

# Biochemical Effects of Aqueous Extract of *Persea americana* (Mill) on the Myocardium of Left Ventricle of High Salt–Fed Adult Wistar Rats

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## Abstract

**Background.** The cardioprotective effects of *Persea americana* extract was investigated on biochemical activities of high salt–fed adult Wistar rats in this study. **Method.** Forty healthy Wistar rats of both sexes weighing 120 to 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 4 weeks. Concomitantly, daily administration of 50, 100, and 150 mg/kg of the *P americana* extract were given orally to groups C and F, D and G, and E and H, respectively, while rats in groups A and B were administered distilled water. Blood samples were taken by cardiac puncture; concentration of sodium ion, potassium ion, nitric oxide, and activity of lactate dehydrogenase were determined. One-way analysis of variance was used to analyze data, followed by Student-Newman-Keuls (SNK) test for multiple comparison. **Results.** Results revealed that concentration of potassium ion and nitric oxide was significantly lower ( $P < .05$ ) in high salt–fed groups. Sodium ion concentration and activity of lactate dehydrogenase were higher in high salt–fed group while *P americana* prevented biochemical perturbations in other experimental groups. **Conclusion.** In conclusion, high salt–diet induced biochemical alterations which were significantly protected by oral administration of *P americana* extract.

## Keywords

high salt–fed rat, *Persea americana*, lactate dehydrogenase, nitric oxide, sodium ion, potassium ion

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Dietary salt is ionic compound composed of sodium chloride, which is 40% sodium and 60% chloride. Though salt is an essential electrolyte, 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.<sup>1</sup> Raised blood pressure is a major risk factor for left ventricular hypertrophy,<sup>2</sup> which in turn is an important predictor of cardiovascular disease.<sup>3</sup> The reduction in salt intake may have a direct effect on left ventricular hypertrophy independent of blood pressure.<sup>4</sup> In experimental animals, it has been established to cause endothelial dysfunction,<sup>5</sup> increased plasma brain natriuretic peptide concentration and perivascular inflammation,<sup>6</sup> as well as deactivation of adenosine triphosphate–sensitive potassium channels and Na<sup>+</sup>-K<sup>+</sup> adenosine triphosphatase pump on the vascular smooth muscle membrane.<sup>7</sup> Current estimates suggest that a large proportion of the population in many developing countries rely heavily on traditional

practice and medicinal plants to meet primary health care needs,<sup>8</sup> the functionality of which is owed to phytochemical components of these plants. Avocado (*Persea americana*) aqueous leaf extract has long been used for a variety of cardiovascular conditions; especially hyperlipidemia.<sup>9</sup> It has been reported to have antihypertensive action; it is indicated to cause vasorelaxation.<sup>10</sup> Avocado (*P americana*) aqueous leaf extract has been reported to be related to decrease of blood pressure in patients with increased systolic pressure.<sup>11</sup> Avocado (*P americana*) aqueous leaf extract has been found to be antihypertensive, this has been observed in healthy

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normotensive and hypertensive rats.<sup>11</sup> Research has shown that avocado (*P americana*) aqueous leaf extract caused transient but significant reduction ( $P < .05$  to  $P < .001$ ) in the systemic arterial blood pressure and heart rates of the anesthetized normotensive and hypertensive rats used. This study points to the beneficial cardioprotective action of avocado (*P americana*) aqueous leaf extract. Hence, the need for more investigation on the effect of *P americana* aqueous leaf extract on the biochemical parameters of high salt-fed Wistar rats.

## Materials and Methods

### Animal Care and Management

Forty adult Wistar rats of both sexes weighing between 120 and 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 were maintained in natural day and night cycle and temperature. The rats were assigned to 8 groups of 5 animals each.

### Plant Material and Preparation of Extract

Fresh avocado (*P 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°C  $\pm$  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.<sup>7,12</sup>

### 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 those in groups B, C, D, and E were fed on the high-salt diet for 4 weeks; concomitantly, daily administration of 50, 100, and 150 mg/kg of *P americana* extract were giving orally to groups C and F, D and G, and E and H, respectively. The extract solution was administered orally, using oral canula and duration of the experiment was 4 weeks.

### Measurement of Serum Electrolytes

Blood samples from each rat was collected separately into clean capped plain tubes and allowed to stand for 30 minutes for clotting to occur. These were then centrifuged at 2500 revolution per minutes

**Table 1.** Effects of *Persea americana* on Relative Heart Weight of Across All Groups, n = 5.<sup>a</sup>

Group	Heart's Absolute Weight (g)	Relative Heart Weight (%) (RAW = HW $\div$ BWAS $\times$ 100)
A (Control)	0.60 $\pm$ 0.045	0.45 $\pm$ 0.013
B (Negative control)	0.74 $\pm$ 0.051	0.61 $\pm$ 0.059
C (50 mg/kg PAE + high salt feed)	0.60 $\pm$ 0.032	0.47 $\pm$ 0.022
D (100 mg/kg PAE + high salt feed)	0.54 $\pm$ 0.068	0.39 $\pm$ 0.083
E (150 mg/kg PAE+ high salt feed)	0.62 $\pm$ 0.058	0.42 $\pm$ 0.061
F (50 mg/kg PAE + standard feed)	0.62 $\pm$ 0.058	0.43 $\pm$ 0.064
G (100 mg/kg PAE+ standard feed)	0.60 $\pm$ 0.032	0.40 $\pm$ 0.046
H (150 mg/kg PAE+ standard feed)	0.60 $\pm$ 0.071	0.41 $\pm$ 0.53

Abbreviations: PAE, *Persea americana* aqueous extract; RAW, relative heart weight; HW, heart weight; BWAS, body weight at sacrifice.

<sup>a</sup>Values are expressed as mean relative heart weight (%)  $\pm$  standard error of the mean (SEM), with no significant difference across all groups ( $P < .05$ ).

for 15 minutes. The serum was extracted into clean test tubes for sodium and potassium analysis. This was measured using flame photometry method at wavelengths 590 nm for sodium and 770 nm for potassium.

**Measurement of Lactate Dehydrogenase.** Blood sample from each rat was collected separately into clean capped plain tubes and allowed to stand for 30 minutes for clotting to occur. These were then centrifuged at 2500 revolution per minutes for 15 minutes. Fresh distilled H<sub>2</sub>O was aspirated and a new gain calibration in flow cell mode was performed. Lactate dehydrogenase was selected and run test screen was programmed; a water blank was run as instructed. The initial absorbance was read after 0.5 minutes simultaneously with the starting of the timer and the absorbance was read again after 1, 2, and 3 minutes.

**Determination of Nitric Oxide Levels in Serum.** At the end of the experiment, the serum was separated from the blood by centrifugation at 3,000  $\times$  g for 10 minutes. The serum nitric oxide (NO) levels were determined using NO assay kit for the colorimetric determination of total nitrite (BioAssays Systems, Hayward, CA, USA).

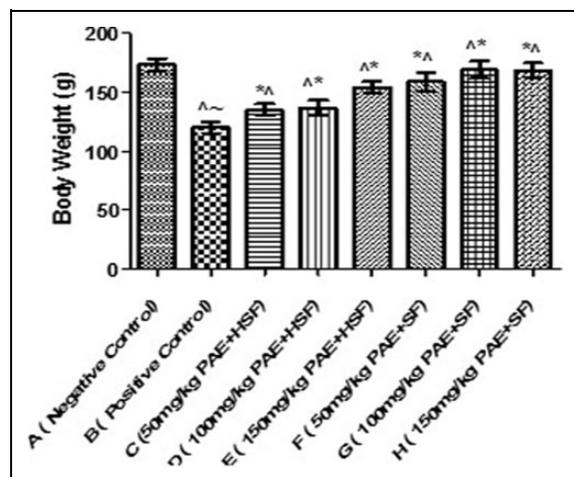
## Results

### Effects of *Persea americana* on Relative Heart Weight of Rats Across All Groups

There was no statistical significant difference in the relative heart weight across all groups ( $P < .05$ ) (Table 1).

## Discussion

The relative heart weight of the high salt diet-fed group was higher than the control group, but the level of difference was not statistically significant ( $P = .175$ ) using analysis of



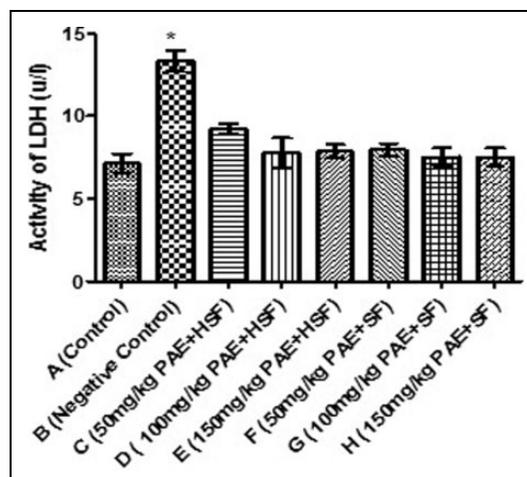
**Figure 1.** Effects of *Persea americana* on left ventricular body weight in rats fed with high salt feed,  $n = 5$ . Values are expressed as body weight (g)  $\pm$  standard error of the mean (SEM). \*Significant difference compared with control. ~Significant difference compared with other treatment groups. ^Significant difference compared with positive control at  $P < .05$ .

variance. The effects of *P americana* on left ventricular body weight in rats fed with high salt feed is shown in Figure 1. This may be attributable to the relatively small sample size used in this study.

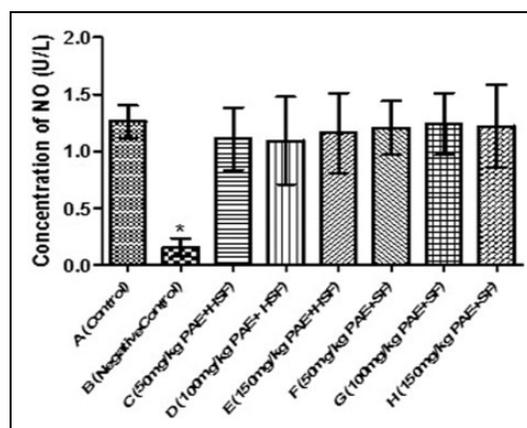
Lactate dehydrogenase is a fairly stable enzyme that has been widely used to evaluate the presence of damage and toxicity of tissue and cells. It is an oxidoreductase that catalyzes the interconversion of pyruvate and lactate with concomitant interconversion of reduced nicotinamide adenine dinucleotide (NADH) and oxidized nicotinamide adenine dinucleotide (NAD<sup>+</sup>).<sup>13</sup> There are different isoforms of lactate dehydrogenase: lactate dehydrogenase-1 is predominant in heart while lactate dehydrogenase-2 is found in the serum. According to previously done research, the presence of lactate dehydrogenase in the tissue is indicative of myocardial integrity and its release signifies myocardial injury.<sup>13</sup> Experimental studies in rats have shown that elevation in the lactate dehydrogenase activity in the serum correlated with a decrease in the activity of cardiac muscle lactate dehydrogenase.<sup>13</sup> The result obtained from the assay of plasma lactate dehydrogenase activity in this current study showed that the activity of lactate dehydrogenase was significantly higher ( $P < .05$ ) in negative control group when compared with the control and all groups (Figure 2); this may be an indication of myocardial injury.

The plasma NO concentration was also significantly lower ( $P < .05$ ) in negative control group when compared with the control and all groups (Figure 3); the high concentration observed in all other experimental groups may be as a result of the inhibition of generation of superoxide in the treated groups, an attribute owed to certain content of *P americana* leave (Persenones A and B, alongside with persin).

High salt intake probably induces the impairment of endothelial function causing atherosclerosis and stimulation of endothelial  $\beta$ -adrenoreceptors.<sup>14</sup> The importance of this



**Figure 2.** Effects of *Persea americana* on left ventricular lactate dehydrogenase (LDH) activity in rats fed with high salt feed,  $n = 5$ . Values are expressed as LDH activity (U/L)  $\pm$  standard error of the mean (SEM). \*Significant difference compared with control at  $P < .05$ .

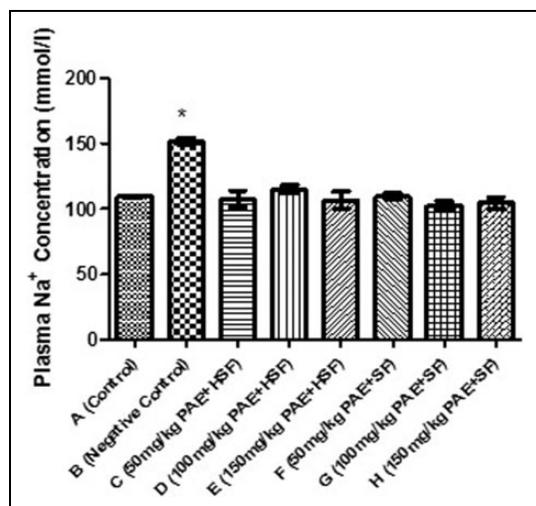


**Figure 3.** Effects of *Persea americana* plasma nitric oxide (NO) concentration,  $n = 5$ . Values are expressed as mean  $\pm$  standard error of the mean (SEM). \*Significant difference when compared with groups F, G, H (extract alone) and control group.

strong molecular connection has been recently demonstrated in animal and human using several studies; Low level of NO probably induced myocardial dysfunction and remodeling.<sup>14</sup> NO is synthesized in cardiomyocytes, endocardial endothelium, coronary endothelium and cardiac nerves by endothelial NO and neuron NO.<sup>14</sup>

NO as an endothelium-derived relaxing factor plays many important roles in physiological regulation of cardiac function, including coronary vasodilation, activation, and modulation of cardiac contractile function, inhibiting platelet, neutrophil adhesion and inhibiting cardiac oxygen consumption.<sup>15-17</sup>

The link between endothelial dysfunction and cardiovascular disease is well established.<sup>18</sup> The modulation of rennin angiotensin-aldosterone and adrenergic system is important in treatment of several pathologies, including cardiovascular disease<sup>19</sup>; it was observed that the activity of lactate dehydrogenase and concentration of NO in plasma were not

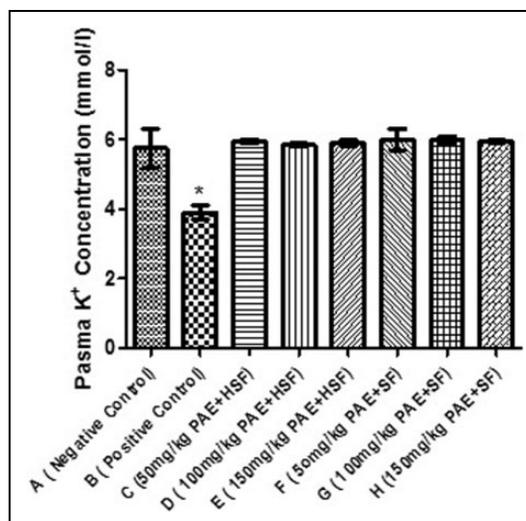


**Figure 4.** Effects of *Persea americana* on plasma sodium ion concentration in rats fed with high salt feed,  $n = 8$ . Values are expressed as mean sodium ion concentration (mmol/L)  $\pm$  standard error of the mean (SEM). \*Significant difference when compared with control and groups F, G, H (extract alone).

significantly different ( $P > .05$ ) when extract alone (F, G, and H) groups were compared with control and high salt-fed + graded doses of extract groups; the cardioprotective potency of *P americana* aqueous extract in these groups complements the report of a previously done study.<sup>11</sup>

*P americana* aqueous extract probably affected the activity of angiotensin-converting enzyme (angiotensin II type I receptor blockers) and  $\beta$ -blockers by inhibiting  $\beta$ -adrenoceptors enhancing NO bioavailability in endothelial cells exerting beneficial effect against myocardial infarction, left ventricular dysfunction, and cardiac remodeling in a dose-dependent manner. Angiotensin-converting enzymes generate cardioprotective effects improving left ventricular function and attenuating fibrosis and hypertrophy.<sup>20</sup>

It was observed in this study that plasma sodium level increased significantly ( $P < .05$ ) in negative control group when compared with control and all groups while there was no difference when control was compared with the extract alone groups (F, G, and H) (Figure 4); This may be due to sodium retention and expansion of extracellular volume as a result of increased activity of angiotensin-converting enzyme (angiotensin II) and stimulation aldosterone release. Angiotensin II has been reported to causes sodium ion retention through stimulation of aldosterone release.<sup>21</sup> High salt feed probably increased renal reabsorption of filtered sodium because of stimulation of several sodium transporters located at the luminal membrane, as well as the sodium pump, which is localized to the basolateral membrane and provides the energy for such transport. A pivotal luminal transporter is sodium-hydrogen exchanger, which resides in the proximal tubule and the thick ascending limb of the loop of Henle, where the bulk of filtered sodium is reabsorbed. The activity of this exchanger is increased in the kidneys of rats in salt loading.<sup>22</sup> Furthermore, a high-sodium intake increases kaliuresis (excretion of



**Figure 5.** Effects of *Persea americana* on plasma potassium ion concentration in rats fed with high salt feed,  $n = 5$ . Values are expressed as mean potassium ion concentration (mmol/L)  $\pm$  standard error of the mean (SEM). \*Significant difference when compared with control.

potassium in urine), especially when sodium reabsorption by the renal cortical collecting tubule is enhanced.<sup>23</sup> It was also observed that plasma potassium level decreased significantly ( $P < .05$ ) in negative control group when compared with control and all groups while there was no difference when control was compared with the extract alone groups (F, G, and H) (Figure 5). Moreover, potassium depletion enhances sodium-hydrogen exchanger by inducing intracellular acidosis and by stimulating the sympathetic nervous system and the renin-angiotensin system.<sup>24</sup> In animals and humans, a low-potassium itself causes renal sodium retention by means of several mechanisms.<sup>23</sup> Potassium deficit in the body as a result of inadequate conservation of potassium by the kidneys and the alimentary tract causes a high fecal potassium loss that exceeds even urinary losses.<sup>23</sup> *P americana* aqueous extract probably induced inhibition of angiotensin-converting enzyme that lead to reduction in production of aldosterone from the adrenal glands, natriuresis, and decreased plasma volume. The inhibition of angiotensin-converting enzyme 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 the protective effect of *P americana* aqueous extract against biochemical perturbations in high salt-fed Wistar rats.<sup>25</sup>

## Conclusion

Using the activities of lactate dehydrogenase, NO, and sodium/ potassium ion concentration as parameters, the results obtained in this study showed that high salt feed induced changes in the left ventricle. Also, the result showed that *P americana* aqueous extract has cardioprotective effects on these perturbations.

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## Author Contributions

AIO initiated the research and was involved in the bulk of the research work, analyses of data, and writing of the article. KOA was involved in the design of the experiment and supervised various stages of the work. He was also responsible for the collection of plant material. OSA was involved in the design of the experiment and proof-read the article. SOS was involved in the writing and editing of article.

## Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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## Ethical Approval

The experiment procedures adopted in this study were in strict compliance with Experimental Animal Care and Use of Laboratory Animals in Biomedical Research, College of Health Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria.

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