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The effect of interaction of *Rauwolfia vomitoria* root bark extract with vitamin E on rats' liver enzymes

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Abstract: In our previous paper we demonstrated the influence of *Rauwolfia vomitoria* root bark extract on cardiac enzymes of normal Wistar rats and the effectiveness of this root bark as a source of antihypertensive agent, as determined by the marker enzymes, would be a useful index for the toxicity of this plant in clinical application. In this paper we present the results of the effect of *Rauwolfia vomitoria* root bark extract and its interaction with vitamin E on the liver function enzymes, such as alkaline phosphatase (ALP), alanine amino transferase (ALT), aspartate amino transferase (AST), gamma glutamyl transferase (γ-GT), and the histological architectures of the liver tissues of normal animals. The results showed significant ($P \leq 0.01$) increase in the activity of AST by the extract. The findings also showed that interaction reduced the activity of enzymes to about normal or less than normal as in the case of γ-GT, ALT, and ALP, indicating the ameliorating effects of vitamin E on the toxicity of *Rauwolfia vomitoria* root bark extract. Histological results also confirmed the ameliorating effect of vitamin E on possible toxicity of this herb. As vitamin E and *Rauwolfia vomitoria* root bark extract already have known advantages in the management of cardiovascular disease, concomitant administration of *Rauwolfia vomitoria* root bark extract with vitamin E may be a better option.

Key words: *Rauwolfia vomitoria*, root bark, vitamin E, liver enzymes, histopathology

Sıçanların karaciğer enzimleri üzerine E vitamini ile *Rauwolfia vomitoria* kök kabuk özütünün etkileşiminin etkisi

Özet: Daha önceki çalışmamızda, normal Wistar sıçanlarının kalp enzimleri üzerine *Rauwolfia vomitoria*'nın kök kabuğu özütünün etkisini ve bu kök kabuğu etkinliğini, marker enzimler tarafından belirlendiği gibi, kan basıncı düşürücü ajan olarak, klinik uygulamada bu bitkinin toksisitesi için faydalı bir dizin olabileceğini gösterdik. Bu çalışmada, *Rauwolfia vomitoria* kök kabuk özütünün etkisi ve E vitamini ile olan etkileşiminin alkalen fosfataz (ALP), alanin amino transferaz (ALT), aspartat amino transferaz (AST), gama glutamat transferaz (γ-GT) gibi karaciğer fonksiyon enzimleri üzerine etkisinin sonuçları ve normal hayvanların karaciğer dokularının histolojik yapısı sunulmuştur. Sonuçlar özütün AST aktivitesinde önemli bir artışa ($P \leq 0,01$) sebep olduğunu göstermiştir. Bulgularda etkileşimin γ-GT, ALT ve ALP'nin durumundaki gibi normal veya normalden daha az enzimlerin aktivitesini azalttığını ve *Rauwolfia vomitoria*' nın kök kabuk özütünün toksikliği üzerine E vitamininin iyileştirici etkileri olduğunu gösterdi. Histolojik sonuçlarda bu bitkinin muhtemel toksikliği üzerine E vitamininin iyileştirici etkisini doğruladı.

Anahtar sözcükler: *Rauwolfia vomitoria*, kök kabuğu, E vitamini, karaciğer enzimleri, histopatoloji

Introduction

Vitamin E is one of the fat soluble vitamins that occur in nature and functions as an antioxidant by intercepting free radicals generated during normal metabolic process and exposure to environmental pollutants. A group of 8 natural antioxidants, 4 tocopherols, and 4 tocotrienols have been found to express the functions of vitamin E (1). The role of this vitamin includes prevention of lipid peroxidation, which is implicated in cardiovascular disease. It is also important in normal reproduction, development of muscles, inhibition of platelet aggregation to enhance vasodilation, and other biochemical functions (2,3). It has also been reported that alpha-tocopherol is the only form of vitamin E that is actively maintained in the human body and is therefore, the form of the vitamin found in large quantities in blood and tissues (4).

Rauwolfia vomitoria Afzer, of Apocynaceae, is a rain forest shrub that grows in Nigeria, having oval leaves with straight venation and clusters of tiny flowers (5). Major phytochemical constituents of this plant include alkaloids, glycosides, polyphenols, and reducing sugars (6). The alkaloids have been reported to include reserpine, a well-known antihypertensive substance found in this plant. However, about 30 additional alkaloids have been detected in tissue cell culture of this plant (7). It is well documented that some herbal extracts have influenced serum enzymes as seen by their activities on experimental animals (5, 8-10).

The mechanism of drug toxicity may involve the generation of free radicals, but often serum enzymes are used as indicators of tissue damage. Liver function enzymes are markers of liver damage, but the effect of interactions of these proteins with chemicals may modify their functional state, hence their activities during biological processes. Plant extracts could be either safe or toxic; as a result, it is always necessary to evaluate possible toxicological effects of plants' extract which are used for medicinal purposes. This study was designed to investigate the effect of concomitant administration of *Rauwolfia vomitoria* root bark extract and vitamin E on the liver functional integrity. To this end, we measured serum enzymes activities and processed the liver for possible histological changes.

Materials and methods

Chemicals and reagents

All reagents and chemicals used in this study were of analytical grade. The enzyme kits (AST, ALT, ALP, γ-GT) were obtained from Randox Laboratory, UK. Dimethylsulphoxide (DMSO) was obtained from Calbiochem, Canada. Absorbances were read using Optima Spectrophotometer SP – 300 from Optima, Inc., USA.

Plant materials

On November 2004, *Rauwolfia vomitoria* roots were obtained from farmland in Ekpene Obo, Esit Eket local government area of the Akwa Ibom State in Nigeria. A botanist from the botanical garden of the University of Calabar authenticated a sample of the plant. A voucher specimen numbered MIA 2004 was submitted to the herbarium of the same University. The roots were cleaned and made free from sand. The fresh air-dried roots were powdered in an electric kitchen blender. 100 g of root powder was extracted twice with 80% ethanol according to the method of Ugochukwu et al. (11). Extraction was repeated with the second batch of root powder to obtain enough extract for the work. The combined filtrate was concentrated using rotary evaporator and the concentrate was dried in a Plus 11 Gallenkamp oven at 45–50 ^{o}C to dryness. Then, 1 g of the extract was re-suspended in 10 mL solution using distilled water and 1 mL of dimethylsulphoxide before use.

Animal treatment

Forty-two albino Wistar rats, weighing 200 - 230 g, were used in this work. The animals were obtained from the animal house of the Biochemistry Department in the University of Calabar. They were maintained under standard laboratory conditions with rat chow (Guinea Feed Ltd., Nigeria) and water ad libitum. All animal experiments were carried out in line with the guidelines of Institutional Animal Ethical Committee. The animals were randomly selected into 6 groups with 7 animals each. Group 1 used as a control and received 0.3 mL of normal saline containing same concentration of DMSO (1:10 v/v) as the test groups. Group 2 was treated with vitamin E (10 IU/kg body weight), while Groups 3 and 4 were treated with 150 and 300 mg/kg body weight/day of *Rauwolfia vomitoria* root extract in 0.3 and 0.6 mL, respectively. Groups 5 and 6 were treated with

concomitant administration of 150 mg/kg body weight/day of *Rauwolfia vomitoria* root extract + vitamin E (10 IU/kg body weight) and 300 mg/kg body weight/day of *Rauwolfia vomitoria* root extract and vitamin E (10 IU/kg body weight) for 7 days. The administration of the extracts and vitamin E was daily by oral intubations between the hours of 10:00 a.m and noon.

Preparation of sera and liver tissues

All experimental animals were anaesthetized using chloroform fumes, 24 h after the last administration of the extract. Blood samples for sera preparation were collected by cardiac puncture into sterile plain tubes. After the blood samples were collected, the animals were dissected and the liver tissue removed for histological study. The liver tissue histological preparation and photomicrograph were done in accordance to the method of Akpanabiatu et al. (12). Thereafter, the animals were sacrificed and disposed of accordingly. Serum samples were extracted from the unclotted blood into sterile plain tubes after centrifugation at 3000 × g for 5 min using a bench top centrifuge (MSE Minor, UK). The sera were then stored in the refrigerator for analyses. All biochemical analyses were carried out within 24 h of sample collection.

All the results were presented as mean ± S.D. Computer assisted two-way ANOVA was used to analyze the statistical significance and $P < 0.05$ was regarded as significant.

Results and discussion

Activities of serum ALP, ALT, AST, GGT and AST/ALT ratio of adult albino wistar rats treated with the 80% ethanol extract of *Rauwolfia vomitoria* root bark and vitamin E

The enzyme activities investigated in the serum of rats treated with *Rauwolfia vomitoria* root bark extract, vitamin E, and the combination of vitamin E and the extract at 150 and 300 mg/kg body weight, respectively, are presented in the table below. The table shows that the administration of vitamin E to normal Wistar albino rats significantly ($P \geq 0.05$) decrease the serum activity of γ-glutamyl transferase (γ-GT). The activity of serum alanine amino transferase (ALT) also had a non-significant decrease. The extract concentration of 300 mg/kg body wt. resulted in lower activities of ALP, ALT and γ-GT. γ-glutamyl transferase was significantly ($P \leq 0.001$) lower at both extract concentration, while AST on the other hand, had significantly ($P \leq 0.01$) higher activity. Upon concomitant administration of vitamin E and 150 and 300 mg/kg body weight/day of *Rauwolfia vomitoria* root bark extract, there were significant decreases in γ-GT, ALT. Furthermore, the activity of AST in the vitamin + extract treated groups were significantly reduced when compared with the groups that received the extract only. However, there was also a non-significant decrease in the serum activity of ALP.

In environments with limited resources , people tend to desperately look for cures due to scarcity or high cost of medicine and have resorted to herbal

Table. Activity of serum ALP, ALT, AST, GGT and AST/ALT ratio of adult albino Wistar rats treated with the 80% ethanol extract of *Rauwolfia vomitoria* root bark and vitamin E.

Parameter /group	Serum ALP (IU/L)	Serum ALT (IU/L)	Serum AST (IU/L)	Serum GGT (IU/L)	AST/ALT ratio
Control	117.69 ± 34.94	21.57 ± 6.23	28.71 ± 6.68	29.49 ± 9.25	1.33
Vitamin E (10 IU/ kg body weight.	117.01 ± 27.01	13.71 ± 4.53	35.57 ± 11.33	18.42 ± 3.95*	2.59
R.V. (150 mg/ kg body weight	118.76 ± 29.79	27.57 ± 8.23	61.71 ± 22.18**	18.95 ± 5.03	2.24
R.V. (300 mg/ kg body weight.	69.83 ± 16.42*	16.43 ± 5.99	66.57 ± 22.50**	10.79 ± 3.80**	4.05
R.V. (150 mg) + Vit.E (10 IU) / kg body weight.	107.03 ± 20.61	7.00 ±1.91**	31.71 ± 13.66	13.91 ± 4.45**	4.53
R.V. (300 mg) + Vit.E (10 IU) / kg body weight.	81.42 ± 18.49	10.71±1.6**	39.85 ± 7.36*	10.72 ± 4.59***	3.72

RV = *Rauwolfia vomitoria* root bark extract; a = mean ± SD of 7 determinations; * = $P \leq 0.05$; ** = $P \leq 0.01$; *** $P \leq 0.001$ compared with control.

therapy without adequate knowledge of the possible side effects. For this group of persons, the beneficial effects of medicinal plants are often overshadowed by their deleterious effects; hence, concerted efforts in the screening of plants for possible toxicity and guidance are needed. The simplest method of assessing toxicity in experimental studies is by enzyme assay, since their activities are often affected by introduction of xenobiotics in biological species. Moreover, the administration of some vitamins that are capable of quenching free radicals may be of special relevance in ameliorating the toxic effects of medicinal plants.

The near control values recorded in the activity of ALP and the significantly lower ALT and γ-GT in the vitamin E control group indicated that there might have been no vitamin E toxicity at the concentration that was given to the experimental animals. The significantly higher AST at the extract concentrations of 150 and 300 mg/kg body wt. is indicative of mitochondrial enzyme induction. The cytoplasmic ALT activity was found to be lower than that of the control, indicating possible enzyme inhibition. Furthermore, the lower than normal activity of γ-GT reported in this study coupled with the low activity of ALT is an indication of nontoxic and the protective effect of this extract on both concentrations. Nada et al. (13), reported values of AST and ALT similar to that reported in this study for animals treated with phytochemicals. The heart tissue may be responsible for the increase in the serum activity of AST and we have earlier reported that application of *Rauwolfia vomitoria* root bark extract may have significant biochemical effect on the myocardium (5). The significant difference between the activities of ALP, ALT, AST, and γ-GT on concomitant administration of the extract and vitamin E showed that the differences are most likely a culmination of the interaction between the extract and vitamin E. Expression of toxicity of xenobiotics is usually determined biochemically by the use of some plasma enzymes. A rise in AST, ALT, ALP, and γ-GT activities are indices of liver cell damage (14-16).

γ-GT and ALT were the most affected and γ-glutamyltransferase, a microsomal enzyme that is widely distributed in the tissue involved in the secretory and absorptive processes, was particularly the bile (15). Liver diseases associated with biliary system that are capable of elevating the activity of γ-glutamyltransferase, may not be connected to the administration of this extract, rather, the extract may be useful in treating such disease conditions. The low activity of γ-glutamyltransferase recorded in this study is indicative of a healthy state of the liver, membrane integrity and functionality of the cells in the experimental animals, since γ-GT is elevated in any type of liver disease (17). Since the body metabolic processes are capable of generating free radicals, it is likely that the only significant rise in enzyme activities measured in this study may not actually be due to the extract, but even if it were, there is hope to remedy this by using the herb in combination with vitamin E. We have numerous reports supporting the preventive, protective, and curative role of natural anti-oxidants, such as vitamin E in a variety of oxidative stress mediated disorders, including myocardial disorders (18).

Effects of *Rauwolfia vomitoria* root bark extract and vitamin E on the liver histology of experimental animals.

Figures 1a to 1f show the histological evaluation of the tissue architectures of the different treatment groups in this study. Figure 1a depicts a typical liver section of normal animals showing the central vein with hepatocytes radiating outward. The hepatocytes also consist of a cytoplasm and prominent nuclei indicating normal cells. In figure 1b, the photomicrograph of a typical section of the liver cell of experimental animals treated with vitamin E is presented. We observed enlarged hepatocytes and some pyknotic cells showing nuclei degeneration compared to sections from the control group.

The histopathological study of a typical liver section of animals treated with 150 mg/kg body weight *Rauwolfia vomitoria* root bark extract as presented in Figure 1c was seen to have enlarged nuclei and degenerated hepatocytes. The cellular integrity was altered by the extract at this concentration and this could result in cell malfunction. Moreover, the increase of cells observed in Figure 1c was in line with normal cellular mechanism of combating xenobiotics. At the dose level of 300 mg/kg body wt. (Figure 1d), the extract resulted in significant increase in hepatocyte population (hyperplasia). The nuclei appeared

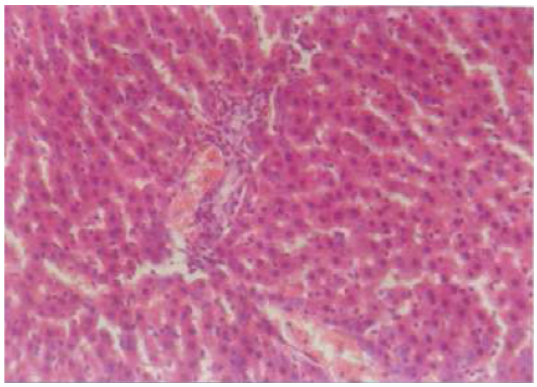


Figure 1a. Photomicrograph of s section (mag. × 40) of a typical normal rat liver of control animal showing normal liver cell architecture.

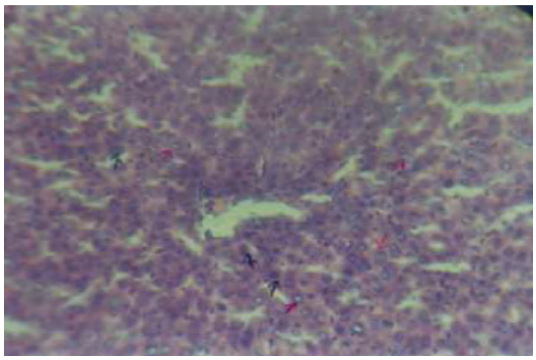


Figure 1b. Photomicrograph of a liver section of vitamin E treated Wistar albino rat showing enlarged and degenerating nuclei as indicated by black and red arrows respectively.

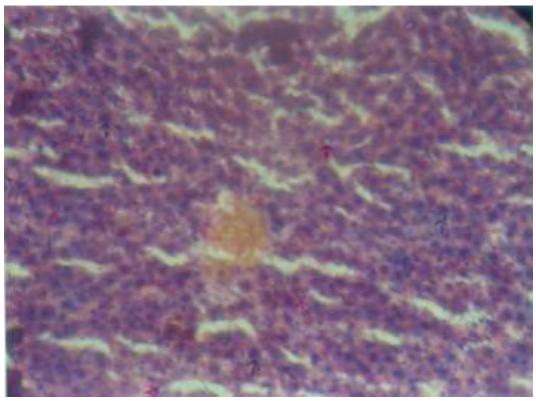


Figure 1c. Photomicrograph of a liver section of Wistar albino rat treated with 150 mg/kg body weight of *Rauwolfia vomitoria* root bark extract showing enlarged nuclei and nuclei degeneration as indicated by black and red arrows respectively. Cell proliferation was noticed in this section.

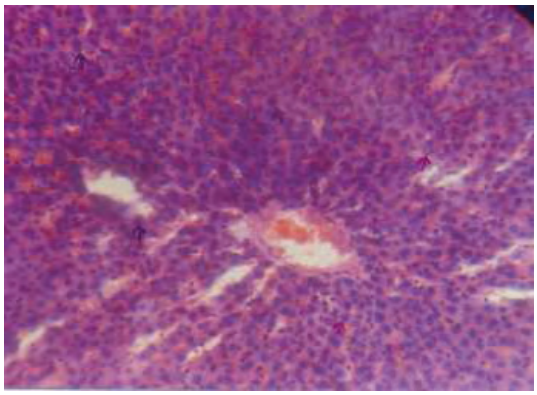


Figure 1d. Photomicrograph of a liver section of Wistar albino rat treated with 300 mg/kg body weight of *Rauwolfia vomitoria* root bark extract showing enlarged nuclei and nuclei degeneration as indicated by black and red arrows, respectively. Cell proliferation was noticed in this section.

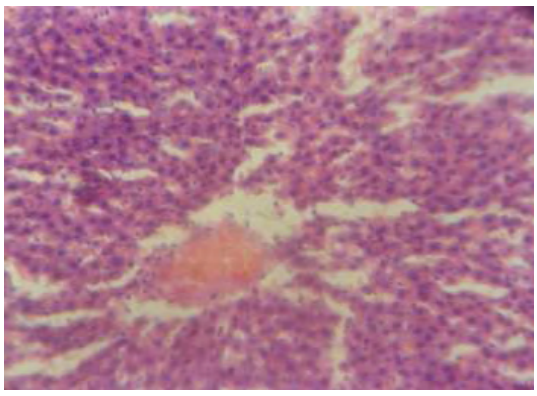


Figure 1e. Photomicrograph of a typical liver section of Wistar albino rat treated with 150 mg/kg body weight of *Rauwolfia vomitoria* root bark extract + vitamin E showing normal hepatocytes compared to the control.

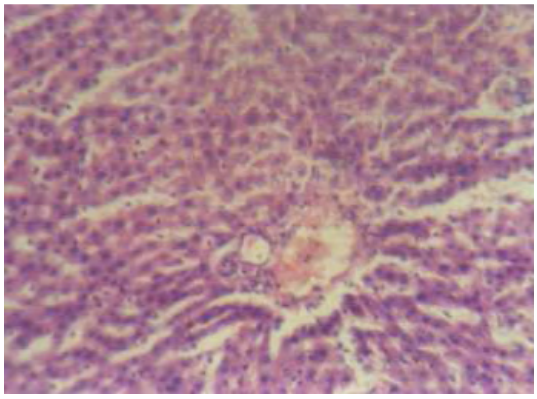


Figure 1f. Photomicrograph of a typical liver section of Wistar albino rat treated with 300 mg/kg body weight of *Rauwolfia vomitoria* root bark extract + vitamin E showing normal hepatocytes compared to the control.

enlarged and degenerative, hence the likelihood of derangement of cellular functions.

The histological preparation of a typical liver section of rats showing the interaction of *Rauwolfia vomitoria* root bark extracts (150 and 300 mg/kg body wt.) with vitamin E are depicted in Figures 1e and 1f. These figures depict normal liver hepatocytes as shown in the control (Figure 1a). The administration of vitamin E was able to restore possible histological damage due to the effects of the extract. Therefore, we would recommend the combination of this herb with vitamin E, considering the role of this herb as antihypertensive agent(s) and vitamin E as useful moderator of cardiovascular complications.

Rauwolfia vomitoria root bark extract (300 mg/kg body wt) and its interactions with both concentrations, 300 and 150 mg/kg body wt. with vitamin E, significantly lowered the activities of ALP, ALT and GGT in the serum of experimental animals. This is in line with the histological report in Figure 1e and 1f. The antioxidant effect of vitamin E is a key factor in hepatocyte membrane integrity and may be the possible mechanism of protection resulting in lower enzymes' activities in this work. In this view, *Rauwolfia vomitoria* at a dose of 300 mg/kg body wt. is better than 150 mg/kg body wt. as it offers higher protection, stabilization of plasma membrane, and may even repair hepatocyte damage. Work is on going for better understanding of the mechanism of action of *Rauwolfia vomitoria* root bark extract and its hepatoprotective activity.

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