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Research Article

Metal Contents and Acute Toxicity of Combined *Vernonia amygdalina* Leaves and *Garcinia kola* Seeds-VAGK, a Herbal and Nutritional Formulation in Male Wistar Rats

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ABSTRACT

While plants have been useful to man as food and medicine, many have the potentials to induce toxicity either singly or in combination, which may result from their phytochemical contents or accumulation of toxic metals. Therefore, this study investigated the metal contents and acute toxicity of *Vernonia amygdalina* (VA) leaves and *Garcinia kola* (GK) seeds in single and combined forms on rat's kidney and liver. Aqueous extracts of these plants were used to investigate their acute toxicity potentials separately and in combined formulation, VAGK, using male Wistar rats. Following previous studies, a limit acute toxicity investigation of VA and GK were carried out. In addition, a full acute toxicity test was performed on VAGK using Lorke's method. The geometric means of doses were used to obtain the acute toxicity value. Histopathological examination was carried out on harvested rats' kidney and liver while metal analysis was performed on the powdered plants. Results showed that all animals survived the limit dose as well as the full acute toxicity tests. The Kidney and liver revealed no notable pathological changes in the tested plants. Concentration of some of the analysed metals (mg/kg) in plants fell within recommended permissible limits except for Pb, Co, Cr and Ni, which were above these limits. In conclusion, although the acute oral toxicity test revealed no mortality and demonstrated no detrimental effects on the kidney and liver of the treated rats, however based on metal results, caution must be taken when using these plants as herbal remedy.

Keywords: *acute toxicity, heavy metals, Vernonia amygdalina, Garcinia kola*

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INTRODUCTION

The liver and the kidney are two essential organs of human body; while the liver is the major organ for detoxification and elimination of endogenous substances such as xenobiotics (Momoh *et al.*, 2015), the kidney is the major route of excretion (Jha, 2010), thus maintaining the body homeostasis. These two organs have been constantly exposed to different toxic chemicals or substances, which are obtained from the environments, water, food and plants resulting in liver and kidney damages (Goyer and Clarkson, 2001; Singh *et al.*,

2011; Arroyo *et al.*, 2012). Plants have been documented to contain some toxic substances or chemicals including metals which may result in various toxicities in the body such as nephrotoxicity, hepatotoxicity, cardiotoxicity, neurotoxicity, and skin toxicity (Nudrat and Naira, 2016).

The toxicity of these metals on human health and their persistence in the environment has been a matter of concern in recent years. Heavy metals may be considered as those groups of elements with potentials to induce toxicity when consumed by humans. The excretion rates of these metals through the kidney are low, and this may result in deleterious effects on

humans even at very low concentrations. Heavy metals have a predisposition to accrue in the food chain, and plants are often the link in their movement from the contaminated soil to humans (Biswas *et al.*, 2018). Of the heavy metals, lead (Pb) and cadmium (Cd) are the most common contaminants of dietary supplements, and their high levels have been reported to have toxicological effect on human health (Sahoo *et al.*, 2010). Other metals such as zinc (Zn), copper (Cu), iron (Fe), cobalt (Co), manganese (Mn), chromium (Cr), nickel (Ni), and magnesium (Mg) amongst others are considered essential nutrients. They are important for the physiological and biochemical functions of the human body (WHO, 1996). However, an increase in their intake above certain acceptable limits may also result in toxicity (Korfali *et al.*, 2013).

For centuries, herbal medicines and their formulations (polyherbal remedies) are widely perceived by the public as being natural, effective, relatively cheaper, accessible, wholesome and elicit minimal side effects (Mayur *et al.*, 2017; Fibrich and Lall, 2018). This supposition may have influenced the indiscriminate use of these formulations to a great extent amongst the rural populace. Several plant-based herbal formulations are often given over a long period of time without dose consideration. More so, there is a dearth of knowledge on the toxic effects that might result from such acute and prolonged usage (Ben-Arye *et al.*, 2016; Hudson *et al.*, 2018). Although, studies have demonstrated the acute toxicity of VA and GK in single forms, however, there is need to determine the full oral toxicity of combined VAGK, a new diherbal formulations (Kagbo and Ejebe, 2009; Atsukwei *et al.*, 2015; Zakaria *et al.*, 2016). Therefore, scientific documentation of the combined VAGK acute oral toxicity is required.

Vernonia amygdalina Del also known as bitter leaf because of its bitter taste, is a vegetable used in preparing traditional soup in Nigeria. It is a shrub of 2-5 m tall with petiolate green leaves of about 6 mm diameter (Ojiako and Nwanjo, 2006) and belongs to the Asteraceae family (Crellin *et al.*, 1989; Farombi and Owoeye, 2011). All parts of this plant have been reported to be medicinally useful (Ojiako and Nwanjo, 2006). In Nigerian ethno-medicine, the roots and the leaves are used to treat fever, hiccups, kidney problems and stomach discomfort (Udochukwu *et al.*, 2015). The aqueous extracts have been used by many herbalists and naturopathic doctors to treat diabetes mellitus, emesis, loss of appetite, induced ambrosia, dysentery and other gastrointestinal tract problems, bacteria and parasitic infections, malarial and cancer amongst others (Crellin *et al.*, 1989; Ezuruike and Prieto, 2014; Udochukwu *et al.*, 2015). Similarly, it has been used in zoo-pharmacology by chimpanzees for treatment of parasitic infections (Huffman, 2003). Previous toxicity studies reported different LD50 values for VA. These include 1122mg/kg in mice (Akah and Okafor, 1992); 500 mg/kg, 1265.22 mg/kg, and 5000.15 mg/kg in rats (Nwanjo, 2005; Ojiako and Nwanjo, 2006; Adiukuet *et al.*, 2012).

Garcinia kola Heckel, popularly known as “bitter kola” is an angiosperm belonging to the family Guttiferae (Farombi and Owoeye, 2011; Adesuyi *et al.*, 2012). It is regarded as a wonder plant because every morphological part of the plant has been established to be of medicinal importance (Adesuyi *et al.*, 2012). The leaves, fruit, seeds, root and bark of the plant

have been used for centuries in traditional medicine to treat ailments such as bronchitis, throat infections, colic, head or chest colds and cough amongst others. It is believed by African medicine practitioners to have purgative, anti-parasitic and anti-microbial properties (Adesuyi *et al.*, 2012). The extract of the seed has been reported to have several pharmacological activities including anti-inflammatory, analgesic, molluscidal, anti-atherogenic, antioxidant and hepato-protective activities (Eleyinmi *et al.*, 2006; Madubunyi, 2010). Consumption of *Garcinia kola* seed has also been shown to reduce the incidence of cardiovascular diseases in human participants (Omeh *et al.*, 2014). The reported LD50 for GK include 6741.43 mg/kg (Udenzie *et al.*, 2012) and >5000 mg/kg (Nworu *et al.*, 2007) in rodents.

In spite of the various utilizations of these plants, there is paucity of data on the toxicological potentials of the combined use of VA and GK. Therefore, this study investigated the metal contents and acute toxicity of *Vernonia amygdalina* leaves and *Garcinia kola* seeds in single and combined forms on rat's kidney and liver over a period of 14 days.

MATERIALS AND METHODS

Collection and Authentication of Plants

Samples used for the acute toxicity assessment included aqueous extract of VA, GK and a combination of VAGK. The VA leaves and GK seeds were obtained from local markets in Ibadan (Bodija and Oje), Oyo State, Nigeria. These plants were identified and authenticated by Mr. Esimekhuai Donatus, a taxonomist of the Department of Botany, University of Ibadan. The voucher specimen numbers were assigned for *Vernonia amygdalina* (UIH-22612) and *Garcinia kola* (UIH-22611) and deposited in the herbarium.

Preparation and Extraction of Plants

Garcinia kola seeds were peeled to remove the seed coat covering the pulp, rinsed and grated into smaller pieces. Both the VA leaves and grated GK seeds were separately air-dried at room temperature to obtain a constant weight. The dried samples were homogenized using a wooden pestle and mortar, and sieved to obtain finely divided powder. Approximately, 200 g each of dried VA and GK samples was extracted in distilled water containing less than 10% absolute methanol in 1:10 (w/v) ratio, to inhibit microbial contamination and growth. The cold maceration lasted for 48 hours and thereafter, the filtrate was then administered orally to the rats. Also, yield from the various aqueous extracts was determined using dried extract samples.

Acute Toxicity Study

A limit acute toxicity investigation was carried out on VA and GK while a full acute toxicity was done for the combined VAGK using a total of 23 male Wistar rats. All rats were acclimatized for five days and ethical approval was obtained before the commencement of the acute toxicity experiment. The method described by Lorke (1983) was used. The limit single dose comprised ten Wistar rats: three rats each for VA and GK, four rats for control. A limit dose of 1600 mg/kg body weight (b.wt) was chosen for VA and GK following previous acute toxicity studies on the plants (Akah and Okafor, 1992;

Ojiako and Nwanjo, 2006; Nworu et al., 2007; Sha'a et al., 2011). The control group received only distilled water by oral route. In the full acute toxicity study, 13 rats were randomly classified into 3 groups comprising two administration phases; Phase I (9 rats) and Phase II (3 rats), and control (1 rat). Phase I is made up of Group 1, Group 2 and Group 3 of three rats each. The doses under this phase include 10, 100, and 1000 mg/kg b.wt of rats respectively. Rats in Phase II groups respectively received 1600, 2900 and 5000 mg/kg b.wt. The average weight of rats used for this toxicity study is 111.5 g.

All rats were fasted overnight prior to administration. The oral administration of aqueous extracts was adopted. Signs of toxicity or death were closely monitored in the test groups for 24 hours. Some of the signs of toxicity investigated include tremors, convulsions, salivation, diarrhoea, lethargy, sleep, restlessness, paw licking, stretching, coma and death. Other observations monitored are skin colour, eyes, mucus membrane, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavioural pattern. Survivors were further observed for 14 days for death. The median lethal dose was calculated from the geometric mean except where no death occurs at the highest dose (5000 mg/kg b.wt). Rats were sacrificed and the kidneys and the livers were harvested immediately before euthanizing the rats. Formalin-fixed slides of the liver and kidney were prepared and stained with haematoxylin and eosin.

Metal analysis was carried out on the sun-dried and powdered samples of VA and GK to investigate the level of occurrence of some metals in these samples. Metals assessed in the samples include Ca, Mg, K, Pb, Cd, Co, Cr and Ni. Plant samples were digested by weighing 1g of the dried plant tissue into a 100 mL berzelius beaker, and adding 5 mL nitric acid (HNO₃) and 2 mL perchloric acid (HClO₄) then heated to a final volume of 3-5 mL. Thereafter, 15 mL of water was added and the digest solution was filtered through an acid-washed

filter paper into a 50 mL volumetric flask. The filter paper was washed with water and the filtrate diluted to volume with deionised water. Using the appropriate hollow cathode lamp, the digest samples were sprayed and read on Buck Atomic Absorption Spectrophotometer (AAS) model 210/211 VGP to determine the metals required.

RESULTS

The yield obtained from the aqueous maceration of the dried leaves of VA, GK and VAGK (1:1) were 2.2%, 12.7% and 7.0% respectively.

Table 1 represents the summary of the acute toxicity investigation of the aqueous extracts of VA, GK and the full acute toxicity test of the combined VAGK. The oral administration of aqueous extract of VA, GK and a combination of VAGK did not result in the physical death of the test rats. This observation was made after 24 hours and 14 days post administration study. However, rats that received high doses (2900 and 5000 mg/kg body weight) of the aqueous extracts of VAGK exhibited few signs of toxicity, which lasted for less than an hour. Some of the observed signs of toxicity included initial restlessness, stretching, lethargy and paw licking.

The absence of physical and observable toxicity coupled with the toxic signs demonstrated by the test rats encouraged the harvesting and investigation of the liver and kidney for histopathological examination. The photomicrographs of the harvested liver and kidney from rats in different dose administration of aqueous extracts of VA, GK and VAGK are presented in Fig. 1 (limit acute toxicity test for VA and GK individually), Fig. 2 (full acute toxicity test on kidney for combined VAGK) and Fig. 3 (full acute toxicity test on liver for combined VAGK).

Table 1:

Acute lethal effect of aqueous extracts of VA and GK and full acute toxicity of the combined VAGK orally administered to Wistar rats

<i>Vernonia amygdalina</i> (VA) plus <i>Garcinia kola</i> (GK) (VA + GK) (1:1)					VA (1600 mg/kg b.wt)		GK (1600 mg/kg b.wt)		Total
Experiment (no of rats)	Dose (mg/kg Body weight (b.wt))	Death after 24 hours	Survival after 14 days	Death	Survival on day 14	Death	Survival on day 14		
Phase I (3)	10	0	3	ND	ND	ND	ND	-	
	100	0	3	ND	ND	ND	ND	-	
	1000	0	3	ND	ND	ND	ND	-	
Phase II (1)	1 600	0	1	0	3	0	3	6	
	2 900	0	1	ND	ND	ND	ND	-	
	5 000	0	1	ND	ND	ND	ND	-	
Control (1 and 3)	0	0	1	0	3	0	3	6	
Total		0/13	13/13	0/6	6/6	0/6	6/6	12/12	

ND: No death

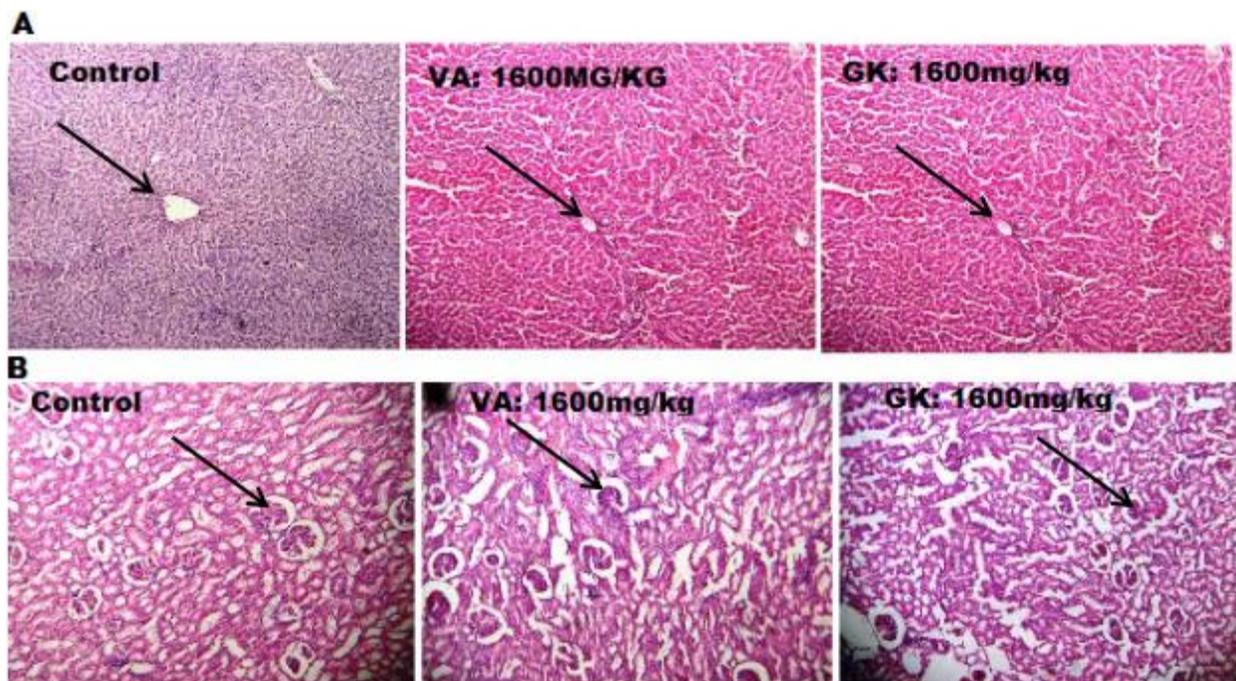


Plate 1:

Representative photomicrographs (magnification x 100) of haematoxylin and eosin-stained sections of the liver (A) and kidney (B) of 1600 mg/kg b.wts of rats (limit acute toxicity test) after the administration of aqueous extracts of VA and GK. In section A, the liver of rats in the control and 1600 mg/kg (VA and GK) groups demonstrated no pathological lesion: the architecture is normal, the central venules and portal tracts appear normal and not congested (black arrow). In section B, the kidney of rats in control and 1600 mg/kg (VA and GK) also demonstrated no pathologies with normal architecture. The renal cortex revealed some glomeruli with shrunk mesangial cells and widened capsular spaces (black arrow).

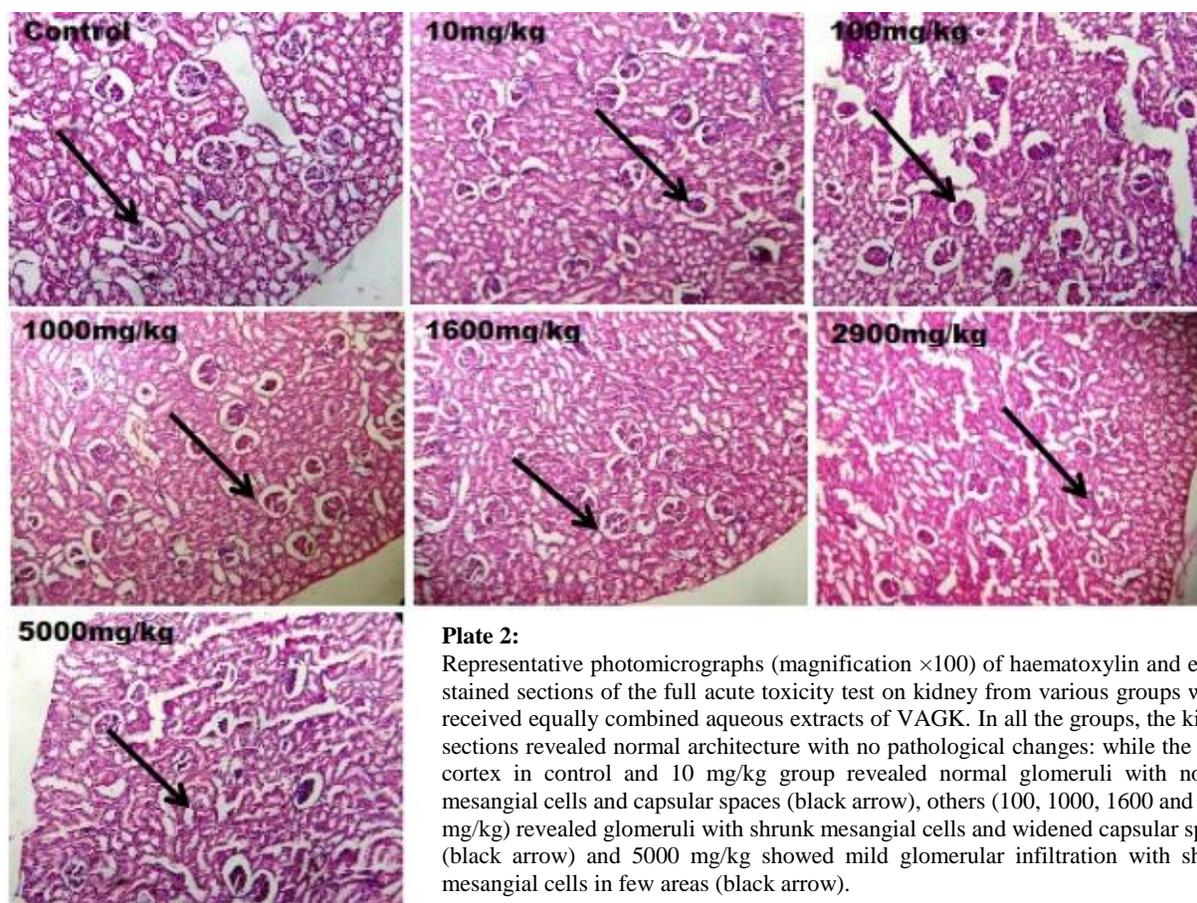


Plate 2:

Representative photomicrographs (magnification ×100) of haematoxylin and eosin-stained sections of the full acute toxicity test on kidney from various groups which received equally combined aqueous extracts of VAGK. In all the groups, the kidney sections revealed normal architecture with no pathological changes: while the renal cortex in control and 10 mg/kg group revealed normal glomeruli with normal mesangial cells and capsular spaces (black arrow), others (100, 1000, 1600 and 2900 mg/kg) revealed glomeruli with shrunk mesangial cells and widened capsular spaces (black arrow) and 5000 mg/kg showed mild glomerular infiltration with shrunk mesangial cells in few areas (black arrow).

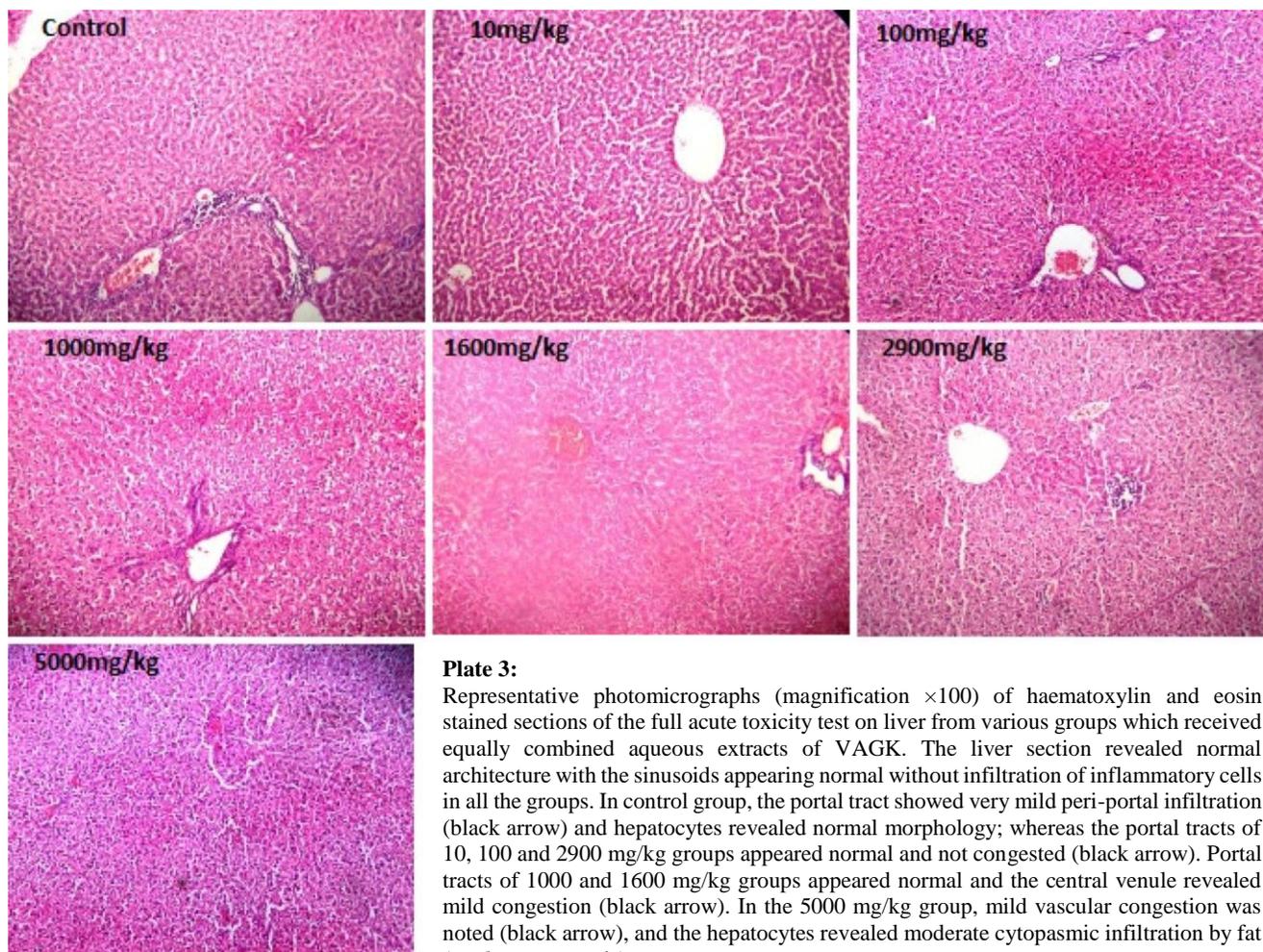


Plate 3:
 Representative photomicrographs (magnification $\times 100$) of haematoxylin and eosin stained sections of the full acute toxicity test on liver from various groups which received equally combined aqueous extracts of VAGK. The liver section revealed normal architecture with the sinusoids appearing normal without infiltration of inflammatory cells in all the groups. In control group, the portal tract showed very mild peri-portal infiltration (black arrow) and hepatocytes revealed normal morphology; whereas the portal tracts of 10, 100 and 2900 mg/kg groups appeared normal and not congested (black arrow). Portal tracts of 1000 and 1600 mg/kg groups appeared normal and the central venule revealed mild congestion (black arrow). In the 5000 mg/kg group, mild vascular congestion was noted (black arrow), and the hepatocytes revealed moderate cytoplasmic infiltration by fat (moderate steatosis).

Table 2:
 Summary of gross pathology of investigated organs (kidney and liver) obtained from test Wistar rats

Organ	Summary of gross pathology							
	Doses (mg/kg body weight of rats)							
	VA	GK	VA + GK					
	1600	1600	10	100	1000	1600	2900	5000
Kidney	NP	mg _i , smc	NP	NP	NP	NP	NP	NP
Liver	NP	mla, msis	NP	mfi, mif	mci, mmvs, hsci	mci, mmvs	mfi	mvc, mci

*NP*No Pathologies; *mg_i*Mild glomerular infiltration; *smc*Shrunk messenger cells in few areas; *mla*Mild Lymphocytes infiltration; *msis*Moderate to severe infiltration of Sinusoids with inflammatory cells; *mfi*Mild to moderate fat infiltration into the hepatocytes in the cytoplasm; *mif*Mild infiltration of fat within the hepatocytes; *mci*moderate cytoplasmic infiltration of inflammatory cells; *mmvs*moderate vesicular steatosis; *hsci*hepatocytes show severe cytoplasmic infiltration by fat; *mvc*mild vesicular congestion

The effects of oral administration of aqueous extracts of VA, GK and VAGK on the liver and kidney as well as the histopathological changes observed and documented are summarized in table 2.

The results of metals such as Pb, Cr and Ni in powdered samples of VA and GK were remarkably above the Joint Food and Agriculture Organization (FAO) and World Health Organization (FAO/WHO) permissible limits (PLs) of 10, 2

and 1.63 mg/dl respectively. Cobalt in GK was above the PLs (0.14 mg/kg) while Co in VA falls within the limit. Only Cd content of both VA and GK was observed within the PLs (0.3 mg/kg) (FAO/WHO, 1984; WHO, 2007; Maobeet *et al.*, 2012) (Table 3).

Table 3:

Heavy metal analysis of powdered test plants

Symbol	Botanical material				Metals analysed			
	Ca (%)	Mg (%)	K (%)	Pb (mg/kg)	Cd (mg/kg)	Co (mg/kg)	Cr (mg/kg)	Ni (mg/kg)
<i>G. kola</i>	0.10	0.08	0.35	22.45	0.25	1.25	8.85	9.35
<i>V. amygdalina</i>	0.71	0.95	0.99	11.35	0.15	0.40	6.25	13.85

DISCUSSION

Although, the use of herbal products including medicinal plants and phytochemicals for prevention and treatment of various health ailments has been in practice from time immemorial (Vashisth *et al.*, 2013; Hong *et al.*, 2015); it is sad to note that the possible toxicities of some of these herbal products and their constituent plants are not fully elucidated. Therefore, this study investigated the metal contents and acute toxicity of *Vernonia amygdalina* leaves, and *Garcinia kola* seeds singly and in combined forms on rats' kidney and liver over a period of 14 days.

In this study, the acute toxicity test of the aqueous extracts of VA leaves and GK seeds demonstrated no noticeable toxicity and mortality in the tested rats. This finding is supported by the studies of Zakaria *et al.* (2016) in VA; Atsukwei *et al.*, (2015) and Kagbo and Ejebe (2009) in GK. The lack of any pathological response in rats that took the individual plant (VA and GK) may mean that the accumulation level of Cd, and especially Pb, was inadequate to induce noticeable toxicity on an acute time scale or other compounds within the formulation enhances the metabolism and excretion of these toxic trace metals from the rat's body.

In order to establish some safety standards for VAGK herbal supplement, a holistic safety profile of the individual plants as well as the diherbal formulation is required as a guide for the management of its applications and usage in herbal preparations. Therefore, VAGK diherbal supplement was subjected to full acute toxicity test since this kind of combination is totally new and yet to receive any scientific investigation. The interaction between the components making up the diherbal formulation may result in toxicity or synergy in therapeutic activity. The acute toxicity was found to be greater than 5000 mg/kg, suggesting VAGK to be safe in rats as described by Lorke (1983). In addition, the absence of death among the tested rats in all the dose groups throughout the 14 days post administration examination supported this claim. To further support this observation for toxicity or mortality, histopathological examination of the VAGK formulation showed very mild pathology in the liver at higher doses of 1000, 1600, 2900 and 5000 mg/kg body weights of tested rats. Whereas, the kidney did not present any pathological change for the individual plant making up VAGK at all doses. Therefore, this study indicates that the aqueous extracts of VAGK do not cause acute toxicity effects on the male Wistar rats at the doses tested up to a maximum dose of 5000 mg/kg body weights of rats.

Similarly, in this study, the aqueous yield obtained from GK, VA and combined VAGK are indicative of their content of water-soluble polar phyto-constituents such as

polyphenolics, bioflavonoids, saponins, alkaloids, glycosides and tannins. These have been reported to be abundant in VA and GK (Mbotto *et al.*, 2009). Plant processing and extraction procedures may introduce some differences in yield. However, the yield obtained for GK in this study is higher than the reported yield for GK in Akerele and colleagues' study (2008). This difference may be due to plant particle size after powdering and length of extraction.

It was observed in this study that higher occurrence of health promoting metals such as Ni, Co, Ca, Mg, and K were found in VA while GK accumulated higher amounts of Cd and Pb. The level of Co in VA falls within the permissible limits (PL) stated in the FAO/WHO guideline (1984), while GK content was above the limit. Similarly, Ni and Cr in both VA and GK were found to be above the limit. Nickel was observed to accumulate more in VA while Cr accumulates more in GK. These findings do not agree with the previous studies where lower values of Cr and higher values of Co and Ni were reported in VA (Kalagbor *et al.*, 2014) and GK (Oti, 2015).

The heavy metal analysis of individual plant recipe making up VAGK indicated that the plant accumulates more Pb than Cd. The level of Cd in VA and GK extracts were below the PL of 0.3 mg/kg, while Pb in VA was lower compared to GK, although both values were found to be above the PLs of 10 mg/kg (FAO/WHO, 1984; WHO, 2007; Maobe, *et al.*, 2012). Our findings agree with other studies from Ibadan, where metal accumulations in plants were reported (Ogundiran and Osibanjo, 2008; Adelekan and Olawode, 2011) but disagree with other studies carried out in other parts of the country by Kalagbor *et al.* (2014) and Oti (2015), where higher Cd and lower Pb were reported in VA and GK respectively. Conversely, in Doherty and colleagues' study, Pb was not detectable while lower value was reported for Cd in VA (Doherty *et al.*, 2012). The variation in the plants' metal contents may be due to soil contents and pollution from water, atmosphere, decentralised and indiscriminate waste disposal, where the plants were grown (Ogundiran and Osibanjo, 2008; Adelekan and Olawode, 2011).

In conclusion, this study revealed that *Vernonia amygdalina* leaves and *Garcinia kola* seed, either in single or in combined formula, is not toxic to rats and did not produce any evidence of mortality in the acute oral toxicity studies. The histology examination revealed no remarkable changes in the kidney and liver, in both control and treated rats. The few mild histopathological changes observed may be associated with the acute exposure to Pb, which was above the international permissible level. Data obtained in this study on the tested plants and formulation may be useful in strengthening the confidence in their safety to humans for the use in the development of herbal supplements. However, it may be necessary to carry out batch quality assessment on

these plants, particularly those from different soil origin as the plants can easily accumulate toxic heavy metals such as lead and cadmium. The recommendation is therefore to introduce batch quality control of heavy metals measurements in medicinal plants value chain.

Conflicts of Interest

There is no conflict of interests in this study.

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