

Pharmacotherapeutic Potential of *Persea americana* and *Vernonia amygdalina* in Modulating Oxidative Stress in a Rodent Model of Experimentally-induced Gestational Hypertension

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Abstract

Background: Gestational hypertension is a major obstetric complication associated with oxidative stress and endothelial dysfunction. The pharmacological potential of natural plant extracts in mitigating these oxidative effects has gained increased research attention. This study investigates the modulatory effects of *Persea americana* (avocado) and *Vernonia amygdalina* (bitter leaf) on oxidative stress biomarkers in lipopolysaccharide (LPS)-induced gestational hypertension in rats.

Methods: Leaves and seeds of *P. americana* and leaves of *V. amygdalina* were collected from Ikere-Ekiti, Nigeria, and authenticated. Extracts were obtained using ethanol maceration. Fifty-four confirmed pregnant albino rats were randomly assigned into nine groups (A–I), with LPS administered intraperitoneally at 0.1 mL to induce hypertension on gestational days 13–15. Concurrent treatment for 7 days was given using different concentrations (100 mg/kg and 200 mg/kg) of plant extracts and Aldoxi (0.036 mg/kg) as standard. Blood samples were collected on gestational days 20–21 for oxidative stress analysis. One-way ANOVA followed by Tukey's post hoc test was used for statistical evaluation at $P \leq 0.05$.

Results: LPS exposure significantly reduced glutathione (GSH) and glutathione peroxidase (GPx), and elevated malondialdehyde (MDA), indicating increased oxidative stress. Treatment with *P. americana* and *V. amygdalina* notably improved GSH and GPx levels, and reduced MDA and oxidized glutathione (GSSG) levels, particularly at 200 mg/kg doses. The effects were comparable to the standard drug Aldoxi.

Conclusion: *Persea americana* and *Vernonia amygdalina* demonstrated significant antioxidant properties, mitigating oxidative stress in LPS-induced gestational hypertension. These findings suggest their potential as adjunct pharmacotherapeutic agents in the management of hypertensive disorders during pregnancy.

Key words: black women; depression; somatic symptoms; cultural competence; mental health; systemic barriers; community support; holistic health; misdiagnosis; healthcare access

Introduction

Gestational hypertension, a subset of hypertensive disorders of pregnancy (HDP), constitutes a major global health challenge and remains a leading cause of maternal and perinatal morbidity and mortality [1]. It is typically

diagnosed after 20 weeks of gestation in previously normotensive women and is characterized by elevated blood pressure without proteinuria. However, in certain cases, it may progress to preeclampsia, a more severe

condition associated with endothelial dysfunction, systemic inflammation, and oxidative stress [2]. The pathophysiological mechanisms underlying gestational hypertension are multifaceted, involving impaired placentation, immune maladaptation, and increased production of reactive oxygen species (ROS), leading to oxidative damage and endothelial injury [3].

Oxidative stress, defined as an imbalance between the production of ROS and the antioxidant defense system, plays a central role in the pathogenesis of gestational hypertension and preeclampsia [4]. Excessive ROS generation disrupts vascular homeostasis, exacerbates inflammation, and impairs nitric oxide (NO) bioavailability, contributing to elevated vascular resistance and hypertension [5]. Animal models have been instrumental in elucidating the underlying mechanisms of gestational hypertension, with lipopolysaccharide (LPS)-induced inflammation being a widely accepted model that mimics the inflammatory milieu and oxidative stress seen in preeclamptic pregnancies [6]. LPS, a potent endotoxin from gram-negative bacteria, triggers toll-like receptor-4 (TLR4)-mediated immune responses, increasing systemic cytokine production and oxidative damage, thereby serving as a reliable inducer of experimental gestational hypertension [7].

Conventional pharmacological interventions for gestational hypertension include antihypertensives such as labetalol, hydralazine, and methyldopa. However, these agents often present limitations due to side effects, safety concerns during pregnancy, and inconsistent efficacy [8]. Consequently, there is growing interest in exploring alternative and complementary therapeutic strategies derived from medicinal plants with antioxidant and anti-inflammatory properties. Phytotherapy offers a promising avenue for developing safer and more effective interventions for managing oxidative stress-related disorders, including gestational hypertension [9].

Among the numerous medicinal plants with potential therapeutic benefits, *Persea americana* (avocado) and *Vernonia amygdalina* (bitter leaf) have garnered attention due to their rich phytochemical profiles and well-documented antioxidant, anti-inflammatory, and antihypertensive activities. *Persea americana* is known to contain bioactive compounds such as flavonoids, polyphenols, vitamins E and C, and oleic acid, which contribute to its antioxidant and cardioprotective properties [10]. Studies have demonstrated that avocado extracts mitigate oxidative stress, modulate lipid profiles, and improve vascular function [11]. Similarly, *Vernonia amygdalina*, a common leafy vegetable in Africa, is rich in flavonoids, saponins, tannins, and sesquiterpene lactones, which possess potent antioxidant and anti-inflammatory effects [12]. Empirical evidence suggests that *Vernonia amygdalina* enhances enzymatic antioxidant activity, reduces lipid peroxidation, and ameliorates blood pressure in hypertensive models [13].

Despite the growing body of literature supporting the individual effects of *P. americana* and *V. amygdalina* on oxidative stress and cardiovascular health, there is a paucity of studies investigating their pharmacotherapeutic potential in the context of gestational hypertension, particularly using established experimental models such as LPS-induced hypertension. Moreover, the synergistic effects of these plants in modulating oxidative stress biomarkers and restoring endothelial function remain largely unexplored. Investigating their combined effects could provide novel insights into the development of plant-based therapeutics that are both efficacious and safe for maternal health.

This study, therefore, seeks to evaluate the pharmacotherapeutic potential of *Persea americana* and *Vernonia amygdalina* in mitigating oxidative stress in a rodent model of LPS-induced gestational hypertension. The findings from this research could contribute significantly to the growing field of phytomedicine, offering alternative treatment strategies for managing pregnancy-related hypertensive disorders.

Materials And Methods

Collection and preparation of samples

Bitter leaves (*Vernonia amygdalina*) and Avocado leaves and seed (*Persea americana*) were sourced locally in Ikere-Ekiti, Ekiti State, Nigeria. They were identified and authenticated at the Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, University of Ibadan, Oyo State, Nigeria and assigned the voucher specimen numbers 2022010 and 2022009 for *V. amygdalina* and *P. americana* respectively. The leaves of the bitter leaf and avocado leaf were detached from the stem. They were rinsed thoroughly with clean water and they were spread on a sack and placed under room temperature for drying. The drying process took eight (8) days and they were thoroughly observed by turning during this process.

The avocado fruits were cut and opened to remove the avocado seed and grated into smaller pieces for an easy drying process. The grated avocado seed was spread on a sack and was placed at room temperature for drying. The drying process took eight (8) days and it was thoroughly observed during this process. The samples were weighed using a weighing balance after. It has dried before it was turned into a powder form. The samples (bitter leaf, avocado leaf etc) were blended using a blending machine and weighed in the laboratory using weighing balance.

Extraction of Samples

The weighed samples were soaked with 95% ethanol for 72 hours in different labelled containers with periodic stirring. After 72 hours, each sample was filtered using the Whatman filter paper and dried. They were preserved at 4 °C in the refrigerator for further analysis.

Experimental Design

Fifty-four female albino rats were obtained from the animal house Faculty of Basic Medical Sciences, College of Medicine, Ekiti State University, Ado Ekiti. They were housed in a plastic cage with steel wire lids, and two male albino rats were introduced into each cage for copulation.

The female albino rat's oestrus cycle was checked in the laboratory after four days using their vaginal smear to confirm pregnancy. Few rats were confirmed pregnant on the fourth day and on the sixth day, the entire fifty-four rats were confirmed pregnant, and the male rats were removed from each cage. The pregnant albino rat was then grouped in another cage (Group A to Group I) with six in each cage. The rats were transported to the Cardio Renal Unit Laboratory, Department of Veterinary Physiology and Biochemistry, Faculty of Veterinary Medicine, College of Medicine, University of Ibadan, Oyo State, Nigeria.

Animal Treatment

Lipopolysaccharide (LPS) was used for the induction of preeclampsia at gestational ages 13 and 14 days of pregnancy. Administration of 0.1 mL of LPS through the intraperitoneal route for 3 consecutive days. Treatment was done concurrently with induction but lasted for 7 days. The treatment was as follows:

Group A: Normal control (Feed and water only)

Group B: LPS only

Group C: LPS + 0.036 mg/kg body weight of Aldoxi (a standard antihypertensive drug)

Group D: LPS + 100 mg/kg body of *V. amygdalina* leaf extract

Group E: LPS + 200 mg/kg body of *V. amygdalina* leaf extract

Group F: LPS + 100 mg/kg body of *P. americana* leaf extract

Group G: LPS + 200 mg/kg body of *P. americana* leaf extract

Group H: LPS + 100 mg/kg body of *P. americana* seed extract

Group I: LPS + 200 mg/kg body of *P. americana* seed extract

At the end of the 7-day treatment period, the animals were sacrificed at gestational ages of 20 and 21 days. Blood samples were obtained by cardiac puncture and dispensed into labelled lithium heparin bottles. The blood

samples were centrifuged at 4000 rpm for 5 minutes to obtain plasma, which was then stored in sterile plastic bottles and refrigerated at -20°C until analysis.

Biochemical Analysis

Oxidative stress parameters were assessed following the procedures outlined by Airaodion et al. [14].

Data Analysis

One-way ANOVA was used to analyze the data, and the Tukey post hoc mean comparison test was employed to see whether there were any

statistically significant differences between the variables. The analyzed data were expressed as the mean and standard deviation of the mean for six replicates. Statistical significance was defined as a P-value of 0.05 or below ($P \leq 0.05$). GraphPad Prism was used for all statistical analyses (version 8.0).

Results

LPS exposure significantly decreased GSH levels in the negative control group (6.04 mmol/L) compared to the normal control (8.42 mmol/L), indicating oxidative stress. Treatment with both plant extracts improved GSH levels, with the 200 mg/kg *P. americana* leaf group (8.19 mmol/L) showing near-normal values (**Figure 1**).

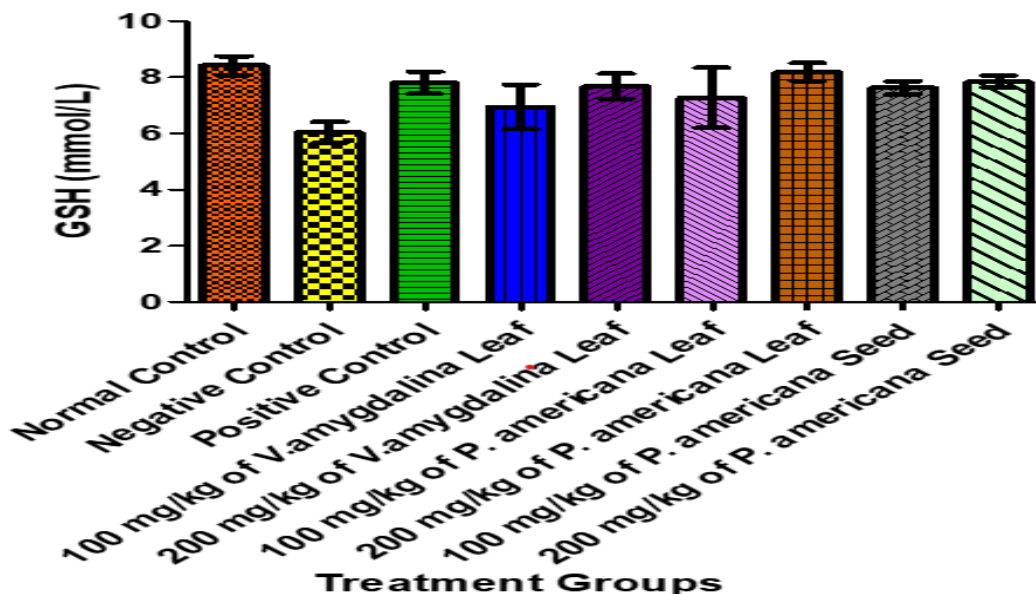


Figure 1: Effect of *Persea americana* and *Vernonia amygdalina* on the Reduced Glutathione (GSH) Level of Lipopolysaccharides-exposed Pregnant Rats

GSSG levels were lowest in the negative control (2.59 mmol/L) and increased in treated groups. The 200 mg/kg *P. americana* leaf group had the highest GSSG (3.01 mmol/L), similar to the normal control (3.70 mmol/L), suggesting effective oxidative balance restoration (**Figure 2**).

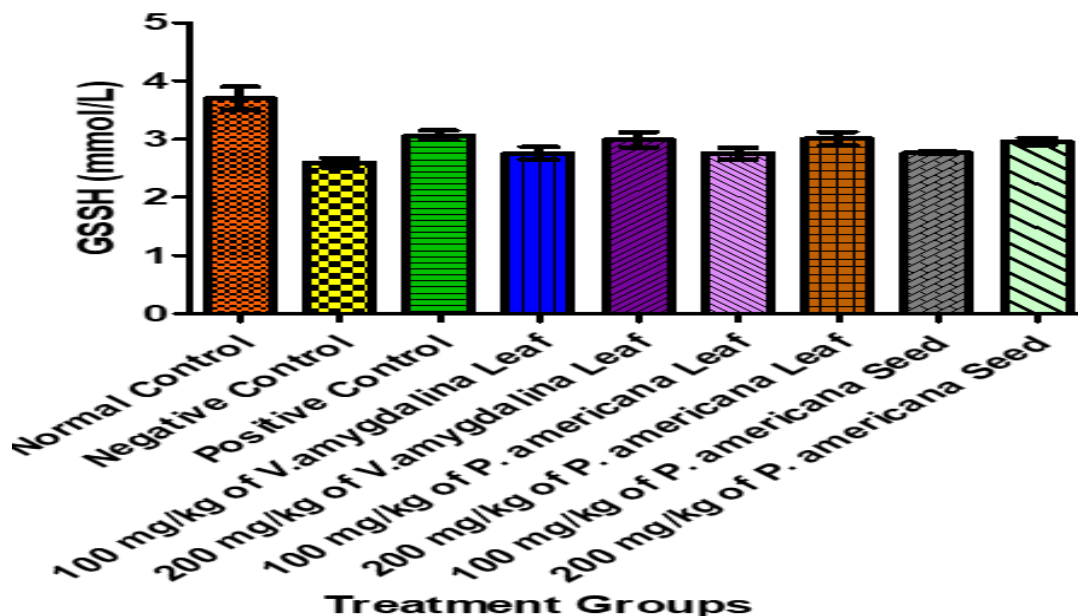


Figure 2: Effect of *Persea americana* and *Vernonia amygdalina* on the Oxidized Glutathione (GSSG) level of Lipopolysaccharides-exposed Pregnant Rats

GPx activity decreased in the negative control (8.76 μ mol/L) but improved with treatment. The 200 mg/kg *P. americana* leaf group exhibited values (11.47 μ mol/L) close to the normal control (12.30 μ mol/L), suggesting enhanced antioxidant defense (**Figure 3**).

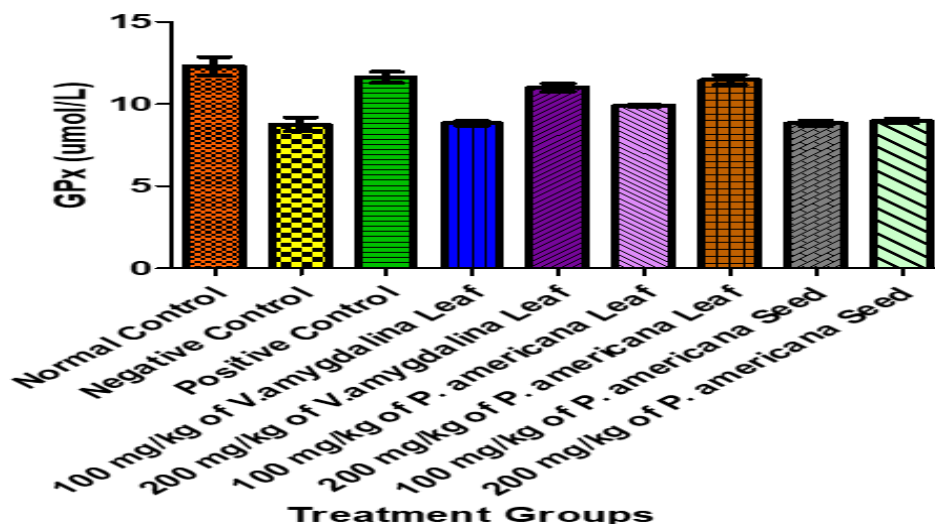


Figure 3: Effect of *Persea americana* and *Vernonia amygdalina* on the Glutathione Peroxidase (GPx) Level of Lipopolysaccharides-exposed Pregnant Rats

MDA, a lipid peroxidation marker, was elevated in the negative control (3.99 $\mu\text{mol/L}$). Treatment with 200 mg/kg *P. americana* seed extract reduced MDA levels to 2.89 $\mu\text{mol/L}$, approximating the normal control (2.74 $\mu\text{mol/L}$), indicating reduced lipid peroxidation (**Figure 4**).

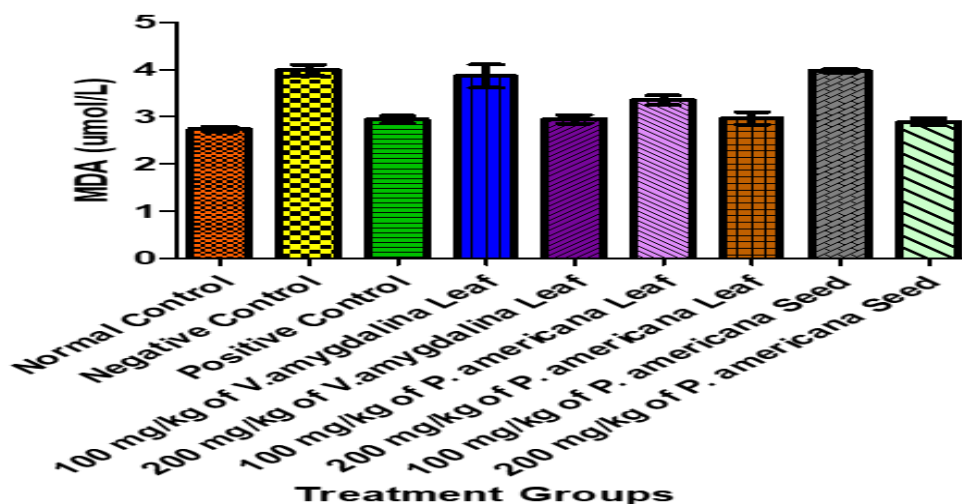


Figure 4: Effect of *Persea americana* and *Vernonia amygdalina* on the Malondialdehyde (MDA) Level of Lipopolysaccharides-exposed Pregnant Rats

Discussion

The present study investigated the pharmacotherapeutic potential of *Persea americana* (avocado) and *Vernonia amygdalina* (bitter leaf) in modulating oxidative stress biomarkers in a rodent model of lipopolysaccharide (LPS)-induced gestational hypertension. Reduced glutathione is a critical antioxidant that neutralizes reactive oxygen species (ROS) and helps maintain redox homeostasis. In this study, LPS exposure led to a substantial decrease in GSH levels in the negative control group (6.04 mmol/L) compared to the normal control (8.42 mmol/L), indicating oxidative stress. However, treatment with 200 mg/kg of *P. americana* leaf (8.19 mmol/L) and seed extract (7.84 mmol/L) showed near-restoration of GSH levels, comparable to the normal control and positive control (7.81 mmol/L).

These findings corroborate previous studies which reported that *P. americana* contains bioactive phytochemicals such as flavonoids, carotenoids, and polyphenols that exhibit antioxidant properties by upregulating endogenous antioxidants like GSH [15][16]. Similarly, *V. amygdalina* leaf extract also elevated GSH levels, particularly at 200 mg/kg (7.69 mmol/L), supporting earlier evidence that its polyphenolic compounds mitigate LPS-induced oxidative damage [17].

The accumulation of GSSG is a marker of oxidative stress due to the conversion of GSH during detoxification of ROS. LPS exposure reduced GSSG levels (2.59 mmol/L) in the negative control, a somewhat paradoxical observation, possibly due to diminished GSH availability. Treatment with *P. americana* and *V. amygdalina* extracts restored GSSG levels closer to normal (e.g., 3.01 mmol/L in 200 mg/kg *P. americana* leaf and 2.99 mmol/L in 200 mg/kg *V. amygdalina* leaf), suggesting improved redox turnover and recovery of the GSH/GSSG cycle.

This finding is consistent with the observations of Ayoola et al. [18], who reported that antioxidant plant extracts could restore GSSG levels through improved GSH synthesis and GPx activity. It also suggests that the bioactive constituents of both plant species aid in preserving glutathione cycling, which is essential for cellular detoxification during gestational hypertension.

GPx plays a central role in catalyzing the reduction of hydrogen peroxide using GSH. The negative control rats exhibited a marked decline in GPx levels (8.76 $\mu\text{mol/L}$) compared to the normal control (12.30 $\mu\text{mol/L}$), indicative of oxidative stress. Treatment with 200 mg/kg of *P. americana* leaf resulted in near-normal GPx levels (11.47 $\mu\text{mol/L}$), surpassing both the positive control (11.62 $\mu\text{mol/L}$) and other treatment groups.

This finding aligns with prior studies showing that *P. americana* extracts enhance GPx expression and activity in oxidative stress models [19]. Furthermore, *V. amygdalina* at 200 mg/kg significantly restored GPx levels (11.02 $\mu\text{mol/L}$), in agreement with reports by Iwalokun et al. [20], who found that bitter leaf extracts could enhance antioxidant enzyme activities in hypertensive rats.

Notably, while the seed extracts of *P. americana* showed some improvement (8.84 and 8.98 $\mu\text{mol/L}$ for 100 and 200 mg/kg, respectively), they were less effective than the leaf extracts, suggesting that leaves may contain more potent antioxidant constituents for GPx modulation.

MDA is a well-established marker of lipid peroxidation and oxidative cell membrane damage. The LPS-exposed rats (negative control) exhibited significantly elevated MDA levels (3.99 $\mu\text{mol/L}$) compared to the normal control (2.74 $\mu\text{mol/L}$), indicating increased oxidative lipid damage. Notably, treatment with 200 mg/kg of *P. americana* seed (2.89 $\mu\text{mol/L}$) and 200 mg/kg of *V. amygdalina* leaf (2.95 $\mu\text{mol/L}$) effectively reduced MDA levels close to normal, suggesting strong lipid peroxidation-inhibitory effects.

These results support previous findings by Ugochukwu and Babady [21] and Oboh et al. [22], which demonstrated that *P. americana* and *V. amygdalina* have protective roles against lipid peroxidation due to their scavenging properties. The presence of compounds like quercetin, catechins, and terpenoids may be responsible for this observed effect.

Overall, both *P. americana* and *V. amygdalina* showed substantial antioxidant effects in modulating oxidative stress markers in LPS-induced gestational hypertension. The leaf extracts, particularly at 200 mg/kg, demonstrated superior efficacy compared to their respective seed or lower-dose counterparts. This suggests a dose-dependent response and highlights the importance of plant part selection in phytotherapy.

The observed ameliorative effects can be attributed to the diverse phytochemical constituents present in the plants, including alkaloids, flavonoids, saponins, and phenolics, which are known to exert protective effects by scavenging free radicals, enhancing antioxidant enzyme activities, and modulating signaling pathways implicated in oxidative stress [23][24].

Conclusion

The findings of this study provide compelling evidence that *Persea americana* and *Vernonia amygdalina* possess significant antioxidant properties capable of modulating oxidative stress in gestational hypertension. These effects are particularly evident in the regulation of GSH/GSSG balance, enhancement of GPx activity, and reduction of lipid peroxidation. The leaf extracts at higher doses (200 mg/kg) showed the most promise, indicating potential for therapeutic application in oxidative stress-associated pregnancy disorders. Future studies should focus on mechanistic elucidation of the signaling pathways involved, as well as clinical validation in human subjects to explore translational potential.

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