Effects of Aqueous Extract of *Persea americana* (Mill.) Leaf on the Myocardium of Left Ventricle of High Salt Fed Adult Wistar Rat

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Abstract

Introduction This study was designed to show that *Persea americana* extract possess the ability to protect the myocardium of left ventricle against injury caused by high salt diet in adult Wistar rats.

Method Forty healthy Wistar rats of both sexes weighing 120–150 g were randomly assigned into 8 groups of 5 rats each (Groups A, B, C, D, E, F, G and H). Rats in groups A, F, G and H were fed with standard laboratory pellets, while groups B, C, D and E were fed on the high-salt diet for four weeks. Concomitantly, daily administration of 50 mg kg-1, 100 mg kg-1 and 150 mg kg-1 of the *Persea americana* extract were given orally to groups C&F, D&G and E&H respectively while rats in groups A and B were administered distilled water. The rats were sacrificed under ketamine anesthesia (30mg/kg i.m). The left ventricle of the heart was excised, processed in paraffin wax and stained with haematoxylin and eosin and Verhoeff-Van Gieson stains. One-way ANOVA was used to analyze data, followed by Student Newman-keuls (SNK) test for multiple comparison.

Result Results revealed that there was statistically significant ($p < 0.05$) difference in body weight change across all experimental groups; which was significantly lower in high salt fed groups. It was revealed that there were morphological alterations in the myocardium of left ventricle in group B while *Persea americana* protected myocardium in other experimental groups.

Conclusion In conclusion, high salt diet induced myocardium alterations which were significantly protected by oral administration of *Persea americana* extract.

Keywords
- myocardium
- high salt fed
- Persea Americana
- left ventricle

Introduction

Cardiovascular disease is a class of disease that involves the heart, the blood vessels (arteries, capillaries, and veins) or both.[1] Cardiovascular disease refers to any disease that affects the cardiovascular system, principally cardiac disease, and peripheral arterial disease.[2] Though the causes of cardiovascular disease are many but hypertension and atherosclerosis are the most common. Also there are numbers of physiological and morphological changes that alter cardiovascular function with aging and this may lead to increased risk of cardiovascular disease even in healthy asymptomatic individuals.[3]

High salt intake has deleterious effect on the body system, one of the main organ systems vulnerable to the adverse effects of excessive sodium in the diet is the cardiovascular system; excess sodium predisposes to high blood pressure (BP);[4] Raised BP is a major risk factor for Left ventricular hypertrophy (LVH)[5]

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and LVH is an important predictor of CVD.[6] Reduction in salt intake may have effect a direct effect on LVH independent of BP.[7]

Various phytochemicals thave been found in *persea americana* leaf these include: saponins that has hemolytic activity and cholesterol binding properties,[8] Tannins noted for astringency and bitter taste, hastening the healing of wounds and inflamed mucus membrane,[9] Flavonoids that prevent oxidative cell damage and have strong anticancer activity and protect against all stages of carcinogenesis, Alkaloids that are used as basic medicinal agents for their analgesic and bactericidal effects,[10] and Phenols which have been extensively research as disease preventive agents.[11]

It has been established that high salt diet causes damage to the cardiovascular system leading to cardiovascular disease especially hypertension; components like such as tannin, flavonin and alkaloids with suggested antihypertensive effects have been discovered in avocado leaf aqueous extract and its use for treatment of hypertension has also been documented, but the effect of avocado aqueous leaf extract on the microanatomy of the myocardium of left ventricle when exposed to high salt has not been fully documented. This study aimed at providing complementary information on the cardio-protective effect of avocado (*Persea americana*) aqueous leaf extract on the myocardium of left ventricle of high salt-fed Wistar rats.

Materials and Methods

Animal Care and Management

Forty adult Wistar rats of both sexes between 120–150 g obtained from Animal Holdings of Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile-Ife were used for this research. The animals were housed in Animal Holdings of Basic Medical Sciences, Obafemi Awolowo University, Ile-Ife. They were maintained on standard laboratory pellet before commencement of the experiment and clean water was provided ad *libitum.* They wsere maintained in natural day and night cycle and temperature. The rats were assigned into eight groups of five animals each.

Plant Material and Preparation of Extract

Fresh avocado (*Persae americana*) leaves were obtained from the avocado fruit trees in the town of Ilesa, Osun State. The leaves specie was authenticated by a taxonomist in the Department of Botany Obafemi Awolowo University, Ile-Ife; the collected plant samples were washed thoroughly with running tap water. The leaves were air-dried at room temperature, the dried leaves were pulverized using an electric blender, soaked in distilled water and kept at 4°C for 48 hours with occasional shaking. The mixture was then filtered and the filtrate was concentrated to gel at 40 $\pm$ 1°C in a rotary evaporator, and then freeze dried. This crude extract was used without further purification. Required dosage was prepared from the freeze dried extract.

Preparation of High Salt Diet

High salt diet containing 8% sodium chloride was prepared specially by replacing 0.3% sodium chloride-containing standard diet with 8% sodium chloride.

Animal treatment

Group A was the control, group B was negative control, while groups C, D, E, F, G and H were the test groups. Rats in groups A, F, G and H were fed with standard laboratory pellets, while groups B, C, D and E were fed on the high-salt diet for four weeks; concomitantly, daily administration of 50 mg kg-1, 100 mg kg-1 and 150 mg kg-1 of *persea americana* extract were giving orally to groups C&F, D&G and E&H respectively. The extract solution was administered orally, using oral cannula and duration of the experiment was 4 weeks.

Measurement of Body Weight

The body weights of the animals were taken using a top loader weighing balance.

Sacrifice of Animals

At the end of the experiment, the rats were sacrificed under ketamine anesthesia. The left ventricles of the heart were excised and weighed.

Histological and Histochemical Analyses

The excised left ventricle was fixed in neutral buffered formalin for 24 hours, and processed using paraffin wax embedding method. Sections of 5 µm thicknesses were produced from the paraffin embedded tissues. Haematoxylin and Eosin method was used to demonstrate the general histoarchitecture of the left ventricle and Verhoef Van-Giesons Stain was used for elastic-collagen fiber differential staining.

Determination of Relative Heart Weight (%)

At sacrifice, the heart weight was determined using a top loader sensitive balance (Mettler-Toledo Garvens GmbH, Giesen, Germany). The relative weight of the heart (%) to the body weight at sacrifice was evaluated.

Photomicrography

Stained sections were viewed under a LEICA research microscope (LEICA DM750, Switzerland) with digital camera attached (LEICA ICC50) and digital photomicrographs were taken at various magnifications.

Quantification of Staining Intensity

Image analysis and processing for Java (ImageJ), was used to analyze and quantify Verhoeff- Van Gieson staining intensity. Imported RGB images were converted to grayscale images on ImageJ. The software quantifies staining intensity by measuring the pixel value of each pixel in grayscale images following threshold of areas of staining activity and converting the pixel value to brightness value or gray value, in a scale of 0 to 255 from less brighter (that is more intensity) to more brighter (that is less intensity).

Statistical Analysis

One-way ANOVA was used to analyze data, followed by Student Newman-Keuls (SNK) test for multiple comparisons. GraphPad Prism 5, Version 5.03 (GraphPad Software, Inc., La Jolla, CA) was used as the statistical package. Statistically significant difference was set at $p < 0.05$.

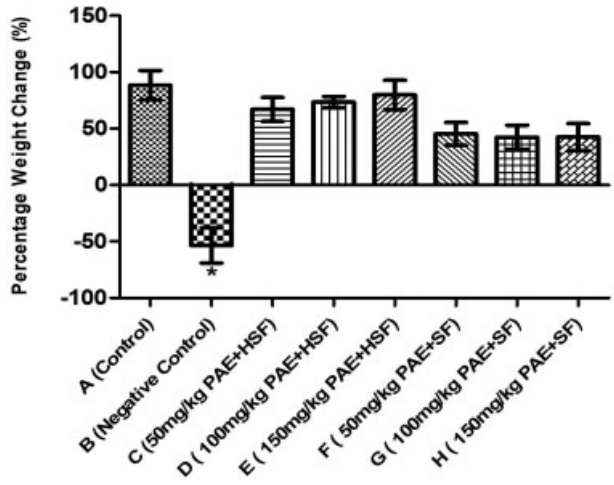

Fig. 1 Effect of *persea americana* on Body Weight Change of Rats fed with High Salt Diet. $n = 8$, values are expressed as mean ± SEM. *statistical difference relative to control at $p < 0.0001$.

Results

Body Weight Change

One way ANOVA revealed that there was statistically significant difference in body weight change across all experimental groups ($p < 0.05$). Post hoc analysis showed that percentage body weight was significantly lower in high salt fed groups; this reduction was reversed in a dose dependent fashion across all treatment groups (►**Fig. 1**).

Histological Findings

Histology showed well arranged clearly branched myocardial fiber with well placed nucleus and no alterations in A Control, E (►**Fig. 2e**), F (►**Fig. 2f**), G (►**Fig. 2 g**), and H (►**Fig. 2h**); Mild distortion of myocardial fiber branching arrangement in C (►**Fig. 2c**) and D (►**Fig. 2d**). The cross-banding pattern of cardiac cells was distorted and there was degeneration of myocardial fiber, and nuclear displacement in high salt-fed group (►**Fig. 2b**). The increase in collagen fiber deposit was evident on histological sections of left ventricle of the rats in the high salt-fed group (►**Fig. 3b**). Collagen content measured by digital densitometry is shown as result of collagen content in each specimen (►**Fig. 4**). The amount of elastic fibers in ►**Figs. 3b–c** were reduced when compared with the control group (►**Fig. 3a**). Elastic fiber in ►**Figs. 3d–h**, were same as in 3a as evident in the intense staining intensity and also elastic fiber content measured by digital densitometry is shown in each specimen (►**Fig. 5**).

Discussion

The effects of concomitant treatment with *Persea americana (Mill)* aqueous extract (PAE) and sodium (NaCl) load via high salt feed (HSF) on the left ventricle of Wistar rats were investigated in this study.

One of the main organ systems vulnerable to the adverse effects of excessive sodium load in the diet is the cardiovascular system;[12] this has been demonstrated in this study.

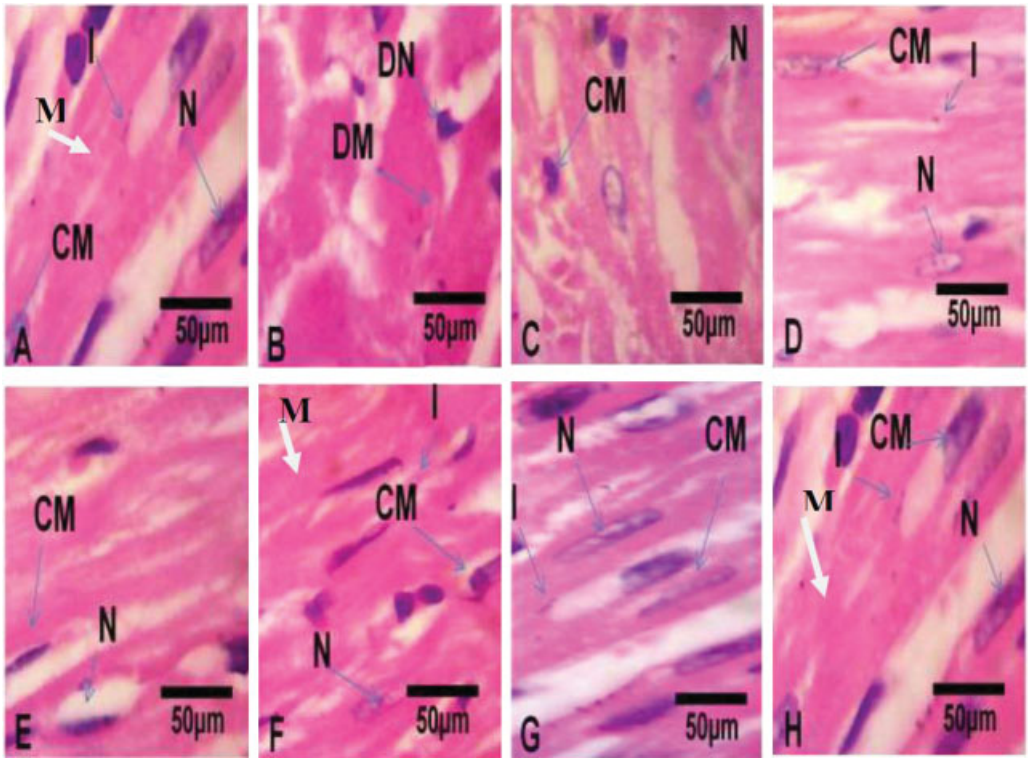

Fig. 2 Shows photomicrographs of left ventricles of experimental group of Wistar rats: A (Control), B (Negative Control), C (50 mg/kg PAE + HSF), D (100 mg/kg PAE + HSF), E (150 mg/kg PAE + HSF), F (50 mg/kg PAE + SF), G (100 mg/kg PAE + SF), H (150 mg/kg PAE + SF) I - Intercalated disc; M – Muscle fiber; CM – cardiomyocytes, N – Nucleus; DM – Degenerated muscle fiber; DN – Displaced Nucleus (H&E x400).

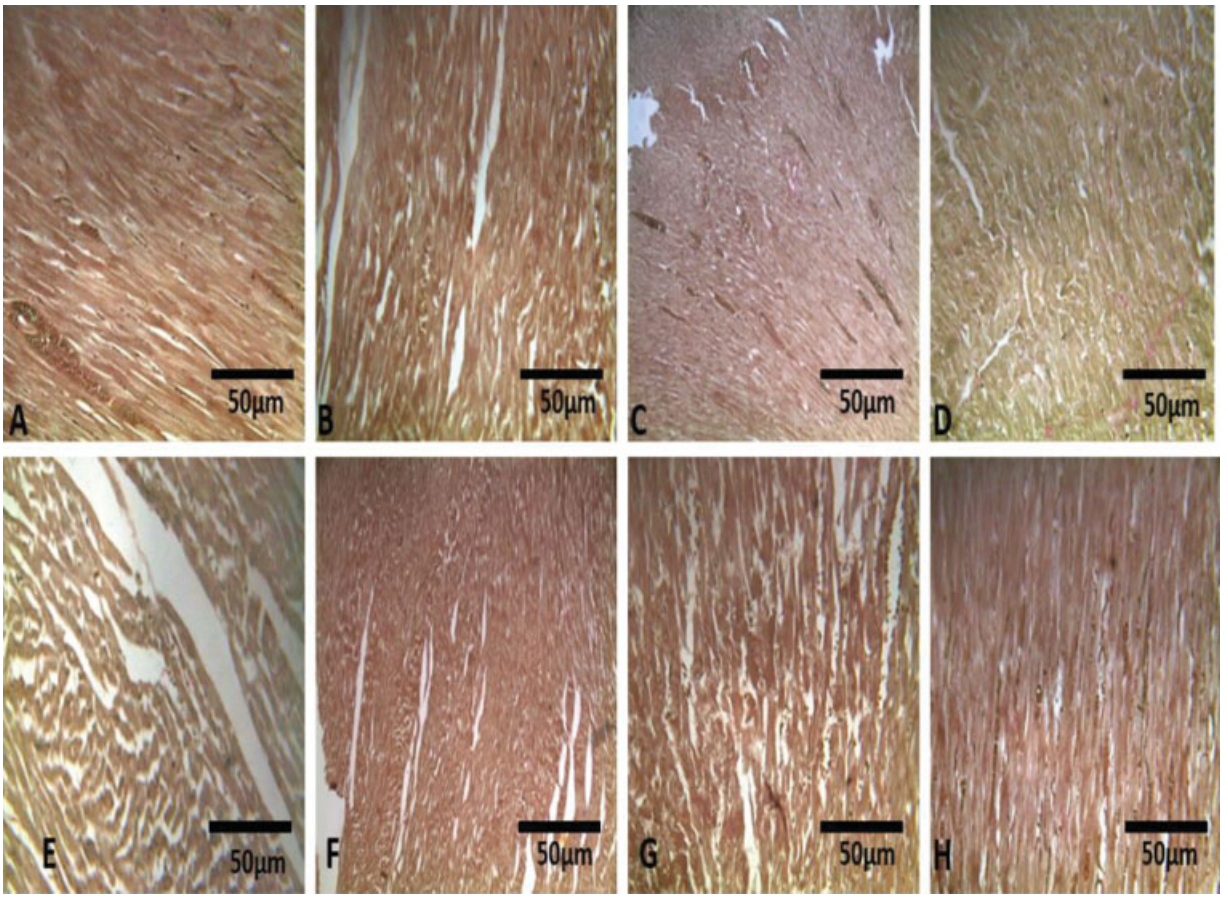

Fig. 3 Shows photomicrographs of left ventricles of experimental group of Wistar rats: A (Control), B (Negative Control), C (50 mg/kg PAE + HSF), D (100 mg/kg PAE + HSF), E (150 mg/kg PAE + HSF), F (50 mg/kg PAE + SF), G (100 mg/kg PAE + SF), H (150 mg/kg PAE + SF) dark spots (elastic fiber), reddish brown color (collagen fiber) (Verhoeff's-Van Gieson's x100).

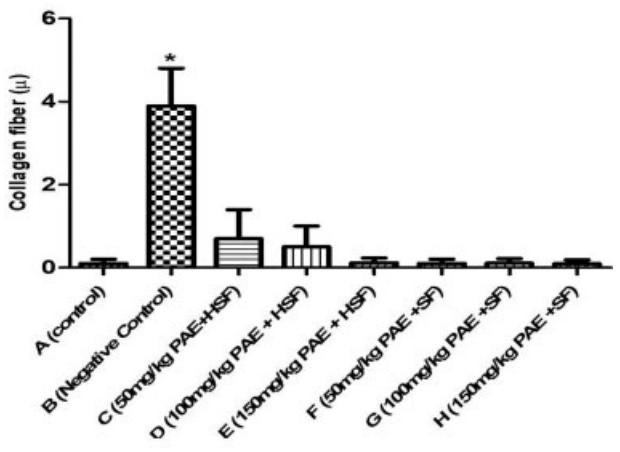

Fig. 4 Elastic fiber content measured by digital densitometry is shown as a result of collagen content in each specimen. Effect of *persea americana* on area of Elastic fiber was determined by digital densitometry recognition using Image J software. *untreated group (Negative control).

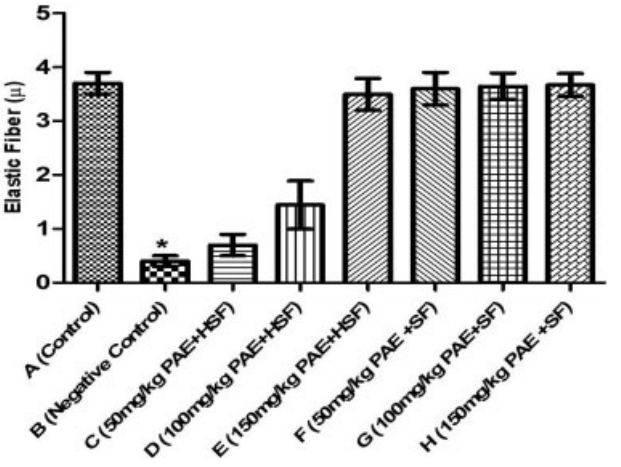

Fig. 5 Collagen content measured by digital densitometry is shown as a result of collagen content in each specimen. Effect of *persea americana* on area of collagen accumulation was determined by digital densitometry recognition using Image J software. *untreated group (Negative control).

A high-sodium diet increases potassium excretion by increasing distal sodium delivery. It has been suggested that potassium depletion inhibits insulin secretion and is associated with glucose intolerance.[13,14]

Another study stated that salt load results in insulin resistance that results in abnormal glucose uptake and energy release.[15] The reduction in weight as documented by this present study may probably be as a result high salt

induced ulceration of the stomach; this is in support of a previously done study.[16] The weight gain in groups treated with HSF+ graded doses of PAE in a dose dependent fashion when compared with negative control group could be as a result of increased glucose uptake.

It has been reported that aqueous extract of *Persea americana* causes reduction of body lipid;[17] it was observed in this study that there was weight loss in groups treated with SF + PAE when compared with control group; this may be as a result of breakdown of body lipid probably by increased catabolism of lipid in the adipose tissue.

This study showed that high salt diet induced left ventricular hypertrophy; in control group, the micrograph showed normal histology of the myocardium with regular striations without histological alterations; these striations were distorted in the negative control group following the administration of high salt feed (4 weeks). High salt induced hypertrophy in the negative control group probably because excess sodium intake induces lactic acid formation by switching on the sodium-induced cellular anaerobic thermogenesis (SICAT).[18] Also, reported that switching on SICAT may result in acid-induced cell death.

High salt may have also induced hypertrophy in the negative control group by enhancement of rennin–angiotensin system probably by increased rennin and aldosterone production that result in vasoconstriction. Increased Angiotensin Converting Enzyme (ACE) activity results in increased plasma level of angiotensin II; angiotensin II has been reported to cause cardiac remodeling.[19]

It was also observed that collagen fiber deposit in negative control group was higher than that of the control and extract alone groups while there was a marked decrease in elastic fiber deposit in negative control group when compared with all groups, elastic fiber deposition is similar in the control and extract alone groups. Ushiki[20] reported that collagen fiber functions to provide structural support while elastic fibers provide resilience and contractility; high collagen fiber deposition in negative control group as observed in this study is thus suggestive of reduced contractility and resilience, and cellular degeneration.

The protective effect of PAE against myocardial damage in this study can be explained by its Angiotensin Converting Enzyme (ACE) inhibition.[21] PAE probably induced inhibition of ACE that lead to reduction in production of aldosterone from the adrenal glands, natriuresis, and decreased plasma volume. The inhibition of ACE activity resulted ultimately in decrease in reabsorption of water and sodium from the distal convoluted tubule resulting in natriuresis and decrease in plasma volume. This may be the mechanism underlying prevention of left ventricular hypertrophy in this study.[22]

Conclusion

The results of this study indicate that high salt diet caused significant histomorphological changes on myocardium of the left ventricle of rats as evidenced by myocardiocyte distortion and ventricular hypertrophy. *Persea americana* extract has protective properties against these perturbations probably via inhibition of renin-angiotensin-aldosterone system. Further studies on the effect of *persea americana* extract on markers of activity of renin-angiotensin- aldosterone system will be required to support this suggestion.

Conflict of Interest

There was no conflict of interest among the authors and every necessary detail was agreed upon during the preparation of the work.

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