Aqueous and Methanolic Extract of *Ocimum Gratissimum* (Linn.) Leaf Reversibly Normalizes the Antioxidant Activities of Rats with Gentamicin-Induced Liver Injury

Oluwadare J. Ogundipe[1], Risquat O. Olaleye [1], Christian E. Imafidon[1], Olaoluwa S. Olukiran[1], Omolola F. Akinpelu[2], Adedapo J. Sanusi[3], A. Oyerinde[4], Oyelade R. Oluwakemi[5]

[1]*Department of Physiological Sciences, Faculty of Basic Medical Sciences, Obafemi Awolowo University, Ile – Ife, Osun State, Nigeria*
[2]*Department of Anatomy, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Ago – Iwoye, Ogun State, Nigeria*
[3]*Department of Biochemistry, Faculty of Sciences, Obafemi Awolowo University, Ile – Ife, Osun State, Nigeria*
[4]*Department of Physiology, Faculty of Basic Medical Sciences, Lead city University, Ibadan, Oyo State, Nigeria*
[5]*Department of Physiology, Faculty of Basic Medical Sciences, University of Ibadan, Ibadan, Oyo State, Nigeria*

Abstract:-

Objective: **the objective of the work is to investigate the ameliorative effect of aqueous and methanolic extract of ocimum gratissimum (linn) on gentamicin-induced liver injury. Forty five adult male Wistar rats were used for this study. They were divided into 5 groups as follows: Group 1 (the control) (n = 5) received distilled water daily by oral route for the whole period of the study. Group 2 (the toxic control) (n = 10) received 100 mg/kg/day of gentamicin i.p. for a week. Groups 3, 4, and 5 (n = 5) were pre-treated with gentamicin as the Group 2 rats, after which they received 100, 200 and 400 mg/kg/day each of AOGL *p.o.*, Groups 6, 7, and 8 (n = 5) were pre-treated with gentamicin as the Group 2 rats, after which they received 100, 200 and 400 mg/kg/day each of MOGL *p.o.*, respectively, for 14 days. Markers of liver function such as ALT, AST, ALP, Total bilirubin, conjugated bilirubin and total protein were determined in the plasma. Oxidative stress markers such as TBARS and GSH were assayed in the tissue homogenate. Gentamicin treatment induced significant ($p < 0.05$) increases in ALT, AST, ALP, Total bilirubin and total protein and TBARS in the toxic control when compared to the control group. Significant decreases ($p < 0.05$) in GSH was also associated with gentamicin administration. Post-treatment with AOGL caused significant increases in and GSH, and significant ($p < 0.05$) decreases in ALT, AST, ALP, Total bilirubin and total protein in the treated groups when compared with the toxic control group. The results of this study indicated that AOGL ameliorated the liver injury caused by gentamicin in rats. Hence, the extracts have the potential of being used for the management of gentamicin-induced liver injury.**

Keywords: ***Ocimum gratissimum*** **leaves extract; Gentamicin; ALT; AST; ALP; Total protein; TBARS; GSH; Liver; rats**.

I. INTRODUCTION

The liver, is a natural chemical factory which neutralizes toxins and aids in the building up of complex molecules from simple substances that are absorbed from the gastro-intestinal tract [1,2]. It is one of the vital organs in the body that performs homeostatic function through detoxification mechanisms. Malfunctioning of the liver can have deteriorating health consequences which sometimes lead to terminal illness [3] due to biological build-up of toxins. Liver injury can result from exposure to various kinds of exogenous compounds or chemicals, either through job demands or way of life [4]. Gentamicin (Gen.) is widely used against serious and life-threatening gram-negative bacterial infection as an abiotic. Its use clinically is limited due to its adverse effect on some vital organs in the body such as kidney, liver etc [5]. Like all aminoglycosides, when gentamicin is given orally, it is not systemically active. This is because it is not absorbed to any appreciable extent from the small intestine of human. It is administered intravenously, intramuscularly or topically to treat infections (5). It appears to be completely eliminated unchanged in the urine. Urine must be collected for many days to recover all of a given dose because the drug binds avidly to certain tissues such as bladder and liver (5). The use of gentamicin despite its clinical benefits has been limited due to its side effects. The main side effects include liver damage that is one of the major factors of liver inefficiency in a significant number of people taking this medication (6-8). Therefore taking these medications face limitations due to the fact that one of the major side effects of Gentamicin is creating hepaotoxicity. 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 (9-11). *Ocimum gratissimum* (OG) is an herbaceous plant that belongs to the family of Labiatae. The plant is mostly found in the tropical areas especially India and West Africa. The flowers and the leaves of this plant are used in preparation of

teas and infusion because it is rich in essential oils [13]. It's used in the treatment of epilepsy, high fever and diarrhea in the coastal area of Nigeria [12]. The decoctions of the leaves are used to treat mental illness in the savannah areas [14]. Despite all these apparently vast medicinal properties of Ocimum gratissimum, till date, available scientific data 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 14 days of administration of aqueous extract of Ocimum gratissimum leaf (AOGL) and methanolic extract of Ocimum gratissimum leaf (MOGL) on the liver function of Wistar rats with gentamicin-induced liver injury.

II. MATERIALS AND METHODS DRUGS

Gentamicin injection 80 mg/2 mL was purchased from Shanxi Shuguang Pharmaceutical Co (Jinzhong, China), while the metabolic cages used for this study were fabricated by Central Technological Laboratory and Workshops, Obafemi Awolowo University, Ile-Ife, Nigeria.

2.1 Extraction Process

Aqueous Extraction

Leaves of Ocimum gratissimum were collected, washed, air dried under shade, and ground into fine powder using a blender. The aqueous extract was prepared by macerating the powdered leaves (323 g) with 3 L of distilled water in an electric shaker for 48 hours. The extract was filtered through Whatmann No. 1 paper (Whatmann International Ltd, Maidstone, England) and evaporated under reduced pressure using a rotary evaporator. The yield (33.60 g) was kept in a bottle with a tight-fitting cover until it was needed for the study.

$$\text{Extraction yield in \%} = \frac{33.60}{323} \text{ x } 100$$

$$= 10.4\%.$$

2.2 Methanolic Extraction

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).

2.3 Animal Care and Management

Forty-five adult male Wistar rats weighing 150 to 200 g were used for this study. They were obtained from the Animal Holdings of the College of Health Sciences, Obafemi Awolowo University, Ile-Ife, and were housed in plastic cages. The rats were kept under natural light/dark cycle and had access to standard rodent pellet diet (Caps Feed PLC, Osogbo, Nigeria) and water ad libitum. They were allowed to acclimatize in the laboratory for 2 weeks before the commencement of the study.

2.4 Experimental Design

The rats were divided into 8 groups as follows: Group 1 (control) consisting of 5 rats received 2 mL/kg/day of distilled water via oral route for the study period. Twenty-four hours after final administration, the rats were euthanized. Group 2 (toxic) consisted of 10 rats, each of which received 100 mg/kg/day of gentamicin (intraperitoneal) for a week and thereafter left untreated (without AOGL and MOGL) for 2 weeks. Five rats each were sacrificed a day after the final gentamicin administration as well as after 14 days of recovery period. Groups 3, 4, 5, 6, 7 and 8 consisted of 5 rats each that were pretreated with gentamicin as group 2 and thereafter received graded doses of AOGL at 100, 200, 400 mg/kg/day and graded doses of MOGL at 100, 200, 400 mg/kg/day per os, respectively for 14 days. Rats from each group were sacrificed a day after the last dose of AOGL and MOGL.

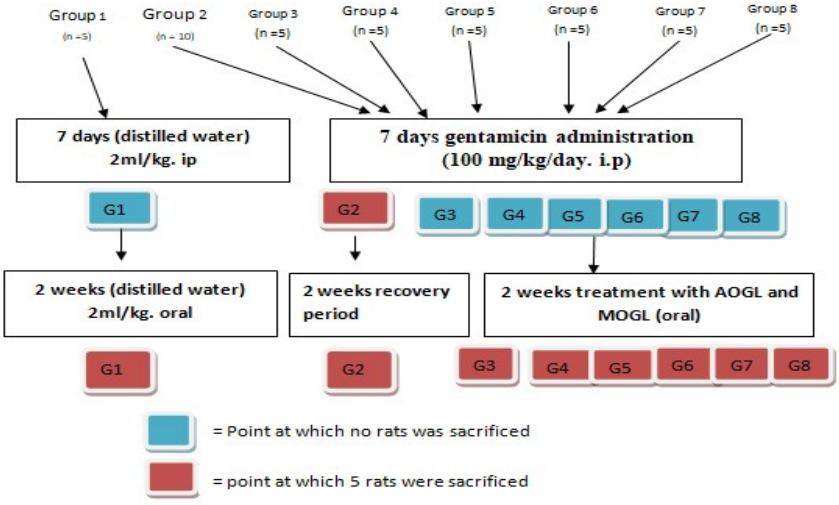

Fig. 1. Experimental Protocol

G1=Group 1 (2 ml/kg of distilled water, both i.p and orally); G2=Group 2 (First 5 rats=100 mg/kg GEN; Remaining 5 rats=5 mg/kg GEN + Recovery); G3=Group 3 (GEN+100 mg/kg AOGL); G4=Group 4 (GEN+200 mg/kg AOGL); G5=Group 5 (GEN+400 mg/kg AOGL); G6=Group 6 (GEN+100 mg/kg MOGL); G7=Group 7 (GEN+200 mg/kg MOGL); G8=Group 8 (GEN+400 mg/kg MOGL); n=number of rats in the group.

2.5 Biochemical Assay

Blood obtained after the rats were euthanized was collected into separate ethylenediaminetetraacetic acid bottles and centrifuged at 4000 rpm for 15 minutes using Cold Centrifuge (Model 8881, Centurion Scientific, West Sussex, England). The resulting plasma was analyzed for some biochemical indices of renal function. The urine samples were similarly subjected to the same procedure.

2.6 Protein Determination

Protein determination was carried out according to the method of Lowry et al11 as described by Holme and Peck.12

2.7 Assessment of Antioxidant Status

Each carefully excised left kidney was weighed and homogenized with 10 mL of sucrose solution (0.25 M) using Electric Homogenizer (SI601001). The homogenate was centrifuged at 3000 rpm for 20 minutes and the supernatant was collected for the assessment of reduced glutathione (GSH) as well as thiobarbituric acid reactive substance (TBARS) levels.

III. RESULTS

3.1 Effects of AOGL and MOGL on hepatic GSH (mM) and TBARS (mM) activities in wistar rats with GEN-induced liver injury.

GEN-induced reduction in hepatic GSH level and was significantly normalized following AOGL and MOGL administration. The AOGL and MOGL treated groups 3, 4, 5 and the GEN recovery group showed no significant difference in these indicators of oxidative stress (at the end of the study) when compared with the control, but the levels were recorded to be significantly higher than the toxic groups (fig 1).

The significantly elevated level of TBARS that accompanied GEN administration was significantly lowered following AOGL and MOGL administration.

The AOGL and MOGL-treated groups 3, 4 , 5 and the GEN recovery group showed significant reductions when compared with the toxic groups, but recorded no significant difference when compared with the control (fig 2).

3.2. Effects of AOGL and MOGL on plasma total bilirubin (µmol/L) and hepatic total protein (mg/mL) levels in wistar rats with GEN-induced liver injury.

Gentamicin administration was associated with significantly elevated level of plasma total bilirubin level. The total bilirubin levels were not significantly lowered in the AOGL and MOGL-treated groups 3, 4 and 5 when compared with both the toxic and toxic recovery groups with no recorded significant difference when these treated groups were compared with the control group (Fig. 3).

Following exposure to GEN, hepatic total protein level was significantly lowered. AOGL and MOGL treatment does not significantly restored hepatic total protein levels to physiological levels when compared with the control, toxic and toxic recovery groups (Fig. 4).

3.3. Effects of AOGL and MOGL on plasma AST (U/L), ALT (U/L) and ALP (U/L) levels in wistar rats with GEN-induced liver injury

The plasma levels of AST, ALT and ALP were significantly elevated following GEN exposure. These levels were, however, restored to physiological levels in the MOGL-treated groups 4 and 5 when compared with the control, toxic and toxic recovery groups (Figs. 2–4).

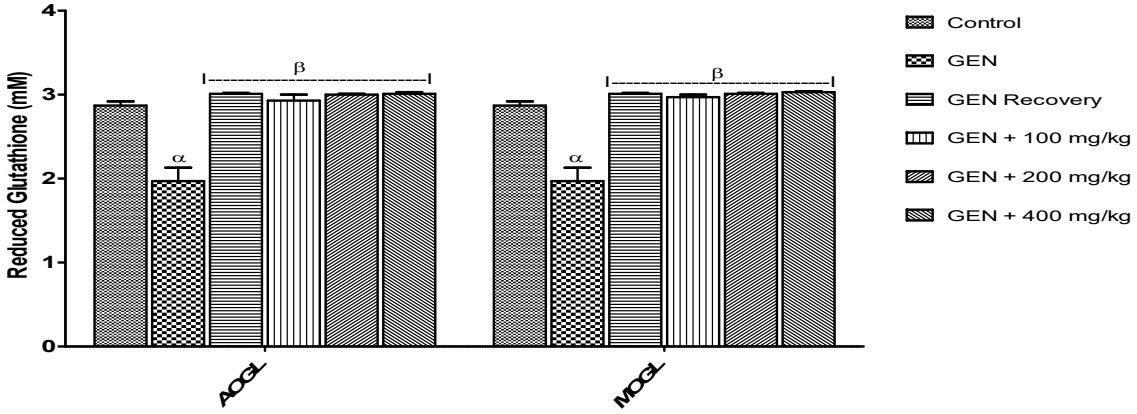

Fig 1. Graph showing the effects of AOGL and MOGL on plasma level of GSH of rats with GEN induced liver injury.
Each bar represents mean ± standard error of mean ($p < 0.05$).
α = significantly different from control group;
β =significantly different from GEN group.

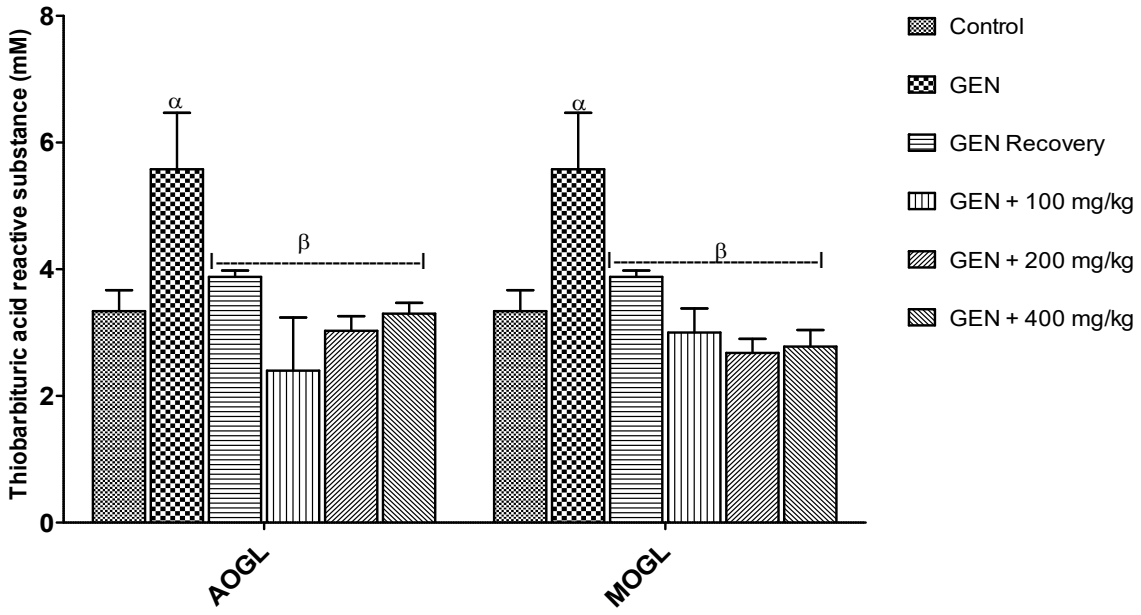

Fig 2. Graph showing the effects of AOGL and MOGL on plasma level of TBARS of rats with GEN induced liver injury.

Each bar represents mean ± standard error of mean ($p < 0.05$).

α = significantly different from control group;

β =significantly different from GEN group.

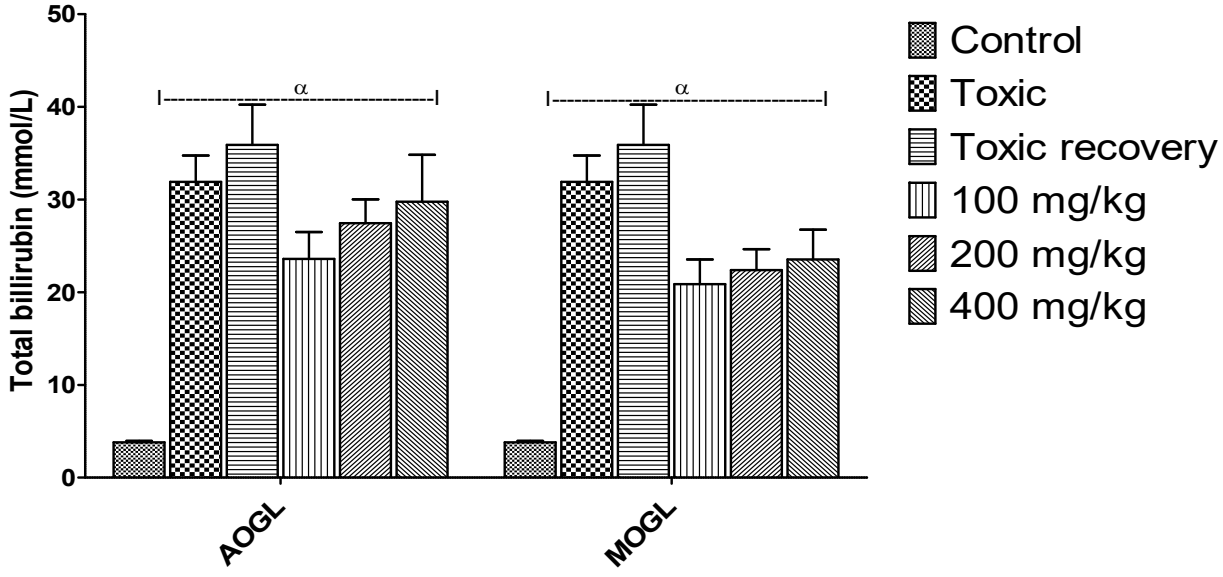

Fig 3. Graph showing the effects of AOGL and MOGL on plasma level of total billirubin of rats with GEN induced liver injury.

Each bar represents mean ± standard error of mean ($p < 0.05$).

α = significantly different from control group

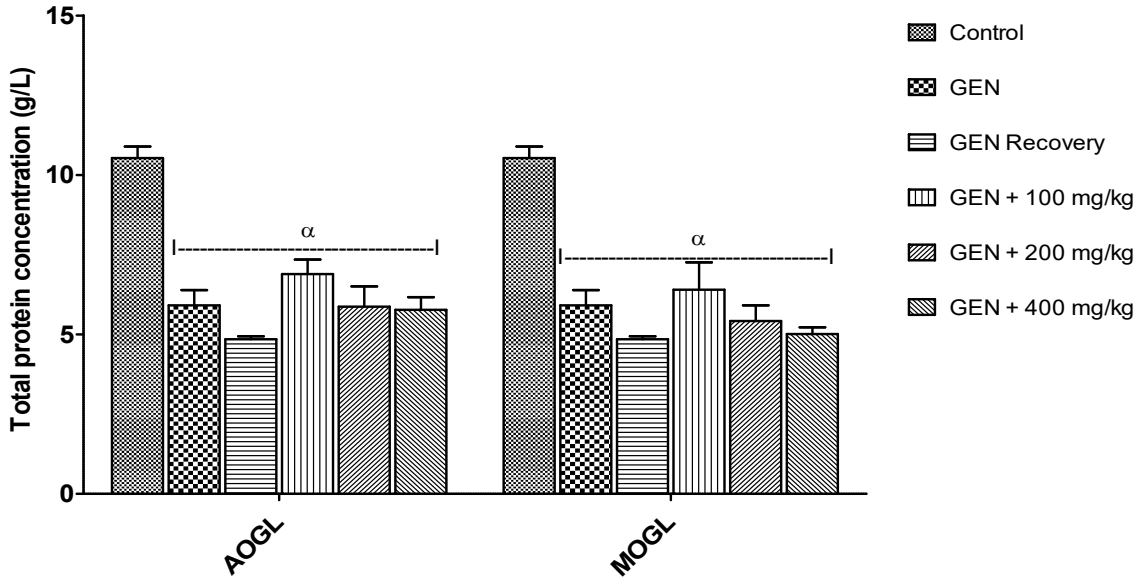

Fig 4. Graph showing the effects of AOGL and MOGL on plasma level of total protein of rats with GEN induced liver injury.

Each bar represents mean ± standard error of mean ($p < 0.05$).

α = significantly different from control group

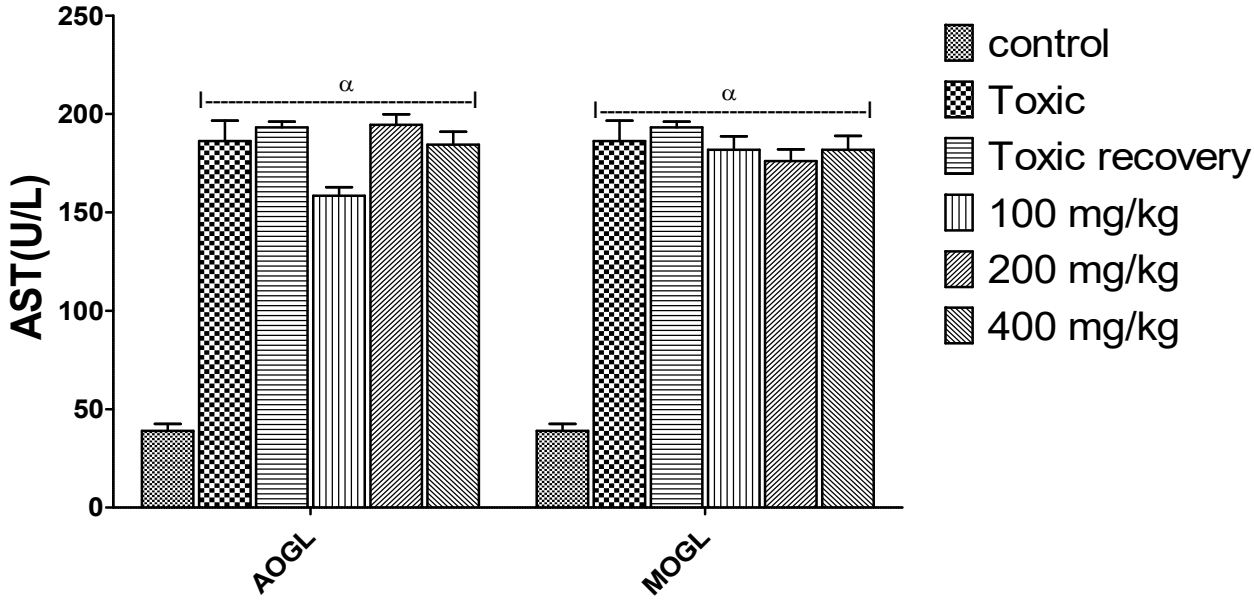

Fig 5. Graph showing the effects of AOGL and MOGL on plasma level of AST of rats with GEN induced liver injury.

Each bar represents mean ± standard error of mean ($p < 0.05$).

α = significantly different from control group

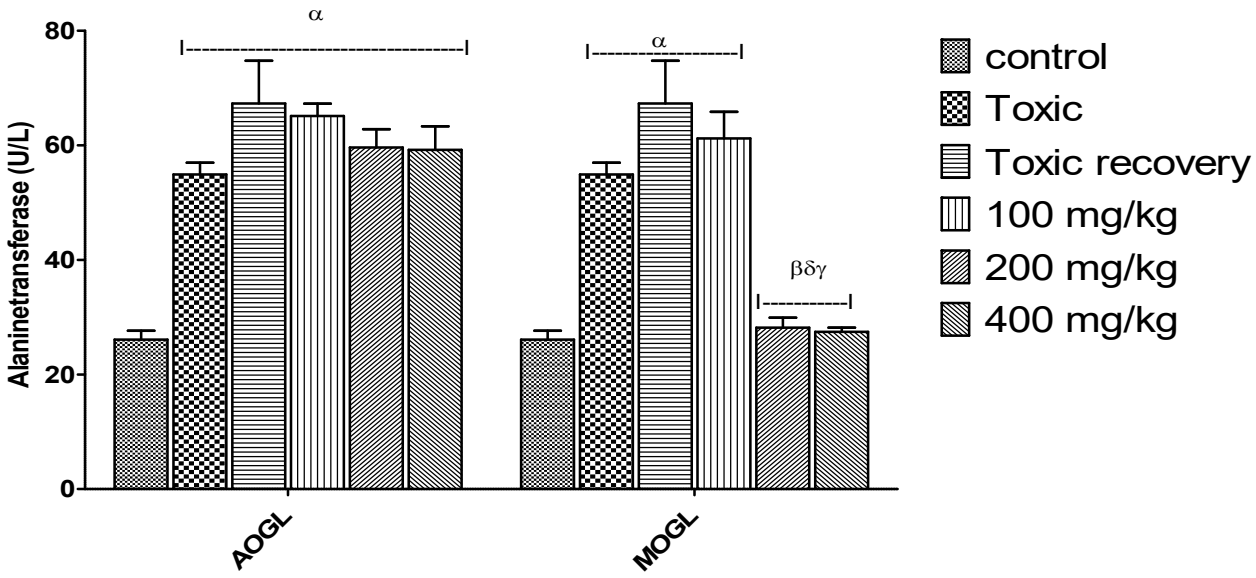

Fig 6. Graph showing the effects of AOGL and MOGL on plasma level of ALT of rats with GEN induced liver injury.

Each bar represents mean ± standard error of mean ($p < 0.05$).

α = significantly different from control group;

β =significantly different from GEN groug;

δ =significantly different from GEN Recovery;

γ =significantly different from GEN + 100mg/kg.

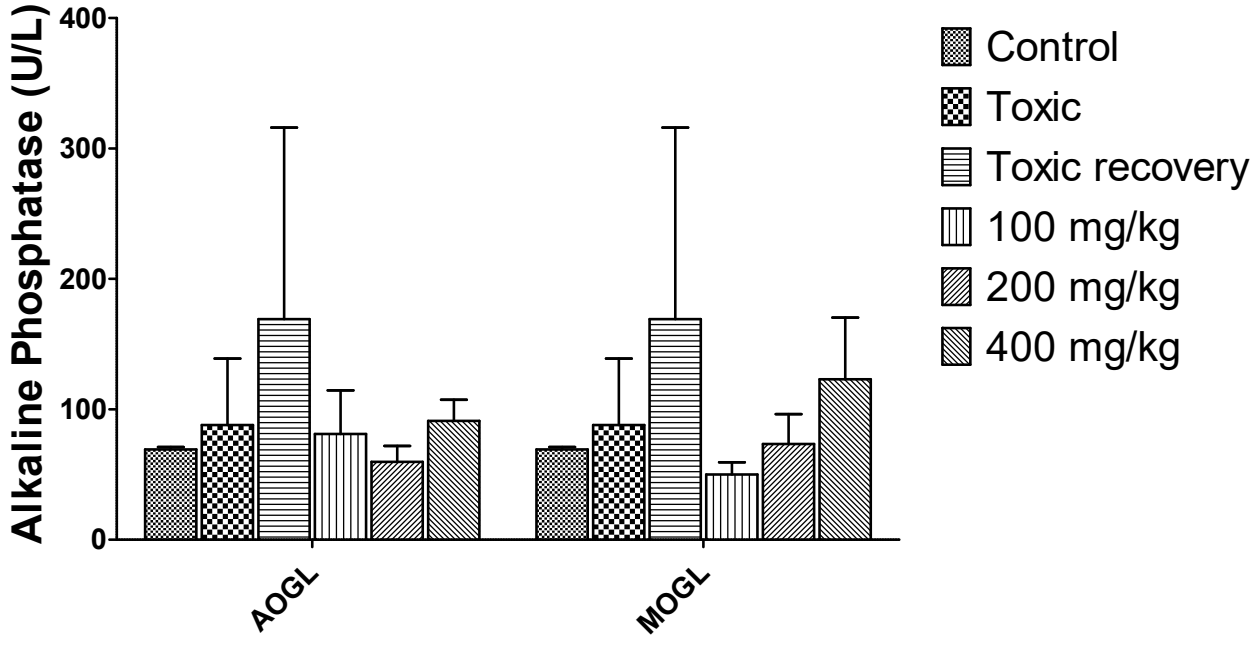

Fig 7. Graph showing the effects of AOGL and MOGL on plasma level of ALP of rats with GEN induced liver injury.

Each bar represents mean ± standard error of mean ($p < 0.05$).

IV. DISCUSSION

This study investigated the effects of two weeks administration of both aqueous and methanolic extract of ocimum gratisimum (linn) leaf (AOGL and MOGL) on gentamicin induced liver injury. Based on the results obtained from this study, it is observed that administration of the graded doses of AOGL and MOGL restores the antioxidant activities and also the liver functions biomarkers. GSH is a non-enzymatic index of oxidative stress [30, 31, 46]. On the other hand, TBARS is an important index of lipid peroxidation [15, 30, 31, 45]. Increased activity of TBARS is directly proportional to the increase degree of injury in biological tissues; hence, it is an index of lipid peroxidation [15, 30]. The deleterious reduction in the activities of GSH in the liver following exposure to GEN toxicity was an indication of oxidative stress due to generation of free radicals. This can be attributed to an increased use of these enzymatic and non-enzymatic biomarkers by the liver to scavenge free radicals in an attempt to restore homeostasis of the antioxidant system and (or) reduced ability of the liver to sustain these lines of defence following ROS generation due to GEN toxicity. These are showed in increased TBARS level which was reflective of a high degree of GEN induced liver injury. The AOGL and MOGL treated groups demonstrated attenuating effects against the GEN-induced oxidative stress and lipid peroxidation. This indicate that the plant has potent antioxidant activities; a finding that supports existing literatures [15–17, 30]. It therefore implies that AOGL and MOGL restores homeostasis of the antioxidant system by conjugating and excreting toxic cellular molecules, detoxification of ROS, sustenance of cellular integrity as well as a possible enhancement of tissue regeneration. The group that was left to self-recover also reverses the alterations in the antioxidant system to physiological levels. This connote that apart from the anti-oxidant ability of these plant on the renal tissue, the kidney its self has the potential to restore it physiological functions following deprivation from GEN for a few weeks.

Total bilirubin is used as an index of bile duct lesion [55] or hepatic injury due to bile duct obstruction [56]. Elevated level of plasma total bilirubin after exposure to GEN toxicity was recorded. The AOGL and MOGL treated groups showed a reduction in the elevated billirubin but not to the physiological level. This insignificant reduction of billirubin level maybe due to the duration of exposure to the plant. This assertion is further buttressed with the fact that the group that was left to self recover could not reverse the alterations in the billirubin level.

The assay for the activities of blood levels of AST, ALT and ALP as important biomarkers of liver function is useful in clinical world. [50–52].

These enzymes reside in the liver cells and are released into the plasma in response to liver cell injury or damage. Following GEN administration, the activities of these liver enzymes were significantly elevated beyond physiological levels. AST and ALT enzymes are concentrated more in the liver [53]. Whereas AST is both mitochondrial (about 80% of total activity) and cytosolic (about 20% of total activity) in location, ALT is solely cytoplasmic [53]. Therefore, the non

potential of the extract to attenuate the GEN-induced derangements in both AST and ALT activities is suggesting that both AOGL and MOGL has no cytoplasmic, cytosolic and mitochondrial effects to bring about the prevention of membrane fragility and reduced leakage of hepatic enzymes into the blood. This may be due to the duration of both AOGL and MOGL administration. The ALP is a cholestatic index [53,54], the AOGL and MOGL-enhanced attenuating effects against elevated ALP levels can be attributed to the extract's potential to reverse cholestatic mechanism(s). This is because cholestasis (obstruction of bile flow) enhances both synthesis and release of hepatic ALP from cell surfaces [53,54].

A contributory self-healing mechanism to liver regeneration process is the stimulation of protein synthesis [57]. A chemical agent can induce adverse changes in the process of protein synthesis; hence, the level of hepatic total protein content can be an important index for the determination of chemically-induced liver injury or dysfunction [54].

The extract's potential to restore physiological levels of liver total protein is indicative of its tissue-regenerative ability an ability that was least expressed in the group that received AOGL and MOGL. This may also due to the duration of this study. This assertion is also buttressed with the fact that the group that was left to self recover could not reverse the alterations in the total protein level.

REFERENCE

[1]. M.G. Palanive, B. Rajkapoor, R.S. Kumar, Hepatoprotective and antioxidant effect of Pisonia aculeate L. Against CCl4-induced hepatic damage in rats, Sci. Pharm. 76 (2008) 203–215.

[2]. A.A. Buraimoh, I.G. Bako, F.B. Ibrahim, Hepatoprotective effect of ethanolic leaves extract of Moringa Oleifera on the histology of paracetamol induced liver damage in wistar rats, Int. J. Anim. Vet. Adv. 3 (2011) 10–13.

[3]. A. Subramoniam, P. Pushpangadan, Development of phytomedicine for liver diseases, Indian J. Pharmacol. 31 (1999) 166–175.

[4]. S.O. Olukiran, R.O. Akomolafe, K.D. Bamitale, A.O. Ajayi, R.E. Okonji, R.A. Bejide, Protective and curative effects of Livolin forte® on carbon tetrachloride-induced liver damage in Wistar rats, J. Exp. Integr. Med. 4 (2013) 57–65.

[5]. Moulds RFW, Jeyasingham MS. Gentamicin: a great way to start. Aust Prescr 2010;33:134–5.

[6]. Masakazu K, Yoshiko E, Masashi E., Acquired resistance of Listeria monocytogenes in and escaped from liver parenchymal cells to gentamicin is caused by being coated with their plasma membrane. Microbes and Infection **16**(3): 237-243 (2014).

[7]. Nayma S, Sadia C S, M Tanveer H P, Jesmine A., Effects of Ashwagandha (Withania somnifera) Root Extract On Some Serum Liver Marker Enzymes (AST, ALT) In *Gentamicin Intoxicated Rats* **7**(1):1-7 (2012).

[8]. Stojiljkovic N, Stoiljkovic M., Micromorphological and histochemical characteristics of a rat's liver treated with gentamicin. *Acta medica Medianae* **45**(3): 24-28 (2006).

[9]. Wojciech L, Vincent LP., Ternary Complexes of Gentamicin with Iron and Lipid Catalyze Formation of Reactive Oxygen Species. *Chemical Research in Toxicology* **18**(12): 357-364 (2005).
[10]. Erin E. Battin J., Sulfur and selenium: A Review of reactiveOxygen species scavenging, glutathione peroxidase and metal-binding antioxidant mechanisms. *Cell Biochem Biophys* **55**: 1-23 (2009).
[11]. Jeffrey WC, Roger GU, Philip GL, Clay TC Acute Hepatocellular Effects of Erythromycin, Gentamicin, and Trospectomycin in the Perfused Rat Liver: Lack of Correlation between Lamellar Body Induction Potency and Cytotoxicity. *Pharmacology & Toxicology.*
[12]. Effraim KD, Jacks TW, Sodipo OA. Histopathological studies on the toxicity of *Ocimum gratissimum* leave extract on some organs of rabbit. Afr J Biomed Res 2003;6:21–5.
[13]. Rabelo M, Souza EP, Soares PMG, Miranda AV, Matos FJA, Criddle DN. Antinociceptive properties of the essential oil of *Ocimum gratissimum* L. (Labiatae) in mice. Braz J Med Biol Res 2003;36:521–4.
[14]. Akinmoladun AC, Ibukun EO, Emmanuel A, Obuotor EM, Farombi EO. Phytochemical constituent and antioxidant activity of extract from the leaves of *Ocimum gratissimum*. Sci Res Essays 2007;2:163–6.
[15]. C.E. Imafidon, R.O. Akomolafe, S.A. Abubakar, D.J. Ogundipe, S.O. Olaoluwa, A.A. Oladele, Amelioration of cadmium-induced nephropathy using polyphenolrich extract of Vernonia amygdalina (Del.) leaves in rat model, Open Access Maced. J. Med. Sci. 3 (2015) 567–577.
[16]. P. Erasto, D.S. Grierson, A.J. Afolayan, Antioxidant constituents in Vernonia amygdalina leaves, Pharm. Biol. 45 (2007) 195–199.
[17]. G.A. Ayoola, H.A.B. Coker, S.A. Adesegun, A.A. Adepoju-Bello, K. Obaweva, E.C. Ezennia, T.O. Atangbayila, Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria, Trop. J. Pharm. Res. 7 (2008) 1019–1024.
[18]. E.M. Arhoghro, K.E. Ekpo, E.O. Anosike, E.O. Ibeh, Effect of aqueous extract of bitter leaf (Vernonia amygdalina Del) on carbon tetrachloride (CCl4) induced liver damage in albino Wistar rats, Eur. J. Sci. Res. 26 (2009) 122–130.
[19]. O. Lolodi, G.E. Eriyamremu, Effects of methanolic extract of Vernonia amygdalina (Common bitter leaf) on lipid peroxidation and antioxidant enzymes in rats exposed to cycasin, Pak. J. Biol. Sci. 16 (2013) 642–646.
[20]. J. Momoh, A.O. Longe, A.O. Damazio, O.O. Eleyowo, Hepatoprotective effects of ethanolic extract of Vernonia amygdalina and Azadiracha indica against acetaminophen-induced hepatotoxicity in Sprague-Dawley male albino rats, Am. J. Pharmacol. Sci. 3 (2015) 79–86.
[21]. M.I. Iwo, S.L. Sjahlim, S.F. Rahmawati, Effect of Vernonia amygdalina Del. Leaf ethanolic extract on intoxicated male Wistar rat liver, Sci. Pharm. 85 (2017) 1–7.
[22]. M.E. Halilu, A. Abubakar, M.K. Garbar, A.A. Isah, Antimicrobial and preliminary phytochemical studies of methanol extract of root bark of Crossopteryx febrifuga (Rubiaceae), J. Appl. Pharm. Sci. 2 (2012) 066–070.
[23]. J.B. Harborne, Phytochemical Methods, 2nd edition, Chapman and Hall, London, 1980, pp. 288–293.
[24]. B.O. Obadoni, P.O. Ochuko, Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria, Global J. Pure Appl. Sci. 8 (2002) 203–208.
[25]. S.E. Allen, H.M. Grinshaw, J.A. Parkinson, C. Quarmbay, Chemical Analysis of Ecological Materials, 1st edition, Blackwell Scientific Publication, London, 1973.
[26]. H. Benmehdi, O. Hasnaoui, O. Benali, F. Salhi, Phytochemical investigation of leaves and fruit extracts of Chamaerops humilis L, J. Mater. Environ. Sci. 3 (2012) 320–337.
[27]. S. Anjali, S. Sheetal, Phytochemical analysis and free radical scavenging potential of herbal and medicinal plant extracts, J. Pharmacogn. Phytochem. 2 (2013) 22–29.
[28]. D. Lorke, A new approach to practical acute toxicity testing, Arch. Toxicol. 54 (1983) 275–287.
[29]. Guide for the Care and Use of Laboratory Animals, 8th edition, (2011) https:// grants.nih.gov/grants/./Guide-for-the-Care-and-use-of-laboratory-animals.pdf (Access date: 5 October 2015).
[30]. C.E. Imafidon, T.R. Olatoye, F.S. Bamidele, O.E. Ojo, K.A. Ademoye, Cadmium-induced testicular toxicity, oxidative stress and histopathology in Wistar rats: sustained effects of polyphenol-rich extract of Vernonia amygdalina (Del.) leaf, J. Interdiscip. Histopathol. 4 (2016) 54–62.
[31]. A.O. Ayoka, A.K. Ademoye, C.E. Imafidon, O.E. Ojo, A.A. Oladele, Aqueous extract of Allium sativum (Linn.) bulbs ameliorated pituitary-testicular injury and dysfunction in Wistar rats with Pb-induced reproductive disturbances, Open Access Maced. J. Med. Sci. 4 (2016) 200–212.
[32]. O.H. Lowry, J.R. Nira, L.A. Farr, J.R. Rose, Protein measurement with the folinphenol reagent, J. Biol. Chem. 193 (1951) 265–275.
[33]. E. Beutler, O. Duron, B.M. Kelly, Improved method for the determination of blood Glutathione, J. Lab. Clin. Med. 61 (1963) 882–888.
[34]. J.M. McCord, I. Fridovich, Superoxide dismutase, an enzymic function for erythrocuprein (hemocuprein), J. Biol. Chem. 244 (1969) 6049–6055.
[35]. K.A. Sinha, Colorimetric assay of catalase, Anal. Biochem. 47 (1971) 389–394.
[36]. H. Ohkawa, N. Ohishi, K. Yagi, Assay for lipid peroxides in animal tissues bythiobarbituric acid reaction, Anal. Biochem. 95 (1979) 351–358.
[37]. Z. Merali, R.L. Singhal, Prevention by zinc of cadmium-induced alterations in pancreatic and hepatic functions, Br. J. Pharmacol. 57 (1976) 573–579.
[38]. A.S. Katherine, M.M. Niamh, R.B. Steve, Hypothalamic regulation of apetite, Expert Rev. Endocrinol. Metab. 3 (2008) 577–592.
[39]. A. Pretto, V.L. Loro, V.M. Morsch, B.S. Moraes, C. Menezes, B. Clasen, L. Hoehne, V. Dressler, Acetylcholinesterase activity, lipid peroxidation, and bioaccumulation in Silver catfish (Rhamdia quelen) exposed to cadmium, Arch. Environ. Contam. Toxicol. 58 (2010) 1008–1014.
[40]. F. Rossana, C. Giovanni, C.G. Maria, D.B. Salvatore, L. Massimo, F. Ida, Bioaccumulation of cadmium and its cytotoxic effect on zebrafish brain vol. 27, Taylor and Francis, 2011, pp. 39–46.
[41]. E.O. Farombi, I.A. Adedara, S.A. Akinrinde, O.O. Ojo, A.S. Eboh, Protective effects of kolaviron and quercetin on cadmium-induced testicular damage and endocrine pathology in rats, Andrologia 44 (2012) 273–284.
[42]. A.B. Steven, H.Z. Robert, W.P. Richard, Relationships between organ weight and body/brain weight in the rat: what is the best analytical endpoint? Toxicol. Pathol. 32 (2004) 448–466.
[43]. Health Benefits of Plant Tannins, (2016) http://www.medibiztv.com/articles/ health-tannins (Access date: 3 January 2016).
[44]. Health Benefits of Flavonoids, (2016) http://www.livestrong.com/article/492244- whatare-the-health-benefits-of-flavonoids/ (Access date: 5 April 2016).
[45]. A.O. Ayoka, O.E. Ojo, C.E. Imafidon, K.A. Ademoye, A.A. Oladele, Nuero-endocrine effects of aqueous extract of Amaranthus viridis (Linn.) leaf in male Wistar rat model of cyclophosphamide-induced reproductive toxicity, J. Toxicol. Rep. 3 (2016) 608–619.
[46]. S. Sunitha, M. Nagaraj, P. Varalakshmi, Hepatoprotective effect of lupeol and lupeol linoleate on tissue antioxidant defence system in cadmium-induced hepatotoxicity in rats, Fitoterapia 72 (2001) 516–523.
[47]. C. Bowler, M.V. Montagu, D. Irize, Superoxide Dismutase and stress tolerance, Annu. Rev. Plant Physiol. Plant Mol. Biol. 43 (1992) 83–116.
[48]. M. Zamocky, P.G. Furtmuller, C. Obinger, Evolution of catalases from bacteria to humans, Antioxid. Redox Signal. 10 (2008) 1527–1548.
[49]. D.E. Heck, M. Shakarjian, H.D. Kim, J.D. Laskin, A.M. Vetrano, Mechanisms of oxidant generation by catalase, Ann. N. Y. Acad. Sci. 1203 (2010) 120–125.

[50]. S.K. Mitra, M.V. Venkataranganna, R. Sundaram, S. Goupmadhavan, Protective effects of HD-03, herbal formulation, against various hepatotoxic agents in rats, J. Ethnopharmacol. 63 (1998) 181–186.
[51]. A.H. Atta, T.A. Elkoly, S.M. Mouneir, K. Gehan, N.A. Alwabel, Z. Shaimaa, Hepatoprotective effects of methanol extracts of Zingiber officinale and Cichorium intybus, Indian J. Pharm. Sci. 72 (2010) 564–570.
[52]. M.I. Kazeem, H.A. Bankole, A.A. Fatai, Protective effect of ginger in normal and carbon-tetrachloride induced hepatotoxic rats, Ann. Biol. Res. 2 (2011) 1–8.
[53]. B.R. Thapa, W. Anuj, Liver function tests and their interpretation, Indian J. Pediatr. 74 (2007) 663–671.
[54]. T.K. Motawi, M.A. Hamed, M.H. Shabana, R.M. Hashem, A.F. Aboul-Naser, Zingiber officinale acts as a nutraceutical agent against liver fibrosis, Nutr. Metab. 8 (2011) 1–11.
[55]. T.B. Leonard, D.A. Neptun, J.A. Popp, Serum gamma glutamyl transferase as a specific indicator of bile duct lesions in the rat liver, Am. J. Pathol. 116 (1984) 262–269.
[56]. S.S. Bun, H. Bun, D. Guedon, C. Rosier, E. Ollivier, Effect of green tea extract on liver functions in Wistar rats, Food Chem. Toxicol. 44 (2006) 1108–1113.
[57]. N. Sharma, S. Shukla, Hepatoprotective potential of aqueous extract of Butea monosperma against CCl4 -induced damage in rats, Exp. Toxicol. Pathol. 63 (2011) 671–676.