nitrogen (BUN), urea clearance, serum creatinine & creatinine clearance by using standard diagnostic kits (Span Diagnostics, Gujarat, India).

Assessment of oxidative stress

Post mitochondrial supernatant preparation (PMS)

Kidneys were, perfused with ice cold saline (0.9% sodium chloride) and homogenized in chilled potassium chloride (1.17%) using a homogenizer. The homogenates were centrifuged at 800 g for 5 minutes at 4°C to separate the nuclear debris. The supernatant so obtained was centrifuged at 10,500 g for 20 minutes at 4°C to get the post mitochondrial supernatant which was used to assay catalase and superoxide dismutase (SOD) activity.

Estimation of lipid peroxidation

The malondialdehyde (MDA) content, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid reacting substances (TBARS) by the method of Okhawa et al. [63]. Briefly, the reaction mixture consisted of 0.2 ml of 8.1% sodium lauryl sulphate, 1.5 ml of 20% acetic acid solution adjusted to pH 3.5 with sodium hydroxide and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid was added to 0.2 ml of 10%(w/v) of PMS. The mixture was brought up to 4.0 ml with distilled water and heated at 95°C for 60 minutes. After cooling with tap water, 1.0 ml distilled water and 5.0 ml of the mixture of n-butanol & pyridine (15:1 v/v) was added and centrifuged. The organic layer was taken out and its absorbance was measured at 532 nm. TBARS were quantified using an extinction coefficient of $1.56 \times 10^5\ M^{-1}/cm^{-1}$ and expressed as nmol of TBARS per mg protein. Tissue protein was estimated using Biuret method of protein assay and the renal MDA content expressed as nanomoles of malondialdehyde per milligram of protein.

Estimation of reduced glutathione

Reduced glutathione (GSH) in the kidneys was assayed by the method of Jollow et al [64]. Briefly, 1.0 ml of PMS (10%) was precipitated with 1.0 ml of sulphosalicylic acid (4%). The samples were kept at 4°C for at least 1 hour and then subjected to centrifugation at 1200 g for 15 minutes at 4°C. The assay mixture contained 0.1 ml filtered aliquot and 2.7 ml phosphate buffer (0.1 M, pH 7.4) in a total volume of 3.0 ml. The yellow colour developed was read immediately at 412 nm on a spectrophotometer.

Estimation of superoxide desmutase(SOD)

SOD activity was assayed by the method of Kono et al[65] The assay system consisted of EDTA 0.1 mM, sodium carbonate 50 mM and 96 mM of nitro blue tetrazolium (NBT). In the cuvette, 2 ml of above mixture, 0.05 ml hydroxylamine and 0.05 ml of PMS were taken and the auto-oxidation of hydroxylamine was observed by measuring the absorbance at 560 nm.

Estimation of catalase

Catalase activity was assayed by the method of Claiborne et al [66]. Briefly, the assay mixture consisted of 1.95 ml phosphate buffer (0.05 M, pH 7.0), 1.0 ml hydrogen peroxide (0.019 M) and 0.05 ml PMS (10%) in a final volume of 3.0 ml. Changes in absorbance were recorded at 240 nm. Catalase activity was calculated in terms of k minutes-1.

Assessment of serum/tissue nitrite concentration

Serum and tissue nitrite was estimated using Greiss reagent and served as an indicator of NO production. 500 µl of Greiss reagent (1:1 solution of 1% sulphanilamide in 5% phosphoric acid and 0.1% napthaylamine diamine dihydrochloric acid in water) was added to suitably diluted 100 µl of plasma and absorbance was measured at 546 nm [67]. Nitrite concentration was calculated using a standard curve for sodium nitrite. Nitrite levels were expressed as µmol/ml in serum and as µmol/mg protein in homogenate.

Histopathological examination

For microscopic evaluation kidney were fixed in 10% neutral phosphatebuffered formalin solution. Following dehydration in ascending series of ethanol (70, 80, 96, 100%), tissue samples were cleared in xylene and embedded in paraffin. Tissue sections of 5 µm were stained with hematoxylin-eosin (H-E). A minimum of 10 fields for each kidney slide were examined and assigned for severity of changes by an observer blinded to the treatments of the animals and assigned for severity of changes using Scores of none (-), mild (+), Moderate (++) and Severe (+++)

Statistical analysis

Results were expressed as mean± SEM. The intergroup variation was measured by one way analysis of variance (ANOVA) followed by Fischer's LSD test. Statistical significance was considered at $p < 0.05$. The statistical analysis was done using the Jandel Sigma Stat Statistical Software version 2.0.

Authors' contributions

Naveen Tirkey, Gangandeep kaur and Garima Vij did all the biochemical estimations in kidney and did the data interpretation after statistical analysis. Kanwaljit Chopra contributed in manuscript preparation.

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