Antidiabetic Activity of *Andrographis paniculata*

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ABSTRACT: The hot water and ethanol extracts of *Andrographis paniculata* (local name Kalomegh) collected from Chittagong exhibited a significant hypoglycemic (blood glucose lowering) activity in both glucose-loaded and alloxan-induced diabetic rats. Oral administration of glucose (1.5 g/kg body weight) increased the blood sugar level while the intraperitonial (ip) administration of alloxan (40 mg/kg body weight) enhanced the blood sugar level much higher than that of the glucose-loaded rats. The hot water (0.8 g/kg b.w.) and ethanol extracts (2 g/kg b.w.) of *A. paniculata* reduced the elevated glucose level by 41.51 and 41.82%, respectively in glucose-loaded rats as compared to the respective diabetic control rats. On the other hand, administration of hot water and ethanol extracts of *A. paniculata* decreased the blood sugar level by 46.21 and 45.13%, respectively in alloxan-induced diabetic rats, when compared with that of diabetic control rats.

Keywords. *Andrographis paniculata*, Glucose-loaded, Alloxan-induced, Rats, Antidiabetic.

INTRODUCTION

Diabetes is a disorder of metabolism due to absolute deficiency or diminished effectiveness of insulin. Due to lack of insulin, hyperglycemia and glycosuria almost invariably occur.[1] It is a fatal health problem in the present world. Diabetes is the fourth-leading cause of death.[2] The diabetic population is rapidly increasing globally, particularly in the developing countries. South Asian region including Bangladesh is the most vulnerable focus. The current worldwide diabetic population is about 150 million, and this will be doubled by 2025.[3] The estimated prevalence of diabetes in Bangladesh is around 4%, which is similar to the average prevalence in many other countries. But the prevalence of impaired glucose tolerance (IGT) here varies between 7.5-10% depending on urban and rural backgrounds.[4] A significant proportion of these patients obviously fail to get proper treatment and medication. Indigenous drugs, since long, have been used for the treatment of diabetes.[5] Hundreds of plants are known to be useful in treating diabetes in different corners of the world. Bangladesh is abundant in antihyperglycemic plants. These species may represent a source of new hypoglycemic compounds for developing better remedies to treat diabetic patients without serious side effects.

Andrographis paniculata (Burm. f.) Wall (=*Justicia paniculata* Burm.f.), locally known as Kalomegh (English name Creat), belonging to the Acanthaceae family, is an annual herb that grows wild in wastelands throughout Bangladesh (particularly in Chittagong hill tracts) and

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occasionally planted in gardens. Previous chemical investigations of *A. paniculata* revealed the occurrence of a resinous bitter substance, kalmeghin, the diterpenes, andrographolide, andrographiside and neoandrographolide. Extracts are known to contain 14-deoxy-11-oxoandrographolide, 14-deoxyandrographolide, 14-deoxy-11,12-idehydroandographolide and14-deoxy-11,14-didehydro-andrographolide, epigenin ethers and various flavonoids, phenols and stigmasterol. On the other hand leaves are reported to contain β-sitosterol glucoside, andrographolide and panicolide, polyphenols, caffeic and chlorogenic acids and a mixture of dicaffeoylquinic acid.[6]

The plant possesses hypoglycemic, cholagogue properties and also used in spleen & liver complaints, diarrhea, dysentery, dyspepsia, helmenthiasis, colic and conspitation.[7,8] Andrographolide and extract of the plant have been shown to be a strong hepatoprotective drug. Husen *et al.*[9] and Zhang *et al.*[10] have reported the antihyperglycemic property of *A. paniculata* in streptozotocin-induced hyperglycemic rats but enough evidence was not available to confirm the hypoglycemic activity of the various extracts on different hyperglycemic conditions. In this paper, we report the hypoglycemic activity of hot water and ethanol extracts of the aerial parts of *A. paniculata* growing in Bangladesh in glucose-loaded hyperglycemic and alloxan-induced diabetic rats.

MATERIALS AND METHODS

Collection of plant material. The aerial parts of *A. paniculata* were collected from plantation area of the BCSIR Laboratories, Chittagong and adjacent hilly regions and were identified at the Plant Taxonomy Division. The plants were cut into small pieces and were dried at room temperature for about 20 days, followed by drying in an oven and were then ground to a coarse powder.

Preparation of plant pxtracts

Hot Water Extract. For preparing water extract the powder of *A. paniculata* (about 2 g, according to need) was mixed with distilled water (1 : 12), boiled for 5-7 minutes, cooled at room temperature and filtered through a filter paper. The liquid (aqueous extract) was then administered to rats through feeding needle.

Calculation of solid content in water extract. 1g of the prepared powder was taken in 250 ml beaker to which 12 ml of distilled water was added and the mixture was heated on a burner for about 5-7 minutes, cooled and filtered through a filter paper. The liquid extract was dried in oven to drive off water. The solid portion thus obtained was measured with an electronic balance.

Ethanol extract. The powder of *A. paniculata* was soaked in ethanol in a closed glass bottle for 7 days. Then the extractive was filtered using a filter paper. The extract, thus obtained was concentrated under reduced pressure at about 45-50 ºC with a rotary vacuum evaporator.

Animal and diet. Adult male and female albino rats obtained from the Animal Breeding Center, BCSIR Laboratory, Chittagong, Bangladesh weighing 200-230 g were used for the study. The rats were acclimatized to standard laboratory conditions (relative humidity 55 ± 5%, temperature 24 ± 1ºC and a 12 h diurnal photoperiod) in galvanized cages (3-6 rats/cage) with replaceable wire-meshed net lid for 7 days before the commencement of the experiment. During the study, all animals were maintained on normal laboratory chow, *ad libitum* water.

Induction of diabetes in rats. In glucose-loaded study, rats were fasted overnight (18 h) before oral feeding of glucose. Glucose at a concentration of 1.5 g/kg b.w. was dissolved in distilled water immediately before administration through feeding needle. Alloxan (40 mg/kg b.w.) was injected intraperitonially and after that, the rats were fasted for 18 hours.

Estimation of blood sugar level (BSL). The level of glucose in blood samples from each of the experimental and control rat was determined by using standard glucose kit essentially following the glucose oxidase-peroxidase (GOD-POD) method.[11] The blood was centrifuged to get a clear supernatant

(serum). 2 µl of serum was taken in 2 ml test solution in a separate test tube. The intensity of the color of the solution was measured spectrophotometrically at 546 nm for quantification of the glucose initially present in the blood specimen.

EXPERIMENTAL PROTOCOL

For glucose-loaded experiments. 28 rats were randomly divided in equal number into four groups (marked I, II, III, IV). One group (Gr-I, 7 rats) received only distilled water and termed as vehicle control group. The three experimental groups (Gr-II, III, IV) were orally administered with 1.5 g/kg b.w glucose solution. Gr-II rats were considered as diabetic control (only glucose), while Gr-III rats received 4 mg/kg b.w. Daonil [Glibenclamide BP tablet, 5mg, a standard market drug for non-insulin dependent diabetes mellitus (NIDDM, type-2) treatment] and served as the positive control (drug treated). Gr-IV was given with either the hot water or ethanol extract at different experimental regimen and was considered as sample treated.

Time schedule for glucose-loaded experiment. All the animals were primarily fasted for 18 hours (given only distilled water) and then glucose solution was given through feeding needle. After 2 hours, distilled water, drug solution and *A. paniculata* extracts, prepared with water were given orally according to rats of respective group. Two hours later, all the animals were anesthetized with diethyl ether and blood sample were collected from cardiac vessel by syringe for every observation in each study.

For alloxan induced experiments. Rats were grouped in an identical manner to glucose-loaded classification. Gr-I rats received only distilled water. Rats of groups-II, III and IV were intraperitonially injected alloxan tetrahydrate (40 mg/kg b.w.). Gr-II rats were considered as diabetic control (only alloxan), Gr-III rats also received 4 mg/kg b.w. Diactin (Glipizide BP tablet 5 mg, a standard drug indicated as an adjunct to diet the control of hypoglycemia in NIDDM) and termed as positive control. Gr-IV rats were treated with hot water or ethanol extract at different experimental observation and were designated as the sample treated group.

Time schedule for alloxan induced experiment. All the animals were injected with alloxan and were fasted for 18 hours. Then standard drug and sample extract were given orally to the rats group wise in every experiment. Two hours later of treatment, blood samples were collected as described before.

Statistical analysis (Calculation). Student's 't' test was formulated for analysis of data from each experimental group. Percentage change in glucose level (increased or decreased) was determined by using the formula:

$$\frac{(\text{Mean from control groups} - \text{Mean from treated groups})}{\text{Mean from control groups}} \times 100$$

RESULTS AND DISCUSSION

Effect of hot water extract of *A. paniculata* on blood sugar level (BSL) of glucose-loaded rats. The effect of hot water extract of *A. paniculata* on BSL of glucose-loaded rats is presented in Table 1. Administration of glucose increased the BSL of rats by 89.47% as compared to vehicle-control rats while the hot water extract of *A. paniculata* significantly ($p < 0.001$) decreased the glucose elevated BSL by 41.51% as compared to diabetic control (glucose-loaded) rats. In the case of standard drug, Daonil treatment, the percent of BSL decrease was 44.70.

Effect of ethanol extract of *A. paniculata* on BSL of glucose-loaded rats. The effect of ethanol extract of *A. paniculata* on BSL of glucose-loaded rats is shown in Table 2. Administration of glucose increased the rats BSL by 87.07% when compared to vehicle-control rat. On the other hand, rats treated with ethanol extract of *A. paniculata* significantly ($p < 0.001$) lowered 41.82% the enhanced BSL as compared to diabetic control rats. In the case of drug (daonil) treatment group, the glucose level was lowered by 45.63%.

Effect of hot water extract of *A. paniculata* on BSL of alloxan-induced rats. Table 3 depicts the

effect of hot water extract of *A. paniculata* on BSL of alloxan-induced diabetic rats. Administration of alloxan increased the BSL of rat by 104.69% as compared to the vehicle control group. On the other hand, rats treated with the hot water extract of *A. paniculata* significantly ($p < 0.001$) lowered the elevated BSL by 46.21% when compared to diabetic control group. In this situation, the standard drug reduced the BSL by 49.66%.

Effect of ethanol extract of *A. paniculata* on BSL of alloxan-induced rats. Table 4 shows the serum blood sugar level in vehicle control, diabetic control (alloxan), standard drug and sample treated groups. Alloxan enhanced the BSL by 104.61% when compared with vehicle-control rats. On the other

Table 1. Effect of hot water extract of *A. paniculata* on blood sugar level (BSL) of glucose-loaded rats.

Group	Treatment	Blood sugar level[a] (Mean±S.D, mg/dl)	Percent changed (Increased/decreased)
I	Vehicle control	60.77 ± 3.28	-
II	Diabetic control	115.14 ± 2.36	89.47 (↑)
III	Drug treated (Daonil)	63.67 ± 3.05	44.70 (↓)
IV	Sample treated	67.35 ± 2.17	41.51 (↓)

[a]Values are Mean ± S.D. (n=7) S.D. = Standard deviation. n = number of rat

Table 2. Effect of ethanol extract of *A. paniculata* on BSL of glucose-loaded rats.

Group	Treatment	Blood sugar level[a] (Mean ± S.D, mg/dl)	Percent changed (Increased/decreased)
I	Vehicle control	61.40 ± 3.34	-
II	Diabetic control	114.86 ± 1.62	87.07 (↑)
III	Drug treated (Daonil)	62.45 ± 4.02	45.63 (↓)
IV	Sample treated	66.83 ± 2.36	41.82 (↓)

[a]Values are Mean ± S.D (n=7); S.D. = Standard deviation. n = number of rat

Table 3. Effect of hot water extract of *A. paniculata* on BSL of alloxan-induced rats.

Group	Treatment	Blood sugar level[a] (Mean ± S.D, mg/dl)	Percent changed (Increased/decreased)
I	Vehicle control	60.22 ± 3.19	-
II	Diabetic control	123.27 ± 3.68	104.69 (↑)
III	Drug treated (Diactin)	62.31 ± 5.18	49.66 (↓)
IV	Sample treated	66.31 ± 4.93	46.21 (↓)

[a]Values are Mean ± S.D (n=7); S.D. = Standard deviation. n = number of rat

Table 4. Effect of ethanol extract of *A. paniculata* on BSL of alloxan-induced rats.

Group	Treatment	Blood sugar level[a] (Mean ± S.D, mg/dl)	Percent changed (Increased/decreased)
I	Vehicle control	61.21 ± 3.25	-
II	Diabetic control	125.24 ± 3.19	104.61 (↑)
III	Drug treated (Diactin)	61.30 ± 3.06	51.05 (↓)
IV	Sample treated	68.72 ± 5.02	45.13 (↓)

[a]Values are Mean ± S.D (n=7); S.D. = Standard deviation. n = number of rat

Table 5. Comparison of the effect of hot water and ethanol extract of *A. paniculata* on blood sugar of glucose-loaded rats.

Group	Treatment	Hot water extract (Mean ± S.D., mg/dl)	Ethanol extract (Mean ± S.D., mg/dl)
I	Vehicle control	60.77 ± 3.28	61.40 ± 3.34
II	Diabetic control	115.14 ± 2.36	114.86 ± 1.62
IV	Sample treated	67.35 ± 2.17	66.83 ± 2.36
Percent decrease		41.51	41.81

Values are Mean ± S.D (n=7); S.D. = Standard deviation.

Table 6. Comparison of the effect of hot water and ethanol extract of *A. paniculata* on blood sugar in alloxan-induced diabetic rats.

Group	Treatment	Hot water extract (Mean ± S.D., mg/dl)	Ethanol extract (Mean ± S.D., mg/dl)
I	Vehicle control	60.22 ± 3.19	61.21 ± 3.25
II	Diabetic control	123.27 ± 3.68	125.54 ± 3.19
IV	Sample treated	66.31 ± 4.93	68.72 ± 5.02
Percent decrease		46.21	45.13

[a]Valuse are Mean ± S.D (n=7); S.D. = Standard deviation. n = number of rat

hand, treatment of rats with ethanol extract of *A. paniculata* significantly ($p < 0.001$) decreased 45.13% the alloxan elevated BSL. Here, the percent of BSL decreasing effect of the standard drug, Diactin was 51.05.

CONCLUSION

It is clearly evident from the study that the aqueous and ethanolic extractives of *A. paniculata* are capable to exhibit significant blood sugar lowering effects in both glucose-loaded and alloxan induced diabetic rat (Tables 1-4). Thus the folk use of this plant in treating diabetes is justified. Moreover, the lowering of blood glucose levels by the aqueous and ethanolic extracts is also comparable. Both extractives are capable to reduce the sugar level almost identically as evident from Tables 5 and 6.

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