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To cite this article: Oluwadare J. Ogundipe, Omolola F. Akinpelu, Abiodun. Oyerinde & Oyelade R. Oluwakemi (2021) *Ocimum gratissimum* (Linn) leaves extract attenuates oxidative stress and liver injury in gentamicin-induced hepatotoxicity in rats, Egyptian Journal of Basic and Applied Sciences, 8:1, 146-155, DOI: [10.1080/2314808X.2021.1922250](https://doi.org/10.1080/2314808X.2021.1922250)

To link to this article: <https://doi.org/10.1080/2314808X.2021.1922250>



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Published online: 05 May 2021.



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Ocimum gratissimum (Linn) leaves extract attenuates oxidative stress and liver injury in gentamicin-induced hepatotoxicity in rats

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ABSTRACT

The adverse effect of gentamicin on tissues like kidney and liver makes its use limited in clinical settings. *Ocimum gratissimum* are known for many medicinal uses. The effect of aqueous extract (AOGL) and methanolic extract (MOGL) of *Ocimum gratissimum* (Linn) on liver following injury induced by gentamicin was investigated in this study. Forty adult (40) male Wistar rats were divided into 8 groups as follows: Group 1 (n = 5) received distilled water daily by oral route. Groups 2, 3, 4, 5, 6, 7 and 8 (n = 5) received 100 mg/kg/day of gentamicin i.p. for a week. After, groups 3, 4, 5, 6, 7 and 8 (n = 5) received 100, 200 and 400 mg/kg/day of AOGL and MOGL p.o, respectively, for 28 days. The rats treated with both AOGL and MOGL for 28 days were left for a recovery period of 14 days. Liver enzymes (ALT, AST and ALP), total bilirubin and total protein were determined in the plasma. TBARS and GSH levels were assayed in the liver homogenate. Gentamicin increases ALT, AST, ALP, total bilirubin and total protein, TBARS and decreases GSH, catalase and SOD of the toxic control when compared to the control group. Post-treatment with AOGL and MOGL in the treated groups caused increases GSH, catalase and SOD and decreases TBARS, ALT, AST, ALP, total bilirubin and total protein when compared with the toxic control group. The results show that AOGL and MOGL are capable of ameliorating liver injury caused by gentamicin in rats.

ARTICLE HISTORY

Received 14 January 2021
Accepted 23 April 2021
Published online

KEYWORDS

Ocimum gratissimum leaves extract; ALT; GSH; gentamicin; liver; rats

INTRODUCTION

Gentamicin (GEN) belongs to the family of antibiotics that are widely used in the treatment of serious and life-threatening gram-negative bacterial infection. Its adverse effect on some vital organs in the body, such as kidney, liver, etc., makes its use limited in clinical settings [1]. The main side effects include liver damage, which is one of the major factors of liver inefficiency in

a significant number of people taking this medication [2,3]. Increased production of Reactive Oxygen Species (ROS), which can be seen after the use of gentamicin in cells, is effective in inducing toxic impacts of this drug on the structure and function of tissues [4–6].

Ocimum gratissimum (OG) is a herbaceous plant that belongs to the family of Labiatae, mostly found in tropical areas, especially India

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and West Africa. It is used in the treatment of epilepsy, high fever and diarrhea in the coastal area of Nigeria [7]. The flowers and the leaves of this plant are used in preparation of teas and infusion because it is rich in essential oils [8]. The decoction of the leaves is used to treat mental illness in the Savannah areas [9].

With all the medicinal attributes of OG, till date, scientific data available are inadequate to infer the hepatocurative and/or hepatotoxic potentials of this plant in models of drug-induced liver injury. Therefore, this study aimed at contributing to the body of existing knowledge by studying the effects of 28 days of administration of aqueous extract of *Ocimum gratissimum* (AOGL) and methanolic extract of *Ocimum gratissimum* (MOGL) leaf on the liver function of Wistar rats with gentamicin-induced liver injury.

Materials and method

Gentamicin injection (80 mg/2 mL) was purchased from Shanxi Shuguang Pharmaceutical Co (Jinzhong, China). OG leaves plucked from a garden at Ede Road Ile-Ife were certified by a taxonomist at the Department of Botany, Obafemi Awolowo University (OAU), Ile-Ife, Osun state, Nigeria. Standard laboratory kits for assaying the markers of liver function were acquired from Randox Laboratory Ltd., United Kingdom.

Extraction process

Aqueous extraction

After air dried, the leaves of OG were ground into powder using a blender. The powdered leaves (323 g) were soaked with 3 L of distilled water in an electric shaker for 48 hours. The extract was filtered through Whatman No. 1 paper (Whatman International Ltd, Maidstone, England) and evaporated under reduced pressure using a rotary evaporator.

Methanolic extraction

After air-dried and blending, the leaves of OG were macerated with 3 L of 90% methanol for 48 hours. The resulting mixture was filtered with Whatman number 1 filter paper, concentrated at 38°C using a Rotary Evaporator (6540--2, Buchi Laboratorium-Technik AG.CH-9230 Flawil/Schweiz, Switzerland). Concentrated solution was freeze-dried to obtain (MOGL).

$$\text{Extraction yield in \%} = \frac{W_2}{W_1} \times 100$$

W2 = weight of the extract after the extraction process

W1 = weight of the extract before the extraction process

$$\text{Extraction yield of AOGL in \%} = \frac{33.60}{323} \times 100 = 10.4\%.$$

$$\text{Extraction yield of MOGL in \%} = \frac{36}{340} \times 100 = 10.6\%.$$

Animal care and management

The forty adult male Wistar rats (100 to 150 g) used for this study were obtained from the Animal Holdings of the College of Health Sciences, Obafemi Awolowo University, Ile-Ife, and were housed in plastic cages. They were kept under natural light/dark cycle (12 hours light and dark) at 27°C and had access to standard rodent pellet diet and water ad libitum. They were left to acclimatize in the laboratory for 2 weeks before the commencement of the study. Ethical clearance was obtained from the Health Research Ethics Committee of the Obafemi Awolowo University Ile-Ife.

Experimental design

The rats were divided into 8 groups as follows: Group 1 (control) rats received 1 mL/kg/day of distilled water via oral route for the study period of 6 weeks. Groups 2 (toxic), 3, 4, 5, 6, 7 and 8, each of which received 100 mg/kg/day of gentamicin (intraperitoneal) for a week and thereafter group 2 rats were left untreated

(without AOGL and MOGL) for 4 weeks. Groups 3, 4, 5, were treated with graded doses of AOGL at 100, 200, 400 mg/kg/day, respectively, while groups 6, 7 and 8 received MOGL at 100, 200, 400 mg/kg/day orally, respectively, for 28 days, after which they were left to recover from the treatments for 14 days. Blood samples were collected a day after the administration of distilled water for group 1, after gentamicin administration and 4 weeks recovery period (withdrawal of gentamicin) for group 2, and after 4 weeks treatment with AOGL and MOGL as well as 14 days of recovery period for group 3, 4, 5, 6, 7 and 8 via retro-orbital plexus into EDTA bottles and centrifuged at 4000 rpm for 15 minutes using Cold Centrifuge (Model 8881, Centurion Scientific, West Sussex, England) to obtain plasma. The plasma was analyzed for some biochemical indices of liver function. After the blood samples collection, animals were sacrificed via cervical dislocation and their liver was excised and weighed (1 g) for liver homogenate.

Study design

	8 days	Week 4	Week 6
Group 1	W	W	W [#]
Group 2	GEN [#]	RT [#]	
Group 3	GEN	100 AOGL [#]	RT [#]
Group 4	GEN	200 AOGL [#]	RT [#]
Group 5	GEN	400 AOGL [#]	RT [#]
Group 6	GEN	100 MOGL [#]	RT [#]
Group 7	GEN	200 MOGL [#]	RT [#]
Group 8	GEN	400 MOGL [#]	RT [#]

W = Distilled water; GEN = Gentamicin; RT = Recovery time; AOGL = Aqueous extract of *Ocimum gratissimum*; Group 1 = control; Group 2 = Toxic control; Group 3 = GEN + 100 mg/kg AOGL; Group 4 = GEN + 200 mg/kg AOGL; Group 5 = GEN + 400 mg/kg AOGL; Group 6 = GEN + 100 mg/kg MOGL; Group 7 = GEN + 200 mg/kg MOGL; Group 8 = GEN + 400 mg/kg MOGL; [#] = Time/point of blood collection.

Measurement of oxidative stress and lipid peroxidation

The rat's liver was carefully excised and weighed. Onegram of it was homogenized

with 10 ml of sucrose solution (0.25 M) using Electric Homogenizer (SI601001). Ten percent phosphate buffer (100 Mm) was prepared at pH of 7.4. The homogenate was centrifuged at 3000 rpm for 20 minutes and the supernatant was collected for the assessment of glutathione activity.

Reduced glutathione (GSH) level was determined by the method of Beutler and coworkers [10], Superoxide dismutase (SOD) by the method of McCord and Fridovich [11], catalase by the method of Sinha [12], while Thiobarbituric acid reactive substances (TBARS), an index of lipid peroxidation was determined as described by Ohkawa et al. [13].

Assessment of liver biomarkers

Assessment of biochemical markers of liver function such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and total bilirubin in the plasma was carried out using Randox standard laboratory kits. The procedures for these tests were as provided in their respective kits. Protein determination was carried out according to the method of Lowry et al., (1951) as described by Holme and Peck (1998).

Statistical analyses

All data were subjected to descriptive and inferential statistics. All values were expressed in Mean $\pm$ Standard error of mean (M $\pm$ SEM) using One-way analysis of variance (ANOVA) with $P < 0.05$, considered statistically significant.

Results

Effects of GEN, AOGL and MOGL on TBARS in Wistar rats

The TBARS level in the liver increases following 7 days Gentamicin administration and was reduced with the intervention of AOGL and

MOGL for 4 weeks except for the 100 mg AOGL. The AOGL, MOGL-treated groups and the GEN recovery group showed reductions when compared with the toxic groups, but no difference when compared with the control except for the 100 mg AOGL. Also for the 2 weeks treatment recovery, AOGL treated recovery groups show no different to the control (Figure 1).

Effects of GEN, AOGL and MOGL on GSH in Wistar rats

Gentamicin administration for 7 days reduces the liver GSH level and was increased back to normal following 4 weeks of AOGL and MOGL intervention. No significant difference in the liver GSH level of AOGL, MOGL-treated groups

and the 2 weeks GEN recovery group when compared with the control was observed. Also for the 2 weeks treatment recovery, MOGL and AOGL recovery show no difference from the control which is more pronounced in the MOGL-treated groups and 100 mg AOGL treatment (Figure 2).

Effects of GEN, AOGL and MOGL on SOD in Wistar rats

Gentamicin administration for 7 days reduces the liver SOD level and was increased back to normal following 4 weeks of AOGL and MOGL intervention. No significant difference in the liver SOD level of AOGL, MOGL-treated groups, 2 weeks treatment recovery and significant

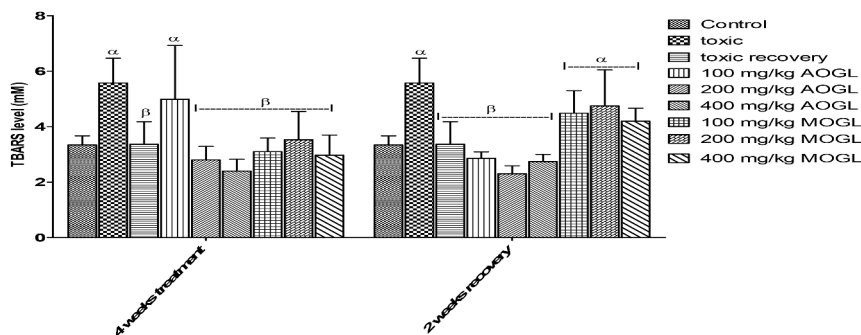


Figure 1. Graph representing the level of TBARS in rats. Each bar represents $M \pm SEM$ ($p < 0.05$). α = significantly different from control group; β = significantly different from GEN group.

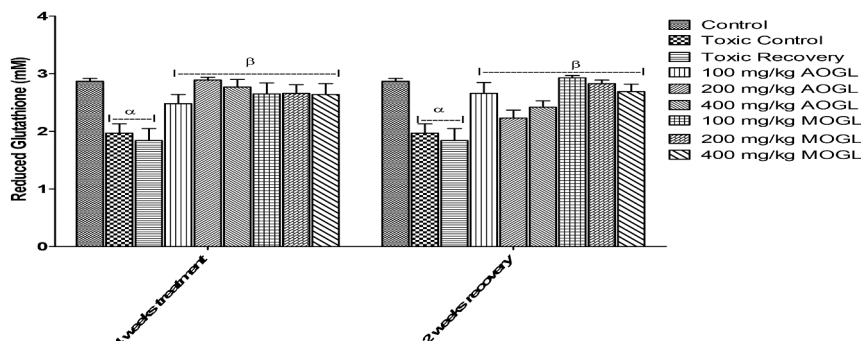


Figure 2. Graph representing the plasma level of GSH of rats. Each bar represents $M \pm SEM$ ($p < 0.05$). α = significantly different from control group; β = significantly different from GEN group.

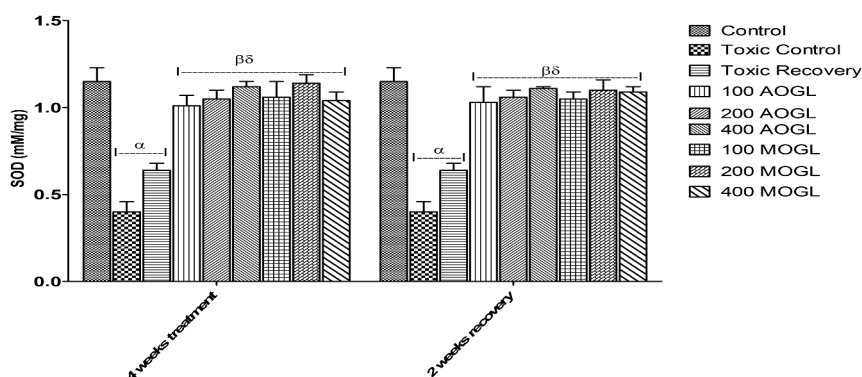


Figure 3. Graph representing the plasma level of GSH of rats. Each bar represents $M \pm SEM$ ($p < 0.05$). α = significantly different from control group; β = significantly different from GEN group. δ = significantly different from GEN Recovery group.

difference in 2 weeks GEN recovery group when compared with the control was observed (Figure 3).

Effects of GEN, AOGL and MOGL on catalase in Wistar rats

Gentamicin administration for 7 days reduces the liver catalase level and was increased back to normal following 4 weeks of AOGL and MOGL intervention. There was no significant difference in the liver catalase level of AOGL, MOGL-treated groups, 2 weeks treatment recovery and a significant difference in the

2 weeks GEN recovery group when compared with the control (Figure 4).

Effects of GEN, AOGL and MOGL on AST levels in Wistar rats

There was a significant increase in plasma AST levels following 7 days GEN exposure. This was, however, reduced insignificantly in the 4 weeks MOGL, AOGL treated and 2 weeks toxic recovery groups when compared with the control group. The same observation was seen in the 2 weeks AOGL and MOGL treatment recovery (Figure 5).

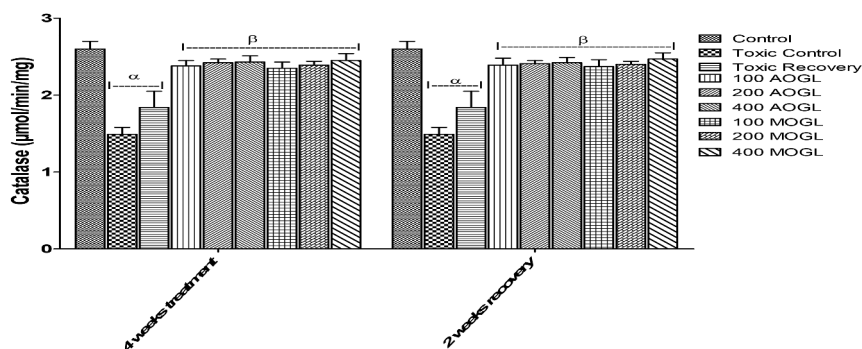


Figure 4. Graph representing the plasma level of GSH of rats. Each bar represents $M \pm SEM$ ($p < 0.05$). α = significantly different from control group; β = significantly different from GEN group. δ = significantly different from GEN Recovery group.

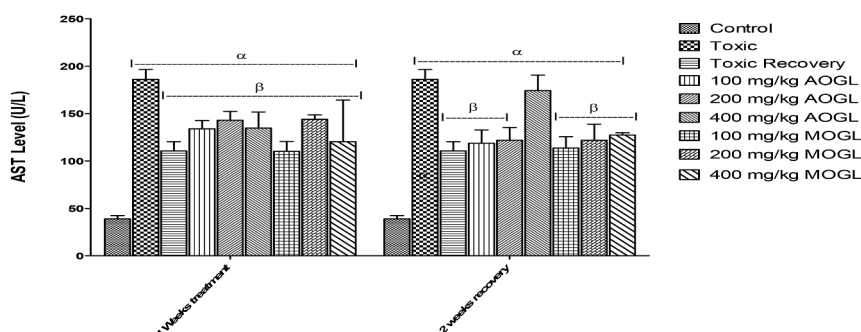


Figure 5. Graph representing the plasma level of AST of rats. Each bar represents $M \pm SEM$ ($p < 0.05$). α = significantly different from control group; β = significantly different from GEN group.

Effects of GEN, AOGL and MOGL on ALP levels in Wistar rats

The plasma level of ALP was elevated following 7 days GEN administration and this was restored in the 4 weeks MOGL-treated groups, 400 mg AOGL-treated group and 2 weeks toxic recovery group when compared with the control group. For the 2 weeks treatment recovery, both MOGL and AOGL reduced significantly when compared with the toxic group (Figure 6).

Effects of GEN, AOGL and MOGL on ALT levels in Wistar rats

The plasma level of ALT was significantly elevated following 7 days GEN administration. This level was restored in the 4 weeks MOGL, AOGL-

treated groups and 2 weeks toxic recovery group when compared with the control group. The same observation was seen in the 2 weeks treatment recovery groups (Figure 7).

Effects of GEN, AOGL and MOGL on total bilirubin levels in Wistar rats

Gentamicin administration for 7 days leads to significant increase in the plasma level of total bilirubin. The total bilirubin levels were insignificantly lowered in the 4 weeks AOGL and MOGL-treated groups when compared with the toxic groups except for the 2 weeks toxic recovery that shows significant difference when compared with the toxic group. This was also observed in the 2 weeks treatment recovery groups (Figure 8).

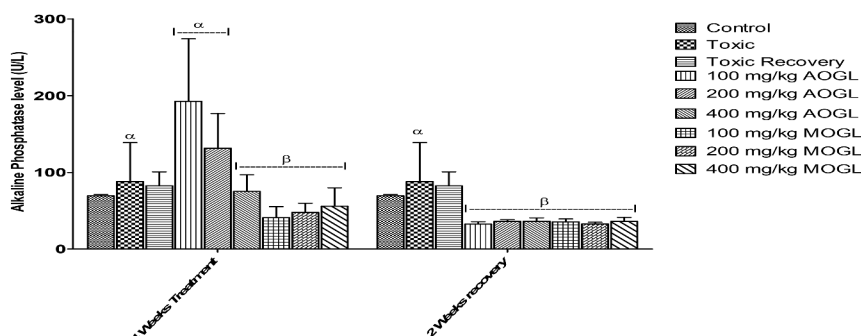


Figure 6. Graph representing the plasma level of ALP of rats. Each bar represents $M \pm SEM$ ($p < 0.05$). α = significantly different from control group; β = significantly different from GEN group.

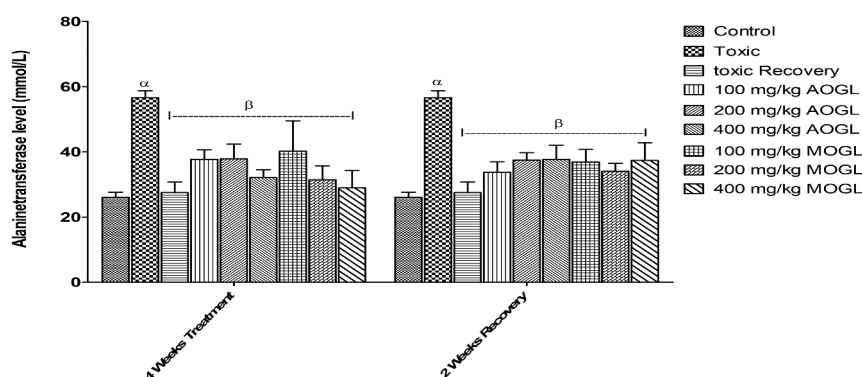


Figure 7. Graph representing the plasma level of ALT in rats. Each bar represents $M \pm SEM$ ($p < 0.05$). α = significantly different from control group; β = significantly different from GEN group.

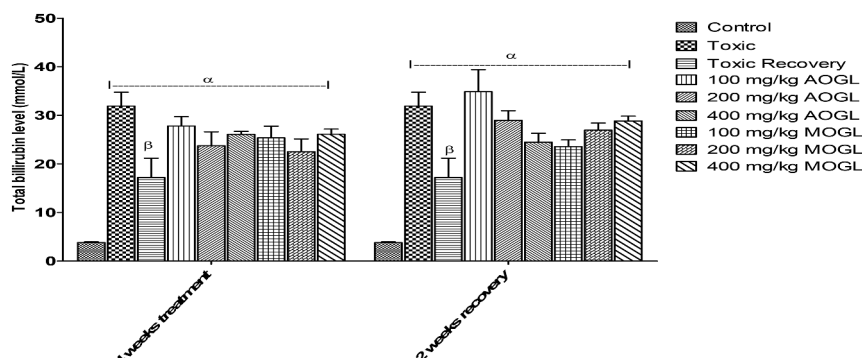


Figure 8. Graph representing the plasma level of total bilirubin in rats. Each bar represents $M \pm SEM$ ($p < 0.05$). α = significantly different from control group; β = significantly different from GEN group.

Effects of GEN, AOGL and MOGL on total protein levels in Wistar rats

Gentamicin administration for 7 days significantly lowered the plasma level of total protein. AOGL and MOGL treatment for 4 weeks does not significantly restore the plasma level of total protein when compared with the control, toxic and toxic recovery groups. The 2 weeks MOGL treatment recovery groups show no significant difference with the control (Figure 9).

Discussion

This study focused on the treatment of gentamicin-induced liver injury with both AOGL and MOGL for a period of 28 days. It has been

reported that aminoglycoside alters liver glycogen phosphorylase activities leading to decrease in liver glycogen content [14]. As obtained from this study, administration of the graded doses of AOGL and MOGL restores the antioxidant activities and also the liver function biomarkers. This was in agreement with previous study that observed two weeks administration of graded doses of both AOGL and MOGL restores antioxidant activities [15]. Increase in the concentration of TBARS [16–18] and reduction in the level of GSH, SOD and catalase [17–20] suggest an injury in biological tissues, which is an indication of free radicals generation leading to oxidative stress, respectively, in the liver following administration of GEN. The reduction in (GSH) level maybe due

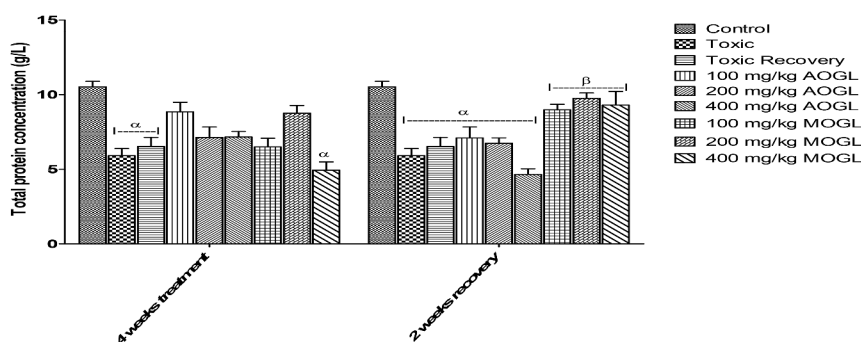


Figure 9. Graph representing the plasma level of Total protein of rats. Each bar represents $M \pm SEM$ ($p < 0.05$). α = significantly different from control group; β = significantly different from GEN group.

to the fact that the liver uses up GSH to mop up the free radicals generated as a result of injury caused by GEN toxicity. The increased (TBARS) level reflected high degree of injury due to GEN treatment. The AOGL- and MOGL-treated groups showed ameliorating effects by reducing the elevated TBARS level and increasing the (GSH, SOD and catalase) level. This indicates that the plant has potent antioxidant activities. This assertion was supported by existing literatures [7,9,17]. The two weeks treatment recovery shows that the extracts have no aftermath effects on the markers of oxidative stress. The group that was left to recover without AOGL and MOGL treatment also reverses the alterations in the antioxidant system when compared with control. This suggests that apart from the antioxidant ability of this plant in the liver tissue, the liver itself has the potential to restore its physiological functions following deprivation from (GEN) for a few weeks.

Gentamicin administration increases the activities of these liver enzymes such as AST, ALT and ALP when compared with control. AST and ALT are enzymes found in the liver [21]. AST is found in mitochondrial and cytosol of the liver cells, while ALT is solely cytoplasmic of the liver cells [21]. Therefore, the ameliorating effect of the extracts in the gentamicin-induced alteration in ALT activities suggests that both AOGL and MOGL have

cytoplasmic effects that restore the integrity of the membrane of the liver cell. This may be due to the duration of both AOGL and MOGL administration. AOGL and MOGL administration restored the elevated ALP levels. This can be attributed to the extract's ability to reverse the blockage of the free flow of bile. This is because (cholestasis) obstruction of bile flow in the liver enhances both synthesis and release of hepatic ALP from cell surfaces [21,22]. These are further buttressed by the two weeks treatment recovery.

Gentamicin toxicity increased plasma level of total bilirubin. This is an indication of hepatic injury caused by bile duct obstruction [23] or lesion [24]. The AOGL- and MOGL-treated groups showed a reduction in the elevated level of bilirubin but not to the control level. This observation was similar to the result obtained during 14 days treatment with the two extracts. This assertion is supported with the fact that the group that was left to recover without the intervention of the extracts and the two weeks treatment recovery could not reverse the alterations in the bilirubin level. This is an indication that the extracts have no positive action on the treatment of hepatic injury or lesion caused by gentamicin administration.

One of the limitations of this study is the failure to look at the photomicrograph of the

rats' liver. This was observed at the end of this study based on the results obtained from the biochemical analyses. Though it was established that 7 days gentamicin administration caused injury to the liver based on the biochemical results, but it is not out of play if this injury can be examined structurally. Therefore, further study should be done putting in mind the structural examination. The self-healing mechanism to liver regeneration process is stimulated by the synthesis of protein [25]. The hepatic total protein level is an important index for the determination of chemically induced liver injury or dysfunction [22]. Gentamicin administration induces adverse changes in the process of protein synthesis by lowering the total protein. The extract's ability to restore the liver total protein to its physiological levels is an indication of its tissue-regenerative ability that was least expressed in the group that received AOGL and MOGL during the cause of treatment. The MOGL treatment recovery showed restoration of liver total protein. This connote that this extract exact its physiologic action on tissue regeneration after its usage.

In conclusion, gentamicin induces liver injury as evident in the alteration of the marker of lipid peroxidation, the antioxidant enzymes of the liver homogenate and the markers of liver function test. Thus, the extracts restored the antioxidant concentration, total protein and cholestasis mechanism of rats with gentamicin-induced liver injury.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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