International Journal of Biomedical Science and Engineering
2024; Vol. 12, No. 1, pp. 1-9
https://doi.org/10.11648/j.ijbse.20241201.11

Research Article

Therapeutic Potential of *Persea americana* Stem Bark in Lipopolysaccharide (LPS) Induced Hepatic Inflammation and Oxidative Stress in Mice

Babatunde Oluwatoyin[1, *], Amuda Mutiu Olasunkanmi[2], Ore Ayokanmi[1]

[1]Department of Physical Sciences, Ajayi Crowther University, Oyo, Nigeria

[2]Department of Pure and Applied Chemistry, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

Abstract

This study investigated the potential of *Persea americana* stem bark for the treatment of inflammation and oxidation in mice. Oxidative stress occurs in the body when there is serious imbalance between the generation of free radicals and the antioxidant defense system, inflammation is a natural defense mechanism against pathogens and it is associated with many pathogenic diseases such as microbial and viral infections, exposure to allergens, radiation and toxic chemicals, autoimmune and chronic diseases, obesity, consumption of alcohol, tobacco use, and a high-calorie diet. Plants offer significant potentials for development of new anti-inflammatory therapies and treatment of diseases associated with inflammation and Oxidation. Stem bark of *Persea ameriacna* was extracted using EtOH/H_2O (80:20 v/v), the extract obtained was subjected to maceration using 3 different solvents, hexane fraction (PAHF), Ethyl-acetate fraction (PAEF) and methanol fraction (PAMF). The methanol fraction came out with the highest yield and was used for the animal study, 30 male mice was divided into 3 different groups, LPS was introduced in one of the groups, PAMF was later administered to the same group, The experiment shows that the extract was able to demonstrate significant reduction in inflammation and also ameliorate oxidation in mice. In conclusion the methanolic fraction of *Persea americana* stem bark might contain beneficial phytochemicals that can be explored as potential anti-inflammatory and oxidative therapy.

Keywords

P. americana, Methanolic Extract, LPS, Anti-inflammation

1. Introduction

Plants have been used by mankind for its therapeutic value. Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources. The plant-based, traditional medicine systems continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care [2]. Epidemiological and experimental evidence has shown that oxidative stress is closely related to chronic diseases such as cancer, diabetics and inflammation [11]. Currently, NSAIDs (non-steroid and inflammatory drugs.) are some of the most commonly used drugs in the world for the

*Corresponding author: talk2mt@yahoo.com (Babatunde Oluwatoyin)

Received: 29 January 2024; **Accepted:** 12 February 2024; **Published:** 2 April 2024

treatment of numerous conditions that involve inflammation. These NSAIDs have adverse effects when used and attention has been shifted medically to the use of traditional medicine and natural products used in folklore medicine as potential alternatives to these drugs, owing to their minimal toxicity effect in the gastrointestinal tract. It is therefore against this background that this study investigated the therapeutic potential of *persea americana* stem bark as alternative for the treatment of oxidation and inflammation in mice.

Persea americana (Avocado pear) is one of the numerous important plants with antimicrobial properties [4]. The seed, flower, leaves and stem bark have a useful application in ethno medicine ranging from treatment of diarrhea, dysentery, toothache, inflammation and antimicrobial [1]. Previous studies on *P. ameriacana* shows that seeds are rich in tannin, carotenoids and phenols [3]. The avocado leaves extract was reported to have antifungal properties and to be toxic to skin worms [5]. Similarly, *P. americana* root and stem bark extract also possess antibacterial activity against *staphylococcus aurens, Escherichia coli. Pseudomonas aeruginosa, serratia marcescence, enterobacter aregenes and Klebsellia pneumonia* which could cause serious infections [7].

Antioxidants are substances capable of preventing or slowing the oxidation of other molecules. They exert their protective action by suppressing the formation of free radicals [8]. Oxidative stress occurs in the body when there is serious imbalance between the generation of free radicals and the antioxidant defense system [9].

Free radical is defined as a reactive molecular species that contain unpaired electron in their outermost orbital. Free radical can be formed from molecules by the homolytic fission of a chemical bond and via redox reactions, which is a far more common process in biological systems [10]. Usually free radicals are very short-lived and derived from two elements: oxygen and nitrogen, thus creating highly reactive molecules like ROS and RNS.

Inflammation is clinically defined as a pathophysiological processes characterized by redness, edemia, fever, pain and loss of function. Currently the use of steroid anti-inflammatory drugs (SAID) and non-steroid anti-inflammatory drugs to treat acute inflammatory disorder, these conventional drugs have not been successful to cure chronic inflammatory disorder such as rheumatoid arthritis (AA) and atropic dermatitis (AD) [11]. Non-steroidal anti-inflammatory drugs (NSAIDs) are group of substances that relieve pain and fever and reduce inflammation [12]. They work by blocking a specific group of enzymes called cyclo-oxygenase enzymes, often abbreviated as COX enzymes. These enzymes are responsible for production of prostaglandins. Prostaglandins are a group of compounds with hormonian effects that control many different processes such as inflammation, blood flow, and the formation of blood clots. (MSDs Musculoskeletal disorder) are injuries and disorders that affects the human body movement or musculoskeletal system such as muscles, tendons, ligaments, nerver, disc and blood vessels. Chronic pain as a result of MSDs is a common reason for consultation in general practice [13]. Diclofenac was the most prescribe NSAIDs, a significant number of patients with MSDs reportedly experienced side effects which were mostly dizziness, abdominal pain, indigestion and gastric ulcer from NSAIDs. Most of them are not informed or unaware of the side effects [14].

Liver inflammation and oxidative stress significantly contribute to various liver diseases such as nonalcoholic fatty liver disease (NAFLD), hepatitis, and cirrhosis. Lipopolysaccharide (LPS) is a potent inducer of inflammation and oxidative stress in the liver and is often used as a model to study liver diseases. However, effective therapeutic interventions to alleviate LPS-induced hepatitis inflammation and oxidative stress remain limited. *Persea americana*, commonly known as avocado, is known for its potential health benefits due to its rich phytochemical composition, including bioactive compounds in the bark of its stems. Therapeutic potential of *Persea americana* stem bark to alleviate LPS-induced liver inflammation and oxidative stress in mice provides an interesting avenue for research and potential clinical applications.

Liver inflammation and oxidative stress are important factors in the development of liver diseases, and lipopolysaccharide (LPS) acts as a reliable trigger of these diseases in experimental models. Despite extensive research, developing effective therapeutic strategies to counter LPS-induced hepatitis inflammation and oxidative stress remains a challenge. The aim of this study is to investigate the therapeutic potential of *Persea americana* stem bark extract in reducing hepatitis inflammation and oxidative stress induced by LPS in mice.

2. Materials and Methods

2.1. Plant Under Study

2.1.1. Taxonomy

1) Kingdom: Plantae
2) Specie: *P.americana*
3) Genus: *Persea*
4) Family: Laurancea
5) Synonyms(s): *Laurus persea L, Persea drymifolia Schlecht, and cham, Persea gatissima Gaertn.f.,*

2.1.2. Common Names

1) Amharic: avocado
2) English: alligator pear, avocado-pear, butter fruit
3) Filipino: avocado
4) French*: avocet, avocatier, zabelbok, zaboka*
5) Current name: *Persea americana*
6) Authority: Miller

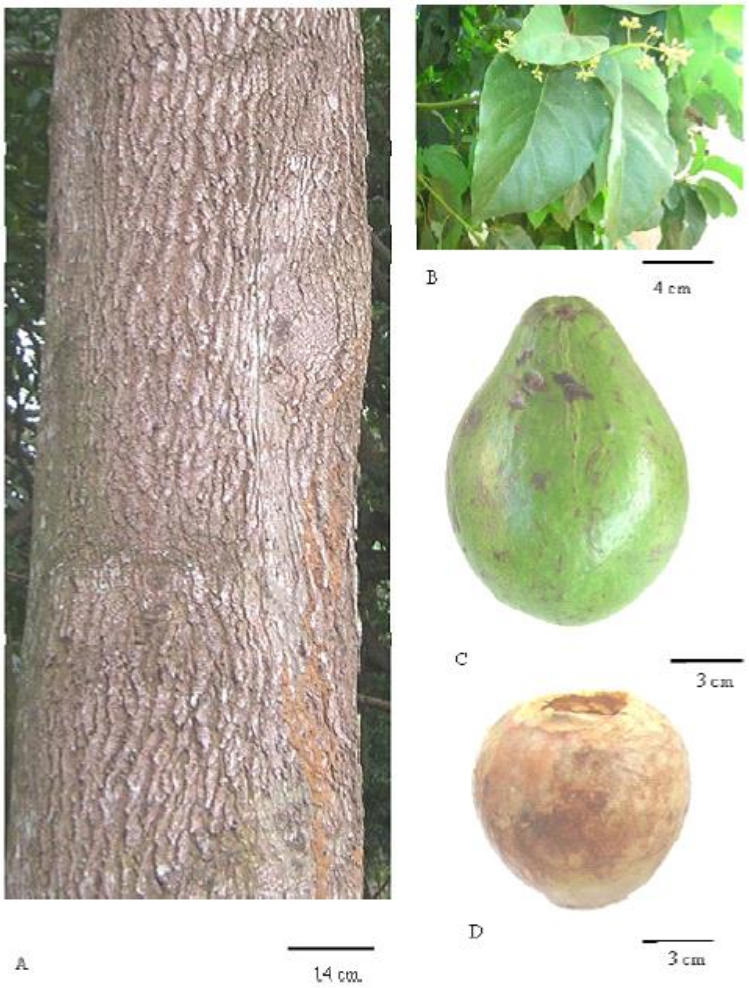

Figure 1. *Diagram of P. americana tree showing the stem bark, leaves, and fruits.*

2.2. Sample Collection and Preparation

Fresh stem back of *Persia americana* was collected from an uncultivated farm land in Aroje, Ogbomosho in Oyo State (Latitude 8^o 07' 60.00"N, Longitude 4^o 14' 60.00" E). The stem back was identified at the Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomoso, with a voucher number (Voucher No. LHO 656) and deposited in the institution's herbarium.

2.3. Animal Study

A total number of 30 male mice were purchased at the animal house in university of Ibadan. They were housed with plastic cages under standard lighting conditions 12 hrs. light/dark cycles, and allowed free access to tap water and standard food and acclimatized for two weeks at the animal house of the department of Biochemistry and are only fed with pellets and water. After the scheduled period of acclimatization, the rats were randomly selected for the administration of test substances into various groups.

2.4. Extraction

The stem was then taken to the laboratory and air-dried at room temperature for three weeks. The stems were pulverized to increase its surface area and allow a better contact of the extracting solvents with the sample for effective extraction.

Extraction was achieved using cold extraction method. Powdered sample (1000g) was soaked with n-hexane (non-polar solvent) in a glass jar. The solvent was decanted and filtered off after 24 hours. The process was repeated three times to remove chlorophyll and other non-polar compounds. The defatted sample was then soaked with 80:20 EtoH/H_2O for 24 hours. The supernatant was carefully decanted and the residue re-soaked with 80:20 EtoH/H_2O until 5 L of supernatant was obtained. The clear supernatant was thereafter concentrated *in vacuo* at 40^oC 0using a rotary evaporator to obtain a crude extract which was kept airtight in readiness for maceration. Crude extract (63.92g) was macerated successively with n-hexane, ethyl acetate and methanol. The methanol fraction represent *Persea americana* Methanolic Frac-

tion, henceforth, PAMF.

2.5. Grouping and Treatment Regimen

Thirty (30) male Balb/c albino mice (20-23 g) were divided into three (3) groups (n = 10 per group) and were treated as follows:

Group I (Control): Mice in this group were given the vehicle once daily for 7 days; and injected Physiological saline (2.5 mL/kg intraperitoneally, i.p.) on day 7.

Group II (Lipopolysaccharide, LPS): Mice in this group were administered 4 mg/kg LPS i.p. on day 7.

Group III (LPS + PAMF): mice in this group were pretreated with 125 mg/kg PAMF once daily for 7 days, and 4 mg/kg LPS i.p. on the 7th day just before receiving the last dose of PAMF. The dose of LPS used (4 mg/kg) has been shown previously to induce hepatic damage and oxidative stress [15].

2.6. Sacrifice of Animals and Preparation of Plasma Sample

The animals were first injected with 0.5 ml ketamine, before they were sedated with chloroform causing muscle relaxation and unconsciousness. Afterwards, the animals were dissected with a dissecting scissors or surgical blade. Blood samples were drawn through cardiac puncture using a syringe. This was then dispensed into EDTA bottle bottles for hematological assay.

The heparinize bottle carrying the blood sample was centrifuged 4000 rpm for 15 minutes. After centrifuging, the plasma was extracted into a sample bottle using a pasture pipette, while the sediment was discarded. The collected plasma samples were stored at 4°C until assay.

2.7. Tissue Collection and Tissue Homogenization

Liver was blotted to dry and weighed. Three mice were subjected to intra-cardiac perfusion, and liver from these animals were fixed in 10% neutral buffered formalin (NBF) for histopathology.

Approximately 0.5 g of liver was minced and homogenized in PBS (10 % w/v). The homogenate was centrifuged at 10 000g for 10 minutes at 4 ℃. The resulting supernatant was collected and stored frozen until used for further analyses.

2.8. Anti-oxidant/2,2-Diphenyl-1-picrylhydrazyl (DPPH assay), 2,2-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic) Acid (ABTS Assay) and Nitric Oxide Radical Scavenging Assay

Various concentrations (1ml) of the methanolic extract was added to 4 ml of 0.1mmol methanolic solution of DPPH. A blank probe was obtained by mixing 4 ml of 0.1mmol methanolic solution of DPPH and 200 µl of distilled water. After 30 mins of incubation in the dark at room temperature, the absorbance was taken at 515 nm against the prepared blank. Inhibition of the free radicals by DPPH in percent (I %) was calculated using the formular:

$$I\ (\%)\ = \frac{(A_{blank} - A_{sample})}{A_{blank}}$$

Ascorbic acid was used as standard control. IC_{50} denote the concentration of sample which is required to scavenge 50% of DPPH free radicals.

The antioxidant potential of the stem bark extract was measured using 2, 2'-azinobis- (3-ethylbenzothiazoline-6-sulfonic acid), [33]. 0.17mM ABTS, 40µg/ml extract, 25µg/ml ascorbic acid and phosphate buffer (pH = 7.4) were mixed together, 3.5 ml of the mixture was placed in a UV-vis spectrophotometer and the absorbance recorded at a wavelength of 734 nm. The antioxidant potential of the leaf extract was measured against the standard.

Different concentrations of the extract were prepared for nitric acid scavenging activity and each one was added to sodium prusside (10MM) in phosphate buffered saline. (0.5ml). Ascorbic acid dissolved in methanol (2 ml) was also added to the mixture and incubated at room temperature for 150 min. 0.5 ml of Griess reagent was added and the absorbance measured at 546 nm.

2.9. Biochemical Analysis

2.9.1. Determination of Plasma Alanine Aminotransferase, (ALT)

Activity of ALT in the serum was determined using ALT assay kit according to the manufacturer's protocol (Fortress® Diagnostics Limited, Atrim UK).

2.9.2. Estimation of Liver Reduced Glutathione (GSH)

Reduced glutathione (GSH) was determined in samples according to [16].

2.9.3. Determination of Liver Superoxide Dismutase, SOD

SOD activity was determined by the method of Sun and [17].

2.9.4. Determination of Liver Myeloperoxidase Activity (MPO)

Myeloperoxidase (MPO) activity, an indicator of polymorphonuclear leukocyte accumulation, was determined according to the modified method of Kim [18].

2.10. Statistical Analysis

Data are presented as the mean ± *SD* of 6 replicates. Analysis of variance (ANOVA) and Turkey's test were performed using Graphpad® Prism 6.0.1 (Graphpad Software, La Jolla, CA). Statistical significance was based on *p* values less than 05.

3. Results

The antioxidant activities of garlic acid and PAMF are presented in Figure 2. The antioxidant activity of garlic acid shows a steady increase against PAMF which initially reduces, before showing a steady increase and attained maximum inhibition at concentration of 150 µg/l.

The data below as Figure 3, indicate that garlic acid used as standard maintain a steady inhibition even at different concentration against the PAMF extract which have a slight increase and a plate. The extract was able to inhibit at maximum concentration of 50 µg/l.

Data presented in Figures 4 and 5 shows a steady increase in the activity of garlic acid which is used as standard for both DPPH and ABTS, while the extract was not able to inhibit adequate oxidation. However, the extract is not active against DPPH and ABTS.

Data presented in Figure 6 shows that the Gallic acid was used as a standard has a constant activity at different concentration, while the extract shows a steady increase in its activity and a plateau while the extract was able to inhibit at maximum concentration of 50 µg/l.

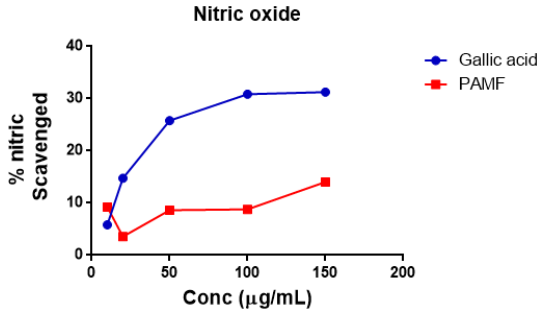

Figure 2. *In-vitro antioxidant activity of PAMF against Nitric oxide, PAMF represent Persea americana methanolic fraction.*

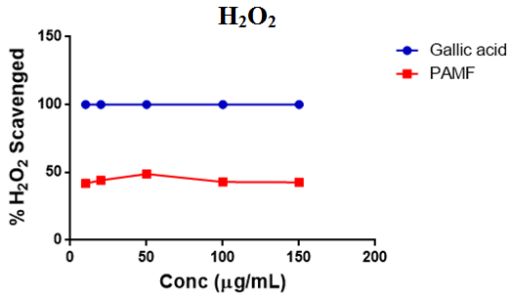

Figure 3. *In-vitro antioxidant activity of PAMF against H_2O_2, PAMF represent Persea americana methanolic fraction.*

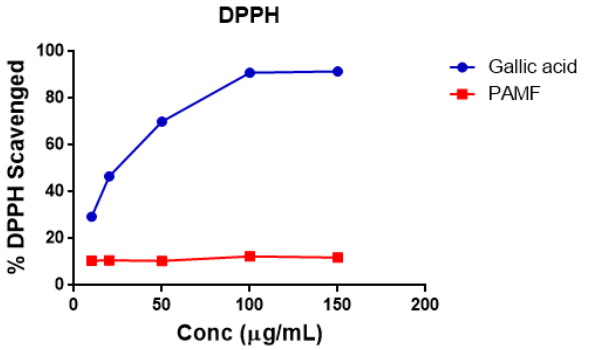

Figure 4. *In-vitro antioxidant activity of PAMF against DPPH, PAMF represent Persea americana methanolic fraction.*

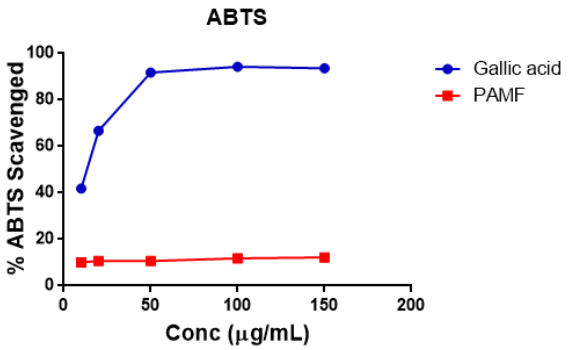

Figure 5. *In-vitro antioxidant activity of PAMF against ABTs, PAMF represent Persea americana methanolic fraction.*

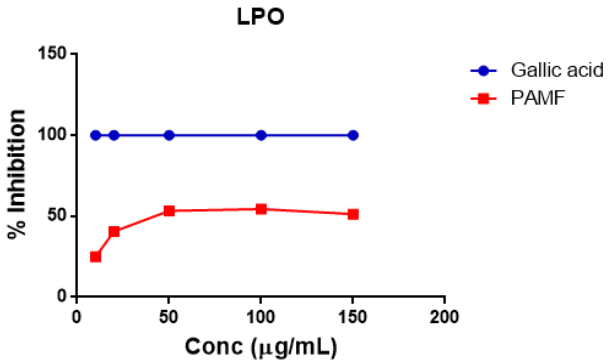

Figure 6. *In-vitro antioxidant activity of PAMF against Lipid peroxidation, PAMF represent Persea americana methanolic fraction.*

Aspartate aminotransferase (AST) and Alanine-Aminotransferase (ALT) are enzymes found mostly in the liver, at low quantity. When the body tissue or an organ such as liver is damaged, additional AST and ALT is released into the blood stream. The amount of AST and ALT releaseed is directly related to the extent of damage done to the tissue. So the data, shows that lipopolysaccharide (LPS) that was introduced causes an increase in the level of AST and ALT respectively, but when the extract was introduced it significantly alters the injury by reducing the effect of LPS. However, the extract was able to ameliorate the damage caused to the liver significantly.

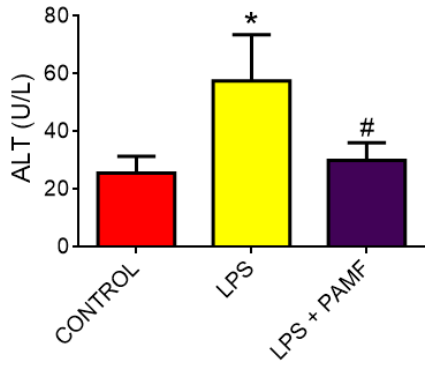

Figure 7. *Protective effect of PAMF on LPS induced increase in plasma alanine aminotransferase (ALT) activities of mice.*

* represent significance difference between control and LPS
# represent significance difference between LPS and LPS+PAMF.
PAMF represent *Persea americana* methanolic fraction

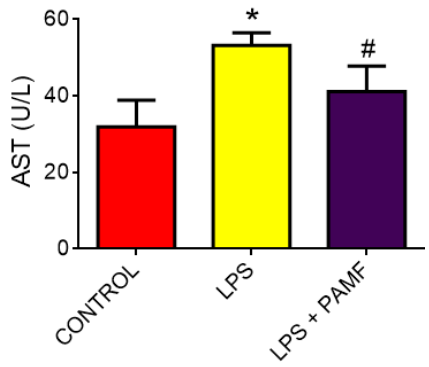

Figure 8. *Protective effect of PAMF on LPS induced increase in plasma aspartate amino transferase (AST) activities of mice.*

* represent significance difference between control and LPS
# represent significance difference between LPS and LPS+PAMF.
PAMF represent *Persea americana* methanolic fraction

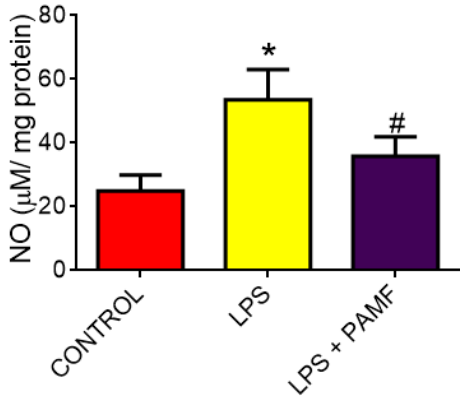

Figure 9. *Protective effect of PAMF on LPS induced inflammation in liver nitric oxide (NO) concentration in mice.*

* represent significance difference between control and LPS
# represent significance difference between LPS and LPS+PAMF.
PAMF represent *Persea americana* methanolic fraction

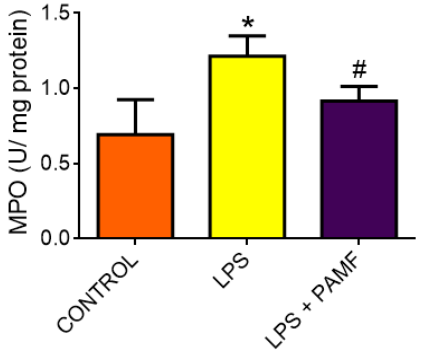

Figure 10. *Protective effect of PAMF on LPS induced inflammation in liver myloperoxidase (MPO) activity of mice.*

* represent significance difference between control and LPS
# represent significance difference between LPS and LPS+PAMF.
PAMF represent *Persea americana* methanolic fraction.

Figures 7 and 8 Nitric oxides play an important role in defense mechanisms, it mediates signaling during several physiological processes and stress response in the cell [32]. From the data presented in Figure 9, increase in nitric oxides possess serious threat to the liver and can lead to damage of the tissues as a result of inflammation, while introduction of the extract causes a significant alteration by reducing the level of injury caused by LPS in the tissue.

Myeloperoxidase (MPO), is white blood cell, derived inflammatory enzyme that measures disease activity from the luminal aspect of the arterial wall. When the artery wall is damaged, or inflamed, MPO is released by invading macrophages where it accumulates [30]. MPO mediates the vascular inflammation that propagates plaque formation [31] and activates protease cascades that are linked to plague vulnerability [29]. The data below as shown in Figure 10, shows that when LPS was introduced, it increases the myloperodase level significantly but introduction of the extract shows significant reduction in the myeloperoxidase level. However, the extract possesses anti-inflammatory agent.

Malodidehyde (MDA) is the principal and most studied product of polyunsaturated fatty acid peroxidation, this aldehyde is highly toxic molecule and should be considered as more than just a marker of lipid peroxidation [28]. Data below (Figure 11), show significant increase in MDA level when treated with LPS which induces oxidative stress, however, application of the extract significantly altered the stress by reducing the MDA levels.

Glutathione (GSH) is one of the body most important and potent antioxidants. Antioxidants are substance that reduces oxidative stress by combating free radicals in the body. Most antioxidants are found in foods. However, glutathione is produced by the body. There was a significant reduction in glutathione level when LPS was introduced compared to the control level. However, the introduction of extract significantly increased glutathione level [26]. Figure 12.

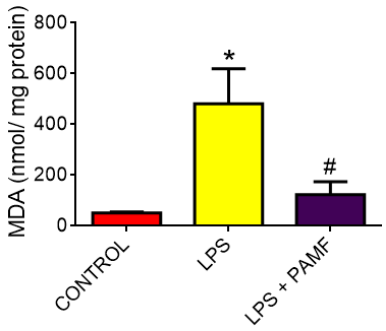

Figure 11. *Protective effect of PAMF on LPS induced oxidation in liver malondidehyde (MDA) level of mice.*

* represent significance difference between control and LPS
# represent significance difference between LPS and LPS+PAMF.
PAMF represent *Persea americana* methanolic fraction

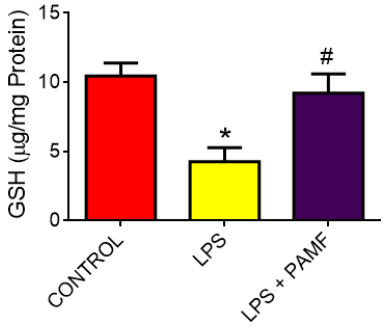

Figure 12. *Protective effect of PAMF on LPS induced oxidation in liver reduced glutathione (GSH) level of mice.*

* represent significance difference between control and LPS
# represent significance difference between LPS and LPS+PAMF.
PAMF represent *Persea americana* methanolic fraction

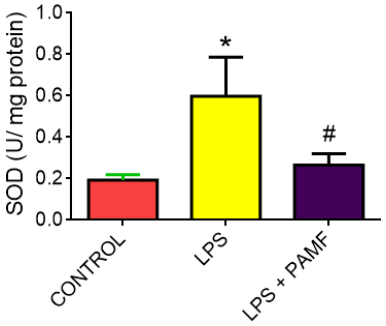

Figure 13. *Protective effect of PAMF on LPS induced oxidation in liver reduced superoxide dismutase (SOD) level of mice.*

* represent significance difference between control and LPS
# represent significance difference between LPS and LPS+PAMF.
PAMF represent *Persea americana* methanolic fraction

Superoxide dismutase (SOD), are group of enzymes that catalyzed the dismutation of superoxide radicals (O^{2-}) to molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) providing cellular defense against reactive oxygen species. The data below Figure 13, shows that there is serious of oxidative damage is present in the tissue, with these damage, the introduction of the extract alter the damage caused by LPS, which signify the extract is rich in antioxidants [27].

4. Discussion

The bioactivity of the extracts was determined at concentrations of 50 µg/ml, 100 µg/ml, 150 µg/ml, and 200 µg/ml and expressed as % inhibition. The percent inhibition of nitric oxide (NO), lipid peroxide (LPO), and hydrogen peroxide (H_2O_2) in the extracts showed concentration-dependent antioxidant activity in the control. The methanolic stem bark extract of P. americana had a comparable reducing capacity compared to the scavenging activity of known standards at all measured concentrations. High antioxidant activity may be related to the plant's curative or therapeutic potential for many diseases claimed in ethno-medicine [6].

DPPH is widely used to evaluate the antioxidant activity of natural products derived from plants and microorganisms [25]. The results of the present study showed that the in vitro radical potential of the extract had the highest radical scavenging activity with IC_{50} values comparable to known standards. The higher the DPPH radical scavenging activity, the lower the IC_{50} value. Therefore, the results presented here suggest that the extracts have lower DPPH and ABTs radical scavenging activities, which are not significant compared to the garlic acid standard.

Nitric oxides play an important role in the inflammatory process, but when concentrations increase they can be directly toxic to tissues, causing vascular damage and other problems. This toxicity increases when it reacts with superoxide radicals to form a second reactive compound, peroxynitrite anion (ONOOH) [24]. This plant extract inhibits the process by removing peroxynitrites. *P. americana* stem bark showed activity comparable to garlic acid. This activity may be related to the presence and concentration of secondary metabolites present in the trunk cortex. Inflammation is a natural defense mechanism against pathogens, including microbial and viral infections, exposure to allergens, radiation and toxic chemicals, autoimmune and chronic diseases, obesity, alcohol consumption, smoking, and high-calorie diets. is associated with many pathogenic diseases. [23]. To investigate this, we developed a mouse model to test whether lipopolysaccharide (LPS)-induced inflammation is altered by methanol extract from *P. americana* stem bark. LPS was chosen as a commonly used stimulus to induce acute inflammation in mouse models [21]. LPS signaling may not be representative of other inflammatory conditions generated by signaling through other pathogen recognition receptors, but it does induce predictable cytokine responses and activation and influx of various cells. It is a well-characterized acute inflammatory stimulus [22].

Mice injected with LPS experienced an acute pulmonary and systemic inflammatory state prior to infection and an

influx of neutrophils into the lungs, which is expected after an acute inflammatory stimulus such as LPS [19]. These findings suggest that inflammation induced by LPS can be ameliorated by *P. americana* stem bark methanolic extract. Examples of this in the literature include studies of old mice with chronic inflammation in both the periphery and the lungs [20]. Although adaptive immune function was not tested in LPS mice, a reduction in inflammation levels was observed when a methanol extract from *P. americana* stem bark was introduced. However, we do not know how LPS mice were able to maintain their levels of inflammation beyond the initial inflammation reduction when our extract was introduced. This may be due to changes in the cellular profile during infection or remodeling of lung granulomas. Alternatively, the influx of neutrophils observed in LPS mice may activate other cells such as dendritic cells, increasing their effectiveness in keeping extractive levels low. The simplest explanation could be that LPS and LPS+PAMF have different inflammatory loads despite ingesting the same dose of methanol extract from *P. americana* stem bark.

5. Conclusion

This study established that the plant offer significant potential for the development of new anti-inflammatory therapies and treatment of diseases associated with LPS induced. Persea Americana stem bark was able to reduce inflammation significantly and also ameliorate oxidative stress. Results from this research validate the ethno-medicinal uses of *Persea americana* for the treatment of several inflammatory ailments by traditional medicine practitioners. Therefore, this research work is an addition to the world data on natural product.

6. Recommendation

This study investigated the potential of the stem bark of *P. ameriana for therapeutic usage for the treatment of inflammation and oxidation in mice. More investigation is required on other parts of the plant to validate the plant usefulness to man.*

Abbreviations

NSAID: Non-Steroid and Inflammatory Drugs
NAFLD: Non-Alcoholic Fatty Liver Disease
LPS: Lipopolysaccharide
ROS: Reactive Nitrogen Specie
Ethanol: EtOH
Water: H_2O
PAHF: Hexane Fraction
PAEF: Ethyl-acetate Fraction
PAMF: Methanol Fraction
SAID: Steroid Anti-Inflammatory Drugs
AA: Rheumatoid Arthritis
AD: Atropic Dermatitis
COX: Cyclo-oxygenase Enzymes
MSD: Musculoskeletal Disorder
ABTS: 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)
NO: Nitric Oxide
PAMF: *Persea americana* Methanolic Fraction
NBF: Neutral Buffered Formalin
ALT: Alanine Aminotransferase
GSH: Reduced Glutathione
SOD: Superoxide Dismutase,
MPO: Myeloperoxidase Activity
DPPH: 2,2-Diphenyl-1-picrylhydrazyl

Authors Contributions

Babatunde O. conceptualize and supervised the research work, Amuda M. O carried out the research work and also source for the materials used, while Ore A. supervised the animal study.

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Edewor-Kuponiyi T. I. (2013). Spectroscopic determination of total phenolic and flavonoid contents, and antioxidant activities of leaves P. Americana. Int. J. Pharm. Sci. vol. 5, issues 3, p. 598-603.

[2] Owolabi M A, Coker H A B, Jaja S I, (2000). Flavonoid metabolites in urine after oral administration of the aqueous extract of P. Americana to rats. *J. Nat. Med.,* 61; p. 200-204.

[3] Owolabi M. O (2010). What are the consistent predictors of generic and specific post stroke health- related quality of life? Cerebrovasc Dis. 29(2), 105-10.

[4] Amuda M. O., Edewor T. I., Ogunyemi O. (2023). Antimicrobial analysis of *Persea americana* leaf. *Am. J. Pharmtech. Res. 13 (01).*

[5] Oelrichs P. B, Ng J. C, Seawright A. A, Ward A, Schaffeler L, MacLeod J. K. (1995). Isolation and identification of a compound from avocado (*Persea americana*) leaves which causes necrosis of the acinar epithelium of the lactating mammary gland and the myocardium. Nat Toxins; 3: 344-9.

[6] Edewor T. I., Agboola P. O., Amuda M. O.,. Mmuo A. I., Owa S. O (2023). Total phenolic, flavonoid and mineral contents of the methanolic leaf extract of Parinari curatellifolia planch, ex benth.

[7] Igbal S., Farooq A. (2005). Anti oxidant properties and components of some commercially available varieties of rice bran in Pakistan. Food chemistry 93: 265-272.

[8] Sivanandham V., (2011). Free radicals in health and diseases-a mini review. Pharmacologyonline. 1: 1062-1077.

[9] Juranek I., Bezek S. (2005). Controversy of free radical hypothesis: reactive oxygen specie-cause or consequence of tissue injury? Gen Physiol. Biophys. 24: 263-278.

[10] Halliwell B. (2008). Are polyphenols antioxidants or pro-oxidants? What do we learn from cell culture and in vivo studies? *Arch Biochem Biophys*. 476: 107-12.

[11] Hyun P. K., Kan H. S., Hyeun W. C., Sam S. K. (2004). Anti-inflammatory plant flavonoids and cellular action mechanisms. J. Pharmacol. Sci. 96, 229-245.

[12] Shen. S. C., Lee W. R., Lin H. Y., Huang H. C., Ko C. H., Yang L. L. (2002). In vitro and in vivo inhibitory activities of rutin, wogonin, and quecetin on LPS induced nitric oxide and postagladin E2 production. Eur. J. Pharmacol. 446: 187-194.

[13] Annika W., Thomas L. M., Kristen M. D, Steve T. (2012). Creatinine inhibit bacteria replication. J. Antibiotic 65(3): 153-156.

[14] Adeyemi O. O, Okpo S. O, Ogunti O. O. (2002). Analgesic and antiinfl ammatory effects of the aqueous extract of leaves of *Persea americana* Mill (Lauraceae). Fitoterapia; 73: 375-80.

[15] Ore Ayokanmiand Oluseyi A. A (2019). Oxidative stress and biomarkers in clinical and experimental models of non-alcoholic fatty liver diseases Medicinal (Kaunas). 55(2): 26.

[16] Jollow D. J., Mitchell J. R., Zampaglione N., Gillete J. R. (1974). Bromobenzene induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as a hepatotoxic metabolite. Pharmacology. 1: 151-169.

[17] Sun M. and Zigman S. (1978). An improved spectrophotometric assay for superoxide dismutate based on epinephrine autoxidation. Anal. Biochem. 90, 81-89.

[18] Kim H. J., Wei Y., Wojtkiewicz G. R, Lee J. Y., Moskowitz M. A., and Chen J. W (2019). Reducing myeloperoxidase activity decreases inflammation and increases cellular protection in ischemic stroke. J. Cereb. Blood flow metab. 39, 1964-1877.

[19] Iqbal A. J., Fisher E. A., Grease D. R. (2016). Inflammation- a critical appreciation of the role of myeloid cells. Microbial. Spectr. 4: http://doi.org/10.1128/micro-biospec. MCHD-0027-2016.

[20] Canan C. H., Gokhale N. S., Caruthers B., Lafuse W. P., Schlesinger L. S., Torrelles J. B., Turner J. (2014). Characterisation of lung inflammation and its impact on macrophage function in aging. J. Leukoc. Biol. 96: 473-487.

[21] Juskewitch J. E., Knudsen B. E., Platt J. L., Nath K. A, Knutson K. L, Brunn G. J., Grande J. P. (2012). LPS induced murine systemic inflammation is driven by parenchymal cell activation and exclusively predicted by early MCP-1 plasma level. Am. J. Phatol. 180: 32-40.

[22] Palsson-McDermott E. M. and Oneill L. A. (2004). Signal transduction by the LPS receptor, Toll- like receptor -4. Immunology. 113: 153-162.

[23] Tarique Hussain, Bie Tan, Yulong Yin, Francois Blachier, Myrlene C. B. Tossou, and Najma Rahu, (2016). Oxidative Stress and Inflammation: What PolyphenolsCan Do for Us? Hindawi Publishing Corporation Oxidative Medicine and Cellular Longevity Volume 2016, Article ID 7432797, 9 pages http://dx.doi.org/10.1155/2016/7432797

[24] Balavione G. G., Geletti Y. V. (1999). Peroxynitrite scavenging by different antioxidants. Part 1: convenient study. Nitric oxide. 3: 40-54. http://doi.org/10.1006/NIOX.1999.0206

[25] Josiah and Bartholomew (2015). In-vitrp antioxidant capacity and free radical scavenging evaluation of active metabolite constituents of newbouldia laevis ethanolic leaf extract. Bio. Res. 48(1): 16. http://doi.org/10.1186/540659-015-0007-x

[26] Yuan L. and Kaplowitz N. (2008). Gluthathione in inner disease and hepatocity. Mol. Aspect Med. In press.

[27] Kim H. W, Murakami A, Nakamura Y, Ohigashi H. (2002). Screening of edible Japanese plants for suppressive effects on phorbol esterinduced superoxide generation in differentiated HL-60 cells and AS52 cells. Cancer Lett; 176: 7-16.

[28] Daniel C., Chihhao C., Ting-chieh H. (2005). Free radical the body killer. National Taichung second high school. Pp 1-7. Retrieved from www.pdffactory.com

[29] Fu X., Kassim S. Y., Park W. C., Heinecke J. W (2001). Hypochlorus acid oxygenated the cysteine switch domain of promatilysm (MMP-7). J. Biochem. 276: 41279-41287.

[30] Tavora F., Mary R., Ling Li, Allen P. B (2009). Monocytes and neutrophils expressing myeloperoxidase occur in fibrous caps and thumbl in unstable coronary plaques. BMC cardiovascular disorder 9(1): 27.

[31] Anderson M. M., Hzan S. L., Hsu F. F., Heinecke J. W (1997). Human neutrophils employs the myeloperoxidase-hydrogen peroxide-chloride system to convert hydroxyl-amino acid into glycolaldehyde, 2-hydroxy-propanal and acrolein. *J. clinical investigation*. 99, 424-432.

[32] Benson-bard A., Courtiuos C., Gauthier A. (2008). Nitric acid in plants. production and cross-take with Ca^{2+} signaling. Mol. Plant. 1: 218-228.

[33] Rice-Evans (2001). Flavonoid antioxidants. Curr. Med. Chem. 8(7): 797-807.