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Sub-acute Toxicity of Rauwolfia vomitoria on the Kidneys and Liver of Wistar Rats: A Histological Study

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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.

Objectives: The present study investigates the toxicological potential of the sub-acute

administration of the aqueous leaf extract of *Rauwolfia vomitoria* (R.V) on the kidney and liver of adult Wistar rats.

Adult male Wistar rats were divided into three groups and orally administered 120 mg/kg R.V (group B), 300 mg/kg R.V (group C) and 1 ml distilled water (Control) for twenty-one days. Histology of the kidney and liver as well as selected indices of renal and hepatic functions were assayed for after completion of the study.

The extract produced a dose dependent increase in the activities of serum Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) and produced no significant difference ($P \leq 0.05$) in the levels of creatinine and urea in the experimental groups when compared to the control group. Histological observation of both organs shows no cyto-architectural difference between the control and experimental groups.

Conclusions: The study demonstrated that the administration of the *R. vomitoria* at these doses has no toxic effect on the kidneys but could impair liver function in Wistar rats.

Key-words: AST, ALT, Urea, Creatinine, Histology.

Introduction

Towards the end of last century, herbal medical practice became mainstreamed throughout the world largely due to the recognition of the value of traditional medical practice particularly of Asian and African origin [1]. Herbs have been shown to have useful significant medicinal effects, both in their natural state, and as a source of pharmaceuticals [2].

With increasing proposals for the integration of traditional medicine into the health care programme in most countries of the World, It would be necessary to investigate the toxicity of medicinal plants used in the traditional medical practice for the treatment of diseases to establish their safety [3].

Rauwolfia vomitoria Afzel (Apocynaceae) is an erect annual herb with leaves, producing small reddish fruits. The plant is found in abundance in many tropical countries of the world, Nigeria included. Its various parts, leaf, bark and root have been used traditionally in herbal medicine for the treatment of coughs, skin infections, malaria, and as an antipsychotic [4, 5].

Reports regarding the effects of the aqueous leaf extract of *R. vomitoria* on the histology and

the biochemical functions of the kidney and liver are scanty in existing literatures. Hence, the present study was undertaken to investigate the effects of the aqueous leaf extract of this plant on the histology and biochemical functions of the kidney and liver in Wistar rats.

Materials and Methods

Location and duration of study

This research work was conducted at the animal holding of the Department of Anatomy, University of Ilorin. This experiment was conducted in accordance to the international ethical standards and approved by the research ethics committee of the College of Health Sciences, University of Ilorin, Nigeria. Animals were acclimatized for 3 weeks prior to the start of extract administration. The administration of extract lasted for twenty-one days.

Preparation of the aqueous leaf extract of *Rauwolfia vomitoria*

Fresh leaves of *Rauwolfia vomitoria* plant were obtained from its tree during midday at Okerube area of Lagos State. Identification was done by Dr. K.S Olorunmaiye at the Herbarium of the Department of Plant Biology, University of Ilorin. The plant material was rinsed; air dried, blended and extracted using a Soxhlet extractor. The blended plant material (100 g) was placed in the Soxhlet chamber and extracted with 1250 cm^3 of distilled water. The concentrated plant material was then evaporated in an oven at a regulated temperature of 40°C. After evaporation, 12 g and 30 g of the dried concentrated extract were dissolved each in 100 ml of distilled water to make a 120 mg/ml and 300 mg/ml aqueous solution of *Rauwolfia vomitoria*.

Animal handling

All animals used for this study have been treated in accordance with the ethics and guidelines of the Institutional Animal Care and Use Committee (IUCAC). Eighteen (18) male adult Wistar rats weighing between 220-250 g were used for the study. They were obtained from the animal house of the department of anatomy, University of Ilorin, Nigeria. The rats were kept in well-ventilated house conditions and given normal rat feed and water *ad libitum.* They were randomly divided into three experimental groups (A, B, and C). Group A which served as control received 1 ml of distilled water orally while groups B and C were administered *R. vomitoria* leaf extract orally at doses of 120 and 300 mg/kg respectively. The extracts were administered daily for a period of 21 days.

Collection of Blood and Biochemical analysis

The rats were sacrificed by cervical dislocation and blood was taken from each rat by cardiac puncture and allowed to clot. Serum samples were extracted by centrifuging the clotted blood at 3000 rpm for 10min after which the serum was separated and stored frozen until needed for analysis. The serum samples were used for biochemical analyses. Serum urea concentration was determined by the method of Veiamin and Vakirtzi [6] while serum creatinine was determined using the Jaffe reaction [7]. Estimation of AST and ALT activities were done using Rietman-Frankel method [8].

Histological studies

The kidneys and liver from each group were fixed in 10% formol saline for 48 h. 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 sections were stained in haematoxylin and eosin. The slides were then examined at magnifications of X400 under a light microscope.

Statistical analysis

The results from the kidney and liver biochemical assays were reported as mean ± standard error of the mean (S.E.M) while statistical significance between groups was done using the ANOVA followed by Tukey's *post hoc* multiple comparison tests. Statistical significance was set at $P<0.05$.

Results and Discussions

There was no significant difference ($P< 0.05$) in urea and creatinine levels between the extracted treated groups and the control group (Table 1). There was a dose dependent increase in the activities of AST and ALT between the extract treated groups and the control group that was significant at ($P< 0.05$) (Table 1).

Table 1: **Activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), Urea, and Creatinine in the serum of rats administered with *R. vomitoria* leaf extract for 21 days.**

Groups	AST (IU/L)	ALT (IU/L)	Urea (mmol/L)	Creatinine (mmol/L)
A (control)	1087.0 ± 62	956 ± 29	1.5 ± 0.2	28.5 ± 2.5
B (120 mg/kg)	1220.5 ± 44.5*	1032.5 ± 2.5*	1.3 ± 0.1	27.5 ± 0.5
C (300 mg/kg)	1289.5 ± 7.5*	1218 ± 3.5*	1.5 ± 0.1	31.0 ± 1.0

Table 1: Effect of the aqueous leaf extract of *R. vomitoria* on serum urea, creatinine, AST, and ALT levels. Data are represented as Mean ± S.E.M (n=6). *Statistically different from the control group.

Histological observations

Histological findings in the control kidney reveal an intact glomeruli, proximal convoluted tubule and distal convoluted tubule (Fig. 1). Similarly in the experimental groups B and C, an intact renal corpuscle, proximal convoluted tubule (PCT) with brush borders, undisrupted distal convoluted tubule is also noticed with clearly seen macula densa (Fig. 2 and 3). Collagen type IV of the basement membrane of the glomerular capillaries is clearly visible in all the groups (Fig. 2 and 3). No cyto-architectural changes were also observed in the liver of the extract treated animals (Fig. 5 and 6) when compared with the control group (Fig. 4). The liver of the extract treated animals reveal no necrosis of any kind. Intact central vein with a regular arrangement of hepatocytes and sinusoids were noticed histologically when compared to the control group.

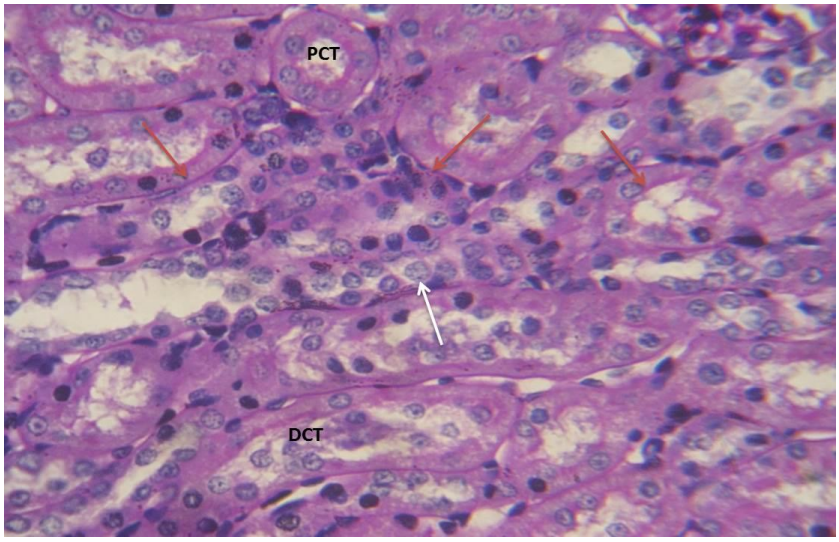

Figure 1: Photomicrograph of the renal cortex of group A animals showing intact proximal convoluted tubule (PCT) with its large cuboidal cells presenting a brush border. The distal convoluted tubule (DCT) is also intact. A macula densa is clearly seen (white arrow). The collagen type IV of the basement membrane of the glomerular capillaries is clearly visible (red arrow). Periodic- acid Schiff stain (X400).

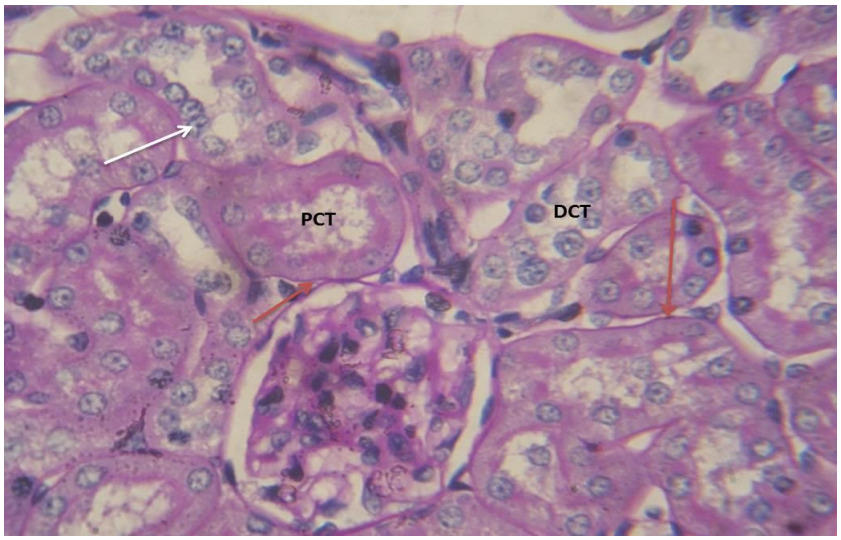

Figure 2: Photomicrograph of the renal cortex of group B animals showing an intact renal corpuscle, proximal convoluted tubule (PCT) with its large cuboidal cells presenting a brush border. The distal convoluted tubule (DCT) is also intact. A macula densa is clearly seen (white arrow). The collagen type IV of the basement membrane of the glomerular capillaries is clearly visible (red arrow). Periodic- acid Schiff stain (X400).

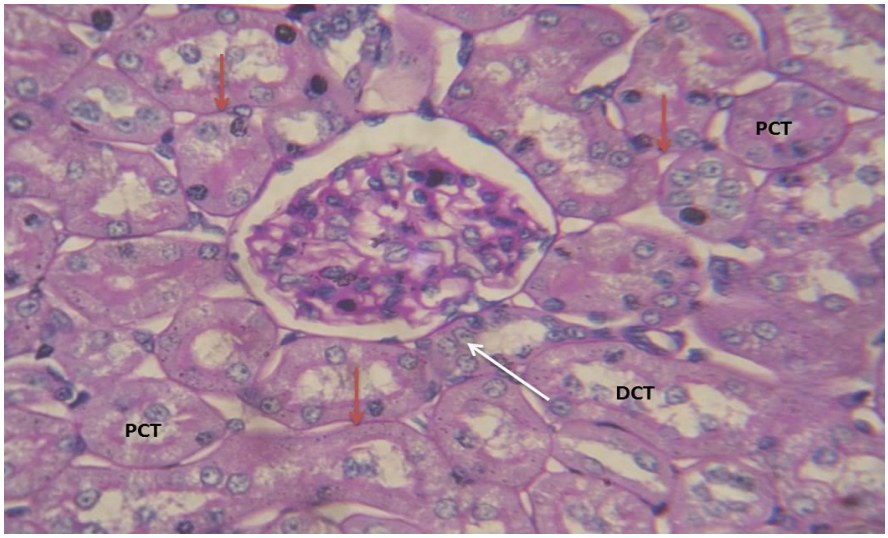

Figure 3: Photomicrograph of the renal cortex of group C animals showing intact an intact renal corpuscle, proximal convoluted tubule (PCT) with its large cuboidal cells presenting a brush border. The distal convoluted tubule (DCT) is also intact. A macula densa is clearly seen (white arrow). The collagen type IV of the basement membrane of the glomerular capillaries is clearly visible (red arrow). Periodic- acid Schiff stain (X400).

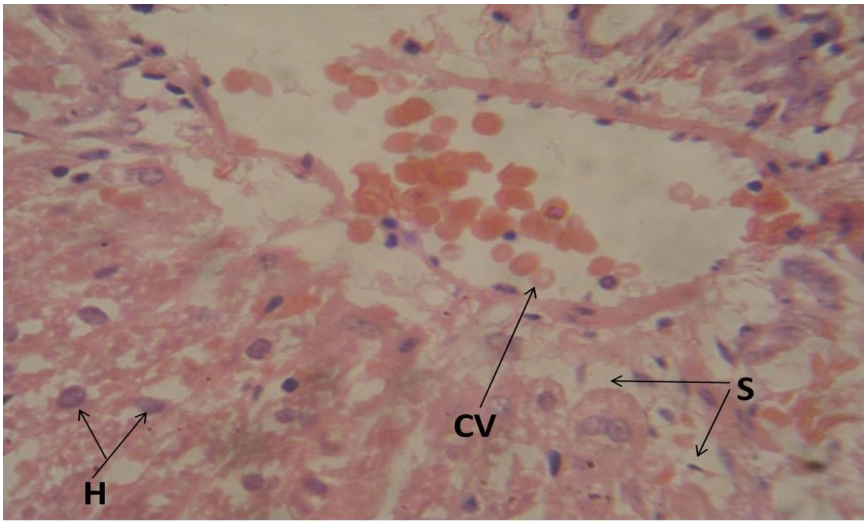

Fig. 4: Photomicrograph of the liver of rats in the control group showing intact central vein (CV) with regular arrangement of hepatocytes (H) and sinusoids (S). H & E (x400).

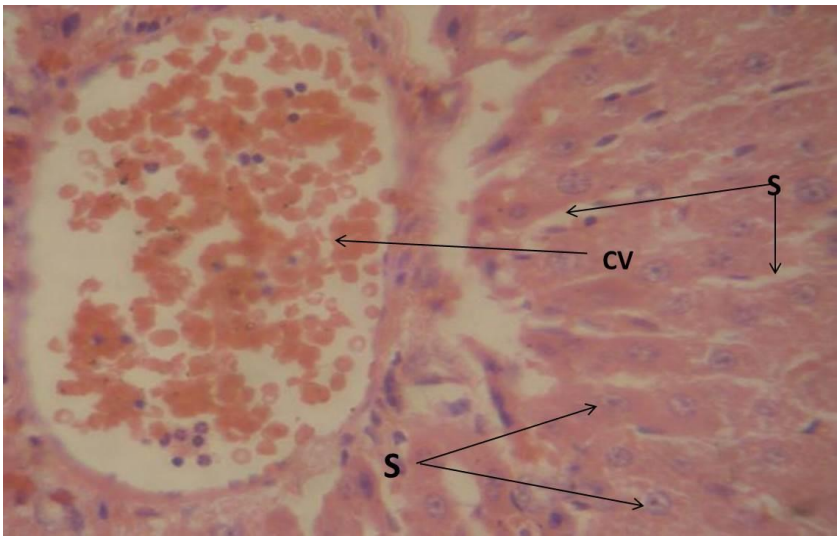

Fig. 5: Photomicrograph of the liver after 21 days administration of 120 mg/kg R. vomitoria in group B animals showing intact central vein (CV) with regular arrangement of hepatocytes (H) and sinusoids (S). H & E (x400).

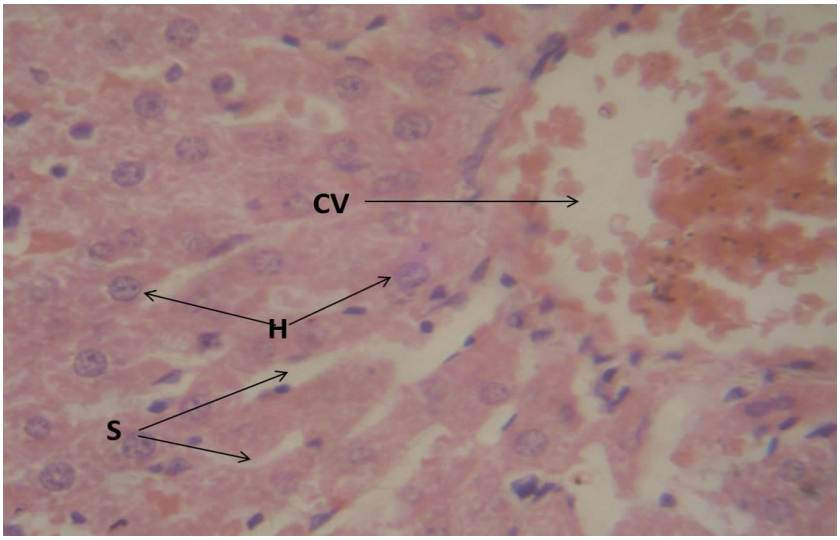

Fig. 6: Photomicrograph of the liver after 21 days administration of 300 mg/kg R. vomitoria in group C animals showing intact central vein (CV) with regular arrangement of hepatocytes (H) and sinusoids (S). H & E (x400).

The use of plants in low and middle income countries has never stopped gaining popularity. In these countries, it is often the main therapeutic system majority of people resort to [9]. Increasing use as oppose to enough scientific evidences on the safety of medicinal plants have raised concerns regarding the safety and detrimental effects of these remedies [10].

Results from this study shows no significant difference ($P<0.05$) in the serum levels of urea and creatinine in the extract treated groups compared with the control as shown in Tables 1. Urea and creatinine are waste products which are passed into the blood stream to be removed by the kidney. Elevation of these waste products in the blood is an indicator of renal function impairment [11, 12]. Serum levels of these metabolites in the test groups were not significantly different from the control, which showed that the extracts did not cause derangement in the cellular activities of the rat's kidneys. Histologically, the renal corpuscles, proximal convoluted tubule and distal convoluted tubules of the extract treated groups show no distortion in their cyto-architecture when compared with the control group.

The administration of the aqueous leaf extract of *R. vomitoria* produced a dose dependent increase in the activities of AST and ALT between the extract treated groups and the control group (Table 1). Different studies have reported toxicity and non-toxicity in the leaf bark and root extracts of *R. vomitoria*. No significant difference was reported in the serum level of ALP, AST and ALT in animals treated with both the leaf and root extracts of *R. vomitoria* for a period of 14 days [3]. The root bark extract of *R. vomitoria* has been reported to have no effect on the activities of ALT but affects the levels of AST significantly [13] and also possess more teratogenic potential than its leaf extract [1].

Results of the current study also focused on liver toxicity using histological and indices of liver functions as indicators. In the current study, serum AST and ALT levels were increased significantly between the control animals and the extract treated animals. AST and ALT are known enzymes used as indicators of liver function [14] and as biomarkers for possible toxicity prediction as significant quantities are found in the serum when the cell membrane becomes leaky or completely ruptured [15].

Thus, the significant increase observed in ALT and AST activities in this study suggests that the sub-acute administration of the aqueous leaf extract of *Rauwolfia vomitoria* did alter the hepatocytes and consequently the metabolism of the rats although the histological findings of the present study reveal no cyto-architectural changes in the liver of both the control and extract treated groups (Figs 4, 5 and 6).

This study demonstrates that the aqueous leaf extract of *Rauwolfia vomitoria* which is used as an antipsychotic, is a medicinal plant with potentially detrimental properties. Precautions

during use especially in higher doses and over longer periods of administration may be necessary in human users.

Conclusion:

Conclusively, results from this study suggest that the sub-acute administration of the aqueous leaf extract of *R. vomitoria* at the doses of 120 mg/kg and 300 mg/kg have no toxic effect on the kidneys but could impair liver function in Wistar rats.

Conflict of Interest: The authors declare no conflict of interest.

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