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Full Length Research Paper

Effect of *Rauwolfia vomitoria* Afzel (Apocynaceae) extract on serum amino transferase and alkaline phosphatase activities and selected indices of liver and kidney functions

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In a risk assessment and safety evaluation, the effect of *Rauwolfia Vomitoria* Afzel (Apocynaceae) extract on serum amino transferase and alkaline phosphatase activities and selected indices of liver and kidney functions were investigated. Ethanolic root and leaf extracts of *R. vomitoria* Afzel (*Apocynaceae*) were administered externally by gastric intubation to two groups of rats at dose 524 mg/kg body weight for a 7 day period. The control received 2 ml of saline for the same period. Biochemical and clinical indices of toxicity; serum aminotransferases, urea, creatinine, total bilrubin, albumin and electrolytes were assessed in serum obtained from treated animals as well as those in the control groups. The extract at the doses administered produced a significant ($p<0.05$) increase in serum aminotransferases [asparate aminotransferase (AST) and alanine aminotransferase (ALT)], alkaline phosphatase activities, serum conjugated bilirubin and urea concentration but a decrease in serum albumin and potassium concentrations relative to controls. The spectrum of changes in biochemical and clinical indices of toxicity were more pronounced with the root extract than the leaf extract. The results of this present work showed that hepatocellulular derangement and poor renal function is more pronounced in the ethanolic root extracts compared with the ethanolic leaf extracts.

Key words: *Rauwolfia vomitoria,* biochemical/clinical-indices, hepatocellular derangement, renal function.

INTRODUCTION

Rauwolfia vomitoria Afzel (Apocynaceae) is a small shrub, which grows to 15 m high and has oval or oblong shiny leaves in whorls and with straight veining and a cluster of inconspicuous white or greenish flowers producing red berries. The wood is white when freshly cut, changes to rose colour on exposure. The roots are tuberous with pale brown cork. The shrub is an ever green perennial plant. It grows in most tropical forest of pacific, South America, Asia and Africa. In Nigeria, it is found near Lagos, Abeokuta, Ibadan, Yakurr, Calabar, Akamkpa and Odukpani LGA of Cross River State and other places (Boun, 1995; Cragg, 1998).

R. vomitoria is a raw material for extraction of isolated alkaloids, preparation of extract with standard alkaloid contents, above all a major source of the bioactive substance-reserpine implicated in the treatment of insanity and hypertension (Blumenthal, 1998; Amole and

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Onabanjo, 2004). Given intramuscularly in dose 2 to 4 mg, it has been used to treat psychosis and schizophrenia. Lately in some rural populations in Nigeria, *R. vomitoria* also enjoys a traditional reputation as a fever reducing agent and antidote for snakebite. The powdered roots are used to treat diarrhea and dysentery, while the latex from the leaves is employed in treatment of parasitic skin disease. Ghanaian and Nigerian healers use the bark in high doses as a powerful emetic and purgative for management of infantile convulsion, jaundice and gastro intestinal troubles.

Toxicology of medicinal plants and their products is an important part of the early and late phase of drug development from medicinal plant (Gamaniel, 2000). The science is also important for safety evaluation and risk assessment. Little information on the toxicity or side effects associated with the use of *R. vomitoria* extracts has been documented. *R. vomitoria* owes its action to the high content of reserpine in the root bark. Generally alkaloids taken along with other plant active agents are less toxic than when taken alone. Only a single report, the significant reduction of red blood cells, which may predispose to anemia, has been reported by Naiho et al. (2004). There is need to sufficiently document the toxicity associated with the use of this herbal extract. Tissue or organ damage may be detected clinically by plasma enzyme assays along with other parameters.

The present study, therefore, reports and also compares the effect of ethanolic leaf and root extracts of *R. vomitoria* on aminotransferase and alkaline phosphatase activities as indices of liver and kidney function tests.

MATERIALS AND METHODS

Plant materials and extract

Fresh leaves and roots of the plant *R. vomitoria* were collected from the forest in Idomi community, Yakurr, Cross River State, Nigeria in the month of October 2004. The leaf and root sample were authenticated by Dr. Mike Eko, Department of Botany, University of Calabar, Calabar and a voucher specimen (code No. MUE 2004) was preserved in the Department of Botany- Herbarium University of Calabar.

The root barks and leaves were dried in shade, and crushed to a coarse powder using a blender. About 155 g each of the powdered root and leaves were macerated in 500 ml of 96% ethanol, agitated using an electric blender and left for 48 h. The liquid extract obtained was concentrated *in* v*acuo* at 4^{o}C. The respective yields (18.19 of the ethanolic root extract and 14.1 g of the ethanolic leaf extract) were stored in a refrigerator at 4^{o}C, until further use. Before use, the materials were each dissolved in 50 ml of 2% Tween saline solution and expressed in terms of dry weight (mg/ml) saline.

Animals

Albino Wistar rats (96-265 g) of either sexes obtained from the animal house of the Departments of Biochemistry and Pharmacology, University of Calabar were used in this study. The rats were kept in plastic cages with wire screen top at room temperature and relative humidity (28 ± 5^{o}C, 46%), with a 12 h light dark cycle. They had free access to drinking water and the standard laboratory feed (Pfizer livestock feed, Aba Nigeria) *ad libitum* and were weighed on weekly basis. Prior to the biological experimentation on animals (rats) the animal protocols were approved by the Animal Ethics Committee of the College of Medical Sciences, University of Calabar, Calabar.

Experimental design and administration of extracts

Twenty-seven (27) albino wistar rats (96-265 g) of either sex were randomly assigned on the basis of weight into three experimental groups of 9 rats each. Group 1 (control) received placebo i.e. 2 ml of 2% Tween saline. Group 2 were gavaged with 542 mg/kg body weight of the ethanolic leaf and ethanolic root extracts respectively in 2.1 ml of the vehicle. The dose was chosen on the basis of the average daily dose for a 70 kg adult (600 mg for reserpine) which is a prescription medicine approved by the Federal Drug Agency. The single dose administration continued daily for seven days between the hours of 8:00 to 10:00 am.

Enzyme and biochemical assays

Twenty-four hours after the last dose administration, the rats were anaesthetized in chloroform vapour and dissected. Whole blood was collected by cardiac puncture from each of the rats into sterile screen cap plain tubes left to stand for an hour to clot and serum samples separated from clot by centrifugation at 3,000 rpm for 10 min. Sera obtained were used for analysis. Asparate aminotransferase (AST: EC. 2.6 1.1) and alanine amino-transferase (ALT: EC. 2.61.2.1) in samples were estimated by use of end point colorimetric diagnostic kit (Randox Laboratories Ltd England) based on Reitman and Frankel (1957). Alkaline phosphatase (ALP) activity was determined by use of sigma diagnostic kits (Sigma dagnostic, USA) and was based on the optimized method as recommended by the German Society for Clinical Chemistry. Serum albumin was determined by the method of Doumas et al. (1975) and bilirubin was estimated by the method of Evelyn and Malloy (1938). Both albumin and bilirubin assay kits were based on the methods of Kaplan (1965) and Henry (1974), respectively. Sodium and potassium ions concentrations in sera were measured using flame photometer as described by Bassir (1971).

Statistical analysis

Results were expressed as mean ± SD. Statistical analysis (compari-son of means of the control trial versus those of the test trial was carried out using Student's t-test; and the results were judged significant if $p<0.05$.

RESULTS AND DISCUSSION

The effects of ethanolic extracts of *R. vomitoria* on body weight of rats are summarized in Table 1. There were statistically significant ($p<0.05$) decreases in body weight of *R. vomitoria* treated rats compared with control. The mean ± SD weight changes were 0.69, 2.74 and 1.74% for the control, ethanolic leaf extract and ethanolic root extract treated groups, respectively. Table 2 presents the

Table 1. Effect of administration of ethanolic extracts of *Rauwolfia vomitoria* on the body weight of rats.

Treatment	Initial body wt. (g)	Final body wt (g)	% Change
Control (2 ml of 2% Tween saline)	231± 29.74	233.25± 29.07	0.69
Ethanolic leaf extract (524 mg/kg Bw.)	139.56 ± 36.68	137.50 ± 35.96	2.74
Ethanolic root extract (254 mg/kg Bw)	146.40 ± 37.43	142.20± 38.34	1.74

Values are mean ± SD, n = 9.

Table 2. Effects of treatment with ethanolic extracts of *Rauwolfia vomitoria* on serum levels of creatinine urea, total bilirubin and albumin.

Treatment	Creatinine (mg/dl)	Urea (mmol/L)	Total bilirubin (mg/dl)	Albumin (Umol/l)
Control (2 ml of 2% Tween saline)	3.20 ± 0.37	7.32 ± 0.96	0.32 ± 0.04	634.68 ± 68.43
Ethanolic leaf extract (524 mg/kg Bw.)	4.14 ± 0.37[a]	5.61 ± 0.77[a]	0.79 ± 0.09[a]	703.14 ± 28.80
Ethanolic root extract (254 mg/kg Bw)	3.48 ± 0.58[a]	12.74 ±3.39[a]	4.25 ± 1.01[b]	481.85 ± 25.68[b]

Values are means ± SD, n = 9.
The superscripts represent significant differences of test groups in comparison with respective controls; [a](P<0.05) and [b](P0.01).

Table 3. Effect of adminstration of ethanol extracts of *Rauwolfia vomitoria* on serum ALT, AST, ALP and selected serum electrolytes.

Treatment	ALP (U/L)	AST (U/L)	ALT (U/L)	Na^+ (mmol/L)	K^+ (mmol/L)
Control (2 ml of 2% Tween saline)	322.46 ± 17.62	21.49 ± 3.7	7.36 ± 2.2	132.00 ± 16.97	4.40 ± 0.14
Ethanolic leaf extract (524 mg/kg Bw)	409.09 ±2.56[a]	19.09 ± 5.5	14.39 ±2.9[a]	156.00 ± 33.94[a]	4.75 ± 0.36[a]
Ethanolic root extract (254 mg/kg Bw)	480.24 ± 15.61[a]	57.08 ±6.2[a]	13.38 ±5.8[a]	136.00 ± 22.63[a]	3.30 ± 0.42[a]

Values are means ± SD, n = 9.
The superscripts represent significant differences of test groups in comparison with respective controls; [a](P<0.05).
ALP, Alkaline phosphatase; ASP, aspartate aminotransferase; and ALT, alanine aminotransferase.

effects on the administration of ethanolic root and leaf extracts of *R. vomitoria* on serum levels of creatinine, urea, total bilirubin and albumin of wistar rats. The treatment with ethanolic root extract produced significant increase in creatinine ($p<0.05$), urea ($p<0.05$), total bilrubin ($p< 0.01$) and a decrease ($p<0.01$) in albumin relative to controls. The effect of the extracts on alkaline phosphatase, aminotransferase activities and electrolyte concentration in serum is summarized in Table 3. With the exception of the AST activities for the ethanolic leaf treated groups, there were significant ($p<0.05$) increases in alkaline phophatase and aminotransferase activities in the groups treated with both leaf and root extracts relative to control but no significant change in Na^+, K^+ electrolytes, except K^+ which was decreased in the group gavaged with ethanolic root extract.

This study was designed to undertake a risk assessment and safety evaluation of extracts of *R. vomitoria* in Wistar rats through assay of biochemical and clinical indices of toxicity. The spectrum of changes in clinical indices of liver and kidney function test obtained here, particularly decreased serum albumin, increase in bilirubin and alkaline phosphatase presented a picture of poor liver function or hepatocellular derangement. Also the increase in urea concentration in serum with the low potassium concentration suggests poor renal function. Additionally, the raised alanine aminotransferase level alongside with the decrease body weight support the toxicity this extract may have on the liver.

The liver is the key site of metabolism of xenobiotics and also plays a role in synthesis of albumin. Liver malfunction will impair its synthetic role and could have led to decreased levels of albumin in serum. Decreased albumin levels have long been used as a measure of liver disease (Mayne, 1994). Increased bilirubin levels in serum has also been observed and it is interesting to note that Naiho et al. (2004) have already reported hemolysis of red blood cells (RBC) and significant decrease in

RBC concentrations following exposure to *R. vomitoria* extract. This will result in elevated billirubin production, which explains the raised bilirubin levels observed in this study. Raised bilirubin levels, particularly total (unconjugated bilirubin) indicates liver malfunction (Baron, 1973).

Enzymes are essential factors, which enable many biochemical reactions that constitute life to proceed in cells of the body. Change in the enzyme concentrations in serum should therefore reflect the state of health, since damaged tissues spill these enzymes into plasma (Kuchman and Moss, 1982). The assessment of functional integrity or lesions on cells has been carried out by monitoring levels of specific enzymes in serum and body fluids. Aminotransferases are cytosolic enzymes widely distributed in tissues with highest concentration in liver and heart but with ALT more specific to the liver and AST to the heart. Damage to the membrane architecture of cells due to exposure to toxicants will lead to their spillage into plasma. The increase in serum ALT therefore implies the toxicity of the extract on the liver. Similarly, alkaline phosphatase is ubiquitous with regard to tissue distribution, but concentrations are high in liver, bone and intestine or placenta. A healthy liver contains alkaline phosphatase and other substances which drain continually through the bile duct. Lobular liver damage may obstruct the flow of bile; subsequently alkaline phosphatase accumulates, and is regurgitated into plasma (Guyton and Hall, 2000). Furthermore, elevation of alkaline phosphatase is almost always due to liver damage (Lefeverkek, 1995).

The kidney excretes end products of body metabolism, drugs, toxins, etc and regulates concentration of H^+, Na^+ K^+ $P0_4^-$ and other ions i.e. water and electrolyte balance via antidiurectic 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. The high levels of serum urea are due to failure of kidney to clear plasma urea.

A comparison of the leaf and root extracts effect showed that the root extract was more toxic than the leaves. The probable reason may be due to the fact that the leaves are generally rich in vitamins, and vitamin C and E are good antioxidants capable of preventing build up of free radicals with subsequent reduction in tissue damage (Abdel-Basset et al., 1997). This may explain the comparatively low toxicity of the leaf extract compared to the root. However, unpublished reports indicated that the leaves contained higher levels of natural toxicant tannin, hydrocynanins and phytates compared to the roots. This may affect bio-availability of mineral elements in the diet.

Conclusion

We conclude from our study that ethanolic extracts of *R. vomitoria* increases the serum activities of aminotransferases and alkaline phosphatases and adversely affect the biochemical and clinical indices of liver and kidney functions. This means that hepato-cellulular derangement and poor renal function is more pronounced in the ethanolic root extract compared to the ethanolic leaf extract.

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