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Anti-Diabetic Activity of the Methanolic Extract of *Blumea lacera* DC (*Asteraceae*) in Alloxan-induced Diabetic Rats

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Anti-diabetic activity of the methanolic extract of *B. lacera* was studied in alloxan-induced (150 mg/kg) in diabetic rats, after oral adminis-tration, at doses of 125, 250, 500, 750 and 1000 mg/kg. A long term hypoglycemic activity of methanolic extract of *B. lacera* was also performed. Biochemical parameters studied included triglyceride, total cholesterol, high density lipoprotein, low density lipoprotein and very low density lipoprotein. Fasting blood glucose and biochemical parameters were studied on the day 0, 7 and 14. The data was compared statistically by employing one way analysis of variance. Oral administration of methanolic extract (500 mg/kg) to alloxan-induced diabetic rats for 14 days duration restored blood glucose levels to normal values. The present study clearly indicated a significant antidiabetic activity of the methanolic extract of *B. lacera*, thus making the plant *B. lacera* more valuable in treating diabetes.

Key Words: Anti-diabetic activity, *Blumea lacera*, Methanolic extract.

INTRODUCTION

B. lacera DC (*Asteraceae*) is an annual plant widely distributed in Asia, has strong odour of turpentine and is used as febrifuge, anthelmintic, antipyretic and diuretic in folk medicines. It is also used to cure bronchitis, blood diseases, fever and to alleviate burning sensations[1].

Diabetes mellitus is the common metabolic disorder characterized by hyperglycemia. There are estimated 143 million people worldwide sufferings from the disease and this is almost five times the estimate 10 years ago[2]. It has been predicted that the number may probably double by the year 2030[3]. Therefore, the human population worldwide appears to be in the midst of an epidemic of diabetes. The introduction of insulin and later oral hypoglycemic agents revolutionized the management of diabetes mellitus. In spite of advances in drug management of diabetes, there are still complications and adverse drug reactions. None of them were unequivocally successful in maintaining normal blood glucose levels and in avoiding complications. In spite of all the advances in therapeutics, diabetes still remains a major cause of morbidity and mortality in the world. Although, there are numerous traditional medicinal plants reported to have hypoglycemic properties many of them have not yet been studied officially for anti-diabetic activity. Further, most of the hypoglycemic agents used in allopathic medicine are reported to have side effects in the long run. Therefore, there is need of public health to search for effective and safe drugs for diabetes[4].

In our previous publication, antibacterial and antioxidant activities of *B. lacera* has been studied[1]. In the present study the methanolic extract of *B. lacera* was evaluated for anti-diabetic activity in alloxan-induced diabetic rats.

EXPERIMENTAL

The fresh whole plants of *B. lacera* voucher no. (GC. Herb. Bot. 125) were collected in the month of March from Government College University, Lahore Campus. The plant was identified at Department of Botany, Government College University, Lahore, Pakistan. The plant material was dried, powdered and soaked in methanol for 7 days at room temperature. The extract was filtered and evaporated using a rotary evaporator (Laborta 4000-Hedolph).

Animals used: Healthy adult (male and female) Swiss albino rats weighing 180 ± 20 g (obtained from Veterinary Research Institute, Lahore) were used in these experiments. The animal experiments were preceded following the internationally accepted ethical guidelines for the care of laboratory animals. The animals were kept in an air-conditioned animal house (22 ± 2 ºC with a 12 h light/dark cycle) in the Department of Zoology, Government College University, Lahore. The animals were given a commercial feed prepared by Purina and allowed tap water *ad libitum*.

TABLE-1
ANTI-DIABETIC EFFECTS OF THE METHANOLIC EXTRACT (ME) OF *Blumea lacera* ON BLOOD GLUCOSE LEVEL IN ALLOXAN-INDUCED DIABETIC RATS

Groups		Mean blood glucose concentration (mg/dl) ± S.E.M			
		0 h	2 h	4 h	6 h
I.	Normal control (0.5 % CMC)	84.0 ± 2.42	82.5 ± 2.30 (1.74 %)	83.1 ± 2.13 (0.98 %)	82.9 ± 2.23 (1.19 %)
II.	Diabetic control (0.5 % CMC)	264.5 ± 2.60	262.0 ± 3.33 (0.97 %)	262.6 ± 2.91 (0.72 %)	263.6 ± 2.58 (0.33 %)
III.	Diabetic + ME (125 mg/kg)	267.0 ± 1.68	258.7 ± 0.84 (3.06 %)	256. 5 ± 1.22 (3.89 %)	259.6 ± 1.35 (2.74 %)
IV.	Diabetic + ME (250 mg/kg)	290.8 ± 2.40	284.1 ± 1.84* (2.27 %)	266.5 ± 1.63* (8.33 %)	268.4 ± 2.81* (7.70 %)
V.	Diabetic + ME (500 mg/kg)	257.6 ± 1.92	246.1 ± 3.09* (4.46 %)	230.2 ± 1.77* (10.62 %)	232.0 ± 2.12* (9.90 %)
VI.	Diabetic + ME (750 mg/kg)	286.7 ± 2.80	271.6 ± 3.44* (5.28 %)	251.5 ± 5.64* (12.25 %)	257.6 ± 2.86* (10.17 %)
VII.	Diabetic + ME (1000 mg/kg)	256.0 ± 2.94	242.9 ± 2.64* (5.08 %)	216.4 ± 3.17* (15.47 %)	224.4 ± 3.31* (12.34 %)
VIII.	Diabetic + tolbutamide (100 mg/kg)	282.8 ± 2.85	237.0 ± 0.90* (16.15 %)	183.9 ± 2.35* (34.93 %)	190.5 ± 1.84* (32.60 %)

n = 6; Values are mean ± S.E.M; CMC: carboxymethyl cellulose; In parenthesis, mean % reduction in blood glucose is shown.; * $p < 0.05$; the mean difference is significant from normal and diabetic control at the 0.05 level.

Induction of diabetes in rats: Diabetes was induced by a single intraperitonial injection of alloxan-monohydrate (single dose of 150 mg/kg) dissolved[5] in freshly prepared normal saline in a volume of 1 mL/kg. After 7 days of alloxan monohydrate administration, rats with blood glucose levels of 250 mg/dl and above were considered as diabetic and were employed in the study.

Determination of immediate effect of methanolic extract of *B. lacera* on blood glucose level: Groups I and II were regarded as normal and diabetic controls respectively. Groups III, IV, V, VI and VII were rendered diabetic and given the methanolic extract of *B. lacera* orally at doses of 125, 250, 500, 750 and 1000 mg/kg respectively. Group VIII was given tolbutamide at a dose of 3 mg/kg orally for each animal. Group I and II the normal and diabetic control received 0.5 % carboxymethyl cellulose (CMC).

Determination of long term effect of methanol extract of *B. lacera* on blood glucose level: Methanolic extract and standard drug tolbutamide (100 mg/kg) were administered once daily, for 2 weeks to alloxan-induced diabetic rats which were divided into three groups (II- IV). Control groups (I and II) as administered orally only vehicle (0.5 % CMC) during 14 days. Group III received methanol extract (500 mg/kg) daily. All the tested materials were suspended in the same vehicle and administered daily for 14 days. The animals were housed under standard laboratory conditions and maintained with free access to water and food during all the experiment. Blood glucose was estimated at days 0, 7 and 14 on both diabetic groups (II- IV) and control group (I) animals. All the experiments were carried out using six animals per group.

Collection of blood samples and determination of blood glucose levels: Blood samples were collected from the vein of tail at 0, 2, 4 and 6 h. Blood glucose concentration (mg/dl) were estimated by using standard kit (Randox Laboratories Crumlin, Co. Antrim, Bt29 4QY, United Kingdom) based on glucose oxidase method. The percentage variation of glycemia for each group was calculated with respect to initial (0 h) level according to:

$$\% \text{ Variation of glycemia} = [(Gi\text{-}Gt)/Gi] \times 100 \%$$

where, Gi is initial glycemia values and Gt is the glycemia value after samples administration[6].

Determination of biochemical parameters: Biochemical parameters; triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were measured using standard kits (Randox Laboratories CrumLin, Co. Antrim, Bt29 4QY, United Kingdom). Low density lipoprotein and very low density lipoprotein were calculated by Friedewalds formula[7] as described below:

(1) VLDL: TG/5; (2) LDL: TC - (HDL + VLDL).

LD_{50}: The LD_{50} of methanolic extract of *B. lacera* was determined in normal Swiss albino rats. After fasting overnight, various doses (500 - 5000 mg/kg) of methanolic extract was given orally to rats divided into six groups each comprising four rats. The animals were observed for 1 h continuously and then hourly for 4 h for any gross behaviour changes and for a further 24 h for any mortality.

Statistical analysis: Statistical analysis was performed using SPSS software package, version 13.0. The values were analyzed by one way analysis of variance (ANOVA) followed by post-hoc (Tukey HSD) test. All the results were expressed as mean ± S.E.M. for six rats in each group. Values of $P < 0.05$ were taken as significant.

RESULTS AND DISCUSSION

Acute effect of methanolic extract on blood glucose: The effects of the methanolic extracts of *B. lacera* on blood glucose are shown in Table-1. Oral administration of the methanolic extracts of *B. lacera* with different doses significantly reduced the hyperglycemia in alloxan-induced diabetic rats. The methanolic extract of *B. lacera* produced a dose-dependent hypoglycemia in alloxan-induced diabetic rats. It produced statistically significant reduction in blood glucose of 8.33 % ($p < 0.05$), 10.62 % ($p < 0.05$), 12.25 % ($p < 0.01$) and 15.47 % at 4th h with doses of 250, 500, 750 and 1000 mg/kg respectively (Table-1). Similarly, the blood glucose lowering activity of methanol extracts at 6th h were 7.70 % ($p < 0.05$), 9.90 % ($p < 0.05$), 10.17 % ($p < 0.05$) and 12.34 % ($p < 0.05$) at the doses of 200, 300, 400 and 500 mg/kg. Tolbutamide (100 mg/kg) produced a significant 34.93 % ($p < 0.01$) and 32.60 % reduction in blood glucose at 4th and 6th h compared to control animals. Normal and diabetic control groups did no show any hypoglycemia in animals. Methanolic extract at the dose of 125 mg/kg did not show any hypoglycemic effect; 3.06, 3.89 and 2.74 % (p: 0.99, 0.78 and 0.97, respectively when compared with normal controls) at 2, 4 and 6 h, respectively. Tolbutamide (100 mg/kg) showed statistically significant hypoglycemic effects and the glucose level comes at 237.0 ±

TABLE-2
EFFECTS OF ORAL ADMINISTRATION OF THE METHANOLIC EXTRACT (ME) OF *Blumea lacera* ON FASTING BLOOD GLUCOSE AND TOTAL LIPID PROFILE IN ALLOXAN INDUCED DIABETIC RATS

Experimental animals	Dose	Mean blood glucose concentration ± S.E.M. (mg/dl) in diabetic animals		
		0^{th} day	7^{th} day	14^{th} day
Fasting blood glucose (mg/dl)				
Normal control	0.5 % CMC	84.6 ± 2.38	86.1 ± 3.12	83.7 ± 2.32
Diabetic control	0.5 % CMC	295.2 ± 2.42	298.6 ± 3.37	299.5 ± 3.33
Diabetic + ME	500 mg/kg	308.2 ± 5.99	185.6 ± 2.60*	115.6 ± 3.59*
Diabetic + tolbutamide	100 mg/kg	286.3 ± 4.12	168.6 ± 3.58*	106.8 ± 3.59*
Total cholesterol (mg/dl)				
Normal control	0.5 % CMC	77.3 ± 3.59	80.0 ± 3.04	79.3 ± 1.12
Diabetic control	0.5 % CMC	136.0 ± 2.78	139.1 ± 3.02	140.7 ± 3.47
Diabetic + ME	500 mg/kg	131.5 ± 4.47	101.3 ± 4.18*	95.6 ± 4.08*
Diabetic + tolbutamide	100 mg/kg	141.4 ± 4.18	111.5 ± 2.73*	90.5 ± 4.91*
Triglyceride (mg/dl)				
Normal control	0.5 % CMC	56.1 ± 3.15	59.8 ± 3.48	61.3 ± 4.04
Diabetic control	0.5 % CMC	104.3 ± 2.47	107.8 ± 3.33	111.9 ± 3.61
Diabetic + ME	500 mg/kg	107.2 ± 5.55	95.9 ± 2.77*	96.2 ± 1.60*
Diabetic + tolbutamide	100 mg/kg	160.7 ± 4.74	141.7 ± 4.77*	113.2 ± 4.27*
High density lipoprotein (mg/dl)				
Normal control	0.5 % CMC	41.1 ± 1.43	42.9 ± 2.98	45.0 ± 2.02
Diabetic control	0.5 % CMC	53.2 ± 2.47	56.7 ± 2.09	54.8 ± 1.01
Diabetic + ME	500 mg/kg	41.2 ± 1.56	49.4 ± 2.34*	56.2 ± 1.96*
Diabetic + tolbutamide	100 mg/kg	39.3 ± 1.34	51.0 ± 3.14*	59.1 ± 0.97*
Low density lipoprotein (mg/dl)				
Normal control	0.5 % CMC	65.3 ± 3.52	64.8 ± 5.72	64.9 ± 2.48
Diabetic control	0.5 % CMC	23.0 ± 3.79	22.7 ± 3.45	28.3 ± 3.45
Diabetic + ME	500 mg/kg	41.8 ± 7.24	28.3 ± 3.12*	24.3 ± 2.96*
Diabetic + tolbutamide	100 mg/kg	93.2 ± 4.93	68.2 ± 6.98*	35.9 ± 4.65*
Very low density lipoprotein (mg/dl)				
Normal control	0.5 % CMC	15.5. ± 0.72	16.0 ± 0.61	15.9 ± 0.22
Diabetic control	0.5 % CMC	27.2 ± 0.56	27.8 ± 0.60	28.1 ± 0.69
Diabetic + ME	500 mg/kg	26.3 ± 0.89	20.3 ± 0.96*	19.1 ± 0.82*
Diabetic + tolbutamide	100 mg/kg	28.3 ± 0.84	22.3 ± 0.55*	18.1 ± 0.98*

n = 6; Values are mean ± S.E.M; Treatment = 14 days, dose 500 mg/kg bw; * $p < 0.05$ (Represents statistical significance *versus* control animals)

0.90, 183.9 ± 2.35 and 190.5 ± 1.84 mg/dl (at 2, 4 and 6 h, respectively) from the initial value of 282.8 ± 2.85 mg/dl at 0 h.

Long term effect of methanolic extract on blood glucose: The long term hypoglycemic effect of methanolic extract on alloxan induced diabetic rats are shown in Table-2. The results demonstrated that oral administration of methanolic extract (500 mg/kg) of *B. lacera* once a day for 14 days produced statistically significant hypoglycemic effects in the alloxan-induced diabetic rats. Fasting blood glucose (FBG) levels were measured on the day 0, 7^{th} and 14^{th}. High fasting blood glucose level exhibited by all diabetic rats on the day 0, compared to normal control rats (group III; methanol extract: 308.2 ± 5.99 mg/kg, group IV, tolbutamide: 286.3 ± 4.12 mg/kg and vehicle: 295.2 ± 2.42 mg/kg) *vs* healthy rats: 84.6 ± 2.38 mg/dl). A significant blood glucose lowering effect (185.6 ± 2.60 mg/dl) was observed by methanol extract on alloxan-induced diabetic rats on the day 7. Finally on the 14^{th} day of the experiments, blood glucose concentration reached almost the normal value of 115.6 ± 3.59 mg/dl ($P < 0.05$; 62.49 ± 1.01 % reduction) from the initial value of blood glucose 308.2 ± 5.99 mg/dl by the regular oral administration of methanol extract (500 mg/kg) once daily. Tolbutamide (100 mg/kg) exhibited statistically significant blood glucose lowering activity, attaining the final blood glucose level of 106.8 ± 3.59 mg/dl, on the day 14 from the initial fasting blood glucose level: 286.3 ± 4.12 mg/dl and 168.6 ± 3.58 mg/dl on 0 and 7th days respectively. Normal and diabetic control did not show any hypoglycemic effects throughout the experiment.

Effect of methanolic extract on total lipid profile: The effect of the administration of methanolic extract for 14 days on total lipid profile is shown in Table-2. Total cholesterol in the diabetic control group was significantly higher (104.3 ± 2.47 mg/dl) compared to (56.1 ± 3.15 mg/dl) the normal control, which remained high at the end of the experiment on 14^{th} day. However, total cholesterol (TC) significantly decreased from 104.3 ± 2.47 mg/dl on 0^{th} day to 107.8 ± 3.33 mg/dl and 111.9 ± 3.61 mg/dl on 7^{th} and 14^{th} day of treatment with the methanolic extract. In the methanolic extract treated group, LDL level was significantly decreased to 28.3 ± 3.12 mg/dl and 24.3 ± 2.96 mg/dl on the 7^{th} and 14^{th} day, respectively from an initial value of 41.8 ± 7.24 mg/dl. Triglyceride levels were also reduced close to the normal values by administration of the extract for 14 days. The values decreased to 101.3 ± 4.18 mg/dl on 7^{th} day and to 95.6 ± 4.08 mg/dl on 14^{th} day from initial value of 131.5 ± 4.47 mg/dl at the beginning of the experiment. Reduction in the VLDL level was also observed in the extract treated group, while no such reduction was seen in the diabetic control group. Treatment with the extract caused significant increase in HDL-cholesterol values from 41.2 ± 1.56 mg/dl on 0 day to 49.4 ± 2.34 mg/dl and 56.2 ± 1.96 mg/dl

on 7th and 14th day, respectively. No significant change in the lipid profile of the animals in the normal control group during the experimental period.

LD_{50}: Experiment was carried out on normal healthy rats. The behaviour of the treated rats appeared normal. No toxic effect was reported up to 5 to 10 times of the effective dose of the methanol extract of *B. lacera* and there were no deaths in any of these groups.

Diabetes mellitus is possibly the world's largest growing metabolic disease[8]. Traditional plant medicines are used throughout the world for a range of diabetic complications. The study of such medicines, might offer a natural key to unlock a diabetologist's pharmacy for the future. Present results showed that tolbutamide reduced blood glucose levels in alloxan-induced diabetic rats. In this study, lower dose (125 mg/kg) did not show any blood glucose lowering activity. While at higher doses of 750 and 1000 mg/kg, the methanolic extract of *B. lacera* exhibited stronger antidiabetic activity with percentage reduction 12.25 ± 1.95 and 15.50 ± 0.59 % at 4th and 6th h in alloxan-induced diabetic rats.

It has been reported that to obtain maximum effect, therapy with plant products should be continued for longer duration[9]. To obtain the maximum effect of methanol extract on the diabetic rats, the extract was administered daily, once at a dose of 500 mg/kg for 14 days, the period which produced a significant reduction on fasting blood glucose of diabetic animals and this effect was more potent after repeated oral administration than after single dose administration. These results confirm the previous findings that the effectiveness of the drugs depends, probably, on the cumulative effect of active principles[10]. Thus, methanol extract effectively controls hyperglycemia and maintain normoglycemia, which may prevent the microvascular complications in diabetes. Diabetes mellitus is usually associated with abnormal levels of serum lipids and such an increase causes the risk factor for coronary heart diseases[11]. The marked increase in blood glucose and associated lipid levels characterize the uninhibited actions of lipolytic hormones on the fat deposits[12]. Lowering of serum lipid concentration through drug therapy or dietary measures seems to decrease the risk of vascular diseases[13]. Increase of lipid, triglyceride and total cholesterol levels in alloxan diabetic rats observed in the present study may be a result of increased breakdown of lipids and mobilization of free fatty acids from the peripheral depots. Regular administration of methanol extract for 14 days normalized lipid profile in diabetic animals. The dose of 500 mg/kg not only lowered the total cholesterol, triglyceride and LDL but also enhanced the LDL-cholesterol, which is considered to be a cardio protective lipid. Thus, the methanol extract of *B. lacera* has significant impact in improving the imbalance in lipoprotein metabolism[12].

These results indicated that the methanol extract of *B. lacera* possesses significant antidiabetic activity. The dose of 500 mg/kg is an effective dose for further *in vivo* studies of *B. lacera*. Administration of methanol extract of *B. lacera* to the diabetic rats for 14 days not only significantly lowered the fasting blood glucose of the diabetic animals to the normal level, but also caused improvement on lipid profile of the diabetic animals. Methanolic extract of *B. lacera* found to have high margin of safety and thus *B. lacera* seems to have a promising value for the development of a potent phytomidicine for diabetes, though further comprehensive pharmacological investigations are needed to elucidate the exact mechanism of action of the *B. lacera* methanolic extract.

Present study clearly indicated a significant antidiabetic activity of the methanol extract of *B. lacera* and can be used as phytomedicine for the control of diabetes. Hence, it might help in preventing diabetic complications. Further works on the fractionation, purification and identification of the active principle(s) present in *B. lacera* will be the next step in our laboratory.

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