Changes in Haematological Parameters Following the Administration of Crude Extract from Tympanotonus fuscatus (Periwinkle) in Rats

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ABSTRACT

Background: The consumption of Periwinkle (Tympanotonus fuscatus) in various meals in Nigeria is on the increase. It is important to ascertain the effects of the various components of our everyday meal on the body functions. Objective: This study was embarked upon to investigate the effect of crude extract from Periwinkle (Tympanotonus fuscatus) on haematological parameters. Results: The graded concentrations of the extract (2.82 - 180.48mgProtein/kg) were administered i.p. to the rats to determine the LD50 value of the extract as preliminary studies. Eighteen albino wistar rats weighing 180 - 240 g were randomly assigned into 3 groups (n = 6), thus; control, low (7mgProtein/ml) and high (52mgProtein/ml) doses extract treated groups. All animals had free access to drinking water and normal rat feed for 6 weeks. The result showed that the extract had a protein content of 10.12 ± 0.00mgProtein/ml and LD50 values of 61.48mgProtein/ml. Both the low and high doses produced significant increase in RBC count (p<0.001) compared with control. The extract treated groups also had significant increase in Hb (p<0.001), PCV (P<0.001), MCH (p<0.001), MCHC (p<0.001) and WBC counts (p<0.001). The results also showed that the extract treated groups had increased platelet count (p<0.001), but decreased mean platelet volume (p<0.001), platelet distribution width (p<0.001) and platelet large cell ratio (p<0.001). Conclusion: The edible molluscan sea food (Periwinkle) probably contains accessory nutrients that enhance blood cells production and reduction in platelet indices in rats. They could therefore serve as alternative source of nutrient.

INTRODUCTION

Seafood, as the name implies are the most useful form of aquatic creatures endowed to us by nature. Seafoods are important sources of protein and are found in different kind of waters. They are of different forms which include fish, roe and shellfish. (Narain and Nunes, 2009). The shellfish is made up of crustaceans, echinoderms and mollusk. Periwinkle belongs to the mollusk family and it forms an important source of nutrient. Edible mollusc are essential for human consumption and their shell is used in making jewelry they also form the primary source of edible protein (Chudler, 2009; Rice, 2004). T. fuscatus contains important nutritive substance such as vitamins, minerals and significant amount of fat called omega-3 fatty acid known to lower cholesterol level in blood, which is important in reducing the incidence of coronary heart disease. It also contains cholesterol, iron, copper and zinc, (Wardlaw and Smith, 2009). It has also been reported that seafoods are excellent sources of selenium, Vit A, Vit D, Vit E, Vit B12, Vit B6, thiamine, proteins and essential fatty acid (Schrimshaw and Young, 1992).

Periwinkles are covered with sea shell, which has a thick wall with up to at least 12 whole separated by sutures. Periwinkle is a marine mollusk dominantly found and thrives better in brackish water that is reach in organic matters and minerals in the riverine areas of Nigeria. They are highly prolific and this has made them a cheap source of protein in many homes when compared to other protein source (Bassey and Ayuk, 2007). It is locally called “Mfi” by the Efiks in Cross River State; “Ihemu” by Rivers/Bayelsa tribes. They are transported to many non-riverine towns and cities where they are use to prepare various palatable dishes in hotels and restaurants across the country Nigeria. The best method to process periwinkle before consumption differs amongst the populace. Most people believe that periwinkle should be thoroughly washed, its pointed end cut off...
and then cooked with its shell because of its perceived medical and nutritive value while others remove the shell.

In the face of paucity in scientific literature on the impact of periwinkle on hematological parameters, considering its vast nutritional composition, it becomes pertinent that this study investigates the effect of chronic ingestion of crude extract of periwinkle on hematological indices using albino Wistar rats.

MATERIALS AND METHODS

Experimental Animals:
Albino Wistar rats (initially weighing between 180 - 240g) were employed for this study. They were purchased from the Animal House of Pharmacology Department, University of Calabar, Nigeria. The animals were treated with normal rat chow and allowed drinking water ad libitum.

Preparation of Aqueous Extract:
The preparation of extract was done according to the method describe by Walker (1977) and Aldeen et al. (1981).

Estimation of Protein Content of the Crude Extract:
Estimation of the protein content of the crude extract (Periwinkle) was done according to the method of Lowry et al. (1951).

Acute Toxicity Test:
Thirty (30) albino Wistar rats weighing 180 – 240g were used for the study. They were randomly assigned one of six groups, (n = 5). They were allowed a week for adaptation. Thereafter, graded doses (0.00, 1.64, 3.27, 6.56, 13.12, 26.24mgProtein/kg, i.p.) were administered to the rats. 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 (Eno et al., 2001).

The Sub-chronic Study:
Eighteen (18) albino Wistar rats weighing between 180 – 240g were used for the study. They were randomly selected and assigned into three groups (A, B and C) of 6 rats per group. They were allowed a week to adapt to the environment. Group A (control) received normal rat chow and drinking water, group B was given a daily dose of 7.0mgProtein/kg (low dose) of the extract, while group C received a daily dose of 52mgProtein/kg (high dose) of the extract i.p. All animals received normal rat feed and drinking water ad libitum. The feeding regimens lasted for six weeks.

Analysis of Haematological Parameters:
Blood samples were collected via cardiac puncture into EDTA capped sample bottles and the full blood analysis was done using automated haematology analyzer SYSMEX model: KX-21N, Serial Number: A6695.

The parameter estimated were: Total and differential white blood cell counts (WBC), red blood cell (RBC) count, hemoglobin concentration (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), red blood cell distribution width standard deviation (RDW-SD), platelet distribution width coefficient of variability (PDW), mean platelet volume (MPV) and platelet large cell ratio (PLCR).

Statistical Analysis:
Data were presented as mean ± SEM. One way analysis of variance (ANOVA) was used to determine significance among variables. It was then followed by a post hoc test (least square deviation, LSD). P < 0.05 was considered significant. Statistical analyses were done with the help of computer software package (Excel and SPSS version) for windows; Microsoft Corporation, USA.

Results:
As shown in table 1, the white blood cell (WBC) count in the low dose (8.04 ± 1.17 x 10^3 cell/µL) and high dose (9.26 ± 1.2 x 10^3 cell/µL) groups were significantly (p<0.05 and p<0.01, respectively) higher compared with the control group (6.98 ± 0.61 x 10^3 cell/µL).

RBC count, Hb concentration, PCV, and platelet counts of the extract treated groups were significantly higher (p<0.01) compared with the control. Values in the control were: RBC, 6.37 ± 0.32 x 10^6 cell/µL; Hb concentration, 10.02 ± 0.62g/dL; PCV, 39.69 ± 0.22%; and platelet count, 362.9 ± 75.32 x 10^3 cell/µL.
In table 2, the changes in mean corpuscular volume (MCV) was of no statistical significance among the different groups, but the mean corpuscular haemoglobin (MCH) in the low dose (21.34 ± 0.45pg) and high dose (23.43 ± 0.50pg) groups were significantly (p<0.001) higher compared with the control (8.40 ± 0.45pg) group. The mean corpuscular hemoglobin concentrations (MCHC) in the low dose (30.26 ± 0.52%) and high dose (30.06 ± 0.65%) groups were significantly (p<0.001) higher compared to the control (29.60 ± 0.24%) group.

The change observed in red cell distribution width (RDW) was not significantly different among the different groups, but the platelet distribution width in the low dose (7.58 ± 0.10) and high dose (6.23 ± 0.24) groups were significantly (p<0.001) lower compared to the control (9.12 ± 0.50) group. Also, the mean platelet volume (MPV) and platelet large cell ratio (PLCR) in low dose and high dose groups were significantly (p<0.01, p<0.001 respectively) lower compared to the control. Table 3

As shown in table 4 for the differential WBC count, Neutrophil count in the low dose (17.2 ± 0.7%) and high dose (14.5 ± 0.3%) groups were significantly (p<0.01) lower compared to the control (31.2 ± 0.6%) group. Also, the eosinophil count in the low dose (3.8 ± 0.4%) and high dose (2.2 ± 0.3%) extract groups were significantly (p<0.05, and p<0.001 respectively) lower compared to the control (4.2 ± 0.4%) group.

On the other hand, lymphocyte count in the low dose (77.0 ± 0.4%) and high dose (82.0 ± 0.6%) extract groups were significantly (p<0.001) higher compared to the control (66.0 ± 0.7%) group.

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<thead>
<tr>
<th>Table 1: Comparison of RBC, Hb, PCV, WBC and platelet count in the control and extract treated groups.</th>
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<tr>
<td><strong>RBC</strong> (x10^6 cell/µL)</td>
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***p<0.001 vs Control, n = 6.

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<th>Table 2: Comparison of red blood cell absolute values (MCV, MCH, MCHC) and indices (RDW) in the control and extract treated groups.</th>
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<td><strong>MCV</strong> (fL)</td>
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***p<0.001 vs Control, n = 6.

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<th>Table 3: Comparison of platelet indices (PDW, MPV and P-LCR) in the control and extract treated groups.</th>
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<td><strong>PDW</strong> (fL)</td>
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***p<0.001; **P<0.01; p<0.05 vs Control, n = 6.

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<th>Table 4: Comparison of differential white blood cell counts in the control and extract treated groups.</th>
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<td><strong>Neutrophils(%)</strong></td>
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***p<0.001; **P<0.01; p<0.05 vs Control, n = 6.

**Discussion:**

This research was aimed at unraveling hematological changes that follows chronic administration of extract from periwinkle. The lethality studies showed high value of LD₅₀ indicating that periwinkle extracts have very wide safety margins and therefore could be relatively non-toxic. No wonder these sea creatures are widely consumed as a source of protein in foods by humans, (Onuoha, 2006).

The protein content as estimated by the method of Lowry et al. (1951) showed that periwinkle extracts have high levels of protein, and protein are known to be very useful for body building, (FAO/WHO/UNU, 1985).

There was an increase in the WBC count in extract-treated animals. The increase was probably due to an increase in the lymphocyte count, as revealed in the differential count studies. In the rats, the lymphocytes are the prevailing white cells (Eno et al., 2001), therefore its increase is expected to increase the total WBC. These extract could probably help to boost the immune processes, since it enhances white blood cell production. However it could also alleviate severe leucopenic conditions. This view is consistent with earlier report that consumption of edible mollusk is of more health benefit (Ndem et al., 2008).

The erythrocyte counts of the rats treated with periwinkle extract was significantly increased and the increase was confirmed by increased PCV and Hb. The increase in Hb concentration and the RBC count is not surprising, since Hb is an integral component of the RBC. It has been reported that sea foods are excellent...
sources of iron, Vit A, Vit B12, Vit B₆ and thiamine (Schrimshaw and Young, 1992). These accessory food substances are known to be the basic requirements necessary for the production of RBC (Guyton and Hall, 2004).

Therefore, this increase in erythrocyte count could be brought about by the presence of these accessory food substances and proteins in the extracts. The vitamins are capable of enhancing erythropoiesis and stimulating the maturation of the erythrocytes (Huebers and Frich, 1987; Guyton and Hall, 2004). Also, the protein in the extract may help facilitate the process of erythropoiesis by enhancing Hb production (Jacobson et al., 1957; Jacobs et al., 1985; Beru, 1986; Krantz, 1991). That the extract also contains erythropoietin-like agents cannot be ruled out. The red cell distribution widths (RDWs) were decreased in extract treated rats. RDW is a numerical measure of the variability in size (anisocytosis) of circulating erythrocytes (Perkins, 2003). This parameter is used in narrowing the differential diagnosis of anemia (Mckenzie, 2003). That the extract decreased the RDW value therefore suggests that the extract could cause the production of RBC that are less variable in size. Therefore, an anemic condition is very unlikely in animals treated with this seafood. The mean corpuscular volume (MCV) which is the average volume of a single RBC size showed no differences between control and extract treated groups. In patience 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) (Tonnesen et al., 1986). It is very likely therefore that the effects of the extracts on the RBC were normocytic since the treated preparations were not different from their control. This is in perfect agreement with the findings in the RDW studies.

Another index for diagnosing anemia is mean corpuscular hemoglobin (MCH) and 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 (Rose and Bentley, 1986). 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 group that received extracts of the edible mollusc (Periwinkle). It is very likely that this extract contains thrombopoietin-like agent(s) or compound capable of causing the release of thrombopoietin (Erslev and Gabuzda, 1979), 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 contain essential fat called omega 3 fatty acid. 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 (FAO/WHO/UNU, 1985; Chajes and Bougnoux, 2003).

In this study, the mean platelet volume (MPV) was reduced following administration of periwinkle extract. MPV is the determinant of platelet function and is found to vary inversely with the platelet count in normal subjects (Nadar et al., 2004; McCabe et al., 2004; Kilicli-Camur et al., 2005) and in chronic vascular disease (Endler et al., 2002), hence the reduction in MPV as in the case in this study, also indicates an increase in platelet count. This finding that the MPV was decreased strongly supports the increase in platelet count observed in this study.

The result obtained for platelet distribution width (PDW) showed a decrease in the extract treated rats. This decrease in PDW is in perfect agreement with the decrease in MPV and 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), (Babu and Basu, 2004). 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 and viable blood platelets, (Babu and Basu, 2004).

**Conclusion:**

The results show that both the low and high doses of the extracts increased WBC, lymphocytes, RBC count, Hb, PCV, MCH and MCHC values but reduced the levels of MPV, PDW and P-LCR in rats. Therefore, edible mollusk (Periwinkle) is safe for consumption by humans, it also contains substances that can help as blood tonic and to boost blood cell production and immunity, hence could serve as a preferred diet supplement.

**REFERENCES**


