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Original Research Article

Renal biomarkers of *Rosemarinus officinalis L.* on gentamicine induced acute kidney injury in adult male albino rats (*Rattus norvegicus*)

Kennedy W. Waswa[1]*, Scolastica Kori[2]

[1]Department of Human Anatomy, School of Medicine, Maseno University, Kenya
[2]Department of Microbiology, School of Medicine, Maseno University, Kenya

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***Correspondence:**
Dr. Kennedy W. Waswa,
Email: kennedywaswa1@gmail.com

ABSTRACT

Background: Gentamicin (GN) drug is a common choice for treating severe gram-negative bacteria infections especially in resource-limited settings where cost effective options are considered. However, its potential effect of causing acute kidney injury (AKI) has limited its wide use in clinical applications. *R. officinalis* (rosemary) is a traditional herb rich in antioxidants and anti-inflammatory properties. Conversely, renal biomarkers effect of Rosmarinus Officinalis (RO) on GN has not yet been elucidated. The main objective was to investigate the renal biomarkers and protective effect of *R. officinalis* against Gentamicin induced acute Kidney injury in adult Albino rats.
Methods: 25 adult male Albino rats were equally divided into five groups (5 Albino rats per group). Control group received rat pellets plus water ad libitum, GN (100 mg/kg/bwt/i.p), low dose RO (RO 100 mg/kg/bwt/po+GN), Medium RO(150 mg/kg/bwt/po+GN), and high dose RO (200 mg/kg/bwt/po+GN) groups. GN was administered intraperitoneal, and RO orally for seven consecutive days. After completion of 24hours of the last drug administration, they were humanely sacrificed, serum biochemical analysis of renal biomarkers (creatinine and urea) was determined.
Results: Gentamicin was able to induced acute kidney injury by increasing levels of creatinine and urea. However, with concurrent administration of high dose of RO, Serum creatinine and urea remained within the normal ranges.
Conclusions: These findings showed that, Concurrent administration of a high dose of RO 200 mg/kg/bwt with Gentamicin protects kidneys against Gentamicin induced injuries.
Keywords: Gentamicin, Acute kidney injury, biomarkers, antioxidant, *Rosmarinus officinalis*, and oxidation.

INTRODUCTION

In surgical ward, majority of infections are usually due to one or more of the gram-negative bacilli. Aminoglycosides have proven to be the most effective among different groups of antimicrobials that have been used.[1] After their introduction into therapeutic practice in aminoglycoside antibiotics such as amikacin and gentamicin are the most commonly used antibiotics worldwide for the treatment of gram-negative bacterial infections. However, serious complications like nephrotoxicity and ototoxicity are dose-Gentamicin (GN) drug is a common choice for treating severe gram-negative bacteria infections especially in resource-limited settings where cost effective options are considered.[1-3] This is because it is efficacious and cost-effective in contrast to other renal-friendly drugs. However, endless use of GN in low-income countries has resulted to a notable increase of chronic kidney diseases and mortality cases due to its nephrotoxicity. GN induce acute kidney injury (AKI) is marked by intricate of occurrences involving elevated levels of serum urea and creatinine levels, alongside austere proximal renal tubular necrosis.[4] Serum blood urea and creatinine form the benchmark of renal biomarkers for detecting acute kidney injury. Biomarkers of GN nephrotoxicity may be detected in urine and blood samples after specific days of

induction.[5] Urea and creatinine are commonly used in developing countries to check for nephrotoxicity associated with drugs. Acute kidney injury is described as an upsurge in serum creatinine and urea concentration, and necrosis of the convoluted tubules.[6] Renal toxicities related with GN is due to its accretion of metabolic wastes in the proximal tubules of kidneys, usually 50 to 100 times in serum.[7] Reduced oxygen metabolites are valuable intermediaries of toxic, immune-mediated, and ischemic tissue impairment. Nephrotoxicity possesses a significant health concern, particularly in developing countries where factors like, herbal medicine, industrialization and environmental toxins exacerbates the risks. The use of Aminoglycosides like GN has contributed to acute kidney injury especially when not monitored or poorly administered.[2] Gentamicin-induced nephrotoxicity is ranked as the third cause of AKI with a prevalence ranging from 18% to 27%.[8] GN causes AKI by generating free reactive oxygen and nitrogenous species, renal damage by apoptosis, and releases of toxic enzymes. The antioxidant properties of *R. officinalis* lamiaceae, can be used to reduce the impact of reactive oxygen species that contributes to AKI. Rosmarinus officinalis (RO) is a native natural plant to the Mediterranean region but currently widely tamed too many countries like Kenya. Leafs of the RO are used as food condiment, traditional medicine for analgesia and stimulant. The benefit of this plant includes, high antioxidants properties like; rosemarinic, carnosic, caffeic and ursonic acids.[9] Proven treatment to Asthma, hepatotoxicity, ischemic heart disease, hypercholesteremia and inflammation.[10] The antioxidants in RO have demonstrated an essential role in deterring and attenuating free radicals, hence protecting people against infections and degenerative conditions. Though there is a lot of data on nephroprotection and attenuation of RO, data on protective capabilities on GN-induced nephrotoxicity is scarce. The researchers currently advocate the use of natural antioxidants due to their safe therapeutics' properties. While RO has been used traditionally as medicine, there is scanty data on its concurrent administration with GN and its effect on kidney structures. serum biochemical effects on kidney structures when is concurrently administered with GN. The study delves into investigated the renal biomarkers to attenuate the effects of RO on gentamicin AKI in adult male albino rats.

METHODS

Study location

The study was done in the month of May 2023 to July 2013 in Kenya 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.

Experimental animals

This study was conducted on the 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. Twenty-five male Albino rats with weight between 150 to 200 grams were acquired from the animal house of the department of zoology, Maseno University. Albino rats were feed on standard rat pellet diet Ad water libitum. Rats were kept in cages at research room with average temperature of 27 degrees Celsius for a period of 2 weeks to acclimatize before administration of study drugs. Strict occupational animal handling procedures were adhered to, from the start to the end of the study.

Experimental design

Post-test only true experimental design was used. 25 male Albino rats were randomly apportioned into two main groups; a control group of 5 animals, and 20 animals, for the experimental group. The 20 albino rats were further randomly categorized into four separate groups of 5 rats respectively. GN group given GN100mg/kg/bwt intraperitoneal (i.p), Low dose group received RO 100 mg/kg/bwt per oral (PO)+GN 100 mg/kg/bwt/ i.p, Medium dose group RO 150 mg/kg/wt/po+GN 100 mg/kg/bwt/i.p, and group 4 High dose group RO 200 mg/kg/bwt/PO+GN 100 mg/kg/bwt/i.p.

Acquisition of study drugs

Gentamicin sulphate B.P 80 mg/2 ml with Batch Number 202103177 was purchased from Crowns Healthcare Company and RO capsules were purchased from SWANSON company Fargo ND 58104USA1-800-437-4148.

Determination of doses for gentamicin sulphate and R. officinalis

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 rosmarinic acid on methotrexate-induced renal-toxicity and hepatotoxicity in Albino rats. A low dose of RO (100 mg/kg/bwt/PO), a medium dose (150 mg/kg/bwt/PO), and a high dose of (200 mg/kg/bwt/PO) were used, as per.[12]

Procedure for drug administration

Doses of GN 100 mg/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 controlled firmly head-down, Anatomical landmarks were identified on the abdomen,

the needle was inserted with 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 R. officinalis capsules doses

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

Procedure of administration of R. 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 ml of demonized water. The albino rat was gently held out of the neck region with the left hand. The rats were wrapped in a tablecloth to avoid soiling the researcher's clothing. The albino rats were 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.

Blood samples collection

After completion of administration of study drugs for seven days, the renal biomarkers were done to check for acute kidney injury caused by GN, and protective effect of RO. Albino rats were anesthetized, 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 then 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 the midline incision made, the kidneys were pushed interiorly and the posterior vena cava was seen between the kidneys. The syringe attached to a 21-gauge needle was inserted to collect 5 ml of the blood sample into a plain Vacutainer BD tube in line with.[13] The blood samples were then centrifuged at 1500 rpm for 5 minutes; serum was decanted into Cry vials, and then stored at -20 °C until biochemical analysis was done.

Serum biochemical analysis

Automated Mindray Chemistry Analyzer Model: BS-200 from Shenzhen Mindray Bio-Medical Electronics Company limited China, was used to determine the levels of Creatinine and Urea for all samples collected for this study.

Data analysis

The collected data was entered into an excel sheet and analysed through SPSS version 25.0. One-way ANOVA and post hoc Bonferroni was used to compare data obtained from experimental and control groups. P values of less than or equal to 0.05 ($p \leq 0.05$) were considered significant.

Ethical approval

The ethical approval to carry out the study was sought from Baraton University of Eastern Africa (UEAB/ISERC/06/01/023), and research license from Nation commission of science technology and innovation (NACOSTI/P/23/24510). All experimental activities were carried out accordance with code of ethics for experimental animals.

Figure 1 (A and B): Showing administration of Gentamicin and *Rosmarinus officinalis*, respectively.

Figure 2: The collection of blood samples via cardiac puncture.

RESULTS

Animal mortality

There was no mortality observed during the period of experiment.

Mean terminal body weight of Albino rats between GN and RO groups.

There was a significant (p=0.0001) increase in the mean terminal weight of rats in high dose of the RO group as compared to the GN group. However, when the mean terminal weight of both medium and low doses of RO was compared to the GN groups, no significant difference was observed at p=0.8660, and 1.000 respectively (Table 1).

Table 1: Showing mean terminal body weight of the albino rats in GN, and high, medium, and low RO groups.

Groups	Body weight Mean±SEM	P value
GN group (GN 100 mg/kg/bwt/i.p)	175.32±0.83	
High dose of RO (200 mg/kg/bwt)+GN (100 mg/kg/bwt/i.p)	203.58±0.89	0.0001
Medium dose of RO (150 mg/kg/bwt)+GN (100 mg/Kg/bwt/i.p)	179.70±1.84	0.8660
Low dose of RO (100 mg/kg)+GN (100 mg/kg/bwt/i.p)	177.01±2.41	1.000

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

Table 2: Showing mean creatinine and urea levels between the control and the GN groups.

Groups	Serum creatinine levels Mean±SEM	Serum urea levels Mean±SEM
Control (feeds+water)	0.5200±0.01378	6.5800±0.04990
GN (GN 100 mg/kg/bwt/i.p)	1.6920±0.06272	12.5020±0.12290
P value	P=0.001	P=0.001

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

Table 3: Showing mean urea and creatinine levels between the GN group and RO groups.

Groups	Serum creatinine levels	P value	Serum urea levels	P value
GN (GN 100 mg/kg/bwt)	1.6920±0.06272	P=0.001	12.5020±0.12290	p≤0.001
High dose (200 mg/kg/bwt)+GN (100mg/kg/bwt/i.p)	0.7100±0.04171	P=0.001	6.5960±0.16863	P=0.001
Medium dose (150 mg/kg/bwt)+GN (100 mg/kg/bwt/i.p)	1.5420±0.05607	P=0.855	12.0180±0.21383	P≤0.408
Low dose (100 mg/kg/bwt)+GN (100 mg/kg/bwt/i.p)	1.6080±0.09047	P=1.000	12.0480±0.17511	P≤0.535

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

Table 4: showing mean Urea and creatinine levels between the control and other RO groups.

Groups	Serum creatinine levels	P value	Serum urea levels	P value
Control	0.5200±0.01378	P=0.0001	6.5800±0.04990	
High dose (200mg/kg/bwt)+GN (100mg/kg/bwt/i.p)	0.7100±0.04171	P=0.329	6.5960±0.16863	P=1.0000
Medium dose (150mg/kg/bwt)+GN (100 mg/kg/bwt/i.p)	1.5420±0.05607	P=0.0001	12.0180±0.21383	P=0.0001
Low dose (100mg/kg/bwt) + GN (200mg/kg/bwt/i.p)	1.6080±0.09047	P=0.0001	12.0480±0.17511	P=0.0001

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

Renal biomarkers

Serum urea and creatinine levels were measured in all the groups and intergroup comparison was made. The adopted normal ranges for urea levels were (4.2-8.97 mmol/l) and the creatinine levels were (0.2-0.8 mg/dl). The serum urea and creatinine levels of the control group were compared to the GN group to confirm the induction of acute kidney injury. The serum urea and creatinine levels of the GN group were compared with other experimental groups that had been given different doses of RO to check for renal protection. There was a significant (p=0.0001) increase in both the urea and creatinine levels in the GN group as compared to the control group (Table 2).

There was a significant (p=0.001) reduction of the serum Urea and Creatinine levels of the High-dose RO as compared to the GN group. There was no significant reduction of creatinine (p=0.855 and p=1.000) and urea (p=0.408 and p=0.535) in the low and medium RO groups respectively when compared with the GN group. (Table 3).

There was no significant difference (p=0.329) and (p=1.0000) in high dose RO of creatinine and serum urea as compared to the control group. There was a significant increase of creatinine (p=0.0001 and p=0.0001) and Urea (p=0.0001 and p=0.0001) in low and Medium RO groups respectively as compared to the control group, respectively (Table 4).

DISCUSSION

Urea is produced when the liver breaks down protein and ammonia, and creatinine is waste product formed during normal wear and tear of the muscles of the body. They are biomarkers commonly used to detect the deterioration of kidney function and estimate the type of kidney injury. This is because they are filtered by the glomerulus and expelled in the urine as nitrogenous waste products hence, their use to assess AKI is vital. GN causes injuries to the cell membrane of the glomeruli and convoluted tubules, distorting its architectural structures and optimally reducing the filtration rate of the kidney. This causes accumulations of nitrogenous wastes and a reduction in renal perfusion. This study found a significant (p=0.001) increase in urea and creatinine levels in the GN groups, low dose RO, and medium dose RO groups, when compared to control groups, which had levels that were within normal ranges (Table 3). The increase in urea levels in the GN groups may have been due to increased production in oxidative stress and inflammatory markers causing damage to epithelial cells of the glomerulus and convoluted tubules. This injury reduces the glomeruli filtration rate, hence the increase of serum and creatinine in the blood. The findings were tandem with that of, when assessing the biochemical parameters in rats before and after nephrotoxicity.[5] Observed reduced levels of urea in rats when assessing the renal biochemical changes. The difference in urea levels noted by Wu might have been due to the use of agents that are metabolized by both the kidney and liver. Therefore, the level is more likely to be reduced.[14]

Unlike Low-dose RO and medium-dose RO, there was a significant (p≤0.001) reduction of serum Urea in the high-dose RO as compared to the GN groups, but no significant difference was recorded with the control groups. This reduction in urea levels was due to increased anti-inflammatory and anti-oxidative activities of *R. officinalis*, which can prevent inflammation and scavenge oxidants produced by GN which could have caused injuries to the glomeruli to reduce filtration rate hence accumulations of nitrogenous wastes in the blood. This finding is in agreement with, study in Brazil on the palliative action of curcumin on GN-induced nephrotoxicity, which showed a reduction in urea and creatinine levels signifying renal protection effect.[9] Curcumin has been used as a reference as curcumin, and *R. Officinalis* have a similar pathway in protecting kidney structures. These findings also concur with observations from other studies.[15,16] Contrary to the above findings, experimental research on the effect of Curcumin on glycerol-induced acute kidney injury in albino rats showed an increase of urea and creatinine between AKI and Corn oil+AKI groups.[5] The increase of these biomarkers could have caused a lack of high-concentration antioxidants in Corn oil to prevent oxidation caused by glycerol, causing retention of nitrogenous wastes.

The rise in creatinine levels was due to damaged renal function and the accumulation of necrotic cells within the glomerular vessels, consequently reducing the filtration rate and leading to nephrotoxicity. Study in Iraq, while evaluating the short-term effect of Malathion pesticide on physiological functional changes in kidneys in mice reported a similar tendency.[17,18] Study in Bangladesh noted similar reports while assessing renal function parameters of rats on treatment with the "SulvajriniVatika" herb.

In this study, it was noted that there was a statistically significant (P≤0.001) reduction in creatinine levels in the high-dose RO groups as compared to the GN groups. However, there was no statistically significant difference with the control groups (Table 2). This signifies the protective anti-oxidative effects of RO on the kidney at high doses unlike in low and medium doses. The antioxidants produced by RO could have counter-reacted with the reactive oxygen radical species and nitrogen oxide that normally destroy glomerulus and kidney tubules, thus interfering with kidney histo-architecture structures that perform filtration and excretion of toxic waste. Consequently, the presence of RO antioxidant activities sped up drug clearance, thus preventing injury and the accumulations of nitrogenous products in the kidney tissues. These findings also concur with those while assessing the antioxidant benefits of garlic in chronic kidney disease.[19,20] This study concentrated solely on blood urea and creatinine levels to evaluate the preventive effect of RO. However, other important biomarkers such as, urine output, KIM-1, and NGAL, which are also crucial in assessing kidney injury were not included in the analysis.

CONCLUSION

The biochemical tests indicated that pre-treatment with RO can effectively prevent kidney structures damage that is associated with Gentamicin drug. The antioxidants in RO are critical in guarding renal tissue from destruction brought by free radicals. RO shows great promise as preventive agent against kidney injury by maintaining kidney structure and function, lowering oxidative stress, and encouraging healthy fluid balance. To validate these results and investigate wider therapeutic uses of RO in renal healthy, more research is necessary.

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