ORIGINAL ARTICLE

Effect of methanolic extract of *Corchorus trilocularis* Linn. (Tiliaceae) in diabetic nephropathy with special reference to antioxidant activity

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Abstract

Aim: Effect of methanolic extract of *Corchorus trilocularis* Linn. in diabetic nephropathy (DN) with special reference to anti-oxidant activity. **Materials and Methods:** *C. trilocularis* Linn. were collected from local medicinal garden of college campus and authentified by botanist. Dried leaves of selected plant were successively extracted using petroleum ether, dichloromethane, ethyl acetate, methanol, and finally water. In the previous study, methanolic extract showed potent activity so we have further selected methanolic extract for the other model. Animals were fasted for overnight then a single intra peritoneal injection of freshly prepared streptozotocin (50 mg/kg dissolved in citrate buffer pH 4.5) was injected and Nephropathy was induced by Ligation of the left renal artery. Blood was collected on 0, 14, and 28 day by retro-orbital on 28 days from eye for the estimation of biochemical parameters. The withdrawn blood was centrifuged at 5000 rpm for 10 min by using centrifuge and plasma was collected and different biochemical parameters were determined by auto analyzer. Some antioxidant parameters, that is, Malondialdehyde, glutathione, Catalase, and superoxide dismutase were also determined. **Results:** Here body weight of rat is decreased in DN group in comparison to normal control group while increased significantly in extract treated group at 28th day in comparison to DN group. Here blood glucose level of DN group was increased in comparison to normal control group while decreased significantly in extract treated group at 14th, 21st, and 28th days in comparison to DN group. **Conclusion:** The overall results of this study have clearly indicated that extract treated in a dose of 200 and 400 mg/kg ameliorate the renal ischemia reperfusion induced nephropathy in diabetic rats. The action of methanolic extract contributed by renoprotective, anti-diabetic, and antioxidant properties of *C. trilocularis* Linn.

Key words: Anti-oxidant, catalase, *Corchorus trilocularis* Linn., Diabetic nephropathy, Malondialdehyde, Methanolic extract, Superoxide dismutase

INTRODUCTION

Diabetic nephropathy (DN) is the most common cause of end-stage renal disease. DN is one of the most frequent life-threatening complications of diabetes mellitus that occurs approximately 30–40%. DN is one of the important micro vascular complications of diabetes mellitus. It is usually attributed to metabolic consequences of abnormal glucose regulations, such as elevated blood and tissue levels of glycosylated proteins and hemodynamic changes within the kidney tissue. Recently, there has been a renewed interest in understanding the role of reactive oxygen species (ROS), which play a key intermediate role in the pathophysiology of DN. Chronic hyperglycemia the main determinant of the initiation and progression DN, not only generates more reactive oxygen metabolites but also attenuates antioxidative mechanisms through non-enzymatic glycation of the scavenging enzymes.[1,2] DN is clinically defined as the progressive development of renal insufficiency in the setting of hyperglycemia. This disease is now the major single cause of end-stage renal failure in many countries. Diabetic kidney

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disease is most frequent cause of end-stage renal disease (ESRD). Unfortunately, the incident of DN as a cause of ESRD is increasing each year.[3]

Based on the literature survey, *Corchorus trilocularis* Linn. has been used traditionally in diabetes and kidney disease. It is scientifically proved plant shows antidiabetic activity (Suppression of carbohydrate absorption in intestine and reduce the activity of α-amylase),[4,5] antioxidant property (free-radical scavenging activity)[6] and also produces nephroprotective effect.[7] Since no such experimental and scientific evidence is available for different extract of this plant in the treatment of DN. So our, main aim of study is to find out its effect in DN.

MATERIALS AND METHODS

Procurement of Plant Material

C. trilocularis Linn. was collected from local medicinal garden of college campus and authentified by botanist and after authentication the herbarium was submitted in the Department of Pharmacognosy of respective college.

Procurement and Selection of Animals

Albino Wistar rats of either sex; weighing between 150 and 200 g were obtained from Central animal house of Pharmacy College. They had given standard pellet diet and water. The protocol for the animal experiments was approved by Institutional Animal Ethical Committee and all the guidelines of animal experiments were adhered with experiments.

Induction of Diabetes[8]

Albino Wistar rats of either sex weighing 150–200 g were taken. Animals were fasted for overnight then a single intra peritoneal injection of freshly prepared Streptozotocin (50 mg/kg dissolved in citrate buffer pH 4.5) was injected. The diabetes was confirmed by estimation of blood glucose level (BGL) at 3rd day. Rats having BGL more than 300 mg/dl were used for nephropathy.

Induction of Nephropathy by Ligation of the Left Renal Artery (LRA)[8]

In this model, following groups were made and each group having six animals.

Group I: Normal control
Group II: Normal nephropathy
Group III: DN (Disease control)
Group IV: DN treated with methanolic extract in a dose of 200 mg/kg
Group V: DN treated with methanolic extract in a dose of 400 mg/kg

Blood Sample Collection

Blood was collected on 0, 14, and 28 days by retro-orbital on 28 days from eye for the estimation of biochemical parameters. The withdrawn blood was centrifuged at 5000 rpm for 10 min by using centrifuge and plasma was collected. This plasma was used for the determination of various parameters.

Urine Collection

Urine was collected on 0, 14, and 28 days with the help of metabolic cages in the graduated tubes and various biochemical parameters such as urine protein and urine creatinine levels were assessed.

Collection of Organs

After 28th day of the drug treatment in DN model, the animals were euthanized by over dose of anesthetic ether and the kidneys were collected for the determination of glutathione (GSH) content, malondialdehyde (MDA) content.

Determination of Biochemical Parameters

The biochemical parameters were estimated by diagnostic kits with the help of auto analyzer (Star track 21).

Determination of Urine Creatinine[9-11]

The adopted method was Modified Jaffe's reaction. Creatinine reacts with alkaline picrate to produce an orange-yellow color (Jaffe' reaction). The absorbance of the orange-yellow color formed is directly proportional to creatinine concentration and is measured photometrically at 500–520 nm.

Mixed well and read initial absorbance (A_1) 20 s after mixing and final absorbance (A_2) 80 s after mixing.

Difference in absorbance = $A_2 - A_1$

$$\text{Creatinine } (\text{mg/dl}) = \frac{\text{Difference in absorbance of test}}{\text{Difference in absorbance of standard}} \times \text{Conc. of standard } (\text{mg/dl})$$

Determination of Blood Urea Nitrogen (BUN)[12,13]

Method

Glutamate dehydrogenase-urease method [Table 1].
Mixed well and aspirate standard followed by sample.
Difference in absorbance = $A_1 - A_2$

$$\text{Urea (mg/dl)} = \frac{\text{Difference inabsorbance of test}}{\text{Difference inabsorbance of standard}} \times \text{Conc. of standard (mg/dl)}$$

Determination of Urine Protein[14,15]

Proteins in the test sample form a blue-purple complex when reacted with a combination of pyrogallol red dye and molybdic acid at pH 2.2. The concentration of the protein in the sample is obtained by measuring the absorbance at 600 nm [Table 2].

Mixed well incubate at 37°C for 10 min. Measure absorbance standard and test against blank at 600 nm within 60 min.

$$\text{Protein conc. (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Conc. of standard (mg/dl)}$$

In vivo Antioxidant Activity[16]

Determination of lipid peroxidation (MDA)

MDA levels, an index of lipid peroxidation, were measured by the double heating method.

Determination of reduced GSH[17]

1 mL of supernatant was precipitated with 1 mL of 4% sulfosalicylic acid and cold digested at 4°C for 1 h. The samples were centrifuged at 1200 × g for 15 min at 4°C. To 1 mL of this supernatant, 2.7 mL of phosphate buffer (0.1M, pH 8) and 0.2 mL of DTNB were added. The yellow color developed was read immediately at 412 nm using ultraviolet spectrophotometer (Shimadzu).

Determination of catalase (CAT)[17]

The assay mixture consisted of 1.95 mL phosphate buffer (0.5 m, ph-7.0), 1.0 mL H_2O_2 (0.019 m) 0.05 mL of Supernatant (10 % w/v) changes in absorbance were recorded at 240 nm. CAT was calculated in term of nmol H_2O_2 consumed/min/mg of protein

Table 1: Procedure for determination of blood urea nitrogen

Pipette into tubes marked	Standard	Test
Working reagent	100 µl	100 µl
Standard	20 µl	-
Test	-	20 µl

Statistical Analysis

The data of activity were analyzed by one-way analysis of variance followed by "Tukey's test" using GraphPad prism version 5.0 software and $P < 0.05$ was consider at statistically significant.

RESULTS

Assessment of Body Weight (g)

Here body weight of rat is decreased in DN group in comparison to normal control group while increased significantly in methanolic extract group at 28th day in comparison to DN group [Table 3].

Assessment of BGL (mg/dl)

Here BGL of DN group was increased in comparison to normal control group while decreased significantly in methanolic extract group at 14th, 21st, and 28th days in comparison to DN group [Table 4].

Assessment of Urine Creatinine (mg/dl)

Here urine creatinine of normal nephropathy and DN group was increased in comparison to normal control group while decreased significantly in Methanolic extract group at 14th and 28th days in comparison to DN group [Table 5].

Assessment of BUN (mg/dl)

Here BUN of normal nephropathy and DN group was increased in comparison to NC group while decreased significantly in 200, 400 group at 14th and 28st days in comparison to DN group. The data is presented in Table 6.

In vivo Antioxidant Activity

Determination of reduced GSH

Here GSH content of normal nephropathy and DN group was decreased in comparison to normal control group while increased significantly in methanolic extract group at 28nd day in comparison to DN group.

Table 2: Procedure for determination of urine protein

Add to tubes marked	Blank	Standard	Test
Sample	-	-	5 µl
Standard	-	5 µl	-
Water	5 µl	-	-
Dye reagent	500 µl	500 µl	500 µl

Table 3: Change in body weight on 0, 7, 14, 21, and 28 days

Group	0 day	7th day	14th day	21st day	28 day
Normal control	94.3±1.11	94.14±1.22	96.2±1.23	97.84±1.22	100.33±1.01
Normal nephropathy	101.8±2.55	91±2.44	101.8±2.29	111.0±3.94	115.71±2.58
DN	104.9±3.48	83.50±2.29	83.50±1.19**	96.83±2.63***	96.33±2.44***
Methanolic extract (200 mg/kg)	106.7±4.21	111.5±2.51	91.3±1.37***	101.9±1.22***	158.41±1.33***
Methanolic extract (400 mg/kg)	105.6±4.22	110.9±2.59	96.3±1.96***	112.6±1.48***	159.66±1.59***

Values are expressed as mean±SEM, *n*=6 *P<0.05 significant, **P<0.01 Moderate significant, ***P<0.001 highly significant with respect to III group. DN: Diabetic nephropathy

Table 4: Change in BGL (mg/dl) on 0, 7, 14, 21, and 28th days

Group	0 day	7th day	14th day	21st day	28th day
Normal control	84.83±2.78	84.83±1.22	85.33±3.22	81.67±4.24	82.87±3.41
Normal nephropathy	83.83±2.88	82.00±0.99	81.50±4.27	82.33±3.32	83.33±3.67
DN	463.6±2.22**	472.3±2.99**	485.4±4.36***	495.3±4.28***	513.4±4.39***
Methanolic extract (200 mg/kg)	473.2±3.66**	404.8±2.13**	331.8±2.29***	275.4±4.44***	180.4±4.28***
Methanolic extract (400 mg/kg)	478.8±3.55**	406.7±6.66**	327.5±2.74***	270.9±4.96***	168.9±3.63***

Values are expressed as mean±SEM, *n*=6, **P<0.01 Moderate significant, ***P<0.001 highly significant with respect to III group. DN: Diabetic nephropathy, BGL: Blood glucose level

Table 5: Change in urine creatinine (mg/dl) on 0, 14, and 28th days

Group	0 day	14th day	28 day
Normal control	0.332±0.022	0.333±0.028	0.332±0.067
Normal nephropathy	0.394±0.078	0.756±0.094	1.085±0.558
DN	0.453±0.095	0.944±0.063**	1.283±0.052***
Methanolic extract (200 mg/kg)	0.457±0.055	0.518±0.064**	0.485±0.078***
Methanolic extract (400 mg/kg)	0.457±0.066	0.442±0.096**	0.435±0.033***

Values are expressed as mean±SEM, *n*=6, *P<0.05 significant, **P<0.01 Moderate significant, ***P<0.001 highly significant with respect to III group. DN: Diabetic nephropathy

Table 6: Change in BUN (mg/dl) on 0, 14, and 28th days

Group	0th day	14th day	28th day
Normal control	14.35±1.33	14.51±2.20	15.01±1.33
Normal nephropathy	14.59±2.66	34.57±1.33**	51.50±2.54***
DN	16.14±3.86	40.13±2.86**	55.03±2.66***
Methanolic extract (200 mg/kg)	17.83±2.66	23.58±2.66**	18.01±3.26***
Methanolic extract (400 mg/kg)	17.55±2.44	20.58±2.74**	16.22±3.33***

Values are expressed as mean±SEM, *n*=6, *P<0.05 significant, **P<0.01 Moderate significant, ***P<0.001 highly significant with respect to III group. DN: Diabetic nephropathy

Determination of lipid peroxidation (MDA)

Here MDA content of normal nephropathy and DN group was increased in comparison to normal control group while decreased significantly in methanolic extract group at 28nd day in comparison to DN group.

Determination of CAT (unit/mg)

Here CAT level of normal nephropathy and DN group was decreased in comparison to normal control group while increased significantly in methanolic extract group at 28nd day in comparison to DN group. The data is presented in Table 7.

DISCUSSION

Hyperglycemia may also cause an increase in vasodilatory prostaglandins, which in turn causes an increase in both renal perfusion and intraglomerular pressure. This ultimately results in hyperfiltration.[18]

C. trilocularis Linn. is used as a medicinal supplement that contributes toward the treatment and prevention of diabetes. *C. trilocularis* Linn. is a rich source of various phyto-constituents, which shows great therapeutic activity. Among,

Table 7: Change in reduced GSH, MDA, SOD, and CAT on 28 day

Group	GSH (µ mol GSH/g)	MDA (µ mol/L)	CAT (Unit/mg)
Normal control	24.04±3.85	0.361±0.02	27.31±1.22
Normal nephropathy	13.85±2.28**	0.747±0.08**	7.19±2.22***
DN	12.48±1.11**	0.812±0.06**	9.51±2.37***
Methanolic extract (200 mg/kg)	21.67±1.29**	0.402±0.09***	24.23±3.64***
Methanolic extract (400 mg/kg)	23.97±1.63**	0.312±0.07***	26.54±3.01***

MDA: Malondialdehyde, GSH: Glutathione, SOD: Superoxide dismutase. DN: Diabetic nephropathy

many constituents Lupeol is one of the major constituent having various therapeutic uses.[19]

The previous scientific literature shows that *C. trilocularis* Linn. significantly lowers the BGL, glycosylated hemoglobin and lipid peroxidation. It is also used for anti-stress, antioxidant activity, and free-radical scavenging activity and it was also found to decrease the MDA level and prevent lipid peroxidation.

In the present study, we found that ischemia cause rapidly progressive nephropathy in diabetic rats. In the diabetic animals, however, this comparatively short period of ischemia cause a substantial loss of renal function. Increased sensitivity to ischemia may be expected from the increase in sodium k^+ ATPase activity and there by the increased oxygen demand along most segments of the nephron in diabetic rats.

In our data revealed that there were marked reduction in the total body weight as well elevation in the kidney weight of the DN group compared to normal control group may be due to an increase in lipid clearance from the body. Methanolic extract in a dose of, 200 and 400 mg/kg treatment showed significantly amelioration in both body and kidney weights in a dose dependent manner. Methanolic extract has a strong antioxidant and free-radical scavenging effect. Increase in the body weight of rats at 28 days in comparison to disease control group and normal nephropathy groups.

C. trilocularis Linn. proved to antidiabetic by investigator and in our study rats treated with Lupeol showed decrease BGL. In our study, extract showed high significant result comparison to decrease more BGL.

After the ischemia, LRA showed significantly rise in plasma creatinine, blood urea nitrogen, urine creatinine, and urine protein with respect to control group, which clearly indicates development of nephropathy or intrinsic acute renal failure.

BUN and creatinine levels are higher in rats with DN than in normal rats. In control rats by extract treatment suggests that extract play a role, either directly or indirectly, in providing protection against DN. From our study, methanolic extract at dose of 200 and 400 mg/kg rendered significant protection against ischemia induced nephropathy which was evident by the decreased plasma, urine creatinine, blood urea nitrogen level, and urine protein.

Oxidative stress and ROS play major role in the development of DN. Oxidative stress is increased in diabetes and the over production of ROS in diabetes is a direct consequence of hyperglycemia. Cellular damage and oxidative stress develop DN. Oxidative stress and subsequently cellular damage due to renal ischemia could decrease the GSH level, CAT level in rats compared to the normal control group. However, treatment of DN with methanolic extract significantly improved GSH, CAT levels. Methanolic extract in a dose of 200 and 400 mg/kg act as a potent antioxidant and free radical scavenger.

Lipid peroxidation products such as MDA are generated under high level of un-scavenged free radicals. These products may be important in the pathogenesis of vascular complication in diabetes mellitus. The increased MDA level may have an important role in pancreatic damage associated with diabetes. Our study showed methanolic extract significantly decrease lipid peroxidation (MDA). This may be another probably mechanism to support our study.

The result of the present study suggests that methanolic extract significantly alter the disease parameters of experimental nephropathy in treated animals.

CONCLUSION

The overall results of this study have clearly indicated that methanolic extract at the dose of 200 and 400 mg/kg ameliorate the renal ischemia reperfusion induced nephropathy in diabetic rats. The action of extract contributed by renoprotective, antidiabetic, and antioxidant properties of selected plant. Further studies are needed to stabiles possible mechanism of action for protective effect of methanolic extract and isolation of active compound is further required.

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