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Assessment of Lipid Profile and Oxidative Stress Markers of *Persea americana* Treated Wistar Rats

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Abstract

The purpose of this study is to assess the effect of ethanolic stem bark extract of *Persea americana* on lipid profile and oxidative stress markers of male Wistar rats. The rats were randomly divided into three groups of five rats each. They were treated with ethanolic extract of *Persea americana* (except the control group) for a period of 21days. Group1 received distilled water. Group2 received 200mg/kg of the extract and Group3 received 400mg/kg of the extract. The administration was done for 21days. At the end, the rats were sacrificed and blood sample was collected and sent to the laboratory for analysis. The data obtained was statistically analysed using SPSS software version 21. The result showed that, there was no significant difference in serum total cholesterol, triglyceride, high density lipoprotein and low density lipoprotein when test groups were compared to control. There was a significant decrease in serum very low density lipoprotein level in Group3 (400mg/kg) when compared to Group1 (Control). There was no significant alteration in glutathione reductase, catalase and superoxide dismutase enzyme activities. There was a significant difference in malondialdehyde level (a decrease). In conclusion, the ethanolic extract of *Persea americana* stem bark exhibited slight hypolipidemic effect because of the significant reduction in Very Low Density Lipoprotein. Also, the extract showed a significant decrease in Malondialdehyde at low dose, which shows that *Persea americana* may have an anti-oxidative property.

Keywords: *Persea Americana*, Wistar rats, ethanolic extract, cholesterol, Malondialdehyde.

INTRODUCTION

Plant products, particularly in underdeveloped nations, play an essential role in healthcare delivery as therapeutic medicines. As a result, phytomedicine has become an important aspect of many countries' healthcare systems. Medicinal plants have a lot of pharmacological bio-resources, and these chemical compounds have a specific physiological activity in the human body (Edeogha *et al.*, 2005). Since prehistoric times, therapeutic plants, often known as medicinal herbs, have been identified and employed in traditional medicine practices. Numerous phytochemicals have been found as having biological activity, either potential or established. The consequences of taking a complete plant as medication, however, are unknown because a single plant has a vast range of phytochemicals. Furthermore, many therapeutic plants' phytochemical content and pharmacological effects, if any, remain unknown due to a lack of rigorous scientific investigation to determine efficacy and safety (Ahn, 2017).

Persea americana which is also referred to as avocado is a tree native to Central America and Mexico and commercially valuable and are cultured in humid and Mediterranean climates throughout the world (Galindo *et al.*, 2007). It belongs to family lauraceae along with cinnamon, camphor and baylaurel. *Persea Americana* seeds have been shown to have antihypertensive, fungicidal and, more recently,

amoebicidal and giardicidal properties (Jimenez *et al.*, 2013). Similarly, fruit and leaf extracts have been tested for mutagenicity in human lymphocytes (Kulkarni *et al.*, 2010).

According to findings from a study that showed that abnormal serum lipids play an important role in the development of atherosclerosis later in life, the lipid profile (total cholesterol, high density lipoprotein, triglyceride, and low density lipoprotein analysed after a 12 to 14 hour fast is of great value in identifying cardiovascular risk factors (Carreras and Ordonez, 2007).

Oxidative stress occurs when there is a mismatch between the systemic expression of reactive oxygen species (ROS) and a biological system's ability to quickly detoxify reactive intermediates or repair the harm they cause. Disturbances in a cell's normal redox state can lead to the creation of peroxides and free radicals, which can harm all of the cell's components, including proteins, lipids, and DNA (Birnboin, 1986). ROS, on the other hand, can be advantageous because the immune system uses them to target and kill infections. Short-term oxidative stress may also play a role in aging prevention by inducing a mechanism known as mitohormesis (Gems and Partridge, 2008). The aim of the study is to assess the lipid and oxidative profiles of *Persea americana* treated Wistar rats.

MATERIALS AND METHODS

Animal Models

Adult male Wistar rats used for this study were bred in the animal house of the Faculty of Basic Medical Sciences, Rivers State University, Nigeria. They were placed in standard cages and acclimatized in two weeks while maintaining them in environmental conditions with proper ventilation and free access to food and water. Generally, the procedures conformed to the established principles for the care and use of laboratory animals published by the National Institute of Health, USA (National Institutes of Health, 1985). Appropriate institutional approval was obtained for this study.

Preparation of Plant Extract

The stem bark of mature *Persea americana* was obtained from the tree in the Rivers State University, Port Harcourt, Rivers State, Nigeria. The plant was identified in the Department of Plant Science and Biotechnology by the taxonomist, with a voucher number RSUPb040. Samples were deposited in the herbarium. The bark was peeled from the stem, washed and dried in the oven at a temperature of 45°C. Using the soxhlet method of extraction, ground stem bark of *Persea americana* was dissolved in 2 litres of distilled water which was measured with a measuring cylinder for complete 24 hours, in a maceration jar. The solution was filtered using a glass funnel and white handkerchief. The handkerchief was spread over the funnel placed in 1000ml beaker, the filtrate was then carefully poured into the white-handkerchief which carefully filtered through the funnel and into the beaker. To obtain a clear filtrate, what-mann filter paper was used for a second filtration.

The clear filtrate was poured into an evaporating dish and placed on a water bath at a temperature of 40-50 degree Celsius where it gradually evaporated leaving the extract in a paste form. The crude extract which was stored in the refrigerator for use was later reconstituted into 200mg and 400mg/kg body weight and used for animal oral experiments.

Experimental Design/Procedure

This study was designed to assess the lipid and oxidative profiles of the stem bark extract of *Persea americana.* Male Wistar rats were divided into 3 groups of 5 rats each. Group one (1) which served as control received distilled water. Group 2 received 200mg/kg of the extract and Group 3 received 400mg/kg of the extract. The extracts were administered orally and once daily throughout the period of the experiment using appropriate animal feeding tubes. Administration lasted for 21 days. The rats were sacrificed under chloroform anaesthesia, after 24 hours of last administered dose.

Blood was taken by cardiac puncture into appropriate sample bottles to determine the serum lipid profile [Total cholesterol (TC), Triglyceride (TG), High density lipoprotein (HDL), Low density lipoprotein (LDL), and Very low density lipoprotein (VLDL)]. The samples were spun for 20 minutes in a serologic manual centrifuge after collection. The biochemical assay was then carried out using 1 mL aliquots of serum. Commercial Labtest Diagnostic kits were used to analyze biochemical parameters, utilizing standard methodologies based on enzymatic and colorimetric methods, spectrophotometry, and in line with the manufacturer's instructions. An automated biochemical analyzer was used to determine the concentrations. The plasma was utilized to calculate oxidative stress indicators according to conventional procedures (Wasowick *et al.*, 1993; Condezo-Hyos *et al.*, 2013; Peskin and Winterbourn 2017; Vives-Bauza *et al.*, 2007; Slaughter and O'Brien, 2000; Goldberg, 1984).

Statistical Analysis

For the statistical analysis, SPSS software, version 21.0 was used. The Mean and Standard Error of Mean (SEM) were calculated. In the comparison of the mean level of serum lipids and plasma antioxidants between the control and test groups, we used the analysis of variance (ANOVA) and then post hoc test least significant difference. All crosses whose probability *(P) value* was less than 0.05 were considered statistically significant.

RESULT

The result for the study is presented in Tables 1-3.

Table 1: Effect of extract of *Persea americana* on the serum Total cholesterol, Triglyceride and High density lipoprotein cholesterol

Groups (mg/kg)	Total Cholesterol (mmol/L)	Triglyceride (mmol/L)	High Density Lipoprotein (mmol/L)
Control	3.68 ± 0.38	1.03± 0.16	1.33± 0.15
200	3.34 ± 0.16	0.83± 0.11	1.38± 0.06
400	3.58 ± 0.19	0.70± 0.04	1.47± 0.11

Values are presented as Mean ± SEM.

Table 2: Effect of extract of *Persea americana* on serum low density lipoprotein and very low density lipoprotein in male Wistar rats

Groups (mg/kg)	Low Density Lipoprotein (mmol/L)	Very Low Density Lipoprotein (mmol/L)
Control	2.82± 0.32	0.47± 0.07
200	2.38 ± 0.13	0.41± 0.04
400	2.42 ± 0.18	0.32± 0.02*

Values are presented as Mean ± SEM. *Differences are considered significant at P<0.05 when compared to the control.

Table 3: Effect of extract of *Persea americana* on some oxidative stress markers

Groups (mg/kg)	Glutathione Reductase (µg/min/mg.protein)	Catalase (Units/mg.protein)	Superoxide Dismutase (Ug/mg.protein)	Malondialdehyde (Umol/mg.protein)
Control	1.49 ± 0.26	5.91 ± 0.51	0.44 ± 0.07	0.39 ± 0.06
200	1.63 ± 0.12	6.17 ± 1.44	0.54 ± 0.05	0.20 ± 0.04*
400	1. 81 ± 0.92	5.70 ± 0.32	0.30 ± 0.03	0.46 ± 0.04

Values are presented as Mean ± SEM. *Differences are considered significant at P<0.05 when compared to the control.

DISCUSSION

The evaluation of antioxidant properties of a plant extract is interesting and important because it is concerned with finding potential new sources of natural antioxidants that act as functional food products. In this study, there was no significant (p<0.05) change in the level of serum total cholesterol, triglyceride and high density lipoprotein cholesterol (Table 1) and low density lipoprotein cholesterol (Table 2), however, there was a significant change (a decrease) in very low density lipoprotein cholesterol level (Table 2). The findings in this study do not agree with the reported findings by Gouegni and Abubakar (2013) showing that *Persea americana* reduced total cholesterol by 24.91 percent, triglycerides by 37.97%, LDL by 59.57% and HDL by 14.54%. The differences could be due to the dosage and duration of administration. However, the researchers also reported a significant decrease in VLDL-c by 47.41% which is in agreement with findings in the present study. The lipid profile is frequently taken into account in the assessment of dyslipidaemia. The extract of *Persea americana* neither had an effect on LDL, which is considered atherogenic nor induced a rise in HDL, which is regarded as cardioprotective (Juhan-Vague *et al.*, 1991). But the extract caused a significant decrease in Very Low Density Lipoprotein, indicating some hypolipidemic effect of the extract in this investigation.

There was no significant alteration of the enzymatic oxidative stress markers in this study. The activities of glutathione reductase, superoxide dismutase and catalase enzymes were not significantly altered. which was not significant. This was observed when the measured enzyme activity in the test group was compared to the control. The level of Malondialdehyde (MDA) was significantly (p<0.05) reduced in the low dose (200mg/kg) extract in this study. Malondialdehyde is a product of lipid peroxidation. Lipid peroxidation is thought to be a good predictor of cell membrane damage caused by reactive oxygen species. Malondialdehyde is a common end-product of polyunsaturated fatty acid peroxidation and is widely used to determine oxidative stress conditions (Raghavendran *et al.*, 2004). An imbalance between the creation of reactive oxygen species and the antioxidant system's ability to detoxify them causes oxidative stress.

The presence of specific phenolic compounds including flavonoids and phenolic acids, which are apparently the principal anti-oxidative chemicals of fruits and vegetables, has been related to the antioxidant characteristic of many plants. Antioxidants such as phenols, flavonoids, and tannins are beneficial in the prevention and treatment of oxidative stress and related illnesses (Brown and Rice-Evans, 1998). *Persea americana's* ability to decrease lipid peroxidation could be attributed to a free radical scavenging mechanism that avoided additional peroxidation and potential cell injury.

CONCLUSION

The results obtained in the present study showed that the ethanolic stem bark extract of *Persea americana* exhibited slight hypolipidemic effect because of its ability to cause a significant reduction in serum concentration of Very Low Density Lipoprotein. Also, the extract caused a significant decrease in the level of Malondialdehyde which shows that *Persea americana* may have the ability to reduce lipid peroxidation, therefore, may possess an anti-oxidative property.

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