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Original Research Article

Histostereological effect of *Rosemarinus officinalis L.* on gentamicin induced acute kidney injury among adult male albino rats (*Rattus norvegicus*)

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ABSTRACT

Background: Acute kidney injury (AKI) is a worldwide public health defy associated with high morbidity, mortality, and high healthcare costs in developing countries. Gentamicin (GN) is an affordable and efficacious first-line drug for managing all gram-negative bacteria, especially in low-income populations. *Rosmarinus officinalis* (RO) is a natural antioxidant can be used to stop upshots of AKI. The study was done to determine the histo-stereological effect of RO on GN induced acute kidney injury among adult male albino rats of *Rattuss norvegicus* species.
Methods: Post-test only true experimental design was used; 25 adult male Albino rats were simple randomly divided into five groups each having 5 Albino rats. Control group received rat pellets plus water ad libitum, GN (100 mg/kg/bwt/*i.p.*), low dose RO (100 mg/kg/bwt/*p.o.* + GN), medium RO (150 mg/kg/bwt/*p.o.* + GN), and high dose RO (200 mg/kg/bwt/*p.o.* + GN) groups. GN was administered intraperitoneal, and RO given orally for seven consecutive days. After completion of 24 hours of the last dose administration, they were humanely sacrificed, and kidney were harvested for histo-stereology studies.
Results: There was no significant difference observed between the high dose RO and the control group. The glomerulus volume of the high dose RO and the controls were comparable unlike for the low and medium doses RO groups.
Conclusions: The study established that co- administration of high dose (200 mg/kg/bwt) of RO with gentamicin can effectively prevent kidney structures damage that is associated with gentamicin drug toxicity.

Keywords: Gentamicin, Acute kidney injury, Histostereology, Antioxidant, *Rosmarinus officinalis*, Oxidation

INTRODUCTION

Gentamicin (GN) drug remains widely used in developing countries as a first-line antibiotic in the management of severe gram-negative infections in low-income countries.[1-3] 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 nephrotoxic effects. 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 nephrotoxic effects. There exists a multifarious occurrence described by an increase in serum creatinine, urea levels, and severe proximal renal tubular necrosis in GN-induced nephrotoxicity.[4] Acute kidney injury is a global and regional public health challenge associated with high morbidity, and mortality. Histological alteration of GN resulted in noticeable glomerulus, tubular, and interstitial changes.

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 use of drugs including GN in the management of gram-negative bacteria.[2] GN causes toxicity 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 many countries in Africa, including Kenya. The benefits of this plant include: treatment for asthma, hepatotoxicity, ischemic heart disease, and hypercholesteremia; and it has antioxidant and anti-inflammatory benefits due to its rosmarinic acid.[5]

RO has antioxidants that perform an essential role in stopping and scavenging of free radicals, thus protecting humans against infections and degenerative diseases.[6] There exists a lot of data on nephroprotection, attenuation, and amelioration of RO, however, data are scarce on protective abilities in GN-induced nephrotoxicity. The researchers currently recommend the use of natural antioxidants because of their safe therapeutics. Despite RO being used as a traditional medicine, there is limited data on its histo-stereology effects on kidney structures when it is concurrently administered with GN. Hence, this study investigated the probable histo-stereological effects of RO as protection against renal organizational changes when administered with GN in adult male Albino rats.

METHODS

Study location

The study was done at Maseno University, located in Maseno-township along Kisumu-Busia Road at the equator of Kisumu County. All experiments that pertained to handling weighing, administration of GN and RO, collecting of blood samples, and harvesting of kidney tissues were done at the School of Physical and Biological Sciences; Department of Zoology which harbors a quality animal house. The preparation of tissues for histological and histostereology studies and analysis was done in the Histology laboratory in the Department of Human Anatomy at the School of Medicine. Photomicrography and processing of biomarkers were done at Masinde Muliro University of Science and Technology, and the University of Nairobi respectively.

Study design

Posttest only true experimental design was used, whereby an intervention was made and results were compared between the experimental groups and control groups.

Experimental animals

This study was conducted on the adult male Albino rat species of *Rattus norvegicus* from a pure breed colony. The albino rats were obtained from the School of Physical and Biological Sciences in the Department of Zoology which has an animal house that can hold a maximum of 250 rats in a given research period. The albino rats were bred in cages that can hold a maximum of 6 rats per cage. The albino rats were used due to their attributed biological and functional relations with human beings. Therefore, the changes that occurred in Albino rats' kidneys are the same changes that can occur in human beings. The reasons were based on the following: one, they have a big litter size, two, reduced cost of keeping the animals, three they have a relatively short gestation period so are abundant and readily available, four, they small are and easily handled during the experimental process. Finally, they can withstand a wide range of medicines used for research purposes.[7] Externally features of these albino rats are that, red eyes with white fur, thus closely resemble Japanese hooded rats and may therefore have a common origin since they are genetically identical.[8]

These rats sexually mature around 4 to 5 weeks in females, and 6-7 weeks in males after birth.[9] Male rats are larger than females at around 9-11 inches in length and have an average weight of between 150 g to 200 g.

Grouping of animals

The 25 rats were randomly apportioned into 2 groups, mainly; a control group of 5 animals, and 20 animals, for the experimental group.

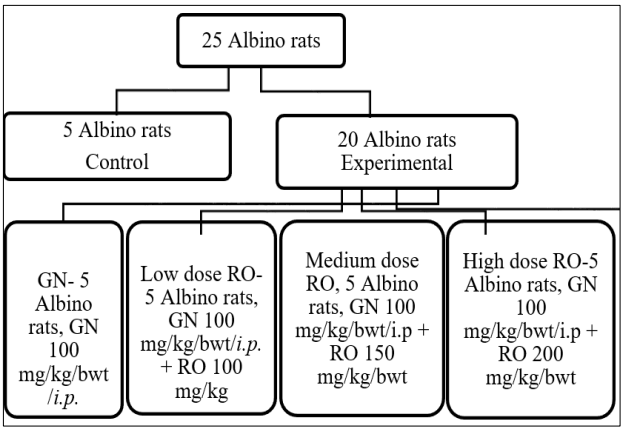

Figure 1: The grouping of Albino rats.

Inclusion criteria

This study used healthy, pure breed male Albino rats that were six to eight weeks old, and weighed 150 to 200 grams. Male Albino rats lack estrogen hormones that is abundant in female, plays molecular and cellular effect, their inclusion could have significantly affected the reliability of RO results.

Exclusion criteria

None-pure Albino rats were excluded.

Study period

The study period was from April 2023 to July 2023.

Feeding of Albino rats

The Albino rats were acclimated for one week in an animal house at a room temperature of 26°C, then 12-hours of light and dark cycles. They were fed on standard rodent pellets that contained the standard diet set by the Academy of Nutrition and Dietetics. It consisting of 4% cellulose, 5% lipid and calories, 20% protein, 72% carbohydrate, 68% starch, 12% lipid, and 54% zinc. They also received water. Feeding took place every morning at 08:00 hour inside the polycarbonate cages as defined.[10]

Determination of GN and RO doses

To induce acute kidney injury, a GN dosage of 100 mg/kg/bwt/day/*i.p.* was administered for seven days.[11] The RO dose was adopted from the previous study on the effects of RO on methotrexate-induced renal-toxicity and hepatotoxicity in Albino rats. A low dose of RO (100 mg/kg/bwt/*p.o.*), a medium dose (150 mg/kg/bwt/*p.o.*), and a high dose of (200 mg/kg/bwt/*p.o.*) were used.[12]

Procedure for administration of gentamicin injection

Doses of GN 100 mg/kg/bwt were calculated then measured using the syringes with a needle, 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 injected 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. The administration was done between 09:00 hours and 10:00 hours for 7 days.

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 2 mls of deionized 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 released into the animal's stomach. The gavage was gently removed and the rats were returned to cages for further observations.

Procedure for harvesting of kidney tissues

Chloroform was placed in a jar that had a close-fitting lid. Albino rats were then placed in for 3-5 minutes to be euthanized. They were removed from the jar, spread on a dissecting board, and fixed with pins while lying on the dorsal. Using forceps and a pair of scissors, made a ventral medial incision from the xiphoidal process to the symphysis pubis to access and excise kidney organs.

Hematoxylin & Eosin (H&E) technique

The kidney organs were immersed in 10% formalin solution for 24 hours. Dehydration was then carried out in aggregate alcohol concentrations (50%, 60%, 70%, 80%, 90%, 95%, and 100% (absolute), cleared in 3 grades of xylene, infiltrated in molted wax to provide internal support, blocked in wax to provide external support, sectioned using a rotary microtome, stained the section in H&E to provide differential account under a light microscope.

Materials used to process tissues specimen for light microscope

Light microscope**,** kidney specimen, 10% formalin solution, paraffin wax, glass slides, 22×22 mm coverslips, automatic tissue processor, dibutylphthalate polystyrene xylene mutant, H&E stains, glass staining jars, rotary microtome, embedding bench, water bath, slide holders, xylene, isopropyl alcohol, tissue take and metal base**.**

Light microscope and slide photography

Material, included, a flash memory card and light microscope, digital camera (32 megapixels), and histological slide.

Procedure for taking photomicrograph using a digital 32-megapixel camera

Photomicrographs were done at Masinde Muliro University of science and Technology; histological slide sections were attached on the stage of the microscope. The focus was attuned using power x10 and x100 to make a diagnosis in a good field view.

Then photographs of the regions were taken in best seen under the focus view using a digital camera. The images were saved in the memory card and then transferred to the computer to determining protective histo-stereological effects of *Rosmarinus officinalis* on GN-induced acute kidney injury among adult male Albino rats.

The photomicrographs were taken and systematically uploaded on the step-analyzer. The volume of the

glomerulus was measured and compared within the groups.

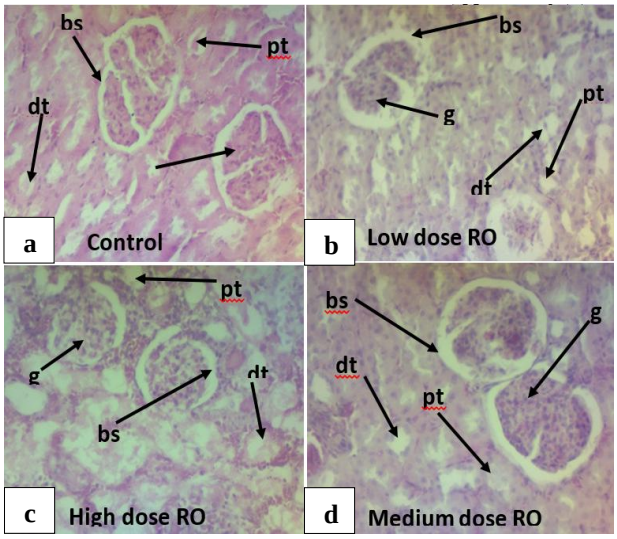

Figure 2 (a-d): Photomicrographs of the control group compared with high, medium, and low doses of RO stained with H&E 100× kidney section.

Key: H-hematoxylin and E eosin, bs-Bowman's space, g-glomerulus, dt-distal convoluted tubule, pt-proximal convoluted tubule

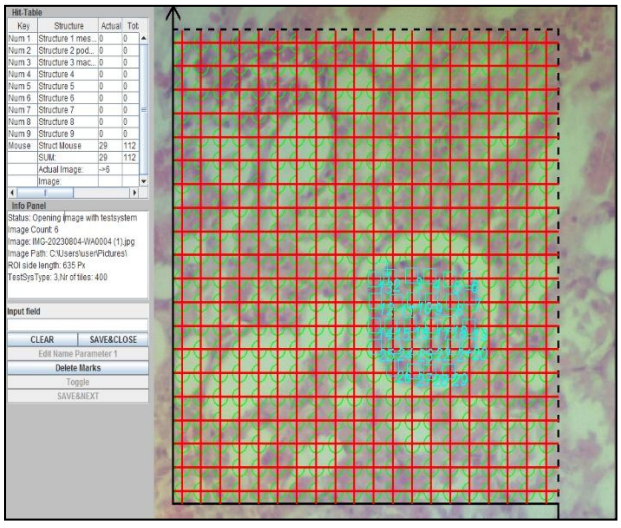

Figure 3: Demonstration of histostereology study using step-analyzer.

Statistical analysis

The data was coded entered into the excel sheet, and then uploaded to the statistical package for the social sciences (SPSS) version 27 (2021).

One-way ANOVA was used to test the means within the groups, and the post hoc Bonferroni was used to test the mean difference between the groups and the p≤0.05 was found to be statistically significant.

RESULTS

The protective histo-stereological changes in the kidney after administration of different doses of Rosmarinus officinalis on GN-induced AKI among adult male Albino rats

The glomerulus volume was measured and means were tested within the groups. The GN group was compared to the control. The high, medium, and low-dose groups were compared to the GN group.

The current study demonstrated a significant (p=0.0001) reduction in the glomerulus volume in the GN group as compared to the control group (Table 1).

Table 1: Mean volume of the glomerulus between control and the GN groups.

Variables	Groups	Mean± SEM	P value
Glomerulus volume (µm^3)	Control group (feeds+water)	6.45± 0.08	≤0.0001
	GN group (GN 100 mg/kg/bwt/i.p.)	5.09± 0.05	

GN=Gentamicin, RO=*Rosmarinus officinalis*, SEM=standard error of the mean

There was no significant difference (p=0.094) in high-dose RO in glomerulus volume as compared to the control group. There was a significant difference between low and medium dose RO as compared to the control group at (p=0.0001 and p=0.0001) respectively (Table 2).

Table 2: Mean volume of the glomerulus between control and high doses, medium, and low RO dose groups.

Variab-les	Groups	Mean± SEM	P value
Glome-rulus volume (µm^3)	Control	6.45± 0.08	
	Low dose (100 mg/kg/bwt) + GN (100 mg/kg/bwt/*i.p.*)	5.16± 0.07	≤0.0001
	Medium dose (150 mg/kg/bwt) + GN (100 mg/kg/bwt/*i.p.*)	5.28± 0.05	≤0.0940
	High dose (200 mg/kg/bwt) + GN (100 mg/kg/bwt/*i.p.*)	6.21± 0.04	≤0.0001

GN=Gentamicin, RO=*Rosmarinus officinalis*, SEM=standard error of the mean

The study also recorded a significant (p≤0.0001) increase in the glomerulus volume in the high-dose RO as compared to the GN group. However, no significant difference (p=1.000 and p=0.333) was observed in low, and medium-doses RO when compared with the GN groups (Table 3).

Table 3: Mean volume of the glomerulus between GN group and high, medium, and low dose RO groups.

Variab-les	Groups	Mean± SEM	P value
Glome-rulus volume (µm³)	GN (GN 100 mg/kg/bwt)	5.09± 0.05	
	Low (100 mg/kg/bwt) + GN (100 mg/kg/bwt) dose	5.16± 0.07	≤1.000
	Medium dose (150 mg/kg/bwt) + GN (100 mg/kg/bwt/*i.p.*)	5.28± 0.05	≤0.333
	High dose (200 mg/kg/bwt) + GN (100 mg/kg/bwt/*i.p.*)	6.21± 0.04	≤0.0001

GN=Gentamicin, RO=*Rosmarinus officinalis*, SEM=standard error of the mean

DISCUSSION

Stereology is a three-dimensional method of obtaining quantitative data from microscopic images using software that analyzes the tissues to give fast, accurate, and precise information. Glomeruli are normally prone to injuries when nephrotoxic drugs such as GN are used without antioxidants hence; its volume capacity is usually affected.[13] The volume of the glomerulus was quantified to ascertain its functional capability to filtrate and excrete nitrogenous products when GN and RO were co-administered. The current study recorded a significant reduction ($p \leq 0.001$) of glomeruli volume of the GN groups as compared to the control groups but recorded no statistically significant difference with the low and medium dose RO groups. The reduction may have been due to oxidative damage causing shrinkage of the glomerulus in these groups (Table 2). This study finding is in tandem with another finding who also recorded a significant reduction ($p \leq 0.01$) in the glomerulus volume when they induced nephrotoxicity with 900 MHZ electromagnet radiation as compared to the control groups that were only given feeds plus water ad libitum.[14] Another study recorded similar study findings by having a significant ($p \leq 0.001$) reduction in the glomerulus volume in the acrylamide treatment group as compared to the control groups that was only given feeds plus water ad libitum.[15] A study also recorded the same findings of significance reduction ($p \leq 0.05$) in the glomerulus volume in the Diclofenac treatment group as compared to the control group that was only given feeds plus water ad libitum.[16]

Other authors disputed the current study findings and observed a significant ($p \leq 0.02$) increase in the glomerulus volume in the cerium oxide 250 mg/kg/bwt treatment groups as compared to the control groups that was only fed on feeds plus water ad libitum and was associated with hypertrophy in glomerulus seen in the affected histological slides.[17] A study found a statistically significant ($p \leq 0.05$) increase in the glomerulus volume in the pomegranate 250 and 500 mg/kg treatment groups as compared to the control groups that were only given feeds plus water ad libitum.[18] This was associated with the flavonoid-inducing vasodilation and infiltration of blood in the glomeruli hence the volume increase. A group of researchers registered a significant ($p=0.0001$) increase in the glomerulus volume in the sesame seeds treatment group as compared to the negative control groups; this was attributed to congestion in the glomeruli hence the volume increase.[19]

The current study observed no statistically significant ($p \geq 0.05$) difference in the glomerulus volume between the high-dose RO groups and the negative control groups, an indication of the reduction of the injurious effects of GN on the kidneys due to the protective anti-oxidative nature of RO (Table 3). Other literature agreed with the current study findings and registered no significant difference ($p \geq 0.05$) in the volume of the glomerulus between the folic acid with electromagnetic treatment groups and the control groups that were only given feeds plus water ad libitum.[14] The association with the antioxidant effect of folic acid on electromagnetic radiation registered no significant difference ($p \geq 0.01$) in the reduction of the glomerulus volume in the acrylamide with vitamin C treatment group as compared to the control group that was only given feeds plus water ad libitum.[20] The associated with the antioxidant effect of vitamin C that prevented rearrangement and fragmentation of the glomerulus volume.[16] Equally recorded no significant difference in glomerulus volume as this could have been attributed presence of antioxidants and anti-inflammatory factors that prevented the occurrence as of segmental necrosis.

Limitations

This study didn't consider the variability in active compounds of *Rosemarinus officinalis*. The chemical composition of rosemary extracts can vary based on growing conditions, extraction methods, and plant age, which might lead to inconsistent results.

CONCLUSION

The study findings show that high dose of RO 200/kg/bwt has protective histo-stereological effect against GN induced nephrotoxicity. Concurrent administration of RO and gentamicin are vivacious in guarding renal tissue from obliteration brought by free radicals. RO shows great promise as preventive agent against kidney injury by maintaining kidney structure and function. There is need to validate these findings, and investigate broader on therapeutic uses of RO in renal healthy.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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