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Pharmacological evaluation of ethanolic extract of *Rhizophora mucronata* flower against streptozotocin-induced diabetic nephropathy in experimental animals

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Abstract
Diabetic nephropathy is a long-term complication of diabetic mellitus. Many experimental evidences suggest that persistent hyperglycemia generates intracellular reactive oxygen species (ROS), which is involved of free radicals in the pathogenesis of diabetes and more importantly in the development of diabetic complications. The objective of the present investigation was to evaluate the antidiabetic activity and protective effect of diabetic-induced nephropathy of ethanolic extract of Rhizophora mucronata flowers (ERMF) on streptozotocin (STZ) generated oxidative stress induced diabetic nephropathy in rats. Forty Wistar rats were indiscriminately distributed into normal control, diabetic untreated and diabetic treated with ERMF (250 and 500 mg/kg per body weight). 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 and total protein along with oxidative stress parameters were examined in different groups of experimental animals. Histopathological examination was also performed for observing cellular changes on kidney tissues. Elevated level of blood sugar and urea whereas lower uric acid, creatinine, and total protein were detected in the STZ-diabetic rats, which were significantly attenuated after ERMF (250 and 500 mg/kg per body weight) administration, dose-dependently. Even various oxidative stress parameters were also drastically changed in a STZ-diabetic rats which were improved by ERMF (250 and 500 mg/kg per body weight). Histopathological study of kidney was supported the potential protective effect of ERMF (250 and 500 mg/kg per body weight). The activity elicited by the extract might be due to its ability to activate antioxidant enzymes. The findings suggest the potential use of the ERMF (250 and 500 mg/kg per body weight) as a novel therapeutically useful nephroprotective, agent.

Keywords: *Rhizophora mucronata* flower, streptozotocin, oxidative stress, total protein, urea, uric acid, nephroprotective

1. Introduction
Diabetic nephropathy is one of the most serious complications of diabetes and the most common cause of end-stage renal failure [1]. At present, diabetic kidney disease affects about 15%–25% of type I diabetes patients and 30%–40% of patients with type II diabetes. Diabetic nephropathy is characterized by specific renal morphological and functional alterations [2, 3]. The early changes in diabetic nephropathy are characterized by an increase in kidney size, glomerular volume and kidney function followed by the accumulation of glomerular extracellular matrix, increased urinary microalbumin excretion, glomerular sclerosis and tubular fibrosis [4, 5]. Last stage overt diabetic nephropathy is clinically characterized by proteinuria, hypertension, and progressive renal insufficiency. Thus, sustained reduction in hyperglycemia will decrease the risk of developing microvascular complications [6, 7].
In modern medicine, the beneficial effects on glycemic levels are well documented; the preventive activity of drugs against progressive nature of diabetes and its complications are modest and not always effective. Treatment with sulfonylureas, biguanides, and insulin possess undesirable side effects [8, 9]. So the management of diabetes without side effects is yet a challenge to the medical system. There is an increasing demand to use the natural products with antidiabetic activity. Plants are useful sources for the development of antidiabetic drugs. Particularly, the use of medicinal plants and oriental medicine prescriptions in modern medicine suffers from the fact that though hundreds of plants are used in the world to prevent or to cure diseases, scientific evidence in terms of modern medicine is lacking in most cases [10, 11]. However, today it is necessary to provide scientific proof as to whether it is justified to use plant active principles or traditional prescriptions.

A search for natural products that have antidiabetic properties and low toxicity are under evaluation.

Mangroves are one of the medicinally important plants produce several phytocompounds or secondary metabolites having significant pharmacological properties and are being used traditionally for treatment of several ailments. *Rhizophora mucronata* is a mangrove (family Rhizophoraceae), commonly known as "red mangrove", used as traditional medicine in the treatment of diarrhea, dysentery, blood in urine, fever, angina, diabetes, hematuria, and hemorrhage. In South Asian countries together with India, the flowers of *R. mucronata* are being used traditionally for the remedy of diabetes [12, 13]. It is abundantly found on the coastal region. *R. mucronata* L. showed the presence of a good amount of polyphenols, like flavonoid, tannin, glycoside, phenolic compounds. Research from Bangladesh revealed the ethanol extract of *Rhizophora mucornata* leaves has significant dose dependant anti-diabetic effects. Recent study reported the anti-diabetic action of hydro-methanolic and hydro-ethanolic extract of *R mucronata* (Sundarban Mangrove) leaves in diabetes model [14-16]. Hence, the present study was undertaken to explore the protective effects of ERMF in streptozotocin (STZ)-induced diabetic nephropathy.

2. Methods

2.1 Experimental animals

Adult male or female Wister rats, weighing 150 to 200g are used in the study. The study protocol is reviewed and approved by the institutional animal ethical committee 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 (38X23X10 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 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.

2.2 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: ERMF have selected 250 and 500mg/kg b.w dose having the good activity against STZ-induced diabetic complications. Starting point of study is animal selection and randomly dividing them into 4 groups (by considering animal body weights).
- STZ Injection 60mg/kg body weight [17].

2.3 Induction of Experimental Diabetes [18].

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 ERMF 250 mg/kg per day by gavage, started 5 days prior to STZ and continued for 16 weeks.
- **Group-4:** Diabetic rats treated with ERMF 500 mg/kg per day by gavage, started 5 days prior to STZ and continued for 16 weeks.

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

2.4 Induction of Diabetes

Diabetes was induced by single injection of streptozotocin (STZ) at a dose of 60 mg/kg, i.p. dissolved in 0.01M citrate buffer (pH 4.5) after 16 h fasting. 72h 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 hours after STZ administration, and thereafter every 4 weeks from orbital plexus by pricking a needle under ketamine anesthesia. Glucose-Oxidase assay method [19] was used to determine the blood glucose. After collecting blood samples were centrifuged for 10 minutes at 3000 rpm. The obtained clear sera are stored at refrigerator for subsequent measurement of blood urea, creatinine, uric acid and total protein levels using colorimetric assay kits.

2.5 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 centrifuged at 5000rpm 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).

2.6 Biochemical estimation of markers of oxidative stress

2.6.1 MDA

According to the method of Esterbauer and Cheeseman (1990), MDA is estimated in terms of TBARS. Homogenized renal tissue (1ml) 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 x 105 $M^{-1}cm^{-1}$) [20].

2.6.2 CAT

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 ^{0}C) [21].

2.6.3 SOD

2.8 ml of sodium carbonate buffer (0.05 mM) and 0.1 ml of tissue homogenate or sucrose (Blank) is incubated at 30 ^{0}C for 45 minutes. Then, the absorbance is adjusted to 0 to sample. Thereafter, the reaction is initiated by adding 10µl of

adrenaline solution (9mM). The change in absorbance is recorded at 480nm for 8-12 minutes. Throughout the assay, the temperature is maintained at 30 ^{0}C. 1 unit of SOD produces approximately 50% of inhibition of auto-oxidation of adrenaline. The results are expressed as unit (U) of SOD activity per mg of tissue [22].

2.6.4 GSH
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 [23].

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

2.8 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 control group using Dunnett's t-test. P value < 0.05 has been considered as statistical significance level.

3. Results
3.1 Effect of ERML on Bodyweight
There is slight decrease of body weights none significantly in antidiabetic treated group (B) when compared with control group (A). There is slight dose-dependent increase of body weights significantly in animals treated with ERMF 250 mg/kg and 500 mg/kg (C, D) when compared with STZ group (B) (Table 1).

Table 1: Effect of ERMF on Body weight

Group	Treatment	Dose	Change In B.W.(G)
A	Control	Vehicle	10.20±3.80
B	Streptozotocin	Streptozotocin Injection 60mg/kg b.w. (i.p.)	-2.22±1.32
G	ERMF	RMF -250mg/kg.+ Streptozotocin 60mg/kg b.w. (i.p.)	7.88±2.83
H	ERMF	RMF -500mg/kg.+ Streptozotocin 60mg/kg b.w. (i.p.)	10.19±3.59

3.2 Effect of ERMF 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 ERMF shows significant (Group C & D) decrease in concentrations of serum urea compared to the Diabetic treated group. ERML 500mg/kg group shows more effective when compared with 250mg/kg group (Table 2).

Table 2: Effect of ERMF 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
ERMF (250mg/kg)*	50.99± 2.38	65.18 ±2.74	80.12 ±1.25	70.14 ±0.99	67.14± 4.78
ERMF(500mg/kg)^{ns}	51.12 ±2.56	61.17± 2.44	68.12 ±3.66	61.14 ±1.72	55.14 ±1.08

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

3.3 Effect of ERMF 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 ERMF shows significant (Group C & D) increase in concentrations of serum uric acid compared to the Diabetic treated group. ERML 500mg/kg group shows more effective when compared with 250mg/kg group (Table 3).

Table 3: Effect of *ERMF* 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
ERMF(250mg/kg)*	2.00 ±1.3	1.55± 0.25	1.25± 1.30	1.38 ±1.28	1.25 ±1.39
ERMF(500mg/kg) ^{ns}	2.15 ±1.05	2.15 ±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 control group (One-way ANOVA followed by Dunnett's multiple comparison test)

3.4 Effect of ERMF on Creatinine
0 week not showing significant differences into all groups and 4 week showing Serum creatinine concentrations are significantly increased (4 week) 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. ERMF 500mg/kg group shows more effective when compared with 250mg/kg group. After 4-week Creatinine shows reverse profile (Table 4).

Table 4: Effect of ERMF 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
ERMF(250mg/kg) ns	0.59± 1.35	1.89 ±1.23	0.65 ±1.23	0.98 ±2.35	0.65 ±1.28
ERMF(500mg/kg) ns	0.67 ±1.37	1.45± 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 control group (One-way ANOVA followed by Dunnett's multiple comparison test)

3.5 Effect of ERMF on Total protein

0 week not showing significant differences into all groups and 4 week 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 ERMF shows significant (Group C & D) increase in concentrations of serum total protein compared to the Diabetic treated group. ERML 500mg/kg group shows more effective when compared with 250mg/kg group (Table 5).

Table 5: Effect of ERMF 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
ERMF(250mg/kg) ns	1.21± 0.58	3.43 ±1.26	5.12 ±1.28	4.45 ±1.23	5.11 ±2.58
ERMF(500mg/kg) ns	1.35 ±1.23	4.12 ±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 control group (One-way ANOVA followed by Dunnett's multiple comparison test)

3.6 Effect of ERMF 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 ERMF shows significant (Group C & D) decrease in concentrations of blood sugar compared to the Diabetic treated group. ERMF 500mg/kg group shows more effective when compared with 250mg/kg group (Table 6).

Table 6: Effect of ERMF 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
ERMF(250mg/kg)*	85.13 ±1.23	180.25 ±1.25	168.25 ±2.58	175.24± 1.28	165.12± 2.36
ERMF(500mg/kg)*	86.15 ±1.23	135.41±1.02	129.25 ±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 control group (One-way ANOVA followed by Dunnett's multiple comparison test)

3.7 Effect of ERMF 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 ERMF significantly (Group C & D) prevented 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 ERMF, 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 7).

Table 7: Effect of ERMF on antioxidant activity in kidney tissues

Parameters	Control	Diabetic	ERMF 250 mg/kg b.w.	ERMF 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
CAT (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

3.8 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 (Figure 1A). The kidneys of untreated diabetic rats showed shrinkage of glomeruli and tubular inflammation (Figure 1B). The groups that were treated with EMRF (250 and 500 mg/kg) demonstrated normal glomerulus, normal basement membrane, and capillaries without any inflammatory cells (Figure 1 C and D).

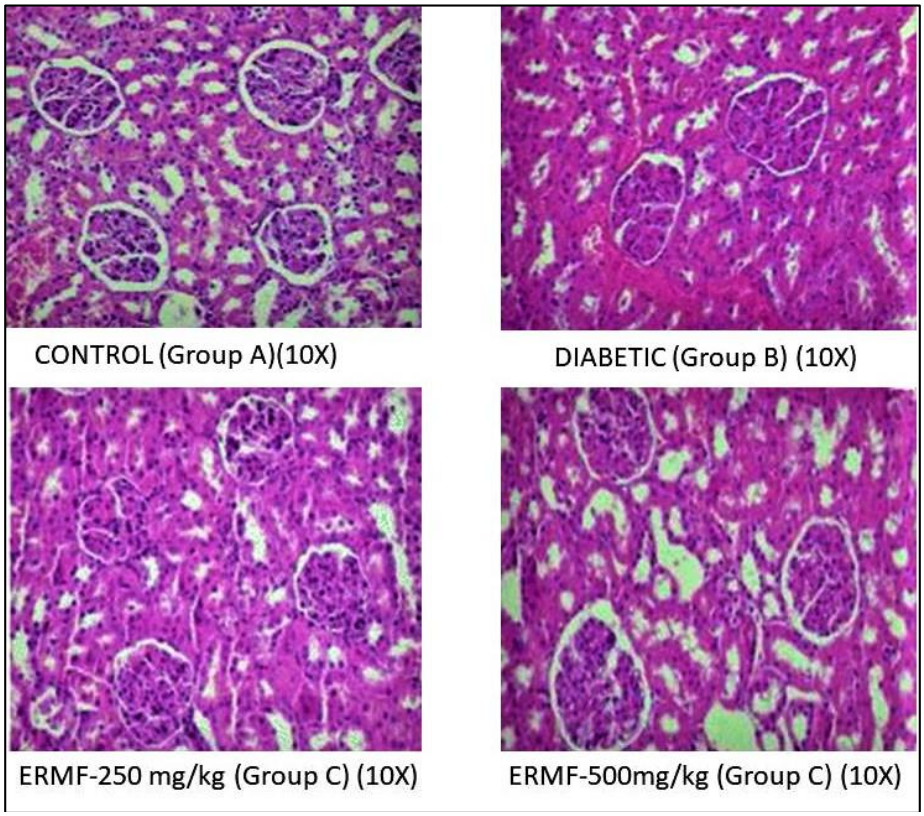

Fig 1: Photomicrographs of histological section of kidney tissue of control and treated rats Group A: Non-diabetic section of kidney showing normal structure with no pathological changes appearance of kidney, normal glomeruli, and tubules, Bowmen's capsule (BC), glomerulus (G), proximal tubule (PT), 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.

4. Discussion

The main function of the kidneys is to excrete the waste products of metabolism and to regulate the body concentration of water and salt. STZ administration elevated renal markers, i.e., serum urea, creatinine, urine glucose, and urine protein and decreased urine urea and urine creatinine which are found responsible for proper maintenance, functioning of kidney, and change in the glomerular filtration rate [24, 25]. These changes were observed in the present study, while EMRF (250 and 500 mg/ kg BW) or decreased the level of fasting blood glucose, serum creatinine, and increased body weight, serum protein, urine urea, and urine creatinine. Hence, our current study confirmed that EMRF showed significant improvement in renal functions. It has been reported that in STZ-induced diabetic rats, the renal pathological changes and deteriorated functions are very similar to human diabetes [26, 27].

STZ are drugs that selectively destroy ß-cells of pancreas and thus induce experimental diabetes. Moreover, it has been reported that in STZ-induced diabetic rats, the renal and liver undergo pathological changes. Glomerular and tubular hypertrophy, increased basement membrane thickness, tubulointerstitial fibrosis, and arteriosclerosis are the pathologic features of diabetic nephropathy [28-30]. Many studies have been shown a significant increase in the rate of kidney cell damage (nephropathy) in diabetes disorders. Finally, this nephropathy reduces the physiological function and changes in the structure of kidney in diabetes disease. Hyperglycemia increases the generation of free radicals by glucose auto-oxidation and the increment of free radicals may lead to kidney cells damage [31, 32].

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 thiol oxidation, mitochondrial endoplasmic reticulum injury, altered homeostasis and irreversible DNA damage characterized by protein adduct formation [33-37]. 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 ERMF, the levels of LPO decreased significantly when compared to diabetic group rats [38, 39].

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 rat is treated with the ERMF the reduction of SOD, CAT activity is increased significantly when compared with induced diabetic rats [40, 41]. 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 ERMF (250 and 500 mg/kg) helped to uplift the GSH depletion induced by STZ. In addition, 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 [42, 43].

The Flower 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 deteriorates the progression of diabetic-induced nephropathy [44-46].

From the overall results of the biochemical and histopathological examinations, it could be inferred that ERMF showed the beneficial effect (especially at dose of 500 mg/kg BW) on renal function in STZ-induced diabetic rats. Further study on ERMF could be extended for the isolation and structure determination of the beneficial effect on renal and liver function principle(s).

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