

## ORIGINAL ARTICLE



# ATTENUATIVE EFFECTS OF *ROSMARINUS OFFICINALIS* ON GENTAMICIN-INDUCED ACUTE KIDNEY INJURY AMONG ADULT MALE ALBINO RATS

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## ABSTRACT

Gentamicin (GN) is one the first-line drugs for managing severe gram-negative bacterial infections in low-income populations, but its association with acute kidney injury (AKI) has limited its use in clinical applications. *Rosmarinus Officinalis* (rosemary) is a traditional herb rich in antioxidants, and anti-inflammatory properties. However, the histological effect of *Rosmarinus Officinalis* (RO) on GN has not yet been explicated. The study investigated the protective histological effect of RO on gentamicin induced acute Kidney injury in adult male albino rats of *Rattus norvegicus*. This study used a total of 25 rats that were systematically randomized into 5 groups of five rats each: Control received rat pellets plus water ad libitum, GN (100mg/kg/bwt/i.p), low dose RO (RO100mg/kg/bwt/po +GN), medium RO(150mg/kg/bwt/po+GN) ,and high dose RO(200mg/kg/bwt/po+GN). GN was administered intraperitoneal and RO orally for seven days. Albino rats were humanely sacrificed after 24hours of the last drug administration, blood collected for biomarkers and kidney harvested for histological examination. Histological findings in the low RO, medium RO, and gentamicin groups; the glomerulus shrunken, proximal convoluted tubules and Bowman's space were dilated, respectively, whereas those in the high-dose RO and control groups were normal and comparable.

This study shows that Co-administration of a high dose of RO attenuates histological effects of Gentamicin-induced AKI among Male albino rats.

**Keywords:** Acute kidney injury, histology, antioxidant, oxidation, *Rosmarinus Officinalis*, and Gentamicin.

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## INTRODUCTION

GN remains widely used in developing countries as a first-line antibiotic in the management of severe gram-negative infections in low-income countries (Hoste et al., 2015; Mehta et al., 2015; Singu et al., 2023). This is because it is very viable and cost-effective when compared to other renal-friendly drugs. Nevertheless, persistent utilization of GN in developing countries has led to a significant prevalence of chronic kidney diseases and mortality attributed its nephrotoxic effect. There exists a complex phenomenon characterized by an increase in serum creatinine, urea levels, and severe proximal renal tubular

necrosis in Gentamicin-induced nephrotoxicity (Alarifi et al., 2012; Duffy et al., 2020). Acute kidney injury is a global and regional public health challenge attributed to high morbidity and mortality. Significant glomerular, tubular, and interstitial alterations were the outcome of GN's histological alteration. These changes include blood capillary occlusion, mesangial hypercellularity, and endothelial cell proliferation. Oxidative stress and other reactive oxygen species are major causes of AKI. Renal toxicity linked with GN is due to its accumulation in the proximal tubules of the kidneys, which is 50 to 100 times

greater than in serum (Selby et al., 2009). Reduced oxygen metabolites are useful mediators of toxic, immune-mediated, and ischemic tissue damage.

The gradual loss of kidney function brought on by medications, herbal remedies, or pollutants from the environment and industry is known as nephrotoxicity. The majority of the population affected are from developing countries due to increased industrialization, use of herbal medicine, environmental and use of drugs including GN in the treatment of gram-negative bacteria (Mehta et al., 2015). However, gentamicin-induced nephrotoxicity remains the number three cause of AKI, with a prevalence of 18-27% (Soni et al., 2011). GN causes AKI by releasing free reactive oxygen and nitrogen oxide radicals, causes tubular renal damage through apoptosis, and releases enzymes that have toxic capacities. The reactive oxygen that causes nephrotoxicity can be reduced using natural *Rosmarinus officinalis* (rosemary). *Rosmarinus officinalis* (RO) is a traditional herb that originated in the Mediterranean

region. However, it has currently been domesticated in so many countries in Africa, including Kenya. The benefit of this plant include; (Ali et al., 2018) it can treat asthma, hepatotoxicity, ischemic heart disease, and hypercholesteremia. (2) it contains rosmarinic acid that has antioxidant and anti-inflammatory benefits (De Oliveira et al., 2019). RO contains antioxidants, which play significant role in inhibiting and scavenging free radicals, thus protecting humans against infections and degenerative diseases. There exists a lot of data on nephroprotection, attenuation, and amelioration of RO, however, data are scarce on protective abilities on gentamicin-induced nephrotoxicity. The researchers currently recommend the use of natural antioxidants because of their safe therapeutics. Despite RO being used as traditional medicine, there is limited data on histological effects on kidney structures when is concurrently administered with GN. Therefore, this study investigated the possible attenuative effects of *Rosmarinus Officinalis* on gentamicin induced acute kidney injury among male albino rats.

## MATERIALS AND METHODS

### Experimental animals

This was a posttest only true experimental study in which 25 male albino rats of species *Rattus norvegicus* were systematically randomly sampled and put into 5 groups. The sample size of 25 male albino rats was arrived at based on (Arifin & Zahiruddin, 2017). The rats were then fed on standard pellets, given water ad libitum and maintained the normal experimental conditions. Strict occupational animal handling procedures were adhered to, from the start to the end of the study.

### Drug administration

Here the control group only received feeds and water ad libitum. To induce acute kidney injury, a GN dosage of 100

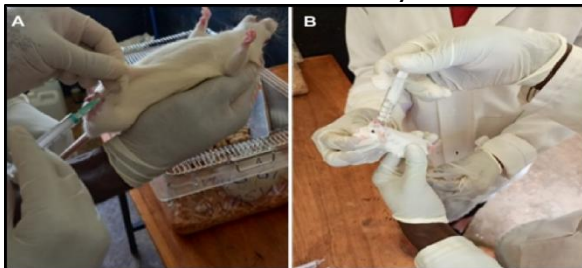
mg/kg/bwt/day/i.p (intraperitoneal) was administered for 7 days (Ghaznavi et al., 2018). The dose of RO was derived from the previous study on the effects of rosmarinic acid on methotrexate-induced nephrotoxicity and hepatotoxicity in Albino rats. A low dose of RO(100mg/kg/bwt/PO), a medium dose(150mg/kg/bwt/PO), and a high dose of (200 mg/kg/bwt/PO) were used, as per (Jafaripour et al., 2021). The renal function test was done after administration of gentamicin and various doses of RO in order to confirm acute kidney injury and if attenuation took place respectively.

### Procedure for drug administration (gentamicin)

Doses of GN100mg/kg/bwt/i.p were measured and put into the syringe with a needle utilized, 70% alcohol swab, and cotton gauze to disinfect the ampule then suck the dosage into the syringe for administration. Rats were removed from the cage and restrained firmly head-down, Anatomical landmarks were identified on the abdomen, the needle was inserted when the bevel facing up into the lower right quadrant of the abdomen towards the head at about 30-to-40-degree angle horizontally, the plug of the syringe was pulled back to create negative pressure before injecting. The needle was pulled out straight and placed needle and syringe into the safety box. Animals were returned to the cage for observation for any further complications.

#### Administration of *Rosmarinus Officinalis* Capsules Doses

The administration was done between 0900 hours and 1000 hours for 7 days.



**Figure 1: Administration of Gentamicin and *Rosmarinus Officinalis*.** Key: A- administration of Gentamicin and B- *Rosmarinus Officinalis*

#### Procedure of Administration of *Rosmarinus Officinalis*

Rosemary capsules were opened and contents were measured on a digital

weighing scale based on the standard weight of each rat. The measured dose was dissolved in 2mls of demonized water. The albino rat was gently held out of the neck region with the left hand. The animal was wrapped in a tablecloth to avoid soiling the researcher's clothing. The animal was then held against the body, with the mouth facing the examiner. The gavage needle was gently inserted into the animal's mouth, gently twisting to pass through strictures of the esophagus and cardiac sphincter, and the RO dose was dropped into the animal's stomach. The Gavage was gently removed and rats were returned to cages for further observation.

#### **Sacrificing and histological preparation**

Concentrated carbon iv oxide was used as anesthesia. An incision was made from the xiphoid process to pubic symphysis. This was done so as to expose the abdominal viscera and be able to easily identify and excise the kidneys. Once the kidneys were excised, both fibrous tissue and adipose tissue were removed. They were then preserved in 10% formalin. Later, they were stained using hematoxylin and eosin stains and observed under a light microscope. Photomicrographs were then taken and key histological changes were noted and described as below. The ethical approval to carry out the study was sought from Baraton University of Eastern Africa (UEAB/ISERC/06/01/023) and NACOSTI licensing No. (NACOSTI/P/23/24510) respectively.

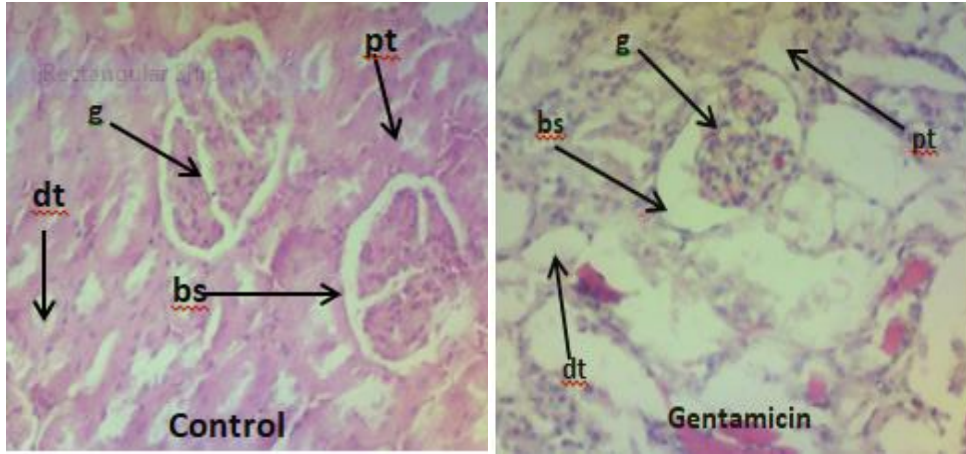
## **RESULTS**

### **Animal Behaviors**

Rats in control and high dose RO group had normal activities throughout the study period while those of the GN, low RO and medium RO had reduced activities from day four of drug administration.

### **Photomicrographs of the control groups.**

The glomerulus, proximal, and distal convoluted for the GN group was compared to the control groups.

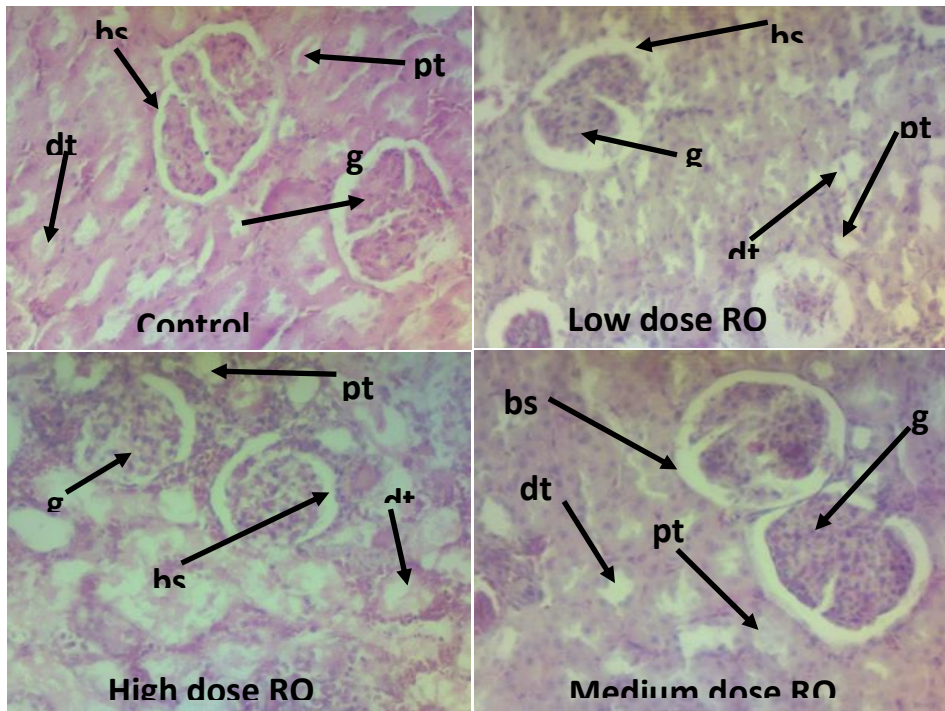


**Figure 2:** photomicrographs of the control and GN group stained with H & E 100× kidney section **Key:** *H & E-* H-hematoxylin and E eosin, *bs-* Bowman's space, *g-* glomerulus, *dt-* distal convoluted tubule, *pt-* proximal convoluted tubule

Control group slide had a normal glomerulus and proximal and distal convoluted tubules. The GN group slide had a shrunken glomerulus with dilated proximal and distal convoluted tubules as compared to the control group. The bowman's space appeared dilated as compared to the control group (Figure 2).

**The photomicrographs of the control compared with low, medium, and high doses RO groups.**

The glomerulus, proximal, and distal convoluted for GN group was compared to high, medium, and low-dose of RO groups.

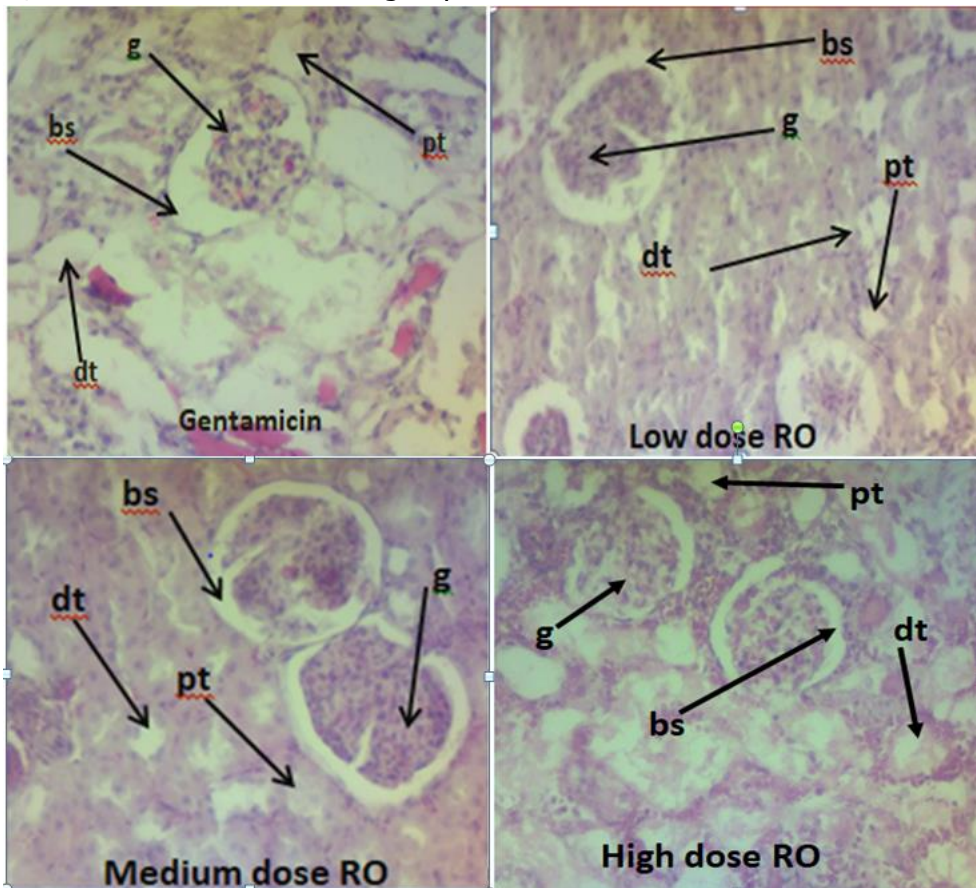


**Figure 3:** photomicrographs of control group compared with high, medium, and low doses of RO stained with H & E 100× kidney section. **Key:** *H-* hematoxylin and *E* eosin, *bs-* Bowman's space, *g-* glomerulus, *dt-* distal convoluted tubule, *pt-* proximal convoluted tubule

The control and high dose RO groups had the normal shape of the glomeruli, proximal and distal convoluted tubules, and normal Bowman's space as compared to low and medium dose RO groups which had a shrunken glomerulus, dilated Bowman's space, dilated proximal and distal convoluted tubules. Bowman's space appeared dilated in low and medium dose RO with variant sizes as compared to the positive group whereas, Bowman's space in high RO appeared narrow as compared to GN, low and medium dose RO groups

**The photomicrographs of the GN group compared with low, medium, and high doses RO groups.**

The GN group, Low, and medium dose RO groups had a shrunken glomerulus with dilated Bowman's space, and proximal and distal convoluted tubules as compared to the High dose RO group which had normal glomerulus shape, normal size of proximal and distal tubules, and normal Bowman's space.



**Figure 4:** Photomicrographs of the control group compared with high, medium, and low doses of RO stained with H & E 100× kidney section. **Key:** *H&E*-hematoxylin and *E* eosin, *bs*-bowman's space, *g*-glomerulus, *dt*- distal convoluted tubule, *pt*-proximal convoluted tubule

**DISCUSSION**

The Bowman's capsule, glomerulus, and convoluted tubules are among the crucial kidney structures whose histological analysis offers important acumens into their morphology and possible changes linked to nephrotoxicity. This study aimed to investigate the protective histological effect of RO on these important renal components when exposed to GN.

The control groups, under light microscope, had normal histological characteristics, such as slightly smaller kidney tubules, normal Bowman's size and space, and well-defined brush borders in the glomeruli. This normal finding is due to lack of exposure to reactive oxygen species of GN that usually alters the histology of the vital organs of the kidney. These findings underlined the structural deviations observed in GN groups and provided a baseline for comparison.

The GN groups revealed distinguished alterations in the glomerulus, essential organ in charge of nitrogenous waste filtration. A common occurrence during nephrotoxicity, glomerular sclerosis, was suggested by the detected shrinkage and distorted boundaries. The buildup of nephrotoxic chemicals could have linked to this structural change, which in turn caused an increase in the production of inflammatory markers and subsequently blockage of glomerular blood vessels. This result is in tandem with earlier study on sildenafil-induced nephrotoxicity conducted by Ali et. al (2018) in which the obvious shrinkage was due to drug accumulation that leads to increased release of inflammatory markers leading to occlusion of glomerular blood capillaries. Padmini & Kumar also made similar observations on histological features of Gentamicin-induced nephrotoxicity.

The present study highlighted distinct dilatation of kidney tubules and the Bowman's space in the GN, low dose RO and medium dose groups. The findings

align with studies conducted by (Mohamed Yousry *et al.*, 2016; Suriyakumari *et al.*, 2016) in India, where they explored the effect Sildenafil on kidneys. Comparison in the observations suggest a shared pathway of nephrotoxicity induced by both sildenafil and Gentamicin. The study implies that the dilatation of renal tubules and enlargement of Bowman's capsule is attributed to the augmented aggregation of inflammatory markers like the cytokines within the blood vessels of the glomerular. This aggregation subsequently reduces the filtration rate of the drugs, increasing the blood pressure in the vessel thus causing dilatation. Previous study has indicated that these changes could have been influenced infiltration of inflammatory cells and the substantial swelling of the epithelial cell when exposed to oxidative stress. This, in turn initiates an elevated production of nitric oxide, which reacts with free radicals, ultimately cause renal injury (Kirbas *et al.*, 2015).

The high-dose groups observed normal histological characteristics, such as slightly smaller kidney tubules, normal Bowman's size and space, and clearly defined brush boundaries in the glomeruli, thus being comparable to the control group's findings. The findings are consistent with those of Anandan & Subramanian (2012), Nieto et al., (2018), and Padmini & Kumar (2012). The study postulates that the presence of a substantial number of antioxidants in the high dose of RO may have shielded the kidney from GN negative effects, hence the decreased tendency to retain drugs in the kidney to cause histological alterations.

## CONCLUSION

In conclusion, high dosages of *Rosmarinus Officinalis* have been shown to attenuate the acute kidney damage caused by Gentamicin in male albino rats in a histologically beneficial way. This suggests that taking *Rosmarinus Officinalis* could be a good way to prevent acute kidney

damage brought on by GN. It's important to remember that more investigation and clinical testing might be required to confirm these results and examine *Rosmarinus Officinalis's* possible use in attenuating acute renal injury in human being. Furthermore, taking into account elements like safety and long-term consequences would help to create a more thorough knowledge of its possible therapeutic application.

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