Original Research Article

Effects of *Persea Americana* Seed Extract on Lipid Profile, Atherogenic and Coronary Risk Indices in Doxorubicin-Injected Male Rats

ABSTRACT

Doxorubicin (DOX) can cause disruption in the lipid profile due to free radical process. The health benefits of avocado seeds stem from essential nutrients and phytochemicals. This study aimed to assess the effects of *Persea americana* seed extract on lipid profile, atherogenic and coronary risk indices in doxorubicin-injected male rats. Thirty-six male wistar rats were divided into six groups: Group A were control rats. Group B rats received DOX alone in over a 5 weeks period. Group C animals received the DOX treatment plus Vitamin E. Groups D, E and F rats received the DOX treatment plus 400 mg/Kg, 800 mg/Kg and 1200 mg/Kg b.wt of the extract respectively. Animals were sacrificed after the treatment period, and serum lipid profile was evaluated. Atherogenic index (AI) and coronary risk index (CRI) were also determined. The results showed that rats given DOX+400 mg/Kg and DOX+1200 mg/Kg b.wt of the extracts were found to benon-significantly lower in LDL(43.54±0.496 and 42.22± 3.399 mg/Kg b.wt respectively) and significantly higher (P = .05) in HDL (51.35±2.888 and 49.02±3.450mg/dl respectively)value when compared to those of the DOX-test and control groups, indicating enhancement of lipid profile. Moreover, the AI and CRI values of the rats given DOX+400 and DOX+800 mg/kg b.wt of the extract were found to be lower compared to the DOX-test and control groups. The findings of this study suggest that the *P. americana* extract has anti-dyslipidemic potentials, hence could prevent aberrant lipid metabolism that leads to hypertension.

Keywords: *Persea americana*, Doxorubicin, anti-dyslipidemic, Cholesterol, Cardiovascular

1.0 INTRODUCTION

“Anthracycline antibiotics like doxorubicin are commonly used in chemotherapy to treat a range of cancers, such as breast cancer, lymphoma, and leukemia”[1]. “Low blood cell counts, mouth ulcers, hair loss, exhaustion, nausea, and vomiting are common side effects of doxorubicin. Another side effect of doxorubicin is cardiotoxicity, which can lead to heart failure, arrhythmias, and other cardiac issues”[2]. “Furthermore, the free radical process caused by doxorubicin might alter the lipid profile, resulting in dyslipidemia and a higher risk of cardiovascular disease”[3].

The body contains fats and fatty substances called lipids, which include triglycerides, cholesterol, and phospholipids. According to [4], these lipids are crucial components of cell membranes and are required for a wide range of physiological processes, including hormone synthesis, energy storage, and cell signaling.Numerous metabolic disorders and health consequences might result from changes in the lipid profile. Doxorubicin is known to cause free radicals, which are very reactive and unstable species that can cause damage to cell membranes and other biomolecules, including lipids.

“The use of medicinal plants in traditional and alternative medicine has garnered a lot of interest worldwide.Numerous plant components, including seeds, leaves, stems, roots, and barks, have been shown to be effective in treating human illnesses”[5]. This is because there is a constant demand for less costly methods of controlling and preventing illness. Additionally, the majority of modern conventional medications are pricy and frequently have negative side effects. Plants are inexpensive, easily accessible resources with therapeutic qualities that have few adverse consequences. In fact, at least one active component in over 25% of the prescription medications that are prescribed comes from plant sources. One of the numerous medicinal plants used to heal

a variety of human ailments is the avocado (*Persea americana*). Nutrients and phytochemicals are abundant in avocados.

"The avocado, as it is usually called, is a Central American edible fruit that may be readily adapted to tropical climates"[6]. "Because of its high nutritional content, which includes vitamin K, dietary fiber, potassium, folic acid, vitamin B6, vitamin C, copper, and moderate calorie level, avocado is regarded as the healthiest fruit in the world. It has been shown to have large quantities of good fats and is one of the most recommended fruits, as well as a diet for body development and a medication for conditions connected to cholesterol"[7]. "An underutilized resource, avocado seeds make up 16% of the weight of an avocado"[8]. "Numerous groups of natural chemicals, including phytosterols, triterpenes, fatty acids, furanoic acids, abscisic acid, proanthocyanidins, flavonoids, and polyphenols, have been discovered through phytochemical analyses of avocado seeds"[9,10]. "The bioactivities of avocado seed extracts, which have many traditional uses in dermatology, have been studied. Others include anti-hyperglycemic, anti-cancer, anti-inflammation, antihypercholesterolemia, anti-oxidant, anti-microbial, and anti-neurogenerative properties"[9]."Native Americans have utilized the seeds to cure diarrhea and dysentery" [11]. "Because it contains a lot of fiber, it helps reduce the effects of cardiovascular disease, obesity, and hypertension. In both human and animal cell lines, avocado seeds and their physiologically active ingredients shown anti-cancer potential against lung and prostate cancer" [12]. "A novel product with additional value and a safe substitute for synthetic substances can be created by investigating the potential of the plant seeds as a prospective source of natural bioactive components"[9]. this study sought to assess the effects of *Persea americana* seed extract on lipid profile, atherogenic and coronary risk indices in doxorubicin-injected male rats.

2.0 Materials and methods

2.1 Materials

2.1.1 Plant sample procurement and identification

Mature avocado fruits were obtained from Eke Amobi rural market in Otolo Nnewi Anambra state, Eastern Nigeria. The seed was separated from the pulp, and was authenticated by a taxonomist from Botany department Nnamdi Azikiwe University, with Habarium number, NAUH-183[A]

2.1.2 Experimental animals

Thirty-six male wistar rats were obtained from the animal facility of the Faculty of Basic Medical Sciences. They were acclimatized for 2weeks, in the animal house, kept in laboratory cages and allowed free access to standard rat feed and water.

2.1.4 Chemicals

All chemicals used for the study were of analytical grade, and was obtained from Sigma Aldrich.

2.2 Methods

2.2.1 Preparation of plant material

The avocado seed was prepared according to the method performed by [13]. The avocado seed was sun-dried and milled, and the powder extracted with cold maceration using 90% ethanol in

ratio 1:2 solid to solvent. Afterwards, the extract was filtered using a linen. The filtrate was concentrated at 60□ using a water bath and dried at 50□ with an oven.

2.2.2 Experimental Design

The animals were divided into six groups as follows

(A) Normal group; was treated with regular feed and water;

(B) Negative control group; was treated with 4 mg/kg b.wt of doxorubicin via intraperitoneal weekly for five weeks

(C) Positive control group; was treated with Vitamin E 100mg/Kg b.wt alternative days, and also 4 mg/kg BW of doxorubicin via intraperitoneal weekly for five weeks

(D) Treatment group I; was treated with 400 mg/kg b.wt of ESEPA alternative days and 4 mg/kg BW of doxorubicin via intraperitoneal weekly for five weeks

(E) Treatment group II; was treated with 800 mg/kg b.wt of ESEPA alternative days and 4 mg/kg BW of doxorubicin via intraperitoneal weekly for five weeks

(F) Treatment group III; was treated with 1200 mg/kg b.wt of ESEPA alternative days and 4 mg/kg b.wt of doxorubicin via intraperitoneal weekly for five weeks

ESEPA = Ethanolic seed extract of *Persea americana*.

On the last day of animal model treatment, all groups get fasting for 18 hrs and then their samples taken for analyses.

2.2.3 Sample collection and preparation

At the end of the experiment, all animals were sacrificed under anesthesia, and blood samples of the animals were collected via ocular puncture, centrifuged at 3000 rpm for 15 min at room temperature to obtain the serum for biochemical analyses.

2.2.4 Determination of serum lipid profile

2.2.4.1 *Total cholesterol*

This was done using UV-VIS spectrometer as recommended by [14]

Procedure

Cholesterol reagent 1 was constituted with 30ml of distilled water. Ten microlitre (10µl) each of distilled water, standard and serum were added into test tubes marked blank, standard test respectively. This was followed by the addition of 1 ml of cholesterol reagent to all the test tubes and incubated at room temperature for 10 min. The absorbance was read at 500 nm.

Calculations:

$$\text{Total Cholesterol} = \frac{\text{Abs Test} - \text{Abs Blank}}{\text{Abs Std} - \text{Abs blank}} \times \text{Conc. of Std (mg/dl)}$$

2.2.4.2 *Determination of serum high density lipoprotein (HDL)*

This was done using standard method as recommended by [14], by using UV-VIS spectrophotometer

Procedure

Two hundred microlitre (200µl) each of sample and standard was added to the test tubes marked test and standard respectively. This was followed by the addition of 500 µl precipitant (phosphotungstate and magnesium ions – Reagent 2) to both test tubes. These were incubated at

room temperature for 10 min. After this, the assay mixtures were centrifuged for 10 min at 400 rpm at room temperature using ultra-modern centrifuge machine (Alphine Medical, England). Fifty Microlitres (50 µl) each of sample and standard supernatants were added into another set of test tubes for test and standard while 50 µl distilled water was added into a test tube for blank. This was followed by the addition of 1 ml of cholesterol reagent1 to all the test tubes. This was mixed and incubated for 10 min at room temperature. Absorbance of test and standard was measured against reagent blank at 500 nm spectrophotometrically using UV-VIS spectrophotometer at room temperature.

Calculations:

Conc. of serum HDL = $\frac{\text{Abs Test} - \text{Abs Blank}}{\text{Abs Std} - \text{Abs blank}}$ × Conc. of Std (mg/dl)

2.2.4.3 *Determination of serum triglycerides (Trigs)*

This was done using known standard method as recommended by [14], by using UV-VIS spectrophotometer.

Procedure

Fifteen mililitres (15 ml) of triglycerides reagent 1a was mixed with the reagent 1b at room temperature and labeled triglyceride reagent. Ten microlitre (10 µl) each of serum and standard triglycerides was added into test tubes marked test and standard respectively. This was followed by the addition of 1 ml of triglycerides reagent to each of the test tubes, including blank test tube and incubated at room temperature for 10 min. The absorbance was read at 500 nm.

Calculations:

The concentration of triglycerides in the serum was calculated using the formula:

Conc. of serum Trigs = $\frac{\text{Abs Test} - \text{Abs Blank}}{\text{Abs Std}} \times$ Conc. of Std (mg/dl)

Abs = Absorbance

2.2.4.4 *Determination of lowdensity lipoprotein (LDL)*

This was calculated from total cholesterol, triglycerides and HDL using a formula model provided by [15].

Concentration of LDL = (TChol) – (HDL) - $\frac{[\text{Trigs}]}{5}$ (mg/dl)

2.2.4.5 *Determination of atherogenic index (AI) and coronary risk index (CRI)*

AI and CRI were determined using the formulae by [16]

AI = LDL/HDL

CRI = T.Chol/HDL

2.2.5 Statistical analysis

Results obtained were expressed as Mean±SEM. One-way analysis of variance (ANOVA) was used to analyze data using statistical product for services and solutions (SPSS) version 25. Duncan multiple range test was used as post-hoc tool to compare the means at $P<0.05$ significance level.

3.0 Results

showed the lipid profile status of the experimental animals. The total cholesterol level of DOX+Vit E Group (group C) was significantly lower than other groups whereas that of DOX+

Extract 800mg/Kg b.wt (group E) was significantly higher than all other groups. The HDL level of groups C was significantly higher and that of group E was significantly lower when compared to those of the other groups. The Triglycerides (Trigs) level of DOX+Extract 400mg/Kg b.wt (group D) was significantly higher than the other groups. The Trigs level of the DOX+Extract 800 mg/Kg b.wt was significantly lower (P = .05) than that of the normal group, but showed no statistical difference with that of DOX+Test group. There was no significant difference in the Trigs level of DOX+Extract 1200 mg/Kg b.wt when compared to the normal control. The LDL level of group C was significantly lower than those of the other groups. However, the LDL values of the DOX+Extract groups (group D, E and F) were lower than those of the DOX+Test (group B) and the normal group (group A).

Table 1: Lipid profile of the experimental animals

Groups	Total Cholesterol	HDL (mg/dl)	Trigs (mg/dl)	LDL (mg/dl)

	(mg/dl)			
Control(A)	122.52±0.229^{C}	43.02±0.377^{AB}	128.61±0.548^{B}	53.63±0.694^{B}
DOX-Test (B)	118.67±1.123^{B}	42.76±0.242^{B}	119.80±1.415^{A}	51.59±1.767^{B}
DOX + 100mg/Kg b.wt. Vit E (C)	104.75±1.383^{A}	63.50±0.866^{D}	122.32±0.353^{A}	30.81±4.311^{A}
DOX + Extract 400mg/Kg b.wt (D)	118.30±0.449^{B}	51.35±2.888^{C}	134.42±0.488^{C}	43.54±0.496^{B}
DOX + Extract 800mg/Kg b.wt (E)	130.68±1.433^{D}	35.52±0.722^{A}	120.20±3.707^{A}	48.75±6.095^{B}
DOX + Extract 1200mg/Kgb.wt (F)	119.77±1.350^{BC}	49.02±3.450^{C}	128.73±1.236^{B}	42.22± 3.399^{B}

Each value represents Mean±SEM of triplicate determinations

Values within a column having the same uppercase letter are not significantly different at *P*= .05.

Table 2 showed the atherogenic index (AI) and the coronary risk index (CRI) of the rats. The AI values of the DOX+Extract 400 mg/Kg b.wt (group D) and DOX+Extract 1200 mg/Kg b.wt. (group F) were found significantly lower (*P* = .05) than the normal control (group A) and DOX-Test groups (group B). However, the Vit E group was significantly lower (*P* = .05) in AI value

than others. The CRI value of group D was significantly lower than those of groups A and B. The CRI of group F was found to be significantly lower (P = .05) than that of the normal control, but non-significantly lower than the DOX+Test group. The Vitamin E group was the lowest in both AI and CRI values whereas the DOX+Extract 800 mg/kg b.wt recorded the highest in both AI and CRI values.

Table 2: Atherogenic index (AI) and Coronary risk index (CRI) of the Animals

Group	AI	CRI
Control(A)	1.25 ± 0.027^{C}	2.85 ± 0.029^{D}
DOX-Test (B)	1.21 ± 0.044^{C}	2.78 ± 0.309^{CD}
DOX + 100mg/Kg b.wt. Vit E (C)	0.49 ± 0.075^{A}	1.65 ± 0.044^{A}
DOX + Extract 400mg/Kg b.wt (D)	0.85 ± 0.039^{B}	2.32 ± 0.141^{B}
DOX + Extract 800mg/Kg b.wt (E)	1.37 ± 0.123^{C}	3.68 ± 0.068^{E}
DOX + Extract 1200mg/Kgb.wt (F)	0.88 ± 0.132^{B}	2.47 ± 0.203^{BC}

Each value represents Mean±SEM of triplicate determinations.

Values within a column having the same uppercase letter are not significantly different at P = 0.05.

Discussion

Cholesterol is an essential structural element of the biological membranes. In addition, it is the precursor of many compounds such as the starting materials for the synthesis of bile acids, steroid hormones, and vitamins among others. Despite this knowledge, high concentration of serum cholesterol increases the risk of developing cardiovascular diseases (CVD) [17]. The total cholesterol and LDL of groups treated with 400 mg/Kg b.wt and 1200 mg/Kg b.wt of the extracts and the Vit. E group were reduced compared to the normal control (Table 1). This could be as a result of the polyphenolic compounds present in the extract. Antioxidants such as flavonoids and tannin play a role in lowering cholesterol, LDL and triglyceride in rats that were induced by doxorubicin [18]. Previous reports have shown that flavonoids can affect the process of LDL cholesterol metabolism by increasing the ability of LDL to bind to its receptors. LDL that is bound to receptors will be metabolized into esters form in the tissues [19,20]. Flavonoids are also known to reduce LDL lipid peroxidation, and reduce the oxidative stress of macrophages by inhibiting cellular oxygenation and activating cellular antioxidants [21]. Thus, flavonoids are natural antioxidants that have the ability to protect against lipid peroxidation in the arteries. They do this by decreasing LDL, inhibiting the formation of foam cells, thereby reducing the risk of atherosclerosis. This study indicated that the DOX+Extract groups showed reduced LDL levels when compared to the DOX+Test group. Therefore, The *P. americana* seed extract might constitute a good candidate for the treatment and management of CVD by lowering serum LDL level. Another risk factor for developing hypertension and other related CVD is the reduced serum level of HDL. This effect of HDL is largely attributed to its central function in the reverse cholesterol transport, a process whereby excess cell cholesterol is taken up and processed by HDL particles for further delivery to the liver for metabolism [22]. Therefore, it is logical that an increase in HDL level can contribute to lower risk of CVD [22]. The results of this study showed

clearly that ethanolic seed extract of *Persea americana* was able to increase the serum level of good cholesterol (i.e HDL) in doxorubicin induced rats treated with 400 mg/kg and 1200 mg/kg of the extract compared to the normal control. With respect to the triglycerides level, there was no significant difference between the group given DOX+1200 mg/Kg b.wt when compared to the normal control. However, the DOX+Extract 800 mg/Kg b.wt was significantly lower (P = .05) than that of the normal control. Low plasma triglyceride (Trig) levels are associated with lower risks of cardiovascular diseases [23]. Similar finding was reported by [24] for *P. americana* seeds in cyclosporine treated rats. The overlapping variation in the effects of the extract concentrations could be as a result of combination effect. Plant extracts show combination effects which could be additive, antagonistic or synergistic at varying concentrations [25]. Additionally, phytochemicals can have complementary and overlapping mechanism of action in the body [26].

The atherogenicity index (AI) characterizes the potential of fatty acids to cause atherosclerosis [27]. The AI indicates the relationship between saturated fatty acids (SFAs) and unsaturated fatty acids (UFAs). SFAs are pro-atherogenic, favouring lipid binding to circulatory and immune cells, while UFAs inhibit plaque buildup and reduce cholesterol [28]. AI is known to be a strong, reliable, and independent predictor of ischemic heart diseases including coronary artery disease and acute myocardial infarction [29,30,31]. The AI values of the Vit E, the DOX+Extract 400 mg/Kg b.wt. and DOX+Extract 1200 mg/Kg b.wt. groups were significantly lower (P = .05) than those of the DOX+Test and normal control groups (Table 2). The coronary risk index (CRI) is a reliable indicator of cardiovascular diseases, especially ischemic heart diseases [32]. CRI values of Vit E and DOX+Extract 400 mg/Kg were found significantly lower than the DOX-Test and control groups. This finding is indicative of cardiovascular health property of the extract. [33]

reported similar result in a related study. They revealed that aqueous extract of *Persea americana* seeds reduced AI and CRI values in diabetic rats. Also, [34] found out that seed extracts of *Citrullus lanatus* and *Persea americana* decreased AI and CRI values in diabetic rats. Moreover, [32] in their study, revealed that *Clerodendrum volubile* ethanol leaf extract reduced AI and CRI levels of doxorubicin-induced rats. The lowering of AI and CRI by the ethanolic seed extract of *P. americana* is indicative of its anti-dyslipidemic effect.

Conclusion

The results of this studysuggest that *Persea americana*seed extract may have an anti-dyslipidemic impact, hence could prevent aberrant lipid metabolism that leads to hypertension. Since dyslipidemia can lead to hypertension, *Persea americana* seeds may be used as part of a dietary plan to treat it.

Ethical Approval :

Ethical clearance was obtained from Animal Research Ethics Committee, Nnamdi Azikiwe University, Awka Anambra State, Nigeria with the reference number NAU/AREC/2024/0068. “All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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