Full Length Research Paper

Effects of *Calliandra portoricensis* extracts on transaminases and creatine kinase of wistar rats challenged with venom of *Echis ocellatus*

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Abstract

Effects of selective solvent extracts of *C. portoricensis* on the aminotransferases and creatine kinase were studied to ascertain their potency in ameliorating the haemotoxicity of venom of carpet viper (*Echis ocellatus*) in wistar rats. A total of 30 wistar rats were used, divided into 6 rats per group of control, venom, venom/flavonoid-rich, venom/polyphenol-rich and venom/whole ethanolic extracts. The control group was given nothing while the other groups were given 200µg venom/rat and 0.5ml of 100mg/100g body weight (b.w) of rat as "antidote" concentration. This "antidote" was given 4-6 hours intramuscularly after the administration of the viperian venom. There was increase in the aminotransferases (P<0.05) in the treatment groups when compared to the control. When compared to the venom-treated and control groups, the *C. portoricensis* groups showed a marked decrease (P<0.05-p<0.01) of creatine kinase. The Aspartate aminotransferase (AST) – Alanine aminotransferase (ALT) ratio showed a marked increase (P<0.01) in the venom group and a corresponding marked decrease (P<0.01) in the flavonoid-rich, polyphenol-rich and whole ethanolic extracts groups. These findings suggest that selective solvent extracts of *C. portoricensis* may be effective in attenuating these enzymes- linked lethality of carpet viper strike.

Keywords: Venom, Extract, Aminotranferases, Creatine kinase, Envenomation.

INTRODUCTION

Snake bite is a major socio-medical problem (Swaroop and Grab, 1954) and Nigeria has the highest mortalities in Africa and Asia due to snake bite (Sawaii and Homma, 1975). In Nigeria, especially in Kaltungo, Gombe State, Taraba, Adamawa, Bauchi, Borno, Yobe and Plateau States, there were 436 snakebite cases in 2000 out of which 40 died. In 2006, 18 deaths occurred out of 1,700 reported cases.The Kaltungo Kingdom is geographically located in a unique ecosystem that favours rapid breeding and multiplication of snake species like the cobra, and the most deadly snake in the world, carpet viper. Because not all victims of snakebites get to hospital, estimates of illness and death caused are not actual figures but are approximates. However, one estimate quoted by World Health Organization (WHO) is that 2.5million occur worldwide each year and 125,000 are fatal (Wagstaff *et al.*, 2006). Carpet viper (*Echis ocellatus*) is the most medically important viper in West Africa (Wagstaff *et al.*, 2006).

Various local tissue alterations accompanying snakebite such as haemorrhage, edema and myonecrosis may result in tissue loss or organ dysfunction (Ownby, 1982; Gutierrez, 1995). These effects develop very rapidly after snake envenomation, making neutralization by anti-venom very difficult, especially if serotherapy is delayed due to either late

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access to medical care or scarcity of anti-venoms (Gutierrez *et al*, 1998). In orthodox medicine, the treatment of snake bites basically involves the use of the following drugs in combination – Polyvalent anti-venom, hydrocortisone, pain reliever (panadol), broad spectrum antibiotics and 5% dextrose saline by intravenous infusion. However, the use of polyvalent anti-venom has many limitations. It is very difficult to handle as preservations must be within $4 - 8^oC$ and therefore requires uninterrupted power supply. As a result of poor power supply in Nigeria, the polyvalent anti-venoms are not readily stored in standard pharmaceutical stores and hospitals and hence not available and accessible to snakebite victims. Where available they are generally very expensive and unaffordable by rural people who make up the greater percentage of snakebite victims. Additionally, polyvalent anti-venom, although life saving contain immunoglobulin pool of unknown antigen specificity and known redundancy which necessitates the delivery of large volumes of heterologous immunoglobulin to the envenomed victim, thus, increasing the risk of anaphylactoid and serum sickness and adverse effects (Wagstaff *et al.*, 2006).

In phytotherapy of snakebites especially of the viperian species traditional herbalists in the south eastern part of Nigeria have found the leaves and roots of *Calliandra portoricensis* very useful and effective in neutralizing viperian venoms. This study therefore is designed to ascertain the effects of selective solvent extracts of *C. portoricensis* on serum aminotranferases and creatine kinase which are indicators in carpet viper toxicity.

MATERIALS AND METHODS

Source of Venom

Lyophilized snake venom of carpet viper was purchased from South African venom suppliers cc;bewild@worldonline.co.za. This venom was preserved as a yellowish crystal kept in a dessicator at 8^oC till it was used.

Source of Plant

Calliandra portoricensis was sourced from the extensive secondary forest of Oji-River in Enugu State where it is used traditionally in the treatment of snakebite of the viperian species. Taxonomically, the plant was identified and confirmed to be *C. portoricensis* by Professor Jonathan C.Okafor, Professor of Botanical Taxonomy, Ebonyi State University, Abakaliki, Nigeria. Voucher specimen "CP-O No.1" has been preserved for reference in the Botany Herbarium of Ebonyi State University, Abakaliki.

Plant Extract

Three hundred and fifty grammes (350g) each of fresh and dry leaves and roots were crushed separately and extracts prepared from them by refluxing in 80% ethanol for 72 hours in a Soxhlet extractor. The extracts were then concentrated in rotary evaporator and dissolved in 0.9% saline for use.

Phytochemical Screening

Qualitative and quantitative screening of the *Calliandra portoricensis* extracts were carried out using the methods of Harborne (1973) for alkaloids, flavonoids, saponin, tannins, polyphenols and reducing compounds; Sofowora (1982) for glycosides; Trease and Evans (1983) for phlobatanins, anthraquines and hydroxymethyl anthraquines. 2g of the concentrated extracts were dissolved in 10ml of 0.9% saline and used for each component of the qualitative and quantitative analysis.

Selective Solvent Extraction

A measured weight of the processed sample was boiled in 100ml of 2M HCL solution under reflux for 40 minutes. After cooling and filtering, the filtrate was treated with equal volume of ethyl acetate. This technique has a preferential selection for flavonoids in the ethyl acetate phase (Harborne, 1973). The total phenols (polyphenols) were extracted from 200mg of sample with 10ml concentrated methanol by the Folin-ciocatean spectrophotometer technique (AOAC, 1990) and the extract analyzed and shown to be rich in polyphenols since methanol has selective extraction capability for phenols.

Animal Treatment

A total of 30 albino wistar rats weighing between 90-120grams were used for this study. The rats were assigned into five treatment groups with group one as control with no venom challenge and extract treatment. Treatment group two was given 0.2ml of 1mg/ml of viperian venom. Groups three, four and five received the same dose of the venom. Four hours after the venom challenge, calculated dose of (0.5ml of 100mg/100g body weight) flavonoid-rich, polyphenol-rich and whole ethanolic extracts were given to the rats in groups three, four and five respectively. Both the venom and the extracts were given intramuscularly. The choice of the intramuscular route was informed by the need to follow or mimic the natural path of snake envenomation in man and animals.

Two hours after the 'medication' with the plant

extracts, the rats were sacrificed by euthanasia and blood samples collected from the various groups via cardiac puncture into sample tubes. The blood samples were allowed to stand for one hour to clot. Serum was later separated from clot by spinning at 5000 revolutions per minute (rpm). Serum was decanted and the quantity collected was used for enzymes (transaminases and creatine kinase) assays.

Aspartate aminotransferase (AST) activity was measured by the method of end point colorimetric assay using diagnostic kits obtained from Randox Laboratories Ltd, Ardmore, United Kingdom. AST was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine.

Alanine aminotransferase (ALT) was similarly measured by method of end-point colorimetric assay with kits supplied by Randox Laboratories Ltd, England. Alanine aminotransferase is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine.

Creatine kinase (CK) was measured by using Spectrum Diagnostic Creatine Kinase reagent. The method was according to the recommendation of the International Federation of Clinical Chemistry (IFCC).

LD$_{50}$ of Plant Extract and Viperian Venom

The determination of the LD$_{50}$ of plant extract and the venom was done using the method of Lorke (1983). LD$_{50}$ was determined to form a basis of dosage for subsequent assays employing sublethal doses of plant extract and venom.

Statistical Analysis

Analysis of variance (ANOVA) was used in analyzing the data generated from this study. Results of the study were expressed as mean ± standard deviation. Data between treatment groups were analyzed using two way analysis of variance. Values of P<0.05, P<0.01 were regarded as being significant.

RESULT

The result of the effect of intramuscularly administered viperian venom and extracts of *Calliandra portoricensis* on serum AST, ALT and CK activities of wistar rats and the AST: ALT ratios are presented in tables 1 and 2.

The data indicated that there was a significant increase in AST activity in the venom-treated rats and further significant increases in the flavonoid-rich, polyphenol-rich and whole ethanolic extract-treated rats when compared to the control (p<0.01). The data showed increases in ALT activities that were dependent on the

type or nature of the extract of *C. portoricensis* namely: flavonoid-rich, polyphenol-rich and whole ethanolic extracts with the increases being statistically significant (p<0.05). The data further showed significant decrease (p<0.05) in Creatine Kinase (CK) in the flavonoid-rich and polyphenol-rich groups and a highly significant decrease (p<0.01) in the whole ethanolic extract group when compared to the venom-treated and control groups of rats.

The AST: ALT ratio for the venom-treated rats as computed (48.50) showed an increase compared with the control value of 34.74 (p<0.05). With the exception of flavonoid-rich extract group which showed non-significant increase from the control value of 34.74 (p>0.05),the polyphenol-rich and whole ethanolic extracts showed statistically significant decrease (p<0.01) from the control value of 34.74.Generally an increase in AST:ALT ratio indicates pathology involving the heart as was the case in the venom-treated rats while a reverse or decrease implicates the liver as would be expected in the detoxification of the venom-polyphenol/whole ethanolic extracts complexation increased the ALT activity thereby decreasing the AST: ALT ratios in the sera of wistar rats in these groups. The veracity of this ratio anchors on the fact that the heart produces more of AST compared to all tissues of the body while the liver does so for ALT. An increase in ALT activity means a decrease in ratio. This ratio provides evidence in support of the complexation of viperian venom with extracts of *C. portoricensis* which the liver tries to detoxify.

DISCUSSION

Viperian venom and various extracts of *C. portoricensis* were administered intramuscularly in wistar rats. After six hours interval AST, ALT and CK enzymes activities were measured in the sera obtained from these rats against appropriate controls. Increases in AST and ALT in the various treatment groups were evident in the sera. The computed AST: ALT ratio showed a significant increase (p<0.01) in the sera of rats treated with viperian venom only. The sera of the animals treated with the venom and flavonoid-rich extract showed a decrease from that of the venom-treated rats. This was not significant (p>0.05). Although results on the effect of viperian venom administration on the aminotransferase activities on tissues have not been reported in literature, however the use of AST: ALT ratio generally to monitor pathologies involving the heart or liver has been reported by Howcroft (1987) and Stroev and Makarova (1989).

According to these reports, an increase in AST: ALT ratio points to pathology involving the heart while the reverse implicates the liver. The increase in this ratio observed in the venom-treated rats therefore provided experimentally determined evidence, at the molecular level, that viperian venom toxicity was cardiovascular.

Table 1. Effects of Treatment on the serum Aspartate Aminotransferase (AST), Alanine Aminotranferase (ALT) and Creatine Kinase (CK) activities on experimental rats (n = 30)

Treatment	AST(u/L) Activity	ALT(u/L) activity	CK(u/L) activity
Control	85.8±4.99	2.47±1.10	651.65±2.92
Venom	130±0	2.68±2.9	693.82±2.54
Flavonoid-rich extract	130±0	3.32±1.7	640.70±2.45
Polyphenol-rich extract	141±7.5	9.9±7.4	597.09±6.08
Whole ethanolic extract	140±10	18.4±9.9	403±1.10

Table 2. Effect of treatments on the Serum AST: ALT Ratio in experimental rats

Enzyme	Enzyme Activities/Ratio				
	Control	Venom	Flavonoid-rich extract	Polyphenol-rich extract	Whole ethanolic extract
AST	85.8±4.99	130.0±0	130.0±0	141±7.5	140±0
ALT	2.47±1.1	2.68±2.9	3.32±1.7	9.9±7.4	18.4±9.9
AST:ALT	34.74	48.50	39.16	14.24	7.61

The explanation for the increase in the activity of these enzymes in the serum of venom-treated group of rats is anchored on the tissue specificity of these two enzymes. According to Witschi (1980), toxicants interact with biochemical machinery of living cells causing subtle changes to vital tissue constituents including the enzymes level in the tissues and the extracellular fluid (ECF). Based on this, measurements of enzyme level in the serum have since been employed as diagnostic tool and the functional status of lesions in cells are carried out by monitoring the levels of specific enzymes in serum or urine (Bauer *et al,* 1968, Ngaha and Plumer, 1977).

Cardiotoxic substances which break down the membrane architecture of the cells bring about the spillage of these cytosolic enzymes into the plasma and their concentration in the plasma rises. Therefore, damage on the heart by the venom led to spillage of the enzymes into the plasma and a consequent rise in their activities. In this study, therefore, the increase in AST and ALT activities and the decrease in serum AST: ALT ratio pointed to increase in the detoxification ability and capacity of the liver to handle the complex of the venom and the extracts of *C. portoricensis* which brought about increase in the ALT activity and the consequent decrease in the AST: ALT ratio in the rats treated with various extracts of *C. portoricensis*.

CONCLUSION

Exposure of wistar rats to viperian venom alters, negatively, their enzyme and other biochemical indices of toxicity. The venom, besides its known haemotoxicity, is cardiotoxic and may bring life to acute fatality if not treated immediately. The alteration of these enzymic parameters accounts for part of viperian venom toxicity. Selective solvent extracts of *C. portoricensis* have been able to reverse these altered enzyme changes to a very large extent and may have been contributory of the plant extracts to the neutralization of carpet viper envenomation in rats.

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