



Therapeutic Effects of *Pterocarpus santalinoides* Stem Bark Aqueous Extract: Evidence from L-NAME-induced Hypertensive Wistar Rats

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Authors' contributions

The present study was carried out in collaboration among all authors. Author DT carried out experiments design, interpretation of results and correction of the final manuscript. Authors CYR, NLTE, AOBF collected materials, developed the research methodology, and wrote the first draft of the manuscript. Authors NTF, GKR and BNMT conducted biochemical assays, performed histological analyses, and carried out statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Ethnopharmacological information indicates that the stem bark of *Pterocarpus santalinoides* is used in traditional medicine to treat many diseases including hypertension. Thus, this study was designed to evaluate the therapeutic effects of the stem bark aqueous extract of *Pterocarpus santalinoides* (AEPS) in L-NAME-induced hypertensive rats (LNHR).

Methodology: Hypertension was induced in normotensive rats by intraperitoneal administration of L-NAME (25 mg/kg/day) for 42 days. Forty-two animals were divided into two main groups: one group of seven rats (group 1) receiving distilled water (10 mL/kg) and another thirty-five rats receiving L-NAME (group 2). After three weeks of treatment, the hypertensive animals (group 2) were divided into five groups of seven rats each. Animals of the first group received distilled water, those of the second group were treated with captopril (20 mg/kg), and the three last groups received the AEPS (50, 100, and 200 mg/kg). These rats were daily treated *per os* with the above substances for three weeks. At the end of the experimental period (42 days), animals were anesthetized and the blood pressure and heart rate were recorded by invasive method. Afterward, the blood, aorta, and heart of each rat were collected for some biochemical and/or histological examination.

Results: L-NAME administration induced hypertension, as associated with left ventricular hypertrophy, dyslipidemia, oxidative stress, hepatic and renal dysfunctions. Aqueous extract administration significantly improved all these metabolic disorders induced by L-NAME. Furthermore, the remodeling of the aortic media after NO deficiency-induced L-NAME has also been improved by *Pterocarpus santalinoides* aqueous extract. The therapeutic effect of AEPS against L-NAME-induced hypertension could probably be due to its antihypertensive, hypolipidemic, and antioxidant properties.

Conclusion: Current results confirmed the empirical use of *Pterocarpus santalinoides* stem bark for the treatment of hypertension in traditional medicine.

Keywords: Hypertension; L-NAME; *Pterocarpus santalinoides*; dyslipidemia; oxidative stress.

1. INTRODUCTION

Hypertension or elevated blood pressure, is a serious medical condition that is constantly on the increase throughout the world. It is a well-defined risk factor for many diseases such as coronary heart diseases, atherosclerosis, and stroke, in addition to kidney and cerebrovascular complications [1,2]. In 2019, it has been estimated that approximately 626 and 652 million of women and men live with this disease, respectively [3]. Also called an insidious “silent killer”, hypertension contributes to about 9.4 million deaths per year worldwide [4]. This disease's severe consequences can be controlled well if detected early and treated appropriately. Although there have been enormous advancements in hypertension research with improved antihypertensive drugs that should lower blood pressure in almost all patients, there is a more uncontrolled disease and the prevalence of hypertension is growing worldwide [5].

Since essential hypertension is part of many important health care problems, it is necessary to investigate its mechanisms in animal models.

The usefulness of animal models for improving the understanding of pathogenesis, prevention, and treatment of hypertension as well as its comorbidities depends on their validity for representing human forms of hypertension. Important unmet needs in this field include the development of models that mimic the discrete hypertensive syndromes that now populate the clinic, resolving ongoing controversies in the pathogenesis of hypertension, and developing new avenues for preventing and treating hypertension and its complications [6]. Based on the premise that deficient nitric oxide (NO) synthesis and its reduced bioavailability are important determinants of hypertension, previous studies defined a model of hypertension induced by long-term NO inhibition [7]. This experimental model was established to investigate not only the role of NO in vascular function and blood pressure regulation but also in the maintenance of homeostasis in the whole cardiovascular system [8]. In addition, the chronic inhibition of nitric oxide synthase (NOS) induces hypertension with concomitant myocardial hypertrophy as a compensatory response in order to adapt the heart function facing a surcharge of pressure or volume [9].

Modern medicine today utilizes active compounds isolated from higher plants, and about 80% of these active ingredients indicate a positive correlation between their modern therapeutic use and traditional uses [10]. However, this modern medicine is very expensive and presents issues related to accessibility, side effects, and effectiveness [11]. Thus, about 70–95% of people in developing countries still largely rely on medicinal plants for their basic health needs [12]. This is not only because of poverty where people cannot afford to buy expensive modern drugs, but traditional medicine has been the trusted, culturally acceptable, affordable and accessible source of health care for African populations for centuries [13,14]. It has been observed that herbal products and alternative herbal therapies play a significant role in decreasing high blood pressure [15,16]. *Pterocarpus santalinoides* which is used in the current study is commonly used in Cameroonian traditional medicine for the treatment of cardiovascular diseases, including hypertension. Previous investigations revealed that the presence of some bioactive compounds such as flavonoids, tannins, saponins, steroids, alkaloids and triterpenoids could be responsible for the vasorelaxant and hypotensive activities of *Pterocarpus santalinoides* stem bark aqueous extract” [17]. Moreover, it has been showed that the same extract of *P. santalinoides* prevented L-NAME induced hypertension in rats by improving endothelial function and antioxidative status [18]. Therefore, the present study was carried out to investigate the therapeutic effects of *Pterocarpus santalinoides* aqueous extract in L-NAME hypertensive Wistar rats (LNHR).

2. MATERIALS AND METHODS

2.1 Chemicals

N⁶ -nitro-L-arginine methyl ester (L-NAME) was supplied from Sigma Aldrich chemical co (St. Louis, MO, USA), heparin choay from Sanofi Aventis (France) and captopril was obtained from Sandoz (Holzkirchen, Germany).

2.2 Preparation of Plant Extract

Fresh stem barks of *Pterocarpus santalinoides* (Fabaceae) were collected at Eseka (Centre Region, Cameroon) in June 2018. The plant was identified at the National Herbarium of Cameroon (NHC), in comparison with the existing voucher specimen registered under the number HNC/42209. Fresh stem barks were dried at room temperature and made into powder with a

motor-driven grinder. The powder sample (500 g) was introduced into 5 L of distilled water and boiled for 30 minutes following the traditional healer’s instructions. The filtrate obtained was dried at 45°C in the drying cupboard and the crude extract powder gave 52.5 g with 10.5% of yield (w/w).

2.3 Animals

Male *Wistar* rats aged 10–12 weeks (180–200 g) were used in this study. These animals were housed in collective plexiglass cages and maintained in the animal house of the Department of Animal Biology and Physiology, Faculty of Science, University of Yaounde I, Cameroon. They were kept under standard laboratory conditions of light (light/dark cycles of 12/12 hours), with free access to normal commercial diet and tap water *ad libitum*. All experimental procedures were approved by the Cameroon National Ethical Committee (authorization number FW-IRB00001954). Investigations using experimental animals were conducted following the internationally accepted principles for laboratory animal use and care of the US guidelines (NIH publication #85-23, revised in 1985).

2.4 Experimental Design

Normotensive *Wistar* male rats (forty-two rats) were randomly divided into two groups: the first group (vehicle, seven rats) received distilled water (1 mL/100 g of body weight, per os), and the second group (thirty-five rats) was treated with L-NAME (25 mg/kg, intraperitoneally) during three weeks [19]. On the 22nd day, the rats of the second group were divided into five groups of 7 rats each. The first group (negative control) received L-NAME (25 mg/kg intraperitoneally) and distilled water (1 mL/100 g of body weight), while the second group (positive control) was treated with L-NAME (25 mg/kg, intraperitoneally) and captopril (20 mg/kg). In addition to L-NAME (25 mg/kg, intraperitoneally), the remaining groups received the plant extract at doses of 50, 100, or 200 mg/kg. The plant extract and captopril were dissolved in distilled water and given daily *per os* to the animals for three weeks.

2.5 Invasive Measurement of Blood Pressure and Heart Rate

At the end of the experimental period, systolic (SAP) and diastolic blood pressure (DAP) and

heart rate (HR) were recorded on anesthetized rats (1.5 g/kg intraperitoneal injection of ethyl carbamate) according to the protocol described by Bilanda et al. [20]. Trachea was exposed and intubated to facilitate spontaneous respiration. Briefly, a polyethylene catheter was inserted into the rat carotid artery and connected to a pressure transducer recording system (RX 104A, BIOPAC Systems Inc., California, USA) for BP and HR measurement after thirty minutes period of stabilization. The mean arterial blood pressure (MAP) was calculated using the following formula used by Bilanda et al. [21]:

$$\text{MAP} = (\text{SAP} + 2 \times \text{DAP}) / 3$$

2.6 Sampling and Biochemical Analysis

After hemodynamic parameters measurements, blood samples were collected on anesthetized rats, through cardiac puncture and were centrifuged at 3000 rpm for 10 min to obtain serum. The serum was collected for biochemical analysis of total cholesterol (Chol), triglycerides (TG), HDL-Cholesterol (HDL-Chol), albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, urea, uric acid, K⁺, and Na⁺ ions levels using commercial diagnostic kits (Fortress, UK indication). Total proteins were obtained using the method of Gornall et al. [22] and LDL-Cholesterol (LDL-Chol) level was determined following the method described by Bilanda et al. [21]. The rats' hearts, aorta, and left ventricle were dissected and weighed. The aorta and heart were homogenized in Mc Ewen physiological solution 20% (w/v). Catalase and superoxide dismutase activities (SOD) were determined respectively according to Sinha [23], and Misra and Fridovich [24] methods, whereas malondialdehyde (MDA), reduced glutathione (GSH) and nitrites levels were assayed using respectively the procedures of Wilbur et al. [25], Ellman et al. [26] and Green et al. [27].

2.7 Quantification of Abdominal Fat Depots and Estimation of Atherogenic and Coronary Risk Indices

After heart and aorta dissection, the abdominal fat of the rats was removed and weighed. The atherogenic index (AI) was calculated as follows formula described by Youmbissi et al. [28]:

$$\text{AI} = (\text{Total cholesterol} - \text{HDL-cholesterol}) / \text{HDL-cholesterol}$$

The coronary risk index (CRI) was calculated according to Jesús et al. [29]:

$$\text{CRI} = \text{Total cholesterol} / \text{HDL-cholesterol}$$

2.8 Histopathological Examination

Different parts of aorta were fixed in 10% formalin for 7 days and embedded in paraffin for microscopically examination under routine Laboratory procedure [8]. Duplicates paraffin sections of 4 µm were prepared and stained with haematoxylin and eosin (H & E) for histological examination.

2.9 Statistical Analysis

All results were expressed as mean ± S.E.M. Data were analysed using one-way analysis of variance (ANOVA) followed by the Tukey test. P value less than 0.05 was considered statistically significant. GraphPad Prism software (version 8.0.1) was used for all analysis.

3. RESULTS

3.1 Effect of *Pterocarpus santalinoides* on Blood Pressure and Heart Rate in L-NAME-induced Hypertensive Rats

The daily administration of L-NAME (25 mg/kg) for 42 days induced a significant increase in systolic blood pressure (SBP), mean blood pressure (MBP), diastolic blood pressure (DBP), and heart rate (HR) by 95.56%, 121.27%, 111.21%, and 19.85% respectively compared with the control rats (Table 1). Administration of *P. santalinoides* (50, 100, and 200 mg/kg) and captopril (a control antihypertensive drug) caused a significant decline of SBP, MBP, and DBP in comparison to those treated only with the L-NAME. The inhibition of the above hemodynamic markers was respectively by 36.85% (p < 0.01), 44.83% (p < 0.001), and 49.43% (p < 0.001) in the group treated with the plant aqueous extract (50 mg/kg) while it was by 42.26% (p < 0.001), 47.92% (p < 0.001) and 51.17% (p < 0.001) in captopril treated rats. Likewise, treatment with captopril or the plant extract (50, 100, and 200 mg/kg) induced a significant decrease in the HR by 16.73%, 14.86%, 13.36%, and 16.73%; respectively when compared to hypertensive rats.

Table 1. Effect of *Pterocarpus santalinoides* on systolic blood pressure, mean blood pressure, diastolic blood pressure and heart rate in L-NAME-induced hypertensive rats

	Control	L-NAME	L-NAME + C 20	L-NAME + E 50	L-NAME + E 100	L-NAME + E 200
SAP (mmHg)	94.27 ± 3.27	184.40 ± 3.30***	106.48 ± 1.59\$\$\$	116.45 ± 5.23\$\$	96.22 ± 3.79\$\$\$	120.83 ± 0.27\$\$
DAP (mmHg)	72.40 ± 3.77	160.20 ± 2.34***	78.22 ± 1.26\$\$\$	81.02 ± 1.43\$\$\$	70.73 ± 3.33\$\$\$	84.55 ± 3.88\$\$\$
MAP (mmHg)	68.69 ± 2.25	168.27 ± 2.55***	87.64 ± 0.81\$\$\$	92.83 ± 2.62\$\$\$	79.23 ± 3.31\$\$\$	96.64 ± 2.61\$\$\$
HR (BPM)	333.16 ± 6.01	399.28 ± 8.30**	332.50 ± 1.84\$	339.93 ± 1.96\$	320.09 ± 2.12\$	345.93 ± 6.85\$

Each value represents mean ± S.E.M. n = 7 rats per group. L-NAME + C 20: rats treated with L-NAME and captopril (20 mg/kg); L-NAME + E 50, E 100, and E 200: rats treated with L-NAME and aqueous extract of *Pterocarpus santalinoides* at the doses of 50, 100, and 200 mg/kg. SAP (Systolic arterial pressure), DAP (Diastolic arterial pressure), MAP (Mean arterial pressure), HR (Heart rate), BPM (Beat per minute). **p < 0.01, ***p < 0.001 compared to control group. \$p < 0.05, \$\$p < 0.01, \$\$\$p < 0.001 compared to L-NAME-induced hypertensive rats

Table 2. Effect of *Pterocarpus santalinoides* aqueous extract on dyslipidemia induced by L-NAME in rats

	Control	L-NAME	L-NAME + C 20	L-NAME + E 50	L-NAME + E 100	L-NAME + E 200
Chol (mg/dL)	125.10 ± 2.91	246.82 ± 6.30***	133.63 ± 3.73\$\$\$	146.90 ± 4.20\$\$	135.92 ± 0.94\$\$	139.11 ± 2.80\$\$
TG (mg/dL)	115.50 ± 2.70	173.33 ± 5.31***	115.30 ± 6.22\$\$	98.54 ± 8.31\$	104.10 ± 8.03\$	126.31 ± 7.60\$
HDL-Chol (mg/dL)	41.12 ± 1.83	23.74 ± 0.89**	36.70 ± 1.01\$	35.10 ± 4.22\$	31.22 ± 2.01\$	32.83 ± 1.91\$
LDL-Chol (mg/dL)	77.81 ± 2.30	245.72 ± 5.50***	90.11 ± 5.02\$\$\$	95.42 ± 2.13\$\$\$	99.40 ± 3.20\$\$\$	106.20 ± 5.33\$\$\$
VLDL-Chol (mg/dL)	23.10 ± 0.93	34.67 ± 2.67***	26.73 ± 1.81\$	29.38 ± 2.12\$	27.18 ± 1.68\$	27.82 ± 2.05\$

Each value represents mean ± S.E.M. n = 7 rats per group. L-NAME + C 20: rats treated with L-NAME and captopril (20 mg/kg); L-NAME + E 50, E 100, and E 200: rats treated with L-NAME and aqueous extract of *Pterocarpus santalinoides* at the doses of 50, 100, and 200 mg/kg. **p < 0.01, ***p < 0.001 compared to control group. \$p < 0.05, \$\$p < 0.01, \$\$\$p < 0.001 compared to L-NAME-induced hypertensive rats

Table 3. Effect of *Pterocarpus santalinoides* aqueous extract on some liver and kidney markers in hypertensive rats

	Control	L-NAME	L-NAME + C 20	L-NAME + E 50	L-NAME + E 100	L-NAME + E 200
Liver markers						
ALT (UI)	16.53 ± 0.14	97.80 ± 6.44***	19.11 ± 1.12\$\$\$	22.09 ± 0.89\$\$\$	21.80 ± 0.36\$\$\$	19.29 ± 1.05\$\$\$
AST (UI)	69.37 ± 4.06	145.81 ± 3.41***	72.27 ± 8.28\$\$\$	86.16 ± 0.82\$\$\$	83.47 ± 0.71\$\$\$	61.01 ± 6.31\$\$\$
Albumin (mg/dL)	35.56 ± 2.61	75.45 ± 2.33***	42.25 ± 3.31\$\$	40.73 ± 1.74\$\$	38.05 ± 3.89\$\$	41.40 ± 1.79\$\$
Proteins (mg/dL)	10.39 ± 0.16	15.49 ± 1.35***	10.28 ± 0.25\$	10.98 ± 0.24\$	10.94 ± 0.07\$	10.42 ± 0.35\$
Kidney markers						
Urea (mg/mL)	53.91 ± 4.88	143.04 ± 0.94***	44.78 ± 2.54\$\$\$	42.17 ± 0.53\$\$\$	46.09 ± 4.48\$\$\$	54.35 ± 4.01\$\$\$
Uric acid (mg/mL)	4.84 ± 0.52	11.62 ± 0.67***	4.53 ± 0.49\$\$\$	6.55 ± 0.57\$\$\$	3.46 ± 0.54\$\$\$	4.04 ± 0.69\$\$\$
Creatinine (mg/dL)	1.48 ± 0.14	3.01 ± 0.04***	1.84 ± 0.26\$\$	1.99 ± 0.22\$\$	1.58 ± 0.12\$\$	2.06 ± 0.12\$\$
Na ⁺ (mMol/L)	75.31 ± 4.57	238.17 ± 5.99***	87.06 ± 6.66\$\$\$	93.79 ± 5.59\$\$\$	88.99 ± 4.09\$\$\$	110.44 ± 4.82\$\$\$
K ⁺ (mMol/L)	2.63 ± 0.34	12.18 ± 0.38***	3.57 ± 0.29\$\$\$	4.89 ± 0.34\$\$\$	4.79 ± 0.29\$\$\$	5.64 ± 0.26\$\$\$

Each value represents mean ± S.E.M. n = 7 rats per group. L-NAME + C 20: rats treated with L-NAME and captopril (20 mg/kg); L-NAME + E 50, E 100, and E 200: rats treated with L-NAME and aqueous extract of *Pterocarpus santalinoides* at the doses of 50, 100, and 200 mg/kg. ***p < 0.001 compared to control group. \$p < 0.05, \$\$p < 0.01, \$\$\$p < 0.001 compared to L-NAME-induced hypertensive rats

3.2 Effect of *Pterocarpus santalinoides* on Relative Weight of Heart and Left Ventricle in L-NAME-induced Hypertensive Rats

The effects of plant extract on the relative weight of heart and left ventricle in L-NAME-induced hypertensive rats are summarized in Fig. 1. Following the treatment of 42 days, the relative weights of hearts and left ventricles in the negative control group were significantly increased ($p < 0.001$) by 75.71% and 103.13% respectively than those of vehicle control rats. However, the administration of the aqueous extract of *Pterocarpus santalinoides* (50, 100, and 200 mg/kg), and captopril significantly reduced the relative weights of hearts and left ventricles when compared to hypertensive rats. The decrease in these relative weights was respectively by 33.33% and 30.77% ($p < 0.01$) in group treated with the extract (50 mg/kg), by 44.72% and 52.31% ($p < 0.001$) in rats treated with the extract (100 mg/kg), by 36.59% and 35.38% ($p < 0.01$) in group treated with the extract (200 mg/kg), and by 40.65% and 46.15% ($p < 0.01$) in rats receiving captopril.

3.3 Effect of *Pterocarpus santalinoides* on Lipid Profile in L-NAME Hypertensive Rats

Table 2 summarizes the effects of *Pterocarpus santalinoides* aqueous extract on some parameters of lipid profile in hypertensive Rats. As compared to vehicle-treated rats, hypertensive rats treated with L-NAME for 6 weeks showed a significant increased ($p < 0.001$) in total cholesterol (Chol, 97.31%), triglycerides (TG, 50.01%), LDL-cholesterol (LDL-Chol, 215.79%), and VLDL-cholesterol (VLDL-Chol, 50.09%) levels as well as a significant decreased ($p < 0.01$) of HDL-cholesterol (HDL-Chol) by 42.30%. However, rats treated with *P. santalinoides* aqueous extract (50, 100, and 200 mg/kg) or captopril (20 mg/kg) exhibited significantly reduced levels of Chol, TG, LDL-Chol levels, and VLDL-Chol as well as a significant increase of HDL level when compared to negative control rats.

3.4 Effect of *Pterocarpus santalinoides* on Abdominal Fats, Atherogenic and Coronary Risk Index in L-NAME-induced Hypertensive Rats

The daily intraperitoneal administration of L-NAME for 42 days resulted in a significant

decrease in abdominal fats by 57.32% as well as in a significant increase in atherogenic and coronary risk index by 326.01% and 242.11% respectively when compared to control rats (Fig. 2). The administration of the plant extract (50, 100, and 200 mg/kg) and captopril (20 mg/kg) significantly prevented ($p < 0.001$) the rise of the above risk index. They decreased the atherogenic and coronary risk index by 63.79% and 59.81%, 65.16% and 58.17%, 65.16% and 59.23%, 72.32% and 65.00% respectively compared to the L-NAME group. Furthermore, captopril and the aqueous extract (50, 100, and 200 mg/kg) induced a significant increase of abdominal fats respectively by 114.29% and 71.43%, 129.71% and 90.29% when compared to L-NAME-induced hypertensive rats.

3.5 Effect of *Pterocarpus santalinoides* on Some Liver and Kidney Functions Markers in L-NAME-induced Hypertensive Rats

As shown in Table 3, the intraperitoneal administration of L-NAME induced a significant increase ($p < 0.001$) in serum albumin and total protein concentrations as well as in ALT (alanine aminotransferase) and AST (aspartate aminotransferase) activities in comparison to normal control rats. The increase in the above serum liver markers was by 112.18%, 49.10%, 491.65%, and 110.22% respectively. Treatment with the aqueous (50, 100, and 200 mg/kg), and captopril significantly improved the L-NAME-induced changes in liver markers. Thus, the extract (50 mg/kg) and captopril (20 mg/kg) significantly decreased ($p < 0.01$) the serum albumin and protein by 46.01% and 44.00% respectively as compared to hypertensive rats. Similarly, the above substances significantly decreased by 77.41% and 80.46% the ALT activities, and by 40.90% and 50.43% the AST activities compared to negative control rats.

The effect of AEPS on renal injuries induced by L-NAME was evaluated by the determination of the serum levels of urea, uric acid, creatinine, Na^+ , and K^+ ions. As presented in Table 3, hypertensive rats exhibited a significant increase ($p < 0.001$) in the serum of the above-mentioned markers respectively by 165.31%, 140.10%, 103.40%, 216.25%, and 363.12% as compared to control rats. However, the treatment of L-NAME-induced hypertensive rats with the aqueous extract of *P. santalinoides* (50, 100, and 200 mg/kg), and captopril (20 mg/kg) significantly restored renal function by decreasing the serum

levels of all markers of kidney function evaluated in the present study. Thus, the decrease in serum urea level ($p < 0.001$) was respectively by 70.53%, 67.80%, 62.01%, and 68.70% while that of uric acid ($p < 0.001$) by 43.62%, 70.21%,

65.20%, and 61.02%. In the same context, the extract (50, 100, and 200 mg/kg), and captopril (20 mg/kg) significantly declined serum creatinine ($p < 0.01$), Na^+ ($p < 0.001$), and K^+ ions ($p < 0.001$) compared to L-NAME group.

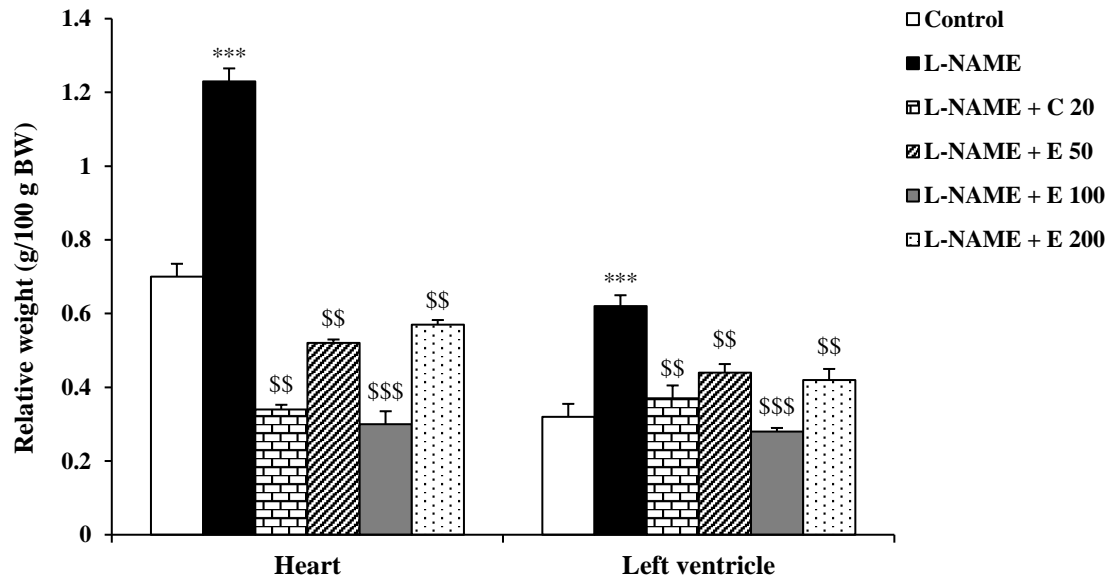


Fig. 1. Effect of *Pterocarpus santalinoides* aqueous extract on heart and left ventricle relative weight in L-NAME-induced hypertensive rats

Each bar represents mean \pm S.E.M. $n = 7$ rats per group. L-NAME + C 20: rats treated with L-NAME and captopril (20 mg/kg); L-NAME + E 50, E 100 and E 200: rats treated with L-NAME and aqueous extract of *Pterocarpus santalinoides* at the doses of 50, 100 and 200 mg/kg. *** $p < 0.001$ compared to control group. \$\$ $p < 0.01$, \$\$\$ $p < 0.001$ compared to L-NAME-induced hypertensive rats. BW: body weight

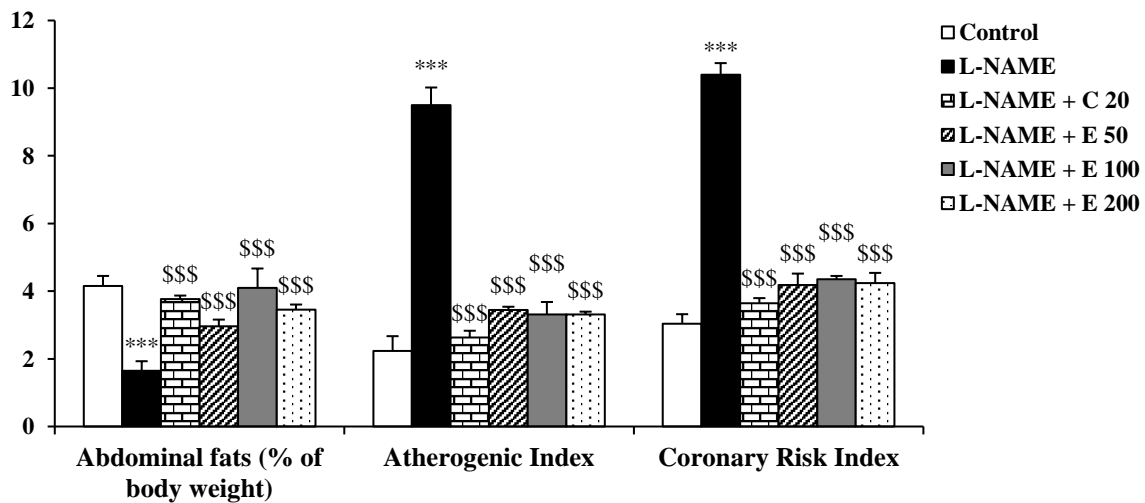


Fig. 2. Effect of *Pterocarpus santalinoides* aqueous extract on abdominal fats, atherogenic and coronary risk index in L-NAME-induced hypertensive rats

Each bar represents mean \pm S.E.M. $n = 7$ rats per group. L-NAME + C 20: rats treated with L-NAME and captopril (20 mg/kg); L-NAME + E 50, E 100, and E 200: rats treated with L-NAME and aqueous extract of *Pterocarpus santalinoides* at the doses of 50, 100, and 200 mg/kg. *** $p < 0.001$ compared to control group. \$\$\$ $p < 0.001$ compared to L-NAME-induced hypertensive rats

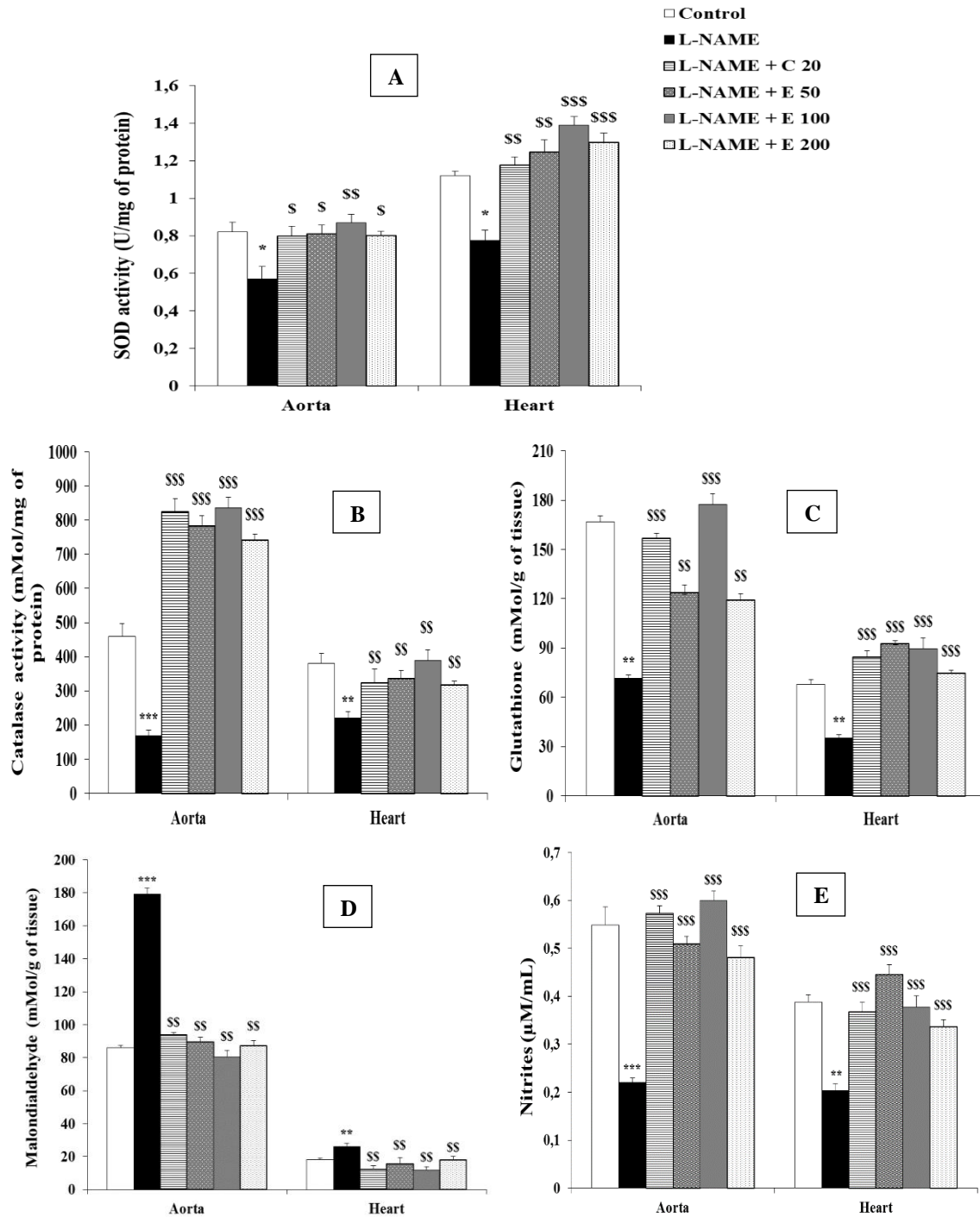


Fig. 3. Effect of *P. santalinoides* aqueous extract on SOD activity (A), catalase activity (B), glutathione level (C), malondialdehyde level (D) and nitrites level (E) in L-NAME-induced hypertensive rats

Each bar represents mean \pm S.E.M. $n = 7$ rats per group. L-NAME + C 20: rats treated with L-NAME and captopril (20 mg/kg); L-NAME + E 50, E 100, and E 200: rats treated with L-NAME and aqueous extract of *Pterocarpus santalinoides* at the doses of 50, 100, and 200 mg/kg. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared to control group. \$ $p < 0.05$, \$\$ $p < 0.01$, \$\$\$ $p < 0.001$ compared to L-NAME-induced hypertensive rats

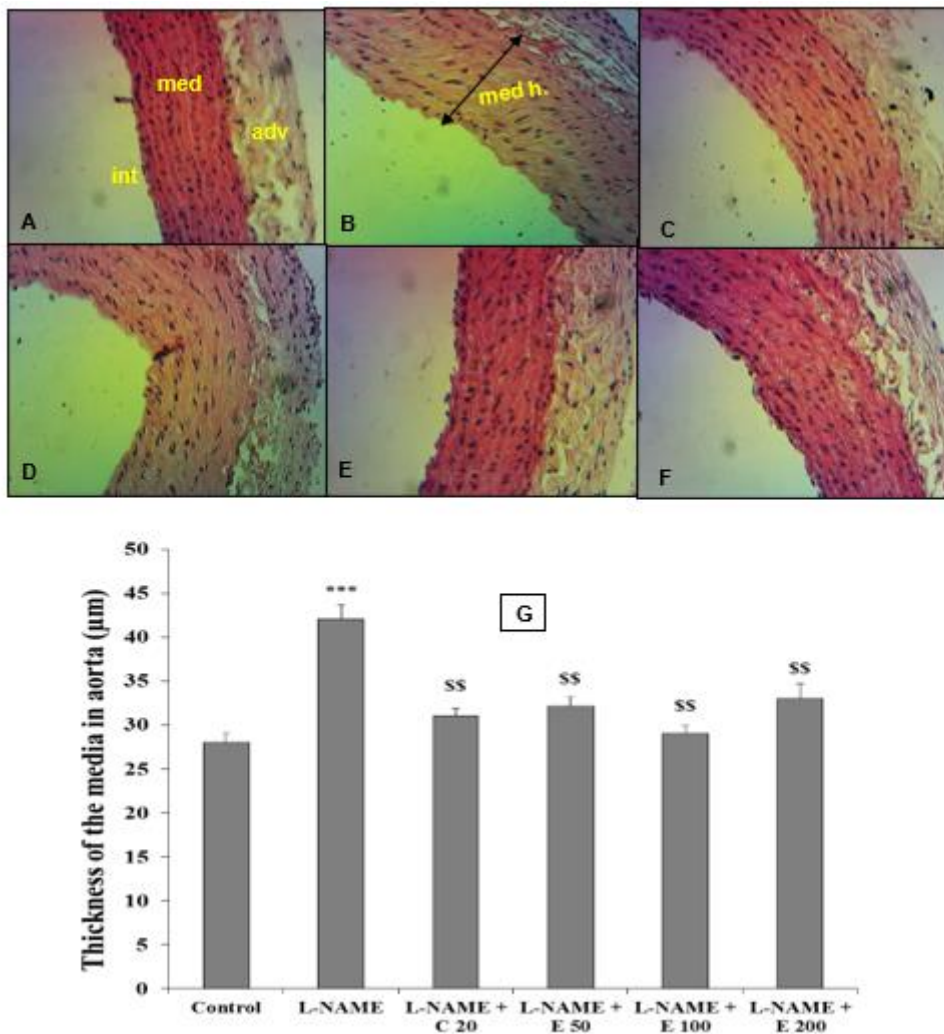


Fig. 4. Photomicrograph showing histopathological changes in the aorta in different groups stained with H & E x 400 (A, B, C, D, E, and F) and media thickness (G)

Each bar represents mean \pm S.E.M. $9 \leq n \leq 15$ per group. A: rats treated with distilled water (10 mL/kg); B: rats treated with N^G -nitro-L-arginine methyl ester (25 mg/kg); C: rats treated with L-NAME and captopril (20 mg/kg); D, E and F: rats treated with L-NAME and aqueous extract of *P. santalinoides* at the doses of 50, 100, and 200 mg/kg. *** $p < 0.001$ compared to control group. \$\$ $p < 0.01$ compared to L-NAME-induced hypertensive rats adv: adventitia; int: intima; med: media; med h.: media hypertrophy

3.6 Effect of *Pterocarpus santalinoides* Aqueous Extract on Oxidative Stress Markers in L-NAME-induced Hypertensive Rats

As shown in Fig. 3A, the activities of superoxide dismutase (SOD) and catalase as well as the reduced glutathione (GSH) level were significantly decreased in aorta and heart of hypertensive rats compared to normal control group. The decrease of the above oxidative stress markers was respectively by 30.50% ($p < 0.05$), 64.44% ($p < 0.001$), and 57.10% ($p < 0.001$) in aorta, and by 30.65% ($p < 0.01$),

42.20% ($p < 0.01$), and 47.89% ($p < 0.001$) in the heart. Treatment of animals with the plant extract or captopril significantly increase the SOD and catalase activities as well as the GSH concentration in aorta and heart in comparison to L-NAME untreated group. The increase activity of SOD in the aorta and heart of animal treated with the aqueous extract was respectively by 42.11% ($p < 0.05$), and 60.42% ($p < 0.01$) at the dose of 50 mg/kg, by 52.63% ($p < 0.01$), and 79.02% ($p < 0.001$) at the dose of 100 mg/kg, and by 40.44% ($p < 0.05$), and 67.31% ($p < 0.001$) at the dose of 200 mg/kg. The increase of catalase activity in both organs induced by the

administration of the aqueous extract was respectively by 365.21% ($p < 0.001$) and 57.80% ($p < 0.01$) at 50 mg/kg, 396.10% ($p < 0.001$) and 79.72% ($p < 0.01$) at 100 mg/kg, by 341.03% ($p < 0.001$), and 45.01% ($p < 0.01$) at 200 mg/kg. Moreover, the plant extract significantly decreased the GSH concentration respectively by 72.81% ($p < 0.01$), 147.92% ($p < 0.001$), and 139.34% ($p < 0.01$) in the aorta and by 162.54%, 152.50%, and 120.71% in the heart ($p < 0.001$) as compared to L-NAME untreated rats.

Daily administration of L-NAME during 6 weeks significantly increased the amount of malondialdehyde (MDA) by 102.30% ($p < 0.001$) in aorta and by 50.04% ($p < 0.01$) in heart as compared to control rats (Fig. 3D). The aqueous extract of *P. santalinoides* (50, 100, and 200 mg/kg) or captopril induced a significant decrease ($p < 0.01$) of MDA in aorta and heart respectively by 50.01% and 39.50%, 55.11% and 54.18%, 51.23% and 31.09%, 47.56% and 52.44% comparatively to L-NAME group.

The results also showed that the administration of L-NAME leads to a significant decrease of nitrites level in aorta and heart respectively by 59.90% ($p < 0.001$), and 47.74% ($p < 0.01$) as compared to control rats (Fig. 3E). In comparison with hypertensive rats, the treatment with plant extract at the doses of 50, 100, and 200 mg/kg increased significantly ($p < 0.001$) nitrites level respectively by 131.80%, 172.70%, and 118.58% in aorta, and by 119.72%, 85.71%, and 66.01% in heart. The administration of captopril (20 mg/kg) reduced significantly ($p < 0.001$) nitrites level by 160.89% in aorta, and by 81.30% in heart as compared to L-NAME-induced hypertensive rats.

3.7 Effect of *P. santalinoides* Aqueous Extract on L-NAME-induced Aortic Remodelling

Fig. 4 shows the effects of *P. santalinoides* on microarchitecture (A, B, C, D, E, and F) and histomorphometry (G) of the aorta. Treatment with L-NAME induced a significant increase ($p < 0.001$) of thickness of the media by 39.91% (Fig. 4B) as compared to control (Fig. 4A). The treatment of L-NAME-induced hypertensive rats with aqueous extract of *Pterocarpus santalinoides* at the different doses of 50, 100 and 200 mg/kg (Fig. 4D, E, F) or captopril (Fig. 4C) significantly inhibited ($p < 0.01$) this rising thickness respectively by 18.72%, 27.54, 17.20% and by 26.03%.

4. DISCUSSION

The present study was investigated to evaluate the therapeutic effects of an aqueous extract of *Pterocarpus santalinoides* (AEPS) in L-NAME-induced hypertensive rats (LNHR). It has been demonstrated that the inhibition or chronic deficit of nitric oxide (NO) in the body is well known to induce severe diseases such as arterial hypertension [30]. Experimental arterial hypertension induced by L-NAME is marked by a reduction of NO/GMPc activity, activation of renin angiotensin aldosterone system (RAAS), and increase of sympathetic activity leading to an increase in vascular peripheral resistance resulting in a rise in blood pressure [30]. In this study, the chronic administration of L-NAME during 42 days to normotensive rats induced a significant increase in arterial blood pressure. Similar results have already been obtained by Fouada et al. and Metchi et al. [8,9]. AEPS administered at the doses of 50, 100, and 200 mg/kg decreased significantly the high blood pressure observed in LNHR, suggesting that this extract might possess phytochemicals that could interfere with mechanisms implicated in L-NAME-induced hypertension. Indeed, phytochemical analysis of this extract revealed the presence of some bioactive compounds such as alkaloids, saponins, triterpenoids and phenols which are well known for their vasorelaxant properties [8]. Thus, the potent antihypertensive effect of this plant extract could be due to its vasorelaxant activity through the increase in NO release after the subchronic administration. These findings are in accordance with our previous studies which showed that the aqueous extract of *Pterocarpus santalinoides* administrated simultaneously prevented L-NAME-induced arterial hypertension in rats [31].

Arterial hypertension is a main risk factor associated with endothelial dysfunction, cardiac alterations, and alteration of hepatic and renal functions [32,33]. Ventricular hypertrophy is a myocardial adaptation mechanism in response to chronic overloading in pressure or volume, leading to the overproduction of angiotensin II [34]. Our results showed that treatment with AEPS at different doses significantly inhibited the left ventricular hypertrophy induced by L-NAME. This inhibition suggests that AEPS could possess cardioprotective activities which might be related to its ability to reduce blood pressure consecutive to the rise in NO [9]. Arterial hypertension is generally associated to dyslipidemia [35]. In this study, administration of

L-NAME induced an increase in blood total cholesterol, triglycerides, LDL-cholesterol, VLDL-cholesterol, and a decrease in HDL-cholesterol. These lipid metabolic disorders were reversed after treatment with AEPS. It has been demonstrated that the blockade of NO synthase by L-NAME could lead to an alteration of lipids metabolism [36]. Nitric oxide plays an important role in the regulation of lipid metabolism. These observations suggest that AEPS would have hypocholesterolemic and hypotriglyceridemic properties, and could also induce an inhibition of 3-hydroxyl-3-methylglutanyl Coenzyme A (HMG-CoA) reductase activity leading to a reduction of the hepatic synthesis and intestinal absorption of cholesterol [37].

It has been demonstrated that hypertension plays an important role in hepatic and renal dysfunctions [38]. The chronic inhibition of nitric oxide induces microvascular changes which lead to perturbations in hepatic and renal perfusion resulting in severe damage in the organs. The nonsufficient perfusion of the liver, which can be observed in several models of hypertension, is marked by an increase in hepatic enzyme activities. The administration of AEPS corrected the increase of albumin levels as well as ASAT and ALAT activities observed in LNHR. These results indicate the protective role of the aqueous extract against liver damage induced by L-NAME. The kidneys play an important role in the balance of water and electrolytes in the body [34]. In this study, AEPS and captopril reduced significantly the rise of urea, uric acid, creatinine, and electrolyte levels observed in L-NAME-induced hypertensive rats. These results suggest that extract can improve glomerular filtration in rats and might interfere with the generation of free radicals and the inhibition of Na⁺-K⁺ ATPase pump. It has been reported that glycosides and phenols present in the extract stimulate the synthesis of the genes responsible for cellular regeneration of renal tissue [39]. This action could contribute to the AEPS effect on maintaining ion balance.

Several studies have shown that hypertension is generally linked to oxidative stress [40]. "As observed in this study, L-NAME induced oxidative status imbalance marked by a rise in malondialdehyde level (MDA), a decrease in NO and reduced glutathione levels, and SOD and catalase activities in aorta and heart. The aqueous extract of *Pterocarpus santalinoides* (50, 100, and 200 mg/kg) reversed all these modifications caused by reactive oxygen species

in hypertensive rats. The decrease of NO in hypertensive rats could be due to a reduction of its biodisponibility by increasing superoxide anion which reacts with NO and forms peroxynitrite [41]. The treatment of L-NAME-induced hypertensive rats with AEPS induced an increase in SOD and catalase activities; these enzymes reduce the level of superoxide anion by converting it into hydrogen peroxide which could be reduced in water [42]. The improvement of antioxidant systems observed after treatment of hypertensive rats with AEPS could be responsible for the decrease in ROS and then the decrease in MDA and glutathione was observed.

Histopathological examination showed arterial wall thickening, due to NO deficiency [43]. Treatment with the plant extract alleviated oxidative stress and subsequently helped to manage hypertension-induced vascular remodeling. *P. santalinoides*, with its antihypertensive and antioxidant effects could inhibit lipids peroxidation, oxidative stress and enhance the bioavailability of NO.

5. CONCLUSION

The present study showed that aqueous extract of *Pterocarpus santalinoides* (AEPS) stem bark can cure L-NAME-induced hypertension in rats. Our results have shown that this medicinal plant could have the potential to lower arterial blood pressure, improve lipid profile, restore hepatic and renal functions as well as antioxidant status and aortic remodeling in L-NAME induced hypertension model in rats. These results which confirmed the empirical use of *Pterocarpus santalinoides* for the management of hypertension, suggest that this medicinal plant should be considered as a new therapeutic tool in the treatment of this silent killer associated with NO deficiency.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image

generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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