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### Evaluation of Antidiabetic Activity of Methanolic Extracts from the Aerial Parts of *Barleria montana* in Streptozotocin Induced Diabetic Rats

Shyam T<sup>1\*</sup>, Ganapaty S<sup>1</sup>

1. College of Pharmaceutical Sciences, Andhra University, Visakhapatnam-530003, A.P, India.  
[E-mail address: shyam14m@gmail.com; Tel: +91-7893194373]

Based on ethnopharmacological information, *Barleria montana* has been used to treat diabetes by the tribals in and around tropical and subtropical areas. But there are no more scientific reports available about the antidiabetic activity of this plant. Hence the study was carried out to ascertain the activity. The plant was extracted with methanol in soxhlet apparatus and the extracts thus obtained were examined for acute toxicity studies in wistar albino rats at different doses upto 2000 mg/kg body weight. The plant extracts were also evaluated for antidiabetic activity at a dose levels of 100, 200 and 400 mg/kg in streptozotocin induced diabetic rats. The plant extracts have not produced any toxic symptoms within the treated animals. The maximum reduction in blood glucose level was observed at 8 and 12<sup>th</sup> hr. after the oral administration of the 400 and 200 mg/kg b. w of methanolic extract of *Barleria montana* aerial parts respectively. From the observations, it was concluded that the reduction of blood glucose levels in diabetic rats was found to be dose dependent and also dependent on duration of action. So it might be useful in the treatment of diabetes without toxicity.

**Keyword:** *Barleria montana*, Acanthaceae, Antidiabetic activity, Streptozotocin

#### 1. Introduction

Diabetes mellitus is a group of metabolic diseases manifested as the presence of higher concentrations of glucose in the blood because of improper production of insulin from pancreas or inactivity of cells to the insulin. It is characterized by recurrent or persistent hyperglycemia, which produces classical symptoms such as polyuria, polydipsia and polyphagia. In view of the formulations with the synthetic drugs caused side effects, the research was initiated with an outcome of bioactive antidiabetic principles from plant origin<sup>[1-3]</sup>.

*Barleria montana* (Family:Acanthaceae) is widely distributed in the hills of exposed slopes, plains among rocks and at higher elevations<sup>[1]</sup>.

The leaf of *Barleria montana* occupied a major place in traditional system of medicine for its usage in the treatment of diabetes, wounds, and cuts. It can also be useful to mitigate hepatic disorders caused by repeated administration of therapeutic drugs<sup>[2,3]</sup>. Root paste when applied externally used for the treatment of rheumatism and joints pain<sup>[4]</sup>. The plant has been reported to possess antioxidant activity and hepatoprotective activity<sup>[5,6]</sup>. The aim of study was to investigate acute toxicity studies and antidiabetic activity of *Barleria montana* aerial parts as the plant being used to treat the symptoms of diabetes by rural people and to provide scientific evidence for further usage of plant in diabetes.

## 2. Materials and Methods

### a. Collection of Plant Material

The plant, *Barleria montana* (2kg), was collected from Tirupati hills in the month of September. The plant was authenticated by Prof.M.Venkaiah, Department of Botany, Andhra University and the specimen was deposited in the herbarium (Voucher specimen number (BM/01)).

### b. Preparation of Extracts

The aerial parts of *Barleria montana* were dried in shade and then powdered in a Wiley mill. The powdered drug was extracted in a soxhlet apparatus for 6 hrs successively with petroleum ether, chloroform and methanol and concentrated in a rotary evaporator which resulted pet ether extract, chloroform extract and methanolic extract. Different dose levels of plant extracts were prepared in 1% sodium carboxy methyl cellulose solution and were used for studies.

### c. Animals

The experimental protocol was approved by the institutional animal ethics committee of Andhra university, Vishakhapatnam , which was registered with Committee for the purpose of control and supervision of experiments on animal (CPCSEA),Govt. of India (registration no.516/01/A/CPCSEA). Wistar albino rats of either sex (150-200 g) were maintained under controlled conditions for all sets of experiments. The rats were allowed to take standard laboratory feed and water ad libitum.

### d. Acute Toxicity Studies

Toxicity studies were conducted as per internationally accepted protocol drawn under OECD guidelines in Wistar albino rats at a dose level of extracts up to 2000 mg/kg b.w. The toxic effect of the methanolic extracts of *Barleria montana* aerial parts were studied at a dose level upto 2000 mg/kg b.w. The animals were also closely examined for signs of intoxication, lethargy, behavioral modification and morbidity [10,11].

### e. Design of Experiment

In this experiment, thirty rats were randomly divided into 5 groups of 6 animals each. The

different doses of extracts were administered orally to the STZ induced diabetic rats. All the extracts were suspended in 1% sodium CMC suspension. In these 5 groups, group 1 served control as they received orally 1% Sodium CMC suspension only. Group 2 was administered with a standard drug Glibenclamide (0.45mg /kg b.w). Group 3,4 and 5 were given orally with the methanolic extracts of *Barleria montana* aerial parts at doses of 100, 200 and 400 mg/kg b.w. The drug treatment was given to the animals and was fasted for 12 hr before estimating the blood glucose level.

### f. Induction of Diabetes

Diabetes was induced by a single intraperitoneal dose of 60 mg/kg of b. w of streptozotocin (STZ) dissolved in 0.1M fresh cold citrate buffer (pH 4.5) into 12 hr fasted rats. The blood samples were taken on third day from retro orbital plexus of the rats for the estimation of blood glucose levels by using the auto analyzer. Rats with diabetes having hyperglycemia (i.e. with blood glucose of 185 to 460 mg/dl) were taken for the experiment<sup>[12-14]</sup>.

### g. Collection of Blood Samples and Serum Glucose Estimation

The blood samples (0.5ml) were collected for every time intervals of 0, 2, 4, 8, 12, 18, and 24<sup>th</sup> hr in 1ml Eppendorf's tubes. Serum was separated by centrifuging at 3000 rpm for 10 minutes. 30 µl of serum sample and 3 ml of working glucose reagent were taken in to a dry and clean test tube and incubated for 10 minutes at 37° C. The pink color developed was measured by using auto analyzer<sup>[15-17]</sup>.

### h. Statistical Analysis

The values were expressed as mean±SEM. The data was subjected to the analysis of variance (one way ANOVA) to determine the significance of changes followed by students “t”-test<sup>[18-20]</sup>. The statistical significance of difference between two independent groups was calculated for the determination of blood glucose levels.

### 3. Results

The extracts did not produce any toxic signs during the observation period for 24 hours in any of the rats they were tested. Hence it was concluded that the extracts are safe upto 2000mg/kg.

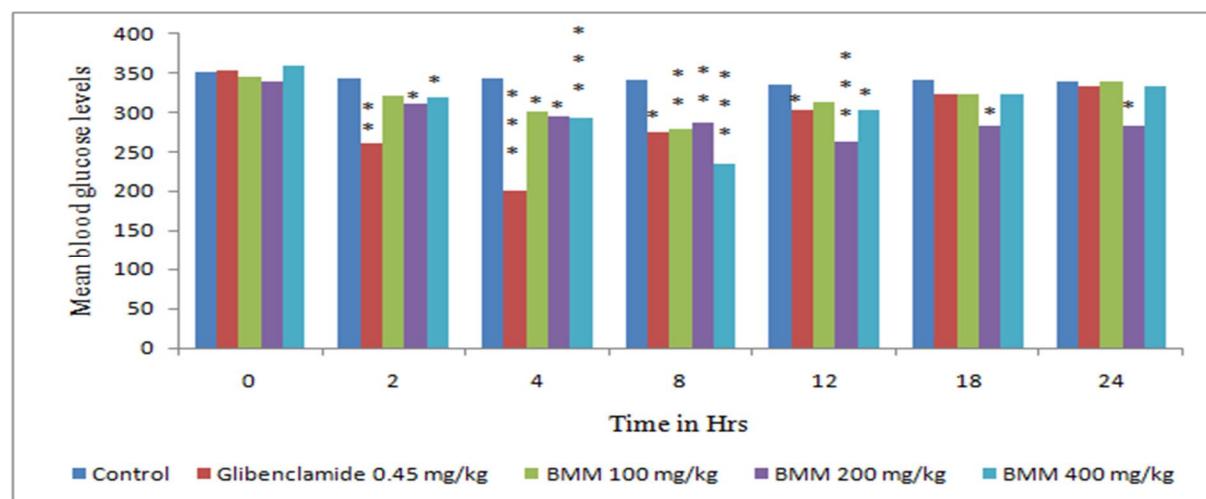
The mean blood glucose levels of control and drug treated animals after oral administration of different doses (100, 200 and 400 mg/kg b.w) of

methanolic extract of *Barleria montana* aerial parts at various time intervals (0, 2, 4, 8, 12, 18 and 24 hrs) are shown in Table 1 and Figure 1. The statistical significance of decrease in blood glucose levels was calculated with respect to initial blood glucose levels. Oral administration of 1% Sodium CMC suspension did not change the blood glucose levels of rats.

**Table 1:** Effect of Methanol extract of *Barleria montana* aerial parts on blood glucose levels (mg/dl) in STZ induced diabetic rats

Group (n=6)	Treatment mg/kg b.w.	Time in hours						
		0	2	4	8	12	18	24
1	Control	352.26± 14.13	343.53± 12.32	343.00± 14.68	341.09± 12.03	335.06± 11.37	341.05± 11.95	339.63± 10.76
2	Glibenclamide (0.45 mg/kg b.w.)	353.29 ± 12.13	261.20± 8.52**	201.93± 5.24***	274.68± 15.41*	302.52 ± 3.47*	323.76± 5.00	333.85± 7.09
3	BMM 100	345.16± 11.23	321.53± 9.94	301.86 ± 11.57*	278.6± 7.84**	314.11± 6.30	322.88± 4.28	339.00± 2.94
4	BMM 200	339.65± 8.93	310.85± 6.05*	294.8± 7.5*	288.30± 7.76**	263.95 ± 3.3***	283.65± 16.94*	282.48± 19.59*
5	BMM 400	359.02 ± 6.17	319.00± 9.75*	293.94± 4.63***	235.04± 2.93***	304.27± 18.89*	324.19± 15.46	332.96± 12.42

Each value is expressed as mean±S.E.M.(n = 6). \* P< 0.05 significant decrease as compared to Zero hr. \*\* P<0.01 More significant decrease as compared to zero hr. \*\*\* P< 0.001 Highly significant decrease as compared to zero hr.



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**Fig 1:** Effect of Methanolic extract of *Barleria montana* aerial parts on blood glucose levels (mg/dl) in STZ induced diabetic rats

The blood glucose levels of diabetic rats treated with Glibenclamide (0.45 mg/kg b.w) showed significant ( $P<0.05$ ) decrease in blood glucose levels at 8 & 12<sup>th</sup> hrs, more significant ( $P<0.01$ ) decrease in blood glucose levels at 2<sup>nd</sup> hr and highly significant ( $P<0.001$ ) decrease in blood glucose levels at 4<sup>th</sup> hr. Nevertheless, the reduction in mean blood glucose levels was no significant at 18 & 24 hrs. After the oral administration of standard drug the mean blood glucose levels were  $353.29\pm12.13$ ,  $261.20\pm8.52$ ,  $201.93\pm5.24$ ,  $274.68\pm15.41$  and  $302.52\pm3.47$  mg/dl at 0, 2, 4, 8 and 12<sup>th</sup> hr respectively. The methanolic extract of the aerial parts of *Barleria montana* at a dose of 100 mg/kg b. w showed significant ( $P<0.05$ ) decrease in blood glucose levels at 4<sup>th</sup> hr and more significant ( $P<0.01$ ) decrease in blood glucose level at 8<sup>th</sup> hr. However, oral administration of 200 mg/kg b. w of methanolic extract of *Barleria montana* showed significant ( $P<0.05$ ) decrease in blood glucose

levels at 2, 4, 18 and 24 hrs, more significant ( $P<0.01$ ) decrease in blood glucose levels at 8<sup>th</sup> hr and highly significant ( $P<0.001$ ) decrease in blood glucose levels at 12<sup>th</sup> hr. Oral administration of 400 mg /kg b. w of methanolic extract of *Barleria montana* aerial parts showed significant ( $P<0.05$ ) decrease in blood glucose levels at 2 & 12<sup>th</sup> hr and highly significant ( $P<0.001$ ) decrease in blood glucose levels at 4<sup>th</sup> and 8<sup>th</sup> hr. The mean blood glucose levels 0, 2, 4, 8 and 12 hrs. After oral administration of 400 mg/kg b. w of methanolic extract of *Barleria montana* aerial parts were  $359.02\pm6.17$ ,  $319.00\pm9.75$ ,  $293.94\pm4.63$ ,  $235.04\pm293$  and  $304.27\pm18.89$  mg/dl respectively.

The mean percent decrease in blood glucose levels produced by the oral administration of different doses (100, 200 and 400 mg/kg b.w) at various time intervals has shown in Table 2 and Figure 2.

**Table 2:** Effect of Methanol extract of *Barleria montana* aerial parts on percent decrease in blood glucose levels in STZ induced diabetic rats

Group (n=6)	Treatment mg/kg b.w.	Time in hours					
		2	4	8	12	18	24
1	Control	2.30±2.02	2.48±2.83	2.90±2.61	4.54±2.92	2.91±2.5 5	3.29±2.3 4
2	Glibenclamide (0.45 mg/kg b.w)	25.47±4.23 **	42.41±2.94* **	21.65±5.62* *	13.81±3.41* *	7.59±4.6 0	4.87±4.1 8
3	BMM 100	6.34±4.36	11.98±4.97	18.63±4.55* *	8.34±4.27	5.87±3.7 6	1.18±3.8 8
4	BMM 200	8.32±1.82* *	13.13±1.63* **	14.41±3.92* **	22.03±2.19* **	15.82±6. 52	16.05±7. 42
5	BMM 400	10.96±3.51 *	18.01±1.87* **	34.45±1.31* **	15.33±4.75* 0*	9.75±3.8 0*	7.22±3.3 0

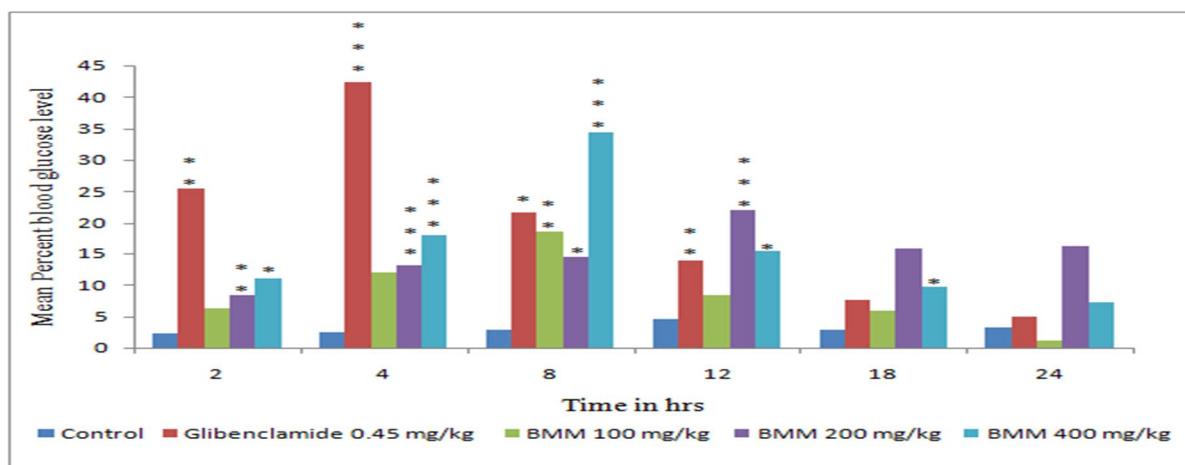
Each value is expressed as mean±S.E.M.(n = 6). \*  $P< 0.05$  significant decrease as compared to Zero hr. \*\*  $P<0.01$  More significant decrease as compared to zero hr. \*\*\*  $P< 0.001$  Highly significant decrease as compared to zero hr.

The mean percent decrease in blood glucose levels was statistically evaluated by comparing with percent reduction of blood glucose levels in the control group at identical times. The mean percent decrease in blood glucose levels produced with 100 mg/kg b. w of methanolic extract of *Barleria montana* aerial parts was found to be  $18.63\pm4.55$  % at 8<sup>th</sup> hr which was statistically significant. However administration of 200 mg/kg

b.w of methanolic extract of *Barleria montana* aerial parts produced significant ( $P<0.05$ ) decrease in blood glucose level at 8<sup>th</sup> hr, more significant ( $P<0.01$ ) decrease in blood glucose level at 2<sup>nd</sup> hr and highly significant ( $P<0.001$ ) decrease in blood glucose level at 4 and 12<sup>th</sup> hrs. The mean percent decrease in blood glucose levels produced by 200 mg/kg b. w of methanolic extract of *Barleria montana* aerial parts were

$8.32\pm1.82\%$ ,  $13.13\pm1.63\%$ ,  $14.41\pm3.92\%$  and  $22.03\pm2.19\%$  at 2, 4, 8 and 12 hrs respectively. The oral administration of 400 mg/kg b. w of methanolic extract of *Barleria montana* aerial parts produced significant ( $P<0.05$ ) decrease in blood glucose level at 2, 12, and 18 hr and highly significant ( $P<0.01$ ) decrease in blood glucose

level at 4 and 8<sup>th</sup> hrs. The mean percent decrease in blood glucose levels produced by 400 mg/kg b. w of methanolic extract of *Barleria montana* aerial parts were  $10.96\pm3.51\%$ ,  $18.01\pm1.87\%$ ,  $34.45\pm1.31\%$ ,  $15.33\pm4.75\%$  and  $9.75\pm3.80\%$  at 2, 4, 8, 12 and 18 hrs respectively.



Each value is expressed as mean $\pm$ S.E.M (n = 6). \*  $P<0.05$  significant decrease as compared to Zero hr. \*\*  $P<0.01$  More significant decrease as compared to zero hr. \*\*\*  $P<0.001$  highly significant decrease as compared to zero hr.

**Fig 2:** Bar diagram showing effect of methanolic extract of *Barleria montana* aerial parts on percent decrease blood glucose level in STZ induced diabetic rats

#### 4. Discussion

The data obtained from the test carried out on STZ diabetic rats clearly showed that the methanolic extracts of *Barleria montana* aerial parts at dose levels of 100, 200 and 400 mg/kg b.w produced a significant reduction of blood glucose level. Comparing the results of 100, 200 and 400 mg/kg b. w methanolic extracts of *Barleria Montana* (aerial parts) in diabetic rats, it was found that the extract the 400 mg/kg b.w showed highly significant ( $P<0.001$ ) decrease in blood glucose levels when compared to control with percentage glycemic change of 34.45% in STZ induced diabetic animals which was comparable with the standard glibenclamide (0.45 mg/kg b.w). The lowest blood glucose levels were observed at 12 and 8<sup>th</sup> hrs after oral administration of 200 and 400 mg/kg b. w of methanolic extract of *Barleria montana* aerial parts. The anti-hyperglycaemic activity of

*Barleria montana* aerial parts was significant up to 24<sup>th</sup> hr at dose of 200 mg/kg b.w. antidiabetic activity of the plant is due to presence of polar compounds available in the methanolic extract. The plant produce reduction of blood glucose levels in diabetic rats was dose dependent manner.

#### 5. Conclusion

From this study, it is concluded that the methanolic extracts of *Barleria Montana* aerial parts possess blood glucose lowering effect in STZ induced diabetic rats. Thus, the folk use of the plant for the control of diabetes may be supported by this study.

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