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Research article

Effects of *Vernonia amygdalina* Delile and *Baccharoides tenoreana* Olive on Biochemical and Histological Markers of Liver and Kidney damage in Alloxan-Induced Diabetic Rats

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ABSTRACT

This study assessed the status of the liver and the kidneys following injury by alloxan monohydrate and treatment with *Vernonia amygdalina* (VA) and *Baccharoides tenoreana* (BT). Total of 30 male Albino Wistar rats assigned into 6 groups (A-F) of 5 rats per group were used. Forty-eight hours following administration of alloxan monohydrate to groups B-F rats, groups C-E were treated with VA, BT and combination of VA and BT respectively while group F rats received glibenclamide. Both groups A and B were administered distilled water. The treatments were daily for 21 days. On day 21, serum samples for determination of hepato-renal biochemical indices were collected. The liver and kidney tissues were also collected. The results indicated significantly elevated activities of ALT, ALP and levels of Creatinine and urea in group B rats (induced untreated) compared to the normal control group while the groups treated with the extracts especially VA showed significantly reduced activities and levels of the analytes when compared to those of the induced untreated group. Liver and kidney photomicrographs of induced untreated rats showed degenerations and necrosis of the hepatocytes and tubular epithelial cells respectively while those of the rats treated with the extracts appeared comparable to those of the normal control rats. Both VA and BT protected the liver and kidney against injury by alloxan monohydrate separately; however, combination of VA and BT did not offer a better protection.

Keywords: *Vernonia amygdalina, Baccharoides tenoreana, Alloxan, Hepato-renal damage markers, Histopathology,*

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INTRODUCTION

The liver and the kidneys are very important organs in the body. The liver cells (hepatocytes) are saddled with the responsibility of biotransformation of xenobiotics, detoxification of harmful substances, storage and immunological functions (Aba and Asuzu, 2015). The kidneys on the other hand, mainly play excretory role and maintains homeostasis. They are important in excretion of urea in the form of urine and in tubular reabsorption of water and electrolytes (Marseric, 2009).

Serum biochemical markers of hepato-renal injuries or dysfunctions have been well studied. Insults to hepatocytes usually manifest in the form of elevated serum activities of certain enzymes that are usually resident in the hepatocytes. These enzymes include: Alanine aminotransferase, Aspartate aminotransferase and alkaline phosphatase (Ramaiah, 2011; Udem and Asogwa, 2011). They can as well manifest in the form of compromised functions. For instance, the inability of the liver to conjugate bilirubin or synthesize albumin will show as unconjugated hyperbilirubinaemia or hypoalbuminaemia respectively (Thapa and Walia, 2007). Creatinine and urea are usually the indicators of renal damage. Under physiologic conditions, the kidneys excrete urea and clear the plasma of creatinine (Marseric, 2009). However, when the kidneys are compromised, the plasma levels of creatinine and urea buildup (Braide and Anika, 2009).

Diabetes mellitus is a metabolic disease that has been shown to affect the kidneys (diabetic nephropathy) and the liver (diabetic hepatopathy) (Sara *et al*., 2012). Alloxan monohydrate is a diabetogenic substance used to induce experimental Diabetes mellitus (Lenson, 2008). It generates free radicals that destroy the pancreatic islet cells including beta cells that are responsible for insulin production. Insulin is a hormone that literally "drags" glucose from the blood circulation to the cells (Akuodor, 2014). Thus reduced insulin production entails accumulation of glucose in the blood (hyperglycemia) which occasions Diabetes mellitus (Lenson, 2008). Studies have also shown that the free radicals generated by alloxan monohydrate are also capable of attacking other organs such as the liver and kidneys (El –Demardash *et al*., 2005).

Vernonia amygdalina and *Baccharoides tenoreana* are tropical African vegetables that belong to the family Asteraceae or Compositae (Burkil, 2000). They are known as bitter leaf in English. However, the latter is less bitter than the former (Ijeh and Egedigwe, 2010). The vegetables have enormous nutritional and medicinal properties. Other researchers reported that *Vernonia amygdalina* possesses phytochemicals such as flavonoids, saponins, tannins, steroids, alkaloids, triterpenoids, reducing sugar and cardiac glycosides while Baccharoides tenoreana possesses saponins, tannins, anthraquinones, cardenolides (Ogundare *et al*., 2010; Usunobun, 2016; Eze *et al*., 2020). When these phytochemicals are ingested by animals, they exhibit various pharmacological and biochemical actions (Usunobun *et al*., 2015). Flavonoids for instance have been reported to interfere with the activities of the enzymes involved in ROS generation, quenching of free radicals, chelating transition metals and rendering them redox inactive in the fenton reaction (Aiyegoro and Okoh, 2009; Omoregie *et al*., 2011).

Studies show that *Vernonia amygdalina* possesses both hypoglycemic and antioxidant activities (Aba and Okenwa-Ani, 2015). Anti-diabetic effects of *Baccharoides tenoreana* have been also documented (Attama *et al*., 2021). This study was undertaken to investigate a possible hepato-renal protection of alloxan-induced rats treated with aqueous extracts of *Vernonia amygdalina* and *Baccharoides tenoreana* separately and in combination and to compare the strengths of such protections.

MATERIALS AND METHODS

Reagents and chemicals: This study was carried out using these chemicals and reagents: Alloxan monohydrate (Sigma Aldrich, UK); Glibenclamide (Hovid Hong Kong); Aminotransferase activity assay kit (ab105135) (Biovison, Abcam company, Cambridge UK); Alkaline phosphatase kit (ab83369) (Biovison, Abcam company, Cambridge UK); Bilirubin assay kit (Sigma Aldrich, UK); Creatinine assay kit (Sigma Aldrich, UK); Urea assay kit (Sigma Aldrich, UK) . All the chemicals used are of good analytical grades.

Experimental animals: Thirty male Albino Wistar rats weighing between 170-190 g were used for this study. The rats were purchased from the laboratory animal house belonging to the Department of Veterinary Physiology and Pharmacology, University of Nigeria Nsukka. They were acclimatized for a period of two weeks, during which they were kept in a stainless wire mesh cage and fed with grower feed (Vital®) and clean water *ad libitum*.

Ethical approval: Ethical approval was given by the Institutional Animal Care and Use Committee, Faculty of Veterinary Medicine, University of Nigeria Nsukka with the approval number: FVM-UNN-IACUC-2020-0266.

Plant collection and extraction: *Vernonia amygdalina* and *Baccharoides tenoreana* leaves were collected and extracted according to the method described in our previous study (Attama *et al*., 2021). Briefly, the leaves were purchased during rainy season from Ogige Market, Nsukka and identified at Bioresources Development and Conservation Programme (BDCP), Aku Road, Nsukka, Enugu State, Nigeria. The Voucher Specimens (*V. amygdalina*: INTERCEED/41 *B. tenoreana*: INTERCEED/2619) were kept at the herbarium. Nsukka is located between Latitude 6˚ 5′ and 6˚ 24′ north and Longitude7˚ 23′ and 7˚ 45′ east in the south-east geopolitical zone (Federal Republic of Nigeria official gazette). The leaves were dried and pulverized into powder after removal of any foreign matter. Cold maceration method using distilled water was used. The pulverized materials were soaked in distilled water for 48 h with intermittent shaking after every 2 h. Thereafter, they were filtered using NO 1 Whiteman filter paper to obtain the filtrate (aqueous extract). The aqueous extract was lyophilized and kept in airtight amber colored bottles and stored in refrigerator at 4 ˚C pending use.

The aqueous leave extracts of VA and BT gave a yield of 12.4 g (6.20% w/w) and 12.7 g (6.35% w/w) respectively.

Experimental protocol: The experimental procedure adopted in this study is similar to the one reported in our previous study [20]. Briefly, the rats (30) were assigned into six (6) groups of five (5) rats in each group. Alloxan monohydrate was intraperitoneally administered to groups B-F at the dose of 160 mg/kg following 16 h fasting, while rats in group A served as normal control. Forty eight hours (48 h) following alloxan monohydrate administration and upon confirmation of fasting blood glucose level ≥126 mg/dl, the rats were treated as shown in Table 1. The treatments were administered daily for 21 days via the oral route. At the end of 21 days duration of the study, serum samples for the assay of biomarkers of hepato-renal damage (Alanine aminotransferase, Alkaline phosphatase, total and direct bilirubin, Creatinine, Urea) were collected. The liver and kidney tissues were also collected for histopathology studies.

Table 1
Experimental grouping and treatments

Group	Treatment
A	Un-induced but treated with 10 ml/kg distilled water (Normal control)
B	Induced and treated with 10 ml/kg distilled water (Negative control)
C	Induced and treated with 200 mg/kg *Vernonia amygdalina*
D	Induced and treated with 200 mg/kg *Baccharoides tenoreana*
E	Induced and treated with 100 mg/kg *Vernonia amygdalina* & 100 mg/kg *Baccharoides tenoreana*
F	Induced and treated with 2 mg/kg Glibenclamide (Standard control)

Sample collections: Blood samples for determination of serum biochemical markers of liver (alanine aminotransferase, alkaline phosphatase, total and direct bilirubin) and kidney (creatinine, urea) damages were collected via the retrobulbar plexus of the medial canthus of the eye. The blood samples were centrifuged at 10,000 g for 10 min. Thereafter, the sera for determination of the parameters were decanted. The liver and kidney tissues were excised for histopathology studies

following humane euthanasia of the rats under chloroform anaesthesia.

Determination of serum biochemical parameters: Serum Alanine aminotransferase (ALT) activity was determined by the Reitman-Frankel spectrophotometric method (Reitman and Frankel, 1957; Colville, 2002; Aba and Okorie- Kanu, 2017). The phenolphthalein monophosphate method was used to determine the alkaline phosphatase (ALP) activity (Klein *et al*., 1960; Coville, 2002)**.** Conjugated and total bilirubins were assayed according to the method of Doumas *et al.* (1971). The creatinine assay was according to the method of Blass *et al.* (1974). Serum urea was assayed according to Urease-Berthelot Method (Fawcett and Scott, 1960; Drury *et al*., 1967).

Histopathological investigation: The histopathological studies of the liver and kidney tissues were done using the method of Drury *et al.* (1967).

Data analysis: The data generated for this study were analyzed using SPSS version 20 of One-way Analysis of Variance (ANOVA). Duncan's Multiple Range post hoc test was used to separate the variant means. $P < 0.05$ was accepted as being significant. Results were expressed as Mean ± standard error of the mean (SEM) and presented in tables.

RESULTS

The Effects of the aqueous leaf extract of *Vernonia amygdalina* and Baccharoides tenoreana on liver and kidney function of alloxan-induced diabetic rats: The alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities of the diabetic untreated rats (group B) were significantly ($p<0.05$) higher than those of the normal control rats (group A) and the group treated with *Vernonia amygdalina*. The ALP activity of the group treated with VA or the combination of VA and BT was statistically similar ($p>0.05$) to that of the negative control rats. The direct bilirubin values were significantly ($p<0.05$) higher in the treated groups compared to the negative control group. The creatinine and urea values of the negative control rats were significantly ($p<0.05$) higher than those of the normal control, VA and glibenclamide-treated groups (Table 2).

The in vitro Antioxidant activity of *Vernonia amygdalina* and Baccharoides tenoreana using DPPH model: Results of the *in vitro* antioxidant activities using DPPH model showed that the antioxidant activities of both VA and VC aqueous extracts were linear in increasing concentration-dependent manner. The antioxidant activities of VA and VC at 400 µg/ml were 60.87% and 48.84% respectively while ascorbic acid; the standard reference recorded an antioxidant activity of 91.68% at 400 µg/ml (Fig 1).

Table 2:
The effects of the aqueous leaf extract of *Vernonia amygdalina* and *Baccharoides tenoreana* on liver and kidney function of alloxan-induced diabetic rats.

GROUP	ALT (Iµ/L)	ALP (Iµ/L)	D.BIL (mg/dl)	T. BIL (mg/dl)	UREA (mg/dl)	CREAT (mg/dl)
A	26.67 ±1.70^{a}	48.00± 2.00^{a}	1.35±0.00^{c}	2.46±0.07^{a}	44.00±3.6^{a}	1.53±0.09^{a}
B	38.33 ± 3.71^{d}	61.67±1.33^{c}	0.92±0.05^{a}	2.84±0.07^{b}	100.00±4.36^{e}	3.00±0.15^{d}
C	28.00 ± 3.21^{b}	52.33±1.67^{b}	1.31±0.06^{bc}	2.57±0.16^{ab}	71.33±5.78^{c}	2.37±0.28^{c}
D	35.33±2.40^{c}	56.33±2.03^{bc}	1.19±0.02^{b}	2.68±0.14^{ab}	83.33±4.81^{d}	2.63±0.12^{cd}
E	35.33 ± 2.40^{c}	59.57±2.60^{c}	1.18±0.02^{b}	2.82±0.00^{ab}	95.00±2.08^{de}	2.67±0.12^{cd}
F	27.33 ±2.20^{ab}	48.00±2.52^{a}	1.33±0.05^{c}	2.55±0.16^{ab}	61.67±2.33^{b}	1.97±0.14^{b}

Superscripts a, b, c, d and e indicate significant difference at P ≤ 0.05 down the columns (across the groups).
ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; D. BIL: Direct Bilirubin; T. BIL: Total Bilirubin; CREAT: Creatinine.

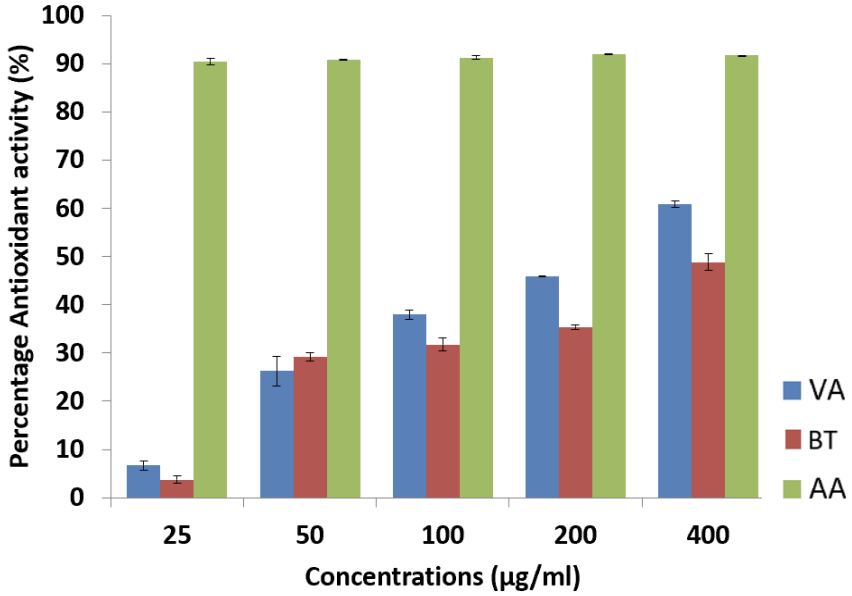

Figure 1:
The *in vitro* Antioxidant activity of *Vernonia amygdalina* and *Baccharoides tenoreana* using DPPH model
VA: *Vernonia amygdalina* aqueous leaf extract
BT: *Baccharoides tenoreana* aqueous leaf extract
AA: Ascorbic Acid

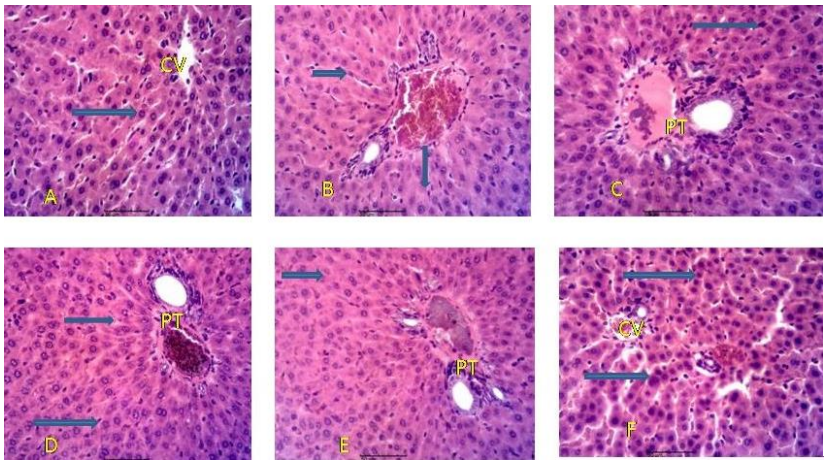

Plate 1
A –Photomicrograph of the liver of group A rats (Normal control) showing central vein (CV) and hepatocytes in cords (arrows); B –Photomicrograph of the liver of group B rats (Diabetic untreated) showing few areas of degeneration and necrosis of hepatocytes (arrows); C -Photomicrograph of the liver of group C rats (Diabetic + 200 mg/kg of *Vernonia amygdalina*) showing the portal triad (PT) and hepatocytes in cords (arrows); D –Photomicrograph of the liver of group D rats (Diabetic + 200 mg/kg of Baccharoides tenoreana) showing some components of the portal triad (PT) and few degenerate hepatocytes (arrows); E –Photomicrograph of the liver of group E rats (Diabetic + 100 mg/kg of *Vernonia amygdalina* & 100 mg/kg of Baccharoides tenoreana) showing the portal triad and few degenerate hepatocytes in cords (arrows); F –Photomicrograph of the liver of group F rats (Diabetic + 2 mg/kg Glibenclamide) showing the central vein and hepatocytes in cords (arrows). H&E X400

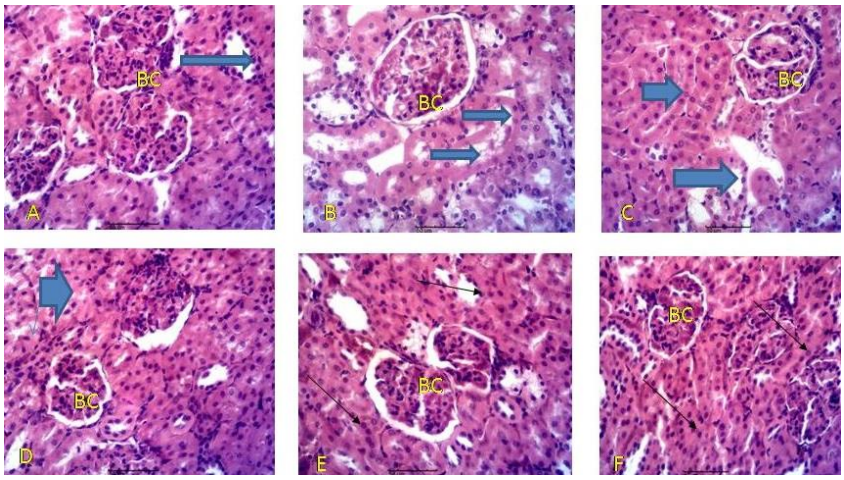

Plate 1
A: Photomicrograph of kidney of group A rats (Normal control) showing the bowman's corpuscles (BC), B: Photomicrograph of kidney of group B rats (Diabetic untreated) showing the bowman's corpuscles (BC) and areas of convoluted tubular epithelial cell necrosis (arrows), C: Photomicrograph of kidney of group C rats (Diabetic + 200 mg/kg of *Vernonia amygdalina*) showing the bowman's corpuscles (BC) and convoluted tubules with normal epithelial cells (arrows), D: Photomicrograph of kidney of group D rats (Diabetic + 200 mg/kg of *Baccharoides tenoreana*) showing the bowman's corpuscles (BC) and convoluted tubules with few necrotic epithelial cells (arrows), E: Photomicrograph of kidney of group E rats (Diabetic + 100 mg/kg of *Vernonia amygdalina* & *Baccharoides tenoreana*) showing the bowman's corpuscles (BC) and convoluted tubules with normal epithelial cells (arrows), F: Photomicrograph of the kidney of group F rats (Diabetic + 2 mg/kg Glibenclamide) showing the bowman's corpuscles (BC) and convoluted tubules with normal epithelial cells (arrows.). H&E X400

The liver photomicrograph of alloxan-induced diabetic rats treated with aqueous leaf extract of *Vernonia amygdalina* and *Baccharoides tenoreana*: The liver photomicrograph of the group B rats showed areas of degeneration and necrosis of the hepatocytes. The hepatocytes of the glibenclamide-treated rats (group F) and those treated with VA (group C) appeared normal and comparable to that of the normal control rats. Few areas of hepatocyte degenerations were seen in the group treated with BT and combinations of VA and BT (Plate 1).

The Kidney photomicrograph of alloxan-induced diabetic rats treated with aqueous leaf extract of *Vernonia amygdalina* and *Baccharoides tenoreana*: The results of the kidney photomicrograph of the diabetic untreated rats showed tubular epithelial degenerations and necrosis. Few areas of degenerations were also seen in the kidney photomicrograph of the rats treated with BT. The renal histo-architecture of the normal control rats were similar to those treated with VA and glibenclamide (Plate 2).

DISCUSSION

Increase in the activities of ALT and ALP in the alloxan-induced and untreated rats (group B) indicate a harmful effect of alloxan monohydrate in the hepatocytes. Studies had earlier shown that the free radicals emanating from alloxan monohydrate could cause harm to the liver (El –Demardash *et al*., 2005). *Vernonia amygdalina* (VA) ameliorated the damaging effects of alloxan monohydrate in the liver as evidenced by reduced activities of ALT and ALP in the VA-treated rats (Onyema *et al*., 2006; Palsamy and Subramanian, 2008). Other researchers have also submitted the ameliorative effects of VA in alloxan-induced diabetic rats (Atangwho *et al*., 2007; Aba and Okenwa- Ani, 2015). Antioxidant and hepatoprotective effects of VA have also been reported (Minari, 2012). The mitigation of hepatic injury by the aqueous extract of VA could be attributed to the antioxidant properties possessed by VA.

The present study reports the antioxidant activity of VA at 400 g/ml using DPPH model to be 60.87%. Antioxidants are known to mop up free radicals or reactive oxygen species (Devasagayam *et al*., 2004). These activities of VA seem to have been dampened when combined with *Baccharoides tenoreana* (BT). We submit that this could probably be because the antioxidant potential of the aqueous extract of the BT was way below that of the VA as also reported by the present study. The aqueous extract of BT recorded antioxidant activity of 48.84% on DPPH photometric assay method.

Direct bilirubin values of all the treated rats were significantly higher when compared to that of the negative

control rats. This indicates that the extracts (VA and BT) improved hepatic function of conjugation. Bilirubin is a byproduct of RBC metabolism. The unconjugated bilirubin is carried by albumin to the liver for conjugation into bilirubin diglucuronide (direct or conjugated bilirubin) (Murray, 2000; Thapa and Walia, 2007). Elevating the values of direct bilirubin by the aqueous extract of VA and BT implies that the extract improved hepatic function.

The values of creatinine and urea were significantly raised in the alloxan-induced rats than in the treated groups. Creatinine and urea are reliable markers of kidney functions (Mukinda and Eagles, 2010). Elevations in the plasma levels of urea and creatinine only portends kidney damage. The rats treated with the extracts (VA and BT) and glibenclamide recorded significantly lower plasma levels of urea and creatinine indicating mitigation of kidney damage. This finding is in agreement with the submissions of other researchers who also reported the positive/curative effects of *Vernonia amygdalina* on the kidneys of diabetic rats (Onyema *et al*., 2006). The rats treated with combination of VA and BT however, did not show a better protection when compared to either of the extracts alone.

The photomicrographs of both the liver and the kidneys of the induced untreated rats (group B) present lesions of degeneration and necrosis of the hepatocytes and renal tubular epithelial cells respectively. This implies that alloxan monohydrate is capable of causing degenerative and necrotic injuries to both the liver and the kidneys. The finding is also in alliance with the submissions of earlier researchers (El – Demardash *et al*., 2005; Onyema *et al*., 2006). The observations of reduced and or absence of lesions in the liver and kidney photomicrographs of the induced and treated groups also show that the aqueous extracts mitigated hepato-renal injuries occasioned by alloxan monohydrate administration. However, the aqueous extract VA appeared to be more effective in protecting injuries to the liver and kidney histo-architectures when compared to its counterpart, the BT alone and in combination with VA.

In conclusion, the aqueous extracts of *Vernonia amygdalina* delile and *Baccharoides tenoreana olive* mitigated hepato-renal injuries occasioned by administration of alloxan monohydrate. The protection given by *Vernonia amygdalina* delile however, was more pronounced compared to that offered by either *Baccharoides tenoreana olive* separately or in combination with *Vernonia amygdalina.*

Author's contributions:

This work was carried out in collaboration between all authors. PE and SC conceptualized this project, IU and PE drafted the experimental design. SC performed the literature search. PE, SC and CU set up all the laboratory experiments, collected the raw data and also drafted the first manuscript. PE and IU managed manuscript revisions. SC and PE performed data analysis. PE, SC, CU and IU participated in final manuscript writing and revisions. All authors read and approved the final manuscript.

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