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Original Research Article

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## Antisickling and Toxicological Evaluation of the Leaves of *Rauwolfia vomitoria* Afzel (Apocynaceae)

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### Abstract

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**Purpose:** *Rauwolfia vomitoria* Afzel (Apocynaceae) is a medicinal plant that is widely used ethnomedically in the treatment of various health conditions. This study was aimed at investigating the claimed antisickling activity as well as establishing the toxicological profile of the leaves of the plant.

**Methods:** Evaluation of the antisickling activity involved the inhibition of sodium metabisulphite-induced sickling of the HbSS red blood cells obtained from confirmed sickle cell patients who were not in crises. Concentrations of the crude extract and its fractions were tested with normal saline and p-hydroxybenzoic acid serving as controls. Acute toxicological evaluation was carried out in mice while 30-day assessment was done in rats.

**Results:** Percentage sickling inhibitions of the aqueous methanol extract of *R. vomitoria* as well as all the fractions

were significant ( $p < 0.05$ ) all through the period of assay compared to normal saline. The LD<sub>50</sub> of the extract in mice was above 5000 mg/kg body weight when administered intraperitoneally. Toxicological evaluation at 250 mg/kg showed essentially normal tissue appearance while at 500 mg/kg, there was mild vascular congestion in virtually all the target organs with additional activation of sinusoidal kupffer cells and periportal lymphocytes in the liver cells.

**Conclusion:** *Rauwolfia vomitoria* has antisickling effects are suggestive of a potential role of the plant in the management of sickle cell disorders and a candidate for further investigations.

**Keywords:** *Rauwolfia vomitoria*, Apocynaceae, sickle cell disorders, toxicological studies.

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**Indexing:** Index Copernicus, African Index Medicus

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### Introduction

Apocynaceae is a family of flowering plants that includes trees, shrubs, herbs and lianas, commonly called the "Dogbane family". Members of the family are native to European, Asia, Australia, Africa and American tropics or subtropics. *Rauwolfia vomitoria* Afzel (Apocynaceae), commonly called Asofeyeje (Yoruba), Akkemta (Ibo) and Wada (Hausa) [1], has been used for various indications such as snake bite, fever, nervous disorders, jaundice, hypertension, cerebral cramps, epilepsy, hemorrhoids, hepatomegaly and most importantly in mentally ill persons amongst others [2].

The major chemical constituents of *R. vomitoria* include reserpine, ajmaline, deserpidine, corynanthine, rescinnamine and yohimbine [3]. Alkaloidal extract of *R. vomitoria* has been reported to have mycobacterial activity [4]. It also has high anti-oxidant activity [4]. A study on anti-prostate

cancer activity of beta-carboline alkaloid enriched extract of *R. vomitoria* revealed that it decreased *in-vitro* cell growth in a dose dependent manner, induced the accumulation of G1 phase cells and it's an effective inhibitor of cell growth in the human prostate cancer cell line, LNCaP [5]. Comparative effect of *R. vomitoria* and chlorpromazine on social behavior reveals that *R. vomitoria* has a high potential as an antipsychotic and may have advantage over chlorpromazine, due to its high selectivity for D<sub>2</sub> receptors on the mesolimbic pathway thus do not cause extra pyramidal side effects such as pseudo-parkinsonism [6].

Sickle Cell Disease (SCD) occurs predominantly in persons of African origin, but it also affects persons of Mediterranean, Carribean, South and Central American, Arab and East Indian origins. In Nigeria, the most common type of SCD is the homozygous (HbSS) form i.e. SCA. Estimates show that 25 % of the Nigerian population are "carriers" of the Sickle cell trait i.e. HbAS [7]. Despite a variety of

antisickling agents acting at different levels of the sickling mechanism, there is still a paucity of antisickling medicines. This is because of the potential toxicities associated with most of these agents. Apart from the general mutagenic and carcinogenic tendencies of gene modifiers, hydroxyurea approved by the US Food and Drugs Administration (FDA) for treatment of adults with sickle cell disease, causes bone marrow suppression which greatly limits its use [8]. On the other hand, this is gradually paving way for the consideration of condiments from natural sources as antisickling remedies. The increasing interest in these condiments is not unconnected with the general innocuous nature of their sources, which most often are herbs and even at times food crops.

Nearly 70 % of the world's population (mainly in the developing countries) relies entirely on such traditional medical therapies as their primary form of health care. Various herbs are also part of the socio-cultural and socio-economic heritage. Even in the present times, rural populations turn to herbal medicine as the most preferred therapeutic source [9]. This present study was designed to investigate *Rauwolfia vomitoria* Afzel (Apocynaceae) for its antisickling activity as well as establish its toxicological profile.

## Materials and Methods

### Preparation of plant extract

The leaves of *Rauwolfia vomitoria* Afzel (Apocynaceae) were collected in Benin City, Edo State, Nigeria. The plants were authenticated by the curator at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City where a voucher specimen was deposited. The fresh leaves were air-dried for 72 h and powdered using an electric mill.

### Animals

Swiss albino mice ( $26.87 \pm 1.64$  g) and Wistar rats ( $207.00 \pm 17.94$  g) of both sexes were obtained from the Animal House, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City. All the animals were kept under standard environmental conditions and were handled according to international protocol for use of animals in experiments [10]. They were fed with standard pellets and tap water *ad libitum*. Ethical approval for the study was obtained from the College of Medicine, University of Benin Animal Ethics Committee (ADM/F. 22A/Vol. viii/349).

### Phytochemical investigation

Chemical tests were employed in the preliminary phytochemical screening for various secondary

metabolites such as tannins (phenazone; iron complex; formaldehyde and modified iron complex tests were carried out on the aqueous extract to detect the presence of hydrolysable, condensed and pseudo tannins); cardiac glycosides (Keller - Killiani and Kedde tests were carried out on the methanolic extract to detect the presence of a deoxy sugar and to indicate the presence of a lactone ring on the cardenolides respectively); alkaloids (Mayer's, Dragendorff's, Wagner's and 1% picric acid reagents to detect the presence of alkaloidal salts and bases), saponins glycosides (frothing of the aqueous extract when shaken and haemolysis test on blood agar plates were carried out to indicate and confirm the presence of saponins); anthracene derivatives (Borntrager's test for combined and free anthraquinones, where aglycones were extracted using chloroform and shaken with dilute ammonia) and cyanogenetic glycosides (sodium picrate paper test were used to test for the presence of hydrocyanic acid in the sample. Conversion to sodium isopurpurate indicates the presence of cyanogenetic glycosides) [11 - 14].

### Extraction and fractionation

The powdered leaves of *R. vomitoria* (5.0 kg) were extracted with methanol-water (50:50). Evaporating the solvent yielded an extract (0.96 kg) which was subsequently re-suspended in water and successively partitioned into petroleum ether (3 x 2L), chloroform (3 x 2L) and n-butanol (3 x 2L).

### Antisickling screening

**HbSS Blood Samples:** HbSS Blood samples were collected by venipuncture from confirmed sickle cell patients not in crises on their clinic days at the Consultant Outpatient Department (COPD) of the University Teaching Hospital, Benin City, Nigeria. None of the patients used was recently transfused with HbAA blood.

**Antisickling activity evaluation:** The evaluation of the leaf extract and fractions of *Rauwolfia vomitoria* for antisickling activities was carried out using a modified method of Moody and co-workers [15]. Venipuncture blood samples from sickle cell anaemia patients not in crises were collected into EDTA bottles. Collected samples were centrifuged to remove the serum. The resulting packed erythrocytes were washed three times with sterile normal saline and centrifuged each time to remove the supernatant. 0.5 ml of the washed erythrocytes were mixed each with 0.5 ml of the different concentrations of the aqueous methanol extract (100, 300 and 500 mg/ml) or fractions (500 mg/ml) in uncovered test tubes. A 5 mg/ml solution of  $\rho$ -hydroxybenzoic acid (PHBA) in normal saline was used as the positive control while normal saline served as negative control. Samples were taken from the different mixtures and the remaining portions of the mixtures incubated for 3

hrs, shaking occasionally. A 2 % sodium metabisulphite (0.5 ml) was added to each mixture to deoxygenate the system, mixed thoroughly and sealed with liquid paraffin. Samples were taken in five replicates from the different mixtures at 0 minutes and at subsequent 30 minutes interval until seven readings were obtained. Each sample was smeared on a microscopic slide, fixed with 95 % methanol, dried and stained with giemsa stain. Each slide was examined under the oil immersion light microscope and counting of 100 red cells in each sample. The numbers of both sickled and unsickled red blood cells were counted and the percentage of unsickled cells determined.

### Toxicological evaluation

Swiss albino mice, divided into 6 groups of 5 animals per group were orally administered the extract at doses of 1, 2, 3, 4 and 5 g/kg. The control group received only the vehicle (normal saline 5 ml/kg). Each group of mice was placed in the test cage for a 30-min habituation period before drug administration. The animals were observed for 10 min for the first 6 h and 10 min each day for the next two days. Lethality and gross toxicological features (convulsion, diarrhea, hyperactivity and pile-erection) were recorded for each group [16]. The animals were further observed for fourteen days.

Wistar rats (30) were randomly distributed into 3 groups of 10 rats each. The first (A) group served as control and received 5 ml/kg of normal saline (vehicle) while the second (B) and third (C) groups received oral doses of 250 and 500 mg/kg per day of the extract respectively for 30 consecutive days. The animals were observed for signs of toxicity (abnormal behaviours, writhing, convulsion, mood, motor activity and general body conditions) for 30 min each day. At the end of 30 days, the rats were sacrificed under chloroform anesthesia. Livers, lungs, hearts and ovaries were removed and preserved in 10 % formaldehyde solution. Each organ was sectioned (6

$\mu$  thick) embedded in paraffin wax and stained with hematoxylin and eosin [17].

### Statistical analysis

Data are expressed as mean  $\pm$  SEM. The differences between the means were analyzed using one way analysis of variance (ANOVA). Values of  $P < 0.05$  were taken to imply statistical significance between compared data.

## Results

### Phytochemical screening

Phytochemical screening of the leaves of *R. vomitoria* for secondary plant metabolites revealed the presence of alkaloids, tannins, flavonoids, saponins and cardiac glycosides (Table 1).

**Table 1:** Phytochemical constituents of *R. vomitoria* leaves

Classes of secondary metabolites	inferences
Alkaloids	+
Tannins	+
Flavonoids	+
Anthracene derivatives	-
Saponin glycosides	+
Cardiac glycosides	+
Cyanogenetic glycosides	-

- = absent; + = present

### Sickling inhibitory activities of crude extracts and fractions of *R. vomitoria*

Percentage sickling inhibition of the various doses of *R. vomitoria* extracts and fractions were significant all through the period of assay ( $p < 0.05$ ) (Table 2).

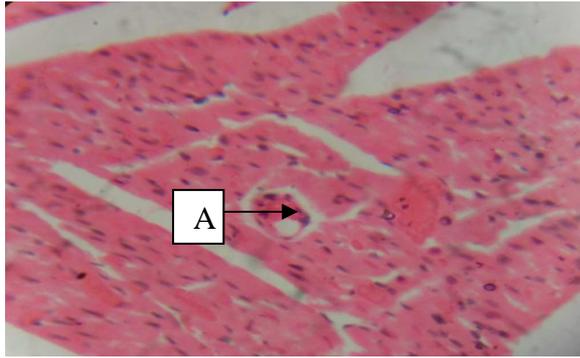
### Toxicological evaluation

The aqueous extract of *R. vomitoria* did not produce any mortality up to the oral dose level of 5 g/kg body weight in mice. There were no changes in behaviour, posture, nature and frequency of stooling, mood and

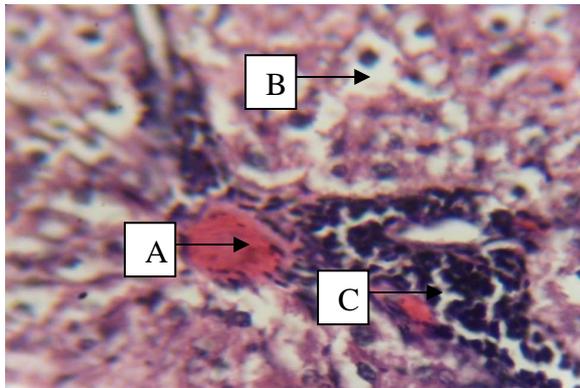
**Table 2:** Sickling inhibitory activities of *Rauwolfia vomitoria* crude extracts and fractions

Incubation time (min)	Percentage inhibition (%)								
	A	B	C	D	E	F	G	H	I
0	35.0 $\pm$ 0.34	40.2 $\pm$ 0.04	79.4 $\pm$ 1.35	67.0 $\pm$ 0.87	67.0 $\pm$ 0.56	71.0 $\pm$ 0.48	70.4 $\pm$ 1.41	69.0 $\pm$ 0.74	41.4 $\pm$ 0.56
30	30.0 $\pm$ 0.25	60.2 $\pm$ 0.23	71.0 $\pm$ 0.68	79.6 $\pm$ 1.44	70.0 $\pm$ 1.62	55.0 $\pm$ 0.99	60.0 $\pm$ 0.36	83.0 $\pm$ 1.21	63.0 $\pm$ 0.29
60	40.0 $\pm$ 0.73	54.0 $\pm$ 1.15	75.2 $\pm$ 1.29	58.0 $\pm$ 0.94	76.6 $\pm$ 0.16	88.4 $\pm$ 0.82	55.0 $\pm$ 0.80	78.2 $\pm$ 0.45	62.0 $\pm$ 1.38
90	40.4 $\pm$ 0.11	50.0 $\pm$ 0.09	82.0 $\pm$ 1.11	69.8 $\pm$ 0.47	67.0 $\pm$ 1.91	67.0 $\pm$ 1.32	72.6 $\pm$ 1.14	75.2 $\pm$ 1.17	72.0 $\pm$ 0.73
120	42.0 $\pm$ 0.68	46.0 $\pm$ 0.74	65.4 $\pm$ 0.94	69.4 $\pm$ 1.62	71.2 $\pm$ 0.64	75.2 $\pm$ 0.46	65.0 $\pm$ 0.36	68.0 $\pm$ 0.62	64.4 $\pm$ 1.27
150	44.0 $\pm$ 0.47	38.2 $\pm$ 1.16	78.0 $\pm$ 1.07	76.0 $\pm$ 1.32	78.0 $\pm$ 0.27	84.0 $\pm$ 1.54	74.0 $\pm$ 1.10	80.0 $\pm$ 0.63	50.0 $\pm$ 0.44
180	40.0 $\pm$ 0.52	32.4 $\pm$ 1.70	66.0 $\pm$ 0.54	63.0 $\pm$ 0.35	55.4 $\pm$ 0.68	84.2 $\pm$ 0.67	78.0 $\pm$ 0.12	68.4 $\pm$ 1.37	45.0 $\pm$ 1.35

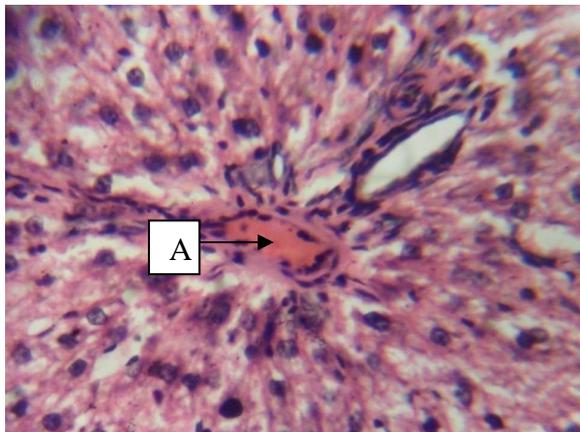
A = blood + normal saline + sodium metabisulphite; B = blood + PHBA + sodium metabisulphite; C = blood + crude extract of *R. vomitoria* leaf at 100 mg/ml + sodium metabisulphite; D = blood + crude extract of *R. vomitoria* leaf at 300 mg/ml + sodium metabisulphite; E = blood + crude extract of *R. vomitoria* leaf at 500 mg/ml + sodium metabisulphite; f = blood + petroleum ether fraction + sodium metabisulphite; G = blood + chloroform fraction + sodium metabisulphite; H = blood + n-butanol fraction + sodium metabisulphite; I = blood + aqueous fraction + sodium metabisulphite



**Figure 1:** Photomicrograph of the heart of rats administered with 500 mg/kg extract of *R. vomitoria* for 30 days showing mild vascular congestion (H&E x 400)



**Figure 2:** Photomicrograph of the liver of rats administered with 250 mg/kg extract of *R. vomitoria* for 30 days showing mild portal congestion A, kupffer cell activation B and lymphocytosis C (H&E x 400)

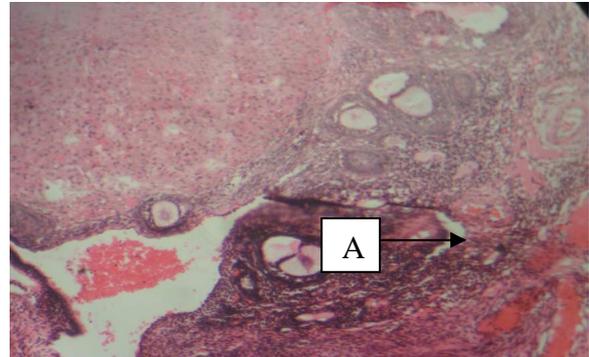


**Figure 3:** Photomicrograph of the liver of rats administered with 500 mg/kg extract of *R. vomitoria* for 30 days showing mild vascular congestion A (H&E x 400)

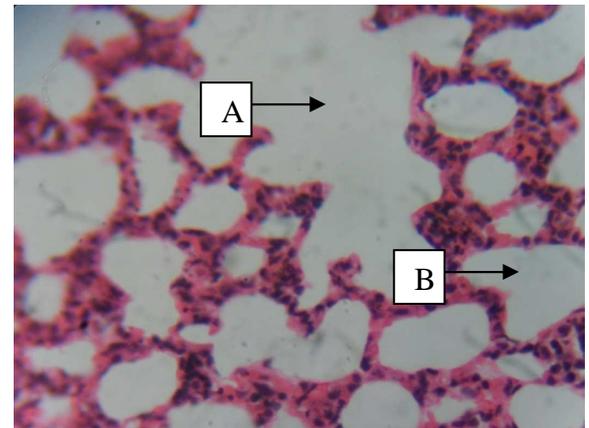
motor activity. The animals did not convulse, exhibit writhing or die.

Daily administration of the extract for 30 days did not produce gross toxicological symptoms or deaths. Histopathology of the heart showed that there was no degeneration of tissues (Figure 1) except for mild vascular congestion at the dose of 500 mg/kg. The liver in the 250 mg/kg *R. vomitoria* treated group (Figure 2) showed mild portal congestion, kupffer

cell activation and lymphocytosis while at 500 mg/kg (Figure 3), there was mild vascular congestion. in the and heart The ovaries of the 500 mg/kg *R. vomitoria* treated group (Figure 4) showed unremarkable alveoli and interstitial spaces. There were no morphological changes in the lungs (Figure 5).



**Figure 4:** Photomicrograph of the ovary of rats administered with 500 mg/kg extract of *R. vomitoria* for 30 days showing moderate stroma congestion A (H&E x 400)



**Figure 5:** Photomicrograph of the lung of rats administered with 500 mg/kg extract of *R. vomitoria* for 30 days showing unremarkable alveoli A and interstitial space B (H&E x 400)

## Discussion

Phytochemical evaluation of *R. vomitoria* revealed the presence of alkaloids, tannins, flavonoids, saponins and cardiac glycosides. Both the aqueous methanol and crude extracts of *R. vomitoria* showed significantly inhibitory effect on sodium metabisulphite-induced sickling of HbSS red blood cells. Toxicological evaluation did not reveal any toxicity of the plant in the laboratory animals.

Some of the compounds detected in the plant are known to possess medicinal properties and health promoting effects [18]. For example, previous investigations have attributed the anti-sickling activity of many medicinal plants used in the management of SCD to their inherent phytochemicals. Anthraquinones, steroidal and cardiac glycosides present in *Cissus populnea* L. contributed to the anti-sickling properties of

Ajawaron, an herbal product that is marketed in Nigeria [15]. Similarly, saponins and flavonoids present in *Hymenocardia acida* leaves were responsible for its anti-sickling activity [19]. Antioxidants have been reported to be major components of medicinal plants with known antisickling activity [20]. Studies demonstrated that anti-oxidant molecules were found to be potent inhibitors of sickle cell hemoglobin polymerization and equally improved the oxidant status of sickled erythrocytes. It is believed that the higher the anti-oxidant property, the higher it's possible anti-sickling effect. Furthermore, a study carried out on the extracts of *R. vomitoria* exhibited high anti-oxidant activity [4]. Thus, the presence of the bioactive compounds can be linked to the antisickling effect being reported. The antisickling activity could be linked to their ability either to inhibit *in-vitro* polymerization of haemoglobin or to some structural modification linked to the environment of haemoglobin by the extracts [21].

Toxicological studies for all herbal medicines including the determination of their median lethal dose  $LD_{50}$  and the absence of adverse alterations or degeneration of tissues following the toxicological evaluation of the plant is indicative of its relative safety and hence it has potential medicinal value for the management of sickle cell anemia.

## Conclusion

On the basis of the results obtained from the pharmacological investigations, it could be said that *Rauwolfia vomitoria* possesses antisickling properties, indicating that it has a role in the treatment of sickle cell disorders and a good candidate for further investigations and can also be considered safe on acute basis.

## Conflict of interest

No conflict of interest associated with this work.

## Contribution to authorship

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. TAA conceived and designed the study. OKO collected and analysed antisickling data. FOA and GIE carried out toxicological works.

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