

Ameliorative Effects of Kolaviron, *Garcinia Kola* (Bitter Kola) Fruit Ethanolic Extract on Lead-Induced Adrenal Gland Toxicity using Adult Wistar-Rats

ABSTRACT

Lead (Pb) toxicity is possibly the most common form of heavy metal intoxication 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 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 300mg/kg/day of Kolaviron for 7 days, and subsequently administered lead-acetate solutions at 50mg/kg/day for 7 days. 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 intoxication. *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

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 behavioral 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 hyperaldosteronism, 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] 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.^[11] The presentation of adrenal insufficiency depends on the rate and extent of adrenal function involvement.^{[9][12]} 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 metabolise free radicals and toxic reactive compounds.^[13]

Plant-derived medicines are attributed to nutraceutical components demonstrable in traditional fruits and herbs attributed to their abundance of secondary metabolites.^[14] 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.^[15] 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.^[16] 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.^{[17][18]} However, this plant demonstrates a complex mixture of biflavonoids, xanthenes, calanolide-type coumarines, and prenylated benzophenones.^[19] Kolaviron is a fraction of defatted ethanol extract containing *Garcinia* biflavonoids GB1, GB2, and kolaflavonone.^{[19][20]} 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 antioxidant action on lipoprotein.^[21]

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.

MATERIALS AND METHODS

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.

Processing and extraction of Kolaviron

The processing of the Fresh *Garcinia kola* fruits was according to the method described by Iwu *et al.* (1985)^[19] 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 defatted 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.

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/day only. Groups C, D and E all received co-administration of lead-acetate solutions at 50mg/kg/day and also 100, 200 and 300mg/kg/day of Kolaviron. Group F received lead-acetate solutions at 50mg/kg/day for the first 7 days and then 300mg/kg/day of Kolaviron for the following 7 days. Extract dosages were adopted and modified from Ajayi *et al.*, (2011)^[22] and Galam *et al.*, (2013)^[23].

Histological Study

24 hours after the last administration, the rats were sacrificed under ketamine (100mg/ml) as anaesthesia. Midline 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.

RESULTS

Confirmation of adrenal toxicity

According to Rana (2014)^[24], 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. 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. The values of the level of cortisol on days 1 and 7 were within the normal ranges of between 15-25mcg/dL. However by day 14, the cortisol hormone level were drastically lowered in groups B, C, D and E to 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 improvement was noticed in groups E and F. Group E had 12.2mcg/dL, while group F had 13.8mcg/dL.(See Figure 1)

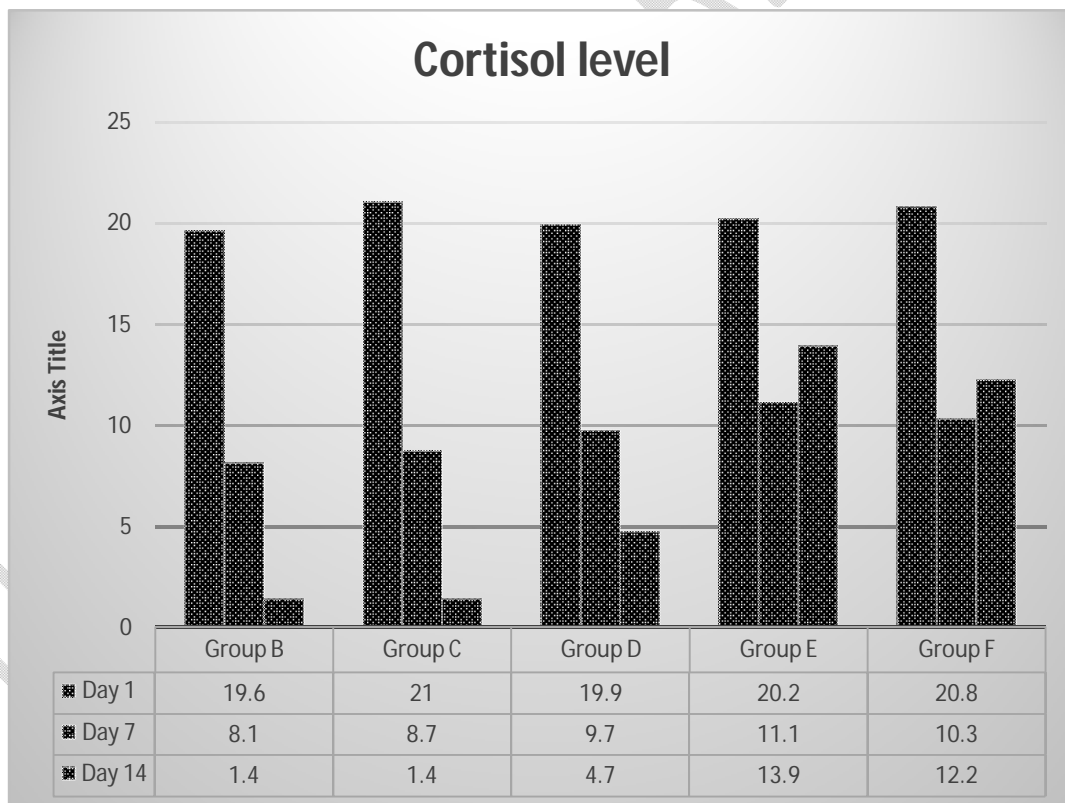


Figure 1. Cortisol level across groups B-F.

Histological Findings

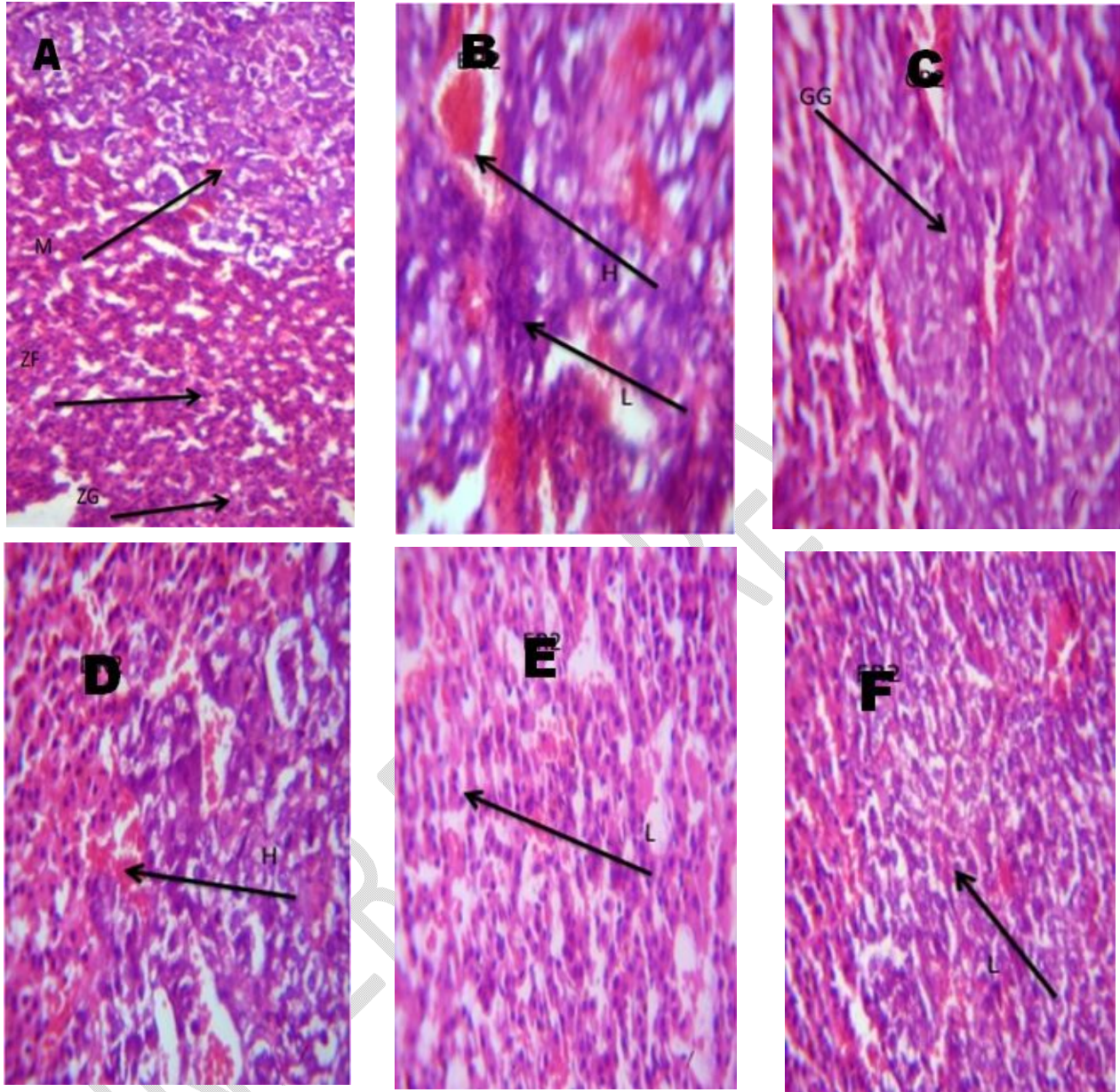


Figure 2 (A): Photomicrograph of the control group. Section of adrenal gland shows the normal adrenal architecture with intact cortex and medulla. The cortex is made up of zona glomerulosa (ZG), zona fasciculata (ZF) and zona reticularis, while the Medulla is depicted as (M). H&E. x400. (B): Untreated group (50mg/kg of lead-acetate). Section of adrenal gland shows severe haemorrhage in both cortex and medulla and chronic infiltration of lymphocyte (L). H&E. x400. (C): Treatment with 100mg/kg of Kolaviron. Section of adrenal gland shows moderate healing with the medulla having a ground glass appearance (GG), non-distinct cell outline with vacuolations, however there are signs of haemorrhage (H) in some area. H&E. x400. (D): Treatment with 200mg/kg of Kolaviron.

Section of adrenal gland shows mild healing. The section still displays severe vacuolations within the medulla, focal area of edematous change and moderate infiltration of lymphocyte and mild haemorrhage (H). H&E. x400. (E): Treatment with 300mg/kg of Kolaviron. Section of adrenal gland shows moderate healing with ground glass appearance of the cytoplasm and mild infiltration of lymphocyte (L). H&E. x400. (F): Animal group treated with 50mg/kg of Pb for 7days and then 300mg/kg of Kolaviron daily for the next 7 days. Section of the adrenal gland shows mild healing with edema, mild infiltration of lymphocyte (L), ground glass appearance and areas of moderate haemorrhage. H&E. x400

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.^{[25][26]}

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

50mg/kg of lead-acetate demonstrated clear signs of pathology on the histology of the adrenal glands in the untreated group relative to the control group with normal histomorphology. The pathologies were evident as severe haemorrhage in both cortex and medulla and chronic infiltration of lymphocyte. An investigation by Wright *et al.*, (1975)^[26] indicated that Pb intoxication triggers the exhaustion of adrenal gland of male Sprague-Dawley rats after their diet was made to contain 1% of lead-acetate.

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 intoxication. 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 (2020)^[27] 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.^{[27]s}

CONCLUSION

Treatment with the Kolaviron demonstrated mild to moderate ameliorative effects on adrenal histomorphologic pathologies to be brought about by Pb intoxication. Thus, *Garcinia kola* is a promising agent that can be engineered to be potent against adrenal gland injuries brought about by Pb intoxication 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

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