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Research Article

EVALUATION OF *IN-VIVO* ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF *COLEUS VETTIVEROIDES JACOB* IN STREPTOZOTOCIN-INDUCED OXIDATIVE STRESS IN RATS

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ABSTRACT

Objective: The aim of the present study was to investigate the antioxidant activities of the methanolic extract of *Coleus vettiveroides Jacob* in streptozotocin-induced **oxidative stress in** rats.

Methods: Animals were treated with methanolic extract of *Coleus vettiveroides Jacob* plant extracts for 14 days and then oxidative stress was induced with a single dose of Streptozotocin 10mg/kg (p.o). Treated with 200 mg/kg & 400 mg/kg (p.o) of methanolic extract of *Coleus vettiveroides Jacob* and determine the Glutathione, SOD, GP_X Catalase, and TBARS levels were determined.

Results: The present study revealed that administration of methanolic extract of *Coleus vettiveroides Jacob* extract showed a significant decrease in thiobarbituric acid reactive substances (TBARS) levels. The treatment also resulted in a significant increase in liver GSH, SOD, CAT, GPx levels when compared with diabetic control rats.

Conclusion: The results clearly suggest that the methanolic extract of *Coleus vettiveroides Jacob* treated group may effectively normalize the impaired antioxidant status in Streptozotocin induced diabetic treated groups.

Keywords: *Coleus vettiveroides Jacob*, Streptozotocin, antioxidant enzymes.

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders characterised by high blood glucose level, resulting from insulin secretion defects, action or both. Long term complications of this illness contribute to increase mortality and morbidity. Diabetes mellitus is the sixth leading cause of death globally**[1].** There is increasing evidence that complications related to diabetes are associated with oxidative stress induced by the generation of free radicals[11] . In diabetes, oxidative stress has been found to be mainly due to an increased production of oxygen free radicals and a sharp reduction of antioxidant defences[12] . Hence, compounds with both hypoglycemic and antioxidative properties would be useful antidiabetic agents. Streptozotocin is often used to induce diabetes mellitus in experimental animals through its toxic effects on pancreatic β-cells. Streptozotocin-induced diabetes mellitus is associated with the generation of reactive oxygen species causing oxidative damage[13]. Diabetics and experimental animal models exhibit high oxidative stress due to persistent and chronic hyperglycaemia, which thereby depletes the activity of antioxidative defense system and thus promotes de novo free radicals generation[14]. Many plant extracts and plant products have been shown to have significant antioxidant activity[15] and useful in treatment of several ill fated diseases including diabetes[16]. Different types of oral hypoglycaemic agents such as biguanides and sulphonylurea are available along with insulin for the treatment of diabetes mellitus**[2]**, but have side effects associated with their uses**[3,4]**. There is a growing interest in herbal remedies because of their effectiveness, minimal side effects in clinical experience and relatively low costs. Herbal drugs or their extracts are prescribed widely, even when their biological active compounds are unknown. Traditionally, a number of plants have been used in various herbal preparations in the management of diabetes and only a few of them have been proven scientifically**[5]**. More than 800 plants have been studied for their antidiabetic potentials**[6,7]** among thousands of plants used in various regions of the world. Even the World Health Organization (WHO) approves the use of plant drugs for different diseases, including diabetes mellitus. Therefore, studies with plant extracts are useful to know their efficacy and mechanism of action and safety. Medicinal plants useful in diabetes were reviewed recently**[8,9]**.

Coleus vettiveroides Jacob (Lamiaceae) is a small profusely branched succulent herb with quandragular stems and branches and deep straw coloured aromatic roots .Leaves are glandular and hairy, broadly ovate with dentate margins and prominent veins in the abaxial side. The whole plant is used in ayurvedic system of medicine for treating varied diseases like leprosy, skin diseases, leucoderma, fever etc **[10].** The formulations are mostly for internal use, and generally indicate a therapeutic activity in cases of G.I disorders like mal-absorption, flatulence, diarrhoea or dysentery and ulcers resulting from such G.I related syndromes. Externally, the Taila and Lepa formulations are used as emollients and plasters over painful areas. The other drugs in such formulations along with which Hrivera is usually added are generally plant drugs containing essential oils with known carminative and analgesic properties. The Present study was taken up to evaluate the antidiabetic activity of *Coleus vettiveroides Jacob* and to establish its therapeutic potential in the treatment of diabetes and its complications.

MATERIALS AND MET HODS

Preparation of the Extract

The entire plant of *Coleus vettiveroides Jacob* was collected from Tirunelveli district, Tamilnadu, India. Taxonomic identification was made from botanical survey of medicinal plants, Siddha Unit, Government of India and Palayamkottai. The dried powdered plant material was extracted with methanol for 72 hours by using soxhlet apparatus[20]. The extract was filtered and concentrated to dryness in vacuum and stored in an air tight container.

Animals

Wistar albino rats (150- 200g) was obtained from RMMCH in Annamalai University at Chidambaram (IAEC Proposal Number-794) were used for the study. The animals were fed with commercial pellets and water ad libitum. The animals were well acclimatized to the standard environmental conditions of temperature (22°C ± 5°C) and humidity (55 ± 5°C) and 12 hrs light/dark cycle throughout the experimental period.

Acute Toxicity Studies

The acute oral toxicity study was carried out as per OECD 423 guidelines (OECD, 2001). The study was approved by the Institutional Animal Ethics Commitee (IAEC). No mortality and no signs of toxicity were found even after administration of a limit dose of 200 mg/kg body weight of extract; hence 1/10th of the dose was

taken as effective dose. Two doses, 200 and 400 mg/kg were selected for the present study to evaluate *in-vivo* antioxidant activity.

Experimental Protocol

The animals were divided into five groups of six animals each. Group I served as normal control treated with normal saline in a dose of 10ml/kg, group II served as a toxic group and was administered Streptozotocin 50mg/kg body weight, Group III served as as a treatment control group and was administered methanolic extract of *Coleus vettiveroides Jacob* at the dose of 200 mg/kg body weight, Group IV a treatment control group and was administered methanolic extract of *Coleus vettiveroides Jacob* at the dose of 400 mg/kg body weight and GroupV served as a standard group and was administered Glibenclamide 10mg/kg body weight.

Dissection and Homogenization

After 14 days of treatment all the rats were sacrificed by cervical decapitation and the blood was collected, heparinised blood were used for anti oxidant enzymes and serum was separated by centrifugation at 2000 rpm for 10 minutes at 4^0C biochemical estimation.

Biochemical Analysis

Thiobarbituric acid reactive substances (TBARS) was determined by the method of Okhawa et al., 1979[17], Reduced glutathione (GSH) (Moron., 1979)[18], Superoxide dismutase (SOD) was determined according to the method of Mc Cord and Fridovich et al., 1971[19] after removing the haemoglobin by the method of Minami and Yoshikawa.,(1979), Catalase (CAT) (Aebi ey al., 1974)[21], glutathione peroxidase (GPx) (Paglia et al., 1967)[22].

Statistical analysis

The results are expressed as mean ± SD. Data were analyzed by one way analysis of variance (ANOVA).

RESULTS

Table shows the concentration of TBARS , GSH, SOD,CAT, Glutathione Peroxidase (GPx) and Glutathione-S-Transferase (GST) in the liver of normal control and experimental groups of rats. The levels of TBARS in Streptozotocin treated rats were significantly higher than normal control rats, whereas Streptozotocin induced rats-treated with the methanolic extract of *Coleus vettiveroides Jacob* and glibenclamide restored the altered values to the near normalcy when compared to group II.

The decreased SOD, CAT, GSH and GPx levels was observed in streptotozin induced rats. After the administration of methanolic extract of *Coleus vettiveroides Jacob* with streptotozin and glibenclamide treated rats were showed significantly increases the levels of SOD,CAT, GSH and GPx levels when compared with group II.

Table 1: Effect of methanolic extract of on *Coleus vettiveroides Jacob on* biochemical parameters

Treatment	SOD (U/gm)	CAT(U/gm)	LPO(nmol/l)	Reduced-GSH (nmol/l)	GPx (g/l hemosylate)
Group 1	120.45±34.03	210.06±48.76	1.22±0.87	21.61±4.78	1.21±0.76
Group 2	56.89±22.78	32.78±10.09	3.02±0.01	10.09±2.78	0.318±0.2
Group 3	59.78±21.34	45.89±18.13	2.99±0.09	12.45±2.34	0.67±0.08
Group 4	168.98±22.00**	187.78±33.45	1.01±0.08**	19.88±3.13**	1.20±0.77**
Group 5	187.92±41.98##	290.88±53.09##	0.99±0.66	19.09±4.55	1.34±0.78

Values are expressed as mean ± SD,*: Compared with diabetic rats, **#** : Compared with normal rats, */ #p<0.05**, **/** ##p<0.01, ***/ ###p<0.001 when compared to STZ control

DISCUSSION

The present study was conducted to evaluate the beneficial effects of methanolic extract of *Coleus vettiveroides Jacob* on antioxidant status in STZ-induced diabetic rats. The intensified free radical production during STZ-mediated experimental diabetes resulted in the elevated levels of lipid peroxides and hydroperoxides by oxidative degradation of polyunsaturated fatty acids. These are unstable, cytotoxic and highly reactive, leading to free radical damage to proteins and DNA and finally cause various diabetes–mediated complications. The degree of tissue damage persuaded by free radicals depends on the balance between free radical generation and the endogenous antioxidant defense mechanism **[23].** One of the most often used biomarker to investigate the oxidative damage on lipids is TBARS a major lipid peroxidation product. It can react with the free amino group of proteins, phospholipids, and nucleic acids leading to structural modification [24]. According to the provided a notable increase in TBARS level in liver was observed in STZ-diabetic rats compared with their respective normal controls. Previous study had reported increased levels of lipid peroxidation in STZ- diabetic rats. However, the oral administration of *Coleus vettiveroides Jacob* extracts to the diabetic group of rats significantly reverted back TBARS levels to near normal values which show the anti-lipid peroxidative property of *Coleus vettiveroides Jacob* extract in experimental diabetes. It had been reported that *Coleus vettiveroides Jacob* extract is an efficient scavenger of OH^- and O^{-}_2 radicals[25].

Numerous studies have revealed lower antioxidant and enhanced peroxidative status in diabetes mellitus[26,29]. SOD, CAT, GPx are enzymes that destroy the peroxides and play a significant role in providing antioxidant defences to an organism. GPx [27], CAT [28]are involved in the elimination of H_2O_2, and SOD acts to dismutate superoxide radicals to H_2O_2 which is then acted upon by GPx. The functions of all three enzymes are interconnected and lowering of their activities results in the accumulation of lipid peroxides and increased oxidative stress in diabetic rats [30]. In the present study the activities of GPx, SOD and CAT in plasma and different tissue organs extracts of the STZ-diabetic rats were significantly lower than their control ones. Impairment of antioxidant machinery may be described by both the damage of antioxidant enzymes caused by protein glycation and consumption by an excess demand[31]. The compromises in enzymatic antioxidant defense systems and alterations in their activities have been implicated in the mechanisms of abnormal tissue function observed in diabetes mellitus [32]. GSH is a major intracellular non protein sulphydral compound and is accepted as the most important intracellular hydrophilic antioxidant [33]. Also, GSH acts as a co-substrate for GPx activity and as a cofactor for many enzymes, stress resistance of many cells is associated with high intracellular levels of GSH. A decreased GSH content may predispose the cells to lower defense against condition of oxidative stress during several degenerative disease conditions including diabetes.

CONCLUSION

In conclusion, the present study showed that the methanolic extract of *Coleus vettiveroides Jacob* possesses potent antioxidant and lipid peroxidation activity can be employed in protecting tissue from the oxidative stress, which may be responsible for its hypoglycemic property.

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