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PROTECTIVE ROLE OF *RHIZOPHORA MUCRONATA* LEAVES AGAINST STREPTOZOTOCIN-INDUCED DIABETIC NEPHROPATHY

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ABSTRACT: Oxidative stress has been recommended as a contributory factor in complication and progression of diabetes. The aim of the study was to evaluate the effects of *Rhizophora mucronata* Leaves on streptozotocin generated oxidative stress induced diabetic nephropathy in rats. Forty Wistar rats were randomly divided into normal control, diabetic untreated and diabetic treated with ethanolic extract of *Rhizophora mucronata* leaves (ERML) at 250 and 500 mg/kg doses, respectively. Experimental diabetic nephropathy was induced by a single intraperitoneal (i.p) administration of STZ 60 mg/kg body weight (b.w.). The body weight, plasma glucose, creatinine, urea, uric acid as well as total protein along with oxidative stress parameters were investigated in various groups of rats. Histopathological analyses were also conducted on the kidney tissues. In STZ treated rats LPO were increased whereas the level of SOD, CAT and GSH were decreased significantly. ERML at 250 and 500 mg/kg, in a dose-dependent manner, significantly improve all levels as compared to diabetic rats. Destruction of the liver architecture of the hepatocytes in the diabetic group showed the signs of necrosis, degeneration, dilatation, and inflammation in the central vein and blood vessels. In the kidney, shrinkage and lesion in Bowman's capsule were observed. ERML (250 and 500 mg/kg) dose-dependently acted as an antioxidant thereby preventing oxidative damage in the diabetic kidney.

INTRODUCTION: Diabetes mellitus is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Sustained hyperglycemia is further associated with long-term damage, dysfunction, and failure of various organs and is a major factor in the development of many complications in patients with diabetes [1, 2].

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Moreover, diabetes is the most common cause of progressive kidney failure leading to dialysis or transplantation. Nephropathy is reported to develop in 30-40% of patients with diabetes and has become a leading cause of end-stage renal failure worldwide.

Diabetic nephropathy is characterized by structural as well as functional abnormalities. Poor glycemic control and accumulation of advanced glycation end products (AGEs) play a significant role in the development of diabetic nephropathy. Furthermore, advanced glycation end products have been implicated in tissue damage associated with diabetic nephropathy [3-5]. The clinical and pathological hallmarks of diabetic nephropathy

include urinary albumin excretion along with the accumulation of extracellular matrix, thickening of basement membranes, mesangial expansion, hypertrophy and glomerular epithelial cell (podocyte) loss within the glomeruli [6]. Patients with diabetic nephropathy have a progressive decline in glomerular function. Antihypertensive agents, particularly those targeting the renin-angiotensin system, such as angiotensin converting enzyme inhibitors, angiotensin receptor-1 antagonists are reported being the most effective treatments for progressive diabetic nephropathy, to date. However, these treatments are not capable of preventing the onset of diabetic nephropathy [3, 7, 8].

Medicinal plants play a significant role in the development of potent therapeutic agents. Herbal medicines have been used to treat various human diseases [9, 10]. Moreover, the demand for herbal medicines is increasing day by day. *Rhizophora mucronata* Lam. (Rhizophoraceae), a true mangrove, is widely distributed along the delta of Indian Sunderbans (21°32 and 22°40 N and between 88°05 and 80°00 E. [11, 12] The bark, root, leaves, fruit, and flowers of *R. mucronata* have been traditionally used as medicine in the coastal areas of Asian subcontinents for treating health ailments such as diabetes, diarrhea, hepatitis, inflammation, and cognitive function [13].

The perspective of its use as anti-diabetic medicine was supported with numerous scientific reports, but more information is still required. The chemical identity of *R. mucronata* has also been carried out, and the presence of seco-labdane diterpenoid (Rhizophora A), phomoxanthone, lupeol, beta-sitosterol, gallic acid, coumarin, quercetin, and tannins. Studies reported that *R. mucronata* extracts had a hypoglycemic, anti-radical, anti-cancer, anti-nociceptive, antiplasmodial, anti-HIV, Peroxisome proliferator-activated receptor-γ antagonistic effect, α-amylase along with α-glucosidase inhibitory effect [14-17]. The fruit flour of *R. mucronata* was used as a functional food for anti-diabetic effect. The alkaloid fractions of *R. mucronata* leaves have been reported with anti-microbial and radical scavenging effects. There are several terpenoids, volatile derivatives, and phenolic constituents identified from the different extracts of this plant. The computational studies expressed that alkaloids of *R. mucronata* efficiently interacted with dipeptidyl peptidase-4 receptor (DPP-IV) receptor, alpha ketoglutarate-dependent dioxygenase, and cyclooxygenase II receptors and inhibit their mode of action [13, 18, 19]. Taking all this into consideration, the objective of the present study was to assess the protective effects of *R. mucronata* leaves in streptozotocin (STZ)-induced diabetic nephropathy.

METHODS:

Experimental Animals: Adult male or female Wister rats, weighing 150 to 200 g are used in the study. The study protocol is reviewed and approved by the institutional animal ethical committee (AMRMCP/06/18-19/IEAC/Ph.D.) and conforms to the Indian national science academy guidelines for the use and care of experimental animals in research. Animals are obtained from the Raghavendra Enterprises, Bangalore. Rats are housed in polyacrylic cages (38 × 23 × 10 cm) with not more than four animals per cage. They are housed in an air-conditioned room and are kept in standard laboratory conditions under natural light and dark cycle (approximately 14 h light / 10 h dark) and maintained humidity 60 ± 5% and an ambient temperature of 25 ± 2 ºC. All experiments are performed between 9:00 am and 4:00 pm. The animals are free to access to standard diet and tap water *ad libitum* and allowed to acclimatize for one week before the experiments. Commercial pellet diet contained 22% Protein, 4% Fat, 4% Fiber, 36% Carbohydrates, and 10% Ash w/w, supplied by Raghavendra Enterprises, Bangalore is used.

Acute Oral Toxicity Study: The procedure is followed by using OECD 423 (Acute Toxic Class Method). The acute toxic class method is a stepwise procedure with four mice of a single-sex per step. Male albino mice weighing 20-35 g are used for the study. The starting dose level of ethanolic extract *R mucronata* leaves (ERML) is 500, 1500, 2500, 5000 mg/kg body weight p.o. Dose-volume is administered to overnight fasted mice *ad libitum*. Food is withheld for further 3-4 hours after administration of EERML and observed for signs for toxicity. The body weight of the mouse before and after administration are noted that changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic, central nervous system, motor activity, and behavior pattern are observed, and also signs of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma are

noted. The onset of toxicity and signs of toxicity also noted (OECD 2002) [20, 21].

Experimental Design:

Compound (Drugs to be Administered) Preparation:

- Extract: Extract is weighed according to rat body weight and dissolved in distilled water.
- Extract Dose Selection: Based on toxicity studies, EERML has selected 250 and 500 mg/kg b.w. dose have good activity against streptozotocin-induced diabetic complications. The starting point of study is animal selection and randomly dividing them into 8 groups (by considering animal body weights).
- Streptozotocin Injection 60 mg/kg body weight [22].

Induction of Experimental Diabetes: [23] Following the induction of diabetes, the animals were randomly allocated into four groups (n =10) and treated for 16 weeks as follows

- **Group 1:** Control (non-diabetic) rats.
- **Group 2:** Diabetic rats with no treatment.
- **Group 3:** Diabetic rats treated with the EERML 250 mg/kg per day by gavage, started 5 days before STZ and continued for 16 weeks.
- **Group 4:** Diabetic rats treated with ERML500 mg/kg per day by gavage, started 5 days before STZ and continued for 16 weeks.

This was a randomized study with concurrent control and appropriate blinding.

Induction of Diabetes: Diabetes was induced by a single injection of streptozotocin (STZ) at a dose of 60 mg/kg, i.p and dissolved in 0.01M citrate buffer (pH 4.5) after 16 h fasting. 72 h after STZ injection diabetes was confirmed in rats showing blood sugar level greater than 250 mg/dL. Animals with blood glucose levels greater than 250 mg/dL were considered for further study.

Sampling and Biochemical Analysis: Blood samples were collected 72 h after STZ administration and after that every 4 weeks from orbital plexus by pricking a needle under ketamine anesthesia. Glucose-Oxidase assay method [24]. It was used to determine blood glucose. After collecting blood samples were centrifuged for 10 minutes at 3000 rpm. The obtained clear sera are stored at the refrigerator for subsequent measurement of blood urea, creatinine, uric acid, and total protein levels using colorimetric assay kits.

Preparation of Tissue Homogenates: The kidneys are removed and dissected free from the surrounding fat and connective tissue. Each tissue is longitudinally sectioned and kept at -8 °C. Subsequently homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4) followed by centrifuging at 5000 rpm for 10 min at 4 °C. The resulting supernatant of each organ is used for the determination of – malondialdehyde (MDA) content (kidney sample) and antioxidant enzyme levels such as superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH).

Biochemical Estimation of Markers of Oxidative Stress:

Malondialdehyde: According to the method of Esterbauer and Cheeseman (1990), MDA is estimated in terms of TBARS. Homogenized renal tissue (1 ml) in 2 ml of normal saline is mixed with 1 ml trichloroacetic acid (20%), 2 ml thiobarbituric acid (0.67%) and heated for 1 h at 100 °C. After cooling, the precipitate is removed by centrifugation. The absorbance of the sample is measured at 535 nm using a blank containing all the reagents except the sample. As 99% TBARS are MDA, so TBARS concentrations of the samples are calculated using the extinction coefficient of MDA (1.56×10^5 $M^{-1}cm^{-1}$). [25]

Catalase: 0.95 ml of 10 mM H_2O_2 in 60 mM phosphate buffer (pH = 7.0), 50 µl of the tissue supernatant is added and the rate of degradation of H_2O_2 is followed at 240 nm per min. Catalase content in terms of U/mg of protein is estimated from the rate of decomposition of H_2O_2 using the formulae $k = 2.303/\Delta t \times \log(A1/A2)$ (A unit of catalase is defined as the quantity which decomposes 1.0 µmole of H_2O_2 per min at pH = 7.0 at 25 ºC). [26]

Superoxide Dismutase: 2.8 ml of sodium carbonate buffer (0.05 mM) and 0.1 ml of tissue homogenate or sucrose (Blank) is incubated at 30

ºC for 45 min. Then, the absorbance is adjusted to 0 to sample. After that, the reaction is initiated by adding 10 µl of adrenaline solution (9 mM). The change in absorbance is recorded at 480 nm for 8-12 min throughout the assay, the temperature is maintained at 30 ºC. 1 unit of SOD produces approximately 50% of inhibition of auto-oxidation of adrenaline. The results are expressed as a unit (U) of SOD activity per mg of tissue [27].

Calculation:

SOD = c × total volume × 1000 / 50 × sample volume × mg protein per ml

Unit: Units/ mg Protein.

Reduced Glutathione: Tissue homogenate 50 µl is diluted with 50 µl of 100 mM phosphate buffer containing 1 mM EDTA. To this mixture, 100 µl of reaction buffer (295 µM 5, 5^1- dithiobis (2-nitrobenzoic acid) (DTNB) made in 10 ml of phosphate buffer) is added and change in absorbance measured at 412 nm for 5 min. Reduced pure GSH is used to obtain a standard curve. Reduced GSH is expressed as µM/GSH/mg tissue [28].

Histopathological Examination: At the end of the experiment, organs are immediately fixed in 10% buffered neutral formalin solution. The tissue is carefully embedded in molten paraffin with the help of metallic blocks, covered with flexible plastic molds and kept under the freezing plate to allow paraffin to solidify. Cross section (5 µm thick) of the fixed renal tissues is separated. These sections are stained with hematoxylin and eosin (H & E) and visualized under a light microscope to study the light microscopic architecture of the dissected organs.

Statistical Analysis: The results are expressed as mean ± standard deviation (SD) differences in groups for biochemical estimations. Statistical analysis is determined by one way – analysis of variance (ANOVA); individual groups are compared with the control group using Dunnett͐s t-test. P value < 0.05 has been considered as a statistical significance level.

RESULTS:

Acute Toxicity Studies: The body weight of the mice`s before and after administration of drugs is noted, and the changes in the body weight are not so prominent. No changes in the skin, fur, eyes, mucous membrane, respiratory, circulatory, autonomic, central nervous system, motor activity, and behavior pattern are observed, and also no sign of tremors, convulsions**,** salivation, diarrhea, lethargy, sleep and coma are noted.

The onset and signs of toxicity are also observed. No mortality is observed. So select the dose levels of 250 mg/kg and 500 mg/kg b.w **Table 1**.

TABLE 1: ACUTE TOXICITY STUDIES FOR ERML

S. no.	Groups	Dose (mg/kg b.w.)	Signs of toxicity	Onset of toxicity	Duration of study
1	ERML	500 mg	No	Nil	14 days
2	ERML	1500 mg	No	Nil	14 days
3	ERML	2500 mg	No	Nil	14 days
4	ERML	5000 mg	No	Nil	14 days

Effect of ERML on Body Weight: There is a slight decrease in body weights none significantly in the anti-diabetic treated group (B) when compared with the control group (A). There is a slight dose-dependent increase of body weights significantly in animals treated with ERML 250 mg/kg and 500 mg/kg (C, D) when compared with STZ group (B) **Table 2**.

TABLE 2: EFFECT OF ERML ON BODY WEIGHT

Group	Treatment	Dose	Change in b.w.(g)
A	Control	Vehicle	10.20 ± 3.80
B	Streptozotocin	Streptozotocin Injection 60 mg/kg b.w. (i.p.)	-2.22 ± 1.32
C	ERML	RML -250 mg/kg. ± Streptozotocin 60 mg/kg b.w. (i.p.)	6.88 ± 2.83
D	ERML	RML -500 mg/kg. ± Streptozotocin 60 mg/kg b.w. (i.p.)	8.19 ± 3.59

Effect of ERML on Urea: 0 week not showing significant differences into all groups and 4 week onwards showing serum urea concentrations are significantly increased in the Diabetic treated group

of animals compared to the normal animals indicating the induction of severe nephrotoxicity. Treatment with the ethanolic extract of ERML shows significant (Group C & D) decrease in concentrations of serum urea compared to the Diabetic treated group. ERML 500 mg/kg group shows more effective when compared with 250 mg/kg group **Table 3**.

TABLE 3: EFFECT OF ERML ON UREA

Group / Treatment	UREA				
	0 week	**4 week**	**8 week**	**12 week**	**16 week**
Control	48.36 ± 3.18	50.13 ± 1.25	49.82 ± 2.56	50.36 ±2.58	47.15 ± 1.02
Diabetic*	50.13 ± 2.35	95.12 ± 1.29	98.15 ± 1.87	99.17 ± 2.56	99.25 ± 1.87
ERML (250 mg/kg)*	48.99 ± 2.38	66.18 ± 2.74	79.12 ± 1.25	69.14 ± 0.99	66.14 ± 4.78
ERML(500 mg/kg)^{ns}	50.12 ± 2.56	60.17 ± 2.44	65.12 ± 3.66	58.14 ± 1.72	52.14 ± 1.08

All values are mean ±S.D. (n=10). p<0.05 all groups are compared with the control group (One-way ANOVA followed by Dunnett's multiple comparison test)

Effect of ERML on Uric acid: 0 week not showing significant differences into all groups and 4 week onwards showing serum uric acid concentrations are significantly decreased in the Diabetic treated group of animals compared to the normal animals indicating the induction of severe nephrotoxicity. Treatment with the ethanolic extract of ERML shows significant (Group C & D) increase in concentrations of serum uric acid compared to the Diabetic treated group. ERML 500 mg/kg group shows more effective when compared with 250 mg/kg group **Table 4**.

TABLE 4: EFFECT OF ERML ON URIC ACID

Group / Treatment	URIC ACID				
	0 week	**4 week**	**8 week**	**12 week**	**16 week**
Control	1.90 ± 0.13	2.01 ± 1.25	1.99 ± 2.15	2.00 ± 1.25	2.15 ± 1.25
Diabetic^{ns}	1.99 ± 0.12	0.99 ± 0.24	0.65 ± 2.08	0.68 ± 1.23	0.87 ± 1.92
ERML (250 mg/kg)*	1.65 ± 1.3	1.51 ± 0.25	1.25 ± 1.30	1.38 ± 1.28	1.25 ± 1.39
ERML (500 mg/kg)^{ns}	1.88 ± 1.05	2.00 ± 1.85	1.89 ± 1.25	1.89 ± 1.34	2.12 ± 0.99

All values are mean ±S.D. (n=10). p<0.05 all groups are compared with the control group (One-way ANOVA followed by Dunnett's multiple comparison test)

Effect of ERML on Creatinine: 0 week not showing significant differences into all groups and 4 weeks showing Serum creatinine concentrations are significantly increased (4 weeks) in the Diabetic treated group of animals compared to the normal animals indicating the induction of severe nephrotoxicity. Treatment with the ethanolic extract of ERML shows significant (Group C & D) decrease in concentrations of serum creatinine compared to the Diabetic treated group. ERML 500 mg/kg group shows more effective when compared with 250 mg/kg group. After 4-week Creatinine shows reverse profile **Table 5**.

TABLE 5: EFFECT OF ERML ON CREATININE

Group / Treatment	CREATININE				
	0 week	**4 week**	**8 week**	**12 week**	**16 week**
Control	0.52 ± 0.15	1.23 ± 0.75	0.99 ± 1.28	1.00 ± 0.25	0.85 ± 1.21
Diabetic ^{ns}	0.65 ± 1.25	2.12 ± 0.28	0.31 ± 0.98	0.54 ± 1.23	0.21 ± 0.25
ERML (250 mg/kg)^{ns}	0.65 ± 1.35	1.99 ± 1.23	0.65 ± 1.23	0.98 ± 2.35	0.65 ± 1.28
ERML (500 mg/kg) ^{ns}	0.77 ± 1.37	1.35 ± 0.99	1.21 ± 1.01	1.00 ± 1.09	0.99 ± 1.24

All values are mean ±S.D. (n=10). p<0.05 all groups are compared with the control group (One-way ANOVA followed by Dunnett's multiple comparison test)

Effect of ERML on Total Protein: 0 week not showing significant differences into all groups and 4 weeks onwards showing Serum total protein concentrations are significantly decreased in the Diabetic treated group of animals compared to the normal animals indicating the induction of severe nephrotoxicity. Treatment with the ethanolic extract of ERML shows significant (Group C & D) increase in concentrations of serum total protein compared to the Diabetic treated group. ERML 500 mg/kg group shows more effective when compared with 250 mg/kg group **Table 6**.

TABLE 6: EFFECT OF ERML ON TOTAL PROTEIN

Group / Treatment	TOTAL PROTEIN				
	0 week	4 week	8 week	12 week	16 week
Control	1.21 ± 0.12	5.54 ± 1.23	6.12 ± 1.04	8.52 ± 1.25	7.99 ± 1.52
Diabetic ^{ns}	1.23 ± 0.35	2.01 ± 1.01	4.12 ± 2.10	3.18 ± 0.17	2.53 ± 1.68
ERML (250mg/kg) ^{ns}	1.61 ± 0.58	4.23 ± 1.26	5.12 ± 1.28	4.45 ± 1.23	5.11 ± 2.58
ERML (500mg/kg) ^{ns}	1.45 ± 1.23	6.12 ± 1.25	7.14 ± 1.25	7.54 ± 1.28	8.00 ± 1.28

All values are mean ±S.D. (n=10). $p<0.05$ all groups are compared with the control group (One-way ANOVA followed by Dunnett's multiple comparison test)

Effect of ERML on Blood Sugar: 0 week not showing significant differences into all groups and 4 week onwards showing blood sugar concentrations are significantly increased in the Diabetic treated group of animals compared to the normal animals indicating the induction of severe diabetes. Treatment with the ethanolic extract of ERML shows significant (Group C & D) decrease in concentrations of blood sugar compared to the Diabetic treated group. ERML 500 mg/kg group shows more effective when compared with 250 mg/kg group **Table 7**.

TABLE 7: EFFECT OF ERML ON BLOOD SUGAR

Group / Treatment	BLOOD SUGAR				
	0 week	4 week	8 week	12 week	16 week
Control	86.17± 1.31	89.12 ± 1.26	90.15 ± 2.87	89.32 ± 1.27	90.12 ± 1.27
Diabetic*	89.12 ± 2.13	250.12 ± 1.87	264.14 ± 2.25	271.12 ± 1.25	287.18 ± 1.34
ERML (250mg/kg)*	82.13 ± 1.23	180.25 ± 1.25	165.25 ± 2.58	175.24 ± 1.28	165.12 ± 2.36
ERML (500mg/kg)*	87.15 ± 1.23	135.41 ± 1.02	123.25 ± 1.28	118.28 ± 1.27	115.23 ± 0.39

All values are mean ±S.D. (n=10). $p<0.05$ all groups are compared with the control group (One-way ANOVA followed by Dunnett's multiple comparison test)

Effect of ERML on Anti-oxidant Parameters: The activity of CAT in the diabetic treated group is significantly decreased when compared to the normal animals (Group A). Treatment with the ethanol extract of ERML significantly (Group C & D) prevented a decrease in the level of catalase activity compared to the diabetic-induced rat (Group B). Renal SOD activity is decreased significantly in the diabetic treated (group B) animals compared to normal group. Treatment with the extract (250 & 500 mg/kg body wt) (Group C & D) significantly elevated the SOD levels as compared to the diabetic-induced (Group B) animals. The GSH level reduced significantly along with increased in MDA concentration in the diabetic treated group as compared to the Group A. On treatment with ethanolic extract of ERML, the GSH level is found to be enhanced significantly and the MDA contents are reduced in Group C and D as compared to the induced group (Group B) **Table 8**.

TABLE 8: EFFECT OF ERML ON ANTIOXIDANT ACTIVITY IN KIDNEY TISSUES

Parameters	Control	Diabetic	ERML 250 mg/kg b.w.	ERML 500 mg/kg b.w.
SOD (units of activity/mg protein)	18.75 ± 2.14	9.44 ± 1.87	13.5 ± 2.22	17.14 ± 2.3
MDA (μM/mg protein)	45.17 ± 3.33	99.53 ± 4.47	66.51 ± 2.55	55.27 ± 3.28
CATALASE (micromoles of H_2O_2 decomposed/mg protein/min)	34.52 ± 2.40	22.6 ± 3.55	29.2 ± 2.38	32.93 ± 2.7
GSH (nM/mg protein)	30.45 ± 2.7	12.80 ± 3.3	26.40 ± 2.9	27.90 ± 2.4

Histopathological Study: Histopathological evaluation of the normal kidney tissue of the non-diabetic rats demonstrated normal structure of glomerulus surrounded by the Bowman's capsule, distal convoluted tubules, and proximal without any inflammatory alterations **Fig. 1A**. The kidneys of untreated diabetic rats showed shrinkage of glomeruli and tubular inflammation **Fig. 1B**. The groups that were treated with EMRL (250 and 500 mg/kg) demonstrated normal glomerulus, normal basement membrane, and capillaries without any inflammatory cells, **Fig. 1C** and **D**.

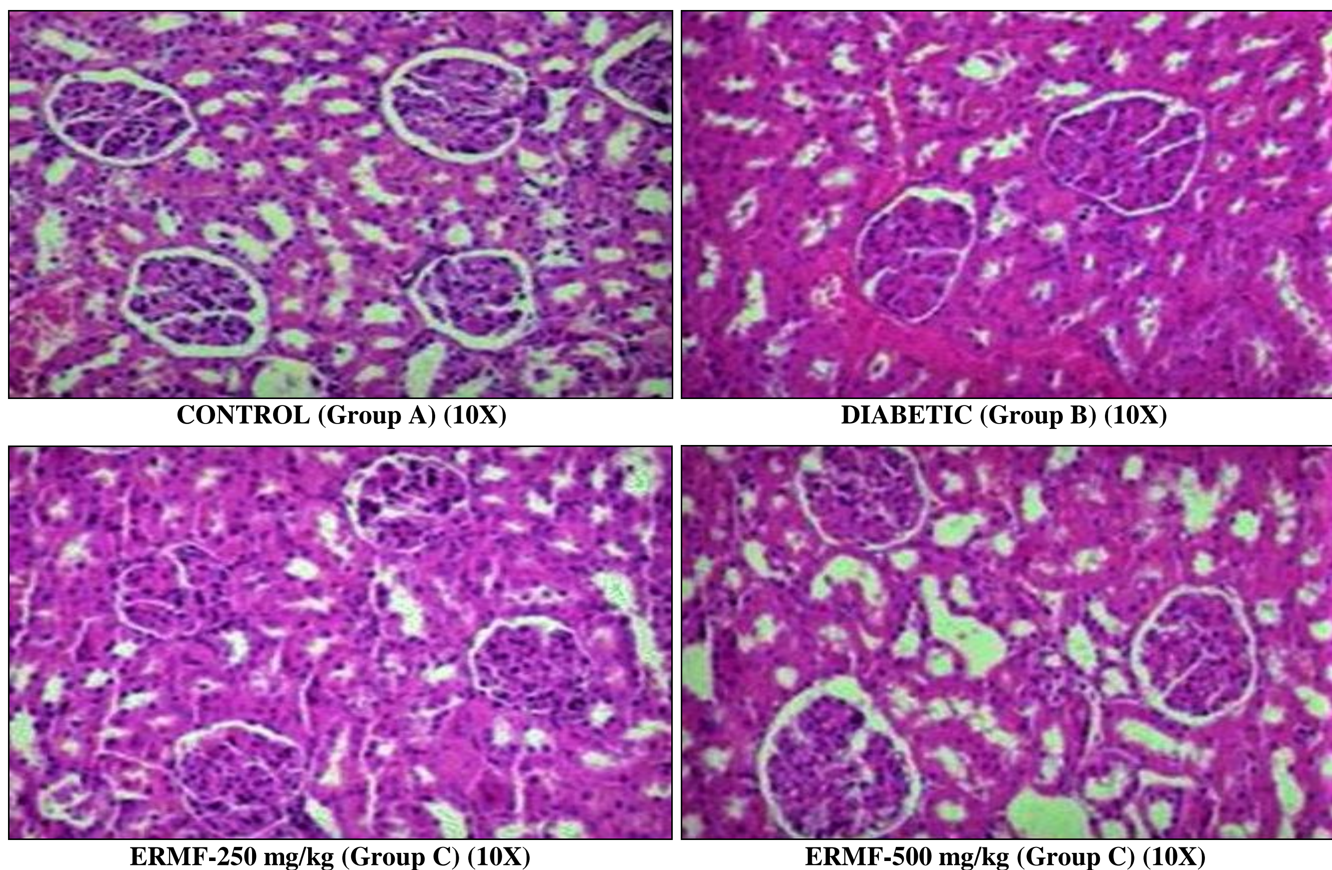

FIG. 1: HISTOPATHOLOGICAL STUDY. Group A: Non-diabetic section of a kidney showing normal structure with no pathological changes appearance of the kidney, normal glomeruli, and tubules, Bowmen's capsule (BC), glomerulus (G), proximal tubule (PT), distal convoluted tubular (DCT), Macula Densa cell (MD). Group B: STZ- induced diabetic kidney showing shrinkage of tubular and inflammation. Group C: ERMF-250 mg/kg treated groups show minor changes in glomerulus with inflammation in cells. Group D: ERMF-500 mg/kg treated groups show normal glomerulus, normal basement membrane, and capillaries, without any inflammatory cells.

DISCUSSION: Streptozotocin prevents DNA synthesis in mammalian and bacterial cells. In bacterial cells, it renders special reaction with cytosine groups, resulting in degeneration and destruction of DNA. The biochemical mechanism results in mammalian cell death. Streptozotocin prevents cellular reproduction with a much smaller dose than the dose needed for inhibiting the substrate connected to the DNA or inhibiting many of the enzymes involved in DNA synthesis. Although, Streptozotocin prevents the entry of cells into mitosis, but no special phase of the cellular cycle is especially sensitive to its mortal effects [29-31]. Streptozotocin, which is used in intravenously form by rapid injection or constant short diffusion, stimulates the tissues. Metabolically, a slight deviation of the glucose-bearing pain from the normal limit has been seen in patients treated with a certain dose of Streptozotocin, which is generally reversible. However, the insulin shock, which is one of its other effects, is irreversible.

In this study, the clinical manifestations and also the amount of glucose, insulin, and C-peptide after using a 60 mg/kg dose of Streptozotocin, ensured the induction of diabetes in rats. Hyperglycemia, hypoinsulinemia, polyphagia, polyuria and polydipsia accompanied by weight loss were seen in adult rats within three days of Streptozotocin treatment and, within one week to ten days, the amounts of the relevant factors were almost stable, which indicates irreversible destruction of Langerhans islets cells moreover, Researchers around the world have used streptozotocin to create experimental diabetes because it is a simple, inexpensive and available method [32-35].

In renal disease, the serum urea accumulates because the rate of serum urea production exceeds the rate of clearance. Elevation of urea and creatinine levels in the serum is taken as the index of nephrotoxicity. Creatinine is mostly derived from endogenous sources by tissue creatinine

breakdown. Thus, serum urea concentration is considered a more reliable renal function predictor than serum creatinine [36-38].

In the present study, administration of single injection of streptozotocin (STZ) at a dose of 60 mg/kg, i.p. to rats resulted in the development of oxidative stress damage in heart, hepatic and renal tissues. Diabetic group rats' shows a significant increase in the serum urea and creatinine concentrations in the when compared to the normal group. Oral administration of ERML (250 and 500 mg/kg) significantly decreased dose-dependently. The level of uric acid & total protein is significantly decreased in the diabetic rats when compared to normal group. Oral administration of ERML (250 and 500 mg/kg) significantly increases the uric acid & total protein levels when compared to the diabetic rats. Thus, oxidative stress and lipid peroxidation are early events related to radicals generated during the hepatic metabolism of STZ. Also, the generation of reactive oxygen species has been proposed as a mechanism by which many chemicals can induce nephrotoxicity. STZ induced oxidative stress results in lipoperoxidation, protein thioloxidation, mitochondrial endoplasmic reticulum injury, altered homeostasis, and irreversible DNA damage characterized by protein adduct formation [39-43].

In recent studies have clearly shown that STZ increases the lipid peroxidation and suppresses the antioxidant defense mechanisms in renal tissue. In the diabetic group animals, the LPO levels are increased significantly when compared to normal control rats. On Administration of ERML, the levels of LPO decreased significantly when compared to diabetic group rats [44, 45].

During diabetic induced complications superoxide radicals are generated at the site of damage and modulate SOD and CAT, resulting in the loss of activity and accumulation of superoxide radical, which increases oxidative stress and damages major organs in the body. SOD and CAT are the most important enzymes involved in ameliorating the effects of oxygen metabolism. The present study also demonstrated that STZ resulted in a decrease in the SOD, CAT activities when compared with normal control rats. It is due to enhanced lipid peroxidation or inactivation of the antioxidative enzymes. When the rat is treated with the ERML the reduction of SOD, CAT activity is increased significantly when compared with induced diabetic rats [46, 47].

Intracellular GSH plays an essential role in detoxification of STZ and prevention of STZ toxicity. The generation of the reactive oxygen species appears as an early event which precedes intracellular GSH depletion and cell damage. STZ also caused a significant decrease in GSH content. Administration of ERML (250 and 500 mg/kg) helped to uplift the GSH depletion induced by STZ. Also, alkaloids have also been reported to strongly inhibit lipid peroxidation induced in isolated tissues via its antioxidant activity. The protection offered by the extract could have been due to the presence of flavonoids and alkaloids [48, 49].

The leaves of the plant have rich in polyphenols and flavonoids due to the presence of these constituents the plant has antioxidant property. Diabetes mellitus is usually conveyed by excessive production of free radicals, hyperglycemia-induced mitochondrial reactive oxygen species production could be a key episode in the progress of diabetic complications. The antioxidant property of the plant has deteriorated the progression of diabetic-induced nephropathy [50-52].

CONCLUSION: The activity elicited by the extract might be due to its ability to activate antioxidant enzymes. The findings suggest the potential use of the ethanolic extract of leaves of *Rhizophora mucronata* as a novel therapeutically useful nephroprotective agent.

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