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In-vivo sub-chronic toxicological evaluation of extract of *Vernonia glaberrima* leaves in experimental rats

Divinelove E. ABU[1,2a], Jennifer N. TIM[2b], Precious IMO-OBASI[1,2c], Thecla O. AYOKA[3], Charles O. NNADI[2*]

[1]*University of Nigeria Teaching Hospital, Department of Pharmacy, P.M.B. 01129, Ituku-Ozalla 400001 Enugu State, Nigeria; abudivinelove@gmail.com*
[2]*University of Nigeria Nsukka, Faculty of Pharmaceutical Sciences, Department of Pharmaceutical and Medicinal Chemistry, 410001, Enugu State, Nigeria; charles.nnadi@unn.edu.ng (*corresponding author); timjennifer6@gmail.com; obasipresh@gmail.com*
[3]*University of Nigeria Nsukka, Faculty of Physical Sciences, Department of Science Laboratory Technology (Biochemistry Unit), 410001, Enugu State, Nigeria; thecla.ayoka@unn.edu.ng*
[a,b,c]*These authors contributed equally to the work*

Abstract

The toxicological profile of a plant is an important ethnomedicinal parameter considered in drug discovery, ethno-therapeutics and safety management in vital organs. The study evaluated the 28-day repeated-dose toxicological profile of moderately polar extract of *Vernonia glaberrima* being investigated as a therapeutic for sleeping sickness and other parasitic infections in experimental rats. Albino rats were divided into four groups (n=10) and the fraction was administered orally once daily at doses of 0 (control), 40, 250 and 1000 mg kg^{-1} day^{-1}. Food intake, body weight, organ-to-body weight, biochemical, hematological, plasma blood chemistry, serum hormone levels and histopathological examinations were evaluated on the 29th day. There was no difference in the clinical signs between the treated and untreated rats. The mean body weight of female rats treated with 250 mg kg^{1} dose increased significantly ($p < 0.05$) on the 20th day with no difference in the food intake. All the changes in the blood chemistry, hematology and serum hormone levels cannot be considered to be of toxicological importance. A statistically significant elevation ($p < 0.05$) was observed in the liver-to-body weight (3.21 to 3.89 g $100g^{1}$) of male rats and in the kidney- (0.67 to 0.98 g $100g^{-1}$) and brain-to-body (0.58 to 0.72 g $100g^{1}$) weights of female rats in the 1000 mg kg^{1} groups. The histopathological examination showed that there was no definitive association between the lesions in the liver and the treatments. The present data, in addition to folklore, has further demonstrated its safety and the need for the isolation of non-toxic bioactive constituents, especially from the dichloromethane fraction.

Keywords: hematology; histopathology; toxicity; *Vernonia glaberrima*

Introduction

Vernonia glaberrima (Welw. Ex O. Hoffm) belonging to the Asteraceae family, is an erect shrub commonly known as the bitter leaf (Abdullahi *et al.*, 2015b). The leaves are grown in Sub-Saharan Africa for

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consumption in vegetable dishes (Hlashwayo *et al.*, 2020). Due to the bitter taste and the enormous pharmacological properties, the leaves are usually pressed in an aqueous solution to squeeze out the bitterness before consumption. The bitterness has been linked to the phytoconstituents of the leaves. Notwithstanding the bitterness, *V. glaberrrima* is a known nutraceutical and functional food (Abdullahi *et al.*, 2015a).

V. glaberrima has documented ethnopharmacological relevance in traditional African medicine (Hlashwayo *et al.*, 2020). The plant is used to treat malaria, migraine, diabetes, psora and dysmenorrhea in ethnomedicine (Muluye *et al.*, 2021). It is also used to relieve nausea, inflammation, vertigo, microbial diseases, body pain, skin cancer and other conditions associated with the skin (Abdullahi *et al.*, 2015a, 2015b, 2015c; Alhassan *et al.*, 2018; Jega *et al.*, 2020; Wouamba *et al.*, 2020; Hlashwayo *et al.*, 2020; Gangas *et al.*, 2021; Muluye *et al.*, 2021). *V. glaberrima* is also a medicinal plant with strong activity against skin cancer and other lesser activities (Toyang and Verpoorte, 2013; Nasir *et al.*, 2017; Abdullahi *et al.*, 2017).

Several studies have linked one phytochemical constituent or the other to some of the nutraceutical benefits (Nnadi *et al.*, 2021). Our previous study had linked the antitrypanosomal activity against *Trypanosoma brucei brucei* to the moderately polar constituents of *V. glaberrima* (Nnadi *et al.*, 2023). Generally, some phytoconstituents are known for their relatively toxic effects especially at high doses due to the synergism of the constituents or polypharmacological properties that usually disrupts cellular signaling on human cells (Schmidt *et al.*, 2007; Nnadi *et al.*, 2020). *Helenium amarum*, *Hymenoxys odorata*, *Centaurea repens*, *Hymenoxys richardsonii* and *Centaurea solstitialis* are a few examples of plants rich in sesquiterpene lactones and other moderately polar phytoconstituents that have documented evidence of livestock poisoning, neurotoxicity and Parkinson-like equine nigropallidal encephalomalacia (Schmidt *et al.*, 2007; Vanderplanck *et al.*, 2020; Gaston *et al.*, 2020).

Although *V. glaberrima* leaves have been in use as vegetables in traditional African medicine with no documented chronic toxicity, the phytoconstituents are usually squeezed out or boiled to reduce the bitter content during processing (Abdullahi *et al.*, 2015a). This local folkloric approach is believed to reduce the high concentration of phytochemical constituents in the vegetable that has the potential to produce toxic effects. Considering these practices and the investigated potential for the antitrypanosomal activity of a fraction of *V. glaberrima*, we evaluated the safety of moderately polar constituents (dichloromethane fraction) using the 28-day repeated dose sub-chronic toxicity study in experimental rats.

Materials and Methods

Plant material

The leaves of *V. glaberrima* were harvested in Nsukka Nigeria by a taxonomist Mr. Felix Nwafor of the Plant Science and Biotechnology Department of the University of Nigeria in Nsukka Nigeria in April 2021. A voucher specimen (ID: PCG/UN/As0972) was deposited at the herbarium of the Institute of Pharmacognosy and Environmental Medicine of the same University.

Experimental rats

The experimental Wister albino rats weighing 140 ± 35 g of either sex utilized for this study were procured from the animal house of the Department of Veterinary Medicine, University of Nigeria Nsukka. The rats were housed in standard environmental conditions with free access to water and food. The use of rats in this study was reviewed and approved by the University of Nigeria Ethical Committee (UN/FEC/Pharm/2021/000022) for teaching and research only.

Extraction and fractionation

The dried leaves were reduced to a coarse powder (1 kg) and cold-macerated successively twice (2 x 2.5 L) in fresh methanol (95% v v^{-1}) each for 48 h, and the extract was filtered and concentrated under reduced pressure. The yield of the dried extract was 16.8% w w^{-1} of the coarsely powdered sample. An amount of 150 g of the extract was re-dispersed in 200 ml aqueous methanol (10 %v v^{-1}) using magnetic stirring and made up to 500 ml in a separating funnel. The dispersion was successively partitioned in equal volume each of n-hexane (Hex), dichloromethane (DCM), ethyl acetate (EtOAc) and n-butanol (But) to yield Hex (6.2 g), DCM (75.9 g), EtOAc (18.4 g) and But (9.8 g) after evaporation under reduced pressure. The DCM fraction was used for this study following previous findings (Nnadi *et al.*, 2021; Nnadi *et al.*, 2023)

Dosing of rats

Three dose levels were selected for this study based on data from previous studies (Abdullahi *et al.*, 2015b). The highest dose was selected to induce some level of toxicity while the lowest dose level was expected to be a No-Observed-Adverse Effect-Level (NOAEL). The other two doses represented 4 and 25 % of the highest dose level. Thus, the dose levels selected were 1000, 250 and 40 mg kg^{-1} body weight per day. The rats were randomly divided into four groups (n=10), each consisting of males (n=5) and females (n=5) and received DCM fractions of the extract or the blank (dimethylsulphoxide) alone daily orally. The groups were marked as A, B, C and D and were treated with 1000, 250, 40 and 0 mg kg^{-1} day^{-1} DCM fraction respectively.

Toxicity study

A 28-day repeated oral dosing approach was adopted for this study (Schmidt *et al.*, 2007). In this study, day 1 of the study represented the first day of dosing. The experimental rats were allowed free access to feed and water throughout the study. Thereafter, the rats were observed daily to establish the baseline for all the parameters and clinical signs were recorded weekly during the treatment. However, animals were fasted overnight before blood collection on the 29th day and before organ harvest on the 31st day of the study. On the 29^{th} day of administration, the male rats were sacrificed using chloroform anaesthesia. The female rats were sacrificed in the same way 32^{nd}-day of administration.

Observation of clinical signs of toxicity

On the 29^{th} day of treatment, several home-cage observations, in-hand observation and open-field assessment were recorded. On the 4^{th} week of administration, the rats were also examined by manipulative tests (Hong *et al.*, 2021).

Bodyweight and body weight gain

The body weights of the rats were recorded on days 1 and 4 during the seven days of acclimatization, on day 1 of the experiment and two times every week thereafter. The fasted body weight of the rats was also weighed immediately before sacrifice for the calculation of organ-to-body weight ratio (Schmidt *et al.*, 2007).

Food intake and food consumption efficiency

The food consumption of the individual rats was measured on days 1 and 4 during the acclimatization period, on day 1 of the study and two times every week thereafter. The mean food intake and food consumption efficiency for each sex/dose level were calculated during each weekly interval and on days 1-28 treatment interval based on body weight gain and food consumption data respectively.

Clinical pathology examinations

On day 29, blood samples were collected via orbital sinus bleeding of the overnight fasted rats under chloroform anaesthesia for hematology and clinical chemistry examinations using heparin anticoagulant (Cros

et al., 2021; Abebe *et al.*, 2021; Osagie-Iweka *et al.*, 2021). A portion of the blood sample was centrifuged to separate the plasma and the non-haemolysed plasma was used for biochemical examination: Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). The other portion was used for hematological examination such as: red blood cell count (RBC), hemoglobin (Hb), hematocrit (HCT), Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), leucocyte count (WBC), differential leukocyte counts; plasma blood chemistry: total bilirubin (TBi), blood creatinine (TCr), total serum protein (TP), blood urea nitrogen (BUN), total cholesterol (CHOL), albumin (ALB) and serum hormone levels: luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin, estradiol, testosterone, thyroid-stimulating hormone (TSH), triiodothyronine (T3) and thyroxine (T4) (Alam *et al.*, 2021; Bim-Jumah *et al.*, 2021; Tassinari *et al.*, 2021).

Necropsy and histopathological examination

On day 31 of the study, the rats were sacrificed and the gross necropsy of all the rats was recorded. Selected organs: liver, kidney, heart, brain, spleen of all the rats sacrificed were weight wet and thereafter preserved in neutral buffered formalin for possible histopathological examination (Nnadi *et al.*, 2020). The preserved tissues were processed and embedded in molten wax, stained with Mayer's hematoxylin and eosin and examined histopathologically on a Motic™ 5.0 megapixels microscope camera at x100 and x160 magnifications.

Results

Clinical observation

All the rats treated with 0, 40, 250 and 1000 mg kg^{-1} of DCM fraction of *V. glaberrima* were examined for treatment-related toxicities. There was no difference in the home-cage signs (convulsion, abnormal movements and posture), in-hand observation (response to handling, skin condition, salivation, lacrimation and abnormal breathing), open field assessment (gait, arousal, tremor, urine and fecal excretion) and manipulative signs (auditory, vision, touch and nociception) between the treated and untreated rats.

Bodyweight and body weight gain

For male and female rats treated with 40, 250 and 1000 mg kg^{-1} of DCM fraction, the mean body weight and the corresponding gain were comparable with the untreated (Figure 1(I)). On the 20^{th} day, the average body weight of female rats treated with 250 mg kg^{-1} dose increased significantly ($p < 0.05$) compared with the untreated group.

Food intake and food consumption efficiency

There was no significant difference in the overall food intake and consumption efficiency of both male and female rats treated with 40, 250 and 1000 mg kg^{-1} DCM fractions compared with the untreated. However, the average food consumption by the female rats was significantly higher compared with the male rats (Figure 1(II)). Some differences in the average consumption were not considered to be of significant toxicological importance.

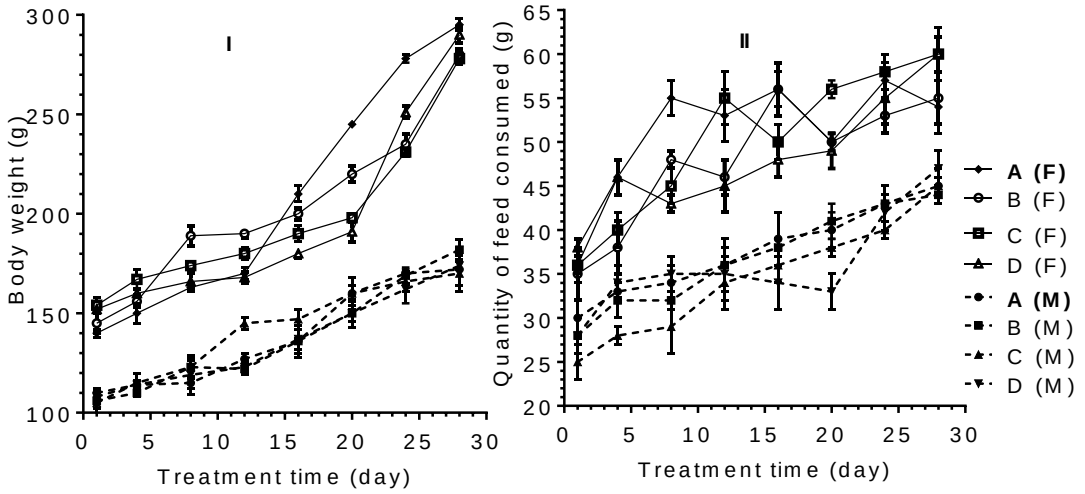

Figure 1. Variation of Mean body weight (I) and Mean food consumption (II) with time; male (M), female (F); *$p < 0.05$ (significant difference from the control group); values are mean ± SEM, Group A (controls)

Blood chemistry, hematology and serum hormone levels

Blood samples of treated rats were used to determine the biochemical, hematological, blood chemistry and hormone level parameters (Table 1). There were no toxicological effects on liver enzymes activities. There was a dose-dependent surge in AST activity in female rats, though cannot be considered to be of toxicological importance. Apart from platelet count that showed a dose-dependent increase, all other hematological parameters did not show any defined trend or treatment-related toxicity. The results of blood chemistry showed that there was no statistical significance ($p < 0.05$) effect in all blood chemistry parameters. The slight changes observed were unrelated to the administered doses and could not be considered to be related to the treatments. The effects of *V. glaberrima* on sex hormone levels appear to be more dose-dependent. FSH and TSH were significantly higher in male rats of group D. Prolactin was significantly upregulated in both male and female rats of groups C and D. In male rats, LH was significantly increased in the 1000 mg kg^{-1} group and T4 was significantly increased in the female group D.

Table 1. Mean clinical-pathological parameters

Parameters	Male				Female			
	A	B	C	D	A	B	C	D
AST (iuL^{-1})	12.1±0.1	11.9±0.2	11.2±0.2	12.9±0.1	11.2±0.3	11.8±0.5	13.1±0.2	13.6±0.4
ALT (iuL^{-1})	10.7±0.1	11.6±0.2	12.2±1.1	11.8±0.8	12.7±0.2	12.4±02	13.1±0.3	12.8±0.7
ALP (iuL^{-1})	56.1±0.2	62.3±1.2	58.6±2.1	65.2±2.4	59.9±0.2	51.8±1.7	64.2±0.1	60.2±1.5
RBC ($x10^{6}\mu L^{-1}$	8.3±0.4	8.2±0.9	8.4±0.1	8.0±0.1	9.2±0.2	8.6±0.6	8.1±0.7	8.2±0.2
Hb ($g\ dL^{-1}$)	32.9±0.2	38.1±0.4	35.0±0.2	33.9±0.3	36.2±0.1	39.1±0.4	36.0±0.5	31.8±0.7
HCT (%)	48.9±0.3	50.1±1.2	45.9±2.1	43.9±0.4	49.7±0.3	45.2±0.8	43.2±0.8	42.6±0.1
MCV (fL)	54.2±0.3	55.8±1.2	59.3±0.3	56.3±0.3	55.8±0.2	52.9±1.7	58.2±0.5	57.2±0.5
MCH (ρg)	20.9±0.1	18.5±0.3	21.0±0.4	18.8±04	19.8±0.3	18.5±0.4	19,4±0.2	18.1±0.6
MCHC ($g\ dL^{-1}$)	33.9±0.1	32.2±0.4	36.8±1.4	38.1±1.6	33.9±1.4	35.2±0.8	36.1±0.6	34.9±0.3
PLT ($x10^{3}\ \mu L^{-1}$)	514±23	567±53	589±87	653±75	548±35	558±87	582±93	635±11
WBC ($x10^{3}\ \mu L^{-1}$)	6.2±0.2	6.2±0.1	6.7±0.8	6.9±0.3	5.8±0.2	6.0±0.5	6.7±0.3	6.8±0.3
Neutrophil (%)	55.9±0.1	54.0±1.1	58.2±2.2	56.8±0.9	58.0±0.2	54.8±0.2	54.1±1.4	55.4±0.9
Lymphocyte (%)	30.0±0.2	33.1±0.4	35.1±0.3	38.3±0.4	34.2±0.5	38.3±0.3	37.2±0.3	40.6±1.1
Monocyte (%)	2.8±0.6	2.4±0.1	2.2±0,0	2.3±0.3	2.4±0.9	2.3±0.1	2.3±0.4	1.5±0.3
Eosinophil (%)	2.2±0.4	1.8±0.2	2.0±0.4	2.5±0.5	2.3±0.3	2.6±0.3	2.6±0.2	2.9±0.1

TBi (x10^{-1}mg dL^{-1})	1.1±0.2	1.3±0.0	1.2±0.2	1.4±0.2	1.0±0.3	1.2±0.3	1.1±0.2	1.4±0.1
TCr (x10^{-1}mg dL^{-1})	2.5±0.2	2.8±0.1	3.0±0.2	2.6±0.2	3.0±0.4	2.6±0.2	2.8±0.1	2.8±0.3
TP (g dL^{-1})	6.0±0.2	5.9±0.2	5.8±0.4	6.2±0.1	6.3±0.3	6.2±0.3	6.4±0.1	6.6±0.3
BUN (mg dL^{-1})	11.1±0.3	10.3±0.3	11.4±0.3	11.8±0.5	12.0±0.2	11.3±0.3	10.8±0.2	10.5±0.3
CHOL (mg dL^{-1})	52.7±3.6	65.2±7.4	53.6±0.9	57.1±9.1	60.6±1.1	66.5±1.2	58.8±9.2	48.2±1.2
ALB (g dL^{-1})	3.8±0.1	3.3±0.2	3.8±0.6	2.5±0.5	3.4±0.3	3.6±0.7	3.2±0.7	3.6±0.9
LH (ng mL^{-1})	19.2±2.1	17.6±0.2	16.7±0.9	11.6±0.3*	16.2±0.3	14.3±0.1	13.7±0.5	12,1±0.8
FSH (ng mL^{-1})	180±45	189±29	200±34	267±38*	167±29	190±96	168±26	178±29
Prolact (ng mL^{-1})	46.1±0.2	83.2±2.2	208±12*	188±34*	25.8±0.3	34.8±0.4	48.2±0.9	66.8±0.2
Estradi (ρg mL^{-1})	189±23	201±10	178±90	220±67	189±38	193±30	206±62	209±59
Testost (ng mL^{-1})	6.6±0.2	6.3±0.3	5.2±0.2	3.1±0.3	0.7±0.2	1.1±0,1	1.0±0.5	12.2±0,.2
TSH (ng mL^{-1})	9.4±0.2	12.6±0.3	12.3±0.9	15.2±0.6*	11.9±0.3	12.9±0.1	11.8±0.3	13.6±0,3
T3 (ng mL^{-1})	0.9±0.3	1.1±0.1	1.1±0.2	1.3±0.2	1.2±0.3	1.4±0.2	1.5±0.3	1.7±0.1
T4 (μg dL^{-1})	4.3±0.1	4.8±0.2	5.2±0.5	5.4±0.3	4.7±0.1	5.0±0.5	5.6±0.3	6.0±0.1*

*$p < 0.05$ (significant difference from the control group); values are mean ± SEM

Organ weight and organ-to-weight

The mean relative weight (organ-to-body weight) for the liver, spleen, heart, kidney and brain were comparable to the controls with few exceptions (Table 2). There was statistically significant rise ($p < 0.05$) in the liver-to-body weight of male rats, the kidney- and brain-to-body weights of female rats in group D.

Table 2. Body and relative organ weights post-treatment with *V. glaberrima*

Organ	Male, Mean body-to-organ weight (g 100g^{-1})				Female, Mean-organ-to-body weight (g 100g^{-1})			
	A	B	C	D	A	B	C	D
BW	172±8	182±15	170±9	172±12	295±23	280±13	278±23	290±14
Liver	3.21±0.01	3.48±0.06	3.57±0.11	3.89±0.12*	0.38±0.11	0.40±0.01	0.45±0.03	0.46±0.11
Kidney	0.66±0.12	0.94±0.12	0.63±0.01	0.72±0.11	0.67±0.04	0.66±0.11	0.66±0.03	0.98±0.02*
Heart	0.17±0.03	0.19±0.00	0.17±0.02	0.18±0.02	0.22±0.04	0.19±0.03	0.18±0.09	0.20±0.05
Brain	0.58±0.01	0.62±0.04	0.64±0.03	0.68±0.07	0.58±0.01	0.66±0.02	0.68±0.02	0.72±0.09*
Spleen	0.22±0.01	0.27±0.02	0.17±0.09	0.18±0.03	0.39±0.04	0.26±0.04	0.18±0.06	0.29±0.07

*$p < 0.05$ (significant difference from the control group); values are mean ± SEM; body weight (BW)

Histopathology

The histopathological examinations of the liver showed no gross lesions in the rats in all the treatment groups (Figure 2). However, in one rat from groups C (male) and D (female), the liver showed vacuolar degeneration and necrosis of the hepatic cells in the centrilobular areas of the hepatic lobules. The affected hepatocytes appeared swollen, with clear vacuolated cytoplasm and pyknotic nuclei. (Group C). Among group D rats also, the liver showed marked vacuolar degeneration and necrosis of the hepatic cells in the centrilobular areas of the hepatic lobules. The affected hepatocytes appeared swollen, with clear vacuolated cytoplasm and pyknotic nuclei. These changes were not observed in other rats in the same groups. There was no definitive association between the lesions observed in the liver and the *V. glaberrima* dosing.

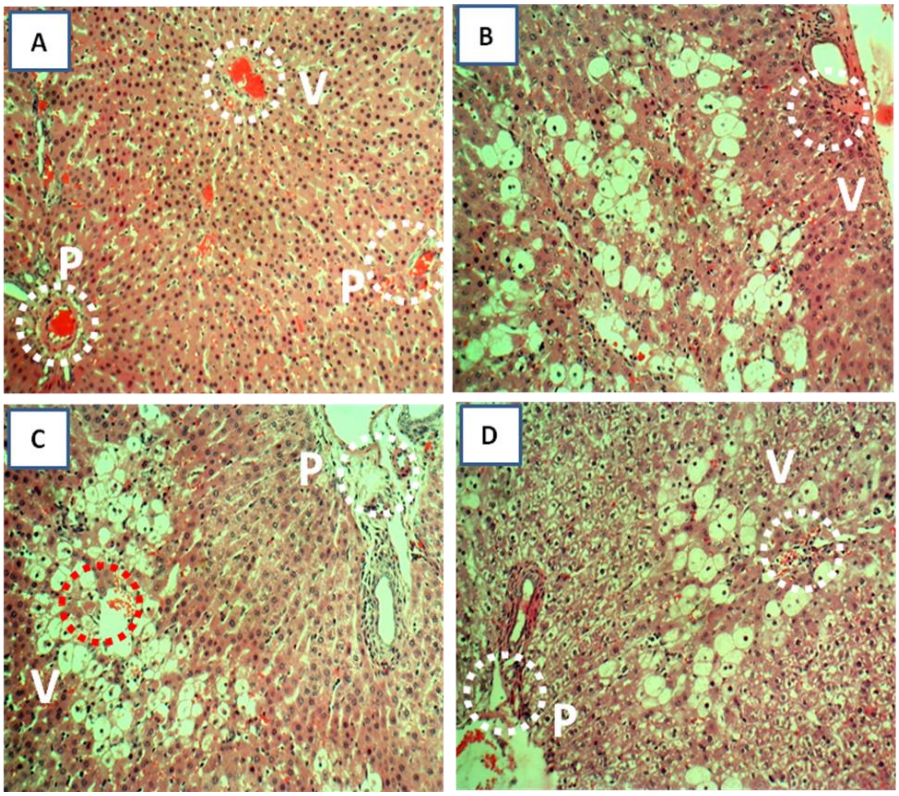

Figure 2. Liver sections of rats treated with *V. glaberrima*; groups A, B, C and D. Portal triads (P); central vein; H & E x 160

Discussion

Many locally available plants in Africa have been of great importance to man since time immemorial for the management of several ailments with or without the actual knowledge of their potential adverse effects. The increasing interest in herbs and herbal products demands that basic toxicological information on the various plant preparations used in the management of diseases should be known. The study evaluated the sub-chronic toxicity potential of repeated doses of a DCM fraction of *V. glaberrima* in rats recently investigated for its antitrypanosomal activity against *Trypanosome brucei brucei*. The understanding of the potential toxicities of the plant on several organs such as the brain, liver, kidney, heart and spleen is vital in the further discovery and development of the bioactive constituents of the plants thereby complementing other known pharmacological and/or ethnomedicinal properties of *V. glaberrima* (Toyang and Verpoorte, 2013; Abdullahi *et al.*, 2015a, 2015b, 2015c; Nasir *et al.*, 2017; Abdullahi *et al.*, 2017; Alhassan *et al.*, 2018; Jega *et al.*, 2020; Wouamba *et al.*, 2020; Hlashwayo *et al.*, 2020; Gangas *et al.*, 2021; Muluye *et al.*, 2021).

Several attempts have been made to understand the toxicity of *V. glaberrima* whole extract considering the consumption level in sub-Saharan Africa. However, these studies have not explored the moderately polar constituents which constitute the bulk phytoconstituents of the plant. A 28-day repeated administration of 40, 250 and 1000 mg kg^{-1} extract to rats elicited no significant adverse toxicological effects on the body weight, food intake and efficiency, clinical pathology, hematology, necrosis and histopathology of the rats attributable to the treatments. Although varied toxicological effects were observed in a few groups of rats treated with the low and mid-doses of extract, these responses were not seen in rats administered with the high doses (1000 mg kg^{-1}) of *V. glaberrima* suggesting that the few observed adverse toxicological effects were not effects of the treatment.

It is imperative to note that the treatments did not cause mortality or any sign of chronic toxicity in the rats dosed for 28 days. All the home-cage, in-hand, open field and manipulative signs recorded did not show any toxicological significant difference with the controls. Apart from the female rats treated with 250 mg kg^{-1} that showed significant weight gain on the 20th day, other rats even at 1000 mg kg^{-1} dose elicited no change in weight, weight gain, or food consumption when compared with the controls.

It is a known fact that kidney impairment may lead to a severe depletion of electrolytes such as Na, K, Ca, Cl and phosphates (Caglayan *et al.*, 2021; Abdel-ghaffar *et al.*, 2021). The overall findings showed that *V. glaberrima* maintained the liver enzyme activities and kidney function biomarkers at the normal physiological baselines. ALP is a biomarker enzyme for the plasma membrane and endoplasmic reticulum which are employed to assess the integrity of the plasma membrane. In addition to the ALP, AST and ALT are also localized in the hepatic cells and are usually pumped into the blood plasma following liver compromise, damage or injury (Bim-Jumah *et al.*, 2021). The no-observed-significant alteration in the AST and ALP levels indicates the potential of this plant to maintain liver integrity, even if it is damaged which agreed with the reports on the ameliorative effect of *V. species* on hepatic function (Barnes *et al.*, 2020). The lack of significant alteration in the hepato-renal biomarkers which support good indicators of hepatic and renal functions, suggests that repeated administration of *V. glaberrima* did not alter hepatocytes and kidneys of rats or the normal metabolism of the rats.

The fluctuations in the plasma blood chemistry parameter: TBi, TCr, TP, BUN, CHOL and ALB did not follow any known trend and could not be attributed to the treatments. However, it is known that most bilirubin is a product of hemoglobin and other hemoproteins breakdown. High plasma bilirubin levels result in jaundice due to the accumulation of bilirubin or its conjugates in body tissues. Creatinine is a metabolite of muscle creatinine, which serum concentration varies with the body's muscle mass. The amount is usually constant so that elevated levels indicate diminished renal function only since it is easily excreted by the kidneys (Achuba and Nwokogba, 2015). Rising serum creatinine and urea is an established indicator of poor glomerular filtration and has been established as a significant clinical marker for kidney dysfunction and loss of kidney integrity (Cros *et al.*, 2021; Abebe *et al.*, 2021; Osagie-Iweka *et al.*, 2021)

The hematopoietic system is one of the most sensitive targets of toxic compounds and is also a vital index of physiological and pathological status in animals. Apart from the dose-dependent elevation in PLT, all other hematological parameters did not show any defined trend or treatment-related toxicity. There were also no dose-dependent or treatment-related changes in all the hematological parameters.

Apart from the hepato-renal and hematological indicators, organ weight variations are other parameters that indicate the pathological conditions in rats. In this study, some organ weight and organ-to-bodyweight in the treatment groups were comparable to control groups with a few exceptions. The liver-to-body weight of male rats, kidney- and brain-to-body weights of female rats treated with 1000 mg kg^{-1} *V. glaberrima* extract were statistically significant ($p < 0.05$) with the control. However, these effects were not significant in the liver-to-body weight of female rats or the kidney- and brain-to-body weights of male rats. It was, therefore, not possible to attribute these findings to any toxicological importance since the integrity of liver and kidney parameters were not altered correspondingly.

To reconcile these data, especially where the present data do not provide definite information about the toxicological effect of *V. glaberrima*, a histopathological examination of the liver was carried out. Apart from a male rat dosed with 250 mg kg^{-1} and a female rat dosed with 1000 mg kg^{-1} that exhibited vacuolar degeneration and necrosis of the hepatocytes in the centrilobular areas of the hepatic lobules, other rats showed clear vacuolated cytoplasm and pyknotic nuclei with no definitive evidence to associate the lesions in the liver with the treatments.

Conclusions

The leaves of *V. glaberrima* have a historical use as vegetables across Africa. Our study indicated no *V. glaberrima*-related adverse toxicological events and non-toxic in rats with a no-observed-adverse-effect level (NOAEL) of 1000 mg kg^{-1} day^{-1} when dosed orally for 28 days. The present data, in addition to folklore, has further demonstrated its safety and the need for the discovery of non-toxic bioactive constituents, especially from the DCM fraction. This is currently ongoing.

Authors' Contributions

Conceptualization: CON and TOA; Data curation: DEA, JNT and PI; Formal analysis: DEA, JNT and PI; Supervision: CON and TOA; Writing - original draft CON and TOA; Writing - review and editing: CON. All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

The use of rats in this study was reviewed and approved by the University of Nigeria Ethical Committee (UN/FEC/Pharm/2021/000022) for teaching and research only.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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