ANTIDIABETIC AND ANTIOXIDANT POTENTIAL OF *COLEUS AROMATICUS* LEAF EXTRACTS IN ALLOXAN INDUCED DIABETIC RATS

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Summary

The present work was carried out to study the effect of *Coleus aromaticus* leaves on blood glucose and antioxidant enzymes levels in alloxan rendered diabetic rats. Alloxan (150 mg/kg body wt. i.p.) induced diabetic rats were treated with aqueous and ethanolic extracts of *Coleus aromaticus* at dose of 200 mg/kg twice daily 12 hours cycle (8:00 am and 8:00 pm) for 8 weeks. The concentration of glucose in the blood and antioxidant enzymes levels viz. superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and malondialdehyde (MDA) in liver homogenate were measured.

Aqueous and ethanolic extract of *Coleus aromaticus* significantly ($p<0.01$ and $p<0.001$) lowered the alloxan mediated hyperglycemia**.** The activity of antioxidant enzymes such as SOD, CAT and GPxase were found to be increased in the liver homogenate of diabetic animals treated with both the extracts of *Coleus aromaticus*. Test extracts also produced significant decrease in MDA level compered to diabetic control. This confirms the antihyperglycaemic and antioxidant activity of *Coleus aromaticus* in alloxan induced diabetic rats. Among the tested extracts, ethanolic extract of *Coleus aromaticus* leaves was found to be having potent antihyperglycemic activity than the aqueous extract.

Key words : *Coleus aromaticus*, Alloxan, SOD and Catalase.

Introduction

Diabetes mellitus is a major endocrine disorder affecting nearly 10% of population all over the world [1]. According to the world ethnobotanical information reports, almost 800 plants may possess antidiabetic potential. Traditional and indigenous methods have been employed in order to prevent diabetes mellitus in India since ancient times. Currently available drugs fail to maintain tight glycemic control over time and are associated with various side effects. Therefore, there is a need to develop newer treatment strategies such as hypoglycemic agents of plant origin as they are known to have fewer adverse effects [2]. Many herbs and plant extracts have been shown to possess significant antidiabetic property [3-5].

Coleus aromaticus belongs to the plant family Labiatae (Mint family) syn. *Coleus amboinicus* or *Plectranthus ambonicus* is commonly known as Indian / country borage. The leaves of this plant are often eaten raw with bread and butter. The leaves of this plant are traditionally used for the treatment of severe bronchitis, asthma, diarrhea, epilepsy, diabetes, renal and vesicle calculi [6]. Literature reports indicate that *Coleus aromaticus* is known to possess antilithiotic,[7] chemopreventive,[8] antiepileptic[9] and antioxidant[10] activites. However, scientific investigation on antidiabetic property of leaves of the title plant has not been documented in the literature so far. Hence, the present study was designed to evaluate antihyperglycemic efficacy on alloxan induced diabetic rats model.

Materials and methods

Collection of plant material

Coleus aromaticus leaves were collected from Sasya Kaashi, a herbal garden maintained by Adichunchanagiri math. B.G.Nagara, Karnataka, India in the months of January to March and the sample was authenticated by botanist. The fresh *Coleus aromaticus* leaves were washed in distilled water to remove extraneous material and dried at 60˚C for two hours.

Preparation of extracts

50 g of cleaned fresh leaves of *Coleus aromaticus* were homogenized with 500ml of distilled water and ethanol separately using pestle and mortar. This was centrifuged at 7000 rpm for 10 minutes.

The clear supernatant was concentrated using rotary evaporator at 38 - 40°C. The yield of aqueous and ethanolic extracts were found to be 8.05 g and 10.24 g of brownish dried material respectively. The extracts were suspended in 3% w/v aqueous gum acacia for oral administration in antidiabetic study.

Experimental animals

Sprague - Dawley male rats (170-200g) were purchased from Sri Venkateshwara animal breeders, Bangalore, India and they were housed individually at 22 ± 1°C in cages with a 12 hours light dark cycle. All rats were allowed free access to the diet and water for 1 week for adaptation to the new environment. Rats were fed with standard laboratory pellets (Hindustan Lever Ltd., India). The experimental protocol was approved by the Institutional Ethics Committee (IAEC) and conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

Chemicals

Alloxan monohydrate was purchased from (IOBA Chemie, Mumbai), EDTA, Acetic acid, Ortho toludine, Ethanol, Chloroform, Hcl, Prrogallol, H_2O_2, Sodium azide, TCA and DTNB.

Induction of diabetes in rats [11]

Diabetes was induced by 150 mg/kg of alloxan administered i.p. in physiological saline. After 7 days blood glucose level was measured to confirm the induction of diabetes. Rats showing fasting blood glucose levels above 200 mg/ dL were selected for the antidiabetic study.

Experimental grouping of animals

The diabetic rats were allocated to 4 groups of 6 animals each

Group I : Normal control - Not given alloxan
Group II : Diabetic control - Alloxan 150 mg/kg + received vehicle
Group III : Alloxan + 200 mg/kg aqueous extract of Coleus aromaticus leaves
Group IV : Alloxan + 200 mg/kg ethanolic extract of Coleus aromaticus leaves

After eight weeks of treatment, the rats were sacrificed by anesthesia using diethyl ether. Blood was collected by cardiac puncture and immediately transferred into tubes containing EDTA. Blood was then centrifuged at 4,000 rpm for 10 min to recover plasma for the estimation of glucose level. Then the liver tissues were excised and subjected to homogenization to measure the activities of ROS scavenging enzymes (SOD, GPX and CAT) and the levels of MDA.

Estimation of plasma glucose level [12]

Plasma glucose was estimated by ortho toludene method.
To 0.5ml of plasma, 3.5 ml of ortho toludene mixture (Ortho toludene in glacial acetic acid) was added and incubated in boiling water bath for 10 min. Cooled and the colour developed was measured at 637 nm in a photo electrical colorimeter. Glucose standard also processed as above and were read against reagent blank.

Estimation of Superoxide dismutase [13]

Superoxide dismutase was assayed by the method of Marklund and Marklund (1974). One ml of tissue homogenate was mixed with 0.25ml of ethanol and 0.15ml of chloroform, kept in a mechanical shaker for 15 minutes and centrifuged. To 0.5ml of the supernatant, 2.0ml of Tris-HCl buffer (0.1M, pH 8.2), 1.5ml of water and 0.5ml of pyrogallol were added. Change in optical density at 0,1,2,3 minutes at 420 nm was read in Photochem colorimeter. Control tubes containing 0.5ml of water was also treated in the similar manner against a buffer blank.

The enzyme activity is expressed as units/mg protein. One enzyme unit corresponds to the amount of enzyme required to bring about 50% inhibition of pyrogallol autooxidation**.**

Estimation of Catalase[14]

Catalase activity was assayed by the method of Sinha (1972). To 0.1 ml of homogenate or lysate, 1.0ml of buffer and 0.5 ml of hydrogen peroxide were added and the time was noted. The reaction was arrested by the addition of 2.0ml of dichromate acetic acid reagent. Standard hydrogen peroxide in the range of 4 to 20 µmoles were taken and treated similarly. The tubes were heated in a boiling water bath for 10 minutes. The green colour developed was read at 570nm using a Photochem colorimeter**.** Catalase activity is expressed as µmoles of H_2O_2 consumed/min/mg protein.

Estimation of Glutathione peroxidise [15]

Glutathione peroxidase was assayed by the method of Rotruck et al. (1973). 0.2 ml each of EDTA, sodium azide, GSH, H_2O_2, 0.4ml of buffer and 0.1ml of tissue homogenate or lysate were mixed and incubated at 37˚C for 10 minutes. The reaction was arrested by the addition of 0.5ml of 10% TCA and the tubes were centrifuged. To 0.5 ml of supernatant, 4.0ml of phosphate solution and 0.5 ml of DTNB were added and the colour developed was immediately read at 420nm using a Photochem colorimeter. Graded amount of standards were also treated similarly. Glutathione peroxidase activity is expressed as µg of glutathione utilized/min/mg protein.

Statistical analysis

Results were presented as Mean ± SEM for 6 rats in each group. The results obtained from the present investigation were subjected statistical analysis using one-way ANOVA, followed by Turkey Kramer Multiple Comparison test.

Results and Discussion

Many plants have been used for the treatment of diabetes mellitus in Indian system of medicine and in other ancient systems of the world. Out of these, only a few have been evaluated as per modern system of medicine. From many such plants only extracts have been prepared and their usefulness evaluated in experimental diabetes in animals. Most of them seem to act directly on the pancreas (pancreatic effect) and stimulate insulin level in the blood. Some have extra pancreatic effect by acting directly on tissues like liver, muscle etc. and alter favorably the activities of the regulatory enzymes of glycolysis, gluconeogenesis and other pathways. Many of its products / chemical constituents are known to possess wide array of medicinal properties.

The present study demonstrated the hypoglycemic effect of aqueous and ethanolic extracts of *Coleus aromaticus* on blood glucose profile and liver antioxidant enzymes activities on alloxan induced diabetic rats. Animals intoxicated with alloxan 150 mg/kg i.p. produced significant increase in hyperglycemia compared to normal control rats. Further, significantly elevated levels of liver antioxidant enzymes SOD, CAT and GPX and lowered level of MDA were also found in diabetic control group.

Table 1: Effect of aqueous and ethanolic leaf extracts of *Coleus aromaticus* on blood glucose and liver antioxidant enzymes levels in alloxan induced diabetic rats

Results are Mean ± SE, n = 6, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ compared to CCl_4 control.

Groups	Blood glucose level mg/dl	SOD Units/mg of protein/g tissue	CATALASE Units/mg of protein/g tissue	GPX Units/mg of protein/g tissue	MDA µg/g tissue
Normal control	93.4 ± 8.94	35.84 ± 2.82	0.007 ± 0.0003	0.018 ± 0.003	70.40 ± 3.80
Diabetic control	250.0 ± 10.20	25.26 ± 2.67	0.003 ± 0.0001	0.009 ± 0.001	220.50 ± 6.50
Diabetic + aqueous extract	190.0 ± 9.50*	31.48 ± 2.40ns	0.005 ± 0.0002***	0.012 ± 0.002ns	180.3 ± 3.40***
Diabetic + ethanolic extract	170.0 ± 8.40**	34.50 ± 1.94*	0.006 ± 0.0002***	0.016 ± 0.002*	150.8 ± 4.50***

Animals treated with test extracts exhibited significant reduction in fasting blood glucose level and reversed the changes in antioxidant enzymes and MDA levels when compared to diabetic control rats. Though the concentrations of SOD and GPX were found to be decreased in considerable manner in rats treated with 200 mg/ kg of aqueous extract compared to diabetic control group, but the results were found to be statistically not significant.

Among the tested extracts, ethanolic extract of *Coleus aromaticus* leaves was found to be having potent antihyperglycemic activity than the aqueous extract. The results are presented in Table – 1. The antidiabetic activity of the both extracts may be due to the presence of phenolic content, which was evident by determination total phenolic content in test extracts.

In conclusion, the above study suggests that antioxidant property of *Coleus aromaticus* leaves strongly inhibit the lipid peroxidation, which could reduce the susceptibility of tissues to alloxan induced oxidative stress.

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