Full Length Research Paper

Effects of *Calliandra portoricensis* extracts on the lipid profile of wistar rats challenged with venom of carpet viper

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Abstract

Effects of whole ethanolic and selective solvent extracts of *Calliandraportoricensis* on lipid profile were carried out to ascertain their potency in reducing or attenuating the haemotoxicity of carpet viper venom in wistar rats. Thirty(30) rats of mixed sexes were randomly assigned into 5 treatment groups of 6 rats each. Group 1 (control) received nothing. Group 2 received viperian venom only. Groups 3, 4 and 5 received same amount of venom in addition to calculated doses of flavonoid-rich, polyphenol-rich and whole ethanolic extracts respectively. The venom and *C. portoricensis* extracts were administered intramuscularly. The rats were sacrificed 4 hours later, serum collected and lipid profile assayed. The result obtained from group 2 showed significant increase (p<0.05) in the serum total cholesterol while groups 3, 4 and 5 showed decreases that were not statistically significant (p>0.05) relative to group1. Triacylglycerols decreased significantly (p<0.01) in groups 3 and 4. While the High Density Lipoprotein decreased significantly in venom-treated group 2, a correspondingly significant increases (p<0.01) occurred also in groups 3, 4 and 5. The effects of the extracts on the very low density lipoprotein (VLDL) were unclear but there was marked reduction in the Low Density Lipoprotein (LDL). The LDL:HDL ratio increased in the venom-treated group 2, while decreases occurred in groups 3, 4 and 5, strongly suggesting a complexation of the venom and extracts of *C. portoricensis* due to increase in High Density Lipoprotein.

Keywords: *Calliandra portoricensis*, Venom, Extracts, Lipid profile.

INTRODUCTION

Nigerians especially in the Nothern and Central parts of the country (Borno, Gombe,Taraba, Adamawa, Bauchi, Yobe and Plateau States) have been victims of lethal snakebites especially of viperian origin. This may be attributed to the rocky and marshy nature of the landscape which favours rapid breeding and multiplication of snake species like cobra, puff-adder and the deadly haemotoxic carpet viper. Snakebite is a major socio-medical problem (Swaroop and Grab, 1954) and Nigeria appears to have the highest mortalities in Africa especially in these northern states according to local hospital reports.

Toxins of venomoussnakes constitute great medical challenge in Africa and Southeast Asia (Wagstaff et al, 2006). 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 2.5 million occur annually and 125,000 are fatal (Wagstaff et al, 2006). Carpet viper (*Echisocellatus*) is the most medically important viper in West Africa (Wagstaff et al, 2006).

Haemolysis, haemorrhage, edema, myonecrosis and various local tissue changes accompanying snakebite may result in tissue loss or organ dysfunction (Ownby,

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1982; Gutierrez, 1995). Because these effects develop very rapidly after snakebite, neutralization of the snake toxin by anti-venoms is very difficult, especially in delayed access to medical care or scarcity of anti-venoms (Gutierrez et al, 1998). This delayed access to medical attention along with non-availability of polyvalent anti-venom (PVA) is a common feature in most snakebite cases in Nigeria and indeed West Africa.

In orthodox medicine, the treatment of snakebites basically involves the use of a combination of polyvalent anti-venom, corticosteroid e.g hydrocortisone, pain reliever, broad spectrum antibiotics and 5% dextrose saline by intravenous infusion. However, the use of PVA is limited by the challenge of preservation at $4-8^0$C because of unreliable power supply in Nigerian rural and even urban communities. This makes storage in standard pharmaceutical stores and hospitals very difficult and not easily accessible. Where available they are very expensive and unaffordable by rural people who make up the greater percentage of snakebite victims. Additionally, PVA, although life saving contain immunoglobulin pool of unknown antigen specificity which is formed in the process of anti-venom production, thus increasing the risk of anaphylaxis (Wagstaff et al, 2006).

In phytotherapy of snakebites especially of the viperian species, traditional herbalists in the South Eastern part of Nigeria claim they have found the leaves and roots of *C. portoricensis* very useful and effective in neutralizing carpet viper venoms. Therefore, the alternative choice of treatment available and accessible to the teeming local Nigerian snakebite victims is phytotherapy.

This study therefore, is designed to ascertain the effects of whole ethanolic and selective solvent extracts of *C. portoricensis*on the lipid profile (one of the indicators of carpet viper venom toxicity) induced by carpet viper venom using wistar rat models.

MATERIALS AND METHODS

Source of Venom

Carpet viper (*Echis ocellatus*) venom used for this study was purchased from South African venom suppliers cc;bewild@worldonline.co.za. It was lyophilized and preserved in a desicator at 8^{oC} until used.

Source of Plant

Calliandra portoricensis was sourced from the extensive secondary forest of Oji-River in Enugu state where it is used traditionally for the treatment of snakebite of viperian species. Taxonomically, this plant was ident-ified and confirmed to be *C. portoricensis by* Professor

Jonathan C. Okafor, Professor of Botanical Taxonomy, Ebonyi State University, Abakaliki, Nigeria.

Plant Extract

Whole ethanolic solvent fractions were prepared from three hundred and fifty grammes (350g) each of fresh and dry leaves and roots of *C. portoricensis* by crushing and refluxing them in 80% ethanol for 72 hours in a Soxhlet extractor. The extracts were then concentrated in a rotary extractor and dissolved in 0.9% saline for use. This extract constituted whole *C. Portoricensis* extract (CP-extract).

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 of flavonoids in the ethyl acetate phase (Harborne, 1973). The total phenols (polyphenols) were extracted from 200mg of the 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 selectively extracts phenols.

Phytochemical Screening

Qualitative and quantitative screening of CP-extracts was carried out using the methods of Harborne (1973) for alkaloid, flavonoids, saponins, tannins, polyphenols and reducing compounds; Sofowora (1982) for glycosides; Trease and Evans (1983) for phlobatannins, anthraquinones and hydroxymethylanthraquinones. 2 g of the concentrated CP-extract were dissolved in 10ml 0f 0.9% saline and 2ml used for each component of the qualitative and quantitative analysis.

Animal Treatment

30 albino wistar rats weighing between 90-120 grammes were used for this study. The rats were randomly divided into five treatment groups. Group 1(control) was given nothing. Group 2 received 0.2ml of 1mg/ml of the venom only. Groups 3, 4 and 5 received the same dose of the venom and four hours after the venom challenge, calculated dose (0.5ml of 100mg/100ml) of flavonoid-rich, polyphenol-rich and crude CP-extracts was given to the rats in groups 3, 4 and 5 respectively. 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 using Chloroform vapour and blood samples collected from the various groups via cardiac puncture into sample tubes. The blood samples were allowed to stand for about one hour to clot. Sera were later separated from the clot by spinning at 5000 revolutions per minute (rpm) and decanted from the sample tubes. The quantities collected were used to determine the liquid profile and consequently the lipid oxidation indices of envenomed rats treated with *C. portoricensis* extracts.

Cholesterol is present in serum as cholesterol esters and free cholesterol. The cholesterol esters present in the serum were hydrolyzed by cholesterol esterase and the cholesterol was then measured by oxidising with cholesterol oxidase to form hydrogen peroxide. The hydrogen peroxide in turn reacted with phenol and 4-aminoantipyrine present to form the red quinoneime dye. The intensity of the dye formed was directly proportional to the level of cholesterol present in the samples. Elevated levels of cholesterol were primarily considered as indication of increased risk of cardiovascular disturbances. This principle was used to ascertain the effect of various CP-extracts on serum cholesterol of rats and comparing the findings with control values. This test by Fortress diagnostic technique was carried out using Easy Way Medical (EWM) Chemistry Analyzer.

Triacylglycerols are the main lipids present in serum. Spectrum Diagnostics liquizyme triglycerides reagent was used for the determination of triacylglycerols using the EWM Chemistry Analyzer.

Direct enzymatic calorimetric method developed by spectrum was used to determine the High Density Lipoprotein (HDL) using the EWM Chemistry Analyzer.

By dividing the serum triacylglycerol value by 5, the Very Low Density Lipoprotein (VLDL) was obtained. This factor of 5 was based on the understanding that fasting subjects with a triacylglycerol concentration of 400mg/dL, the VLDL to total serum triacylglycerol ratio is fixed relatively at 1:5.

By the Friendwalds relationship, the estimation of Low Density Lipoprotein (LDL) was derived from the difference between the total cholesterol and the sum of HDL and VLDL.

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). The LD$_{50}$ of *C. portoricensis* was carried out in two stages which clearly determined the dose of extract which caused 50% of the experimental mice to die. The geometric mean of the non-lethal dose and the lowest

lethal dose was calculated as the LD$_{50}$ (Lorke,1983). The geometric mean gave a value of 150mg/kg body weight (b.w). Similar procedure determined the LD$_{50}$ of the venom to be 250µg /kg b.w. LD$_{50}$ was determined to form a basis of dosage for subsequent assays employing sub-lethal doses of plant extract and venom.

Statistical Analysis

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

RESULTS

Table 1 presents the effect of treatment on lipid profile of experimental wistar rats.

The flavonoid-rich, polyphenol-rich and whole ethanolic CP-extract treated groups had lower total cholesterol values than the control. However, the decreases were not statistically significant ($p>0.05$). The venom-treated group showed a significant increase in the same serum total cholesterol index ($p<0.05$).

Effects of treatment on the serum triacyglycerols showed a steady increase that was significant ($p<0.05$) in all the groups when compared to the control with the exception of the flavonoid-rich extract treated group which showed a statistically significant decrease ($p<0.01$) in the triacylglycerols content of the serum in the rats.

The effect on the HDL of the venom-treated animal showed a marked significant decrease ($p<0.01$) and the flavonoid-rich, polyphenol-rich and whole CP-extracts treated groups also showed correspondingly significant increase ($p<0.01$) in the HDL in the experimental rats. (Table 2)

There was a statistically significant increase in the LDL:HDL ratio in the venom-treated group of rats ($p<0.01$).This marked increase which predisposes victims to coronary artery disease may be contributory to the cardiovascular disturbances associated with viperian envenomation. The ratios in the flavonoid-rich, polyphenol-rich and whole CP-extracts treated groups showed statistically downward decreases ($p<0.01$) strongly suggesting a complexation of the venom and extract fractions especially the polyphenol-rich extract due to the increase in "good" cholesterol (HDL) and the decrease in LDL.

DISCUSSION

The focus of this study was to assess wh-ether intramuscular administration of whole ethanolic CP-

Table 1. Effects of Treatment on the Lipid Profile of Experimental Rats in mg/dL (n=30)

Extract Treatment	Total Cholesterol	Triacylglycerols (TG)	HDL	VLDL	LDL
Control	125.4±0.08	81.63±1.90	44.74±1.03	16.33±0.38	64.33±1.33
Venom	134.2±0.09	86.25±1.51	11.17±5.70	17.25±0.30	105.78±5.91
Flavonoid-rich extract	114.6±0.09	51.99±1.46	72.24±1.16	10.40±0.29	31.96±0.29
Polyphenol-rich extract	115.8±0.10	103.32±6.52	107.39±1.59	20.66±1.30	12.25±2.79
Whole ethanolic extract	123.1±0.13	95.72±1.12	165.13±1.13	19.14±0.23	61.17±1.23

Table 2. Effects of Treatment on LDL and HDL Activities in Experimental Rats and their calculated ratios

Test Serum Lipid	Control	Venom	Flavonoid-rich extract	Polyphenol-rich extract	Whole Ethanolic extract
LDL (mg/dL)	64.33±1.33	105.78±5.91	31.96±0.26	12.25±2.79	61.17±1.23
HDL (mg/dL)	44.74±1.03	11.17±5.70	72.24±1.16	107.39±1.59	165.13±1.13
LDL: HDL	1.44	9.47	0.44	0.11	0.37

extract and selective solvent fractions induced any changes in serum lipid profile and the LDL:HDL ratio in rats challenged with a calculated dose of viperian venom. The information obtained from here would be useful in addressing the reduction of haemotoxicity by the plant extracts of the carpet viper venom. The total cholesterol, Triacylglycerol (TG), VLDL, LDL and LDL:HDL ratio were significantly elevated ($p<0.05$ to $p<0.01$) in the venom-treated group of rats excepting the HDL where marked decrease occurred ($p<0.01$) when compared to the control. With the exception of the flavonoid-rich treated group of rats with a mean TG concentration of 51.99±1.46 ($p<0.01$), all other groups increased in TG concentration when compared to the control. This may be attributed to the high concentration of flavonoid in this group of extract and hence showed significant anti-oxidant effect on the TG. Of all the phytochemical components it is believed that compounds of a flavonoid structure should manifest a relatively high degree of biological activity (Ibekwe, 2005). The activity is conferred on them by the chemical nature of the flavonoid molecule which contains reactive hydroxyl (-OH) and methyl ($-CH_3$) substitutes. The ability of compounds of this type to chalconize, thereby forming quinines and other structures capable of interacting with specific enzymatic functional groups in a biological system is indicative of their high degree of biological activity (Martin, 1995). Compounds with these groups may play a role in the anti-oxidant properties of flavonoid-rich extract of *C. portoricensis* by reducing peroxaidation activity in the TG. The reduction in TG concentration by flavonoid-rich extract could be one of the ways *C. portoricensis* contributes to disabling

and inactivating the viperian venom. Additionally, the flavonoid-rich treated group of rats decreased significantly ($p<0.01$) VLDL concentration. This may point also to the anti-oxidant role of flavonoid-rich extract of *C. portoricensis* in protecting envenomed cells from the damaging effects of reactive oxygen species (ROS), such as singlet oxygen, superoxide, peroxyl and hydroxyl radicals and peroxynitrite. An imbalance between anti-oxidants and ROS would result in oxidative stress leading to cellular damage. The flavonoid-rich extract, along with vitamins C and E which abound quantitatively in the plant extract might have helped in reducing the cellular damage and improving the anti-oxidant defense system following challenge by carpet viper venom in experimental rats. High intake of vitamin E have been associated with reduced risk of coronary heart disease in man probably due to anti-oxidant property (Rimm et al,1993).There was also significant increase ($p<0.01$) in HDL in the selective solvent and CP-extract treated groups with a corresponding decrease in the LDL:HDL ratio ($p<0.01$). The LDL also decreased in the groups treated with whole ethanolic CP-extract and selective solvent fractions. These decreases in concentration of LDL and the computed LDL:HDL ratio would suggest synergistic interaction of the flavonoid-rich and polyphenol-rich extracts in bringing down the concentration of the 'bad' cholesterol (VLDL and LDL) and minimizing the cardiotoxicity, haemopathies and shock which accompany carpet viper envenomation.

It was observed from this study that the LDL:HDL ratio in the group treated with venom alone was highly increased ($p<0.001$). It is known that high serum

LDL:HDL ratio predisposes to artherogenesis (Mayes,1990; Gaw and Shepherd, 1997). The marked increase in this ratio in the venom-treated group indicated tendency towards atherogenesis. This process appeared to have been impeded by the whole ethanolic CP-extract and selective solvent fractions. The effect of the venom, besides the haemopathies, appeared to be direct on the heart through increased contractile force. Increased contractile force beyond physiological limits that cardiac wall tension can sustain over-distends cardiac ventricles and their attempt to pump blood into the systemic circulation. As large part of the myocardium is deprived of adequate blood supply, hypoxia and defective nutrition result. Hypoxia induces a sequence of biochemical events beginning with reduction of available ATP levels (Macsween and Whalley, 1992). Cells then switch to anaerobic glycolysis to maintain energy, generating excess lactic acid in the process and a drop in pH.

Lack of ATP causes failure of sodium pump mechanism and consequently sodium accumulates in cells and drags water into them causing inflammation of cells. The re-instatement of blood supply after a long period of hypoxia leads to generation of free radicals by damaged tissues and peroxidation of membranes occurs (Sinatra and Demarco, 1995). The breakdown of membranes leads to leakage of cellular contents into extracellular fluid spaces including cytosolic enzymes, typically the aminotransferases. Acid hydrolytic enzymes of lysosomes leak into and digest cytosolic nuclear components. Necrosis is the end result of this cardiac damage (Macsween and Whalley, 1992) induced by viperian venom.

The use of whole ethanolic CP-extract and selective solvent fractions in experimental rats appeared to have suppressed all these biochemical and pathophysiological derangements brought about by the intramuscular injection of carpet viper venom. The effect of these fractions especially in lowering lipid levels in envenomed rats with elevated plasma lipid levels, the possible exploitation of this property for therapeutic benefits in carpet viper envenomation may have been unravelled and this finding seems novel.

CONCLUSION

Exposure of wistar rats to viperian venom alters negatively their lipid profiles and other biochemical indices of toxicity. The whole ethanolic CP-extract and the selective solvent fractions significantly lowered the high lipid oxidation indices usually induced by the viperian venom, hence a protection against cardiotoxicity, haemopathies and shock that are normal consequencies in carpet viper envenomation. The significant reduction in the lipid parameters (Total Cholesterol, TG, VLDL and LDL) and the elevation in HDL had shown that *C. portoricensis* extracts had a neutralizing effect on the

viperian venom. Additionally, the decrease in LDL: HDL ratio also proved that whole ethanolic CP-extract and selective solvent fractions removed the possibility of atherogenesis which viperian venom can predispose a victim to after recovery. In fact epidemiological studies indicate that high plasma HDL levels are strongly correlated with a low incidence of cardiovascular complication (Voet and Voet, 1990), lending support to the findings of this study.

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