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Alteration of Lipid Profile and Serum Electrolytes Following Chronic Consumption of Periwinkle Extract in Rats

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ABSTRACT

Periwinkles are important edible seafood essential to life. They are cheap and easily affordable sources of protein, although they are harvested from river waters that are made of variety of pollutants. This research therefore sought to investigate any possible alterations in some biochemical parameters of albino Wistar rats by periwinkle intake. Fifteen albino Wistar rats weighing between 200-250 g were assigned into 3 groups of 5 rats each and housed singly in metabolic cages. The rats took normal rat pellets and drinking water *ad libitum*. The 2 test groups received different doses (low dose -7.0 mg mL⁻¹ and high dose -52 mg mL⁻¹) of periwinkle extract orally, once daily. The control group received a daily oral administration of 0.6 mL of normal saline as vehicle. The feeding regimens lasted for 6 weeks, after which blood samples were collected for estimation of some biochemical parameters. Results showed that mean values of the lipid profile in control group were: TC (1.44±0.05 mg dL⁻¹), TG (0.78±0.16 mg dL⁻¹), HDL-c (0.47±0.02 mg dL⁻¹), LDL-c (1.20±0.01 mg dL⁻¹) and VLDL-c (0.35±0.01 mg dL⁻¹). For the electrolytes, control values were: Na⁺ (133.0±0.66 mmol L⁻¹), K⁺ (5.74±0.09 mmol L⁻¹), Cl⁻ (103.4±0.75 mmol L⁻¹), HCO₃⁻ (22.0±0.32 mmol L⁻¹) and Ca²⁺ (1.02±0.07 mmol L⁻¹). Rats placed on both low and high doses of the periwinkle extract had significant (p<0.01) increases in HDL-c, K⁺, HCO₃⁻ and Ca²⁺ when compared with control group. The periwinkle extract fed rats also had significant reduction in Na⁺ (p<0.001) and Cl⁻ (p<0.001) concentrations compared with the control. Total cholesterol, triglyceride, LDL-c and VLDL-c concentrations were also significantly (p<0.001) reduced in the periwinkle extract fed groups compared with control. Previous study had shown that periwinkle (*T. fuscatus*) contains omega 3 fatty acid, which could be attributed to the beneficial effect of the extract on lipid profile and serum electrolytes observed in this study. Hence, chronic consumption of periwinkle could help alleviate dyslipidemia and other conditions associated with deleterious alterations in electrolyte concentrations. This beneficial effect of the extract on lipid profile and serum electrolytes was supported by multivariate analysis, which showed that statistically there was a significant difference in lipid profile among the different groups, F (10, 16) = 15.578, p<0.0001; Will's Lambda = 0.009, partial Eta Sq. = 0.907 and in serum electrolyte concentrations among the different groups, F (10, 16) = 222.958, p<0.0001; Wilk's Lambda = 0.000, partial Eta Sq. = 0.993.

Key words: Periwinkle, blood, electrolytes, fatty acid, edible mollusk

INTRODUCTION

Periwinkles are important seafood that is very essential to life (Narain and Nunes, 2007). Periwinkle is considered to be the most common and dominant mollusks in the brakish water of

West Africa (Nickles, 1950). They are highly prolific and are the cheapest source of protein (Okon and Ayuk, 2007), they are locally called “Mfi” by the Efiks and “Themu” by Rivers/Bayelsa tribes. Periwinkles are found in riverine waters with salinity 1.5%, pH 6.5-7.1 and temperature between 27 and 34°.

Periwinkles contain important vitamin such as vitamins A, B, D, E and thiamine and other nutrients like iron, copper, zinc, selenium, calcium, magnesium, phosphorus, potassium, proteins and essential fatty acid (Scrimshaw and Young, 1992). They also possess majorly 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 (Wardlaw and Smith, 2009). This omega 3 fatty acid also increases the concentration of HDL-c (Mensink *et al.*, 2003) in blood. High density lipoprotein plays a vital role in the body which include reduction of inflammation, protection against oxidation of low density lipoproteins, interfere positively with blood clotting and decrease the accumulation of atherosclerosis within the walls of the artery (Sirtori, 2006). Over decades, now we have been eating and benefitting from this seafood without recourse to other health conditions. It has been shown that periwinkle extract increases RBC, Hb, PCV, MCH, MCHC, WBC and platelet count (Archibong *et al.*, 2014) and also seafoods exposed to hydrocarbon possess health hazard to man who is the final consumer (Clinton *et al.*, 2009).

Taking cognizance of the impending danger of domestic and industrial waste and the effect of their toxicity on our water bodies, it therefore become pertinent for us to study the influence of this periwinkle components on rat biochemical parameters since most of our seafood evolve from these water bodies that serve as a dumping ground for these waste.

MATERIALS AND METHODS

Experimental animals and protocol: Fifteen male albino Wistar rats weighing between 200-250 g 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 metabolic cage which was cleaned daily. They were randomly selected and assigned to three groups thus the control, Low Dose (LD) and High Dose (HD) groups of five rats each. The test doses were selected based on pre-determined LD₅₀ values (Archibong *et al.*, 2014) 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⁻¹ of the extracts daily while, the high dose groups received 52 mg mL⁻¹ of the extracts daily. The control group received 0.6 mL 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. 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 the method described by Walker (1977) and Aldeen *et al.* (1981) and as used by Archibong *et al.* (2014). 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 homogenized for 5 min using tissue blender. The homogenate was then dissolved in 100 mL of saline (0.9% NAC). After dissolving the homogenate, it was then centrifuged for 10 min using 10,000 rpm. The supernatant was then poured into a clean container via filter paper fitted funnel and this formed the stock solution of 1 g mL⁻¹.

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 (1986). A 5 mL syringe, attached to a sterilized needle was used to collect the blood samples from the heart. About 4-5 mL blood was collected from each rat into separate sample bottles and allowed to stay for 30 min to enhance clotting. It was then centrifuged at 2,500 rpm for 15 min 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 biochemical properties.

Determination of serum lipids (lipid profile)

Determination of total cholesterol: The determination of total cholesterol was carried out as described by Siedel *et al.* (1983). Cholesterol esters are hydrolyzed by cholesterol esterase to produce cholesterol and fatty acids. The cholesterol is oxidized by cholesterol oxidase to cholesterone and hydrogen peroxide. The H_2O_2 is later hydrolyzed by peroxidase to form water and oxygen. The oxygen then reacts with 4-aminoantipyrine which is the chromogen to form quinoneimine. The colour intensity of the solution is 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 colour produced was read colorimetrically at 540 nm. According to Siedel *et al.* (1983) total cholesterol can be calculated by:

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

Determination of triglyceride: The determination of triglyceride was done as described by Negele *et al.* (1992), triglyceride in the sample was hydrolyzed by lipoprotein lipase to glycerol and free fatty acids. Glycerol is phosphorylated by the kinase to form glycerol-3-phosphate and ATP. The glycerol phosphate is then oxidized by glycerol phosphate oxidase to dihydroxyacetone phosphate and H_2O_2 . The H_2O_2 is hydrolyzed by peroxidase to form H_2O and O_2 . The O_2 then react with 4-amino-antipyrine and phenol to form the colour complex quinoneimine. The samples were mixed and incubated for 10 min in a water bath at 37°C. The colour produced was read colorimetrically at 540 nm According to Negele *et al.* (1992) triglyceride can be calculated by:

$$\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of standard (2.3 mmol L}^{-1}\text{)}$$

Determination of high density lipoprotein cholesterol: The determination of high density lipoprotein cholesterol was carried out as described by Siedel *et al.* (1983). The HDL-cholesterol is a precipitate off apo protein 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 rpm. The samples were mixed and incubated for 10 min in a water bath at 37°C. According to Siedel *et al.* (1983) high density lipoprotein cholesterol can be calculated by:

$$\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \text{Concentration of standard (1.3 mmol L}^{-1}\text{)}$$

Final result is multiplied by the dilution factor 3.0.

Determination of low and very low density lipoprotein: Determination of low and very low density lipoprotein concentrations were based on Friedewald formula.

$$\text{LDL}_C = \text{Total Cholesterol} - (\text{HDL}_C + \text{VLDL}_C)$$

$$\text{VLDL}_{C2.22} = \text{Triglyceride}$$

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 by end point calorimetric titration following the method of Kolthoff and Coetzee (1957). Serum bicarbonate (HCO_3^-) concentration was measured by the modified method of Forrester *et al.* (1976).

RESULT

Lipid profile in the different experimental groups: As shown in Table 1, the total cholesterol concentration was significantly ($p < 0.001$) lower in the low dose ($1.39 \pm 0.01 \text{ mg dL}^{-1}$) and high dose ($1.36 \pm 0.02 \text{ mg dL}^{-1}$) extract fed groups compared with the control ($1.44 \pm 0.05 \text{ mg dL}^{-1}$) group. Triglyceride concentration also was significantly ($p < 0.001$) lower in the low dose ($0.78 \pm 0.01 \text{ mg dL}^{-1}$) and high dose ($0.64 \pm 0.02 \text{ mg dL}^{-1}$) extract fed groups compared with the control ($0.78 \pm 0.16 \text{ mg dL}^{-1}$) group, respectively.

The rats placed on the low dose and high dose had significantly increased high density lipoprotein cholesterol concentration (0.51 ± 0.01 and $0.55 \pm 0.01 \text{ mg dL}^{-1}$, respectively) compared with the control group ($0.47 \pm 0.02 \text{ mg dL}^{-1}$) group.

Significantly lower concentrations of low density lipoprotein cholesterol was recorded in the low ($1.19 \pm 0.02 \text{ mg dL}^{-1}$) and high dose ($1.10 \pm 0.03 \text{ mg dL}^{-1}$), ($p < 0.001$) extract fed groups compared with the control.

No significant statistical differences were observed in the concentrations of very low density lipoprotein cholesterol among the different experimental groups.

Multivariate analysis showed statistically that there was a significant difference in lipid profile among the different groups, $F(10, 16) = 15.578$, $p < 0.0001$; Wilk's Lambda = 0.009, partial Eta Sq. = 0.907.

Serum electrolytes concentrations of the different experimental groups: As shown in Table 2, serum sodium concentration was significantly ($p < 0.001$) lower in the low dose ($124.0 \pm 0.63 \text{ mmol L}^{-1}$) and high dose ($123.0 \pm 0.89 \text{ mmol L}^{-1}$) periwinkle extract fed groups compared with the control ($133.0 \pm 0.66 \text{ mmol L}^{-1}$) group.

Table 1: Lipid profile in the different experimental groups

Parameters	TC	TG	HDL-c	LDL-c	VLDL-c
			(mg dL^{-1})		
Control	1.44 ± 0.05	0.78 ± 0.16	0.47 ± 0.02	1.20 ± 0.01	0.35 ± 0.01
Low dose	$1.39 \pm 0.01^{***}$	0.78 ± 0.01	$0.51 \pm 0.01^{***}$	1.19 ± 0.02	0.35 ± 0.00
High dose	$1.36 \pm 0.02^{***}$	$0.64 \pm 0.02^{***}$	0.55 ± 0.01	$1.10 \pm 0.03^{***}$	0.31 ± 0.00

TC: Total cholesterol, TG: Triglyceride, HDL-c: High density lipoprotein cholesterol, LDL-c: Low density lipoprotein cholesterol, VLDL-c: Very low density lipoprotein cholesterol, ***Significant at $p < 0.001$

Table 2: Serum electrolyte in the different experimental groups

Parameters	Na^+	K^+	Cl^-	HCO_3^-	Ca^{2+}
			(mmol L^{-1})		
Control	133.0 ± 0.66	5.74 ± 0.09	103.4 ± 0.75	22.0 ± 0.32	1.02 ± 0.07
Low dose	$124.0 \pm 0.63^{***}$	6.00 ± 0.07	$94.4 \pm 0.75^{***}$	24.0 ± 0.63	$1.50 \pm 0.04^{***}$
High dose	$123.0 \pm 0.89^{***}$	$6.70 \pm 0.05^{***}$	$89.4 \pm 0.40^{***}$	$26.7 \pm 0.54^{**}$	$1.64 \pm 0.02^{***}$

***Significant at $p < 0.001$

The serum potassium concentration was significantly ($p < 0.001$) higher in the low dose ($6.0 \pm 0.07 \text{ mmol L}^{-1}$) and high dose ($6.7 \pm 0.05 \text{ mmol L}^{-1}$) periwinkle extract fed group compared with the control group ($5.74 \pm 0.09 \text{ mmol L}^{-1}$).

The low and high doses periwinkle extract fed rats also had significantly ($p < 0.001$) lowered chloride ion concentrations (94.4 ± 0.75 and $89.4 \pm 0.40 \text{ mmol L}^{-1}$) compared with the control group ($103.4 \pm 0.75 \text{ mmol L}^{-1}$) group.

Significant ($p < 0.001$) increase in serum bicarbonate ion concentrations were observed in the low dose ($24.0 \pm 0.63 \text{ mmol L}^{-1}$) and high dose ($26.7 \pm 0.54 \text{ mmol L}^{-1}$) groups compared with the control ($22.0 \pm 0.32 \text{ mmol L}^{-1}$) group.

Also, serum calcium ion concentrations increased significantly in the low dose ($1.50 \pm 0.04 \text{ mmol L}^{-1}$, $p < 0.001$) and high dose ($1.64 \pm 0.02 \text{ mmol L}^{-1}$, $p < 0.01$) periwinkle extract fed groups compared with the control ($1.02 \pm 0.07 \text{ mmol L}^{-1}$) group.

Multivariate analysis showed statistically that there was a significant difference in serum electrolyte concentrations among the different groups, $F(10, 16) = 222.958$, $p < 0.0001$; Wilk's Lambda = 0.000, partial Eta Sq. = 0.993.

DISCUSSION

Consumption of edible seafood is of immense benefit to health because of its high content of protein, unsaturated fatty acid and polyunsaturated fatty acid especially omega 3 fatty acid (Sidhu, 2003).

Result obtained from this investigation on serum lipid concentrations revealed that periwinkle extract caused a significant reduction in total cholesterol, triglyceride and low density lipoprotein level and an increase in high density lipoprotein level in albino Wistar rats. Raised levels of serum total cholesterol, triglycerides and low density lipoprotein cholesterol are possible indication of coronary heart attack, risk of heart disease and stroke (Drummond and Brefere, 2013). This result is supported by two studies previously carried out first by Sun *et al.* (2015), which revealed that administration of fenofibrate therapy decreased TG level and also ameliorate system oxidation and inflammation, secondly by Mashmoul *et al.* (2014), which revealed that extract of saffron and crocin administration reduced TC and TG levels and are useful in the prevention of dyslipidemia and obesity. The mechanism of action of nutraceuticals and other related food components on lipid profile and their contribution in lipid disorder management have been reviewed by Scicchitano *et al.* (2014).

The ability of the extract of periwinkle to reduce these bad cholesterol in the blood shows that their consumption would be beneficial to health. Periwinkle extract has been reported to contain omega 3 fatty acid which is believed to mediate the decrease in the concentrations of bad cholesterol (Davidson, 2008) and this is useful in promoting the clearance of triglyceride from blood (Tayyab *et al.*, 1991).

The increase in HDL-c observed in the periwinkle extract fed groups could also be attributed to omega 3 component of the extract (Mensink *et al.*, 2003), 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 periwinkle 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. There was also a decrease in chloride concentration, sodium

and chloride ions are always transported alongside (Ganong, 1991). The extract recipients had a significant increase in potassium ion concentration, possibly because of the decrease in serum sodium ions occasion by its 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 (Kaplan, 2002), or it may be that the extracts contain huge amount of potassium ion (Walker *et al.*, 1977).

There was an increase in HCO_3^- concentration in serum of the periwinkle extract recipients. Bicarbonate is produced by the pancreas and liver to neutralize the acidic pH produced by the acid in the gastrointestinal tract. Bicarbonate ions also maintain the acid-base buffering system of the blood.

Finally, extract of periwinkle also produced elevated plasma Ca^{2+} , this may be useful in preventing bone resorption and other related conditions associated with calcium deficiency.

CONCLUSION

In conclusion, chronic consumption of periwinkle (*Tympanotonus fuscatus*) is very useful to health in that its consumption could not predispose people to dyslipidemia or other conditions that may arise from adverse alteration in electrolyte concentrations.

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