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EVALUATION OF *IN-VIVO* ANTIOXIDANT ACTIVITY OF URSOLIC ACID ACETATE ISOLATED FROM 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 in -vivo antioxidant activities of Ursolic acid acetate isolated from methanolic extract of *Coleus vettiveroides (jacob*) in streptozotocin-induced oxidative stress in rats. **Methods:** Animals were treated with Ursolic acid acetate isolated from methanolic extract of *Coleus vettiveroides (jacob)* for 14 days and then oxidative stress was induced with a single dose of Streptozotocin 50 mg/kg (p.o). Treated with 50 mg/kg (p.o) of Ursolic acid acetate isolated from methanolic extract of *Coleus vettiveroides (jacob)* and to determine the Glutathione, SOD, GP_X Catalase, and TBARS levels were determined. **Results:** The present study revealed that administration of Ursolic acid acetate isolated from methanolic extract of *Coleus vettiveroides (jacob)* 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 Ursolic acid acetate isolated from 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, Ursolic acid acetate.

INTRODUCTION

Type 2 diabetes mellitus (DM) is a metabolic disorder that is a major health problem worldwide (Cusi.et al.,2009). TheWorld Health Organization has reported that the global prevalence of diabetes will increase, from 2.8% in 2000 to 4.4% in 2030 (wild et al.,2004). Free radicals are reactive molecules produced naturally in the human body during metabolic reactions. High levels of free radicals damage cellular proteins, membrane lipids, and nucleic acids, and eventually lead to cell death. Free radicals play an important role in the pathogenesis of many chronic diseases, including atherosclerosis, myocardial failure, immune diseases, and type 2 diabetes. Free radicals include reactive oxygen species (ROS) and reactive nitrogen species (Tesfamariam et al.,1994). In healthy subjects, antioxidant compounds counter the effects of free radicals (wild et al.,2004). Antioxidants, which are produced either endogenously or are derived from dietary sources, are categorized into two groups: enzymatic and non-enzymatic. Catalase, superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase, tyrodoxin reductase, ariel esterase, and paraoxonase are included in the enzymatic group, while the non-enzymatic group includes vitamins A, C, and E, carotenoids, glutathione,flavonoids, other compounds such as α-lipoic acid and coenzyme Q10, and copper, zinc, magnesium, and selenium (Esteghamati et al.,2008). Oxidative stress is defined as the increased generation of free radicals and/or the impaired compensatory response of endogenous antioxidant defenses, both observed in type 2 diabetes (Betteridge et al.,2000). Oxidative stress is a pathologic condition resulting from either increased production of free radicals or decreased levels of antioxidants. Hyperglycemia, by the promotion of lipid peroxidation of low-density lipoprotein (LDL) can result in the production of free radicals (Maritim et al.,2003). Diabetes is a metabolic disorder and is generally accompanied by increased levels of free radicals and decreased concentration or activity of antioxidants. Studies have shown that serum concentrations of SOD and other antioxidants such as vitamin E and α-lipoic acid are decreased in type 2 diabetic patients. There is evidence that deficiency of catalase in erythrocytes is associated with increased risk of diabetes (Tesfamariam et al.,1994). There is considerable evidence that oxidative stress plays a key role in insulin resistance, impaired insulin secretion and many of the complications of diabetes such as micro-macro vascular damage (Ahmed et al.,2005).

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 (Raghunatha et al**.,**1994).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 Ursolic acid acetate isolated from *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 kollidam, Nagapattinam 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(Harborne et al.,1984). 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 ($22^{o}C \pm 5^{o}C$) and humidity ($55 \pm 5^{o}C$) 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 50 mg/kg body weight of Ursolic acid acetate isolated from MECVJ.

Experimental Protocol

The animals were divided into four 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 Ursolic acid acetate isolated from methanolic extract of *Coleus vettiveroides (jacob)* at the dose of 50 mg/kg body weight, Group IV 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 antioxidant 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, Reduced glutathione (GSH) (Moron.,et al., 1979), Superoxide dismutase (SOD) was determined according to the method of Mc Cord et al., 1971,after removing the haemoglobin by the method of Minami and Yoshikawa.,(1979), Catalase (CAT) (Aebi et al., 1974)' glutathione peroxidase (GPx) (Paglia et al., 1967).

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) 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 Ursolic acid acetate isolated from 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 Ursolic acid acetate isolated from 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 Ursolic acid acetate isolated from 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	118.95±33.53	208.56±47.26	0.3±0.1	20.11±3.28	1.11±0.33
Group 2	55.39±21.28	31.28±8.59	1.52±0.01	8.59±1.28	0.217±0.1
Group 3	167.48±20.5	186.28±31.95	1.01±0.06	18.38±1.63	1.10±0.33
Group 4	185.5±39.68**	289.12±51.088	0.78±0.42**	18.02±0.58**	1.10±0.54**

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 Ursolic acid acetate isolated from 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 (Davi et al**.,**2005). 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.(Pandey et al.,2010). 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 Ursolic acid acetate isolated from *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 Ursolic acid acetate isolated from *Coleus vettiveroides (Jacob)* extract in experimental diabetes. It had been reported that *Coleus vettiveroides(Jacob)* extract is an efficient scavenger of $OH^{\cdot -}$ and $O^{\cdot -}_2$ radicals (Mahmoud et al.,2011).

Numerous studies have revealed lower antioxidant and enhanced peroxidative status in diabetes mellitus (Punitha et al.,2005). SOD, CAT, GPx are enzymes that destroy the peroxides and play a significant role in providing antioxidant defences to an organism. GPx (Chen et al.,1999), CAT (.Liedias et al.,1998), 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 (.Kaleem et al.,2006). 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 (Davi et al.,2005). 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 (Martin et al.,2003) GSH is a major intracellular non protein sulphydral compound and is accepted as the most important intracellular hydrophilic antioxidant. 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 (Melov et al.,2002).

CONCLUSION

In conclusion, the present study showed that the Ursolic acid acetate isolated from 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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