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Protective Effect of *Solanum surattense* Leaf-Extract on Blood Glucose, Oxidative Stress and Hepatic Marker Enzymes in STZ-Diabetic Rats

M. Sridevi, G. Chandramohan and K.V. Pugalendi
Department of Biochemistry and Biotechnology, Faculty of Science,
Annamalai University, Annamalainagar-608 002, Tamilnadu, India

Abstract: In this study, the protective effect of alcoholic leaf extract of *Solanum surattense* on blood glucose and redox status in streptozotocin (STZ)-induced diabetic rats has been evaluated. Diabetes was induced in adult male albino Wistar rats weighing 180-200 g, by the administration of STZ (40 mg kg^{-1} body weight) intraperitoneally. The diabetic rats showed elevated levels of blood glucose and a significant decrease in plasma insulin levels. The increased levels of lipid peroxidation markers such as thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD) and lipid hydroperoxides (LOOH) were observed in diabetic rats. The activities of enzymatic antioxidants (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glucose 6-phosphate dehydrogenase) and the levels of non-enzymatic antioxidants, (reduced glutathione (GSH) and vitamin C) decreased, whereas, vitamin E increased in the erythrocytes of diabetic rats. Plasma hepatic marker enzymes also increased in STZ-diabetic rats. Oral administration of *S. surattense* leaf extract (100 mg kg^{-1} b.wt.) reversed these parameters towards normalcy. Thus, our results indicate that the *S. surattense* leaf extract possesses antihyperglycemic, antilipidperoxidative and hepatoprotective and antioxidant potential in diabetic rats.

Key words: *Solanum surattense,* diabetes mellitus, antihyperglycemic activity, lipid peroxidation, antioxidants, streptozotocin

INTRODUCTION

Increased oxidative stress is a widely accepted factor in the development and progression of diabetes and its complications. Hyperglycemia-induced glucose oxidation initiates lipids peroxidation and non-enzymatic glycation of proteins, which, inturn lead to enhanced production of reactive oxygen species (ROS). Abnormally high levels of free radicals and subsequent decline of antioxidant defense mechanisms lead to oxidative damage of cells resulting in cell injury (Nishikawa *et al*., 2000). Moreover, reactive oxygen species have also been implicated in the mechanism of damage of red blood cells (RBC) (Rice-Evans, 1986; Vives Corrons *et al*., 1995; Rao *et al*., 2003). Non-enzymic antioxidants such as vitamins C, E and glutathione (GSH) and enzymatic systems like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) play a role in alleviating tissue damage due to the formation of free radicals (Klepac *et al*., 2005).

A variety of plant preparations have been mentioned in Ayurveda and other indigenous systems of medicine, which are claimed to be useful in the treatment of diabetes. World Health Organization (WHO, 1980) has suggested the evaluation of potential plants as effective therapeutic agents, especially in areas, where we lack safe modern drugs.

Among natural plants, *Solanum surattense* (synonym-*S. xanthocarpum*) commonly known as Indian night shade or yellow berried night shade has been used traditionally for curing various ailments

Corresponding Author: Dr. K.V. Pugalendi, Department of Biochemistry and Biotechnology, Annamalai University, Annamalainagar-608 002, Tamilnadu, India Tel: +91-4144-238343 Fax: +91-4144-234191

such as fever, cough, asthma and diabetes in South Indian traditional medicines (Nadkarni, 1954; Kirtikar and Basu, 1975). The antidiabetic potential of the fruit was studied in diabetic rats (Gupta *et al*., 2005; Kar *et al*., 2006). The present investigation has been carried out to shed light on the effect of *S. surattense* leaf-extract on antidiabetic effect and erythrocytes antioxidant status and hepatic markers.

MATERIALS AND METHODS

Chemicals

Streptozotocin was obtained from Sigma-Aldrich Co. (St. Louis, Missouri, USA). All other chemicals used were of analytical grade obtained from E. Merck and HIMEDIA, Mumbai, India.

Plant Material

Leaves of *Solanum surattense* were collected from local areas of Chidambaram Tamil Nadu, India, October 2006. The plant was botanically identified and authenticated in the Department of Botany, Annamalai University, Annamalainagar, Chidambaram, Tamil Nadu, India and a voucher specimen (AU 189) was deposited at the herbarium of botany.

Preparation of Leaf Extract

The plant leaf was shade dried at room temperature (30±2°C) and the dried leaf was ground into fine powder using pulverizer. The powdered part was sieved and kept in deep freezer until the time of use. One hundred grams of dry fine powder was suspended in 400 mL of ethanol for 72 h. The extract was filtered using a muslin cloth and concentrated at 40±5 °C, refrigerated and used within two months.

Animals

Male albino Wistar rats (weighing 180-200 g) were procured from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University and maintained in an air-conditioned room (25±1°C) with a 12 h light/12 h dark cycle. Feed and water were provided *ad libitum*. All the experimental study were conducted in Department of Biochemistry, Faculty of Science, Annamalai University, in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH, 1985) and the experimental study was approved by the Ethical Committee of Rajah Muthiah Medical College and Hospital (Reg No. 160/1999/CPCSEA), Annamalainagar.

Experimental Induction of Diabetes

After an overnight fast, the animals were made diabetic by an intraperitoneal injection of streptozotocin (STZ, 40 mg kg^{-1} b.wt.) in a freshly prepared citrate buffer (0.1 M, pH 4.5). STZ injected animals were given 20% glucose solution for 24 h to prevent initial drug-induced hypoglycemic mortality. These animals exhibited massive glycosuria (determined by Benedict's qualitative test) and hyperglycemia within a few days. Diabetes was confirmed by measuring the fasting blood glucose concentration, 96 h after induction. Albino rats with a blood glucose level above 220 mg dL^{-1} were considered diabetic and were used in the experiment.

Experimental Protocol

The animals were randomly divided into five groups of six animals each. *S. surattense* leaf extract was suspended in 2% gum acacia (vehicle solution) and fed by intragastric tube daily for 45 days.

Group-I : Normal control (2% gum acacia)
Group-II : Normal control + *S. surattense* (100 mg kg^{-1} b.wt.) in 2% gum acacia.
Group-III : Diabetic control
Group-IV : Diabetic rats + *S. surattense* (100 mg kg^{-1} b.wt.) in 2% gum acacia.
Group-V : Diabetic rats + glibenclamide (600 μg kg^{-1} b.wt.) in 2% gum acacia.

After 45 days, the animals were fasted for 12 h, anaesthetized between 8:00 am to 9:00 am using ketamine (24 mg kg^{-1} b.wt, intramuscular injection) and sacrificed by decapitation. Blood was collected in heparinised tubes and the plasma was separated by centrifugation at 1200 x g for 15 min. The buffy coat was removed and the erythrocytes were washed three times with physiological saline. Aliquots of erythrocytes were kept at 4°C until analysis.

Biochemical Analysis

Glucose was estimated by the method of Trinder (1969) using a reagent kit. Insulin in the rat plasma was measured by the method of Burgi *et al.* (1988) Erythrocyte TBARS, LOOH and CD were estimated by the methods of Niehaus and Samuelson (1968), Jiang *et al.* (1992) and Klein (1979), respectively. The activities of SOD, CAT, GPx and glucose 6-phosphate dehydrogenase were measured by the methods of Kakkar *et al.* (1984), Sinha (1972), Rotruck *et al.* (1973) and Bergmeyer (1984), respectively. The nonenzymic antioxidants GSH, vitamin C and vitamin E were estimated by the methods of Ellman (1959), Roe and Kuether (1943) and Baker *et al.* (1980), respectively. The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated (by using commercially available kits), by the method of Reitman and Frankel (1957). The activities of alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) were estimated by the method Kind and King (1954) method and Rosalki and Rau (1972), respectively.

Phytochemical Analysis

The ethanolic extract analysis of *S. surattense* by Harborne (1984), method showed the presence of alkaloids, flavonoids, tannins, glycosides, triterpenoids and sterols in laboratory.

Statistical Analysis

Statistical evaluation was done using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) by using Statistical Package of Social Science (SPSS) 10.0 for Windows. Significance level was set at $p<0.05$.

RESULTS

Effect of 45 Day Oral Administration of *S. surattense* on Body Weight, Blood Glucose and Insulin Level in Normal and STZ-Diabetic Rats

Blood glucose level was elevated and body weight and insulin level decreased significantly in diabetic rats (Table 1). Administration of *S. surattense* and glibenclamide for 45 days decreased significantly blood glucose level and improved body weight and insulin level in diabetic rats.

Effect of *S. surattense* on Lipid Peroxidative Markers (TBARS, LOOH and CD) in the Erythrocytes of Normal and STZ-Diabetic Rats

The levels of lipid peroxidative markers levels increased significantly in diabetic rats Table 2. Administration of *S. surattense* and glibenclamide brought these values towards normalcy.

Table 1: Effect of *S. surattense* leaf-extract on body weight, blood glucose and insulin in normal and STZ-diabetic rats

	Body weight (g)		Glucose (mg dL^{-1})		
Groups	Day 0	Day 45	Day 0	Day 45	Insulin (μU mL^{-1})
Normal	179.33±4.16	201.79±8.40^{a}	68.65±2.07	75.32±7.59^{a}	14.39±1.31^{a}
Normal + *S. surattense* (100 mg kg^{-1} b.wt.)	185.20±5.10	202.93±7.00^{a}	67.02±4.94	74.45±7.76^{a}	14.29±1.30a
Diabetic control	181.33±5.56	141.70±9.92^{b}	250.25±15.80	291.38±19.20^{b}	4.53±0.41^{b}
Diabetes + *S. surattense* (100 mg kg^{-1} b.wt.)	184.50±5.09	170.04±5.50^{c}	247.95±17.92	140.57±12.82^{c}	8.97±0.80^{c}
Diabetes + glibenclamide (600 μg kg^{-1} b.wt.)	187.12±6.00	192.72±7.57^{a}	254.09±14.60	105.22±8.68^{d}	13.88±1.26^{a}

Values are means±SD of 6 rats from each group, Values not sharing a common superscript differ significantly at $p<0.05$ DMRT

Table 2: Effect of *S. surattense* leaf-extract on lipid peroxidative markers in the erythrocytes of normal and STZ- diabetic rats

	Hemolysate		
Groups	TBARS (nmol mg^{-1} protein)	LOOH (μmol mg^{-1} protein)	CD (ratio of 240/214 nm)
Normal	1.73±0.16^{a}	1.20±0.06^{a}	0.73±0.06^{a}
Normal + *S. surattense* (100 mg kg^{-1} b.wt.)	1.54±0.18^{a}	1.16±0.06^{a}	0.69±0.06^{a}
Diabetic control	4.13±0.31^{b}	1.50±0.11^{b}	0.90±0.08^{b}
Diabetic + *S. surattense* (100 mg kg^{-1} b.wt.)	3.23±0.31^{c}	1.35±0.09^{c}	0.84±0.07^{c}
Diabetic + glibenclamide (600 μg kg^{-1} b.wt.)	2.47±0.20^{d}	1.24±0.11^{c}	0.74±0.06^{a}

Values are means±SD from six rats in each group. Values not sharing a common superscript differ significantly at $p<0.05$ (DMRT)

Table 3: Effect of *S. surattense* leaf-extract on the activities of enzymatic antioxidants in the erythrocytes of normal and STZ-induced diabetic rats

	Hemolysate		
Groups	SOD (U^{x}/mg Hb)	CAT (U^{y}/mg Hb)	GPx (U^{z}/mg Hb)
Normal	7.02±0.64^{a}	181.50±7.28^{a}	14.94±1.39^{a}
Normal + *S. surattense* (100 mg kg^{-1} b.wt.)	7.25±0.52^{a}	186.50±10.60^{a}	15.24±0.97^{a}
Diabetic control	4.17±0.47^{b}	104.55±10.4^{b}	7.96±0.67^{b}
Diabetic + *S. surattense* (100 mg kg^{-1} b.wt.)	5.68±0.51^{c}	140.15±10.95^{c}	10.27±0.77^{c}
Diabetic + glibenclamide (600 μg kg^{-1} b.wt.)	6.46±0.59^{ac}	162.99±10.14^{d}	12.43±0.74^{d}

Values are means±SD from six rats in each group. Values not sharing a common superscript differ significantly at $p<0.05$ (DMRT), U^{x}- one unit of SOD is defined as the amount of enzyme, which gives 50% inhibition of NBT reduction in 1 min, U^{y}- one unit of CAT is defined as the μmole of hydrogen peroxide consumed per min, U^{z}- one unit of GPx is defined as the μg of glutathione consumed per min

Effect of *S. surattense* on the Activities of Enzymatic Antioxidants (SOD, CAT and GPx) in the Erythrocytes of Normal and Diabetic Rats

The activities of enzymatic antioxidants decreased in diabetic rats Table 3 and administration of *S. surattense* and glibenclamide brought these values closer to normalcy.

Effect of *S. surattense* on the Levels of Non-enzymatic Antioxidants (Vitamin E, Vitamin C and GSH) and Glucose 6-phosphate Dehydrogenase Activity in the Erythrocytes of Normal and Diabetic Rats

Diabetic animals showed a significant decrease in vitamin C, GSH levels and glucose 6-phosphate dehydrogenase activity and an increase in vitamin E level (Table 4). Administration of *S. surattense* and glibenclamide brought these values closer to normalcy.

Table 4: Effect of *S. surattense* leaf-extract on non-enzymatic antioxidants in the erythrocytes of normal and STZ-induced diabetic rats

	Hemolysate			
Groups	Vitamin E (μg mg^{-1} of Hb)	Vitamin C (μg mg^{-1} of Hb)	GSH (mg g^{-1} of Hb)	Glucose 6-phosphate dehydrogenase (U*/g Hb)
Normal	1.33±0.13^{a}	1.81±0.07^{a}	76.31±5.61^{a}	4.28±0.30^{a}
Normal + *S. surattense* (100 mg kg^{-1} b.wt.)	1.49±0.08^{ad}	2.04±0.11^{c}	77.14±5.99^{a}	4.64±0.39^{a}
Diabetic control	2.99±0.24^{b}	0.92±0.09^{b}	46.96±4.27^{b}	2.94±0.26^{b}
Diabetic + *S. surattense* (100 mg kg^{-1} b.wt.)	1.96±0.18^{c}	1.24±0.12^{d}	61.03±4.25^{c}	3.40±0.31^{c}
Diabetic + glibenclamide (600 μg kg^{-1} b.wt.)	1.62±0.13^{d}	1.61±0.13^{e}	69.16±6.57^{d}	4.30±0.37^{a}

Values are means±SD from six rats in each group. Values not sharing a common superscript differ significantly at $p<0.05$ (DMRT). U*- nmole of NADPH formed per min

Table 5: Effect of *Solanum surattense* leaf-extract on plasma hepatic marker enzymes in normal and STZ- diabetic rats

Groups	ALT (IU*/L)	AST (IU*/L)	ALP (IU#/L)	γ-GT (IU@/L)
Normal	26.30±2.08^{a}	72.12±2.98^{a}	79.74±3.02^{a}	15.97±1.56^{a}
Normal + *S. surattense* (100 mg kg^{-1})	24.20±2.36^{a}	70.65±3.25^{a}	77.96±5.01^{a}	15.21±1.16^{a}
Diabetic control	61.70±2.49^{b}	120.97±9.32^{b}	140.57±10.89^{b}	27.16±1.85^{b}
Diabetes+ *S. surattense* (100 mg kg^{-1})	49.18±2.19^{c}	96.21±9.50^{c}	105.47±8.10^{c}	20.23±1.42^{c}
Diabetes+glibenclamide (600 μg kg^{-1} b.wt.)	29.33±2.56^{d}	80.06±4.05^{d}	83.97±5.63^{a}	16.39±1.20^{a,c}

Values are means±SD of 6 rats from each group, Values not sharing a common superscript differ significantly at $p<0.05$ DMRT, * μmol of pyruvate liberated per h, # μmol of phenol liberated per min, @ μmol of p-nitroanilide liberated per min

Effect of *S. surattense* on the Hepatic Markers of Normal and Diabetic Rats

Diabetic rats showed significant increase In the activities of AST, ALT, ALP and γ-GT (Table 5) and treatment with *S. surattense* and glibenclamide markedly decreased these values towards normalcy.

DISCUSSION

Free radicals may play an important role in the causation and complication of diabetes mellitus. The increased oxidative stress and accompanying decrease in antioxidants may be related to the causation of diabetes. STZ, a diabetogenic compound, selectively destroys beta cells of pancreas resulting in a hyperglycemia state (Takasu *et al*., 1991). The antihyperglycemic activity of the *S. surattense* leaf extract was associated with decrease in blood glucose and increase in plasma insulin (Sridevi *et al*., 2007). The exact mechanism of action of the extract is not known, but the decrease of glucose, could be due to increased pancreatic secretion of insulin from existing β-cells. In this context, a number of other plants have also been reported to exert antihyperglycemic activity through insulin stimulatory activity (Chattopadhyay, 1999; Senthil Kumar *et al*., 2006; Kar *et al*., 2006). Phytochemical analysis of the leaf-extract shows the presence of alkaloids, flavonoids, tannins, glycosides, triterpenoids and sterols, which are in agreement with Rahman *et al*. (2003). Thus, the presence of flavonoids may exert its antihyperglycemic effect (Coskum *et al*., 2005).

STZ-induced diabetes is characterized by severe weight loss (Al-Shamaony *et al*., 1994) which was observed in the present study. *S. surattense* and glibenclamide administration controlled the body weight loss in diabetic animals, though *S. surattense* did not normalize the body weight completely.

The decrease in body weight in diabetic rats might be the result of protein wasting due to unavailability of carbohydrate as an energy source (Chen and Ianuzzo, 1982). The treated groups enhanced glucose metabolism and thus, improved the body weight in STZ-diabetic rats.

Tremendous increase in lipid peroxidation in erythrocytes observed in diabetic rats is attributed to chronic hyperglycemia that generates superoxide and hydroxyl radicals (Wolff *et al*., 1987). Increased lipid peroxidation in erythrocytes is known to cause decreased cell survival, altered membrane lipid asymmetry, hypercoagulability, increased adhesivity to the endothelium (Jain *et al*., 1990) and focal occlusion (Raman and Biswas, 2002). Endogenous lipid peroxidation products were increased in the erythrocytes of the diabetic patients (Augustyniak *et al*., 1996) and STZ-diabetic rats (Prakasam *et al*., 2003). The increased concentration of lipid peroxidation products could be due to the increased production or decreased destruction of ROS. These levels decreased significantly in *S. surattense* treated rats, which might be due to the presence of lupeol, a triterpene, present in the callus of *S. surattense* (Heble *et al*., 1970). Previous study shows that lupeol significantly reduce lipid peroxidation by its ability to scavenge the free radicals (Preetha *et al*., 2006).

Antioxidant enzymes, SOD, CAT and GPx form the first line of defense against ROS and decreased activities were observed in the erythrocytes of STZ diabetic rats (Matakovics *et al*., 1998; Sharma *et al*., 2000; Mahindrakar *et al*., 2007). SOD scavenges superoxide anion to form H_2O_2 and diminishes the toxic effects derived from secondary reaction. CAT and GPx are involved in the reduction of H_2O_2 and thus protect the erythrocytes from ROS. Increased activities of SOD, CAT and GPx after treatment with *S. surattense* extract may be due to the presence of lupeol, an antioxidant.

Vitamin C is a well-known antioxidant and quenches the singlet molecular oxygen in aqueous medium (Chou and Khan, 1983). The decreased level of ascorbic acid may be due to increased utilization against ROS and/or decrease in the non protein thiols like GSH as it is required for the recycling of vitamin C (Jin *et al*., 2000). Treatment with the *S. surattense* extract increased the level of vitamin C.

The most important antioxidant in the cell membrane is α-tocopherol (Garg *et al*., 1996). It interrupts the chain reaction of lipid peroxidation by reacting with lipid peroxyl radical thus protecting the cell structures against damage (Niki *et al*., 1995) Increased level of α-tocopherol found in the STZ diabetic rats in this study may be due to the release of membrane bound α-tocopherol from damaged cell membrane. Administration of *S. surattense* significantly decreased the level of vitamin E.

GSH plays a pivotal role in the protection of cells against free radicals. Decreased GSH in hyperglycemia is due to decreased formation, GSH formation requires NADPH and Glutathione Reductase (GR) (Garg *et al*., 1996). NADPH level in the cell is maintained by the action of glucose-6-phosphate dehydrogenase through HMP shunt, which plays an important role in the maintenance of NADPH/NADP ratio and in the regeneration of GSH from GSSG (Jain, 1998) in the cell. Reduced availability of NADPH may be due to decrease in the activity of glucose 6-phosphate dehydrogenase and hence, decreased level of GSH. Administration *S. surattense* caused an increase in the activity of glucose-6-phosphate dehydrogenase; thereby increasing NADPH levels and, in turn, the GR activity. Thus GSH is replenished by the administration of *S. surattense*, which may, in turn, maintain the antioxidant status in the erythrocytes of diabetic rats.

Increased activities of AST, ALT, ALP and γ-GT are used as the indices of liver damage. Increased enzyme activities were observed in diabetic rats (Ramesh and Pugalendi, 2006). The increase in the activities of these enzymes is due to the leakage of these enzymes from the cytosol into the blood stream (Tawta, 2000). Treatment with *S. surattense* reduced activities of these enzymes and thus inhibits the liver damage induced by STZ. Similar findings are consistent with Kar *et al*. (2006) that the fruit extract of *Solanum xanthocarpum* reduced the elevated hepatic markers against cellular damage.

CONCLUSIONS

The present investigation reveals that the alcoholic leaf-extract of *S. surattense* possesses antidiabetic effect against STZ-diabetic rats. It also exerts protective effect against lipid peroxidation and enhances cellular antioxidant defense, thereby reducing diabetic complications. Further, isolation of the active compound(s) responsible for the above activities is underway.

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