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Extract of Edible Seafood - Egeria Radiata (Clam) Boosts Blood Parameters in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author ANA wrote the first draft of the manuscript, managed the literature search author OEO performed the statistical analysis and manuscript editing authors AAA, SUU and AEE contributed in carrying out the feeding regimens and analysis of blood samples author FNB designed the study and wrote the protocol. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: This study investigates the effect of consumption of an edible seafood - Egeria Radiata (Clam) on hematological parameters in rats.
Study Design: Albino Wistar rats were assigned to 3 groups of 5 rats each.
Place and Duration of Study: Department of Physiology, University of Calabar, Nigeria.
Methodology: The protein content of the extract was estimated as 24.60±.1mg protein / mL. Graded doses of the extract (1.64 – 104.96mg protein / kg) were administered (i.p) in rats to determine the LD_{50} value of the extract. Based on the LD_{50} value (56.36mg/kg), two test doses (low dose - 7.0 mg protein / mL and high dose - 52mg protein / mL) were selected and administered to two groups of rats orally and daily for six weeks, while a third group of rats served as the control, n = 5. Blood samples were obtained from all the rats via cardiac puncture for the analysis of the various hematological indices.
Results: Both the low and high doses of the extract produced significant increases in RBC count (P<.001), Hb (P<.001) and PCV (P<.001) compared with control. MCH (P<.001), MCHC (P<.001), total WBC count (P<.001) and platelet count (P<.001) were also increased in the extract groups. The extract groups had significant reductions in mean platelet volume (P<.001), platelet distribution width (P<.001) and platelet large cell ratio (P<.001) compared with control.

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Conclusion: In conclusion, an edible molluscan sea animal (Clam) probably contains food substances that enhance erythropoiesis, leukocytosis and thrombopoiesis. Clam could therefore serve as preferred and stable supplementary diets.

Keywords: Egeria radiata (clam); blood parameters; full blood count; rat.

1. INTRODUCTION

Seafood has been a major source of nutrient from time immemorial. Sea foods are important sources of protein and are dispersed throughout the world in different kind of waters and have been divided into three types, which include fish, Roe and Shellfish [1]. The shellfish is made up of crustaceans, echinoderms and mollusk. Clam belongs to the mollusk family and it is a potentially important source of nutrients which include iron, iodine, selenium, vit A, vit D, vit E, vit B12, vit B6, proteins and essential fatty acid. Edible mollusk is used for human consumption and their shell is used in making jewelry they also constitute the main source of edible protein [2].

Nutritional evaluation of edible mollusc in Nigeria indicates that its protein content and elementary composition are quite high and are comparable with whole hen's egg [3]. From the foregoing is evident that edible mollusc is a rich source of protein, vitamins and essential fatty acids particularly the omega-3 fatty acid, which are useful in the prevention of cardiovascular disease [3], as well as managing protein energy malnutrition, that is, a deficiency in quality protein common among the developing countries. There is a paucity of scientific literature on the effect of consumption of this edible mollusc on hematological parameters. This study therefore provides background information on the actual amount of protein present in Egeria radiata, and reports upon its impact on hematological parameters. The findings should contribute to addressing the issue of Protein Energy Malnutrition and may inform relevant communities regarding this cheap source of edible protein.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Albino Wistar rats (initially weighing between 180 – 240g) were utilized for this study. They were purchased from the Animal Science of Pharmacology Departments, University of Calabar, Nigeria. The animals were fed with normal rat chow (manufactured by Pfizer feeds, Aba – Nigeria) and allowed drinking water ad libitum. The average weight of chow consumed by each group was 18.32±0.38 g per day. They were kept under standard environmental conditions of 12 hours dark and 12 hours light cycles.

2.2 Preparation of the Aqueous Extract

The preparation of extract was done according to the method describe by Walker [4] and modified by Aldeen et al. [5].

100 grams of the clean, fresh samples of clam was weighed and homogenized for 5 minutes using tissue blender. The homogenate was then dissolved in 100ml of saline (0.9% NaCl). Thereafter, the solution was centrifuge for 10 minutes at 10,000 revolutions per minutes.

The supernatant was then poured into a clean container via filter paper and funnel, and this formed the stock solution of 1g/ml.

2.2.1 Estimation of protein content of the crude extract

Estimation of the protein content of the crude extract (clam) was done according to the method of Lowry et al. [6]

Egg albumin (0.25 grams) was weighed and dissolved in 250ml of distilled water (1mg/ml). From this concentration (1mg/ml) a wide range (0.1 - 1.0mg/ml) of concentrations of the egg albumin was prepared by serial dilutions. Ten different concentrations were prepared each in a test tube. Four chemical reagents marked A, B, C and D were also prepared fresh. Reagent A contained 2% Na_2CO_3 in 0.1M NaOH. B, 0.5% $CuSO_4.5H_2O$ in 1% Na-K tartrate. C, a mixture of 50ml of reagent A with 1ml of B and D Lipart Follins reagent in 2 parts of distilled water. One millilitres of each sample of egg albumin was drawn using a pipette and added to 10ml of reagent C, mixed and allowed to stand at room temperature for 10min. After 10min, 1ml of reagent D was quickly added and mixed immediately. The mixture was allowed to stand for another 10min for colour to develop. Eight of such experiments were carried out and the absorbance in each tube was read at 750nm in a spectrophotometer in order to prepare standard curve. The optical density (OD) of the extract was determined. This procedure was repeated for the ten different preparations of the extract (same stock concentration). The mean OD of the diluted extract preparations was then calculated. A standard curve was constructed for the egg albumin (OD against egg albumin concentration). Regression line (for x as the dependent variable) was fitted into the curve. From this standard curve, the protein content of the extract was extrapolated.

2.3 Acute Toxicity Test

Thirty albino Wistar rats weighing between 180 – 240g were used for the study. They were randomly selected and assigned to six groups, each group containing five animals. They were allowed a week for adaptation. Thereafter, each group received one of the following doses (0, 1.64, 3.28, 6.56, 13.12, 26.24, 52.48 and 104.96mg protein/kg respectively) of extract intraperitoneally. The control group had an equivalent volume of normal saline also intraperitoneally. 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 log10 of the dose of the extract [7].

2.4 The Sub-chronic Study

Fifteen male albino Wistar rats weighing between 180 – 240g were used for the study. They were randomly selected and assigned to three groups of 5 rats each. They were allowed a week for adaptation. Group A (control) received normal rat chow and drinking water, group B (low dose) were given a daily dose of 7.0mg protein/kg, i.p of the extract, while group C (high dose) received a daily dose of 52 mg protein/kg, I. p. All animals received food and drinking water ad libitum. The feeding regimens lasted for six weeks.

2.5 Collection of Blood Samples and Analysis of Hematological Parameters

Blood samples were collected via cardiac puncture into EDTA capped bottles and the full blood analysis was done using automated hematology analyzer Sysmex model: kx-21N, Serial Number: A6695.

The machine has a standard calibration according to the manufacturer's instruction (Coulter Electronic, 1979) using normal human blood and with complete profile for red blood cell (RBC) count, total white blood cell (WBC) count, differential WBC count, haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red blood cell distribution width (RDW), mean platelet volume (MPV), platelet distribution width (PDW) and platelet large cell ratio (P-LCR).

2.6 Statistical Analysis of Data

Data are presented as mean ± SEM. Data were analyzed using one-way analysis of variance (ANOVA) and then followed by post hoc test (least square deviation). Data analysis was done with the help of computer software (Excel and SPSS version 17.0 for windows). P-values of less than 0.05 was considered as significant.

3. RESULTS AND DISCUSSION

3.1 Results

As shown in Table 1, the total white blood cell count of the low dose (10.14 ±0.42cell/mm) and high dose (11.02±1.58) extracts treated groups were significantly ($P<0.05$) higher compared with the control group.

The red blood cell count of the low dose (7.32±0.37) and high dose (8.05±0.33) extract groups were significantly ($P<0.001$) higher than that of the control (6.61±0.05) group. The Hemoglobin concentrations in the low dose (10.02±0.48) and high dose (13.72±0.27) extract groups were also significantly ($P<0.001$) compared with control (7.87±0.09). The packed cell volume in the control, low and high dose groups were (40.92±0.22), (45.69±1.83) and (52.22±1.16) respectively. PCV was significantly ($p<0.01$) higher in the extract groups compared with control group.

No significant differences were observed in the mean corpuscular among the different groups. The MCH and MCHC in the low dose and high dose extract groups were significantly ($P<0.001$) higher compared with the control group.

The platelet count in the control, low and high dose groups were 350.00±81.07, 431.9±67.17 and high dose 551.17±50.94 $x10^3/\mu L$ respectively. It was significantly ($p<0.01$) higher in the extract groups compared with control.

The change in red cell distribution width (RDW) was not statistically different among groups, but the platelet distribution width (PDW), mean platelet volume (MPV) and a platelet large cell ratio (P-LCR) were significantly lower in the extract groups compared with control.

Table 1. Red blood cell indices of the different experimental groups

	RBC ($x10^6$/µL)	Hb (g/dL)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RDW-SD (fL)
Control	6.61±0.05	10.87±0.22	40.92±0.22	62.04±0.18	18.46±0.18	29.82±0 .20	35.58±0.60
Low dose	7.32±0.37	12.02±0.48	45.69±1.83	63.54±2.31	20.12±0.67	30.10±0 .53	35.12±1.15
High dose	8.05±0.33	13.72±0.27	52.22±1.16	63.90±1.62	26.87±0.59	30.50±0 .18	33.50±2.60

Values are expressed as mean ±SEM, n = 5

Table 2. Total and differential white blood cell count of the different experimental group

	Total WBC ($x10^3$/µL)	Neutrophils (%)	Eosinophils (%)	Lymphocytes (%)	Monocytes (%)
Control	8.64±0.42	26.20±0.36	2.50±0.20	71.20±0.95	0.20±0.00
Low dose	10.14±1.57	21.50±0.36	1.50±0.20	77.00±0.66	0.00±0.00
High dose	11.02±1.58	19.20±0.20	0.80±0.80	80.00±0.9 6	0.20±0.00

Values are expressed as mean ±SEM, n = 5

Table 3. Platelet and platelet indices of the different experimental groups

	Platelet count ($x10^3$/µL)	PDW (fL)	MPV (%)	P-LCR (%)
Control	350.00±81.07	8.54±0.36	6.74±0.18	6.38±0.95
Low dose	431.90±76.17	7.87±0.62	5.34±0.31	5.23±0.23
High dose	551.17±50.94	6.11±0.27	4.34±0.19	3.45±0.3 3

Values are expressed as mean ±SEM, n = 5

3.2 Discussion

In this study, the impact of chronic administration of clam extract in hematological parameters was investigated. The lethality studies showed high values of LD50 indicating that clam extracts have very wide safety margins and hence could be relatively non- toxic. This is consistent with the observation that these sea creatures are widely used as a source of protein in foods consumed by humans [8].

The protein content as estimated by the method of Lowry et al. [6] showed that clam extract has high protein content and protein are important for sound nutrtiion [9]. The extract-treated animals exhibited an increase in the WBC count, attributed to an increase in the absolute lymphocyte count, as revealed in the differential leukocyte count studies. In the rats, the lymphocytes are the predominant white cells in the peripheral circulation [7]. Therefore an increase in lymphocyte count is expected to increase the total WBC. These extracts may boost immune processes, although this was not specifically tested in these experiments. One could also speculate that consumption of clams could also alleviate severe leucopenia. These findings are consistent with earlier reports that consumption of edible mollusk does have health benefits [10].

The erythrocyte counts of the rats treated with clam extract was significantly increased as compared to control animals and the increase was confirmed by an increase in PCV and Hb. The increase in Hb concentration and the RBC count is not surprising, since Hb is a component of major protein of the RBC. It has been reported that sea foods are excellent sources of Fe, Vit A, Vit B12, Vit B6 and thiamine [3]. These substances are known to be

among the basic requirements necessary for the production of normal RBC [11]. Hence, the increase in erythrocyte count observed in these experiments could be the result of the presence of these specific substances in the clam extracts, although the specific content inducing the increased hematologic parameters observed were not characterized in these experiments. Some vitamins are capable of enhancing erythropoiesis and stimulating the growth of the erythrocytes [12,11]. Likewise, protein in the extract may help facilitate the process of erythropoiesis by enhancing Hb production [13,14,15,16]. That the extract may also contain erythropoietin-like agents cannot be ruled out.

The Red Cell Distribution Width (RDW) was decreased in extract treated rats. The RDW is a numerical measure of the variability in size (degree of anisocytosis) of circulating erythrocytes [17]. This parameter is used clinically in narrowing the differential diagnosis of anemia [18]. That the extract decreased the RDW value therefore suggests that the extract could cause the production of RBCs that are less variable in size.

The mean corpuscular volume (MCV) which is the average volume of a single RBC size showed no difference between treated and untreated animal preparation. In patients with anemia, it is the MCV measurement that allows classification as microcytic (MCV below normal range), normocytic (MCV within normal range) or macrocytic (MCV above normal range) [19]. The extract did not have any significant effect on the size of the RBCs. This is in complete agreement with the findings in the RDW studies. Another index for diagnosing anemia is the mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC). Both parameters (MCH and MCHC) showed significant increases in the extracts-treated groups. Low MCHC is an indicator of hypochromia in early iron deficiency and also MCH falls as the hypochromia develops [20]. Therefore, this extract may contain agents capable of enhancing Hb production which is responsible for the increased MCHC in treated animals. Also, the possibility of hypochromia occurring in the extract treated animals is very unlikely.

Results obtained from platelet count showed that there were thrombocytosis in the group that received extracts of the edible mollusc (clam). It is very likely that this extract contains thrombopoietin-like agent(s) or compound capable of causing the release of thrombopoietin [21], in the same way as the kidney is stimulated to release erythropoietin. Despite the high levels of platelets, intravascular clotting did not seem to occur because seafood contains essential fat called omega 3 fatty acids. This omega 3 fatty acids form a different pattern of prostaglandin that diminishes intravascular clotting, reducing the number of stickiness of blood cells, thereby making them more flexible so that they flow more smoothly [9,22]. In this study, the mean platelet volume (MPV) was reduced following administration of clam extract. The MPV is the determinant of platelet function and is found to vary inversely with the platelet count in normal subjects [23,24,25] and in chronic vascular disease [26], hence the reduction in the MPV as in the case in this study, also indicate an increase in platelet counts. This finding that the MPV was decreased strongly supports the increase in platelet count also observed in this study.

The result obtained for the platelet Distribution Width (PDW) showed a decrease in the extract treated rats. This decrease in PDW is in perfect agreement with the decrease in the MPV and an increase in platelet count as would be expected. PDW has been found to be of some use in distinguishing essential thrombocythaemia (PDW increase) from reactive thrombocytosis (PDW normal) [27]. In this study, the P-LCR value was decreased. This result further supports the PDW and platelet count results, suggesting that the extract causes the production of normal a viable blood platelet [27].

4. CONCLUSION

In conclusion, clam extract has a high protein content and LD50 value, both the low and high doses of the extracts produced significant increases in RBC count, WBC, platelet, Hb, PCV, MCH and MCHC. Edible mollusk (clam) could therefore be very safe for consumption, it also contains substances that can help as a blood tonic and to boost immunity, thus it could serve as preferred diet.

CONSENT

Not applicable.

ETHICAL APPROVAL

For manuscripts involving animal experiments, authors may use the following wordings for this section “all authors hereby declare that "principles of laboratory animal care" (nih publication no. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee”

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COMPETING INTERESTS

Authors declare that there are no competing interests exist.

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