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CHEMICAL COMPOSITION AND HISTOLOGICAL EFFECTS OF HYDRO-ALCOHOL ROOT EXTRACT OF *Rauwolfia vomitoria* AFZEL, (APOCYNACEAE) IN ALBINO WISTAR RATS

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ABSTRACT

Herbal recipes containing *R. vomitoria* Afze (Apocynaceae) and its extracts are used in herbal medicine for the treatment of several ailments including sickle cell disease. The study evaluated chemical composition, and effects of hydro-alcohol extract of *R. vomitoria* root (HAE) on body weight and cellular architecture of target organs in albino wistar rats. High performance liquid chromatography fingerprinting of HAE was carried out, followed by subacute toxicity testing and weight monitoring of study animals at 125, 250, and 500 mg/kg/day of HAE for 14 days. Alkaloids, flavonoid, phenolics and saponins were present in HAE. The repeated administration of HAE at 250 and 500 mg/kg/day was associated with significant ($p<0.05$) reduction of body weight. Histologically, all tested doses increased blood flow to all the organs compared to the control, and without abnormality in cellular architecture.The HAE appears safe at low doses but repeated administration of high doses needs further safety evaluation.

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KEYWORDS

Rauwolfia vomitoria, chemical composition, histological, toxicity.

INTRODUCTION

The world population is expected to reach approximately 8.2 billion by the year 2025, out which 6.5 billion individuals are dependent on medicinal plants for their diverse bioactive compounds for the prevention and management of febrile illnesses [1]. The bioactive compounds contained in medicinal plants possess useful therapeutic properties, as well as harmful effects, thus may elicit adverse herbal/drug reactions especially if not used appropriately [2]. *Rauwolfia vomitoria* is an evergreen perennial shrub whose root extract has been found useful in folklore medicine as treatment for snake bites, fever, insect stings, mania, epilepsy, hypertension, amongst other uses [3]. The reported use of herbal remedy containing the root of *Rauwolfia vomitoria* and its possible use as antisickling agent [4] necessitates further investigation of its toxicities in target organs of albino wistar rats, since its use as antisickling agent may involve chronic administration.

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MATERIALS AND METHODS

The plant material

The roots of *R. vomitoria* were collected from the Botanical Garden of University of Ibadan, Nigeria. The specimen was authenticated by a plant scientist at National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja and assigned a voucher number – NIPRD/H/6715. The voucher specimen was deposited in the herbarium of NIPRD, Idu, Abuja for future reference. The *R. vomitoria* roots were carefully cleaned, air-dried under shade, and ground to coarse powder using mortar and pestle before further pulverisation with an electric blender. The powdered root was extracted in a mixture of solvents consisting of distilled water and 95% ethanol according to methods already described in the literature [4] to obtain a hydro-alcohol extract (HAE) which was used for the experiment.

Experimental animals

Albino Wistar rats weighing 168 ± 29 g (Mean ± SD) of equal sexes were obtained from the Animal House of the Faculty of Pharmacy, University of Benin, Benin-City, Nigeria. The animals were kept in standard plastic cages at room temperature, exposed to natural lighting, and had free access to water and feeds (Livestock Feeds Ltd, Ibadan, Nigeria) according to international protocols for use of laboratory animals in experiments [5] Ethical approval for the experiments was obtained (EC/FP/021/01) from the Ethical Committee, Faculty of Pharmacy, University of Benin, Nigeria.

HPLC Analysis of HAE

Equipment

A Shimadzu HPLC system equipped with photodiode array detector (Kyoto, Japan), Auto-sampler (SIL-20A), Solvent delivery pump (LC-8518), High-pressure switching valve, UV detector (LC-8518), and N2000 chromatography software.

Column: RP-C18 column (150 mm X 4.6 mm) packed with 5 μm diameter particles.

Reference Compounds

Quercetin, kaempferol, isorhamnetin, ascorbic acid, gallic acid, catechin, methyl-gallate, caffeic acid, syringic acid, ellagic acid, chlorogenic acid, nicotine, strychnine, rutin, quinine, caffeine, pyrogallol, tannins, and saponins.

Preparation of reference compounds and HAE samples for HPLC analysis

An amount of each reference compound (0.001 g) (Table 1) was vigorously shaken with 10 ml of 70% methanol for 10 min using vortex mixer and the mixture sieved into the 5 ml sample bottle using a Cosmonice Filter.

A little amount of HAE (0.1 g) was mixed with 10 ml of 70% methanol and the mixture allowed to stand for one hour in a covered test tube. The supernatant liquid was decanted, centrifuged and filtered into a 5 ml sample bottle using a Cosmonice Filter. Table 1 shows the chromatographic conditions.

Table 1: Chromatographic conditions for HPLC analysis

PHYTOCHEMICAL GROUP	MOBILE PHASE	COLUMN TEMPERATURE	DETECTOR UV WAVELENGHT	RETENTION TIME
Flavonoids	Acetonitrile/Water/Formic acid (25:74:1)	40ºC	450 nm	12 min
Alkaloids	Methanol/Acetonitrile/Water (70:20:10)	35ºC	340 nm	7 min
Saponins	Acetonitrile/water (70:30)	40ºC	230 nm	6 min
Phenolics	Acetonitrile/Water/Acetic acid (19:80:1)	40ºC	372 nm	6 min

The injection volume was 40 μL, the flow rate was 0.6 mL/min, and the mobile phase was filtered through a membrane filter (0.45 μm) and then degassed by an ultrasonic sound before use.

The HAE and reference compound samples were prepared in the same mobile phase of HPLC, while the standard calibration curves were generated in the concentration range of 0.0125 to 0.200 mg/ml.

Handling of animals

The experimental rats were randomly distributed into four groups of eight rats per group (4 males and 4 females). The rats in group A were given 0.5 mL of 10% Tween 80 daily via the oral route while rats in groups B, C, and D were orally administered 125, 250, and 500 mg/kg/day of HAE respectively for fourteen days. All rats were monitored for changes in body weight. All the rats that survived were fasted overnight and then sacrificed on day 15.

Histology

The target organs namely the brain, heart, lungs, liver, spleen and kidneys from each group of rats including those that died before end of experiment were removed, placed on filter paper for 10 min before weighing [6]. The weighed organs were fixed in 10%v/v formaldehyde in saline solution for 48 h after which they were embedded in paraffin wax. The tissues were completely dehydrated in graded concentrations of ethanol. Sections (5 μm thick) were prepared and stained with haematoxylin and eosin dyes (H&E) and viewed under a light microscope (Olympus, Germany) at x100 magnification [7] by a histopathologist. Photomicrographs were prepared using Aekins Microscopic Camera UK [8].

Analysis

The chromatographic peaks were confirmed by comparing its retention time with those of reference standards and quantification was performed by peak integration using the external standard method [9]. The photomicrographs of all investigated doses were compared with the controls in all the target organs and the changes in cellular architecture was qualitatively noted, and documented. All computable measurements are stated as the mean ± standard error of mean (SEM). Relationship between groups was examined using one-way analysis of variance followed by Tukey's *post hoc* test. Data analysis was performed using GraphPad Prism software version 6 (GraphPad Prism, USA). $P < 0.05$ indicates statistically significant difference [4].

Statistical Analysis

The chromatographic peaks were confirmed by comparing its retention time with those of reference standards and quantification was performed by peak integration using the external standard method [9]. The photomicrographs of all investigated doses were compared with the controls in all the target organs and the changes in cellular architecture was qualitatively noted, and documented. All computable measurements are stated as the mean ± standard error of mean (SEM). Relationship between groups was examined using one-way analysis of variance followed by Tukey's *post hoc* test. Data analysis was performed using GraphPad Prism software version 6 (GraphPad Prism, USA). $P < 0.05$ indicates statistically significant difference [4].

RESULTS

HPLC Analysis

The result of the HPLC analysis confirmed the presence of alkaloids, flavonoid, phenolics and saponins. Alkaloids identified include nicotine, caffeine, and strychnine. The flavonoids consist of caffeic acid, quinic acid, rutine, quarcetin, chlorogenic acid, and Salicylic acid. HPLC fingerprinting also identified ascorbic acid, gallic acid, catechin, rutin, ferrulic acid, and apigenin. The saponin appeared as a distinct peak on the chromatogram while other thirteen compounds in this group of biomolecules though appearing with different retention times, area under curve, and concentration, their peaks were not too distinct (Figures 1-4).

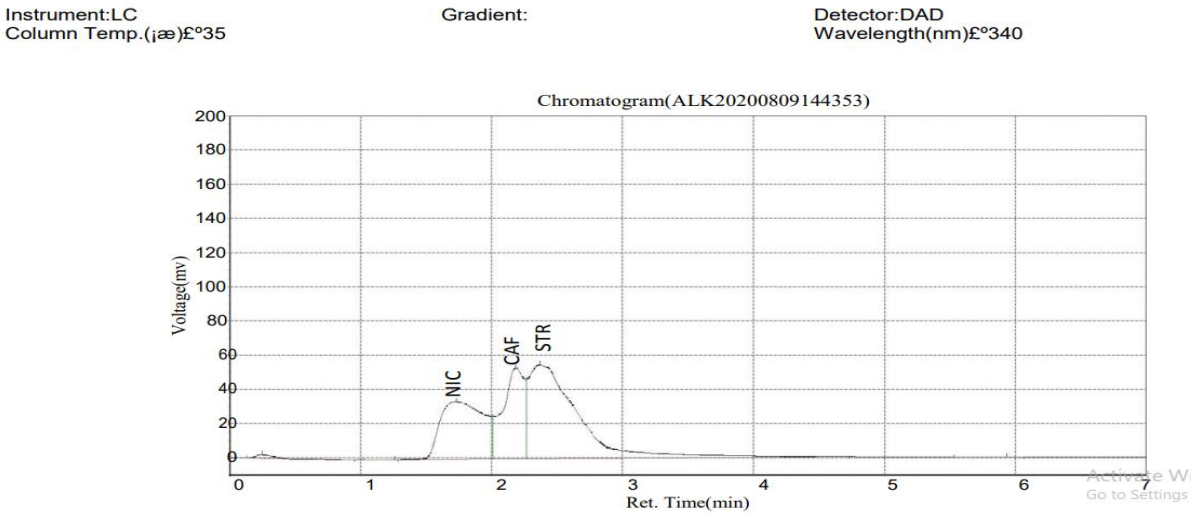

Figure 1: Chromatogram of alkaloids components of hydro-alcohol extract of *R. vomitoria* root.

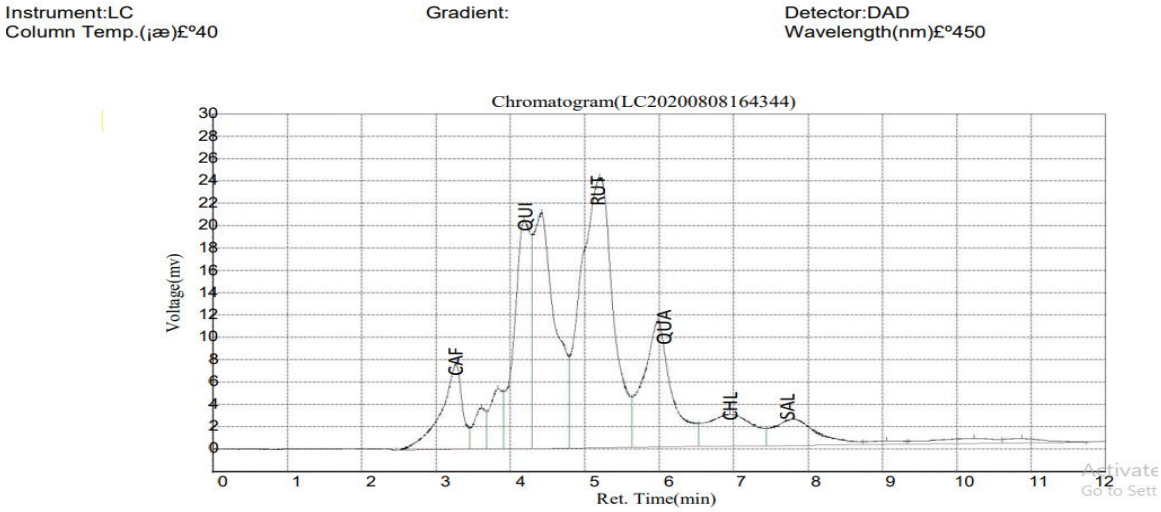

Figure 2: Chromatogram of flavonoids content of hydro-alcohol extract of *R. vomitoria* root.

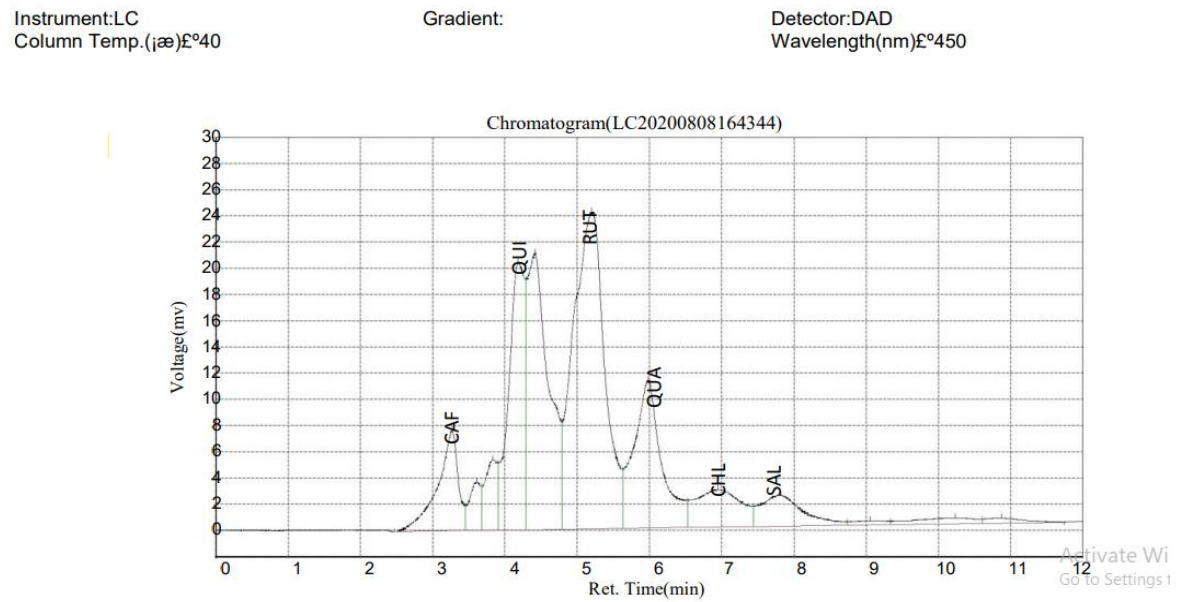

Figure 3: Chromatogram of phenolic components of hydro-alcohol extract of *R. vomitoria* root

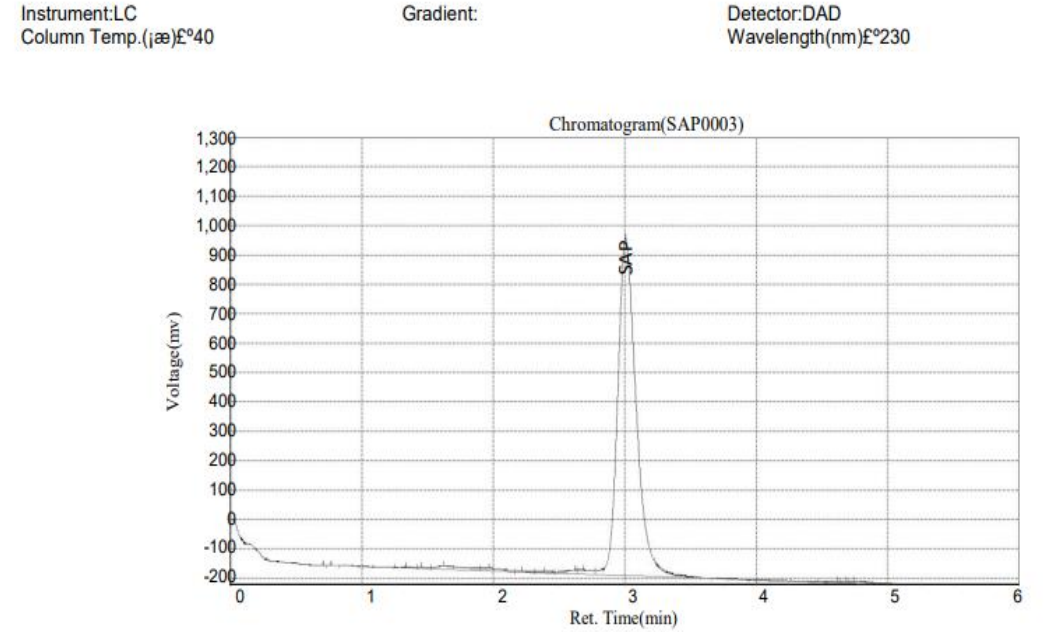

Figure 4: Chromatogram of saponin content of hydro-alcohol extract of *R. vomitoria* root

Effect of HAE on body weight

As shown in Figure 5, there was significant ($p < 0.05$) body weight reduction on the eighth and eleventh day in the group of rats that received 500 mg/kg/day of HAE. The rats that received 250 mg/kg/day of HAE also showed significant body weight reduction on days 11 and 14. All the rats that received 125 mg/kg/day did not experience a significant change in body weight throughout the duration of the experiment.

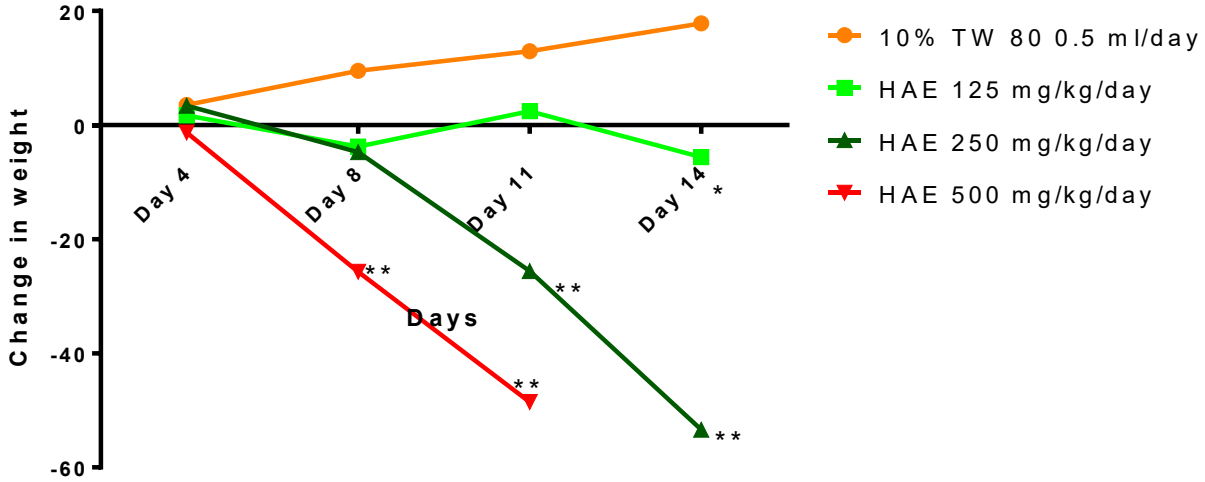

Figure 5: Changes in weight of rats given daily doses of hydro-alcohol extract (HAE) of *R. vomitoria*. *p < 0.05, **p < 0.005. (TW: Tween 80; n = 6 – 8**;** 500 mg/kg/day group was terminated on day 11)

Histological findings in organs

The Liver: Figure 6 shows the histopathological effects of HAE on rat liver. The hepatocytes and sinusoids in all the treated groups and control were normal with no sign of injury or damage e.g., fatty liver, necrosis and hepatitis. Activation of Kupffer cells was observed in all the treated groups and control. However, all animals in the 500 mg/kg/day group exhibited less florid activation of Kupffer cells compared to other treated groups. There was increased blood flow and dilatation of the portal vein in all the treatment groups.

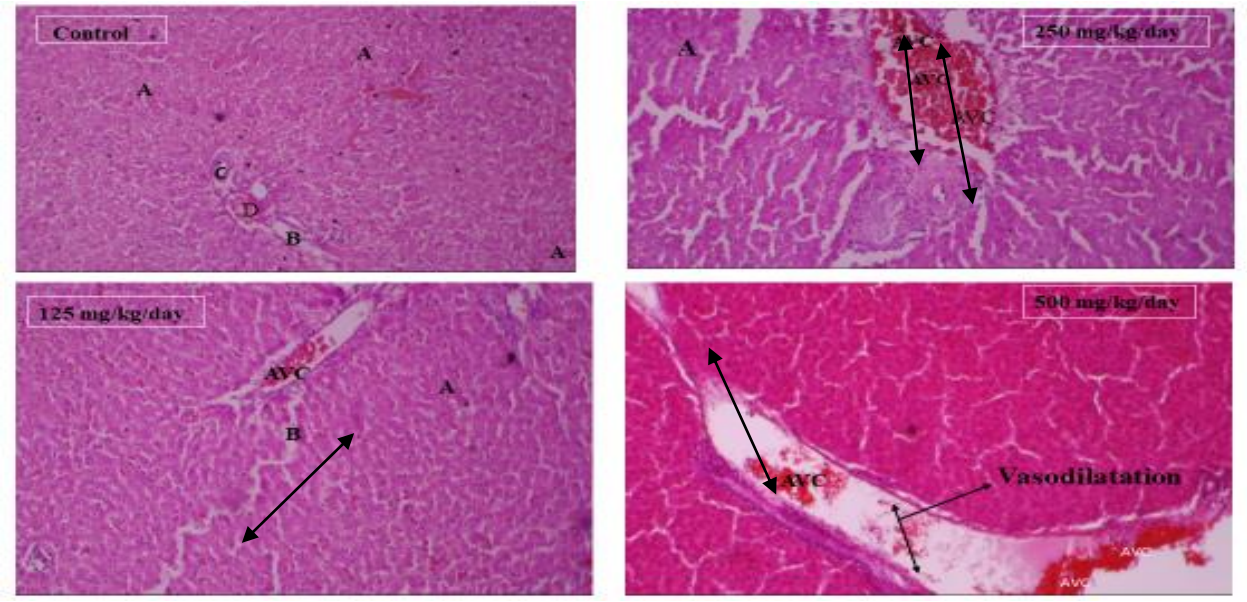

Figure 6: Photomicrographs of liver from rats treated with HAE of *R. vomitoria* for 14 days. A: hepatocytes, B: sinusoids, C: bile duct, D: portal vein, AVC: active venous congestion. H&E x100. The arrows depict the progression of the vasodilation.

The Heart: Histological assessment of the heart did not reveal any abnormality in the architecture of the cardiac fibers, coronary arteries and interstitial spaces (Figure 7). There was no sign of myocardial infarction or ischaemia. While HAE induced vascular hypertrophy in the rats given 250 mg/kg/day, signs of active interstitial and vascular congestion were seen in the rat given 125 and 250 mg/kg/day showing

increased blood flow to the heart. There were patent coronary arteries at 250 and 500 mg/kg/day.

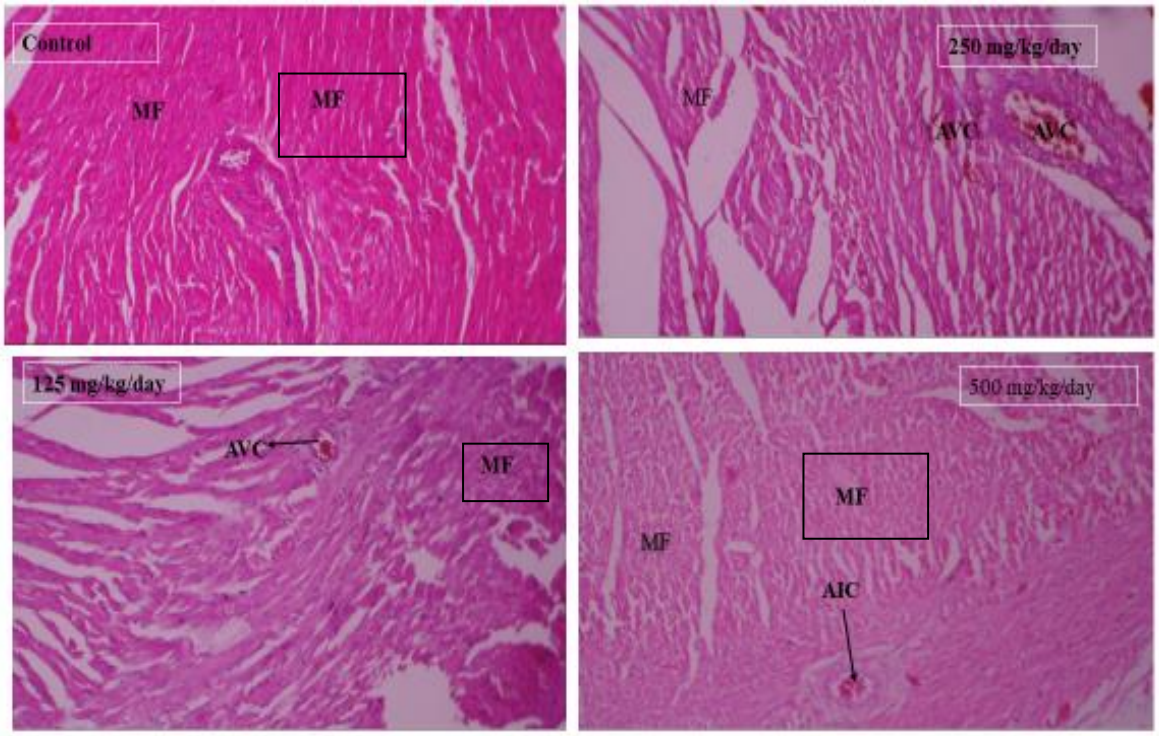

Figure 7: Photomicrographs of heart from rats treated with HAE of *R. vomitoria* for 14 days. AIC: active interstitial congestion, AVC: active venous congestion, MF: normal myocardial fibres. H&E x100

The Lungs: There was remarkable dilation of the bronchi, expansion of the alveoli and active interstitial congestion in all the HAE-treated group but there was no obvious abnormality (Figure 8). There was increased blood flow in the group given 250 mg/kg/day and activation of the immune system in group that received 125 mg/kg/day (Figure 8).

The Kidney: The architecture of renal corpuscles and tubules of the kidneys was normal in all the treated groups and control but there was increased blood flow due to vasodilatation in all the treated groups which was not seen in the control (Figure 9).

The Spleen: Figure 10 showed that HAE induced vascularization of the splenic tissues and activation of the lymphoid follicle, red and white pulp receiving increased boost from the 125 to 500 mg/kg/day groups compared to the control but optimal follicular activation occurred at 125 mg/kg. There was marked dilatation of splenic arteriole in group D animals (500 mg/kg/day).

The Brain: The molecular, Purkinje and granular layers of the cerebellum were well outlined and normal in all the groups of rats treated with HAE. There was evidence of increased blood flow in all the HAE-treated groups compared with the controls (Figure 11).

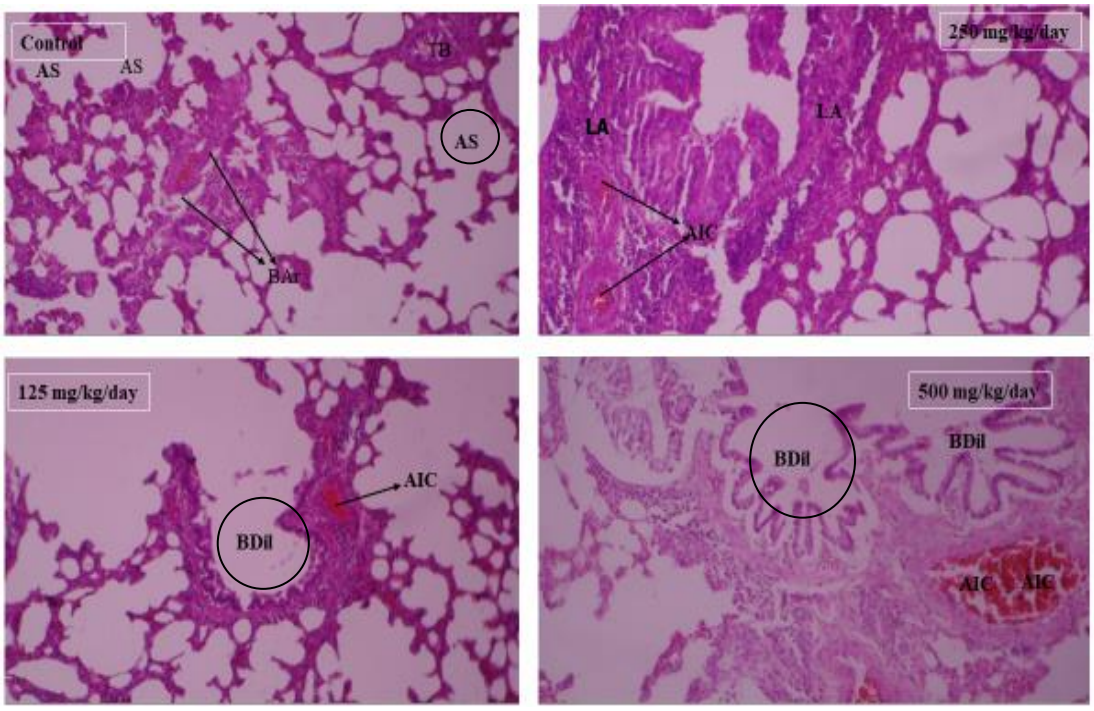

Figure 8: Photomicrographs of lungs from rats treated with HAE of *R. vomitoria* for 14 days. AIC: active interstitial congestion, AS: alveolar spaces, BAr: bronchial artery, LA: lymphoid activation, BDil: bronchial dilatation, TB: Terminal bronchiole. H&E x100

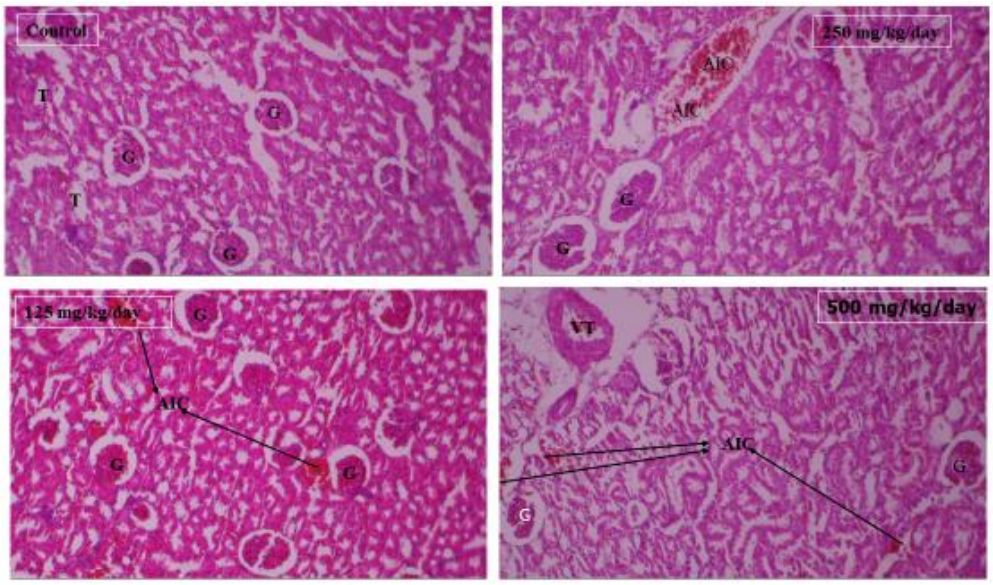

Figure 9: Photomicrographs of kidney from rats treated with HAE of *R. vomitoria* for 14 days. AIC: active interstitial congestion, G: glomerulus, T: tubule, VT: vascular tubule. H&E x100

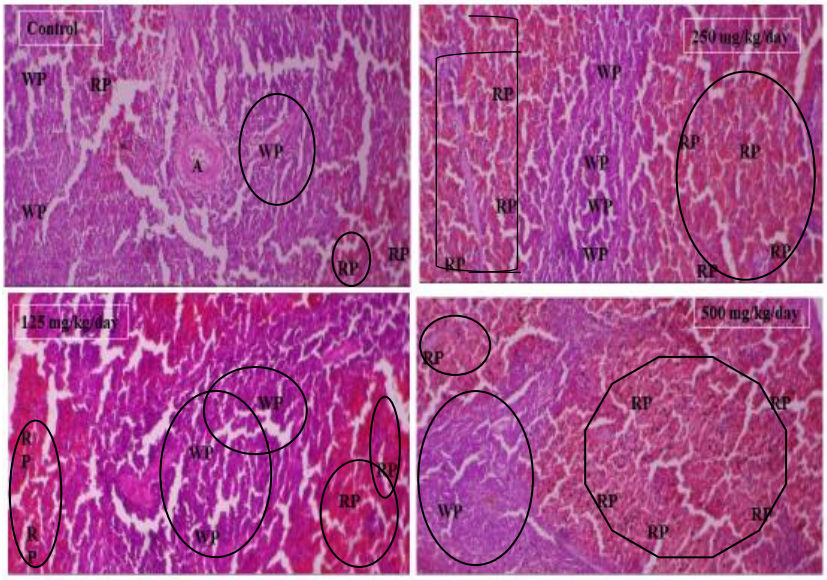

Figure 10: Photomicrographs of spleen from rats treated with HAE of *R. vomitoria* for 14 days. A: arteriole, WP: white pulp, RP: red pulp. Expansion of the red pulp with increase in dosage. H&E x100

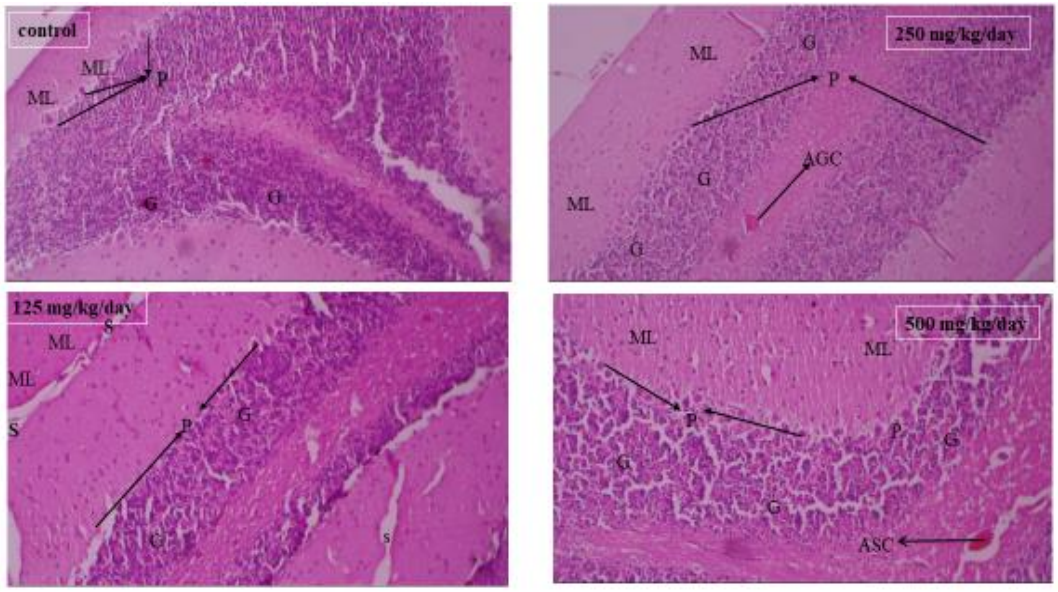

Figure 11: Photomicrographs of brain from rats treated with HAE of *R. vomitoria* for 14 days. AGC: active glial congestion, ASC: active sulcal congestion, Granular layer, ML: molecular layer, P: Purkinje layer, S: sulcus. H&E x100

DISCUSSION

The hydro-alcohol extract of *R. vomitoria* root (HAE) contains alkaloids, flavonoid, phenolics and saponins. The constituents identified in this study are consistent with those earlier reported [10, 11]. However, the results of our study differ slightly with the findings of another study [12] which in addition to the aforementioned constituents also detected tannins and terpenoids in the ethanol extract of *R. vomitoria* root. These phytochemicals were not detected in our study probably due to differences in test protocols used for the qualitative analysis.

The significant reduction in body weight among the experimental animals that received higher doses of HAE compared to those that received 125 mg/kg/day and control indicated that repeated administration of HAE at higher doses have deleterious effects on bodyweight as previously reported [13]. Available

reports suggest that loss of weight in experimental animals may be due to loss of appetite and reduction in water intake caused by the activity of reserpine, which is a major alkaloid of *R. vomitoria*. Reserpine produces loss of appetite by depleting monoamine neurotransmitters, including norepinephrine, dopamine, and serotonin, in the central nervous system (CNS), particularly within brain regions that regulate hunger and satiety, such as the hypothalamus. [14]

The histopathological examination revealed that the hepatocytes and sinusoids in all the treated groups and control were normal with no sign of injury or damage such as fatty liver, necrosis and hepatitis. The activation of Kupffer cells as observed in all the treated groups and control signified mobilization of the local immune system which may be due to gut derived endotoxins/lipopolysacharide [15] or fatty acid component of Tween 80 [16] which was administered to the control 14group and used for reconstituting HAE for administration to the treatment groups.

The histological examination of the heart, lungs, kidney, spleen and brain further appear to confirm the safety of HAE as there were no abnormalities in the architecture of all the organs examined in all the treated groups when compared to the control. Furthermore, signs of vasodilatation and vascular congestion observed in all the treated groups of animals in this study are consistent with observations made by Abere *et al.* [17] showing that HAE increased blood flow to all the organs examined which may be due to the blood pressure lowering effects for which the extract is known [18]. However, the constituents of HAE eliciting the observed vasodilatation, active congestion, and the mechanisms of action is not clear, except that the dilatation and active congestion are dose dependent. The consequence of excessive vasodilatation may lead to anaphylaxis [19] and septic shock [20] if the dilatation is caused or preceded by inflammation due to increased vascular permeability or excessive release of vasodilator substances to deal with systemic infections. Available evidence also showed that venous congestion can lead to severe organ dysfunction with elevated oxidative stress [21] that may not reveal changes in cardiac function [22]

CONCLUSION

The results of this study revealed that hydro-alcohol extract of *R. vomitoria* root contains alkaloids, flavonoids, phenolics, and saponins. The normal architecture of organ sections in all the treated animal groups indicates that HAE is safe at the doses investigated but increased venous congestion, vasodilatation, and weight loss at higher doses suggests chronic use should be limited to lower doses.

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AUTHORS' CONTRIBUTION

VOA conceived and designed study, performed animal studies, collated and analyzed study results, and drafted the manuscript. RIO designed study, performed animal studies, and supervised the study. GIE analyzed the histological slides, EA performed the HPLC analysis, OAS supervised the study and manuscript drafting. All the authors proofread and approved the manuscript draft.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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