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Treatment with methanolic extract of *Ocimum gratissimum* (Linn.) leaf reversibly normalizes urine protein-creatinine ratio in Wistar rat model of gentamicin-induced kidney injury

Olaleye R.O[1], Akomolafe R.O[1], Imafidon E.C[1]*, Ogundipe D.J[1], Olukiran S.O[1], Oladele A.A[2]

[1]Department of Physiological Sciences, Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. [2]Department of Medical Laboratory Sciences, College of Medicine, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria.

*Corresponding author: staywithchris@gmail.com

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ABSTRACT

Background: Due to its rapid onset of action, high antibacterial efficacy and low cost, gentamicin (GEN) is still been used (particularly in underdeveloped and developing countries) despite its nephrotoxic antibiotic effects. Experimental ethno-botanical evaluations are imperative in a world with increasing incidence of kidney injury; a condition that is expensive to treat or manage. **Aim:** Effects of methanolic extract of *Ocimum gratissimum* (Linn.) leaf (MOGL) on urine protein-creatinine ratio (UPC) was determined in rats with GEN-induced kidney injury. **Methods:** This study involved the use of 50 rats such that graded doses of MOGL (100, 200 and 400 mg/kg p.o.) were administered following 8 days exposure to GEN (100 mg/kg i.p) and comparison were made against the control, toxic, 2 weeks and 4 weeks MOGL treatment groups at $p < 0.05$. **Results:** GEN induced nephrotoxicity as depicted by significant perturbations in plasma and urine levels of creatinine and total protein ($p < 0.05$); significantly lowered creatinine clearance and abnormal elevations of UPC as well as deleterious alterations of the kidney antioxidant system (GSH and TBARS) ($p < 0.05$). Histopathological examination showed glomerular atrophy, formation of densely eosinophilic/ colloid cast within the tubules and severe loss of cellular constituents in the medullary interstitium. When compared with the control, these conditions were significantly normalized after 2 week MOGL treatment ($p < 0.05$) with a deleterious reversal from normalcy at 4 week MOGL treatment ($p < 0.05$). **Conclusion:** This study substantiated that MOGL has therapeutic potentials that becomes deleterious after sub-chronic administration in rat model of GEN-induced kidney injury.

Key words: Gentamicin, renal function test, *Ocimum gratissimum*, urine protein-creatinine ratio, creatinine clearance, antioxidant system

INTRODUCTION

Nephrotoxicity has been implicated in the use of gentamicin, an amino glycoside antibiotic commonly used in the treatment of life-threatening gram negative bacterial infections.[1,2] Despite the introduction of less nephrotoxic antibiotics against gram negative infections, gentamicin is still been used clinically due to its rapid onset of action, high antibacterial

efficacy and low cost (relative affordability),[1] particularly in underdeveloped and developing countries. Several studies have shown that reactive oxygen species (ROS) may be implicated in gentamicin-induced renal dysfunction.[3,4] This involves free radical generation and reduction in antioxidant defense mechanisms with consequent decline in glomerular filtration rate.[3,4] The pathophysiological mechanism of this drug resulted in a hypothesis that a possible reversal or, at least, amelioration of its nephrotoxic effects can be achieved using a potent antioxidant. Engaging ethno-botanical approach, this study aimed at testing this hypothesis with a focus on the urine protein-creatinine ratio (UPC); an important index of renal function in clinical models of kidney injury.

UPC is an important clinical index used to quantify and monitor significant levels of proteinuria.[5] For an apparently healthy individual or one with stable kidney injury, creatinine excretion is constant. Hence, UPC (a quantitative test), allows for effective monitoring of the progression of clinical models of renal condition as it trends the level of proteinuria.[5,6]

It is a known fact that plant derived medicines is easily available and relatively affordable for a common man. Besides, they are relatively safer than synthetic alternatives and provide inspiration for novel drugs development.[7,8] Commonly known as “scent leaf”, *Ocimum gratissimum* is a perennial plant used as spice in food.[9] It is a shrub that prefers moist and fertile soil during growth but will tolerate drought after flowering.[9,10] This plant is reputed to possess several medicinal properties ranging from antimicrobial, anti-helminthic and insect repellant activities.[10,11] It also experimentally evaluated for treatment of upper respiratory tract infections, headache, diarrhea, pneumonia, cough, fever and conjunctivitis[13] as well as treatment or management of epilepsy and mental illness [14, 15]. Nevertheless, there is dearth of literature on the effects of this plant in models of nephrotoxicity or kidney injury.

There is a gradual but increasing incidence of various forms of nephrotoxicity or kidney injury. In Nigeria, about 27–30 million people have kidney disease, with an incidence of about 15,000 new cases every year and prevalence of about 45,000 living with kidney failure annually.[16,17] The cost of management or treatment of the various forms of kidney injury is usually affordable, only, to the rich; a number that constitute a microscopic few when compared with the general population in underdeveloped and developing countries. There is, therefore, an increasing need to exploit the health benefits of natural products of plant origin.

Our literature survey revealed that there is no experimental evidence on the effects of *Ocimum gratissimum* (Linn.) leaf on urine protein-creatinine ratio despite evidences portraying this plant to have a wide range of health-beneficial effects. This study was carried out with an attempt to bridge this gap in knowledge using animal (Wistar rat) model of drug-induced kidney injury.

METHODOLOGY

Plant material, drug, laboratory kits and metabolic cages

Fresh leaves of *Ocimum gratissimum* were obtained from a garden in Ife-Ibadan Toll Gate Area of Ile Ife and thereafter identified by the Chief Herbarium Officer, Mr Gabriel Ademoriyo of the Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. A voucher specimen with reference number IFE - 17491 was deposited at the Herbarium.

Gentamicin injection (80mg/2ml) was purchased from Shanxi Shuguang Pharmaceutical Co., Limited, China. Standard laboratory kits for plasma and urine biochemical analyses were purchased from Randox Laboratories Limited, United Kingdom while metabolic cages were fabricated by Central Technological Laboratory and Workshops (CTLW), OAU, Ile-Ife, Osun State, Nigeria.

Extraction process

Leaves of *Ocimum gratissimum* were air-dried, blended and thereafter macerated with 90% methanol. The resulting mixture was filtered with Whatman number 1 filter paper, concentrated at 38°C using a Rotary Evaporator (6540-2, Buchi Laboratorum-Technik AG.CH-9230 Flawil/Schweiz, Switzerland). Concentrated

solution was freeze-dried to obtain Methanolic extract of *Ocimum gratissimum* leaf (MOGL).

Animal management, experimental protocol and dose regimen

Fifty male Wistar rats of about 2 months–2 months and 2 weeks old (weighing 120-150g) were divided into five groups as follows; Group 1 consisted of 5 rats that received distilled water (0.2 ml/100g i.p.) for 8 consecutive days and thereafter received the same amount via oral route for 4 weeks before they were sacrificed. Group 2 consisted of 15 rats that received 8 days administration of gentamicin (100 mg/kg/day) after which 5 rats were sacrificed. Thereafter, 5 rats were each sacrificed after 2 weeks and 4 weeks of recovery period. Groups 3, 4 and 5 (each consisted of 10 rats) were each treated with gentamicin as group 2 and thereafter received graded doses of the extract at 100, 200 and 400 mg/kg respectively via oral route at 0.2 ml/100g. Thereafter, 5 rats from each group were sacrificed after 2 weeks and 4 weeks of treatment with MOGL (figure 1). After the rats were euthanized, blood samples were collected by cardiac puncture into separate EDTA bottles and thereafter centrifuged at 4000 rpm for 15 minutes using a cold centrifuge (Centurium Scientific, Model 8881) set at –4 °C. Plasma obtained was decanted with a sterile syringe into separate plain bottles for biochemical assays. The left kidney of each rat was excised into a cooler for homogenate preparation while the right kidney was fixed in 10 % formal saline solution for histopathological examination using hematoxylin-eosin (H and E) staining technique.

Percentage weight change and relative kidney weight

Weekly body weight was measured using a digital weighing balance (Hanson, China) while relative kidney weight (RKW) was measured using the formula below:

$$RKW\ (\%) = \frac{\text{Weight of left kidney + Weight of right kidney}}{\text{Final body weight}} \times 100\%$$ [19]

Biochemical assays of plasma and urine

The plasma and urine levels of creatinine were determined as described in the laboratory protocols provided by Randox kit while the total protein was determined by Lowry's method.[20]

However, creatinine clearance was calculated using conventional formula as follows;

Clearance = U_cV/P_c *(ml/min)*

Where U_c = *Urine concentration of creatinine;*

V = *Urine flow rate = Amount of urine/time (seconds);*

P_c = *Plasma concentration of creatinine.*

Urine protein-creatinine ratio (UPC) was calculated as follows;

$$UPC = \frac{\text{Protein concentration in urine}}{\text{Creatinine concentration in urine}}$$

Note: Conversion from dl/ml to mg/g was made using the following conversion system;

1dl = 100g[19,21]

Assessment of oxidative stress indicators

The left kidney of each rat was carefully excised, defatted and weighed. Using an electric homogenizer (SI601001), the kidney tissue was homogenized with 10 ml of sucrose solution (0.25 M). Thereafter, 10% homogenate in phosphate buffer (100 mM) was prepared at pH 7.4. The homogenate was centrifuged at 3000 rpm for 20 minutes and the supernatant was collected for the assessment of the following indicators of oxidative stress. Reduced glutathione (GSH) level was determined by the method of Beutler *et al*.[22] while the activity of thiobarbituric acid reactive substances (TBARS) was determined by the method of Ohkawa *et al*.[23]

Histopathological examination

The right kidney of each rat was fixed in 10% formal-saline solution. Thereafter, sections of about 7 – 8 µm thick were taken, dehydrated in graded alcohol and embedded in paraffin wax. Using Hematoxylin and Eosin (H & E) staining technique, photomicrographs were taken using Leica DM 750 camera microscope at x400 magnification.

Ethics

All experimental protocols were in strict compliance with the guideline for animal research, as detailed in the NIH Guidelines for the Care and Use of Laboratory Animals [18] and approved by local Institutional Research Committee.

Statistical analysis

The data obtained were expressed as mean ± standard error of mean (S.E.M) using one-way analysis of variance. Data were thereafter subjected to Tukey's post-hoc test for multiple comparison and values at $p< 0.05$ were considered statistically significant. Analyses were carried out using Microsoft excel (2007 package) and Graph pad prism 5.03 (Graph Pad Software Inc., CA, USA).

RESULTS

Effects of MOGL on Relative Kidney Weight [RKW (%)] of rats with gentamicin-induced kidney injury

Group 1 (control) showed significantly lower RKW (0.55 ± 0.01) when compared with the toxic subgroup (1.09 ± 0.07) ($p<0.0001$) as well as 2 and 4 weeks toxic recovery subgroups of group 2 (1.05 ± 0.06 and 0.84 ± 0.07 respectively) ($p=0.04$). Following 2 weeks of MOGL treatment, groups 3, 4 and 5 still maintained a significantly higher RKW (0.70 ± 0.01; 0.72 ± 0.01 and 0.79 ± 0.02 respectively) when compared with group 1 (0.55 ± 0.01) ($p<0.001$) but significantly lower when compared with toxic subgroup (1.09 ± 0.07) ($p<0.0001$) and 2 weeks recovery subgroup of group 2 (1.05 ± 0.06) ($p<0.0001$). Also, 4 weeks treatment with MOGL was associated with a significantly higher RKW in groups 3, 4 and 5 (0.75 ± 0.02; 0.80 ± 0.04 and 0.78 ± 0.04 respectively) when compared with group 1 (0.55 ± 0.01) ($p<0.0001$) but significantly lower when compared with 2 weeks recovery subgroup of group 2 (1.05 ± 0.06) ($p=0.0004$) (figure 2).

Effects of MOGL on percentage body weight change (% BWC) of rats with gentamicin-induced kidney injury

Two weeks post-exposure to gentamicin treatment was associated with a significantly higher % BWC in both the toxic and 2 week MOGL-treated groups 3, 4 and 5 when compared with the control ($p< 0.05$). The same is true when the control (group 1) is compared with 4 weeks toxic recovery group and 4 week MOGL-treated groups ($p< 0.05$). The significantly higher % BWC that accompanied MOGL treatment following gentamicin administration was shown to be dose-dependent, with the highest dose (400 mg/kg) having the highest effect (figure 3).

Effects of MOGL on plasma and urine levels of creatinine (mg/dl) in rats with gentamicin-induced kidney injury

As shown in table 1, the plasma creatinine level was significantly higher in the toxic and toxic recovery subgroups of group 2 when compared with group 1 ($p=0.004$). However, following 2 weeks treatment with MOGL, the MOGL-treated groups 3, 4 and 5 showed no significant difference in plasma creatinine level when compared with group 1 ($p=0.55$). The levels in these groups were significantly lower than the toxic and 2 weeks toxic recovery subgroups of group 2 ($p=0.012$). After 4 weeks treatment with MOGL, the MOGL-treated groups 3, 4 and 5 showed significantly higher levels of plasma creatinine when compared with their corresponding 2 weeks MOGL-treated groups ($p=0.0004$) and group 1 ($p<0.0001$).

Urine creatinine level was significantly lowered in toxic and toxic recovery subgroups of group 2 when compared with group 1 ($p=0.0069$). MOGL-treated groups 3, 4 and 5 showed no significant difference when compared with group 1 ($p=0.56$) after 2 weeks of MOGL treatment following exposure to gentamicin toxicity. After 4 weeks of MOGL treatment, the urine creatinine level was significantly lower in the MOGL-treated groups 3, 4 and 5 when compared with their corresponding 2 weeks MOGL-treated groups ($p=0.0093$) as well as group 1 (0.0014). The 4 weeks MOGL-treated groups 3, 4 and 5 showed no significant difference in urine creatinine level when compared with the toxic and toxic recovery subgroups of group 2 ($p=0.0048$) (table 1).

Effects of MOGL on creatinine clearance (ml/min) of rats with gentamicin-induced kidney injury

Creatinine clearance (CrCl) in the toxic subgroup (0.49 ± 0.15) as well as 2 and 4 weeks toxic recovery subgroups of group 2 (0.76 ± 0.12 and 0.54 ± 0.17 respectively) was significantly lower than that of group 1 (3.40 ± 0.18) ($p<0.0001$) following gentamicin administration. Although 2 weeks MOGL treatment was associated with a significantly lower CrCl in groups 3, 4 and 5 (1.96 ± 0.19; 1.68 ± 0.14 and

1.44 ± 0.15 respectively) when compared with group 1 (3.40 ± 0.18) (p<0.0001), this was showed to be significantly higher when compared with the toxic (0.49 ± 0.15) (p<0.0001) and 2 weeks toxic recovery (0.76 ± 0.12) (p=0.0003) subgroups of group 2. After 4 weeks of MOGL treatment to rats with gentamicin-induced kidney injury, CrCl was shown to be significantly reversed to toxic levels as there was no significant difference in the MOGL-treated groups 3, 4 and 5 (0.62 ± 0.16; 0.64 ± 0.17 and 0.62 ± 0.19 respectively) when compared with toxic (0.49 ± 0.14) (p=0.92), 2 and 4 weeks toxic recovery subgroups (0.76 ± 0.12 and 0.54 ± 0.17 respectively) (p=0.92 and 0.98 respectively) of group 2. Also, these MOGL-treated groups showed significantly lowered CrCl when compared with 100 mg/kg MOGL (1.96 ± 0.19) (p=0.0001), 200 mg/kg MOGL (1.68 ± 0.14) (p=0.0006) and 400 mg/kg MOGL (1.44 ± 0.15) (p=0.0064) of the 2 weeks treatment period (figure 4).

Effects of MOGL on plasma and urine levels of total protein (mg/ml) in rats with gentamicin-induced kidney injury

Table 2 shows a significantly lower plasma total protein level in the toxic and toxic recovery subgroups of group 2 when compared with group 1 (p=0.0007) as well as 2 weeks MOGL-treated groups 3, 4 and 5 (p=0.044). After 4 weeks treatment with MOGL, the MOGL-treated groups 3, 4 and 5 showed no significant difference in plasma total protein level when compared with their corresponding 2 weeks MOGL-treated groups (p=0.472) but was significantly lower than group 1 (p=0.009).

Following exposure to gentamicin toxicity, urine total protein of the rats was significantly higher in the toxic and toxic recovery subgroups of group 2 when compared with group 1 (p=0.004) and 2 weeks MOGL-treated groups 3, 4 and 5 (p<0.0001). The 2 weeks MOGL-treated groups 3, 4 and 5 showed no significant difference when compared to group 1 (p=0.1913). After 4 weeks treatment with MOGL, the MOGL-treated groups 3, 4 and 5 showed significantly higher urine total protein when compared with their corresponding 2 weeks MOGL-treated groups (p=0.0028) and group 1 (p=0.0026) (table 1).

Effects of MOGL on urine protein-creatinine ratio (mg/g) of rats with gentamicin-induced kidney injury

Urine protein-creatinine ratio (UPC) was significantly higher in toxic subgroup 2 (10.20 ± 0.13) as well as 2 and 4 weeks recovery subgroups of group 2 (6.88 ± 0.12 and 6.21 ± 0.12 respectively) when compared with group 1 (2.01 ± 0.12) (p<0.0001). Following 2 weeks treatment with MOGL, groups 3, 4 and 5 (1.75 ± 0.11; 2.04 ± 0.11 and 2.23 ± 0.11 respectively) showed no significant difference in UPC when compared with group 1 (2.01 ± 0.12) (p=0.06). However, these MOGL-treated groups showed a significantly lower UPC when compared with the toxic subgroup (10.20 ± 0.13) as well as 2 and 4 weeks recovery subgroups of group 2 (6.88 ± 0.12 and 6.21 ± 0.12 respectively) (p<0.0001). After 4 weeks of MOGL treatment, the MOGL-treated groups 3, 4 and 5 (6.18 ± 0.13; 5.53 ± 0.12 and 4.82 ± 0.12 respectively) showed a significantly higher UPC when compared with group 1 (p<0.0001) as well as when compared with 2 weeks MOGL-treated groups 3, 4 and 5 (1.75 ± 0.11; 2.04 ± 0.11 and 2.23 ± 0.11 respectively) (p<0.0001) (figure 5).

Effects of MOGL on the activity of thiobarbituric acid reactive substances [TBARS x 10^{-6} (mmol/mg protein)] and level of reduced glutathione [GSH (µg/mg protein)] in the kidney homogenate of rats with gentamicin-induced kidney injury

TBARS activity in the kidney homogenate of the rats was significantly higher in the toxic and toxic recovery subgroups of group 2 when compared with group 1 (p<0.0001) and 2 weeks MOGL-treated groups 3, 4 and 5 (p<0.0001). The 2 weeks MOGL-treated groups 3, 4 and 5 showed no significant difference when compared to group 1 (p=0.8698). After 4 weeks of MOGL treatment, the MOGL-treated groups 3, 4 and 5 showed a significantly higher TBARS activity when compared with their corresponding 2 weeks MOGL-treated groups (p=0.0137) and group 1 (p=0.0201) (table 3).

Table 3 shows a significantly lower GSH in the toxic and toxic recovery subgroups of group 2 when compared with group 1 (0.0004) and 2 weeks MOGL-treated groups 3, 4 and 5 (p=0.0002). The 2 weeks MOGL-treated groups 3, 4 and 5 showed no significant difference

when compared to group 1 (p=0.9761) while 4 weeks MOGL-treated groups 3, 4 and 5 showed no significant difference in GSH level when compared with their corresponding 2 weeks MOGL-treated groups (p=0.3361).

Histological effects of MOGL on the kidney of rats with gentamicin-induced kidney injury

Following exposure to gentamicin toxicity, the toxic subgroup of group 2 showed evidence of atrophic glomerulus (green arrow), signs of inflammation with formation of densely eosinophilic/ colloid cast within the lumen of the renal tubules (red arrow). This subgroup also revealed severe loss of cellular constituents in the medullary interstitium (double black arrow head). The 2 weeks and 4 weeks recovery subgroups of group 2 also revealed evidence of severe loss of cellular constituents (double black arrow head), formation of colloid casts (red arrow) as well as atrophic glomeruli (green arrow), with the 4 week recovery subgroup showing the best histoarchitecture in the toxic subgroups of group 2 when compared with group 1 (figure 6).

Two week treatment with MOGL was associated with appreciable improvement in the kidney histoarchitecture of the rats in groups 3, 4 and 5 when compared with the toxic subgroups of group 2. However, there was evidence of increased Bowman's capsular space in groups 3 and 4 after treatment with MOGL for 2 weeks. Also, group 4 showed evidence of cellular constituents' loss (double black arrow head) (figure 6).

Four week MOGL treatment showed evidence of severe loss of cellular constituents (double black arrow head) in the kidney histology of the rats, atrophic glomerulus (green arrow) and apparent inflammatory cells in the proximal convoluted tubule (orange arrow). In general, the kidney histoarchitecture of rats treated with MOGL for 4 week showed lesser improvement when compared with their corresponding 2 week MOGL-treated groups but more improvement when compared with the toxic subgroups of group 2 (figure 6).

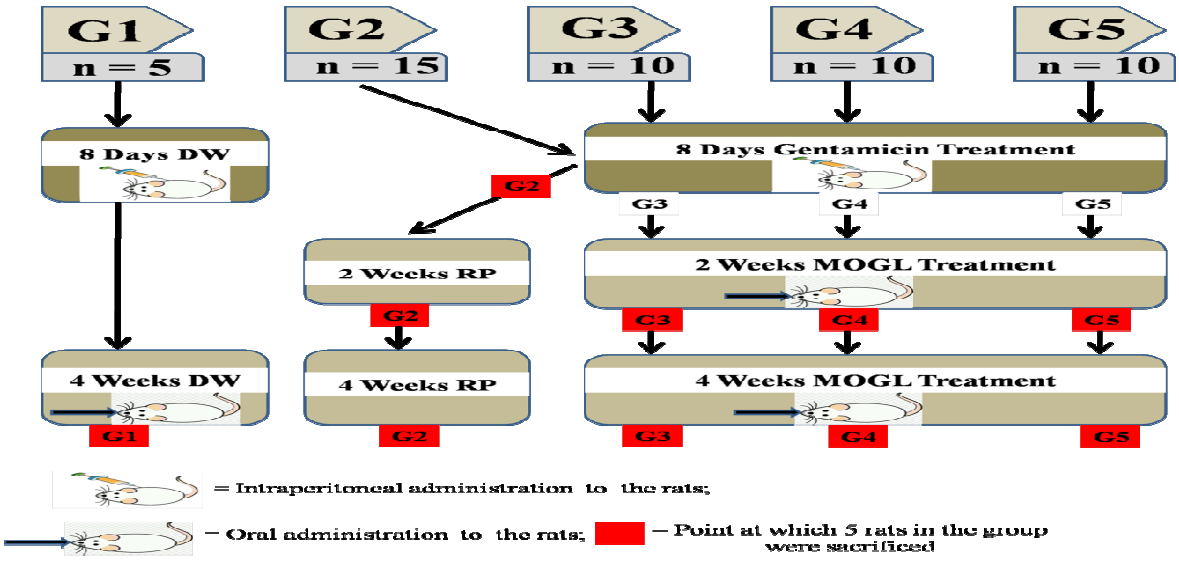

Figure 1: Dose regimen and experimental protocol

MOGL = Methanolic extract of *Ocimum graticimum* Leaf; **G1 =** Group 1 (Control)**; G2 =** Group 2 (Toxic group & Toxic + Recovery group)**; G3** = Group 3 (Toxic + 100 mg/kg MOGL); **G4** = Group 4 (Toxic + 200 mg/kg MOGL); **G5** = Group 5 (Toxic + 400 mg/kg MOGL); **n** = Total number of rats in the group; **DW** = Distilled water; **RP** = Recovery period.

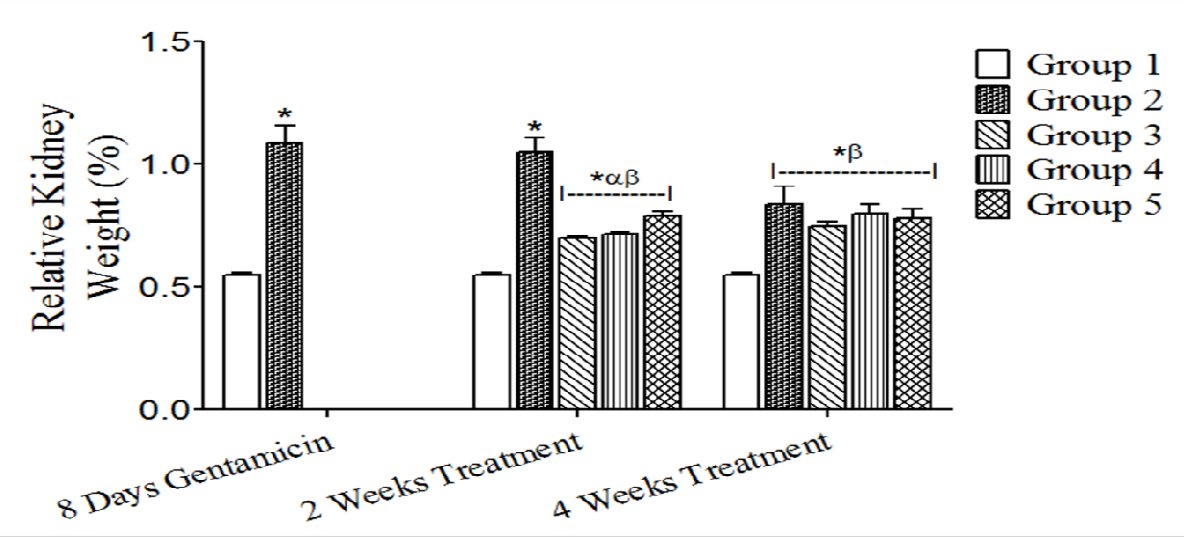

Figure 2: Graph showing the effects of MOGL on Relative Kidney Weight (%) of rats with gentamicin-induced kidney injury

Each bar represents mean ± standard error of mean (S.E.M) and is significant at $p < 0.05$. ***** = significantly different from group 1 (control); **α** = significantly different from toxic subgroup 2; **β** = significantly different from 2weeks recovery subgroup 2.

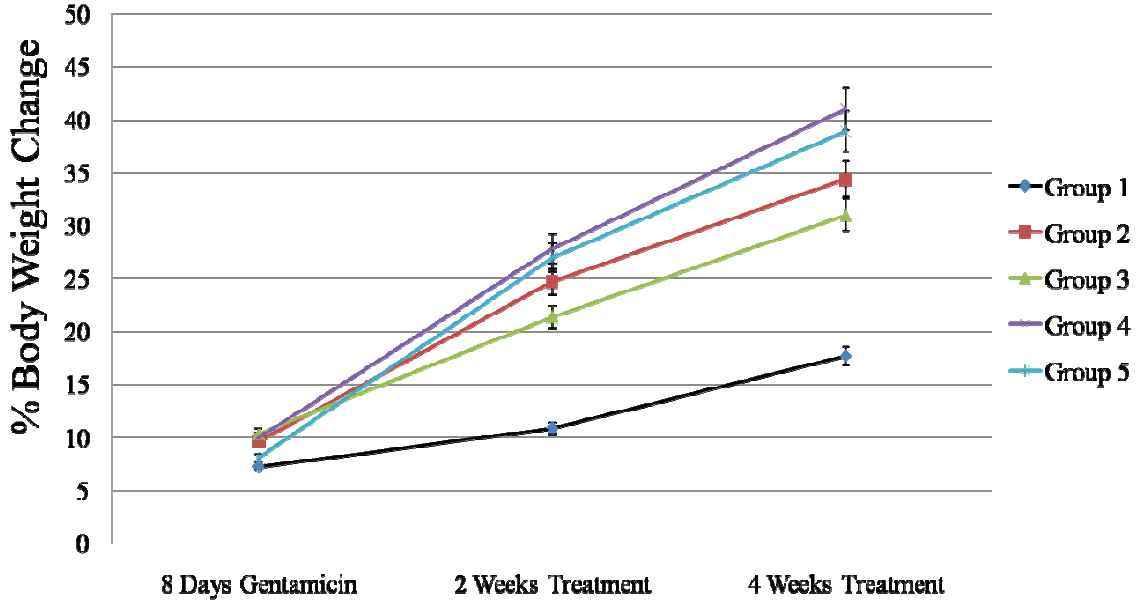

Figure 3: Graph showing the effects of MOGL on Percentage Body Weight Change (% BWC) of rats with gentamicin-induced kidney injury
Group 1 = Control; Group 2 = Toxic; Group 3 = Toxic + 100 mg/kg MOGL; Group 4 = Toxic + 200 mg/kg MOGL; Group 5 = Toxic + 400 mg/kg MOGL.

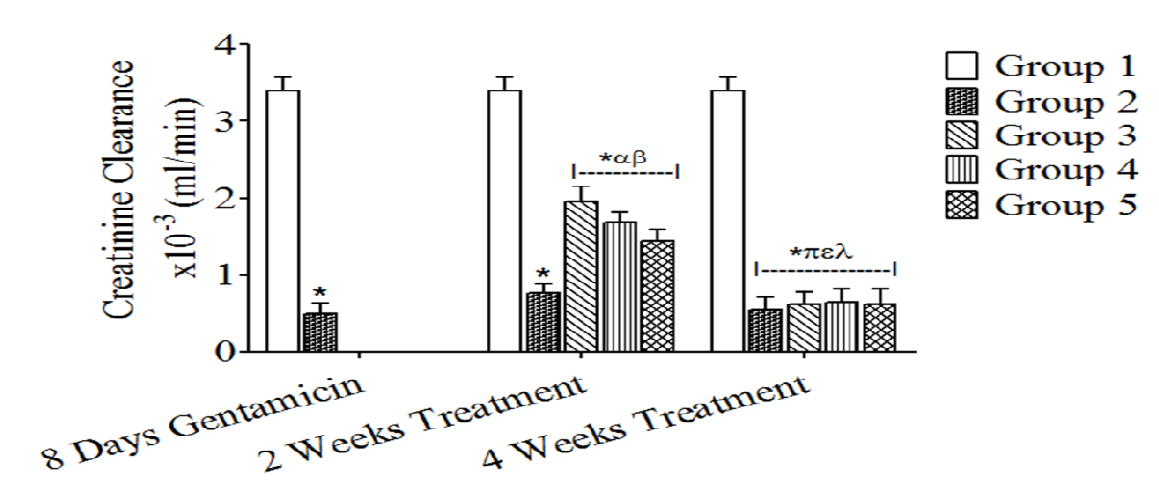

Figure 4: Graph showing the effects of MOGL on creatinine clearance (ml/min) of rats with gentamicin-induced kidney injury

Each bar represents mean ± S.E.M and is significant at $p < 0.05$. ***** = significantly different from group 1 (control); **α** = significantly different from toxic subgroup 2; **β** = significantly different from 2weeks recovery subgroup 2; **π** = significantly different from 2 weeks 100 mg/kg MOGL treatment; **ε** = significantly different from 2 weeks 200 mg/kg MOGL treatment; **λ** = significantly different from 2 weeks 400 mg/kg MOGL treatment.

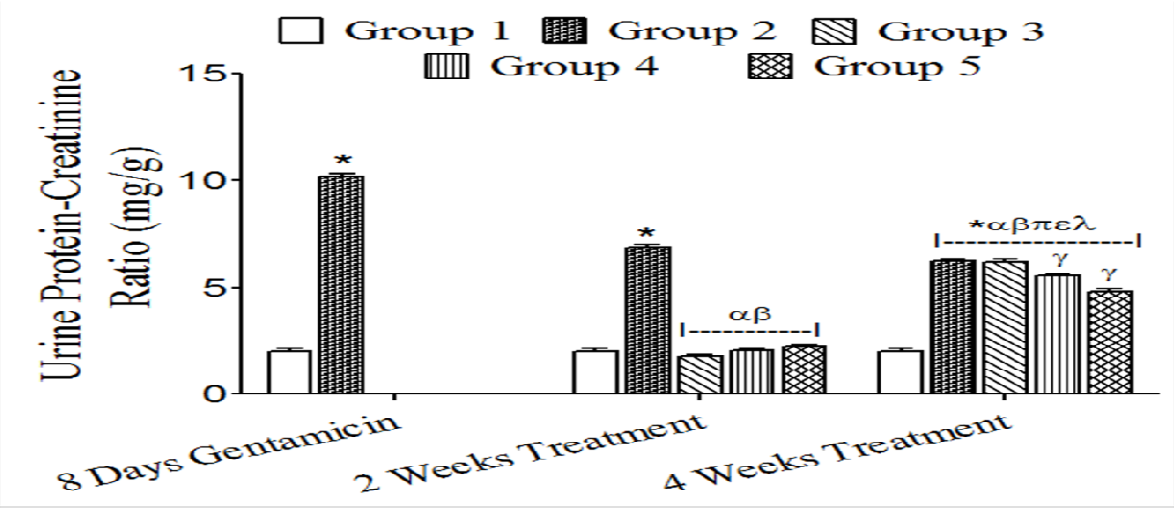

Figure 5: Graph showing the effects of MOGL on urine protein-creatinine ratio (mg/g) of rats with gentamicin-induced kidney injury

Each bar represents mean ± S.E.M and is significant at $p < 0.05$. ***** = significantly different from group 1 (control); **α** = significantly different from toxic subgroup 2; **β** = significantly different from 2weeks recovery subgroup 2; **γ** = significantly different from 4weeks recovery subgroup 2; **π** = significantly different from 2 weeks 100 mg/kg MOGL treatment; **ε** = significantly different from 2 weeks 200 mg/kg MOGL treatment; **λ** = significantly different from 2 weeks 400 mg/kg MOGL treatment.

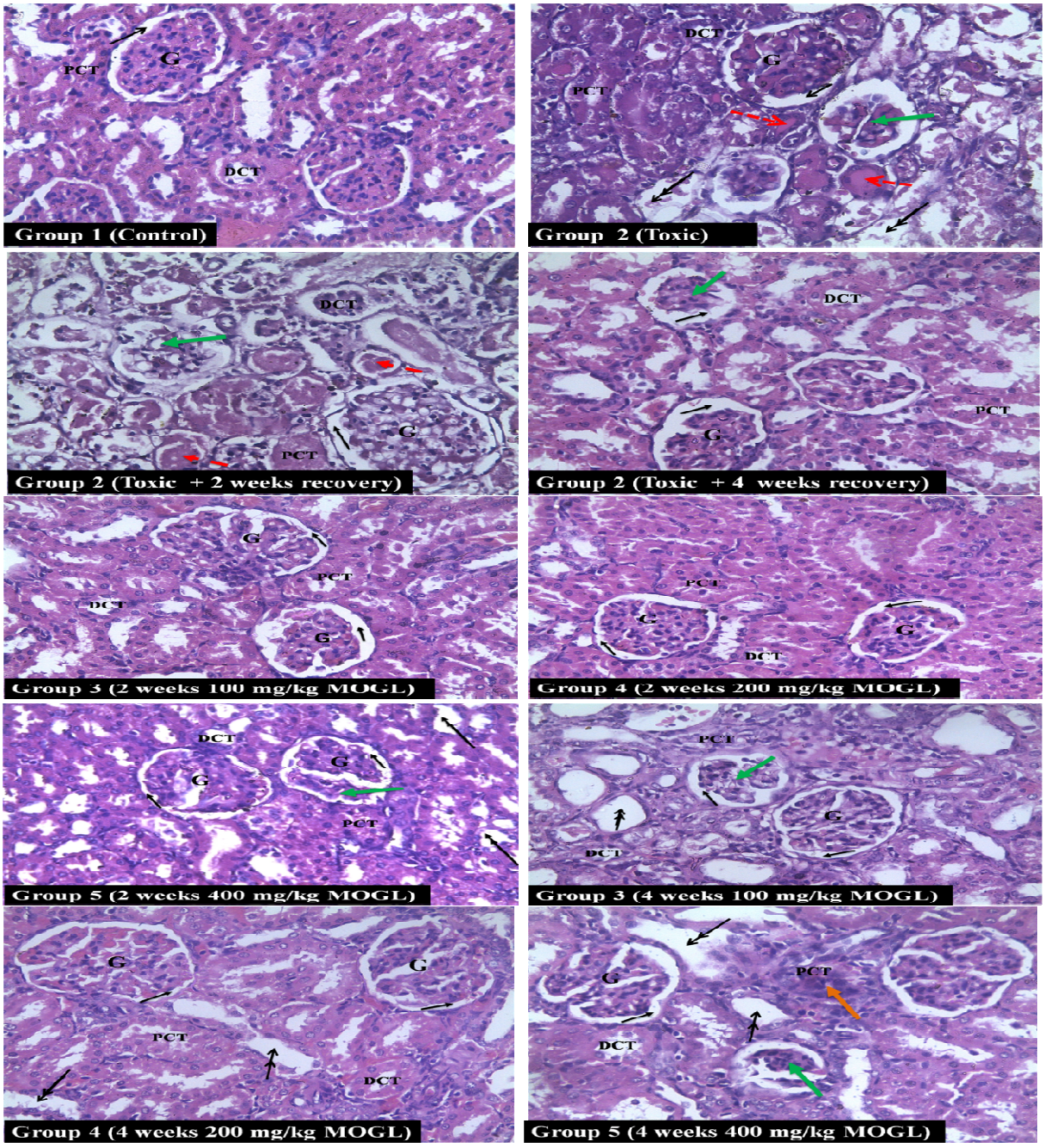

Figure 6: Histological effects of MOGL on the kidney of rats with gentamicin-induced kidney injury (magnification x400)

G = glomerulus; **PCT** = proximal convoluted tubule; **DCT** = distal convoluted tubule; **Black arrow** = Bowman's capsular space; **Red arrow** = densely eosinophilic (colloid) cast; **Green arrow** = Atrophic glomerulus; **Double black arrow head** = severe vacuolation of medullary interstitium; **Orange arrow** = apparent inflammatory cells in the proximal convoluted tubule.

Table 1: Table showing the effects of MOGL on plasma and urine levels of creatinine in rats with gentamicin-induced kidney injury

CREATININE LEVEL					
	Plasma Creatinine (mg/dl)				
Groups ⟶	Group	Group 2	Group 3	Group 4	Group 5
Distilled water (0.2 ml/100g)	+	-	-	-	-
Gentamicin (100 mg/kg)	-	228.50 ± 18.84*	+	+	+
2 weeks MOGL treatment	-	-	93.79 ± 7.97$^{\alpha\beta}$	72.33 ± 9.40$^{\alpha\beta}$	80.90 ± 15.11$^{\alpha\beta}$
4 weeks MOGL treatment	-	-	166.40 ± 11.41*$^{\alpha\beta\gamma\pi\varepsilon\lambda}$	158.20 ± 12.25*$^{\alpha\beta\gamma\pi\varepsilon\lambda}$	107.30 ± 10.33*$^{\alpha\beta\gamma\pi\varepsilon\lambda}$
2 weeks recovery period	-	189.10 ± 20.57*	-	-	-
4 weeks recovery period	70.32 ± 12.1	172.90 ± 14.83*	-	-	-
	Urine Creatinine (mg/dl)				
Groups ⟶	Group 1	Group 2	Group 3	Group 4	Group 5
Distilled water (0.2 ml/100g)	+	-	-	-	-
Gentamicin (100 mg/kg)	-	88.79 ± 10.25*	+	+	+
2 weeks MOGL treatment	-	-	301.90 ± 37.61$^{\alpha\beta}$	291.10 ± 31.80	226.20 ± 35.09$^{\alpha\beta}$
4 weeks MOGL treatment	-	-	117.65 ± 21.94*$^{\pi\varepsilon\lambda}$	125.79 ± 23.40*$^{\pi\varepsilon\lambda}$	115.84 ± 23.57*$^{\pi\varepsilon\lambda}$
2 weeks recovery period	-	123.76 ± 21.96*	-	-	-
4 weeks recovery period	334.10 ± 62.72	126.20 ± 35.09*	-	-	-

Each value represents mean ± S.E.M and is significant at $p < 0.05$. ***** = significantly different from group 1 (control); **α** = significantly different from toxic subgroup 2; **β** = significantly different from 2weeks recovery subgroup 2; **γ** = significantly different from 4weeks recovery subgroup 2; **π** = significantly different from 2 weeks 100 mg/kg MOGL treatment; **ε** = significantly different from 2 weeks 200 mg/kg MOGL treatment; **λ** = significantly different from 2 weeks 400 mg/kg MOGL treatment; **+** = Applicable; **-** = Not applicable.

Table 2: Table showing the effects of MOGL on plasma and urine levels of total protein in rats with gentamicin-induced kidney injury

TOTAL PROTEIN					
	Plasma Total Protein (mg/ml)				
Groups ⟶	Group 1	Group 2	Group 3	Group 4	Group 5
Distilled water (0.2 ml/100g	+	-	-	-	-
Gentamicin (100 mg/kg)	-	5.91 ± 0.48*	+	+	+
2 weeks MOGL treatment	-	-	8.40 ± 0.36*$^{\alpha\beta}$	7.42 ± 0.49*$^{\alpha}$	7.01 ± 0.21*$^{\alpha}$
4 weeks MOGL treatment	-	-	6.93 ± 0.58*	6.76 ± 0.52*	6.20 ± 0.55*
2 weeks recovery period	-	6.53 ± 0.61*	-	-	-
4 weeks recovery period	9.80 ± 0.47	6.97 ± 0.29*	-	-	-
	Urine Total Protein (mg/ml)				
Groups ⟶	Group 1	Group 2	Group 3	Group 4	Group 5
Distilled water (0.2 ml/100g	+	-	-	-	-
Gentamicin (100 mg/kg)	-	9.06 ± 0.41*	+	+	+
2 weeks MOGL treatment	-	-	5.28 ± 0.41$^{\alpha\beta}$	5.95 ± 0.47$^{\alpha\beta}$	5.05 ± 0.42$^{\alpha\beta}$
4 weeks MOGL treatment	-	-	7.27 ± 0.32*$^{\alpha\beta\gamma\pi\varepsilon\lambda}$	6.96 ± 0.44*$^{\alpha\beta\gamma\pi\varepsilon\lambda}$	5.58 ± 0.28*$^{\alpha\beta\gamma\pi\varepsilon\lambda}$
2 weeks recovery period	-	8.52 ± 0.26*	-	-	-
4 weeks recovery period	6.34 ± 0.48	7.84 ± 0.35*	-	-	-

Each value represents mean ± S.E.M and is significant at $p < 0.05$. ***** = significantly different from group 1 (control); **α** = significantly different from toxic subgroup 2; **β** = significantly different from 2weeks recovery subgroup 2; **γ** = significantly different from 4weeks recovery subgroup 2; **π** = significantly different from 2 weeks 100 mg/kg MOGL treatment; **ε** = significantly different from 2 weeks 200 mg/kg MOGL treatment; **λ** = significantly different from 2 weeks 400 mg/kg MOGL treatment; **+** = Applicable; **-** = Not applicable.

Table 3: Table showing the effects of MOGL on the activity of thiobarbituric acid reactive substances (TBARS) and level of reduced glutathione (GSH) in the kidney homogenate of rats with gentamicin-induced kidney injury

ANTIOXIDANT STATUS					
	TBARS x 10^{-6} (mmol/mg protein)				
Groups ⟶	Group 1	Group 2	Group 3	Group 4	Group 5
Distilled water (0.2 ml/100g)	+	-	-	-	-
Gentamicin (100 mg/kg)	-	19.84 ± 0.87*	+	+	+
2 weeks MOGL treatment	-	-	3.11 ± 0.84$^{\alpha\beta}$	3.54 ± 0.70$^{\alpha\beta}$	3.35 ± 0.21$^{\alpha\beta}$
4 weeks MOGL treatment	-	-	3.38 ± 0.98*$^{\alpha\beta\gamma\pi\lambda}$	3.69 ± 0.58*$^{\alpha\beta\gamma\pi\lambda}$	0.48 ± 0.13*$^{\alpha\beta\gamma\pi\lambda}$
2 weeks recovery period	-	11.28 ± 1.00*	-	-	-
4 weeks recovery period	3.19 ± 0.24	5.87 ± 1.00*	-	-	-
	GSH (µg/mg protein)				
Groups ⟶	Group 1	Group 2	Group 3	Group 4	Group 5
Distilled water (0.2 ml/100g)	+	-	-	-	-
Gentamicin (100 mg/kg)	-	1.84 ± 0.21*	+	+	+
2 weeks MOGL treatment	-	-	2.97 ± 0.13$^{\alpha\beta}$	3.01 ± 0.11αβ	3.03 ± 0.12αβ
4 weeks MOGL treatment	-	-	2.65 ± 0.19$^{\alpha\beta\gamma}$	2.66 ± 0.15$^{\alpha\beta\gamma}$	2.64 ± 0.19$^{\alpha\beta\gamma}$
2 weeks recovery period	-	2.14 ± 0.13*	-	-	-
4 weeks recovery period	3.03 ± 0.06	2.30 ± 0.15*	-	-	-

Each value represents mean ± S.E.M and is significant at $p < 0.05$. ***** = significantly different from group 1 (control); **α** = significantly different from toxic subgroup 2; **β** = significantly different from 2weeks recovery subgroup 2; **γ** = significantly different from 4weeks recovery subgroup 2; **π** = significantly different from 2 weeks 100 mg/kg MOGL treatment; **ε** = significantly different from 2 weeks 200 mg/kg MOGL treatment; **λ** = significantly different from 2 weeks 400 mg/kg MOGL treatment; **+** = Applicable; **-** = Not applicable.

DISCUSSION

Akinmoladun *et al.*[15] investigated the phytochemical constituents of both aqueous and methanolic extract of *Ocimum gratissimum* leaf and showed that it possess antioxidant activity. Phytochemicals such as tannins, flavonoids and cardiac glycosides were positive in methanolic extract of *Ocimum graticimum* (Linn.) leaf (MOGL) and were found to have a DPPH scavenging activity of 84.6 % at 250µg/ml and a reductive potential of about 0.77 at 100µg/ml. These values were comparable with gallic acid equivalent at 91.4 % and ascorbic acid 0.79 at 60 µg/ml as standard for DPPH scavenging activity and reductive potential respectively.[15]

In the absence of morphological changes, significant differences in organ weights between treated and untreated animals may occur and this can be a sensitive indicator of an effect of an experimental compound or xenobiotics.[24] In order to avoid any complication that may arise as a result of differences in body weight between groups, the ratio of body weight to organ weight (generally described as relative organ weight) is commonly used for analysis.[24] Consistent with the findings of Jain and Somoni[25] is an increase in relative kidney weight (RKW) that was associated with

gentamicin toxicity, as shown in this study. Also, according to Erdem *et al.*[26] significant increase in RKW following gentamicin administration may have resulted from inflammatory response with a consequent increase in kidney size. In this study, the representative photomicrograph of the toxic group showed signs of inflammation with formation of densely eosinophilic/ colloid cast within the lumen of the renal tubules, despite loss of some cellular constituents in the medullary interstitium. When compared with the toxic model, both 2 and 4 week MOGL treatments showed a significantly lowered RKW but was significantly higher when compared with the control. This, at least, showed an anti-inflammatory potential of MOGL with a consequent apparent disappearance of inflammatory evidence and colloid casts in the kidney histoarchitecture; an activity that may be attributed to the presence of tannins and flavonoids in the extract. These phytochemicals are reputed to posses both antioxidant and anti-inflammatory properties.[27,28,29]
Previous study on MOGL by Iweala and Obidoa[30] showed appreciable histological changes in the rats' intestines, revealing larger goblet cells as well as increased villi. Increased intestinal villi facilitate increased nutrient absorption due to increase in intestinal surface area.[31] It is possible that MOGL enhanced increased food absorption in the treated groups, consequently resulting in dose-dependent weight gain in the rats; hence, the observed dose-dependent increase in percentage body weight change.

Nephro-restorative potential of MOGL was depicted after 2 week MOGL administration to the rats with gentamicin-induced kidney injury as evidenced by a significantly lower plasma creatinine and a significantly higher urine creatinine when compared with the toxic model. Prolonged administration of MOGL significantly reversed the plasma and urine levels of creatinine from normalcy. A significant increase in plasma creatinine with a corresponding decrease in urine creatinine is a feature associated with a compromise of kidney function, particularly filtering capacity.[32] Hence, creatinine clearance is an index of kidney filtering capacity. Compared with the toxic model, although creatinine clearance was significantly higher in the MOGL-treated groups following 2 week treatment, there was no significant difference shown after 4 weeks treatment with MOGL. This indicates a high risk profile of decline in renal clearance following prolonged administration of MOGL in Wistar rat model of gentamicin-induced kidney injury. Subject to further investigation, a possible explanation for this possible reversal from normalcy following prolonged administration could be *tolerance* and *half life*. It implies that, possibly in biological systems, tolerance to MOGL reduces with prolong administration apparently due to its high half life. This suggests that sufficient amount of MOGL could have still been present in circulation after previous administration consequently making prolonged administration of additional amounts of toxic effects rather than therapeutic. Hence, the activity of MOGL was observed to reverse from therapeutic effects following administration beyond sub-chronic level. This assertion is corroborated by the findings of Anigbogu and Uzoaga[33] as well as Nwagwu *et al*.[34] who reported that *Ocimun gratissimum* potentiates nephrotoxic effect when administered for a long period of time.

In clinical models of kidney injury, minimal change nephropathy has been found to be associated with loss of negative charges that are normally present in the glomerular capillary basement membrane. Like-charge repels, therefore the physiologic relevance of the negatively charged basement membrane of the glomerular capillaries is to repel the negatively charged plasma proteins.[35] Loss of normal negative charges in this basement membrane allows protein, especially albumin, to pass through the glomerular membrane with ease.[35] Although apparently normal rats express physiologic proteinuria[19,36,37] as shown by the control group, the level of urinary protein excretion was significantly elevated after exposure to gentamicin toxicity. Besides the loss of negative charges in the glomerular basement membrane, gentamicin-induced increase in the histomorphometry of the glomerular filtration barrier could be an additional possible explanation for the observed high urine protein excretion. An increased capacity to express proteinuria is directly proportional to increased sizes of the glomerular filtration features which include Bowman's capsular space, thickness

and size of the glomerulus and Bowman's capsule[19] as well as the endothelia *fenestrae* of the glomerular capillaries. The representative photomicrographs depict evidence of increased Bowman's capsular space which can be a contributory factor to the observed proteinuria. In clinical models of kidney injury, an increased urine protein-creatinine ratio (UPC) is an index of declining renal function.[38] UPC allows for effective monitoring of the progression of clinical models of renal condition as it trends the level of proteinuria.[5,6] It is a convenient and apparently accurate index for diagnosing non-benign proteinuria.[38,39] When compared with the control, 2 week MOGL treatment significantly restored UPC homeostasis in the rat model, a condition that was deleteriously reversed following 4 week administration of MOGL. This further buttresses the fact that, in biological systems, administration of MOGL beyond sub-chronic level is an abuse and is therefore toxic at this duration. The 2 week restorative potential of MOGL on the deleterious alterations in urine and plasma level of total protein can be attributed to its ability to integrate and or inhibit loss of protein-repelling negative charges in the glomerular capillary basement membrane as well as appreciably enhance regenerative ability of the kidney tissue; although fractions of the phytochemical responsible for this activity needs to be investigated. The tubular epithelium of the kidney is composed of polarized mature cells that have the capacity to regenerate, following acute kidney injury.[40] After injury occurs, these cells rapidly lose their brush borders and de-differentiate into a more mesenchymal phenotype. The de-differentiated cells migrate into the regions where cell necrosis, apoptosis or detachment has resulted in denudation of the tubular basement membranes; they proliferate and eventually re-differentiate into an epithelial phenotype, completing the repair process.[40] Accordingly, the representative photomicrographs show appreciable improvement in kidney histoarchitecture following 2 week treatment with MOGL. Worthy of note is the fact that literatures exist on the regenerative potential of the kidney in Wistar rat models of xenobiotics-induced kidney injury.[17,41-42]

The potential of MOGL to restore homeostasis in the antioxidant system of biological system was well expressed by the level of reduced glutathione (GSH) and activity of thiobarbituric reactive acid substances (TBARS) in the MOGL-treated groups following exposure to gentamicin toxicity. GSH is a major antioxidant that offers protection against free radical attack.[43] It is a sulfhydryl peptide involved in the protection of normal cell structure and function by maintaining redox homeostasis, quenching of free radicals, participation in detoxification reaction and regeneration of other antioxidants.[43] Determination of changes in the concentration of GSH, therefore, provides an alternative method of monitoring oxidative damage in the kidney.[44] Depletion of GSH may be an overall toxic manifestation of gentamicin. Also, the elevated TBARS activity, a measure of lipid peroxidation and oxidative stress, was significantly lowered following 2 week MOGL treatment. Apparently, the activity of MOGL explains its antioxidant and anti-peroxidation properties that are associated with nephro-protection against gentamicin-induced kidney injury. A study by Ujowundo *et al.* [45] reported that these abilities of *Ocimum gratissimum* plant are made possible by the presence of flavonoids and phenolic compounds which have the ability to scavenge free radicals.

It is recommended that prolonged-consecutive consumption of this plant, beyond sub-chronic level, be avoided as it expresses toxic rather than therapeutic potentials at this duration. There is need for a future study of the extract fractions for the biochemical basis of its therapeutic effects. Also, to better elucidate the extract's mechanism of action (with a view to maximizing its health benefits), it is imperative to conduct an advance study at a molecular level. Apparently, the effects of this extract (cheap and accessible) provides a ray of hope on the horizon in models of drug-induced kidney injury in a world with increasing incidence of renal disease which is often associated with high cost of management and or treatment. Nevertheless, in order to maximize the therapeutic potential of MOGL, administration should be with a note of caution.

CONCLUSION

In conclusion, with attributes of antioxidant, possible anti-inflammatory and membrane stabilizing effects, treatment with methanolic

extract of *Ocimum gratissimum* (Linn.) leaf shows therapeutic potentials only at sub-chronic administration as it reversibly normalizes urine protein-creatinine ratio (UPC) in Wistar rat model of gentamicin-induced kidney injury. Therefore, a high risk profile of renal dysfunction is not unlikely with administration beyond sub-chronic level in models of kidney injury.

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