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ROLE OF *SESAMUM ALATUM L.* ON NEPHROPATHY IN DIABETIC RATS

Rajkiran Ellandala*[1], Nusrath Yasmeen [1], T. Shashank [1], K. Sujatha [2], Keerthi Prathapagiri [3]

Department of Pharmacology, Care College of Pharmacy [1], Warangal, Andhra Pradesh, India
Deparment of Pharmacognosy, Care College of Pharmacy [2], Warangal, Andhra Pradesh, India
Department of Medicinal Chemistry, University College of Pharmaceutical Sciences, Kakatiaya University [3], Warangal, Andhra Pradesh, India

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Correspondence to Author:

Rajkiran Ellandala

Department of Pharmacology, Care College of Pharmacy, Ogulapur, Warangal, Andhra Pradesh, India

ABSTRACT

Background: Type 2 Diabetes is a metabolic disorder, characterized by chronic hyperglycemia. It results from insulin resistance, a condition in which the body cells fail to use insulin properly. In Ayurveda *Sesamum alatum L.* (Pedaliaceae) has been used for the treatment of Diabetes. In this study, the antidiabetic activity of the Methanolic leaf extract of S. alatum was investigated, and the renal protective activity of the extract was also evaluated in diabetic rats.

Materials & Methods: Antidiabetic activity of the Methanolic Leaf Extract of *Sesamum alatum* (MESA) was evaluated in the Streptozotocin (65mg/kg) induced Male albino rats. Blood was collected from the Retro orbital plexus of rats' before and after the administration of MESA. Activity of 300 and 500 mg/kg doses of MESA were evaluated for a period of 30days. Urine samples were collected and analyzed for Albumin, BUN, Creatinine and Lipid parameters. In addition, Kidney MDA levels, Antioxidant enzymes (SOD, CAT) and Non-Enzymatic (GSH) levels were measured.

Results: Dose-dependent reduction in blood glucose levels were observed in treated group & it can be comparable with that of Standard drug, Glibenclamide. Significant Renoprotective activity was observed in the MESA treated group. MESA (500mg/kg) has shown the antidiabetic activity by improving the levels of GSH, SOD, CAT and has decreased the levels of MDA in kidney. And the other Blood and urine parameters were normalized in treated groups.

Conclusion: Present study clearly supported significant Antidiabetic Renoprotective activity of *Sesamum alatum*. Further investigation is needed to determine the exact Phytoconstituent(s) which are responsible for the Antidiabetic activity.

INTRODUCTION: Diabetes is a metabolic disorder characterized by Chronic Hyperglycemia, disturbances of carbohydrate, fat, protein metabolism results from defects in Insulin action. Type 2 DM is a major health problem affecting majority of people throughout the world population. Still no modern medicine has reached the satisfactory level in the treatment of non-insulin dependent type II diabetes [1]. Diabetes mellitus includes micro and macro vascular complications [2]. Here oxidative stress plays the key role in the pathogenesis of Nephropathy. It is characterized by the elevated arterial blood pressure, and decreased glomerular filtration rate along with persistent albuminuria [3].

In Ayurveda, diabetes is named as 'Madhumeha', where it is mentioned that a variety of plant extracts can cure the diabetes in which most of them are experimentally evaluated for its activity [4-6]. Traditional medicines are extensively used as medicine in ayurveda for the better control and management of Diabetes mellitus. The WHO also recommends the evaluation of traditional plant extracts for the treatment of diabetes as they have less side effects

and possess better glycemic control over the synthetic medicines.

Sesamum alatum L. belongs to the family of Pedaliaceae. It was traditionally used in the ayurvedic medicine. Sesamum has wider range of activities its seeds (black) are medicinally preferred. Folk medicine explains the renal protective activity of *Sesamum alatum*.

MATERIALS AND METHODS:

Preparation of Plant Extracts (MESA): The plant was identified and authenticated by a botanist from Department of Botany, Kakatiya University, Warangal. The dried powdered leaves (1kg) of *Sesamum alatum* were used for the Methanolic extraction by using Soxhlet's apparatus. The obtained Methanolic leaf extract of *Sesamum alatum* (MESA) was evaporated to dryness by using rotary evaporator pump.

Drugs and Chemicals: All the solvents and chemicals were collected from the Merck industries, Hyderabad. The chemicals used were of analytical grade.

Animals: Male Wistar albino rats weighing 180-210gm were procured from the Mahaveer Enterprises, Hyderabad. They were acclimatized for a week. And free access was allowed to standard diet, and maintained at room temperature, 60±5% relative humidity, light period (12h light/12 h dark), water and *ad libitum.*

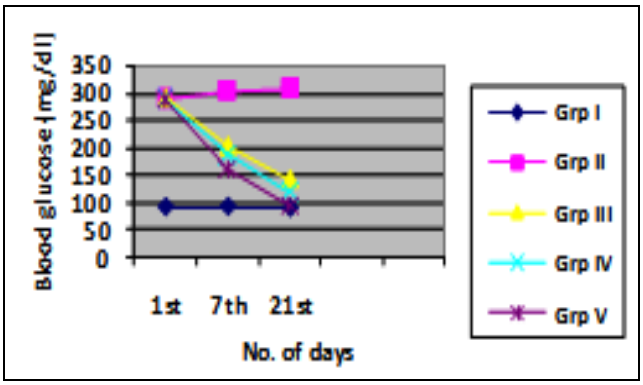
Induction of Diabetes: Rats were made diabetic by intra peritoneal injection of Streptozotocin (dissolved in 0.05 M of Citrate buffer, pH 4.5), 65mg/kg body weight [7]. After a week, rats, which showed blood glucose levels >280 mg/dl were considered as diabetic and were used for further studies.

Experimental design: Thirty rats were divided into 5 groups 6 rats per each group (n=6):

- Group I: Normal (negative control)
- Group II: positive control (STZ 65 mg/kg body weight treated)
- Group III: STZ (65 mg/kg body wt) + MESA (300mg/kg body wt)
- Group IV: STZ (65 mg/kg body wt) + MESA (500mg/kg body wt)
- Group V: STZ (65 mg/kg body wt) + Glibenclamide (600 µg/kg body wt)

The entire experiment was carried out in 30days. The leaf extracts, Glibenclamide and vehicle solution were administered orally. Group I served as the negative control which received vehicle only, Group II-V served as diabetic control, Group II which does not receive any extract or standard drug but was previously treated with STZ(65mg/kg body wt). Group III-IV was given a fixed dose of leaf extracts (300mg/kg, p.o) and (500mg/kg, p.o) respectively. Group V receives standard drug Glibenclamide.

Estimation of Blood glucose levels and Glucose Tolerance Test (GTT): Plasma blood glucose levels are estimated by using commercial kits. Blood samples were collected on 0^{th} day, 7^{th} day, and 21st day through the retro orbital sinus. And plasma blood glucose levels were estimated (**Table 1, Graph 1**).



GRAPH 1: FBG LEVEL

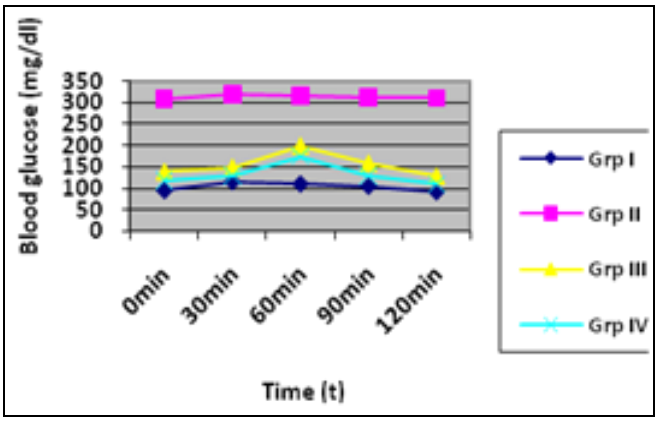
GTT test was carried out 24 hrs before sacrificing control and treated groups. It explains the ability to respond to a glucose challenge. The rats were fasted overnight before the test. And blood samples were collected from the retro orbital sinus. The rats were intraperitoneally loaded with glucose (2gm/kg) after the drug treatment. Blood samples were collected at 30, 60, 90, 120 min by puncturing retro orbital sinus after the glucose loading.

Statistical analysis: All the data were analyzed by using ANOVA and Dunnet's t-test, and were expressed in

mean ± SEM (Standard Error Mean). Difference between groups was considered significant at p<0.01.

RESULTS:

Blood glucose & Glucose tolerance test: The plasma blood glucose levels were significantly lowered by the administration of MESA. The effects of MESA (300 and 500mg/kg body wt) on GTT were explained in **Graph 2**. Plasma glucose level of Group II was significantly increased when compared to that of negative control (Group I). Administration of MESA (300 and 500mg/kg body wt) resulted in the significant decrease in the plasma blood glucose levels when compared to that of Group II.



GRAPH 2: GLUCOSE TOLERANCE TEST

Estimation of BUN, Creatinine and Albumin: Group II animals showed significant increase in the levels of BUN and Creatinine when compared to that of normal group (Group I). Administration of MESA produced significant decrease in the levels of BUN, Creatinine levels [8, 9]. At the end of the study, Urinary albumin levels were significantly increased in Group II untreated animals when compared to that of normal group. Administrations of MESA (300, 500mg/kg body wt) produced marked reduction in the Urinary albumin excretion (**Table 2**).

Estimation of Plasma Lipid Parameters: Serum cholesterol, LDL-C, triglyceride (TG) levels are significantly increased in diabetic control group (Group II) to that of normal group, whereas, HDL-C levels significantly decreased in diabetic control group when compared to that of normal group. Administration of MESA (300, 500mg/kg body wt) significantly improved these levels to the normal level (**Table 3**).

Lipid Peroxidation & Antioxidant System (SOD & CAT) in Kidney: GSH levels, Antioxidant enzyme (SOD, CAT) levels, MDA levels of control and treated groups are shown in **Table 4** [10-12]. Significant decrease of GSH, SOD and CAT levels, significant increase in the levels of MDA were observed in the untreated group (Group II). Administration of MESA (300, 500mg/kg body wt) normalizes the levels of enzymes of antioxidant system and increases the GSH levels, decreases the MDA levels in the treated groups (Group III-IV) (**Table 4**).

TABLE 1: EFFECT OF ORAL ADMINISTRATION OF METHANOLIC EXTRACT OF *S. A* ON FBG LEVELS FOR 30DAYS

	1st day	7th day	21st day
Group I	90.45±1.41	91.21±2.33	88.66±2.76
Group II	290.15±3.44	303.91±2.53	308.71±3.11
Group III	292.15±2.98	202.37±3.96*	140.98±3.58**
Group IV	290.65±1.43	187.99±4.17**	119.77±4.22***
Group V	288.05±4.36	160.29±3.49**	94.33±3.66***

The values represent the means ± SEM for six rats in each group. p-values are calculated based on the ANOVA with Dunnet's t-test. *p<0.05 compared to diabetic control group; **p<0.01 compared to diabetic control group; ***p<0.001 compared to diabetic control group

TABLE 2: EFFECT OF METHANOLIC LEAF EXTRACT OF S.A ON SERUM UREA AND SERUM CREATININE, URINARY ALBUMIN LEVELS.

Parameters	Group I	Group II	Group III	Group IV	Group V
Serum urea (mg/dl)	30.49±1.59	49.27±2.78	43.36±3.12*	34.18±2.10**	31.31±2.65**
Serum Cretinine (mg/dl)	0.47±0.06	0.52±0.04	0.46±0.05*	0.50±0.08**	0.50±0.10***
Albumin (g/dl)	3.30±0.10	2.84±0.30	2.90±0.14*	3.20±0.08**	3.33±0.31**
BUN (mg/dl)	18.98±2.20	30.78±0.97	26.65±0.56**	19.86±0.59**	25.57±2.65**

Values are expressed as Mean±SEM values for six rats in each group. Diabetic control rats were compared with normal rats. Diabetic + MESA 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 3: EFFECT OF MESA ON LIPID PROFILE

	Group I	Group II	Group III	Group IV	Group V
Cholesterol (mg/dl)	145.36±3.2	276.16±10.5	189±32.2.5	163.46±5.6	148.42±5.3
Triglyceride (mg/dl)	71.70±2.81	129.50±5.50	99.40±5.86	97.20±5.96*	90.50±5.76*
HDL-C (mg/dl)	36.83±2.5	30.00±1.9	34.22±4.3*	36.63±1.5*	38.73±1.5*
LDL-C (mg/dl)	91.32±1.2	189±12.4	120.27±1.4*	93.65±3.6*	92.35±3.1*

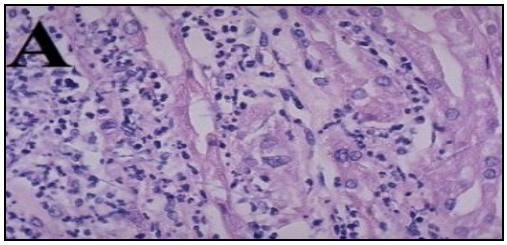
Values are expressed as Mean±SEM values for six rats in each group. *p<0.01 (ANOVA with Dunnet's t-test). Diabetic control rats were compared with the normal and treated group were compared with the diabetic control group

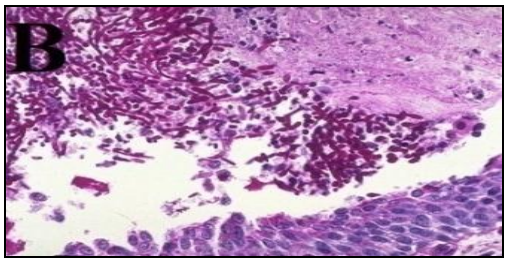
TABLE 4: EFFECT OF METHANOLIC LEAF EXTRACT OF SESAMUM ALATUM ON MDA, GSH, CATALASE, SOD LEVELS IN KIDNEY.

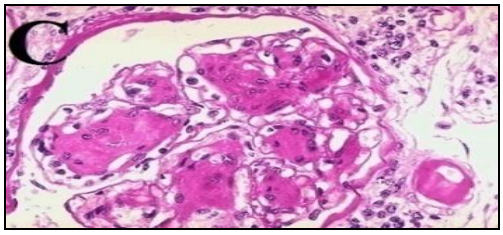
	Group I	Group II	Group III	Group IV	Group V
MDA (nM/100g tissue)	1.33±0.09	2.34±1.16	1.71±0.97*	1.47±0.60***	1.43±0.71***
GSH (µmol/g)	35.53±1.87	16.75±127[a]	26.78±129[a,b]	27.42±148[a,b]	30.62±1.73[b]
CAT(U/mg)	38.33±1.22	20.55±1.75	25.69±1.20*	36.57±1.41**	35.24±1.81**
SOD(U/mg)	27.33±1.19	11.89±0.43[a]	19.01±0.37[b]	24.70±1.47[b]	26.26±2.08[b]

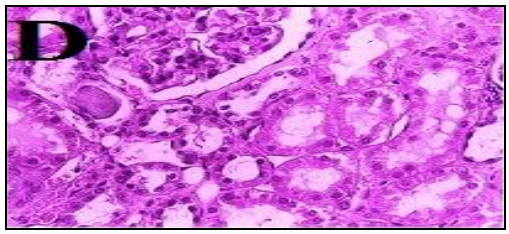
Values are expressed as Mean±SEM values for six rats in each group. *p<0.05; **p<0.01; ***p<0.001 with resepect to the Diabetic control group (ANOVA with Dunnet's t-test). [a]: Significantly different from control group; [b]:Significantly different from diabetic control group

Histopathology of Kidney: By the histo pathological sections of the rat kidney, degenerations in the kidney cortex and hemorrhage in the interstitial area was observed. The MESA treated group shows the good control over the degeneration of the kidney (**Fig: A-E**).









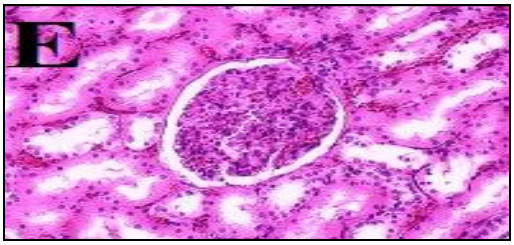


FIG. A-E: HISTOPATHOLOGY OF KIDNEY

DISCUSSION: Diabetic mellitus is the world's largest growing metabolic syndrome and Type 2 diabetes is a chronic disease marked by the high levels (abnormal levels) of blood glucose which is non-insulin dependent type of diabetes. Type 2 DM is often referred as a group of metabolic disorder which damages various organs, leading to complications such as Diabetic Nephropathy, Retinopathy, Neuropathy other Heart diseases etc., it is estimated that the number of adults suffering from any form of diabetes or its complications will increase to 366 million in 2030 (where as 285 million of people affected in 2010) [13], so

that, the need for the more appropriate therapy increases [14]. In Ayurveda, large numbers of Herbs are mentioned which posses hypoglycemic or antidiabetic, but their use in the modern therapy needs the evaluation of activity by using recent methods/techniques. The aqueous defatted seed extraction of *Sesamum indicum* already has shown Hypoglycemic and Hypolipidemic activity [15].

Present study revealed that the methanolic leaf extract of *Sesamum alatum* L. exhibit significant hypolipidemic, hypoglycemic and renal protective effects in STZ induced diabetic rats. In this study the main important basal parameter is Fasting Blood glucose level (FBG) for the monitoring of diabetes [16]. In the group IV MESA causes the hypoglycemic and antihyperglycemic effects along with that of Hypolipidemic effect by lowering the fasting blood glucose levels, and LDL-C, TG levels.

Kidney excretes the metabolic wastes, which includes Urea, Uric acid Creatinine and other ions. By the removal of these metabolic wastes it maintains the optimum balance in the body fluids. In the renal damage associated with diabetes, the increased levels of these metabolites were observed [17]. Due to the uncontrolled blood glucose levels, these metabolites may deposit in the vital organs such as Kidneys, the toxic concentration of blood sugar damages the kidney tissue. This leads to altered kidney function in the patients, causing Diabetic nephropathy. MESA has shown the renal protective action in treated groups, and the decreased levels of these metabolic wastes were observed in the treated groups.

The selective marker of glomerular injury is Urinary albumin levels. During the period of study for 5 weeks normal animals showed a rise in Urinary albumin levels. This indicates progressive nephropathy [18, 19]. The treatment with MESA has shown the protective effect on the elevated Urinary albumin level. The effect was more with MESA 500mg/kg than 300mg/kg. And it indicates that the extract shows the protective effect on the glomerular damage due to the diabetes.

Diabetes usually associated with the increased plasma lipid levels, which is the main risk factor for the development of Coronary heart disease [20, 21]. Through the drug therapy or by the dietary supplements decreased levels of these markers will lower the risk of vascular diseases [22]. Increased levels of Total Cholesterol, Triglycerides, LDL-C was observed in the untreated group where as the MESA treated groups has shown the significant decrease in the levels of these markers and increased levels of HDL-C were observed, the cholesterol which plays the important role in the transport of cholesterol from the peripheral cells to the liver. The cytotoxic actions were observed in the diabetic animals due to the administration of the Streptozotocin. The cytotoxic nature of this chemical is responsible for degeneration of the β-cells of Pancreas. This is due to the generation of the free radicals at the intracellular level. This has been demonstrated both in vivo and in vitro [23, 24].

Reactive oxygen species are the free radicals which generate in the diabetes and its complications in due to which it damages the tissue [25]. The compounds having the free radical scavenging activity may improve the Hyperglycemic condition and other diabetic complications. In this study, MESA shows the significant free radical scavenging activity and restores the GSH, SOD, CAT levels in the body and lowers the MDA levels.

CONCLUSION: Methanolic leaf extract of *Sesamum alatum* L. (MESA) is demonstrated to have potential attenuation effect on diabetic nephropathy through its anti-oxidant effect. From the above results MESA (500 mg/kg body wt) has shown the desirable protective effects than the MESA (300 mg/kg).

From the above results, MESA showed the therapeutic significance in amelioration of hyperlipidemia and prevention of metabolic syndrome in diabetic patients. Further investigations are necessary to determine the exact Phytoconstituent(s) which are responsible for the anti diabetic activity.

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