EFFECTS OF INTERACTION OF VITAMIN A AND *RAUWOLFIA VOMITORIA* ROOT BARK EXTRACT ON MARKER ENZYMES OF CARDIAC DISEASES

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ABSTRACT

Serum creatine kinase (CK), creatine kinase isoenzyme (CK-MB) and aspartate aminotransferase (AST) are key players in the diagnostic study of cardiac complications. These enzymes are specific diagnostic markers of myocardial infarction and hypertension is a disease condition characterized with a wide range of complications, including myocardial infarction. In this study, we determined the effects of interaction of vitamin A and *Rauwolfia vomitoria* (RV) root bark extract on marker enzymes of cardiac diseases. CK and CK-MB activities had significant decrease in the group of animals with concomitant administration of vitamin A (40 IU/kg body wt.) and 150 mg/kg body wt. of RV root bark extract. At the interaction of vitamin A with 300 mg/kg body wt. RV root bark extract, CK-MB only showed significant ($p \geq 0.05$) decrease while CK decreased insignificantly. Also at the interaction of vitamin A with 300 mg/kg body wt. RV root bark extract, AST increased significantly but decreased significantly at the interaction of vitamin A (40 IU/kg body wt.) and 150 mg/kg body wt. of RV root bark extract. Our findings showed that vitamin A dose did not lower the activities of cardiac marker enzymes. However, concomitant administration of RV root bark extract at 150 mg/kg body wt. with vitamin A shows significant reduction in the activities of CK, CK-MB and AST. These findings suggest that interaction of vitamin A with RV root bark extract would be a meaningful ethno-pharmaceutical approach in the management of myocardial infarction and treatment of hypertension.

KEY WORDS

Rauwolfia vomitoria, Vitamin A, Interactions, Cardiac enzymes, Hypertension, Myocardial infarction.

INTRODUCTION

It is well known that some cardiac enzymes are affected by drugs, especially antihypertensive drugs/herbs. The catalytic activities of enzymes are also modulated by agent(s) acting as antioxidants or pro-oxidants. The combination of vitamin A and plant extract may reveal some biochemical interactions in enzyme study, which in turn may result in some benefits to man. It is also a known fact that drug-drug interaction may

exacerbate or ameliorate drug metabolic effects. Equally multi-drug therapy has been a useful practice in medicine. Over expression of a particular enzyme in multi-drug therapy may create a variety of unfavorable effects, which may turn out to be the primary cause of some diseases in both human and animals. On the other hand, under expression of enzyme activity may be of benefits or not as this could be a resultant effect of enzyme inhibition.

Most enzymes that participate in the biochemistry of heart muscles include creatine kinase (CK), creatine kinase isoenzyme (CK-MB), aspartate amino-transferase (AST) and lactate dehydrogenase (LDH). The activities of these enzymes have been reported as markers in myocardial infarction, which is a cardiac complication in hypertension. Marker enzymes of myocardial infarction used in clinical diagnosis have been reported to express high serum activities in hypertension

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(1, 2, 3). Herbal extract have also been reported to modulate the activities of cardiac enzymes (4).

Rauwolfia vomitoria (RV) is a herb whose phytochemical constituents are potent medicine. Reserpine, an alkaloid extracted from this herb is the first modern drug used in the treatment of hypertension (5, 6). The presence of polyphenols in *Rauwolfia vomitoria* root bark extract may be another advantage of using this herb since polyphenols are important antioxidants in cardiovascular research (7-10). Functional antioxidant agents of plant origin play significant roles in ameliorating tissue toxicity in both human and animals. *Rauwolfia vomitoria* root bark extract have been reported to reduce the activity of cardiac enzymes (4). Several authors have reported on the effects of herbal extracts on serum enzymes activities (11-15).

Vitamin A, β -carotene and other carotenoids are members of a family of antioxidant vitamins that are known to protect against oxidative stress in the tissue of human and animals. Vitamin A is a generic terminology for a large number of related compounds. Retinol and retinal are often referred to as preformed vitamin A while β -carotene and other carotenoids that can be converted by the body into retinol are referred to as pro-vitamin A carotenoids (16, 17). Hundreds of different carotenoids are synthesized by plants, but only 10 % of them are provitamin A carotenoids (16).

Recently, focus in biochemical research has shifted in favour of vitamin A antioxidant activity, hence the need to study the interactions of herbal extracts with vitamin A. The main thrust of this investigation is the study of interaction of vitamin A with *Rauwolfia vomitoria* root bark extract on marker enzymes of myocardial infarction in Wistar albino rats. The results of this work will provide additional information on herb-drug interaction as a useful approach to herbal drug therapy.

MATERIALS AND METHODS

Chemicals and reagents: All reagents and chemicals used in this study were of analytical grade. The enzyme kits were obtained from Randox Laboratory, UK. Dimethylsulphoxide (DMSO) was obtained from Calbiochem, Canada. Vitamin A was obtained from a pharmacy shop (Kamel Pharmacy shop, Calabar, Nigeria). Absorbances were read using Optima Spectrophotometer SP – 300 (Optima, Inc. Chicago, USA)

Plant materials: *Rauwolfia vomitoria* roots were obtained from a farm land in Ekpene Obo, Esit Eket local Government Area of Akwa Ibom State, Nigeria in the month of November, 2004.

The botanist in 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 leaf powder was extracted twice with 80 % ethanol according to the method of Ugochukwu (12). 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 °C to dryness. 1 g of the extract was re-suspended in 10ml solution using distilled water and 1 ml of dimethylsulphoxide before use.

Animal treatment: Forty two *albino* rats of the Wistar strain weighing 200 - 230 g were used in this work. The animals were obtained from the animal house, Biochemistry department, 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 of 7 animals each. Group 1 animals were the normal control and received normal saline containing same concentration of DMSO as the test groups. Group 2 was treated with vitamin A (40 IU/kg body weight) while groups 3 and 4 were treated with 150 and 300 mg/kg body weight/day of *Rauwolfia vomitoria* root bark 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 bark extract + vitamin A (40 IU/kg body weight) and 300 mg/kg body weight/day of *Rauwolfia vomitoria* root bark extract + vitamin A (40 IU/kg body weight), respectively for 7 days. The administration of the extract and vitamin A was by daily oral intubations between the hours of 10 am and noon.

Animal sacrifice and preparation of sera for analysis: 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. Thereafter, the animals were sacrificed and disposed accordingly. Serum samples were extracted from the clotted blood into sterile plain tubes after centrifugation at 3000 x g for 5 min using a bench top centrifuge (MSE Minor, England). The sera were 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 $\pm$ SD and standard

Student's 't' - test was employed to assess statistical significance. $P < 0.05$ was regarded as significant.

RESULTS

The summary of the effects of interactions of vitamin A (40 IU/kg body wt.) with *Rauwolfia vomitoria* root bark extract (150 and 300 mg/kg body wt., respectively) on the marker enzymes of the myocardium is depicted in Table 1. The enzymes activity of creatine kinase and creatine kinase isoenzyme decreased significantly ($p \geq 0.05$) in rats following the respective administration of 150 and 300 mg/kg body wt. of the root bark extract of *Rauwolfia vomitoria*. In addition, concomitant administration of vitamin A (40 IU/kg body wt.) with 150 mg/kg body weight of the root bark extract of *Rauwolfia vomitoria* decreases significantly the serum activity of creatine kinase and creatine kinase isoenzyme. While the combination of vitamin A with 300 mg/kg body weight decreased insignificantly serum creatine kinase, the creatine kinase isoenzyme activity was significantly reduced ($p \geq 0.05$). In Table 1, the activity of serum aspartate amino transferase (AST) was significantly higher ($p \leq 0.01$) except in animals of the group that was given vitamin A and 150 mg/kg body weight of *Rauwolfia vomitoria* root bark extract, respectively. Animals treated with vitamin A in this investigation did not show any difference in the CK-MB and AST activities when compared with the control animals. However, CK activity was slightly lower in vitamin A treatment group compared to control group. The results of our investigation suggest that treatment with *Rauwolfia vomitoria* root bark extract and concomitant administration of the extract and vitamin A are likely to be of significance to those on these medications. The synergetic effect of the interaction in of vitamin A and RV is of biochemical benefits in terms of creatine kinase isoenzyme sensitivity.

DISCUSSION

Enzymes are packaged in cellular compartments as integral component of the tissue and their activities are normal in normal physiological condition. The study of enzyme compartmentalization enhances the association of enzymes to specific tissue in which they are predominantly domiciled. The integrity of such tissue can therefore be measured by the activity of tissue enzymes. Enzymes leak from injured tissues and are released into the serum following the same mechanism reported by Ichihara and Shirai (18). Marker enzymes in the infarct myocardium cell are likely to be released following the characteristic mechanism as that reported for liver AST (18). Clinical knowledge of the activity of drug metabolizing enzymes and marker enzymes is an important biochemical tool in the study of toxicity or beneficial effects of foreign compounds. The experimental evidences of the relationship between enzyme activity and antihypertensive drugs such as enalapril, atenolol and extract of *Rauwolfia vomitoria* root bark have been reported (4, 19).

Marker enzymes of myocardial infarction (CK, CK-MB, AST and LDH) are predominantly resident in cardiac tissue, though tissue such as skeletal muscle has expressed CK-MB at pathological value (20). The integrity of cardiac tissue in drug biotransformation and metabolism is highly associated with the level of CK, CK-MB and AST. In situations where there is high activity of these enzymes in the blood due to oxidative damage, the administration of antioxidant vitamin such as vitamin A may ameliorate tissue dysfunction since antioxidant vitamins are known to improve tissue integrity.

In this study, we observed that concomitant gavaging of normal Wistar albino rats with vitamin A (40 IU/kg bd. wt.) and root bark extract of *Rauwolfia vomitoria* (150 mg/kg bd. wt.) resulted

Table 1 : Effects of vitamin A interaction with the 80 % ethanol extract of *Rauwolfia vomitoria* root bark on serum cardiac enzymes activity of adult albino Wistar rats

Parameter (IU/L) ^a /group	Serum creatine kinase	Serum creatine kinase isoenzyme	Serum aspartate amino transferase
Control	180.34 ± 33.05	89.70 ± 37.484	28.71 ± 6.68
Vitamin A (40 IU/kg body weight)	168.57 ± 31.054	90.33 ± 17.73	29.64 ± 9.13
R.V. (150 mg/kg body weight)	107.23 ± 26.63**	43.41 ± 10.75*	61.71 ± 22.18*
R.V. (300 mg/kg body weight)	97.20 ± 27.25**	70.09 ± 11.34*	66.57 ± 22.50*
R.V. (150 mg) + vit.A (40 IU) / kg body weight	100.76 ± 40.18**	53.15 ± 17.41*	28.14 ± 9.21
R.V. (300 mg) + vit.A (40 IU) / kg body weight)	153.03 ± 49.29	39.06 ± 10.28*	51.43 ± 27.60*

RV = *Rauwolfia vomitoria* root bark extract; a = mean ± SD of 7 determinations; * $p \leq 0.05$; ** $p \leq 0.01$.

in a significant decrease in serum activity of CK and CK-MB. At the dose level of 300mg/kg body wt. RV root bark extract, the combination of vitamin A and RV did not significantly change the activity of CK. However, CK-MB changed significantly and this may be due to selective sensitivity of these enzymes to physiological changes. It is also interesting to note that vitamin A administration alone did not affect the activities of CK, CK-MB and AST significantly when compared with the control animals. However, the highly significant decrease in the activity of CK-MB when the extract of RV root bark and vitamin A were administered concomitantly is considered to be the result of the interaction between the two drugs.

Aspartate amino transferase activity was significantly higher ($P \leq 0.05$) when the root bark extract of RV was administered at 150 and 300 mg/kg body wt. but significantly lower at 150 mg/kg body wt. + vitamin A (40 IU)/kg body wt. combination therapy. This is to say that interaction of vitamin A with this herb is likely to be of health benefit in situations where there are raised cardiac enzyme markers. Earlier, we reported that application of *Rauwolfia vomitoria* root bark extract in the treatment of hypertension may have significant effect on myocardial infarction (4). The combination of this extract with vitamin A in this work has revealed the antioxidant activity of the RV root bark extract. This may be attributed to the presence of biologically active phytochemical constituents of RV root bark such as polyphenols.

In conclusion, it can be said that while extract of RV root bark is a useful medication in cardiac treatment, the combination of this herbal extract with vitamin A may be of more biochemical significance. Toxicopathological study of the heart muscle under experimental treatment with *Rauwolfia vomitoria* root bark extract, and its interaction with vitamin A is ongoing in our laboratory.

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