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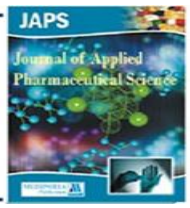

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Effects of *Calliandra Portoricensis* Extracts on the Haematological Indices of Wistar Rats Challenged With Venom of *Echis Ocellatus*

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ABSTRACT

Effect of whole ethanolic and selective solvent extracts of C. portoricensis plant was carried out in wistar rats to ascertain their efficacy in ameliorating or attenuating the haemotoxicity of venom of carpet viper. 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 was given nothing while the other groups were given 200µg venom/rat and 0.5 ml 100mg/100g body weight (b.w.) of rat as "anti-dote" concentration. This "antidote" was given intramuscularly 4-6 hours after the administration of the venom. The result obtained showed significant increase ($P<0.05$) in the haemoglobin concentration, red blood cell count, mean corpuscular volume, white blood cell count especially in neutrophils and marked decrease ($P<0.05$) in platelet parameters. These findings suggest that whole ethanolic and selective solvent extract of C. portoricensis may be effective in ameliorating and indeed reversing the haemotoxicity occasioned by carpet viper haemotoxin in wistar rats.

Keywords: *Calliandra portoricensis*, Venom, Blood, Haemotoxin, Extract.

INTRODUCTION

The incidence of snakebite in our rural and some parts of urban areas has been on the rise and Nigeria appears to have the highest mortalities in Africa and Asia (Sawaii and Homma, 1975). This constitutes a socio-medical problem (Swaroop and Grab, 1954). The incidence of snakebite appears, as a result of the unique ecosystem, to be more marked in the North eastern and central parts of Nigeria where breeding and multiplication of snakes are favoured. Because not all victims of snakebites get to hospital, estimates of illness and death caused are not actual figures but are approximates. However, World Health Organisation (WHO) stated that 2.5million cases occur worldwide each year and 125,000 are fatal (Wagstaff *et al*, 2006). Serious envenomations by venomous snakes give rise to haemorrhage, edema and myonecrosis which results in organ dysfunction and tissue loss (Ownby, 1982; Gutierrez, 1995).

If these alterations are not handled promptly as a result of late access to medical care or scarcity of anti-venoms (Gutierrez *et al*, 1998) death supervenes in a few hours. A known conventional medical procedure using polyvalent anti-venom (PVA) is normally used to neutralize the toxins in the venom. However, preservation of the PVA in rural setting is almost impossible as a result of unreliable power supply; where available the PVA is very expensive and beyond the reach of rural victims.

Over the years, traditional herbalists have developed recipes in the south eastern part of Nigeria for the effective handling of carpet viper venoms. This recipe which is ethanolic extract of *C. portoricensis* is specific for haemotoxin of snakebites that occasion haemotocixity (eg. the haemotoxin of pit viper like the rattlesnake and true vipers such as the puff adder and the carpet viper). According to traditional herbalists, the secondary metabolites (bioactive principles) of *C. portoricensis* do not have effect on the neurotoxin of elapine snakes like cobras, mambas and coral snakes.

This study therefore is designed to ascertain the effects of whole ethanolic and selective solvent extracts of *C. portoricensis* on haematological parameters which are indicators of viperian venom 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 inside a tube surrounded by blueish larger silica gel to maintain a dry environment. This was then kept in a dessicator at 8^0C till it was used.

Source of Plant

Calliandra portoricensis was sourced from the extensive secondary forest of Oji-River in Enugu State, Nigeria 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 them 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 (tables I and II). 2gms of the concentrated extracts were dissolved in 10ml of 0.9% saline and used for each component of the qualitative and quantitative analysis.

Table. 1: Qualitative Constituents of Fresh *Calliandra portoricensis*

S/No	Chemical Constituents	Fresh Root	Fresh Leaf	Fresh mixed sample
1	Alkaloids	+	+	++
2	Glycosides	+	+	+
3	Saponins	+	-	+
4	Tannins	-	+	+
5	Flavonoids	++	++	++
6	Reducing compounds	++	++	++
7	Polyphenols	++	+++	+++
8	Phlobatannins	-	-	-
9	Anthraquines	-	-	-
10	Hydoxymethyl anthraquine	+	+	-

	Keys:
+	Present
++	Present in moderately high level
+++	Present in high level
-	Absent

Table. 2: Qualitative Constituents Of Dry *Calliandra portoricensis*:

S/No	Chemical Constituents	Dry Root	Dry Leaf	Dry mixed sample
1	Alkaloids	+	+	+
2	Glycosides	+	+	++
3	Saponins	+	+	+
4	Tannins	-	-	-
5	Flavonoids	++	+	+
6	Reducing compounds	++	++	+
7	Polyphenols	++	++	+++
8	Phlobatannins	+	-	-
9	Anthraquines	-	+	+
10	Hydoxymethyl anthraquine	-	-	-

	Keys:
+	Present
++	Present in moderately high level
+++	Present in high level
-	Absent

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 (0.5ml of 100mg/100g body weight) of

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 sample tubes contained anticoagulant Ethylene diamine tetra acetate (EDTA) to prevent blood cloting and allow accurate measurement of the blood parameters. The following blood parameters of white blood cells, red blood cells and platelets were determined in the various treatment groups using haematology analyzer (BC-2600) of Bio-medical Electronics Co. Ltd. UK as shown in tables VII, VIII and IX.

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 (tables III, IV, V and VI).

Table. 3: Determination Of Ld_{50} Of *C. Portoricensis* (Stage 1).

Substance	Doses (Mg/Kg B.W)	Mortality
Ethanolic Extract	10,000	3/3
Ethanolic Extract	625	3/3
Ethanolic Extract	39	1/3

Table. 4: Determination Of Ld_{50} Of *C. Portoricensis* (Stage 2).

Substance	Doses (mg/kg b.w)	Mortality
Ethanolic extract	5	0/2
Ethanolic extract	39	0/2
Ethanolic extract	150	0/2

Table. 5: Determination Of Ld_{50} Of Venom (Stage 1) **.**

Substance	Doses (Mg/Kg B.W)	Mortality
Ethanolic Extract	1000	3/3
Ethanolic Extract	675	3/3
Ethanolic Extract	250	1/3

Table. 6: Determination Of Ld_{50} Of Venom (Stage 2).

Substance	Doses (µg/kg b.w)	Mortality
Ethanolic extract	15	0/2
Ethanolic extract	55	0/2
Ethanolic extract	250	0/2

The LD_{50} of *C. portoricensis* which was carried out in 2 stages (tables III and IV) clearly determined the dose of the extract at which 50% of the experimental animals died. The geometric mean of the non-lethal dose and the lowest lethal dose was calculated as the LD_{50} (Lorke, 1983).This geometric mean gave a value of 150mg/kg body weight (b.w).The LD_{50} of the venom was similarly determined and gave a value of 250µg/kg b.w (V andVI).

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.

RESULTS

The outcome of this study revealed a statistically significant decrease in haemoglobin concentration (HGB) in venom-treated group of rats ($P<0.05$) and even more statistically significant increase ($P<0.01$) in the *C. portoricensis* treated groups when compared to the former. However this increase, though very significant, never got HGB values up to the values of the control group. The red cell numbers showed a significant increase ($P<0.05$) in the venom-treated rats while the extract treated groups maintained red blood cell counts close to the control. The effect on the packed cell volume (PCV) which is the haematocrit value expressed as the percentage of cellular elements with that of the whole blood had no significant impact, though the mean corpuscular volume increased significantly ($P<0.05$). There were little or no significant changes in the mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) in all the groups of the experimental rats (table VII).

The result of treatments on WBC indices showed statistically significant increase ($P<0.05$) of WBC in all the groups. The mean values for the lymphocytes in differential counting were 5.3 ± 1.0, 4.15±1.39, 4.22 ± 2.13, 3.83 ± 1.07 and 4.26 ± 2.61 for the control, venom-treated and extract-treated groups respectively. The granulocytes, mainly the neutrophils showed a preponderant increase that is statistically significant ($P<0.01$) compared to the lymphocytes value which is not significant ($P>0.05$). The same effects were observed in the percentage values with lymphocytes showing a significant decrease ($P<0.05$) while the neutrophils showed significant increase.

The values of platelet count showed a significant decrease ($P<0.01$) while no significant change ($P>0.05$) were observed in the mean platelet volume (MPV) and the platelet distribution width (PDW). However the plateletcrit (PCT%) decreased significantly in all the treatment groups when compared to the control. See tables VII – IX for details.

TABLE. 7: Effects of Treatment on Red Blood Cells of Experimental Rats (n=30) .

Blood type	Control	Venom	Flavonoid-rich extract	Polyphenol-rich extract	Whole Ethanolic Extract
HGB g/L	177.33±6.81	13.3±8.74	161.±9.72	192.5±6.80	146.6±3.80
RBC x 10^{12}/L	7.77±0.6	9.21 ±0.43	7.78±0.76	7.96±0.82	7.39±2.58
HCT% (PCV)	44.7±3.2	52.45±0.94	46.11±3.4	52.4±1.93	46.94±4.71
MCV (fL)	57.75±2.7	54.72±1.36	57.6±2.9	58.4±0.53	57.96±3.2
MCH (pg)	17.12±0.48	17.4±0.33	18.1±0.46	17.82±0.63	17.74±1.3
MCHC g/L	297±6.84	312±10.91	313.67±9.11	310.3±10.4	317.2±6.05

HGB = Haemoglobin concentration; MCH = Mean Cell Haemoglobin
RBC = Red Blood Cell; HCT = Haematocrit
Pg = (Pico gram or micromicro gram); MCHC = Mean Cell Haemoglobin concentration
MCV = Mean corpuscular volume

8: Effects of Treatment on White Blood Cells of Experimental Rats (n=30).

Blood type	Control	Venom	Flavonoid-rich extract	Polyphenol-rich extract	Whole ethanolic extract
WBC x 10^9/L	10.3 ± 1.94	14.1±5.08	10.5±4.21	15 ±2.49	13.86±2.3
Lymph x 10^9/L	5.3±1.0	4.15±1.39	4.22±2.13	3.83±1.07	4.26±2.61
Gran # 10^9/L	3.4±1.7	7.52±4.72	5.5±2.85	15.2±6.2	8.82±4.5
Lymph%	52.03±3.17	29.9±4.20	38.9±4.50	19.6±4.83	29.54±4.69
Gran %	38.35±4.86	61.9±4.08	55.05±5.05	74.35±5.28	64.54±4.69

WBC = White Blood Cells, Lymph = Lymphocyte, Gran = Granulocyte

TABLE. 9 : Effects of Treatment on platelets of Experimental Rats (n=30)

Blood type	Control	Venom	Flavonoid-rich extract	Polyphenol-rich extract	Whole ethanolic extract
PLT x 10^9/L	551 ±1.70	416.67±1.32	237.8±1.96	205±1.33	111.6±0.87
MPV(fL)	8.57±0.44	9.12±0.44	10.6±0.55	9.1±0.78	9.86±0.55
PDW	14.67±0.23	14.77±0.23	14.82±0.67	14.7±0.55	14.92±0.19
PCT %	0.47±0.13	0.37±0.10	0.235±0.16	0.269±0.09	0.106±0.09

PLT = Platelets MPV = Mean Platelets Volume
PCT% = Plateletcrit PDW = Platelets Distribution Width

DISCUSSION

The outcome of the haematological studies revealed a significant increase in HGB (P<0.01) which was decreased significantly (P<0.01) in venom-treated rats. The object of measuring haemoglobin was to estimate the oxygen carrying capacity of blood in the face of envenomation of viperian haematoxin, in addition to providing an assessment of the erythropoetic status of the reticuloendothelial system. The results assisted in the detection of deficiency in haemoglobin (anaemia) or excess of haemoglobin (polycythemia) (Baker and Silverton, 1985). The total RBC count rose significantly (P<0.05) in the venom-treated group but there was also a statistically significant reversal almost to the normal values of the control rats in the various extracts groups. The venom caused a significant increase in haematocrit percentage (PCV) P<0.01 which the various extracts, excepting the polyphenol-rich group, decreased close to the control value but this was not significant (P>0.05).

The mean cell volume (MCV) was also significantly decreased (P<0.05) by the venom but reversals that were significant were achieved by the various extracts (P<0.05). The MCH values for all the groups showed an increase from the control value but these were not significant (P>0.05). The MCHC in grams per litre increased in the venom-treated group though insignificantly (P>0.05) while the various extracts significantly increased (P<0.05) the MCHC especially the whole ethanolic extract group. The results agreed to some extent with reports of other authors who have assessed these parameters in anaemic and normal rats. For instance the HCT% (PCV) values of between 38.6% and 45% for normal rats and values below 30% for anaemic rats have been reported (Ifere, 1986; Chen and Chiang, 1981).

Similarly blood haemoglobin concentration of 12.0g/100ml (130.0g/L) had been reported for normal rats by Underwood (1977). The increases in HCT% (PCV) of the extracts groups of rats compared with the control and the elevated values of HGB, coupled with the significant elevation in RBC count compared with their respective control in this study meant that the various extracts restored the blood parameters in the experimental rats to normal. The use of HCT% (PCV), HGB and RBC count data to arrive at this conclusion of the restoration of the normal blood indices is justified because anaemia which is defined as a state of lower than normal concentration of haemoglobin, percentage packed cell volume or the number of red cells per cubic millimeter was eliminated by the various extracts. Haematological alterations inducing anaemia according to Stockman (1981) could manifest as significant deficits in immune competence and other derangement of physiological indices.

The second finding in this study was that the increase in RBC and WBC counts especially the granulocytes supported the fact that extracts of *C. portoricensis* could stimulate erythropoesis and white cell production by a mechanism which is not yet known. This study showed that the extracts administration altered haematological indices by preventing anaemia which the viperian venom was expected to produce. It is presumed that the extracts made available methyl groups which could be transmethylated and metabolized into tetrahydrofolic acid. The provision of one carbon unit in form of terahydrofolate which is utilized during erythropoesis (Eteng, 1999) may be the point at which the extracts affect erythropoesis.

CONCLUSION

Exposure of wistar rats to viperian venom alters negatively their haematological parameters among other biochemical indices of toxicity. The venom is both cardiotoxic and haemotoxic and, may bring life to an acute fatality if not treated immediately. The alteration of haematological indices with significant elevation in RBC and WBC counts along with the increase in blood haemoglobin affirms that *C. portoricensis* extracts reversed the anaemia associated with the haemolysis and bleeding encountered in carpet viper strike. Although the mechanism by which *C. portoricensis* extracts stimulate erythropoesis is unknown, the extracts have been able to reverse the altered haemotological parameters to a very large extent. This study in rat model, if extrapolated to man and large animals, may serve useful purpose in the treatment of snakebites in Nigeria.

REFERENCES

AOAC. Official Method of Analysis of the Association of Analytical Chemists. AOAC. Washington D. C. (1990) 223 – 225.

Baker FJ and Silverton RE. Introduction to Medical Laboratory Technology, 6th Ed. Butterworths London (1985) 320 – 330.

Chen, L.T., Chiang, P. K. Intrasplenic P. A. In normal and induced anaemic rats Am. J. Haemotol 1981; 11: 403 – 407.

Eteng MU. Biochemical Aspects of Theobromine Toxicity in Rats. Unpublished PhD Thesis. University of Calabar, Calabar (1999).

Gutierrez JM. Clinical Toxicology of Snakebite in Central America. In: Meier. J. White (Eds). Handbook of clinical toxicology of animal venoms and poison. CRC Press, Florida (1995) 645 -665

Gutierrez J. M., Leon, G., Rojas, G., Lomonte, B., Rucavado, A., Chaves, T. Neutralization of local tissue damage induced by *Bothrops asper* (terciopelo) snake venom. Toxicon 1998; 36: 1529 -1538 (Pubmed).

Harborne JB. Phytochemical Methods. A guide to modern technique of plant analysis. Chapman and Hall, London (1973) 188 -190.

Ifere GO. Adenosine triphosphate activity (Total ATPase and Na^+-K^+) in red blood cells from iron deficient rats. MSc Thesis, University of Calabar, Calabar, Cross River State, Nigeria (1986).

Ownby CL. Pathology of rattle snake envenomation. In Tu AT (Ed). Rattlesnake venoms. Their action and treatment. Marcel Dekker, New York (1982) 163 – 209.

Sawaii Y., Homma, M. Snakebites in India. The snake. 1975; 7, 35 – 40.

Sofowora EA. Medicinal Plants and Traditional Medicine in Africa. John Wiley and Sons, New York (1982) 43 - 46.

Stockman J. A. Infections and Iron: too much of a good thing? Am. J. Dis. Child. 1981; 135: 18 – 20.

Swaroop S., Grab, B. Snakebite mortality in the World. Bull WHO. 1954; 61, 949 – 956.

Trease GE and Evans EC. Pharmacology (12th ed). Bailliere Tindall, London (1983)

Underwood EJ. Trace elements in human and animal nutrition, 4th ed. Academic Press, New York (1977) 31 – 32.

Wagstaff S. C., Laing G. D., Theakston D. G., Papaspyridis C., Harrison R. A. Bioinformatics and Multiepitope DNA Immunization to Design Rational Snake antivenom. Plos Medicine. 2006; 3 (6): e 184.