
ENHANCED ANTIOXIDANT AND OXIDATIVE LOWERING PROPERTIES OF AQUEOUS LEAF EXTRACT OF PERSEA AMERICANA (AVOCADO PEAR) IN NORMAL RABBITS

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Abstract

There is an increase in the use of herbal medicine (26; 17) and alongside a growing knowledge on the use of Persea Americana in the treatment of diseases. The study examined the effect of aqueous leaf extract of Persea americana (Avogado pear) on lipid peroxidation level and antioxidant enzymes in normal rabbits. Twelve New Zealand white rabbits weighing between 1.2kg to 1.8kg were used for this study. They were housed in clean disinfected hutches under standard conditions of temperature, humidity and light and acclimatized on growers mash for a week after which they were weighed and randomly assigned to two groups of six rabbits each. Rabbits in group one served as the control group. Animals in group two (test group) were given 2ml/kg body weight aqueous leaf extract of Persea Americana using cannula. All the animals had free access to feed (growers mash) and water throughout the duration of the experiment which lasted for twenty eight (28) days. Fresh feed was provided daily while stale remnants were discarded. The rabbits were weighed weekly to allow for adjustments in administration of the extract. Lipid peroxidation in form of malondialdehyde (MDA) was determined in the serum by using the method of Buege and Aust (1978). The glutathione reductase activity was measured by the method of Tietze (1969). Superoxide dismutase activity was measured by the method of Misra and Fridovich (1972). Glutathione Peroxidase activity was measured according to the procedure of Rotruck et al (1973) with slight modification. Analysis was done by student's t-test. Values that were less than 0.05 were accepted as significant. Results obtained from this study showed that aqueous leaf extract of Persea americana significantly ($P < 0.05$) decreased lipid peroxidation level (0.26 ± 0.24) and glutathione reductase activity (0.36 ± 0.24) and significantly ($P < 0.05$) increased super oxide dismutase activity (4.72 ± 0.56) as well as glutathione peroxidase activity (8.70 ± 0.33). These results suggest that aqueous leaf extract of Persia americana will be beneficial for the treatment of diseases.

Keywords: *Persea americana, lipid peroxidation, glutathione reductase, super oxide dismutase*

INTRODUCTION

A medicinal plant is a plant in which one or more of its organs contain substances that can be used for therapeutic purposes (9). Plants from different botanical sources have been used by many traditional medicine practitioners for the treatment of numerous diseases (44). One of such plants is *Persea Americana* that belongs to the family Lauraceae and commonly called Avocado pear, alligator pear or Mexican Avocado (30). The medicinal relevance of the various parts of *Persea Americana* plant is enormous (18). Previous studies have shown that *Persea Americana* possesses a catalogue of pharmacological activities. The leaf extract has anti ulcer properties (48 ; 37), hypotensive activity (1), hepatoprotective effects (16), analgesic and anti inflammatory properties (2). Anti convulsant property (36), vasorelaxant property (38) and anti hyperlipidaemic effect (25). The seed extract has antimicrobial activity (23), antihypertensive and hyperlipidaemic property (24), Hypolipidaemic and hypocholesterolic effect (33). The stem bark extract has hypoglycaemic property (21). There is an increase in the use of herbal medicine (26; 17) and alongside a growing knowledge on the use of *Persea Americana* in the treatment of diseases. This study is thus aimed to investigate the effect of aqueous leaf extract of *Persea Americana* on lipid peroxidation level and activities of antioxidant enzymes in normal rabbits.

MATERIALS AND METHODS

Experimental animals

Twelve (12) New Zealand white rabbits weighing between 1.2kg to 1.8kg were used for this study. They were obtained from the animal house of the College of Medicine, Faculty of Basic Medical Sciences, Ambrose Ali University, Ekpoma, Edo State, Nigeria. They were housed in clean disinfected hutches under standard conditions of temperature, humidity and light. They were fed with standard diet (pelleted growers mash) and distilled water. The animals were acclimatized for a period of one week to the new environment.

The experimental protocols were according to our Institutional Animal Ethics Committee guidelines as well as internationally accepted practices for use and care of laboratory animals as contained in US guidelines (32).

Chemicals/Reagents

All reagents/chemicals used were of analytical grades.

Medicinal plant

The *Persea Americana* leaves were collected from Idumebo, Ekpoma, and were identified by experts in the Department of Botany, Ambrose Alli University, Ekpoma, Edo state.

Extraction and preparation of plant extracts

Fresh leaves of *Persea Americana* were washed with clean water, sun dried for a period of three weeks and cut into small pieces. They were then pulverized to powder using an electric blender. Weighed with the aid of a weighing balance, 280g of the sample (leaves) was extracted separately in 2800ml of aqueous (distilled water) in cold percolation by maceration technique under room temperature. This was followed by periodic stirring then the macerated sample was filtered with cheese cloth to eliminate particles after 72hrs. The filtrate collected was then concentrated by applying direct heat (pasteurization method) for 4hrs to yield a

brown concentrate which was allowed to cool. This was preserved in the freezer at -21°C until used. To determine the actual volume (ml) to administer, 10ml of the boiled extract was introduced into a small beaker and placed in a water bath and then evaporated to dryness. This was weighed using a weighing balance. The difference in weight of the dry and clean beaker before evaporation and the beaker + 10ml of extract after evaporation was calculated accordingly and recorded as 303.2mg i.e 303.2mg/10ml of extract.

Experimental procedure

Twelve (12) New Zealand white rabbits weighing between 1.2kg to 1.8kg were used for this study. They were obtained from the animal house of the College of medicine, Faculty of Basic Medical Sciences, Ambrose Ali University, Ekpoma, Edo State, Nigeria. They were housed in clean disinfected hutches under standard conditions of temperature, humidity and light and acclimatized on growers mash for a week after which they were weighed and randomly assigned to two groups of six rabbits each. Rabbits in group one served as the control group. Animals in group two (test group) were given 2ml/kg body weight aqueous leaf extract of *Persea Americana*, two times daily (morning and evening) using cannula. All the animals had free access to feed (growers mash) and water throughout duration of the experiment which lasted for twenty eight (28) days. Fresh feed was provided daily while stale remnants were discarded. At the end of the experiment, blood was collected from the tail of the animals into immobilized lithium heparin bottles for analysis.

Blood Collection

The rabbit was restrained while the tail was cleansed with a ball of cotton wool soaked in methylated spirit. The tail of rabbit was gently and repeatedly massaged towards the tip following a vaseline smear. The red tip of the tail was then slightly and carefully incised with a new and sterilized blade and further massaged gently as the blood trickled into immobilized fluoride oxalate sample tubes. Cotton wool soaked in methylated spirit was again used to cleanse the incised area of the tail.

Blood samples collected were subjected to centrifugation for 10 minutes at 3,000 g to obtain the serum for lipid peroxidation, glutathione reductase, super oxide dismutase and glutathione peroxidase assays. Analysis was carried out immediately after centrifugation (41).

Biochemical Assays

Determination of Lipid Peroxidation

Malondialdehyde (MDA) is one of the most well studied markers of lipid peroxidation (Ho *et al.*, 2013). Lipid peroxidation in form of malondialdehyde (MDA) was determined in the serum by using the method of Buege and Aust (1978).

Principle:

The thiobarbituric acid was used to measure lipid peroxidation after reaction of the sample with TBA, increase in absorbance at 532 nm was measured.

Procedure:

Exactly two millilitres (2ml) of glacial acetic acid and 2ml of 1% thiobarbituric acid were added to 0.2ml of the supernatant (sample). The tube was stoppered loosely and immersed in boiling water for 15min and swirled slightly at intervals. The mixture was cooled and centrifuged for 10mins at 5000g. The absorbance was read at 532nm using the reagent blank.

Lipid peroxidation in units/g of wet tissue was calculated with a molar extinction co-efficient of $1.56 \times 10^5 \text{M}^{-1}$

Estimation of glutathione reductase activity

The glutathione reductase activity was measured by the method of Tietze (1969).

Procedure

2.5ml of sample were mixed with 1.5ml of 0.6mM 5,5-dithiobis-2-nitrobenzoic acid. Conjugate was measured at 412nm.

Detemination of total superoxide dismutase activity

Superoxide dismutase activity was measured by the method of Misra and Fridovich (1972)

Procedure

The blank tube was prepared by putting 2.7ml of 0.05M carbonate buffer into a tube. The test of sample tube was prepared by putting 2.5ml of carbonate buffer into a tube then adding 0.2ml of the sample. 0.3ml of 0.3mM adrenalin was added to each tube to initiate the reaction. The content of the tube was read at 480nm after one minute interval.

Estimation of glutathione Peroxidase activity

Glutathione Peroxidase activity was measure according to the procedure of Rotruck et al (1973) with slight modification.

Procedure

To 6ml of phosphate buffer in a test tube was added 1.5ml of NaN_3 (sodium azide), followed by 2.5ml of glutathione and then 0.05ml of hydrogen peroxide and 0.25ml of sample. The reaction mixture was incubated for 3 minutes at 37°C after which 0.25ml of TCA was added and the final mixture centrifuged at 3000 rpm for 5 minutes. To 1ml of the supernatant, 2ml of K_2HPO_4 and 1ml of DTNB were added and absorbance read against reagent blank containing 1ml of distilled water, 2ml of K_2HPO_4 and 1ml of DTNB at 412nm.

Statistical Analysis

All values are quoted as the mean \pm SEM. Data were analyzed using the student's t-test. Values that were less than 0.05 were accepted as significant.

RESULTS

Results of the analyses of serum samples for lipid peroxidation level and antioxidant enzymes in normal rabbits treated with aqueous leaf extract of *Persea Americana* are presented in table 1. Aqueous leaf extract of *Persea Americana* significantly ($P < 0.05$) decreased lipid peroxidation level and glutathione reductase activity and significantly ($P < 0.05$) increased super oxide dismutase activity as well as glutathione peroxidase activity.

Table 1: Plasma Malondialdehyde level, glutathione reductase activity, superoxide dismutase activity and glutathione peroxidase activity in normal rabbits treated with aqueous leaf extract of *Persea americana*

	GROUP 1 (CONTROL)	GROUP 2 (TEST)
MDA (mmoles/mg protein)	0.92±0.39	0.26±0.24*
GLUTATHIONE REDUCTASE (U/L)	0.87±0.39	0.36±0.24*
SUPER OXIDE DISMUTASE (U/mg Protein)	1.88±0.03	4.72±0.56*
GLUTATHIONE PEROXIDASE (U/L)	3.20±0.55	8.70±0.33*

Values are expressed as mean ± SEM * P<0.05 compared to control, n = 6 per group

DISCUSSION

Reactive oxygen species are produced as a normal product of cellular aerobic metabolism (39; 12) and occurs continuously in cells (27). When they are produced in excess or when cellular defenses are not able to metabolise them, they produce oxidative damage to lipids, proteins, carbohydrate and nucleic acids (12). Oxidative damage to lipids results in lipid peroxidation (12). Lipid peroxidation is one of the most investigated consequences of reactive oxygen specie actions on membrane structure and function (28). Lipid peroxidation have gained importance in recent times due to its central role in various physiological conditions as well as its implication in a variety of disorders such as cancer, diabetes, cardiovascular diseases, aging and other degenerative diseases in humans (19; 4). Malondialdehyde is one of the end products of lipid peroxidation and is a good indicator of the degree of lipid peroxidation (3). A rise in malondialdehyde indicates serious damage to cell membrane (40). The reduced malondialdehyde level observed in the group 2 rabbits upon administration of the extract indicates that the extract could prevent cellular damage and diseases linked to lipid peroxidation like diabetes. This is in agreement with the work done by Temitope et al (2018) who reported that *Persia Americana* leaf and fruit parts aqueous extract reduced malondialdehyde production in a dose dependent pattern with the leave showing anti lipid peroxidation strength.

Antioxidant enzymes which include superoxide dismutase, glutathione peroxidase and catalase are the first line of defense against superoxide and hydrogen peroxide (28). They act in concert to protect the organism from oxidative damage (Halliwell and \ (20) there by reducing the risk of degenerative diseases (35). The mechanism behind antioxidants actions have been emphasized to involve the scavenging of free radicals and activation of antioxidant enzymes (5). Studies have shown that flavonoids and other plant phytochemicals have antioxidant activity and could lower cellular oxidative stress and have protective role against degenerative diseases (42; 13), Aqueous leaf extract of *Persia Americana* is reported to contain various bioactive compounds such as flavonoids alkaloids, tannin and polyphenols which are known for their antioxidant activities (34; 11; 2). Results obtained from this study showed that aqueous leaf extract of *Persia Americana* significantly (P<0.05) increased the

activities of super oxide dismutase and glutathione peroxidase. This reflects increased defense against oxidative stress indicating that *Persia Americana* may protect against oxidative damage and diseases linked to them,

Glutathione reductase is not directly an antioxidant but it plays a crucial role in cellular defense. Glutathione which is the antioxidant get oxidized by oxidants leading to glutathione disulfide formation. The glutathione disulfide is subsequently returned to its reduced state by glutathione reductase (8), Significantly ($P < 0.05$) reduced levels of glutathione reductase was observed in this study. The reason for this is however not clear.

Attention is being focused on the use of herbal medicines for the treatment of diabetes (45). Studies have shown that *Persia Americana* is a valuable alternative for the control of diabetes mellitus (29). The leaves of *Persia Americana* have been reported to be an effective antidiabetic by traditional medicine practitioners of Ibibio tribe of southern Nigeria (7). Moussa, (2008) reported that diabetic patients suffer from enhanced oxidative stress and decreased antioxidant system. Results obtained from this study showed that aqueous leaf extract of *Persia Americana* significantly ($P < 0.05$) decreased lipid peroxidation level and glutathione reductase activity but significantly ($P < 0.05$) increased the activities of super oxide dismutase and glutathione peroxidase. These results suggest that aqueous leaf extract of *Persia Americana* may be beneficial for the treatment of diabetes mellitus and therefore support the rationale behind the use of *Persia Americana* in traditional medicine for the treatment of diabetes.

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