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Anti-hypertensive Evaluation of n-Hexane and Hydro-ethanol Fruit Peel Extracts of *Persea americana* Mill. (Lauraceae) in albino rats

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ABSTRACT

The fruits of *Persea americana* are consumed in Nigeria and are reported to have antihypertensive properties in folklore use. Hypertension is currently one of the major risk factors for cardiovascular diseases that lead to high morbidity and mortality globally. The present study was designed to evaluate the antihypertensive effects of n-Hexane and hydro-ethanol extracts of the fruit peel of *P. americana* on high-salt diet-induced hypertension rats. The antihypertensive investigation of the extracts at different doses (100, 200, and 400 mg/kg) showed a remarkable dose-dependent effect. At 100 mg/kg, the systolic and diastolic blood pressure (SBP/DBP) for n-hexane and hydro-ethanol extracts were 124.28/103.83 mmHg and 122.72/104.78 mmHg, with pulse pressure (PP) of 20.45 and 17.94 mmHg, respectively. At 200 mg/kg, the SBP/DBP were 125.01/112.54 mmHg and 119.92/110.69 mmHg with PP of 12.47 and 9.23 mmHg, respectively. At 400 mg/kg, the SBP/DBP were 117.66/98.8 mmHg and 135.88/121.94 mmHg, with PP of 8.86 and 13.94 mmHg, respectively. The standard drug nifedipine gave SBP/DBP of 105.17/87.72 mmHg with PP of 17.45 mmHg, while other standards gave higher SBP/DBP values; HCT (138.28/126.81 mmHg), telmisartan (174.94/141.29 mmHg) and enalapril (128.99/115.08 mmHg). The results from the highest dose of the n-Hexane extract (400 mg/kg) compared favourably with that of the standard (nifedipine) which shows that the extract might be acting through a similar mechanism as that of nifedipine. The reduction of BP in this study could be due to the presence of identified secondary metabolites in the plant.

Keywords: *Persea americana*, Phytochemical Investigation, Antihypertensive, n-Hexane, Hydro-ethanolic Extracts.

Introduction

Natural products have been a source of medicinal agents since time immemorial and a remarkable number of modern drugs have been derived from natural sources especially plants. Natural products will continue to play a crucial role in meeting this demand through the expanded investigation of the world's biodiversity, much of which remains unexplored. *Persea americana* which belongs to the family Lauraceae is one of the emerging plants of interest in the management of hypertension.[1,2] It is commonly known as avocado pear and is widely distributed in tropical countries. The edible fruit pulp has been reported to have wound healing properties,[3-5] hepatoprotective effects,[4-6] and anticancer properties.[7]
The aqueous leaf extract of the plant has shown analgesic and anti-inflammatory effects,[4,8] anticonvulsant,[9] hypoglycemic and hypocholesterolaemic,[10] vasorelaxant and blood pressure lowering[1,11] activities in animal studies. The leaf extract is used to treat hypertension and induce diuresis in Brazilian ethno medicine.[12] The aqueous stem-bark extract of the plant is used by Traditional Medicine Practitioners in Nigeria for the treatment of parasitic skin diseases.[1] Studies on the antihypertensive properties of the plant have been reported on the leaf extract.[1,4,5] However, herbalists in Nigeria have through oral communication confirmed that the aqueous seed extract is equally effective in the treatment of hypertension. Hypertension is currently one of the major risk factors for cardiovascular, neurological and renal events.[5] It is well known that hypertension can often lead to lethal complications if left untreated.[13] Consequently, the continued search for alternative antihypertensive agents of natural origin, with fewer side effects but greater effectiveness, necessitated evaluation of *Persea americana* for possible antihypertensive potential. Therefore, there is urgent need to develop new and effective drugs for the treatment of hypertension.[5] This study is designed to evaluate the phytochemical constituents and antihypertensive effect of the Hydro-ethanol and n-Hexane rind extracts of *Persea americana* on rats.

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Materials and Methods

Collection and Identification of Plant Material
The fruits of *Persea americana* were collected at Ketu axis of Lagos State. The plant samples were identified and authenticated by plant taxonomist from Department of Biological Science, University of Lagos, Nigeria with the herbarium number of 7500 and were deposited at Department of Pharmaceutical Chemistry, University of Lagos, Nigeria.

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Preparation of Plant Extract
The fruit peels were removed and washed thoroughly with distilled water. It was air-dried and pulverized to powder using a grinding machine. The grounded rinds were then extracted via two extraction processes, which are; soxhlet extraction process and cold maceration extraction process using n-hexane and 50% hydro-ethanol, respectively. The two extracts were concentrated using the rotary evaporator, and further dried in an oven at 40ºC and then kept in air-tight amber bottles in a refrigerator until further analysis.

Phytochemical Screening for Secondary Metabolites
Phytochemical screening were carried out on both the n-Hexane and 50% hydro-ethanol extracts of the fruit peel of *P. americana* for the presence of secondary metabolites such as alkaloids, tannins, steroids, flavonoids, saponins, anthraquinones and reducing sugars using standard procedures.[14-16]

Experimental Animals
The antihypertensive activity of the extracts was carried out on 55 female Wistar rats aged 6 weeks and weighing 70-90 g prior to the experiment. The albino rats were housed in standard environmental conditions at 12/12 hr light/dark natural cycle in the College of Medicine Animal House, University of Lagos, Nigeria. All albino rats had free access to standard growers feed and water *ad libitum.*

Ethical Consideration
All animal treatment procedures used in the present study were approved by the ethical committee of the University of Lagos, Nigeria (Ref N^{o}: #2365).

Experimental Design
The antihypertensive activity of the extracts (n-Hexane and 50% Hydro-ethanol) of fruit peels of *P. americana* was evaluated by salt-induced hypertension model previously described by Sofola and Balogun.[17,18] In this method, the rats were placed on a high-salt diet at a dose of 1 mg/kg of sodium chloride ($NaCl_2$) and cadmium chloride ($CdCl_2$) for 6 weeks. A total of 55 rats were randomly divided into eleven groups of five rats each. The experiments were conducted separately based on the high-salt diet used (i.e. $NaCl_2$ and $CdCl_2$) on two different extracts (hydro-ethanol and n-Hexane extract) and standard drugs.
The first group (negative control) received distilled water (1 mL/kg/day) by oral gavage. Groups 2, 3, 4, 5, 6 and 7 were treated with extracts at different concentrations (100, 200, 400 mg/kg), while groups 8, 9, 10 and 11 were treated with standard antihypertensive drugs (Nifedipine 20mg/kg, Enalapril 50 mg/kg, HCT 25 mg/kg and Telmisartan 5 mg/kg) orally for six weeks. At the end of the investigation, blood pressure (systolic blood pressure, diastolic blood pressure, mean arterial blood pressure and pulse pressure) and heart rate of all the albino rats were measured as described by Balogun *et al.*[18] Invasive blood pressure measurement was carried out via arterial cannulation. The albino rats were anaesthetized with a solution of 25% (w/v) urethane and 1% (w/v) α-chloralose injected intraperitoneally at a dose of 5 mL/kg body weight. The anaesthetized rat was placed on its back on the operating table, the limbs were fastened to the table, and the trachea was exposed and cannulated.[19,20]
The blood pressure measurements were obtained by cannulation of one carotid artery. A polyethylene cannula filled with 1% heparinised saline was inserted into the artery, tied in place, and connected via a pressure transducer (model SP 844, Physiological Pressure Transducer. AD Instruments) that was attached through MLAC11 Grass adapter cable to a computerized data acquisition system with LabChart-7 pro software (Power Lab-4/24T, model MLT844/P; AD Instruments Pty Ltd., Castle Hill, Australia). The LabChart-7 Pro software computes the Heart Rate by applying the cyclic measurement function, which is a channel calculation that analyzes periodic blood pressure waveforms in real-time. Data of the detected cycles are displayed as a continuous data-trace for HR in another channel of the data acquisition system. Recordings were taken at a sampling frequency of 5/seconds.[17,18,20]

Statistics Analysis
All the data obtained from the pharmacological experiments were subjected to statistical analysis, and values were reported as means ± standard deviation using GraphPad software 2003 version.

Results and Discussion

Phytochemical screening
The results of the phytochemical screening for secondary metabolites of both n-Hexane and 50% Hydro-ethanol extracts of *Persea americana* fruit peel are presented in Table 1 below.
The results of phytochemical screening of this study showed the presence of alkaloids, flavonoids, tannins, steroids and reducing sugars in both the extracts which is in agreement with the results obtained by Noorul *et al*,[2] Yasir *et al*,[4] Dzeufiet *et al*,[5] and Evans.[15] The antihypertensive effects of these extracts may be due to the presence of the above phytochemical components which are known for their vasorelaxant and cardioprotective activities.[5]
Hypertension also known as high blood pressure is a long-term medical condition in which the blood pressure in the arteries is persistently elevated.[21] Long term high blood pressure, however, is a major risk factor for coronary artery disease, stroke, cardiac failure, cardiac hypertrophy, peripheral vascular disease, vision loss, renal dysfunction and impaired endothelium-dependent relaxations as co-morbid diseases with BP.[22,23]
The antihypertensive studies were carried out on both n-Hexane and 50% hydro-ethanol extracts of *Persea americana* using an *in vivo* studies on rats. The systolic blood pressure(SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MABP), and heart rate (HR) of the $CdCl_2$ and NaCl-induced hypertensive rats on extracts at different concentrations (400, 200, 100 mg/kg) and standard drugs (enalapril, nifedipine, HCT and telmisartan) are presented in figures 1 and 2, respectively. Chronic consumption of high-salt diet (NaCl and $CdCl_2$) at a dose of 1 mg/kg for 6 weeks significantly increased systolic BP, diastolic BP and heart rate as compared with rats receiving distilled water (negative control group). The increase of blood pressure and heart rate induced by NaCl and $CdCl_2$ consumption was significantly reduced in groups treated with hydro-ethanol and n-hexane extracts at various concentrations. This result discloses that the extracts are effective as antihypertensive agent with a dose-dependent activity which is in agreement with the results obtained by Sofola *et al.* and Balogun *et al*.[17,18]
At 100 mg/kg, the systolic and diastolic blood pressure for n-Hexane and hydro-ethanol was 124.28/103.83mmHg and 122.72/104.78 mmHg with a pulse pressure of 20.45 BPM and 17.94 BPM respectively. At 200 mg/kg the systolic and diastolic blood pressure for n-Hexane and 50% ethanol was 125.01/112.54 and 119.92/110.69 with a pulse pressure of 12.47 and 9.23, respectively. At 400 mg/kg the SBP/DBP for n-Hexan e and hyro-ethanol was 117.66/98.8 and 135.88/121.94 mmHg with a pulse pressure of 18.86 and 13.94 mmHg, respectively, which when compared to the mean average of

Table 1: Phytochemical constituents of n-Hexane and Hydro-ethanol fruit peel extracts of *P. americana*

Phytochemical	Inference	
	n-Hexane	Hydro-Ethanol
Alkaloids	+	+
Flavonoids	+	+
Free Anthraquinones	-	-
Combined Anthraquinones	-	-
Saponins	+	+
Steroids	+	+
Tannins	+	+
Reducing sugars	+	-

+ = Present; - = Absent

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the result obtained after three weeks of salt-diet (SBP/DBP = 143.35/126.66 mmHg with a pulse pressure of 16.64 mmHg and heart rate of 420 bpm) showed a remarkable reduction in the blood pressure. The standard drug Nifedipine gave a result of 105.17/87.72 mmHg with a pulse pressure of 17.45 mmHg. Hydrochlorothiazide gave 138.28/126.81 mmHg with 11.47 mmHg as pulse pressure, Telmisartan gave 174.94/141.29 mmHg with a pulse pressure of 33.65 mmHg and Enalapril gave 128.99/115.08 mmHg with a pulse pressure of 19.33 mmHg.

The untreated rat gave 138.17/118.84 and 19.33 as pulse pressure. From this result, it can be deduced that n-Hexane extract led to a tremendous decrease in blood pressure compared to hydro-ethanol extract.

The results obtained from the highest concentration (400 mg/kg) of the extracts showed similar result with that of standard nifedipine which means that the extract may be acting through a similar mechanism as nifedipine by blocking calcium channel leading to vasodilatation, preservation of depressor mechanism and attenuation of reflex response.[24,25] Most standard drugs used in this study showed a rapid decrease in blood pressure although the most effective was Nifedipine.

The results obtained from cadmium chloride-induced hypertensive rats also showed similar antihypertensive activity with the sodium chloride as discussed above (Figure 2). At a dose of 100 mg/kg the systolic and diastolic blood pressure for n-Hexane and 50% hydro-ethanol was 131.16/120.78 and 125.37/105.73 with a pulse pressure of 10.60 and 19.65, respectively. At 200 mg/kg the systolic and diastolic blood pressure for n-Hexane and 50% ethanol is 126.04/117.77 and 124.77/111.12 with a pulse pressure of 8.27 and 13.65, respectively. At 400 mg/kg the systolic and diastolic blood pressure for n-Hexane and 50% hydro-ethanol extracts were 120.26/108.2 and 113.18/107.83 with pulse pressure of 11.83 and 5.35, respectively, as compared to results obtained from cadmium chloride induction which was 146.55/120.6 with pulse pressure of 21.25.

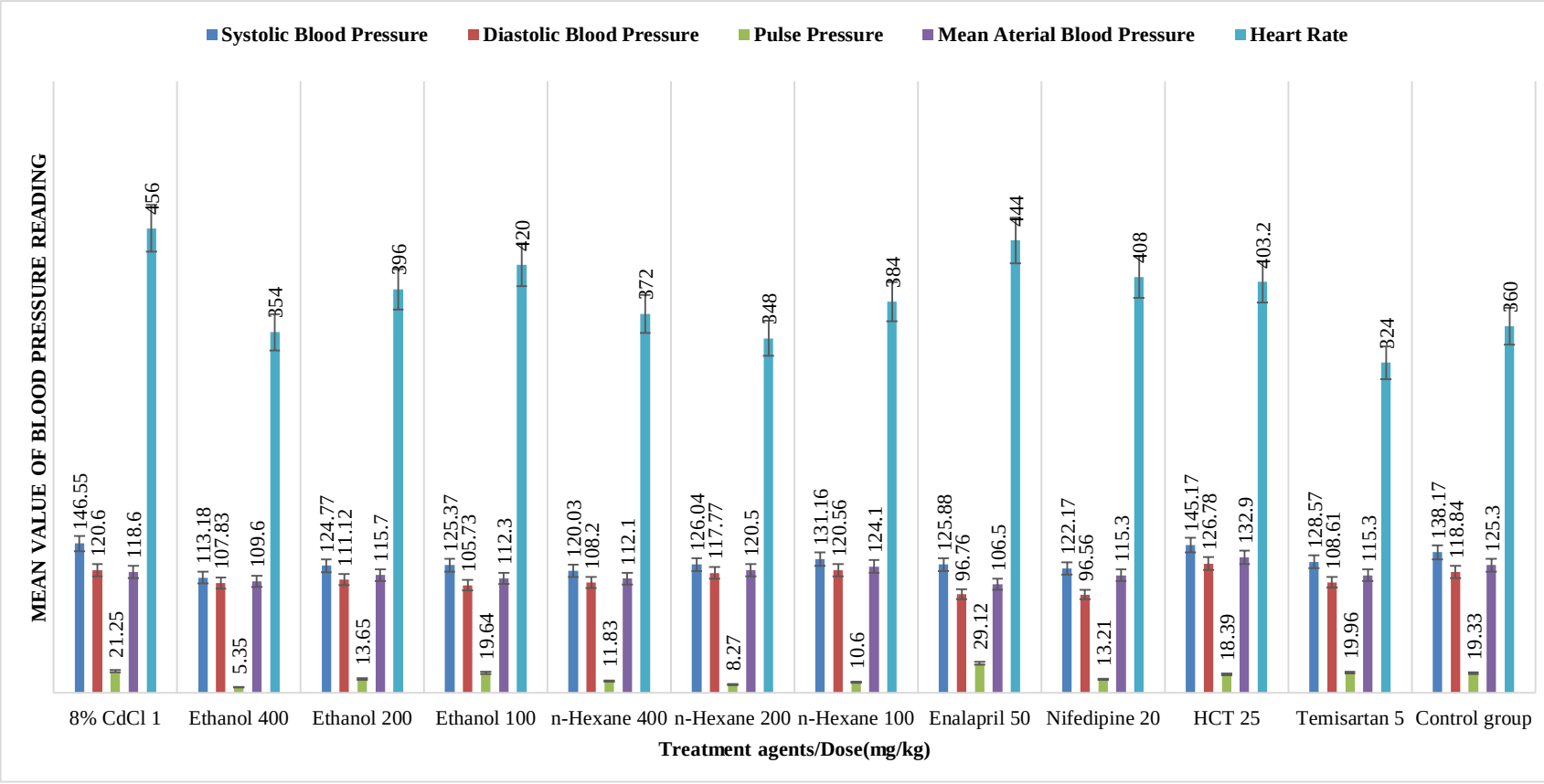

Figure 1: A chart showing systolic blood pressure, diastolic blood pressure, Pulse pressure, Mean arterial blood pressure and Heart rate of the antihypertensive evaluation of the n-Hexane and 50% hydro-ethanolic extract of *Persea americana* in comparism with the standard drugs on cadmium chloride-induced hypertensive rats.

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The standard drug Nifedipine gave results of 122.17/96.56 mmHg with pulse pressure of 13.21 mmHg. Hydrochlorothiazide gave 145.17/126.78 mmHg with 18.38 mmHg as pulse pressure, Telmisartan gave 128.57/108.61 mmHg; pulse pressure 19.96 mmHg and Enalapril gave 125.88/96.76 mmHg with a pulse pressure of 29.12 mmHg. The untreated rats gave 138.17/118.84 mmHg and 19.33 mmHg as pulse pressure. Nifedipine still showed the most effective antihypertensive activity in cadmium chloride induced hypertensive rats. Comparing the results obtained from n-Hexane and 50% hydro-ethanol extracts of *P. americana* fruit peels with the standard drugs, from high-salt induced hypertensive rats, both showed a significant decrease in blood pressure. The extracts have also shown similar mechanisms of action with enalapril (ACE inhibitors, diuretic),[26] HCT (diuretic, inhibits the NaCl cotransporter system).[27] The antihypertensive effects of this plant were as a result of the presence of these identified phytochemicals present in the plant.[2,4,5,15]

Bioassay-guided isolation, purification and characterization of the active compound(s) is recommended for further investigation in order to ascertain lead compound that may be used for pre-clinical study.

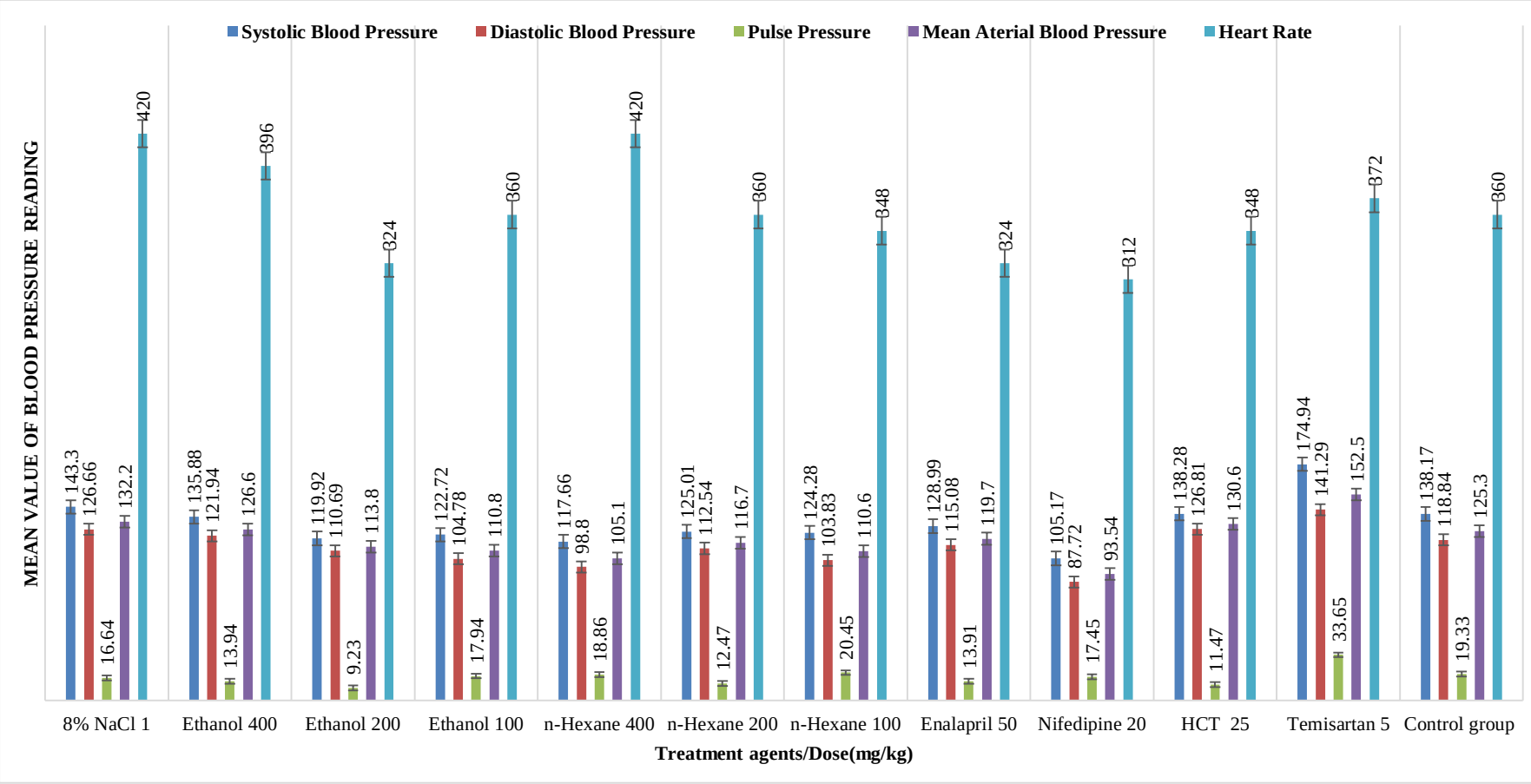

Figure 2: A chart showing systolic blood pressure, diastolic blood pressure, Pulse pressure, Mean arterial blood pressure and Heart rate of the antihypertensive evaluation of the n-Hexane and 50% hydro-ethanolic extracts of *Persea americana* in comparison with the standard drugs on sodium chloride-induced hypertensive rats.

Conclusion

The fruit peel extract of *P. americana* has shown a remarkable antihypertensive property in albino rats having similar results with standard antihypertensive drug (nifedipine). The result has suggested that the extract may have similar mechanism of action at highest concentration (400 mg/kg) as that of nifedipine (calcium channel blocker). The antihypertensive effect of the extracts can be attributed to the presence of phytochemicals identified in the plant.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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