Antidiabetic and antioxidant potentials of *Euphorbia hirta* leaves extract studied in streptozotocin-induced experimental diabetes in rats

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Abstract. *Euphorbia hirta,* commonly known as asthma weed, is a popular folk remedy for the treatment of various ailments. Recent studies have indicated that plant has potent antioxidant properties. As part of an ongoing programme to validate the use of some reputed herbs in Indian traditional medicines, the present study was aimed to evaluate the antidiabetic and antioxidant potentials of *E. hirta* leaves in streptozotocin-induced experimental diabetes in rats. Oral administration of *E. hirta* leaves extract (300 mg/kg b.w./rat/day) for a period of 30 days indicated the antidiabetic nature of the leaves extract. Determination of the lipid peroxides, hydroperoxides, and both enzymatic and non-enzymatic antioxidants evidenced the antioxidant potential of the leaves extract. Assay of enzymes such as serum aspartate transaminase (AST), serum alanine transaminase (ALT) and serum alkaline phosphatase (ALP) revealed the non-toxic nature of *E. hirta* leaves. The hypoglycemic activity of the leaves extract was comparable with gliclazide, a standard reference drug.

Key words: Antidiabetic — Antioxidant — *Euphorbia hirta* — STZ-induced diabetes — Gliclazide

Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; CAT, catalase; GPx, glutathione peroxidase; GST, glutathione-S-transferase; LSD, least significant difference; SOD, superoxide dismutase; STZ, streptozotocin.

Introduction

Diabetes mellitus is a complex multisystemic metabolic disorder as old as mankind that has reached epidemic proportions worldwide (Zimmet et al. 2001). It is characterized by persistent hyperglycemia as a result of relative or absolute lack of insulin or the actions of insulin. Hyperglycemia affects the metabolism of carbohydrates, protein and fat leading to structural changes in a wide range of cells subsequently leading to long-term complications and premature death. The prevalence of diabetes is strongly associated with a sedentary lifestyle and high calorie-nutrition and obesity (King et al. 1998). Currently available drugs for the treatment of diabetes such as sulfonylureas, biguanides, α-glucosidase inhibitors, thiazolidenediones and insulin have a number of limitations such as adverse effects and high rates of secondary failure.

The situation warrants the active search for the novel therapeutic agents preferably from natural sources.

Plants are omnipresent in the earth's environment and have been used for a large range of purposes including medicine, nutrition, flavoring, beverages, dyeing, repellants, fragrances, cosmetics, charms and various industrial uses (Smith and Winder 1996). India is one of the twelve mega-biodiversity countries of the world having rich vegetation with a wide variety of plants with medicinal values. However, only few of them received scientific validation.

Euphorbia hirta is an herbaceous wild plant from the *Euphorbiaceae* family widely used in the Indian traditional medicine for various ailments. The plant is distributed in southern Western Ghats of India and Northern east coast of Tamil Nadu (Abdul Rahman et al. 2007). The plant has a worldwide distribution and its common names include cat's hair, asthma weed and milk weed. Its local name in the southern part of India is "Amman pacharisi". Whole plant or the leaf extract obtained from *E. hirta* is known to have multitude actions in the biological system. The plant is traditionally used as treatment of asthma and respiratory

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tract inflammation, cough, chronic bronchitis and other pulmonary disorders and proven pre-clinically for anti-inflammation, asthma, wound healing and diarrhea (Ogbulie et al. 2007).

The antidiabetic and free radical property of *E. hirta* flower extract has been studied in alloxan-induced diabetic mice (Kumar et al. 2010). However, literature survey revealed that no systematic studies have been carried out on antidiabetic effect of *E. hirta* leaves in experimental models. The aerial parts of the plants are reported to contain flavonoids such as quercetin, Euphorbianin and tannins (Euphorbins) (Aqil 1999). Several triterpenes, polyphenols and phytosterols have also been investigated (Sandeep Patil et al. 2009). Since the plant is reported to contain many biologically active ingredients with known pharmacological actions, the present study was conducted to explore the antidiabetic and antioxidant effects of oral administration of *E. hirta* leaves extract in STZ-induced experimental diabetic animal models.

Materials and Methods

Plant material

Fresh, mature *E. hirta* leaves were collected from a plant in Thiruvannamalai (Tamilnadu, India). Leaves were selectively removed using forceps. The plant was identified and authenticated by Dr. V. Kaviyarasan from Centre for Advanced studies in Botany, University of Madras. A voucher specimen has been deposited in the department herbarium.

Preparation of plant extract

The *E. hirta* leaves were shadow dried at room temperature and finely powdered in an electrical grinder and stored at 5°C until further use. The absence of flavonoids like quercetin and rutin in the aqueous extract is due to their non polar nature. Hence, the extraction was performed using less polar solvents (Loh et al. 2009). *E. hirta* leaves powder was delipidated with petroleum ether (60–80°C) for overnight. It was then filtered, and filtrate was discarded and the residue was extracted with ethanol (90% V/V) by soxhalation. Ethanol was evaporated in a rotary evaporator at 40–50°C under reduced pressure.

Phytochemical screening

Leaves extract was subjected to preliminary phytochemical screening for the presence of various plant constituents. The presence of alkaloids were inferred using Dragendroff's reagent; flavonoids by Shinoda's test; tannins with Ferric chloride test; and saponins by the production of stable foam and steroids with Libermann-Burchard reagent (Harborne 1998; Kokate 2005).

Experimental animals

Male albino Wistar rats weighing (160–180 g) were purchased from Tamilnadu Veterinary and Animal Sciences University (Chennai, India). The rats were housed in polypropylene cages lined with husk. It was renewed for every 24 h. The rats were fed with commercial pelleted rats chow (Hindustan Lever Ltd., Bangalore, India), composition of 5% fat, 21% protein, 55% nitrogen-free extract, and 4% fiber (w/w) with adequate vitamin levels for the animals and had free access to water. The experimental rats were maintained in a controlled environment (12/12 h light/dark cycle and temperature (30 ± 2°C). The experiments were designed and conducted in strict accordance with the ethical norms approved by Ministry of Social justices and Environment, Government of India and Institutional Animal Ethical Committee guidelines (IAEC NO: 01/055/09). The rats were acclimatized for one week before starting the experiments.

Experimental design

Rats were fasted overnight and experimental diabetes was induced by intraperitoneal injection of streptozotocin (STZ) with a single dose of 50 mg/kg body weight (b.w.). STZ was dissolved in freshly prepared 0.1 M cold citrate buffer, pH 4.5 (Rakieten et al. 1963). The control rats were injected with the citrate buffer. Because STZ is capable of inducing fatal hypoglycemia as a result of massive pancreatic insulin release, STZ-treated rats were allowed to drink 10% glucose solution overnight to overcome the drug-induced hypoglycemia (Palsamy and Subramanian 2008). Neither death nor any other adverse effect was observed. After 3 days for development and aggravation of diabetes, rats with moderate diabetes (i.e., blood glucose concentration above 250 mg/dl) that exhibited glycosuria and hyperglycemia were selected for the experiment (Cánepa et al. 1990).

The animals were divided into four groups, comprising of six animals in each group as follows: control rats (group I), STZ-induced diabetic rats (group II), diabetic rats treated with *E. hirta* leaves extract (300 mg/kg b.w./rat/day) in aqueous solution orally for 30 days (group III), and diabetic rats given a single dose of gliclazide (5 mg/kg b.w.) in aqueous solution orally for 30 days (group IV) (Pulido et al. 1997).

The change in body weight gain in all groups of rats was recorded at regular intervals. After 30 days of treatment, rats were deprived of food overnight and sacrificed by cervical dislocation. Blood was collected in sterilized tubes with and without anticoagulant.

Oral glucose tolerance test

At the end of the experimental period, fasting blood samples were collected from all groups of rats. Four more blood samples were collected at 30, 60, 90 and 120 min intervals after the oral administration of glucose solution at a dosage of 2 g/kg b.w. and the blood samples were collected with EDTA for the estimation of glucose.

Biochemical parameters

The biochemical parameters analyzed include blood glucose, urea, plasma insulin, protein, lipid peroxides, hydroperoxides, enzymatic and non enzymatic antioxidants. Serum creatinine and uric acid levels were also determined. The activities of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were assayed in the serum of control and experimental groups of animals. For the analyses of the above parameters commercially available kits and routine standardized procedures were adopted.

Statistical analysis

The results were expressed as mean ± SD of six rats *per* group and statistical significance was evaluated by one-way ANOVA using SPSS (version 19.0) program followed by the post hoc test, least significant difference (LSD). Values were considered statistically significant when $p < 0.05$.

Results

The preliminary phytochemical analysis of *E. hirta* leaves extract showed a positive response for the presence of alkaloids, flavonoids, glycosides, tannins, saponins and triterpenoids.

The effect of *E. hirta* leaves extract as well as gliclazide supplementation on glucose tolerance in control and experimental groups of rats receiving an oral glucose load is shown in Figure 1. The blood glucose concentration in the control rats was elevated to a maximum value at 60 min and declined to near basal levels at 120 min, whereas, in STZ-induced diabetic rats, the peak increase in blood glucose level was noticed even after 60 min and remained high over the next 60 min. Oral treatment with *E. hirta* leaves extract as well as gliclazide to diabetic rats elicited significant ($p < 0.05$) decrease in blood glucose level at 60 min when compared with untreated diabetic rats.

Table 1 shows the effect of *E. hirta* leaves extract on the levels of biochemical and hematological parameters in the control and experimental groups of rats. The observed elevated levels of blood glucose, glycosylated hemoglobin, urea, uric acid and creatinine in the diabetic group of rats were reverted to near normal levels by the oral administration of *E. hirta* leaves extract. Conversely, the decreased levels of plasma insulin, hemoglobin and total protein in diabetic group of rats were elevated by the administration of *E. hirta* leaves extract to diabetic rats for 30 days. Urine
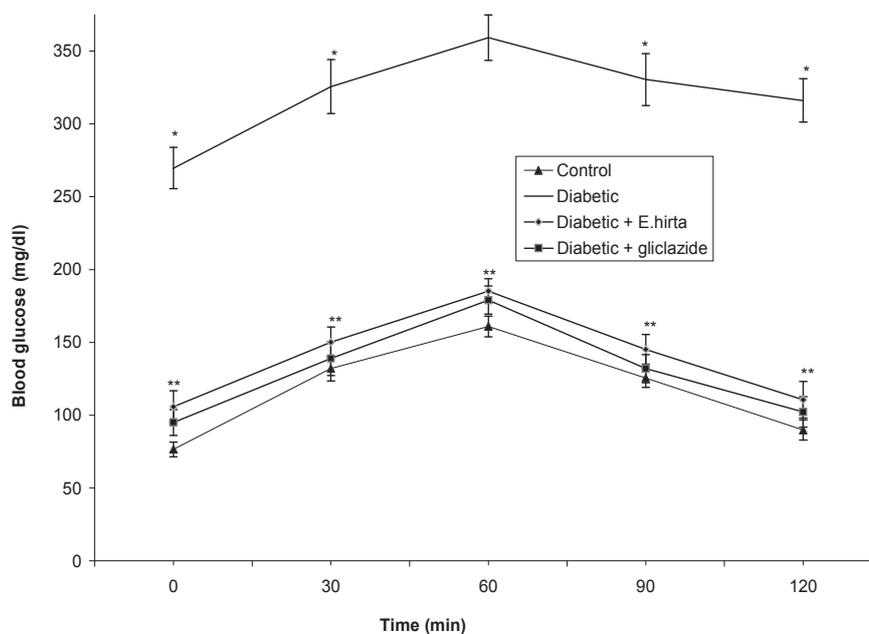

Figure 1. Effect of *E. hirta* leaves extract on the levels of blood glucose after an oral glucose load in the experimental groups of rats. Results are expressed as mean ± S. D., *n* = 6. One-way ANOVA followed by post hoc test LSD. *p* < 0.05. The results were compared with [*]control, [**]diabetic control.

Table 1. Effect of *E. hirta* leaves extract on the levels of biochemical and hematological parameters in the control and experimental groups of rats

Parameters	Control	Diabetic control	Diabetic + *E. hirta*	Diabetic + gliclazide
Blood glucose (mg/dl)	92.54 ± 6.30	301.51 ± 39.17[*]	95.56 ± 9.85[**]	94.12 ± 5.67[**]
Plasma insulin (µU/ml)	12.53 ± 0.92	4.97 ± 0.28[*]	9.04 ± 0.16[**]	10.42 ± 0.82[**]
Hemoglobin (g/dl)	13.01 ± 4.17	8.11 ± 2.03[*]	11.29 ± 2.97[**]	11.86 ± 2.54[**]
Glycosylated hemoglobin (%Hb)	5.86 ± 1.74	13.11 ± 4.05[*]	6.99 ± 1.17[**]	6.39 ± 0.83[**]
Total protein (g/dl)	8.23 ± 0.94	4.82 ± 0.46[*]	7.87 ± 0.43[**]	8.01 ± 0.64[**]
Blood urea (mg/dl)	19.36 ± 2.41	36.43 ± 2.87[*]	24.81 ± 1.96[**]	23.67 ± 1.09[**]
Serum creatinine (mg/dl)	0.69 ± 0.07	1.40 ± 0.09[*]	0.79 ± 0.04[**]	0.63 ± 0.03[**]
Serum uric acid (mg/dl)	2.98 ± 0.28	7.53 ± 0.47[*]	4.36 ± 0.40[**]	3.73 ± 0.31[**]
Urine sugar	0	+++	0	0

+++ indicates more than 2% sugar. Results are expressed as mean ± S.D., $n = 6$. One-way ANOVA followed by post hoc test LSD; $p < 0.05$. The results were compared with [*]control; [**]diabetic control.

sugar which is observed in the diabetic group of rats was absent in leaves extract as well as gliclazide-treated diabetic group of rats. The results are comparable with gliclazide, an oral hypoglycemic drug.

Table 2 depicts the effect of *E. hirta* leaves extract on the levels of lipid peroxides and hydroperoxides in the plasma of control and experimental groups of rats. The levels of lipid peroxides and hydroperoxides are found to be elevated in plasma of diabetic group of rats. Oral administration of *E. hirta* leaves extract as well as gliclazide significantly decreased ($p < 0.05$) the levels of lipid peroxides and hydroperoxides to near normalcy.

Table 3 depicts the effect of *E. hirta* leaves extract on activities of enzymatic and non-enzymatic antioxidants in plasma of control and experimental groups of rats. Oral treatment with *E. hirta* leaves extract to STZ-induced diabetic rats resulted in significant increased ($p < 0.05$) activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione-S-transferase (GST) enzymes as well as showed a significant increase ($p < 0.05$) in levels of the non-enzymatic antioxidants.

Table 4 depicts the effect of *E. hirta* leaves extract on the activities of serum AST, ALT and ALP in control and experimental group of rats. Oral administration of *E. hirta* leaves extract significantly decreased ($p < 0.05$) the activities of these enzymes.

Discussion

STZ, an antibiotic produced by *Streptomyces achromogenes,* has been widely used for inducing diabetes in the experimental animals through its cytotoxic effects on pancreatic β cells (Rakieten 1963). The mechanism behind is that STZ is preferentially uptaken by pancreatic beta cell *via* GLUT2 transporter and causes DNA alkylation followed by the activation of poly ADP ribosylation leading to depletion of cytosolic concentration of NAD^+ and ATP. Enhanced ATP dephosphorylation after STZ treatment supplies substrate for xanthine oxidase resulting in the formation of superoxide radicals and also NO moiety is liberated from STZ leading to the destruction of β cells by necrosis (Szkudelski 2001).

Gliclazide is a second generation sulphonylurea derivative and is used as a reference drug to compare the efficacy of oral hypoglycemic drugs (Pulido et al. 1997). The acute toxicity studies indicated the non-toxic nature of *E. hirta* leaves extract.

The drug showed optimum activity at 300 mg/kg b.w and further increase in extract dose did not result in a further significant decrease in blood glucose levels, thus it appears that unlike insulin and other oral hypoglycemic drugs overdose of the drug may not cause hypoglycemia (data not shown).

The oral glucose tolerance test (OGTT) measures the body's ability to utilize glucose, the body's main source of energy. The

Table 2. Effect of *E. hirta* leaves extract on the levels of lipid peroxides and hydroperoxides in the plasma of control and experimental groups of rats

Parameters	Control	Diabetic control	Diabetic + *E. hirta*	Diabetic + gliclazide
Lipid peroxides (nmol/ml)	3.42 ± 0.83	7.23 ± 0.87[*]	4.16 ± 0.68[**]	3.94 ± 0.31[**]
Hydroperoxide (10^{-5} mmol/dl)	7.61 ± 1.62	12.93 ± 1.84[*]	9.33 ± 1.67[**]	8.95 ± 1.58[**]

Results are expressed as mean ± S.D., $n = 6$. One-way ANOVA followed by post hoc test LSD; $p < 0.05$. The results were compared with [*]control; [**]diabetic control.

Table 3. Effect of *E. hirta* leaves extract on activities of enzymatic and non-enzymatic antioxidants in plasma of control and experimental groups of rats

Parameters	Control	Diabetic control	Diabetic + *E. hirta*	Diabetic + gliclazide
SOD	12.45 ± 1.41	5.73 ± 0.74[*]	9.79 ± 1.41[**]	8.54 ± 1.18[**]
CAT	79.27 ± 4.17	34.23 ± 2.84[*]	72.21 ± 4.39[**]	70.93 ± 4.82[**]
GPx	8.87 ± 0.72	4.93 ± 1.03[*]	7.41 ± 0.53[**]	7.22 ± 0.49[**]
GST	7.07 ± 1.22	3.64 ± 0.85[*]	6.18 ± 0.81[**]	5.98 ± 0.79[**]
α-tocopherol (mg/dl)	3.82 ± 0.91	1.79 ± 0.23[*]	2.32 ± 0.84[**]	2.41 ± 0.21[**]
Vitamin C (mg/dl)	1.84 ± 0.31	0.58 ± 0.12[*]	1.43 ± 0.34[**]	1.59 ± 0.17[**]
Ceruloplasmin (mg/dl)	25.88 ± 2.74	15.84 ± 1.14[*]	20.13 ± 2.71[**]	19.27 ± 1.68[**]
Reduced glutathione (mg/dl)	28.19 ± 4.82	16.72 ± 2.35[*]	23.93 ± 4.11[**]	24.88 ± 4.64[**]

Enzyme activities are expressed as: 50% inhibition of epinephrine auto oxidation/min/mg protein for SOD; μmol of hydrogen peroxide decomposed/min/mg protein for CAT; μmol of glutathione oxidized/min/mg protein for GPx; nmol of CDNB conjugate formed/min/mg protein for GST. Results are expressed as mean ± S.D., $n = 6$. One-way ANOVA followed by post hoc test LSD; $p < 0.05$. The results were compared with [*]control; [**]diabetic control.

impaired glucose tolerance observed in STZ-induced diabetic group of rats were altered to near normal by the treatment with *E. hirta* leaves extract which indicates the insulin stimulatory effect of *E. hirta* leaves extract from remnant β cells.

Blood glucose is a biochemical index widely used for the diagnosis and monitoring the prognosis of diabetes mellitus. Deficiency of insulin causes hyperglycemic status as a result of excessive production of endogenous glucose by hepatic as well as extra hepatic tissues through gluconeogenic and glycogenolytic pathways and reduced utilization of glucose by various tissues, a classical state of diabetes mellitus (Soling and Kleineke 1976). In the present study, oral treatment with *E. hirta* leaves extract as well as gliclazide lowered the level of blood glucose and improved the insulin level in STZ-induced diabetic rats.

The possible mechanism by which *E. hirta* leaves extract brings about its antidiabetic action may be by potentiating the insulin effect through the stimulation of insulin release from remnant pancreatic β cells or its release from the bound form. In the above context a number of other plants have also shown similar mechanism (Arulselvan et al. 2006; Mahadeva Rao and Subramanian 2009).

The reduced glucose levels suggested that *E. hirta* leaves extract might exert insulin like action on peripheral tissues by

either promoting glucose uptake metabolism or by inhibiting hepatic gluconeogenesis (Gray et al. 2000).

The level of total hemoglobin is found to be decreased in the diabetic group of rats and this may be due to increased formation of glycosylated hemoglobin. Glycosylated hemoglobin is considered as a golden marker for accurate and reliable measurement of fasting glucose. Chronic hyperglycemia results in glycosylation in which excess glucose nonenzymatically reacts with hemoglobin to form glycosylated hemoglobin (Koenig et al. 1976). This condition favors reduction in the level of hemoglobin and concomitant elevation in glycosylated hemoglobin, which is directly proportional to supraphysiological glucose (Alyassin and Ibrahim 1981). The observed decrease in the levels of glycosylated hemoglobin in diabetic rats treated with *E. hirta* leaves extract may be due to its hypoglycemic activity.

Urea is the chief end product of protein catabolism in the body. During the diabetic conditions, the catabolism of both hepatic as well as plasma proteins leads to the excessive accumulation of urea nitrogen in the blood. Moreover, the nitrogen homeostasis is also altered by elevated peripheral release of nitrogenous substances as well as elevated hepatic elimination of urea nitrogen. This results in the negative nitrogen balance. Oral administration of *E. hirta* leaves ex-

Table 4. Effect of *E. hirta* leaves extract on the activities of serum AST, ALT and ALP in control and experimental group of rats

Biochemical markers	Control	Diabetic control	Diabetic + *E. hirta*	Diabetic+ gliclazide
Serum AST	76.61 ± 2.45	133.50 ± 2.54[*]	81.13 ± 1.80[**]	78.42 ± 3.38[**]
Serum ALT	16.22 ± 0.73	35.21 ± 1.74[*]	15.23 ± 0.55[**]	14.42 ± 0.91[**]
Serum ALP	68.93 ± 1.11	107.79 ± 3.02[*]	69.29 ± 1.93[**]	71.79 ± 2.50[**]

Enzyme activities are expressed as: AST and ALT – μmol of pyruvate liberated/h/mg of protein; ALP – μmoles of phenol liberated/min/mg of protein. Results are expressed as mean ± S.D., $n = 6$. One-way ANOVA followed by post hoc test LSD; $p < 0.05$. The results were compared with [*]control; [**]diabetic control.

tract as well as gliclazide to experimental diabetic group of rats significantly normalized the altered levels of blood urea suggesting the beneficial role of *E. hirta* leaves in protein metabolism.

During STZ-induced experimental diabetes, the level of purines is elevated due to accelerated muscle wasting. These accumulated purines are the main source for the production of uric acid by the activity of xanthine oxidase (Anwar and Meki 2003). Various studies evidenced that increased oxidative stress is closely related to diabetes and its vascular complications (Baynes 1991). The elevated levels of circulating uric acid level may be an indicator that the body is trying to protect itself from the deleterious effects of free radicals by increasing the products of endogenous antioxidants, such as uric acid. Uric acid prevents oxidative modification of endothelial enzymes and preserves the ability of endothelium to mediate vascular dilatation in the face of oxidative stress (Becker 1993). In the present study, the increased level of serum uric acid observed in diabetic rats was restored to near normal by the administration of *E. hirta* leaves extract as well as gliclazide indicating the free radical scavenging activity of *E. hirta* leaves.

The level of total proteins is found to decrease in diabetic group of rats. The deficiency of insulin leads to defective amino acid/protein metabolism, which may be a more important factor than hyperglycemia in the etiology of some diabetic complications (Rosenlund 1993). STZ-induced diabetes in rat model indicates several alterations of amino acid metabolism, which may be attributed to increased muscle proteolysis, reduced protein synthesis, an energy-dependent process in the liver, and stimulated hepatic gluconeogenesis utilizing gluconeogenic amino acids (Fando et al. 1985). This results in the decrease of total protein content in STZ-induced rats. Oral administration of *E. hirta* leaves extract to diabetic rats significantly inhibits proteolysis caused by insulin deficiency and improves total protein levels to near normalcy. This property of *E. hirta* leaves extract is comparable to that of gliclazide Sunil et al. (2010) have reported that total protein level was found to be increased in STZ-induced diabetic mice treated with leaf, stem and flower extracts of *E. hirta.*

Serum creatinine was found to be increased in diabetic group of rats. Serum creatinine concentration is not only used to assess the impairment of kidney function but also in clinical chemistry as an end point to detect treatment related toxic effects of substances on kidney tissues (Bräunlich et al. 1997). However, administration of *E. hirta* leaves extract to diabetic group of rats significantly normalized their levels indicating the renal protective nature that occurs due to hyperglycemia.

Diabetes is associated with significant oxidative stress and that oxidative damage to tissues may be a contributory factor to several diabetic complications. As there is a strong correlation between oxidative stress and incidence of diabe-

tes, antioxidant supplementation gears up the detoxification machinery thereby playing a pivotal role in the treatment of diabetes mellitus (Osawa and Kato 2005). STZ is known to generate free radicals and these free radicals undergo dismutation to hydrogen peroxide. Subsequently, highly reactive hydrogen radicals are formed by the Fenton reaction. The action of reactive oxygen species (ROS) with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of β cells (Szkudelski 2001).

The levels of lipid peroxides and hydroperoxides are found to be elevated in plasma of diabetic group of rats. Hydroperoxides are potentially toxic molecules that are capable of demolishing enzymes and cell membranes (Wang et al. 1996). The elevation in the level of hydroperoxides may be due to declined activities of antioxidant enzymes, which is a favorable cause for abandoned production of free radicals and subsequent production of lipid hydroperoxides (Matkovics et al. 1997). Oral administration of *E. hirta* leaves extract as well as gliclazide significantly decreased the levels of lipid peroxides and hydroperoxides to near normalcy indicating the free radical scavenging action of *E. hirta* leaves. This antioxidant activity might be due to the presence of phytochemical constituents e.g. flavonoid in the *E. hirta* leaves which acts as a strong superoxide radical and singlet oxygen quenchers. These observations suggest that *E. hirta* leaves extract possess antioxidant potential.

The activities of enzymatic antioxidants and the levels of non-enzymatic antioxidants in plasma in diabetic group of rats were found to be decreased. Enzymatic antioxidants are involved in detoxification of free radicals and peroxides formed during oxidative stress, including diabetes. Enzymatic antioxidants such as SOD, CAT, GPx, and GST are crucial cellular components of antioxidant defense system in the body, thus playing a crucial role in the maintenance of a balanced redox status (Evan and Littlewood 1998). Diminished activities of enzymatic antioxidants in diabetic rats have been reported (Miyazaki et al. 2007). The results of the present study indicated that oral treatment with *E. hirta* leaves extract to STZ-induced diabetic rats resulted in increased activities of SOD, CAT, GPx, and GST enzymes. This may be attributed to the free radical scavenging and antidiabetic activities of the extract of *E. hirta* leaves.

Vitamins C and E and reduced glutathione are known to be non-enzymatic antioxidants that are found to be decreased in diabetic state because of their free-radical scavenging property (Garg and Bansal 2000). The declined levels of vitamins C and E and reduced glutathione were due to the increased production of free radicals (Fang et al. 2002). However, oral administration of *E. hirta* leaves extract to diabetic group of rats showed a significant increase in levels of these non-enzymatic antioxidants, thereby suggesting the free radical scavenging potential of *E. hirta* leaves extract which in turn may be responsible for its antioxidant property.

Ohaeri (2001) found that liver was necrotized in STZ-induced diabetic rats. Therefore the observed increase in the activities of AST, ALT and ALP in the serum of diabetic rats might be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream. On the other hand, treatment of the diabetic rats with *E. hirta* leaves extract caused reduction in the activity of these enzymes in plasma when compared to the diabetic group and consequently alleviate the liver damage.

A number of phytochemical constituents have been detected in the *E. hirta* Linn; tannins, gallic acid, quercitin, phenols, alcohols, alkaloids (Adedapo et al. 2005). A variety of herbs and herbal extracts contain different phytochemicals with biological activity that can be of valuable therapeutic index. Phytochemicals such as saponins, terpenoids, flavonoids and tannins found to inhibit cancer cell proliferation, regulate inflammatory, immune response and protect against lipid peroxidation (Liu 2003). Rupasinghe et al. (2003) have reported that saponins possess hypocholesterolemic, antidiabetic, antitumor, antiviral, antioxidative, anticarcinogenic, immunostimulatory and hepatoprotective properties. Phenolic compounds have cholesterol lowering effect (Leontowicz et al. 2002); flavonoids increases the insulin secretion from the β cells of pancreas and this may explain the antidiabetic potential of the plants (Hii and Howell 1985).

In conclusion, the observed antidiabetic and antioxidant properties of the *E. hirta* leaves might be due to the synergistic effect of biologically active phytochemicals present in the leaves extract.

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