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Evaluating the Protective and Ameliorative Effects of Kolaviron on Lead-induced Adrenal Gland Toxicity in Adult Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Lead (Pb) toxicity is possibly the most common form of heavy metal poisoning in the human body due to the abundant global distribution and utilization of Pb. Pb has no functional role in the body. Its only effect on human organs is regarded as harmful. The adrenal gland, one of the most common endocrine organs associated with chemically induced lesions, has its fair share of organ damage from Pb toxicity. Interestingly, some herbs and fruits notably have ameliorative actions against organs damaged due to Pb and other heavy metal toxicity. Kolaviron, the active constituent of Garcinia kola has such an effect on glands, but substantial studies are yet to be done concerning the adrenal gland. Hence, this study aims to investigate the ameliorative effects of the ethanolic extract of Garcinia kola fruit (Kolaviron) on lead-induced adrenal gland toxicity using adult Wistar

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rats. Thirty (30) healthy adult Wistar rats weighing 160-200g were grouped into six (n=5). Group A (control) was placed on a diet of food and water ad libitum. Group B (untreated) received lead-acetate solutions at 50mg/kg/day for 14 days. Groups C, D, and E received co-administration of lead-acetate solutions at 50mg/kg/day for 7 days and 100, 200, and 300mg/kg/day of Kolaviron consecutively for 14 days. Group F received lead-acetate solutions at 50mg/kg/day for 7 days only, and subsequently for a period of 7 days, 300mg/kg/day of Kolaviron was administered. The route of all administrations was oral and the experiment lasted for 14 days. The rats were sacrificed under ketamine (100mg/ml) as anesthesia 24 hours after their last treatment. Their adrenal glands were carefully harvested, fixed for histological analysis, and stained with Hematoxylin and Eosin dyes. Relative to the control and untreated groups, treatment with increasing doses of Kolaviron demonstrated mild to moderate ameliorative effects on the pathologic damages noted to be brought about by Pb toxicity. Garcinia kola is a promising agent with potency against adrenal gland injuries from heavy metal intoxication in the human body.

Keywords: Garcinia kola; kolaviron; adrenal gland, lead toxicity.

1. INTRODUCTION

Lead (Pb) is a naturally occurring heavy metal with no beneficial function to the human body [1]. In its smallest amounts, it is toxic, due to its reaction with both acid and base to form covalent bonds that further cause its accumulation within soft tissues and bones [1,2]. This affects the general health of affected individuals, ranging from behavioural problems to brain damage, cardiovascular complications, renal failure, and endocrine problems, to mention the least. The removal of Pb from paint and gasoline since the 1970s markedly reduced the incidence of Pb toxicity [1]. However, Pb exposure is still a public health concern that targets mostly the lower socio-economic class [2]. The rise of industrial development has also led to a rise in environmental pollution by heavy metals which threatens the lives of living creatures in various ways [3].

Pb is one of the major heavy metals that have the most damaging effects on human health and is well-recognized as one of the most hazardous and insidious poisons [4,5]. Some reports show a relationship between Pb toxicity and elevation of peripheral blood pressure, though the mechanism of action is unknown [6]. Some scholars which include Campbell et al. [7] reports a positive correlation between blood Pb concentration and plasma aldosterone concentration in individuals with Pb exposure, citing increased production of aldosterone as perhaps the mechanism of action responsible for elevated blood pressure. Aldosterone is a mineralocorticoid hormone primarily produced in the zona glomerulosa of the adrenal gland in humans to influence the balance of salt and water [7]. In hyper-aldosteronism, there is an uncontrolled production of aldosterone due to a primary tumor in the adrenal gland. This is postulated to be the same mechanism through which Pb toxicity affects the adrenal gland, leading to increased secretion of aldosterone and other adrenal hormones; thus adrenal toxicity [7]. Also, hypo-functioning of the adrenal gland can result due to Pb toxicity [8]. This is characterized by the destruction of the adrenal gland due to Pb accumulation leading to adrenal insufficiency [9]. The adrenal gland is reported to be one of the most common endocrine organs associated with chemically induced lesions [10,11]. The response of the adrenal cortex to tissue injury includes; degeneration (characterised by vacuolization and granulation), necrosis, and haemorrhage [11]. It regulates several essential physiological functions in the adult organism through the production of steroids and catecholamines. In adrenal insufficiency, catecholamines are mostly affected which may clinically present as generalized muscle weakness, loss of appetite, fatigue, nausea, vomiting, abdominal pain, weight loss, dizziness, tachycardia, and/or postural hypotension [12]. The presentation of adrenal insufficiency depends on the rate and extent of adrenal function involvement [9,13]. Some of the reasons why the adrenal gland may be highly sensitive to Pb toxicity is due to specific biochemical features including high lipophilicity due to fatty acid contents such as steroid hormones, high vascularization, and the presence of cytochrome P450 enzymes that metabolize free radicals and toxic reactive compounds [14].

Plant-derived medicine is attributed to nutraceutical components demonstrable in traditional fruits and herbs attributed to their abundance of secondary metabolites [15]. Plant-derived medicines are beneficial due to

properties such as being primarily effective, having a relatively low toxicity status making them relatively safe for consumption, high accessibility to the population as primary health care, and affordability [16] One such plant is *Garcinia kola* commonly called “bitter kola”. *Garcinia kola,* an edible seed/fruit, is a member of the Guttiferae species found throughout West and Central Africa. As a plant, almost all components of bitter kola is an important and utilized in traditional herbal medicine worldwide [17]. Phytochemical screening of “bitter kola” reveals it is a rich source of carbohydrates, proteins, and other anti-inflammatory, antioxidant, antiviral, and anti-carcinogenic components, despite having small amounts of anti-nutrients such as tannin, phytate, and oxalate [18,19]. However, this plant demonstrates a complex mixture of biflavonoids, xanthones, calanolide-type coumarines, and prenylated benzophenones [20]. Kolaviron is a fraction of defatted ethanol extract containing *Garcinia* biflavonoids GB1, GB2, and kolaflavonone [20,21]. The ability of Kolaviron to inhibit hydroxy and superoxide anion radicals, which are known to play important role in the process of lipid peroxidation has been reported recently. Thus it has an established anti-oxidant action on lipoprotein [22].

These imply that Kolaviron could have ameliorative effects on the adrenal gland in the event of adrenal toxicity. Therefore, Kolaviron is a compelling candidate to examine in the restoration of the morphology of glandular organs such as the adrenal gland.

2. MATERIALS AND METHODS

2.1 Procurement of Plant Materials

Fresh *Garcinia kola* fruits were bought from an open market in Enugu State, Nigeria. These fruits were authenticated by a taxonomist at the department of Plant Sciences, Faculty of Agricultural Science, Enugu State University of Science and Technology.

2.2 Processing and Extraction of Kolaviron

The processing of the Fresh *Garcinia kola* fruits was according to the method described by Iwu *et al.* (1985) [20]. 5kg of fresh *Garcinia kola* fruits were peeled, then cut into small pieces and air dried under shade at room temperature for 10 days. The dried fruits were ground into fine powder and then extracted with n-hexane, using a soxhlet extractor for 24 hours at room temperature. The de-fatted dried mass was repacked and further extracted with absolute ethanol using a soxhlet extractor for 24 hours at room temperature. The extract was concentrated and diluted to twice its volume with distilled water and extracted with ethylacetate which yielded a golden yellow solid; Kolaviron.

2.3 Animals Grouping and Experimental Design

Thirty (30) healthy adult Wistar rats within the range of 160-200g were used for this study. They were housed in a well cross-ventilated cage system and allowed easy access to food and water, under standard laboratory conditions and handling in the Animal house facility of the Enugu State University of Science and Technology, College of Medicine, Parklane, Enugu.

All treatments were administered via oral route and the experiment lasted for 14 days. The experimental animals were divided into six groups (n=5). Group A served as the control group and was placed on a normal diet of food and water *ad libitum*. Group B received lead-acetate solutions at 50mg/kg/bwt/day only. Groups C, D and E all received co-administration of lead-acetate solutions at 50mg/kg/bwt/day and also 100, 200 and 300mg/kg/bwt/day of Kolaviron. Group F received lead-acetate solutions at 50mg/kg/bwt/day for the first 7 days and then 300mg/kg/bwt/day of Kolaviron for the following 7 days. Extract dosages were adopted and modified from Ajayi et al. [23] and Galam et al. [24].

2.4 Confirmation of Adrenal Toxicity

Adrenal toxicity was confirmed by evaluation of serum cortisol level which may be influenced by disruption of its secretion [25]. The serum cortisol level were evaluated twice per day on days 1, 7 and 14, between 7am-8am in the morning when cortisol levels are at their highest and also between 4pm-6pm in the evening when the levels are much lower.

2.5 Histological Study

24 hours after the last administration, the rats were sacrificed under ketamine (100mg/ml) as anaesthesia. Mid-line abdominal incisions were made on the animals and their adrenal glands were carefully harvested, washed with saline and then fixed in 10% formalin, then labelled accordingly in plastic containers for 72 hours prior to processing. The fixed tissues were processed using the standard protocols for

histological tissue processing and stained with hematoxylin and eosin for histological studies. Photomicrographs of the organized slides were taken using Amscope 14MP USB 3.0 digital microscope camera at x400 magnification.

3. RESULTS

3.1 Result of Treatment on Cortisol

The values of the level of cortisol on day 1 were within the normal ranges of between 15-25mcg/dL. However, by day 7, the cortisol hormone levels were drastically lowered in all groups. With the continuation of treatment, the level of cortisol in groups B, C, and D markedly dropped to abnormal values way below 14mcg/dL. Groups B and C had an average cortisol level of 1.4mcg/dL, while group D had an average of 4.7mcg/dL. However, slight improvements were noted in groups E and F. Group E had 14.9mcg/dL, while group F had 13.7mcg/dL (See Fig. 1).

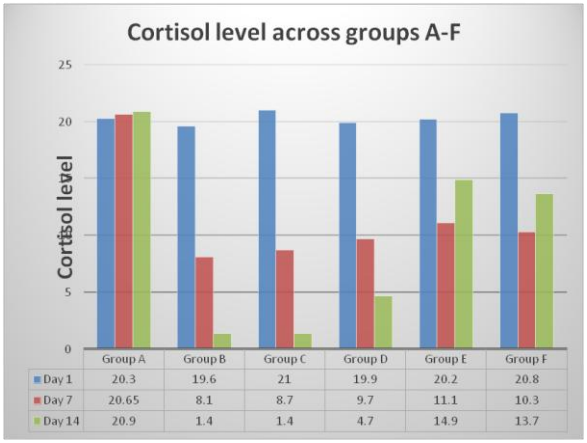

Fig. 1. Cortisol level across groups A-F

Table 1. Mean Serum Cortisol level across groups A-F in 14 days

	Day 1 (Mean ± SD)	Day 7 (Mean ± SD)	Day 14 (Mean ± SD)
Group A (Normal Control)	20.3 ± 0.47	20.65 ± 0.31	20.9± 0.62
Group B (Positive Control)	19.6± 0.74	8.1 ± 0.34[a]	1.4 ± 0.33[a]
Group C (Low Dose)	21 ± 0.45	8.7 ± 0.57[a]	1.4 ± 0.21[a]
Group D (Medium Dose)	19.9 ± 0.31	9.7 ± 0.61[a]	4.7 ± 0.69[a]
Group E (High Dose)	20.2 ± 0.56	11.1 ± 0.32[ab]	14.9 ± 0.41[ab]
Group F (Lead + High Dose)	20.8 ± 0.31	10.3 ± 0.81[a]	13.7 ± 0.54[ab]

[a]P≤0.05, significantly different from normal control; [b]P≤0.05, significantly different from positive control

3.2 Result of Treatment on Adrenal Tissues (Histological Findings)

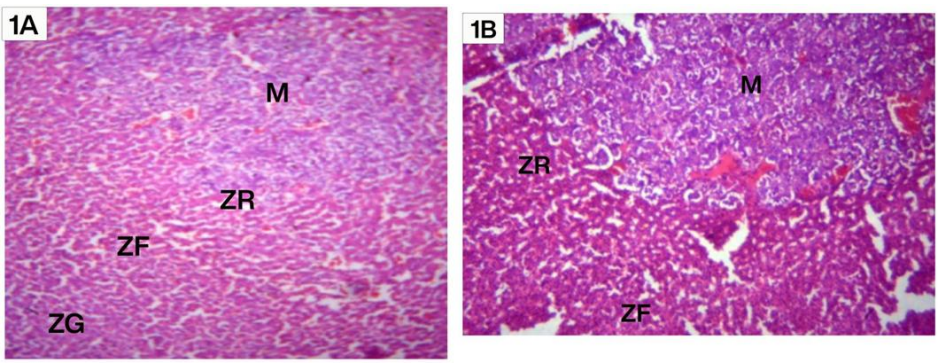

Fig. 2. Photomicrograph of a section of the adrenal gland of the control group that received feed and water only. General normal tissue appearance of adrenal architecture with cortex and medulla (M). The cortex is made up of zona glomerulosa (ZG), zona fasciculata (ZF) and zona reticularis (1A x200 and 1B x400)

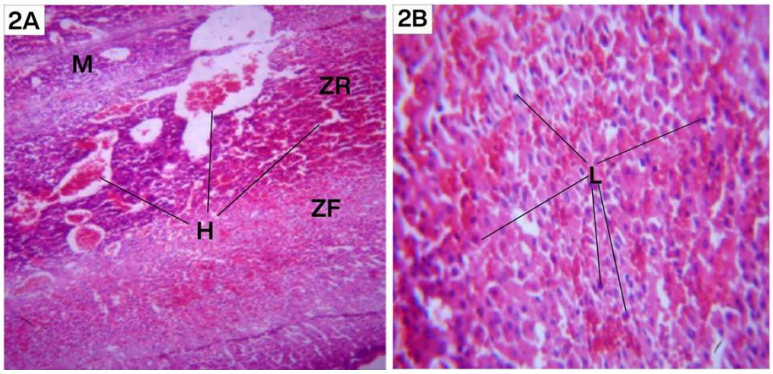

Fig. 3. Photomicrograph of a section of the adrenal gland of group B that received lead only. This demonstrates severe adrenal architectural distortion with vacuolizations and haemorrhage (H) in both cortex and medulla (M). There is also infiltration of lymphocytes as seen in 2B, (2A x200 and 2B x400)

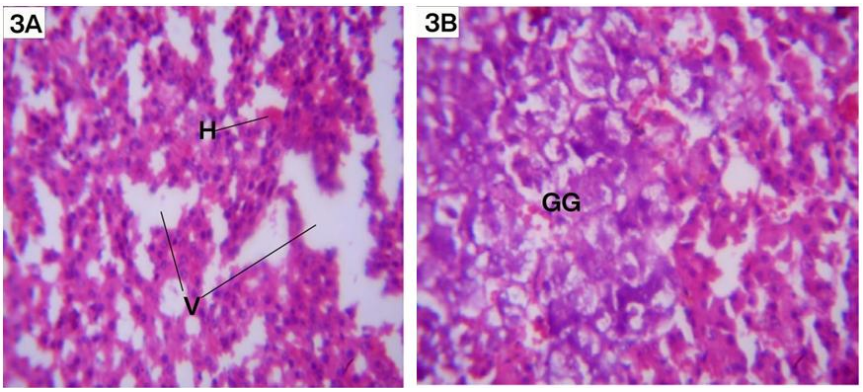

Fig. 4. Photomicrograph of a section of the adrenal gland of group C that received lead and treated with low dose of Kolaviron. 3A shows the cortex focusing on the Zone Reticularis and Zona Fasciculata. This demonstrates moderate architectural distortion with severe vacuolizations (V), and moderate haemorrhage (H). 3B demonstrates the medulla showing a non-distinct cell outline (GG). (3A x400 and 3B x400)

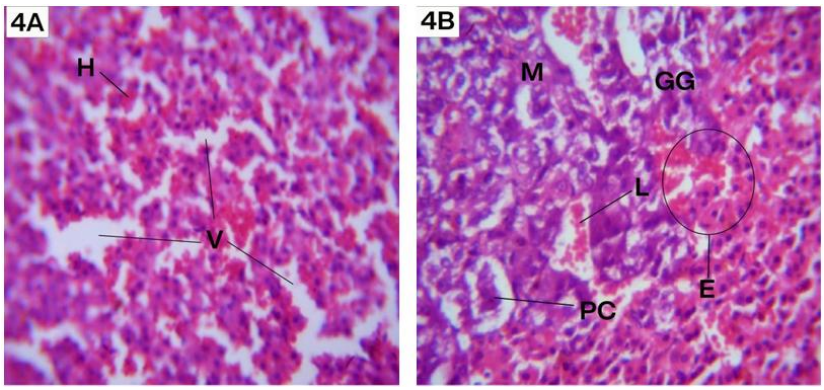

Fig. 5. Photomicrograph of a section of the adrenal gland of group D that received lead and treated with medium dose of Kolaviron. 4A shows the cortex focusing on the Zone Reticularis and Zona Fasciculata. This demonstrates moderate architectural distortion characterised by sparse vacuolizations (V), and moderate haemorrhage (H) suggesting a healing process. 4B demonstrates the medulla (M) shows oedematous changes (E), moderate lymphocyte (L) and plasma cell (PC) infiltrations and non-distinct cell outline (GG). (4A x400 and 4B x400)

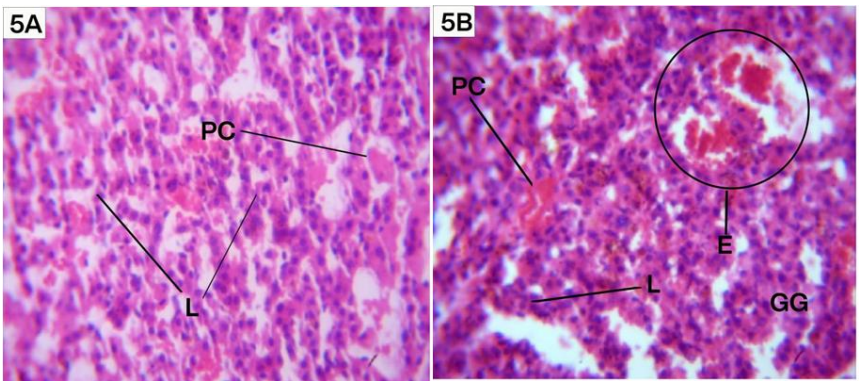

Fig. 6. Photomicrograph of a section of the adrenal gland of group E that received lead and treated with high dose of Kolaviron. 5A shows the cortex with mild architectural distortion, lymphocyte (L) and plasma cell (PC) infiltrations, suggesting a healing process. 5B demonstrates the medulla with non-distinct cell outline (GG), mild oedematous changes (E), moderate lymphocyte (L) and plasma cell (PC) infiltrations. (5A x400 and 5B x400)

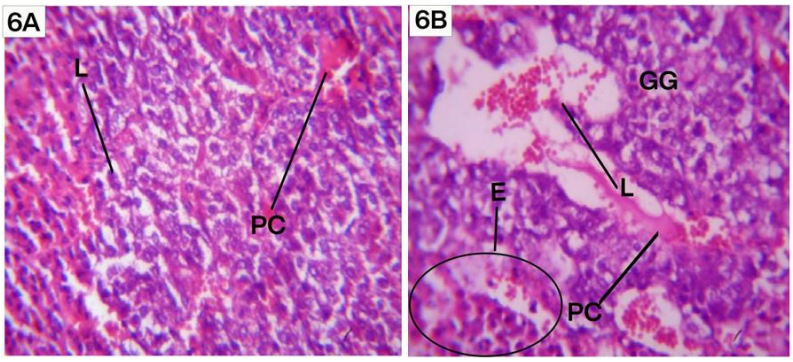

Fig. 7. Photomicrograph of a section of the adrenal gland of group F that received lead for 7 days only before receiving high dose of Kolaviron for 7 days. 5A and 5B shows the cortex and medulla respectively with severe architectural distortion characterised by severe lymphocyte (L) and plasma cell (PC) infiltrations. 5B demonstrates the medulla only showing non-distinct cell outline (GG) and severe oedematous changes (E). (5A x400 and 5B x400)

4. DISCUSSION

Both environmental and occupational exposure to lead (Pb) has shown to have negative effects on the different organs of the body including organs concerned with the endocrine system [26,27]. According to Rana [25], in adrenal toxicity there is release of catecholamines, disruption of cortisol secretion and suppressed cortical responses. Therefore, evaluation of one or more of these parameters serves as a tool in the confirmation of adrenal toxicity. In this study, we evaluated Pb toxicity to the adrenal gland using the level of serum cortisol. The drastic reduction in the level of serum cortisol at day 7 post-induction across groups B-F was a positive indication of adrenal gland toxicity and when compared to the normal control, there was a statistically significant difference. Similar report by Fortin et al. [27] showed a decrease in serum cortisol level with increase in lead serum level/toxicity. This postulates that Pb toxicity causes a negative effect on the adrenal gland evidenced by disruption of cortisol secretion. However, group E which received high dose of Kolaviron showed significant differences to both the normal and positive control groups. This could possibly suggest that Kolaviron has both protective and ameliorative effects on the adrenal gland with regards to Pb toxicity [28]. The response of the adrenal gland to Kolaviron on day 14 is a clear indication of dosage-response activity. Groups C and D had no significant difference from the positive control, however groups E and F had statistically significant difference to both the normal and positive control due to their treatment with high doses of Kolaviron. Hence, suggestive of the fact that

increase in the dosage of Kolaviron may be the key to its ameliorative activities on the adrenal gland.

Recent histology findings of the effect of Kolaviron on the adrenal cortex and medulla has shown no cyto-architecture distortions in previous studies [28], thus eliciting a dose-dependent inhibition of oxidation of linolenic acid, reduction of damages to both proteins and lipids [29]. In this study, we observed that cyto-architectural distortions and tissue appearance of both the adrenal cortex and medulla was somewhat related to the dosage of Kolaviron administered. In the control groups, group A demonstrated a normal tissue appearance in both the cortex and medulla, however that was not the case in group B (positive control). Group B exhibited extensive and severe architectural distortions, characterised by severe vacuolizations, haemorrhaging and lymphocyte infiltrations in both the cortex and medulla. This was a clear indication of lead toxicity of the adrenal glands as reported by Mohameda & Abol, 2011 [30,31]. These were the basis used in the evaluation of the other groups (C-F). An investigation by Wright et al. [30] indicated that Pb toxicity triggers the exhaustion of adrenal gland of male Sprague-Dawley rats after their diet was made to contain 1% of lead-acetate.

In groups that received Kolaviron treatment, the response observed was dose-dependent. Animals in groups C, D, and E were induced and treated with daily doses of lead and Kolaviron respectively for a period of 14 days. This ensured that both protective and ameliorative effects of Kolaviron on lead toxicity of the adrenal gland were both evaluated. However in group F, treatment with Kolaviron for 7 days was initiated after Pb administration of 7 days ended. This evaluated the ameliorative properties of Kolaviron on lead toxicity of the adrenal gland only. Group C demonstrated severe architectural distortion with vacuolizations, lymphocyte infiltration and haemorrhage in the cortex with the medulla characterized by a non-distinct cell outline. This was the same outcome demonstrated in the positive control thus suggesting Pb toxicity to the adrenal gland without any form of protection or amelioration. Group D demonstrated a moderate architectural distortion with features of sparse vacuolization and moderate haemorrhage. This suggests that a healing process is in place with the presence of oedematous changes, moderate lymphocyte and plasma-cell infiltrations, however, a non-distinct cell outline suggests unresolved toxicity. In groups E and F, the difference in the commencement and duration of Kolaviron treatment may have been the reason for the disparity in adrenal cyto-architecture. Group E is characterised by mild cortical distortion, lymphocyte and plasma infiltration, with mild oedematous changes. This demonstrates an advanced healing process, unlike group F where there is a severe architectural distortion with severe oedematous changes, lymphocyte and plasma cell infiltrations, Group F histology findings suggests initial stages of a healing process.

Daily co-treatment of 50mg/kg of lead-acetate with increasing doses of Kolaviron, demonstrated mild to moderate ameliorative effects on the pathologic damages thought to be brought about by Pb toxicity. Also, treatment with 300mg/kg/day of Kolaviron for 7 days, after 7 days of administering lead-acetate solutions at 50mg/kg also showed similar results with mild to moderate ameliorative effects. This ameliorative property can be linked to the antioxidant property of *Garcinia kola*.

Osifo and Iyawe [28] recorded about the antioxidant properties of *Garcinia kola* in their study as its ingestion reduced adrenal oxidative stress status through the stimulation of tissue antioxidants activities. Ingestion of *Garcinia kola* stimulated the weight of the adrenal gland and also its tissue SOD and CAT but inhibited MDA and protein in a dose dependent fashion [29].

This study depicted the injurious consequences of Pb toxicity on the Adrenal gland and demonstrated the ameliorative capabilities of Kolaviron of *Garcinia kola* on the adrenal gland.

5. CONCLUSION

Treatment with the Kolaviron demonstrated mild to moderate ameliorative effects on adrenal histo-morphologic pathologies brought about by Pb toxicity. This insinuates potential protective and attenuating properties of kolaviron to the adrenal gland and further buttressing the need to consider *Garcinia kola*as a promising agent that can be engineered to be potent against adrenal gland toxicity from Pb and other heavy metals.

CONSENT

It is not applicable

ETHICAL APPROVAL

Ethical approval was given for this study and all procedures/protocols were conducted in accordance with the guidelines of the Institutional Animal Ethics Committee (IAEC) of the Faculty of Basic Medical Sciences, College of Medicine, Enugu State University of Science and Technology (ESUT) Enugu State, Nigeria. ESUCOM/FBMS/ETR/2020/032.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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