ANTI-HYPERGLYCEMIC ACTIVITY OF *AJUGA BRACTEOSA* LEAVES IN ALLOXAN INDUCED DIABETIC CHICKS

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ABSTRACT

Present study was carried out to investigate a local medicinal plant, *Ajuga bracteosa* for its anti-hyperglycemic activity in alloxan induced diabetic chicks. A total of eighteen domestic chicks (Gallus gallus domesticus) of either sex were divided into three groups (A, B and C) of six animals each. For the induction of diabetes in group A and group B, chicks were administered with alloxan (120 mg/kg i.p.) for one week. After the confirmation of diabetes, done by glucose test kit; Group A chicks were considered as diabetic control, Group B chicks as diabetic chicks treated with *Ajuga bracteosa* leaves extract (100 mg/kg, orally, 30 days daily) and Group C chicks as normal control. On different intervals of days (1st, 10th, 20th and 30th), blood samples were taken from the wing's vein and glucose level was evaluated. Data were analyzed by using one-way analysis of variance, $p<0.05$ was considered statistically significant. The Group B diabetic chicks treated with the extract of *Ajuga bracteosa* leaves showed significant ($p<0.05$) reduction in blood glucose level as compared to Group A diabetic control chicks. The result obtained in the study indicates the potent anti-hyperglycemic activity of the *Ajuga bracteosa* leaves extract in alloxan-induced diabetic chicks.

KEYWORDS: Anti-hyperglycemic, alloxan, *Ajuga bracteosa*, Diabetes

1. Introduction

Diabetes mellitus (DM) is a group of metabolic disorder characterized by hyperglycemia. DM occurs due to defects in insulin secretion, insulin action or both.[1] The presence of DM confers increased the risk of many devastating complications. It is considered as one of the five leading cause of death in the world.[2] Insulin and various types of hypoglycemic agents such as biguanides and sulfonylureas are available for the treatment of diabetes. However, none of these medications is ideal due to toxic side effects and in some cases domination of response after prolong use.[3] The main disadvantages of the currently available drugs are that they have to be given throughout the life and produces side effects in many cases.

Therefore, Inspite 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.[4] Although several medicinal plants have gained importance for the treatment of DM and many remain to be scientifically investigated.[5] Therefore, plant materials are continuously scrutinized and explored for their effect as anti-hyperglycemic agents.

Ajuga bracteosa is an important medicinal plant of Himalaya region. It is a praised medicinal, aromatic, villous, soft and decumbent herb which is about 10–30 cm in height.[6] It is found on grassland, exposed slopes and open field in temperate and subtropical region of the world [7] at an elevation of 1300m to 2400 m. [8] *Ajuga bracteosa* is known from different vernacular names. It is commonly called "Bungle" in English, "Nilkanthi" in Sanskrit and 'Ratpatiya' in Hindi.

It has been utilized in medicine since ancient times and has tremendous medicinal potential because of the presence of active ingredients. Phytochemically, it contains various compounds such as neo-clerodane diterpenoids, flavonol glycosides, iridoid glycosides, ergosterol-5,8-endoperoxide and phytoecdysones.[9-12] In ethno medicine its use is reported as anthelmintic, astringent, antibacterial, antifungal, anti-inflammatory, anti-hyperglycemic and it also remediates intestinal ailments.[9] This study was conducted to investigate the anti-hyperglycemic activity of *Ajuga bracteosa* leaves in alloxan induced diabetic chicks.

2. Materials and Methods

2.1.Plant Material Collection and preparation

Aerial part of *Ajuga bracteosa* was collected from the local areas of Lohaghat 29°25'0" N, 80°6'0" E (Uttarakhand, India) for the study. Its leaves were allowed to dry under shade for 5-7 days and coarsely powdered. 500 gm powder of shade dried leaves of the plant were mixed with hydroalcoholic solvent containing 80% of water and 20% of alcohol, hot extraction of this mixture was carried out by Soxhlet Apparatus. The extract thus obtained was further freeze dried before use.

2.2.Animals

Healthy Domestic chicks - Croiler chabra **(***Gallus gallus domesticus*), approximately 2-3 weeks old weighing 100 ± 20 gm of either sex, were selected for the study. Chicks were maintained and acclimatized according to the laboratory conditions of the animal house in the department. The animals were housed in battery cages under laboratory conditions at existing room temperature and relative humidity. They were offered commercial food (Starter, Grower and Finisher) purchased from the market (Godrej Company) and tap water ad libitum. All protocols were approved by the Institutional Animal Ethics Committee (IAEC), Department of Pharmaceutical Science, Bhimtal, Kumaun University, Nainital and the member secretary, CPCSEA, Ministry of Environment, Forest and Climate Change, Government of India. The animals were kept under standard conditions throughout the experiment to reduce the error. Minimum number of animals was used to obtain reliable results.

2.3.Chemicals

Alloxan was purchased from Sigma Chemicals, St. Louis, USA. Glucose test kit was procured from ARKRAY Healthcare Pvt. Ltd. Surat, INDIA. All other chemicals used in this study were of analytical grade and highest purity procured from standard commercial sources in India.

2.4.Induction of Diabetes

DM was induced in chicks by administration of alloxan monohydrate (120 mg/ kg body weight, i.p.). After one week, blood samples were collected from the wing's vein, and serum glucose levels were estimated using glucose test kit supplied by ARKRAY Healthcare Pvt. Ltd. (Surat, India). Animals with fasting blood glucose levels ≥ 250 mg/dL were selected as diabetic chicks for further study.

2.5.Experimental Design and Treatments

The chicks were divided into three groups (A, B and C) randomly, each containing six chicks. After one week diabetes was induced in chicks of group A and B with alloxan (120 mg/ kg body weight, i.p.). Chicks with a blood glucose level ≥ 250 mg/dL were considered to be diabetic. Group A chicks served as Diabetic Control while Group B served as diabetic chicks treated with hydroalcoholic extract of *Ajuga bracteosa* leaves (100 mg/kg, orally, 30 days daily); Group C chicks were served as Normal control.
The treatment was given in fasting state and food was provided after dosing the animals. During experiment on different intervals of days blood sample were collected from wing's vein for blood glucose analysis.

2.6.Blood Glucose Levels

The blood samples were collected from wing's vein on 1st, 10th, 20th and 30th day after one hour of the plant extract administration for blood glucose level evaluation. Estimation of blood glucose level was done using a glucose test kit supplied by ARKRAY Healthcare Pvt. Ltd. (Surat, India).

2.7.Statistical Analysis

The mean values of the blood glucose taken at different intervals of days for diabetic control, diabetic treated with *Ajuga bracteosa* and normal control chicks were analyzed for the statistical significance using the student's t-test by one-way analysis of variance (ANOVA). $P<0.05$ were considered statistically significant when compared with diabetic control.

3. Results

Administration of alloxan causes significant increase in blood glucose level in group A and B compared to group C normal control chicks. However, Group B diabetic chicks treated with *Ajuga bracteosa* showed a significant reduction ($p<0.05$) in blood glucose levels compared to diabetic control group A chicks [Table 1][Figure 1].

Table 1: Effect of extract of *Ajuga bracteosa* leaves on fasting blood glucose level in alloxan induced diabetic chicks

Group	Blood glucose level (mg/dl)			
	1st	10th	20th	30th
A	262±2.31*	269±2.65*	282±3.21*	303.7±3.48*
B	259±1.53**	250.6±0.67**	244±1.16**	226±2.08**
C	161±3.61	161.3±2.91	162±3.47	161.3±2.41

Group A- Diabetic control, Group B- Diabetic + *Ajuga bracteosa*, Group C- Normal control. Values as mean ± S.E. (n= 3). * Significantly different ($p<0.05$) when compared with group C chicks. **Significantly different ($p<0.05$) when compared with group A chicks.

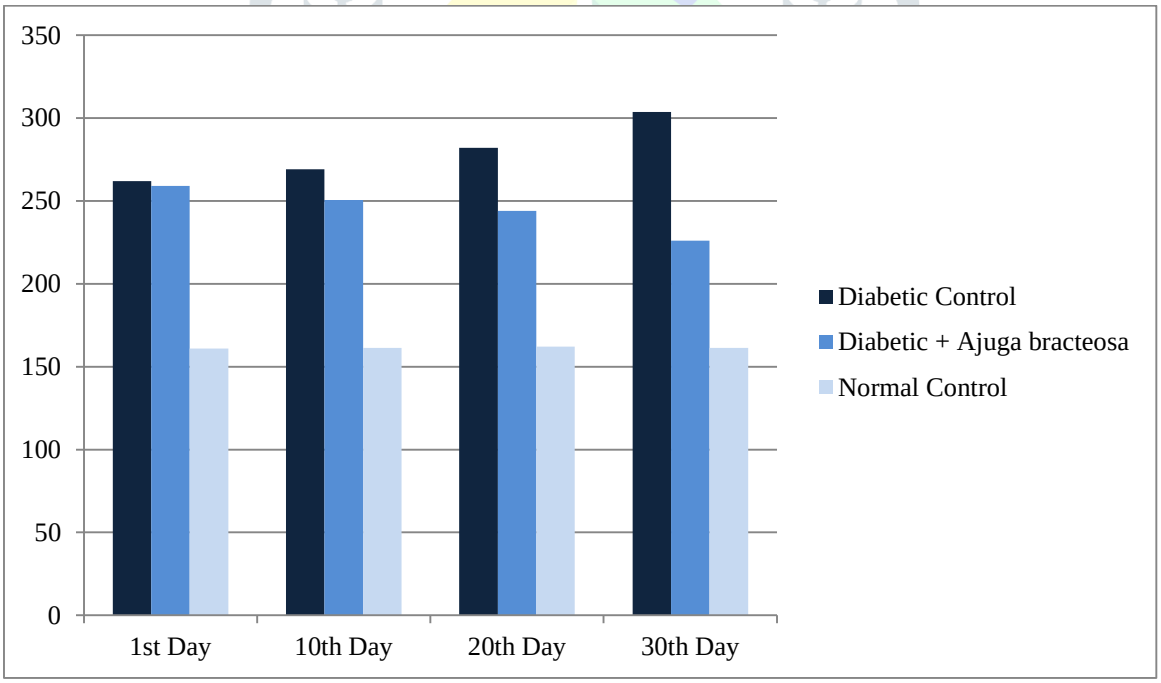

Figure 1: Graphical representation for anti-hyperglycemic activity of *Ajuga bracteosa* in alloxan induced diabetic chicks.

4. Discussion

This investigation aims to investigate the anti-hyperglycemic activity of *Ajuga bracteosa* in alloxan induced diabetic chicks. Alloxan induces diabetes by being selectively toxic to the beta-cells of the islets of Langerhans which secrets insulin, a hormone which regulates blood glucose concentration, thus,

consequently resulting in the accumulation of free glucose in the blood. [13] It is known that alloxan induces free radical formation by redox reaction that causes tissue injury and make beta-cells to degranulate and consequently degenerate. [14] The administration of hydroalcoholic extract of *Ajuga bracteosa* leaves causes a significant ($p<0.05$) decrease in blood glucose level in the extract treated diabetic group (Group B) when compared to the diabetic control group (Group A) on 1st, 10th, 20th and 30th day of the experiment.

The reduction in blood glucose level by most bioactive compounds from plants might occur through one of several mechanisms including increased repair or proliferation of beta-cells and stimulation of insulin secretion. [15] This mechanism of action depicted the underlying principle through which the hydroalcoholic extract of *Ajuga bracteosa* reduced the blood glucose concentration in alloxan induced diabetic chicks. Our experimental pharmacology data shows that *Ajuga bracteosa* have potent anti-hyperglycemic activity which is in accordance with the finding of Gupta *et al*. [16] and Harsahay Meena et al. [17] where glucose level has been shown reduced by the use of this herb.

It has been also proved that the protection of the diabetes in extract treated rats may be attributed to the antioxidant activity found in the essential oil [18] and the aerial part of the plant. [19] Alcoholic extract of *Ajuga bracteosa* leaves also help in restore hematological parameters in diabetic chicks. [20] 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. [21]

A significant number of compounds have been isolated from various species of the *Ajuga* herb including; sterols (ajugalactone, β-sitosterol, γ-sitosterol, stigmasterol,), phenolic components, arabinose, cerotic acid, ecdysterone, phytoecdysteroids (phytoecdysteroid, Cyasterone, ajugalactone, ajugasterone A-C), flavonol glycosides, triterpenoid (ergosterol-5,8-endoperoxide), iridoid glycoside (8-O-acetylharpagide, 6,8-diacetylharpagide, Ajureptoside, 8-acetylherpagide, Herpagide), neoclerodane-diterpenes and diterpenes (ajugarins I, II, IV and V). [22,23] The result reveals that hydroalcoholic extract of *Ajuga bracteosa* is a potent anti-hyperglycemic agent and can be explored as natural remedy for the diabetes.

5. Conclusion

There is a keen interest of researchers and pharmacists in natural products and the use of medicinal plants for the treatments of diabetes and its complications. Many medicinal plants were used for the treatment of diabetes throughout the world for a long time. As these natural products have minimal or no side effects and is also less expensive than the chemically synthesized drugs; therefore, in recent years more research work is done in this area. In our study we have selected *Ajuga bracteosa* as an anti-hyperglycemic agent for the study of alloxan induced diabetes in chicks. Our results shows the potent anti-hyperglycemic activity of *Ajuga bracteosa* leaves extract on alloxan induced diabetic chicks, as its administration in diabetic chicks showed a significant ($p<0.05$) decrease in the level of blood glucose as compared to diabetic control chicks. Hence, there is a great chance that the future studies will broaden our knowledge about *Ajuga bracteosa* as a potent anti-hyperglycemic agent.

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