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Research Article

**EVALUATION OF RENAL PROTECTIVE EFFECTS OF *DESMODIUM GANGETICUM L.*
IN STREPTOZOTOCIN - INDUCED DIABETIC RATS****Nusrath Yasmeen^{1*}, Rajkiran Ellandala¹, K. Sujatha² and R. Veenavamshee³**¹ Department of Pharmacology, Care College of Pharmacy, Warangal, Andhra Pradesh, India.² Department of Pharmacognosy, Care College of Pharmacy, Warangal, Andhra Pradesh, India.³ Department of Pharmacology, Shadaan College of Pharmacy, Hyderabad, Andhra Pradesh, India.*Corresponding Author: nishu_yasmeen@yahoo.com**ABSTRACT**

Desmodium gangeticum L. (Family: Fabaceae) is an erect, semi-woody herb widely used in Ayurveda for the treatment of various disorders. Animals allocated into six groups of six animals per each. The treatment of Streptozotocin(65mg/kg) induced diabetic rats with Whole Plant extract of *Desmodium gangeticum (DGE) L.*(100, 200 and 400mg/kg body weight) for 30days showed the decreased levels of LipidPeroxidation markers such as Malondialdehyde (MDA) . The activities of enzymatic antioxidants SuperoxideDismutase(SOD), Catalase(CAT), Glutathione Peroxidase(GPx) and levels of non-enzymatic antioxidants reduced Glutathione(GSH) increased in diabetic treated rats. And Blood Urea Nitrogen (BUN), Serum Creatinine & Urea levels were estimated. The results of this study showed that Ethanollic DGE has antidiabetic activity along with that of antioxidant activity and renal protective effects.

Keywords: Diabetes Mellitus, Lipid peroxidation, Antioxidant markers, renal markers.**INTRODUCTION**

Diabetes mellitus(DM) is a multi-factorial disease which is characterized by hyperglycemia, lipoprotein abnormalities, raised basal metabolic rate, defect in reactive oxygen species scavenging enzymes and altered intermediary metabolism of major food substances. Diabetes is a major degenerative disease in the world today, affecting at least 15 million people and having complications which include hypertension, atherosclerosis and microcirculatory disorders¹.

Antioxidants play an important role in protecting the human body against damage by reactive oxygen species. Increased oxidative stress has been postulated in the diabetic state. Oxidative stress in diabetes coexists with a reduction in the antioxidant status, which can increase the deleterious effects of free radicals². In diabetes, oxidative stress seems caused by both increased production of ROS,

sharp reduction in antioxidant defenses and altered cellular redox status.

Hyperglycemia may lead to an increased generation of free radicals via multiple mechanisms. Patients with diabetes may be especially prone to acute and chronic oxidative stress which enhances the development of late diabetic complications. Although the source of this oxidative stress remains unclear, it has been suggested that the chronic hyperglycemia in diabetes enhances the production of ROS from glucose auto-oxidation, protein glycation and glycooxidation, which leads to tissue damage. Also, cumulative episodes of acute hyperglycemia (fasting or postprandial hyperglycemia) can be source of acute oxidative stress³.

Elevated generation of free radicals resulting in the consumption of antioxidant defense components may lead to disruption of cellular

functions and oxidative damage to membranes and may enhance susceptibility to lipid peroxidation. Under physiological conditions, a widespread antioxidant defense system protects the body against the adverse effects of free radical production. The efficiency of this defense mechanism is altered in diabetes and, therefore, the ineffective scavenging of free radicals may play a crucial role in determining tissue damage.

Diabetes is becoming the third "KILLER" of mankind, after cancer and cardiovascular diseases because of its high prevalence, morbidity and mortality⁴.

For this cogent resource, therefore, there is a great need to search for an acceptable, cheap and safe blood glucose lowering oral hypoglycemic agents that would be effective in treatment of diabetes and devoid of serious side effects, interest has thus been shifted to use of other alternative medicine⁵.

Traditional medicines and extracts from medicinal plants have been extensively used as alternative medicine for better control and management of Diabetes Mellitus. Medicinal plants are continued to be a powerful source for new drugs, now contributing about 90% of the newly discovered pharmaceuticals. Traditional medicines provide better health coverage for 80% of the world population, especially in the developing countries⁶.

Desmodium gangeticum L. is an erect, semi-woody herb or under shrub up to 2m high of galleried forest and old clearings and on damp sites. It is found in tropical and sub-tropical climatic conditions. It belongs to family Fabaceae⁷.

As the evidence of earlier studies shows that the whole plant of *Desmodium gangeticum* L. possesses flavonoids, tannins, glycosides which are the major chemical constituents responsible for exhibiting antioxidant activity, the present study has been undertaken to evaluate the protective effects of ethanolic extract of whole plant of *Desmodium gangeticum* L. in kidney tissues in streptozotocin induced oxidative stress.

MATERIALS AND METHODS

Collection of drug: The plant *Desmodium gangeticum* was collected from the forest area of Chittoor district in AP (India) during the month of October. Botanist of Sri. Venkateshwara University, Tirupathi, authenticated the plant.

Drugs and chemicals

All the drugs and chemicals were obtained from SigmaChemical Co. (St. Louis,MO,USA). All solvents were of analytical grade and were obtained from Sd. Fine Chemicals, Mumbai,India.

Preparation of the DGE

Dried and powdered whole plant material of *Desmodium gangeticum* was purchased from a commercial source (Madhavchetty). The powdered material was soaked with 70% ethanol overnight in Soxhlet thimble. The residue in the R.B flask was transferred into a beaker and was concentrated under reduced vacuum pressure to give an average yield of 70% (w/w). Solutions of the *Desmodium gangeticum* extract (DGE) were prepared freshly for the pharmacological studies.

Animals

The male wistar albino rats (150-200gms) were procured from Shadan Animal Husbandary and from Sai Animal Distributors, Musheerabad. The animals were acclimatized for 1week. They were fed with commercial pelleted rats chow and were given free access to water ad libitum throughout the study. The animals were handled gently to avoid giving them too much stress, which could result in an increased adrenal output.

Induction of Diabetes

Diabetes was induced by administering intraperitoneal injection of a freshly prepared solution of Streptozotocin (65mg/kg b.w) in 0.1M cold citrate buffer (PH4.5) to the overnight fasted rats. Since Streptozotocin is capable of producing fatal hypoglycemia as a result of massive pancreatic release of insulin, the rats were kept on 5% glucose for next 24hrs to prevent hypoglycemia⁸⁻⁹.

Experimental Design

Forty rats were used and were classified into 6 groups (6 animals/ group; n=6).

Untreated:

- Group I : Normal

STZ treated groups

- Group II: Diabetic (Positive) control
- Group III: DGE 100mg/kg b.w
- Group IV: DGE 200mg/kg b.w
- Group V: DGE 400mg/kg b.w
- Group VI: Glibenclamide treated (600µg/kg b.w)

Out of forty rats, six were retained as normal group and remaining 34 animals were induced diabetes using STZ (65mg/kg b.w) through i.p. Initial blood glucose levels were estimated followed by estimations on 3rd, 5th and 7th day. After a period of one week i.e., on seventh day the rats with blood glucose levels >280mg/dl were considered diabetic and used for research work.

The three different doses of drug were suspended with 2% Gum Acacia and given to the Group III to V. The above treatment was given for thirty days. At the end of the treatment i.e., after 30 days the animals were fasted overnight. Blood was taken from the retro-orbital plexus under mild chloroform anesthesia. Blood sugar level was evaluated by the method of Glucose Oxidase Peroxidase method. Serum was then separated from blood and was used for estimation of Plasma renal marker enzyme levels.

The animals were then sacrificed by cervical dislocation. The tissues (kidney) were removed and cleared off blood, rinsed in ice-cold physiological saline. Small portions of tissue were fixed in 10% neutral Formalin solution for histology. The remaining tissue was homogenized by tissue homogenizer with Teflon pestle at 4°C in 0.1M Tris-HCl buffer at pH7.4. The homogenate of kidney were centrifuged in a cooling centrifuge (500 X g) separately to remove the debris and supernatant was kept at -20°C and used for biochemical analysis.

BIOCHEMICAL ESTIMATION

Determination of Blood Glucose levels

Blood glucose levels were checked at weekly intervals during the duration of the experiment. Blood samples for blood glucose determination were collected from tail tip at intervals of 7, 14, 21, 28 days. The blood glucose levels were detected by Glucose – Oxidase Principle (*Beach and Turner*). And results were reported as mg/dl¹⁰.

Estimation of Serum Urea, Creatinine and BUN

The plasma levels of Urea estimated by using the method of Berthelot using EndPoint Assay technique¹¹. Creatinine was estimated by using Modified Jaffe's reaction by initial Rate Assay Technique¹². Blood Urea Nitrogen was estimated by method of Berthelot using Endpoint Assay technique¹³.

Estimation of Antioxidant Markers

A. Malondialdehyde (MDA) levels

The activity of MDA was estimated by Okhawa et al.,¹⁴ with minor modification TBARS was estimated by reaction with thiobarbituric acid in the presence of butylated hydroxytoluene and measuring the absorbance at 535 nm of the pink coloured chromogen formed against reagent blank. MDA levels were expressed as mmol of MDA/mg protein or nm/100gm tissue.

B. Superoxide Dismutase levels (SOD)

The activity of Superoxide Dismutase was estimated by the method of Mc Cord and Fridvoich¹⁵. These are metalloenzymes, the amount of SOD present in cellular and extracellular environments are crucial for the prevention of diseases linked to oxidative stress. Absorbance of the blue color formed were measured again. Percent of inhibition was calculated after comparing absorbance of sample with the absorbance of control (the tube containing no enzyme activity). The volume of the sample required to scavenge 50% of the generated superoxide cation was considered as 1 unit of enzyme activity and expressed in U/protein.

C. Estimation of Catalase (CAT)

The activity of Catalase was estimated by the method of Aebi. H¹⁶. Catalase activity was measured by using the rate of decomposition of H₂O₂ and the values are expressed in nmol H₂O₂ consumed U/mg protein.

D. Estimation of Reduced Glutathione (GSH)

The activity of Reduced Glutathione was estimated by the method of Ellman GL¹⁷. Total reduced glutathione was measured by the use of DTNB assay using Elman's method wherein the incubation mixture at 37 °C contained 0.08M sodium phosphate (pH7.0), 0.08M EDTA, 1.0mM sodium azide, 0.4nM GSH and 0.25mM H₂O₂. GSH was determined at 3-min intervals using DTNB and is expressed as n.mol/mg protein.

E. Estimation of Glutathione Peroxidase (GPx)

The activity of Glutathione Peroxidase was estimated by the method of Paglia and Valentine¹⁸. GPx, an enzyme with Selenium, works with Glutathione in the decomposition

of H₂O₂ or other organic hydroperoxides to non-toxic products at the expense of GSH. Reduced activities of GPx may result from radical induced inactivation and glycation of enzymes.

One unit of enzyme activity was defined as decrease in GSH 0.001/mm after subtraction of the decrease GSH per minute for the non-enzymatic reaction and is expressed as units/mg protein.

Statistical analysis

Values were represented as Mean \pm S.E.M. and data was analyzed by ANOVA followed by Dunnet's test using WINKS STAT SOFTWARE.

RESULTS

The present study was designed to evaluate the renal protective activity of ethanolic extract of whole plant of "*Desmodium gangeticum* L." (DGE) in Streptozotocin (at dose of 65mg/kg) induced diabetic rats. In this study the various antioxidant parameters, FBG levels, plasma renal marker levels were used for the assessment of renal protective activity of "*Desmodium gangeticum* L.".

The effect of the extract on fasting blood glucose levels was observed, using STZ at dose of 65 mg/kg bodyweight. After one week, it was observed that there was marked elevation in fasting blood glucose levels. The diabetic rats with FBG>280mg/dl were selected for further studies.

The blood glucose level in diabetic control group significantly increased and was higher than those of the control group. On the other hand, oral administration of ethanolic extract of whole plant of "*Desmodium gangeticum*" for 30 days was found to lower the blood glucose levels significantly in a dose dependent manner in treated diabetic groups when compared with those of the diabetic group.

Oral administration of "*Desmodium gangeticum*" for 30 days showed significant reduction in MDA levels (1.71 \pm 0.97, 1.63 \pm 0.82, 1.47 \pm 0.60), and significant increase in levels of GSH(62.32 \pm 2.89, 71.23 \pm 3.14, 91.33 \pm 2.89), Catalase(22.37 \pm 1.26, 34.65 \pm 1.20, 35.24 \pm 1.81), SOD(10.22 \pm 2.11, 16.37 \pm 2.40, 18.91 \pm 1.21), GPx (5.22 \pm .44, 5.54 \pm 0.21,5.97 \pm 0.76) at doses of 100, 200 and 400 mg/kg respectively (Table 1-3 and Graph I).

Light Microscopy study of KIDNEY

Light microscopy of kidney sections of diabetic rats showed degenerations in proximal tubules epithelial cells in the cortex of kidneys, hemorrhage in the interstitial area and periglomerular lympholytic infiltration and hyalinization of the arterioles.

Examination of kidneys of the diabetic rats treated with whole plant of extract DGE indicated that the kidneys appeared more or less as control. Fig 1: (A-F)

DISCUSSION

DM is probably the fastest growing metabolic disease in the world and as the knowledge of multifactorial or heterogeneous nature of the disease increases so does the need for more challenging and appropriate therapies^[19]. Traditional plant remedies have been used for centuries in the treatment of diabetes but only a few have been scientifically evaluated. Therefore the present study was done to investigate the effect of ethanolic extract of whole plant of *Desmodium gangeticum* on biomarkers of oxidative stress, and renal markers in Diabetic rats²⁰.

Oxidative Stress in diabetes co-exists with the decrease in antioxidant status which can increase the deleterious effects of free radicals .Evidence has accumulated indicating that the generation of reactive oxygen species (Oxidative stress) may play an important role in the etiology of diabetic complications²¹.

Glibenclamide is a standard antidiabetic drug used. It has been involved in stimulating insulin secretion from pancreatic β -cells principally by inhibiting ATP sensitive K_{ATP} channels in the plasma membrane.

Antioxidant enzymes, Super oxide dismutase, Catalase, Reduced Glutathione and Glutathione peroxidase form the first hue of defense against ROS and decreased activities were observed in the STZ diabetic rats.

In this study, we have found that poor glycemic control in diabetic patients was also associated with decreased free radical scavenging activity. In hyperglycemia, glucose undergoes auto-oxidation and Produces free radicals that in turn lead to peroxidation of lipids in lipoproteins. Elevated Levels of lipid peroxidation, as seen in diabetic patients are clear manifestation of excessive formation of free radicals resulting in tissue damage^{22,23}. The activity of super oxide dismutase was found to be lower in diabetic patients when

compared to normal. This decrease in activity could result from activation of the enzyme by H_2O_2 or by glycation of the enzyme, which are known to occur during diabetes. Super oxide dismutase scavenges superoxide anion to form H_2O_2 and diminishes the toxic effects derived from secondary reaction. The activity of Super oxide dismutase was found to be lowered in diabetic controlled rats.

Catalase is a haeme protein, which catalyses the reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals. This decrease in Catalase activity could result from inactivation by glycation of the enzyme²⁴. The increase in SOD activity may indirectly play an important protective role in preserving the activity of Catalase. The reduced activities of SOD and CAT in kidney have been observed during diabetes.

Glutathione peroxidase, an enzyme with selenium, works with Glutathione in the decomposition of H_2O_2 or other organic hydroperoxides to non-toxic products at the expense of GSH. Reduced activities of Glutathione peroxidase may result from radical induced inactivation and glycation of enzymes. Further, insufficient availability of GSH may also reduce the activity of GPx. Reduced activities of GPx in kidney have been observed during diabetes and this may result in a number of deleterious effects due to accumulation of toxic products.

Increased activities of SOD, CAT and GPx after treatment with *Desmodium gangeticum* extract may be due to the presence of flavonoids and isoflavonoids, an antioxidant.

Glutathione is a tripeptide normally present at high concentrations intracellularly, and constitutes the major reducing capacity of cytoplasm. Decreased level of GSH in kidney during diabetes represents its increased utilization due to oxidative stress²⁵. 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). Reduced availability of NADPH may be due to decrease in the activity of glucose 6-phosphate dehydrogenase and hence, decreased level of GSH. Administration of *Desmodium gangeticum* 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 *Desmodium gangeticum*, which may, in turn, maintain the antioxidant status in the tissues of diabetic rats. This indicates that the extract can reduce the oxidative stress leading to less degradation of GSH, or have both effects.

The increases of serum Creatinine, Urea levels are considered as obvious indicators for kidney damage and dysfunction. The diabetic hyperglycemia induces elevations of blood levels of creatinine urea which are considered as significant markers of renal dysfunction.

In our study, it was observed that the levels of antioxidant enzymes (SOD, CAT, GPx, and GSH) and the lipid peroxidation levels were decreased in kidney of diabetic rats. The plasma renal markers also showed changes in levels. In diabetic rats treated with ethanolic extract of *Desmodium gangeticum* (DGE), a significant increase in activity of these enzymes was observed, that is the levels were brought back to normal by DGE, indicate oxidative stress elicited by STZ had been nullified due to the effect of the extract. This might reflect the antioxidant potency of ethanolic extract, which by reducing blood glucose levels prevented glycation and inactivation of enzymes.

CONCLUSION

Present study was conducted to evaluate the renal protective effects of Ethanolic extract of whole plant of *Desmodium gangeticum* (DGE) in Streptozotocin induced diabetic rats. Glibenclamide was used as the standard drug. Improvement in the Renal marker enzymes levels by *Desmodium gangeticum* reveals that the plant has Renal protective potential with anti-diabetic activity. The ability of *Desmodium gangeticum* to reduce oxidative stress may help to prevent diabetic complications. The whole plant of *Desmodium gangeticum* was found to have Alkaloids, Flavonoids, Isoflavonoids. And probably the antioxidant and antidiabetic effect of ethanolic extract of whole plant of *Desmodium gangeticum* could be due to the presence of Flavonoids, Isoflavonoids and Alkaloids.

Further studies should be undertaken to identify the active hypoglycemic compounds and investigate the mechanism of hypoglycemia and anti-oxidant actions of whole plant of *Desmodium gangeticum*.

Table 1: Effect of oral administration of ethanolic extract of D.G on FBG levels for 30 days

Sample	1 st day	7 th day	15 th day	21 st day	28 th day
Group I	79.77±1.72	74.23±3.64	78.68±5.27	75.66±2.46	76.12±1.66
Group II	295.27±3.44	308.91±2.53	312.46±3.16	313.71±3.11	319.34±3.67
Group III	215.15±2.98	198.01±3.44	155.66±4.23	122.01±2.98	99.98±2.11*
Group IV	210.15±2.86	167.48±2.97	127.11±3.09*	108.47±2.77*	89.67±3.43
Group V	210.65±1.43	145.76±5.11	106.24±3.00*	99.45±3.11**	79.42±2.06*
Group VI	201.03±4.36	140.37±4.61	99.56±2.76**	89.67±2.87**	78.34±2.66**

Values are expressed as Mean ± SEM values for six rats in each group. Diabetic control rats were compared with normal control rats. Diabetic + "Desmodium gangeticum" and Diabetic + Glibenclamide treated were compared with diabetic controlled rats. P<0.05, *P<0.01, **P<0.001 with respect to the Diabetic Control Group (ANOVA with Dunnet's t-test)

Table 2: Effect of ethanolic extract of whole plant of Desmodium gangeticum on MDA, GSH, Catalase, SOD, GPx levels in Kidney

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
MDA ^a	1.33 ± 0.09	2.34 ± 1.16	1.71 ± 0.97*	1.63 ± 0.82*	1.47 ± 0.60***	1.43 ± 0.71***
GSH ^b	118.77 ± 2.31	46.17 ± 2.47	62.32 ± 2.89 *	71.23 ± 3.14 *	91.33 ± 2.89 **	95.17 ± 2.55**
CAT ^c	38.33 ± 1.22	20.55 ± 1.75	27.37 ± 1.26*	31.65 ± 1.20 *	35.24 ± 1.81**	37.57 ± 1.41 **
SOD ^d	20.61 ± 1.21	8.95 ± 0.18	10.22 ± 2.11*	16.37 ± 2.40 **	18.91 ± 1.21 ***	19.42 ± 1.46***
GPX ^e	7.33 ± 0.14	4.99 ± 1.01	5.22 ± 0.44 *	5.54 ± 0.21*	5.97 ± 0.76**	6.97 ± 0.58 ***

Values are expressed as Mean ± SEM values for Six rats in each group. Diabetic control rats were compared with normal control rats. Diabetic + Desmodium gangeticum and Diabetic + Glibenclamide treated were compared with diabetic controlled rats.

*P<0.05, *P<0.01, ***P<0.001 with respect to the Diabetic Control Group(ANOVA with Dunnet's t-test).

^aMDA= nM/100g tissue;

^bGSH= nM of DTNB conjugated/mg protein ;

^cCAT= U/mg protein;

^dSOD= U/mg protein;

^eGPX= U/min/mg protein

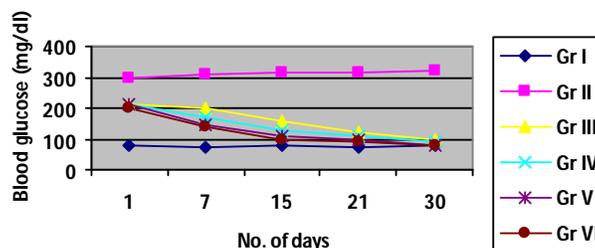
Table 3: Effect of ethanolic extract of whole plant of Desmodium gangeticum on Serum Urea and Serum Creatinine levels

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
Serum Urea(mg/dl)	29.49 ± 1.59	48.27 ± 2.78	42.36 ± 3.12	39.27 ± 2.72*	33.18 ± 2.10**	31.31 ± 2.65**
Serum Creatinine(mg/dl)	0.73 ± 0.04	1.98 ± 0.05	1.90 ± 0.21	1.68 ± 0.07 *	1.37 ± 0.06**	1.01 ± 0.03***
BUN (mg/dl)	17.98 ± 2.20	31.78 ± 0.97	27.65 ± 0.56	20.68 ± 0.59**	20.68 ± 0.59**	26.57 ± 2.65**

Values are expressed as Mean ± SEM values for Six rats in each group. Diabetic control rats were compared with normal control rats. Diabetic + Desmodium gangeticum and Diabetic + Glibenclamide treated were compared with diabetic controlled rats.

P<0.05, *P<0.01, ***P<0.001 with respect to the Diabetic Control Group(ANOVA with Dunnet's t-test).

Graph I: FBG levels levels



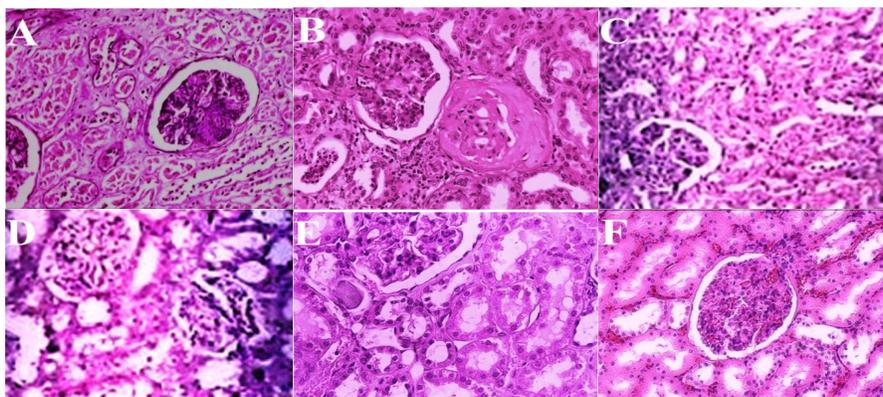


Fig. 1: (A-F): Kidney histological section of Normal(A), DC(B), DGE 100mg/kg(C), DGE 200mg/kg(D), DGE 400mg/kg(E), STD (F)

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