



Biochemical and Haematological Effects of Acute and Sub-acute Administration of Ethyl Acetate Fraction of *Vernonia amygdalina* Leaf in Laboratory Rodents

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ABSTRACT

Background: *Vernonia amygdalina*, commonly known as bitter leaves, is a staple vegetable in the culinary traditions of many equatorial African societies. Traditionally, these leaves have been used to manage psychiatric disorders, malaria, and diabetes, and are also recognised for boosting the immune system and providing antioxidant benefits.

Objectives: This study aimed to evaluate the acute and sub-acute toxicological effects of the ethyl acetate fraction derived from *Vernonia amygdalina*, using male and female mice for the acute study and female rats for the sub-acute study.

Methods: The toxicological assessments were conducted in accordance with the protocols established by the Organisation for Economic Cooperation and Development (OECD guidelines 423 and 407). In the acute phase of the study, a solitary dose of 2000 mg/kg of *Vernonia amygdalina* was administered via the oral route to both male and female mice. Observations included mortality rates, alterations in behaviour, and fluctuations in body weight. In the sub-acute phase, female rats were subjected to oral administration of *Vernonia amygdalina* at dosages of 100, 200, and 400 mg/kg over a duration of 28 days. Evaluative measures included mortality, behavioural alterations, changes in weight, as well as biochemical, haematological, and histopathological analyses.

Results: The administration of *Vernonia amygdalina* did not elicit any observable alterations in behaviour or weight, and no mortality was recorded. Similarly, in the sub-acute toxicity evaluation, we noted an absence of behavioural changes, weight fluctuations, or mortality, alongside no statistically significant differences in all assessed parameters.

Conclusion: The ethyl acetate fraction of *V. amygdalina* showed no significant adverse effects on behaviour, body weight, haematological, or biochemical indices in both acute and sub-acute studies, though mild histological changes were observed at higher doses (400 mg/kg). These findings suggest a wide safety margin but recommend caution at elevated doses.

Keywords: *Vernonia amygdalina*, Acute toxicity, Sub-acute Toxicity

INTRODUCTION

The utilisation of traditional remedies is prevalent in the sub-Saharan African region, and consultations with traditional healers continue to constitute a fundamental aspect of healthcare for numerous individuals due to factors such as preference, economic viability, and restricted access to hospitals and contemporary healthcare professionals (Homsy *et al.*, 1999). The application of these botanical substances in healthcare provision, particularly in resource-constrained environments, is experiencing an upward trajectory, with an estimated 80% of the global populace depending on traditional medicine for primary healthcare services (Ekor, 2014). Despite the promising potential demonstrated by these plants, which have garnered significant global demand, apprehensions regarding their safety persist (Obidike and Salawu, 2013). Herbal products are predominantly perceived as safe or of minimal

toxicity, attributable to their extensive historical usage by humans (Ibrahim *et al.*, 2016).

Nonetheless, recent surveys have revealed that a considerable number of these products utilised in traditional medicine have exhibited adverse effects (Koduru *et al.*, 2006). Given that safety remains a critical concern associated with the application of medicinal plants, it is imperative to undertake toxicity assessments to determine their safety profiles. Due to its distinctly bitter taste, *Vernonia amygdalina* is often dubbed the "bitter leaf" in English. In Africa, it is known by various names such as ewuro (Yoruba), onugbu (Igbo), and oriwo (Edo). This intriguing plant belongs to the Asteraceae family and manifests as a petite shrub flourishing in the tropical expanses of Africa. *V. amygdalina* generally reaches an impressive height between 2 and 5 meters. Its leaves sport an elliptical shape and can extend to a length of up to 20 centimetres. The bark of this plant is notably rough (Ijeh and Ejike, 2011). Global academic and pharmaceutical interest in this plant has significantly expanded due to its broad spectrum of traditional applications. Despite its wide use, there is limited toxicological data on solvent fractions of *V. amygdalina*, particularly the ethyl acetate fraction known to contain bioactive phytochemicals. Therefore, this study was specifically designed to investigate the acute and sub-acute effects of the ethyl acetate fraction of *Vernonia amygdalina* on biochemical and haematological parameters in rodent models.

MATERIALS AND METHODS

Plant preparation and extraction: Fresh foliage of *Vernonia amygdalina* (VA) was procured from the University of Ibadan, situated in Ibadan, Oyo State, Nigeria. The identification and authentication of the botanical specimen were conducted at the herbarium section of the Forest Reserve Institute of Nigeria (FRIN) located in Ibadan, Oyo State, Nigeria, with ethical approval reference number: UI-ACUREC/139-1119/18. These specimens were subsequently air-dried and subjected to pulverisation. The pulverised *V. amygdalina* (1.2 kg) was immersed in 4.5 litres of methanol for 72 hours. The resultant mixture underwent filtration, and the filtrate was concentrated utilising a rotary evaporator at a maximum temperature of 45°C to yield the crude aqueous extract of the plant, which was further fractionated into n-hexane

and ethyl acetate fractions and stored in a refrigerator at 4°C for subsequent application. Following reconstitution in clean water at the appropriate concentration, the ethyl acetate fraction of *Vernonia amygdalina* was labelled as EAVA and was administered orally.

2.2 Experimental Animals and Treatment:

This investigation utilised male and female mice weighing 20-25 grams, and female rats weighing 70-80 grams. All animals were sourced from the Animal House at the College of Medicine, University of Ibadan, Nigeria, and maintained under optimal environmental conditions with *ad libitum* access to standard rodent pellets and water. For the acute toxicity assay, animals were divided into two groups (n=3 each): Group 1 received normal saline (10 mL/kg), while Group 2 was administered 2000 mg/kg of the ethyl acetate extract of *Vernonia amygdalina* (EAVA). In the sub-acute toxicity assessment (n=5), Group 1 continued to receive normal saline (10 mL/kg) as the control. Groups 2, 3, and 4 received EAVA at dosages of 100 mg/kg, 200 mg/kg, and 400 mg/kg, respectively. All experimental protocols adhered to the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (NIH, 1985).

2.3. Acute and sub-acute toxicity studies

2.3.1 Acute toxicity study

Acute oral toxicity of the ethyl fraction of *Vernonia amygdalina* was evaluated in both male and female mice as recommended by the OECD guideline (Organisation for Economic Cooperation and Development, 2001). According to Guidance OECD 423, these procedures were performed using 3 animals in both sexes. Animals of the test group received a single dose of 2000 mg/kg of ethyl fraction of *Vernonia amygdalina*, dissolved in water. As the vehicle, regular saline was given to the control group. The dose used in the experiment of acute toxicity was selected from one of four fixed levels: 5, 50, 300, or 2000 mg/kg, as described in the OECD guide. Animals were then monitored every day for 14 days and six and twelve hours after the treatment. Lethargy, locomotion, salivation, diarrhoea, tremors, convulsions, and abdominal contortions were taken into consideration.

Daily measurements of the animals' body weight and food intake were made during the study. Following 14 days of treatment, animals were fasted for 8 hours and subsequently sacrificed. Organs were then excised and weighed.

2.3.2 Sub-acute toxicity

The sub-acute toxicity study was created with some minor adjustments by OECD 407 (Organisation for Economic Cooperation and Development, 1995). The fraction was dissolved in water and administered by gavage once a day for 28 days. The animals' body weight and food intake were measured every day during the study period. On day 28 of the study, each animal had a short 8-hour fast before blood samples were drawn and put into two tubes: one with EDTA, an anticoagulant, and the other without. The kidney, liver, brain, stomach, heart, spleen, lungs, and uterus were then removed, dissected, and preserved in a 10% formalin solution after the animals were put to sleep.

2.3.3. Open field test

Twenty-four hours before sacrifice, the open-field test was conducted as previously described by Ogunmiluyi *et al.* (2024) to evaluate spontaneous exploratory behaviour and identify any motor disturbances induced by *Vernonia amygdalina* administration. The apparatus was a plywood open field with 30 cm high walls. Its 45 cm x 45 cm floor was divided into a 3 x 3 grid of nine squares using a permanent marker. Each animal was placed in the centre of the apparatus and observed for five minutes, with the number of lines crossed by all four paws recorded as the primary measure (Walsh and Cummins, 1976).

2.4. Assessment of haematological parameters

The haematology samples were placed in bottles that contained ethylene diamine tetra-acetic acid (EDTA), an anticoagulant. The haematological analyses were performed using an automated haematology analyser (Mytaic 22, Germany). The following parameters are analysed: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), erythrocytes (RBC), total and differential leukocytes (WBC), haemoglobin (Hgb), hematocrit (HCT), and platelet count (PLT).

2.5. Evaluation of biochemical parameters in serum

Blood was carefully placed in plain bottles for biochemical analysis to prevent hemolysis of the blood cells. The sample of blood was centrifuged to extract the blood serum. Standard procedures for aspartate aminotransferase (AST), alanine aminotransferase (ALT), Alanine phosphatase (ALP), Gamma-glutamyltransferase (GGT) and creatinine were used in the biochemical investigations.

2.6. Statistical Analysis

The mean ± S.E.M. was used to express the results. One-way Analysis of Variance (ANOVA) was used to analyse variance and the Student T test and Newman–Keuls multiple comparisons test were used. Statistical significance was defined as $P<0.05$. Prism software (Graph-Pad Software) was used for all statistical analyses.

3. RESULTS

3.1. Effect of *Vernonia amygdalina* on weight gained in acute toxicity test in male and female mice

Table 1 below shows the effect of the Ethyl acetate fraction of *Vernonia amygdalina* (EAVA) on the percentage increase in weight of mice using a single dose of 2000 mg/kg of EAVA. At varying dosages, the ethyl acetate fraction of *Vernonia amygdalina* did not significantly differ from the control. At the different dosages, these doses exhibited no toxicity.

Table 1: EAVA administration's impact on mice's weight gain during acute toxicity; results are shown as means ± SEM (n=3). Data analysis was done with the Student's test. $p\leq0.05$

Group	Body weight (g)		
	Initial weight (g)	Final weight (g)	Weight gained
Male			
Control (10 ml/ kg)	22.3±0.6	24.7±0.3	2.3±0.3
EAVA (2000 mg/ kg)	21.7±0.3	25.3±0.8	3.7±0.6
Female			
Control (10ml/ kg)	18.7±0.3	23.3±0.8	4.3±0.8
EAVA (2000mg/ kg)	18.7±1.2	23.7±1.2	4±0.0

3.2. Effect of Ethyl acetate fraction of *Vernonia amygdalina* activities on Open Field Test in acute toxicity test in male and female mice

Fig. 1 and 2 demonstrate the potential of treating animals with 2000 mg/kg of *Vernonia amygdalina*. The acute toxicity test showed no discernible difference in the number of lines crossed between the EAVA-treated and control groups.

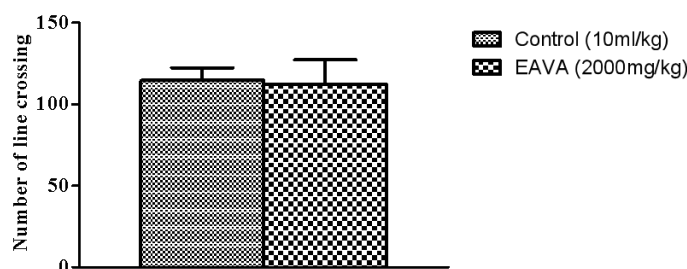


Fig 1: Results are expressed as Means $\pm$ SEM (n=3). The impact of ethyl acetate fraction of *Vernonia amygdalina* administration on the quantity of line crossings in male mice's acute toxicity test during the Open Field Test (OFT). Data were analysed using Student's t-test. $p \leq 0.05$

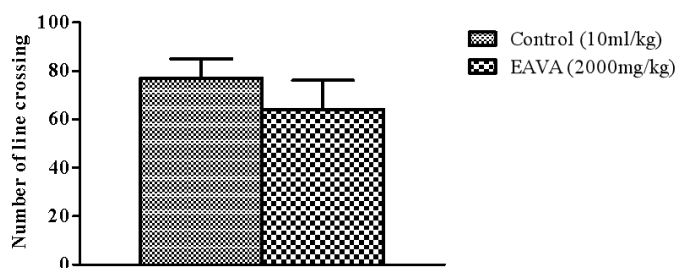


Fig. 2: Results are expressed as Means $\pm$ SEM (n=3). The impact of ethyl acetate fraction of *Vernonia amygdalina* administration on the quantity of line crossings in female mice's acute toxicity test during the Open Field Test (OFT). Data were analysed using the Student t- test. $p \leq 0.05$

3.3. Effects of the ethyl acetate fraction of *Vernonia amygdalina* on the relative weight of organs in mice in an acute toxicity test

The impact of a single dose of 2000 mg/kg of *Vernonia amygdalina* (EAVA) on the relative organ weight of mice is displayed in Table 2 below. When compared to control, the ethyl acetate fraction of *Vernonia amygdalina* (EAVA) at various dosages did not exhibit any discernible variations. These doses showed no toxicity at the various doses.

3.4 *Vernonia amygdalina*'s impact on female rats' weight gain during a sub-acute toxicity test

The impact of ethyl acetate fraction of *Vernonia amygdalina* on the percentage increase in weight in female rats administered 100 mg/kg, 200 mg/kg, and 400 mg/kg of EAVA is displayed in Table 3 below. When compared to control, the ethyl acetate fraction of *Vernonia amygdalina* (EAVA) at various dosages did not exhibit any discernible variations. At all doses, these doses exhibited no toxicity.

Table 2: The results are presented as means $\pm$ SEM (n=3) and show the effect of ethyl acetate fraction of *Vernonia amygdalina* administration on the relative organ weight of mice in acute toxicity. The Student T test was used to analyse the data. $p \leq 0.05$.

Organs	Control 10ml/kg	EAVA2000mg/kg
Male		
Kidney	0.67 $\pm$ 0.23	0.82 $\pm$ 0.06
Liver	4.12 $\pm$ 0.45	5.25 $\pm$ 0.42
Lungs	0.82 $\pm$ 0.04	0.62 $\pm$ 0.07
Brain	1.65 $\pm$ 0.08	1.61 $\pm$ 0.07
Stomach	1.38 $\pm$ 0.31	0.48 $\pm$ 0.10
Heart	1.86 $\pm$ 2.53	1.83 $\pm$ 1.16
Spleen	0.41 $\pm$ 0.02	0.53 $\pm$ 0.10
Female		
Kidney	0.56 $\pm$ 0.07	0.55 $\pm$ 0.01
Liver	5.48 $\pm$ 1.17	5.76 $\pm$ 0.27
Lungs	0.73 $\pm$ 0.11	0.64 $\pm$ 0.09
Brain	1.58 $\pm$ 0.03	1.45 $\pm$ 0.37
Stomach	1.26 $\pm$ 0.35	1.57 $\pm$ 0.11
Heart	1.89 $\pm$ 1.30	0.98 $\pm$ 0.02
Spleen	0.95 $\pm$ 0.28	0.96 $\pm$ 0.24

Table 3: Effect of ethyl acetate fraction of *Vernonia amygdalina* administration on percentage increase in rats in sub-acute toxicity; the results are expressed as means $\pm$ SEM (n=5). Data were analysed using One-way ANOVA followed by Newman-Keuls' post hoc test. $p \leq 0.05$

Group	Body weight (g)		Weight gained
	Initial weight (g)	Final weight (g)	
Control (10ml/kg)	60.4 $\pm$ 2.4	108.2 $\pm$ 4.1	47.8 $\pm$ 3.4
EAVA(100mg/kg)	58.3 $\pm$ 10.7	97.6 $\pm$ 17.6	39.7 $\pm$ 7.4
EAVA (200mg/kg)	59.5 $\pm$ 11.5	100.6 $\pm$ 19.2	41.5 $\pm$ 7.5
EAVA (400mg/kg)	65.2 $\pm$ 2.6	112 $\pm$ 3.6	47 $\pm$ 4.1

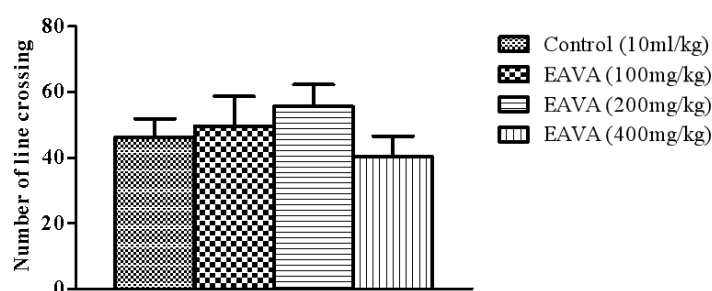


Fig. 3: Ethyl acetate fraction of *Vernonia amygdalina* administration's impact on the quantity of line crossings in female rats' Open Field Test (OFT) sub-acute toxicity test. Means $\pm$ SEM (n=5) are used to express the results. One-way ANOVA and the Newman-Keuls post hoc test were used to analyse the data. $p \leq 0.05$

3.5. Effect of Ethyl Acetate Fraction of *Vernonia amygdalina* activities on Open Field Test in sub-acute toxicity test in female rats

Fig. 3 illustrates the potential of ethyl acetate fraction of *Vernonia amygdalina* treatment with 100 mg/kg, 200 mg/kg, and 400 mg/kg of EAVA. When comparing the number of lines crossed in animals treated with *Vernonia amygdalina* to the control group in a sub-acute toxicity test.

3.6. Effects of the ethyl acetate fraction of *Vernonia amygdalina* on the relative weight of organs in female rats in the sub-acute toxicity test

The impact of the ethyl acetate fraction of *Vernonia amygdalina* (EAVA) at 100, 200, and 400 mg/kg on the relative organ weight of female rats is displayed in the table below. When compared to control, the ethyl acetate fraction of *Vernonia amygdalina* (EAVA) at various dosages did not exhibit any discernible variations. These doses showed no toxicity at the various doses.

3.7. Effects of sub-acute administration of ethyl acetate fraction of *Vernonia amygdalina*

The following table displays the impact of administering 100, 200, and 400 mg/kg of the ethyl acetate fraction of *Vernonia amygdalina* (EAVA) to female rats over 28 days on their haematological and biochemical parameters. When compared to control, the ethyl acetate fraction of *Vernonia amygdalina* (EAVA) at various dosages did not exhibit any discernible variations. At the different doses, these doses did not exhibit any toxicity.

Fig 4. shows the photomicrograph of sections of female kidneys and livers in control and treated groups (100 X). Glomeruli, tubules and interstitium show no visible lesion in the kidney, and the Liver showed Closely-packed hepatic plates with no visible lesion. There is moderate Kupffer cell hyperplasia in the kidney, and the liver section showed mild inflammatory infiltration within the lobules of the liver at 200 mg/kg of the ethyl acetate fraction of *Vernonia amygdalina* in both organs. 100 mg/kg and 400 mg/kg were similar to the control.

Table 4: Ethyl acetate fraction of *Vernonia amygdalina* administration's impact on the rat's relative organ weight in sub-acute toxicity. Means $\pm$ SEM (n=5) are used to express the results. One-way ANOVA was used to analyse the data, and then the Newman-Keuls post hoc test was used.

Organs	Control 10ml/ kg	EAVA 100 mg/ kg	EAVA 200mg/ kg	EAVA 400mg/ kg
Kidney	0.31 $\pm$ 0.01	0.30 $\pm$ 0.04	0.32 $\pm$ 0.02	0.31 $\pm$ 0.04
Liver	3.60 $\pm$ 0.31	3.09 $\pm$ 0.73	3.48 $\pm$ 0.13	3.62 $\pm$ 0.29
Lungs	0.79 $\pm$ 0.19	1.05 $\pm$ 0.07	0.89 $\pm$ 0.09	0.89 $\pm$ 0.07
Brain	1.21 $\pm$ 0.05	1.06 $\pm$ 0.09	1.09 $\pm$ 0.05	1.27 $\pm$ 0.07
Stomach	0.91 $\pm$ 0.04	0.93 $\pm$ 0.05	0.78 $\pm$ 0.04	0.91 $\pm$ 0.05
Heart	0.38 $\pm$ 0.02	0.43 $\pm$ 0.04	0.39 $\pm$ 0.02	0.37 $\pm$ 0.01
Spleen	0.63 $\pm$ 0.04	0.54 $\pm$ 0.07	0.44 $\pm$ 0.05	0.51 $\pm$ 0.09
Uterus	0.14 $\pm$ 0.03	0.13 $\pm$ 0.04	0.19 $\pm$ 0.02	0.18 $\pm$ 0.01

Table 5: Impact of sub-acute administration of ethyl acetate fraction of *Vernonia amygdalina* on female rats' haematological parameters. Means $\pm$ SEM (n=5) are used to express the results. One-way ANOVA was used to analyse the data, and then the Newman-Keuls post hoc test was used

Variables	Control 10ml/kg	EAVA 100mg/kg	EAVA 200mg/kg	EAVA 400mg/kg
PCV	38.8 $\pm$ 0.58	39.0 $\pm$ 1.73	40.8 $\pm$ 0.66	40.0 $\pm$ 2.28
H.b	12.8 $\pm$ 0.21	12.92 $\pm$ 0.59	13.52 $\pm$ 0.17	13.4 $\pm$ 0.83
RBC	6.37 $\pm$ 0.04	6.44 $\pm$ 0.34	6.43 $\pm$ 0.03	6.52 $\pm$ 0.38
WBC	4910 $\pm$ 741.05	6940 $\pm$ 878.56	4920 $\pm$ 251.35	5340 $\pm$ 846.95
Platelet	164400 $\pm$ 32053.76	168800 $\pm$ 20926.03	159600 $\pm$ 15901.88	132800 $\pm$ 7982.21
Lymphocytes	66.0 $\pm$ 2.16	60.8 $\pm$ 2.29	66.4 $\pm$ 2.06	63.6 $\pm$ 2.85
Neutrophils	30.0 $\pm$ 2.38	34.8 $\pm$ 1.98	29.8 $\pm$ 1.90	32.2 $\pm$ 2.92
Monocytes	1.80 $\pm$ 0.37	2.0 $\pm$ 0.32	2.2 $\pm$ 0.37	2.2 $\pm$ 0.37
Eosinophil	1.8 $\pm$ 0.37	2.4 $\pm$ 0.39	1.6 $\pm$ 0.39	2.0 $\pm$ 0.45
MCV	60.63 $\pm$ 0.58	60.724 $\pm$ 0.90	63.56 $\pm$ 1.03	61.412 $\pm$ 0.72
MCHC	32.99 $\pm$ 0.24	33.13 $\pm$ 0.18	33.16 $\pm$ 0.46	33.46 $\pm$ 0.26
MCH	20.18 $\pm$ 0.34	20.12 $\pm$ 0.36	21.02 $\pm$ 0.18	20.55 $\pm$ 0.33

WBC = White Blood Cells; RBC = Red Blood Cells; Hb = Haemoglobin; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Haemoglobin; MCHC = Mean Corpuscular Haemoglobin Concentration; PCV = Packed cell volume. Values represent the mean $\pm$ SEM (n=5). There is no significant difference ($p < 0.05$).

Table 6: Impact of sub-acute administration ethyl acetate fraction of *Vernonia amygdalina* on female rat biochemical parameters. Means $\pm$ SEM (n=5) are used to express the results. One-way ANOVA was used to analyse the data, and then the Newman-Keuls post hoc test was used.

Variables	Control 10ml/kg	EAVA 100mg/kg	EAVA 200mg/kg	EAVA 400mg/kg
AST	40.6 $\pm$ 1.16	40.4 $\pm$ 1.36	43.8 $\pm$ 2.22	38.4 $\pm$ 1.39
ALT	30.6 $\pm$ 0.51	29.2 $\pm$ 1.39	31.0 $\pm$ 1.58	29.4 $\pm$ 0.68
ALP	118.4 $\pm$ 3.49	123.6 $\pm$ 5.91	130.2 $\pm$ 2.70	130.6 $\pm$ 4.07
GGT	3.88 $\pm$ 0.28	3.28 $\pm$ 0.24	3.82 $\pm$ 0.31	3.6 $\pm$ 0.19
Creatinine	0.66 $\pm$ 0.05	0.64 $\pm$ 0.02	0.82 $\pm$ 0.07	0.66 $\pm$ 0.04

Values represent the mean $\pm$ SEM (n=5). AST (aspartate aminotransferase), ALP (alanine phosphatase), ALT (alanine aminotransferase), GGT (Gamma-glutamyltransferase). There is no significant difference ($p \leq 0.05$).

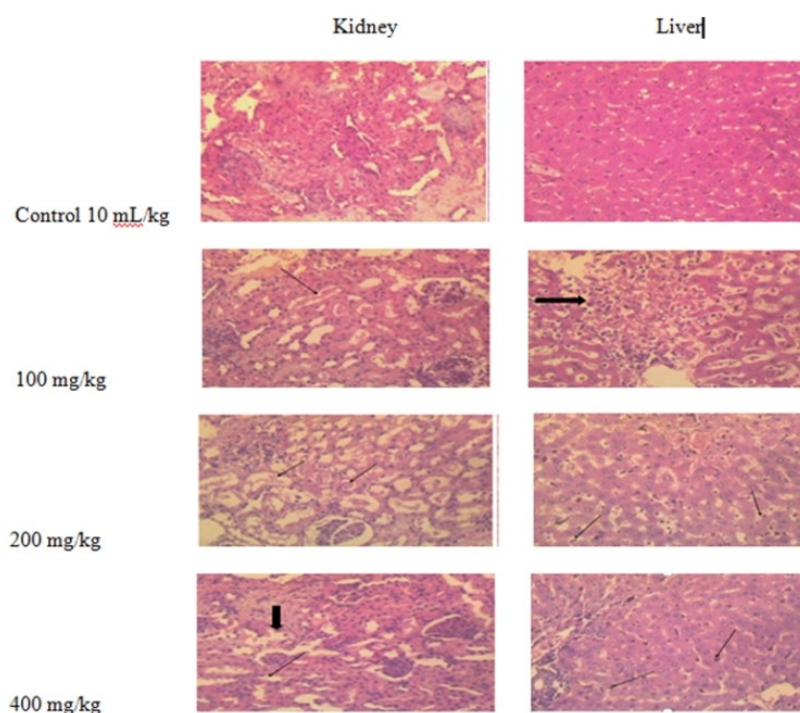


Fig 4. Photomicrograph of sections of female kidneys and livers in control and treated groups (100 X).

4. DISCUSSION

This study investigated the acute and subacute oral toxicity of the ethyl acetate fraction of *Vernonia amygdalina* (EAVA). A single acute oral dose of 2000 mg/kg EAVA administered to mice resulted in no observed adverse effects across both acute (24 hours and 14 days) and sub-acute (28 days with graded doses of 100–400 mg/kg) toxicity tests, no changes in motility, body weight, or organ weight were detected in male or female mice. Furthermore, treated mice and rats exhibited no behavioural changes compared to control groups throughout the study. Behavioural alterations were specifically assessed using the Open Field Test, a standard method for evaluating locomotor activity in animals (Geerse et al., 2006). Despite normal fluctuations in body weight, no deaths occurred during the 28-day sub-acute experiment.

Haematological parameter analysis is crucial for assessing the toxicity of drug substances, including plant extracts (Ibrahim *et al.*, 2016). Changes in the hematopoietic system (blood cell formation) are highly predictive of human toxicity when extrapolated from animal studies (Olson *et al.*, 2000). All blood cells (erythrocytes, leukocytes, and thrombocytes) originate from pluripotent stem cells (Cavanaugh, 2003). In this study, 28-day administration of EAVA to female rats resulted in no significant changes across all blood parameters. The absence of discernible differences between treated and control groups, even at varying doses, indicates

no adverse effects on haematological parameters.

Biochemical parameters, such as aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT), are established biomarkers for predicting potential toxicity. Elevated blood levels of these enzymes typically indicate liver damage associated with hepatocellular injury or membrane dysfunction. In this study, administration of EAVA (100–400 mg/kg) did not significantly alter these liver enzyme levels compared to the control group.

Kidneys regulate blood creatinine, making it a reliable indicator of renal function; elevated levels commonly signify compromised kidney function due to inadequate clearance. This study found no discernible differences in creatinine levels between the treated and control groups, suggesting no adverse impact on kidney function.

Gamma-glutamyltransferase (GGT), primarily found in the kidneys, liver, and pancreas, is a highly sensitive marker for liver disease, particularly biliary obstruction. It often rises earlier and persists longer than other liver enzymes like AST, ALT, and ALP. However, in this study, no significant differences in GGT levels were observed between the treated and control groups.

Histopathological examination revealed mild inflammation in the liver and moderate Kupffer cell hyperplasia in the kidneys of female rats at the 400 mg/kg dose. Conversely, the 100 mg/kg and 200 mg/kg doses of EAVA showed no significant histopathological differences from the control. A significant challenge in traditional medicine is the limited clinical and scientific evidence supporting the safety and efficacy of its preparations, often due to a lack of toxicological and mechanistic evaluations (Saidou *et al.*, 2007). While many plants are not inherently toxic, improper use, especially when combined with other medications or in the presence of contraindications, can lead to serious adverse effects (Silveira *et al.*, 2008). The liver and kidney are among the vital organs in the human body, and are highly susceptible to a wide array of metabolic, toxic, microbial, circulatory, and neoplastic injury (Crawford and Liu, 2010). In most cases, liver and kidney diseases start as a gradual and subtle process in which clinical detection and manifestation could occur weeks, months, or even many years following the onset of injury. Therefore, at the point at which most patients with hepatic and renal dysfunction are referred to clinicians, they already have chronic organ disease (Ugiagbe and Udoh, 2013). Injury associated with the consumption of herbal medicines is referred to as 'herb-induced injury and this injury occurs rarely in only a few susceptible individuals (Pantano *et al.*, 2017).

One of the reasons for the toxicity of the herbal drug (Bitter leaves) is that the cultivation and growth of the plants are inevitably affected by their surrounding environment. Large differences may exist in the compositions of the same kind of medicinal materials from different places of origin. Patients often opt to increase dosages for treatment when the content of effective ingredients is low. In this case, the toxic components ingested by patients also increase, thus resulting in both liver and kidney damage. When the soil, air, and water of the habitat of the *Vernonia amygdalina* plants are polluted, adverse phenomena, such as heavy metal concentrations exceeding standards may occur (Gao and Shi, 2020).

Vernonia amygdalina does not contain heavy metals, but when *Vernonia amygdalina* is cultivated in an industrial-contaminated area, it may contain heavy metals like mercury and lead. The long-term use of contaminated *Vernonia amygdalina* can cause the accumulation of mercury and other heavy metals in the kidneys and liver may cause damage (Wang *et al.*, 2015b). Clinically, acute renal function damage is often caused by the misuse of a large dose of herbal medicine contaminated with heavy metals, and chronic renal function damage is caused by taking a single or compound preparation for a long time as part of an excessive or conventional dose.

Vernonia amygdalina contains a high concentration of alkaloids and other phytochemical constituents. The long-term administration of high doses of total alkaloids can lead to oxidative damage hepatic and renal toxicity in rats (Sun, 2017). This condition may be caused by the large amount of reactive oxygen species (ROS) produced by the body after the drug acts on the kidney, thus directly damaging the kidney cells. The possible mechanism may be as follows: ROS reacts with the macromolecular substances of the cell membrane to form lipid peroxide, thus damaging the oxidative phosphorylation process, making the energy metabolism abnormal, and finally leading to abnormal mitochondrial function, reduced SOD and XOD secretion, cell damage, and apoptosis (Huang and Sun, 2010).

V. amygdalina contains anthraquinone compounds, although not a major constituent, but long-term use of *V. amygdalina* may cause both liver and renal damage under certain conditions, and this was in accordance with a study conducted by Yan *et al.* (2006; Liu *et al.*, 2017; Deng *et al.*, 2018). In another study conducted by Hu *et al.* (2019) reported that the long-term administration of *R. palmatum* L. and its anthraquinone compounds causes certain toxicity to the kidney of mice. The mechanisms underlying its potential toxicity may be the imbalance in the glutathione antioxidant system, the induction of excessive oxidation, the triggering of an inflammatory reaction, the activation of the expression of caspase-3, and the induction of cell apoptosis.

CONCLUSION

Vernonia amygdalina's ethyl acetate fraction demonstrated non-toxicity at both acute and sub-acute exposure conditions based on most behavioural, physical, haematological, and biochemical parameters, although mild hepatic and renal histopathological changes at higher doses warrant further chronic toxicity and mechanistic studies.

REFERENCES

- Abdulkareem, F.B., & Odukoya, O.A. (2016). Sub-acute and chronic toxicity profiles of Markhamia tomentosa ethanolic leaf extract in rats. *Journal of Ethnopharmacol.* 193,68e75.
- Baravalle, C., Salvetti, N. R., Mira, G. A., Pezzone, N., & Orteaga, H.H. (2006). Microscopic characterisation of follicular structures in Leotrozole-induced Polycystic ovarian syndrome in the rat. *Arch. Medical Research.* 37, 830e839.
- Cavanaugh, B. M. (2003). Nurse's Manual of Laboratory and Diagnostic Tests. FA Davis Company, Philadelphia
- Crawford, J.M.; Liu, C. Liver and biliary tract. In Robbins Pathologic Basis of Disease, 8th ed.; Kumar, V., Abbas, A.K., Fausto, N., Aster, J.C., Eds.; W.B. Saunders Co.: Philadelphia, PA, USA, 2010; pp. 833–890.

- Deng, N., Yi, Y., Liang, A. H., Li, C. Y., Zhao, Y., Pan, C., et al. (2018). Mechanism of Nephrotoxicity of Rhubarb in Rats. *China J. Chin. Mater. Med.* 43, 2777–2783. doi: 10.19540/j.cnki.cjcm.20180314.001
- Ekor, M., (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Front. Pharmacol.* 4, 177.
- Geerse, G., Gurp L.C.A., Wiegant V.M., & Stam R. (2006). Individual reactivity to the open-field predicts the expression of Stress-induced behavioural and somatic pain sensitisation. *Behavioural Brain Research* 174, 112–118.
- Guo, X. L., and Li, J. F. (2001). A case of chronic renal failure caused by taking cinnabar. *Pract. Pharm. Clin. Rem.* 4, 139. doi: 10.14053/j.cnki.ppcr.2001.03.039
- Homsy, J.K.E., Kabatesi D., Mubiru F., Kwamya L., Tusuba C., Kasolo S., Mwebe D., Ssentamu L., Okello M., & King R. (1999): Evaluating herbal medicine for the management of malaria
- Hu, Y. F., Huang, W. Y., Li, Y. Q., Luo, Y., Jiang, Q., Liang, Y. S., et al. (2019). Mechanism of Rhein on Renal Toxicity of Mice. *Chin. J. Exp. Tradit. Med. Formulae.* 25, 54–59. doi: 10.13422/j.cnki.syfjx.20190821
- Huang, W., and Sun, R. (2010). Pathological Damage Mechanism of Rats' Nephrotoxicity caused by Alcohol-Extracted Components of Herba Leonuri. *Chin. J. Exp. Tradit. Med. Formulae.* 16, 111–114. doi: 10.13422/j.cnki.syfjx.2010.09.070
- Ibrahim, M. B., Sowemimo, A. A., Sofidiya, M. O., Badmos, K. B., Fagayinbo, M. S., Abdulkareem, F. B., & Odukoya, O. A. (2016). Subacute and chronic toxicity profiles of *Markhamia tomentosa* ethanolic leaf extract in rats. *Journal of Ethnopharmacol.* 193, 68–75.
- Ijeh, I. I., Ejike, C. E. C. (2011). Current perspectives on the medicinal potential of *Vernonia amygdalina* Del. *Journal of Medicinal Plant Research*, 5(7), 1051–1061.
- Liu, P., Wei, H. W., Chang, J. H., Miao, G. X., Liu, X. G., Li, Z. S., et al. (2017). Oral colon-specific drug delivery system reduces the nephrotoxicity of rhubarb anthraquinones when they produce purgative efficacy. *Exp. Ther. Med.* 14, 3589–3601. doi: 10.3892/etm.2017.4959
- Obidike, I., & Salawu, O. (2013). Screening of Herbal Medicines for Potential Toxicities: Pharmacology, Toxicology and Pharmaceutical Science; *New Insight in Toxicity and Drug Testing. Pub. InTech*, pp. 63–67. Chapter 4.
- Ogunmiluyi, O. E., Naiho, A.O., Emojevwe, V. O., Oladele, T. S., Adebisi, K. A., Siyanbade, J. A., & Akinola, A. O. (2024). Zinc/or Vitamin E Supplementation Mitigates Oxidative Stress, Neuroinflammation, Neurochemical Changes and Behavioural Deficits in Male Wistar Rats Exposed to Bonny Light Crude Oil. *J Toxicol.* 2024 Dec 21;2024:9317271. Doi: 10.1155/jt/9317271. PMID: 39734606; PMCID: PMC11681987
- Olson, H., Betton, G., Robinson, D., Thomas, K., Monro, A., Kolaja, G., Lilly, P., Sanders, J., Sipes, G., Bracken, W., & Dorato, M. (2000). Concordance of the toxicity of pharmaceuticals in humans and in animals. *Regul. Toxicol. Pharmacol.* 32 (1), 56–67
- Pantano, F.; Mannocchi, G.; Marinelli, E.; Gentili, S.; Graziano, S.; Busardò, F.P.; di Luca, N.M. Hepatotoxicity induced by greater celandine (*Chelidonium majus* L.): A review of the literature. *Eur. Rev. Med. Pharmacol. Sci.* 2017, 21, 2146–2152.
- Saidou, Y., Bilbis, L.S., Lawal, M., Isezuo, S.A., Hassan, S.W., & Abbas, A.Y. (2007). Acute and subchronic toxicity of crude aqueous extract of *Albizia chevalieri* Harms (Leguminosae). *Asian J. Biochem.* 2, 224–236.
- Silveira, P. F., Bandeira, M. A. M., & Arrais, P. S. D. (2008). Pharmacovigilance and adverse reactions to the medicinal plants and herbal drugs: a reality. *Brazilian Journal of Pharmacognosy* 18, 618–626.
- Sun, X. Q. (2017). Research on Nephrotoxicity of Total Alkaloids of *Leonuri Herba* and Its Mechanism. *Shandong Univer. Tradit. Chin. Med.* 20–45.
- Ugiagbe, E.E.; Udoh, M.O. The histopathological pattern of liver biopsies at the University of Benin Teaching Hospital. *Niger. J. Clin. Pract.* 2013, 16, 526–529.
- Walsh, R.N., & Cummins, R. A. (1976): The open-field test: a critical review. *Psychological Bulletin* 83, 482–504.
- Wang, Y., Wang, D. P., Wu, J., Wang, B. H., Wang, L. J., Gao, X., et al. (2015b). Cinnabar induces renal inflammation and fibrogenesis in rats. *Biomed. Res. Int.* 2015, 280958. doi: 10.1155/2015/280958
- WHO (2003): Assessment and monitoring of anti-malarial drug efficacy for the treatment of uncomplicated falciparum malaria. Geneva.
- Yan, M., Zhang, L. Y., Sun, L. X., Jiang, Z. Z., and Xiao, X. H. (2006). Nephrotoxicity study of total rhubarb anthraquinones on Sprague Dawley rats using DNA microarrays. *J. Ethnopharmacol.* 107, 308–311. doi: 10.1016/j.jep.2006.03.03