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Antioxidative and Haematopoietic Potential of *Pelusios sinuatus* (Turtle) Blood on Myelosuppressed Wistar Albino Rats

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NFN and UNO presented the concept. CIF, NFN and UNO wrote the article. NFN administered the project and provided funding and. UNO curated data. CIF validated the results. OEC managed software. EEC and OEC formated analysis and resources. OEC did statistical analysis.

Key words
Anaemia, Cyclophosphamide, Myelosuppression, Turtle blood, Ginovera

ABSTRACT

This study investigated the antioxidative and haematopoietic potency of the blood of *Pelusios sinuatus* (turtle) on cyclophosphamide induced myelosuppression in rats. Blood constituents were determined to identify the presence of vitamins and minerals. Thirty albino rats used were grouped into six comprising of five rats each and each group except group A was induced with cyclophosphamide. Group A received normal saline, group B remained untreated, group C, D, E and F were respectively treated with standard haematopoietic drug, Ginovera (5 mg/kg body weight), 50 and 100 mg/kg body weight of raw blood and 100 mg/kg body weight of cooked blood respectively. Determination of the constituents of turtle blood revealed the presence of vitamins such as Vitamin A, B_6, B_9 and B_{12} and minerals such as Selenium, Iron and Zinc. After the induction of myelosuppression, there was a significant ($p<0.05$) decrease in haematological parameters and a significant ($p<0.05$) increase in the concentrations and activities of oxidative stress biomarkers. However, treatment with the blood of *P. sinuatus* reversed the effect of cyclophosphamide on the haematological parameters and the oxidative stress biomarkers. These results suggest that the *P. sinuatus* blood exhibits both haematopoietic and antioxidative potential and could be a good source for the amelioration of leucopenia and anaemia.

INTRODUCTION

The use of plant and animal extracts, products, and even secretions for human health management is a worldwide phenomenon, dating far back in history. It co-evolved with human evolution, reached its peak in medieval medicine, and still exists in folklore medicine across the globe (Loko *et al*., 2019; Mtewa *et al*., 2019). Animals and their products have constituted an important portion of the Mediterranean pharmacopeia for millennia. Collectively recognized as zootherapeutic remedies today, many of these ancient therapies have persisted in current day traditional medicine and even become integrated into modern pharmaceuticals (Souto *et al*., 2018). The utilization of animal parts and its secretion in the preparation of several products employed in diverse ways for health care delivery via traditional medical practices enjoys very wide acceptance across Nigeria (Ajagun *et al*., 2017; Alade *et al*., 2018). In South-Western and Northern part of Nigeria, 55 and 22 animal species have been reported in the treatment of different diseases (Ajagun *et al*., 2017). Zootherapies have been studied in Latin America, Africa, Europe and East Asia. Skin, bones, blood, meat, feathers, faeces of both wild and domesticated animals and their products are used in treating different diseases (Jugli *et al*., 2020; Mtewa *et al*., 2019). Although much have been accomplished using plants and its derivatives in folklore medicine and authenticated scientifically (Kar *et al*., 2019; Luo *et al*., 2019; Zhang *et al*., 2020), however, the use of animal parts, products and secretions derived from different organs of their bodies for human health management is still underexploited in our current day medical practices and begs for scientific validations.

Raw turtle and tortoise blood, mixed with substances such as milk (which served as a vehicle) is used to treat anaemia in traditional medical practices in Africa, precisely in Nsukka in Enugu State of Nigeria, but this has not been proven scientifically.

Based on this conception, this study intends to ascertain the potency of both raw and cooked form of the turtle blood to treat myelosupression, with a view of scientifically authenticating its option as a promising candidate for drug formulation and disease management in modern medical practices. Oral administration of blood prevents agglutination which is one of the major challenges

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of blood transfusion. Oral administration circumvents the immune system because the given substance (blood) is exposed to digestive enzymes. In the GIT, the blood is digested upon contacts with salivary amylases and gastrointestinal fluid enzymes to amino acids and other blood components. These products of digestion are not foreign to the body and when absorbed, they exert haematopoetic effect. Moreover, due to the immunological function of the intestine, there is also the added advantage of eliminating any pathogen that may be present in the substance (blood) (Gaucher *et al*., 2010).

Hence, this study investigated the antioxidative and haematopoietic potency of both raw and cooked *Pelusios sinuatus* (turtle) blood on myelosuppressed wistar albino rats.

MATERIALS AND METHODS

Collection of turtles and their blood samples

Four turtles of the species *P. sinuatus* (females) and within the age of 3 to 5 years used in this study were purchased from Ibagwa-Aka Market in Nsukka L. G. A. in Enugu State of Nigeria. The turtles were identified and authenticated at the Zoology garden, of the Department of Zoology and Environmental Biotechnology, University of Nigeria, Nsukka by a zoologist. They were then, kept in a neat and conducive cage and observed for a period of two weeks to ensure they were of good health. They were fed with vegetables and fruits and inspected daily during this period.

The turtle was kept on a wooden stand with its bottom placed very close to a heat source (burning coal) and as a result the turtle sensing danger was forced to bring out its head and immediately its head was successfully grabbed using a clip-like tool. An ocular puncture was made at the appropriate anatomical site in the eyes, and using a non-heparinized microcapillary tube and the blood that gushes out was carefully collected into tubes. Half of the blood obtained was cooked in a water bath at 71.1°C for 3 min. The cooked blood was weighed and dissolved with tween 80. The raw turtle blood was dissolved in normal saline solution and tween 80 was used to make a stock concentration of the raw/cooked blood solution. The tween 80 solution used was to aid the dissolution of the blood in the normal saline while also acting as a vehicle for the blood.

Maintenance of rats and induction of myelosuppression

Thirty albino rats (*Rattus norvegicus*) of average body weight of 148 g used in this study were purchased from the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were housed in standard cages under clean environmental conditions (23±1°C, with 55±5 % humidity and a 12 h/12 h light/dark cycles), given standard feeds (vital) and allowed to acclamitise for 14 days. All animal experiments were in compliance with National Institute of Health Guide for the care and use of Laboratory animals.

Induction of myelosuppression

The rats were administered 75 mg/kg body weight of cyclophosphamide (CP) to induce myelosuppression via intraperitoneal (IP) injection and the method used was a slight modification of the method of Manjarekar *et al*. (2000). Blood samples of rats in each group were collected and haematological parameters listed below were determined. Then CP was administered to each of the rats in the groups. Three days later, blood samples of the rat were collected and haematological parameters determined. This was followed by oral administration of different concentrations of *P. s*. blood sample and Ginovera (5 mg/kg) multivitamin capsule as a standard for 7 days. On the thirteenth day (from the day of CP administration), the blood samples of the animals were collected and the haematological parameters determined.

Experimental design

In the CRD method of experimental design, thirty Wistar albino rats used in this study were grouped into six namely A, B, C, D, E and F comprising of five rats each. Cyclophosphamide (CP) a myelosuppressive drug (suppresses the bone marrow resulting in corresponding decrease in blood cells) was administered intraperitoneally to rats in all the groups except group A rats. Group A rats served as the normal control i.e., was treated with normal saline while group B rats served as the positive control for, they were not treated. Groups C, D, E and F were treated respectively with standard haematopoietic drug [Ginovera (5 mg/kg) multivitamin capsule stimulates the bone marrow to produce blood cells and is used to boost blood], 50 and 100 mg/kg of raw *P. s*. blood and 100 mg/kg of cooked P. s. blood.

Collection of rats blood samples

Blood collected from the rats by ocular puncture were put into EDTA anti-coagulated and plain non anti-coagulated tubes, respectively. Plasma and serum to be used in haematological and biochemical assays where obtained respectively, by allowing the blood samples on both test tube to centrifuge at 3,000 rpm for 10 min using a table centrifuge. In each case the clear supernatant were carefully aspirated using a syringe and needle, and were stored in clean bottles for subsequent uses.

Determination of mineral and vitamin constituent of turtle blood

Serum concentrations of inorganic ions and vitamin composition were determined according to the method described by AOAC (2005).

Determination of rat haematological parameters

In the haematological assay, the following parameters were determined, white blood cell (WBC) count, red blood cell (RBC) count, percentage packed cell volume (PCV), haemoglobin (Hb) concentration, lymphocyte (lymph) count, granulocyte (Gran) count, monocyte (Monoc) count and platelet count. The parameters were determined by automated haematology system analyzer (ADVIA 60 Open Tubes; Bayer Corporation, Tarrytown, Newyork, USA).

Assay of antioxidant and lipid peroxidation enzymes in rats

The superoxide dismutase (SOD) and catalase (CAT) activity, and malondialdehyde (MDA) level were determined respectively by the methods of Fridovish (1989), Sinha (1972) and Farombi *et al*. (2002).

Statistical analysis

The haematological and antioxidative data obtained were analyzed using computer software known as Statistical Product and Solution Service (SPSS) version 23. One-way analysis of variance (ANOVA) was used for comparison across the different groups and differences were considered significant at $p < 0.05$. Duncan multiple comparison Post Hoc tests was used to separate and compare the mean.

RESULTS

Vitamin and mineral constituent of turtle blood

Figure 1 shows vitamin and mineral composition of the *P. sinuatus* blood in which Vit. B_9 and Vit. A were found to be predominant while Vitamin B_2, B_6 and B_{12} where in minimal concentration. Similarly, selenium was predominant as compared to Fe and Zn.

Antioxidative characteristics of rat blood

The effect of *P. sinuatus* blood on the catalase (CAT) and superoxide dismutase (SOD) activities of Cyclophosphamide (CP) induced rats was observed and the result recorded in Table I. A significant ($p < 0.05$) reduction in CAT was observed in CP induced rats treated with 50 and 100 mg/kg RPsB as compared to the standard control. The SOD activity was significantly reduced in 100 mg/kg RPsB treatment while 50 mg/kg RPsB and 100 mg/kg CPsB treatments recorded similar SOD activity with the standard control. The serum malondialdehyde (MDA) level of CP induced rats treated with different concentrations of *P. sinuatus* blood was significantly lower as compared to the untreated group (positive control).

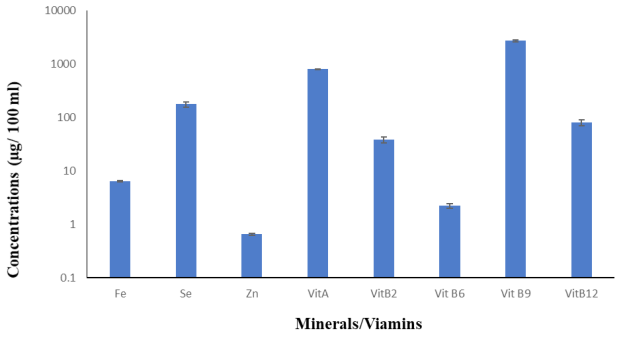

Fig. 1. Vitamin and mineral composition of the blood of *Pelusios sinuatus* (turtle).

Table I. Effect of raw and cooked blood of *P. sinuatus* on Catalase (CAT) and superoxide dismutase (SOD) activities and malondialdehyde (MDA) concentration of myelosuppressed Wistar albino rats.

Groups	CAT (IU/L)	MDA (µmol/l)	SOD (IU/L)
Normal control	1.46±0.26^{a}	10.99±0.27^{a}	1.46±0.32^{ab}
Positive control	3.71±0.54^{b}	11.43±0.01^{b}	2.25±0.62^{c}
Standard control	1.29±0.09^{a}	10.86±0.17^{a}	1.90±0.19^{bc}
50mg/kg RPsB	1.38±0.12^{a}	10.96±0.06^{a}	1.65±0.16^{ab}
100mg/kg RPsB	1.65±0.39^{a}	10.96±0.17^{a}	1.26±0.33^{a}
100mg/kg CPsB	1.35±0.08^{a}	11.02±0.21^{a}	1.90±0.20^{ab}

Values are mean±SD, (n=5). Values in the same column having different superscripts differ significantly ($p<0.05$). CAT, catalase; MDA, malondialdehyde; SOD, superoxide dismutase; RPsB, raw *Pelusios sinuatus* blood; CPsB, cooked *P. sinuatus* blood.

Haematological assays

The result of the haematological assays as presented on Table II shows a significant ($p<0.05$) decrease in concentration of the treatments as compared to the normal control after induction. However, treatment with Ginovera multivitamin drug (5 mg/kg body weight) and *P. sinuatus* blood was seen to have reversed the effect of cyclophosphamide on the haematological parameters. After treatment, there was a significant ($p<0.05$) increase in WBC and RBC across the treatment groups as compared to the positive control. The Hb concentration significantly ($P < 0.05$) increased with 100 mg/kg RPsB as compared to the normal, positive and standard controls (Table II). The significantly ($P < 0.05$) high leucocyte

differentials observed in the normal control group after induction indicated the effect of the induction of CP on the experimental rats. However, a significant effect of *P. sinuatus* blood was observed through the increased leucocyte differential levels following treatment with *P. sinuatus* blood as compared to the positive control (Table III). The results as presented in Table III shows the platelet count of CP induced rats treated with *P. sinuatus* blood. A significant reduction in the number of platelet as compared to the normal control after induction and a significant increase as compared to the positive control after treatment was observed (Table III).

DISCUSSION

The result of this study showed that the blood of *P. sinuatus* is rich in some essential vitamins such as vitamin A, B_2, B_6, B_9 and B_{12} and minerals such as selenium, iron and zinc. This corresponds with the findings of Tardy *et al.* (2020) who stated that minerals are inorganic substances present in body tissues and fluids and are necessary for the physicochemical processes essential to life. Minerals of animal origin could be said to be better than that from plant origin for such minerals cannot be chelated. The presence of zinc, iron and vitamin B_{12} is a clear indication that they interrelate in haemoglobin synthesis and red blood cell formation and this could be attributed to the observed increase in Hb and RBC. Ahmeda *et al.* (2020) reported the action of selenium in protecting the tissues against oxidative damage and this could be responsible for the decrease in oxidative parameters as depicted in the Table I.

From the result shown in Table II induction with cyclophosphamide showed a reduction in the white blood cells when compared to the normal and this could be as a result of immunosuppression caused by the cyclophosphamide (Yang *et al.*, 2018; Kar *et al.*, 2019) but on treatment with *P. sinuatus* blood in the treatment groups, a recovery in the white blood cell count was observed probably due to the presence of selenium in the blood being administered. The increase in white blood cell count of the treated group could also be as a result of the high quantity of vitamin A which is directly involved in the formation of bone marrow through the initiation of stem cells (blood cells) therefore increasing the white blood cell count (De Anglis *et al.*, 2018).

Table II. Effect of turtle blood on haematological parameters of myelosuppressed Wistar albino rats.

Groups	WBC (10^9)	RBC (10^3 cell/l)	PCV (%)	Hb (g/dl)
On induction				
Normal control	5.92±1.18^{a}	6.30±1.32^{a}	52.91±6.26^{a}	13.48±2.28^{a}
Positive control	4.64±1.20^{a}	6.30±0.53^{a}	52.01±2.90^{a}	12.33±0.81^{a}
Standard control	5.52±0.35^{a}	5.81±1.71^{a}	51.20±2.62^{a}	13.53±0.82^{a}
50 mg/kg RPsB	5.37±0.23^{a}	6.11±0.48^{a}	52.85±3.74^{a}	13.95±0.68^{a}
100 mg/kg RPsB	5.90±1.49^{a}	6.30±0.49^{a}	52.51±1.73^{a}	13.63±0.56^{a}
100 mg/kg CPsB	5.43±0.42^{a}	6.12±0.61^{a}	52.50±5.71^{a}	13.83±1.42^{a}
After induction				
Normal control	5.92±1.18^{b}	6.30±1.32^{b}	52.91±6.26^{b}	11.95±0.26^{b}
Positive control	1.64±0.20^{a}	3.30±0.53^{a}	23.01±2.90^{a}	5.83±1.61^{a}
Standard control	1.52±0.35^{a}	3.81±0.71^{a}	23.20±2.62^{a}	4.28±1.14^{a}
50 mg/kg RPsB	1.37±0.23^{a}	3.11±0.48^{a}	24.85±3.74^{a}	8.85±0.74^{a}
100 mg/kg RPsB	1.90±0.49^{a}	3.30±0.49^{a}	23.51±1.73^{a}	2.29±1.90^{a}
100 mg/kg CPsB	1.43±0.42^{a}	3.12±0.61^{a}	24.50±5.71^{a}	5.40±2.25^{a}
After treatment				
Normal control	5.82±1.40^{d}	8.30±0.58^{c}	51.01±6.02^{c}	13.48±2.28^{c,d}
Positive control	1.48±0.46^{a}	3.11±1.96^{a}	23.03±1.18^{a}	1.23±0.51^{a}
Standard control	3.50±0.67^{b}	4.43±0.06^{a,b}	46.01±0.40^{b}	11.53±0.82^{b}
50 mg/kg RPsB	3.17±0.03^{b}	4.46±0.07^{b}	47.06±2.60^{b,c}	12.95±0.68^{b,c}
100 mg/kg RPsB	3.22±0.08^{b}	4.83±1.07^{b}	48.10±1.73^{b,c}	15.63±0.56^{e}
100 mg/kg CPsB	4.01±0.05^{c}	4.64±0.02^{b}	47.82±1.74^{b,c}	14.83±1.42^{d,e}

Values are mean±SD, (n=5). Values in the same column having different superscripts differ significantly ($p<0.05$). WBC, white blood cell; RBC, red blood cell; PCV, packed cell volume; Hb, haemoglobin; RPsB, raw *Pelusios sinuatus* blood; CPsB, cooked *P. sinuatus* blood.

Table III. Effect of turtle blood on Leucocyte differentials and platelet count of myelosuppressed Wistar albino rats.

Groups	Lymph. (10^9/L)	Gran. (10^9/L)	Monoc. (10^9/L)	Plate. count (10^9L)
On induction				
Normal control	71.58±16.98^a	40.10±4.14^a	18.33±16.12^a	597.50±17.50^a
Positive control	69.83±18.90^a	38.98±8.63^a	13.20±4.00^a	601.25±14.08^a
Standard control	68.43±5.88^a	35.80±870^a	12.00±5.35^a	590.50±230.68^a
50 mg/kg RPsB	69.43±8.40^a	43.24±4.84^a	23.30±9.29^a	587.50±10.11^a
100 mg/kg RPsB	70.13±1.68^a	39.23±4.71^a	22.65±5.65^a	613.50±11.69^a
100 mg/kg CPsB	69.25±6.13^a	35.55±4.25^a	18.20±9.78^a	586.50±13.46^a
After induction				
Normal control	70.02±13.2^b	43.75±6.60^b	19.48±2.26^c	554.74±77.08^b
Positive control	42.75±1.26^a	9.70±4.06^a	9.88±0.70^a	74.24±34.12^a
Standard control	42.00±13.76^a	6.75±1.37^a	14.75±1.72^b	35.00±13.15^a
50 mg/kg RPsB	51.50±4.43^a	9.00±3.97^a	12.50±1.68^b	15.50±9.04^a
100 mg/kg RPsB	41.25±7.41^a	12.25±5.85^a	14.87±0.85^b	29.50±21.20^a
100 mg/kg CPsB	48.008.04^a	14.00±6.32^a	12.25±2.22^b	38.75±21.09^a
After treatment				
Normal control	71.58±16.98^b	40.10±4.14^b	18.33±1.12^b	597.50±17.50^b
Positive control	33.83±18.90^a	4.98±8.63^a	8.20±0.00^a	40.95±12.57^a
Standard control	68.43±5.88^b	35.80±870^b	12.00±2.35^a	590.50±98.68^b
50 mg/kg RPsB	69.43±8.40^b	43.24±4.84^b	23.30±1.29^c	587.50±10.11^b
100 mg/kg RPsB	70.13±1.68^b	39.23±4.71^b	22.65±1.65^c	613.50±11.69^b
100 mg/kg CPsB	69.25±6.13^b	35.55±4.25^b	18.20±0.78^b	586.50±13.46^b

Values are mean±SD, (n=5). Values in the same column having different superscripts differ significantly ($p<0.05$). Lymph, Lymphocyte; Monoc, Monocytes; Plate, Platelet; RPsB, raw *Pelusios sinuatus* blood; CPsB, cooked *P. sinuatus* blood.

As depicted in the table above, red blood cell count in group B showed a significant decrease when compared to the normal control after induction with cyclophosphamide but on treatment with *P. sinuatus* blood, a significant increase was observed. This increase could be attributed to the presence of Vit B_{12} in the blood of *P. sinuatus* and is involved in erythropoiesis (Waheed and Elzouki, 2018). The packed cell volume of the groups decreased after induction however treatment with *P. sinuatus* blood reversed the effect. There was a difference in the PCV count on administration of cooked and raw blood as the cooked blood gave higher PCV count than the raw blood; however, this difference was not significant ($p>0.05$).

Moreover, induction of myellosuppression with cyclophosphamide reduced the levels haemoglobin, lymphocyte, granulocyte, platelet, eosinophil and monocytes. This decrease confirms the capability of cyclophosphamide as an immunosuppressive agent as stated by Zhang *et al*. (2020). However, treatment with *P. sinuatus* blood reversed the effect. From this, it could be deduced that the administered blood has a haematopoietic property that was able to enhance the increase of these haematological parameters.

The result above showed a significant increase in the activities of SOD, CAT after induction in the positive control group however, treatment with *P. sinuatus* blood resulted in a significant increase in the treated groups. This increase could be as a result of the presence of selenium and zinc which are cofactors of anti-oxidant enzymes (Larvie *et al*., 2019).

CONCLUSION

Therefore it could be inferred that the blood of *Pelusios sinuatus* exhibits both haematopoietic and antioxidative potential and could be a good source and possibly substitute for the amelioration of leucopenia and anaemia, validating its use in folklore medicine.

Statement of conflict of interest

The authors have declared no conflict of interest.

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