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Liver Enzymes and Hematological Effect of Sub-chronic Periwinkle (*Pachymelania aurita*) and Rock Snail (*Thais coronata*) Consumption in Anaemic Albino Rats

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To investigate the effects of sub chronic consumption of Periwinkle and Rock snail extracts on liver enzymes and blood in anaemic albino rats was the objective of the study. Twenty-five male and female albino rats were randomly used in the study and were divided into three groups. Anaemia was first introduced into twenty rats after strict corn-meal feeding for three weeks. Group one (5 rats) served as control (normal rat feed and water); group two (10 rats)-anaemic+rat feed and periwinkle extract; group three (10 rats)-anaemic+rat feed and rocksnail extracts. After three weeks, the rats were sacrificed and their blood taken for analysis. In the test for anemia, the result indicated that the rat was anaemic. There was decrease in the blood parameters (Red blood cell, White blood cell, hemoglobin, Pack cell volume) when compared with control at $p<0.001$. The results for serum AST, ALT and ALP level from anaemic-rock snail fed rats revealed that there was significant decrease ($p<0.05$) in liver enzyme level when compared with the control. This was similar with anaemic-periwinkle extract-fed rats except in ALP level which was significantly increased ($p<0.05$) when compared with control. Similarly, there was significant increase in the blood parameters (RBCs, WBCs, Hb Concentration and PCV) and also differential WBC counts from anaemic extracts-fed rats when compared with control rats. This result reveals that subchronic consumption of rock snail and periwinkle extracts on anemic liver enzymes shows no deleterious effect on the liver enzymes and there was also improved state of the blood cells counts.

Key words: Periwinkle, rocksnail, liver-enzyme, subchronic consumption

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INTRODUCTION

Periwinkles (Pachymelania aurita) and Rock snail (Thais coronata) are one of the most overlooked and underrated members of the seafood family. They are what is called "the poor man's escargot". It represents one of the most important shellfish's resources of the world. In West Africa, periwinkles are the most dominant of the aquatic mollusks, occurring widely in both fresh and brackish water (Buchanan, 1954). The term shellfishes comprise crustaceans (shrimps, crabs, lobsters, crawfish etc) and mollusks (bivalves, squids, snails etc.) and possess single (univalve) or double (bivalve) shells for a covering. Considerable amounts of periwinkles are obtained daily for food. Periwinkles have been found to be rich in protein and carbohydrates. Periwinkles have been reported to be one of the preferred pollution bio-monitors because of their sedentary and bottom feeding habits which make them good accumulators of heavy metals and polycyclic aromatic hydrocarbons (Wilson *et al*., 1992; Jack *et al*., 1996). Periwinkles are mass-consumer products constituting relatively cheap animal protein in Calabar and are one of the many delicacies in the Nigeria cuisines (cooking style). Pachymelania aurita and Thais coronata are commercially valuable shellfishes and their collection and marketing form an important industry in the Niger Delta. Their value competes favorably with those of domestic livestock and fish (Dambo, 1993). Thais coronatas are easily found in the mangrove areas, sandy beaches and muddy sandy substrate areas.

Human diet is major player in the development of degenerative human disease such as cardiovascular disease and the mechanism is multifaceted. Consumption of food rich in saturated fats has been associated with degenerative diseases such as cancer, coronary heart disease and cerebro-vascular disease (Renaud and de Lorgeril, 1992; Stephen and Wald, 1990; Lapinskas, 2001). Due to westernization, dietary pattern seem to favor the consumption of more omega-6 fatty-acids (groundnut oil, vegetable oil, soya-bean oil, etc).-though also good for the body than omega-3 fatty acids. This imbalance has therefore resulted in high rate of heart disease, cancer, obesity, autoimmune disease, allergies, diabetes and depression (Thurnham, 1997; Renaud and de Lorgeril, 1992). In Nigeria, especially among the rich, congestive heart failure, hypertension and other degenerative disease may be attributed to reduced intake of balanced ratio of omega-3 and omega-6 fatty acids enriched diet.

Although, these mollusks shellfishes are good and cheap sources of protein, they could also harbor a lot of food poisoning organisms that pose serious health hazards to man, since they contain permissible nutrients that support microbial growth. Therefore, considering the massive consumption/demand of edible shellfishes, its commercial and industrial importance, this present work was undertaken to investigate the effects of subchronic consumption of these extracts-Pachymelania aurita (periwinkle) and Thais coronata (rock snail) on liver enzymes and some haematological parameters in anaemic albino rats as not much information or literature exists about this subject.

MATERIALS AND METHODS

Animals: Twenty-five adult male and female Albino Wister rats were obtained from the Animal house of the Department of Zoology, Faculty of Biological Sciences, University of Calabar, Calabar. The rats weighing 160-220g were housed each in metabolic cages. The beddings were changed daily to keep the environment clean and conducive for the experimental animals. The animals were fed with standard rat feed and allowed free access to water and allowed to acclimatize for 7 days before experiment. All animals were cared for according to the rules and regulations of the Institute of Animals Ethics Committee (IAEC).

Instrument/chemicals used: Instruments: Chemical Weighing Balance, Blender, Oven, Micro Haematocrit Reader, Sahli's Haemoglobinometer Set, Heparanized Capillary Tube, Micro-Haematocrit Centrifuge, Metabolic Cages, Distilled Water, Cotton Wool, Disposable Syringes, Hand Gloves, Spectrometer.

Chemicals: Chloroform, Biuret Reagent, EDTA, Drablam's Solution.

Preparation of extract: Fresh periwinkles and rocksnails were purchased from Calabar Main Market (Watt Market), Calabar, Cross River State, Nigeria. The edible portions of both animals were removed from their shells by cracking the shells with a stone. The edible portions were washed with warm water and 100 g of each sea food was weighed and oven dried at temperature of 50°C overnight. Fifty grams of the oven dried samples were blended using electric blender and were stored in separate containers each and labeled for identification. Then, 80 mg protein kg^{-1} of rock snail was taken from the blended sample and mixed with rat feed and fed daily to the rats for 3 weeks. Also 40 mg protein kg^{-1} of the periwinkle was taken from the blended sample, added with rat feed and administered to the rat daily for 3 weeks.

Induction of Anaemia in the rats: After one week of acclimatization, the rats were subjected to only 10 mg kg^{-1} corn meal and water for three weeks. The rats were proven anaemic due to decrease in RBC (Red Blood Cell) counts, PCV (pack cell volume) and haemoglobin when compared to the control.

Experimental design: The experimental animals of 25 rats were divided into 3 groups. 10 rats each for extract treated rats and 5 rats for control. After one week of acclimatization: Group 1 animals served as control, fed with rat pellets only; Group 2 were anaemic+rat pellets enriched periwinkle extracts; Group 3 were anaemic+rat pellets enriched rocksnail extracts. The animals had free access to drinking water. After the 3 weeks, the animals were sacrificed and their blood samples collected for analysis.

Collection of blood sample: After administration of the corn meal for three weeks, the animal fasted for 18 h. The rats were subjected to unconsciousness after inhaling chloroform anaesthesia (3.5% soaked in cotton wool). The abdomens of the rats were cut open to view the heart. With the aid of 5 mL syringe connected to a needle, the blood samples were easily gotten by aspiration via cardiac puncture from the left ventricle. The blood is then emptied into the EDTA (ethylene diamine tetra-acetic acid) bottle. The EDTA prevents blood coagulation. Part of the blood was put into a lithium heparanized sample bottle for liver enzyme analysis and stored at 4°C to maintain enzyme activity.

Determination of haematological parameters: Haematology profile which covers Pack Cell Volume (PCV), White Blood Cell (WBC) and red blood cell counts, was determined. Haemoglobin was measured using the method of Alexander and Griffith (1993). Differential WBC counts were determined using Synchron CX5 auto analyzer according to the manufacturer protocol.

Liver enzyme determination: Liver enzymes were measured with enzymatic calorimetric method using commercial kits from Randox laboratories, UK according to the manufacturer's principle.

Statistical analysis: The results were presented as Mean±SEM (Standard Error of Mean). The set of data were analyzed using one-way ANOVA (Analysis of variance) and Independent T-test using Statistical Package for Social Sciences (SPSS) software-version 18. p-value less than 0.05 was considered as statistically significant.

RESULTS

The effects of extract treated rats on anaemic liver enzymes presented in Table 1 showed significant reduction ($p<0.05$) in AST levels in Rock snail and Periwinkle extracts-treated rats when compared with the control rats. More so, ALT levels in the extracts treated rats were significantly reduced when compared to the control at ($p<0.05$). Serum ALP levels in the periwinkle treated rats was significantly ($p<0.05$) increased when compared with control. The increase was about 23.34%. Rock snail extract treated-rats however showed significant ($p<0.05$) low levels of ALP when compared with control. The decrease was about 9.06%.

Table 2 showed a presentation of extract treated rats on blood indices on anaemic rats. Rock snail treated rats showed significant high levels of Red blood cells, White blood cells, Hemoglobin concentration (at $p<0.001$, respectively) and Pack cell volume ($p<0.01$) when compared with the control. Periwinkle-treated rats also showed significant increases at various probability levels of RBCs, WBCs, Hb Concentration and PCV when compared with control. Periwinkle treated rats had the highest level of different blood cells when compared with other groups, followed with rocksnail treated rats. Similarly, differential White blood cells counts (Neutrophil, Eosinophil, Basophil, Lymphocyte and Monocyte) showed significant increases when compared with the control at various probability levels ($p<0.05$, $p<0.001$, $p<0.01$, $p<0.01$, $p<0.001$). This is shown in Table 3.

Table 1: Effect of extracts treated rats on liver enzymes

Groups	AST (IU L^{-1})	ALT (IU L^{-1})	ALP (IU L^{-1})
Control	128.00±3.60	48.20±1.39	57.40±1.36
Group 2	97.40±2.63*	26.40±1.63*	52.20±1.16*
Group 3	108.20±2.40*	38.80±2.76*	70.80±2.33*

*$p<0.05$ vs. control

Table 2: Effect of extracts treated rats on blood indices

Groups	RBC($\times10^{12}$ L^{-1})	WBC ($\times10^{9}$ L^{-10})	Hb (g dL^{-1})	PCV (%)
Control	2.50±0.01	2.40±0.72	12.24±0.20	30.90±0.60
Group 2	5.71±0.21*	5.42±0.20*	15.98±0.30*	38.18±0.25*
Group 3	6.40±0.37*	6.06±0.22*	18.84±0.25*	45.00±0.61*

*$p<0.05$ vs. control

Table 3: Effect of extracts treated rats on differential WBC counts

Groups	Neutrophils (%)	Eosinophil (%)	Basophil (%)	Lymphocyte (%)	Monocyte (%)
Control	31.20±2.35	0.72±0.14	0.50±0.20	21.41±1.33	2.25±0.12
Group 2	59.20±0.18*	3.51±0.10*	0.80±0.63*	41.20±0.42*	7.10±0.23*
Group 3	60.40±0.37*	5.00±1.23*	0.90±0.26*	40.50±1.25*	7.20±0.23*

*$p<0.05$ vs. control

DISCUSSION

Liver enzymes are proteins that speed up chemical reactions in the liver and perform specific functions. Some of these enzymes leave the liver and enter the blood when the liver is injured. When liver enzymes are elevated, or decreased, it is a sign of liver impairment that cannot be ignored. Inflamed or injured liver cells leak higher than normal amount of certain chemicals including liver enzymes in the blood stream which can result in elevated liver enzymes on blood test. Liver enzymes may be elevated even if here is no damage to the liver. ALP could be elevated due to damaged bones not damaged liver. Also ALT and AST level may be elevated due to damaged heart not liver (http://www.chemocare.com/).

The body needs iron, folate and vitamin B12 to produce sufficient number of healthy blood cells. A diet lacking in these key nutrients can cause decrease in blood cells production. Therefore introduction of only corn meal to the experimental animals is likely to cause anaemia as observed in the experimental animals.

The levels of liver enzymes in the control group and experimental animals are in line with the normal ranges except AST (Aspartate Transaminase) which was beyond 5-40 IU/L (normal range). This is likely due the presence of damage from one or more tissues other than the liver leading to increase of AST in blood as AST is produced from variety of tissues.

The decrease of AST, ALT and ALP liver enzymes in the experimental animals is likely due to the presence of iron deficiency anemia even with the introduction of the seafood extracts as iron deficiency is also seen as leading cause of decreased liver enzymes (Palande, 2011). Iron deficiency may be due to an increased iron requirement, decreased dietary intake of iron, diminished iron absorption or utilization, blood loss or a combination of factors. Apparently, iron-deficiency is caused by a shortage of the element, iron in the body. The bone-marrow needs iron to make haemoglobin for Red Blood Cells (RBCs) production. Therefore lack of iron as seen in the strict corn meal administered to the experimental animals is expected to cause iron deficiency anaemia. In this study, the RBCs were diminished and thereby needs extra folic-acid and iron supplements to produce the haemoglobin it needs to make more RBCs.

The high level ALP in the periwinkle extract-fed rats is probably due to mild rapid growth of bone since it is produced by bone-forming cells called osteoblasts, thereby increases it level in blood though not beyond the normal range.

Following results of haematological parameters from extract-treated rats when compared with control, the increase in Red blood cell, Pack Cell Volume (PCV) and Haemoglobin concentration was expected. This is similar to a study conducted in 2008 by Ndem *et al*. (2008) where there was marked increase in Hb concentration, PCV and RBC due to the seafood effect on the experimental animals. Seafood contains iron which carries oxygen in the blood. It also contains a high amount of protein and omega-3 fatty acid (Ndem *et al*., 2008). Omega-3 fatty acid is a polyunsaturated fatty acid with high health benefits as it contains vitamin B, Vitamin E, iron zinc and magnesium. This is known to reduce high cholesterol thus preventing heart diseases. Eating eight ounces/week of seafood is highly recommended.

The significant increase in differential WBC counts was also expected as the almighty seafood is known to contain zinc. Zinc as been shown to enhance phagocytic activity of macrophages and neutrophils (Thurnham, 1997) which helps in the building of immune system making the body function effectively as it serves as primary defense mechanism invading foreign bodies. Its supplementation for 1-2 months has been reported to restore immune responses, reduce the incidence of infections and prolong survival (Thurnham, 1997).

In African and Asian cuisine, periwinkle is considered a delicacy. Its meat is high in protein and low in fat. This could be the reason for a marked increase in result when compared with rocksnail extracts (http://www.ifood.tv/photo/periwinkle).

This result reveals that subchronic consumption of rock snail and periwinkle extracts on anemic liver enzymes shows no deleterious effect on the liver enzymes. Though there showed decrease in liver enzymes when compared with control, there still fell within the normal ranges. Seafood greatly influences the haematological indices by increasing the blood cells and also the differential WBC counts.

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