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Edible Seafood –*Thais coronata* (Rock Snail) Extract Boosts RBC, PCV, Hb, Platelets, WBC and Lymphocytes Counts in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ANA and AEE designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors AAA, SUU and VOO managed the literature searches, experimental feeding, and the experimental process while author OEO analyzed the research data, read and edited the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

This study sought to elucidate the impact of consumption of crude extract of an edible seafood, *Thais coronata* (rock snail), on hematological parameters in male rats of albino

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Wistar strain. Crude extract of *T. coronata* was prepared from fresh edible samples, and its protein content was thereafter estimated following standard procedure. LD_{50} value of the extract was also determined using graded doses (2.82, 5.64, 11.28, 22.56, 45.12, 90.24 and 180.48mgProtein/Kg i.p.) of the extract in rats. Eighteen albino Wistar rats were divided into three groups of 6 rats each (n=6), the animals eat either normal rat chow, low (7.0mgProtein/ml), and/or high (52.0mgProtein/ml) doses of the extract for six weeks. Thereafter, blood samples were obtained from each rat via cardiac puncture to estimate the various blood parameters. The results revealed that the average protein content of the extract was 4.17±0.01mg Protein/ml, while the LD_{50} values was 88.98mgProtein/ml. RBC count, Hb, PCV, MCH, MCHC, platelet, total WBCs and lymphocyte counts increased significantly in rock snail fed groups ($p<0.01$); while mean platelet volume, platelet distribution width and platelet large cell ratio decreased significantly in rock snail fed groups ($p<0.001$). In conclusion, edible seafood–*Thais coronata* (rock snail) is very safe for consumption and contains vital nutrients that boosts production of blood cells, hence could serve as an essential food supplement.

Keywords: Thais coronata (rock snail); blood cells; edible seafood; rats.

1. INTRODUCTION

Seafood is the most useful form of aquatic creatures endowed to us by nature. They are important sources of edible protein and are found in different kind of waters. They are different types of seafood, some of which are fish, roe and shellfish [1]. Crustaceans, echinoderms and mollusk make up the shell fish. Rock snail belongs to the mollusk family. *Thais coronata* (rock snail) otherwise locally called *Nkonko* by the Efiks in Nigeria, are tropical fresh water snail from the family *Muricidae*. The world's largest fresh water snails are found in South America (Cuba, Brazil), Central America, USA (Califonia), Asia (Philippines, Hawai, Taiwan, Japan, Indonesia) and Africa (Nigeria), and occur mostly in tropical and subtropical localities. Rock snail contains important nutrients like iron, iodine, selenium, Vit. A, Vit. D, Vit. E, Vit. B12, Vit. B6, proteins and essential fatty acid. Edible molluscs are essential for human consumption and their shell is used in making jewelry [2,3,4,5].

Nutrition evaluation of edible molluscs in Nigeria indicates that mollusc has high protein content and elemental composition [6]. Moreover, it has been reported that edible molluscs could serve as rich sources of essential fatty acids like the omega- 3 fatty acid, which is useful in the management of deficiency in quality protein common among the developing countries. The omega-3 fatty acids are also involved in the prevention of cardiovascular diseases [7,8,9]. Hence, the national nutrition and health programme (PNNS) in France recommends consumption of these sea-foods twice a week especially for people who have heart attacks [9]. Fish nutrients has been reported to depress tumour necrosis factor-a in cultured human macrophages [10].

Other benefits of seafood are that they have antioxidant property, lowers the arterial blood pressure; they elevate HDL-c and lower LDL-c levels in the body and they also enhance tissue lipoprotein lipase activities. Seafood also provides negligible amounts of trans-fats, dietary fibres and sugars [11,12,13,14,15,16,17,18].

Most food consumed by the human body are digested and assimilated into the blood stream and these food may affect the blood cells in varying ways [19]. Blood is a tissue which

consists of fluid plasma in which are suspended a number of formed elements (erythrocyte, leucocyte and thrombocytyes). The blood cells exist at fairly constant levels, suggesting the existence of feedback regulatory mechanism for the [20]. The effect of rock snail on the homeostasis of the blood cells lack scientific evidence. This study is justifiable because it will add to scientific knowledge, the impact of consumption of rock snail on haematological parameters, most especially on the platelet indices like MPV, P-LCR, and PDW which are major determinants of cardiovascular heart diseases.

Therefore, the aim of this study is to provide a background information on the actual amount of protein present in *Thais coronata* (rock snail), and to elucidate the impact of consumption of this edible sea-food on haematological parameters in rats.

2. MATERIALS AND METHODS

2.1 Experimental Animals

The experimental animals used in this study were male rats of the albino Wistar strain (initially weighing between 180–240g), the animals were obtained from the Animal Sciences and Pharmacology Departments, University of Calabar, Nigeria. The animals were allowed one week to adapt to their new environment (i.e. the research laboratory of Physiology Department, University of Calabar, Nigeria). They were housed in wooden cages with adequate ventilation at room temperature, under the lighting condition of twelve (12) hours light and dark cycle. The animals were handled under a standard guideline for care and use of laboratory animals as promulgated by Canadian council of animal's care [21].

2.2 Preparation of the Aqueous Extract of Rock Snail

The preparation of rock snail extract was done standard procedure [22,23].

Fresh samples of edible seafood (rock snail) were obtained from a local market (called Watt Market) in Calabar, Nigeria, between the months of January to March. They were washed and rinsed in clean warm tap water to remove debris. The samples (100g) were pulverized and 100ml of normal saline (0.9% NaCl solution) was added to it, then centrifuge at 10,000 revolutions per minutes for 10 minutes. The supernatant was extracted using a 10mL syringe as the stock solution of (1g/mL).

2.3 Estimation of Protein Content of Rock Snail Extract

The protein content of the extract was estimated a standard method [24]. Serial dilutions (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0mg/mL) of egg albumin was prepared from a stock concentration of 0.001g/mL (i.e. 0.25g of egg albumin per 250ml of distilled water). Fresh reagents A, B, C and D were prepared. The compositions of the reagents were:

A- 2% Na_2CO_3 in 0.1M NaOH.
B- 0.5% $CuSO_4.5H_2O$ in 1% Na-K tartrate.
C- 50ml of reagent A + 1ml of reagent B and
D- Lipart Follins reagent in 2 parts of distilled water.

Egg albumin (1mL) was added into 10mL of reagent C, agitated. After 10 minutes. 1ml of reagent D was added and allowed to stay for distinct colour to appear in 10minutes. A

standard curve was plotted from the absorbance of 8 different trials at 750nm using a spectrophotometer. The optical density (OD) of 10 readings were also taken. A standard regression curve was plotted for the egg albumin concentration (OD against egg albumin concentration) and regression line fitted into the curve. The protein content of the extract was extrapolated from the standard curve.

2.3 Acute Toxicity Test to Determine LD_{50} Value of Rock Snail

Acute toxicity study was conducted on forty (40) albino Wistar rats (final body weights between 180–240g) randomly assigned into eight groups of 5 rats each. They were kept in the research laboratory of Physiology Department, University of Calabar-Nigeria for a week to enable them adapt to the new environment. Thereafter, each group received one of the following doses (0, 2.82, 5.64, 11.28, 22.56, 45.12, 90.24 and 180.48mgProtein/kg respectively) of extract i.p. The control group received equivalent volume of normal saline i.p. They were all returned to their home cages and allowed free access to food and drinking water. The mortality in each group was assessed 24 hours after administration of the extract. The percentage mortalities were converted to probits and plotted against the $\log_{10}$ of the dose of the extract [25].

2.4 The Sub-chronic Feeding Study

The sub-chronic feeding study was done using eighteen (18) albino Wistar rats, the final body weight of the animals ranged from 180–240g. The animals were assigned into 3 groups of 6 rats each. Group 1 (control) took normal rat chow (Pfizer grower feed) without rock snail extract. Group 2 received normal rat chow + low dose (7.0mgProtein/kg) of the rock snail. Group 3 received normal rat chow + high dose (52mgProtein/kg, i.p.) of rock snail. All the animals drank clean tap water *ad libitum*. The feeding regimens lasted for a period of six weeks.

2.5 Analysis of Haematological Parameters

Upon the expiration of the feeding regimens, the animals were made unconscious by chloroform (3.5% soaked in cotton wool) inhalation. Their thoracic cages were dissected and blood samples were obtained via cardiac puncture into EDTA capped bottles. Complete blood count was done on the blood samples using automated blood analyzer (Sysmex Model: kx-21N, Serial Number A6695).

The machine was pre-calibrated with a potential to record the different blood parameters.

2.6 Statistical Analysis

Data were presented as mean±SEM (standard error of mean). One way analysis of variance (ANOVA) was used to compared among the different groups. It was then followed by a post hoc test (least significant difference) to determine significant differences between two groups or variables. Microsoft Excel for Windows (2007) and statistical package for social sciences (version 17.0, Chicago, Il, USA) were used to run the statistical analysis. $p<0.05$ was accepted for significant.

3. RESULTS

3.1 Protein Concentration of Rock Snail

The amount of protein (mg/ml) in the rock snail extract was 4.17±0.01mgProtein/mL.

3.2 Acute Lethality Studies (LD_{50})

The LD_{50} value from extract of edible mollusk, rock snail, was 88.98mgProtein/ml, this is shown in Fig. 1.

3.3 Haematological Parameters

As shown in Table 1, the red blood cell count of the low dose (7.89±0.04 $x10^6$cell/µL) and high dose (8.03±0.10$x10^6$cell/µL) extract fed groups were significantly ($p<0.001$)higher compared with the control group which had a mean RBC count of 6.61±0.05$x10^6$cell/µL. The Hb concentration of the control group was 9.32±0.09g/dl, significantly ($p<0.001$) higher values were observed in the Hb concentrations of animals fed with low dose (11.76±0.20g/dl) and high dose (13.72±0.22g/dl) of the extracts. Also, PCV values in the low dose (43.58±2.56%) and the high dose (46.67±2.18%) extract fed groups were significantly ($p<0.001$) higher compared with control values (40.92±2.22%).

Also shown in Table 1 are results for absolute values of red blood cell. The changes observed in MCV following extract feeding were not statistically significant, but mean values of MCH and MCHC were significantly ($p<0.001$) elevated in the extract fed groups compared with controls.

The platelet count in the low dose (513.62±40.29$x10^3$cell/µL) and high dose (605.12±26.02$x10^3$cell/µL) extract fed groups were significantly ($p<0.001$) elevated compared with the control values of 350.00±81.07 $x10^3$cell/µL, see Table 2.

The change in RDW-SD following extract administration did not show any statistical differences, Table 2.

The platelet large cell ratio (P-LCR), platelet distribution width (PDW) and mean platelet volume (MPV) in the extract recipients were significantly ($p<0.05$) lower compared with controls, this result is presented in Table 2.

As shown in Table 3, the total WBC count of the control group was 8.64±0.42$x10^3$cell/µ, it was significantly higher in the low dose (10.07±0.42$x10^3$cell/µL; $p<0.05$) and high dose (10.92±0.40$x10^3$cell/µL; $p<0.01$) extract recipients compared with the control.

The results obtained for differential WBC count is also shown in Table 3. The neutrophil and eosinophil counts in the extract fed groups were significantly ($p<0.01$) lower compared with the control, but the lymphocyte were significantly ($p<0.001$) higher in extract fed groups compared with the control group. No significant statistical changes were observed in mean values of basophils and monocytes counts following extract administration.

Table 1. Comparison of RBC count, Hb, PCV and their absolute values in control and rock snail extract fed groups

Variable	RBC count (x10^6 cell/µL)	Hb (g/dL)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
Control	6.61±0.05	9.32±0.09	40.92±2.22	62.04±0.18	18.46±0.18	29.82±0.20
Low dose	7.89±0.04***	11.76±0.20***	43.58±2.56***	62.80±0.33	20.23±0.18***	32.16±0.14***
High dose	8.03±0.10***	13.72±0.22***	46.67±2.18***	63.45±0.49	24.56±0.17***	31.12±0.28***

*Values are expressed as mean±SEM, n=6; *p<0.05, **p<0.01, ***p<0.001 vs control*

Table 2. Comparison of platelet count and platelet indices in control and rock snail extract fed groups

Variable	Platelet count (x10^3 cell/µL)	RDW-SD (fL)	PDW (fL)	MPV (fL)	P-LCR (%)
Control	350.00±81.07	35.58±0.60	8.54±0.38	6.74±0.18	6.38±0.76
Low dose	513.62±40.29***	34.54±0.79	7.98±0.07	6.23±0.05*	5.12±0.18**
High dose	605.12±26.02***	32.25±0.11	6.24±0.25***	5.12±0.12***	3.89±0.45***

*Values are expressed as mean±SEM, n=6; *p<0.05, **p<0.01, ***p<0.001 vs control*

Table 3. Comparison of total and differential WBC counts in control and rock snail extract fed groups

Variable	Total WBC (x10^3 cell/µL)	Lymphocytes (%)	Neutrophils (%)	Eosinophils (%)	Basophils (%)	Monocytes (%)
Control	8.64±0.42	70.0±0.7	26.0±0.5	3.8±0.2	0.0±0.0	0.2±0.2
Low dose	10.07±0.42*	75.0± 0.8***	22.6±0.9	2.4±0.2*	0.0±0.0	0.0±0.0
High dose	10.92±0.40**	75.6±0.6***	22.4±0.5**	2.0±0.2***	0.0±0.0	0.0±0.0

*Values are expressed as mean±SEM, n=6; *p<0.05, **p<0.01, ***p<0.001 vs control*

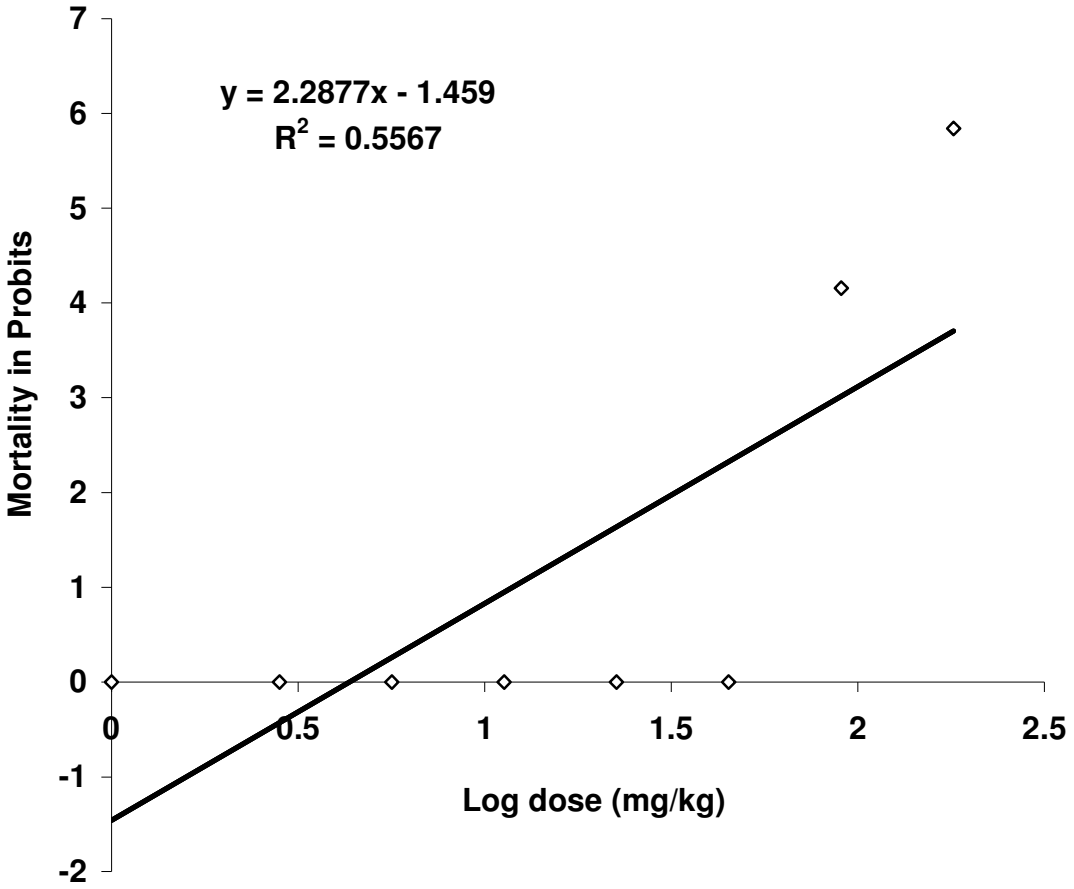

Fig. 1. Lethality study for determination of LD50 for rock snail in rat (LD50=88.98mgProtein/Ml)

4. DISCUSSION

Rock snail is staple food consumed by most people near the riverine areas as a cheap and ready source of protein [26]. Protein is known to be very useful ingredient for repairs and renewal of cells of the body [27]. But due to paucity in scientific literature on the impact of this edible sea food on blood parameters, it was the aim of the research work to investigate the hematological changes that could occur following chronic administration of rock snail extract in rats. Results obtained from this study revealed some interesting findings. Results of lethality studies conducted on the rock snail showed the extract had a high LD_{50} value, an indication that rock snail extract could be relatively non-toxic to the body when consumed because of the wide safety margin. The protein content of the extract was also high from our estimation.

From our study, there were significant increases in the RBC, Hb, PCV, platelets, total WBCs, lymphocyte counts, MCH, and MCHC of extract-treated animals relative to the control group.

These increases in platelets, lymphocyte, total WBC, RBC count alongside PCV and Hb, could probably be due to the rich nutritional values of the extract. Previous research indicate

the sea food have ample nutrients like Fe, Vit A, Vit B_{12}, Vit B_6, thiamine and protein [28]. These food substances are basic requirements for the production of RBC by enhancing erythropoiesis and stimulating the maturation of the erythrocytes [29,20].

The high levels of platelets induced by the extract might not predispose to intravascular clotting because of the abundance of omega-3 fatty acid in this seafood. Omega-3 fatty acids is a precursor for certain prostaglandins that diminish intravascular clotting and reduce the aggregation or clumping of blood cells, thereby making them more flexible so that they flow more smoothly [27,30].

Results of this study also revealed significant reductions in platelet large cell ratio (P-LCR), mean platelet volume (MPV), and platelet Distribution Width (PDW) in the extract fed rats. MPV and PDW determinants platelet function and is found to vary inversely with the platelet. Count in normal subjects [31-33] and also a useful index for chronic vascular disease [34].

RDW is a numerical measure of the variability in size (anisocytosis) of circulating erythrocytes [35]. This parameter is used in narrowing the differential diagnosis of anemia [36]. Our results revealed significant reductions in red-cell distribution width (RDW) in the extract fed rats. That the extract decreased the RDW value therefore suggests that the extract caused production of less variable RBC.

The mean corpuscular volume (MCV) which is the average volume of a single RBC size showed no differences between treated and untreated animal preparation, indicating that the extract lead to production of normocytic cells. Low MCHC indicates hypochromic anaemia in early iron deficiency [37]. Both parameters (MCH and MCHC) showed significant increases in the extracts-treated groups.

5. CONCLUSION

From our results, we conclude that rock snail has high protein content and LD_{50} value making it safe for consumption. The extract is capable of boosting Hb, RBC, lymphocytes, total WBC and platelet counts production, rock snail extract also decreases P-LCR, MPV and PDW values in rats. Hence, consumption of rock snail could be beneficial as a blood booster. However, further study is recommended to ascertain the clinical implication of this result.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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