

Ajuga bracteosa: A central Himalayan plant shows potent hypoglycemic effect in rats

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Abstract

Background: In the present study, a central Himalayan herb; *Ajuga bracteosa* Wall. ex Benth. (Neelkanthi, Ratapaati) was investigated for the probable hypoglycemic activity underlying in it.

Material and Method: The hydroalcoholic extract (80:20) of the leaves of the plant was tested in diabetic rats experimentally induced by streptozotocin (50 mg/kg). Hydroalcoholic extract of *Ajuga bracteosa* was administered orally @ 100 mg/ kg body wt for 14 days to treat the diabetic rats.

Results and Conclusion: On 14th day, the glucose concentration in the *A. bracteosa* treated group was significantly decreased (P<0.01) as compared to untreated diabetic rats. The body weight gain in the extract treated group was also significantly higher than that of untreated diabetic rats which was comparable to negative control rats.

Keywords: *Ajuga bracteosa*, Hypoglycemic, Rat.

Introduction

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin action or both⁽¹⁾ or when the body cannot effectively use the insulin it produces. Hyperglycemia is the common effect of the diabetes and over time it leads to serious damage to many of the body's systems, especially the nerves and blood vessels and causes secondary complications affecting eyes, kidneys, nerves and arteries.⁽²⁻⁴⁾

In 2014, 8.5% of adults aged 18 years and older had diabetes according to WHO report on diabetes. In 2012 diabetes was the direct cause of 1.5 million deaths and high blood glucose was the cause of another 2.2 million deaths and its prevalence has been rising more rapidly in middle- and low-income countries.⁽⁵⁾ The first WHO *Global report on diabetes 2016* demonstrates that the number of adults living with diabetes has almost quadrupled since 1980 to 422 million adults and projects the diabetes as 7th leading cause of death in 2030.⁽⁶⁾ Based on a report by Shaw *et al.* (2010),⁽⁷⁾ diabetes among adults is expected to increase from 285 million in the year 2010 to 439 million or more by the year 2030. It has been proposed that approximately 87 million Indians will be affected by DM by the year 2030.⁽⁷⁾

In conventional therapy, IDDM is treated with exogenous insulin and NIDDM with oral hypoglycemic agents.⁽⁸⁾ Besides insulin, the most widely used medication for diabetes are oral hypoglycemic drugs including insulin sensitizers (biguanides, thiazolidinediones), insulin secretagogues (sulfonylureas, meglitinides), α -glucosidase inhibitors, incretin agonists and dipeptidyl peptidase-4 inhibitors.⁽⁹⁾ The clinical uses of the current drugs produce unpleasant side effects such as severe hypoglycemia, lactic acidosis, peripheral edema and abdominal discomfort.⁽⁹⁾ Therefore, the search for new antidiabetic agents with more effectiveness and less side effects has been

continued for which medicinal plants have always been an important source.

Before the advent of insulin injections and other pharmaceutical preparations, the treatment of diabetes was mainly based upon medicinal plants and herbs. In spite of availability of many oral hypoglycemic agents for the treatment of diabetes, in the last few years, use of herbal medicines for diabetes has been increased greatly and these drugs are gaining popularity both in developed and developing countries because of their natural origin and less side effects.⁽¹⁰⁾ Therefore, plant materials are continuously scrutinized and explored for their effect as hypoglycemic agents. The potential role of the medicinal plants as hypoglycemic agents has been reviewed by several authors, supported by the ethnobotanical surveys and traditional medicines of different cultures.⁽¹¹⁻¹⁶⁾

Ajuga bracteosa Wall. ex Benth. is a perennial hairy herb and belongs to family *Labiatae Lamiaceae*. It is distributed in subtropical and temperate regions from Kashmir to Bhutan, Pakistan, Afghanistan, China, Malaysia, western Himalayas, plains of Punjab and upper gangetic plains of India at an altitude of 1300 m.⁽¹⁷⁾ In ayurveda it is commonly called 'Nilkanthi' (sanskrit) while in kumaon region of uttarakhand is called as 'Ratapaati'. Many compounds like gamma-sitosterol, beta-sitosterol, triacontanyl docosanoate, and tetracosanoic acid have been isolated from arial part.⁽¹⁸⁾ Other constituents like phenolic components, bitter components, arabinose, cerotic acid, palmitic acid along with glucosidic constituents, D-glucoside and anthocynidin-glucosides have been found.⁽¹⁹⁾ The aqueous extract of leaves shows diuretic, stimulant action, aperient and febrifugal.⁽²⁰⁾ A decoction of the leaves of the herb is used in the traditional medicine for a number of diseases including diabetes, hypertension, fever, malaria and stomach pain.⁽²¹⁾ Although there exist ethnobotanical reports as a hypoglycemic plant but

scientific report about its efficacy is rarely reported. The aim of the present study was to investigate the hypoglycemic effect of hydroalcoholic extract in streptozotocin (STZ)-induced diabetic rats.

Materials & Method

Plant Material: The fresh leaves of the plant *Ajuga bracteosa* were used for the preparation of hydroalcoholic extract and were collected from Pithoragarh region of Uttarakhand (India).

Preparation of extracts: For preparation of extract, 500 gm powder of shade dried leaves of the plant was mixed with hydroalcoholic solvent containing 80% water and 20% alcohol. Hot extraction of this mixture was carried out by Soxhlet apparatus. The extract thus obtained from Soxhlet apparatus was further freeze dried before any use.

Animals: Eighteen male Wistar rats (200–250 g) were obtained from animal facility of our Institute. After randomization and prior to use in the experiment, were acclimatized for 7 days 12 h light/dark cycle. The animals were housed in polypropylene cages with temperature maintained at $25 \pm 2^\circ\text{C}$ and humidity 50-70 %. Rats were given standard chow diet (Ashirwad Feeds, Chandigarh) throughout the experiment and water was also provided ad libitum. The study was approved by Institutional Animal Ethical Committee constituted by Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Animal Welfare Cell, Ministry of Environment, Forests and Climatic change, Government of India, Delhi. Animals were randomized into three groups comprising six rats in each: Group I: control animals, normal drinking water; Group II: streptozotocin @50 mg/kg, i.p.; Group III: streptozotocin @50 mg/kg, i.p and treatment with *A bracteosa* extract @ 100 mg/kg, orally.

Induction of diabetes: Diabetes in the rats was induced by a single intra peritoneal (ip) injection of freshly prepared streptozotocin (STZ) solution (50 mg/kg in

injectable normal saline) to rats fasted overnight. Diabetes was identified by measuring random plasma glucose levels 48 h post STZ injection. Animals showing blood glucose level more than 200 were considered as diabetic.

Experiment: For induction of diabetes in the animals, Streptozotocin was procured from M/S Sigma Aldrich chemicals and administered @ 50 mg/kg b wt by i.p. route in group II and III. Hydroalcoholic extract of *A bracteosa* leaves was administered orally @ 100 mg/ kg to treat the diabetic rats for 14 days. The dose of the extract was selected on the basis of use of the crude plant leaves by regional folks. Blood glucose level was recorded on 7th and 14th day of the experiment. The blood samples were collected from tail vein and measurement of glucose concentration was done by Glucometer (Accu-Chek Active, Roche Diagnostics India Pvt. Ltd, Mumbai). The average food intake and average body weight after 14 day were also observed in all the three groups for comparison of the extract effect.

Results were expressed as mean \pm SEM. All Data were analyzed by ANNOVA and Dunnett's test. $p < 0.01$ or less was considered significant.

Results

Effect on food intake and average body weight: The effect of different treatments on average food intake and body weight is depicted in Table 1. Food intake of individual animal in all the three groups was recorded on 0th and 14th day of the experiment. A increase rate of food intake in streptozotocin treated group and a decrease food intake in extract treated group was observed as compared to control rats. Body weight was also decreased in STZ treated group after 14 days, however body weight increased in extract treated group as par as control group.

Table 1: Effect of *Ajuga bracteosa* leave extract on body weight and food intake of rat

Group	Body weight (gm)		Food intake (gm)	
	0 th day	14 th day	0 th day	14 th day
Control	198.8 \pm 8.78	212.8 \pm 8.095	20.3 \pm 0.41	21.6 \pm 0.53
Streptozocin	191.8 \pm 14.72	174 \pm 8.21*	17.4 \pm 1.17	25.8 \pm 0.51
Streptozocin+ Extract	214 \pm 10.83	229.8 \pm 9.78	26.3 \pm 1.13	23.1 \pm 1.73

*significant as compared to control at 14 day ($P < 0.01$)

Effect on blood Glucose concentration: The data showing blood glucose level at different time interval before and after treatment is presented in Table 2. Glucose concentration of all the three groups was measured by Glucometer at 0 hr (before STZ), 48 hr after STZ and after 14 days of extract treatment. The administration of hydroalcoholic Ratapaati leaves extract was able to significantly decrease the glucose concentration after 7 day which were further slightly decreased after 14 days, however the glucose concentration could not be reached upto the normal level. The glucose concentration in control and the untreated STZ group remained nearly unaffected after 14 days.

Table 2: Effect of *Ajuga bracteosa* leave extract on blood glucose level in rat

Groups	Glucose Concentration (mg/dl)			
	0 hr (Before STZ)	48 hr after STZ	7 days	14 days
Control	108.4±4.21	103.6±4.057	110.4±3.84	105.6±3.24
STZ control	106.4±6.69	587.2±12.34*	588.4±8.76	593.2±9.04*
STZ+Extract	104.6±6.68	347.6±22.61*	197.6±19.87	181.0±20.11* ^a

*significant as compared to control (P<0.01), ^aSignificant as compared to column 3

Discussion

The present manuscript discusses about the hypoglycemic/ antidiabetic effects of the hydroalcoholic leaf extract of *Ajuga bracteosa* in streptozotocin-induced-diabetic rats. The results of the study have shown that the hydroalcoholic extract of *Ajuga bracteosa* leaves at the dose of 100 mg/kg body weight for 14 days has a marked hypoglycemic activity by lowering the blood glucose levels in STZ-induced-diabetic rats.

In our study the body weight of the group II diabetic rats decreased significantly as compared to control. This is supported by the fact that streptozotocin induced diabetes causes a severe loss in body weight^(22,23) and leads to loss of body weight due to the increased muscle wasting and loss of tissue proteins.^(24,25) The treatment of the ABHL extract to group III STZ induced diabetic rat increased the body weight significantly as compared to group II and this body weight gain was similar to than in group I (control).

In the present study the untreated diabetic rats showed marked increased in blood glucose level as compared to control rats confirming the diabetes. Where in untreated diabetic rats the blood glucose level remained unchanged after 14 days, in diabetic rats treated with *A. bracteosa* the glucose level reduced to almost half after 14 days treatment extract showing the hypoglycemic effect. Khan and Shechter, (1991)⁽²⁶⁾ have suggested that a 25% reduction in blood glucose levels is considered a significant hypoglycemic effect. Our experimental pharmacology data confirm that *A. bracteosa* shows clear hypoglycemic activity which is in accordance with finding of Gupta *et al.* (2008)⁽²⁷⁾ where glucose level has been shown reduced by use of this herb.

Experimental evidences suggest the involvement of free radicals in the pathogenesis of diabetes⁽²⁸⁾ and that diabetes is associated with an increased production of ROS, including superoxide radical, hydrogen peroxide and hydroxyl radical.⁽²⁹⁻³²⁾ The protection of the diabetes in extract treated rats may be attributed to the antioxidant activity found in the essential oil⁽³³⁾ and the aerial part of the plant.⁽³⁴⁾ Also several phytochemical constituents particularly phenols, flavonols, terpenoids, polyphenols in the plant have been found by many researchers and these polyphenols have been reported to act as antioxidants in plant.⁽³⁵⁾ N-acetyl-L-Cysteine, a well-known antioxidant, has also been shown to prevent

hyperglycemia in STZ injected diabetic animals through reduced oxidative stress and restoring β cell function.⁽³⁶⁾

The findings of the present study reveals that the hydroalcoholic extract of *Ajuga bracteosa* is a potent hypoglycemic agent and can be explored as herbal remedy for the diabetes/ hypoglycaemia. Though, the work presented in the study is preliminary, it gives a path and field to be focused on. Further studies can be undertaken to determine the active ingredients in the extract and to elucidate the mechanism at the cellular and molecular levels in detail.

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