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ANTIHYPERGLYCEMIC AND ANTIOXIDANT POTENTIALS OF *SESBANIA GRANDIFLORA* LEAVES STUDIED IN STZ INDUCED EXPERIMENTAL DIABETIC RATS

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ABSTRACT: Diabetes mellitus is a metabolic syndrome involving severe insulin dysfunction in conjugation with gross abnormalities in glucose homeostasis and lipid metabolism, which has been affecting several millions of population all over the world. Despite the introduction of hypoglycemic agents from natural as well as synthetic sources, diabetes and its secondary complications continue to be a major health problem for the medical fraternity. *Sesbania grandiflora* L. Pers. is an Indian medicinal plant which belongs to family *Leguminosae* possesses a wide array of beneficial and pharmacological properties. In the present study an attempt has been made to evaluate the antidiabetic and antioxidant nature of *Sesbania grandiflora* leaves. Diabetes was induced by single intraperitoneal injection of streptozotocin (45mg/Kg b.wt). Diabetic rats orally treated with leaves extract (300 mg/kg b.w/day) for 30 days resulted in significant ($p<0.05$) decrease in the levels of blood glucose, glycosylated hemoglobin, blood urea, serum uric acid, serum creatinine and diminished activities of pathophysiological enzymes such as aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP). The levels of glycogen content and the activities of glycogen metabolizing enzymes were normalized in diabetic rats treated with leaves extract. The elevated oxidative stress marker and diminished antioxidant status were normalized indicating the antioxidant potential of leaves extract. The results of the present study indicate that the leaves extract possess both antidiabetic and antioxidant potent which could be attributed to the presence of pharmacologically active ingredients such as vitamins, flavonoids, saponins, tannins, diterpenes, triterpenoids, glycosides and phenols in the leaves.

INTRODUCTION: Diabetes mellitus describes a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion (type 1), insulin action (type 2), or both.

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The unprecedented economic development and rapid urbanization have led to a shift in health problems from communicable to non-communicable diseases [1].

Nature has been a rich source of medicinal agents for thousands of years and an impressive number of modern drugs have been originally isolated from natural sources, many based on their use in traditional medicine. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times [2].

Herbal drugs with anti-diabetic activity are yet to be successfully formulated as modern medicines, even though they have been acclaimed for their therapeutic properties in the traditional systems of medicine [3].

Many Indian plants have been investigated for their beneficial use in different types of diabetes and reports occur in numerous scientific journals. Ayurveda and other traditional medicinal system for the treatment of diabetes describe a number of plants used as herbal drugs. Hence, they play an important role as an alternative medicine due to less side effects, availability, accessibility and affordability.

In the series of medicinal plants, *Sesbania* grandiflora is one such plant that lacks scientific scrutiny. *Sesbania grandiflora*, *L.* is a short lived, fast growing soft wooded tree which belongs to family *Papilonaceae*[4]. It is commonly called as 'sesbania' and 'agathi'[5]. It grows up to 6-9 m height. It has been used as an important dietary nutritive source and often planted for its edible flowers and pods in Southeast Asian countries[6]. It is believed to have originated either in India or Southeast Asia and grows primarily in hot and humid tropical areas of the world. The other names include agathi, agati sesbania, August flower, Australian corkwood tree, flamingo bill, sesban, swamp pea, tiger tongue, West Indian pea, white dragon tree etc [7].

Sesbania grandiflora possess hepatoprotective [8], anticancer activity [9]. For congenital bronchitis or cold in babies, leaf juice mixed with honey is often recommended [10] (Nadkarni, 1982). All parts of this unique plant are useful and have a wide spectrum of medicinal properties [11]. The leaves possess analgestic, antipyretic, anti-inflammatory and antioxidant properties [12, 13, 14]. In the absence of systemic studies in the literature, the present study was aimed to investigate the antidiabetic and antioxidant properties of leaves extract when administered orally in experimentally induced diabetes in rats.

MATERIALS AND METHODS:

Experimental Animals: Male albino Wistar rats (150-180 g) were purchased from TANUVAS, Madavaram, Chennai. The rats were housed in polypropylene cages lined with husk and kept in centralized Animal house facility, University of Madras, Guindy campus, Chennai. The husk was renewed every 24 hours. The rats were fed with commercial pelleted rats chow (VRK Nutritional Solutions, Maharashtra, India) and had free access to water. The experimental rats were maintained in a controlled environment (12:12 hours light/dark cycle) and temperature (30 ± 2° C). The experiments were designed and conducted in accordance with the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines for the investigation of experimental pain in conscious rats. The rats were acclimatized for one week before initiating the experiments.

Plant Material: The leaves of *Sesbania grandiflora* were collected from a local plantation near chengalpet. The leaves were identified and authenticated and a voucher specimen was deposited at the Centre for Advanced studies in Botany, University of Madras, Chennai. The leaves were washed for any contaminants, dried thoroughly under shade and powdered in a pulverizer and stored in an airtight container at 5°C until further use.

Preparation of Plant Extract: The powdered leaves were delipidated with petroleum ether (60 - 80°C) for overnight. It was then filtered and soxhalation was performed with 95% Ethanol. Ethanol was evaporated in a rotary evaporator at 40 – 50°C under reduced pressure. The yield was 14.7g.

Preliminary Phytochemical Screening: The ethanolic extract of *Sesbania grandiflora* leaves were subjected to preliminary phytochemical screening of various plant constituents[15, 16].

Experimental Induction of Diabetes: Diabetes was induced in overnight fasted rats by single intraperitoneal injection of streptozotocin (45 mg/kg b.w) dissolved in freshly prepared 0.1M of ice cold citrate buffer (pH 4.5) [17]. Since, STZ is capable of inducing fatal hypoglycemia due to massive pancreatic insulin release, the rats were provided with 10% glucose solution after 6 h of

STZ administration for the next 24 h to overcome drug induced hypoglycemia[18]. Neither death nor any other adverse effect was observed. After a week time, for the development and aggravation of diabetes, rats with moderate diabetes (i.e. fasting blood glucose concentration, >250 mg/dl) that exhibited hyperglycemia and glycosuria were selected for further experimentation.

Experimental Protocol: The rats were grouped into 4 groups, comprising of 6 rats in each group as follows:

Group I : Control rats

Group II : STZ induced diabetic Rats.

Group III : Diabetic Rats treated with *Sesbania grandiflora leaves* extract (300 mg/Kg bw/day) in aqueous solution orally for 30 days.

Group IV : Diabetic rats treated with gliclazide (5mg/Kg b.w /day) in aqueous solution orally for 30 days.

During the experimental period, body weight and blood glucose levels of all the rats were determined at regular intervals. At the end of the experimental period, the rats were fasted overnight, anaesthetized, and sacrificed by cervical decapitation. The blood was collected with or without anticoagulant for plasma or serum separation respectively.

The liver and pancreatic tissues were dissected out and washed in ice-cold saline, which is then used for further experimental studies.

Oral Glucose Tolerance Test (OGTT): At the end of the experimental period, fasting blood samples were taken from all the groups of rats to perform oral glucose tolerance test. Four more blood samples were collected at 30, 60, 90 and 120 min intervals after an oral administration of glucose solution at a dosage of 2 g kg^{-1} body weight. All the blood samples were collected with EDTA for the estimation of glucose.

Biochemical parameters: Blood glucose level was estimated by the method of glucose oxidase/peroxidase method [19] using a commercial kit (Span Diagnostic Chemicals, India) and urea [20]. Glycosylated hemoglobin was estimated [21]. Plasma was used for protein assay [22]. Urine sugar was detected using urine strips. Serum was used for the determination of creatinine [23] and uric acid [24]. The activities of aspartate transaminase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP) were assayed [25, 26].

Preparation of Tissue Homogenate: The liver and pancreatic tissues were excised, rinsed in ice- cold saline. Known amount of the tissues were homogenized in Tris–HCl buffer (100 mM, pH 7.4) at 4°C, in a Potter–Elvehjem homogenizer with a Teflon pestle at 600 rpm for 3 min. The homogenate was then centrifuged at 12,000-×g for 30 min at 4°C. The supernatant was collected as tissue homogenate, which was used to assay various parameters. The protein content in the tissue homogenate was estimated. A portion of wet liver tissue was used for the estimation of glycogen content [27]. Glycogen synthase, glycogen phosphorylase activities were assayed in liver tissues [28, 29].

Assay of antioxidant status: The levels of lipid peroxides were determined in plasma and tissue homogenate [30, 31]. The activities of enzymatic antioxidants such as SOD, catalase and GPx were assayed in the tissue homogenate of control and experimental groups of rats [32, 33, 34]. The levels of non-enzymatic antioxidants such as vitamin C, vitamin E, and GSH were also determined [35, 36, 37].

Statistical Analysis: The values were expressed as mean ± S.D for six rats in each group. All data were analyzed with SPSS/16.0 student software. Hypothesis testing method included one way analysis of variance (ANOVA) followed by post hoc testing performed with least significant difference (LSD) test. A Value of $P < 0.05$ was considered as significant.

RESULTS: Table 1 shows the qualitative analysis of phytochemicals present in the ethanolic extract of *Sesbania grandiflora* leaves. From the preliminary phytochemical screening, it was found that the *Sesbania grandiflora leaves* extract contains flavonoids, saponins, tannins, diterpenes, triterpenoids, glycosides and phenols.

TABLE 1: PHYTOCHEMICAL SCREENING OF *SESBANIA GRANDIFLORA* LEAF EXTRACT

Phytoconstituents	Inference
Alkaloids	-
Flavonoids	+
Saponins	+
Tannins	+
Phytosterol	+
Diterpenes	+
Triterpenoids	-
Glycosides	+
Anthraquinones	-
Phenols	+

Table 2 shows the observed levels of body weight in control and experimental group of animals. The body weight of control rats was progressively increased whereas there was a significant decrease in the body weight of STZ induced diabetic rats. Diabetic rats treated with *Sesbania grandiflora leaves* extract as well as gliclazide for 30 days showed a significant improvement in body weight.

TABLE 2: EFFECT OF *SESBANIA GRANDIFLORA* EXTRACT ON CHANGES IN BODY WEIGHT OF EXPERIMENTAL GROUPS OF RATS AFTER 30 DAYS TREATMENT

Groups	Body weight (g)	
	Initial	Final
Control	165.59 ± 2.91	229.15 ± 3.86
Diabetic	172.37 ± 2.71	149.08 ± 4.24*
Diabetic + *Sesbania grandiflora* extract	170.68 ± 3.06	190.11 ± 3.98@
Diabetic + gliclazide	168.59 ± 3.50	187.95 ± 4.12@

Values are given as mean ± SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, @ compared with diabetic rats.

Table 3 depicts the levels of blood glucose in certain durations after the oral administration of glucose (2g/Kg body weight) in normal and experimental groups of rats. In control rats, the blood glucose level reached the maximum peak at 60 min after the glucose has been loaded and then was gradually reverted back to near normal levels after 120 min.

TABLE 3: EFFECT OF *SESBANIA GRANDIFLORA* EXTRACT ON THE BLOOD GLUCOSE LEVEL (MG/DL) IN THE EXPERIMENTAL GROUPS OF RATS RECEIVING AN ORAL GLUCOSE LOAD

Groups	Fasting	30 min	60 min	90 min	120 min
Control	86.49 ± 8.72	185.37 ± 12.49	220.13 ± 19.54	169.82 ± 14.73	112.09 ± 12.86
Diabetic	276.91 ± 20.43*	335.82 ± 22.51*	386.44 ± 26.58*	348.29 ± 25.16*	309.64 ± 22.78*
Diabetic + *Sesbania grandiflora* extract	159.68 ± 15.26@	220.52 ± 17.58@	279.60 ± 25.38@	215.71 ± 18.62@	180.74 ± 16.25@
Diabetic + gliclazide	149.82 ± 12.86@	226.54 ± 20.17@	281.42 ± 22.59@	190.37 ± 19.77@	168.58 ± 15.99@

Unit: mg/dL; Values are given as mean ± SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, @ compared with diabetic rats.

Table 4 depicts the levels of blood glucose, glycosylated hemoglobin and urine sugar. STZ induced diabetic rats showed a significant elevation in the levels of blood glucose, presence of urine sugar and a simultaneous increase in glycosylated hemoglobin. Oral administration of ethanolic extract of *Sesbania grandiflora leaves* to the diabetic group of rats significantly reduced the levels of blood glucose and glycosylated hemoglobin. Urine sugar which was present in the diabetic group of rats was found to be absent in rats treated with the extract.

TABLE 4: EFFECT OF *SESBANIA GRANDIFLORA* EXTRACT ON THE LEVELS OF BLOOD GLUCOSE, GLYCOSYLATED HEMOGLOBIN, AND URINE SUGAR IN THE EXPERIMENTAL GROUPS OF RATS

Groups	Glucose (mg/dl)	Glycosylated hemoglobin (%)	Urine sugar
Control	89.61 ± 10.72	6.51 ± 1.91	Nil
Diabetic	280.36 ± 20.15*	12.85 ± 3.46*	+++
Diabetic + *Sesbania grandiflora* extract	164.27 ± 16.92@	8.05 ± 1.69@	Nil
Diabetic + gliclazide	155.82 ± 19.34@	7.92 ± 1.89@	Nil

Values are given as mean ± SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, @ compared with diabetic rats.

Table 5 depicts the levels of total protein, blood urea, uric acid and serum creatinine in control and experimental groups of rats. In STZ induced diabetic rats, there was a significant decrease in the total protein and increase in the levels of urea, uric acid and creatinine when compared with the control group of rats. Administration of an ethanolic extract of *Sesbania grandiflora leaves* as well as the standard drug, gliclazide to the diabetic group of rats significantly decreased the levels of blood urea, uric acid, serum creatinine and increased the levels of total protein.

TABLE 5: EFFECT OF *SESBANIA GRANDIFLORA* EXTRACT ON THE LEVELS OF PROTEIN, UREA, CREATININE AND URIC ACID IN PLASMA OF EXPERIMENTAL GROUPS OF RATS

Groups	Protein (g/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Control	8.18 ± 1.79	25.94 ± 3.10	0.76 ± 0.08	2.74 ± 0.91
Diabetic	5.69 ± 1.15*	55.68 ± 5.77*	2.51 ± 0.59*	5.44 ± 1.71*
Diabetic + *Sesbania grandiflora* extract	7.24 ± 1.42[@]	30.21 ± 5.28[@]	1.09 ± 0.31[@]	3.51 ± 1.28[@]
Diabetic + gliclazide	7.38 ± 1.57[@]	34.49 ± 4.98[@]	1.40 ± 0.39[@]	3.72 ± 1.35[@]

Values are given as mean ± SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, [@] compared with diabetic rats.

Table 6 depicts the levels the levels of glycogen content and activities of glycogen synthase and glycogen phosphorylase in liver tissues control and experimental groups of rats. A significant decline in the glycogen level as well as in the glycogen synthase activity and a concomitant increase in the activity of glycogen phosphorylase were noted in the liver of diabetic group of rats. Oral administration of *Sesbania grandiflora leaves* extract as well gliclazide to diabetic rats restored the level of glycogen and the activities of glycogen synthase, glycogen phosphorylase to near normalcy when compared to control group of rats.

TABLE 6: EFFECT OF *SESBANIA GRANDIFLORA* EXTRACT ON THE LEVELS OF LIVER GLYCOGEN CONTENT IN THE EXPERIMENTAL GROUPS OF RATS

Groups	Glycogen	Glycogen synthase	Glycogen phosporylase
Control	42.96 ± 5.42	794.28 ± 59.71	576.18 ± 36.22
Diabetic	25.38 ± 3.99*	539.75 ± 36.45*	899.37 ± 65.16 *
Diabetic + *Sesbania grandiflora* extract	36.75 ± 4.18[@]	696.78 ± 33.59 [@]	654.25 ± 40.17 [@]
Diabetic + gliclazide	37.26 ± 4.92[@]	677.85 ± 40.27 [@]	681.34 ± 45.91[@]

Units are expressed as: mg/g wet tissue for glycogen, µmoles of UDP formed/h/mg protein for glycogen synthase and µmoles of Pi liberated/h/mg protein for glycogen phosphorylase. Values are given as mean ± SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, [@] compared with diabetic rats.

Table 7 depicts the levels of AST, ALT and ALP in the control and experimental group of rats. Diabetic rats showed a significant elevation in the levels of aspartate transaminase, alanine transaminase and alkaline phosphatase when compared with the control group of rats. Administration of *Sesbania grandiflora leaves* extract and gliclazide to the diabetic rats resulted in a significant decrease in the levels of these markers.

TABLE 7: EFFECT OF *SESBANIA GRANDIFLORA* EXTRACT ON THE ACTIVITY OF AST, ALT AND ALP IN THE SERUM OF EXPERIMENTAL GROUPS OF RATS

Groups	AST	ALT	ALP
Control	40.67 ± 8.21	20.35 ± 3.17	70.49 ± 10.91
Diabetic	109.75 ± 12.86*	68.74 ± 10.38*	202.35 ± 20.72*
Diabetic + *Sesbania grandiflora* extract	72.38 ± 10.41[@]	35.26 ± 7.14[@]	98.56 ± 12.94[@]
Diabetic + gliclazide	81.74 ± 12.86[@]	30.99 ± 8.12[@]	102.78 ± 10.85[@]

The enzyme activities are expressed as: AST and ALT µmoles of pyruvate liberated /h/mg of protein; ALP µmoles of phenol liberated/min/mg of protein. Values are given as mean ± SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, [@] compared with diabetic rats.

The levels of TBARS in the plasma and pancreas of control as well as experimental group of rats are presented in **Table 8**. STZ induced diabetic rats showed marked increase in the levels of TBARS when compared to control rats. Treatment of *Sesbania grandiflora leaves* extract to diabetic rats showed a significant decrease in the levels of TBARS.

TABLE 8: EFFECT OF *SESBANIA GRANDIFLORA* EXTRACT ON THE LEVEL OF TBARS IN PLASMA AND PANCREAS, OF EXPERIMENTAL GROUPS OF RATS

Groups	TBARS	
	Plasma	Pancreas
Control	3.42 ± 0.91	28.57 ± 5.64
Diabetic	7.54 ± 2.18*	72.89 ± 12.35*
Diabetic + *Sesbania grandiflora* extract	4.89 ± 1.52$^{@}$	40.27 ± 9.58$^{@}$
Diabetic + gliclazide	5.14 ± 1.98$^{@}$	39.52 ± 10.85$^{@}$

Units: mM/100 g in tissues; nM/ml in plasma. Values are given as mean ± SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, $^{@}$ compared with diabetic rats.

Table 9 and 10 illustrates the activities of enzymatic and non-enzymatic antioxidants in pancreas as well as plasma of control and experimental group of rats. In STZ induced diabetic rats, there was a significant reduction in the activities of enzymatic and non-enzymatic antioxidants in pancreas and plasma respectively. Treatment of *Sesbania grandiflora leaves* extract to the diabetic rats showed improvement in the activities of enzymatic and non-enzymatic antioxidants.

TABLE 9: EFFECT OF *SESBANIA GRANDIFLORA* EXTRACT ON THE ACTIVITY OF SOD, CATALASE AND GPX, AND THE LEVEL OF GSH IN PANCREAS OF EXPERIMENTAL GROUPS OF RATS

Groups	SOD	Catalase	GPx	GSH
Control	4.55 ± 1.37	18.64 ± 2.85	6.49 ± 1.71	24.97 ± 2.54
Diabetic	1.95 ± 0.98*	8.12 ± 2.79*	3.58 ± 0.91*	10.75 ± 2.12*
Diabetic + *Sesbania grandiflora* extract	3.29 ± 1.45$^{@}$	14.69 ± 2.27$^{@}$	4.86 ± 1.21$^{@}$	19.55 ± 2.37$^{@}$
Diabetic + gliclazide	3.33 ± 1.81$^{@}$	13.79 ± 3.10$^{@}$	4.51 ± 1.38$^{@}$	20.18 ± 3.42$^{@}$

Activity is expressed as: 50% of inhibition of epinephrine autooxidation/min/mg of protein for SOD; µmoles of hydrogen peroxide decomposed/min/mg of protein for catalase; µmoles of glutathione oxidized/min/mg of protein for GPx; mg/100 g tissue for GSH. Values are given as mean ± SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, $^{@}$ compared with diabetic rats.

TABLE 10: EFFECT OF *SESBANIA GRANDIFLORA* EXTRACT ON THE LEVELS OF VITAMIN C, VITAMIN E, CERULOPLASMIN AND GSH IN PLASMA OF EXPERIMENTAL GROUPS OF RATS

Groups	Vitamin C	Vitamin E	Ceruloplasmin	GSH
Control	1.36 ± 0.12	0.65 ± 0.07	13.71 ± 1.52	28.93 ± 2.74
Diabetic	0.52 ± 0.09*	0.30 ± 0.04*	5.18 ± 0.95*	12.86 ± 2.17*
Diabetic + *Sesbania grandiflora* extract	0.94 ± 0.11$^{@}$	0.57 ± 0.08$^{@}$	10.21 ± 1.59$^{@}$	20.72 ± 2.95$^{@}$
Diabetic + gliclazide	0.89 ± 0.12$^{@}$	0.59 ± 0.04$^{@}$	9.76 ± 1.91$^{@}$	22.10 ± 3.18$^{@}$

Units: mg/dl. Values are given as mean ± SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, $^{@}$ compared with diabetic rats.

DISCUSSION: Plants provide an extraordinary source of natural medicines for various ailments. Moreover, secondary metabolites of plant origin serve as an invaluable chemical library for drug discovery and current medicinal chemistry in the pharmaceutical industry. The medicinal properties of plants lie in the phytoingredients that exert distinct physiological activities in the human body. Over the past 25 years, 50% of prescription drugs have been developed from natural products and their derivatives[38]. In the present study, phytochemical analysis on *Sesbania grandiflora leaves* extract indicated the presence of flavonoids, saponins, tannins, diterpenes, triterpenoids, glycosides and phenols. These phytochemicals were speculated to account for the observed

pharmacological effects of the extract. The leaves contain essential amino acids, minerals, vitamins such as A, E, C, thiamine, riboflavin and nicotinic acid in addition to secondary phytochemicals such as pectin, triterpenoid, tannin, glycosides and saponin [39, 40].

The leaves extract was reported to be non-toxic. It has been reported that the oral administration of leaves extract even at the maximum single dose of 2000 mg/kg body weight was found to be safe, since no mortality [41].

STZ acts as a cytotoxin for beta-cells of the islet of langerhans, causes diabetes by inducing β-cell necrosis [42]. Diabetic rats exhibit gradual weight loss as compared with the normal group. This process is due to muscle wasting and depletion of protein in tissues. A decrease in body weight was observed in diabetic group indicating the increased proteolysis. Diabetic rats treated with *Sesbania grandiflora leaves* extract for 30 days showed a significant improvement in body weight as compared to diabetic animals, which shows the beneficial effects of the extract in controlling the muscular wasting.

Diabetes mellitus is characterized by decreased glucose tolerance due to low secretion of insulin or its action. Oral glucose tolerance test (OGTT) is a test of immense value in favor of using fasting plasma glucose concentration to facilitate the diagnosis of diabetes mellitus. OGTT revealed that the blood glucose levels in control rats reach peak at 60 minutes after the oral glucose load and gradually return backs to normal levels after 120 minutes. In diabetic rats, the peak increases in blood glucose concentration was observed after 60 minutes and remained high over the next 60 minutes. However, oral treatment with leaves extract showed definite lower peak blood glucose values, 60 minutes after glucose load also gives lower values almost at the end of 120 minutes indicating the improved glucose tolerance in diabetic rats treated with *Sesbania grandiflora* leaves extract.

Diabetes mellitus is characterized by persistent hyperglycemia which results from reduced glucose utilization by various tissues. STZ induction causes specific damage in cells and thus exerts a pronounced increase in blood glucose concentration. It is well established that gliclazide is used as an antihyperglycemic drug, which stimulate the insulin secretion from pancreas and it is often used as a standard drug in STZ induced diabetic models to compare the antidiabetic property of various plant extracts. Oral administration of with *Sesbania grandiflora* leaves extract to STZ induced diabetic rats resulted in significant reduction in blood glucose level indicating the hypoglycemic nature of the leaves extract.

Persistent hyperglycemia results in glycation of hemoglobin that leads to the formation of glycosylated hemoglobin[43]. Glycosylated hemoglobin is an easily measurable biochemical marker that strongly correlates with the level of ambient glycemia during a 2- to 3-month period and is a more accurate and reliable measure than fasting blood glucose level [44]. The concentration of glycosylated hemoglobin strongly predicts the risk of eye, kidney and neural disease in diabetes mellitus and is regarded as a key target for the diagnosis and prognosis of diabetes-related complications [45].

Oral treatment with *Sesbania grandiflora* leaves extract significantly decreased the levels of glycosylated hemoglobin, suggesting that it may prevent oxidative damage caused by the glycation reaction in diabetic conditions. These results on the levels of glucose and glycosylated hemoglobin indicate the beneficial effects of in the maintenance of glucose homeostasis. Urine sugar which was present in diabetic rats was found to be absent in the rats treated with the extract indicating the improved glycemic control.

The biochemical parameters such as Urea, Creatinine and Uric acid are considered as significant markers for renal dysfunction. Renal dysfunction is one of the pathophysiological condition occurs in diabetic condition. Urea is the end product of protein catabolism. The diabetic animals manifest a negative nitrogen balance related to proteolysis in muscles and other tissues which is coupled with lowered protein synthesis [46] and increased protein catabolism accelerates urea synthesis thereby resulting in hyperuremia.

The accelerated proteolysis of uncontrolled diabetes occurs as a result of deranged glucagon mediated regulation of cyclic AMP formation in insulin deficiency [47]. This readily accounts for the observed decrease in the total protein content in diabetes mellitus. The serum creatinine concentration is the variable used not only to assess impairment of kidney function but also to detect the toxic effects of certain compounds derived from medicinal plants on kidney, in order to determine its efficacy in the treatment of diabetic rats. Serum uric acid is significantly associated with the risk of diabetes. Serum uric acid has been shown to be associated with oxidative stress and production of tumour necrosis factor [48].

In addition, a recent study in rats showed that fructose-induced hyperuricemia plays a pathogenic role in metabolic syndrome [49] (Nakagawa, 2006). Thus, lowering uric acid may be a novel treatment target for preventing diabetes. The levels of urea, serum creatinine and uric acid were restored to near normalcy by treatment with *Sesbania grandiflora* leaves extract as well as gliclazide in STZ induced diabetic rats.

Liver plays a unique role in controlling carbohydrate metabolism by maintaining glucose concentrations in a normal range over both short and long periods of time. Liver produces glucose by breaking down glycogen (glycogenolysis) and by *de novo* synthesis of glucose (gluconeogenesis) from non-carbohydrate precursors such as lactate, amino acids and glycerol. Glycogen is the primary intracellular storable form of glucose and its availability in various tissues is a direct manifestation of insulin action as insulin facilitates intracellular glycogen deposition by stimulating the activity of glycogen synthase and inhibiting glycogen phosphorylase [50].

Glycogen synthase is a crucial and rate-limiting enzyme which catalyzes the transfer of glucose from UDP-glucose to glycogen. Glycogen phosphorylase is a rate-limiting enzyme of glycogenolysis and is regulated by phosphorylation and by allosteric binding of AMP, ATP, glucose-6-phosphate and glucose[51]. In diabetes, the glycogen levels, glycogen synthase activity and responsiveness to insulin signaling are diminished and glycogen phosphorylase activity is significantly increased. Oral administration of leaves extract to diabetic rats restored the glycogen content and the activities of glycogen metabolizing enzymes demonstrating the role of leaves extract in the regulation of glycogen metabolism. Earlier, we have reported similar findings with *Murraya koenigii* leaves extract [52].

AST and AST are the intracellular enzymes that have escaped into the blood stream and serve as a clinical index of tissue injury chiefly hepatocyte as well as renal injury. ALP acts as a marker of biliary function and cholestasis. It is assumed that elevation in the levels of serum ALT, AST and ALP are considered as predictors of diabetes [53]. The increased activities of ALT, AST and ALP in the serum of diabetic rats may be primarily due to the leakage of these enzymes from liver as well as kidney into the blood stream[54]. Oral administration of leaves extract to diabetic group of rats showed a notable decline in the activity of these enzymes to their basal levels, indicating its non-toxic as well as tissue protective nature.

Oxidative stress is associated with the molecular mechanism of the decreased insulin biosynthesis and secretion, which is the main etiology of glucose toxicity. Because pancreatic islet cells show extremely weak manifestation of antioxidative machinery [55, 56], it is thought that the pancreas may be more susceptible to oxidative stress than other tissues and organs. Several conditions are known to disturb the balance between ROS production and cellular defense mechanisms.

The elevated cytotoxic and highly reactive oxidative stress markers such as lipid peroxides causes oxidative damage to proteins and DNA and the reduced cellular nonenzymatic and enzymatic antioxidant levels in diabetic conditions further increases the severity of tissue dysfunction resulting in decreased insulin synthesis, secretion, and finally resulting in β cell death.

In the present study, the elevated levels of lipid peroxides in plasma and pancreatic tissues of diabetic rats were significantly altered upon oral administration of the leaves extract which demonstrates the anti-lipid peroxidative property of the leaves extract.

Furthermore, the levels of activities of enzymatic antioxidants such as SOD, CAT, GPx, and GST were significantly improved in extract treated diabetic rats. Also, the plasma levels of nonenzymatic antioxidants such as vitamin C, vitamin E, reduced glutathione, and ceruloplasmin are found to be increased. The observed improvement in the antioxidant status reflects the antioxidant property of the leaves extract.

CONCLUSION: The results of the present study shows that *Sesbania grandiflora* leaves extract possess antidiabetic and antioxidant nature. Phytochemical screening indicated the presence of pharmacologically active ingredients in the leaves. The improved glycemic control is evident from the results of OGTT. The improvement in body weight gain indicates the beneficial effect of the leaves in controlling muscle wasting. The leaves extract significantly normalizes the biochemical alterations that occurred during diabetic mellitus.

The normalization in the activities of pathophysiological enzymes indicates the non-toxic nature of the leaves extract. The improved enzymatic and non-enzymatic antioxidant status indicates the antioxidant property of the leaves extract. In conclusion, the observed antidiabetic and antioxidant property could be due to the presence of biologically active ingredients in the leaves. Thus, the study provides a scientific rationale for the use of Sesbania *grandiflora* leaves in the traditional system of medicine. Extraction, isolation and identification of active ingredients from the leaves may provide valuable lead molecules with wide range of medicinal values.

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