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*For further information about this article or if you need reprints, please contact:*

A.N. Archibong  
Department of Physiology,  
Faculty of Basic Medical Sciences,  
College of Medical Sciences,  
University of Calabar, P.M.B. 1115,  
Calabar, Nigeria  
Tel: +2348063074679

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## Effect of *Egeriaradiata* (Clam) Extract on Biochemical Parameters of Albino Wistar Rats

Archibong Nsa Archibong, Akwari AdaAk, Ofem Effiong Ofem,  
Irene Okon Bassey, Samuel Usoh Ukwani and Asim Ekaha Eno

Edible seafood (Clam) are important nutritive diet because they contain great quantities of protein and essential fatty acid (alpha linolenic (Omega 3), linoleic (omega-6) and oleic (omega 9) fatty acid) etc which are essential for healthy living, therefore this research seeks to investigate the effect of this dietary seafood on biochemical parameters of albino Wistar rats. Fifteen albino Wistar rats weighing between 200-250 g were assigned into 3 groups of 5 rats each in metabolic cages and were given rat feed and drinking water *ad libitum*. Two test doses (low dose-7.0 mg mL<sup>-1</sup> and high dose-52 mg mL<sup>-1</sup>) base on previously obtained LD<sub>50</sub> were selected and administered to two groups of rats orally, while the third group of rats served as the control, n = 5. At the end of six weeks blood samples were obtained from all the rats via cardiac puncture for the analysis of the various biochemical parameters. Both the low and high doses of the extract treated groups produced significant increase in total protein (p<0.001), globulin (p<0.001) and HDLC (p<0.001) compared with control. The k (p<0.001), Hco (p<0.01) and Ca (p<0.001) ion concentrations were also increased significantly in the extract treated groups compared with the control. The extract treated groups had significantly reduced ALT (p<0.001), ALP (p<0.001), Na<sup>+</sup> (p<0.001) and Cl<sup>-</sup> (p<0.001) compared with control. Also TC (p<0.001), TG (p<0.001) and LDL (p<0.001) were significantly reduced in the extract treated groups. Consumption of dietary clam is of great benefit, because it protect the hepatocytes and also prevents one from being predispose to atherogenesis or dyslipidaemic conditions.

**Key words:** Dietary clam, haematology, biochemical parameters, fatty acid, edible seafood

## INTRODUCTION

Edible seafood are important sources of dietary nutrients and delicacies right from the origin of man (Szabo, 2001). In developing countries, seafood constitute a cheap source of non conventional protein (Omenwa *et al.*, 2011) with an amino acid profile similar to that of the meat and a good example is clam (*Egeria radiata*).

Clam (*Egeria radiata*) is said to possess great quantities of protein (Chapman, 1997; Bames, 1980; Kohl, 2005; Malu *et al.*, 2009) and essential fatty acid which include alpha linolenic (Omega 3), linoleic (omega-6) and oleic (omega 9) fatty acid (Linder, 1992), they also possess varieties of essential trace elements such as Fe, Cu, P, Ca, Hco, Mg, Zn, Hg, I, Se, thiamine and vitamins (A and D) (Rice, 2004; Chapman *et al.*, 2007; Chudler, 2009). Edible seafood and their shell serves agricultural, medicinal, religious, commercial and economical purposes (Szabo, 2001).

Clam (*Egeria radiata*) commonly known as Nkop by the Efiks belongs to the class bivalvia (Clench and Turner, 1956). They feed on planktons and occur in fresh water. Its presence extends from the Gulf of Guinea, Congo, Ghana, Cameroon to Nigeria (Cross River) where they occur in seasons ranging from November to April. *Egeria radiata* are commonly found in river waters with the following characteristics, water temperature (31°C), pH (5.1), free carbon dioxide (5.7 mg L<sup>-1</sup>), total alkalinity (35.0 mg L<sup>-1</sup>) and salinity (0.051%).

The fatty-acid content in clam is high in polyunsaturated fat, particularly those which are attributed to reducing blood cholesterol (Robson, 2006; Narain and Nunes, 2007). The eicosanoids obtained from its omega 3 fatty acid are known to decrease blood pressure, decrease inflammation and secretion of the stomach, decrease platelet aggregation and formation of blood clots, decrease allergic reaction and cell proliferation. Omega 3 fat has been shown to possess natural antioxidants which helps to prevent thickening and damaging of artery walls and also slow down the wearing of cartilage that leads to osteo-arthritis (Rice, 2004). Omega 3 fatty acid helps to boost brain power and reduce behavioral problems in adult while improving reading skills and ability to concentrate in children (Mark, 2010). The eicosanoid derived from omega-6 fatty acid also play an important role in such body function as blood pressure, labor, blood clotting, immune response etc (Bagga *et al.*, 2002; Chajes and Bougnoux, 2003). Edible seafood has been shown to increase Red Blood Cell (RBC) count, White Blood Cell (WBC) count, hemoglobin concentration (Hb), platelet count and Packed Cell Volume (PCV) (Archibong *et al.*, 2014).

Over one billion people especially those living around the riverine areas, rely on edible seafood as their primary source of protein and essential nutrients and there are reports of its health benefit (Archibong *et al.*, 2014). However the environmental conditions and constituents of this edible seafood may pose another threat on its nutritive constituents since oil spillage, salinity and hydrocarbon level of the water can equally affect the nutritional components of these clam harvested from this water bodies. This study is therefore aim at investigating the effect of this dietary clam extract on biochemical parameters of albino wister rats.

## 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 house 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 base on pre-determined LD<sub>50</sub> values and on serial dilution of the stock solution. The extract was added into a small amount of the feed base on the weight of each rat. The low dose groups received 7 mg mL<sup>-1</sup> of the extracts daily, while the high dose groups received 52 mg mL<sup>-1</sup> 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 use for the various experiments. Handling of the animals all ethical standards laid down in the 1964 declaration of Helsinki were strictly adhered to.

**Collection of clam sample:** Fresh samples of the clam was purchased from a local market (Watt Market) in Calabar.

**Preparation of the aqueous extract:** The preparation of aqueous extract was done according to the method described by Walker (2011) and Aldeen *et al.* (1981) and as used by Archibong *et al.* (2014) Clam 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 clam was weighed out respectively and homogenize for 5 min 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 min using 10,000 revolutions per min. The supernatant was then

poured into a clean container via filter paper fitted funnel and this formed the stock solution of 1 g mL<sup>-1</sup>.

**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 revolutions min<sup>-1</sup> 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 parameters.

**Determination of serum proteins:** The serum protein (albumin, globulin and total protein) was determined as follows.

**Total protein (Burette method):** The peptide bond in protein has affinity for burette reagent. In alkaline medium, CuSO<sub>4</sub> in the burette reagent reacts with the peptide bond of protein to give a blue colour complex. The colour produced is proportional to the concentration of protein in the sample. The samples were thoroughly mixed and incubated in a water bath at 37°C for 10 min. After which they were then removed from the water bath and read colorimetrically at 540 nm (Savory *et al.*, 1976).

**Serum albumin (Bromocresol green method):** In an acidic medium bromocresol green dye binds to albumin to give a violet green colouration. Samples were mixed thoroughly and incubated at room temperature for 5 min and read colorimetrically at 620 nm (Savory *et al.*, 1976).

#### Globulin

##### Calculation:

$$\text{Total protein} - \text{Albumin} = \text{Globulin}$$

#### Determination of liver enzymes

**Determination of alkaline (ALP) phosphatase:** ALP was analyzed as shown below (Bowers and McComb, 1966). 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, initial absorbance read after 1 min. The reaction was allowed to stand for 3 min and the absorbance read again at 405 nm (Bowers and McComb, 1966). Alkaline phosphate activity was calculated from:

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

Where:

UL = Unit of alkaline phosphatase affinity

A = Change in absorbance

**Determination of aspartate (ALT) and alanine transferase (AST):** Serum AST and ALT levels were determined, using endpoint colorimetric-diagnostic kit (Reitman and Frankel, 1957) (Randox Laboratories, UK). The pyruvate produced by transamination reaction between L-alanine and ketoglutarate reacts with 2, 4, dinitrophenyl hydrazine to give a colored hydrazone 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.

#### Determination of serum lipids (lipid profile)

**Determination of total cholesterol:** The determination of total cholesterol was carried out as shown below (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<sub>2</sub>O<sub>2</sub> 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 (Siedel *et al.*, 1983).

##### Calculation:

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

**Determination of triglyceride:** The determination of triglyceride was analyzed as shown below (Negele *et al.*, 1992). Triglyceride in the sample was hydrolysed 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<sub>2</sub>O<sub>2</sub>. The H<sub>2</sub>O<sub>2</sub> is hydrolysed by peroxidase to form H<sub>2</sub>O and O<sub>2</sub>. The O<sub>2</sub> 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 (Negele *et al.*, 1992).

**Calculation:**

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

**Determination of high density lipoprotein cholesterol:**

The determination of high density lipoprotein cholesterol was analysed as shown below (Siedel *et al.*, 1983). 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 min<sup>-1</sup>. The samples were mixed and incubated for 10 min in a water bath at 37°C (Siedel *et al.*, 1983).

**Calculations:**

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

Final result is multiplied by the dilution factor 3.0.

**Determination of low and very low density lipoprotein:**

Low and very low density lipoprotein concentrations were calculated using the Fried wald formular (Friedewald *et al.*, 1972):

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

$$\text{VLDL}_C = \frac{\text{Triglyceride}}{2.22}$$

**Determination of serum electrolytes:** Serum Na<sup>+</sup> and K<sup>+</sup> concentrations were determined using a flame photometer (Model 410C, Petracourt Ltd, England). Serum Cl<sup>-</sup> concentration was determined using the end point calorimetric titration method (Kolthoff and Coetzee, 1957). Serum bicarbonate (HCO<sub>3</sub><sup>-</sup>) concentration was measured using the modified method (Forrester *et al.*, 1976).

**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 0.001 were considered statistically significant.

**RESULTS**

**Analysis of serum protein:** As shown in Table 1, the total protein concentration was significantly (p<0.001) increased in the low dose and high dose (64.0±0.31 and 68.4±1.39 g dL<sup>-1</sup>) extract treated groups in comparison with the control (61.2±0.68 g dL<sup>-1</sup>) group, respectively.

The albumin concentration showed no significant difference between the low dose (38.0±0.21 g dL<sup>-1</sup>), high dose (39.0±0.32 g dL<sup>-1</sup>) and control (37.1±0.41 g dL<sup>-1</sup>) group, respectively.

The globulin concentration was significantly (p<0.001) increased in the low dose and high dose (27.0±1.43 and 28.6±1.6 g dL<sup>-1</sup>) extract treated groups in comparison with the control (22.6±0.71 g dL<sup>-1</sup>) group, respectively.

**Analysis of serum enzymes:** As shown in Table 2, the alanine aminotransferase enzyme concentration was significantly (p<0.05 and 0.001) decreased in the low dose and high dose (76.0±1.4 and 55.2±1.3 IU L<sup>-1</sup>) extract treated groups in comparison with the control (78.6±0.37 IU L<sup>-1</sup>) group, respectively.

The alkaline phosphatase enzyme concentration was significantly (p<0.05 and 0.001) decreased in the low dose and high dose (80.4±1.10 and 70.8±1.02 IU L<sup>-1</sup>) extract treated groups in comparison with the control (87.2±0.75 IU L<sup>-1</sup>) group, respectively. The difference in Aspartate aminotransferase enzyme concentration was of no statistical significance among the three groups.

**Analysis of lipid profile:** As shown in Table 3, the total cholesterol concentration was significantly (p<0.001) decreased in the low dose and high dose (1.30±0.66 and 1.24±0.05 mg dL<sup>-1</sup>) extract treated groups in comparison with the control (1.40±0.03 mg dL<sup>-1</sup>) group, respectively.

Table 1: Serum protein in the different experimental groups

Variables	Total protein	Albumin	Globulin
	----- (g dL <sup>-1</sup> ) -----		
Control	61.2±0.68	37.1±0.41	22.6±0.71
Low dose	64.0±0.31	38.0±0.21	27.0±1.43***
High dose	68.4±1.39***	39.0±0.32	28.6±1.60***

Values are represented as Mean±SEM. \*\*\*p<0.001 vs control

Table 2: Serum enzyme in the different experimental groups

Variables	ALT	ALP	AST
	----- (IU L <sup>-1</sup> ) -----		
Control	78.6±0.37	87.2±0.75	110.8±0.21
Low dose	76.0±1.4*	80.4±1.10*	110.5±0.32
High dose	55.2±1.3***	70.8±1.02***	110.0±0.22

Values are represented as Mean±SEM. \*p<0.05, \*\*\*p<0.001 vs control, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase, AST: Aspartate aminotransferase

Table 3: Lipid profile in the different experimental groups

Variables	Tc	TG	HDLc (mg dL <sup>-1</sup> )	LDLc	VLDLc
Control	1.40±0.03	0.67±0.02	0.66±0.02	1.03±0.02	0.30±0.01
Low dose	1.30±0.66***	0.40±0.04***	0.69±0.01***	0.79±0.03***	0.18±0.01***
High dose	1.24±0.05***	0.35±0.02***	0.73±0.01	0.67±0.03***	0.17±0.04***

Values are represented as Mean±SEM. \*\*\*p<0.001 vs control, TC: Total cholesterol, TG: Triglyceride, HDLc: High density lipid cholesterol, LDLc: Low density lipid cholesterol, VLDLc: Very low density lipid cholesterol

Table 4: Serum electrolyte in the different experimental groups

Variables	Na <sup>+</sup>	K <sup>+</sup>	Cl <sup>-</sup> (mmol L <sup>-1</sup> )	HCO <sub>3</sub> <sup>-</sup>	Ca <sup>2+</sup>
Control	136.6±0.87	5.74±0.04	102.8±0.49	22.2±0.37	1.08±0.05
Low dose	120.0±0.66***	5.90±0.07***	94.0±0.49***	24.2±0.37**	1.40±0.04***
High dose	100.0±1.85***	6.00±0.14***	82.0±0.75***	25.6±0.75**	1.70±0.05***

Values are represented as Mean±SEM. \*\*p<0.01, \*\*\*p<0.001 vs control, Na<sup>+</sup>: Sodium, K<sup>+</sup>: Potassium, Cl<sup>-</sup>: Chlorine, HCO<sub>3</sub><sup>-</sup>: Bicarbonate, Ca<sup>2+</sup>: Calcium

The triglyceride concentration was significantly (p<0.001) decreased in the low dose and high dose (0.40±0.04 and 0.35±0.02 mg dL<sup>-1</sup>) extract treated groups in comparison with the control (0.67±0.02 mg dL<sup>-1</sup>) group, respectively.

The high density lipoprotein cholesterol concentration was significantly (p<0.001) increased in the low dose and high dose (0.69±0.01 and 0.73±0.01 mg dL<sup>-1</sup>) extract treated groups in comparison with the control (0.66±0.02 mg dL<sup>-1</sup>) group, respectively.

The low density lipoprotein cholesterol concentration was significantly (p<0.001) reduced in the low dose and high dose (0.79±0.03 and 0.67±0.03 mg dL<sup>-1</sup>) extract treated groups in comparison with the control (1.03±0.02 mg dL<sup>-1</sup>) group, respectively.

The very low density lipoprotein cholesterol concentration was significantly (p<0.001) reduced in the low dose and high dose (0.18±0.01 and 0.17±0.04 mg dL<sup>-1</sup>) extract treated groups in comparison with the control (0.30±0.01 mg dL<sup>-1</sup>) group, respectively.

**Analysis of serum electrolytes:** As shown in Table 4, the sodium (Na) concentration was significantly (p<0.001) reduced in the low dose and high dose (120.0±0.66 and 100.0±1.85 mmol L<sup>-1</sup>) group in comparison with the control (136.6±0.87 mmol L<sup>-1</sup>) group, respectively.

The Potassium (K) concentration was significantly (p<0.001) increased in the low dose and high dose (5.90±0.07 and 6.00±0.14 mmol L<sup>-1</sup>) extract treated groups in comparison with the control (5.74±0.04 mmol L<sup>-1</sup>) group, respectively.

The chloride (Cl) concentration was significantly (p<0.001) reduced in the low dose and high dose (94.0±0.49 and 82.0±0.75 mmol L<sup>-1</sup>) extract treated groups in comparison the control (102.8±0.49 mmol L<sup>-1</sup>) group, respectively.

The bicarbonate (HCO<sub>3</sub><sup>-</sup>) concentration was significantly (p<0.001) increased in the low dose and high

dose (24.2±0.37 and 25.6±0.75 mmol L<sup>-1</sup>) groups in comparison with the control (22.2±0.37 mmol L<sup>-1</sup>) group, respectively.

The serum calcium (Ca<sup>2+</sup>) concentration was significantly (p<0.001 and 0.01) increased in the low dose and high dose (1.4±0.04 and 1.70±0.05 mmol L<sup>-1</sup>) extract treated groups in comparison with the control (1.08±0.05 mmol L<sup>-1</sup>) group, respectively.

## DISCUSSION

The effects of crude extracts of dietary clam on biochemical properties has reveals so many wonders as discussed below.

The result reveals that the extract significantly increased Total Protein (TP) and Globulin concentration but did not significantly alter the albumin concentration. TP represent the sum of albumin and globulin. Increased globulin concentration in plasma may imply that the immune system was stimulated (Adebayo-Tayo *et al.*, 2006; Burtis *et al.*, 2006), since globulin is formed almost entirely in the lymphoid tissue and constitute the antibodies used in the immune system. The serum albumin concentration reveal that the liver cells were not damaged (Peters, 1996), since albumin concentration is decreased in both chronic and acute liver disease (Burtis *et al.*, 2006).

The serum enzyme results revealed that the hepatocytes or liver tissues in general benefited from the extract administration since ALT was significantly decreased 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 (Panteghini, 1990; Darling, 2011). Therefore, lowered serum ALP confirms that the extracts may not have damaging effects on the liver cells and bone and this confirms the result of the serum albumin.

The lipid profile result has reveal that the high presence of omega-3 fatty acids in the clam extract is of immense benefit because it has natural in-built anti-oxidants and have been found to lower TG and TC concentration as observed in the result (Kakafika *et al.*, 2011). Clam have also been reported to contain less saturated fat (Robson, 2006).

As observed from the result the extract was shown to significantly increase high density lipoprotein cholesterol (HDL-c) being the good cholesterol (Arai *et al.*, 2000) showing to what extent the extract is of benefit. Low density lipoprotein cholesterol (LDL-c) and Very low density lipoprotein cholesterol (VLDL-c) were significantly reduced following extract administration, this may possibly be due to lowered TC and TG (Ndem *et al.*, 2008; Mora *et al.*, 2011), sinceLDLc and VLDLc, are calculated from TC and TG, respectively (Friedewald *et al.*, 1972). These finding point to the fact that consumption of dietary clam would not predispose to atherogenesis or dyslipidaemic conditions.

The serum electrolytes result reveals that clam extract reduced sodium ion concentration, suggesting that dietary clam has low sodium content. Similar effect of the extracts on chloride ion concentration is not surprising because increased sodium reabsorption is normally accompanied with chloride ion reabsorption (Ganong, 1991). Potassium ion is the major cation inside the cell. Clam extract increased potassium ion concentration this could probably be mediating its effect via the Na<sup>+</sup>/K<sup>+</sup> pump on the cells membrane which pumps these ions in opposite direction (Kaplan, 2002), or probably the extracts are rich in potassium ion (Walker, 1977). Studies have also shown that ionic content of clam can be affected by the source of water the animal is harvested from (Adebayo-Tayo *et al.*, 2006). There was an increase in HCO<sub>3</sub><sup>-</sup> concentration in plasma. Bicarbonate is produced by the pancreas in order to neutralize food mixed with stomach acid leaving the stomach and entering the small intestine. It maintains the acid-base buffering system of the blood. The results also revealed elevated plasma Ca<sup>2+</sup> level caused by dietary clam. This could be explained from the fact that generally, dietary clam is rich in Ca<sup>2+</sup> (Rice, 2004; Zemel *et al.*, 2005). In conclusion consumption of clam extract is of great benefit because it prevents one from being predispose to atherogenesis or dyslipidaemic conditions they also offers desirable effects on serum electrolytes.

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