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Comparative Toxicity Studies of *Rauwolfia vomitoria* Leaf and Root Extracts in Wistar Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author EOAT designed the study, performed the statistical analysis, wrote the protocol and revised the manuscript. Author AII wrote the first draft of the manuscript, carried out the study, while author IEMN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: *Rauwolfia vomitoria* Afzel (Apocynaceae) is used in the African traditional medical practice for the management of various diseases such as a cough, malaria and as an anti-psychotic. The present study investigates the toxicological potential of the administration of the leaf and root extracts of *Rauwolfia vomitoria* on the brain, kidney and liver of male Wistar rats.
Materials and Methods: The toxicity study was carried out by Lorke's method. For the acute study, thirteen groups of 5 rats each were orally administered 10, 100 and 1000 mg/kg of aqueous and ethanol extracts of leaf and root of *R. vomitoria.* Mortality was recorded after 24h. For the subacute, thirteen groups of five rats each were orally given doses of 1600, 2900 and 5000 mg aqueous and ethanol extracts of leaf and root of *R. vomitoria*. Observation continued for 2weeks after administration to check for any mortality arising from delayed toxicity. In the end, surviving animals were sacrificed and pathological changes were observed.
Results: There was a dose-dependent increase in the liver enzymes in the subchronic and subacute. Histological studies reveal an area of inflammations in the liver subacute study. No significant differences were noticed in the concentration of urea, creatinine and the electrolytes among the various group and control. There were no marked cellular changes in the kidney tissue.

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Convulsion was observed in a subacute study among the animals at a high dose of 5000 mg of the root extract.
Conclusion: The use of the leaf and root extracts of *Rauwolfia vomitoria* is safe to the liver, kidney and brain when used at low doses over a long time but harmful when used at high doses over a short time.

Keywords: Rauwolfia vomitoria; leaf and root extracts; toxicity; liver enzymes; rats.

1. INTRODUCTION

Rauwolfia vomitoria Afzel (Apocyanaceae) is one of the medicinal plants that have served all through the ages as the mainstay in the treatment and preservation of human health [1,2]. It is a rainforest shrub having oval leaves and straight venation and clusters of tiny flowers producing red berries. It grows in the tropical forest of Pacific, South America, Asia and Africa [3,4]. The plant has many common names like serpent wood, serpent root, and swizzle stick. In Nigeria, it is known as 'asofeyeje' in Yoruba, 'wada' in Hausa,' akanta' in Ibo, 'akata' in Bini and "utoenyin" in Efik languages, respectively [5]. The parts that are commonly used for herbal remedies are roots, root bark, leaves and stem-bark [6]. From 1931, Indian doctors researched on possible utilization of *Rauwolfia vomitoria* in neuro-psychiatry. The extract from this plant was first extracted by Swiss Chemists in 1952 and becomes the first natural neuroleptic [4]. Today, this plant is still the source of a lot of drugs used in psychiatry [3].

Extensive studies carried out on its chemical properties showed that the plant contained more than 50 active indole alkaloids, each possessing remarkable pharmacological activities. The pharmaceutical derivatives are used mainly as antihypertensive and sedative drugs. Its sedative property is attributed to its ability to balance body response to stress, anxiety and to increase oxygen delivery to the brain [7,8]. The roots and the leaves of *Rauwolfia vomitoria* are brewed as a tea and used traditionally for the treatment of hypertension, insanity, and cholera. *Rauwolfia vomitoria* has been associated with CNS depression [9], reduce neurobehavioural activity and distort the cytoarchitecture of the cerebellum [10]. The leaf and root extracts of *Rauwolfia vomitoria* have been reported not to have deleterious effects on the kidney and liver functions in rats [11].

With the current increasing proposals for the incorporation of traditional medicine into the healthcare programmes in most countries of the World, it would be of urgent necessity to investigate the toxicity of medicinal plants used in the traditional medical practice for the treatment of diseases to establish their safety for consumption [11]. Reports regarding the effects of the various leaf and root extracts of *R. vomitoria* on the histological and biochemical functions of the brain, kidney and liver are scanty in existing literature. Hence, the present study was carried out to investigate the comparative effects of the leaf and root extracts of this plant and to ascertain its toxicity on the histology and biochemical functions of the brain, kidney and liver in Wistar rats.

2. MATERIALS AND METHODS

2.1 Collection of *Rauwolfia vomitoria* and Identification

Fresh leaves and root of *R. vomitoria* were obtained from Lagos State Polytechnic farm, Lagos, Nigeria. The stem barks were identified and authenticated with voucher number LUH 6213 by Professor J.D. Olowokudejo of the Department of Botany, University of Lagos, Nigeria.

2.2 Preparation of the Leaf and Root Extracts

The roots and leaves were cleaned, diced into smaller pieces and were air dried at room temperature for two weeks. They were blended and extracted using a Soxhlet extractor. The extract was concentrated using a rotary evaporator and preserved at 4°C for further use.

2.3 Animal Handling

Sixty-five albino Wistar rats (185.64±7.03 g) were used for this study. The rats were obtained from the laboratory animal centre of the College of Medicine, University of Lagos, and were acclimatized for 2 weeks. They were fed with rat chow and water *ad libitum*.

2.4 Acute Toxicity Study

The toxicity study was carried out by the method of Lorke [12]. Thirteen groups of five rats each were orally administered 10, 100 and 1000 mg/kg of aqueous and ethanol fractions of root and leaf of *R. vomitoria.* The treated rats were monitored for 2h for mortality, general behavior and after 24h, mortality was recorded.

2.5 Sub Acute Toxicity Study

Thirteen groups of 5 rats each were orally administered doses 1600, 2900 and 5000 mg /100 g body weight aqueous and ethanol extracts of leaf and root of *R. vomitoria*. Observation continued for 2 weeks after administration to check for any mortality arising from delayed toxicity. Parameters observed after dosing include: tremors, diarrhea, death and other general behaviour. At the end, surviving animals were sacrificed and pathological changes were observed.

2.6 Collection of Organs and Blood

The rats were sacrificed by cervical dislocation and the brain, liver and kidney were collected, carefully excised and fixed in 10% formal saline in universal sample bottles. Blood samples were collected by orbital puncture into lithium heparin bottles, centrifuged at 3000 rpm for 10 min and the plasma collected for biochemical studies.

2.7 Biochemical Analysis

Liver Function Test: L-alanine, L-aspartate aminotransferases and alkaline phosphatase activities were determined using Randox kits according to manufacturer's protocol.

Kidney function test: Electrolyte, urea and creatinine were determined using diagnostic kits according to manufacturer's protocol.

2.8 Histological Studies

The brain, kidney and liver from each group were fixed in 10% formal saline for 48h. The organs were dehydrated in ascending grades of alcohol, cleared in xylene, embedded in paraffin and sectioned. The kidney sections were stained in periodic acid Schiff, while liver and brain sections were stained in haematoxylin and eosin. The slides were examined at magnifications of X400 under a light microscope.

2.9 Statistical Analysis

Statistical significance was established using one way analysis of variance (ANOVA) and data were reported as mean ± standard deviation. The level of statistical significance was taken as $P<0.05$. GraphPad prism 6.0 was used.

3. RESULTS

During the sub-acute toxicity study, only the ethanol root extract showed mortality in all concentrations. At the highest concentrations of 1000 mg/kg of the acute study and 5000 mg/kg of the sub-acute, the other extract showed no mortality. Hence LD_{50} value is greater than 5000 mg/kg body weight for the aqueous root, aqueous leaf and ethanol leaf. From the Probit curve, the LD_{50} for the root ethanol is 3162 mg/kg.

Table 1. Liver function of rats administered root extract of *Rauwolfia vomitoria* for 14 days

Group/concentration(mg/kg)	AST(IU/L)	ALT(IU/L)	ALP(IU/L)
RA1600	180. 0±28.28*	65.0±7. 07	61.50±2. 12
RE1600	140.0±63.64	65.0±21.21	69.0±2. 83
RA2500	195. 0±35.36*	45.0±7. 07	57.50±14. 83
RE2500	155. 0±49.50	35.0±7. 07	84.50±9.19
RA5000	130. 0±0. 00	115.0±7. 07*	64.0±4. 24.
RE5000	215. 0±7. 07*	115.0±7. 07*	79.50±19. 09
CONTROL	75. 00±7. 07	50.0±7. 07	68.50±17. 68

RA=Root Aqueous, RE= Root Ethanol
**= Values are significant at p<0. 05.*
AST=Aspartate amino transferase, ALT= Alanine amino transferase,
ALP= alkaline phosphate

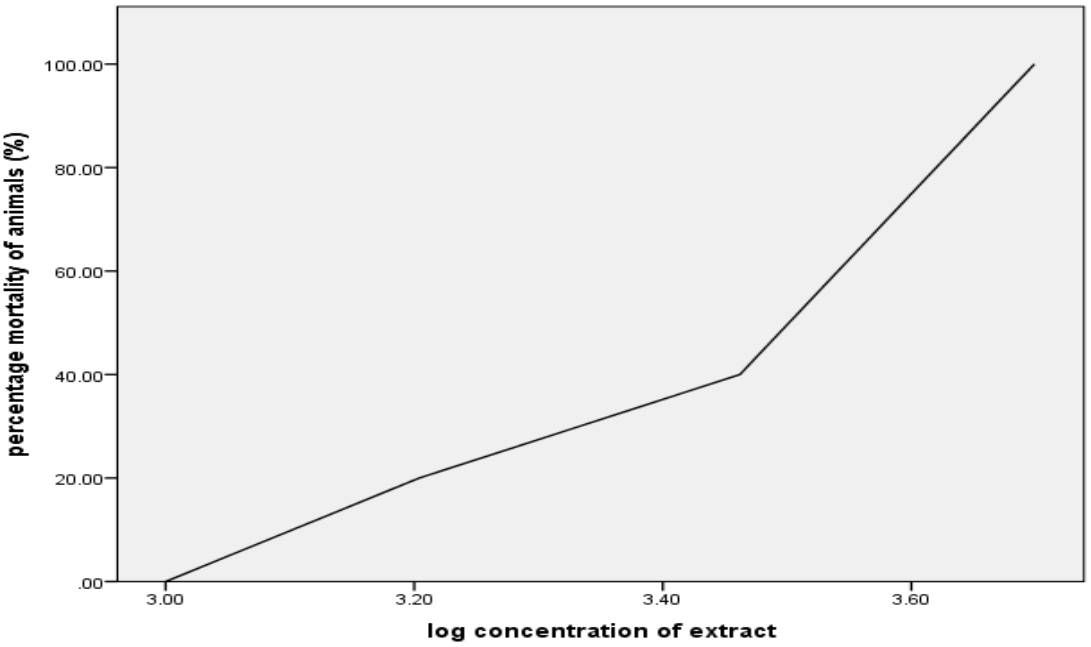

Fig. 1. Probit curve showing LD_{50} for ethanolic root extract of *Rauwolfia vomitora*
LD_{50} = 3.50; Antilog of 3.50 = 3162.2mg/kg; LD_{50}= 3162mg/kg

Table 2. Liver function of rats administered leaf extract of *Rauwolfia vomitoria* for 14 days

Group/concentration(mg/kg)	AST(IU/L)	ALT(IU/L)	ALP(IU/L)
LA1600	245. 0±63.64*	65. 0±7. 07	67. 00±5.66
LE1600	110. 0±14.14*	75. 0±21.21	69. 00±5.66
LA2500	195. 0±7. 071*	80. 0±14.14*	69.50±4.95
LE2500	230. 0±28.28*	65. 0±7. 07	75.50±7.78
LA5000	95. 00±7. 07*	105. 0±7. 07*	74.50±15.41
LE5000	165. 0±21.21*	125. 0±7. 07*	71. 00±12.73
CONTROL	75. 00±7. 07	50. 0±0. 00	68.50±17.68

LA=Leaf Aqueous, and LE=Leaf Ethanol Extracts
**= Values are significant at p<0.05. AST=Aspartate amino transferase, ALT=Alanine amino transferase, ALP=Alkaline Phosphatase*

Table 3. Electrolyte levels of rats administered root extract of *Rauwolfia vomitoria* for subacute toxicity

Group/concentration(mg/kg)	$K^{+mEq/l}$	$Na^{+mEq/l}$	$Cl^{-mEq/l}$	$HCO_3^{-mmol/l}$
RA1600	4.35±0.78	140.5±0.71	102.0±1.41	23.0±0.00
RE1600	4.65±1.20	141.5±2.12	105.5±4.95	23.0±1.41
RA2500	4.80±0.42	145.5±2.12	103.5±4.95	24.0±1.41
RE2500	4.40±0.0	141.0±1.41	105.0±4.24	23.0±0.00
RA5000	5.15±0.35	145.0±0.21	107.5±2.12	24.5±0.71
RE5000	4.05±0.21	141.5±0.71	103.5±2.12	22.5±0.71
CONTROL	5.00±0.14	140.5±0.71	103.5±0.71	24.0±0.00

RA=Root Aqueous, RE= Root Ethanol
** = Values are significant at p<0.05.*
K^+=Potassium, Na^+=Sodium, Cl^-=Chloride, HCO_3^-=Bicarbonate

Table 4. Electrolyte levels of rats administered leaf extract of *Rauwolfia vomitoria* for subacute toxicity

Group/concentration(mg/kg)	K^{+}mEg/l	Na^{+}mEg/l	Cl^{-}mEg/l	HCO_3^{-}mmol/l
LA1600	7.15±3. 04	147.5±6.36	107. 0±2.83	24.5±0.71
LE1600	5.45±1. 06	144.5±3.54	111. 0±4.24	25.0±1.41
LA2500	5. 00±0.28	141.5±2.12	107. 0±4.24	23.0±1.41
LE2500	4.45±0. 07	141.5±0.71	104. 0±0. 00	24.0±0. 00
LA5000	4.25±0. 07	141.5±0.71	103. 0±1.41	23.5±0.71
LE5000	4.10±0.14	143. 0±1.41	103.5±3.54	24. 0±0.00
CONTROL	5.00±0.14	140.5±0.71	103.5±0.71	24. 0±0.00

LA=Leaf Aqueous, LE= Leaf Ethanol

** = Values are significant at p<0.05.*

K^{+}=Potassium, Na^{+}=Sodium, Cl^{-}=Chloride, HCO_3^{-}=Bicarbonate

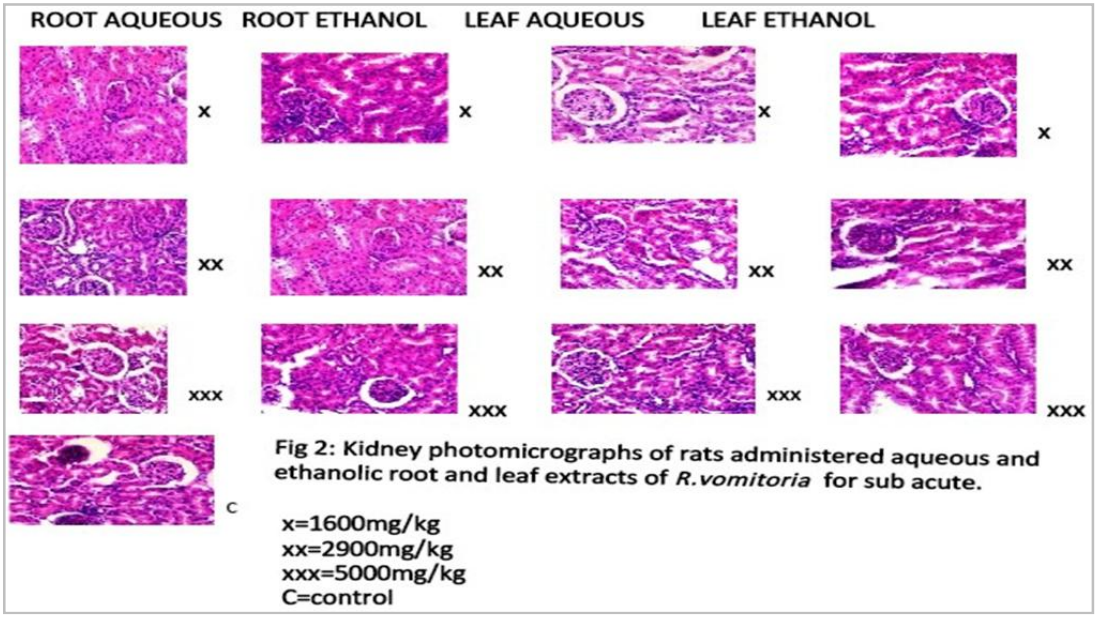

Fig 2: Kidney photomicrographs of rats administered aqueous and ethanolic root and leaf extracts of *R.vomitoria* for sub acute.

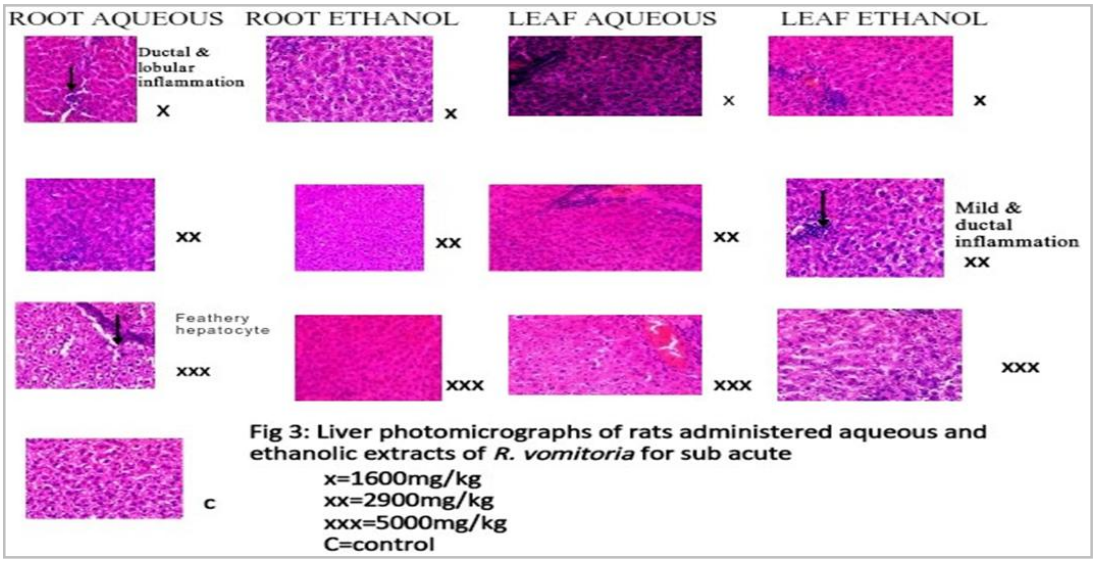

Fig 3: Liver photomicrographs of rats administered aqueous and ethanolic extracts of *R. vomitoria* for sub acute

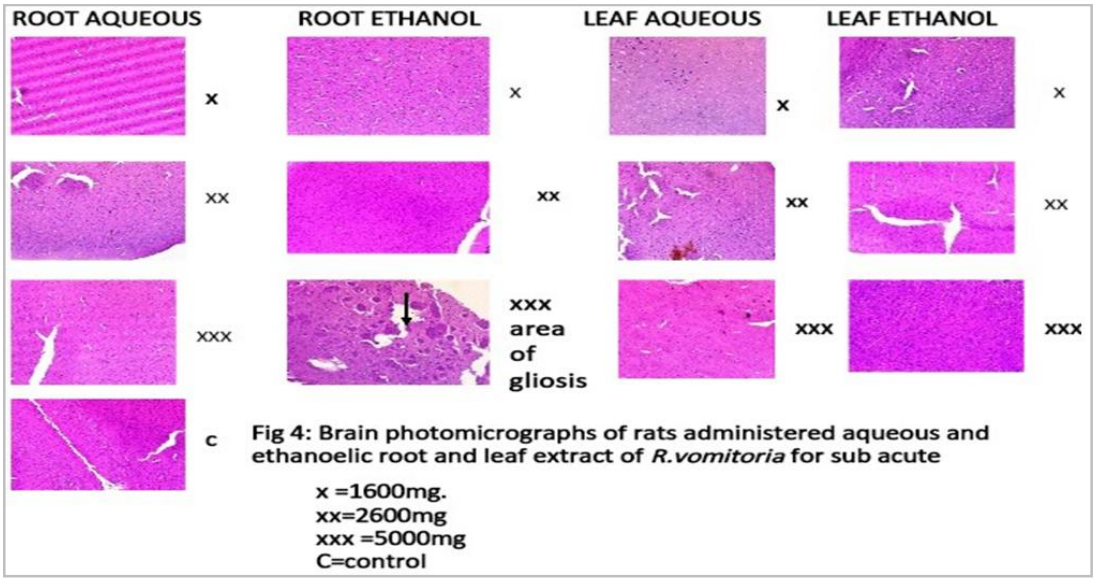

Fig 4: Brain photomicrographs of rats administered aqueous and ethanoelic root and leaf extract of *R.vomitoria* for sub acute

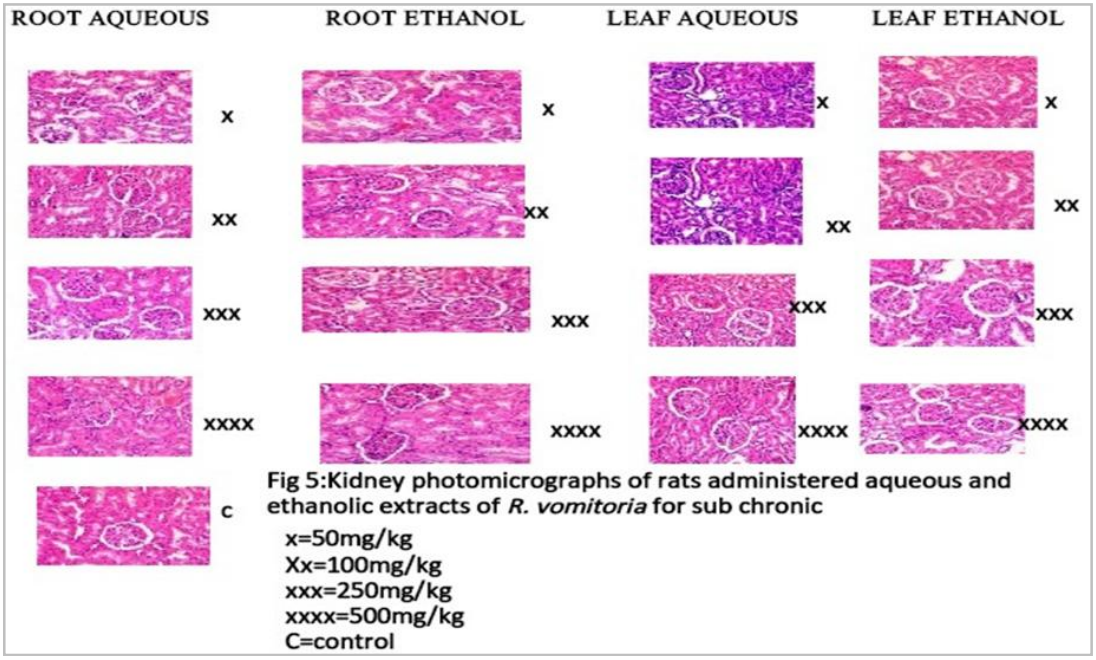

Fig 5:Kidney photomicrographs of rats administered aqueous and ethanolic extracts of *R. vomitoria* for sub chronic

Table 5. Kidney function of rats administered root extract of *Rauwolfia vomitoria* for 14 days

Group/concentration(mg/kg)	Urea(mol/l)	Creatinine(mol/l)
RA1600	34. 0±1.41	1.55±0.21
RE1600	40. 0±2.83	1.60±0.28
RA2500	41.5±6.36	1.80±0.14
RE2500	31.5±6.36	1.20±0. 00
RA2500	38.5±10.6	1.55±0.50
RE2500	39. 0±0. 00	1.65±0. 07
CONTROL	43. 0±0. 00	1.35±0. 07

RA=Root Aqueous, RE = Root Ethanol Extracts

⋆ *= Values are significant at p<0.05*

Table 6. Kidney function of rats administered leaf extract of *R. vomitoria* for subacute toxicity

Group/concentration(mg/kg)	Urea(mol/l)	Creatinine(mol/l)
LA1600	33. 0±4.24	1.45±0.21
LE1600	35.5±0.71	1.45±0. 07
LA2500	35. 0±0.71	1.55±0.21
LE2500	34.5±1.41	1.35±0.70
LA5000	37. 0±5.66	1.40±014
LE5000	14.77±2.41	1.60±4.53
CONTROL	43. 0±0.00	1.35±0.07

RA=Root Aqueous, RE = Root Ethanol Extracts
** = Values are significant at p<0.05*

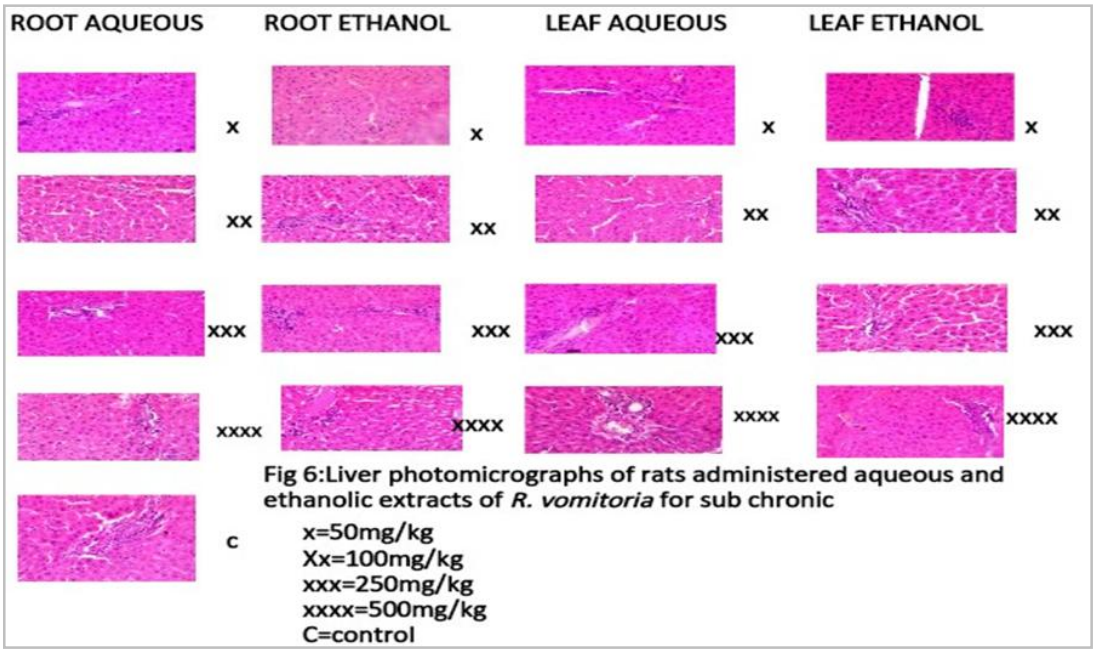

Fig 6:Liver photomicrographs of rats administered aqueous and ethanolic extracts of *R. vomitoria* for sub chronic

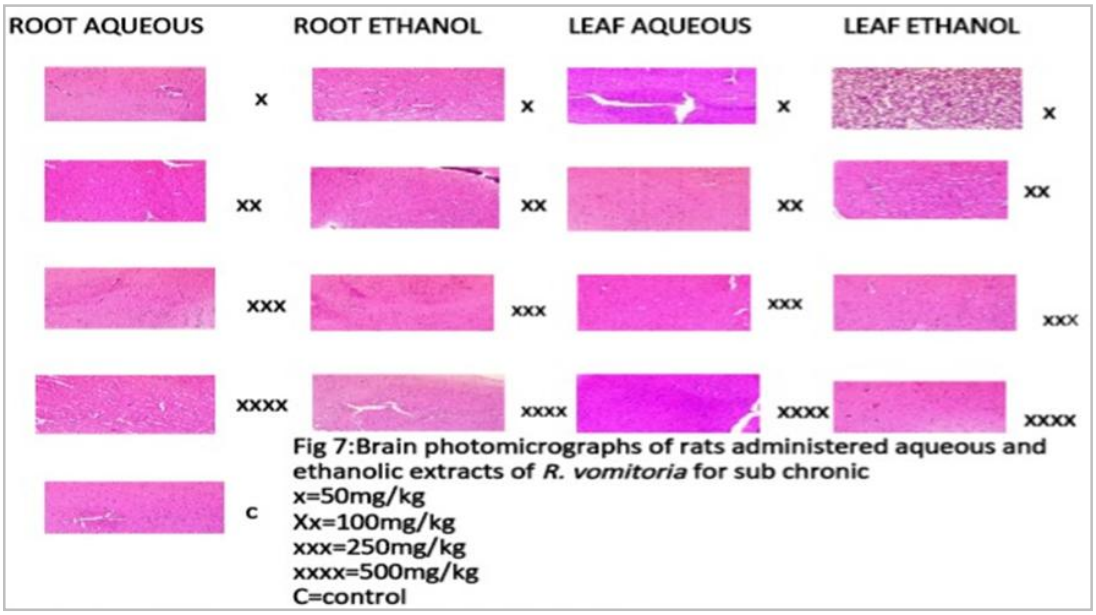

Fig 7:Brain photomicrographs of rats administered aqueous and ethanolic extracts of *R. vomitoria* for sub chronic

4. DISCUSSION

Evaluation of blood parameters in animal toxicity studies is of great importance to report any alterations in those parameters and evaluate the relative risk to the hematopoietic system when extrapolating those findings to humans [13]. Determining certain blood biochemical parameters and investigating major toxic effects on specific tissues, specifically the kidney, brain and liver, may provide useful information regarding the mechanisms of toxicity of /and otherwise safe and therapeutic agent [1,14,15].

Enzymes are essential factors, which enable many biochemistry of life to proceed in the body cells. Change in the enzyme concentrations in plasma should therefore reflect the state of health, since any damage to the tissues tend to spill these enzymes into plasma [16,17]. Amino transferases are cytosolic enzymes widely distributed in tissues with highest concentration in liver and heart, but ALT is more specific to the liver and AST to the heart. ALT and AST are two liver enzymes used majorly as indicators of liver function [18] and as biomarkers for possible toxicity prediction. Significant quantities are found in the serum when the cell membrane becomes leaky or completely ruptured [19]. Some studies, however have reported the toxicity and non-toxicity potential of the leaf and root extracts of *R. vomitoria*. During the sub acute phase, there was significant difference in the activities of AST and ALT in animals treated with both the leaf and root extracts of *R. vomitoria*. The increase of ALT, AST activities and inflammations noticed in the histological findings during the sub acute study reveals that the hepatocytes are impaired by the root and leaf extracts. This is in agreement with the work of Ibrahim et al. [20].

The kidney play a good role in excreting end products of body metabolism, drugs, toxins, etc and regulates the concentration of H+, Na+, K+, $P0_4^-$ and other ions, i.e. water and electrolyte balance via anti- diuretic hormone. Renal function test evaluates the severity of renal diseases and the functional state of the kidney. Creatinine, urea and electrolytes are common parameters often measured to assess the state of the kidney. Results from this study shows no significant difference ($p \leq 0.05$) in the serum levels of urea and creatinine in the extract treated groups compared with the control as shown in Table 5. Urea and creatinine are waste products which are passed into the bloodstream to be removed by the kidney. Elevation of these waste products in the blood is a pointer to renal function impairment [21]. Serum levels of these metabolites in the test groups were not significantly different from the control, which showed that the extracts do not cause derangement in the cellular activities of the rat kidneys. There was no significant change in the electrolytes showing that the kidneys were not affected by the extracts at various concentrations. Histologically, the renal corpuscles, proximal convoluted tubule and distal convoluted tubules of the various extracts treated groups show no distortion in their cyto-architecture when compared with the control group. This shows that the extract is safe for the kidney. This finding is in agreement with the previous reports of Ibrahim et al. [21], Ebuehi [22] and Kamal et al. [23].

The area of gliosis seen in the histological studies of the brain shows that the neurons are affected because the glial cells which supply nutrients are impaired. The mortality noted across all groups sub-acutely administered with doses (1600-5000 mg/kg) of the ethanol root fraction suggests that *R. vomitoria* has to be taken with precaution. The cellular architecture of the brain, liver and kidney of rats administered root and leaf extract of *R. vomitoria* revealed no marked damage during the sub-chronic study [12]. This shows that the plant, when used sub-acutely, is not safe, but its usage sub-chronically is safe.

5. CONCLUSION

The aqueous and ethanol root and leaf extracts of *Rauwolfia vomitoria* administered in the study at various concentrations were not deleterious to the kidney, but are likely to impair the liver when used at high doses. Though used as an antipsychotic, it has potentially detrimental properties when used in high concentrations. Therefore, precautions should be taken when it is used for therapeutic purpose.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical permission has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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