Original Research Article

Therapeutic effects of Pterocarpus santalinoides stem barks aqueous extract: Evidence from L-NAME-induced hypertensive Wistar Rats

ABSTRACT

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

Methodology: Hypertension was induced in rats by intraperitonealadministration of L-NAME (25 mg/kg/day) for 42 days. Forty-twoanimals were divided into two main groups: one group of seven rats (group 1) receivingdistilled 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 three lastgroups 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, animals were anesthetised and the blood pressure and heart rate were recorded by invasivemethod. Afterwards, the blood and the heart of each were collected for some biochemical and/or histological examination.

Results:L-NAME administration inducedhypertension; as associated with left ventricular hypertrophy,dyslipidemia, oxidative stress, hepatic and renal dysfunctions. The administration of the aqueous extract significantly improved all these metabolic disorders induced by L-NAME. Furthermore, the remodelling of the aortic media subsequent to NO deficiency induced L-NAME has also beenimproved by *Pterocarpus santalinoides* aqueous extract.The therapeutic effect of AEPSagainst L-NAME-induced hypertension couldprobablydue to its antihypertensive, hypolipidemic, and antioxidant properties.

Conclusion: Current results confirmed 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 which 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” [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 toabout 9.4 million deaths per year worldwide [4].“This disease, whose consequences can be severe, 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 usefulnessofanimal 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, the resolution of ongoing controversies in the pathogenesis of hypertension, and the development of new avenues for preventing and treating hypertension and its complications[6].On the basis of 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 wasestablished 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 the traditional uses"[10]. However, this modern medicine is very expensive and presents issues related to the accessibility, side effects and effectiveness [11]. Thus, about 70–95% 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 played a significant role in decreasing high blood pressure[15, 16]. "*Pterocarpus santalinoides*which is used in the current study;is commonly used in Camerooniantraditional medicine for the treatment of cardiovasculardiseases 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 wascarried out to investigate the therapeutic effectsof *Pterocarpus santalinoides* aqueous extract in L-NAMEhypertensive Wistar rats (LNHR).

2. MATERIAL AND METHODS

2.1 Chemicals

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

2.2 Preparation of Plant Extract

Fresh stem barks of *Pterocarpus santalinoides*(Fabaceae)were collectedat Eseka (Centre Region, Cameroon) in June 2018. The plant was identified at the National Herbarium of Cameroon (NHC), in comparison with theexisting voucher's specimen registered under the number HNC/42209. Fresh stembarks were dried at room temperature and made into powder with a motor-driven grinder. Thepowder sample (500 g) was introduced into 5 L of distilled water and boiled during30 minutes following traditional healer's instructions. The filtrate obtained was dried at 45°C in drying-cupboard and the crude extractpowder 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 plexiglasscages and maintained in the animal house of the Department of Animal Biology and Physiology, Faculty of Science, University ofYaounde 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 six 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*al*. 2017 *et*[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*. 2019 [21]:

MAP= (SAP +2 x 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.* 2019[21]. The rats' hearts, aorta, and left ventricle were dissected and weighed. The aorta and heartwere homogenized in Mc Even physiological solution 20 % (w/v). Catalase and superoxide dismutase activities (SOD) were determined respectively according to Sinha [23], and Misra and Fridovish [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 weighted. The atherogenic index (AI) was calculated as follows formula described by Youmbissi*et al.* [28]:

AI= (Total cholesterol - HDL-cholesterol) / HDL-cholesterol

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

CRI= Total cholesterol / 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 significantly increased of systolic blood pressure (SBP), mean blood pressure (MBP), diastolic blood pressure (DBP), and heart rate (HR) by 95.56 %, 121.27 %, 111.21 %, and19.85 %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 significantdecline of SBP, MBP and DBP in comparison to treated only with L-NAME group. The inhibition of the above hemodynamic markers was respectively by 36.85 % ($p < 0.01$), by 44.83 % ($p < 0.001$) and by 49.43 % ($p < 0.001$) in 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/kginduced a significant decrease in the HR by 16.73 %, 14.86 %, 13.36 %, and 16.73 %; respectivelywhen 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.

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 Figure 1. Following the treatment of 42days, the relative weights of hearts and left ventricles in negative control groupwere significantly increased ($p < 0.001$) by 75.71 % and 103.13 % respectively than those ofvehicle control rats. However, the administration of the aqueous extract of *Pterocarpus santalinoides*(50, 100, and 200 mg/kg), andcaptopril significantly reduced the relative weights of hearts and left ventricles when comparedtohypertensive 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.

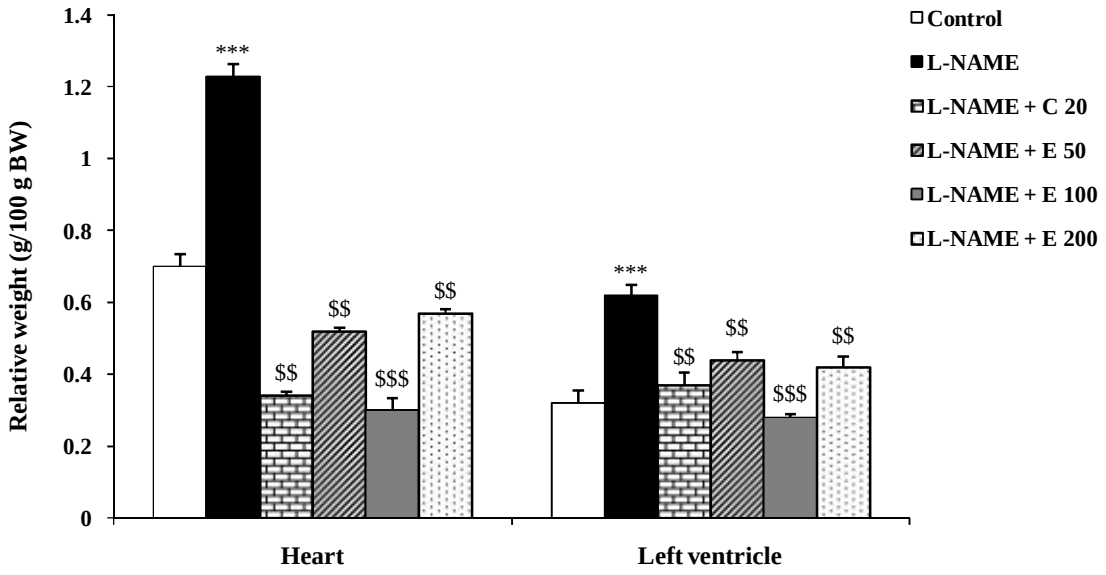


Figure 1: Effect of *Pterocarpus santalinoides*aqueous extract on heart and left ventricle relative weight in L-NAME-induced hypertensive rats

Each bar 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. $$p < 0.01, $$$p < 0.001 compared to L-NAME-induced hypertensive rats.

BW: body weight

3.3 Effect of *Pterocarpus santalinoides* on lipid profile inL-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 negativecontrol 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.

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

The daily intraperitonealadministration of L-NAME for 42 days resulted in a significant decrease in abdominal fats by 57.32 % as well as in a significant increasein atherogenic and coronary risk index by 326.01 % and 242.11 % respectively when compared to control rats (Figure 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 decreasedthe 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-NAMEgroup. 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.

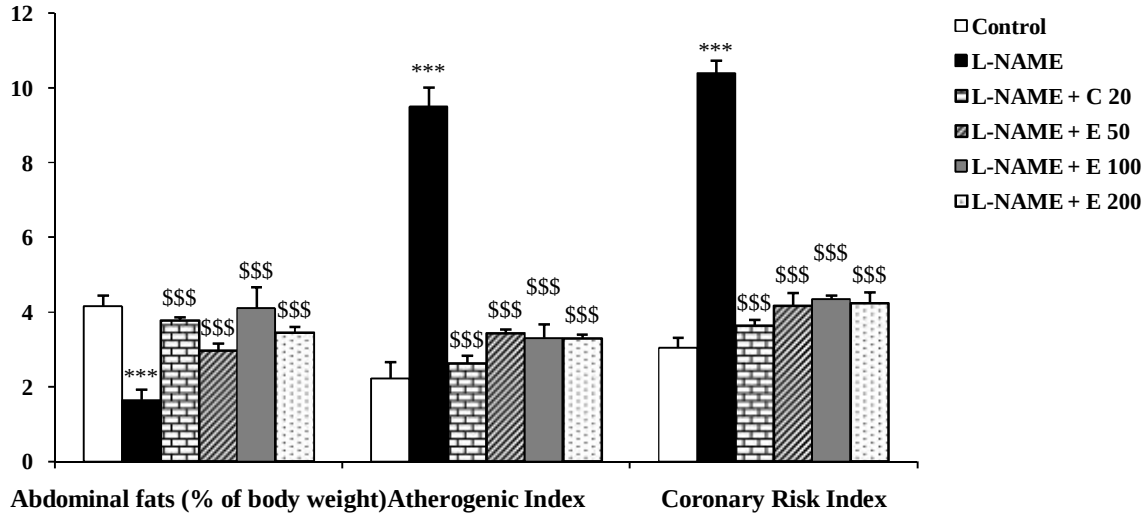


Figure 2: Effect of *Pterocarpus santalinoides*aqueous extract on abdominal fats, atherogenic and coronary risk index in L-NAME-induced hypertensive rats

Each bar represents mean ± 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.

3.5 Effect of *Pterocarpus santalinoides* onsome liverand kidney functionsmarkers inL-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 wasby 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.

Table 3: Effect of *Pterocarpus santalinoides*aqueous extract on some liver and kidneymarkers inhypertensive 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.

3.6 Effect of *Pterocarpus santalinoides*Aqueous Extract on oxidative stress markers in L-NAME-induced Hypertensive Rats

As shown in Figure 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 aorta and by 162.54 %, 152.50 %, and 120.71 % in 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 (Figure 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 (Figure 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.

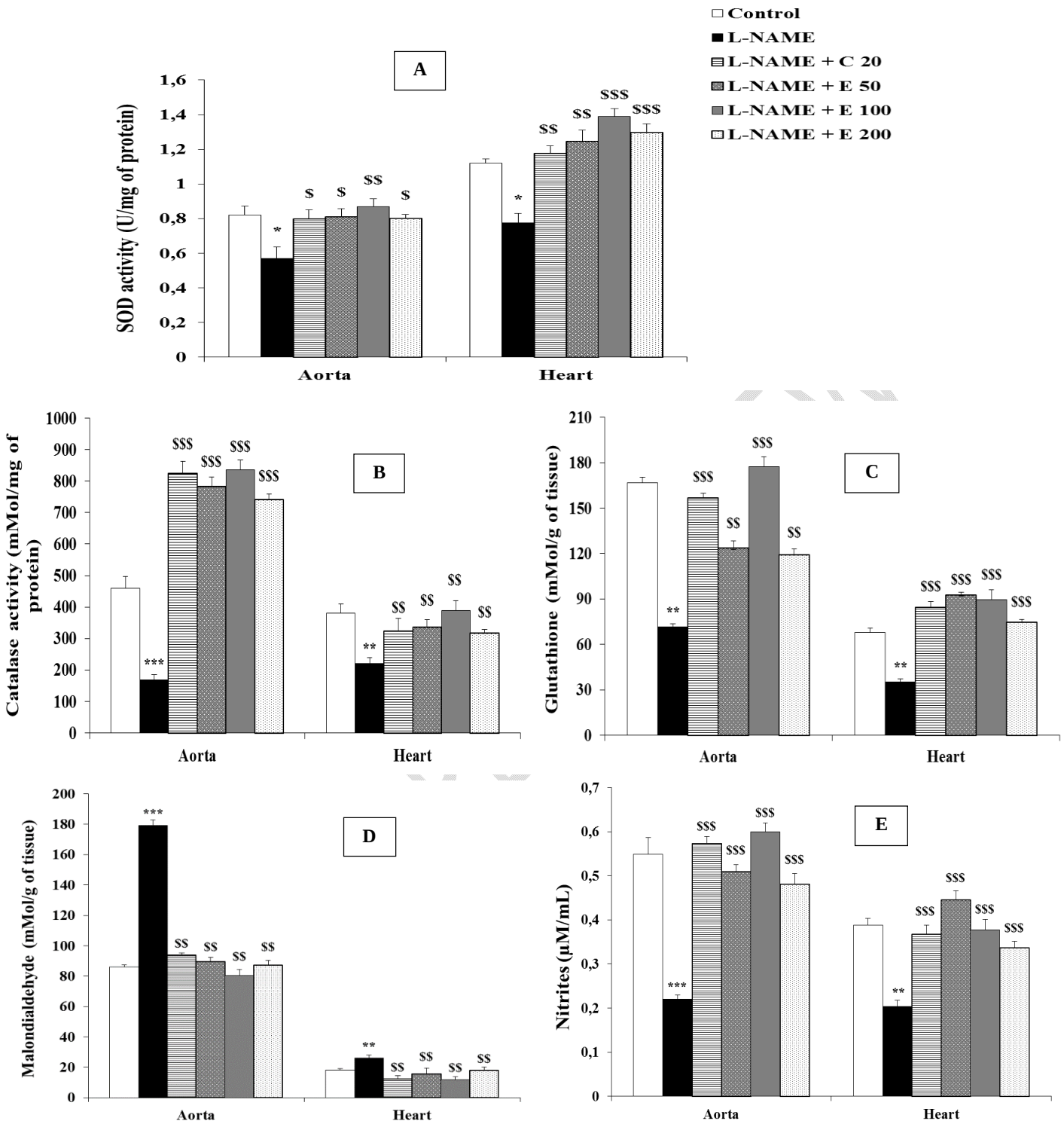
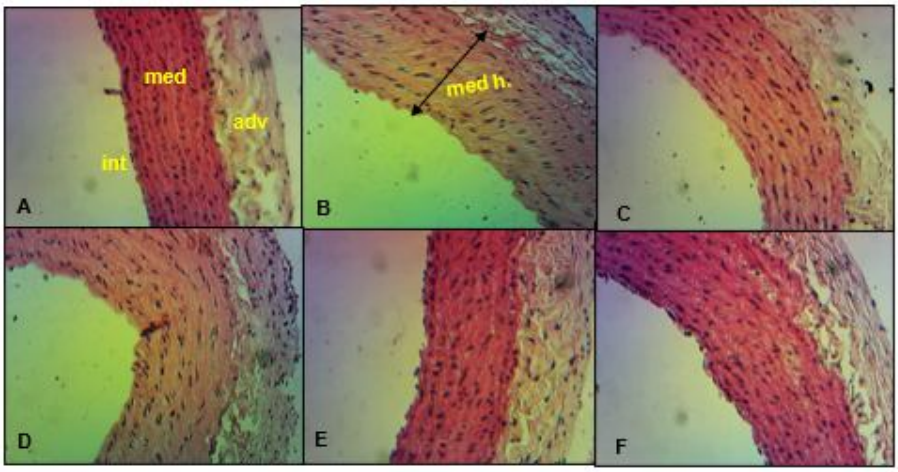


Figure 3: Effect of *P. santalinoides*aqueous extract on SOD activity (A), catalase activity (B), glutathione level (C), malondialdehyde level (D) and nitrites level (E)in L-NAME-induced hypertensive rats

Each bar represents mean ± 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

3.7 Effect of *P. santalinoides*aqueous extract on L-NAME-induced Aortic Remodelling

Figure 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 % (Figure4B) as compared to control (Figure 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 (Figure 4D, E, F) or captopril (Figure 4C) significantly inhibited ($p < 0.01$) this risingthickness respectively by 18.72 %, 27.54, 17.20 % and by 26.03 %.



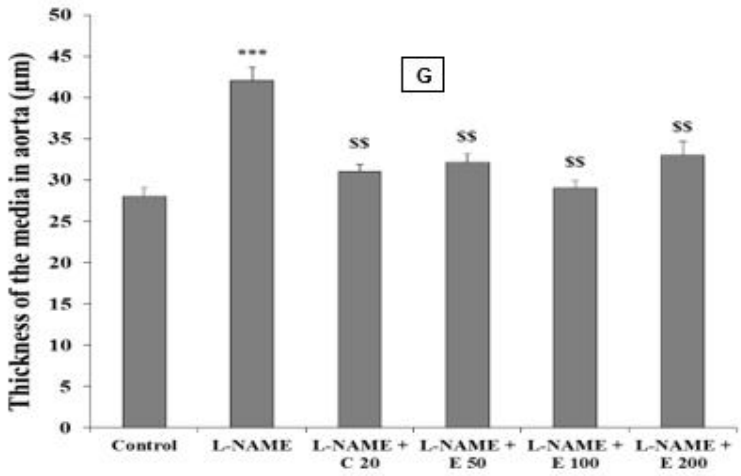


Figure 4: Photomicrograph showing histopathological changes in the aorta in different groups stained with H & E x 400 (A, B, C, D, E, and F) and media thickness (G).
Each bar represents mean ± S.E.M. 9 ≤ n ≤ = 15 per group. A: rats treated with distilled water (10 mL/kg); B: rats treated with N^G-nitro-L-arginine methyl ester (25 mg/kg); C: rats treated with L-NAME and captopril (20 mg/kg); D, Eand 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.

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/GMPcactivity, 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 of arterial blood pressure. Similar results have already been obtained byFouda *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 ofsome 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 preventedL-NAME-induced arterial hypertension in rats”[31].

Arterial hypertension is a main risk factor associated to 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 overproduction of angiotensin II [34]. Our results showed thattreatment with AEPS at different doses significantly inhibited the left ventricular hypertrophy induced byL-NAME. This inhibition suggests that AEPScouldpossess 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]. In fact, “the 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 reduction of the hepatic synthesis and intestinal absorption of cholesterol” [37].

It has been demonstrated that hypertension plays an important role on 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 damages in the organs. The nonsufficient perfusion of 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 level as well as ASAT and ALAT activities observed in LNHR. These results indicate the protective role of the aqueous extract against liver damages induced by L-NAME. The kidneys play an important role in the balance of water and electrolytes in the body” [34]. In thisstudy, “AEPS and captopril reduced significantly the

rise of urea, uric acid, creatinine and electrolytes 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 theinhibition of Na^{+}-K^{+} ATPase pump. It has been reported thatglycosides and phenols present in the extract stimulate the synthesis ofthe genes responsible of cellular regeneration of renal tissue" [39]. Thisaction could contribute to the AEPS effect on maintaining ionsbalance.

"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 which NO and form peroxynitrite"[41]. The treatment of L-NAME-induced hypertensive rats with AEPS induced an increase of SOD and catalase activities; theses enzymes reduce the level of superoxide anion by converting it into hydrogen peroxide which could bereduce 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]. In fact, treatment with the plant extract alleviated oxidative stress and subsequently helps to manage hypertension-induced vascular remodelling. *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 aqueousextract of *Pterocarpus santalinoides*(AEPS) stem bark is able to cure L-NAME-induced hypertension in rats. Our results have shown that this medicinal plant could have potential to lower arterial blood pressure, to improve lipid profile, to restore hepatic and renal functions as well as antioxidant status and aortic remodelling in L-NAME induced hypertension model in rats. These results which confirmed the empirical use of *Pterocarpus santalinoides*for the management 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.

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.

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