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Effects of *Rauwolfia vomitoria* on the Haematological Indices and Hippocampal Histology of Wistar Rats

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ABSTRACT
Rauwolfia vomitoria (Rv) has been reported to cause neurodegeneration in the cerebral cortex and cerebellum of experimental Wistar rats. Here we report the effect of varying concentrations of Rv on the haematological indices, hippocampal histology and immunohistochemistry of Wistar rats. Twenty (20) rats randomized into four groups of five rats received 20% of tween 80 1 ml (control) and Rv 100 mg, 200 mg and 300 mg per kilogram body weight of rats respectively (treatment groups) for fourteen days via orogavage. Results show that MCV was significantly ($p<0.05$) increased when Rv 300 mg group was compared to control and is significantly ($p<0.05$) increased when group 4 is compared to the control; group 2 and group 4 is significantly ($p<0.05$) decreased compared to group 2 (Rv 100 mg); group 1 (control) and group 2 (Rv 100 mg) is significantly ($p<0.05$) increased compared to group 3 (Rv 200 mg). H&E revealed mostly increasing levels of hydropic vacuolations in the treated groups compared to control; while the immunolabeling marker for neuroinflammation and neurotoxicity showed that there was a down regulation of glial fibrillary acidic protein (GFAP) expression in the entire treated groups compared to the control. Our findings conclude that *Rauwolfia vomitoria* alters haematological parameters; increases WBC in a dose dependent concentration, increases hydropic vacuolations in the neuropil with mild neuronal shrinkage but down regulates the expression of glial fibrillary acidic protein in the hippocampus CA1 region of Wistar rats.

Keywords: *Rauwolfia vomitoria,* Haematology, Hippocampal distortions, Astrogliosis

INTRODUCTION
Globally 40,000 to 50,000 plants species are used for medicinal or aromatic purposes in traditional and modern medical systems [1]. One of the principal tenets of modern herbal medicine is that within a plant several compounds act on each other, either moderating or opposing, or enhancing in an additive or a synergistic action, whereby the combination of constituents is greater than would have been expected from the sum of individual contributions [2]. Small fractures of herbal plants have been investigated for their toxicological profile although some have been widely used in clinical trials and therapies.

Rauwolfia vomitoria (Rv) is one of such herbal plants, with common names such as African serpent wood, African snakeroot or swizzle stick. Phytochemical constituents in this plant include; alkaloids, glycosides, polyphenols, and reducing sugars, and some of the active alkaloids are; rauwolfine, reserpine, rescinnamine, serpentine, ajmaline serpentinine, steroid-serposterol and saponin [3, 4]. *Rv* possesses antisickling properties and is considered safe on acute basis [5]. Low doses of Rv (25 and 50 mg/kg) improve the number, transit and motility of sperm cells in the epididymis and vas deferens, while high doses (100 and 200 mg/kg) increase the production of sperm in the testis [6]. *Rv* leaf extract at low and high doses did not induce toxicity to the spleen of adult Wistar rats [7], possessed protective properties that could prevent damage to the kidneys doses via its antioxidant property [8]. Sub-acute administration of the aqueous leaf extract of *Rv* at 120 mg/kg and 300 mg/kg had no toxic effect on the kidneys, but could impair liver function in Wistar rats [9]. Also leaves extract administered with vitamin C showed some hypolipidemic and antioxidant potentials in high fat fed rats [10]. This study investigated the effect of *Rauwolfia vomitoria* on haematological, and histological distortions on the hippocampus of Wistar Rats.

MATERIALS AND METHODS
Plant Extract
Fresh roots of *Rauwolfia vomitoria* were collected from the environs of University of Uyo, Uyo. The plant sample was exposed to dry at room temperature for 7 days. The plant was identified in the Botany Department, Faculty of Science, University of Uyo, Uyo, Nigeria. The dried leaves were ground into powder and the powdered sample, weighing 1150 g, was extracted in 80% ethanol. Extraction in ethanol gave a percentage yield of 90 g, and was stored in a

refrigerator (Thermocool Nig. Ltd), for use during the experiment. Stock solution was prepared by dissolving 1 gram of ethanolic extract in 10 ml DMSO to give a concentration of 100 mg/ml respectively.

Study approval

Twenty albino Wistar rats (180 - 200g) were purchased from the animal house, College of Health Sciences, University of Uyo and housed in standard cages of five rats per cage. Animal procedures were carried out at the animal house of the University of Uyo, and performed according to the guidelines of animal research, with prior approval by the Research Ethics Committee of the College of Health Sciences of the University of Uyo. All animals were maintained under pathogen-free conditions with an artificial 12-hour dark-light cycle, with free access to food and water *ad libitum*. Animal procedures carried out were performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals [11].

Experimental Design

Experimental rats were randomly selected into four groups of five animals each; group 1 served as the control while groups 2, 3 and 4 were orally administered 100 mg, 200 mg and 300 mg respectively of *Rauwolfia vomitoria* root bark extract per kilogram body weight of the animals being an approximate estimate for 10%, 20% and 30% of acute toxicity based on oral route administration in mice. Stock solution from which individual dosage was taken from was prepared daily and administered orally. At the end of 14 days, the rats were fasted overnight and then sacrificed under Ketamine anaesthesia**. w**hole blood was collected by cardiac puncture with sterile needles, placed into EDTA containing sample tube**s** for haematological studies, while animals for tissue processing were intracardially perfused with formal saline and then immediately with 4% paraformaldehyde until fixed. Brain tissues were excised, cleaned by blotting with filter paper and fixed in 4% buffered paraformaldehyde for histological examination.

Haematological Studies

Haematological parameters were determined using automated haematological analyzer (Sysmex® Analyzer KX-21N, Sysmex Corporation, Japan) and read outs were printed out and analyzed.

Histological Studies

Paraffin embedded brains were sectioned to reveal the hippocampal CA region and processed for light microscopy [12], and likewise same embedded tissues sectioned at 5 microns thick were processed to reveal glial fibrillary acidic protein expression [13]. Paraffin embedded tissue cut at 5 microns thick was allowed to heat on hot plate for 1 hour, then sections were taken to xylene, alcohols and finally water respectively. Antigen retrieval method was performed using citric acid solution pH 6.0 in a pressure cooker for 15 minutes. Sections were equilibrated by gently displacing hot *citric acid with running tap water for 3 minutes.* Blocking of peroxidises in tissue sections was done using peroxidise block for 15 minutes and then washed for 2 minutes with phosphate buffered saline (PBS) with tween 20. Blocking of protein was then performed with Novocastra® protein block for 15 minutes. Tissue section was then washed for 2 minutes with PBS, then incubated with primary antibody e.g., goat anti-Glial fibrillary acidic protein (GFAP) 1 in 100 dilution for 45 minutes, washed in PBS for 3 minutes and later added rabbit anti-goat secondary antibody for 15 minutes. Tissue section was then washed twice with PBS. Polymer was thereafter added and allowed for 15 minutes, washed twice with PBS and then added the diaminobenzidine (DAB) chromogen diluted 1 in 100 with the DAB substrate for 15 minutes, and then washed with water and counterstained for 2 minutes in Haematoxylin. Again the tissue section was washed, dehydrated, cleared and mounted in DPX mountant.

Statistical analysis

The results are presented as mean ± standard error of mean (SEM) and were analyzed for statistical significance by one-way analysis of variance (ANOVA). The values with $p < 0.05$ were considered statistically significant.

RESULTS

In Table 1 is presented the haematological indices where treatment groups (Rv 100 mg/kg, 200 mg/kg and 300 mg/kg) are compared to the control group. WBC, RBC, HGB, and HCT were statistically ($p>0.05$) insignificant when treatment groups were compared to the control. However, MCV is significantly ($p<0.05$) increased when group 4 (Rv 300 mg/kg) is compared to the control; a, b when group 2 and group 4 is significantly ($p<0.05$) decreased compared to group 2; c, d when group 1 and group 2 is significantly ($p<0.05$) increased compared to group 3.

In Figure 1 (A-D) is shown the paraffin embedded section stained with haematoxylin and eosin of the hippocampus CA1. In 2A is the control with normal cytoarchitecture (not affected); 2B shows moderate neuronal shrinkage with onset of sparse vacuolations (mildly affected); 2C had more prominent vacuolations and sparse neuronal shrinkage (moderately affected); 2D has similar prominent vacuolations and mild neuronal shrinkage onset of neurodegeneration and fewer neuronal loss of cell density (moderately affected).

In Figure 2 (A-D) is shown the immunolabeling marker for neuroinflammation and neurotoxicity via the expression of glial fibrillary acidic protein (GFAP). 2A shows normal expression of GFAP in a control hippocampal CA1 section with the absence of dendritic thickening or astrocytic cell body swellings (+ mildly expressed); 2B shows depressed GFAP expression (+ low to mildly expressed); 2C shows intense immunopositivity with foci astrocyte cell body thickening and dendrites (+ low stained); 2D shows very weak stain intensity for GFAP (+ low stained).

Table 1. Effect of *Rauwolfia vomitoria* on Haematological Indices in Albino Wistar Rats

Parameters	Control	Rv 100 mg/kg	Rv 200 mg/kg	Rv 300 mg/kg	P value
WBC ($X10^3$/UL)	8.00±1.67	12.10±1.47^{NS}	14.20±2.43^{NS}	11.00±0.89^{NS}	0.117
RBC ($X10^3$/UL)	7.36±0.18	8.77±0.55^{NS}	7.61±0.15^{NS}	8.26±0.43^{NS}	0.060
HGB (g/dL)	12.56±0.37	14.54±0.80^{NS}	13.38±0.29^{NS}	13.54±0.57^{NS}	0.123
HCT (%)	52.76±1.19	61.2±3.16^{NS}	53.76±1.74^{NS}	55.88±2.83^{NS}	0.094
MCV (fL)	71.76±0.41	69.94±0.93^{NS}	70.58±1.20^{NS}	67.72±1.00*	0.047
MCH (pg)	17.06±0.09	16.62±0.19^{a}	17.6±0.17^{NS}	16.42±0.27^{b}	0.002
MCHC (g/dL)	23.78±0.19^{c}	23.74±0.10^{d}	24.94±0.45^{d}	24.28±0.28^{NS}	0.030
PLT ($X10^3$/UL)	941.6±55.17	875.6±123.12^{a}	536.2±107.70^{NS}	942.2±67.20^{b}	0.019

* is significantly ($p<0.05$) increased when group 4 is compared to the control; a, b when group 2 and group 4 is significantly ($p<0.05$) decreased compared to group 2; c, d when group 1 and group 2 is significantly ($p<0.05$) increased compared to group 3.

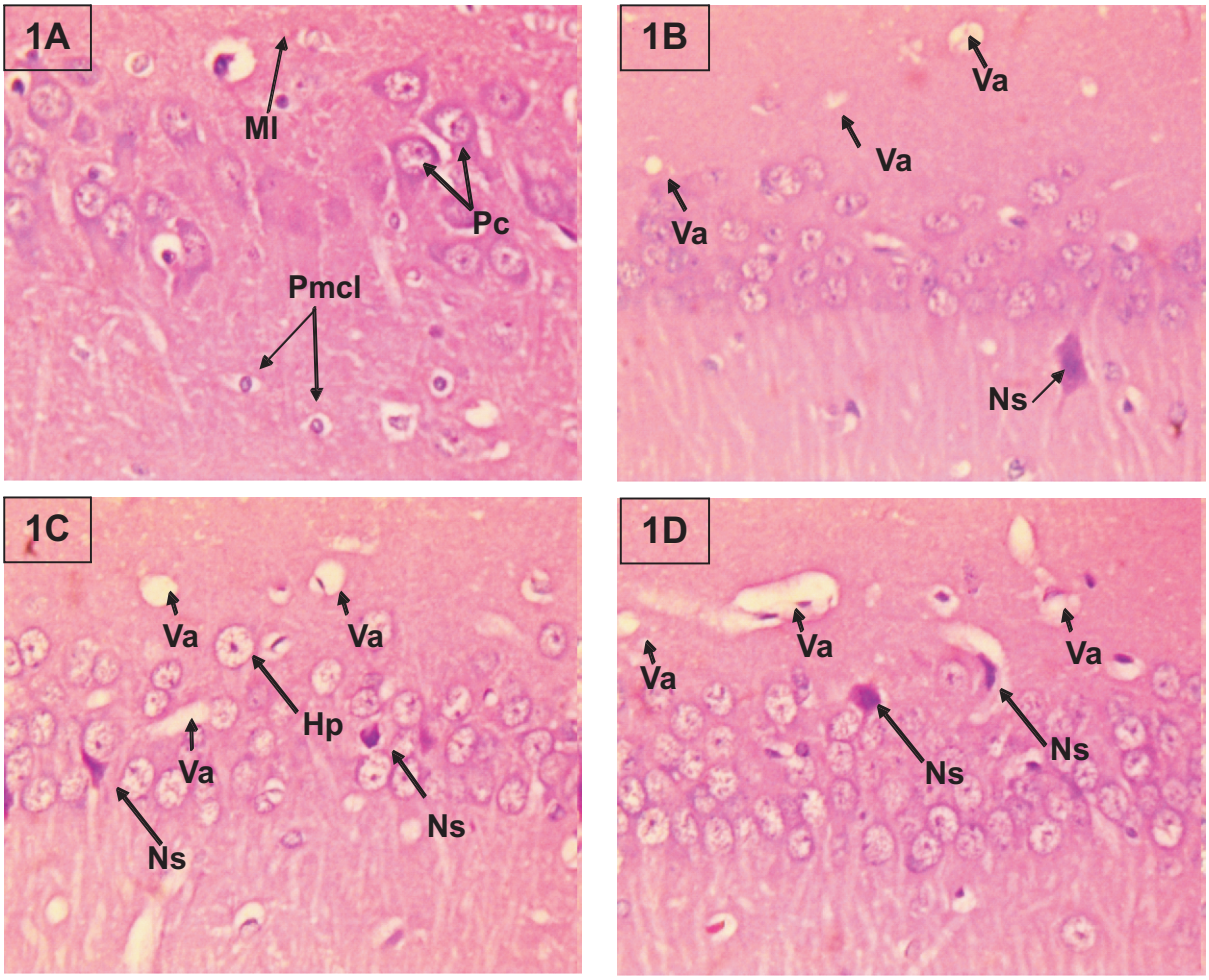

Figure 1: (A-D). Photomicrograph of the CA1 section of the hippocampus of the control group showed three layers; molecular layer (Ml), pyramidal layer (Pl), and polymorphic cell layer (Pmcl) H&E, x400. Va – vacuolation; Hp – Hypertrophy; Ns – Neuronal shrinkage

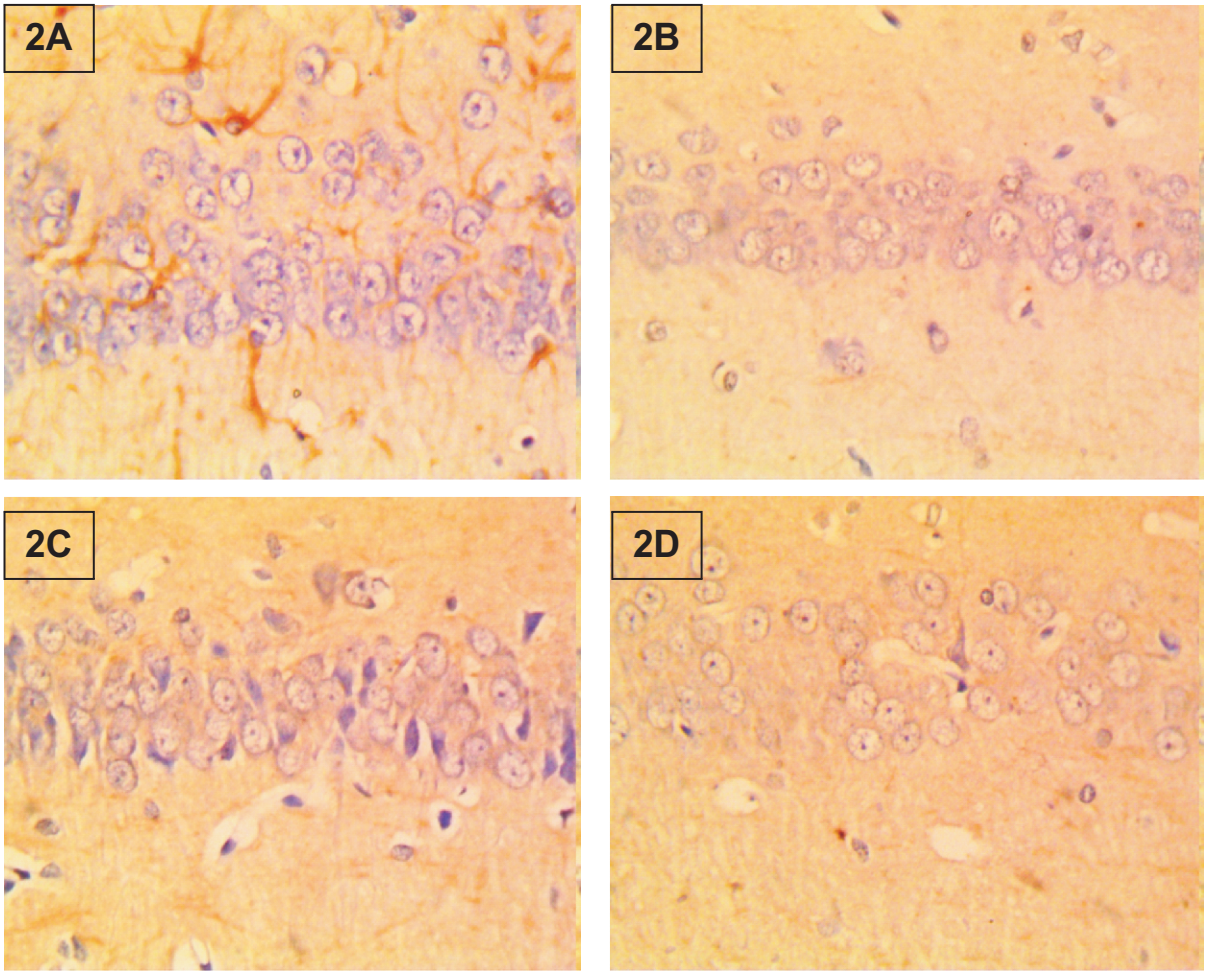

Figure 2: (A-D). Photomicrograph of the CA1 section of the hippocampus of the control and treatment groups showing three layers; molecular layer (Ml), pyramidal layer (Pl), and polymorphic cell layer (Pml) H &E, x400.

DISCUSSION

White blood cell (WBC) mildly increased in the treatment groups (Rv 100 mg/kg, 200 mg/kg and 300 mg/kg) compared with the control group in Table 1, but was not statistically significant. WBC increases in infection, inflammation and tissue damage, however WBC and neutrophil counts do not always indicate disease severity [14] though elevated WBC is associated with higher risk for most disorders [15].

The gradual sparse-like to wide-spread hydropic vacuolations with little foci neuronal shrinkage observed in the treatment groups compared to the control may indicate an early onset of some neurotoxic trauma to the pyramidal neurons and the neuropil. [16] reported that *Rv* administered singly may cause hypertrophied cerebral cortical cellswith few pyknotic cells in rats. Ethanolic root bark of Rv is reportedly more neurotoxic than the leaf extract and may cause neuronal degeneration in the cerebellum of the Wistar rats [17], this corroborates the results of this study in which the treated groups (Fig. 1B-1D) showed atrophy and vacuolations in the parenchyma. Studies have demonstrated that there may be molecules of varying activity in a plant (which may or may not act synergistically), but when the compounds are isolated, overall activity may be lost [18, 19]. Polyvalence however describes the range of biological activities that a plant extract may exhibit that contribute to the overall observed clinical effects, whereas synergism applies to only one pharmacological function [18].

The hippocampus is part of a system that plays a critical role in the encoding and retrieval of long-term memory for facts and events. The system is vital for this "declarative" or "explicit" form of memory, but is not involved in other forms of long term memory, in nonmnemonic aspects of cognition, or in immediate (or "working") memory. Its involvement in declarative memory is not permanent, but is time-limited in nature [20]. Interestingly in Figure 2 (C-D) the expression of glial fibrillary acidic protein was down regulated compared to control (A). The mechanism leading to decreased expression of GFAP remains unclear. However, it is suggested that the role of the active principle of the extract, reserpine which causes the sedative or tranquilizing activity of the plant via the depletion of serotonin stores in the brain tissues might

be affected [21]. Decreased GFAP expression is invariably associated with detrimental conditions in the central nervous system [22], including depression [23] with functional impairment of the hippocampus.

In conclusion, the ethanolic extract of *Rauwolfia vomitoria* mildly increases WBCs in dose dependent concentrations; causes prominent vacuolations and mild neuronal shrinkage in the hippocampal neuropil and down regulates the expression of glial fibrillary acidic protein in the hippocampal CA1 region of Wistar rats.

Conflict of interest
None is declared.

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