International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2022.1401.1411

Research Article
Antihypertensive Effect of the lyophilized Aqueous Extract of *Lannea microcarpa* in L-NAME-Induced Hypertensive Wistar Rats

[1,2]Nintie San Ga Yasmina Valerie Ida Traore, [1]Lazare Belemnaba, [1]Mathieu Nitiema, [1,2]Christian Windingoudi Rimwagna Ouedraogo, [2]Tata Kadiatou Traore, [1]Souleymane Compaore, [1]Salfo Ouedraogo, [1]Noufou Ouedraogo and [1]Sylvin Ouedraogo

[1]Research Institute for Health Sciences, National Centre for Scientific and Technological Research, 03 BP 7047 Ouagadougou 03, Burkina Faso
[2]Drug Development Laboratory, Center for Training, Research and Expertise in Medicine Sciences, Doctoral School of Sciences and Health (ED2S), Joseph Ki-Zerbo University, 03 BP 7021 Ouagadougou 03, Burkina Faso

Abstract

Background and Objective: *Lannea microcarpa* is widely used in traditional medicine against hypertension. This study evaluates the effect of aqueous extract of *Lannea microcarpa* (LMaq) on normotensive rats and its chronic antihypertensive effects in the L-NAME-induced hypertensive model. **Materials and Methods:** The effect of LMaq (5 and 25 mg kg^{-1}), Captopril (5 mg kg^{-1}) and NaCl 0.9% on arterial pressure were studied by daily oral administration in normotensive rats for 4 weeks. Daily oral administration of L-NAME (40 mg kg^{-1}) for two consecutive weeks resulted in hypertensive rats. For evaluation of antihypertensive effects, hypertensive animals were then treated with either LMaq (5 or 25 mg kg^{-1}) or captopril (5 mg kg^{-1}) in combination with L-NAME for the next 2 weeks. Blood pressure parameters were measured by a tail-cuff system. **Results:** In normotensive rats, daily administration of LMaq did not have a significant effect on their blood pressure compared to that of the control group. Daily administration of L-NAME induces an average mean progressive increase in systolic blood pressure (SBP) from the 1st week (106.91±7.28 mmHg) to 171.16±0.41 mmHg after 2 weeks in LMaq and Captopril groups, respectively. In L-NAME-induced hypertensive rats, LMaq and Captopril significantly decreased their average mean SBP to 112.25±2.59 mmHg only after four days of treatment. Some significant modifications were noted for creatinine, alanine, etc., parameters but the autopsy revealed any change in the appearance of the noble organs. **Conclusion:** These results suggested that LMaq has significant antihypertensive effects in L-NAME-induced hypertensive rats comparable to those of Captopril.

Key words: *Lannea microcarpa*, non-invasive, cardiovascular disease, life expectancy, hypertension, alanine, captopril

Citation: Traore, N.S.G.Y.V., L. Belemnaba, M. Nitiema, C.W.R. Ouedraogo and T.K. Traore *et al*., 2022. Antihypertensive effect of the lyophilized aqueous extract of *Lannea microcarpa* in L-NAME-induced hypertensive Wistar rats. Int. J. Pharmacol., 18: 1401-1411.

Corresponding Author: Lazare Belemnaba, Research Institute for Health Sciences, National Centre for Scientific and Technological Research, 03 BP 7047 Ouagadougou 03, Burkina Faso

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

High blood pressure or hypertension, is a major public health problem in Burkina Faso like in other countries in Africa and the world[1]. The normal Blood Pressure (BP) of an adult is set at 120 mmHg for the systolic blood pressure (SBP) and is set at 80 mmHg for the diastolic blood pressure (PAD). High blood pressure is an abnormal and prolonged increase in the pressure exerted against the walls of blood vessels. It's corresponding to an SBP $\geq$140 mmHg and/or a DBP $\geq$90 mmHg following repeated examination[2,3]. This disease is the main risk factor for Cardiovascular Disease (CVD) and stroke with more than 9 million deaths recorded in 2010[4,5]. Moreover, this pathology is a serious disease because not only is it silent but also, it considerably reduces the life expectancy of the affected subject if it is not detected in time[6]. And for the WHO, reducing the prevalence of hypertension by 33% between 2010 and 2030 is enclosed as one of its global priorities[7]. As a result, hypertension can lead to cerebral (dizziness), cardiac (palpitations, chest pain) complications and can even accelerate to the end-stage kidney disease[8-10]. In addition, studies have shown as early as the 2000s that 26.4% of the world's adult population was hypertensive and this proportion is expected to reach 29.2% by 2025[11]. According to the WHO, the prevalence of hypertension varies according to the regions, the countries and according to the standard of living of the populations. Indeed, WHO estimates that the prevalence of hypertension is highest in the African Region, with around 46% of adults aged 25 and over suffering from hypertension compared to 40% elsewhere in the world[12]. In addition, the highest rates were recorded in Eritrea (19.3%) and Seychelles (39.6%) according to the WHO STEP reports in 2015 (STEP wise surveillance approach). Likewise, in a systematic review on hypertension, it emerges that if nothing is done, hypertensive people could reach 216.8 million by 2030 while it was 54.6 million, 92.3 million and 130.2 million, respectively in 1990, 2000 and 2010. In 2021, the highest prevalence was estimated at 27% in the African region (WHO, 2021). According to the Burkina Faso Ministry of Health in 2013, its overall prevalence was estimated at 17.6% among the population aged 25-64[13]. Moreover, for the WHO, around 1.5 million people are at high risk for hypertension in this country where Cardiovascular Disease (CVD) is responsible for around 20,600 each year. In addition, a third of adults living with hypertension in Burkina Faso, constitute a real public health problem in this country like other countries in Africa and the world[14]. Unfortunately, its treatment in modern medicine remains expensive, lifelong and difficult to access for most populations in developing countries, especially in Sub-Saharan Africa. Also, many authors have noted the persistence of problems related either to the efficacy or to secondary and/or undesirable events of modern antihypertensive drugs (such as angina pectoris, myocardial infarction, premature mortality, disabilities, etc.) contributing to negatively impact patient adherence. Moreover, several studies also reveal that treatments based on combination therapies or multi-drug regimens as well as the adoption of a healthy lifestyle encounter many difficulties in adhering in hypertensive patients. Hence a renewed interest of patients in traditional medicine and pharmacopoeia in low-income countries as well as in industrialized countries for the regulation of their blood pressure parameters[15].

However, the efficacy and/or safety of traditional medicines have not always been scientifically proven. Thus, to cope, among other things, with the cost of treatment and the undesirable effects of modern products but also to verify the effectiveness and safety of traditional remedies, ethnopharmacological surveys were carried out by researchers from the Research Institute in Health Sciences (IRSS) from traditional health practitioners in the provinces of Ioba and Passoré to identify the medicinal plants they use for hypertension management. As a result of these investigations, a recipe based on the bark of the trunk of *Lannea microcarpa* Engl. and K. Krause (Anacardiaceae) also called African grape[16] and widely used for the traditional treatment of hypertension was discovered. Chemistry, pharmacology and toxicology studies were then carried out on various extracts from this species to evaluate their vasorelaxant and antihypertensive effects and their inhibition capacity of Cyclic nucleotide phosphodiesterases[17]. Other studies have also shown that the lyophilized aqueous extract of *Lannea microcarpa* can oppose the installation of hypertension caused by angiotensin II[18]. But so far, no evaluation of its antihypertensive effect on a model of hypertension induced by N_{ω}-nitro-L-arginine methyl ester (L-NAME) has yet been carried out. Hence the interest of the present study, which evaluated the antihypertensive activity of the lyophilized aqueous extract of the bark of the trunk of *Lannea microcarpa* Engl and K. Krause (LMaq) in L-NAME-induced hypertensive Wistar rat and its impacts on vital organs of treated animals.

MATERIAL AND METHODS

Study area: This experimental study was carried out at the Research Institute for Health Sciences (IRSS, Ouagadougou, Burkina Faso) during the period from February 01-April 31, 2020.

Ethical consideration: This study was conducted according to the ethical rules in this area. All experimental procedures were following 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. It was performed according to the protocol validated by other authors with the approval of the Joseph KI-ZERBO University Ethics Committee (Protocol number: CE-UOI/2019-04)[19].

Chemicals and reagents: N_{ω}-Nitro-L-arginine methyl ester hydrochloride (L-NAME) and sodium chloride (NaCl) were purchased from Sigma Aldrich (France). Captopril Denk 25 were from Denk Pharma GmbH and C_o (Germany). A biochemical analysis Kit was purchased from Teco Diagnostics.

Plant material: The trunk barks from *Lannea microcarpa* Engl and K. Krause were collected at 20 km in the Northeast of Ouagadougou (Burkina Faso) at Loumbila in the savannah zone (January, 2015). A sample was collected and then authenticated by a botanist from the "Herbier National du Burkina (HNBU)" located at the National Center for Scientific and Technological Research (CNRST, Burkina Faso) in where a voucher number HNBU 361 was obtained and then deposited. The collected sample was dried in the open air, without dust or sunlight and then reduced to powder using a mechanical grinder. The lyophilized aqueous decoction of *Lannea macrocarpa* (LMaq) was prepared from the obtained powder.

Extract formation: For that, 100 g of the trunk barks powder from *Lannea microcarpa* were mixed with 500 mL distilled water and a decoction was carried out for 30 min. After a cooling period, the aqueous decoction extract was filtered and centrifuged using a centrifuge (CHRIST, ALPHA 1-2 LD plus) at 2000 rpm for 5 min. After that, the supernatant was collected, concentrated and then frozen before lyophilized.

***In vivo* model of hypertension and experimental protocol**

Experimental animals: Forty-two female normotensive Wistar rat about 3 months of age and weighing between 180 and 200 g (average of 221.69±16.39 g) from the CIRDES (International Center for Research and Development on Livestock in the Subhumid Zone, Burkina Faso) animal facility were used. Animals were then placed in plastic cages and acclimatized at the Research Institute for Health Sciences/ National Centre for Scientific and Technological Research (IRSS/CNRST) pet store for 2 weeks until the experiment started. All used animals were healthy and received an adequate diet in a quality environment (21-25°C, 12:12 hrs light-dark cycle, humidity level between 40-60%). They were free to access food and drinking water *ad libitum*. During the experiment, rats' food and water were weighed at the same time each week.

Induction of experimental hypertension: Hypertension was induced using the L-NAME-induced hypertensive model. For this purpose, all groups of animals to which hypertension has to be induced, have received daily, orally and at the same time, a solution of L-NAME (40 mg/kg/day) for 14 days (2 weeks). Subacute oral L-NAME intake is well known to cause organ damage within the cardiovascular and kidney systems leading to hypertension by chronic inhibition of nitric oxide synthase[19,20]. This experimental model has already been validated in our laboratories[21].

Experimental procedure: Precautions were taken for minimized or avoid pain and distress during the experimental procedure.

The experimental animals were first isolated and then acclimatized for 1 week. They were then divided into 07 groups of six animals each.

During the following week, the systolic, diastolic and heart rate parameters of each group were evaluated to confirm their normotensive state. The treatments were carried out as follows:

- **Group 1:** Group 1 received once a day, an oral administration of NaCl 0.9% for 4 weeks and was represented as the control for the study
- **Group 2:** Rats were given a daily *per os* administration of L-NAME at a dose of 40 mg/kg/day (L-NAME 40) for 4 weeks
- **Group 3-5:** All rats from these groups were received for 4 weeks, a daily administration of L-NAME at a dose of 40 mg/kg/day. Specifically, after the first 2 weeks of L-NAME administration, these groups were treated simultaneously with captopril (5 mg/kg/day: L-NAME+ Captopril 5), LMaq at 5 mg/kg/day (5 mg/kg/day: L-NAME+LMaq 5) and LMaq at 25 mg/kg/day (L-NAME+ LMaq 25) for the following 2 weeks, respectively
- **Group 6 and 7:** Rats were given for 4 weeks, a daily *per os* administration of LMaq of 5 mg/kg/day (LMaq 5) and 25 mg/kg/day (LMaq 25) respectively for Group 6 and 7

During this study, all reagents were prepared in NaCl 0.9%. Moreover, the average rat body gain weight as well as their water and food consumption, were determined

during the experimental period for assessing the potential effect of LMaq treatment on them.

Blood pressure and heart rate measurement: The methods of measuring blood pressure parameters have been previously described[21]. Briefly, it consisted of measuring the SBP, the DBP and the heart rate (HR) of all rats for 5 consecutive weeks at the same time, using non-invasive tail-cuff plethysmography (58 500 Blood Pressure Recorder System, Ugo Basile).

For that, groups of six rats were placed in a heating box for rat and each rat was individually placed in a holder. Their blood pressure was measured by the tail-cuff plethysmography method after a 30 min acclimatisation in the heating box for rat at 37°C. For that, when animals were relaxed and became calm in their holder, the tail-cuff was inflated to a pressure well above the expected systolic pressure (195 mmHg) and then slowly released and the pulse was recorded by the BP recorder analyzer.

The mean values of four measurements were considered after excluding the extreme values linked to background noise or the uncontrolled and sudden movements of the experimental animals.

Autopsy method and analysis of biochemical parameters

Autopsy practice: At the end of the experiment, an autopsy was applied. For this, rats were weighed, then fasted for 16 hrs and finally humanely sacrificed the next day. Thus, each animal was weighed again on the day of sacrifice and general anaesthesia intraperitoneally with ketamine 150 mg kg^{-1} body weight was applied. Once asleep, each animal was placed in the supine position on a dissection board followed by an opening in the abdomen to expose the different organs.

A cardiac puncture was then carefully performed for biochemical analysis. The noble organs such as the heart, the lungs, the liver, the spleen, the kidneys and the ovaries were removed and then freed of residual blood using an adsorbent paper. Subsequently, careful macroscopic observation of these nobles' organs was performed and then they were weighed with an analytical balance. At the end of the autopsy, a photograph of the various organs was taken and then conserved for ulterior investigations.

Measurement of serum biochemical parameters: The method of biochemical analysis has been previously described[21]. Briefly, blood samples collected after the autopsy were transferred into hemolysis tubes and centrifuged for 10 min (3,000 rpm) in a ROTOFIX 32A bench-top centrifuge (from Hettich Zentrifugen, Germany). The sera were then collected for the biochemical analysis. The Mindray BS-300 from China which was an automatic biochemistry analyzer has been used for the determination of the biochemical parameter which was glucose, calcium, magnesium, total cholesterol, creatinine, chlorides, phosphorus, uric acid, Alanine Aminotransferase (AALT), Aspartate Aminotransferase (ASAT) and total protein.

Statistical analysis: Statistical analysis was performed using Prism-7 software for Windows and results were compared using analysis of variance (One-way and Two-way ANOVA) followed by Bonferroni multiple comparison post-tests or Dunnett's. All data were expressed as Means±Standard error of the mean. The differences were considered statistically significant with a $p<0.05$.

RESULTS

LMaq effect on blood pressure parameters in L-NAME-induced hypertensive rat: The effects of LMaq on systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) parameters obtained during the study were shown in Fig. 1-2 and Table 1.

Effects of LMaq on systolic blood pressure in L-NAME-induced hypertensive rat: The blood pressure of treated rats was measured by the indirect tail-cuff method twice a week ie the 4th day followed by the 3rd day of each week for 5 weeks. Results presented in Fig. 1 showed LMaq effects on systolic blood pressure (SBP) of daily treated animals with

Table 1: Effect of LMaq on change in heart rate for each study group (bpm) (n = 6)

	Treatments						
Weeks	NaCl 0.9%	L-NAME	L-NAME+Captopril 5	L-NAME+LMaq 5	L-NAME+LMaq 25	LMaq 5	LMaq 25
1	640.74±32.27	411.88±21.59	507.47±39.90	565.02±64.63	540.35±48.55	551.05±46.32	524.26±28.02
2	527.61±41.53	460.15±42.23	510.07±49.72	719.52±113.1	573.5±64.08	622.65±56.98	505.80±73.54
3	529.26±33.45	517.16±37.89	511.48±15.31	578.13±39.44	515.44±58.84	545.06±80.53	510.45±51.86
4	508.91±45.21	527.68±32.07	471.55±44.79	565.51±47.34	546.64±59.11	539.29±66.04	490.12±53.43

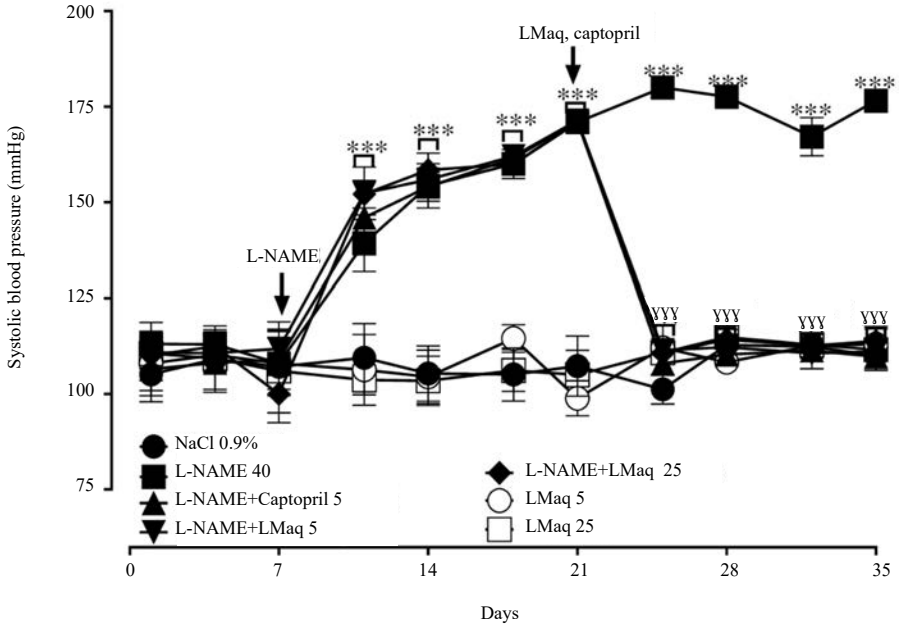

Fig. 1: Effect of LMaq (5 and 25 mg kg^{-1}) and captopril 5 on the SBP of hypertensive rats induced by L-NAME 40 mg kg^{-1}
***p<0.001 vs. 0.9% Nacl and γγγp<0.001 vs. L-NAME (n = 6)

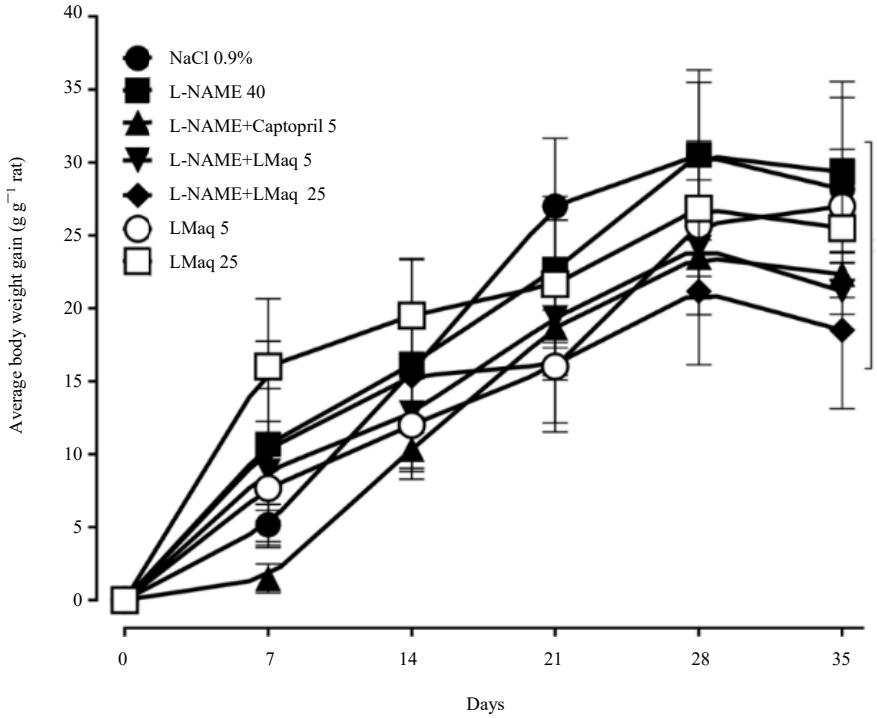

Fig. 2: Effect of LMaq on the average body weight gain of treated animals (n = 6)

LMaq or L-NAME alone and or concomitantly with L-NAME plus LMaq or Captopril for 5 weeks.

As presented in Fig. 1, no significant differences in SBP were observed between all groups during the week before treatments (Week 0: Day 1-7). Indeed, during inclusion (week 0:Day 1), all groups of animals had normal SBP which were of 105.10±5.79, 113.06±4.68, 106.51±4.51, 110.87±2.68, 110.12±2.03, 108.00±5.83, 110.60±2.92

mmHg for the lots NaCl 0.9%, L-NAME 40, L-NAME+Captopril 5, L-NAME+LMaq 5, L-NAME+LMaq 25, LMaq 5 and LMaq 25, respectively.

Likewise as shown in Fig. 1, on the 7th day (Week 0: Day 7) of taking systolic parameters, no significant change was observed in any animal group. The values were of 107.14±5.86, 108.00±5.83, 108.92±5.94, 111.83±5.50, 99.97±6.19, 107.99±6.43 and 106.03±8.53 mmHg, respectively for the groups NaCl 0.9%, L-NAME 40, L-NAME+ Captopril 5, L-NAME+LMaq 5, L-NAME+LMaq 25, LMaq 5 and LMaq 25.

Administration of L-NAME 40 for 2 consecutive weeks (days 7-21) caused a statistically significant increase in SBP of treated lots with values reaching 170.93±0.57, 171.17±0.42, 171.40±0.29 and 170.91±0.39 mmHg, respectively for the L-NAME 40, L-NAME+Captopril 5, L-NAME+LMaq 5 and L-NAME+LMaq 25 groups after the 2 weeks (day 21) compared to the normal control group (NaCl 0.9% group with 107.26±6.51 mmHg).

After hypertension installation in these groups of animals, concomitant treatment with L-NAME+Captopril 5 or L-NAME+LMaq 5 or further L-NAME+LMaq 25 brought their raised SBP values to normal pressure values respectively of 107.95±1.79, 111.45±5.43 and 110.66±2.05 mmHg. It should be noted that the return to these normal pressure values was observed after 4 days of treatment both in the presence of Captopril taken as a reference molecule and in the presence of LMaq 5 and LMaq 25 without significant difference.

In addition, no change in SBP was observed in the groups which received LMaq only (5 or 25) during the 4 weeks of treatment compared to the NaCl 0.9% control group.

Effect of LMaq on the heart rate of L-NAME-induced hypertensive rat: The average effect of LMaq on the heart rate of the study animals was shown in Table 1. Analysis of this Table 1 showed that the heart rate values (week 0: day 1-7) were 640.74±32.27 beats per minute (bpm), 411.88±21.59, 507.47±39.90, 565.02±64.63, 540.35±48.55, 551.05±46.32 and 524.26±28.02 bpm, respectively for the NaCl 0.9%, L-NAME, L-NAME+Captopril, L-NAME+LMaq 5, L-NAME+LMaq 25, LMaq 5 and LMaq 25 groups on the 7th day of inclusion.

A slight increase in the heart rate of the L-NAME 40 group was observed throughout the study (from 411.88±21.59 bpm to 527.68±32.07 bpm), likewise, a slight decrease in the heart rate of the NaCl 0.9% group has been noted (from 640.74±32.27 bpm to 508.91±45.21 bpm). However, no statistically significant variation in heart rate was observed in all treated lots compared to that of the NaCl 0.9% control lot.

LMaq effect on treated animals: The assessment of the impact of LMaq treatment was carried out during treatment. The parameters of concern were its impact on body mass, water consumption and food consumption. In addition, the impact of LMaq on the weight of noble organs and the biochemical parameters after sacrifice and autopsy were evaluated.

Effect of LMaq on the body weight gain: The change in body mass of different lots of control rats treated daily with NaCl 0.9%, L-NAME 40 and or LMaq (5 and 25) groups during the last 4 weeks of the experiment was shown in Fig. 3a-f. The results showed that there was no statistically significant difference in body mass gain between the 07 lots of rats during the study.

During inclusion, the averages weight of animals was 223.33±16.78, 216.17±18.17, 228.33±13.33, 221.00±21.33, 220.83±16.83, 224.17±18.22 and 218.00±9.33 g, respectively for NaCl 0.9%, L-NAME, L-NAME+Captopril 5 or L-NAME+LMaq 5 or even L-NAME+LMaq 25, LMaq 5 and LMaq 25 groups.

The average gains in weight (per g of rat) were 15.67±5.33 g, 16.17±11.17 g, 10.33±3.78 g, 12.83±8.17 g, 15.33±6.11 g, 12.00±6.00 g and 19.50±7.00 g and at the 2nd week of inclusion for the NaCl 0.9%, L-NAME, L-NAME+Captopril 5 or L-NAME+LMaq 5 or even L-NAME+ LMaq 25, LMaq 5 and LMaq 25, respectively. At the end of the 4th week of inclusion, these mean weight gains further increased by 30.50±9.67, 30.50±9.00, 23.50±5.50, 24.17±9.83, 21.17±8.22, 25.67±9.44 and 26.83±9.17 g, respectively for these respective lots compared to their weight 2 weeks before (Fig. 3).

Effect of LMaq treatment on animal water consumption: The daily water consumption measured for the 07 lots of rats during the 5 weeks of the study showed no statistically significant variation between the different treated groups compared to the normal control group (NaCl 0.9%). The daily water consumption (in mL/rat/day) of the lots of rats during the study period as shown in Table 2. In addition, no significant difference was observed between the different stages of weekly water consumption of the same lot from weeks 1-5.

Effect of LMaq treatment on food consumption: The average weekly food consumption of food for the lots of rats during the 5 weeks of the study was shown in Table 3. The food consumption of the different lots of the experiment was not modified by the different treatments. Indeed, there was no significant change in the average food consumption compared to the control NaCl 0.9% group.

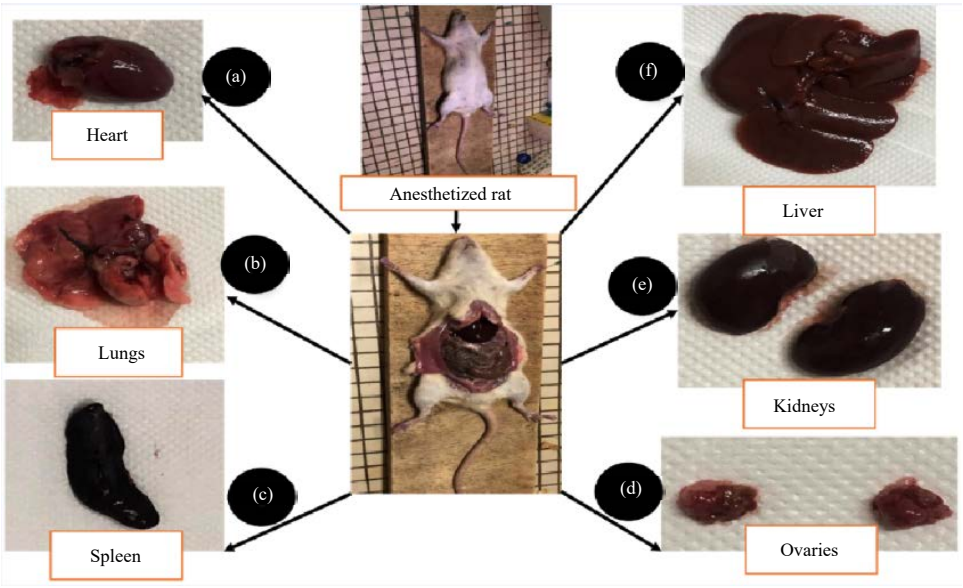

Fig. 3(a-f): Autopsy of a Wister rat showing the organs intact at the end of the experiment (a) Heart, (b) Lungs (c) Spleen (d) Ovaries, (e) Kidneys and (f) Liver

Table 2: Effect of LMaq on the mean daily water consumption of the treated animals (mL/rat/day) (n = 6)

	Weeks				
Treatments	1 (mL/rat/day)	2 (mL/rat/day)	3 (mL/rat/day)	4 (mL/rat/day)	5 (mL/rat/day)
NaCl 0.9%	10.48±7.50	40.48±4.64	36.31±7.87	35.71±7.93	35.12±7.48
L-NAME	47.02±3.96	45.71±6.64	43.45±7.55	36.90±8.82	39.88±5.10
L-NAME+Captopril 5	46.43±6.10	38.10±4.45	39.29±5.82	33.93±6.56	35.71±5.10
L-NAME-LMaq 5	41.90±6.83	36.90±5.06	42.86±5.22	38.69±5.75	33.33±8.33
L-NAME-LMaq 25	45.71±4.94	45.24±2.88	40.48±5.75	45.24±8.48	38.69±4.93
LMaq 5	42.38±3.06	42.26±3.75	44.05±5.82	41.67±9.32	40.48±5.78
LMaq 25	35.95±6.41	35.12±5.30	32.14±2.03	33.33±7.98	35.12±3.74

Table 3: Effect of LMaq on the average weekly food consumption of the study animals (g/rat/week) (n = 6)

	Weeks				
Treatments	1 (g/rat/week)	2 (g/rat/week)	3 (g/rat/week)	4 (g/rat/week)	5 (g/rat/week)
NaCl 0.9%	152.17	151.00	146.17	151.33	144.17
L-NAME	140.00	122.67	152.50	137.83	122.33
L-NAME+Captopril 5	148.00	164.33	158.17	147.83	144.50
L-NAME-LMaq 5	128.50	136.33	129.33	127.50	142.50
L-NAME-LMaq 25	128.17	131.33	133.17	119.83	129.33
LMaq 5	137.83	139.17	143.17	134.83	137.83
LMaq 25	128.00	131.17	127.00	121.00	123.67

Impact of the administration of LMaq on the appearance and average organs weight of rats after autopsy: At the end of the experiment, an autopsy was performed and Fig. 3a-f showed the appearance of the noble organs involved in hypertension, namely the heart, lungs, spleen, liver and ovaries of the studied animals. Macroscopic observation showed that there was no perceptible negative impact on these different organs, both in the NaCl 0.9% group and in the treated lots.

The mean target organ weight of control rats (NaCl 0.9%) and treated with L-NAME and or LMaq (5 and 25) of body weight was shown in Table 4. No statistically significant change was observed between the organs of the 06 treated rats groups compared to the control NaCl 0.9% group.

In addition, no alteration was macroscopically detectable on the different organs of the study lots. Indeed, any change in colour or appearance between the organs compared to those of the control NaCl 0.9% group was observed.

Table 4: Effect of LMaq on the mean relative weight of the different organs removed after 4 weeks of treatment (n = 6)

Organs	NaCl 0.9%	L-NAME	L-NAME+Captopril 5	L-NAME-LMaq 5	L-NAME-LMaq 25	LMaq 5	LMaq 25
Heart	0.81±0.12	0.67±0.09	0.70±0.06	0.84±0.08	0.82±0.13	0.88±0.19	0.78±0.06
Lungs	1.10±0.16	0.95±0.09	0.94±0.08	1.26±0.19	1.24±0.08	1.27±0.22	1.14±0.08
Kidneys	1.53±0.13	1.29±0.16	1.32±0.15	1.66±0.23	1.52±0.18	1.81±0.36	1.61±0.27
Liver	7.38±1.04	6.27±0.41	6.94±0.80	7.64±1.19	7.34±0.83	8.64±0.91	7.08±1.11
Spleen	0.54±0.09	0.45±0.09	0.46±0.07	0.69±0.15	0.72±0.13	0.70±0.30	0.53±0.15
Ovaries	0.10±0.02	0.08±0.02	0.08±0.02	0.14±0.06	0.12±0.02	0.14±0.04	0.14±0.03

Table 5: Effect of LMaq on the biochemical parameters hepatic, renal and electrolytes in animals' serum after 4 weeks of treatment

	Treatments						
Parameters	NaCl 0.9%	L-NAME	L-NAME+Captopril 5	L-NAME-LMaq 5	L-NAME-LMaq 25	LMaq 5	LMaq 25
Hepatic							
ASAT (µmol L^{-1})	189.83±14.33	206.67±13.56	206.33±24.90	171.50±24.60	175.17±60.59	227.20±62.81	229.67±76.08
ALAT (IU L^{-1})	66.50±10.41	90.50±6.75***	70.33±19.95##	77.17±23.50##	64.33±15.98###	73.40±24.29##	54.33±16.02##
CT (g L^{-1})	1.81±0.38	2.14±0.75	2.27±0.32*	2.43±0.42**	1.55±0.38	1.82±0.41	1.59±0.24
TG (mmol L^{-1})	0.31±0.17	0.31±0.18	0.38±0.16	0.37±0.08	0.36±0.12	0.48±0.17	0.35±0.17
Renal							
PT (g L^{-1})	67.35±7.73	71.08±5.46	80.25±6.82	72.38±14.83	72.08±9.30	82.48±12.73	77.23±11.13
CREAT (µmol L^{-1})	84.23±6.80	101.05±13.74	106.50±16.40**	92.52±14.18	67.10±13.90###•+	63.84±27.51*•###•+	70.65±8.80#•+
GLY (µmol L^{-1})	6.33±2.00	6.35±2.83	5.80±1.26	6.04±1.24	7.55±2.35	9.38±3.49	8.04±2.81
UREA (mmol L^{-1})	6.67±1.55	5.40±1.11	8.24±0.90***•#	7.08±1.39	6.95±1.75	6.73±1.15#	8.47±1.56
UA (µmol L^{-1})	151.25±84.69	123.66±64.33	163.05±51.86	172.16±45.99	187.68±91.62	253.92±72.60**•###•+	226.40±51.80**•###•+
Electrolytes							
Cl^- (mmol L^{-1})	116.83±5.12	122.33±7.74	130.67±9.16	128.17±17.57	123.50±11.26	126.20±9.52	127.83±6.15
PO_4^{2-} (mmol L^{-1})	4.64±0.90	4.94±0.87	4.37±0.88	4.37±1.08	4.46±1.79	5.92±0.42	5.95±1.85
Ca^{2+} (mg dL^{-1})	3.62±0.66	3.20±1.12	4.49±0.72***•#	4.38±0.24**	3.35±0.57+	3.57±0.42+	3.20±0.28+
Mg^{2+} (mmol L^{-1})	2.16±0.61	1.73±0.26***•	2.21±0.20*•##	2.27±0.40***•	2.40±0.28***•###	2.74±0.35###	2.70±0.37###

ASAT: Aspartate aminotransferase, ALAT: Alanine aminotransferase, CT: Total cholesterol, TG: Triglycerides, PT: Total protein, CREAT: Creatinemia, GLY: Glycemia, UREA: Urea, AU: Uric acid, Cl^-: Chlorine, PO_4^{2-}:Phosphorus, Ca^{2+}: Calcium ion, Mg^{2+}: Magnesium ion, *p<0.05, **p<0.01, ***p<0.001, #p<0.05, ##p<0.01, ###p<0.001, +p<0.05 and symbol±represents the accuracy of the measurement (n = 6)

Effect of LMaq treatment on serum biochemical parameters: The effects of the administration of LMaq on the biochemical parameters of the animals were listed in Table 5. Results showed that a daily oral administration for 4 weeks of LMaq at doses of 5 and 25 mg kg^{-1} body weight did not lead to significant changes in rat's blood serum parameters specially ASAT, TG and total proteins (PT), GLY, Cl^- and PHOS. However, at the level of certain parameters of liver function [alanine aminotransferase, total cholesterol (CT) or renal function (creatinine, urea, uric acid) and or electrolytes (calcium, magnesium), some statistically significant differences were observed compared to the control NaCl 0.9% group (Table 5).

In terms of liver function parameters (ASAT, ALAT, TG, CT), results showed no statistically significant difference in the levels of ASAT and serum TG were observed between the 06 treated lots compared to the control lot (NaCl 0.9%) (Table 5). However, there was a significant increase in the serum ALT level only in the L-NAME group (90.50±6.75 IU L^{-1}) compared to the NaCl 0.9% group (66.50±10.41 IU L^{-1}). Likewise, a significant difference was observed in the L-NAME+Captopril 5, NAME+LMaq 5, L-NAME+LMaq 25, LMaq 5 and LMaq 25 groups compared to the L-NAME group (Table 5).

Moreover, an increase in CT was observed in the lot which received L-NAME plus Captopril 5 (2.27±0.32 g L^{-1}) and as well as in the lot that received concomitantly L-NAME plus LMaq 5 (2.43±0.42 g L^{-1}) compared to the NaCl 0.9% group (1.81±0.38 g L^{-1}). In terms of renal function parameters (PT, CREAT, GLY, UREE, AU), the analysis showed no statistically significant difference in serum PT and GLY levels between the 06 treated groups compared to the control NaCl 0.9% group (Table 5). However, an increase in CREAT was noted in the L-NAME plus Captopril 5 group (106.50±16.40 µmol L^{-1}) compared to the lot NaCl 0.9% (84.23±6.80 µmol L^{-1}). These rates also remain higher than those of the other treated lots, for which there was no statistically significant difference compared to the NaCl 0.9% group. Moreover, an increase in Urea was also observed in the L-NAME plus Captopril group (8.24±0.90 mmol L^{-1}) compared to the NaCl 0.9% group (6.67±1.55 mmol L^{-1}). This rate and that of the L-NAME plus LMaq 5 also remain higher than the urea rate of the group that received only L-NAME. In addition, there is a slight decrease in AU but it's not statistically significant in the group that received only L-NAME (123.66±64.33 µmol L^{-1}) compared to the NaCl 0.9% group (151.25±84.69 µmol L^{-1}) as well as a statistically significant increase in the lots which received only

LMaq (5 mg kg^{-1}, 25 mg kg^{-1} with, respectively 253.92±72.60, 226.40±51.80 µmol L^{-1}). The UA levels of the LMaq (5, 25 mg) group also remain higher than those of groups that received only L-NAME or L-NAME combined with Captopril (Table 5).

In terms of electrolytes, results showed no statistically significant difference in the Cl^- and PHOS levels between the 06 treated lots compared to the control NaCl 0.9% group. However, an increase in Ca^{2+} was noted in the lot which received L-NAME combined with Captopril (4.49±0.72 mg dL^{-1}) and L-NAME combined with LMaq 5 (4.38±0.24 mg dL^{-1}) compared to the NaCl 0.9% group (3.62±0.66 mg dL^{-1}). The Ca^{2+} level of the L-NAME plus Captopril group also remains higher than those from the L-NAME, L-NAME+LMaq 25 and LMaq (5, 25 mg) groups. Moreover, a decrease in Mg^{2+} in the L-NAME 40 only (1.73±0.26 mmol L^{-1}) and an increase in the L-NAME plus captopril group (2.21±0.20 mmol L^{-1}), L-NAME plus LMaq (5, 25 mg) (2.27±0.40 and 2.40±0.28 mmol L^{-1}, respectively) compared to NaCl 0.9% group (2.16±0.61 mmol L^{-1}). The Mg^{2+} level in the group that received only L-NAME remains lower than those of the L-NAME+Captopril group, 25 mg L-NAME+LMaq and LMaq (5, 25 mg) (Table 5).

DISCUSSION

Lannea microcarpa is a plant widely used in traditional medicine in Burkina Faso, especially for the treatment of high blood pressure[16,17]. The lyophilized aqueous extract obtained from the bark of the trunk of this plant species has an endothelium-independent and antihypertensive vasodilator properties[18]. Note that hypertension corresponds to a pathological rise in blood pressure (BP). It is mainly due to two main causes which are the reduction in the size of the blood transport system and the increase in plasma volume. The latter are the consequences of three main factors: Excess angiotensin II (Ang II), catecholamines and ionic imbalance by hydro-sodium retention[22]. In this study, L-NAME was used for the induction of hypertension in normotensive Wistar rats. Indeed, studies have shown that inhibited chronically nitric oxide synthase by the L-NAME lead to hypertension and is a well-established model of experimental hypertension which causes organ damage within the cardiovascular system[19,23,24] and kidneys[20,25,26]. It should be remembered that studies have also shown that the acute administration of L-NAME is at the base of a surge of hypertension in experimental animals but in this case, the renin-angiotensin system is not used very much which augurs that in our case, the involvement way of Ang II was priority[27].

The present results showed that oral administration of L-NAME at 40 mg/kg/day has caused a significant increase in the BP of treated rats compared to those of the control NaCl 0.9 group. These results are following those of other authors which show that L-NAME is an inducer of hypertension and cardiovascular disease [28-31]. This could be explained by the fact that L-NAME inhibits the activity of endothelial Nitric Oxide Synthase (eNOS) responsible for an intracellular decrease in Nitric Oxide (NO). This action should be the basis of a decrease in the concentration of cyclic Guanosine Monophosphate (cGMP) which in turn causes a blockage of the reuptake of free calcium in the cells, a slowing of the secretion of renin and an increase in Ang II[32].

All these mechanisms increase the concentration of intracellular Ca^{2+} which lead to a cascade of events at the origin of vasoconstriction and therefore an increase in BP [33-36]. However, other scientific works have shown that the simple inhibition of NO production alone does not justify the mechanism of hypertension induced by L-NAME. Nevertheless, a consequent decrease in vasodilation function followed by an increase in contraction in different parts of the vascular tree due to several secondary messengers is considered the primary factor responsible for the increase in blood pressure parameters [37-39]. Moreover, studies have shown that overproduction of reactive oxygen species and/or prostaglandins of the eicosanoid superfamily were responsible for the genesis of L-NAME-induced hypertension. Also, uninterrupted and increased activity of the sympathetic system as well as that of the renin-angiotensin-aldosterone system, is known to be a good model for induction of hypertension[38,40]. However, the concomitant administration of LMaq (5 or 25) and L-NAME during the study caused a return of the SBP values to normal values only after 4 days of treatment in hypertensive rats compared to those in the control NaCl 0.9 group. This return to normal SBP values showed that LMaq would be able to reverse the hypertensive effects induced by L-NAME. These results could be attributable on the one hand to the endothelium-independent vasorelaxant properties of the LMaq extract insofar as authors have already shown that activity[41]. On the other hand, they are similar to those of other authors which reported that an ethyl acetate extract of *Lannea microcarpa* (obtained after fractionation of the aqueous extract of *Lannea microcarpa*) inhibited the hypertensive effects of Ang II in a model of arterial hypertension induced by Ang II and resulted in an inhibition of the onset of arterial hypertension in rats[18]. In addition, the action of the extract on Blood Pressure could be explained by the presence of chemical compounds in

LMaq, namely triterpenoids, coumarins, saponins, anthocyanins and tannins[18]. Indeed, to only cites two cas, authors have already shown that tannins are involved in the inhibition of the Ang II reconversion enzyme which increase the bioavailability of NO by providing a surplus of L-arginine. But also, that coumarins contribute to the thinning of the blood among others by their venotonic, coronary vasodilator and anticoagulant properties, which would facilitate blood circulation and therefore lower the BP of the arteries[42,43].

During the study, it has been also noted that captopril (5 mg/kg/day) inhibited the hypertensive effects induced by L-NAME which could be explained by its ability to inhibit the Ang II converting enzyme responsible for the reduction of systolic parameters in hypertensive rats. More interesting still, the antihypertensive profile of LMaq (5 or 25) is superimposed on that of captopril, suggesting that LMaq with only 5 mg kg^{-1} could be assimilated in terms of efficacy to that of captopril (5 mg kg^{-1}). Moreover, authors have already shown that captopril reverses the systolic parameters of arterial hypertension induced by L-NAME in just 24 hrs of administration, all of which have not yet been investigated for LMaq.

Assessment of the impact of LMaq on Wistar rats revealed that it did not cause significant changes in their body mass gain or in their water or food intake compared to that of the control group. These results were in line with those of the authors who had shown that the ethyl acetate fraction of the aqueous extract of *Lannea microcarpa* also had no impact on the indicators of the general condition of the animals such as body weight, water consumption and food consumption after daily oral administration for 4 weeks up to a dose of 1000 mg kg^{-1} [44]. In addition, analysis of the body mass weight values and the macroscopic aspect of the organs removed after autopsy have shown any significant difference compared to those of the control group, which would mean that LMaq did not have any noticeable negative impact on the kidney, heart, spleen, liver, ovaries and lungs of treated animals. These results are similar to those of other authors which had shown that LAMIC, a prototype antihypertensive drug based on LMaq, did not cause damage to vital organs after a 28 days subacute oral administration[17]. Likewise, the determination of the biochemical parameters of the blood serum obtained by cardiac puncture of the LMaq groups (5 and 25 mg kg^{-1}) did not show any significant change in the levels of electrolytes (Cl^-, PO_{42}^-), glycemia, on the hepatic function parameters (ALAT, ASAT) and certain renal function parameters (PT) compared to those of the control NaCl 0.9% group. This suggests that LMaq would not upset the ionic balance (Cl^-, PO_{42}^-), impaired liver function and impact total protein (PT) levels in kidney function or blood sugar. This ensures the safety of using LMaq. These results corroborate those of other authors which had shown that LAMIC did not induce notable effects on biochemical parameters such as total proteins, glucose, phosphate, chlorine and transaminases[17]. However, the present results found a significant increase in the levels of Ca^{2+}, Mg^{2+}, CREAT and AU in the groups received concomitantly, L-NAME plus Captopril compared to those of the NaCl 0.9% group. These results were similar to those of certain authors who reported that angiotensin II converting enzyme inhibitors including captopril would have diuretic effects and could increase levels of creatine, Ca^{2+}, Mg^{2+} in urea and AU[45]. LMaq (5 mg kg^{-1}) which showed an antihypertensive effect comparable to that of captopril (5 mg kg^{-1}) in this study could also be justified by its potential action on diuresis since a previous study had shown that this extract was endowed with a diuretic property[44].

Likewise, a significant increase in ALT and triglycerides was observed in lot given L-NAME 40 alone, which was in line with those of authors who claim that L-NAME increases the levels of TG and ALAT. More specifically, damage in liver tissue is known to be associated with the elevated serum ALT level. However, in the absence of L-NAME, LMaq has no impact on these parameters and more interestingly, this extract like captopril seems to correct this dysfunction.

CONCLUSION

In summary, the present study demonstrated preclinically, the antihypertensive effect of the lyophilized aqueous extract of *Lannea microcarpa* in L-NAME-induced hypertensive Wistar rat. The efficacy of LMaq at a dose of 5 mg kg^{-1} was comparable to that of Captopril 5 mg kg^{-1} which is an effective benchmark antihypertensive agent already used in hospitals. Thus, all data supported the use of *Lannea microcarpa* in traditional settings as an antihypertensive agent. In addition, they reinforce those already existing on the antihypertensive phytomedicine prototype LAMIC available and which is awaiting a clinical trial in humans.

SIGNIFICANCE STATEMENT

The bark of the trunk of *Lannea microcarpa* Engl. and K. Krause (Anacardiaceae) was widely used for the traditional treatment of hypertension. Several studies have already shown the preventive effect of angiotensin II induced-hypertensive in mice. For this extract LMaq from *Lannea macrocarpa,* Engl and K. Krause, no evaluation of its antihypertensive effect on a model of hypertension induced

by Nω-nitro-L-arginine methyl ester (L-NAME) has yet been carried out. Thus, this study constitutes the first time and hence its originality.

REFERENCES

1. Sarki, A.M., C.U. Nduka, S. Stranges, N.B. Kandala and O.A. Uthman, 2015. Prevalence of hypertension in low- and middle-income countries: A systematic review and meta-analysis. Medicine, Vol. 94. 10.1097/MD.0000000000001959.
2. Unger, T., C. Borghi, F. Charchar, N.A. Khan and N.R. Poulter *et al*., 2020. 2020 international society of hypertension global hypertension practice guidelines. Hypertension, 75: 1334-1357.
3. Abarca-Gomez, L., Z.A. Abdeen, Z.A. Hamid, N.M. Abu-Rmeileh and B. Acosta-Cazares *et al*., 2017. Worldwide trends in body-mass index, underweight, overweight and obesity from 1975 to 2016: A pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents and adults. Lancet, 390: 2627-2642.
4. Kaplan, J., D.M. Ward, R.J. Crisp and C.C. Philpott, 2006. Iron-dependent metabolic remodeling in *S. cerevisiae*. Biochim. Biophys. Acta (BBA)-Mol. Cell Res., 1763: 646-651.
5. Lim, S.S., T. Vos, A.D. Flaxman, G. Danaei and K. Shibuya *et al*., 2013. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: A systematic analysis for the global burden of disease study 2010. Lancet, 380: 2224-2260.
6. Bell, J.G., E.E. MacKinlay, J.R. Dick, D.J. MacDonald, R.M. Boyle and A.C.A. Glen, 2004. Essential fatty acids and phospholipase A_2 in autistic spectrum disorders. Prostaglandins Leukot. Essent. Fatty Acids, 71: 201-204.
7. World Health Organisation, 2021. Hypertension. https://www.who.int/news-room/fact-sheets/detail/ hypertension
8. Meissner, A., 2016. Hypertension and the brain: A risk factor for more than heart disease. Cerebrovascular Dis., 42: 255-262.
9. Ku, E., B.J. Lee, J. Wei and M.R. Weir, 2019. Hypertension in CKD: Core curriculum 2019. Am. J. Kidney Dis., 74: 120-131.
10. Fuchs, F.D. and P.K. Whelton, 2020. High blood pressure and cardiovascular disease. Hypertension, 75: 285-292.
11. Kearney, P.M., M. Whelton, K. Reynolds, P. Munter, P.K. Whelton and J. He, 2005. Global burden of hypertension: Analysis of worldwide data. Lancet, 365: 217-223.
12. Campbell, N.R.C., D.T. Lackland, L. Lisheng, X.H. Zhang and P.M. Nilsson, M.L. Niebylski and World Hypertension League Executive, 2015. The world hypertension league: Where now and where to in salt reduction. Cardiovasc. Diagn. Ther., 5: 238-242.
13. Kambiré, Y., G. Kinda, G.R.C. Milllogo, L. Konaté and I. Diallo *et al*., 2017. Contribution of ambulatory blood pressure monitoring to the management of arterial hypertension at Blaise Compaoré University Hospital. World J. Cardiovasc. Dis., 7: 442-450.
14. Soubeiga, J.K., T. Millogo, B.W. Bicaba, B. Doulougou and S. Kouanda, 2017. Prevalence and factors associated with hypertension in Burkina Faso: A countrywide cross-sectional study. BMC Public Health, Vol. 17. 10.1186/s12889-016-3926-8.
15. Noubiap, J.J., J.J. Bigna, U.F. Nyaga, A.M. Jingi, A.D. Kaze, J.R. Nansseu and J.F. Domgue, 2019. The burden of hypertensive disorders of pregnancy in Africa: A systematic review and meta-analysis. J. Clin. Hypertens., 21: 479-488.
16. Belemnaba, L., M. Nitiema, S. Traoré, N. Somé, A. Traoré, S. Ouédraogo and I.P. Guissou, 2014. Search for plants with antihypertensive potential in the biodiversity of Burkina Faso. Pharmacopoeia Traditional Afr. Med., 17: 33-40.
17. Lazare, B., S. Madi, G.O. Geoffroy, T.T. Kadiatou and N. Mathieu *et al*., 2019. Antioxidant properties and subchronic toxicity of the standardized extract of LAMIC, a phytomedicine prototype based on aqueous extracts from trunk bark of *Lannea microcarpa* Engl and K. krause. J. Drug Delivery Ther., 9: 1-8.
18. Nitiéma, M., M. Koala, L. Belemnaba, J.C.W. Ouédraogo and S. Ouédraogo *et al*., 2019. Endothelium-independent vasorelaxant effects of anthocyanins-enriched extract from *Odontonema strictum* (Nees) Kuntze (Acanthaceae) flowers: Ca^{2+} channels involvement. Eur. J. Med. Plants, Vol. 29. 10.9734/ejmp/2019/v29i330155.
19. Torres-Narváez, J.C., I. Pérez-Torres, V. Castrejón-Téllez, E. Varela-López and V.H. Oidor-Chan *et al*., 2019. The role of the activation of the TRPV1 receptor and of nitric oxide in changes in endothelial and cardiac function and biomarker levels in hypertensive rats. Int. J. Environ. Res. Public Health, Vol. 16. 10.3390/ijerph16193576.
20. Yadav, V.R., B. Teng and S.J. Mustafa, 2019. Enhanced A1 adenosine receptor-induced vascular contractions in mesenteric artery and aorta of in L-NAME mouse model of hypertension. Eur. J. Pharmacol., 842: 111-117.
21. Belemnaba, L., M. Nitiéma, S. Ilboudo, G.G. Ouédraogo and N. Ouédraogo*et al*., 2021. Preclinical evaluation of the antihypertensive effect of an aqueous extract of *Anogeissus leiocarpa* (DC) Guill et Perr. bark of trunk in L-NAME-induced hypertensive rat. J. Exp. Pharmacol., 13: 739-754.
22. Stanko, P., T. Baka, K. Repova, S. Aziriova and K. Krajcirovicova *et al*., 2020. Ivabradine ameliorates kidney fibrosis in L-NAME-induced hypertension. Front. Med., Vol. 7. 10.3389/fmed. 2020.00325.
23. Sonoda, K., K. Ohtake, H. Uchida, J. Ito and M. Uchida *et al*., 2017. Dietary nitrite supplementation attenuates cardiac remodeling in L-NAME-induced hypertensive rats. Nitric Oxide, 67: 1-9.

24. Babál, P., O. Pechánová, I. Bernátová and S. Stvrtina, 1997. Chronic inhibition of NO synthesis produces myocardial fibrosis and arterial media hyperplasia. Histol. Histopathol., 12: 623-629.
25. Holécyová, A., J. Török, I. Bernátová and O. Pechánová, 1996. Restriction of nitric oxide rather than elevated blood pressure is responsible for alterations of vascular responses in nitric oxide-deficient hypertension. Physiol. Res., 45: 317-321.
26. Ignarro, L.J., 2002. Nitric oxide as a unique signaling molecule in the vascular system: A historical overview. J. Physiol. Pharmacol., 53: 503-514.
27. Nyadjeu, P., E.P. Nguelefack-Mbuyo, A.D. Atsamo, T.B. Nguelefack, A.B. Dongmo and A. Kamanyi, 2013. Acute and chronic antihypertensive effects of *Cinnamomum zeylanicum* stem bark methanol extract in L-NAME-induced hypertensive rat. BMC Complementary Altern. Med., Vol. 13. 10.1186/1472-6882-13-27.
28. Campbell, D.J., 2006. L-NAME hypertension: Trying to fit the pieces together. J. Hypertens., 24: 33-36.
29. Tarsitano, C.A.B., V.A. Paffaro, J.R. Pauli, G.H. da Silva and M.J. Saad *et al*., 2007. Hepatic morphological alterations, glycogen content and cytochrome P450 activities in rats treated chronically with N^{ω}-nitro-L-arginine methyl ester (L-NAME). Cell Tissue Res., 329: 45-58.
30. Chen, J.X., K.Y. Xue, J.J. Xin, X. Yan and R.L. Li *et al*., 2019. 5-Lipoxagenase deficiency attenuates L-NAME-induced hypertension and vascular remodeling. Biochim. Biophys. Acta (BBA)-Mol. Basis Dis., 1865: 2379-2392.
31. Rajeshwari, T., B. Raja, J. Manivannan, T. Silambarasan and T. Dhanalakshmi, 2014. Valproic acid prevents the deregulation of lipid metabolism and renal renin-angiotensin system in L-NAME induced nitric oxide deficient hypertensive rats. Environ. Toxicol. Pharmacol., 37: 936-945.
32. Zhao, Y., S. Flavahan, S.W. Leung, A. Xu, P.M. Vanhoutte and N.A. Flavahan, 2015. Elevated pressure causes endothelial dysfunction in mouse carotid arteries by increasing local angiotensin signaling. Am. J. Physiol. Heart Circulatory Physiol., 308: H358-H363.
33. Fardella, M.D.C. and J.A. Rodriguez-Portales, 1995. Intracellular calcium and blood pressure: Comparison between primary hyperparathyroidism and essential hypertension. J. Endocrinol. Invest., 18: 827-832.
34. Alexandraa, S., M. Alexandraa, S. Franziskaa, K. Fadia, V. Rolandb, Z. Waltera and T. Martin, 2005. Sustained increase of extracellular calcium concentration causes arterial vasoconstriction in humans. J. Hypertens., 23: 2049-2054.
35. Amberg, G.C. and M.F. Navedo, 2013. Calcium dynamics in vascular smooth muscle. Microcirculation, 20: 281-289.
36. Zhu, Y., J. Qu, L. He, F. Zhang, Z. Zhou, S. Yang and Y. Zhou, 2019. Calcium in vascular smooth muscle cell elasticity and adhesion: Novel insights into the mechanism of action. Front. Physiol., Vol. 10. 10.3389/fphys.2019.00852.
37. Wilson, C., X. Zhang, C. Buckley, H.R. Heathcote, M.D. Lee and J.G. McCarron, 2019. Increased vascular contractility in hypertension results from impaired endothelial calcium signaling. Hypertension, 74: 1200-1214.
38. Bunaim, M.K., Y. Kamisah, M.N.M. Mustazil, J.S.F. Zuhair, A.H. Juliana and N. Muhammad, 2021. *Centella asiatica* (L.) Urb. prevents hypertension and protects the heart in chronic nitric oxide deficiency rat model. Front. Pharmacol., Vol. 12. 10.3389/fphar.2021.742562.
39. Brown, I.A.M., L. Diederich, M.E. Good, L.J. DeLalio and S.A. Murphy *et al*., 2018. Vascular smooth muscle remodeling in conductive and resistance arteries in hypertension. Arteriosclerosis Thrombosis Vasc. Biol., 38: 1969-1985.
40. Guimarães, D.D., J.C. Cruz, A. Carvalho-Galvão, Z. Zhuge and S.M. Marques *et al*., 2019. Dietary nitrate reduces blood pressure in rats with angiotensin II-induced hypertension via mechanisms that involve reduction of sympathetic hyperactivity. Hypertension, 73: 839-848.
41. Ouedraogo, S., L. Belemnaba, H. Zague, A. Traoré and M. Lompo *et al*., 2010. Endothelium-independent vasorelaxation by extract and fractions from *Lannea microcarpa* Engl. and K. Krause (Anacardiaceae): Possible involvement of phosphodiesterase inhibition. Int. J. Pharmacol., 4: 9-16.
42. Venugopala, K.N., V. Rashmi and B. Odhav, 2013. Review on natural coumarin lead compounds for their pharmacological activity. BioMed Res. Int., Vol. 2013. 10.1155/2013/963248.
43. Banikazemi, Z., S.M. Mirazimi, F. Dashti, M.R. Mazandaranian and M. Akbari *et al*., 2021. Coumarins and gastrointestinal cancer: A new therapeutic option? Front. Oncol., Vol. 11. 10.3389/fonc.2021.752784.
44. Mathieu, N., B. Lazare, O. Salfo, O. Noufou, O. Sylvin and G.I. Pierre, 2018. Diuretic activity of aqueous decoction extract andethyl acetate fraction of *Lannea microcarpa* Engl. and K. Krause (Anacardiaceae) trunk barks in wistar rats. World J. Pharm. Res., 7: 39-51.
45. Leary, W.P., A.J. Reyes, T. Acosta-Barrios and B. Maharaj, 1985. Captopril once daily in patients with essential hypertension and hyperuricaemia. S. Afr. Med. J., 68: 642-644.