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EFFECT OF THE AQUEOUS EXTRACT OF SESBANIA GRANDIFLORA LINN IN ALLOXAN INDUCED DIABETES IN ALBINO RATS

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ABSTRACT

Diabetes mellitus is the syndrome of disturbed energy homeostatis. It has become a major public health challenge in all over the world. The present study was designed to evaluate the antidiabetic potential of aqueous extract of *Sesbania grandiflora* Linn. Wistar strain of albino rats were divided into six groups of six rats each. The groups were Group I (Normal control), Group II (Alloxan induced diabetic control (150 mg/ kg.b.wt)), Group III (Alloxan + *Sesbania grandiflora* Linn (500 mg/ kg.b.wt), Group IV (Alloxan + *Sesbania grandiflora* Linn (750 mg/ kg/b.wt), Group V (Alloxan + Glibenclamide (200 mg/ kg.b.wt), Group VI (plant treated (750 kg/b.wt)) respectively. After the experimental period of 45 days, the blood and tissue samples were collected and used for analyzing various biochemical and enzymatic parameters such as plasma glucose, serum insulin, glcosylated hemoglobin, hepatic glycogen, glucokinase, glucose-6-phosphatase, serum marker enzymes (AST, ALT and ALP). Group II rats showed significant increase in the level of plasma glucose, glycosylated hemoglobin, glucose-6-phosphatase and serum marker enzymes (AST, ALT and ALP) and decrease in insulin, hepatic glycogen, glucokinase. Oral administration of *Sesbania grandiflora* Linn aqueous extract to diabetic rats restored all the biochemical parameters and a serum marker enzyme level which depicts the promising antidiabetic effect of *Sesbania grandiflora* Linn.

KEYWORDS: *Sesbania grandiflora* Linn, Diabetes, Alloxan, glucokinase, glucose-6-phosphatase.

INTRODUCTION

Diabetes is a chronic metabolic disorder that is characterised by either the insufficient production or the lack of response to a key regulatory hormone of metabolism - insulin. Diabetes has been correctly labelled as the "Silent epidemic" as its non-dramatic insidious and chronic nature often masks the menace inflicted by the disease. The incapacitation, negative impact and quality of life of patients is spread over years coping with the life-changing troubles. [1] Almost 1.3% of the population suffers from this disease throughout the world and the number is increasing by 6% per year. Approximately 300,000 deaths each year are attributed to diabetes. The prevalence of diabetes for all age groups worldwide was estimated to be 2.8% in 2000 and is projected to rise to 4.4% in 2030. [2] Insulin is the principal hormone that regulates uptake of glucose into most cells from the blood. The cells poorly respond to effects of insulin when its supply is low. It leads to high blood glucose and metabolic disorders. The deficiency on insulin can be experimentally induced. Alloxan is an oxygenated pyrimidine derivative and a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus (called "Alloxan diabetes) in these animals with characteristics similar to type I diabetes in humans. Alloxan may exert its diabetogenic effect by damaging the β-cell membrane, thereby increasing its permeability. Its destructive effect on the β-cells of the pancreas and causes a massive reduction in insulin release by the destruction of β-cell of the islets of langerhans, thereby inducing hyperglycemia. [3] Traditional medicines derived from plants are used by about 60% of the world population. There has been an exponential growth in the field of herbal medicine and herbal drugs are gaining popularity both in developing and developed countries because of their natural origin and less side effects. [4] *Sesbania grandiflora* Linn commononly called agathi, is a fast growing tree with a typical adult height of between 3 and 5 m. Leaves are aperients, tonic, diuretic and laxative. The juice of the leaves as also that of the flowers is used in nasal catarrh and headache. A poultice made from leaf juice is applied to bruises. Seeds are used as emmenagogue. The juice of the root is given with honey as an expectorant. A paste of equal quantities of roots of red flowered agathi and stramonium is applied to painful swellings. A paste of the roots in water is applied in rheumatism. Agathi bark is astringmant, bitter and tonic and a febrifuge. A decoction of the bark in small doses is taken

against diarrohea and dysentery. Pounded bark is externally applied to cure scabies. In ayurvedic medicine the leaves are utilized for the treatment of epileptic fits and clinical research supports the anticonvulsive activity of agathi leaves. [5] The present study was designed to investigate the antidiabetic efficacy of aqueous extracts of *Sesbania grandiflora* Linn in alloxan induced diabetic rats.

MATERIALS AND METHODS

Identification and Authentication

Plant source selected for the present study was *Sesbania grandiflora* Linn (leaves). Plant was collected from Trichy, identified with the help of Flora of Presidency of Madras and authenticated with the specimen deposited at RAPINAT Herbarium, Department of Botany, St. Joseph's College, Trichy.

Preparation of the Extract

The plant material was shade dried and coarsely powdered with electrical blender. 200 gm of the plant powder was mixed with 1200 ml of water. Then it was boiled until it was reduced to one third and filtered. The filtrate was evaporated to dryness. The extract obtained was subjected to pre-clinical screening.

Experimental Animals

Healthy adult wistar strains of albino rats of both sexes, two to three months old, weighing 150-200 gm were used as experimental models for the present study. The animals were allowed to acclimatize to laboratory conditions for a period of 10 days prior to the experiment. Animals were housed in standard condition of 12 hours light/dark cycle and at an ambient temperature at 23 ± 2ºC with 65 ± 5% humidity. Animals were fed with standard rat chew pellet and water *ad libitum*.

Induction of Diabetes in Rats

Diabetes mellitus was induced in normoglycemic albino rats starved for 16 hours. 150mg/kg body weight of alloxan monohydrate was dissolved in physiological saline and injected intraperitoneally. [6] This dose of alloxan produced persistent hyperglycemia after 4 days as revealed by determination of urine sugar by BQR method. The diabetes induced rats were chosen and grouped for further studies. The parameters studied were plasma Glucose [7], Serum insulin [8], Glycosylated Hb [9], Hepatic glycogen [10], Glucokinase [11], Glucose-6-phosphatase [11], serum marker enzymes (AST, ALT and ALP) [12]

Experimental Design

Animals were divided into six groups of six rats (both sex) each. The experimental design given below has been followed for the present study. Group I served as normal control and was given normal saline only. Group II was maintained as disease control. Diabetes was induced by an intraperitoneal injection of alloxan at a dose of 150 mg/ kg bw as a single dose. Group III & IV were induced with alloxan and treated with aqueous extract of *Sesbania grandiflora* Linn at a dose of 500 & 750 mg/kg b.wt respectively for 45 days orally. Group V was induced with alloxan and treated with a standard drug glibenclamide at a dose of 200 mg/kg b.wt for 45 days orally. Group VI received the aqueous extract of *Sesbania grandiflora* Linn only at a dose of 750 mg/kg b.wt respectively for 45 days orally. After the experimental period animals were sacrificed by cervical decapitation. Blood was collected and serum was separated by centrifuge at 3000 rpm for 10 minutes. Liver was dissected out and washed in ice-cold saline. Liver tissue was homogenized in 0.1 M phosphate buffer, pH 7.4 and used for studying various parameters.

Statistical Analysis

All the results were expressed as mean ± S.E. The data were statistically analyzed by one-way analysis of variance (ANOVA) and p values < 0.05 were considered as significant.

RESULTS

Table 1 shows the level of blood glucose and serum insulin of the experimental models. It shows significant increase in the blood glucose and low level of serum insulin in alloxan induced rats when compared to normal groups. Oral administration of aqueous extracts of *Sesbania grandiflora* Linn showed marked decrease in blood glucose level with subsequent increase in serum insulin which was comparable to the glibenclamide treated group.

Table 1: Estimation of Serum Glucose and Insulin in Experimental Models.

GROUPS	I	II	III	IV	V	VI
Glucose (mg/dl)	86.3±1.01	248.21±0.66*	129.51±0.86	108.47± 0.78	87.51±1.03**	81.79±1.01**
Insulin (µIU/ml)	3.34± 0.08	1.10* ± 0.96	2.17 ± 0.54	6.30**± 1.03	4.12± 0.84	3.70±0.91

Values are mean ± SEM (n=6),

* *p<0.05 statistically significant when compared with normal control*

** *p<0.05 statistically significant when compared with Alloxan group*

Table 2 shows the level of glycosylated Hb and hepatic glycogen in the experimental animals. The group II animals show an elevated level of glycosylated Hb and low level of hepatic glycogen. Diabetic rats treated with aqueous extract exhibited decreased glycosylated Hb level with significant increase in hepatic glycogen.

Table 2: Estimation of Glycosylated Hemoglobin and Hepatic Glycogen in the Animals

GROUPS	I	II	III	IV	V	VI
Glycosylated hemoglobin (%)	3.91±1.04	8.4±0.98*	4.5±1.23	3.7±0.96**	5.42±1.08	3.69±0.67
Hepatic glycogen(mg/g)	38.31±0.85	11.71±0.42*	24.52±0.31	32.44±0.73**	39.50±0.91**	30.86±1.13**

Values are mean ± SEM (n=6),

* *p<0.05 statistically significant when compared with normal control*

** *p<0.05 statistically significant when compared with Alloxan group*

The activity of glucokinase, glucose-6-phosphatase, Aspartate transaminase (AST), Alanine transaminase (ALT) and alkaline phosphatase (ALP) in experimental animals are depicted in Table 3. The disease control animals showed an elevation in the activity of glucose-6-phosphatase, Aspartate transaminase (AST), Alanine transaminase (ALT) and alkaline phosphatase (ALP) which was reversed to near normal in Group III & group IV rats.

Table 3: Assay of glucose metabolizing and serum marker enzymes in the animal models

GROUPS	I	II	III	IV	V	VI
Glucokinase (µmol/mg protein)	135± 4.2	98.92± 1.89*	103.4± 2.78	130.2± 3.12**	132± 3.67**	140± 3.21
Glucose-6-phosphatase (u/mg of protein)	7.7± 0.67	18.92± 0.77*	9.24± 1.11**	8.01± 0.44**	9.1± 1.08**	7.1± 1.15**
AST(IU/L)	7.12± 0.28	94.34± 2.41*	28.33± 0.64**	11.32± 0.96**	15.45± 1.23**	10.71± 0.54
ALT(IU/L)	12.4± 0.29	109.91± 1.82*	42.46± 1.38**	16.76± 1.21**	18.95± 1.13**	14.51± 0.12**
ALP(IU/L)	9.94± 0.96	83.59± 0.87*	34.97± 2.01**	13.58± 0.74**	15.82± 0.95**	11.23± 0.54

Values are mean ± SEM (n=6),

* *p<0.05 statistically significant when compared with normal control*

** *p<0.05 statistically significant when compared with Alloxan group*

DISCUSSION

Diabetes mellitus is the syndrome of disturbed energy homeostatis, caused by an abnormal metabolism of carbohydrates, protein and fats. Alloxan, a betacytotoxin causes a massive

reduction in insulin release by the destruction of β cells of the islets of langerhans and thereby induce hyperglycemia. [13] Excessive amount of glucose in the blood induces peripheral glucose consumption and control the production of glucose through other mechanisms. Insulin deficiency occurs in alloxan induced diabetic rats leading to alterations in the carbohydrate metabolism such as elevated blood glucose and reduced level of insulin. [14] The possible hypoglycemic mechanism of *Sesbania grandiflora* Linn may be through potentiation of pancreatic secretion of insulin from β cells of islets or due to enhanced transport of blood glucose to the peripheral tissue.

Glycosylated hemoglobin is produced through the glycosylation of hemoglobin. It is formed progressively and irreversibly over a period of time and is stable till the life of the RBC and is unaffected by diet, insulin or exercise on the day of testing. Excess glucose present in the blood reacts with hemoglobin and form glycosylated hemoglobin. Therefore, Glyosylated hemoglobin can be used as an excellent marker and standard diagnostic for estimating the degree of protein glycation during diabetes mellitus. Protein glycation is a non enzymatic reaction between excess glucose present in the blood and free amino groups on the globin component of hemoglobin. 15] The glycosylated hemoglobin was found to be increased in diabetes mellitus and the amount of increase is directly proportional to that of fasting blood glucose level. *Sesbania grandiflora* Linn plant extract significantly decreased the glycosylated hemoglobin level which reflects in the decreased protein glycation.

The regulation of glycogen metabolism occurs by the multifunctional enzyme glycogen synthase and glycogen phosphorylase that play a major role in the glycogen metabolism. [16] The reduced glycogen store in diabetic rats has been attributed to reduced activity of glycogen synthase and increased activity of glycogen phosphorylase. Administration of alloxan induces insulin deficiency which in turn leads to enhanced glycogenolysis and depletion of glycogen content. In the present study the experimental diabetic rats the level of hepatic glycogen was restored on treatment with *Sesbania grandiflora* Linn which may be due to the activation of glycogen synthase and inhibition of glycogen phosphorylase. Glucokinase is the prime enzyme catalyzing glucose phosphorylation. Impairment of glucokinase activity suggests impaired oxidation of glucose via glycolysis leading to its accumulation resulting in hyperglycemia. Insulin increases hepatic glycolysis by increasing the activity and amount of key enzyme glucokinase. Glucokinase is universally present in cells of all types. Glucokinase catalyses the conversion of glucose to glucose-6-phosphate

and plays a central role in the maintenance of glucose homeostatis.[17]Glucose-6-phosphatse is one of the important regulatory enzymes in gluconeogenesis. In diabetic animals, the enzyme levels were observed to increase. The increased activities of glucose-6-phosphatase in liver of the alloxan induced diabetic rats may be due to insulin insuffiency. Hence, the hepatic tissues were incapable of utilizing peripheral glucose and increase the synthesis of glucose-6-pjhosphatase to enhance gluconeogenesis. [18]

Decreased glucokinase and increased glucose-6-phosphatase activity was observed in diabetic rats which may be due to insulin deficiency. Treatment with *Sesbania graniflora* Linn extract restored the activity of both glucokinase and glucose-6-phosphatase.The serum AST and ALT levels increase as a result of metabolic changes in the liver such as administration of toxin, cirrhosis of the liver, hepatitis and liver cancer including diabetes. Similarly in the present study, it was observed that the level of serum AST and ALT in alloxan induced diabetic rats were elevated. It may be due to leaking out of enzymes from the tissues and migrating into the circulation by the adverse effect of alloxan. [19] The measurement of enzyme activity of alkaline phosphatase is of clinical and toxicological importance and changes in their activities are indicative of tissue damage by toxicants. [20]In this study, the serum ALP increased considerably in alloxan induced diabetic rats. Elevated level of this enzyme in diabetic control may be due to extensive damage to liver in the experimental animals by alloxan. Administration of plant extract to alloxan induced diabetic rats reduced the levels of serum marker enzymes AST, ALT and ALP, which indicates that the plant extract protects the liver from the adverse effects of alloxan.

CONCLUSION

Studies revealed that aqueous leaf extract of *Sesbania grandiflora* Linn can be considered as an important addition to the therapeutic armamentarium for the treatment of diabetes. Further studies can be undertaken at the cellular and molecular level, which may further elucidate its mechanism in detail.

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