COMPARATIVE EFFECT OF TWO EDIBLE SEA-FOOD, *P. PALLUDOSA* AND *E. RADIATA* ON SERUM LIPID PROFILE AND ATHEROGENIC INDICES IN RATS

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SUMMARY

Background: *Pomacea palludosa* (apple snail) and clams (*Ergeria radiate*) are staple edible mollusks, rich in minerals, vitamins, proteins and omega-3-fatty acids (involved in the prevention of cardiovascular diseases).
Aim: The study was conducted to ascertain whether or not consumption of sea foods will predispose to dyslipidaemia.
Materials and Methods: Rats were fed on Basal, reference casein, *P. palludosa* and *E. radiate* and plain soup diets.
Results: Results revealed significant ($p<0.05$) decreases in serum total and LDL cholesterols in *P. palludosa* diet recipients, all the diets increased ($p<0.05$) HDL-c vs basal diet. *P. palludosa* diet also resulted in significant ($p<0.05$) lowering of cardiac risk ratio and atherogenic coefficient, while *E. radiata* diet reduced the atherogenic index of plasma.
Conclusion: In conclusion, *E. radiate* and *P. palludosa* diet would not predispose to atherogenesis since it favored the good cholesterol (HDL-c). Nevertheless, *P. palludosa* has desirable influence on serum lipids, and protects better against atherogenesis than *E. radiata*.

Key words: Diets, *E. radiata, P. palludosa*, lipid profile, atherogenic indices, rats.

INTRODUCTION

Pomacea palludosa (apple snail) are tropical fresh water snail from the family ampullaridae (sometimes referred to as pilidae), while clams (*Ergeria radiate*) are bivalves mollusks with two shells that provide protection to the soft body[1]. There are over 15,000 different species of these edible sea foods worldwide[2,3].

These edible sea foods have long been the focus of nutritional studies; nutritionists consider them important sources of high quality protein, minerals, vitamin D and essential fatty acids including omega-3-fatty acids[1,4-7]. Omega-3 fatty acids are involved in the prevention of cardiovascular diseases[8]. Hence, the national nutrition and health program (PNNS) in France recommends consumption of these sea-foods twice a week especially for people who have heart attacks and other cardiovascular disease.

Cardiovascular disease is one of the world's leading causes of death. One of the major risk factors for the development of cardiovascular disease is dyslipidemia, which may be primary or associated with hypertension, diabetes mellitus and obesity[9-11]. Dyslipidemia usually involve elevated plasma levels of triglycerides, total, LDL-c and VLDL-c cholesterol and a low level of HDL-c cholesterol[12,13]. Therefore, any nutritional and pharmacologic intervention that reduces or normalizes non-HDL-c metabolism may be useful for reducing the risk of cardiovascular diseases[13].

Whether or not the consumption of these edible sea foods can predispose to or ameliorate dyslipidemia is the main aim of this investigation.

MATERIALS AND METHODS

Collection and preparation of *E. radiate* and *P. palludosa* samples

Fresh samples of *Pomecia palludosa* used for this study were harvested and bought from a riverine fresh water habitat at Idomi, Yakurr, Central Cross River State, Nigeria. Some were bought from a local market at Aningheje in Akampka Local Government Area of Cross River State, Nigeria. While fresh samples of *Ergeria radiata* were harvested from Calabar Itu bridge beach market in Akwa Ibom and Watt market in Calabar, Cross River State, Nigeria. The samples were collected between the months of January to March, 2009.

Soon after collection, the samples were within hours conveyed to the Laboratory of Biochemistry Department, University of Calabar for processing. The samples were washed with clean tap water to remove sand and other particles. Each edible portion of the *Ergeria radiata* and *P. palludosa* were removed from their calcerous shells, for *E. radiata*, the edible portion was removed by making a bilateral incision to expose their content of the stomach which was flushed out with clean tap water and then dried and for *P. Palludosa*, the apple shaped shell was cracked after steeping in hot water for 5 minutes and the edible portion removed. :er removing the edible portions, the samples were washed, pooled together and divided into two portions, one portion remained fresh sun-dried until it was crispy and powdered. The other portion was cooked and oven dried at 60°C until it was crispy.

Preparation of experimental diets

Tables 1 and 2 show the different experimental diets used in this study, each *Ergeria radiata* and *Pomecia palludosa* was used prepare experimental soup as used locally. Briefly, whole meat extracted from their shells, washed thoroughly to remove possible contaminants were used to prepare the delicacy known as "okro soup".

Experimental animals and feeding regimens

Forty (40) albino Wistar rats were randomly assigned into 5 groups of 8 rats each. The animals were obtained from the animal house of Biochemistry Department, University of Calabar, Nigeria. The animals in each group were placed on different experimental diets, thus: group 1 was placed on basal diet; group 2 on reference diet; group 3 on 23% *E. radiata* diet; group 4 on 23% *P. palludosa* diet and group 5 on plain soup diet. All animals were allowed free access to drinking water; the feeding regimens lasted for 21 days. The animals were housed in well ventilated rooms under appropriate environmental conditions.

All experiments were conducted in line with approved guidelines of the local ethics committee and were therefore performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Collection of blood samples from the rats

At the end of the experimental period, each rat (non-fasted) was anaesthetized in chloroforom vapour in a dessicator and immediately the thorax was dissected using forceps and scissors. Blood samples were collected by cardiac puncture using sterile syringe and needle into plain sample bottles. The blood samples were allowed to stay for 2 hours for effective clotting to occur before it was subjected to centrifugation at 3000rpm for 10 minutes using a bench top centrifuge, MSE England. Thereafter, serum was obtained as supernatant of the clotted blood in plain sample tubes. Sera obtained from the respective samples were carefully removed using Pasteur pipettes into clean, dry, labeled plastic specimen bottle. These were kept frozen in a refrigerator until when used for biochemical analysis of serum lipids.

Assessment of serum lipid profile

Serum total cholesterol, triglyceride, HDL-c cholesterol were estimated by the colorimetric end point, increasing reaction method, CHOD-PAP of Dialab kit method at the University of Calabar Teaching, Calabar.

LDL-Cholesterol was calculated from triglyceride concentration using the Friedewald's *et al.,* equation[14]. However, according to Chawla[1] (1999), since triglyceride levels in the samples did not exceed 390mg/dL, the LDL-Cholesterol was calculated from the following formula:

LDL-cholesterol (mg/dL) = Total cholesterol – (Triglyceride – HDL-cholesterol)[5]

VLDL-Cholesterol was estimated by using the following formula:

VLDL-cholesterol(mg/dL)= Triglycerides[5].

The relationship is based on the fact that the ration of VLDL and triglycerides in serum is fixed relatively at 1:5 in fasting subjects with triglycerides concentration not exceeding 400mg/dL.

Calculation of atherogenic indices

The atherogenic indices were calculated as follows:

Cardiac Risk Ratio (CRR) = TC / HDL-c[16]

Atherogenic Coefficient (AC) = (TC – HDLC) / HDL-c[17].

Atherogenic Index of Plasma (AIP) = log (TG / HDL-c)[18]

Statistical analysis

All data were analyzed using the statistical package for social sciences (SPSS) version 17.0 built by Microsoft corporation, USA. The data were analyzed by one way ANOVA and significant ones followed with a post-hoc (LSD) test between groups. All data were expressed as mean ± SEM and probability tested at 95% level of significant ($p<0.05$).

RESULTS

Serum total cholesterol (TC) concentrations

The values of total cholesterol obtained were 103.88 ± 0.84 mg/dL, 105.09 ± 0.96 mg/dL, 100.29 ± 0.12mg/dL, 119.29 ± 0.12mg/dL, 114.39 ± 1.69mg/dL for animals fed on basal diet, reference casein diet, *P. palludosa*, *E. radiata* and plain soup diet. The results indicate that there was significant increase in total cholesterol in *E. radiata* soup and plain soup compared to basal, reference and *P. palludosa* diets fed groups. TC levels of animals placed on basal and *P. palludosa* diet were in turn significantly lower compared to reference casein diet, Table 3.

Serum triacylglycerol (TG) concentrations

The value obtained for serum triacylglycerol were 73.03 ± 0.01 mg/dL, 56.03 ± 0.01mg/dL, 77.03 ± 0.01mg/dL, 73.90 ± 2.59mg/dL and 68.29 ± 0.12mg/dL for animals placed on basal, reference, *P. palludosa, E. radiata* and plain soup diets respectively. The values show that there was a significant increase in TG levels in reference diet fed group compared with other groups ($p<0.05$), Table 3.

Serum high density lipoprotein (HDL) concentrations

The following values were obtained for serum HDL: basal diet 36.03 ± 0.01mg/dl, reference casein diet 38.02 ± 0.01mg/dL, *P. palludosa* diet 38.02 ± 0.01mg/dL, *E. radiata* diet 38.33 ± 0.05mg/dL and plain soups diet 39.01± 0.01mg/dL. The data for HDL-cholesterol in all the groups did not differ significantly, although it was lowest in the basal diet fed group relative to other groups, Table 3

Serum low density lipoprotein (LDL) concentrations

Serum LDL concentration for the basal diet fed group was 52.29 ± 0.12mg/dL, LDL was significantly higher in *E. radiata* and plain diets fed groups compared with the other experimental groups (p<0.05). Animals placed on *P. palludosa* diet had the least serum LDL concentration, which was 46.76 ± 0.17mg/dL, this result is also shown in table 3.

Serum very low density lipoprotein (VLDL) concentrations

The following values were obtained for serum VLDL: 14.50 ± 0.09mg/dL, 11.38 ± 0.17mg/dL, 15.25 ± 0.13mg/dL, 14.29 ± 0.12mg/dL, and 13.44 ± 0.21mg/dL for basal, reference casein, *P. palludosa*, E. radiata and plain soup diets respectively. The VLDL-cholesterol was significantly lower in animals placed on reference diet compared with all other groups (p<0.05), Table 3.

Atherogenic indices

The values for cardiac risk ratio and atherogenic coefficient were significantly higher in the experimental group placed on *E. radiata* and plain soup diets compared with other groups, p<0.05. The atherogenic index of plasma of animals placed on basal, *P. palludosa* and *E. radiata* diets were comparable to each other, but was significantly lower in the reference diet fed group compared to other groups, Table 4.

Table 1: Ingredient composition of soup diets

Ingredients	Quantity (g)
Crayfish	50g
Pepper	0.6g
Magi	0.5g
Onions	10g
Okro	5g
Palm oil (red oil)	60ml

Table 2: Diet composition of experimental diets for lipid profile determination

Composition	Basal (g)	Casein (g)	PPS (g)	ERS (g)	Plain soup (g)
Corn starch	370	230	230	230	230
Sucrose	50	50	50	50	50
Glucose	50	50	50	50	50
Cellulose	50	50	50	50	50
Corn oil	50	50	50	50	50
Mineral mixture	20	20	20	20	20
Vitamin mixture	10	10	10	10	10
Casein	-	140	-	-	-
Pomecia palludosa	-	-	140	-	-
Ergeria radiata	-	-	-	140	-
Plain soup	-	-	-	-	140
Total	600	600	600	600	600

Table 3: Lipid profile of rat placed on the various experimental diets (mg/dL)

Parameters	**Basal Diet**	**Reference Diet**	**PP Soup**	**ER Soup**	**Plain Soup**	**F- ratio**	**Level of significant**
TC	103.88 ±0.84	105.09 ±0.96	100.29 ±0.12	119.29 ±0.12	114.39 ±1.69	555.954	0.001
TG	73.03 ±0.01	56.03 ±0.01	77.03 ±0.01	73.90 ±2.59	68.29 ±0.12	403.604	0.001
VLDL-c	14.50 ±0.09	11.38 ±0.17	15.25 ±0.13	14.29 ±0.12	13.44 ±0.21	770.889	0.001
HDL-c	36.03 ±0.01	38.02 ±0.01	38.02 ±0.01	38.33 ±0.05	39.01 ±0.01	19881.560	0.001
LDL-c	52.29 ±0.12	54.79 ±0.12	46.76 ±0.17	66.46 ±0.17	60.04 ±0.02	25676.659	0.001

Values are expressed as mean ±SEM, n = 8

Table 4: Atherogenic indices of rat placed on the various experimental diets

Parameters	Basal Diet	Reference Diet	PP Soup	ER Soup	Plain Soup	F- ratio	Level of significant
Cardiac risk ratio	2.89 ±0.009	2.77 ±0.009	2.64 ±0.002	3.11 ±0.15	2.93 ±0.27	401.568	0.001
Atherogenic coefficient	1.89 ±0.009	1.77 ±0.009	1.64 ±0.002	2.11 ±0.003	1.93 ±0.15	401.568	0.001
Atherogenic index of Plasma	0.31 ±0.00	0.17 ±0.00	0.31 ±0.00	0.28 ±0.005	0.21 ±0.00	577.681	0.001

Values are expressed as mean ±SEM, n = 8

DISCUSSION

In this study, the impact of consumption of two edible sea foods on serum lipid profile and atherogenic indices was investigated in albino Wistar rats. Results obtained from our study indicate that individuals who consume clam and rock snail in their diet would not be predisposed to dyslipidemia because of the tendency of these sea foods to favour serum HDL-c synthesis. Sea food have been shown as rich sources of edible nutrients including omega-3-fatty acids (about 1648mg per serving), which are involved in the prevention of cardiovascular diseases.[1,5-8].

The red palm oil had briefly been shown to lower the plasma levels of TG, VLDL and atherogenic index, and also enhancing the levels of HDL-c in experimental animals.

Elevated level of total cholesterol in the blood is a strong indicator and risk factor for coronary heart disease[19]. Our findings show that *P. palludosa* did not alter serum TC levels significantly, but *E. radiata* did alter serum TC level by increasing it. This shows that *P. palludosa* is better at protecting the heart compared with *E. radiata*. This effect may be attributable to the abundant omega-3 fatty acid in the seafood.

Increased plasma levels of LDL and VLDL cholesterol is often found in hypertension[20] and diabetes mellitus[9,13,12] and is a risk factor for cardiovascular disease[19,21,22,23]. In this study, we observed that the serum levels of VLDL cholesterol were not significantly altered in animals fed on *P. palludosa* and *E. radiata*. But, animals fed on *E. radiata* exhibited higher values of serum LDL compared with other groups. This indicates that *P. palludosa* may over better protection on the heart and other organs of the body like blood vessels than *E. radiata.*

A high serum TG concentration is both an independent and synergistic risk factor for cardiovascular diseases[11,18] and is often associated with hypertension,[24] abnormal lipoprotein metabolism, obesity, insulin resistance and diabetes mellitus[25,13]. The TG levels of the group placed on the edible sea foods were comparable with values obtained for animals placed on the basal diet, but not with animals placed on reference casein diet, because values obtained for edible sea food groups were significantly higher compared values obtained for the reference casein diet. The ability of the seafood to stabilize the TG levels could also be due to the abundance of omega-3 fatty acids and other vital nutrient like zinc in the sea foods.

Low serum levels of HDL cholesterol is a risk factor for cardiovascular diseases, as documented by some scholars.[12,16] Low serum levels of HDL-c are usually expressed in hypertensive[24] and in diabetic[9] patients. In this study, the sea food consumed by the experimental animals increased plasma HDL cholesterol levels significantly compared with values obtained for the basal diet, values obtained for the reference diet were comparative with those of the sea foods. The effect of the sea food on promoting serum HDL-c biosynthesis may again be mediated by the omega-3 fatty acid in these sea foods. Increase in plasma HDL cholesterol levels has been considered to reduce risk in coronary heart disease[12]. This cardio-protective effect of HDL-c is via the enhancement of reverse cholesterol transport by scavenging excess cholesterol from peripheral tissues, which it esterifies with the aid of lecithin: cholesterol acyl transferase, and delivers to the liver and steroidogenic organs for subsequent synthesis of bile acids and lipoproteins, and eventual elimination from the body[27,19] and inhibiting the oxidation of LDL as well as the atherogenic effects of oxidized LDL by virtue of its antioxidant[28] and anti-inflammatory property[29].

Atherogenic indices are strong determinants of the risk of coronary heart disease; the higher the value, the higher the risk of developing cardiovascular disease and vice versa[17,30,11]. In this study, we observed that *P. palludosa* significantly reduced cardiac risk ratio (CRR) and atherogenic coefficient, while atherogenic index was reduced in *E. radiata* fed rats. Low levels of atherogenic indices protect against coronary heart disease.

In conclusion, results of this investigation reveals that *Ergeria radiate* and *Pomecia palludosa* consumption would not predispose their consumers to dyslipidaemia and atherogenesis since it favored the biosynthesis of the good cholesterol (HDL-c). Meanwhile, *P. palludosa* appears to have a better effect on lipid profile and protect better against atherogenesis than *E. radiata*.

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