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DIETARY PERIWINKLE AND ASSOCIATED EFFECT ON SOME SERUM BIOMARKERS OF LIVER FUNCTION

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ABSTRACT

Dietary periwinkle (*Tympanotonus fuscatus)* is natural seafood with very rich vitamins and nutrients, but there is little or no literature on its effect on different biomarkers of liver function, hence the need for this study. Twenty one albino Wistar rats weighing between 200-250g were assigned into 3 groups of 7 rats each, housed singly in metabolic cages. Rats in control group took normal rat pellet and drinking water *ad libitum*. The 2 test groups were administered two different doses (low dose (7.0mg/kg b. w) and high dose (52mg/kg b. w) of periwinkle extract. At the end of six weeks feeding period, blood samples were obtained from all the rats via cardiac puncture for biochemical analysis. The liver was also harvested for histological study. The result of the analysis showed that rats placed on both the low and high doses of the extract had significant increases in total protein ($p<0.001$) and globulin ($p<0.001$) compared with control group. The extract fed rats also had significantly reduced ALT ($P<0.001$) and ALP ($P<0.001$) levels compared with the control. The extract had no effect on the cytoarchitecture of the liver. This study has therefore revealed that consumption of *Tympanotonus fuscatus* has no adverse effect on health but rather offers a protection, as reflected by the result of the serum protein, serum enzyme and even the histological studies. In conclusion, dietary periwinkle (*Tympanotonus fuscatus)* extract has hepato-protective potentials and its consumption should therefore be encouraged.

KEYWORDS: Periwinkle, Liver, Enzymes, Proteins, Omega 3 fatty acid.

1. INTRODUCTION

The liver is an important internal organ which performs many functions.[2] They include secretion of bile, sythethic function, metabolic function, excretory function, heat production, hemopoietic function, hemolitic function, defence and detoxification function[26], clotting factors and albuminsynthesis.[19] The human liver contains thousands of enzymes that help necessitate chemical reactions in the body.[6, 19] As observed the liver plays a very important role in life and therefore contributes a whole lot to our wellbeing. The most commonly used indicators of liver functions are the Alanine aminotransferase (ALT), Aspartate aminotransferase (AST)[24] and alkaline phosphatase concentration. Variations in the concentrations of these enzymes have many implications. Increased levels of ALT and AST are indications of hepatocellular disease, active cirrhosis, metastatic liver tumor, toxic hepatitis, severe, pancreatitis, myocardial infarction (heart attack), trauma, severe burns, acute hemolytic anemia, crushing injuries and shock.[6] Alkaline phosphatase is the most frequently used test to detect obstruction in the biliary system. Elevation of this enzyme may be found inbiliary tumor, gallstone disease, alcohol abuse and drug-induce hepatitis.[21]

Taking cognizance of how useful the liver is to our daily living it is pertinent for us to investigate how vulnerable this liver can be with respect to dietary periwinkle consumption. Because dietary periwinkle is the most common, prolific and cheapest source of protein.[13, 18] they are readily available and are consumed on daily bases. They are locally called "Mfi" by the Efiks, and "Ihemu" by Rivers/Bayelsa tribes[7], and contain important vitamins and nutrients such as Vitamin (A, B, D, E,) and thiamine which are useful in improving hematological parameters.[3] Others include iron, cupper, zinc, selenium, calcium, magnesium, phosphorus, potassium, proteins and essential fatty acid (omega-3 fatty acid)[20], which is important in improving serum lipid profile and electrolytes[1] thereby reducing the incidence of coronary heart disease.[10]

MATERIALS AND METHODS

Experimental animals and protocol

Twenty one male albino Wistar rats weighing between 200-250g obtained from the animal house of

Pharmacology and Animal Science Departments of the University of Calabar, Nigeria, were employed for the study. Each animal was housed in separate clean metabolic cage. They were randomly selected and assigned to three groups thus: Control, low dose (LD) and high dose (HD) groups of seven rats each. The test doses were selected based on pre-determined LD_{50} values[3] and on serial dilution of the stock solution. The low dose groups received 7mg/kg bw of the extracts daily while the high dose groups received 52mg/kg bw of the extracts daily. The control group received 0.6ml of normal saline daily. All animals had access to food and water *ad libitum*. The feeding period lasted for 6 weeks, after which the animals were used for the various experiments. Let me mention here that in the handling of the animals all ethical standards laid down in the 1964 declaration of Helsinki were strictly adhered to.

Preparation of the aqueous extract

The preparation of aqueous extract of periwinkle was done according to standard procedure[17, 22] as used by Archibong *et al*.[3] Fresh periwinkle 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 periwinkle was weighed out respectively and homogenised for 5 minutes using tissue blender. The homogenate was then dissolved in 100ml of saline (0.9% NaCl). After dissolving the homogenate, it was then centrifuged for 10 minutes using 10,000 revolutions per minutes. The supernatant was then poured into a clean container via filter paper fitted funnel, and this formed the stock solution of 1g/ml.

Collection of blood samples and measurement of biochemical parameters

The animals were made unconscious using chloroform anesthesia. The blood samples were collected via cardiac puncture, a method modified by Ohwada.[16] A 5ml syringe, attached to a sterilized needle was used to collect the blood samples from the heart. About 4-5ml 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 hematocrit centrifuge. The serum was collected into clean test tubes and were then used for the estimation of various parameters.

Determination of liver enzymes

ALP was measured according to standard procedure.[11] P-nitrophenyl phosphate was hydrolysed to phosphate and p-nitrophenol in the presence of ALP. A calculated amount of sample 0.01ml in a test tube was mixed with reagent (0.5ml) containing the substrate p nitrophenyl phosphate and kept at room temperature. The solution was mixed, initial absorbance read after 1 minute. The reaction was allowed to stand for 3 minutes and the absorbance read again at 405nm. Alkaline phosphate activity was calculated from.

UL = 2760 x ΔA nm/minute micro

Where UL = Unit of alkaline phosphatase affinity
ΔA = Change in absorbance

Serum AST and ALT levels were determined using endpoint colorimetric diagnostic kit (Randox Labouratories, UK) based on Reitman and Frankel's method.[27] The pyruvate produced by transamination reaction between L-alanine and ketoglutarate reacts with 2, 4, dinitrophenyl hydrazine to give a coloured hydrazone, and was used to measure alanine aminotransferase activity. The oxaloacetate hydrazone formed with 2, 4 dinitrophenyl hydrazine was used to measure aspartate aminotransfarase (AST). Both ALT and AST were read at 540nm wavelength.[27]

Determination of serum proteins

Total protein and albumin was determined by the method as reported by Savory et al.[14] while Globulin was mathematically calculated as below.

Globulin = Total Protein – Albumin

Histological studies

After the last day of administration of the extract, all the rats were anaesthetized with chlorofom (CHCL3).The animals were sacrificed using chlorofom inhalation method.An incision was made throught the abdomen up to the thorax, the liver was extracted and preserved in 10 percent buffered formaline and was processed for the histological studies.The harvested tissues were processed using routine Haemotoxylin and Eosin method and embedded in parafin wax, sectioned and some section stained with Haemotoxylin and Eosin staining method for this histological studies.

Statistical analysis

Data were 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). $p<0.05$ and 0.001 were considered statistically significant.

RESULTS

Analysis of Serum Enzymes

As shown in table 1. The alanine aminotransferase enzyme concentration (IU/L) was significantly ($p<0.05$ and $p<0.001$ respectively) lower in the low dose (74.4±1.33 IU/L) and high dose (68.0±0.32 IU/L) group compared with the control (78.0 ± 0.24 IU/L) group.

The alkaline phosphatase enzyme concentration (IU/L) also was significantly ($p<0.05$ and $p<0.01$ respectively) lower in the low dose (83.0±1.36 IU/L) and high dose (76.0±0.51 IU/L) groups compared with the control (87.4 ± 0.75 IU/L) group. No significant statistical differences were observed in aspartate aminotransferase enzyme concentration (IU/L) among the different experimental groups.

Analysis of serum protein

As shown in Table 2. The total protein concentration was significantly ($p< 0.001$) higherin the low dose (67.0±0.64 g/dL) and high dose ((68.0±0.55 g/dL)groups compared with values obtained for the control(65.0±0.32 g/dL). There was no significant difference in the albumin concentration among the different experimental groups.

The globulin concentration was significantly ($p<0.001$) higher in the low dose (26.3±0.51g/dL) and high dose(26.3± 0.80 g/dL) groups compared with the control (25.9±1.05 g/dL) group respectively.

Histological Analysisof the effect of extracts on the liver

Plate 1: Shows the photomicrographs of a section of the liver from control and the periwinkle fed groups showing normal histological features of the liver.

Group A: The section of the liver from the control group showed a preserved architecture consisting of a central vein (CV) and hepatocyte (HP) radiating outward. The hepatocytes are prominent with eosinophilic cytoplasm and basophilic nuclei. The sinusoidal spaces (SS) are prominent and empty.The portal traid (PT) are prominent consisting of portal vein (PV), hepatic artery (HA) and bile duct(BD)

Group B: Section of the Liver from the extract treated (52mg/Kg) group. The section shows prominent congested central vein and hepatocyte radiating outward. The hepatocyte have eosinphilic cytoplasm and basophilic nuclei. The sinusoidal spaces are dilated and congested. The portal traid consists of bile duct, hepatic artery and portal vein. The limiting plate are intact.

Table 1: Serum enzyme in the different experimental groups.

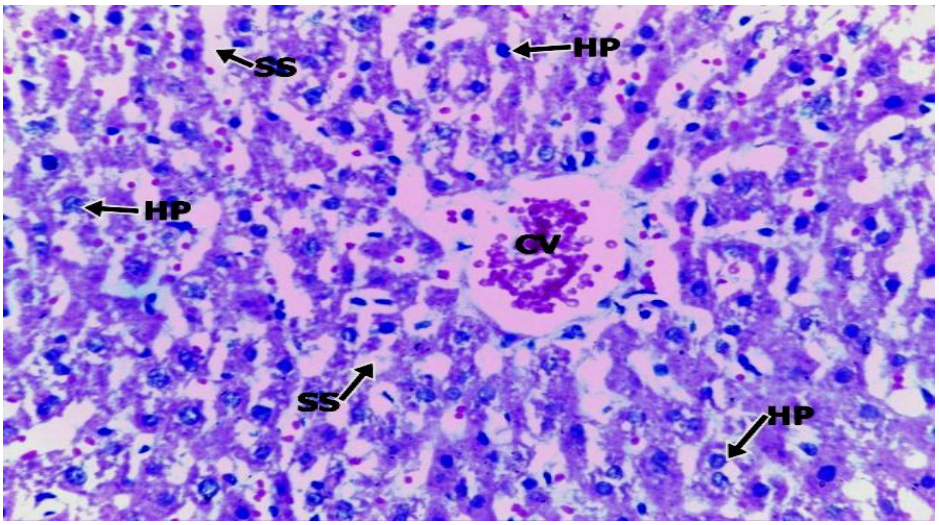
	ALT (IU/L)	ALP (IU/L)	AST (IU/L)
Control	78.0±0.24	87.4±0.75	105.3±21.0
Low dose	74.4±1.33*	83.0±1.36*	104.0±10.3
High dose	68.0±0.32***	76.0±0.51**	103.1±4.12

Values are represented as Mean ± SEM. *P < 0.05, P <0.01, ***P < 0.001 vs Control

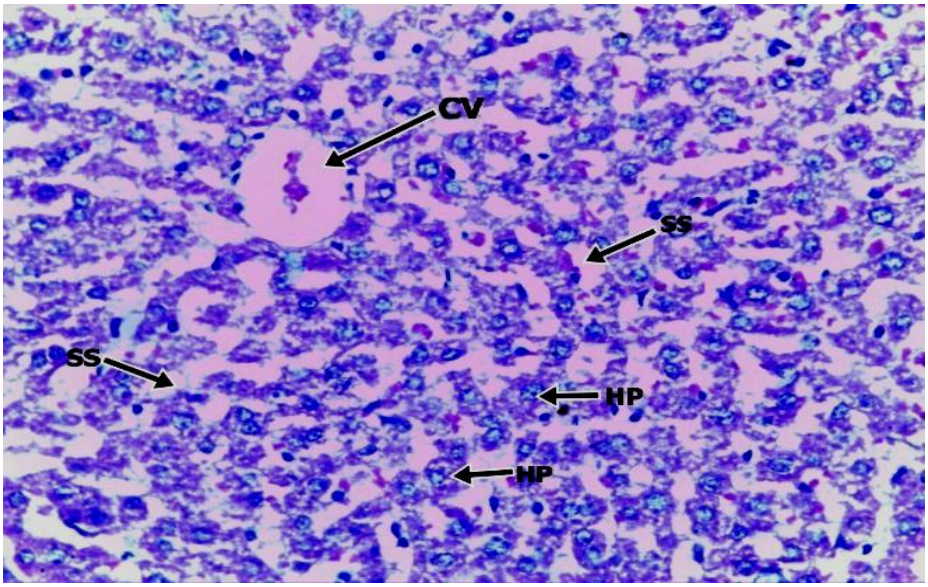
Table 2: Serum protein in the different experimental groups.

	Total Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
Control	65.0±0.32	39.1±2.14	25.9±1.05
Low dose	67.0±0.64***	40.1±1.23	26.3±0.51
High dose	68.0±0.55***	41.7±4.10	26.3±0.80***

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



Control – A



Periwinkle fed group - B

PLATE 1: Photomicrographs of a cross section of the liver tissues in A- control and B- periwinkle fed (52mg/mL) groups. Showing normal cytoarchitecture, CV- central vein, HP- hepatocytes, SS – sinosoids.

Periwinkle B
A cross sectional area of photomicrograph of the liver tissues of the different experimental groups stained with H & E.
Magnification = x400

DISCUSSION

The result has shown that consumption of dietary periwinkle *(Tympanotonus fuscatus)*is not hazardous tothe liver tissues, as it does no harm to the cytoarchitecture of the liver. This is being corroborated by the results obtained from the serum enzyme, serum protein and histological study.

The result shows that the periwinkle extract fed rats had significantly lowered serum alanine aminotransferase (ALT) and alkaline phosphatase (ALP) concentrations compared with that of the control group. This result is supported by previous research[12] wish shows that dietary periwinkle is capable of decreasing serum ALP and ALT.[12] This may be due to its active components, omega-3 fatty acid which is known to be Hepatoprotective.[15] The serum concentrations of ALP and ALT are increased in conditions of kidney, pancreatic, heart and liver damage.

Also the result shows that the extract significantly increased Total Protein (TP) and Globulin concentration, this result is being supported by previous research published by Archibong *et al*.[4] which reported increase in Total protein and globulin level following administration of seafood. Increased globulin concentration in plasma is an indication of immune system stimulation[5, 8], since globulin is formed almost entirely in the lymphoid tissue and constitute the antibodies that forms the immune system. The elevated globulin level also indicates that the extract could play an important role in the prevention of microcytic hypochromic anemia since globulin is involved in iron transportation in blood. The serum albumin concentration revealed that the liver cells were not damaged[23], since albumin concentration is decreased in both chronic and acute liver disease.[8]

The histological study as shown on the plate has further confirmed that consumption of dietary periwinkle does not have any adverse effect on the health of the liver tissues as evident in the similarity between the cyto-architecture of the control and periwinkle extract fed rats. This may in part be due to the potent effect of omega 3 fatty acid component of dietary periwinkle. Omega-3 fatty acid is very essential as it plays a major role in ameliorating nonalcoholic fatty liver disease[25] and regulates hepatic lipid metabolism, adipose tissue function and inflammation.[9] Therefore, *Tympanotonus fuscatus* extract exhibits hepato-protective effect and its consumption therefore should be encouraged.

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AUTHORS CONTRIBUTION

This work was carried out in collaboration between all authors. Author ANA and JPK designed and wrote the protocol. SAL wrote the first draft of the manuscript, managed the literature and performed the statistical analysis, author AUI edited the manuscript, while author MSK contributed in carrying out the feeding regimens and analysis of blood samples. All authors read and approved the final manuscript.

Conflict of interest statement

The Authors declare no conflict of interest.

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