



Received on 27 October, 2011; received in revised form 03 February, 2012; accepted 23 February, 2012

COMPARATIVE STUDY OF HYPOGLYCEMIC EFFECT OF ETHANOLIC AND HOT WATER EXTRACTS OF *ANDROGRAPHIS PANICULATA* IN ALLOXAN INDUCED RAT

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Keywords:

Andrographis paniculata,
alloxan-induced,
antidiabetic,
rats,
blood sugar level

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ABSTRACT

The aim of present study was to investigate the potential roles of ethanolic and hot water extracts of *Andrographis paniculata* on reducing the blood sugar level (BSL) in alloxan- induced rats. Oral administration of ethanolic (2 g/kg b. w.) and hot water (0.8 g/kg b. w.) extracts of *Andrographis paniculata* showed the antidiabetic properties and decreased the blood glucose level by 33.71% ($p < 0.0001$) and 39.69% ($p < 0.0001$) respectively in alloxan induced (40mg/kg b.w.) rats. The results were also compared with that of diabetic control rats.

INTRODUCTION: *Andrographis paniculata* (Burm. F.) is a member of the family Acanthaceae and has been used for centuries in Asia to treat not only one, but several types of illness¹. Extensive research has revealed that the plant has a wide range of pharmaceutical activities. Hepatoprotective², antiviral³, antimalarial⁴, antithrombotic⁵, anti-clotting⁶ and cardiovascular activities⁷ of *Andrographis paniculata* has been identified. The plant also acts as anti-inflammatory⁸ and immunostimulant⁹.

The plant is also safe and efficacious treatment for the relief of symptoms of uncomplicated upper respiratory tract infection¹⁰. The gastroprotective effect was observed due to the presence of flavonoids in the hydroalcoholic extract of *Andrographis paniculata*¹¹. The presence of diterpenoids such as 14-deoxyandrographolide and 14-deoxy-11, 12-didehydroandrographolide is responsible for the cardioprotective activity of *Andrographis paniculata*¹². The leaves contain the highest amount of andrographolide (2.39%), the most medicinally active

phytochemical in the plant, while the seeds contain the lowest¹³. The formation of free radicals such as superoxide, hydroxyl radicals, lipid peroxidation and nitric oxide in in-vivo system was inhibited by the plant¹⁴. The antihyperglycemic property of *Andrographis paniculata* have been reported in streptozotocin-induced hyperglycemic rats but enough evidence was not available to confirm the hypoglycemic activity of the various extracts on different hyperglycemic conditions¹⁵. It is the aim of the present study to investigate the potential roles of ethanolic and hot water extract of *Andrographis paniculata* in lowering blood sugar level in alloxan-induced diabetic rats.

MATERIALS AND METHODS:

Collection of Plant Material: *Andrographis paniculata* plants were collected from the Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka. The fresh leaves of *Andrographis paniculata* were cut into small pieces which were subjected to dry at room

temperature using ceiling fan and homogenized to fine powder and stored in airtight containers.

Preparation of Hot Water Extract: 2 gm of the powder material was mixed with water and the mixture was boiled and cooled at room temperature and filtered through a filter paper. The filtrate was considered as hot water extract.

Preparation of Ethanolic Extract: 10 gm of powder of *Andrographis paniculata* was macerated with ethanol containing glass bottle which was kept for two weeks. The mixture was filtered using a filter paper and the filtrate was concentrated under reduced pressure with a rotary vacuum evaporator.

Animal and Diet: Adult male and female albino rats weighing 180 to 200g collected from BCSIR-Laboratory, Dhaka were used in the entire study. The animals were kept at standard laboratory conditions (temperature $24 \pm 1^\circ\text{C}$, relative humidity $55 \pm 5\%$, and a 12 hour photoperiod) for one week before the commencement of the experiment. During the entire period of study, the rats were supplied with semi purified basal diet water *ad libitum*.

Induction of diabetes in rats: In alloxan-induced experiment, Alloxan (40 mg/kg b.w.) was injected intraperitoneally in order to induce diabetes in rats and after that, the rats were fasted for 18 hours.

Determination of Blood Sugar Level (BSL): A standard glucose kit was used to determine the concentration of glucose in blood samples collected from the alloxan-induced diabetic rats and control rats¹⁶. The centrifugation method was carried out to get a clear supernatant (serum). 2 μ l of supernatant was mixed with 2ml of the test solution and the intensity of the solution was measured spectrophotometrically at 546nm for quantification of the glucose present in blood specimen.

Experimental Protocol:

TABLE 1: EFFECT OF HOT WATER EXTRACT OF ANDROGRAPHIS PANICULATA ON BLOOD SUGAR LEVEL (BSL) OF ALLOXAN-INDUCED RATS

Group (n)	Treatment	Blood sugar level* (mg/dl)	% changed (increased/decreased)
A	Normal control	60.77 \pm 3.07	-
B	Diabetic control	110.59 \pm 6.27	81.98 (increased)
C	Drug treatment (Dionil)	62.83 \pm 5.59	43.18 (decreased)
D	Sample treatment	73.31 \pm 3.90	33.71 (decreased)

*Values are Mean \pm S.D. (n=5); S.D. =Standard deviation, n=number of rat

For Alloxan-induced experiments: 20 albino (male) rats were randomly divided into four (marked A, B, C, D) different experimental groups (five rats per group). Group-A rats received only distilled water. Rats of Group-B, C and D were intraperitoneally injected alloxan tetrahydrate (40 mg/kg b.w.). Group-B rats were considered as diabetic control (only alloxan). Group-C rats were dosed Dionil (Gliclazide BP 5 mg) tablets [standard drug for diabetic treatment] (4.0 mg/kg b.w. orally) and served as positive control. Group-D rats were treated with either hot water or ethanolic extract (orally) at different experimental regimen. All the rats were injected with alloxan and fasted for 18 hours. *Andrographis paniculata* leaves extract and standard drug were given orally to alloxan-induced 18 hours fasted rats. After 2 hours of drug treatment, all the rats were anaesthetized with diethyl ether and blood samples were collected from heart of each experimental group for this study.

Statistical analysis: All values were also expressed as Mean \pm SD (standard deviation). All data from each treated and control group was analyzed by using by Student's "t" test. P-values less than 0.05 were considered to be significant. Percentage increase or decrease in glucose level was determined by using the formula:

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

RESULTS AND DISCUSSION:

Effect of hot water extract of *Andrographis paniculata* on BSL of alloxan-induced rats: Table 1 shows the effect of hot water extract of *Andrographis paniculata* on BSL of alloxan-induced diabetic rats. Administration of alloxan increased the BSL of rat by 81.98% as compared to the normal control group. On the other hand, rats treated with the hot water extract of *Andrographis paniculata* significantly ($p < 0.0001$) lowered the elevated BSL by 33.71% when compared to diabetic control group. In this situation, the standard drug reduced the BSL by 43.18% ($p < 0.0001$).

Effect of ethanolic extract of *Andrographis paniculata* on BSL of alloxan-induced rats: Table 2 shows the serum blood sugar level in vehicle control, diabetic control (alloxan), standard drug and sample treated groups. Alloxan enhanced the BSL by 94.65% (<0.0001) when compared with the normal control rats. On the other hand, treatment of rats with ethanolic extracts

of *Andrographis paniculata* significantly decreased (39.69%, $p < 0.0001$) the alloxan elevated BSL. Here, the percent of BSL lowering effect of the standard drug of Dionil was 47.91 (<0.0037). The comparison of antidiabetic effect of hot water and ethanolic extract of *Andrographis paniculata* on BSL of alloxan-induced rats is shown in Table 3.

TABLE 2: EFFECT OF ETHANOLIC EXTRACT OF *ANDROGRAPHIS PANICULATA* ON BLOOD SUGAR LEVEL (BSL) OF ALLOXAN-INDUCED RATS

Group (n)	Treatment	Blood sugar level* (mg/dl)	% changed (increased/decreased)
A	Normal control	59.88 ± 3.25	-
B	Diabetic control	116.56 ± 2.95	94.65 (increased)
C	Drug treatment (Dionil)	60.71 ± 2.75	47.91 (decreased)
D	Sample treatment	70.29 ± 1.05	39.69 (decreased)

*Values are Mean ± S.D. (n=5); S.D. =Standard deviation, n=number of rat

TABLE 3: COMPARISON OF THE EFFECT OF HOT WATER AND ETHANOLIC EXTRACT OF *ANDROGRAPHIS PANICULATA* ON BLOOD SUGAR LEVEL (BSL) OF ALLOXAN-INDUCED RATS

Group (n)	Treatment	Hot water extract (Mean ± S.D., mg/dl)	Ethanol extract (Mean ± S.D., mg/dl)
A	Normal control	60.77 ± 3.068	59.88 ± 3.25
B	Diabetic control	110.59 ± 6.277	116.56 ± 2.95
C	Sample treatment	73.31 ± 3.90	70.29 ± 17.05
	% decrease	33.71	39.69

*Values are Mean ± S.D. (n=5); S.D. =Standard deviation, n=number of rat

CONCLUSION: The findings of the study showed that the hot water and ethanolic extracts of *Andrographis paniculata* had successfully reduced the blood sugar level in alloxan induced rats. The present study results clearly indicate that ethanolic extract of *Andrographis paniculata* possesses hypoglycemic activity. Finally, this research work reveals a scientific basis for its use in the treatment of diabetes. Further investigation is required to establish its therapeutic effects in diabetes mellitus.

ACKNOWLEDGEMENTS: The authors wish to thank to the authority of University of Science and Technology, Chittagong for conducting the research work.

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