



# RESTORATIVE EFFECTS OF *AZADICARACHTA INDICA* ON THE KIDNEY HISTOMORPHOMETRY IN CISPLATIN-TREATED WISTER ALBINO RATS

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## ABSTRACT

**Background:** Cisplatin is used for treatment of soft tissue tumors and has been associated with nephrotoxicity. *Azadirachta indica* is a local herb that has been associated with attenuation and counteracting the nephrotoxic effects of cisplatin. However, there is inadequate histomorphometric evidence to support this potential. This study therefore aims to evaluate the possible Restorative Effects of *Azadirachta indica* on Kidney Histomorphometry in Cisplatin-Treated Wister Albino Rats. **Methods:** Twenty-five Wister Albino Rats of weight 250-400g were randomly selected into five groups of control or experimental with each group containing five animals. The control group only fed on water *ad libitum*. Experimental group one was administered with intraperitoneal injection 0.28mg/kg of cisplatin, other three experimental groups were given a constant dose of intraperitoneal 0.28mg/kg of cisplatin on day one followed by per oral 6.67mg/kg/bwt/day experimental group two, 5mg/kg/bwt/day experimental group three and 3.33mg/kg/bwt/day experimental group four of *azadirachta indica* on day five for seven days. On day thirteen all animals were euthanized. The kidneys were histologically prepared. Photomicrographs were taken and uploaded on stepanizer for histostereology. **Results:** The glomerulus and the Bowmans capsule of experimental group one, two and four had a significant ( $P \leq 0.0001$ ) reduction and increased respectively, only the glomerulus space for the experimental group two had significant ( $P \leq 0.002$ ) increase as compared to the control. **Conclusion:** Out of the three doses of *azadirachta indica*, the medium dose of 5.0mg/kg was able to restore the histomorphometry and histostereology of the kidney on administration of 0.28mg/kg of cisplatin.

**Keywords:** Restorative, histomorphometry, *Azadirachta indica*, Cisplatin, histostereology

**DOI:** <https://dx.doi.org/10.4314/aja.v12i2.13>

## INTRODUCTION

*Azadirachta indica* is a herbal medicine used traditionally and is believed to treat several conditions such as intestinal worms, diabetes, skin condition and others (Diabaté et al., 2014). The herb is believed to have an Indian origin and is locally found abundant in Kenya (Yao et al., 2007). It's believed to have nephroprotective effects against cisplatin (Abdel Moneim et al., 2014). The therapeutic effect of *azadirachta indica* is dependent on its active components such as ascorbate, nimboline and azadirachtin responsible for the antioxidant effects

(Carpenter, 2020). *Azadirachta indica* traps the free radical oxygen in the circulation hence attenuating or counteracting the effects of cisplatin (Abireh et al., 2020).

Nephrotoxicity is the process where the kidney is damaged when it has been introduced by a toxic substance (Al-Naimi et al., 2019). Cisplatin is metabolized in the kidney causing nephrotoxicity (Miller et al., 2010).

Kidney disease is among the leading cause of death with a prevalence of 13% worldwide

(Lv & Zhang, 2019) and over 4 million of Kenyans are suffering from renal disease. Cisplatin is a cytotoxic drug used alongside other chemotherapy medications. Despite cisplatin being effective, 20-30% of patients using it develop kidney disease (Burns et al., 2021). So, cisplatin is used with precaution and sometimes restricted because of its known side effect of nephrotoxicity. Among the elderly, the incidence is high up to 65%

of the patient's taking cisplatin develop nephrotoxicity (Latcha et al., 2016). Cisplatin produces free radical oxygen that damages the mitochondria at the tubules of the nephrons hence causing nephrotoxicity (Zhang et al., 2021). *Azadirachta indica* destroys the free radical oxygen in the circulation thus helping to attenuate and counteract the effects of cisplatin.

## MATERIALS AND METHODS

The study was conducted at a medical institution and followed all the necessary research ethical approvals with the following reference numbers UEAB/ISERC/01/2023 and NACOSTI/P/23/23376. The sample size of twenty-five Wister albino rats was calculated using the modified human resource formulae and were randomly separated into five groups of five animals each. All animals were put in cages for seven days for acclimatization after that the control group was only fed on water *adlibitum* while other experimental groups were given a constant dose of cisplatin of 0.28mg/kg once intraperitoneally to induce nephrotoxicity. After five days, renal function test was done to confirm kidney injury for all the experimental groups. The experimental group one only received 0.28mg/kg of cisplatin, experimental group two, three and four were given a constant dose of 0.28mg/kg of cisplatin and after five days were administered with different doses of *azadirachta indica* of 6.67mg/kg/bwt/day, 5.0mg/kg/bwt/day and 3.33mg/kg/bwt/day respectively and this was adopted from (Iman et al., 2021; Jacob et al., 2022; Perše, 2021) this was given for seven days for restoration of the kidney damage and after

which the rats were euthanized. The kidneys were dissected, the cortical regions were fixed, dehydrated, embedded, sectioned, stained with hematoxylin and eosin, photomicrographs were taken and reported as per the group. The photomicrographs were taken at  $\times 100$  magnification. Simple random sampling was used in selecting the photomicrographs that were further uploaded on the stepanizer stereological tool for measuring the surface area of the glomerulus space, glomerulus and the bowman's capsule.

The surface area was calculated as follows; the total number of point grid within the specified region multiplied by estimated surface area of the point grid divided by the linear magnification (Tschanz et al., 2011). The measurements of the surface areas of the glomerulus, bowman's capsule and glomerulus space obtained from the stepanizer were put into an Excel sheet and uploaded into the SPSS. One way ANOVA and post hoc test was used in comparing data from the experimental group to control group where the P-value of less or equal to 0.05 was found to be of statistical significance at 95% confidence interval.

## RESULTS

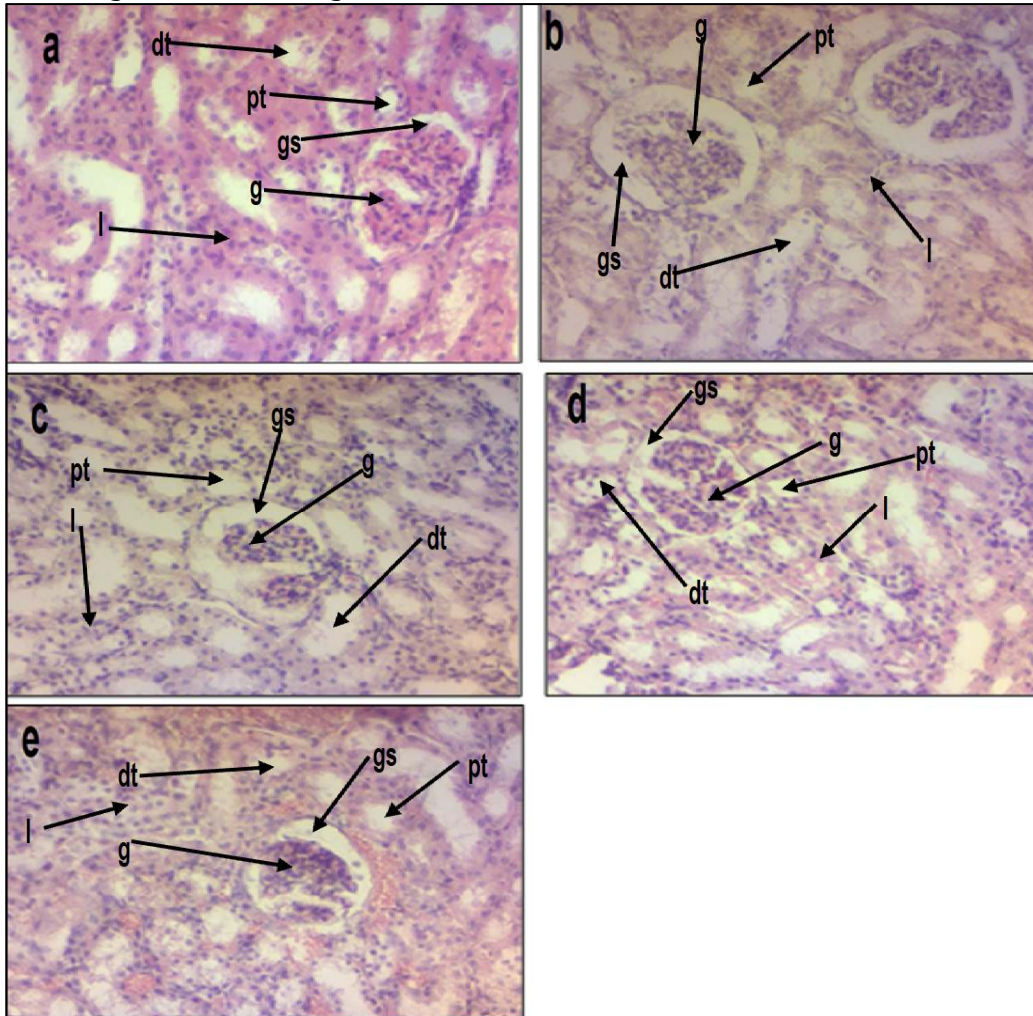
### **Restorative histo- structural changes of the kidney on administration of cisplatin followed by different doses of *Azadirachta indica***

In the photomicrographs b, c and e had tubular necrosis of the distal and proximal

convoluted tubules, shown by shrinking of their epithelium and necrotic tissues both in the right and the left kidneys. There also was marked renal corpuscle involvement shown by necrosis of the parietal layer of the bowman's capsules and shrinking of the

glomerulus capillaries mostly on the right kidney as compared to the left one. In the photomicrographs a and d had normal microscopic features of the renes, the renal corpuscle is normal with no necrotic or apoptotic signs in both the right and left kidney with the glomerulus having and

irregular in shape. The tubules are normal with no dilatation of the epithelium in both the right and left kidney. The renal interstitial is normal with no vacuolation, necrotic, apoptotic cells and fibrosis in both the right and left kidney. (Figure 1.1)



**FIGURE 1.1:** Photo-Micrographs of the Control and Experimental groups stained with H&E  $\times 100$  of the kidney section **Key:** a- control group, b- experimental group one, c- experimental group two, d- experimental group three, e- experimental group four. g – glomerulus, gs- glomerulus space, pt- proximal convoluted tubules, dt- distal convoluted tubules and I- interstitial part.

**Histo- stereological analysis**

The surface area of the renal tubules of control and experimental group could not be measured as the epithelial cells of some of the tubules in study group were disintegrated and distorted. The surface area of the glomerulus, glomerulus space and the

bowman capsule were determined using the stepanizer stereological tool.

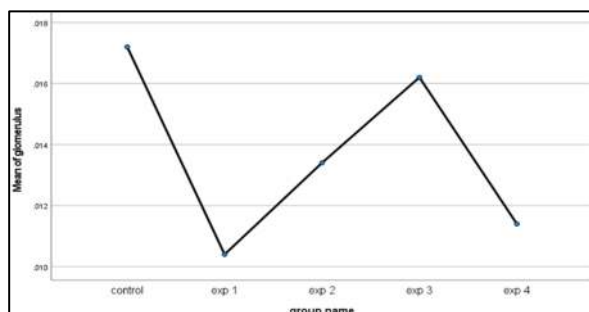


FIGURE 1.2: Mean surface area of the glomerulus of the control and the experimental groups Key: control (water+ feeds), exp 1- Experimental group one (0.28mg/kg Cisplatin only), exp 2- Experimental group two 0.28mg/kg Cisplatin + Azadirachta indica 6.67mg/kg), exp 3- Experimental group three (0.28mg/kg Cisplatin + Azadirachta indica 5mg/kg), exp 4- Experimental group four (0.28mg/kg Cisplatin + Azadirachta indica 0.33mg/kg).

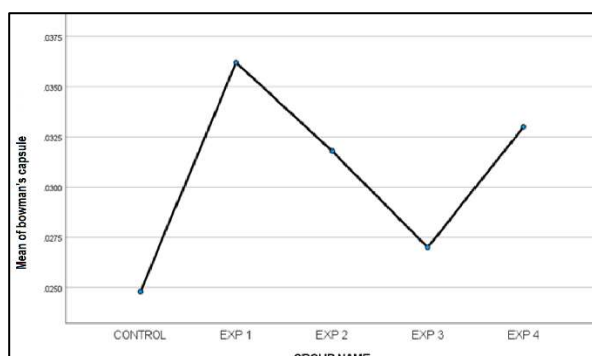


FIGURE 1.3: Mean surface area of the Bowman's capsule of the control and the experimental groups. Key: control (water+ feeds), exp 1- Experimental group one (0.28mg/kg Cisplatin only), exp 2- Experimental group two 0.28mg/kg Cisplatin + Azadirachta indica 6.67mg/kg), exp 3- Experimental group three (0.28mg/kg Cisplatin + Azadirachta indica 5mg/kg), exp 4- Experimental group four (0.28mg/kg Cisplatin + Azadirachta indica 0.33mg/kg).

Renal histo-architectural changes are associated with nephrotoxicity. In the current study, there was a significant shrinkage of the glomerulus, and dilatation of proximal and distal convoluted tubules in the experimental group one, two and four as compared to the control group. This might be due to the circulation of free oxygen radical, endoplasmic and intracellular stress, and mitochondrial damage induced by cisplatin toxicity that led to necrosis of the parietal layer of the Bowman's capsules, destruction of the basement membrane of

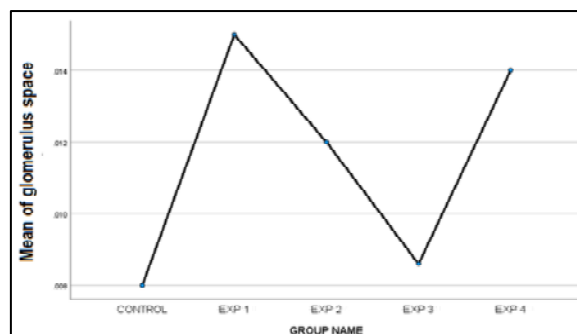


FIGURE 1.4: Mean surface area of the Bowman's capsule of the control and the experimental groups. Key: control (water+ feeds), exp 1- Experimental group one (0.28mg/kg Cisplatin only), exp 2- Experimental group two 0.28mg/kg Cisplatin + Azadirachta indica 6.67mg/kg), exp 3- Experimental group three (0.28mg/kg Cisplatin + Azadirachta indica 5mg/kg), exp 4- Experimental group four (0.28mg/kg Cisplatin + Azadirachta indica 0.33mg/kg).

The surface area of the glomerulus of experimental group one, two and four had a significant reduction ( $P < 0.0001$ ). The surface area of the glomerulus space and Bowman's capsule significantly ( $P < 0.0001$ ) increase in Experimental group one, two and four, only glomerulus space of the Experimental two had a significant increase ( $P \leq 0.002$ ). The P value was tested between the mean difference of surface area of the glomerulus, glomerulus space and the Bowman's capsule for the control and Experimental one, two, three and four using one way ANOVA and post hoc test (Figure 1.2, 1.3, 1.4).

## DISCUSSION

the glomerulus, and tubular necrosis. From the literature reviewed (Al-Kahtani et al., 2014; Ibraheim & Eldamaty, 2018; Kumar Singh & Singh Karchuli, 2014) most authors recorded significant changes in the glomerulus, proximal, and distal convoluted tubules in cisplatin-induced nephrotoxicity, in line with the present findings. However, (Albalawi et al., 2023) reported contrary findings when they noted significant involvement of the interstitial part of the kidney as seen by the deposition of collagen fibrils following gentamycin-induced

nephrotoxicity. This may be due to inflammation of the intestinal part as a result of gentamycin toxicity. There was no significant change in the histo-architecture of experimental group three as compared to the control group, this perhaps demonstrated restoration of the glomerulus, distal, and proximal convoluted tubules. This study's findings concurred with those of (Abireh et al., 2020) who recorded similar results after treatment of ibuprofen-induced nephrotoxicity with *Azadirachta indica*.

The renal corpuscle, the surface area of the glomerulus, bowman's capsule and glomerulus space can be associated with the histo-stereological changes in assessing drug-induced nephrotoxicity. There was a significance increase in the surface area of the bowman's capsule in the experimental group one, two and four as compared to the control group. This may perhaps be due to the destruction of its parietal layer. The current study findings are in tandem with the results of

(Koca et al., 2013; Sasaki et al., 2018; Sobolev et al., 2021) who recorded a significant increase in the surface area of bowman's capsule after introduction of - induction of a nephrotoxic agent. There was no significance increase in the surface area of the bowman's capsule of the experimental group three as compared to the control group and this may suggest restoration. There was a significance reduction in the surface area of the glomerulus in the experimental group one, group two, four as compared to the control. This may be due to the destruction of the basement membrane or loss of appetite that led to dehydration. Results from Dixit *et al.*, (2014) also recorded a significant reduction in the surface area of the glomerulus following monosodium

glutamate-induced nephrotoxicity, which he suggested a reduction in glomeruli as a result of exudation of contents of capillaries, high cellular proliferation, and hyalinization.

There was a significance increase in the surface area of glomerulus space in the experimental group one, two and four as compared to the control group. This could have been due to the shrinking of the glomerulus and dilatation of the bowman's capsule. Previous studies from the literature reviewed (Owagboriaye et al., 2022; Tobar et al., 2013) also recorded a significance increase in the enlargement of glomerulus space as a result of hyperfiltration of the glomerulus space and degeneration of the glomerulus capillaries when exposed to nephrotoxic agents. There was no significance increase in the surface area of the glomerulus space of the experimental three as compared to the control thus this may suggest reconstructive restoration.

## CONCLUSION

Out of the three (Low, Medium & high) doses, the medium dose of *Azadirachta indica* was able to restore cisplatin-induced nephrotoxicity among the Wister albino rats. There was a significance increase in the surface area of the bowman's capsule, glomerulus space and reduction in glomerulus in cisplatin-induced nephrotoxicity among the Wister albino rats.

## ETHICAL APPROVAL

The research ethical clearance was obtained from Baraton University of East Africa and licensed by Kenya, National commission for science, Technology and innovation (NACOSTI) with the following reference numbers UEAB/ISERC/01/2023 and NACOSTI/P/23/23376 respectively.

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