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Ameliorative effect of alcoholic extract of *Shorea robusta* resin on kidney injury in diabetic rats

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Abstract
Nephropathy is the major consequence in long term hyperglycemia. Herbal extracts impart a range of important pharmacological action that may retard the progressive decline in renal function in diabetes. This present work was conducted to explore the ameliorative effect of ethanolic extract of *S. robusta* resin (SRE) in in long term hyperglycemia linked kidney failure in rats as it has phenolic components which show anti-oxidative properties. Resin was extracted with 70% ethanol using a Soxhlet assembly and concentrated under rotary evaporator. Diabetes was induced in male Wistar rats by single intraperitoneal injection of streptozotocin (60 mg/kg). Animals were treated once daily for eight weeks as follows: normal control with aqueous vehicle (0.5% CMC), normal with SRE (300 mg/kg), Diabetic control with vehicle and Diabetic with SRE (30,100 and 300 mg/kg). Animals from each group were sacrificed and collected the granulation tissue which was used for the estimation of antioxidant enzyme activity and lipid peroxidation by spectrophotometer. SRE treated animals exhibited markedly improved activity of SOD and catalase, also reduced the MDA and NO as compared to diabetic control. So, the herbal extract has shown significant effect to prevent severity of kidney injury in diabetic rats. Findings emphasis the use of this plant as herbal medicine in diabetes and other relevant ailments.

Keywords: Diabetes, Kidney, *Shorea*, Antioxidant

Introduction
Diabetic nephropathy clinically manifested a syndrome that consist of albuminuria, regression in renal function, and risk of cardiovascular disease. Clinically, albuminuria is the suitable biomarker for the early diagnosis of diabetic nephropathy. The histopathological changes in kidney include thickening of the glomerular basement membrane, glomerular hypertrophy and nodular lesions of glomerulus (Mauer *et al.*, 1984) [13]. Mostly management is recommended to combat diabetic nephropathy i.e. diet therapy, blood pressure, and lipid control, (Kitada *et al.*, 2014) [10]. However, a substantial body of evidence suggests that herbal extracts possess a range of important pharmacological properties that may retard the progressive decline in renal function in diabetes (Yokozawa *et al.*, 2004) [16]. Studies suggest that plant extracts with hypoglycemic properties alleviate kidney complications associated with clinical diabetes mellitus (Li *et al.*, 2007) [11] and reduce the severity of nephropathy in experimental animals. Indeed, Chinese herbal extracts indicate therapeutic potential against diabetic nephropathy in terms of their effects on associated diabetic metabolic disorders. The mechanisms of extracts that influence kidney function at molecular, physiological, and biochemical levels might be useful in the treatment of diabetes associated renal impairment. The importance of medicinal herbs as potential source of safer, effective, and cheaper remedies for treating diabetes, the present work is being designed on an ethnomedicine *Shorea robusta* Gaertn f. (Dipterocarpaceae).

Materials and Methods
Extract preparation
Resin of *Shorea robusta* was procured from Bhubaneswar. It was ground into fine powder then extracted with 70% ethanol using a Soxhlet apparatus under reflux for 72 h. Under reduced pressure it was evaporated to dryness in rotary vacuum evaporator at 40 °C and then kept in vacuum desiccators for complete removal of solvent. The yield of the extract was calculated. Hereafter, extract was stored at 4 °C until use and to be considered as 'SRE'.

Animals

Healthy male Wistar rats (200-220 g) were procured from LAR Section, ICAR -IVRI, Izatnagar. The experimental protocols were according to the guidelines published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996) for the Care and Use of Laboratory Animals.

Induction of diabetes

Freshly prepared streptozotocin (STZ) in citrate buffer pH 4.5 was used to induce hyperglycemia by single intraperitoneal injection @ 60 mg/kg to overnight off feed rats those showing fasting blood glucose level in a normal range. Although, control group received only equal volume of citrate buffer to nullify its effect. After 72 h interval blood glucose was examined by tail pricking using digital glucometer. Rats showing blood glucose on or above 300 mg/dl were selected for study and trial was started after 15 days of observation period.

Experimental design

The animals were randomly designed into six groups of animals as follows: NC (normal control rats treated with vehicle), DC (diabetic control rats treated with vehicle), NE (normal rats treated with SRE 300 mg/kg), DE1, DE2 and DE3 (diabetic rats treated with SRE 30, 100 and 300 mg/kg, respectively). Extract was dissolved in vehicle containing 0.5% carboxymethylcellulose and given orally once in a day by using 16G gastric gavages for eight weeks.

Sample Collection

At the end of 8th week rats were sacrificed with diethyl ether. Thereafter, both kidneys were removed, weighed and the tissue was homogenized in ice-cold PBS. Concentration of Protein was estimated according to the Lowry's kit method (GeNei, Merck). The aliquots were stored at -80 °C for antioxidant parameters.

Biochemical parameters

Super oxide dismutase (SOD) activity

Super oxide dismutase activity was estimated by the method of Madesh and Balsubramanium (1998) [12]. It involves the production of superoxide by auto oxidation of pyrogallol and inhibition of superoxide dependent reduction of the tetrazolium dye (MTT) [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] to its formazan. The mixture has 0.65 ml PBS (pH 7.4), 30 µl MTT (1.25 mM), 10 µl sample and 75 µl pyrogallol (100 mM). Then mixture was incubated at room temperature for five min and the reaction was caused by adding 0.75 ml of DMSO which solubilizes formazan. Finally, the absorbance was taken at 570 nm against the blank and the activity was expressed as U/mg protein.

Catalase (CAT) activity

Catalase activity was described by Aebi (1984) [1] as follows: Sample (0.1ml) was added to 1.9 ml of 50 mM phosphate buffer (1.5:1 v/v of 50mM Na2HPO4 pH 7 and 50mM KH_2PO_4) in a test tube then content is transferred to cuvette. The reaction was started after adding 1 ml of H_2O_2 directly into cuvette (10 mM: 0.1 ml of 30% H_2O_2 was diluted to 100ml phosphate buffer). Optical density was measured at every 15 sec for 1 min at 240 nm against blank. The CAT activity was estimated using milimolar extinction coefficient of H_2O_2 (0.071 mmol cm^{-1}). Finally, the activity was expressed as U/mg protein.

Malondialdehyde (MDA)

Lipid per-oxidation in kidney tissue was estimated by TBARS method of Buege and Aust (1978) [4] using thiobarbituric acid reactive substances. In brief, 0.1 ml of sample in 200 mM Tris-HCl buffer, pH 7.5 was treated with 2 ml of (1:1:1) TBA-TCA-HCl reagent (TBA 0.37%, 0.25N HCl and 15% TCA) and kept in water bath for 15 min and cooled then centrifuged at 2000 rpm for 10 min. The absorbance of clear supernatant was taken at 535 nm against the blank. Enzyme concentration was estimated using molar extinction coefficient of MDA (1.56 X 105 M-1 cm-1) and expressed as nmol/mg protein.

Nitric oxide (NO)

Nitrite measurement is an indirect indicator of NO production in the tissue lysate. Briefly, it was assessed in 100 µl sample and taking equal volume of Griess reagent (1% sulfanilamide and 0.1% naphthylenediamine in 5% phosphoric acid). After incubation at room temperature for 10 min, the absorbance was taken at 550 nm in a microplate reader (SpectraMax Multi-mode). The nitrite concentration was estimated from extrapolation of calibration curve was made with standard sodium nitrite (0-100 µM).

Statistical analysis

The Statistical significance was interpreted by applying one-way ANOVA followed by Tukey's multiple comparison test using the GraphPad Prism v5.03 software program (San Diego, California USA). Findings were expressed as mean ± S.E. and the difference between the treatment groups and control were considered significant at $p<0.05$ statistically.

Results

Findings shows significant reduction in SOD and catalase expression in kidney homogenate from diabetic rats as compared to control. Study revealed that the SRE exhibit a significant increase in SOD and catalase activity in diabetic rats treated with 300 mg/kg dose of extract compared to control. MDA and NO level were significantly increased in diabetic control as compared to normal control. Rats treated with extract exhibited a concentration based significant lower MDA and NO as compared to control (Fig 1).

Discussions

Diabetic nephropathy is a complication associated with long time hyperglycemia, and the chances of nephropathy among diabetic patients is higher than non-diabetic. It is described as a serious microvascular disease, which can damage kidney function, and eventually lead to end-stage renal impairment. The basic pathological alteration in diabetic nephropathy comprises glomerular cell proliferation leads to glomerulosclerosis, sequestration of inflammatory mediators and accumulation of extracellular matrix (Duran-Salgado and Rubio-Guerra, 2014) [6]. Additionally, greater number of immune mediated cells gather and accumulate into the kidney tissue, resulting further release of growth factors and pro-inflammatory cytokines. Thereby, enhancing the inflammatory response and continuously exacerbating renal stromal fibrosis and kidney tissue damage (Bhattacharya *et al*., 2013) [2]. In the earliest stage, proteinuria is the foremost clinical manifestation. Renal impairment is not traditionally considered a disease that is associated with the immune system; however, a study has shown that augmentation of inflammatory mechanisms and the innate immune system are

important in its pathogenesis (Wang *et al*., 2008) [15]. Earlier studies have shown that the important role of inflammatory pathways in diabetic nephropathy (Chen *et al*., 2014) [5].

Higher glucose concentration leads to excessive production of ROS through uncontrolled glucose autoxidation (Hunt *et al.* 1990) [8], which emphasize progressive biological damage and has been implicated in the pathogenesis of diabetes mellitus in humans (Brownlee 2001) [3]. According to Gumieniczek *et al.* (2002) [7], Hyperglycemia causes a depression in glutathione or vitamin C like natural antioxidant defense agents. The increase in the levels of ROS and free radicals cause damage to the cellular structures such as genetic material, enzymes and cell membrane. Herbal extract increases superoxide dismutase level, which catalyzes the dismutation of O-2 into oxygen and H2O2, therefore, decreases generation of ROS and oxidative stress (Ponrasu *et al.* 2013) [14]. Analysis of malondialdehyde is an index of lipid peroxidation (Kakkar *et al*. 1998) [9]. Free radical production increases the peroxidation of lipid molecules.

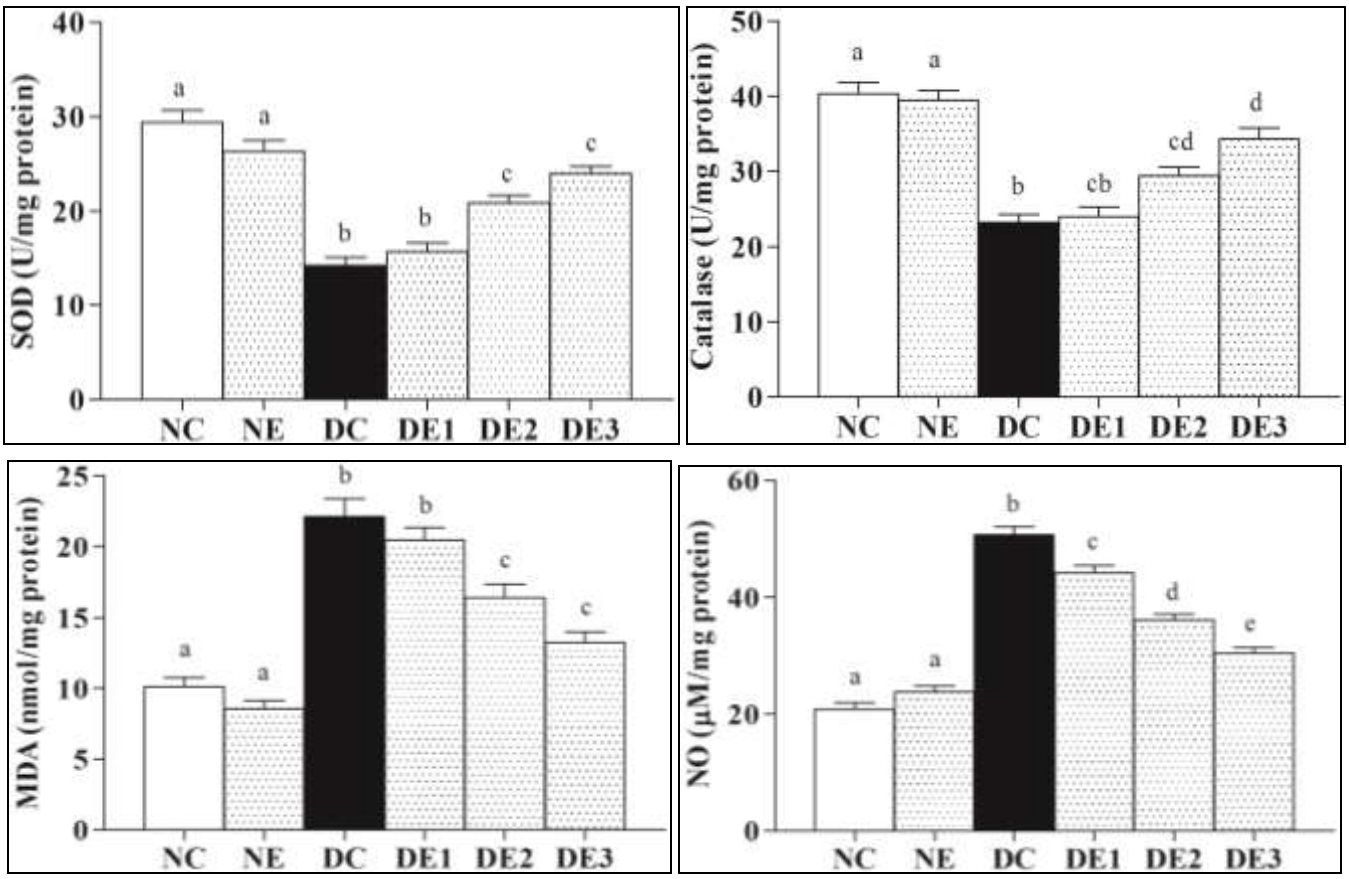

Fig 1: Effects of extract on superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA) and nitric oxide (NO) in kidney tissue lysate from different groups after eight weeks of treatment. Different superscripts (a, b, c, d) differ significantly (p<0.05). Data are presented as mean ± S.E.

Hyperglycemia causes the significant reduction in SOD and catalase enzyme activity in homogenate from diabetic rats as compared to normal control. Present study also shown that extract treated diabetic rats exhibited an elevated SOD and inhibited MDA significantly. Therefore, SRE significantly improved the antioxidant status in the hyperglycemic impaired kidney at some extent. Over all findings showed that the treatment with resin extract improved significant outcome probably by emphasizing ROS scavenging and strengthening antioxidants owing to resin loaded with phenolic and flavonoid contents. In the present study, lipid peroxidation is significantly lower in extract treated group, as compared to control, which manifested that SRE prevents lipid peroxidation by scavenging free radicals. Thus, SRE possesses antioxidant and free radical scavenging property, as well as enhancing the activities of other antioxidants, such as superoxide dismutase, catalase, and reduced glutathione.

Conclusion

In conclusion, the results of current study demonstrate that *Shorea robusta* resin might improve the renal function by reducing oxidative stress or ROS in diabetic rat kidney.

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