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Research Article

Alteration in biochemical indices following administration of seafood (*Thais coronata*) extract

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ABSTRACT

Seafood consumption has been a way of life to most people especially those that leave in riverine areas, because seafoods are known to contain many nutrients that are essential for healthy living. Consequently, this research therefore seeks to investigate the effect of these nutritive components of *Thais coronata* on biochemical indices of albino Wistar rats. Fourty five male albino Wister rats weighing between 180-220 g were assigned into 3 groups of fifteen rats each in metabolic cages and were given rat feed and drinking water *ad libitum*. Two test doses (low dose 7.0 mg protein/ml and high dose 52 mg 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 = 15. At the expiration of the feeding period, blood samples were obtained from all the rats via cardiac puncture for the analysis of the various biochemical indices. Both the low and high doses of the extract produced significant increases in HDLc ($P<0.001$) compared with control. k ($P<0.001$), HCO_3^- ($P<0.01$) and Ca^{2+} ($P<0.001$) were also significantly increased in the extract treated groups. The extract groups had significant reductions in ALT ($P<0.001$), ALP ($P<0.001$), Na^+ ($P<0.001$) and Cl^- ($P<0.001$) compared with control. Also Tc ($P<0.001$), TG ($P<0.001$), LDL ($P<0.001$) and VLDLc ($P<0.001$) were significantly decreased in the extract treated group. In conclusion seafood consumption is of immense benefit to health because it serves to regulate the lipid profile, electrolytes and enzyme concentrations in blood.

Keywords: Rock snail; *Thais coronata*; Biochemical indices; Vitamin; Omega-3 fatty acid.

1. INTRODUCTION

Seafood consumption is a way of life to those that cherish it and it is yet to be discovered by those who don't know about it. Seafood constitutes important and readily available sources of edible nutrients and is found in different kind of waters. There exist different types of Seafoods but one of the most common one is *Thais coronata* (Rock snail) a strong member of the mollusca phylum [1] from the family Muricidae. *Thais coronata* the world's largest fresh water snails are locally known as Nkonko by the Efiks in Nigeria. They occur basically in tropical and subtropical localities in different parts of the world including Nigeria (Calabar) Cuba, Brazil, Central America, USA (California), Philippines, Hawaii, Taiwan, Japan and Indonesia. *Thais coronata* is very rich in iron, iodine, selenium, Vit. A, Vit. D, Vit. E, Vit. B12,

Vit. B6, proteins and essential fatty acid. They are essential for human consumption and their shell is used in making jewelry [2-5].

Nutrition evaluation of seafood in Nigeria indicates that it has high protein content and elemental composition [6]. Moreover, it has been reported that seafood consumption enhance blood production [7] and serves as a rich source of essential fatty acids like the omega-3 fatty acid, which is important in reducing the incidence of coronary heart disease [8] and preventing other diseases [9-11]. Seafoods are known to have antioxidant property which is essential in lowering of arterial blood pressure; they were shown to elevate HDL-c and lower LDL-c levels in blood [12, 13] and they also enhance tissue lipoprotein lipase activities. Seafood also provides negligible amounts of trans-fats, dietary fibres and sugars [14-19].

2. MATERIALS AND METHODS

2.1. Experimental animals

Forty-five (45) male albino Wistar rats weighing initially between 180 to 220 g obtained from the animal house of the Department of Physiology, University of Calabar, Nigeria were employed for this study for 6 weeks. The animals were allowed free access to their feed and drinking water. The rats were weighed before commencement of the feeding experiment and thereafter were weighed daily. Ethical approval was obtained from the Ethics Committee of the Faculty of Basic Medical Sciences, University of Calabar, Nigeria. They were nursed under control of environmental conditions in accordance with international standard [43].

2.2. Collection of Rock snail sample

Fresh samples of the Rock snail were purchased from the local markets (watt market) in Calabar.

2.3. Preparation of the aqueous extract

The preparation of aqueous extract was done according to the method described by Walker [20] and Aldeen et al. [21] as used by Archibong et al. [22]. Fresh Rock snail was obtained from Watt Market Calabar and was rinsed in water to remove leaves and debris on different occasions. One hundred grams of the fresh rock snail was weighed out respectively and homogenised for 5 minutes using tissue blender. The homogenate was then dissolved in 100 ml of saline (0.9% NaCl). After dissolving the homogenate, it was then centrifuged for 10 minutes using 10,000 revolutions per minute. The supernatant was then poured into a clean container via filter paper fitted funnel, and this formed the stock solution of 1 g/ml.

2.4. Experimental design

Fourty five (45) male albino Wistar rats were randomly selected and assigned to three groups thus the control, low dose (LD) and high dose (HD) groups of fifteen (15) rats each. The test doses were selected based on pre-determined LD_{50} values and on serial dilution of the stock solution. The extract was added into a small amount of the feed based on the weight of each rat. The low dose groups received 7 mg/ml of the extracts daily while the high dose groups received 50 mg/ml of the extracts daily. The control group received 0.6 ml of normal saline daily. All the animals had free access to food and drinking water and the experiment lasted for six weeks.

2.5. Collection of blood plasma samples

The animals were made unconscious using chloroform anesthesia. The blood samples were collected via cardiac puncture, a method modified by Ohwada [23]. A 5 ml syringe, attached to a sterilized needle was used to collect the blood samples from the heart and then emptied into plane sample bottles. The blood samples were then used for the estimation of various levels of plasma constituents.

2.6. Preparation and extraction of serum

About 4-5 ml blood was collected from each rat into separate sample bottles and allowed to stay for 30 minutes to enhance clotting. It was then centrifuged at 2,500 revolutions/min for 15 minutes with the help of the micro hematocrite centrifuge. The serums were collected into clean test tubes for

the analysis of the various biochemical indices.

2.7. Determination of liver enzymes

2.7.1. Determination of alkaline phosphatase (ALP)

ALP was analyzed according to the method as described by Bowers and McComb [24]. The p-nitrophenyl phosphate was hydrolyzed to phosphate and p-nitrophenol in the presence of ALP. A calculated amount of sample 0.01 ml in a test tube was mixed with reagent (0.5 ml) containing the substrate p-nitrophenyl phosphate and kept at room temperature. The solution was mixed and initial absorbance read after 1 min. The reaction was allowed to stand for 3 min and the absorbance read again at 405 nm [24]. Alkaline phosphate activity was calculated from the following formular:

$$UL = \frac{2760 \times A\ nm}{min\ micro}$$

Where: UL = Unit of alkaline phosphatase affinity, A = Change in absorbance.

2.7.2. Determination of aspartate transferase (AST) and alanine transferase (ALT)

Serum AST and ALT levels were determined, using endpoint colorimetric-diagnostic kit from Randox Laboratories, UK [25]. The pyruvate produced by transamination reaction between L-alanine and ketoglutarate reacts with 2,4-dinitrophenyl hydrazine to give a colored hydrazine and was used to measure alanine aminotransferase activity. The oxaloacetate hydrazone formed with 2,4-dinitrophenyl hydrazine was used to measure aspartate aminotransferase (AST). Both ALT and AST were read at 540 nm wavelength.

2.8. Determination of serum lipids (lipid profile)

2.8.1. Determination of total cholesterol

The determination of total cholesterol was carried out as demonstrated by Siedel et al. [26]. Cholesterol esters were hydrolyzed by cholesterol esterase to produce cholesterol and fatty acids. The cholesterol was oxidized by cholesterol oxidase to cholesterone and hydrogen peroxide. The H_2O_2 was later hydrolyzed by peroxidase to form water and oxygen. The oxygen then reacted with 4-amino-antipyrine, which is the chromogen to form quino-neimine. The color intensity of the solution was proportional to the concentration of cholesterol in the sample. The samples were mixed and incubated for 10 min in a water bath at 37°C. The color produced was read colorimetrically at 540 nm [26].

Calculation:

$$\frac{\text{Absorbance of test} \times \text{Concentration of standard (5.2 mmol } l^{-1})}{\text{Absorbance of standard}}$$

2.8.2. Determination of triglyceride

The determination of triglyceride was analyzed as demonstrated by Negele et al. [27]. Triglyceride in the sample was hydrolysed by lipoprotein lipase to glycerol and free fatty acids. Glycerol was phosphorylated by the kinase to form glycerol-3-phosphate and ATP. The glycerol phosphate was then oxidized by glycerol phosphate oxidase to dihydroxyacetone phosphate and H_2O_2. The H_2O_2 was hydrolysed by peroxidase to form H_2O and O_2. The O_2 then reacted with 4-amino-antipyrine and phenol to form the color complex quinoneimine. The samples were mixed and incu-bated for 10 min in a water bath at 37°C. The color produce was read colorimetrically at 540 nm [27].

Calculation:

$$\frac{\text{Absorbance of test} \times \text{Concentration of standard (2.3 mmol } l^{-})}{\text{Absorbance of standard}}$$

2.8.3. Determination of high density lipoprotein

The determination of high density lipoprotein cholesterol was analysed as demonstrated by [26]. The HDL-cholesterol is a precipitate off apoprotein B-containing lipoprotein using a mixture of sodium phosphotungstic acid and magnesium chloride. The samples were mixed thoroughly and allowed to stand at room temperature for 15 min and later centrifuged at 3000 revolutions per min. The samples were mixed and incubated for 10 min in a water bath at 37°C [26].

Calculations:

$$\frac{\text{Absorbance of test} \times \text{Concentration of standard (1.3 mmol } l^{-})}{\text{Absorbance of standard}}$$

Final result was multiplied by the dilution factor 3.0.

2.8.4. Determination of low and very low density lipoprotein

Low and very low density lipoprotein concentrations were calculated using the Friedwald formular [28]:

LDL_c = Total cholesterol - (HDL_c+$VLDL_c$)

VLDL = Triglyceride/2.22

2.9. Determination of serum electrolytes

Serum Na^+ and K^+ concentrations were determined using a flame photometer (Model 410C, Petracourt Ltd, England). Serum Cl^- concentration was determined using the end point calorimetric titration method [29]. Serum bicarbonate (HCO_3) concentration was measured using the modified method [30].

2.10. Statistical analysis

Data was presented as Mean ± SEM. The student's t test was employed to compare two sets of data. Three or more variables were compared with one-way analysis of variance (ANOVA). The $p<0.05$ and $p<0.001$ were considered statistically significant.

3. RESULTS

3.1. Analysis of serum enzymes

As shown in Table 1 the alanine transferase enzyme concentration was significantly lower ($p<0.05$ and 0.001) in the low dose (59.0 ± 2.1*) and high dose (43.1±2.81) groups than the control (77.8± 0.37) group, respectively.

The alkaline phosphatase enzyme concentration was significantly lower ($p<0.05$ and 0.001) in the low dose (75.0±1.20) and high dose (71.0±1.41) groups than the control (86.6±0.75) group, respectively.

The difference in aspartate transferase enzyme concentration was of no statistical significance among the three groups.

Table 1. Serum enzymes in the different experimental groups.

	ALT (IU/L)	ALP (IU/L)	AST (IU/L)
Control	77.8±0.37	86.6±0.75	105.0±0.23
Low dose	59.0±2.1*	75.01±1.20	104.2±0.14
High dose	43.1±2.81***	71.0±1.41*	103.2±0.43

Values are represented as Mean ± SEM. *P < 0.05, ***P < 0.001 vs Control.

3.2. Analysis of lipid profile

As shown in Table 2 the total cholesterol concentration was significantly lower ($p<0.001$) in the low dose (1.15±0.04) and high dose (1.03±0.03) extract treated groups than the control (1.32±0.04) group, respectively.

The triglyceride concentration was significantly lower ($p<0.001$) in the low dose (0.31±0.01) and high dose (0.30±0.01) extract treated groups than the control (0.65±0.02) group, respectively.

The high density lipoprotein cholesterol concentration was significantly higher ($p<0.001$) in the low dose (0.67±0.04) and high dose (0.69±0.01) extract treated groups than the control (0.64±0.02) group, respectively.

The low density lipoprotein cholesterol concentration was significantly lower ($p<0.001$) in the low dose (0.63±0.01) and high dose (0.47±0.01) extract treated groups than the control (1.97±0.03) group, respectively.

The very low density lipoprotein cholesterol concentration was significantly lower ($p<0.001$) in the low dose (0.15±0.03) and high dose (0.13±0.05) extract treated groups than the control (0.29±0.01) group, respectively.

3.3. Analysis of serum electrolytes

As shown in Table 3 the sodium (Na) concentration was significantly lower ($p<0.001$) in the low dose (125.6±0.75) and high dose (123.6± 0.45) groups than the control (136.2±1.00) group, respectively.

The pottassium (K) concentration was significantly higher ($p<0.001$) in the low dose (7.0±0.14) and high dose (7.10±0.21) groups than the control (5.66±0.07) group, respectively.

Table 2. Lipid profile in the different experimental groups.

	Tc(mg/dL)	TG (mg/dL)	HDLc (mg/dL)	LDLc (mg/dL)	VLDLc (mg/dL)
Control	1.32±0.04	0.65±0.02	0.64±0.02	1.97±0.03	0.29±0.01
Low dose	1.15±0.04***	0.33±0.01***	0.67±0.04**	0.63±0.01***	0.15±0.03***
High dose	1.03±0.03***	0.31±0.01***	0.69±0.01***	0.47±0.01***	0.13±0.05***

Values are represented as Mean ± SEM. **P < 0.01, ***P < 0.001 vs Control.

Table 3. Serum electrolyte in the different experimental groups.

	Na^+ mmol/L	K^+ mmol/L	Cl^- mmol/L	HCO_3 mmol/L	Ca^{2+} mmol/L
Control	136.2±1.00	5.66±0.07	101.4±0.75	25.1±0.37	0.94±0.04
Low dose	125.6±0.75***	7.0±0.14***	95.0±0.75***	27.2±0.43**	1.35±0.04***
High dose	123.6±0.45***	7.10±0.21***	80.0±0.63***	29.0±0.37**	1.75±0.02***

Values are represented as Mean ± SEM. **p<0.01, ***p<0.001 vs Control. Na^+: Sodium, K^+: Potassium, Cl^-: Chlorine, HCO_3^-: Bicarbonate, Ca^{2+}: Calcium.

The chloride (Cl) concentration was significantly lower (p<0.001) in the low dose (95.0±0.75) and high dose (80.0±0.63) groups than the control (101.4±0.75) group, respectively.

The bicarbonate (HCO_3) concentration was significantly higher (p<0.001) in the low dose (27.2±0.43) and high dose (29.0±0.37) groups than the control (25.1±0.37) group, respectively.

The serum calcium (Ca^{2+}) concentration was significantly higher (p<0.001 and 0.01) in the low dose (1.35±0.04) and high dose (1.75±0.02) groups than the control (0.94±0.04) group, respectively.

4. DISCUSSION

This study was meant to investigate the effect of crude extract of *Thais coronata* on some biochemical indices of albino Wister rats and the results we got were quite amazing. The serum enzyme result revealed that the crude extract was able to reduce the level of ALT and ALP in a dose dependent manner. This is an indication that the hepatocytes or liver tissues in general benefited from the extract administration and ALT is a more specific and stronger indicator of liver cell damage than AST, also ALT is found primarily in the liver and AST is found in many other organs of the body besides the liver [31, 32]. Therefore, lowered serum ALP confirms that the extracts may not have damaging effects on the liver cells and bone.

Consumption of edible seafood was found to be of immense benefit to health because of its high content of unsaturated fatty acid and polyunsaturated fatty acid especially omega-3 fatty acid [33]. Here administration of *Thais coronata* extract was shown to cause significant reduction in total cholesterol, triglyceride and low density lipoprotein level and an increase in high density lipoprotein level in albino Wistar rats.

This result conforms with various studies previously carried out [13] which revealed that edible seafood are capable of boosting high density lipoprotein level, [34] which revealed that administration of fenofibrate therapy decreased TG level and also ameliorate system oxidation and inflammation [35] which revealed that extract of saffron and crocin administration reduced TC and TG levels and are useful in the prevention of dyslipidemia and obesity.

Raised levels of serum total cholesterol, triglycerides and low density lipoprotein cholesterol are possible indicators of coronary heart attack, risk of heart disease and stroke [36]. The ability of the extract of rock snail to reduce these bad cholesterols in the blood shows that their consumption would be beneficial to health. Rock snail extract has been reported to contain omega-3 fatty acid which is believed to mediate the decrease in the concentrations of these bad cholesterol [37] and this is useful in promoting the clearance of triglyceride from blood [38] also the mechanism of action of

nutraceuticals on lipid profile is further being reviewed [39].

The increase in HDL-c observed in the rock snail extract fed groups could also be attributed to omega-3 component of the extract [12], which is equally important because HDL-c is the good cholesterol that function in preventing the accumulation of bad cholesterol and ameliorating the risk of heart disease

The serum electrolyte result has revealed that there was a reduction of sodium ion concentration following the administration of rock snail extract, this may be due to the low concentration of sodium in the extract, or possibly due to the ability of the extract to potentiate excretion of sodium ions from the body. This was followed by a decrease in chloride concentration since sodium and chloride ions are always transported alongside [40]. This result is also very important because elevated Na^+ concentration predisposes one to high blood pressure [41], it therefore means that consumption of *Thais coronata* extract may be important in preventing high blood pressure. The extract treated group also had a significant increase in potassium ion concentration, this may be brought about by the decrease in serum sodium ions occasion by it excretion and reabsorption of potassium ions, since sodium and potassium ions are always exchanged in alternate manner by the Na^+/K^+ pump along the cell membrane [42]

There was an increase in HCO_3 concentration in the rock snail extract treated group. It is well known that bicarbonate is essential in neutralizing the acidic pH produce by the acid in the gastrointestinal tract [41]. Bicarbonate ions also maintain the acid-base buffering system of the blood. Finally, extract treated group also produced elevated plasma Ca^{2+}, this may be useful in preventing bone resorption and other related conditions associated with calcium deficiency.

5. CONCLUSION

Seafood consumption is of immense benefit to health because it serves to regulate the lipid profile, electrolytes and enzyme concentrations in blood.

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AUTHORS' CONTRIBUTION

AAN wrote the initial draft of the manuscript; AEO and AAA designed the study, OEO did the statistical analysis while, IOB and SUK proof read, and edited the word. All authors were involved in the execution of the research plan. The final manuscript has been read and approved by all authors.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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