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**Original Research Article**

# Toxicological evaluation of hydro-alcohol root extract of *Rauwolfia vomitoria* Afzel (Apocynaceae)

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## Abstract

***Purpose:*** *To investigate the phytochemical content and toxicity profile of Rauwolfia vomitoria Afze (Apocynaceae).*
***Method:*** *Qualitative phytochemical screening of hydro-alcohol extract (HAE) of R. vomitoria roots was carried out followed by acute toxicity evaluation in nine Swiss albino Wistar rats using two phases of Lorke's method. In the 14 days subacute study, thirty-two Swiss albino rats were randomly divided into 4 groups of 8 rats each. Group A received 0.5 mL Tween 80 daily and served as control, while groups B, C and D was administered 125, 250 and 500 mg/kg/day of HAE respectively. All the rats were monitored daily for signs of toxicity. Selected haematological and biochemical parameters were assessed at the termination of experiment.*
***Results:*** *Alkaloids, cardiac glycosides, flavonoids and saponins were present in HAE. The estimated oral median lethal dose ($LD_{50}$) was greater than 5000 mg/kg. Daily administration of HAE for 14 days resulted in 15.63 % mortality of animals across the groups by day 14 with significant loss in total body weight in group D animals on day 8 ($p < 0.05$), and in group C animals on day 11 ($p < 0.05$) and day 15 ($p < 0.001$). The dose of 500 mg/kg/day significantly ($p < 0.05$) increased haemoglobin concentration but reduced ($p < 0.05$) white blood cell count, while the dose of 250 mg/kg/day significantly ($p < 0.05$) reduced alkaline phosphatase levels but increased serum albumin levels ($p < 0.05$).*
***Conclusion:*** *Rauwolfia vomitoria may be safe on acute basis but repeated administration of high doses over many days needs to be done with caution.*

***Keywords:*** *Rauwolfia vomitoria, Sickle cell, Alkaloids, Flavonoids, Saponins, Toxicity*



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## INTRODUCTION

*Rauwolfia vomitoria* Afzel, (Apocynaceae) is known by many names such as *asofeyeje* by the Yorubas in western Nigeria, *akkemta* by the Igbos, *wada* by the Hausas, *mmoneba* by the Efiks, and *utoenyin* by the Ibibios [1]. It is an evergreen perennial shrub found mainly in the wild in Africa with height reaching up to 15 m, with oblong or oval shiny leaves, depending on the location [2]. The constituents of *Rauwolfia vomitoria* is reported to possess antipsychotic, antihypertensive, antidiarrheal [2] and anti-sickling effects [1,3] amongst other useful

pharmacological activities, while the safety of *R. vomitoria* has attracted global concern.

The reported anti-sickling property of a plant recipe containing *R. vomitoria* root and leaf extract [1,3] necessitated further evaluation of the safety profile of its hydro-alcohol extract in the present study since management or prophylaxis of sickle cell disease require that doses of selected therapeutic agent are safe both for short time and chronic use across all age groups.

## EXPERIMENTAL

### Plant material

Roots of *R. vomitoria* were collected between August and September 2015 from the Botanical Garden of University of Ibadan, Ibadan, Oyo State, Nigeria. The specimen was authenticated by Dr Grace Ugbabe of the Department Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development, (NIPRD), Idu, Abuja. The specimen was assigned a voucher no. NIPRD/H/6715 and kept in the Herbarium of NIPRD, at Idu, Abuja. The roots were carefully cleaned, air-dried under shade and ground to coarse powder using mortar and pestle before further pulverisation.

### Preparation of hydro-alcohol extract (HAE) of *R. vomitoria*

The method of Egunyomi *et al* [3] with slight modification was used. The powdered roots of *R. vomitoria* (800 g) was soaked in a mixture of solvents (3 L of distilled water and 3.6 L of 95 % ethanol) inside a 5 L sealed flat bottom flask for five days with intermittent stirring using a spatula. The extract was thereafter filtered with Whatman filter paper and the filtrate concentrated to dryness on a water bath at 70 ºC to obtain the extract which was subsequently stored in a labelled sample bottle at 4 ºC.

### Animals

Swiss albino Wistar rats, weighing 167.69 ± 29.57 g (mean ± SD), of equal sexes were obtained from the Animal House of the Faculty of Pharmacy, University of Benin, Benin-City, Nigeria. They were kept in standard plastic cages and had free access to water and feeds (Livestock Feeds Ltd, Ibadan, Nigeria). All the animals were exposed to natural lighting, maintained at room temperature (30 °C) and handled according to international protocols for use of laboratory animals in experiments [4]. Ethical approval (EC/FP/021/01) was obtained from the Ethical Committee, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. Chemicals were sourced from reputable manufacturers such as Sigma-Aldrich and reagents were freshly prepared before use.

### Preliminary phytochemical screening

Preliminary phytochemical screening to identify the class of secondary metabolites in HAE was done using the methods described in literature [5].

### Oral acute toxicity test

The oral median lethal dose ($LD_{50}$) was estimated using the method of Lorke [6]. In the first phase, nine (09) rats of both sexes were randomly assigned to three groups (n = 3) labelled A, B, and C. With the aid of an orogastric tube, animals in groups A, B and C were administered 10, 100 and 1000 mg/kg of HAE, respectively. The animals were observed continuously for the first four hours after dosing, after 24 h and subsequently, after 72 h for signs of toxicity such as changes in behaviour (paw-licking, salivation, stretching, mood, motor activity and gnawing), posture, nature and frequency of stooling, and mortality. The absence of death in this phase necessitated the next. For the second phase, three rats were randomly assigned to three groups (n = 1). With the aid of an orogastric tube, doses of 1600, 2900 and 5000 mg/kg of HAE were administered to each group, respectively. The animals were observed as was done in phase 1 above. The $LD_{50}$ value was calculated using Eq 1.

$$LD_{50} = \sqrt{(D_0 \times D_{100})} \quad \text{..... (1)}$$

Where: $D_0$ = Highest dose that gave no mortality and $D_{100}$ = Lowest dose that produced mortality

### Oral sub-acute toxicity test

Sub-acute toxicity testing was carried out using the method of Ozolua *et al* [9] with slight modification. Thirty-two (32) rats were randomly allotted into 4 groups of 8 rats each (4 males and 4 females). The rats in group A were administered 0.5 mL of 10 % Tween 80 daily while rats in groups B, C and D received 125, 250 and 500 mg/kg/day of HAE, respectively for fourteen days orally via orogastric tube. All the rats were monitored daily for signs of toxicity such as depression and death. Rats that survived were fasted overnight prior to sacrificing on day 15. Animals were anaesthetized in an

airtight glass chamber saturated with chloroform and a portion of blood (5 mL) was drawn from the abdominal aorta of each rat, from which 1 mL was collected into ethylene diamine-tetra-acetic acid (EDTA)-containing bottle for haematological analysis. The remaining portion of blood (4 mL) was collected into a plain bottle and used for biochemical analysis.

### Haematological analysis

Automatic analyser (ERMA PCE-210, Japan) was calibrated and programmed to analyse the blood samples in EDTA bottles for the following parameters: red blood cell count (RBC), haemoglobin concentration (Hgb), haematocrit (HCT), white blood cell count (WBC), platelets count (PLT); lymphocytes count (LY), monocytes count (MO) and mean platelet volume (MPV).

### Biochemical analysis

The blood samples in the plain bottles were allowed to clot at room temperature for 4 h and the resulting serum (1 mL) was analysed using automatic clinical chemistry analyzer (Architect $_{C}$4000, Abbott Diagnostics, Japan) for alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (TBil), conjugated bilirubin (CBil), total proteins (TP), albumin (Alb), creatinine and urea levels. Automatic analyser (Selectra Pro S, ELITech, USA) was used to determine the concentration of sodium, potassium, bicarbonates and chloride ions in each serum sample.

### Statistical analyses

All results were expressed as mean ± standard error of mean (SEM). Comparisons were made between groups by use of one-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test. All data were analysed using GraphPad Prism software version 6 (GraphPad Prism, USA). $P < 0.05$ indicates statistically significant difference.

## RESULTS

### Phytochemical and extraction yield

Phytochemical screening of hydro-alcohol extract of *R. vomitoria* root (HAE) showed that it contained alkaloids, cardiac glycosides, flavonoids and saponins (Table 1). The hydro-alcohol extraction yield was 5.62 % w/w.

**Table 1:** Classes of secondary plant metabolites identified in the hydro-alcohol extract of *R. vomitoria* roots

| Phytochemical Test | Qualitative |
|---|---|
| Alkaloid | + |
| Cardiac glycosides | + |
| Flavonoids | + |
| Saponins | + |
| Tannins | - |
| Glycosides | - |
| Anthraquinones | - |
| Phlobotannins | - |

***Note:*** - = absent, + = present

### Oral acute toxicity

There was no lethality at acute oral doses of 10, 100 and 1000 mg/kg in rats given HAE (Table 2). The rats did not exhibit any significant sign of toxicity such as writhing, diarrhoea, hyper-motility and aggression. Mild depressant activity was observed in the rats given 1000 mg/kg but they recovered within 48 h. The rats manifested toxicological signs of restlessness (writhing) after administration of extract at 1600, 2900 and 5000 mg/kg, but became calm 2 h afterwards. After 24 h, all the rats manifested signs of depression (hypo-activity) as evident from their shut eyes, orbital red patches, immobility and lack of interest in the environment. The absence of death at 5000 mg/kg after 48 h indicated that the $LD_{50}$ of HAE was greater than 5000 mg/kg.

### Oral sub-acute toxicity

The signs of depression such as loss of corneal reflex/partial eye closure were more observable in rats given 500 mg/kg/day than those given 250 mg/kg/day after one week of HAE administration. During the experiment, mortality was recorded in all treated groups, made up of two rats each in 250 and 500 mg/kg/day groups on days 9 and 11, respectively and one rat in 125 mg/kg/day group on day 13. No death was observed among the rats in control group (Table 3).

### Effects on haematological indices

There was a significant ($p < 0.05$) increase in haemoglobin concentration in the group of animals that received 500 mg/kg/day of HAE compared to the animals in control group. Other red blood cell (RBC) parameters such as RBC count, haematocrit levels and mean corpuscular haemoglobin concentration in all treated groups were not significantly different from those of control (Table 4 a). The decrease in white blood cell counts within the 500 mg/kg/day group was significant ($p < 0.05$) compared to control.

**Table 2:** Oral acute toxicity effects of the hydro-alcohol extract of *R. vomitoria* roots in Wistar rats

| Phase | Dose (mg/kg) | Writhing | Depression | Diarrhoea | Tremor | Death | Others |
|---|---|---|---|---|---|---|---|
| I | 10 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 |
| | 100 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 |
| | 1000 | 0/3 | 3/3 | 0/3 | 0/3 | 0/3 | 0/3 |
| II | 1600 | 1/1 | 1/1 | 0/1 | 0/1 | 0/1 | 0/1 |
| | 2900 | 1/1 | 1/1 | 0/1 | 0/1 | 0/1 | 0/1 |
| | 5000 | 1/1 | 1/1 | 0/1 | 0/1 | 0/1 | 0/1 |

Numerator = number of animals affected. Denominator = number of animals in the group. $LD_{50}$ > 5000 mg/kg (*p.o.*)

**Table 3:** Toxicological effects following oral daily doses (x14 days) of HAE of *R. vomitoria* in Wistar rats

| Group | Mortality | | | Symptoms |
|---|---|---|---|---|
| | *Number* | *(%)* | *Latency (Days)* | |
| Control | 0 | 0 | | None |
| 125 mg/kg/day | 2 | 25 | 9, 11 | None |
| 250 mg/kg/day | 1 | 13 | 13 | Anorexia, hypoactivity, loss of corneal reflex |
| 500 mg/kg/day | 2 | 25 | 9, 11 | Anorexia, hypoactivity, loss of corneal reflex |

### Effects on haematological indices

There was a significant ($p < 0.05$) increase in haemoglobin concentration in the group of animals that received 500 mg/kg/day of HAE compared to the animals in control group. Other red blood cell (RBC) parameters such as RBC count, haematocrit levels and mean corpuscular haemoglobin concentration in all treated groups were not significantly different from those of control (Table 4). The decrease in white blood cell counts within the 500 mg/kg/day group was significant ($p < 0.05$) compared to control. The mean platelet volume (MPV) and lymphocytes count were significantly decreased ($p < 0.001$) in the groups that received 250 and 500 mg/kg/day compared to control, although there was no significant ($p > 0.05$) change in platelet and monocytes counts. The decrease in lymphocyte count among treated groups was observed to be dose-dependent (Table 4).

### Effect on enzymes and proteins

The level of alkaline phosphatase (ALP) was significantly ($p < 0.05$) lower in the group of rats that received 250 mg/kg/day of HAE but levels of other serum enzymes (aspartate aminotransferase and alanine amino-transferase) in all treated groups were not significantly ($p > 0.05$) different from those of control (Table 5). Also, albumin levels were significantly ($p < 0.05$) higher in 250 mg/kg/day group when compared to control. Other serum proteins such as total bilirubin, conjugated bilirubin and total protein were not significantly ($p > 0.05$) different compared to control (Table 6).

### Effect on electrolytes, urea and creatinine

The levels of sodium ions ($Na^+$) in the group that received 500 mg/kg/day were significantly elevated compared to control (($p < 0.05$; Table 6 a) but there were no significant changes in the levels of other electrolytes, urea and creatinine in all other treatment groups in comparison with control group (($p > 0.05$; Table 7).

## DISCUSSION

The hydro-alcohol extract (HAE) of *R. vomitoria* root contains alkaloids, saponins, cardiac glycosides and flavonoids. The constituents identified in this study were also detected by Abere *et al* [1]. However, the area of divergence was likely due to the number and differences in test protocols used for qualitative analysis.

The calculated oral $LD_{50}$ obtained for HAE of *R. vomitoria* roots in this present study was greater than 5000 mg/kg and is consistent with the results obtained from Abere *et al* which evaluated the acute toxicity of *R. vomitoria* leaves in mice [1]. The result is also similar to the findings of Ebuehi *et al* that estimated $LD_{50}$ of aqueous extract of *R. vomitoria* root in rats using the Lorke method [8]. However, the $LD_{50}$ obtained for the root extract in this study is at variance with the value estimated by Ebuehi *et al* for the ethanol extract of the *R. vomitoria* root using the Probit curve analysis technique [8].

**Table 4:** Effects of oral daily doses (x14 days) of HAE of *R. vomitoria* on blood parameters

| Group | RBC (x10$^6$/µL) | HgB (g/dL) | HCT (%) | MCH (pg) | PLT (10$^3$/ul) | WBC (x103/ul | LY (% | MO (% | MPV (fl) |
|---|---|---|---|---|---|---|---|---|---|
| Control | 6.02±0.23 | 13.89±0.50 | 38.81±1.73 | 23.08±0.45 | 375.40±64.5 | 13.08±2.0 | 7.16±0.52 | 1.78±0.48 | 3.74±0.10 |
| 125 mg/kg/day | 5.71±0.89 | 14.44±1.37 | 35.76±5.36 | 31.80±8.23 | 548.90±83.5 | 9.11±0.97 | 5.69±0.82 | 0.91±0.25 | 3.53±0.16 |
| 250 mg/kg/day | 6.98±0.46 | 16.10±0.78 | 41.95±2.78 | 23.15±0.50 | 474.70±30.3 | 7.18±0.97 | 3.70±0.64 | 0.88±0.31 | 3.12±0.04 |
| 500 mg/kg/day | 7.57±0.26 | 17.50±0.67***** | 50.75±1.51 | 23.05±0.19 | 650.00±86.4 | 4.25±1.54 | 2.83±1.39 | 0.40±0.07 | 3.15±0.03 |

**P* < 0.05 vs Control. RBC: red blood count; HgB: haemoglobin; HCT: haematocrit; MCH: mean cell haemoglobin, PLT: platelet; WBC: white blood count; LY: lymphocyte; MO: monocyte

**Table 5:** Effects of oral daily doses (x14 days) of HAE of *R. vomitoria* on some serum enzymes

| Enzyme | Control | 125 mg/kg/day | 250 mg/kg/day | 500 mg/kg/day |
|---|---|---|---|---|
| ALP (iU/L) | 691.9±64.5 | 479.6±79.5 | 407.0±61.25***** | 456.3±23.4 |
| AST (iU/L) | 42.3±4.2 | 45.2±3.5 | 48.0±4.0 | 62.7±10.1 |
| ALT (iU/L) | 80.6±10.0 | 73.7±15.3 | 82.4±4.9 | 60.5±20.7 |

**P* < 0.05 vs Control. ALP: Alkaline phosphatase, AST: Aspartate aminotransferase, ALT: Alanine amino-transferase

**Table 6:** Effects of oral daily doses (x14 days) of HAE of *R. vomitoria* on serum proteins

| Parameter | Control | 125 mg/kg/day | 250 mg/kg/day | 500 mg/kg/day |
|---|---|---|---|---|
| Total bilirubin (µm/L) | 0.33±0.05 | 0.34±0.06 | 0.34±0.04 | 0.37±0.09 |
| Conjugated bilirubin (µm/L) | 0.15±0.02 | 0.14±0.03 | 0.16±0.02 | 0.13±0.03 |
| Total protein (g/dL) | 0.61±0.02 | 0.62±0.04 | 0.62±0.03 | 0.60±0.04 |
| Albumin (g/dL) | 3.94±0.11 | 4.36±0.11 | 4.54±0.16***** | 4.30±0.25 |

**P* < 0.05 vs Control

**Table 7:** Effects of oral daily dose (x14 days) of HAE of *R. vomitoria* on serum electrolytes

| Group | $Na^+$ (mol/L) | $K^+$ (mol/L) | $HCO_3$ (mol/L) | $Cl^-$ (mol/L) | Creatinine (µmol/L) | Urea (mol/L) |
|---|---|---|---|---|---|---|
| Control | 143.80±0.56 | 6.79±0.20 | 28.00±1.40 | 108.50±0.68 | 0.61±0.02 | 38.00±1.75 |
| 125 mg/kg/day | 145.80±1.60 | 6.84±0.33 | 26.40±0.51 | 108.00±0.95 | 0.62±0.04 | 36.00±1.34 |
| 250 mg/kg/day | 146.00±1.18 | 6.93±0.21 | 26.00±0.63 | 108.80±1.66 | 0.62±0.04 | 44.67±5.24 |
| 500 mg/kg/day | 151.50±3.70***** | 7.63±0.42 | 28.75±0.75 | 112.30±2.39 | 0.60±0.04 | 37.50±2.39 |

**P* < 0.05 vs Control

Based on OECD guidelines and the Hodge and Sterner scale classification [9], the HAE of *R. vomitoria* may be regarded as practically safe for consumption. In the oral sub-acute toxicity test results, the signs of depressant activity such as sedation and loss of corneal reflex observed in animals treated with 250 and 500 mg/kg/day of HAE nine days after the commencement of experiment suggest the involvement of the central nervous system (CNS) in the extract's toxic effect and the ability of the active phytochemicals to cross the blood-brain barrier. These symptoms and mortality recorded in all the treatment groups were not observed in the findings of Abere *et al* [1] but are consistent with that of Ebuehi *et al* [8] which may probably be due to differences in the test protocol. Doses used in the sub-acute test (one-tenth, one-twentieth, and one-fortieth of the maximum dose of 5000 mg/kg used for the $LD_{50}$ evaluation), appeared toxic as indicated by mortality.

The significant increase in hemoglobin concentration and the lack of significant difference in other hematological indices such as red blood cell count, hematocrit level and mean cell hemoglobin in all treated groups when compared to control as observed in this study is at variance with the findings of Isaiah *et al* [10] in which 150 mg/kg of the root bark extract exerted significant increase in hemoglobin concentration, red blood cell count and hematocrit level. The decrease in white blood cell count observed in this study may be due to toxic effect of HAE of *R. vomitoria* root on the immune system and is consistent with the findings of Isaiah *et al* [10] who reported a decrease in white blood cell count, lymphocyte count and mean platelet volume following administration of 150 and 300 mg/kg of ethanol extract of *R. vomitoria* bark to Albino Wistar rats. However, the findings differ from that of Bonheur *et al* [11] which reported elevated white blood cell count, lymphocyte and platelet counts following administration of 900 mg/kg of aqueous extract of *R. vomitoria* stem bark to male Wistar rats. The difference in dosage of administration, as well as part of the plant used, could be the reason for the different observations.

Although higher levels of ALT and AST are often diagnostic of underlying cellular injuries, this was not the case in this study as the levels of the two enzymes - ALT and AST were not significantly different between treated and control groups [7]. The absence of a significant change in the levels of ALT and AST in all treated groups could imply the safety of HAE of *R. vomitoria* in the hepatic system. The safety of HAE at the dose investigated is further confirmed by the absence of significant changes in the levels of total bilirubin, conjugated bilirubin and total protein. Although the levels of albumin were elevated in animals that received 250 mg/kg of HAE, this increase was not dose-dependent. However, increased albumin levels have been associated with improved liver health [2]. Research evidence also revealed that elevated albumin levels could be a result of dehydration, infections, congenital disorders, liver injury, malnutrition, chronic inflammatory disease, and diminished protein intake among other physiological and biochemical factors [12]. The reduction in the levels of ALP as obtained in this study is at variance with the findings of Eteng *et al* [2] but agrees with the observations of Ezejindu *et al* [13] possibly due to differences in the dose of ethanol extract of *R. vomitoria* root administered.

Furthermore, the estimated serum concentration of sodium, potassium, chloride and bicarbonate electrolytes obtained in this study are all within the reference values of 141 - 150 mmol/L, 5.2 - 7.8 mmol/L, 99 - 114 mmol/L, and 24 - 31 mmol/L, respectively [14]. Although the serum concentration of sodium ion was significantly elevated in animals that received 500 mg/kg of HAE, suggestive of the potential ability of HAE to increase blood pressure, the change in serum concentrations of other electrolytes, especially potassium, was not significant, thus implying that HAE may possess cardio-protective activities [15].

The concentration of serum creatinine in healthy rats is usually in the range of 0.4 – 0.8 mg/dL [16] while the reference values for serum urea concentrations in rats vary, depending on the dietary protein content and dietary intake of protein [17], but usually within the range of 15 – 26 mg/dL [16]. The unremarkable variations in serum urea concentration of all treated animals may be due to their attempts to adapt to the effects of HAE which was not achieved within the 14 days of experiment [18]. Although the levels of serum urea in this study are higher than reference values in all treated animals and controls, the change in creatinine and urea levels in treated groups was not significant compared to controls, thus implying that HAE did not exert any deleterious effect on the renal function.

## CONCLUSION

The hydro-alcohol extract (HAE) of *R. vomitoria* root contains alkaloids, cardiac glycosides, flavonoids and saponins. The oral $LD_{50}$ > 5000

mg/kg shows that the extract appears safe on acute basis but sub-acute doses used in the present study all appear toxic, thus indicating the need for monitoring of serum electrolytes, protein and liver function.

## DECLARATIONS

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None provided.

### *Ethical approval*

Ethical approval (EC/FP/021/01) was obtained from the Ethical Committee, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

### *Availability of data and materials*

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### *Conflict of Interest*

No conflict of interest associated with this work.

### *Contribution of Authors*

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Valentine Adegoke designed the study. Valentine Adegoke, Emmar Okpakpor and Dickson Uwaya carried out the experiments and collection of data. Valentine Adegoke and Raymond Ozolua analysed and interpreted the data. Valentine Adegoke drafted the manuscript. Raymond Ozolua and Oluwakanyinsola Salawu supervised the experiments, data collection, analysis, interpretation of data and discussion of findings. All authors read and approved the manuscript for publication.



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