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Ameliorative Effect of Aqueous Extract of Dried Seeds of *Persea americana* on Lipid Profile of Adult Wistar Rats on High Cholesterol Diet

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Abstract

Persea americana is a major tropical fruit that has been known to contains phytosterol, a bioactive compound which may play a vital role in the reduction of total cholesterol and LDL cholesterol in humans. Hence, this study investigated the therapeutic and protective effect of aqueous extract of the seed of *Persea americana* on the cholesterol, triglyceride, low density lipoprotein, high density lipoprotein on the blood and the histological changes induced by high cholesterol diet on the heart and blood vessels of adult wistar rats. Twenty-five (25) adult wistar rats were divided into five (5) groups of five each (Group A, B, C, D, E). Group A served as negative control and received growers marsh and water only, Group B served as the positive control and received growers marsh mixed with egg yolk, Group C, and D served as treatment groups and received growers marsh mixed with egg yolk and extract of *Persea americana* at doses of 250mg/kg and 500mg/kg respectively. Experimental animals in Group E also received growers marsh mixed with egg yolk but the administration of the extract commenced after the fourth week. At the end of 6 weeks, the rats were sacrificed, blood was collected from each rats in the different groups into plain bottles for lipid profile. The heart and blood vessel of rats were harvested and fixed using 10% formal saline for histological study. The rats that were fed with high cholesterol without *Persea americana*, exhibited atherosclerosis in the intima of coronary arteries of the heart and blood vessels. Myocardiac injury was also observed though it was not severe enough to cause myocardiac infarction. Administration of *Persea americana* seed extract helped to ameliorate these changes that were observed in the heart and blood vessels as a result of high cholesterol diet in-take.

Key words: *Persea americana*, lipid profile, high cholesterol diet

Introduction

Avocado (*Pearsea americiana*) is a major tropical fruit as it contains vitamins that are fat soluble and proteins unlike other fruits [1]. The pulp is known to contain about 13.5%-24% lipids, 0.8%-4.8% carbohydrate, 1.0-3.0% protein, 0.8%-1.5% ash, 1.4%-3.0% fiber and energy density between 140 and 228kcal [2]. The presence of a significant level of Potassium (33mg/100g) makes it a good regulator of muscle activity and offers protection against cardiovascular diseases [3]. It is also recognized that it serves as a good source of glutathione which is a powerful antioxidant that acts on potentially carcinogenic compounds [4]. In addition to these, it contains phytosterol, a bioactive compound which is known to play a vital role in the reduction of total cholesterol and LDL cholesterol in humans [5,6] by inhibiting intestinal absorption of cholesterol and decreasing its hepatic synthesis. Avocado contains other important bioactive compounds such as β-sitosterol known for its special effects on immunity therefore helps in the treatment of diseases such as cancer, HIV and infections by suppressing carcinogenesis and strengthening the immune system [7]. β-sitosterol has also been reported to aid weight loss by reducing compulsive eating binge and fat accumulation in the abdominal region [8,9]. The ability of avocado to play a role in cholesterol reduction may be useful in the treatment of cardiovascular

diseases such as hypertension. Hence, this study aimed at evaluating the changes caused by *Pearsea americiana* on the histomorphology of the heart and blood vessels of adult wistar rats that were fed with egg yolk.

Materials and Method

Preparation of plant extract

The fruits were cut open with a sharp knife to harvest its seeds. These seeds were then chopped in small pieces and sun-dried for two days. They were then mashed into coarse powder, using mortar and pestle and 500g of the powder was then soaked in 1.5 liters of distilled water for 48 hours and then filtered with cheesecloth sieve. The aqueous extract filtrate was then concentrated in a rotary evaporator and further dried in an oven of 30^0C for 3 days. The dried extract was then stored within an air-tight bottle in a refrigerator until required for the study.

Chemical Reagents

Ethanol (BDH chemical LTD Poole England), formaldehyde (Sigma Aldrich Laboratory Chemical LTD GMBD, Germany) and xylene (BDH chemical LTD Poole England) were used for this study while Haematoxylin was prepared by adding 100mls of absolute alcohol, 100mls of glycerin, 100mls of distilled water, 100mls of acetic acid and 10g of potassium dichromate. Eosin was also prepared by adding 95% alcohol 640mls, 160mls of distilled water and Eosin 2g.

Experimental Animals

Twenty-five (25) adult Wistar rats of both sexes weighing between 200-280g were used for this study. The rats were purchased from Department of Anatomy, School of Basic Medical Sciences University of Benin, Benin City, Nigeria and kept in cages in the departmental animal house where the research was carried out. They were acclimatized for two weeks, fed with growers' marsh and water *ad libitum.*

Ethical Consideration

Experimental protocol and procedure was approved by the Animal ethical committee of University of Benin, Benin city, Edo State, Nigeria which also conformed to guidelines in the Principle of Laboratory Animal care (NH 1985)

Experimental Design

Animals were divided into five (5) groups of five of rats each as shown in Table 1 below.

At the end of 6 weeks treatment, rats were weighed then sacrificed by anesthesia using chloroform.

Histological Procedure

Processing of tissues using routine biopsy method was employed. Tissue sections were treated with traditional haematoxylin and eosin stains. Tissue blocks from the heart and blood vessels were fixed in 10% neutral formalin after which they were dehydrated using alcohol and cleaned in xylene. The samples were embedded in paraffin wax and thin sections cut at 5 microns. The sections were then stained with haematoxylin for 15 minutes, differentiated with 1% acid alcohol, counter stained in eosin for 2 mins and mounted with DPX. The sections were viewed under the microscope at x400 magnification and photomicrographs taken.

Blood Lipid Profile

Blood samples were also obtained from the descending abdominal aorta and homogenized in a plain bottle for lipid profile screening. The blood samples collected were centrifuged at 3000 revolution/minute using a table-top centrifuge (Shanghai Surgical Instrument Factory, Shanghai, China) at 37◦C for 15minutes to separate the serum. Total cholesterol (TC), triglyceride (TG), low density lipoproteins (LDL), high density lipoprotein (HDL) were assayed using Randox Diagnostic Kits.

Table 1. Summary of Different Groups

Groups	Treatment administered	Amount of extract	Purpose
GROUP A	Growers marsh + water only	No extract	Negative control
GROUP B	Growers marsh + egg yolk + water	No extract	Positive control
GROUP C	Growers marsh +egg yolk + water	250mg/kg of the extract	Protective effect with low dose
GROUP D	Growers marsh + egg yolk+ water	500mg/kg of the extract	Protective effect at high dose
GROUP E	Growers marsh mixed with egg yolk + water (for 4weeks) before administering extract	500mg/kg of the extract	Therapeutic effect at high dose

Table showing the grouping, treatment and the purpose of each group.

Statistical Analysis

Results were presented as Mean ± Standard Error of Mean (SEM) using Microsoft Excel (2010) and Statistical Package for Social Sciences (SPSS) version for windows 7. Appropriate analyses were done using one way Analysis of Variance (ANOVA). P value < 0.05 was taken as statistically significant.

Results

Table 2 showed the phytochemical result which revealed the presence of tannins, saponnins, flavonoids and alkaloids in aqueous seed extract of avocado. Some of these phytochemicals such as flavoids have been reported to be used in the treatment of allergic, inflammatory, thrombolytic and cardiovascular disorders by acting on the arachidonic acid [10]. They were also reported to be very powerful antioxidant that can help to reduce LDL, cholesterol against degradation and reduces platelet aggregation [11,12,13].

Table 3 showed the lipid profile of animals in the control and experimental groups. The mean cholesterol levels of the group expressed in mg/dL for the control group was 89.50±0.50; Group B (without Extract) 102.25±0.70; Group C (250mg/kg of body weight) 91.00±1.00; Group D (500mg/kg of body weight) 112.20±0.70 and Group E (500mg/kg of body weight after four weeks) 89.75±1.00. The mean serum concentration of triglycerides (TG), low density lipoproteins (LDL) and high density lipoproteins (HDL) of animals administered the same concentration of extract were also shown in Table 2.

Result showed that the concentration of TG in the different groups expressed in mg/dl was: Group A 65.00±0.90; Group B 66.00±0.70; Group C 72.75±0.90; Group D 79.00±0.70 and Group E 87.00±0.90. This value increased along the group but was statistically significant in groups C, D and E when compared to that of the control group at P < 0.05. The concentration of HDL expressed in mg/dL was 41.00±0.40 for animals in Group A, 46.75±0.70 for that of Group B, 44.50±0.30 Group C, 45.60±0.50 Group D and 42.00±0.30 for that of Group E. These values were not statistically significantly different in all the groups. While the LDL significantly increased in Groups B and D but reduced in other groups compared to that of the control group**.**

Table 2. Phytochemical Profile

Glycoside	*Persea Americana* seed (%)
Tannins	21.68±0.03
Saponins	39..03±0.02
Flavonoids	23.00±0.01
Alkaloids	10.43±0.20
Cyanogenic	5.86±0.12

Values in the table are represented as mean ±SEM (n=3)

Table 3. Plasma Lipid Profile

GROUPS	CHOLESTEROL (mg/dl)	TRIGLYCERIDES (mg/dl)	HDL (mg/dl)	LDL(mg/dl)
Group A	89.50 ± 0.50^{ace}	65.00 ± 0.90^{a}	41.00 ± 0.40^{a}	35.50 ± 0.50^{ac}
Group B	102.25 ± 0.70^{bd}	66.00 ± 0.70^{ab}	46.75 ± 0.70^{a}	42.50 ± 1.00^{b}
Group C	91.00 ± 1.00^{ce}	72.75 ± 0.90^{c}	44.50 ± 0.30^{a}	31.75 ± 0.80^{c}
Group D	112.20 ± 0.70^{d}	79.00 ± 0.70^{d}	45.60 ± 0.50^{a}	50.80 ± 0.30^{d}
Group E	89.75 ± 1.00^{e}	87.00 ± 0.90^{e}	42.00 ± 0.30^{a}	30.75 ± 0.10^{ce}

Values are represented as mean ± SEM (n=4). Values with different superscripts are significantly different (P<0.05) at 95% confidence interval.

Histomorphological Studies of the Heart

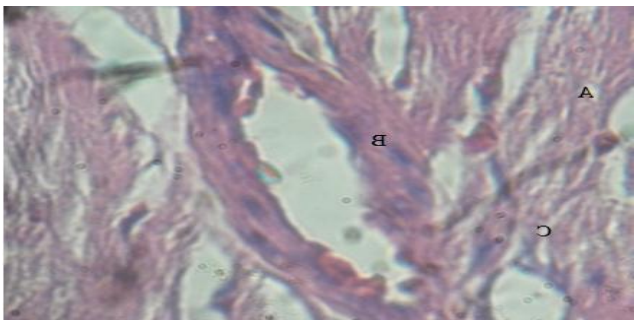

Fig. 1. Control. Rat heart composed of A, bundles of myocardiac fibres, B, coronary vessel and C, interstitial space (H&E x 400).

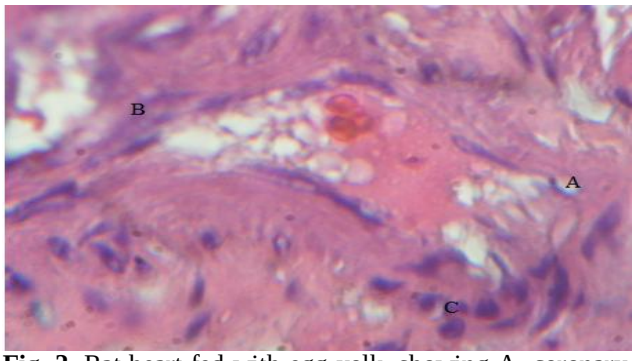

Fig. 2. Rat heart fed with egg yolk, showing A, coronary artery with patchy intimal fat vacuoles, B, luminal occlusion and C, mural infiltrates of chronic inflammatory cells (H&E x 400).

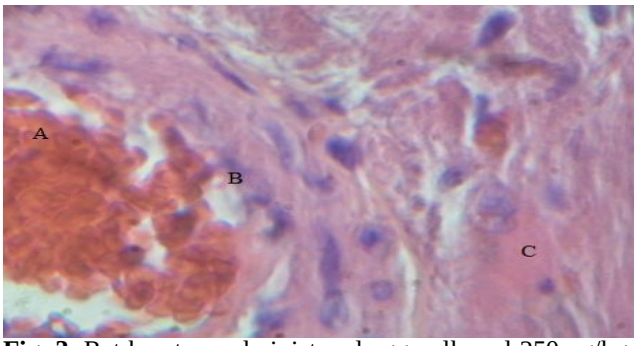

Fig. 3. Rat heart co-administered egg yolk and 250mg/kg P. Americana showing A, mild coronary vascular congestion, B, fairly normal intima and C, normal myocardium (H&E x 400).

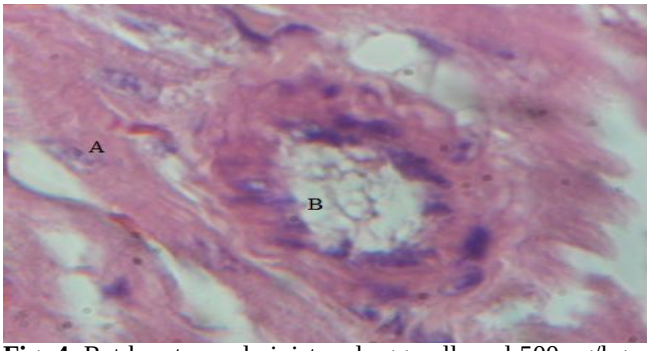

Fig. 4. Rat heart co-administered egg yolk and 500mg/kg P. Americana showing A, fairly normal myocardium and B, fairly normal vascular intima (H&E x 400).

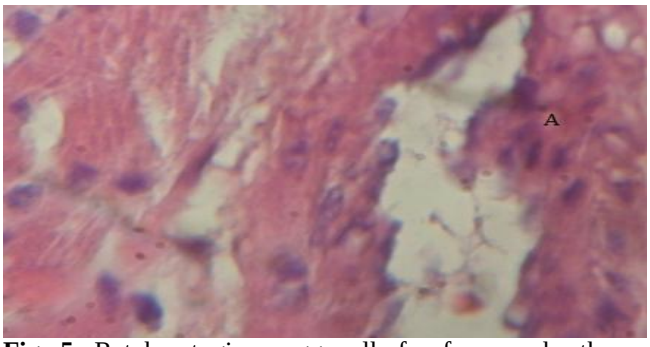

Fig. 5. Rat heart given egg yolk for four weeks then 500mg/kg extract for 2 weeks showing A, focal medial hypertrophy (H&E x 400).

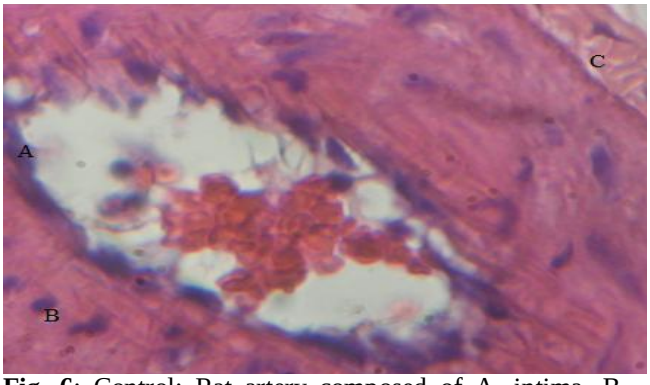

Fig. 6: Control: Rat artery composed of A, intima, B, media and C, adventitia (H&E x 400)

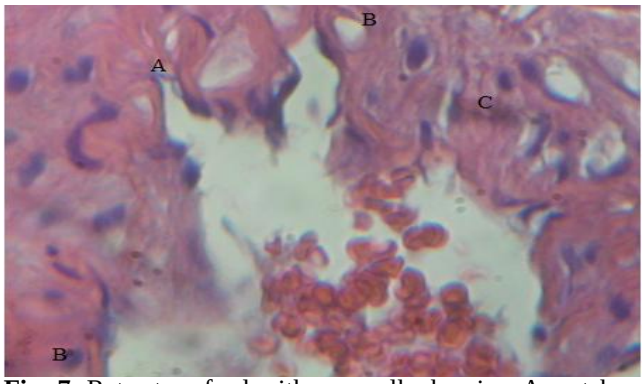

Fig. 7. Rat artery feed with egg yolk showing A, patchy intimal ulceration, B, fat vacuoles, C, medial hypertrophy and D, mild infiltrates of chronic inflammatory cells (H&E x 400)

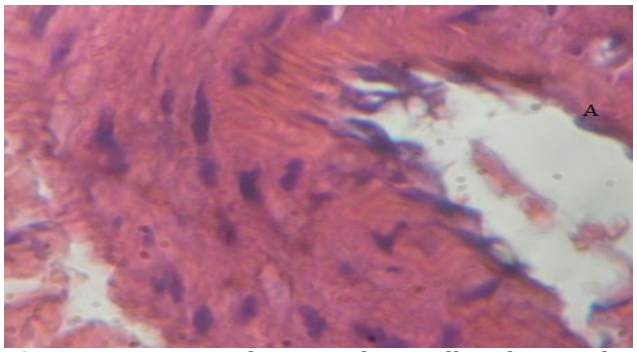

Fig. 8. Rat artery co-administered egg yolk and 250mg/kg extract showing A, normal vascular intima (H&E x 400).

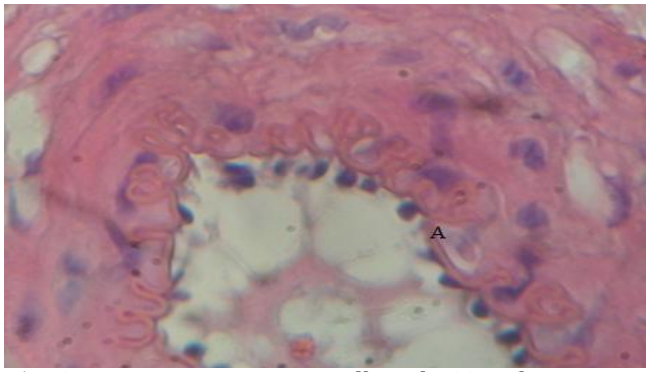

Fig. 9. Rat artery given egg yolk and 500mg/kg extract showing A, normal vascular intima (H&E x 400).

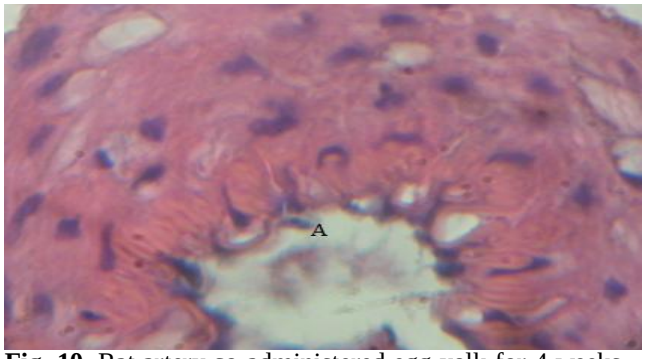

Fig. 10. Rat artery co-administered egg yolk for 4 weeks, then 500mg/kg extract for 2 weeks showing A, focal intimal erosion (H&E x 400).

Discussion

Atherosclerosis, strongly related to cardiovascular disease has been reported to be caused by abnormal high dietary cholesterol [14]. This study evaluated the changes caused by aqueous seed extract of *Persea Americana* on lipid profile and histomorphology of the heart and blood vessels in adult wistar rats fed with egg yolk.

This was in consonance with the report that plasma TG increased after consumption of 250g of avocado for 4 weeks [15] and suggested that it may be due to high carbohydrate content of diet taken by the subjects [16] but was not in agreement with other researchers that reported a decrease in plasma TG linking it to the role of lipoprotein lipase enzyme that breaks down long-chain saturated fatty acids [17][18] and fatty acid released by the TG are incorporated into HDL thereby increasing the level of HDL in plasma [19][20]. This is a suggested mechanism whereby HDL increased and LDL decreased in this study as oleic acid present can reduce plasma concentration of LDL, being the major substrate for Acyl-CoA. The Liver enzyme, cholesterol acyltransferase (ACAT) catalyses the formation of cholesterol esters from cholesterol, free cholesterol is rapidly esterified which does not cause the suppression of LDL receptors but rather favours the uptake of LDL, thereby reducing its plasma concentration.

Ingestion of egg yolk induced pathological lesions of atheroscerosis on the coronary vascular wall and branches of the aorta, these effects were ameliorated by administration of avocado pear as shown in this study.

In conclusion, this study showed that ingestion of high cholesterol diet such as egg yolk caused atherosclerotic lesions on the intima of the coronary vessels of the heart and branches of the aorta and also consequent myocardiac injury although the damages that occurred in the blood vessels might not be severe enough to cause myocardial infarction. However treatment with avocado revealed its protective and high potency therapeutic effect with minimal injury on both the heart and aorta.

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