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Acute Oral Toxicity and Vasorelaxant Effects of Hydroethanolic Extract from *Lannea microcarpa* Engl. & K. Krause (Anacardiaceae) Trunk Barks in Mice Aortas: Possible Involvement of Intracellular Ca^{2+} Mobilization

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Abstract

Lannea microcrapa Engl. & K. Krause (Anacardiaceae) is a fruit and medicinal plant widely used in Burkina Faso. This plant is traditionally used in the treatment of hypertension. The aim of the present study was to evaluate the vasorelaxant effects of the hydroethanolic extract from *Lannea microcarpa* trunk barks (HE_ELM) on the aorta isolated from NMRI mice. Phytochemical screening by HPTLC, assay of phenolic and flavonoid compounds, assessment of antioxidant activity (DPPH, ABTS, FRAP, and LPO), and myography of HE_ELM (1 - 2000 µg/mL) on mice thoracic aortas in the presence and absence of endothelium were carried out. Endothelium-dependent and endothelium-independant vasodilation were assessed by cumulative addition of Ach (1 nM - 10 µM) on aortic rings precontracted with the thromboxane analogue A2 agonist, 9,11-dideoxy9α,11α-methanoepoxy PGF2α (U46619). L-NAME was used to verify the involvement of NO production in the relaxation mechanism of the extract. Acute oral toxicity of HE_ELM was also evaluated. A phytochemical study revealed the presence of tannins, flavonoids, sterols and triterpenes, saponosides, and high levels of total phenolics and flavonoids. These compounds are thought to be responsible for the extract's antioxidant and vasorelaxant properties. HE_ELM demonstrated significant

antioxidant potential and induced aortic relaxation. Indeed, pharmacological parameters gave EC_{50} values ranging from 596.45 ± 95.82 µg/mL to 749.48 ± 133.40 µg/mL and Emax values from 85.51% ± 9.59% to 96.81% ± 8.60% for the three conditions of vasodilation of the extract ($p > 0.05$). A complete antagonism of the contractile effect of U46619 was noted with 1 mg/mL HE_ELM. These results suggest that HE_ELM induces aortic relaxation through a concentration-dependent, endothelium-independent mechanism, possibly involving intracellular calcium mobilization of vascular cells. Acute oral toxicity tests of HE_ELM (2000 mg/kg) showed no mortality or adverse effects, suggesting the extract's safety and potential as a therapeutic agent for hypertension. This discovery scientifically validates the use of the plant in alternative medicine to treat hypertension.

Keywords

Lannea macrocarpa, Hydroethanolic Extract, Phytoconstituents, Antioxidant, Vasorelaxant Effects, Safety

1. Introduction

Hypertension is one of the most widespread and modifiable risk factors for cardiovascular disease in the world [1]. Epidemiological studies over the last decade have shown that cardiovascular disease is the world's leading cause of death and disability [2]. High blood pressure is expanding. In 2025, it is estimated that 1.56 billion of the world's population will be affected [3]. In Africa, cardiovascular disease (CVD) has reached almost epidemic proportions. Hypertension is the leading risk factor for mortality from cardiovascular disease, cerebrovascular disease, and stroke [2] [4]. The pathophysiological mechanism behind this disorder is multifactorial and includes vascular dysfunction, oxidative stress, inflammation, and the renin-angiotensin system [4] [5] [6]. Hypertension is characterized by a chronic abnormal rise in blood pressure (greater than or equal to 140/90 mmHg), in which increased vascular tone plays a major role in maintaining high blood pressure [6]. However, despite improvements in antihypertensive treatments, 20% to 30% of hypertensive patients are resistant to at least three antihypertensive drugs [7]. The use of non-pharmacological treatments, in particular the administration of nutraceutical supplements based on medicinal plants, traditional medicine, and pharmacopeia to lower blood pressure, has developed in recent years [6] [8]. In this context, 80% of the African population uses traditional medicine to treat themselves, including hypertension. In addition, natural medicines are an alternative to synthetic drugs [8] [9]. It has been suggested that a plant-based diet has a protective effect on the cardiovascular system. Polyphenols are one of the plant components most studied in this context [10], by promoting endothelium-dependent and endothelium-independent vasorelaxation and improving the lipid profile, antioxidant defenses and mitochondrial function [1] [6]. In addition to their use in alternative medicine, plants

are a useful matrix for identifying phytochemical compounds, which are then optimized by the pharmaceutical industry [8] [11]. Burkina Faso offers a great botanical treasure trove, given its geographical and climatic diversity, and is a major source of potentially therapeutic plants for the treatment of hypertension [12] [13]. Among the multitude of medicinal plants, traditional remedies prepared from the bark, roots, leaves and fruit of *Lannea microcarpa* Engl K. Krause (Anacardiaceae) are used to treat a wide range of conditions, including high blood pressure [6] [12] [14]. From a pharmacological point of view, *Lannea microcarpa* trunk barks extract has antioxidant activity and can reduce the harmful effects of oxidative stress on cells [6] [15]. The extracts also have anti-inflammatory properties [16] and induce vascular relaxation via inhibition of phosphodiesterases and intracellular calcium influx [6] [17]. They also have antihypertensive properties by counteracting the hypertensive effects of Ang II and L-NAME [6] [14]. However, depending on the polarity of the extraction solvent, the biological properties of plant extracts vary. A number of questions needed to be answered: does HE_ELM contain phytochemicals that are thought to be responsible for its antioxidant and vasodilatory properties? Is HE_ELM safe to use? This study was therefore undertaken to elucidate the phytoconstituents, pharmacological properties, and safety of the hydroethanol extract of *Lannea macrocarpa* trunk barks in the treatment of arterial hypertension.

2. Material and Methods

2.1. Material

2.1.1. Chemicals and Reagents

Chloroform, Methanol, Ethyl acetate, formic acid, Hexane, Dimethyl sulfoxide, NEU reagent, aluminum trichloride, iron chloride, ferric trichloride, Folin Ciocalteu reagent (FCR), sulphuric anysaldehyde reagent, sodium phosphate dibasic, monobasic potassium phosphate, 15-lipoxygenase (EC 1.13.11.12), linoleic acid, sodium bicarbonate, potassium hexacyanoferrate, trichloroacetic acid (TCA), thiobarbituric acid (TBA), hydrogen peroxide solution, 2,2'-azino bis-[3-éthylbenzothiazoline-6-sulfonique] (ABTS), 2,2-diphenyl-β-picrylhydrazyl (DPPH), L-NAME (Nω-Nitro-L-arginine methyl ester) and potassium persulfate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Gallic acid, Quercetin, and Trolox were supplied by Sigma Aldrich. Silica gel TLC plates F 254 grade was from Macherey-Nagel (Germany).

2.1.2. Plant Material

The stem barks of *Lannea microcarpa* were collected in June 2021 in the Loumbila region, 15 km East of Ouagadougou (Burkina Faso). After identification of the plant, reference specimen No. 361 was prepared and deposited at the "Département Environnement et Forêts/Centre National de la Recherche Scientifique et Technologique" (DEF/CNRST), Ouagadougou, Burkina Faso. The collected sample was dried in the open air, protected from sunlight and dust, and then ground to a powder. The powder obtained was used to prepare an extract for chemical and

biological investigation.

2.1.3. Experimental Animals

Male and female NMRI (Naval Medicinal Research Institute) mice, aged 3 months and weighing between 25 - 30 g, from the animal house of the "Institut de Recherche en Sciences de la Santé/Centre National de la Recherche Scientifique et Technologique (IRSS/CNRST), Burkina Faso" were used. The animals were placed in an enclosure at a temperature of 21˚C - 23˚C with a relative humidity of 50% - 60% and subjected to the light/dark cycle of 12 h/12h according to the rearing conditions of these species. Water and standard laboratory pellets enriched with proteins (29%) were provided for satiation and experiments were carried out following the procedures of the Guide of Good Practices in Animal Experimentation under the Declaration of Helsinki. Furthermore, all experimental animal procedures have been performed by the Guide for the Care and Use of Laboratory Animals of the US National Institutes of Health and the EU Directive 2010/63/EU for animal experiments [6] [8]. They were used for toxicological and pharmacological testing of the prepared extract.

2.2. Methods

2.2.1. Phytochemical Study

1) Preparation of Hydroethanolic Maceration Extract

Extraction was carried out using the maceration exhaustion technique described [18]. Briefly, one hundred (100) g of each plant powder was macerated in 500 mL of 80% ethanol (absolute Ethanol/Water; 80/20; v/v) for 24 h at room temperature. After 24 h, the mixture was filtered using cotton wool and then Wattman filter paper. Each residual pomace was reworked twice under the same conditions with 80% ethanol. The filtrate obtained was concentrated in a rotavapor (ELECTRONIC MICROPROCESSOR CONTROLLER CPS ventilated type) under reduced pressure at a constant temperature of 60˚C and then frozen and freeze-dried. The hydroethanolic extract (10.35%), EH_ELM was then recovered and stored in a hermetically sealed plastic bottle protected from light and humidity for the various phytochemical, toxicological, and pharmacological tests.

2) Phytoconstituents Revealed by High-Performance Thin-Layer Chromatography

High-performance thin-layer chromatography (HPTLC) was used to detect flavonoids and tannins in the extracts. It was carried out on chromatoplates (60 F_{254}, 10 × 5 cm, glass support 10 × 20 cm, Merck) following the literature [8]. Approximately 20 µL of extract was streaked with a semi-automatic sample dispenser (CAMAG, Linomat 5, Switzerland) along the baseline 8 mm from the bottom edge of the plate. After deposition and drying, the plates were placed in a tank containing eluent previously saturated (20 × 10 cm, saturation time: 30 min). The solvent system used depended on the metabolite to be identified: Ethyl acetate/formic acid/H_2O, (8/2/1 v/v/v/) for flavonoids; Ethyl acetate/formic

acid/H_2O (18/2/4/2/1 v/v/v/v) for tannins; Ethyl acetate/hexane (8/2 v/v) for Sterol-triterpenes and Hexane/ethyl acetate/methanol (10/5/5 v/v/v) for Saponosides. After migration over 8 cm in length, the plates were dried, and Neu reagent for flavonoids, Sulphuric anisaldehyde reagent for Saponosides, Liebermann and Burchard reagent for Sterol-triterpenes and 5% $FeCl_3$ for tannins revealed the chromatographic profiles. The profiles were then observed under visible light (tannins) and at UV wavelengths of 366 nm.

3) Determination of Total Phenolic Compounds

The total phenolic content of the hydroethanolic extract (HE_ELM) was determined using the Folin-Ciocalteu Reagent (FCR) [18]. Gallic acid was used as the reference compound to produce the standard curve. Briefly, 25 µL of sample at a concentration of 1 mg/mL was mixed with 125 µL of FCR. 100 µL of 7.5% w/v sodium carbonate solution was added to the mixture. After one (1) hour, absorbance at 760 nm was measured using a microplate reader (Spectro UV, Epoch Biotek, USA). Results were expressed as mg gallic acid equivalent (GAE)/g dry extract.

4) Determination of Flavonoid Compound

Total flavonoid content was assessed using an aluminum chloride reagent [18]. A standard calibration curve was plotted with quercetin as the reference. Briefly, 100 µL of HE_ELM (1 mg/mL) was mixed with 100 µL of a 2% w/v aluminium trichloride solution. After 10 min, absorbance was measured using a mass spectrophotometer. After 10 min, absorbance at 415 nm was measured using a spectrophotometer (Epoch Biotek, USA). Results were expressed as mg quercetin equivalent (QE)/g dry extract.

2.2.2. Assessment of Antioxidant Properties

1) DPPH• Essay

Free radical scavenging activity by hydroethanolic extract of *Lannea microcrapa* trunk barks and Trolox was performed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) as previously described [6]. Briefly, the absorbance of 10 µL of samples and Trolox added to 200 µL of DPPH (0.04 mg/mL) was measured at 490 nm after 30 min of incubation in the dark at room temperature using a Bio-Rad spectrophotometer (model 680, Japan). The result was expressed as antioxidant capacity equivalent to Trolox. As a function of sample concentration, a DPPH•-inhibition percentage curve was plotted. The percentage inhibition of the DPPH radical was calculated using the following formula:

$$\text{Inhibition (\%)} = [(Ac - Ae)/Ac] \times 100;$$

Ae and Ac represent the absorbances of the extract/ascorbic acid and the control (DPPH solution without sample). The concentration required to inhibit 50% of DPPH (IC_{50}) was determined on the curve.

Anti-radical power (ARP) was determined by the formula: $ARP = 1/IC_{50}$; ARP: Anti Radical Power; IC_{50}: 50% inhibitory concentration expressed in µg/mL.

2) Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was performed on the hydroethanolic extract of *L. microcrapa* trunk barks and Trolox as previously described [6]. The mixture of 0.5 mL samples with 1.25 mL phosphate buffer and 1.25 mL potassium hexacyanoferrate aqueous solution (1%) was incubated for 30 min at 50˚C. Next, 1.25 mL of trichloroacetic acid (10%) was added and centrifuged at 3000×g for 10 min. Distilled water (0.625 mL) and $FeCl_3$ solution (0.125 mL, 0.1%) were added to the supernatant (0.625 mL), and absorbance was measured at 700 nm using a spectrophotometer (Agilent, Santa Clara, CA) equipped with ChemStation UV-visible software. Trolox was used to plot the calibration curve. The FRAP activity of the samples was expressed as mol Trolox equivalent/gram dry extract.

3) ABTS•$^+$ Assay

The ABTS free radical scavenging activity of HE_ELM and Trolox was assessed using the procedure described previously [18]. In a volume of 5 mL of distilled water, 19.2 mg of ABTS were dissolved. A mass of 3.312 mg potassium persulphate was added to the ABTS solution at 3.84 mg/mL. After adding the potassium persulphate, the solution was left for 16 h in the dark at room temperature before use. On the day of the experiment, 4.5 mL of the mixture was diluted in 220 mL of absolute ethanol. The range of 8 dilutions to be tested was prepared from the parent concentration of the samples (1 mg/mL). On a 96-well microplate, 200 µL of ABTS solution mixed with 20 µL of the extract or reference was added to each well. After incubation for 30 min at 25˚C, absorbances were read against a blank at 415 nm using an Agilent 8453 spectrophotometer with ChemStation UV-visible software. Measurements were performed in triplicate. The percentage inhibition of absorbance at 415 nm was calculated according to the formula:

$$\% \text{ Inhibition} = [(A0 - A)/A0] \times 100.$$

A0 is the absorbance of the control; A is the absorbance of the sample.

The absorbance inhibition curve as a function of the concentration of the extract or reference substance (Trolox) was constructed to determine the 50% inhibitory concentration (IC_{50}). Anti-radical power (ARP) was determined using the formula: ARP = ($1/CI_{50}$); ARP: Anti-radical power; IC_{50}: 50% inhibitory concentration expressed in µg/mL.

4) Lipid Peroxidation Inhibition (LPO) Assay

The lipid peroxidation activity of rat liver was determined using 2-thiobarbituric acid [18]. $FeCl_2$-H_2O_2 was used to induce peroxidation of liver homogenate. A 0.2 mL volume of HE_ELM at a concentration of 1.5 mg/mL was mixed with 1 mL of 1% rat liver homogenate, then 50 µL $FeCl_2$ (0.5 mM) and 50 µL H_2O_2 (0.5 mM) were added. The mixture was incubated at 37˚C for 60 min, then 1 mL trichloroacetic acid (15%) and 1 mL 2-thiobarbituric acid (0.67%) were added. The mixture was heated in boiling water for 15 min. The experiment was performed in triplicate and the absorbance was read at 532 nm. Trolox was used as the reference product. The percentage inhibition was calculated using the following formula: Percentage inhibition (%) = [1 − (A1 − A2)/A0] × 100.

A1 is the absorbance of the control (without sample); A2 is the absorbance with the sample; A0 is the absorbance without liver homogenate.

2.2.3. Pharmacological Study Using Vasorelaxant Test

1) Preparation of Mice Thoracic Aortic Rings for Isometric Tension Recording

This method consists of verifying vascular reactivity in the presence of different pharmacological substances. The experiment on *ex-vivo* vasorelaxant effects was carried out with the thoracic aorta isolated from mice using the method previously described [8] [9]. Male mice were sacrificed by cervical dislocation and the thoracic aorta was removed. The aorta was placed in a petri dish containing Krebs solution and freed of adherent tissue. Segments of aorta (2 mm long) were mounted in myograph isolated organ vessels (Danish Myo Technology 620M, Aarhus, Denmark) filled with Krebs solution (in mM): 130 NaCl, 4.9 $NaHCO_3$, 3.7 KCl, 1.2 $MgSO_4$ $7H_2O$, 1.6 $CaCl_2$ H_2O, 1.2 KH_2PO_4 and 11 D-glucose. The Krebs solution (pH 7.4) was maintained at 37˚C and aerated with a mixture from a pneumatic pump. Two tungsten wires were inserted through the vessel lumen to hold each aortic ring between the vessel hooks. Mechanical activity was recorded isometrically by a force transducer connected to one of the two tungsten wires; the other wire was attached to a support carried by a micromanipulator screw allowing the voltage to be varied in mN.

2) Carrying out the Test

The aortic rings were stretched with a passive wall tension of 5 mN for 60 min. During this period, the rings were washed every 15 min. After stabilisation, the aortic rings were contracted by the addition of KCl (80 mM). Once the contraction plateau was reached, cumulative concentrations (10^{-9} - 3.10^{-7} M) of U46619 were added. The maximum tension of the tissue was then recorded and considered as 100% contraction of the ring. Once maximum contraction had been reached, the rings were successively rinsed 3 times with Krebs solution followed by an hour's rinsing at 20 min intervals. After returning to their baseline tension, HE_ELM was accumulated with increasing concentrations of ACh (10^{-9} - 10^{-5} M) after pre-contraction of the arteries with U46619 (80% of maximum contractile response). Rat aorta rings were considered to have functional endothelium when relaxation to ACh was greater than or equal to 80%. When relaxation of the aortic rings was less than 10%, the rings were considered endothelium-denuded. After checking the integrity of the endothelium, the rings were rinsed as before and recontracted with 80% of the maximum contractile response of U46619. At the contraction plateau, a cumulative increase in extract concentration (1 - 2000 µg/mL) on aortic rings in the presence and absence of endothelium was performed. Nω-Nitro-L-arginine methyl ester (L-NAME) is an inhibitor of nitric oxide synthase (NOS). It was incubated with endothelium-intact aortic rings at a concentration of 10^{-4} M for 20 min to test the ability of the extract to induce NO• production. The rings were then pre-contracted with U46619 and released by the addition of cumulative concentrations of the extract (1 - 2000

µg/mL). The antagonistic effect of the most active extract (1000 µg/mL) on the contraction of mouse aortic rings after incubation for 20 min followed by cumulative concentrations of U46619 (10^{-9} M - 3.10^{-7} M).

2.2.4. Acute Oral Toxicity

The acute oral toxicity test for HE_ELM was performed on female NMRI (Naval Medicinal Research Institute) mice in accordance with OECD guideline 423 [19] [20]. Two (02) batches, each consisting of three mice, were made up and placed separately in polypropylene cages: a control batch of three mice and a test batch of three mice. After fasting for 3 h, the extract was administered by gavage using an esophageal tube in a single dose to the test mice. A dose of 2000 mg/kg body weight (bw) of the extract was chosen as the starting dose. The white control batch received the solvent for dissolving the extract (distilled water, 10 mL/kg). The mice were observed individually for 2 h after administration, at the end of which food was restored. They were then observed twice daily for a period of 14 days to monitor for mortality and toxidrome (signs of toxicity) such as changes in skin and fur, eyes, mucous membranes, convulsions, salivation, diarrhea, sleep, and coma. The weight of each mouse and the quantities of water and food consumed were measured every 2 days during the 2 weeks of experimentation. On day 15, the mice were sacrificed and a necropsy was performed on the organs (liver, kidneys, lungs, spleen, and heart) then weighed. The relative weight of each organ was calculated [(Organ weight (g)/Fasting mouse weight on the day of sacrifice (g)) ×100]. This test was repeated after the mice were sacrificed under the same conditions as the 2 other batches (test batch and control batch).

2.3. Statistical Analysis

The experimental values were calculated by considering the maximum contraction produced by U46619 of each segment equal to 100%. The baseline tension before addition of U46619 was considered as 0%. The raw data have been normalized to the control (vehicle). The concentration-response curves were constructed using GraphPad Prism 8.4.3 Software, San Diego, CA, and two pharmacological parameters were obtained: the maximal effect generated by the agonist (Emax) and a concentration of agonist producing 50% of the maximum response (EC_{50}). Statistical comparisons were performed using one-way ANOVA or two-way ANOVA. Post hoc test was performed using Bonferroni's test analysis to compare all the groups. A p-value less than 0.05 was considered as statistically significant.

3. Results

3.1. Phytochemical Investigation

3.1.1. Compounds Revealed by HPTLC

The phytochemical analysis of HE_ELM highlighted the presence of saponosids, steroids and triterpenes, flavonoids, and tannins (**Figure 1**).

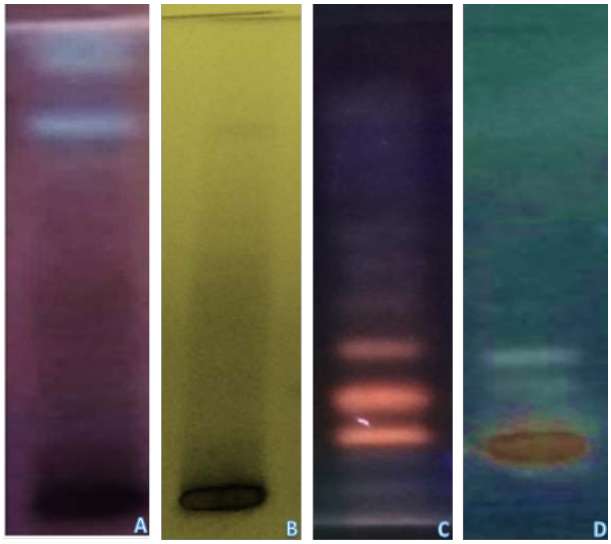

Figure 1. Phytochemical profile of hydroethanolic extract of *Lannea microcrapa* trunk barks revealed by HPTLC. (A) Saponosides; (B) Tannins; (C) Flavonoids; (D) Sterols-triterpenes.

3.1.2. Total Phenolic and Flavonoid Contents in *L. microcarpa* Extract

The contents of total phenolics and flavonoids in the HE_ELM were expressed as milligram equivalent of tannic acid per gram of dry extract for total phenolics (mg EAT/g ES) and as milligram equivalent of Quercetin per gram of dry extract (mg EQ/g ES) for flavonoids. The results for total phenolics and flavonoids in the extract were 58.88 ± 0.37 mg EAT/g ES and 22.5 ± 1.49 mg EQ/mg respectively.

3.2. Antioxidant Activity of Extract

The antioxidant activity of HE_ELM is shown in **Table 1**. The percentage inhibition of the ABTS test was 13.39 ± 2.27 µg/mL with a better activity for Trolox (2.04 ± 0.12 µg/mL) ($p < 0.001$). As for the reduction of the DPPH radical, the inhibition percentages were 7.43 ± 1.73 µg/mL for the extract, and 5.06 ± 0.05 µg/mL for Trolox. There was no significant difference between the DPPH radical inhibition percentages of the two substances. The ferric ion reduction capacity (FRAP) of the extract varied from 883.75 ± 11.5 mmol EAA/g. The lipid peroxidation inhibitory (LPO) power of the extracts was expressed as a percentage (%) (at 100 µg/mL) with 52.64% ± 7.14% for extract, and 48.11% ± 3.88% for the Trolox.

3.3. Pharmacological Properties of Extract

3.3.1. *Ex-Vivo* Vasodilatory Effects of HE_ELM Extract on Mice Aortic Rings

The hydroethanolic extract of *Lannea microcarpa* trunk barks (HE_ELM) was tested on mice arteries to assess its vasorelaxant effects. The **Figure 2** shows the recording of the vasorelaxant effect of HE_ELM on the aorta of NMRI mice. The results show a concentration-dependent relaxation (1 - 2000 µg/mL) in the pres-

ence and absence of functional endothelium in arteries pre-contracted at U46619. The same was true for the relaxation of aortic rings pre-incubated with L-NAME (**Figure 3A**). The 50% effective concentrations (EC_{50}) and maximum effects (E_{max}) were determined for each relaxation curve. **Figure 3B** shows the maximum effects (E_{max}) of HE_ELM on mice aortas of 94.91% ± 7.07%, 96.81% ± 8.60% and 85.51% ± 9.59% respectively in the presence of endothelium, in the presence of endothelium pre-incubated with L-NAME and in the absence of endothelium. The EC_{50} for HE_ELM were 596.45 ± 95.82 µg/mL, 718.65 ± 151.43 µg/mL and 749.48 ± 133.40 µg/mL respectively in the presence of endothelium, in the presence of endothelium preincubated with L-NAME and in the absence of endothelium (**Figure 3C**). No statistically significant differences were observed for the two pharmacological parameters.

3.3.2. *Ex-Vivo* Evaluation of the Antagonistic Effect of HE_ELM on Aortic Contraction in Mice Induced by U46619

Figure 4 illustrates the antagonistic effect of HE_ELM on U46619-induced contraction. The results showed that HE_ELM significantly inhibited compared to control ($p < 0.001$) and almost completely (**Figure 5A**) the contraction induced by cumulative U46619 (10^{-9} M - 3.10^{-7} M). E_{max} was 0.97 ± 0.77 mN/mm for aortic rings pre-incubated with HE_ELM versus 6.42 ± 0.38 mN/mm for control (**Figure 5B**).

Table 1. *In vitro* antioxidant activity of hydroethanolic extract of *L. microcrapa* trunk barks.

Extract	ABTS		DPPH		FRAP	LPO
	IC_{50} (µg/mL)	ARP	IC_{50} (µg/mL)	ARP	mmol EAA/g	Inhibition (%) (at 100 µg/mL)
HE_ELM	13.39 ± 2.27***	0.08	7.43 ± 1.73	0.14	883.75 ± 11.5	52.64% ± 7.14%
Trolox	2.04 ± 0.12	0.49	5.06 ± 0.15	0.19	-----	48.11 ± 3.88

IC_{50}: Inhibition concentration 50%; ARP: Anti-free radical power; n = 3; ***$p < 0.001$ vs Trolox; EAA: Ascorbic acid equivalent.

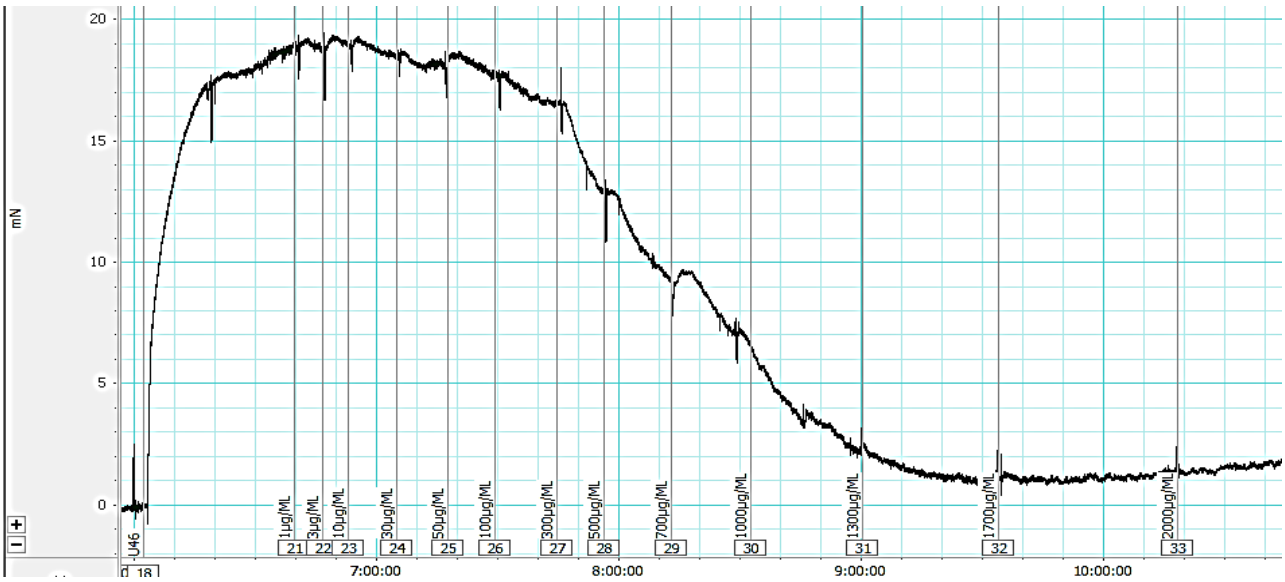

Figure 2. Illustration of the vasorelaxant effect of HE_ELM on a mouse aortic ring pre-contracted with U46619.

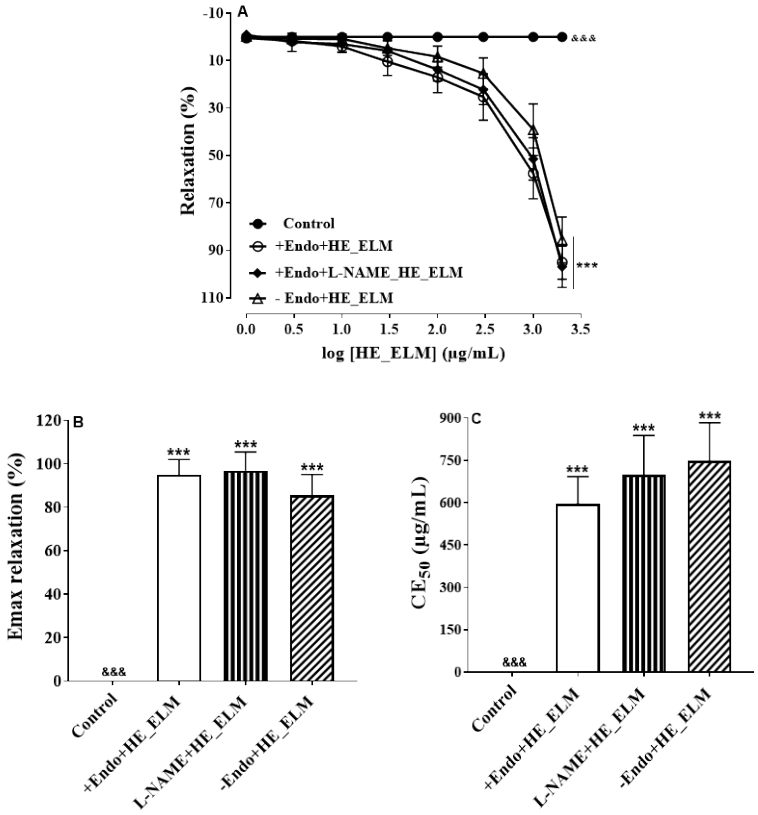

Figure 3. Cumulative concentration-response curves of HE_ELM on endothelium-intact and -denuded aortic rings pre-contracted with U46619 (A). Histograms of Emax (B) and EC_{50} (C) effects respectively of HE_ELM extract, in the presence/absence of endothelium and pre-incubation with L-NAME on mice aortic rings pre-contracted at U46619. Results are expressed as mean ± SEM; ***$p < 0.001$ vs control; $^{\&\&\&}p < 0.001$ vs HE_ELM; n = 6 - 7.

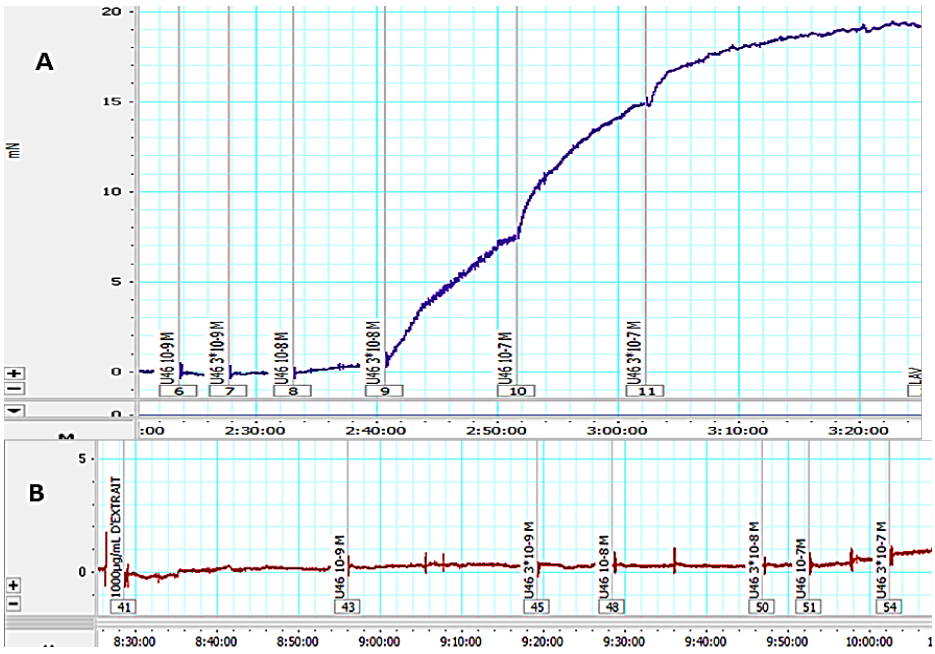

Figure 4. Illustration of the antagonistic effect of the contracting agent U46619 in the absence (A), and presence (B) of HE_ELM on mice aortic rings.

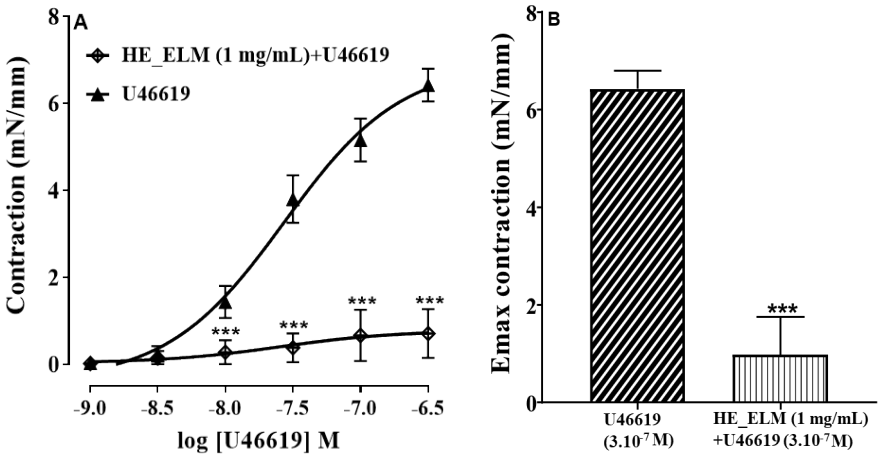

Figure 5. Inhibitory effect of HE_HLM (1 mg/mL) on contractions induced by U46619 (3×10^{-7} M) in mice aortic rings (antagonist effect curves (A) and E_{max} (B)). Results are expressed as mean ± SEM; n = 6 - 7; ***p < 0.001 *vs* U46619.

3.4. Acute Oral Toxicity

3.4.1. Mortality and Toxidrome

The dose of 2000 mg/kg body weight (bw) showed no mortality or remarkable behavioral changes in female mice at the first and second stages of administration of the HE_ELM (Table 2).

3.4.2. Changes in Body Weight, Food, and Water Consumption of Mice

Figure 6 shows the mean weight gain, feed consumption (g/g of mice), and water consumption (mL/g of mice) for 14 days in female mice given a vehicle (1% Tween 80), and a single dose (2000 mg/kg) of the HE_ELM. There was no statistically significant difference in body weight gain between the treated and control batches. The same was true for feed and water consumption ($p > 0.05$).

3.4.3. Macroscopic Observation, and Relative Organ Weights of Mice

Fresh macroscopic examination of vital organs such as the heart, lungs, liver, kidneys, and spleen of control mice and mice treated with HE_ELM (2000 mg/kg) showed that there were no lesions, nor any change in color or appearance of the various organs. Table 3 shows the relative organ weights of batches of control mice and mice treated with the extract. No statistically significant variation was observed between the relative organ weights of control and treated batches.

4. Discussion

Herbal alternative medicine is widely used to treat a variety of conditions. This practice has been identified as complementary to modern medicine and can therefore be recommended in the treatment of cardiovascular disease [21]. *L. microcarpa* trunk bark is widely used in the treatment of hypertension [12] [14]. The aim of this study was therefore to provide scientific evidence for the widespread use of this plant in the treatment of hypertension. Phytochemical analysis of the hydroethanolic extract of *Lannea microcarpa* trunk bark (HE_ELM) revealed

Table 2. Mortality of female mice administered a single dose (2000 mg/kg) of extract (n = 6).

Extract administered	Mortality	
	1st test	2nd test
Control (1% Tween 80)	0/3	0/3
HE_ELM (2000 mg/kg)	0/3	0/3
Excitement	-	-
Sleepiness	-	-
Hair standing up	-	-
Lack of appetite	-	-
Diarrhea	-	-
Vomiting	-	-
Hyperventilation	-	-

Table 3. Relative weights of vital organs of control and hydroethanolic extract of *L. microcrapa* trunk barks (HE_ELM) treated mice; n = 6.

	Relative average organ weight (g/100 g bw)				
	Heart	**Kidneys**	**Lungs**	**Liver**	**Spleen**
Control	0.52 ± 0.0.3	1.17 ± 0.03	0.82 ± 0.04	5.08 ± 0.23	0.42 ± 0.03
HE_ELM (2 g/kg)	0.48 ± 0.02	1.26 ± 0.09	0.86 ± 0.06	5.15 ± 0.0.05	0.43 ± 0.04

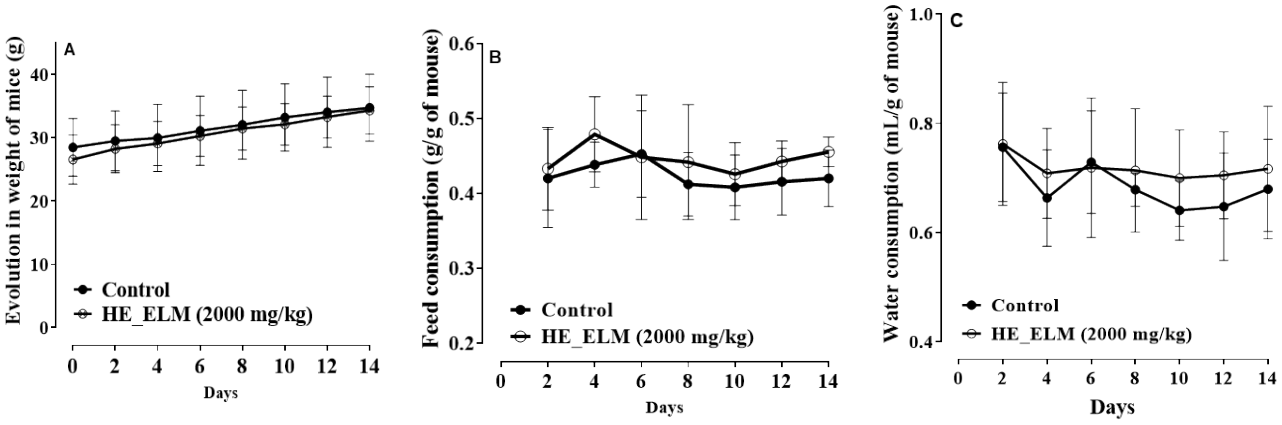

Figure 6. Change in weight (A) and feed (B) and water (C) consumption of female mice from extract test and control batches during 14 days of follow-up; n = 6.

the presence of flavonoids, saponosides, sterols, terpenes and tannins. These results corroborate phytochemical findings in the aqueous decoction and ethyl acetate fraction of the plant's trunk bark. The authors also found anthracenosides, coumarin derivatives, reducing compounds and anthocyanins [6] [17] [22]. These compounds were also found in the trunk bark of *Lannea velutina* A. Rich (Anacardiaceae) [23]. According to the literature, saponosides [24], tannins

[6] [8], sterols and triterpenes [8] [25] [26] have diuretic, vasodilatory and antihypertensive properties, both on the conductance arteries of normotensive and spontaneously hypertensive rats (SHR). In addition, plant extracts rich in polyphenols and flavonoids can induce endothelium-dependent relaxation due to their ability to promote endothelial NO• formation and EDHF-mediated vasodilation [8] [27]. In the metabolic process, oxygen undergoes a series of reduction steps leading to the production of free radicals represented by the superoxide (O^{2-}), oxygen radical (O^{2-}), hydroxyl (OH-), alkoxyradical (RO-) and peroxyl radical (ROO-). To combat the latter, natural antioxidants exert their protective effect by activating the Nrf2 (NF-E2-related factor 2) signalling pathway, which regulates the endogenous antioxidant defence system by stimulating the expression of antioxidants and detoxification enzymes [28]. The phytochemicals revealed have been shown to stabilize the reactive oxygen species known as peroxynitrite [18]. The properties of phenolic compounds, in particular flavonoids, tannins and triterpenes, are mediated by the scavenging of free radicals, the inhibition of certain pro-inflammatory enzymes, the chelation of heavy metals involved in the production of free radicals and the regulation or protection of the antioxidant defense system [29] [30]. In addition, natural antioxidants exert actions that go beyond their ability to counter oxidative stress, including intracellular signaling cascades. The imbalance between oxidizing species and antioxidant compounds can increase harmful species that induce structural modification and functional modulation of proteins, lipids and nucleic acids [31]. This situation ultimately leads to a number of complex diseases, in particular chronic inflammatory diseases and cardiovascular diseases [32]. HE_ELM could therefore help combat hypertension, the spearhead of cardiovascular disease. In fact, these results are in line with the literature that has documented that freeze-dried aqueous extract and its ethyl acetate fraction of trunk barks [6] [15], the leaves and fruits of *L. microcarpa* [11] [33] [34] have properties against oxidative stress. In addition, in the management of cardiovascular diseases, including hypertension, blood vessels play a key role in the search for pharmacological targets. On this scientific basis, the beneficial effects of HE_ELM were investigated *ex-vivo* on the thoracic aorta of mice. Acetylcholine (ACh) was therefore used in this study to test the integrity of the vascular endothelium. In vessels with intact endothelium pre-contracted with U46619, a thromboxane A2 (TXA2) analogue known for its potent and stable vasoconstrictive effect on vascular smooth muscle. ACh induced vasodilation via the release of NO• [6] [8]. Experimental results showed that HE_ELM (1 - 2000 µg/mL) produced concentration-dependent vasorelaxation in isolated mouse thoracic aorta in the presence of endothelium pre-contracted by U46619. The vasodilatory mechanism of the extract was also investigated in aortic rings with intact endothelium in the presence of L-NAME. Vasorelaxation can be induced by the endothelium through the production of NO•, prostacyclin or endothelium-derived hyperpolarising factors (EDHF) on the one hand and direct action on smooth muscle cells on the other [35]. Incubation of aortic rings with L-NAME, a competitive inhibitor of endothelial NO

synthase, showed that NO•, a major contributor to vasorelaxing factors in the endothelium, did not influence vascular relaxation in HE_ELM with an E_{max} of 96.81% ± 8.60% and an EC_{50} of 718.65 ± 151.43 µg/mL. Pre-incubation of the rings with L-NAME did not affect the vasodilatory effect of the extract. Also, in the absence of endothelium, vasorelaxation of HE_ELM was similar to that in the presence of endothelium. This was verified by the determination of E_{max} and EC_{50}, which were not statistically significant between the three conditions of vascular relaxation of the extract. HE_ELM therefore has an endothelium-independent effect. The convincing explanation for this observation would be linked to the ability of HE_ELM to orchestrate a decrease in $[Ca^{2+}]i$ concentration in VSMCs via transmembrane efflux [10]. U46619's ability to bind to G protein-coupled receptors (GPCRs) induces a progressive and sustained contraction of vascular smooth muscle. This contraction involves increasing cytosolic calcium either by releasing calcium stored in the sarcoplasmic reticulum (SR) or by modulating transmembrane calcium channels leading to the entry of extracellular calcium [9]. HE_ELM, also contains sterols and triterpenes, tannins, flavonoids and saponosides. These compounds are known for their vasorelaxant properties [8]. These results corroborated previous studies carried out on the plant highlighting the endothelium-independent vascular component of the antihypertensive properties of extracts derived from the bark of the plant's trunk in an *ex vivo* model [17]. The other part of this study focused on the ability of HE_ELM to block the release of intracellular calcium ($[Ca^{2+}]i$). Aortic rings were incubated with 1 mg/mL HE_ELM and then supplemented with U46619 (10^{-9} - 3.10^{-7} M). The results showed that the extract almost completely blocked the release of $[Ca^{2+}]i$ in vascular smooth muscle cells. Previous work had shown that this vasorelaxation is partly due to the inhibition of phosphodiesterases (PDE_1, PDE_3, and PDE_5) and the release of $[Ca^{2+}]i$ into the cytoplasm, an essential mechanism for cell contraction [6]. Interestingly, HE_ELM offers advantages in the treatment of cardiovascular diseases, including arterial hypertension characterised by endothelial dysfunction. In fact, HE_ELM is said to be able to reduce blood pressure levels because its action is directly on smooth muscle, compared with substances whose action is mediated by vasorelaxant factors derived from the endothelium. This study reinforces the claim that extracts of this plant have antihypertensive effects [6] [14] being formulated as an antihypertensive phytomedicine, LAMIC [15]. However, a health product can only be used when it is both effective and free from toxicity. So, the use of plant extracts in alternative medicine requires vigilance about their safety. With this in mind, HE_ELM has been tested for acute oral toxicity. The results showed that acute oral administration of HE_ELM (2000 mg/kg bw) produced no mortality or visible changes in behaviour or any other physiological activity and indicated that the LD_{50} of this extract was greater than 5000 mg/kg bw in NMRI female mice, according to the United Nations Globally Harmonised System [36]. These results showed that HE_ELM can be used in practically non-toxic. This finding is in agreement with previous work involving oral administration of the aqueous infusion showed no mortality up to a dose of

3000 mg/kg and no clinicals signs of toxicity for 14 days of observation in Wistar rats [37]. Subacute toxicity for 28 days showed that the freeze-dried aqueous decoction of the plant's trunk bark was non-toxic up to a dose of 1000 mg/kg [38]. The subchronic toxicity of LAMIC, a prototype antihypertensive phytomedicine based on the aqueous decoction of the bark from the trunks of the plant, in Wistar rats was non-toxic up to a dose of 1500 mg/kg body weight administered daily for 90 days [15].

5. Conclusion

Our study showed that the hydroethanolic extract of *Lannea microcarpa* trunk barks has antioxidant activity and a powerful concentration-dependent and endothelium-independent vasorelaxant effect on aortic rings isolated from mice pre-contracted at U46619. The presence of flavonoids, tannins, saponosides, sterols and triterpenes in the extract could account for its antioxidant, and vasorelaxant properties. From a toxicological point of view, the extract is non-hazardous. The vasodilatory properties observed on aortic rings suggest the possible use of *L. microcarpa* in the treatment of cardiovascular disease, even in cases of endothelial dysfunction. These findings will help to scientifically validate the traditional therapeutic uses of this plant in the treatment of hypertension.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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