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RESEARCH ARTICLE

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Effects of n-hexane fraction of *Piper guineense seed extract* on N$^{\omega}$-nitro-L-arginine methyl ester hydrochloride-induced hypertension in rats

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Abstract

This study aimed to investigate the effects of the n-hexane fraction of the ethanolic seed extract of PG (NFESEPG) on hypertension induced by Nω-nitro-L-arginine methyl ester (L-NAME) in rats. Specifically, the study examined the impact of NFESEPG on blood pressure, oxidative stress markers, NO concentration, angiotensin-converting enzyme (ACE) and arginase activities, and cardiac biomarkers in hypertensive rats. The study involved collecting, identifying, and processing the PG plant to obtain the ethanolic seed extract. The extract was then partitioned with solvents to isolate the n-hexane fraction. Hypertension was induced in rats by oral administration of L-NAME for 10 days, while concurrent treatment with NFESEPG at two doses (200 and 400 mg/kg/day) was administered orally. Blood pressure was measured using a noninvasive tail-cuff method, and various biochemical parameters were assessed. Treatment with both doses of NFESEPG significantly reduced systolic and diastolic blood pressure in L-NAME-induced hypertensive rats. Additionally, NFESEPG administration increased NO concentration and decreased ACE and arginase activities, malondialdehyde (MDA) levels, and cardiac biomarkers in hypertensive rats. The findings indicate that NFESEPG effectively lowered blood pressure in hypertensive rats induced by L-NAME, potentially through mechanisms involving the modulation of oxidative stress, NO bioavailability, and cardiac biomarkers. These results suggest the therapeutic potential of NFESEPG in managing hypertension and related cardiovascular complications.

KEYWORDS

hypertension, nitric oxide, N$^{\omega}$-nitro-L-arginine methyl ester hydrochloride, oxidative stress, *Piper guineense*

1 | INTRODUCTION

Hypertension is characterized by a persistent increase in systolic blood pressure (BP) to 140 mmHg or higher and/or diastolic BP to 90 mmHg or higher.[1] According to World Health Statistics, cardiovascular diseases (CVDs) are the main cause of non-communicable diseases (NCD), responsible for 31% of global deaths.[1] Hypertension, a known risk factor for CVDs, is linked to approximately 17 million deaths annually,[2] contributing to conditions such as kidney failure, stroke, heart disease, and premature mortality. Despite being asymptomatic in its early stages, hypertension often goes undiagnosed.[3] Endothelial dysfunction (ED) is frequently observed in early hypertension and is identified as a primary contributing factor.[4] Studies suggest that oxidative stress plays a role in hypertension development by deactivating nitric oxide (NO),[5,6] resulting in an imbalance between NO and endothelial vasoconstrictors, ultimately leading to endothelial dysfunction and hypertension.[1]

Various techniques have been utilized in animal models to induce hypertension. Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME), an analog of L-arginine, serves as a competitive inhibitor of nonspecific nitric oxide synthases (NOS).[7] L-NAME is commonly employed to establish a hypertensive model deficient in NO. It is widely recognized that inhibiting nitric oxide biosynthesis through the administration of L-NAME in vivo leads to endothelial dysfunction and vasoconstriction, consequently resulting in hypertension.[8]

Medicinal plants are globally acknowledged as fundamental for health maintenance and treatment. Given the epidemic proportions of chronic degenerative diseases, their treatment is crucial. *Piper guineense* (PG), also referred to as African black pepper, is renowned for its medicinal and health-promoting properties, including the treatment and prevention of morning sickness, allergies, runny nose, and colds.[9] PG, a climbing perennial plant of the *Piperaceae* family, has been recognized for its therapeutic potential in managing various diseases within traditional medicine systems.[10,11] Beyond its pharmacological benefits, PG is noted for its nutritional properties, which enhance food flavor.[10]

Despite the known pharmacological benefits of PG, there is a notable research gap regarding its potential effects on hypertension, particularly when induced by L-NAME. While previous studies have explored the broad therapeutic applications of PG, specific investigations into its impact on blood pressure, oxidative stress, and related cardiac biomarkers in the context of hypertension remain limited. Therefore, the primary aim of this study was to investigate the impact of the n-hexane fraction of the ethanolic seed extract of PG (NFESEPG) on blood pressure, oxidative stress, and hypertension-related biomarkers in rats induced with hypertension by L-NAME. This study seeks to bridge this gap by providing insights into the mechanisms through which NFESEPG might offer therapeutic benefits for managing hypertension and related cardiovascular complications.

Significance statement

This study investigates the potential therapeutic effects of the n-hexane fraction of *Piper guineense* seed extract (NFESEPG) on L-NAME-induced hypertension in rats. The findings reveal that NFESEPG significantly reduces blood pressure and improves oxidative stress markers, NO concentration, and cardiac biomarkers in hypertensive rats. This suggests that NFESEPG may offer a promising avenue for managing hypertension and related cardiovascular complications through its antioxidative and vasodilatory properties. The study contributes to understanding natural remedies for hypertension and underscores the importance of exploring plant-derived compounds for cardiovascular health management.

2 | MATERIALS AND METHODS

2.1 | Collection and identification of plant material

Wet seeds of *Piper guineense* were obtained from Owode-Ede market, Ede, Osun State, and authenticated at the Herbarium Section, Department of Botany, Obafemi Awolowo University, Ile-Ife (Specimen code IFE-18042).

2.2 | Preparation of crude extract

The seeds were air-dried for 72 h and ground into a fine powder. 500 g of the powder was soaked in 2.5 L of absolute ethanol for 72 h, with periodic agitation. The mixture was filtered, and the filtrate was concentrated under reduced pressure at 38–40°C using a rotary evaporator (PCE-E6000 series).

2.3 | Fractionation of crude extract

The n-hexane fraction, showing the highest activity, was selected for further study. Following Abubakar and Haque,[12] 90.71 g of crude extract was mixed with distilled water and fractionated with 500 ml of n-hexane, added twice, shaken, and settled. The aqueous layer was removed, and the n-hexane layer was collected, labeled, and stored. This method was also applied to dichloromethane, ethyl acetate, and butanol fractions. All fractions were evaporated under reduced pressure at 38–40°C, labeled, and stored, yielding 48.2% w/w n-hexane, 2.5% w/w dichloromethane, 2.1% w/w ethyl acetate, and 1.2% w/w butanol.

2.4 | Gas Chromatography-Mass Spectrometry (GC-MS)

GC-MS was performed using capillary columns treated with a 5% phenyl methyl siloxane solution. The column had a length of 30 m, an internal diameter of 0.2 µm, and a thickness of 250 µm. GC-MS parameters included an ion source temperature of 25°C (E10), an interface temperature of 300°C, and a pressure of 16:2 psi with an outlet time of 1.8 mm. Samples were injected in split mode with a 1 µl injection volume and a split ratio of 1:50, at an injection temperature of 300°C. The initial column temperature was set to 35°C for 5 min, followed by a decrease to 15°C at a rate of 4°C per minute. Subsequently, the temperature was ramped up to 250°C at a rate of 20°C per minute and held for 5 min, resulting in a total elution time of 47.5 min.

Data acquisition and instrument control were managed using the supplier's mass spectroscopy software. Compounds were identified by comparing their mass spectra with standard spectra from the NIST collection (NISTII).

2.5 | Ethical declaration

All animal procedures adhered to the National Institutes Guide for the Care and Use of Laboratory Animals and were approved by the Adeleke University Ethical Review Committee (AUERC/2023/04/66B/01).

2.6 | Experimental animals

Forty-two male rats (220–250 g) were housed under controlled conditions at the University of Ibadan. They were given standard rat chow and water throughout the 18-day study period.

2.7 | Experimental design and induction of hypertension

After a 7-day acclimatization, rats were divided into seven groups ($n = 6$ each). Group 1 served as the normal control. Group 2 received L-NAME (40 mg/kg) to induce hypertension.[13] Groups 3 and 4 received 200 mg/kg and 400 mg/kg of n-hexane PG extract,[14] respectively. Groups 5 and 6 received ʟL-NAME with 200 mg/kg and 400 mg/kg of n-hexane PG extract,[13] respectively. Group 7 received ʟL-NAME and Lisinopril (10 mg/kg).

To assess the study's statistical power, a power analysis was performed using G*Power software. Parameters included:

- Effect size (Cohen's d) estimated at 0.5, representing a medium effect size.
- Significance level (α) set at 0.05.
- Total sample size of 42 rats across seven groups.
- Number of groups set to seven.
- Desired power (1 - β) targeted at 0.80, indicating an 80% chance of detecting true effects if they exist.

2.8 | Determination of blood pressure

Systolic and diastolic blood pressure were measured using a noninvasive tail-cuff CODA system at baseline, after hypertension induction, and posttreatment.

2.9 | Preparation of blood plasma

Blood samples were centrifuged at 3000 rpm for 10 min, and the plasma was stored at −20°C for further analyses.

2.10 | Preparation of heart homogenate

Heart tissue was homogenized in phosphate buffer (pH 6.8), centrifuged, and the supernatant was stored at 4°C for biochemical assays.

2.10.1 | In vivo antioxidant parameters

Determination of glutathione peroxidase (U/L)

Following Hemmadi,[15] reagents including sodium phosphate buffer, sodium azide, β-NADPH, glutathione reductase, reduced glutathione, and hydrogen peroxide were used to measure enzyme activity.

Determination of reduced glutathione (GSH) (mM)

Following Ellman,[16] samples were deproteinized and combined with Ellman's reagent, with absorbance measured at 412 nm.

Determination of superoxide dismutase (SOD) (U/ml)

Following Misra and Fridovich,[17] the reaction mixture included carbonate buffer and adrenaline, with absorbance measured at 480 nm.

Determination of catalase (U/ml)

Following Slaughter and O'Brien,[18] hydrogen peroxide was quantified via catalyzed oxidation of TMB, with absorbance measured at 653 nm.

2.10.2 | In vivo hypertension markers

Determination of nitric oxide (µM)

Following Bryan and Grisham,[19] Griess reagent was used to determine NO levels, with absorbance measured at 548 nm.

Determination of angiotensin converting enzyme (ACE) (ng/ml)

Using Cushman and Cheung[20] method, ACE activity was measured with absorbance at 228 nm.

Determination of arginase (U/L)

Arginase activity was measured by quantifying urea formation with Ehrlich's reagent, with absorbance at 450 nm.

Determination of lipid peroxidation (μM)

Following Ohkawa et al.,[21] malondialdehyde (MDA) levels were determined using TBA, with absorbance at 532 nm.

2.10.3 | Estimation of cardiac biomarkers

Determination of creatine kinase myocardial band (CK-MB) (U/L)

Following Witt and Trendelenburg,[22] CK-MB activity was measured using an Agappe Diagnostic Kit.

Troponin I (ng/ml)

Serum troponin I was measured using an ELISA kit as per Spectra I Co. protocol.

Lactate dehydrogenase (LDH) (U/L)

LDH activity was measured using an Agappe Diagnostic Kit.

Histo-morphological examination

Hearts were fixed in formalin, sectioned, and stained with hematoxylin and eosin. Tissue integrity was examined under a biological microscope.

Data analysis

Results were expressed as mean ± SD. One-way ANOVA and post hoc Tukey's test were performed using GraphPad Prism (Version 6.0), with $p < .05$ considered significant.

3 | RESULTS

3.1 | GC-MS identification of selected compounds in NFESEPG

Twelve peaks were found in the GC-MS analysis of NFESEPG, and these correspond to the bioactive compounds that were identified by comparing their mass spectral fragmentation patterns, peak retention times, peak areas (%), and heights (%) to those of the known compounds listed in the National Institute of Standards and Technology's NIST (2022) library. These results are displayed in Table 1. High biological activity classes of chemicals were identified by GC-MS analysis.

All the constituents of the sample were eluted within 15.636 min and 21.704 min. The sample was dominated by a class of terpenes, called sesquiterpene hydrocarbon, accounting for a total of 81.58% of the total percentage of the identified constituents. The dominant compound was (S)-β-Bisabolene (42.38%), followed by Caryophyllene (11.14%), then γ-elemene (7.80%) and, Copaene (7.29%). Also, 1-ethenyl-1-methyl-2-Cyclohexane (6.77%), and β-chamigrene (6.20%). While other identified constituents were below 5.62% in Table 1.

TABLE 1 Gas Chromatography-Mass Spectrometry (GC-MS) identification of compounds in n-hexane fraction of the ethanolic seed extract of PG (NFESEPG).

Peak	R – Time	Area %	Name/Library
1	15.636	1.67	Copaene
2	16.186	6.77	Cyclohexane, 1-ethenyl-1-methyl-2
3	17.285	11.14	Caryophyllene
4	17.712	7.80	γ-Elemene
5	18.476	4.13	(Z) - 7,11-dimethyl -3-methylene-1,6,10-Dodecatriene (β-farnesene)
6	18.922	6.20	3,7,7-trimethyl-11-methylene-, (-)- Spiro [5.5] undec-2-ene (β-chamigrene)
7	19.504	1.76	1-(1,5-dimethyl-4-hexenyl)-4-methyl-Benzene (α-curcumene)
8	19.756	4.97	1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-Naphthalene (α-selinene)
9	20.067	4.14	1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethyl)-Naphthalene ((+)-Valencene)
10	20.526	42.38	1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-Cyclohexene ((S)-β-Bisabolene)
11	21.057	5.62	Copaene
12	21.704	3.42	cis-α-Bisabolene

3.2 | Preinduction, induction and post-induction measurement of blood pressure

3.2.1 | Diastolic blood pressure (mmHg) before induction

Figure 1 represents the result of the diastolic blood pressure before induction with L-NAME. There was no significant difference ($p < .05$) between the normal control group and all other groups.

3.2.2 | Systolic blood pressure (mmHg) before induction

Figure 2 represents the result of the systolic blood pressure before induction of L-NAME. There was no significant difference ($p < .05$) between the normal control group and all other groups.

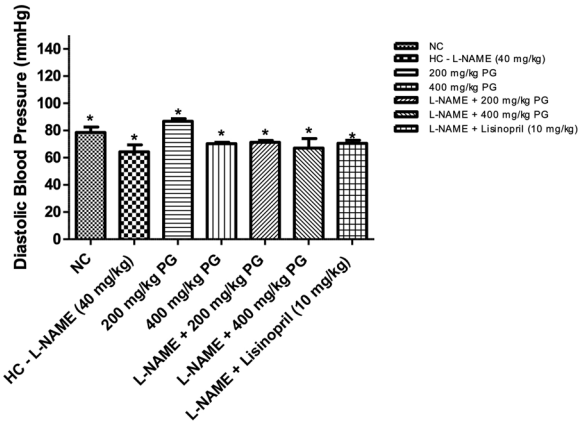

FIGURE 1 The diastolic blood pressure before induction of L-NAME of n-hexane fraction the ethanolic extracts of PG. Values represent mean ± standard deviation of replicate readings ($n = 6$). Values with the same symbol ("$_*$") along the same column are not significantly different ($p < .05$).

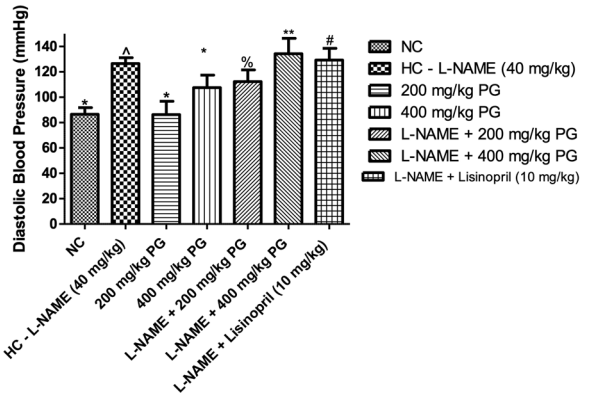

FIGURE 3 The diastolic blood pressure at induction of L-NAME of n-hexane fraction the ethanolic extracts of PG. Values represent mean ± standard deviation of replicate readings ($n = 6$). Values with the same symbol ("$_*$") along the same column are not significantly different ($p < .05$). "$_{**}$," "#," "%," and "^" means significantly different from normal control group at $p < .05$.

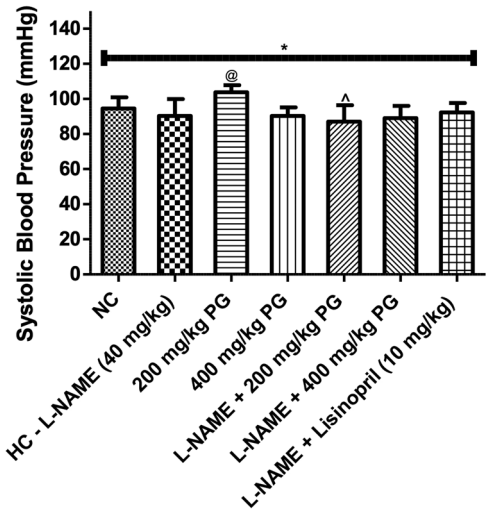

FIGURE 2 The systolic blood pressure before induction of L-NAME of n-hexane fraction the ethanolic extracts of PG. Values represent mean ± standard deviation of replicate readings ($n = 6$). Values with the same symbol along the same column are not significantly different ($p < .05$).

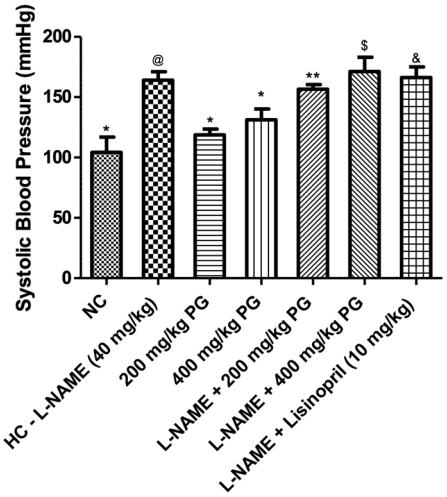

FIGURE 4 The systolic blood pressure at induction of L-NAME of n-hexane fraction the ethanolic extracts of PG. Values represent mean ± standard deviation of replicate readings ($n = 6$). Values with the same symbol ('$_*$') along the same column are not significantly different ($p < .05$), "$_{**}$," "$," "&," and "@," means significantly different from the normal control group at $p < .05$.

3.3 | Blood pressure measurement after induction of hypertension with L-NAME

3.3.1 | Diastolic blood pressure (mmHg) at induction

Figure 3 represents the result of the diastolic blood pressure at induction of L-NAME. The diastolic pressure of the rats was elevated after induction with L-NAME. There was significant difference ($p < .05$) between the normal control group and the hypertensive control, L-NAME + 200 mg/kg, L-NAME + 400 mg/kg, and L-NAME + lisinopril groups. There was no significant difference ($p < .05$) between normal control group, 200 mg/kg group. and 400 mg/kg.

3.3.2 | Systolic blood pressure (mmHg) at induction

Figure 4 represents the result of the systolic blood pressure at induction of L-NAME. The diastolic pressure of the rats was elevated after induction with L-NAME. There was significant difference ($p < .05$) between the normal control group and the hypertensive control, L-NAME + 200 mg/kg, L-NAME + 400 mg/kg, and L-NAME +

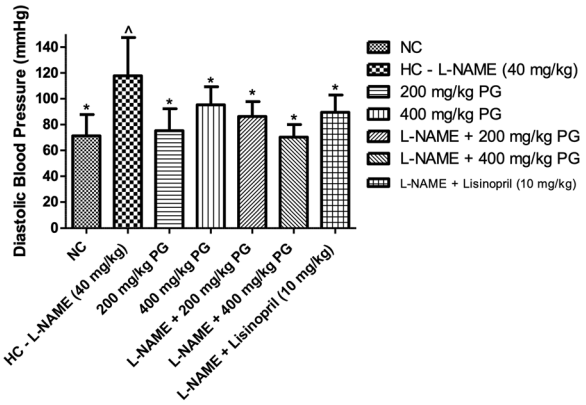

FIGURE 5 The diastolic blood pressure after induction and treatment (post-induction) of L-NAME of n-hexane fraction the ethanolic extracts of *Piper guineense*. Values represent mean ± standard deviation of replicate readings ($n = 6$). Values with the same symbol along the same column are not significantly different ($p < .05$), "^" means significantly different from the normal control group at $p < .05$.

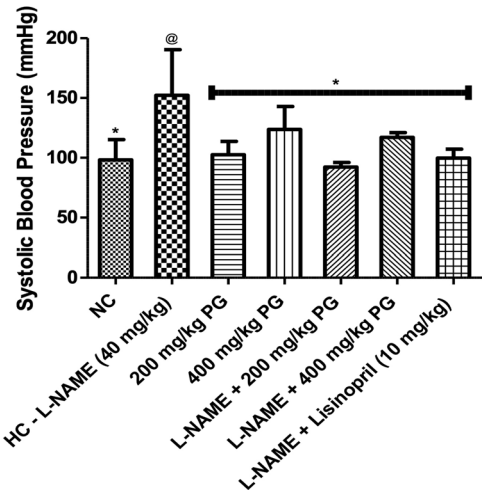

FIGURE 6 The systolic blood pressure after induction and treatment (post-induction) of L-NAME of n-hexane fraction the ethanolic extracts of PG. Values represent mean ± standard deviation of replicate readings ($n = 6$). Values with the same symbol along the same column are not significantly different ($p < .05$), "@," means significantly different from the normal control group at $p < .05$.

lisinopril groups. There was no significant difference ($p < .05$) between normal control group, 200 mg/kg group. and 400 mg/kg.

3.4 | Post-Induction

3.4.1 | Diastolic blood pressure (mmHg) at induction

Figure 5 represents the result of the diastolic blood pressure after induction with L-NAME and treatment with the plant extract. The diastolic pressure of the rats remained elevated in the hypertensive control group after induction with L-NAME. There was an increase in significant difference ($p < .05$) between the normal control group and the hypertensive control group. There was no significant difference ($p < .05$) between normal control group, 200 mg/kg, 400 mg/kg, L-NAME + 200 mg/kg, L-NAME + 400 mg/kg, and L-NAME + lisinopril groups.

3.4.2 | Systolic blood pressure (mmHg) before induction

Figure 6 represents the result of systolic blood pressure after induction with L-NAME and treatment with the plant extract. The diastolic pressure of the rats remained elevated in the hypertensive control group after induction with L-NAME. There was increase in significant difference ($p < .05$) between the normal control group and the hypertensive control group. There was no significant difference ($p < .05$) between normal control group, 200 mg/kg, 400 mg/kg, L-NAME + 200 mg/kg, L-NAME + 400 mg/kg, and L-NAME + lisinopril groups.

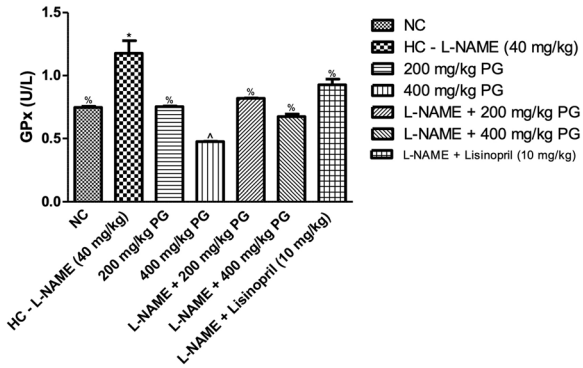

FIGURE 7 The effect of administration of n-hexane fraction of PG on glutathione peroxidase. Values represent mean ± standard deviation of replicate readings ($n = 6$). Values with the same symbol along the same column are not significantly different ($p < .05$), "%," "^," and "^^" means significantly different from normal control group at $p < .05$.

3.5 | The effect of oral administration of n-hexane fraction of PG on in vivo antioxidant parameters

3.5.1 | The effect of oral administration of n-hexane fraction of PG on glutathione peroxidase (UL^{-1})

The effect of the administration of the n-hexane fraction of PG on the activity of glutathione peroxidase is shown in Figure 7. The result shows that glutathione was reduced in the hypertensive control group induced with L-NAME compared to the normal control. There was a significant difference ($p < .05$) between the normal control group and the hypertensive control group, 200 mg/kg, 400 mg/kg

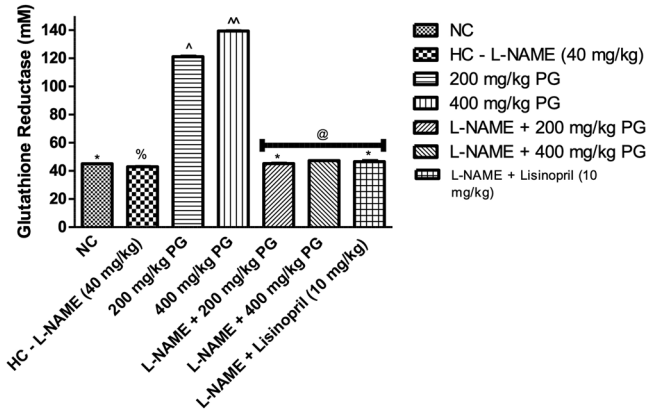

FIGURE 8 The effect of administration of n-hexane fraction of PG on the concentration of glutathione reductase. Values represent mean ± standard deviation of replicate readings ($n = 6$). Values with the same symbol (%) along the same column are not significantly different ($p < .05$), "*," and "^" means significantly different from normal control group at $p < .05$.

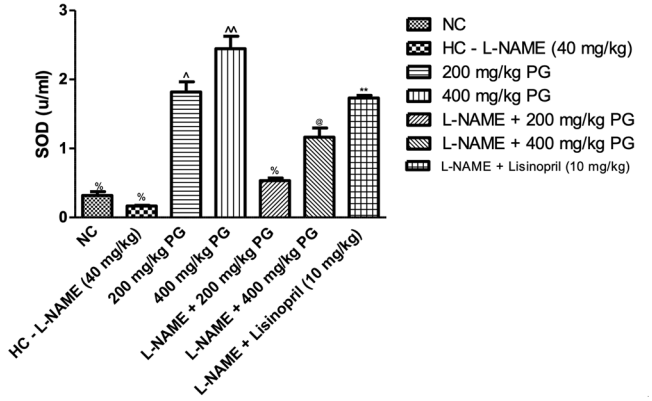

FIGURE 9 The effect of administration of n-hexane fraction of PG on the activity of superoxide dismutase. Values represent mean ± standard deviation of replicate readings ($n = 6$). Values with the same symbol along the same column are not significantly different ($p < .05$), "*," "**," "^," and "^^" means significantly different from normal control group at $p < .05$.

groups. There was also no significant difference ($p < .05$) between the normal control group, L-NAME + 200 mg/kg, and L-NAME + lisinopril.

3.5.2 | The effect of oral administration of n-hexane fraction of PG on glutathione reductase (mM)

The effect of administration of n-hexane fraction of PG on the concentration of glutathione reductase is shown in Figure 8. The result shows the concentration of glutathione reductase was elevated in the hypertensive control group induced with L-NAME compared to the normal control. There was increase significant difference ($p < .05$) between the normal control group and the hypertensive control group. There was decrease significant difference ($p < .05$) between the normal control and 400 mg/kg groups. There was no significant difference ($p < .05$) between the normal control group, 200 mg/kg group, L-NAME + 200 mg/kg, L-NAME + 400 mg/kg, and L-NAME + lisinopril.

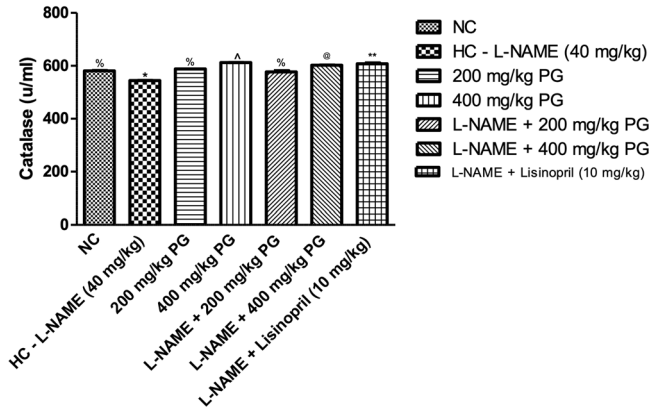

FIGURE 10 The effect of administration of n-hexane fraction the ethanolic extracts of PG on Catalase. Values represent mean ± standard deviation of replicate readings ($n = 6$). Values with the same symbol (%) along the same column are not significantly different ($p < .05$), "*," "**," "^," and "@" means significantly different from normal control group at $p < .05$.

3.5.3 | The effect of oral administration of n-hexane fraction of PG on superoxide dismutase (uml^{-1})

The effect of administration of n-hexane fraction of PG on the activity of superoxide dismutase is shown in Figure 9. The result shows that superoxide dismutase was reduced in the hypertensive control group induced with L-NAME but not significantly different ($p < .05$) when compared to the normal control. There was also no significant difference ($p < .05$) between the normal control group and L-NAME + 200 mg/kg group. There was increase in significant difference ($p < .05$) between the normal control group and the 200 mg/kg, 400 mg/kg, L-NAME + 400 mg/kg and L-NAME + lisinopril groups.

3.5.4 | The effect of oral administration of n-hexane fraction of PG on catalase (uml^{-1})

The effect of administration of n-hexane fraction of PG on the activity of catalase is shown in Figure 10. The result shows that catalase was reduced in the hypertensive control group induced with L-NAME compared to the normal control. There was significant difference ($p < .05$) between the normal control group and the hypertensive control group, 400 mg/kg, L-NAME + 400 mg/kg, and L-NAME + lisinopril groups. There was no significant difference ($p < .05$) between the normal control group, 200 mg/kg, and L-NAME + 200 mg/kg group.

3.6 | Effects of administration of n-hexane fraction of PG on hypertension biomarkers

3.6.1 | The effect of oral administration of n-hexane fraction of PG on the concentration of nitric oxide (μM)

The effect of oral administration of n-hexane fraction of PG on the concentration of nitric oxide is shown in Figure 11. The result shows that nitric oxide synthetase was reduced in the hypertensive control group compared to the normal control. There was significant difference ($p < .05$) between the normal control group and the hypertensive control group, 400 mg/kg, L-NAME + 400 mg/kg, and L-NAME + lisinopril groups. There was no significant difference ($p < .05$) between the normal control group, 200 mg/kg, and L-NAME + 200 mg/kg group.

3.6.2 | The effect of oral administration of n-hexane fraction of PG on the activity of angiotensin converting enzyme (ng/ml)

The effect of oral administration of n-hexane fraction of PG on the activity of angiotensin converting enzyme is shown in Figure 12. The result shows that angiotensin converting enzyme was elevated in the hypertensive control group compared to the normal control. There was increase in significant difference ($p < .05$) between the normal control group and the hypertensive control group, 200 mg/kg, L-NAME + 200 mg/kg, L-NAME + 400 mg/kg, and L-NAME + lisinopril groups. There was no significant difference ($p < .05$) between the normal control group and 400 mg/kg group.

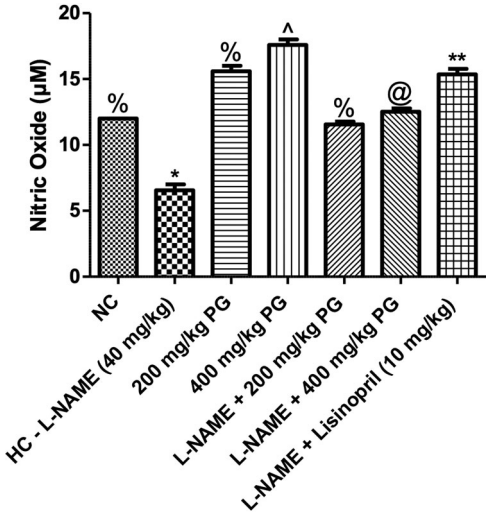

FIGURE 11 The effect of oral administration of n-hexane fraction of PG on the concentration of Nitric Oxide. Values represent mean ± standard deviation of replicate readings ($n = 6$). Values with the same symbol (%) along the same column are not significantly different ($p < .05$), "*," "**," "^," and "@" means significantly different from normal control group at $p < .05$.

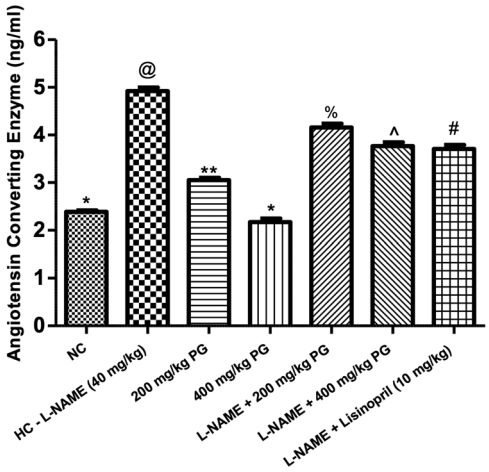

FIGURE 12 The effect of oral administration of n-hexane fraction of PG on the activity of angiotensin converting enzyme. Values represent mean ± standard deviation of replicate readings ($n = 6$). Values with the same symbol (*) along the same column are not significantly different ($p < .05$), "**," "%," "^," "#," and "@" means significantly different from the normal control group at $p < .05$.

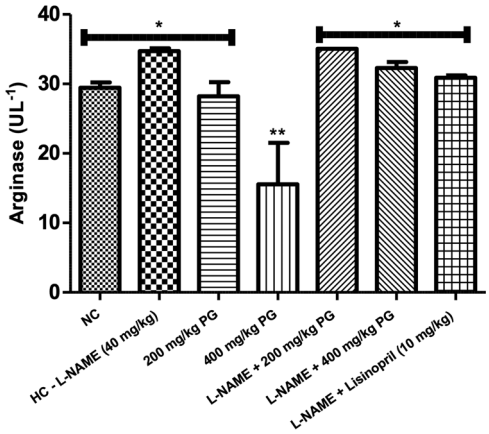

FIGURE 13 The effect of oral administration of n-hexane fraction of PG on the activity of arginase. Values with the same symbol (*) along the same column are not significantly different ($p < .05$), "**" means significantly different from the normal control group, and cardiac group at $p < .05$.

3.6.3 | The effect of oral administration of n-hexane fraction of PG on the activity of arginase (U/L)

The effect of oral administration of the n-hexane fraction of PG on the activity of arginase is shown in Figure 13. The result shows that arginase was slightly elevated in the hypertensive control group compared to the normal control. There was no significant difference ($p < .05$) between the normal control group, hypertensive control

group, 200 mg/kg, L-NAME + 200 mg/kg, L-NAME + 400 mg/kg, and L-NAME + lisinopril groups. There was a decrease in significant difference ($p < .05$) between the normal control group and the 400 mg/kg group.

3.6.4 | The effect of oral administration of n-hexane fraction of PG on the concentration of malondialdehyde lipid peroxidation (μM)

The effect of oral administration of n-hexane fraction of PG on the concentration of malondialdehyde is shown in Figure 14. The result shows that the concentration of malondialdehyde was elevated in the hypertensive control group induced compared to the normal control. There was increase in significant difference ($p < .05$) between the control group and the hypertensive control group, 400 mg/kg, L-NAME + 200 mg/kg, L-NAME + 400 mg/kg, and L-NAME + lisinopril groups. There was no significant difference ($p < .05$) between the control group and the 200 mg/kg group.

3.7 | Cardiac biomarkers estimation

3.7.1 | The effect of oral administration of n-hexane fraction of PG on the activity of creatine kinase myocardial band (Ck-Mb) (UL^{-1})

The effect of oral administration of the n-hexane fraction of PG on the activity of Ck-Mb is shown in Figure 15. The result shows that Ck-Mb was elevated in the hypertensive control group compared to the normal control. There was an increase in significant difference ($p < .05$) between the normal control group and the hypertensive control group. There was no significant difference ($p < .05$) between the normal control group, 200 mg/kg, 400 mg/kg, L-NAME + 200 mg/kg, L-NAME + 400 mg/kg, and L-NAME + lisinopril groups.

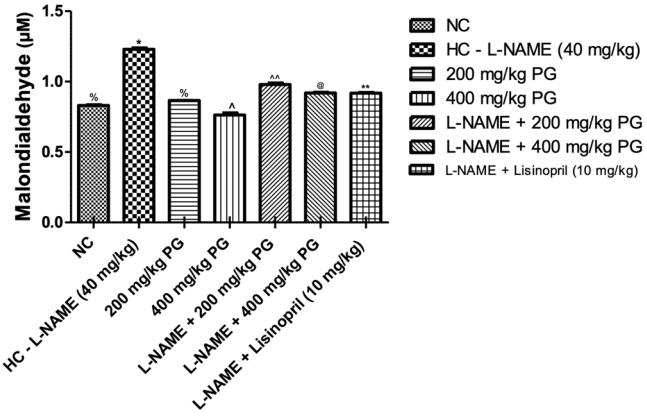

FIGURE 14 The Effect of Oral Administration of n-hexane fraction of PG on the Concentration of Malondialdehyde. Values represent mean ± standard deviation of replicate readings ($n = 6$). Values with the same symbol (%) along the same column are not significantly different ($p < .05$), "*," "**," "^," "^^," and "@" means significantly different from control group at $p < .05$.

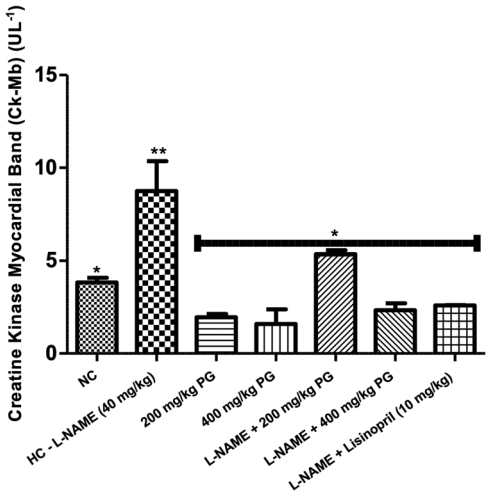

FIGURE 15 The effect of oral administration of n-hexane fraction of PG on the activity of Ck-Mb. Values represent mean ± standard deviation of replicate readings ($n = 6$). Values with the same symbol (*) along the same column are not significantly different ($p < .05$), "**" means significantly different from the normal control group at $p < .05$.

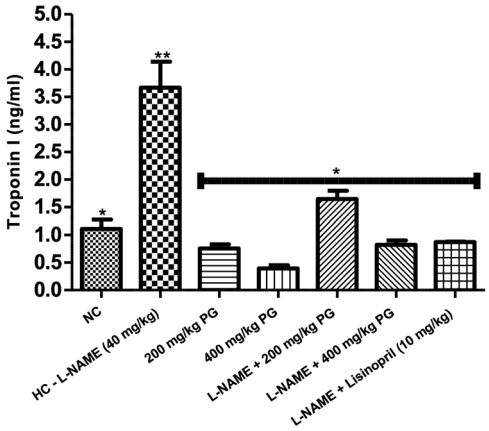

FIGURE 16 The effect of subacute oral administration of n-hexane fraction of the ethanolic extracts of PG on troponin I. Values represent mean ± standard deviation of replicate readings ($n = 6$). Values with the same symbol (*) along the same column are not significantly different ($p < .05$), "**" means significantly different from the normal control group at $p < .05$.

3.7.2 | The effect of oral administration of n-hexane fraction of PG on the concentration of troponin I (ng/ml)

The effect of subacute oral administration of the n-hexane fraction of the ethanolic extracts of PG on troponin I is shown in Figure 16. The result shows that troponin I was elevated in the hypertensive control group induced with L-NAME compared to the normal control. There

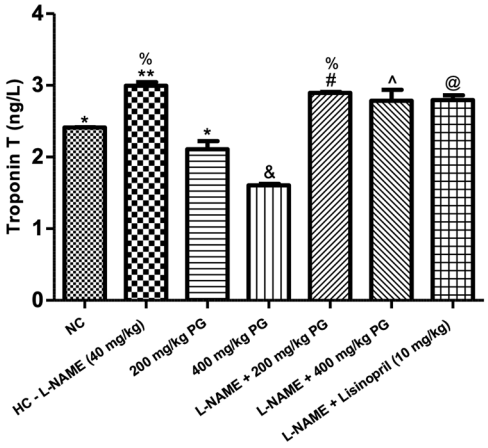

FIGURE 17 The effect of subacute oral administration of n-hexane fraction of the ethanolic extracts of PG on troponin T. Values represent mean ± standard deviation of replicate readings ($n = 6$). Values represent mean ± standard deviation of replicate readings ($n = 6$). Values with the same symbol (*, %) along the same column are not significantly different ($p < .05$), "**," "&," "%," "#," "@," and "^" means significantly different from the normal control group at $p < .05$.

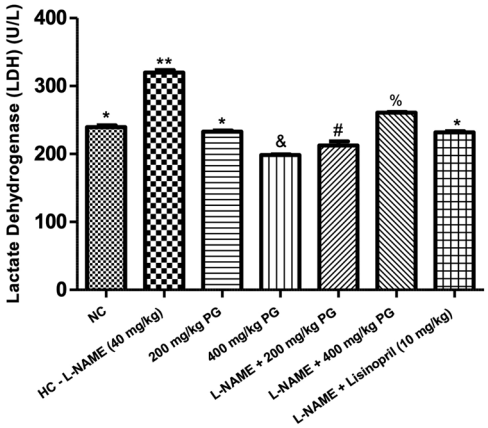

FIGURE 18 The effect of subacute oral administration of n-hexane fraction of the ethanolic extracts of PG on lactate dehydrogenase. Values represent mean ± standard deviation of replicate readings ($n = 6$). Values with the same symbol (*) along the same column are not significantly different ($p < .05$), "**," "%," "&," and "#" means significantly different from the normal control group at $p < .05$.

was an increase in significant difference ($p < .05$) between the normal control group and the hypertensive control group. There was no significant difference ($p < .05$) between the normal control group, 200 mg/kg, 400 mg/kg, L-NAME + 200 mg/kg, L-NAME + 400 mg/kg, and L-NAME + lisinopril groups.

3.7.3 | The effect of oral administration of n-hexane fraction of PG on the concentration of troponin T (ng/L)

The effect of subacute oral administration of the n-hexane fraction of the ethanolic extracts of PG on troponin T is shown in Figure 17. The result shows that troponin T was elevated in the hypertensive control group induced with L-NAME compared to the normal control. There was an increase in the significant difference ($p < .05$) between the normal control group, hypertensive control, 400 mg/kg, L-NAME + 200 mg/kg, L-NAME + 400 mg/kg, and L-NAME + lisinopril groups. There was no significant difference ($p < .05$) between the normal control group and the 200 mg/kg group.

3.7.4 | The effect of oral administration of n-hexane fraction of PG on the activity of lactate dehydrogenase (LDH) (UL^{-1})

The effect of subacute oral administration of the n-hexane fraction of the ethanolic extracts of PG on lactate dehydrogenase is shown in Figure 18. The result shows that lactate dehydrogenase was elevated in the hypertensive control group induced with L-NAME compared to the normal control. There was an increase in the significant difference ($p < .05$) between the normal control group, the hypertensive control group, 400 mg/kg, L-NAME + 200 mg/kg and the L-NAME + 400 mg/kg groups. There was no significant difference ($p < .05$) between the normal control group, 200 mg/kg group and the L_NAME + Lisinopril group.

3.8 | Histo-morphological examination of n-hexane fraction of the ethanolic extracts of PG

The histomorphological examination of the heart structures presented in Figure 19 reveals significant differences among the normal control, hypertensive control group, 200 mg/kg, 400 mg/kg, L-NAME + 200 mg/kg, L-NAME + 400 mg/kg, and L-NAME + lisinopril groups. Microscopic analysis revealed that the heart tissue from the normal control group exhibited no alterations in cell arrangement or adverse effects when observed under various magnifications. The cardiac muscle tissues displayed well-organized fibers, indicative of their healthy state. Conversely, in the hypertensive control group, there was severe disruption observed in the arrangement of cardiac muscle fibers. However, in the 200 mg/kg and 400 mg/kg groups, the cardiac muscle tissues appeared well-arranged with no signs of damage. The L-NAME + 200 mg/kg, L-NAME + 400 mg/kg, and L-NAME + lisinopril groups exhibited indications of recovery.

4 | DISCUSSION

Hypertension stands as the predominant cardiovascular ailment,[23] with its pathogenesis proposed to involve various mechanisms such as renal salt and water handling impairment, augmented angiotensin II production via the renin-angiotensin-aldosterone system (RAAS),

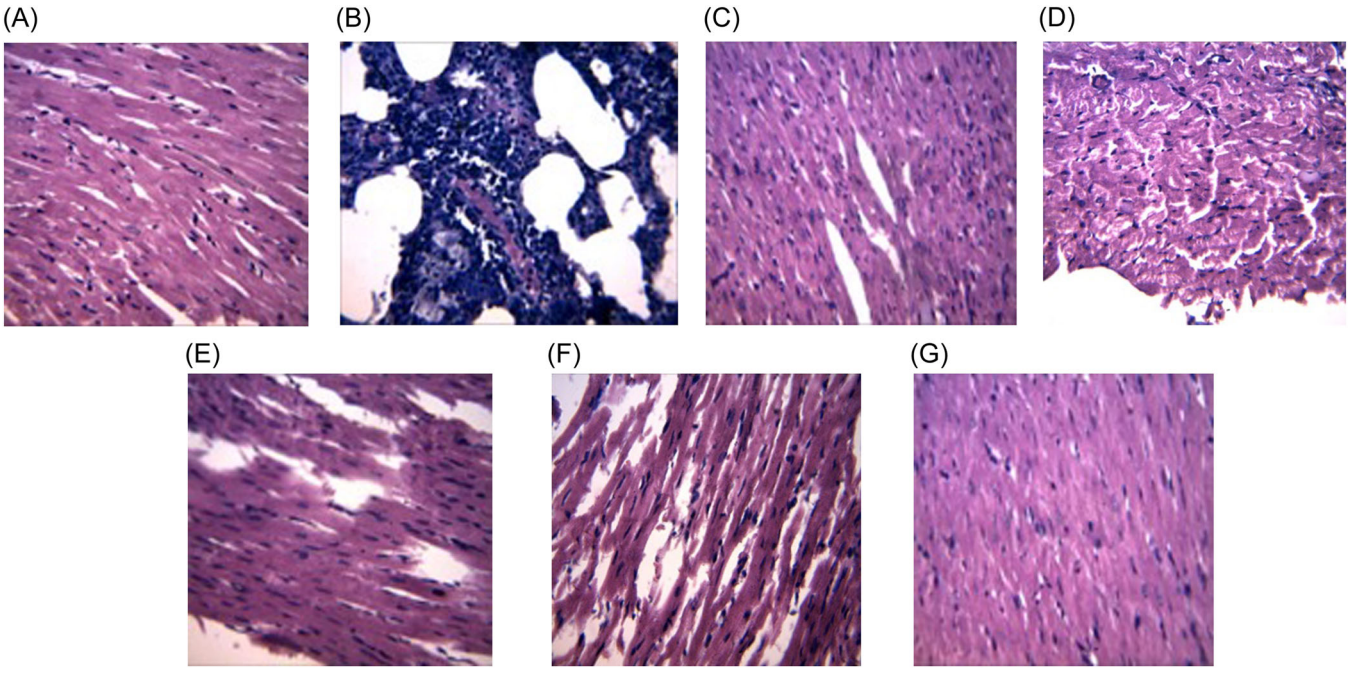

FIGURE 19 Photomicrograph of the heart of the (A) normal control, (B) the hypertensive control group, (C) 200 mg/kg, (D) 400 mg/kg, (E) L-NAME + 200 mg/kg, (f) L-NAME + 400 mg/kg, and (g) L-NAME + lisinopril groups. N.B: Normal control (A): No lesion visible. Hypertensive control (B): Lesion visible indicating damage in the heart tissue. 200 mg/kg (C): No lesion visible. 400 mg/kg (D): No lesion visible. L-NAME + 200 mg/kg (E): No lesion visible. L-NAME + 400 mg/kg (F): No lesion visible. L-NAME + lisinopril (G): No lesion visible

abnormalities in the sympathetic system, and heightened vascular oxidative stress and inflammation.[24,25]

L-NAME has been documented to induce arterial hypertension by depleting nitric oxide (NO). Additionally, in animals treated with L-NAME, cardiac hypertrophy is observed as a compensatory response to prolonged elevated blood pressure.[26] In our investigation, L-NAME administration notably raised ($p < .05$) both systolic and diastolic blood pressure in rats, as measured using the noninvasive tail-cuff method, aligning with earlier findings reported by Vogel and Vogel.[27]

The pathology of hypertension involves the production of reactive oxygen species. Previous studies have demonstrated an increase in O_2- and superoxide-producing enzymes in various models of systemic hypertension, irrespective of the induction method.[28,29] Besides NO synthesis inhibition, L-NAME induces hypertension by provoking oxidative stress. It also disrupts the renin-angiotensin system (RAS), leading to heightened angiotensin II expression and renal dysfunction.[26]

The analyzed sample predominantly consisted of sesquiterpene hydrocarbons, notably comprising 81.58% of the identified constituents. These compounds are essential oils (EOs) derived from various plant parts, known for their volatile nature and concentrated bioactive properties. They encompass a wide array of biological effects, including anticancer, antibacterial, smooth muscle cell relaxation (relevant to cardiovascular health), anti-inflammatory, antioxidant, and antiallergic properties, as outlined in studies by.[30]

The most abundant compound identified was (S)-β-Bisabolene, constituting 42.38% of the sample. This compound belongs to the bisabolane class of monocyclic sesquiterpenoids, recognized for their broad therapeutic potential. Studies, such as those by Li et al.,[31] have documented its antibacterial, anti-inflammatory, and cytotoxic properties, supported by both in vitro and in vivo investigations. The versatility of bisabolane-type sesquiterpenoids in traditional medicine underscores their significant role in therapeutic applications.

Polymorphisms in glutathione peroxidase (GPx) have been associated with an elevated risk of coronary artery disease, stroke, and cerebral thrombosis. Among smokers, the heightened risk of coronary heart disease has been linked to glutathione S-transferase polymorphisms.[32] Following L-NAME administration, there was a significant increase ($p < .05$) in GPx activity in the hypertensive group compared to the control group. However, the NFESEPG notably reduced GPx activity, indicating its potential to shield against oxidative stress and decrease the conversion of GSH to GSSG. Glutathione reductase (GSH) serves a critical role in defending against oxidative stress by scavenging reactive oxygen species (ROS).[33] GSH can interact with various free radicals, including hydroxyl radicals, hypochlorous acid, superoxide, and peroxynitrite radicals, and neutralizes hydrogen peroxide, acting as the primary cellular defense mechanism against oxygen-reactive species.[33] In our study, the NFESEPG notably augmented GSH levels, indicating its capacity to counteract ROS and alleviate ROS-associated hypertension. GSH plays a pivotal role in the cardiovascular system as a vital antioxidant that reinstates intracellular redox balance and

prevents the deactivation of nitric oxide produced by the endothelium, thus averting abnormal vasomotor reactivity in individuals with coronary spastic angina.[33]

The excessive production of angiotensin II leads to increased vascular superoxide (O_2-) formation through increased expression of NADPH-dependent oxidase in aortic smooth muscle cells.[34] The excessive O_2- reacts rapidly with NO to form peroxynitrite (ONOO−). Peroxynitrite is a strong pro-oxidant molecule which causes lipid peroxidation and tissue injury.[35] In this study, the hypertensive group had decreased superoxide dismutase, but the group treated with the NFESEPG had increased activity of superoxide dismutase. The ability of the NFESEPG to mop up superoxide adds to its ability to ameliorate reactive oxygen species (ROS) mediated hypertension.

Catalase deficiency or malfunctioning is associated with many diseases such as diabetes mellitus, vitiligo, cardiovascular diseases, Wilson disease, hypertension, anemia, some dermatological disorders, Alzheimer's disease, bipolar disorder, and schizophrenia, and its hydrogen peroxide catabolism protects the cells from oxidative assault.[36] In this study, the hypertensive group had decreased catalase activity, but the group treated with the NFESEPG had increased activity of catalase (Figure 10). The ability of the NFESEPG to break down hydrogen peroxide to water adds to its ability to ameliorate reactive oxygen species (ROS) mediated hypertension.

Several reports have analyzed the reason for L-NAME-induced arterial hypertension, which is said to be due to a deficiency of NO that has been reported to control coronary vascular tone.[37] This decrease in endothelium-dependent NO arterial dilatation is related to the risk of coronary ischemia and infarction.[38] The chronic elevation of NO synthase also caused myocardial infarction in rats. The blockade of NO synthase by L-NAME results in increased serum cholesterol levels in rats42. Given these reports, it is hypothesized that decreased NO levels may be a risk factor for coronary disease and myocardial infarction.

The impact of NFESEPG on NO concentration indicated a notable reduction ($p < .05$) in NO levels in the L-NAME treated group, signifying an elevation in arginase activity induced by L-NAME. This rise in arginase activity is associated with vasoconstriction and NO level reduction, consistent with findings by Paredes et al.[37] Conversely, treatment with NFESEPG significantly augmented ($p < .05$) NO concentrations in the treated groups, suggesting the extract's potential to contribute to normal cardiovascular function and blood pressure regulation.

Angiotensin Converting Enzyme (ACE) inhibition is a primary target for antihypertensive food-derived phenolics, proposed as an alternative to pharmaceuticals.[39] Hypertension's etiology involves Angiotensin II (ANG II) production facilitated by ACE, a potent vasoconstrictor. Persistent ANG II production exerts pressure, leading to elevated blood pressure.[39] Oral L-NAME administration activated the renin-angiotensin system by increasing ACE (Figure 4.29). However, the results of this study indicated a significant reduction ($p < .05$) in ACE levels due to NFESEPG treatment. Phenolics, either alone or in combination with other compounds, are known ACE inhibitors.[40] The decline in ACE following NFESEPG treatment may be attributed to the bioactivity of compounds such as bisabolene, caryophyllene, copaene, and other terpenes (essential oils) present in the extract.[41]

Arginase catalyzes the conversion of L-arginine to urea and ornithine, with intracellular competition observed between eNOS and arginase enzymes for their shared substrate, L-arginine.[42] Elevated arginase activity can hinder endothelium-dependent vasorelaxation by reducing L-arginine availability to endothelial nitric oxide synthase (eNOS), consequently decreasing NO production and uncoupling eNOS function. This study evidenced increased arginase activity in hypertensive rats treated with L-NAME (Figure 13), consistent with previous reports indicating arginase upregulation and reduced NO in hypertension.[43] However, pretreatment with NFESEPG mitigated the rise in arginase activity in hypertensive rats, potentially attributable to the activity of phenolic compounds (essential oils - terpenes) present in NFESEPG, as reported by Bhullar et al.[40]

Malondialdehyde (MDA) is present in various bodily fluids and tissues, serving as a commonly utilized measure for lipid peroxidation and oxidative stress assessment.[44] In this study, a notable increase ($p < .05$) in MDA levels was observed in hypertensive rats induced by L-NAME (Figure 14), underscoring the significance of oxidative stress in hypertension pathophysiology.[45] Treatment with NFESEPG in L-NAME-induced hypertensive rats resulted in a reduction of MDA levels. The decline in MDA levels in the NFESEPG-treated group may be attributed to the antioxidant properties of PG, which has been demonstrated to possess robust antioxidant activity,[44] possibly due to its bioactive compound content.

Biological markers like endogenous enzymes are organ-specific and are released from damaged organs during necrosis.[46] Cardiac injury biomarkers such as Troponin I, Troponin T, and CK-Mb are released into serum or perfusate during myocardial damage.[47] L-NAME administration in this study led to notable increases in plasma cardiac marker enzyme activities (CK-Mb and LDH). However, treatment with NFESEPG significantly reduced ($p < .05$) CK-Mb and LDH activities. These cellular enzyme levels in the blood are directly linked to the integrity of cardiac cell membranes.[47] Thus, NFESEPG's inhibition of L-NAME-induced elevation of plasma marker enzymes (CK-MB and LDH) may be attributed to its action in maintaining cardiac membrane integrity, aligning with previous observations that cardioprotective compounds mitigate altered biomarkers.[47]

Cardiac troponins serve as prognostic markers in acute or chronic heart failure, remaining at low levels unless there is myocardial necrosis or cell death.[48] Recent studies suggest that cardiac troponins may predict adverse outcomes in hypertensive patients.[48,49] Elevated serum troponin levels likely signify significant cardiac involvement in hypertensive conditions.[48] Oral L-NAME administration resulted in a significant increase ($p < .05$) in Troponin I and Troponin T levels (Figures 16 and 17 respectively), consistent with findings by Adamcova et al.[49]

The histo-morphological examination of the structures of the heart (Figure 19) showed that there were visible lesions indicating damage in the heart tissue. This suggests that an increase in the troponin levels indicated damage to the heart. In this study, the results revealed that the NFESEPG caused a significant reduction

($p < .05$) in the concentration of troponin levels and activities of Ck-Mb and LDH. The histo-morphological examination of the structures of the heart revealed no lesion visible in the control group, and other groups treated with NFESEPG. This indicates the potential of NFESEPG to protect the cardiac organ against myocardial injury and acute myocardial infarction.

5 | CONCLUSION

The bioactive compounds in the n-hexane fraction of the ethanolic seed extract of PG are primarily terpenes. This study demonstrates that NFESEPG has antioxidant properties that can help mitigate oxidative stress-related complications in hypertension. Additionally, the study shows that NFESEPG can lower blood pressure, thereby alleviating hypertension. NFESEPG increased NO concentration and reduced the activity of ACE, arginase, and the levels of MDA. It also decreased the activity of cardiac biomarkers, indicating cardioprotective properties. Furthermore, NFESEPG improved heart morphology in hypertensive conditions.

AUTHOR CONTRIBUTIONS

The author contributions are as follows: Oyedepo T.A. conceptualized the study; Olopade E.O. curated the data, conducted formal analysis, performed investigation, and contributed to methodology and visualization; Adetoun Elizabeth Morakinyo administered the project; Oyedepo T.A. supervised the study and validated the findings; while Alao J.O. contributed to writing the original draft and reviewed and edited the manuscript.

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DATA AVAILABILITY STATEMENT

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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