

# Ameliorative Effects of MnO Nanoparticles on Acute Kidney Injury Model in Rats

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## Abstract

**Aims:** Acute kidney injury (AKI) is described as an abrupt and generally reversible damage of renal function that occurs over days or weeks. Nearly 20% of nephrotoxicity is associated with the use of antimicrobials and antibiotics. **Materials and Methods:** The evaluation of the possible protective effect of MnO nanoparticles (NPs) as well as explore their toxic effects on the kidneys of rat models. MnO NPs were prepared and evaluated for their physicochemical properties. The effect of MnO NPs was compared with gentamicin. **Results and Discussion:** *In vivo* data showed no toxicity according to zootechnical parameters, blood biochemistry, and organ system toxicity in MnO NP treatment groups. The protective effect of the MnO NPs group as compared to the gentamicin treatment group was observed. **Conclusion:** Hence, using the MnO NPs will help improve the AKI values.

**Key words:** Acute kidney injury, biochemical parameters, hematology, MnO nanoparticle

## INTRODUCTION

Kidney injury is categorized into acute kidney injury (AKI) and chronic kidney injury. AKI is described as a rapid and generally reversible loss of renal function that occurs over days or weeks. AKI is determined based on serum creatinine levels or urine production. Serum creatinine is more significant than 0.3 mg/dL (26.4 μmol/L), with 50% increases from the baseline, whereas urine production is <0.5 mL/kg/h for more than 6 h in AKI.<sup>[1,2]</sup> More than 13 million people are affected by AKI every single year, with a frequency of 33.7% in children and 21.6% in adults in a single trial of hospitalization. AKI has a high mortality and morbidity rate. 1.7 million/year is the mortality rate of AKI with 13.8% in children and 23.9% in adults.<sup>[3,4]</sup> It has been studied that 20% of nephrotoxicity occurred because of different medications.

Some medications, such as antimicrobials and antibiotics, have been considered AKI-associated medication. These medications cause structural and functional renal abnormalities. Acute renal failure and acute tubular necrosis mainly occur

because of ischemia. There are various antibiotics, such as sulfonamides tetracyclines, vancomycin, daptomycin, beta-lactams, fluoroquinolones, and aminoglycosides, which can harmfully affect kidney function.<sup>[5,6]</sup> Aminoglycosides are the major cause of kidney injury, which occurs due to changes in multiple factors.<sup>[7]</sup> It can induce renal vasoconstriction, leading to decreased renal blood supply and ultimately prone to renal ischemia. Aminoglycosides are excreted through the kidney, so these are concentrated in the proximal tubular cells of the kidney and are associated with tubular epithelial cell necrosis. Almost 5–25% of cases of AKI have been linked with aminoglycosides after 5–7 days of its treatment.<sup>[8]</sup> AKI is also associated with different risk factors, such as age, chronic kidney disease, diabetes, iodinated contrast, liver disease, hypoalbuminemia, nephrotoxic antibiotics, congestive heart failure, hypotension, and sepsis.<sup>[9]</sup> Among

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these aminoglycosides, gentamicin causes nephrotoxicity and ototoxicity, which occurs at its lowest dose. Nephrotoxicity by gentamicin is associated with urinary accumulation, reduced blood flow, and dilution dysfunction.<sup>[10]</sup> Gentamicin is responsible for acute renal failure and tubular necrosis, accounting for 85% of the occurrences. The rate of nephrotoxicity due to the use of gentamicin is approximately 1.7–58% of patients. Gentamicin is an effective antibiotic measured by the World Health Organization.<sup>[11]</sup> It is used for the treatment of lethal infections, particularly those that are triggered by Gram-negative bacteria. Gentamicin is used only for a short period because it causes acute renal injury, which varies between 2% and 55% of patients. Gentamicin is responsible for renal dysfunction when given up to 30% of patients for more than 7 days. The efforts to prevent gentamicin-inducing AKI have failed, so its use in clinical settings is restricted due to its toxicity.<sup>[12]</sup> There is decreased concentration of antioxidant markers such as superoxide dismutase (SOD), catalase, glutathione (GSH), and glutathione peroxidase, and higher formation of reactive oxygen species (ROS) for instance hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), hydroxyl radical (OH<sup>-</sup>), and superoxide anion (O<sup>-2</sup>) associated with gentamicin induced-nephrotoxicity. Moreover, gentamicin is also linked with inflammation, tubular necrosis oxidative stress, and reduced glomerular filtration rate.<sup>[6,13]</sup>

MnO is an essential microelement biological role in mammals for growth regulation, participating as a cofactor in several metabolic pathways and helping to maintain fragile homeostasis.<sup>[14]</sup> It is also environmentally friendly, low cost, easy to prepare well, and necessary for the activity of many enzymes. Nanoparticles (NPs) are progressively being cast off to overcome the biological barriers in the body and substantially improve the delivery of drugs to specific organs or tissues.<sup>[15]</sup> The development of NPs is to improve the balance between the safety and efficacy of drug compounds.<sup>[16]</sup> MnO NPs were developed by the thermal decomposition method. Furthermore, it was reported that MnO NPs ameliorated the nephrotoxicity induced by antibiotics in rats through their antioxidant effect.<sup>[17]</sup> Ameliorative effects of MnO NPs on the AKI model in rats on zootechnical parameters, anthropometry, renal and liver health biomarkers, serum electrolytes, serum lipid biomarkers, serum and kidney redox status, and kidney histology were determined.

## MATERIALS AND METHODS

### Preparation of MnO NPs

MnO NPs were prepared by the previously reported method.<sup>[18]</sup> Briefly, the thermal decomposition of the precursor was performed in the furnace under a constant air atmosphere at 550°C for 3 h. Just about 1 g of precursor powder was kept in an alumina crucible without pressing into an open 40 mm diameter and 20 mm height. To avoid the dispersion of powder at high temperatures and releasing of gasses because of precursor decomposition, aluminum foil is used to cover

the crucible. Scanning electron microscope (SEM) images were taken for a better understanding of particle morphology.

### Experimental animals

Adult rats were purchased from the breeding center. The animals were caged in standard cages which were adorned by wood chip bedding and kept under standard laboratory conditions (alternate 12-h light/dark cycle, temperature 24 ± 3°C, and relative humidity around 60%) at the animal house of the Northern Border University. Animals were freely allowed to standard pelleted feed and fresh tap water *ad libitum* throughout the experiments. Animal handling was carried out as stated by the university ethical procedures for the caution and use of laboratory animals. The study procedure was approved by the Bioethical Committee at Northern Border University (HAP-09-A-043).

### Experimental design

Following the acclimatization period of 1 week under standard laboratory conditions, animals ( $n = 30$ ) were randomly divided into six groups with equal numbers ( $n = 5$ ). Group-I (Control group): The rats of this group were treated with 0.9% NaCl (0.5 mL/day) intraperitoneally and oral route (0.5 mL/day) for 12 sequential days. Group-II (MnO-NP): The rats of this group will be treated with MnO-NP orally. Group-III (GM): The rats of this group will be treated with GM (100 mg/kg) intraperitoneally and 0.9% NaCl (0.5 mL/day) orally. Group-IV (Treated): The rats of this group will be treated with GM (100 mg/kg) intraperitoneally and MnO-NP (Dose 1) orally. Group-V (Treated): The rats of this group will be treated with GM (100 mg/kg) intraperitoneally and MnO-NP (Dose 2) orally. Group-VI (Treated): The rats of this group will be treated with GM (100 mg/kg) intraperitoneally and MnO-NP (Dose 3) orally.

### Parameters

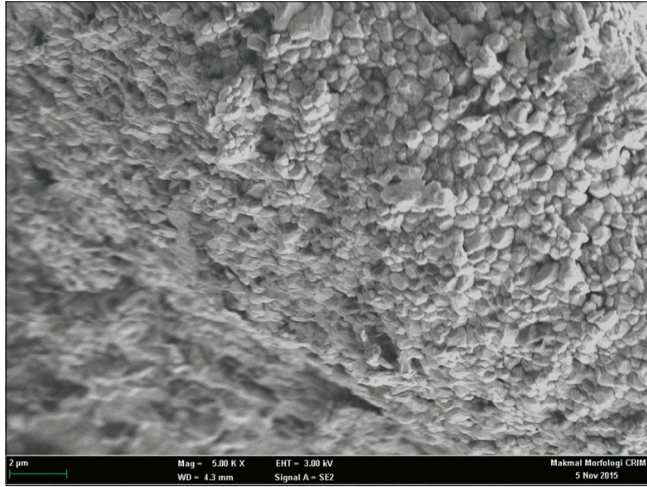
Zootechnical parameters (feed intake, water intake, body weights [BW], organ weights, anthropometry).

Water intake will be measured by the difference between the final volume and the remaining volume over 24 h with the help of a measuring cylinder. This volume will be considered daily water intake for every animal in each group. Feed intake can be measured by the difference between the amount of feed supplemented and the leftover amount of feed. BW and anthropometry measurements will be measured weekly.

### Serum urea, creatinine, aniline transaminase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP)

Blood samples will be collected through the intracardiac route and will be placed in plane vials. Thereafter,

centrifugation will be carried out @ 1500 rpm for 10 min at a temperature of 4°C. The serum thus obtained will be stored at -80°C for analytical purposes. Serum urea, creatinine, ALT, AST, and ALP will be measured using commercially available kits corresponding to the manufacturer's guide from humans (diagnostics worldwide), (Wiesbaden, Germany).<sup>[19]</sup>



**Figure 1:** Scanning electron microscope images of MnO nanoparticles

### Serum electrolytes

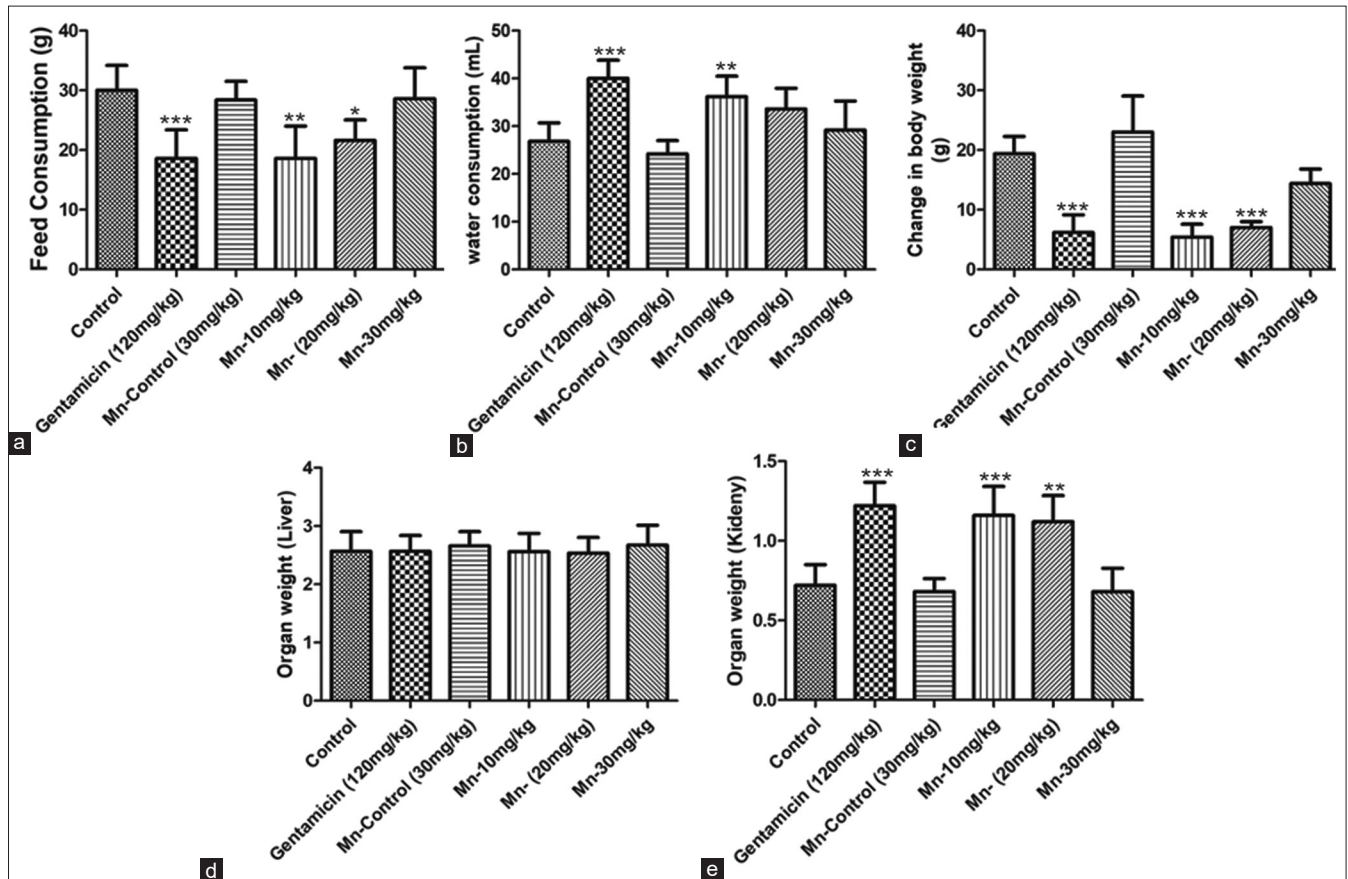
Serum electrolytes (sodium, potassium, and chloride) will be measured by spectrophotometry using kits.<sup>[20]</sup>

### Serum and kidney malondialdehyde (MDA)

A total of 100 µL serum samples will be added in four test tubes. 100 µL SDS (8.1%), 750 µL of 20% acetic acid (pH3.5), and 750 µL TBA 0.8% solutions will be added in test tubes. Gently mix and 350 µL distilled water will be added. These test tubes will be heated for 60 min at 95°C and cool. 1.5 mL N-butanol and 500 µL distilled water will be added and shaken vigorously. Then test tubes will be centrifuged for 10 min at 4000 rpm. The absorbance of the sample will be measured by spectrophotometry at 532 nm wavelength. Serum MDA concentration will be measured by a standard curve.

### Serum and kidney SOD

SOD activity will be measured using a SOD assay kit (Cayman Chemical Company, USA). The absorbance of the sample will be measured at 440–460 nm wavelengths. The sample will be run 2 or 3 times the average will be calculated.



**Figure 2:** Changes in feed consumption (a), water consumption (b), body weight (c), organ weight, liver (d), organ weight and kidney (e), in the rat model of the control group as compared to the control positive and control negative treatment group. Data means ± standard deviation (n=5)



## Serum and kidney catalase

Catalase activity will be calculated by incubating the sample in 1.0 mL substrate which will be prepared by 65  $\mu\text{mol/mL}$  hydrogen peroxide in 60  $\text{mmol/L}$  sodium-potassium phosphate buffer, pH 7.4 for 3 min at 37°C. Ammonium molybdate will be added to stop the reaction. The absorbance of a yellow compound will be measured at 374 nm.<sup>[21]</sup>

## Histopathology of the kidney

After blood sampling, the animals will be euthanized and after a longitudinal incision through the abdominal cavity, the cavity will be explored for kidneys. Then both kidneys will be excised, and tissue sampling will be carried out. 4  $\mu\text{m}$  slices of the kidney will be removed. The tissue sample will be fixed in 10% neutral buffered formalin. The sample will be dried and then incorporated with paraffin wax. Thin slices will be microtome and stained.

## Statistical analysis

All the tests were carried out in triplicate for proximate analysis. Data obtained were analyzed by analysis of variance using the software GraphPad PrismStatistics version 6.0.

## RESULTS AND DISCUSSION

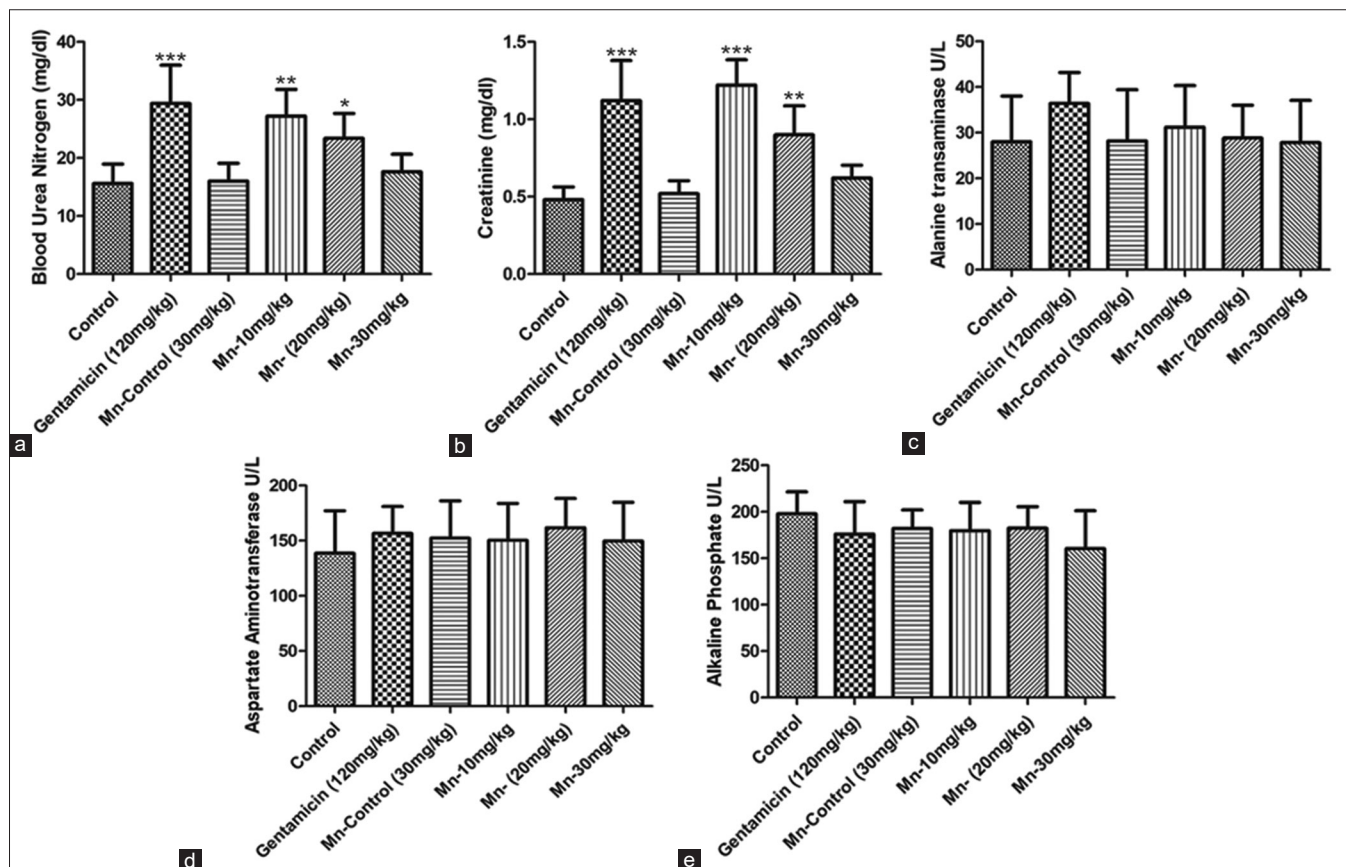
### Preparation and characterization of MnO NPs

The thermal decomposition of the precursor method was used to prepare the NPs. Particle size and morphology of MnO NPs were evaluated by SEM images, Figure 1. The diameter of the NPs ranges from 25 to 35 nm.<sup>[22]</sup> Moreover, it was observed that NPs showed strong attractive forces as well as a tetragonal crystal shape.

### Zootechnical parameters (BW, feed intake, water intake, organ weights, and anthropometry)

The BW of an individual rat was recorded on day 1 and thereafter on day 12. Animals were individually caged and feed consumption was calculated by subtracting the amount of feed remaining with the feed added initially. Significant changes in BW were observed in the treatment group of gentamicin (Group-II) ( $P < 0.05$ ), whereas non-significant changes in BW were observed in Group-VI treating with high doses of MnO NPs [Figure 2a] ( $P > 0.05$ ).

It has been observed that zootechnical parameters indicate feed efficacy.<sup>[23]</sup> Similarly significant changes in water consumption have been observed in the animals treated with



**Figure 3:** Serum urea (a), creatinine (b), aniline transaminase (c), aspartate aminotransferase (d), and alkaline phosphatase (e), Data means  $\pm$  standard deviation ( $n=5$ )

gentamicin whereas no change in water consumption was observed in the higher dose treatment group [Figure 2b].<sup>[24]</sup> All the animals in each group were individually weighed and a significant decrease in BW was found in animals belonging to Groups II, IV, and V, whereas no significant weight loss was observed in Group VI.<sup>[25]</sup> Animals were sacrificed and their liver and kidneys were collected. It has been reported that toxic dose affects organ weight.<sup>[26]</sup> In the current work, the absolute weight of the liver did not significantly vary between the groups as compared to the control [Figures 2c-e]. However, significant decreases in the weight of kidneys were observed in Group-II, IV, and V, indicating the toxic effect of gentamicin on the kidneys. Whereas the protective effect of MnO NPs at the dose of 30 mg/kg was observed in this study.

### Serum urea, creatinine, ALT, AST, and ALP

The biochemical parameters are present in Figure 3a. Blood urea nitrogen (BUN) describes the number of nitrogen atoms in the blood. It has been reported that changes in blood parameters and enzyme activity indicate cell damage, organ function as well as toxicity.<sup>[27]</sup> Moreover, in the previous study, the protective effect of MnO NPs was observed in cells treated with H<sub>2</sub>O<sub>2</sub> for 6 h.<sup>[28]</sup> For BUN, a significant

increase was observed in Group II, Group IV, and Group V as compared to the control group (Group I) ( $P < 0.05$ ), whereas the protective effect of the higher dose of MnO NPs (Group-VI) was observed.

The present data are in line with the previously published result which showed a significant increase in creatinine levels in the gentamicin treatment group.<sup>[29]</sup> The effects of gentamicin and protective responses of the higher dose of MnO NPs (Group-VI) are presented in [Figure 3b] whereas a significant increase in serum creatinine level was observed in Group-IV and Group-V, which received a low dose of MnO NPs.<sup>[30]</sup> ALT is the main enzyme of the liver which releases in case of hepatotoxicity [Figure 3c]; no significant differences were found between any other drug treatment groups and the control ( $P > 0.05$ ). AST analysis revealed no significant changes in [Figure 3d]. ALP (liver produces this enzyme, in case of biliary obstruction), no significant changes were observed in groups as compared to the control group ( $P > 0.05$ ) [Figure 3e].<sup>[31]</sup>

### Serum electrolytes

Serum electrolytes are the most important ions in the body that play a significant function in maintaining acid-base

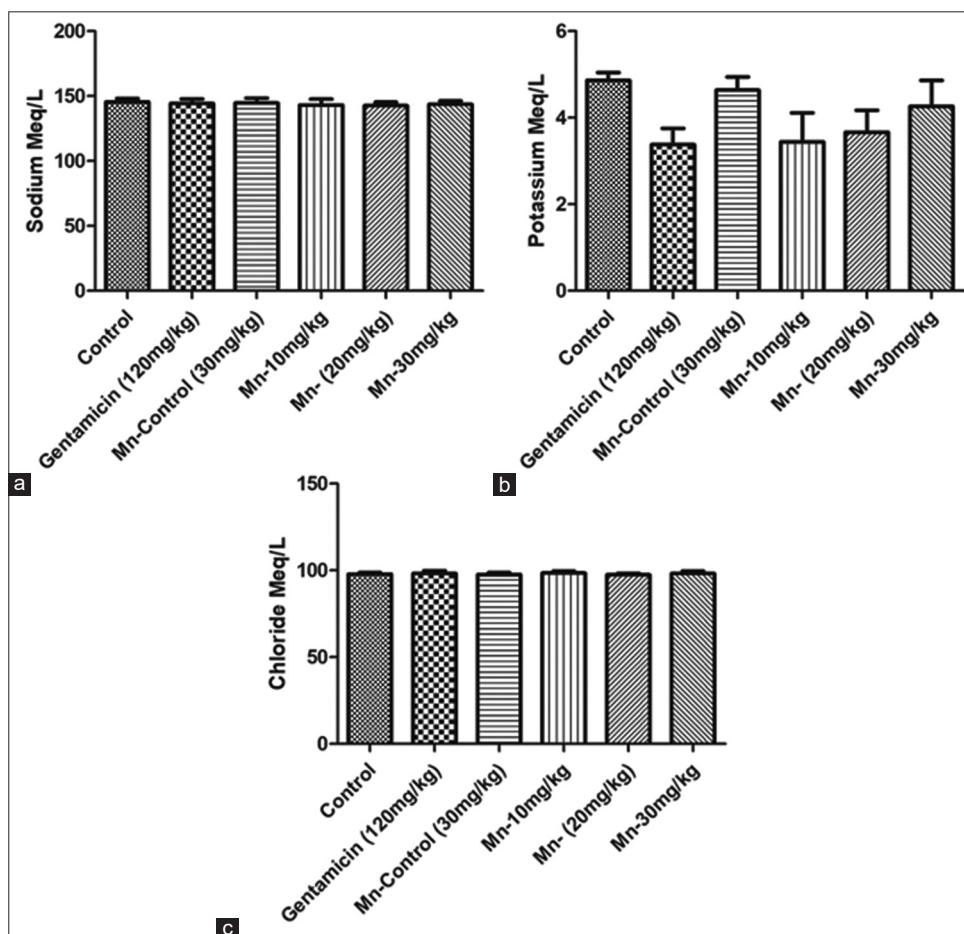


Figure 4: Serum electrolyte sodium (a), potassium (b), and chloride (c)

balance through homeostasis and its counter correlation with bicarbonates. The kidney and gastrointestinal tract are the foremost mediators of homeostasis of chloride ions. The proximal tubule acts as most of the electrolyte handling site through its paracellular and transcellular responsible for reabsorption of more than 50% of filtered chloride.<sup>[32,33]</sup> The results showed no significant change ( $P > 0.05$ ) in serum sodium and chloride concentration whereas significant decrease ( $P < 0.05$ ) in serum potassium concentration in rats treated with gentamicin compared with the control group [Figure 4a-c].

### Serum and kidney MDA

Another important mechanism in undergoes in case of gentamicin toxicity is the formation of superoxide and hydroxyl anion which ultimately stop the electron transport chain<sup>[34]</sup> and cause frightening modifications in cell structure and function.<sup>[35]</sup> A significant increase in serum MDA was observed in gentamicin and low-dose treatment group IV [Figure 5a], whereas a significant increase in kidney MDA

in Group-II, Group-IV, and Group-V was observed [Figure 5b] ( $P < 0.05$ ), however, Group-VI showed the protective effect of MnO NPs.

### Serum SODs and kidney SODs

Oxidative stress plays a pivotal role in the development of nephropathy. Whereas SODs are important antioxidant enzymes that scavenge oxygen radicals through oxidation/reduction of transition metal ions present.<sup>[36]</sup> Results of this study showed a significant increase in serum and kidney %SOD, which indicates that oxidative stress has been generated in the gentamicin treatment group ( $P < 0.05$ ) Figure 6.

Serum SOD did not show any significant decrease in the high and low treatment group with MnO NPs. On the other hand, a slight significant decrease in kidney SOD% was observed in the treatment group with less dose but no significant decrease in the high dose treatment group ( $P > 0.05$ ) was observed.

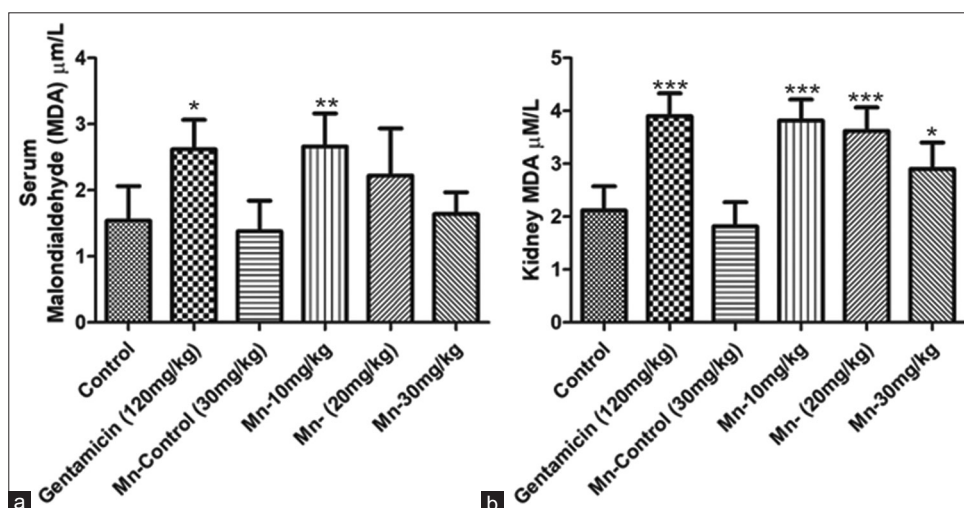


Figure 5: (a) serum malondialdehyde and (b) kidney malondialdehyde. Data means  $\pm$  standard deviation ( $n=5$ )

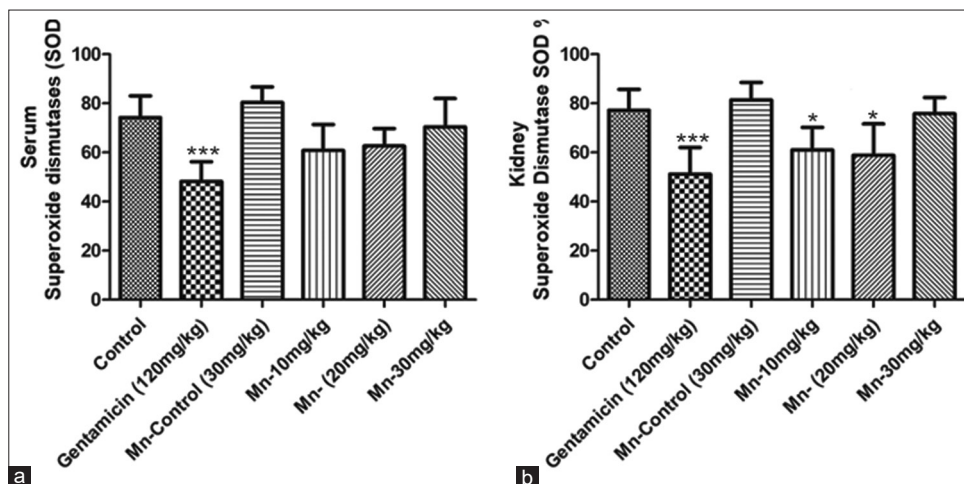


Figure 6: (a) serum superoxide dismutase and (b) % of kidney superoxide dismutases. Data means  $\pm$  standard deviation ( $n=5$ )



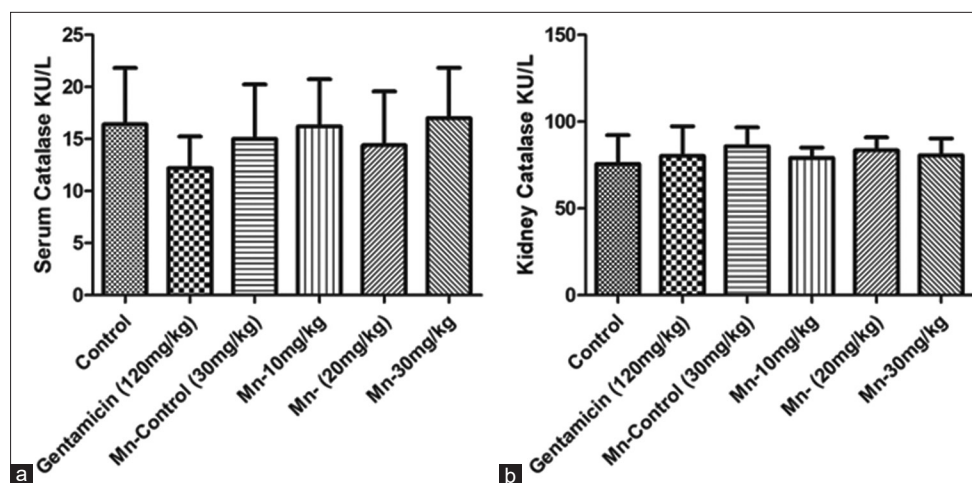


Figure 7: (a) serum catalase and (b) kidney catalase level. Data means  $\pm$  standard deviation ( $n=5$ )

### Serum catalase and kidney catalase

Figure 7a and b showed a non-significant ( $P > 0.05$ ) reduction in the serum catalase level in both the treatment and control group indicating that both control positive and no effect on control negative groups were observed. The shielding impact of MnO NPs on the animal model is in evident the previous data.<sup>[30]</sup>

## CONCLUSION

The present study was conducted to estimate the shielding effect of MnO NPs. Nano-sized MnO particles were synthesized through the thermal decomposition method. The *in vivo* study on Sprague-Dawley rats showed the protective effect of MnO NPs on the gentamicin treatment groups. Various results are evident that inflammation nor any significant change was observed. No modifications in BW and water consumption of animals were recorded, indicating the absence of detrimental impact of the NPs. Serum superoxide and catalase data suggested good biocompatibility of MnO NPs and their shielding role at the cellular level due to ROS-scavenging potential.

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