

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

ABSTRACT

Rauwolfia vomitoria Afzel (Apocynaceae) is used in the African traditional medical practice for the management of various diseases such as 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. 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 1000mg/kg of aqueous and ethanol extracts of leaf and root of *R.vomitoria*. Mortality was recorded after 24h. For the sub acute, thirteen groups of five rats each were orally given doses 1600, 2900 and 5000mg 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. At the end, surviving animals were sacrificed and pathological changes were observed. There was a dose - dependent increase in the liver enzymes in the sub chronic and sub acute. Histological studies reveal area of inflammations in the liver sub acute 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. Convulsion was observed in sub acute study among the animals at high dose of 5000mg of the root extract. The use of the leaf and roof 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

30 INTRODUCTION

31 *Rauwolfia vomitoria* Afzel (Apocyanaceae) is one of the medicinal plants that have served all
 32 through the ages as the mainstay in the treatment and preservation of human health (Ebuehi,
 33 2012, Ekong *et al.*, 2013). It is a rain forest shrub having oval leaves and straight venation and
 34 clusters of tiny flowers producing red berries. It grows in the tropical forest of Pacific, South
 35 America, Asia and Africa (Kutilek and Prinz, 2007, Amole and Onabanjo, 1999a). The plant
 36 has many common names like serpent wood, serpent snake root, and swizzle stick . In Nigeria, it
 37 is known as ‘asofeyeje’ in Yoruba, ‘wada’ in Hausa,’ akanta’ in Ibo, ‘akata’ in Bini and
 38 “utoenyin” in Efik languages, respectively (Amole and Onabanjo, 1999b). The parts that are
 39 commonly used for herbal remedies are roots, root bark, leaves and stem-bark (Gill, 1992). From
 40 1931, Indian doctors researched on possible utilization of *Rauwolfia vomitoria* in neuro-
 41 psychiatry. The extract from this plant was first extracted by Swiss Chemists in 1952 and
 42 becomes the first natural neuroleptic (Amole and Onabanjo,1999a). Today, this plant is still the
 43 source of a lot of drugs used in psychiatry (Kutilek and Prinz, 2007).

44 Extensive studies carried out on its chemical properties showed that the plant contained more
 45 than 50 active indole alkaloids, each possessing remarkable pharmacological activities. The
 46 pharmaceutical derivatives are used mainly as antihypertensive and sedative drugs. Its
 47 sedative property is attributed to its ability to balance body response to stress, anxiety and to
 48 increase oxygen delivery to the brain (Harbourne, 1992, Dietrich, 1983). The roots and the
 49 leaves of *Rauwolfia vomitoria* are brewed as tea and used traditionally for treatment of
 50 hypertension, insanity, and cholera (Shavorov, 1965). *Rauwolfia vomitoria* has been
 51 associated with CNS depression (Ellenborn *et al.*,1988), reduce neurobehavioural activity and
 52 distort the cytoarchitecture of the cerebellum (Mokutima *et al.*, 2009). The leaf and root
 53 extracts of *Rauwolfia vomitoria* have been reported to have untoward effects (safe) on
 54 kidney and liver functions in rats (Oyewole *et al.*, 2007).

55 With the current increasing proposals for the incorporation of traditional medicine into the
56 health care programmes in most countries of the World, it would be of urgent necessity to
57 investigate the toxicity of medicinal plants used in the traditional medical practice for the
58 treatment of diseases to establish their safety for consumption (Oyewole *et al.*, 2008). Reports
59 regarding the effects of the various leaf and root extracts of *R. vomitoria* on the histological
60 and biochemical functions of the brain, kidney and liver are scanty in existing literature.
61 Hence, the present study was carried out to investigate the comparative effects of the leaf and
62 root extracts of this plant and to ascertain its toxicity on the histology and biochemical
63 functions of the brain, kidney and liver in Wistar rats.

64 MATERIALS AND METHODS

65 Collection of *Rauwolfia vomitoria* and Identification

66 Fresh leaves and root of *R.vomitoria* were obtained from Lagos State Polytechnic farm,
67 Lagos, Nigeria. The stem barks were identified and authenticated with voucher number LUH
68 6213 by Professor J.D. Olowokudejo of the Department of Botany, University of Lagos,
69 Nigeria.

70 Preparation of the Leaf and Root Extracts.

71 The roots and leaves were cleaned, diced into smaller pieces and were air dried at room
72 temperature for two weeks. They were blended and extracted using a Soxhlet extractor. The
73 extract was concentrated using a rotary evaporator and preserved at 4⁰C for further use.

74 Animal Handling

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

78 Acute Toxicity Study

79 The toxicity study was carried out by the method of Lorke (1983). Thirteen groups of five
80 rats each were orally administered 10, 100 and 1000mg/kg of aqueous and ethanol fractions
81 of root and leaf of *R.vomitoria*. The treated rats were monitored for 2h for mortality, general
82 behavior and after 24h, mortality was recorded.

83 **Sub acute Toxicity Study**

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

90 **Collection of Organs and Blood**

91 The rats were sacrificed by cervical dislocation and the brain, liver and kidney were
92 collected, carefully excised and fixed in 10% formal saline in universal sample bottles. Blood
93 samples were collected by orbital puncture into lithium heparin bottles, centrifuged at
94 3000rpm for 10min and the plasma collected for biochemical studies.

95 **Biochemical Analysis**

96 **Liver Function Test**

97 L-alanine, L-aspartate aminotransferases and alkaline phosphatase activities were determined
98 using Randox kits according to manufacturer's protocol.

99 **Kidney Function Test**

100 Electrolyte, urea and creatinine were determined using diagnostic kits according to
101 manufacturer's protocol.

102 **Histological studies**

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

108 **Statistical Analysis**

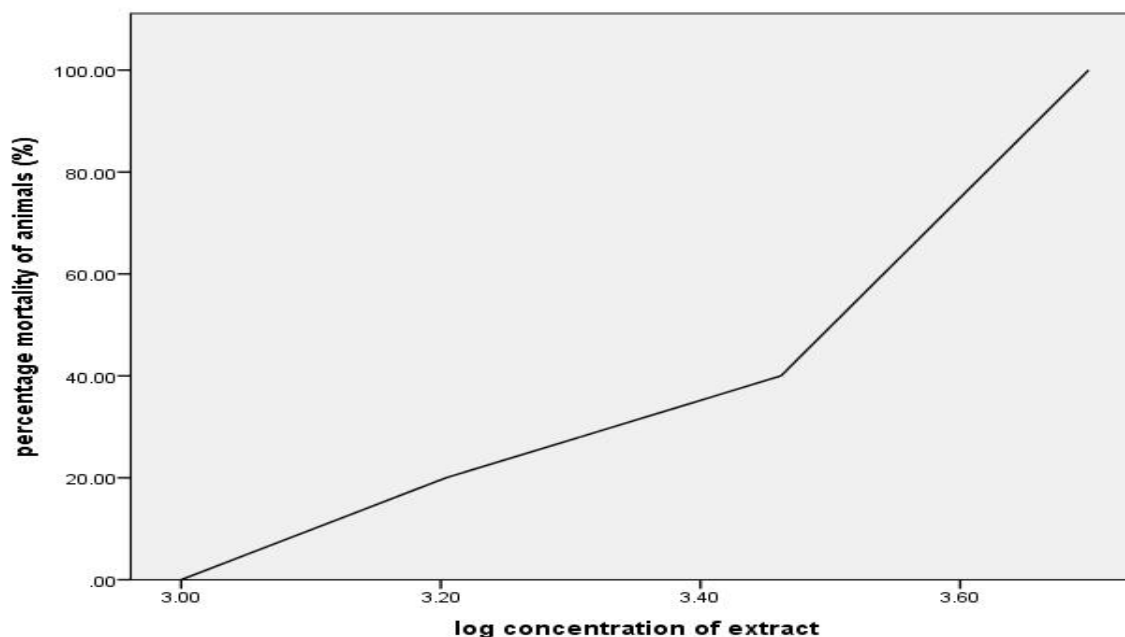
109 Statistical significance was established using one way analysis of variance (ANOVA) and
110 data were reported as mean $\pm$ standard deviation. The level of statistical significance was
111 taken as $P < 0.05$. GraphPad prism 6.0 was used.

112 **RESULTS**

113 During the sub-acute toxicity study, only the ethanol root extract showed mortality in all
114 concentrations. At the highest concentrations of 1000mg/kg of the acute study and
115 5000mg/kg of the sub-acute, the other extract showed no mortality. Hence LD₅₀ value is
116 greater than 5000mg/kg body weight for the root aqueous, leaf aqueous, and leaf ethanol.
117 From the Probit curve, the LD₅₀ for the root ethanol is 3162mg/kg.

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$$LD_{50} = 3.50$$

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$$\text{Antilog of } 3.50 = 3162.2 \text{ mg/kg}$$

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$$LD_{50} = 3162 \text{ mg/kg}$$

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Fig 1: Probit curve showing LD₅₀ for ethanolic root extract of *Rauwolfia*

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vomitara

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

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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

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RA=Root Aqueous, RE= Root Ethanol

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*= Values are significant at $p < 0.05$. AST=Aspartate amino transferase, ALT= Alanine amino transferase, ALP= alkaline phosphate.

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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 ⁺ meq/l	Na ⁺ meq/l	Cl ⁻ meq/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, HCO₃⁻=Bicarbonate

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 $p<0.05$.

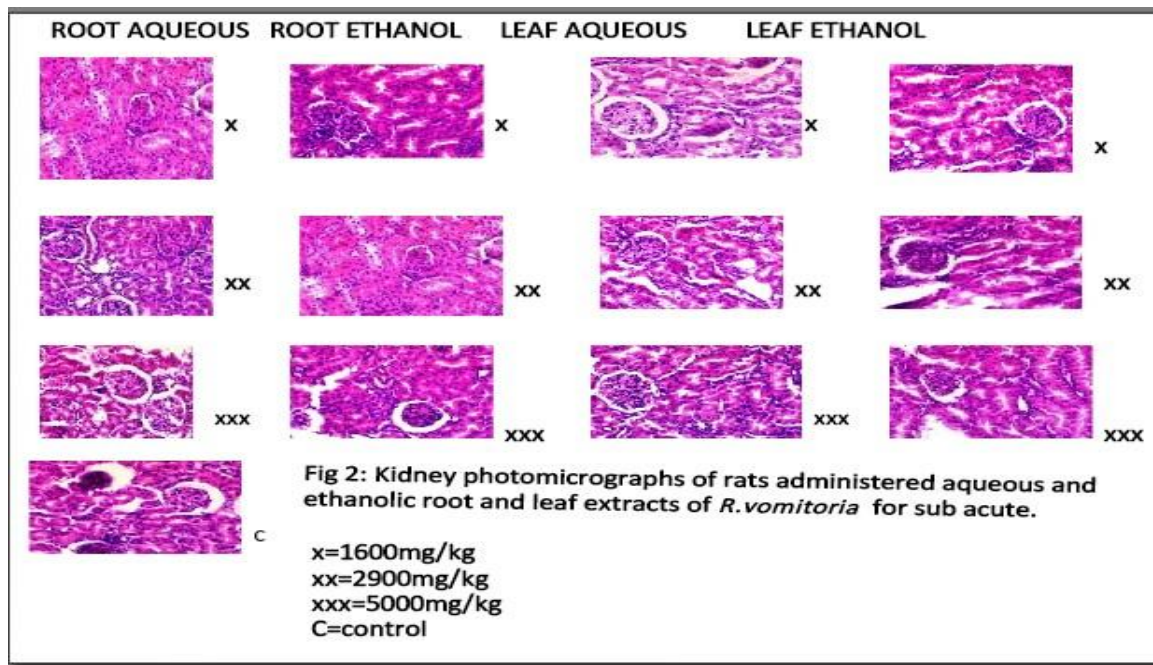
Table 6: Kidney function of rats administered leaf extract of *R.vomitoria* for subacute toxicity

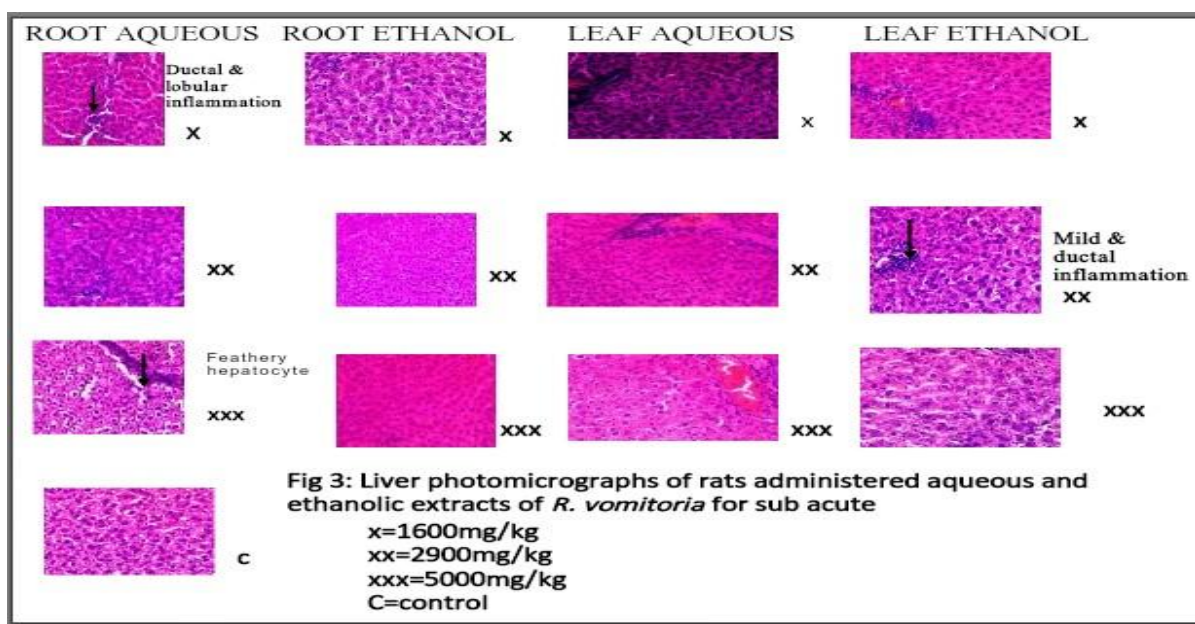
Group/ concentration(mg/kg)	Urea(mol/l)	Creatinine(mol/l)
LA1600	33.0±4.24	1.45±0.21
LE1600	35.5±0.71	1.45±0.07

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

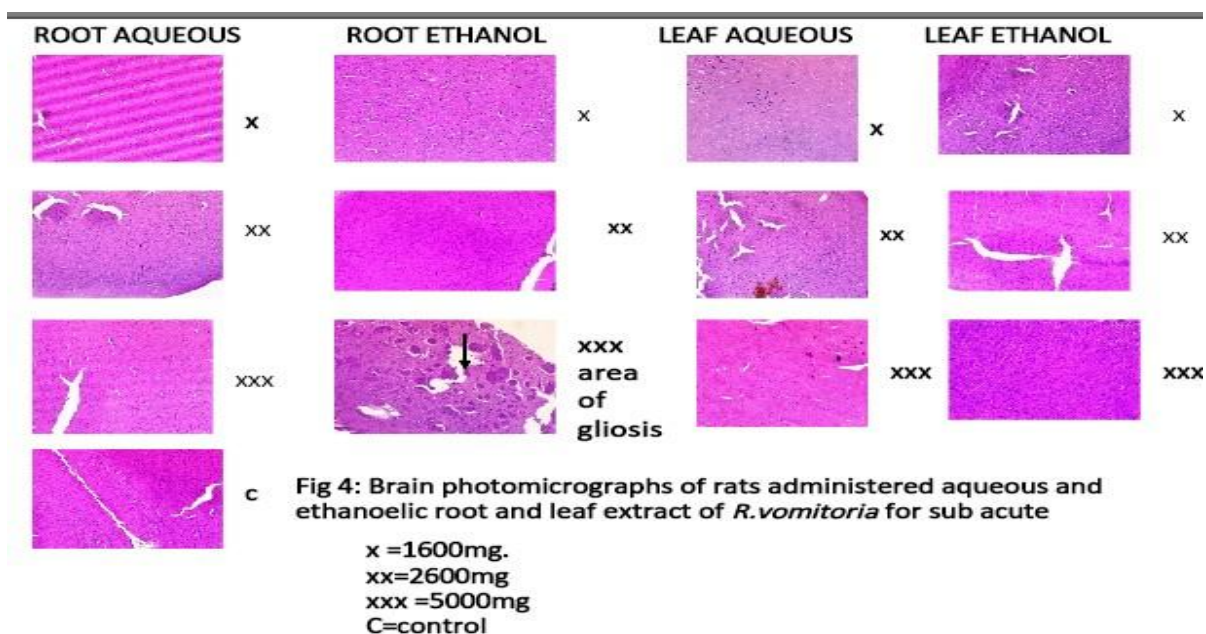
RA=Root Aqueous, RE = Root Ethanol Extracts

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

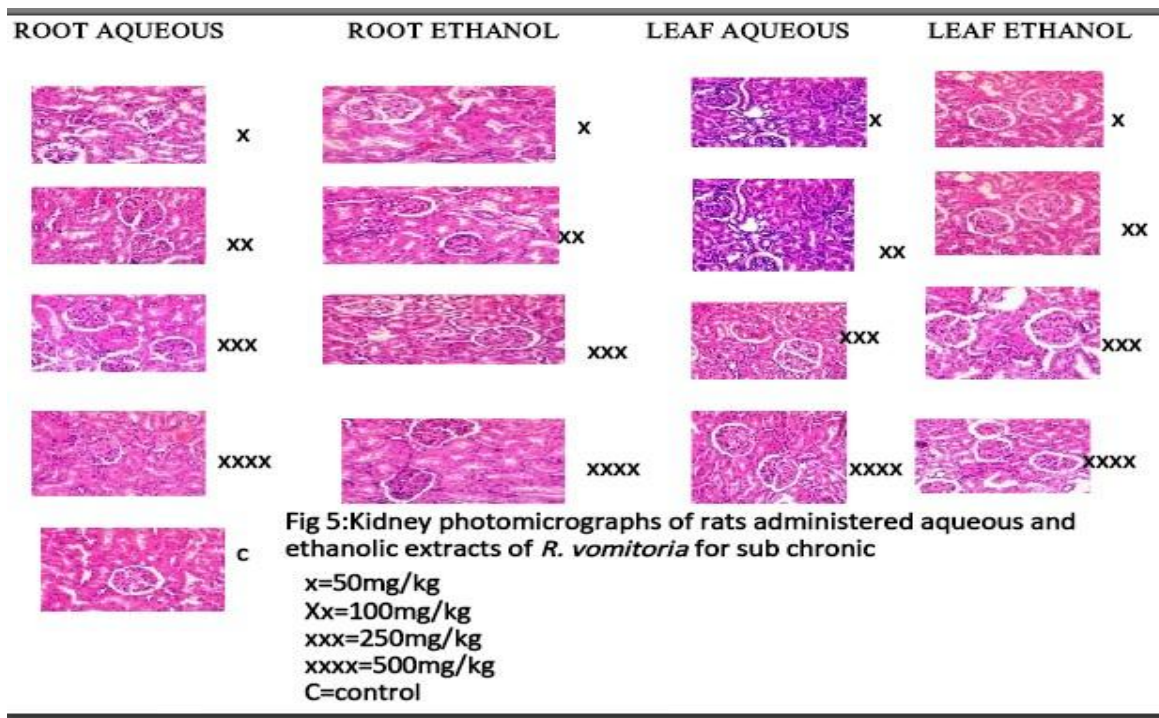




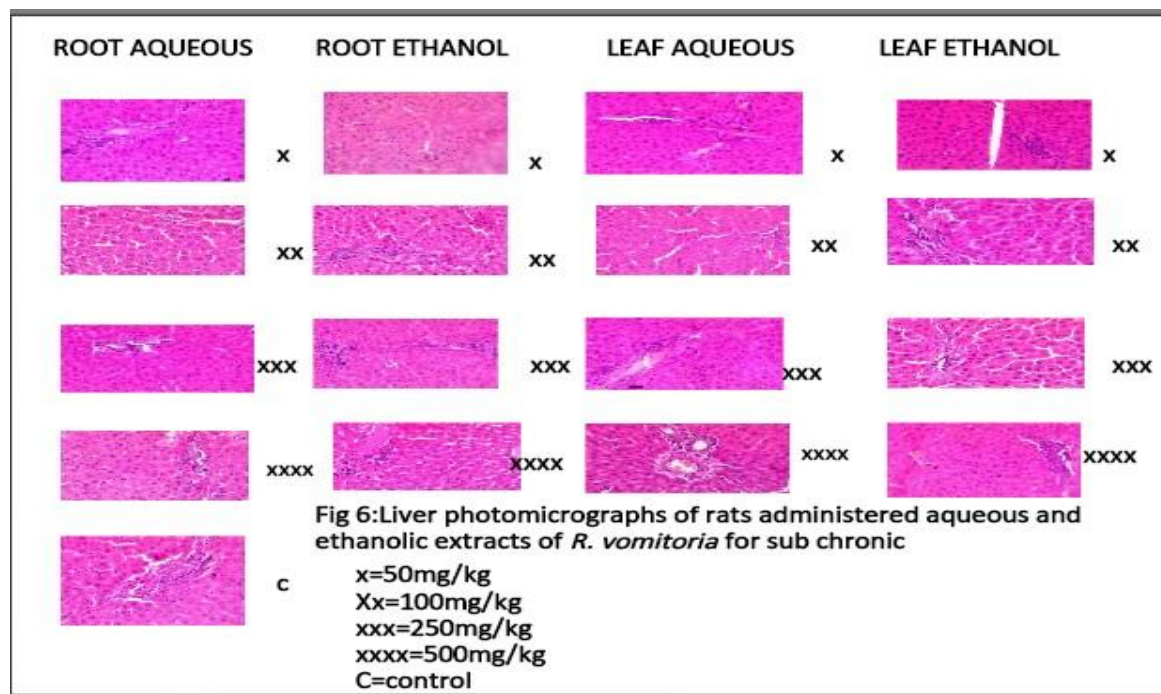
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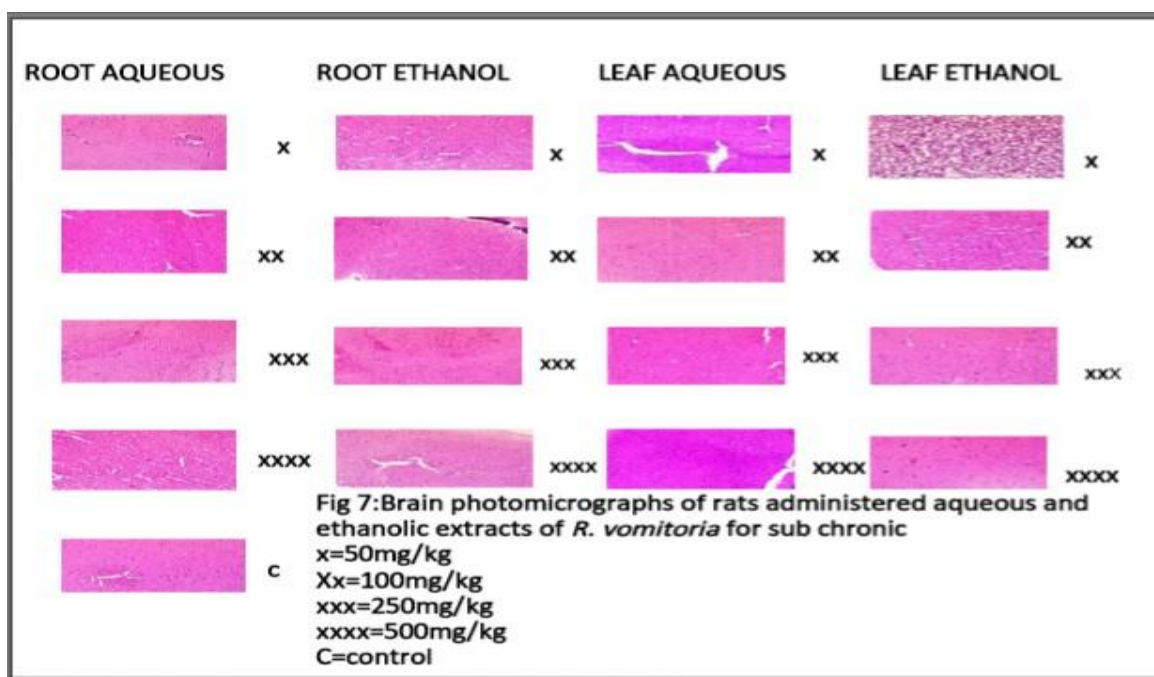
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DISCUSSION

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 (Ahmad *et al.*, 2012). 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 (Yamthe *et al.*, 2012, Ebuehi and Aleshinoye, 2010).

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 (Kuchman and Moss, 1982, Akpanabiatu *et al.*, 2009). 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

212 indicators of liver function (Hilaly *et al.*, 2004) and as biomarkers for possible toxicity
213 prediction. Significant quantities are found in the serum when the cell membrane becomes
214 leaky or completely ruptured (Rahman *et al.*, 2001). Some studies, however have reported the
215 toxicity and non-toxicity potential of the leaf and root extracts of *R. vomitoria*. During the
216 sub acute phase, there was significant difference in the activities of AST and ALT in animals
217 treated with both the leaf and root extracts of *R. vomitoria*. The increase of ALT, AST
218 activities and inflammations noticed in the histological findings during the sub acute study
219 reveals that the hepatocytes are impaired by the root and leaf extracts. This is in agreement
220 with the work of Ibrahim *et al.* (2014).

221 The kidney play a good role in excreting end products of body metabolism, drugs, toxins, etc
222 and regulates concentration of H⁺, Na⁺, K⁺, PO₄⁻ and other ions, i.e. water and electrolyte
223 balance via anti- diurectic hormone. Renal function test evaluates the severity of renal
224 diseases and the functional state of the kidney. Creatinine, urea and electrolytes are common
225 parameters often measured to assess the state of the kidney. Results from this study shows no
226 significant difference ($P \leq 0.05$) in the serum levels of urea and creatinine in the extract treated
227 groups compared with the control as shown in Table 5. Urea and creatinine are waste
228 products which are passed into the blood stream to be removed by the kidney. Elevation of
229 these waste products in the blood is a pointer to renal function impairment (Cameron *et al.*,
230 1998). Serum levels of these metabolites in the test groups were not significantly different
231 from the control, which showed that the extracts do not cause derangement in the cellular
232 activities of the rat kidneys. There was no significant change in the electrolytes showing that
233 the kidneys were not affected by the extracts at various concentrations. Histologically, the
234 renal corpuscles, proximal convoluted tubule and distal convoluted tubules of the various
235 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 report of Ibrahim *et al.*(2015).

The area of gliosis seen in the histological studies of the brain shows that the neurons are affected because the glial cells which supplies nutrients are impaired. The mortality noted across all groups sub-acutely administered with doses (1600-5000mg/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. This shows that the plant when used sub-acutely is not safe, but its usage sub-chronically is safe.

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.

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