



# PROTECTIVE EFFECT OF *ROSEMARINUS OFFICINALIS* ON GENTAMICIN–INDUCED ACUTE KIDNEY INJURY IN ADULT MALE ALBINO RATS.

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## ABSTRACT

**Background:** Gentamicin (GN) drug is one of the first-line drugs for managing severe gram-negative bacteria. Its association with acute kidney injury (AKI) has restricted its use due to oxidation damage to kidney architectural structures. *Rosmarinus Officinalis* (rosemary) is a natural antioxidant available and affordable in many developing countries. Antioxidants can be used to prevent oxidation that causes AKI in GN use. This study was undertaken to evaluate the protective gross morphological effect of *Rosmarinus Officinalis* (RO) on Gentamicin-induced acute Kidney injury in male albino rats' species of *Rattus norvegicus*. **Methodology:** The study was done in Kenya at Maseno University. A posttest true experimental design was used, and a sample size of 25 Albino rats was calculated using a resource-modified equation. Rats were randomly sampled into 5 groups, each with 5 albino rats. The negative control group received a standard rat diet plus water, the positive control received GN100,mg/kg/bwt/i.p, low-dose RO, medium-dose RO, and high-dose RO groups received 100,150 and 200 mg/kg/bwt of RO orally, respectively, and were co-administered with GN 100mg/kg/bwt/i.p. At the end of day seven, they were humanely sacrificed, and gross morphometric were taken. **Results:** It was observed that the mean weight of the rat, weight, and volume of the kidney increased significantly ( $p=0.001$ ) in high-dose RO group as compared to the positive control. The mean length and thickness in high-dose RO group increased significantly ( $p=0.0001$ ) as compared to the positive control. **Conclusion:** This present study shows that co-administration of a high dose of RO has a protective effect on gross morphology against Gentamicin-induced AKI among male albino rats.

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

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## INTRODUCTION

Gentamicin (GN) drug remain widely used in developing countries as one of the first-line antibiotics in the management of severe gram-negative infections (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. However, continuous use of GN in developing countries has led to a high incidence of chronic kidney diseases and mortality-related cases due to its cause of nephrotoxicity. There exists a complex

phenomenon characterized by an increase in serum creatinine and urea levels, and severe proximal renal tubular necrosis in Gentamicin induced nephrotoxicity (Duffy et al., 2020). Acute kidney injury is a global and regional public health challenge attributed to high morbidity and mortality. These changes include blood capillary occlusion, mesangial hyper cellularity, and endothelial cell proliferation (Alarifi et al., 2012). 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.

Nephrotoxicity is the progressive loss of kidney function either due to medicine, herbal concoctions, or industrial and environmental toxins. The majority of the population affected is from developing countries due to increased industrialization, the use of herbal medicine, environmental factors, and the use of drugs including GN in the treatment of gram-negative bacteria (Mehta et al., 2015). However, gentamicin-induced toxicity remains the number-three cause of AKI, with a prevalence of 18-27% (Soni et al., 2011). GN causes AKI by oxidation: it releases free reactive oxygen and nitrogen oxide radicals, causing tubular renal damage through apoptosis, and releases enzymes that have toxic effects. 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 benefits of this plant include: (Wasung et al., 2015) (1) treatment for asthma, hepatotoxicity, ischemic heart disease, and hypercholesteremia. (2) it has antioxidant and anti-inflammatory benefits due to its rosmarinic acid component (De Oliveira et al., 2019). RO has antioxidants that play an important role in inhibiting and scavenging free radicals, thus protecting humans against infections and degenerative diseases. There is a lot of data on renal protection, attenuation, and amelioration of RO, however, data are scarce on protective morphological abilities in gentamicin-induced acute kidney injury. Therefore, this study investigated the possible effects of *Rosmarinus Officinalis* on protection against renal structural changes when administered with Gentamicin in adult male Albino rats.

## MATERIALS AND METHODS

This was a posttest true experimental study in which 25 male Albino rats of the species *Rattus norvegicus* weighing 150-250g were used, and each group had 5 rats, respectively. The modified human resource equation (Arifin & Zahiruddin, 2017) was used to calculate the sample size and allocation of animals to each group. Systematic random sampling was used to select animals that had good health and attained the desired weight. Every fourth rat was picked from a target population of 150 rats in the animal house. They were kept in polycarbonate cages for one week to acclimatize at a temperature of 26°C and two cycles of light and dark; twelve hours apart. Feed and water were given under strict hygiene conditions, with the researcher safeguarding standard occupational animal handling measures.

Gentamicin sulphate B.P 80mg/2ml batch number 202103177 was purchased from Crowns Healthcare company, and RO capsules were acquired from Western cosmetics company SWANSON Fargo ND 58104USA\*1-800-437-4148.

The negative control group received a standard rat diet plus water only, positive control group received GN100mg/kg/bwt/i.p for seven days; low dose RO, medium dose RO and high dose RO groups received 100,150 and 200mg/kg/bwt orally respectively (Medić et al., 2019) and were co-administered with GN 100mg/kg/bwt/i.p for seven days. The experiment lasted seven days, and weight was monitored daily. On day seven, the terminal body weight of each albino rat was taken, humanely sacrificed, and gross morphometric were taken. Chloroform was

used as anesthesia, and then a vertical incision was made from the xiphoid process to the pubic symphysis to expose the viscera and the kidney. A cardiac puncture was done to collect blood samples. Thereafter, creatinine and urea levels were measured to confirm AKI. The kidneys were identified and excised; the fibrous capsule and adipose tissue were removed from the kidneys.

Gross morphological studies were then carried out, whereby the volume of the organs was determined using Archimedes' principles, weight was determined by digital

weighing scale, whereas, length, width, and thickness were determined by use of a digital Vanier caliper. The following dimensions of the right and left kidneys were taken: width measured from the medial to the lateral border. Length: measured from the upper to the lower pole. Thickness: measured from the visceral to the parietal surface.

The ethical approval to carry out this study was obtained from Baraton University of Eastern Africa (UEAB/ISERC/06/01/2023) and NACOSTI (NACOSTI/P/23/24510).

## RESULTS

### **Behavioral changes and mortality**

Rats in the positive control group had reduced activity from day four of drug administration, while others had normal activities. No mortality was recorded during the entire process of the experiment.

### **Gross morphologic effects**

The measured terminal body weight of Wister albino rats and the volume, thickness, length, weight and width of the kidneys were compared within the groups.

Mean terminal body weight of the Wister albino rats between controls and high, medium and low dose RO groups.

There was a significant ( $p=0.001$ ) reduction in the mean-terminal weight of rats in positive control group as compared to the negative control group. There was a significant ( $p= 0.001$ ) increase in the mean terminal weight of rats in high RO doses as compared to the positive control group. However, when the mean terminal weight of both medium, and low doses of RO was compared to the positive control group, no significant difference was observed at ( $p= 0.866$ , and  $1.00$  respectively. (Table 1).

### **Gross Morphometric effects**

There was a significant ( $p = 0.003$ ,  $0.0001$ ,  $0.0001$ ,  $0.0001$ ,  $0.0001$ ) reduction in

weight, volume, thickness, length, and width, respectively, of the right kidney of the positive control group as compared to the negative control group. (Table 2).

There was a significant ( $p = 0.0001$ ) reduction in the weight, volume, thickness, length, and width of the left kidney of the positive control group compared to the negative control group. (Table 3)

There were significant ( $p= 0.001$ ,  $0.0001$ ,  $0.0001$ ,  $0.0001$ ,  $0.0001$ ) increases in weight, width, length, volume, and thickness respectively the right kidney of the high doses as compared to the positive control. However, no significant difference was observed when the positive control group was compared with medium and low dose RO groups. (Table 4).

There were significant ( $p = 0.0001$ ,  $0.0001$ ,  $0.0001$ ,  $0.0001$ ,  $0.0001$ ) increases in weight, width, length, volume, and thickness, respectively, in the left kidney of the high dose as compared to the positive control group. However, no significant difference was observed when the positive control group was compared with medium and low-dose RO. (Table 5).

### **Histological effects**

The glomerulus, proximal, and distal convoluted for the positive control group

were compared to the negative control groups. The negative control group slide had a normal glomerulus and proximal and distal convoluted tubules. The positive control slide had a shrunk glomerulus with dilated proximal and distal convoluted tubules as compared to the negative control group. The Bowman`s space appeared dilated as compared to the negative control group (Figure 1).

**Biochemical marker changes**

There was a significant (p = 0.0001) increase in both the urea and creatinine levels in the positive control group as compared to the negative control group. (Table 6). Positive control group was administered with 100 mg/kg/bwt/i.p. The high, medium, and low dose groups were given a constant dose of GN100 mg/kg/bwt/i.p + 200, 150, and 100 mg/kg/bwt of RO, respectively, for protection against AKI.

Table 1: Mean terminal body weight of the Albino Rats

Groups	Body weight Mean ± SEM	P value
Negative Control group (feeds + water)	203.38 ± 2.02	0.001
Positive Control (GN100mg/kg/bwt/i.p)	175.32 ± 0.83	0.001
High dose of RO (200mg/kg/bwt) +GN (100mg/kg/bwt/i.p)	203.58 ± 0.89	0.001
Medium dose of RO (150mg/kg/bwt) +GN (100mg/Kg/bwt/i.p)	179.70 ± 1.84	0.866
The low dose of RO (100mg/kg) +GN (100mg/Kg/bwt/i.p)	177.01 ± 2.41	1.000

GN= Gentamicin, RO= Rosmarinus Officinalis, SEM=Standard Error of the mean.

Table 2: Mean weight, volume, thickness, length, and width of the right kidneys between the Negative and positive control groups.

Groups	Weight of kidney Mean ± SEM	Volume of kidney Mean ± SEM	Thickness of kidney Mean ± SEM	Length of kidney Mean ± SEM	Width of kidney Mean ± SEM
Negative Control (feeds + water)	1.07 ± 0.06	2.04 ± 0.06	3.92±0.07	14.44 ±0.02	9.78 ± 0.05
Positive Control (GN100mg/kg/bwt/i.p)	0.79 ±0.03	1.03 ±0.01	2.05 ±0.02	12.77 ±0.21	7.87± 0.25
P Value	0.003	0.0001	0.0001	0.0001	0.0001

Negative Control was only given feeds plus + water; the Positive Control group was administered with GN100mg/kg/bwt/i.p

Table 3. Mean weight, volume, thickness, length, and width of the left kidney between the negative and positive control Groups

Groups	Weight of kidney Mean ± SEM	Volume of kidney Mean ± SEM	Thickness of kidney Mean ± SEM	Length of kidney Mean ± SEM	Width of kidney Mean ± SEM
Control (negative)	0.91 ±0.03	1.51 ±0.05	3.56 ±0.10	13.79 ±0.09	9.01 ±0.10
Positive Control (GN100mg/kg/bwt/i.p)	0.62 ±0.01	0.79 ±0.06	2.41 ±0.12	11.33 ±0.17	6.93±0.20
P Value	0.0001	0.0001	0.0001	0.0001	0.0001

Negative control was given feeds + water, and the Positive Control was administered with GN100mg/kg/bwt/i.p

Table 4: Mean weight, volume, thickness, length, and width of the right kidney between the positive control and high, medium, and low RO dose groups.

Groups	Weight of kidney Mean ± SEM	Volume of kidney Mean ± SEM	Thickness of kidney Mean ± SEM	Length of kidney Mean ± SEM	Width of kidney Mean ± SEM
Positive Control (GN100mg/kg/bwt/i.p)	0.79 ± 0.03	1.03 ± 0.01	2.05 ± 0.02	12.77 ± 0.21	7.87 ± 0.25

High dose of RO (200mg/kg/bwt) + GN (100mg/kg/bwt/i.p)	1.05±0.07	1.80±0.18	3.85±0.06	14.26± 0.07	9.83± 0.18
Medium dose of RO (150mg/kg/bwt) + GN (100mg/Kg/bwt/i.p)	0.84±0.03	1.24±0.09	2.33 ± 0.18	12.87± 0.17	8.38± 0.18
The low dose of RO (100mg/kg) +GN (100mg/Kg/bwt/i.p)	0.77±0.02	1.02±0.02	2.38 ± 0.17	12.50 ± 0.17	7.98 ± 0.29

Positive Control was administered with 100mg/kg/bwt/i.p. The high, medium, and low dose groups were given a constant dose of GN100mg/kg/bwt/i.p + 200, 150, and 100mg/kg/bwt of RO respectively for protection against AKI.

Table 5: Mean weight, volume, thickness, length, and width of the left kidney between the positive control and high, medium, and low doses Group.

Groups	Weight of kidney Mean ± SEM	Volume of kidney Mean ± SEM	Thickness of kidney Mean ± SEM	Length of kidney Mean ± SEM	Width of kidney Mean ± SEM
Positive Control (GN100mg/kg/bwt/i.p)	0.62 ± 0.01	0.79 ± 0.06	2.41 ± 0.12	11.33 ± 0.17	6.93± 0.20
High dose of RO (200mg/kg/bwt) + GN (100mg/kg/bwt/i.p)	0.92±0.05	1.39 ±0.05	3.38 ±0.16	13.85 ± 0.28	9.37 ± 0.16
Medium dose of RO (150mg/kg/bwt) + GN (100mg/Kg/bwt/i.p)	0.69 ±0.03	0.98 ±0.01	2.62 ± 0.19	12.18 ± 0.33	7.51 ± 0.19
Low dose of RO (100mg/kg) +GN (100mg/Kg/bwt/i.p)	0.71 ±0.02	0.83 ±0.06	2.85 ± 0.36	11.78 ± 0.10	7.37 ±0.36

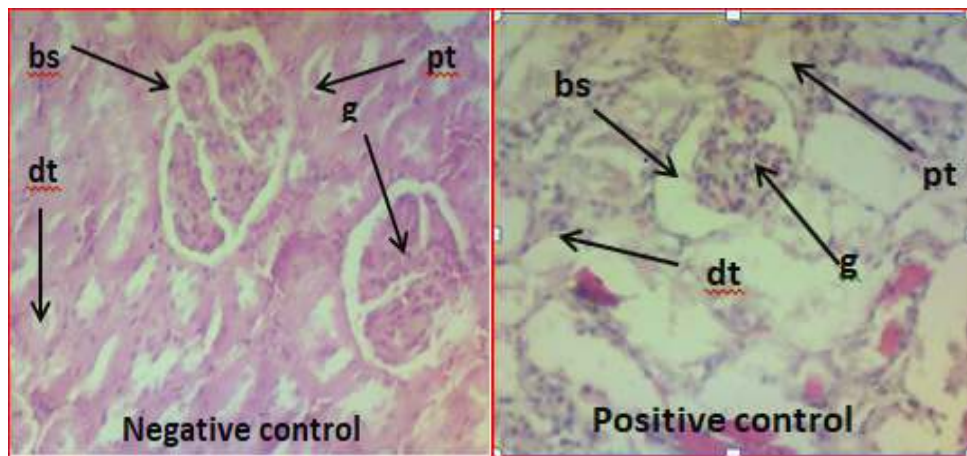


Figure 1: photomicrographs of the negative control and positive control stained with H & E 100× kidney section. H & E – hematoxylin and eosin, g-glomerulus, dt- distal convoluted tubule, pt-proximal convoluted tubule. Negative Control was only given feeds + water, the positive control group was administered GN100mg/kg/bwt/i.p to induce AKI

Table 6: Mean Creatinine and Urea levels between the negative control and the positive control groups

Groups	Serum Creatinine levels Mean ± SEM	Serum Urea levels Mean ± SEM
Negative Control (feeds + water)	0.5200 ± 0.01378	6.5800 ± 0.04990
Positive Control (100mg/kg/bwt/i.p)	1.6920 ± 0.06272	12.5020 ± 0.12290
P VALUE	<i>P = 0.001</i>	<i>P = 0.001</i>

GN= Gentamicin, RO= Rosmarinus Officinalis, SEM=Standard Error of the mean.

## DISCUSSION

There was a significant ( $p=0.001$ ) reduction in the mean-terminal body weight of rats in the positive group as compared to the negative control group. These findings are similar to the observations of (Udupa & Prakash, 2019) who found similar weight reduction. This study postulates that the reduction in the mean terminal weight of rats in the positive control group might have been due to the toxic effect of its high dose. The abnormal accumulation of drug substances within the proximal tubules and Bowman's capsule leads to reduced filtration and excretion of metabolic wastes. This waste might cause a reduced appetite and hence interfere with the weight gain process.

It was observed that there was a significant ( $P= 0.001$ ) increase in the mean terminal body weight of rats in high doses of RO group as compared to the positive control group. The mean terminal body weight of both medium and low doses of RO were comparable with the positive control group, while the high dose RO was comparable with the negative control group as shown by the respective significant differences in (Table1). These suggest that RO could have provided more protective effects to the kidneys at the high dosage than at the low and medium dosages. These findings agree with (Nusier et al., 2007; Sedighi et al., 2015) who made similar observations while assessing the preventive and protective effects of *Rosmarinus Officinalis*. The noted increase in weight could have been due to the protective effect of *Rosmarinus Officinalis* as it contains antioxidants that prevents the release of reactive renal oxidant substances.

It was observed that there was a significant ( $p = 0.001, 0.0001, 0.0001, 0.0001, 0.0001$ ) reduction in weight, volume, thickness, length, and width, respectively, of the right and left kidneys of the positive control group as compared to the negative control group (Table 4 and 5). These findings are in

tandem with (Abdelsameea et al., 2016) while assessing Cilostazol attenuation effects on gentamicin-induced nephrotoxicity in rats. This significant weight reduction might have been due to the toxic effects of gentamicin, which is known to release inflammatory markers and oxidative substances during toxicity. These substances interfere with fiber generation and congest blood glomerular capillaries, which leads to reduced weight. The reduction in volume, width, length, and thickness might have been caused by histo-architectural changes occurring that might affect the filtration capabilities of the kidney which leads to drug accumulation and obvious nephrotoxicity. The reduction in kidney weight might also have been due to increased oxidative stress during nephrotoxicity which is similar to observations in Iran while assessing the effects of infliximab on renal injury due to methotrexate in rats (Kirbas et al., 2015), and (Hegazy et al., 2021) at Zagazig University when assessing paracetamol effects on kidney morphological structure. These two drugs are used as a comparison as they have similar methods of causing acute kidney injury as gentamicin.

There was an increase in weight, volume, thickness, length, and width, respectively, of the right and left kidneys of the High dose RO group as compared to the positive control group, and normal weight was observed when compared with the negative control. These similar findings, which signify renal protection were documented by (Jafaripour et al., 2021) on the protective effects of RO on methotrexate-induced hepatorenal toxicity. The increase in weight could have been due to the antioxidants present in RO: eriocitrin, rosmarinic acid, caffeic acid, carnosic acid, apigenin, luteolin, and quercetin (Ramadan., 2019). These antioxidants could have scavenged the reactive oxidants of gentamicin, hence offering renal protection.

On histological changes in the kidney, several parameters were observed, which included the glomerulus, Bowman's space, proximal convoluted tubule, and distal convoluted tubule. It was observed that in the negative control group, the glomerulus appeared normal with well-defined brush borders, the Bowman's capsule size was normal, while the kidney tubules appeared slightly small. These are kidney normal findings on histological observation under a light microscope. In the positive control group, it was observed that the glomerulus had shrunk and the borders appeared distorted. The study postulates that this change in the structure of the glomerulus may be due to glomerulus sclerosis, that occurs during nephrotoxicity that alters the shape of the glomerulus. This observation concurs with (Ali et al., 2018) on sildenafil-induced nephrotoxicity 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, 2012) made similar observations on the histological features of Gentamicin-induced nephrotoxicity.

There was a significant ( $p=0.001$ ) increase in both the urea and creatinine levels in the Positive control group as compared to the Negative control group (Table 6). (Liu et al.,

2018) observed a similar trend when assessing the biochemical parameter in rats before and after nephrotoxicity. (Wu et al., 2017) recorded reduced levels of urea in rats when assessing the renal biochemical changes. The slight increase in urea levels as seen in positive control group may have been due to increased production in inflammatory markers and oxidative stress and epithelial cell damage that greatly contribute to toxicity hence AKI. The difference in urea levels noted by Wu might be due to use of agents that are metabolized by both kidney and liver therefore the level is more likely to reduce.

### CONCLUSION

Therefore, it can be concluded that high dose *Rosmarinus Officinalis* has kidney protective gross morphological benefits against Gentamicin-induced acute kidney injury among male albino rats. Therefore, it can be used to attenuate the nephrotoxic effects of Gentamicin in the future.

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