs. control.

glucose (2 g/L) + MBR (0.025 g/mL). Group IV recieved tyrode solution with glucose (2 g/L) + Insulin (0.25 IU/mL) + MBR (0.025 g/mL). Following incubation, the hemidiaphragm were taken out and weighed. The glucose content of the incubated medium was measured by GOD/POD, enzymatic method. Glucose uptake was calculated as the difference between the initial and final glucose content in the incubation medium.

Statistical evaluation

Data were expressed as means ± standard error mean. Statistical comparisons were made using one-way ANOVA followed by Newman-Keuls multiple comparison test. The blood sugar levels following administration of each extract at each dose and standard were compared with those of control at each time point.

RESULTS

MBR at the dose of 0.1 and 0.3 g/kg produced a progressive reduction in serum glucose levels at 6h and 8 h ($p < 0.01$) followed by an increase in glucose level at the end of 12 h. From this study, it is evident that the significant decrease in serum glucose levels at 8 h of two doses is comparable to that of the standard. Results are presented in Table 1. The results presented in Table 2 show that the non-diabetic control rats produced rapid increase in the serum glucose levels followed by a progressive decline until they nearly reached control values at the end of 120 min of investigation. MBR 0.1 and 0.3 g/kg decreased the increase in blood glucose

levels significantly ($p < 0.01$, $p < 0.001$). The maximum glucose tolerance was observed at 90 min for both doses of MBR, while in case of standard, the glucose levels reached the fasting values at the end of 60 min. The results shown in Table 3 indicate that 0.1 and 0.3 g/kg dose of MBR significantly ($p<0.001$) reduced the hyperglycemia induced by alloxan. The percent of reduction in blood glucose levels was maximum at the 8h for MBR groups. Antihyperglycemic activity of the MBR 0.3 g/kg was more than that of 0.1 g/kg at the end of every 2 h. Further, the effect of high dose was comparable to that of the standard. The high and low doses of MBR reduced the blood glucose levels to the extent of 13.11% and 27.47%, while standard 19.80% at the 8h. *In-vitro* experiments, the insulin increased the glucose utilization of an isolated rat hemidiaphragm. When compared MBR 0.025 g/ml to the insulin 0.25 IU/ml ($p < 0.001$) and the combination of insulin and MBR ($p < 0.01$) did not show a marked increase of glucose uptake (Table 4).

DISCUSSION

Diabetes mellitus may be regarded as the world's largest growing metabolic disease, and as the knowledge on the heterogeneity of this disorder is advanced, the need for more appropriate therapy increases. Traditional plant medicines are being used throughout the world for diabetis. The study of such medicines might offer a natural key to unlock a diabetologist's pharmacy for the future.

The MBR was used to study the effect in normoglycemic and hyperglycemic rats. From the

results obtained, it is obvious that the MBR produced a statistically significant decrease in blood glucose concentration in both normoglycemic and alloxan-induced hyperglycemic rats. The results obtained were moderately consistent with the studies carried out by Saeed *et al* [6] on the aqueous extract of mesocarp of *B. roxburghii* in alloxan-induced diabetic rats. An emphasize is laid on glucose homeostasis as a severe hypoglycemia can result in life threatening situation. Therefore, lesser hypoglycemic effect with the MBR in normoglycemic animals compared to hyperglycemic animals is a desirable feature.

No histological studies were carried out and it is not possible to explain the detailed mechanism of antidiabetic action of MBR. However, since our results showed that glimeperide immediately reduced the blood glucose levels in hyperglycemic animals, the state of diabetes is not severe. Alloxan-treated animals receiving the MBR showed normalization of blood glucose levels in comparison to the control and this could be due to the possibility that some -cells are still surviving to exert their insulin releasing effect by MBR. Moreover, like sulphonylureas, oral administration of MBR produced hypoglycemia in normal animals. This suggests that the mode of action of the active constituent(s) of MBR is probably mediated by an enhanced secretion of insulin, like sulphonylureas. The *in-vitro* studies suggested that MBR probably has no direct insulin-like effect, which may enhance the peripheral utilization of glucose.

In conclusion, the results of the present study show that *B.roxburghii* kernals possess active constituents like saponins capable of lowering blood glucose levels and hence provide some scientific evidence for the folk use of this drug. Further detailed investigation on the search of *B.roxburghii is* necessary to prove and develope it as an herbal antidiabetic drug.

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