



Antihyperglycemic activity of *Biophytum sensitivum* (L.) DC in alloxan diabetic rats

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SUMMARY

The study was to evaluate the antihyperglycemic activity of *Biophytum sensitivum* (L.) DC in different extracts. Albino Wistar rats with alloxan hydrate induced diabetes were divided into 7 groups of 6 each. Both aqueous and methanolic extract of *Biophytum sensitivum* were prepared and given individually at different doses to different batches of rats (both normal and diabetic rats) after an overnight fast. Methanolic extract at the dose of 200 mg/kg body weight showed maximum blood glucose lowering effect in diabetic rats. The same dosages did not produce any hypoglycemic activity in normal rats. The antihyperglycemic activity of *Biophytum sensitivum* was compared with a standard drug Glibenclamide, an oral hypoglycemic agent. The above results suggest that maximum hypoglycemic effect was found only with a dose of up to 200 mg/kg b.w. of methanolic extract which is therefore the optimum dose for hypoglycemia and was used in all the experiments of the present study.

Key words: *Biophytum sensitivum*; Hypoglycemia; Alloxan hydrate; *Diabetic mellitus*

INTRODUCTION

Diabetes mellitus is a syndrome characterized by chronic hyperglycemia and disturbances of carbohydrate, fat, and protein metabolism associated with absolute or relative deficiencies in insulin secretion and/or insulin action (Bennett, 1994). The characteristic symptoms of diabetes are polyuria, polydipsia, polyphagia, pruritus, unexpected weight loss, etc. In 2006, according to the World Health Organization, at least 171 million people world wide suffer from diabetes and WHO report suggests that over 19% of the world's diabetic population. Patients are generally classified as

either Type I (juvenile-onset diabetes) or Type II (maturity-onset diabetes) diabetics (Rang *et al.*, 2003). For both types of diabetes, control of blood glucose levels, lipid levels, blood pressure and weight will reduce the risk of vascular problems and associated disease (Williams, 1994). There is an increasing demand by patients to use the natural products with antidiabetic activity, due to the side effects associated with the use of insulin and oral hypoglycemic agents (OHAs) (Pour, 1974; Holman and Turner, 1991; Kameswara *et al.*, 1997; Kameswara *et al.*, 2001). Available literature shows that there are more than 400 plant species showing hypoglycemic activity (Akhtar *et al.*, 1981; Mukherjee, 1981; Oliver, 1986; Ivorra *et al.*, 1989; Rai, 1995) and presently several laboratories are involved in isolating new herbal OHAs. Though some of the plants are reputed in the indigenous systems of medicine for

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their activities, their efficacy remains to be scientifically established.

Since ancient times, plants have been an exemplary source of medicine. Ayurveda and other Indian literatures mention the use of plants in the treatment of various human ailments. *Biophytum sensitivum* (L.) DC. syn. *Oxalis sensitivum* L. (family-Oxalidaceae), commonly known in the local languages as “Life Plant”, is a folk medicine used against diabetes. Different tribal groups have been using the plant for a long time as a source of crude drug. *Biophytum sensitivum* is a mesophyte growing in slightly moist places. They are distributed in tropical and sub-tropical regions of the world, almost throughout India. It is common in roadsides and in cultivated grounds and is seasonal. Puri reported the insulinotropic activity of *Biophytum sensitivum*, which is also widely used in Nepal as a traditional medicinal plant, in diabetic rabbits (Puri, 2001).

MATERIALS AND METHODS

Plant material

The plant sample has been collected from the alluvial region of the district of Paschim Medinipur, West Bengal, India. It has been taxonomically identified and authenticated by the Botanical Survey of India, Kolkata. It is an annual herb and is profusely available during the post monsoon season. The use of this plant in traditional medicine has been reported by various authors (Anonymous, 2004).

Preparation of extract

Methanolic extract

The plant was washed; oven dried at 60°C, pulverized and passed through mesh no. 100. The powdered plant was then extracted with petroleum ether by percolation at room temperature. The dried powder was further extracted in methanol by heating in Soxhlet extracting apparatus for 20 h.

Aqueous extract

Another part of dried pulverized plant powdered was soaked in water overnight and homogenized. The homogenate was centrifuged at 3,000 rpm. for 15 min. After 10 min, the suspension was filtered and the filtrate was evaporated to dryness on a rotary vacuum evaporator (Hahn Shin Science Co. - 2001 N) at 40°C and finally freeze dried. Both the extracts were stored in a refrigerator at 4°C for further studies. The yield of the aqueous and methanol extracts was of 10.2% and 8% respectively (w/w in terms of dried starting material).

Animal

Young adult, male Wistar albino rats weighing 150 - 200 g or more were used with the approval of the University Animal Ethics Committee. The animals were allowed to acclimatize for at least 7 days while they were fed a standard pellet diet (Hindustan Lever, Kolkata) and water *ad libitum* and were maintained at 24 - 28°C temperature, 60 - 70% relative humidity, and 12 h day and night cycle.

Induction of diabetes

Diabetes was induced in 16 h fasted rats within 5 days by the single intraperitoneal (i.p.) administration of ice cold Alloxan hydrate [2,4,5,6-(1H, 3H)-Pyrimidinetatrone hydrate] dissolved in 0.9% saline solution at 125 mg /kg body weight (b.w). The injection volume was prepared to contain 0.3 ml/kg b.w. (Chatterjee, 1993). After 4 days rats with marked hypoglycemic (fasting blood glucose (fbg) > 250 mg/dl) were selected and used for the study. All the animals were allowed free access to tap water and pellet diet and maintained at room temperature in Tarson cages.

Assessment of hypoglycemic effect

Blood glucose levels were determined using a Glucometer Monitor-G™ (One Touch Ultra blood glucose monitoring system from Zydus Pathline (Cadila Health Care). Ability of both the extracts to lower blood glucose, body weight, food intake &

liquid intake in the normal or the hypoglycemic animals reflects its hypoglycemic effect.

Experimental design

Different batches of rats were used for studying the effects of aqueous and methanol fraction of *Biophytum sensitivum*. In every batch the rats were divided into 7 groups and each group consisted of 6 rats.

- Group 1: Normal untreated rats (received normal saline solution 0.9 % NaCl w/v, 5 ml/kg)
- Group 2: Diabetic untreated rats.
- Group 3: Diabetic rats treated with 100 mg/kg-b.w. of plant extract (meth/aquos)
- Group 4: Diabetic rats treated with 200 mg/kg-b.w. of plant extract (meth/aquos)
- Group 5: Normal rats treated with 100 mg/kg b.w. of plant extract (meth/aquos)
- Group 6: Normal rats treated with 200 mg/kg b.w. of plant extract (meth/aquos)
- Group 7: Diabetic rats treated with 200 mg/kg b.w. of Glibenclamide

The effect of both the extracts on alloxan induced diabetic rats were determined by measuring blood glucose levels, food and liquid intake amount and changes in body weights (Ewart *et al.*, 1975).

The plant extracts dissolved in distilled water was fed to rats with overnight fasting, by gastric intubations, using a force-feeding needle. The diabetic rats (Group 2) and normal untreated rats (Group 1) received distilled water by using the same route. Blood samples were collected for the measurement of blood glucose from the tail vein at 0, 2, 4, 6, 18 and 24 h after feeding the plant extract. The results were compared with those of the 7th group of rats which was treated with 200 mg/kg b.w. of glibenclamide (OHA) (Sun Pharmaceutical Comp. India.).

Acute toxicity study

Albino male mice of 10 animals per group and

weighting 20 - 25 g were administered graded dose (100, 200, 400 and 4,000 mg/kg, oral) of the methanol extract and aqueous extract of *Biophytum sensitivum*. After administration of the both extract the mice were observed for toxic effects up to 48th h of treatment. The toxicological effects were observed in term of mortality expressed as LD₅₀. The number of animals dying during the period was noted (Ghosh, 1984). The LD₅₀ of the extracts was calculated by the method of Litchfield and Wilcoxon (Litchfield, 1959).

Statistical analysis

The percentage change in the plant-induced glycemia was calculated as a time function by applying the formula:

$$(G_t) - (G_o)/G_o \times 100$$

Where G_o is the initial glycemia and G_t is glycemia at the 't' h (t = 0, 2, 4, 6, 18 and 24 h) (Puri, 2001). This parameter was used as an index of the hypoglycemic activity. Data were expressed as mean ± S.E. Differences between the test and control groups of animals were evaluated using ANOVA followed by Dunnett's test. P < 0.05 was considered as statistical significant.

RESULTS

Assessment of acute toxicity

Mortality was not observed up to 4,000 mg/kg b.w in any of the groups treated with methanolic and aqueous extracts of *Biophytum sensitivum* (L.) DC. Thus, the extract appears to be generally safe.

Effect of both extract (aqueous and methanolic) on body weight, food and liquid intake

The body weight, food and liquid intake were measured and summarized in Table 1. The initial body weights were similar in normal and diabetic groups, whereas the final body weights were significantly (P < 0.05) decreased in diabetic control

(Group 2) when compared with normal saline treated control (Group 1). At the same time, there was no significant difference in the body weights in both the extract treated diabetic rats (Groups 5 and 6). Food and fluid intake amount were significantly ($P < 0.05$) higher in diabetic group than the normal (Table 1).

Effect of different doses and different fraction of *Biophytum sensitivum* on fasting blood glucose levels (fbg)

The effect of different doses of methanolic fraction

of *Biophytum sensitivum* on fasting blood glucose levels of both normal and diabetic rats are given in Table 2. The effect of aqueous fraction of the plant extract is given Table 3. The maximal effective dose of the extract was found out by trial. A dose response study was carried out, wherein different doses of both extracts (aqueous and methanolic) were orally administrated and decrease in plasma glucose levels were observed.

The fasting blood glucose levels of diabetic on treated rats (Group 2) were significantly higher than the fasting blood glucose levels of normal

Table 1. Effect of methanolic and aqueous extracts of *Biophytum sensitivum* on body weight, food and liquid intake in rats during treatment

Groups	Treatment	Dose (mg/kg)	Body weight (g)		Food intake (g/rat/day)	Liquid intake (ml/rat/day)
			Initial	Final		
Group 1	Normal [0.9% NaCl (w/v)]	-	179.2 ± 1.2	191.6 ± 3.5	11.12 ± 5.3	16.33 ± 0.22
Group 2	Diabetic (alloxan)	125	180.4 ± 4.3 ^a	159.2 ± 2.2 ^a	16.88 ± 2.3 ^a	32.14 ± 0.55 ^a
Group 3	Alloxan + Meth.ext.	125 + 100	164.4 ± 5.8 ^b	192.2 ± 3.2 ^b	14.24 ± 1.6 ^a	28.10 ± 0.18 ^a
Group 4	Alloxan + Meth.ext.	125 + 200	177.3 ± 4.1 ^b	193.1 ± 4.3 ^b	12.14 ± 1.8 ^a	26.67 ± 0.12 ^a
Group 5	Alloxan + Aq. ext.	125 + 100	169.2 ± 1.2 ^b	191.6 ± 4.2 ^b	15.32 ± 4.4 ^a	26.67 ± 0.11 ^a
Group 6	Alloxan + Aq. ext.	125 + 200	182.3 ± 2.3 ^b	194.5 ± 1.2 ^b	16.88 ± 2.2 ^a	24.81 ± 0.21 ^a

Values are Mean ± S.E. (n = 6). ^aStatistically significant when compared to diabetic Gr. 2 ($P < 0.01$), ^bStatistically significant when compared to diabetic Gr. 2 ($P < 0.05$). The results were analyzed by ANOVA followed by Dunnett's test.

Table 2. Effect of different doses of methanolic extract of *Biophytum sensitivum* (L.) DC on fasting blood glucose levels (mg/dl) in normal and diabetic rats (mean ± S.E.)

Group	Dose (mg/kg, b.w.)	Blood glucose level at different hours after the treatment					
		0 h	2 h	4 h	6 h	18 h	24 h
Group 1	-	73.33 ± 1.14 ^a	72.66 ± 0.84 ^a	73.16 ± 1.05 ^a	73.5 ± 1.05 ^a	72.3 ± 0.08 ^a	72.66 ± 2.48 ^a
Group 2	125	275.5 ± 13.71	275.66 ± 11.70	274.5 ± 11.79	271.5 ± 12.78	277.16 ± 13.37	291.16 ± 6.81
Group 3	100	225.33 ± 2.15 ^b	215.83 ± 4.49 ^b (-4.21%)	194.33 ± 0.36 ^b (-13.75%)	169.33 ± 2.95 ^b (-24.62%)	162.83 ± 2.64 ^b (-27.73%)	189.33 ± 3.93 ^b (-15.93%)
Group 4	200	243.83 ± 4.04 ^b	203.5 ± 3.62 ^b (-16.54%)	177.8 ± 2.35 ^b (-27.08%)	161.5 ± 2.5 ^b (-33.76%)	176.16 ± 1.75 ^b (-27.7%)	190.60 ± 2.01 ^b (-21.80%)
Group 5	100	73.2 ± 5.5 ^a	77.5 ± 4.4 ^a	72.5 ± 5.9 ^a	73.3 ± 6.8 ^a	77.4 ± 5.4 ^a	72.0 ± 6.3 ^a
Group 6	200	72.1 ± 3.3 ^a	69.3 ± 5.4 ^a	68.1 ± 1.2 ^a	72.5 ± 2.5 ^a	75.2 ± 2.8 ^a	69.0 ± 3.8 ^a
Group 7	200	250.9.3 ^b	220.6 ± 14.6 ^b	204.6 ± 14.7 ^b	153.0 ± 13.6 ^b	171.0 ± 12.3 ^b (-31.73%)	161.0 ± 11.3 ^b

^aComparison of fbg level at 0 h of Group 1 rats with 2 to 24 h fbg levels. Again fbg level of Group 5 and 6 diabetic rats have been compared with that of Group 1 rats (normal untreated). ^bComparison of blood glucose level in 24th h for the rats in group 3, 4 and 5 (positive control) with Group 2 diabetic induced rats (negative control). Figures in the parentheses show percentage of decrease (-) in the blood glucose level. The results were analyzed by ANOVA followed by Dunnett's test.

Table 3. Effect of different doses of aqueous extract of *Biophytum sensitivum* (L.) DC on fasting blood glucose levels (mg/dl) in normal and diabetic rats (mean \pm S.E.)

Group	Doses (mg/kg, b.w.)	Blood glucose level at different hours after the treatment					
		0 h	2 h	4 h	6 h	18 h	24 h
Group 1	-	73.16 \pm 0.7 ^a	73.33 \pm 0.49 ^a	74.5 \pm 0.67 ^a	73.0 \pm 0.57 ^a	72.83 \pm 1.16 ^a	72.0 \pm 2.26 ^a
Group 2	125	262.83 \pm 7.81	264.33 \pm 7.89	263.5 \pm 7.63	255.66 \pm 6.01	255.83 \pm 7.18	256.60 \pm 7.56
Group 3	100	258.66 \pm 6.28 ^b	257.16 \pm 5.95 ^b (-0.57%)	255.83 \pm 4.67 ^b (-1.09%)	250.83 \pm 4.25 ^b (-3.02%)	243.66 \pm 4.58 ^b (-5.80%)	250.16 \pm 3.77 ^b (-3.29%)
Group 4	200	252.83 \pm 4.7 ^b	248.16 \pm 4.6 ^b (-1.84%)	237.66 \pm 4.62 ^b (-6.00%)	229.33 \pm 5.57 ^b (-9.29%)	233.83 \pm 3.33 ^b (-7.51%)	244.16 \pm 3.52 ^b (-3.42%)
Group 5	100	73.46 \pm 0.45 ^a	73.00 \pm 1.16 ^a	72.0 \pm 2.16 ^a	74.0 \pm 0.12 ^a	73.15 \pm 1.14 ^a	72.89 \pm 2.5 ^a
Group 6	200	73.11 \pm 2.5 ^a	72.83 \pm 1.26 ^a	75.11 \pm 0.25 ^a	73.12 \pm 0.19 ^a	72.98 \pm 0.93 ^a	73.45 \pm 1.16 ^a
Group 7	200	250.5 \pm 9.3 ^b	220.6 \pm 14.6 ^b	204.6 \pm 14.7 ^b	153.0 \pm 13.6 ^b	171.0 \pm 12.3 ^b (-31.73%)	161.0 \pm 11.3 ^b

^aComparison of fbg level at 0 hrs. of Group 1 rats with 2 to 24 h fbg levels. Again fbg level of Group 5 and 6 diabetic rats have been compared with that of Group 1 rats (normal untreated). ^bComparison of blood glucose level in 24th h for the rats in group 3, 4 and 5 (positive control) with Group 2 diabetic induced rats (negative control). Figures in the parentheses show percentage of decrease (-) in the blood glucose level. The results were analyzed by ANOVA followed by Dunnett's test.

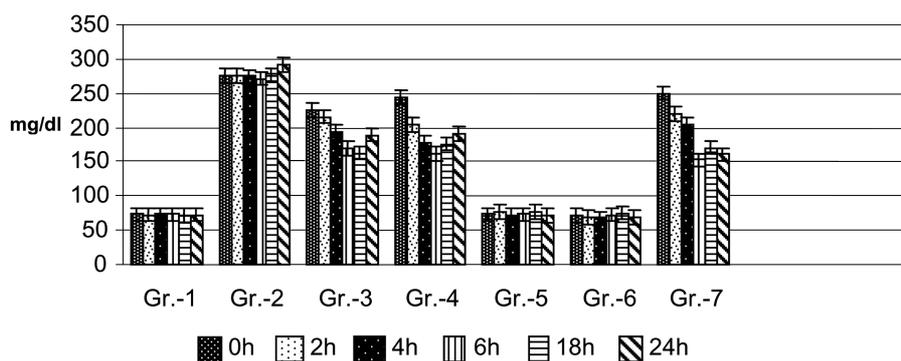


Fig. 1. Effect of different doses of methanolic extract of *B. sensitivum* fbg level (mg/dl) in normal and diabetic rats (0 - 24 h). Gr. 1: Normal untreated rats (received normal saline solution 0.9% NaCl w/v, 5 ml/kg), Gr. 2: Diabetic untreated rats, Gr. 3: Diabetic rats treated with 100 mg/kg b.w. of plant extract, Gr. 4: Diabetic rats treated with 200 mg/kg b.w. of plant extract, Gr. 5: Normal rats treated with 100 mg/kg b.w. of plant extract, Gr. 6: Normal rats treated with 200 mg/kg b.w. of plant extract, Gr. 7: Diabetic rats treated with 200 mg/kg b.w. of Glibenclamide.

untreated rats (Group 1). Normal rats (Group 5 and 6) treated with different (100 mg/kg b.w. and 200 mg/kg b.w.) doses of aqueous extract and methanolic extract itself had no hypoglycemic activity.

The methanolic extract at the dose of 200 mg/kg b.w. showed a maximum decrease (33.76%) in the blood glucose levels in the diabetic rats after 6 hours of drug administration and 100 mg/kg showed 24.62 % decrease in the blood glucose

levels of the diabetic rats on 6 hours of drug administration. Fig. 1 shows effect of different doses of methanolic extract of *B. sensitivum* on fasting blood glucose level of normal and diabetic rats. The results clearly show best performance of the drug on the 6th h of its administration. 200 mg/kg body weights have a better result in comparison to that of 100mg/kg b.w. (Table 1).

Effect of different doses of methanolic extract (Table 2) of the drug on the fbg level on the 6th hour

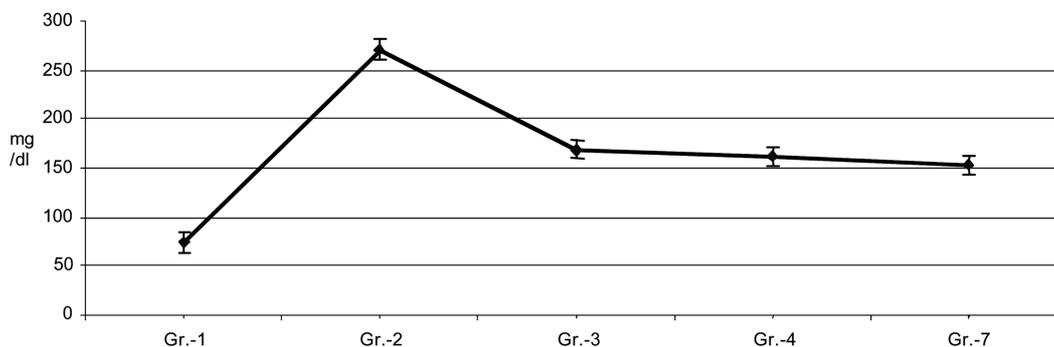


Fig. 2. Effect of different doses of methanolic extract of *B. sensitivum* only 6th h on fbg level (mg/dl).

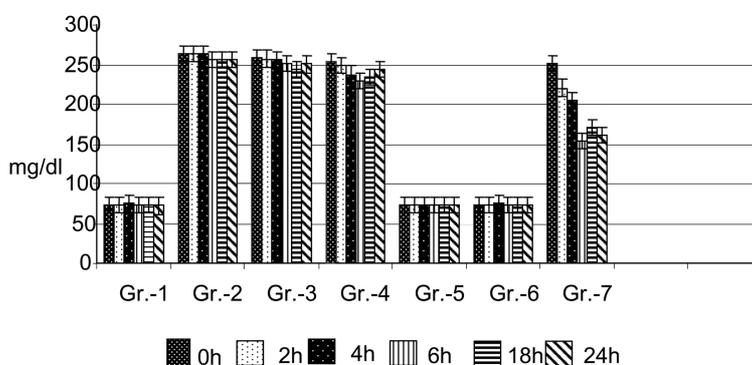


Fig. 3. Effect of different doses of aqueous extract of *B. sensitivum* fbg levels (mg/dl) in normal and diabetic rats (0 - 24 h). Gr. 1: Normal untreated rats (received normal saline solution 0.9% NaCl w/v, 5 ml/kg), Gr. 2: Diabetic untreated rats, Gr. 3: Diabetic rats treated with 100 mg/kg b.w. of plant extract, Gr. 4: Diabetic rats treated with 200 mg/kg b.w. of plant extract, Gr. 5: Normal rats treated with 100 mg/kg b.w. of plant extract, Gr. 6: Normal rats treated with 200 mg/kg b.w. of plant extract, Gr. 7: Diabetic rats treated with 200 mg/kg b.w. of Glibenclamide.

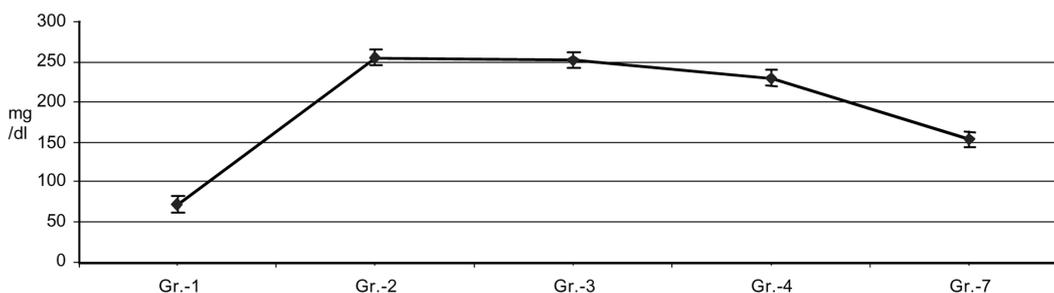


Fig. 4. Effect of different doses of aqueous extract of *B. sensitivum* only 6th h only on fbg level.

only has been given in Fig. 2. The nature of the graph establishes the fact that a higher dose of methanolic extract (200 mg/kg b.w.) has almost similar impact with that of the standard drug, Glibenclamide.

Aqueous extract shows 9.29% fall of blood

glucose level at 200 mg/kg body weight and 3.02% decrease of blood glucose levels at 100 mg/kg body weight. Fig. 3 and 4 show the impact of aqueous and that of the methanolic extract. Although the latter dose caused a decrease but it was less than that 200 mg/kg b.w. of methanolic

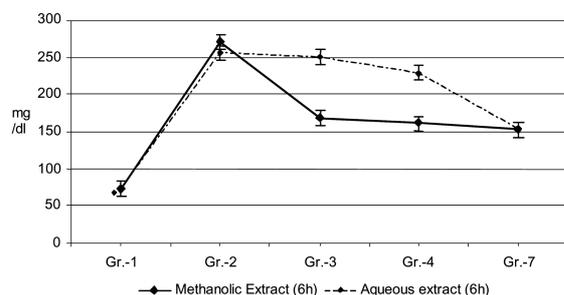


Fig. 5. Comparative study of methanolic and aqueous extract of *B. sensitivum* on the 6th h of drug administration.

extract caused the greatest decrease in the blood glucose level. Treatment with Glibenclamide at a dose of 200 mg/kg body weight (Group 7) of diabetic rats resulted into a 31.73% of decrease on 18th h of drug administration. The results were thus obtained by using both the extracts in Fig. 5. The nature of the graph shows a more efficient impact of the plant drug in methanolic extract on 6th hour of its administration of the drug than its aqueous extract.

DISCUSSION

Biophytum sensitivum significantly decreases the blood glucose level elevated by application of alloxan. Alloxan destroys the pancreatic β -cell and raises blood glucose concentration. *Biophytum sensitivum* lowered blood sugar levels in alloxanized rats, an indication that the extract has non-pancreatic effects. The observation is in agreement with the use of *Biophytum sensitivum* in folklore diabetes management. In this study the methanolic extract at the dose 200 mg/kg b.w. produces a significant reduction in the blood glucose of diabetic rats, but it has no hypoglycemic effect in normal rats. The blood glucose lowering effect of the methanolic extract of *Biophytum sensitivum* in diabetic rats is 20.16% higher after 6 h of drug administration than that of the oral hypoglycemic agent glibenclamide. The change of body weight shows that rats given both extracts have a significant effect in controlling

the loss of body weight, which is caused during diabetes. The activities were dose dependent.

In the present study, we observed a maximum lowering effect of blood glucose of 33.76% by using 200 mg/kg b.w. of methanolic extract, compared to aqueous extract of *Biophytum sensitivum* in the diabetic rats. The aqueous extract lowered the blood glucose level 9.29% at 200 mg/kg b.w. compared with 100 mg/kg b.w. aqueous extract.

In our present study the methanolic extract of *Biophytum sensitivum* produced the maximal blood glucose lowering activity in diabetic rats after 6th h but no hypoglycemic activity was observed in normal rats. The antihyperglycemic activity of *Biophytum sensitivum* may be due to its stimulating effect on the remnant β -cell or improvement in insulin action at cellular levels or it could also be due to the insulin like effect of the active principle(s) present in the extract. The methanolic extract of this plant is currently being screened to isolate, purify and characterize the anti-hyperglycemic principle.

Puri (2001) studied the aqueous extract of crude drug and got almost similar results as that of the present study. Our study in addition to the above findings, established the fact that methanolic extract of the crude drug is almost 34% more effective than its aqueous extract and is as effective as glibenclamide, which is a standard drug for diabetic mellitus (Rai, 1995).

The above results suggest that maximum hypoglycemic effect was found only with a dose of up to 200 mg/kg of methanolic extract, suggesting that may be the optimum dose for hypoglycemic and was used in all the experiments of the present study.

Further pharmacological and biochemical investigations are underway to elucidate the mechanism of the antidiabetic effect of *Biophytum sensitivum*.

ACKNOWLEDGEMENTS

Authors wish to thank *Herbicare Health Care Bioherbal Research Foundation* for the financial help rendered.

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