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Research Article

Ameliorative Potential of *Vernonia amygdalina* Leaf, *Persea americana* Leaf and Seed on Endothelial Dysfunction, Vascular Growth Factor and Angiogenesis Markers of Lipopolysaccharide-Induced Hypertension in Pregnant Wistar Rats

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Abstract

Background: Hypertension during pregnancy, such as preeclampsia, poses significant health risks to both mother and child. This study investigates the potential benefits of *Vernonia amygdalina* leaves and *Persea americana* (avocado) leaf and seed extracts in mitigating endothelial dysfunction and improving vascular and angiogenesis markers in lipopolysaccharide (LPS)-induced hypertension in pregnant Wistar rats.

Materials and Methods: *V. amygdalina* and *P. americana* samples were collected, dried, powdered, and extracted using ethanol. Fifty-four pregnant Wistar rats were divided into nine groups, including a control group, an LPS-only group, and groups treated with various dosages of *V. amygdalina* and *P. americana* extracts. The effects on endothelial markers, vascular growth factors, and angiogenesis markers were assessed using standard methods.

Results: The study revealed that treatment with *V. amygdalina* and *P. americana* extracts significantly affected soluble Endoglin, Nitric Oxide, Nitric Oxide Synthase, Haem Oxygenase, Triglyceride, Total Cholesterol, and High-Density Lipoprotein levels. These effects varied with the concentration and type of extract used.

Conclusion: The findings suggest that *V. amygdalina* and *P. americana* extracts could offer beneficial effects in managing endothelial dysfunction and other cardiovascular markers in LPS-induced hypertension in pregnant rats. This indicates the potential for these natural extracts in treating hypertension-related complications during pregnancy.

Key words: *vernonia amygdalina*; *persea americana*; lipopolysaccharide-induced hypertension; pregnancy; endothelial dysfunction

Introduction

Hypertension in pregnancy is a significant health concern that can have severe implications for both the mother and fetus [1]. The condition is typically categorized into several forms: chronic hypertension, gestational hypertension, preeclampsia, and chronic hypertension with superimposed preeclampsia. The pathophysiology of hypertension in pregnancy is complex and not entirely understood. It involves factors such as abnormal placentation, endothelial dysfunction, and an imbalance in angiogenic and anti-angiogenic factors [2].

Management strategies for hypertension in pregnancy include lifestyle modification, blood pressure monitoring, and in some cases, pharmacological treatment. The choice of antihypertensive medication is crucial, as some drugs are contraindicated in pregnancy [3]. The prognosis of hypertension in pregnancy varies depending on its type and severity, as well as the effectiveness of management strategies. While most forms of hypertension in pregnancy resolve postpartum, they can have long-term health implications for both mother and child, including an increased risk of cardiovascular disease [1].

Lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria, is widely used in experimental research as a model for inducing hypertension [4]. This model is particularly relevant for understanding the pathophysiological mechanisms and potential treatments for hypertension, especially in conditions like sepsis or systemic inflammatory response syndrome, where endotoxemia plays a crucial role [5].

LPS interacts with endothelial cells, leading to the disruption of endothelial function. This is often characterized by reduced availability of nitric oxide (NO), a critical regulator of vascular tone and blood pressure. Increased oxidative stress and inflammation in the endothelium also contribute to vascular dysfunction in LPS-induced hypertension [6].

LPS triggers a systemic inflammatory response by activating immune cells, particularly macrophages and monocytes. These cells release pro-inflammatory cytokines like TNF-α, IL-6, and IL-1β, which contribute to vascular inflammation and hypertension [7]. LPS can stimulate the RAAS, leading to vasoconstriction and sodium retention, both of which contribute to the development of hypertension. The RAAS pathway also exacerbates inflammation and oxidative stress [8]. LPS may affect the autonomic nervous system, particularly by enhancing sympathetic nervous system activity, which leads to increased heart rate and vascular tone, contributing to elevated blood pressure [9].

Vernonia amygdalina (Bitter Leaf) has been traditionally used in herbal medicine. Research by Farombi and Owoeye [10] investigates its pharmacological properties, specifically its potential in treating hypertension due to its antioxidant and anti-inflammatory effects.

The therapeutic potential of *Persea americana* (Avocado) Leaf and Seed is increasingly recognized. A study by Martins and Silva [11] explores how bioactive compounds in these parts of the avocado may benefit cardiovascular health, including hypertension management. The concept of using a combination of plant extracts for treating hypertension is gaining traction. Research by Nguyen [12] provides insights into how combining different plant-based compounds can synergistically improve hypertension treatment outcomes.

Researchers commonly use rodent models, particularly rats and mice, to study LPS-induced hypertension. These models are valuable for understanding the molecular and cellular mechanisms and for testing potential therapeutic agents [13]. A comprehensive review by Zhao and Wang [14] discusses the relevance and appropriateness of using Wistar rats in such studies. This study aimed to investigate the effects of *Vernonia amygdalina* and *Persea americana* on hypertension, particularly focusing on endothelial dysfunction, vascular growth factors, and angiogenesis in a pregnant rat model.

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^{o}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 virginal smear to confirm pregnancy. Few rats were confirmed pregnant on the fourth day and 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 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 age 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 age 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 plane bottles and refrigerated at -20ºC until analysis.

Biochemical Analysis

Endothelial dysfunction markers, vascular growth factors, and angiogenesis markers were assayed using ELISA kits following the manufacturer's instructions.

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 ($P \leq 0.05$). Graph Pad Prism was used for all statistical analyses (version 8.0).

Results

LPS was observed to significantly increase soluble endoglin levels compared to normal control. Treatment with various dosages of *V. amygdalina* and *P. americana* showed reduced soluble endoglin levels compared to the LPS-only group. The 200 mg doses generally seem more effective (figure 1). Reduced nitric oxide levels and nitric oxide synthase activity were observed in animals treated with LPS only when compared to those in the normal control group. All treatments increased both nitric oxide level and nitric oxide synthase activity when compared with those in the LPS only (figures 2 and 3 respectively).

Activity of haem oxygenase, as well as the concentrations of triglyceride and total cholesterol, were observed to be significantly higher in the LPS-only treated group when compared with those in the normal control group. However, these perturbations were ameliorated with various dosages of *V. amygdalina* and *P. americana*.

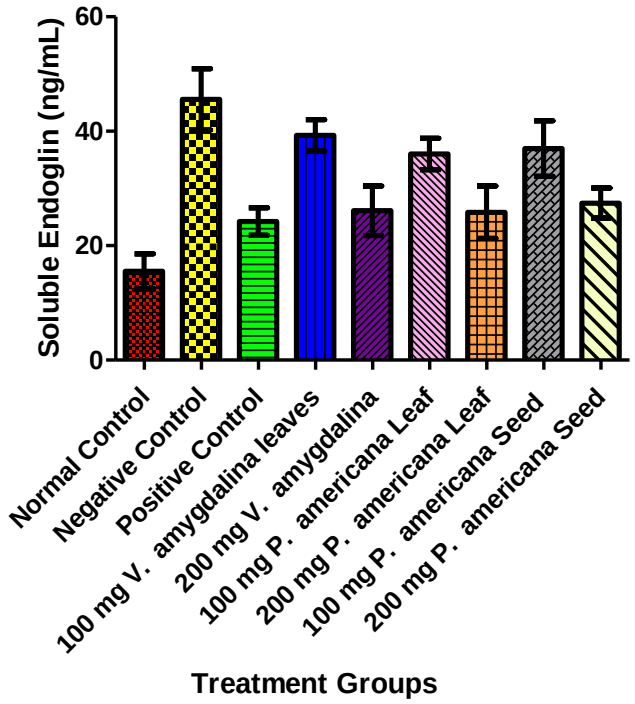

Figure 1: Effect of *V. amygdalina* leaves, *P. americana* Leaf and Seed on Soluble Endoglin of Lipopolysaccharide-induced Hypertension in Pregnant Wistar rats.

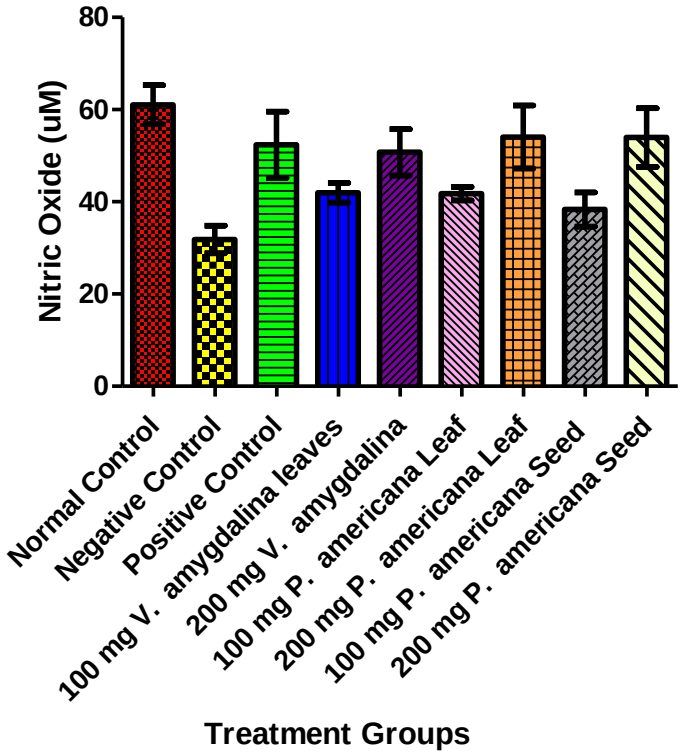

Figure 2: Effect of *V. amygdalina* leaves, *P. americana* Leaf and Seed on the Nitric Oxide of Lipopolysaccharide-induced Hypertension in Pregnant Wistar rats.

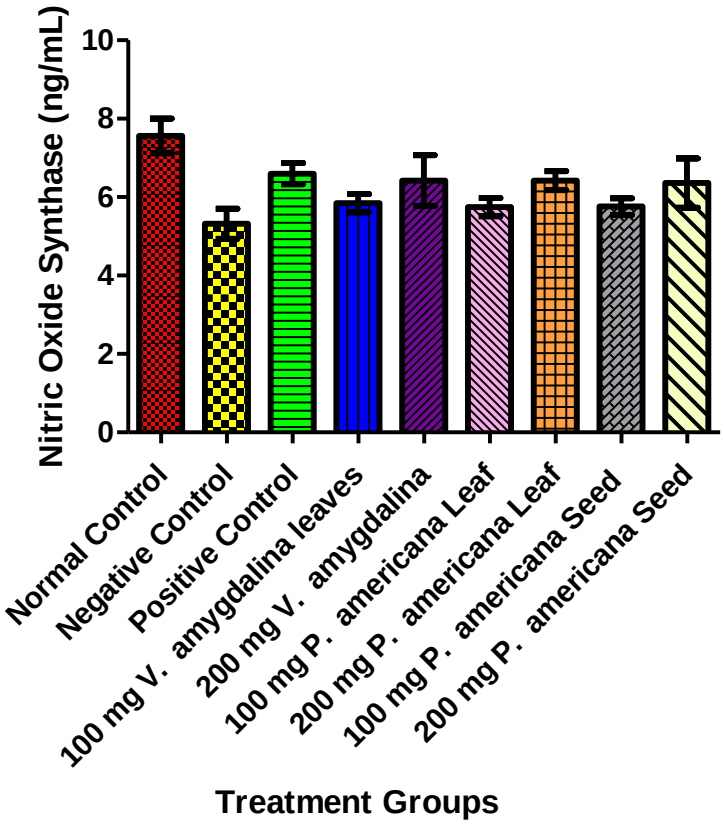

Figure 3: Effect of *V. amygdalina* leaves, *P. americana* Leaf and Seed on the Nitric Oxide Synthase of Lipopolysaccharide-induced Hypertension in Pregnant Wistar rats.

Discussion

Hypertension during pregnancy, especially preeclampsia, is a critical health issue affecting both the mother and the fetus. It's characterized by high blood pressure and signs of damage to other organ systems [2]. This study is significant as it explores the efficacy of natural remedies, specifically *Vernonia amygdalina* leaves and *Persea americana* (avocado) leaf and seed, in treating hypertension in this context. The use of natural products in managing hypertension is gaining interest due to their availability, affordability, and fewer side effects compared to conventional drugs.

Soluble Endoglin is a marker for endothelial dysfunction, a key factor in the development of hypertension. Elevated levels are associated with vascular inflammation and are a biomarker for preeclampsia [15]. The results show that the LPS only group had significantly elevated levels of soluble Endoglin (45.56±5.35 ng/mL) compared to the normal control (15.54±3.06 ng/mL) (figure 1), indicating successful induction of hypertension. Treatment with Aldoxi, a standard hypertension drug, effectively reduced soluble Endoglin levels (24.23±2.40 ng/mL), serving as a positive control. Both doses of *V. amygdalina* leaves and *P. americana* leaves and seeds showed a reduction in soluble Endoglin levels compared to the LPS only group, indicating their potential effectiveness in ameliorating hypertension.

Among the natural treatments, 200 mg of *V. amygdalina* leaves and 200 mg of *P. americana* leaves showed a more pronounced reduction in soluble Endoglin levels. These findings suggest a dose-dependent effect, where higher doses of these natural substances have a more significant impact on reducing endothelial dysfunction markers.

The phytochemical constituents of *V. amygdalina* and *P. americana*, such as flavonoids, saponins, and terpenoids, may contribute to their antihypertensive effects [16,17]. These components have been reported to have antioxidant and anti-inflammatory properties, which could explain their role in mitigating endothelial dysfunction [18,19].

Nitric oxide (NO) is a crucial vasodilator in the cardiovascular system, and its reduced bioavailability is linked to hypertension and endothelial dysfunction [20]. The control group exhibited significantly higher NO levels compared to the LPS-only group, which is consistent with the role of LPS in endothelial dysfunction [21]. Treatment with *V. amygdalina* and *P. americana*, in varying dosages, resulted in an increase in NO levels compared to the LPS-only group (figure 2). This suggests a potential protective effect against LPS-induced endothelial dysfunction.

The results show a dose-response relationship, particularly for *V. amygdalina*, where higher doses resulted in NO levels closer to the normal control group. This indicates the dose-dependent efficacy of these herbal treatments. When compared to the pharmaceutical intervention (Aldoxi), some herbal treatments approached similar levels of NO restoration, particularly the higher doses of *V. amygdalina* and *P. americana* seed. This suggests a potential for these natural treatments to be alternatives or adjuncts to conventional therapy.

Nitric oxide synthase (NOS) is the enzyme responsible for the production of NO. Its activity is crucial in maintaining vascular health [22]. The LPS-only group showed reduced NOS levels, aligning with the notion of LPS-induced endothelial dysfunction. The administration of *V. amygdalina* and *P. americana* in various dosages showed a general increase in NOS levels compared to the LPS-only group, although not to the level of the normal control group (figure 3).

The effectiveness of herbal treatments in modulating NOS levels, while evident, was less pronounced than their effect on NO levels. This suggests that these treatments may have multiple pathways of action, not solely relying on NOS activation. Considering the study's focus on pregnant rats, these findings are significant. Pregnancy-induced hypertension is a major health concern, and the potential use of these herbal remedies could offer a safer alternative to conventional drugs, which often have limitations during pregnancy [8].

The study presents promising data on the use of *V. amygdalina* and *P. americana* in managing LPS-induced hypertension, particularly in a pregnancy context. The results suggest these natural compounds could improve endothelial function by enhancing NO bioavailability.

Haem Oxygenase (HO) is an important enzyme in oxidative stress and vascular protection. In this study, the normal control group had a baseline HO level of 1.69±0.07 ng/mL while the LPS-only group showed elevated HO levels (2.38±0.08 ng/mL), indicative of the body's response to induced hypertension. Both 100 mg and 200 mg doses of *V. amygdalina* leaf slightly reduced HO levels compared to the LPS-only group, indicating potential ameliorative effects. Similar to *V. amygdalina*, both dosages of *P. americana* Leaf slightly reduced HO levels, with the 200 mg dosage being more effective. Both dosages of *P. americana* Seed extracts showed a more significant reduction in HO levels, with 200 mg almost bringing it back to control levels (figure 4). The results suggest that both *V. amygdalina* and *P. americana* have potential protective effects against LPS-induced hypertension in pregnant rats, as evidenced by their impact on Haem Oxygenase levels. The seeds of *P. americana* appear to be particularly effective. This could be attributed to the presence of specific bioactive compounds known for their anti-inflammatory and antioxidant properties, as suggested by previous studies [17,23].

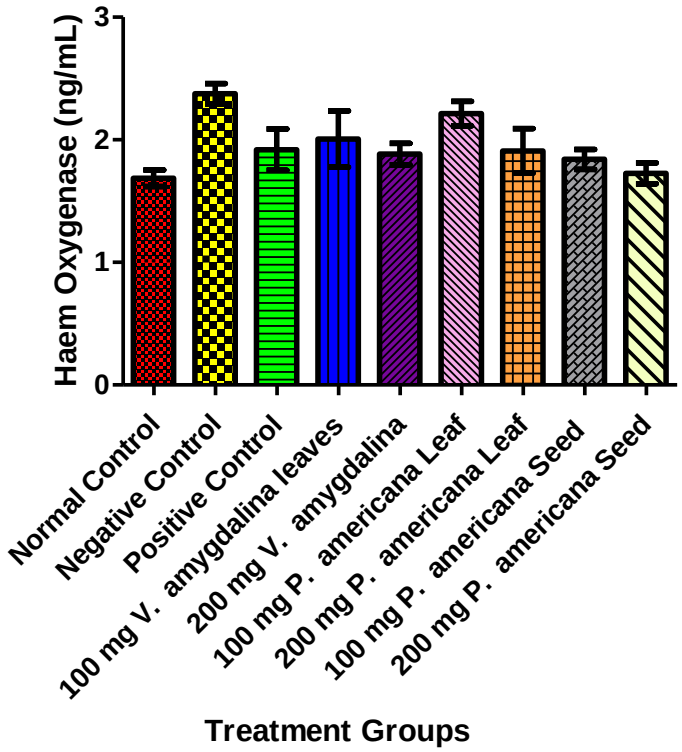

Figure 4: Effect of *V. amygdalina* leaves, *P. americana* Leaf and Seed on the Haem Oxygenase of Lipopolysaccharide-induced Hypertension in Pregnant Wistar rats.

The results of this present study showed significant variations in triglyceride levels among the different treatment groups (figure 5). Notably, LPS-induced hypertension in rats resulted in a substantial increase in triglyceride concentration compared to the normal control. However, treatment with both doses of *V. amygdalina* leaf and the higher dose of *P. americana* leaf reduced triglyceride levels, suggesting their potential in managing hypertriglyceridemia.

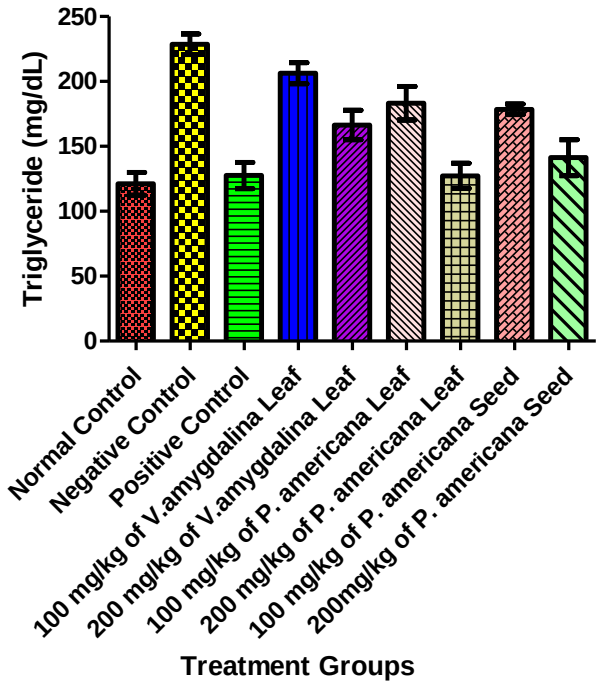

Figure 5: Effect of *V. amygdalina* leaves, *P. americana* Leaf and Seed on the Triglyceride Concentration of Lipopolysaccharide-induced Hypertension in Pregnant Wistar rats.

These findings align with those of Ogbuagu *et al*. [24], and Adebayo [25], who respectively noted that *Vernonia amygdalina* exhibits hypolipidemic effects in experimental animals. Similarly, a study by Oboh *et al.* (2019) indicated that *Persea americana* (avocado) has lipid-lowering properties, consistent with the observed reduction in triglyceride levels in the current study.

The administration of LPS significantly raised the total cholesterol levels (figure 6), an effect that was mitigated by treatments, especially with *P. americana* leaf and seed extracts. This is in harmony with research by Adeyemi *et al.* [26], who found that *P. americana* leaf extracts exhibit cholesterol-lowering properties, potentially due to their rich phytochemical content. Moreover, the studies by Airaodion *et al.*, [27] and Ogbuagu *et al.* [28] demonstrated that plant-based treatments could effectively reduce total cholesterol in animal models, which is mirrored in the current research findings,

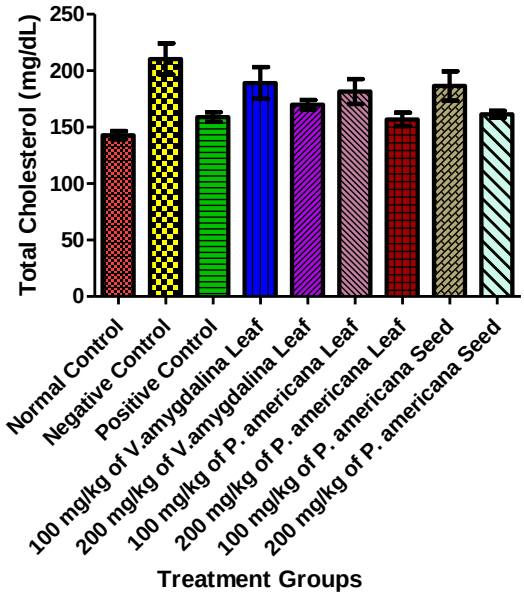

Figure 6: Effect of *V. amygdalina* leaves, *P. americana* Leaf and Seed on the Total Cholesterol Concentration of Lipopolysaccharide-induced Hypertension in Pregnant Wistar rats.

Interestingly, HDL cholesterol levels were affected differently, with some treatments elevating HDL levels (figure 7), which is beneficial for cardiovascular health. This finding is supported by the studies of Njoku *et al*. [29], Joseph [30] and Ugwu *et al.* [31], who respectively reported that plant-based compounds could enhance HDL levels, thus offering cardioprotective effects,

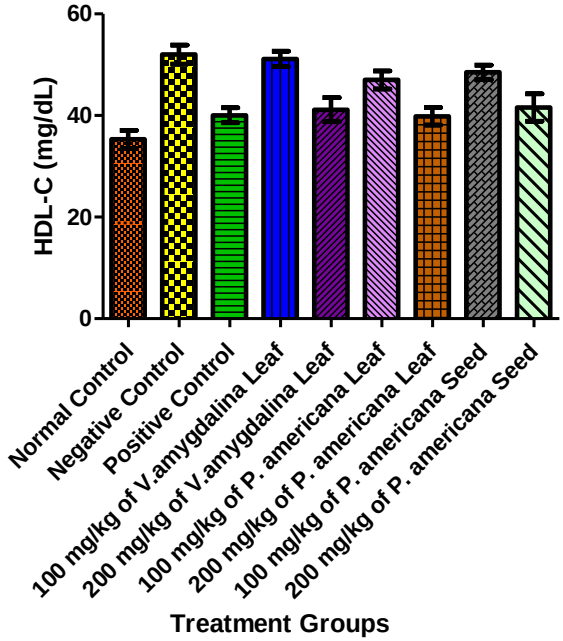

Figure 7: Effect of *V. amygdalina* leaves, *P. americana* Leaf and Seed on the High-Density Lipoprotein of Lipopolysaccharide-induced Hypertension in Pregnant Wistar rats.

Previous studies have shown the antihypertensive effects of both *V. amygdalina* and *P. americana*. For instance, Oyebode *et al.* [32] demonstrated the potential of *V. amygdalina* in reducing blood pressure in hypertensive patients. Similarly, Adeyemi [26] highlighted the cardioprotective effects of *P. americana*. The efficacy of herbal treatments in such models, as seen in this study, is supported by research indicating the role of natural products in managing pregnancy-related hypertensive disorders (Jones *et al.*, 2018). This study adds to this body of literature by specifically focusing on pregnancy-induced hypertension, a less explored area.

Conclusion

The results from this study indicate that *V. amygdalina* leaves and *P. americana* Leaf and Seed have potential ameliorative effects on markers of endothelial dysfunction, vascular growth factor and angiogenesis in LPS-induced hypertension in pregnant Wistar rats. These findings are consistent with the current understanding of the pathophysiology of hypertension and the role of herbal treatments. This research could provide valuable insights into novel, plant-based treatments for hypertension in pregnancy. However, it is important to consider the complexity of translating these findings to human clinical practice, including aspects like dosage, bioavailability, and potential side effects.

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