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Antihypertensive effect of the stem bark aqueous extract of *Garcinia lucida* Vesque (Clusiaceae) in L-NAME-treated rats: Contribution of endothelium-dependent and -independent vasorelaxation

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ABSTRACT

Garcinia lucida is used in Cameroonian folk medicine to handle a variety of ailments, including arterial hypertension. This study aimed at determining the phytochemical profile and the antihypertensive effect of the stem bark aqueous extract of *G. lucida* (AEGL). AEGL was subjected to LC-MS analysis, and its effect (75, 150, and 300 mg/kg/day; by gavage) was evaluated against Nω-nitro-L-arginine methyl ester (L-NAME; 40 mg/kg)-induced hypertension in adult male Wistar rats for four consecutive weeks. Blood pressure and heart rate were monitored weekly using tail-cuff plethysmography. The vasorelaxant effect of cumulative concentrations (3–10–30–100–300 μg/mL) of AEGL was examined on endothelium-intact and denuded thoracic aorta rings which were precontracted with KCl (90 mM) or norepinephrine (NE; 10^{-5} M), and in the absence or presence of L-NAME (10^{-4} M), indomethacin (10^{-5} M), methylene blue (10^{-6} M), tetraethylammonium (TEA, 5×10^{-6} M), glibenclamide (10×10^{-6} M) or propranolol (5×10^{-6} M). The influence of AEGL on the response to NE, KCl, and $CaCl_2$ was also investigated. Six compounds, including Garcinia biflavonoids GB1 and GB2, were identified. AEGL prevented the development of hypertension ($p < 0.01$ and $p < 0.001$) without affecting the heart rate. AEGL induced a concentration-dependent relaxation of aortic rings precontracted with NE ($EC_{50} = 7.915$ μg/mL) that was significantly inhibited by the removal of the endothelium, L-NAME, or methylene blue ($p < 0.05$–0.001). Indomethacin, propranolol, TEA, and glibenclamide did not affect AEGL-evoked vasorelaxation. Preincubation of aortic rings with AEGL reduced the magnitude of contraction elicited by $CaCl_2$ but did not alter that of KCl or NE. AEGL possesses an antihypertensive effect that is mediated by both endothelium-dependent and endothelium-independent mechanisms. The activation of the NO/sGC/cGMP pathway accounts for the endothelium-dependent vasorelaxation. These pharmacological effects of AEGL could be attributed to the presence of the Garcinia biflavonoids GB1 and GB2.

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1. Introduction

Arterial hypertension (AHT) is a common and highly prevalent condition that continuously degrades the health of patients, and thus represents a true socio-economic plague. It is one of the foremost causes of death and disability globally, with a considerable impact in Sub-Saharan African countries [1]. It has been reported that people of African descent are more likely to have AHT, target organ damage, and cardiovascular mortality at a younger age than other ethnic groups [1,2]. Worse, the burden of AHT has switched from high-income countries to low- and middle-income countries, including Sub-Saharan Africa, for the past few decades [3]. In Cameroon, the actual prevalence is estimated to be 31.1% [4].

AHT is an important risk factor for the occurrence of several diseases like heart failure, chronic kidney disease, coronary artery diseases, atherosclerosis, myocardial infarction and stroke [5]. Vascular tone, which is directly affected by the vascular endothelium, is a crucial element in blood pressure control [6]. Structural and functional alterations at the vascular level characterized by endothelial dysfunction play a paramount role in the development of AHT [7]. Endothelial dysfunction occurs as a result of an imbalance between vasoconstrictor and vasorelaxant factors produced by the endothelium in favor of the vasoconstrictors and is responsible for increased peripheral resistance [7,8]. Among the vasorelaxant factors released by the endothelium, nitric oxide (NO) is the most important and the decrease in its bioavailability is considered as the main cause of endothelial dysfunction [7]. Chronic inhibition of NO production by N^{ω}-nitro-L-arginine methyl ester (L-NAME) is an experimental animal model that perfectly mimics this clinical condition of essential hypertension [9–11]. As such, the model is widely used for the evaluation of antihypertensive properties of therapeutics candidates.

Adequate reduction of AHT to non-hypertensive levels is known to reduce the risk for cardiovascular events and all-cause mortality by 20%–40% [12]. However, in many Sub-Saharan Africa countries, including Cameroon, AHT is still poorly controlled mainly because most African hypertensive patients are resistant to pharmacological interventions [1]. In addition, people living in remote areas have poor or no access to medical care and medication. Hence, justifying their reliance on medicinal plants for their primary health care. These plants, due to the large number of secondary metabolites they possess, could serve as potential sources of new antihypertensive drugs.

Garcinia lucida Vesque (Clusiaceae) is commonly known as “essok” in Cameroon. It is a small evergreen dioecious tree, reaching 12–15 m in height and 25–30 cm in diameter at breast height [13]. In Cameroonian folk medicine, *G. lucida* is used to cure stomach ache, gynecological pains, snake bites, and to prevent food poisoning [13]. It is also used for the management of hypertension [14]. *G. lucida* has been reported to display antimicrobial, cytotoxic, and *in vitro* antioxidant activities [15,16]. In a previous study, it was shown that the aqueous extract of *G. lucida* was capable of preventing the increase of blood pressure in adenine-induced chronic kidney disease in rats [17]. Whether *G. lucida* can be effective against a model of hypertension characterized by endothelial dysfunction is unknown. The present study sought to evaluate the effect of the stem bark aqueous extract of *G. lucida* against N^{ω}-nitro-L-arginine methyl ester (L-NAME)-induced hypertension in rats and to further determine the related mechanisms of action.

2. Materials and methods

2.1. Reagents

Roth supplied D (+)-glucose and sodium chloride (NaCl). L-NAME was bought from Enzo Life Sciences (Switzerland). The following items were acquired from Sigma-Aldrich: potassium chloride (KCl), calcium chloride ($CaCl_2$), magnesium sulfate ($MgSO_4$), sodium hydrogenocarbonate ($NaHCO_3$), magnesium chloride ($MgCl_2$), methylene blue, norepinephrine, carbachol, propranolol, indomethacin, and glibenclamide (Germany). The supplier of captopril was Denk Pharma GmbH & Co. (Germany). Fluka provided the tetraethylammonium (TEA), EGTA, and ethyl carbamate.

2.2. Plant collection and extraction

The fresh bark of *Garcinia lucida* was harvested in the South Region of Cameroon, precisely at Mvengue. A plant sample was deposited at the Cameroon national herbarium and authenticated by comparison with a previously collected and registered voucher specimen (57193/HNC). The shade-dried bark was finely minced into powder and served for the preparation of the aqueous extract of *G. lucida* (AEGL) according to Sonfack et al. [17].

2.3. Liquid chromatography–mass spectrometry (LC-MS) analysis

A high-resolution mass spectra using QTOF spectrometer (Bruker, Germany) outfitted with a HESI source was used to determine the phytochemical content of AEGL. The Na formate was used as a calibrant, and the spectrometer was run in positive mode (mass range: 100–1500, scan rate: 1.00 Hz) with automatic gain control to give high-accuracy mass readings within 0.40 ppm variation. A 200 °C capillary temperature and a 4.5 kV spray voltage were used. As a sheath gas, nitrogen was employed at a flow rate of 10 L/min. Thermo Fisher’s Ultimate 3000 UHPLC system, which includes an LC-pump, Diode Array Detector (DAD) (: 190–600 nm), autosampler (injection volume: 10 mL), and column oven (40 °C), was coupled to the spectrometer. The separation was conducted using a Synergi MAX-RP 100A (50 × 2 mm, 2.5 m particle size) with a H_2O (containing 0.1% formic acid) (A)/acetonitrile (containing 0.1% formic acid) (B) gradient (flow rate 500 μL/min, injection volume 10 μL). The following gradient program was used to analyze the samples: the system was in an isocratic state at 95% A for 1.5 min, followed by a linear gradient to 100% B over 6 min, an isocratic state at 100%

B for 2 min, then return to 90% A for 1 min, and 1 min of equilibration.

2.4. Animals

Male Wistar rats aged 3–4 months and weighing 170–250 g were used for the determination of the antihypertensive effect, while rats of both sexes, the same strain, weight, and age were used for vasorelaxant activities. They were raised at the animal house of the Laboratory of Animal Physiology and Phytopharmacology, University of Dschang (Cameroon), with free access to standard laboratory feed and water. All animal procedures were done in accordance with the standard ethical guidelines for animal use and care as described by the law 2010/63/EU on the protection of animals used for scientific purposes and approved by the institutional ethical committee of the University of Douala (N° 2043CEI-UDo/06/2019/T).

2.5. Evaluation of the antihypertensive effects of Garcinia lucida

Male rats were randomly assigned into six groups of 6 rats each and treated for four consecutive weeks as follows:

group 1: Naive distilled water (10 mL/kg) once a day,
group 2: L-NAME,
group 3: L- NAME + captopril 20 mg/kg,
group 4: L- NAME + AEGL 75 mg/kg,
group 5: L-NAME + AEGL 150 mg/kg,
group 6: L-NAME + AEGL 300 mg/kg.

L-NAME was used at the dose of 40 mg/kg/day and was concomitantly administered by gavage with other drugs. The extract dosages were estimated as follows: the equivalent daily dose of *G. lucida* traditionally used for an adult weighing an average of 60 kg was found to be 24.19 mg/kg. This dose was multiplied by 6.2, which is the Km ratio used to convert the rat dose to the human dose according to Nair and Jacob [18]. The dose obtained for rats was 149.98 mg/kg/day (≈150 mg/kg). This dose was further divided by 2 to obtain the lower dose (75 mg/kg) and multiplied by 2 to obtain the higher dose (300 mg/kg).

Animals were acclimatized for three consecutive weeks (once a week) to blood pressure and heart rate recording. Thereafter, blood pressure and heart rate were recorded at baseline and at the end of each experimental week using non-invasive tail-cuff plethysmography (IITC Life Science MRBP tail-cuff blood pressure system). On day 28 of the experiment, animals were treated and placed in metabolic cages for the 24-h urine sample collection. After measuring the volume, urine samples were centrifuged at 3000 rpm for 10 min at 4 °C (Loncare TGL-16 M refrigerated centrifuge). The supernatant was aliquoted and stored at −20 °C for the determination of Na^+, K^+, creatinine, and proteins. On day 29, animals were anesthetized with ethyl carbamate (1.5 g/kg, i.p.). A blood sample was collected via the abdominal aorta in tubes previously coated with heparin and centrifuged at 3000 rpm/min for 15 min. Plasma was collected and stored at −20 °C for the measurement of creatinine. The heart, thoracic aorta, and kidneys were excised, freed of fats and connective tissues, washed in physiological saline, and weighed.

2.6. Biochemical analysis

Na^+, K^+, and Cl^- were determined using an electrolyte analyzer (Biolyte 2000, Na^+, K^+, Cl^-, and Li^+). A kinetic variant of the Jaffe method was used to assay plasma and urine creatinine level. Optical densities were read at 492 nm, 30 and 90 s after mixing 100 μL of sample with 1 mL of Jaffe reagent. Glomerular filtration rate (GFR) was estimated using creatinine clearance according to the following formula:

$$GFR = U \times V/P \times 1440$$

U = urine creatinine, V = 24 h urine volume, P = plasma creatinine and 1440 = time in seconds corresponding to 24 h.

2.7. In vitro vasorelaxant effect of the aqueous extract of Garcinia lucida

2.7.1. Preparation of rat isolated aortic rings

The aorta rings were harvested and mounted as previously described by Nguelefack-Mbuyo et al. [19]. Rats were euthanized via cervical dislocation, and the thoracic aorta was quickly and meticulously dissected out and put in physiological Krebs solution at 37 °C. After removing fat and connective tissue, the aorta was cut into rings of approximately 3–4 mm in length. In some experiments, a tiny catheter was used to gently rub the arterial lumen interior to mechanically remove the endothelium. Five aortic rings from five separate animals were used in each experiment. The aortic rings were hung on two stainless hooks in a 10 mL organ chamber maintained at 37 °C and filled with aerated Krebs solution (95% O_2 and 5% CO_2; pH = 7.4) with the following composition (in mM): NaCl 118.0, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 11.1. A force-displacement transducer (MDE, Heidelberg) was used to measure tension changes isometrically with a 1.5 g resting tension applied to the ring. The transducer was coupled to a computer equipped with a SPELL Advanced Kymograph Data Acquisition software (MDE Heidelberg, Germany). A vessel ring was equilibrated for 60 min during which the physiological Krebs solution was changed every 15 min. Prior to each experiment, the

functional integrity of the endothelium was assessed by examining the vasorelaxant response to 10^{-5} M carbachol on aortic ring precontraction with 10^{-5} M norepinephrine (NE). The endothelium was considered intact when more than 80% relaxation was obtained and destroyed when carbachol failed to induce vasorelaxation.

2.7.2. Effect of Garcinia lucida on norepinephrine or KCl-induced contraction

After the assessment of the functional integrity of the endothelium, the aorta ring was washed and precontracted with NE (10^{-5} M) or KCl (90 mM) and challenged with cumulative concentrations (3-10-30-100-300 μg/mL) of *G. lucida* after a steady contraction. The effect of each concentration was examined until it reaches a plateau before adding the next concentration. Carbachol at a concentration range of 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} M and nifedipine (10^{-9}, 3×10^{-8}, 10^{-7}, 3×10^{-7}, and 10^{-6} M) were used as reference drugs.

2.7.3. Involvement of endothelial factors

To assess the role of endothelium-derived relaxing factors, two experimental sets were performed: on the one hand, the effect of *G. lucida* was examined on endothelium-denuded aortic rings precontracted with NE. On the other hand, the effect of AEGL was evaluated on intact aortic rings preincubated with L-NAME (a nitric oxide synthase inhibitor, 10^{-4} M), indomethacin (a cyclooxygenase inhibitor, 10^{-6} M), or methylene blue (a soluble guanylyl cyclase inhibitor, 10^{-5} M), before contraction with NE. Rings were preincubated with each inhibitor for 20 min, then contracted with NE [20] before being challenged by cumulative concentrations of AEGL.

2.7.4. Contribution of adrenergic receptors

These sets of experiments examined whether AEGL could serve as an antagonist of the alpha-1 or an agonist of the beta-2 adrenergic receptors. For alpha-1 antagonist testing, the concentration-response curve to NE (10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} M) was constructed in the presence or absence of AEGL (7.91 or 15.83 μg/mL, corresponding to EC_{50} and 2xEC_{50}, respectively) or moxisylyte (an α1-adrenergic antagonist, 10^{-6} M). Aortic rings were exposed for 20 min to the tested substances, before being challenged with cumulative concentrations of NE. To test the interaction with the beta-2 adrenergic receptors, the vasorelaxant effect of AEGL was examined in the presence of propranolol (a β-adrenergic receptors blocker, 5×10^{-6} M). Aortic rings were incubated with propranolol for 20 min before NE-induced contraction [21].

2.7.5. Effect of AEGL on potassium channels and calcium influx

To assess the involvement of K^+ channels, endothelium-denuded aortic rings were incubated with TEA (a nonselective K^+ channel blocker, 5×10^{-6} M) and glibenclamide (a K_{ATP} channel blocker, 10×10^{-6} M) for 20 min prior to contraction with NE. Cumulative concentrations of the plant extract were then added to the medium [22].

In the second set of experiments, the contractile response to KCl (90 mM) was measured in the presence or absence of 18.01 μg/mL of *G. lucida* extract. This concentration of the extract corresponds to the EC_{50} value obtained previously during the investigation of the vasorelaxant effect of the plant extract on rings precontracted with KCl [23]. To test the effect of AEGL on calcium influx, endothelium-denuded aortic rings were initially contracted with KCl (90 mM) in a normal Krebs solution. The contraction obtained was used as a reference and considered to be 100%. Rings were then washed and incubated in a Ca^{2+}-free hyperpotassic Krebs solution containing 1 mM ethylene glycol tetraacetic acid (EGTA) for 30 min to remove residual calcium from the tissue. While proceeding, the solution was changed every 10 min. After this, the concentration-response curve to $CaCl_2$ (0.75×10^{-3}, 1.5×10^{-3}, 3×10^{-3}, 6×10^{-3}, and 12×10^{-3} M) was constructed in the absence or presence of the plant extract (18.01 μg/mL) or nifedipine (10^{-5} M) [22].

2.8. Statistical analysis

GraphPad Prism 8.4.2. software was used to perform statistical analysis. All the results were expressed as means ± standard error of the mean. The mean ± SEM were used to express all the results. Heart rate, organ weight, Na^+, and K^+ data collected at a single time point were analyzed using a one-way ANOVA and the Tukey post hoc test. Data collected repeatedly like blood pressure were analyzed with a two-way ANOVA with repeated measures followed by a Bonferroni post hoc test. Relaxation responses were expressed as the percentage reversal of the force generated by the contractile agent. EC_{50} was determined after the logarithmic transformation of the concentration-response curve. Data with two independent variables (concentration-response relationships) were analyzed with a two-way ANOVA followed by Bonferroni's post-test, except data on the inhibitory effect of the plant extract against KCl-induced contraction, which was analyzed using a paired Student "t" test. The statistical significance level was set at $p < 0.05$.

3. Results

3.1. LC-MS analysis

Compounds identified by LC-MS analysis are presented in Fig. 1. The combination of retention time (RT), data from mass spectrometry and the literature allows the identification of a total of 6 compounds in the aqueous extract of *G. lucida*. They were mainly flavonoids, xanthones and terpenes. Compound **1** (RT 2.9 min, [M+H]+*m/z* 465.1734) was identified as isomucronulatol-7-O-β-D-glucoside [24], compound 2 (RT 3.6 min, [M+H]+*m/z* 575.1199) was identified as 3,8″-Binaringenin [25], compound **3** ([M+H]+*m/z* 575.1193) showed a spike at 3.7 min and was identified as Garcinia biflavonoid 2 (GB2) [26,27], compound **4** appears at the RT 3.8

min with a [M+H]+*m/z* of 559.1251 and was identified as Garcinia biflavonoid 1 (GB1) [26,27], compound **5** (RT 4.1 min, [M+H]+ *m/z* 573.1041) was identified as 2,3-Dihydro-6,6‴biluteolin commonly known as Hegoflavone B [28], and compound **6** ([M+H]+*m/z* 443.3874) showed a spike at 5.7 min and was identified as Butelin [29].

3.2. Effects of aqueous extract of Garcinia lucida extract on blood pressure

As depicted in Fig. 2A and C, the chronic oral administration of L-NAME alone induced a progressive and significant ($p < 0.001$) increase in rat systolic (SBP), diastolic (DBP), and mean blood pressure (MABP). Concomitant treatment with AEGL significantly inhibited the rise in blood pressure elicited by L-NAME only at doses of 75 and 300 mg/kg. To have an overview of the global activity of each treatment, the area under the curve was calculated. Results in Fig. 2B and D shows that L-NAME treatment drastically increased SBP, DBP, and MABP. Only the minimal and maximal doses of AEGL used in this study were able to reduce this blood pressure surge ($p < 0.05$ and $p < 0.01$) with the dose of 300 mg/kg being the most effective. Captopril used as the reference drug partially inhibits the development of hypertension in L-NAME-treated rats ($p < 0.01$ and $p < 0.001$) (Fig. 2B and D).

3.3. Effects of the aqueous extract of Garcinia lucida on heart rate, organs' weight and renal function

No significant ($p > 0.05$) change in heart rate was observed among the different experimental groups, as shown in Table 1. Long-term L-NAME administration resulted in a 59.60% increase in aorta mass ($p < 0.01$). Treatment with the plant extract mitigated this effect. Indeed, the plant extract at the dose of 75 mg/kg significantly ($p < 0.05$) prevented the increase in aorta mass. Regardless of the treatment regimen, the heart mass did not change. When administered alone, L-NAME induced a non-significant ($p > 0.05$) increase in kidney mass. Groups that received L-NAME in combination with the plant extract or captopril, had a normal kidney mass (Table 1). With regard to renal function, L-NAME neither when administered alone nor when combined with the plant extract or captopril significantly affected the urinary volume, natriuresis, kaliuresis, chloruresis, and GFR (Table 2).

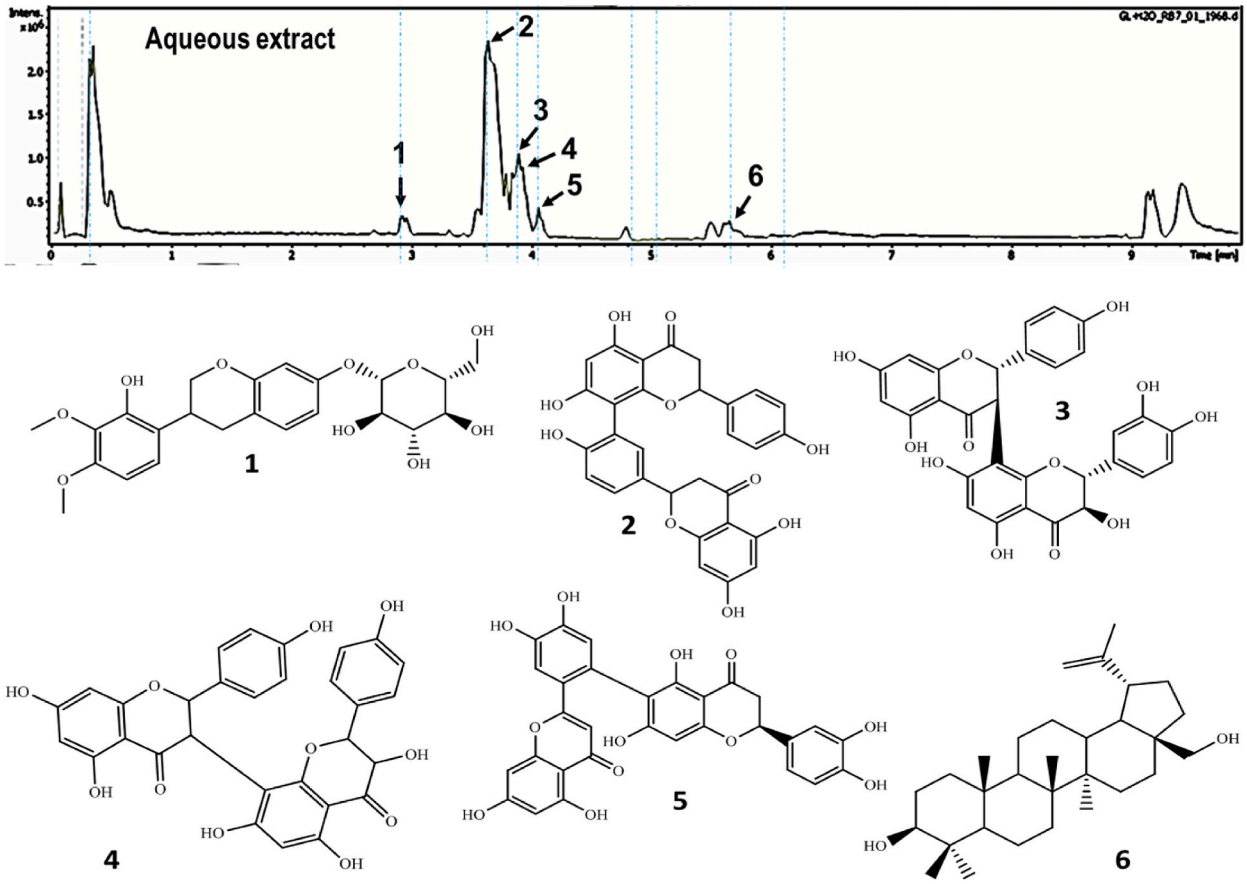

Fig. 1. Phytochemical analysis of aqueous extract from the stem bark of *Garcinia lucida* showing the total ion chromatograms and 6 compounds likely present in the plant extracts. 1: Isomucronulatol-7-*O*-*β*-*D*-glucopyranoside; 2: 3″,8″-Binaringenin; 3: Garcinia biflavonoid 2 (GB2); 4: Garcinia biflavonoid 1 (GB1); 5: Hegoflavone B (2,3-Dihydro-6,6″ biluteolin); 6: Butelin.

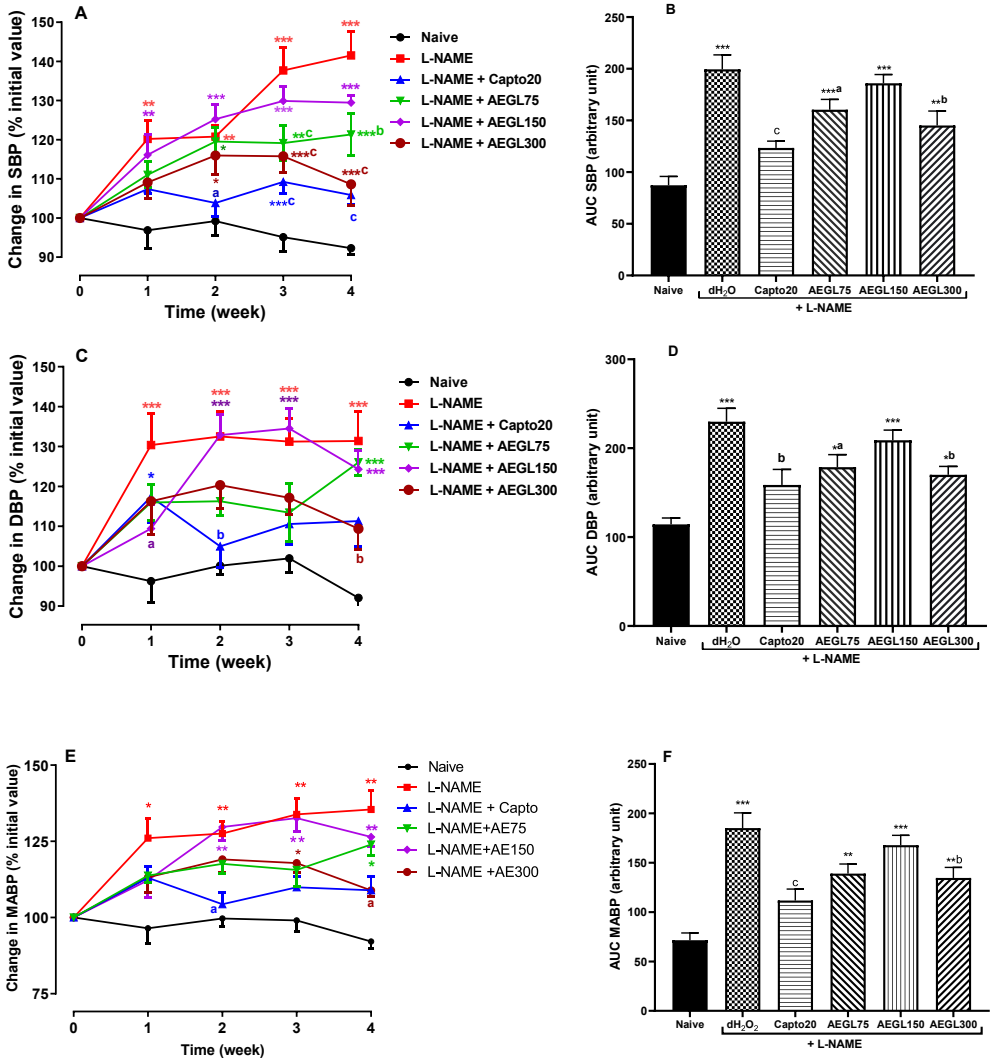

Fig. 2. Antihypertensive effect of aqueous extract from the stem bark of *Garcinia lucida* on systolic (A and B), diastolic (C and D), and mean (E and F) blood pressure in rats concomitantly treated with L-NAME. All values are expressed as mean ± SEM (n = 6). *p < 0.05, **p < 0.01, ***p < 0.001 compared to naive group. [a]p < 0.05, [b]p < 0.01, [c]p < 0.001 compared to L-NAME group Data were analyzed with two-way ANOVA with repeated measures followed by Bonferroni post-test. Capto: captopril; AE: Aqueous extract; the numbers represent the different doses used, AUC: Area under curve.

Table 1
Effects of the aqueous extract of the stem bark of *Garcinia lucida* on heart rate and organs' mass.

Experimental groups	Heart rate (Beats/min)	Aorta weight (mg/100 g BW)	Heart weight (g/100 g BW)	Kidney weight (g/100 g BW)
Naive	358.5 ± 9.77	20.47 ± 1.27	0.32 ± 0.01	0.57 ± 0.01
L-NAME	370.50 ± 11.43	32.67 ± 1.24***	0.33 ± 0.01	0.62 ± 0.01
L-NAME + captopril (20 mg/kg)	372.80 ± 15.10	28.09 ± 2.87	0.29 ± 0.01[a]	0.57 ± 0.01
L-NAME + aqueous extract (75 mg/kg)	365.70 ± 16.98	24.54 ± 1.03[a]	0.32 ± 0.01	0.57 ± 0.02
L-NAME + aqueous extract (150 mg/kg)	365.10 ± 14.12	28.23 ± 2.53*	0.32 ± 0.02	0.57 ± 0.02
L-NAME + aqueous extract (300 mg/kg)	358.6 ± 7.30	26.37 ± 0.77	0.31 ± 0.01	0.59 ± 0.01

All values are expressed as mean ± SEM. (n = 6) *p < 0.05, ***p < 0.001 significant difference compared to Naïve. [a]p < 0.05 significant difference compared to L-NAME group. Data were analyzed with one-way ANOVA, followed by Tukey's multiple comparison test.

3.4. Effects of the aqueous extract from Garcinia lucida against norepinephrine and KCl-induced contraction

AEGL induced a concentration-dependent relaxation of intact aortic rings precontracted with NE or KCl with respective EC_{50} values of 7.92 μg/mL and 18.01 μg/mL. It is worth noticing that the plant extract was more effective against NE-induced contraction than KCl-induced contraction. The maximum effect (E_{max}) obtained was 99.87 ± 2.63% and 29.73 ± 3.70% respectively, for NE and KCl-evoked contraction (Fig. 3A and Table 2). As depicted in Fig. 3B, nifedipine induced a concentration-dependent relaxant effect on aortic rings contracted either with NE or KCl in a similar manner, although a significant ($p < 0.05$) difference was observed at the lower concentration (10^{-3} mM).

3.5. Role of endothelial factors in the vasorelaxant response to Garcinia lucida

As depicted in Fig. 4A, the removal of the endothelium or the pretreatment of intact aortic rings with L-NAME or methylene blue, significantly ($p < 0.05$ to $p < 0.001$) reduced the vasorelaxant response to AEGL. The EC_{50} value of the plant extract increased from 7.92 μg/mL with intact aortic rings to 53.94 μg/mL, 52.66 μg/mL, and 58.94 μg/mL, respectively, when the endothelium was denuded, pretreated with L-NAME or methylene blue (Table 3). Indomethacin (Fig. 4B) had no significant effect on the vasorelaxant effect of the plant extract. The vasorelaxant effect of carbachol was also significantly ($p < 0.05$ and $p < 0.001$) inhibited by L-NAME (Fig. 4D).

3.6. Involment of adrenergic receptors in the vasorelaxant effect of Garcinia lucida

As depicted in Fig. 5A, the concentration-response curve to NE did not change significantly ($p > 0.05$) when aortic rings were preincubated with the plant extract at concentrations of 7.92 μg/mL (EC_{50}) or 15.83 μg/mL ($EC_{50} \times 2$). However, a shift of the curve to the right was observed with no difference on the E_{max}. On the contrary, moxisylyte (an α_1-adrenergic antagonist) caused a significant ($p < 0.01$ and $p < 0.001$) shift to the right of the NE curve.

Pretreatment of aortic rings with propranolol, a β-adrenergic blocker, had no effect on the vasorelaxant effect of *G. lucida* (Fig. 5B).

3.7. Effect of aqueous extract of Garcinia lucida on calcium influx and on potassium channels

The preincubation of the plant extract did not significantly affect the response to KCl. However, it is worth noticing that when the plant extract was used at a lower concentration (18.01 μg/mL = EC_{50} value), the aortic ring contractile response to KCl was rather reduced than when it was used at a higher concentration ($EC_{50} \times 2$) (Fig. 6A). Moreover, the addition of cumulative concentrations of $CaCl_2$ in the organ chamber induced a progressive increase in aortic ring tension, ranging from 62.22 ± 11.57% to 151.11 ± 15.15% of the KCl contraction. Pretreatment with *G. lucida* or nifedipine significantly inhibited $CaCl_2$-induced contraction. In the presence of these two inhibitors, the tension developed significantly reduced ($p < 0.001$) by up to 72% with the plant extract and 94% with nifedipine (Fig. 6C). The preincubation of aortic rings with TEA or glibenclamide did not significantly ($p > 0.05$) affect the concentration-response curve for *G. lucida* (Fig. 6B).

4. Discussion

The present study investigated the antihypertensive effect of the aqueous extract from the stem bark of *G. lucida* (AEGL) and its related mechanisms. The phytochemical composition of this plant extract was also examined. L-NAME-induced hypertension is a widely used and well-accepted model of experimental hypertension as it mimics human essential hypertension. The results of this study showed that chronic administration of L-NAME alone resulted in a significant and progressive increase in systolic and diastolic blood pressure with no change in heart rate. This result is in agreement with previous findings [30,31]. Treatment with AEGL or captopril significantly prevented the rise in blood pressure elicited by L-NAME and did not affect heart rate suggesting that the antihypertensive effect of AEGL is not associated with bradycardia. It has been demonstrated that kolaviron, a mixture of Garcinia biflavonoids, made of GB1, GB2, and kolaflavanone, attenuates the elevation in blood pressure in salt-induced hypertensive rats [32]. Although kolaflavanone was not identified in AEGL, its antihypertensive effects could be ascribed to both Garcinia biflavonoids GB1 and GB2. Further studies are needed to ascertain this hypothesis.

The administration of the plant extract induced a non-dose-dependent antihypertensive effect. In fact, the low and high doses

Table 2
Effects of aqueous extract from the stem bark of *Garcinia lucida* on urine output, electrolytes, and glomerular filtration rate (GFR).

Experimental groups	Urine volume (mL/100 g)	Na+(mEq/100 g)	K^+ (mEq/100 g)	Cl^-(mEq/100g)	GFR (mL/min)
Naive	4.85 ± 0.57	2.55 ± 0.48	1.25 ± 0.24	3.45 ± 0.64	0.02 ± 0.01
L-NAME	5.30 ± 0.94	2.94 ± 1.14	1.15 ± 0.44	4.49 ± 1.30	0.03 ± 0.01
L-NAME + captopril (20 mg/kg)	6.09 ± 0.96	2.20 ± 0.39	0.98 ± 0.16	2.99 ± 0,53	0.03 ± 0.01
L-NAME + aqueous extract (75 mg/kg)	3.19 ± 0.54	1.12 ± 0.33	0.89 ± 0.25	2.08 ± 0.51	0.01 ± 0.00
L-NAME + aqueous extract (150 mg/kg)	5.07 ± 0.48	2.35 ± 0.24	1.03 ± 0.11	3.24 ± 0.29	0.02 ± 0.03
L-NAME + aqueous extract (300 mg/kg)	6.19 ± 0.71	2.22 ± 0.52	0.95 ± 0.19	2.97 ± 0,69	0.04 ± 0.01

All values are expressed as mean ± SEM. (n = 6). Data were analyzed with one way ANOVA, followed by Tukey's multiple comparison test.

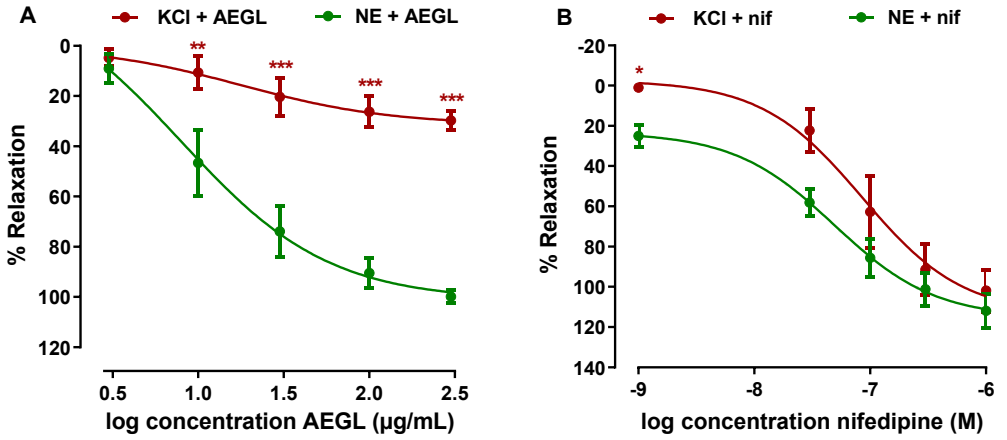

Fig. 3. Effect of cumulative concentration of the aqueous extract from the stem bark of *Garcinia lucida* (A) or nifedipine (B) on intact aortic rings precontracted with KCl (90 mM) or norepinephrine (NE; 10^{-5} M). Each point represents the mean ± SEM of five different experiments. Data were analyzed using ANOVA two-way with Bonferroni. $^{**}p < 0.01$ and $^{***}p < 0.001$, significantly different compared to norepinephrine curve. AEGL: aqueous extract of *Garcinia lucida*; Nif: nifedipine.

produced more effective antihypertensive effects than the medium dose, resulting in an inverted U-shape curve typical of a hormetic response [33]. This hormetic response is assumed to be mediated by a family of receptors with opposing receptor sub-types or through multiple antagonistic signaling pathways [33]. Thus, it can be thought that the aqueous extract from the stem bark extract of *G. lucida* may act through similar mechanisms.

MABP is the average blood pressure value through a single cardiac cycle [34] that ensures good organ perfusion. According to Kandil et al. [34], measuring MABP is a more reliable parameter in the definition of hypertension. In this study, the chronic administration of L-NAME drastically increased MABP, which was significantly prevented by the administration of the plant extract at the dose of 300 mg/kg. This result evidences the potential of *G. lucida* in the management of hypertension.

The L-NAME model has been associated with structural changes at the cardiac, aortic, and kidney levels [35,36]. Left ventricular hypertrophy has been reported by some authors [31,35], whereas others found no change in left ventricular mass [37,38]. In the present study, no significant change in left ventricular mass was observed among the different experimental groups, but an increase in aorta and kidney mass was noticed. Aortic and kidney hypertrophy, as observed in this study following L-NAME exposure, was prevented by both *G. lucida* extract and captopril.

One of the important parameters that interfere with blood pressure homeostasis is renal function. In fact, salt excretion contributes to the regulation of blood volume and hence the regulation of blood pressure. Thus, it is recommended to prescribe diuretics as an initial antihypertensive treatment in both the black and nonblack general population [5]. Besides, antihypertensive drugs should be able to prevent renal dysfunction. In order to determine whether AEGL could possess diuretic effects or protect renal function, urine volume, Na^+, K^+, and GFR were assessed in treated animals. The results showed that none of the treatment protocols caused a significant change in the parameters studied. These results suggest that, the antihypertensive effect exhibited by *G. lucida* is not related to diuretic activity.

Increased vascular tone is a hallmark of arterial hypertension, especially in essential type, and therapeutic strategies aim at lowering vascular tone in hypertensive patients. Results from the present study showed that AEGL does not possess a diuretic effect and cannot reduce heart rate. It is then likely that the antihypertensive effect of AEGL is mediated by its ability to reduce vascular resistance. To test this hypothesis, we explored the vasodilatory effect of *G. lucida* on rat aortic rings precontracted with norepinephrine (NE) or KCl. The results show that the aqueous extract of *G. lucida* induced a concentration-dependent vasorelaxant response to NE and KCl evoked-contraction. However, this vasorelaxant effect was more pronounced in NE-induced contraction, suggesting that AEGL acts mainly through the metabotropic signaling pathway [39].

The vascular endothelium plays a central role in the regulation of vascular tone through the release of various vasoactive factors, including nitric oxide (NO) and prostacyclin, and endothelium-derived contracting factors such as endothelin-1 and thromboxane [40, 41]. To assess the contribution of the endothelium to the vasorelaxant activity of AEGL, it was tested on endothelium-denuded aortic rings. The relaxant effect of AEGL was partially inhibited by the removal of the endothelium, demonstrating the implication of the endothelium in its vasorelaxant response.

In order to determine which endothelial factor mediates the endothelium-dependent vasorelaxant effect of the plant extract, the concentration-response curve to *G. lucida* was constructed in the presence of L-NAME or indomethacin. It was observed that L-NAME inhibited the vasorelaxant effect of AEGL similar to the removal of the endothelium, while indomethacin had no effect. It could then be concluded that NO is the sole endothelial-derived relaxing factor that mediates the endothelium-dependent relaxant effect of AEGL.

NO is a well-known endothelial factor that activates a soluble guanylate cyclase to produce cyclic guanosine monophosphate (cGMP) [6,40,41]. The produced cGMP further activates its downstream signaling protein, protein kinase G (PKG), which causes

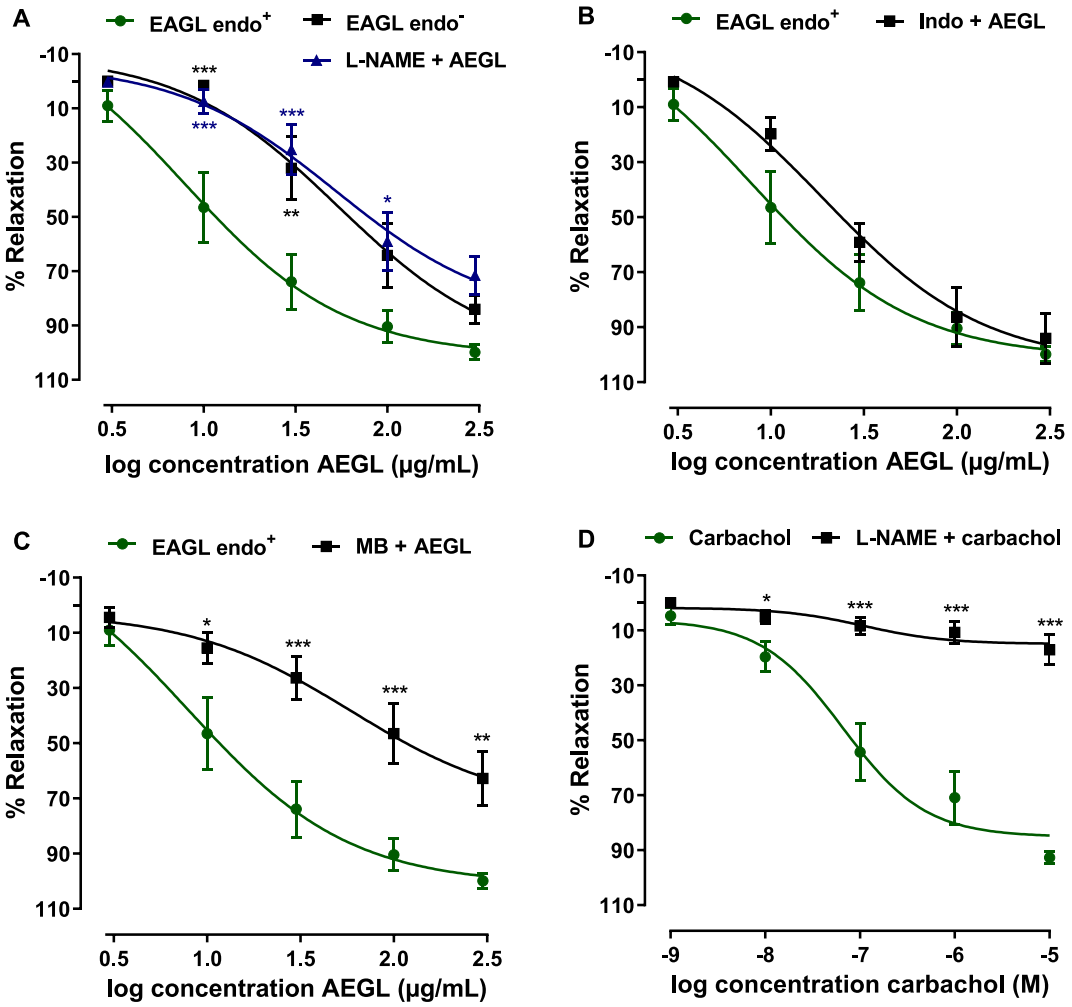

Fig. 4. Effect of endothelium removal, L-NAME (10^{-4} M; panel A), indomethacin (10^{-5} M: panel B), and methylene blue (10^{-6} M; panel C) on relaxation elicited by *Garcinia lucida*. Panel D presents the effect of L-NAME on carbachol-induced relaxation. Each point represents the mean ± SEM of five different experiments. Data were analyzed using ANOVA two-way with Bonferroni. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$, significantly different compared to the effect of the control (AEGL). AEGL: aqueous extract of *Garcinia lucida*; endo^{+}: with intact endothelium; endo^{-}: with endothelium denuded; Indo: indomethacin; MB. Methylene blue; Carb: carbachol.

Table 3
EC_{50} and E_{max} of the aqueous extract from the stem bark of *Garcinia lucida* on norepinephrine (NE) or KCl-induced aortic ring contraction in the presence or absence of some inhibitors.

NE-induced contraction	EC_{50} (µg/mL)	E_{max} (%)
AEGL (endo+)	7.92	99.87 ± 2.63
AEGL (endo-)	53.94	84.05 ± 5.17
Preincubation with L-NAME + AEGL (endo+)	52.66	71.54 ± 6.95
Preincubation with Indo + AEGL (endo+)	18.95	94.07 ± 8.85
Preincubation with MB + AEGL (endo+)	58.94	62.78 ± 9.65
Preincubation with Prop + AEGL (endo+)	13.50	98.71 ± 0.84
Preincubation with TEA + AEGL (endo-)	68.67	98.41 ± 5.38
KCl-induced contraction		
AEGL (endo+)	18.01	29.73 ± 3.70

AEGL: aqueous extract *Garcinia lucida*; endo+: with intact endothelium; endo-: without endothelium; Indo: indomethacin; MB: methylene blue; NE: norepinephrine; Prop: Propranolol; TEA: tetraethylammonium.

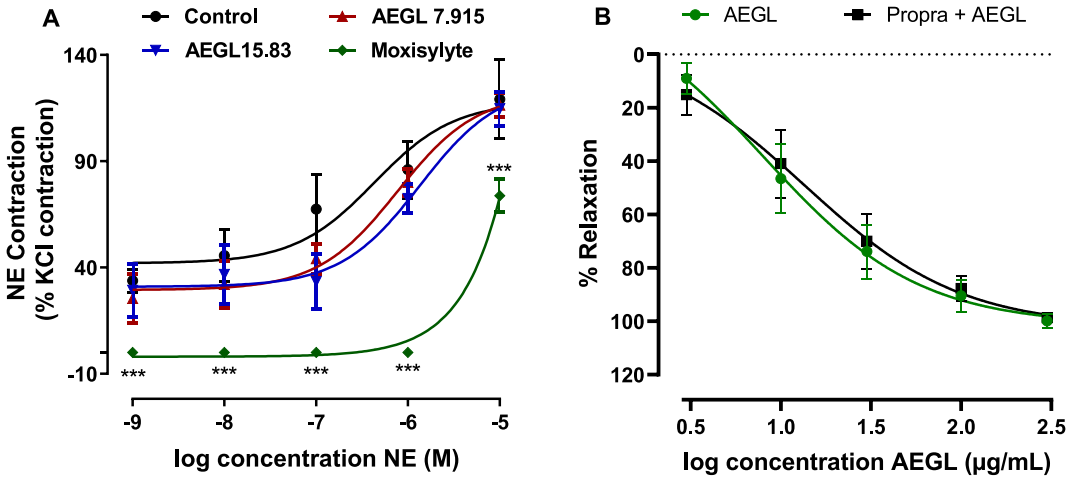

Fig. 5. Effect of the aqueous extract of *Garcinia lucida* (7.915 and 15.83 µg/mL), or moxisylyte (10^{-6} M) on the contractile response to norepinephrine (A) and effect of propranolol (5×10^{-6} M) on vasorelaxation elicited by *Garcinia lucida* (B). Each point represents the mean ± SEM of five different experiments. Data were analyzed using ANOVA two-way with Bonferroni post-test. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$, significantly different compared to the control (NE and AEGL). AEGL: aqueous extract of *Garcinia lucida;* NE: norepinephrine; Prop: propranolol.

vasorelaxation by either activating potassium channels, reducing Ca^{2+} influx, or, decreasing the sensitivity of contractile proteins to Ca^{2+} [23]. We investigated whether AEGL could interfere with any stage of NO downstream signaling, beginning with guanylate cyclase. The role of soluble guanylate cyclase was investigated by incubating the aorta rings with methylene blue, a soluble guanylate cyclase (sGC) inhibitor. Methylene blue significantly reduced the vasorelaxant effect of *G. lucida* aqueous extract. This result supports the hypothesis that AEGL may act through the activation of the NO/sGC/cGMP signaling pathway to induce vasorelaxation. But further studies are needed to confirm this hypothesis.

The fact that the vasorelaxant effect of the plant extract was not totally abolished by the removal of the endothelium or by the pretreatment of aortic rings with L-NAME prompted the hypothesis that other mechanisms may account for the vasorelaxant effect of the plant extract. That might be a direct effect on vascular smooth muscles, including adrenergic receptors, and potassium or calcium channels. We investigated the implication of adrenergic receptors using two different protocols. In the first protocol, the aortic rings were pretreated with the plant extract prior to the introduction of cumulative concentrations of NE, and in the second experiment, the effect of the plant was antagonized with propranolol, a non-specific beta-adrenergic receptor blocker. AEGL did not significantly alter the vasocontractile response to NE, although a slight shift to the right of the NE response was observed. Also, propranolol did not affect the vasorelaxant response to *G. lucida*. Taken together, these two results clearly show that the vasorelaxant effect of the plant extract does not involve the blocking of alpha-1 adrenergic receptors or the activation of beta-adrenergic receptors.

Potassium channels have been shown to play an important role in the regulation of vascular tone [41]. It has been demonstrated in this study that the blockade of potassium channels with the nonselective calcium activator K^+ channel blocker, TEA [41,42] or with the ATP-dependent potassium channel inhibitor, glibenclamide, did not change the response to the plant extract suggesting that AEGL does not exert its vasorelaxant effect through the activation of potassium channels.

Vascular smooth muscle contraction elicited by KCl results from an increase in intracellular calcium through the activation of voltage-dependent calcium channels following membrane depolarization [43]. In the course of this study, it was noticed that the plant extract relaxed aortic rings precontracted with KCl but, surprisingly, failed to inhibit vascular smooth muscle contraction elicited by KCl. This finding suggests that AEGL may either interfere with the intracellular release of calcium or the sensitivity of the contractile machinery but not with the calcium influx. This hypothesis was further tested by constructing the concentration-response curve for $CaCl_2$ using aortic rings preincubated with the plant extract. Conversely, the preincubation of the aortic ring with AEGL, similar to nifedipine (an L-type voltage-gated calcium channel blocker), greatly reduced the amplitude of contraction elicited by $CaCl_2$, suggesting that the vasorelaxant effect of AEGL is also mediated by the inhibition of calcium influx. Additional studies are needed to understand the calcium-dependent relaxation of AEGL.

However, Kolaviron, a mixture of GB1, GB2, and kolaflavanone as mentioned earlier, has been shown to exhibit both endothelium-dependent and endothelium-independent vasorelaxation [44]. The endothelium-independent pathway of kolaviron was achieved through the blockade of extracellular calcium influx. Although kolaflavanone was not identified in AEGL, it can be thought that the vasorelaxant activity of this extract may be related at least to the presence of GB1 and GB2.

Taking it all together, we can conclude that AEGL possesses endothelium- and non-endothelium-dependent vasorelaxant activities that mediate its antihypertensive effect. The endothelium-dependent effect relies on the NO-guanylate cyclase pathway, while the mechanism of the non-endothelium-dependent activity remains to be clarified.

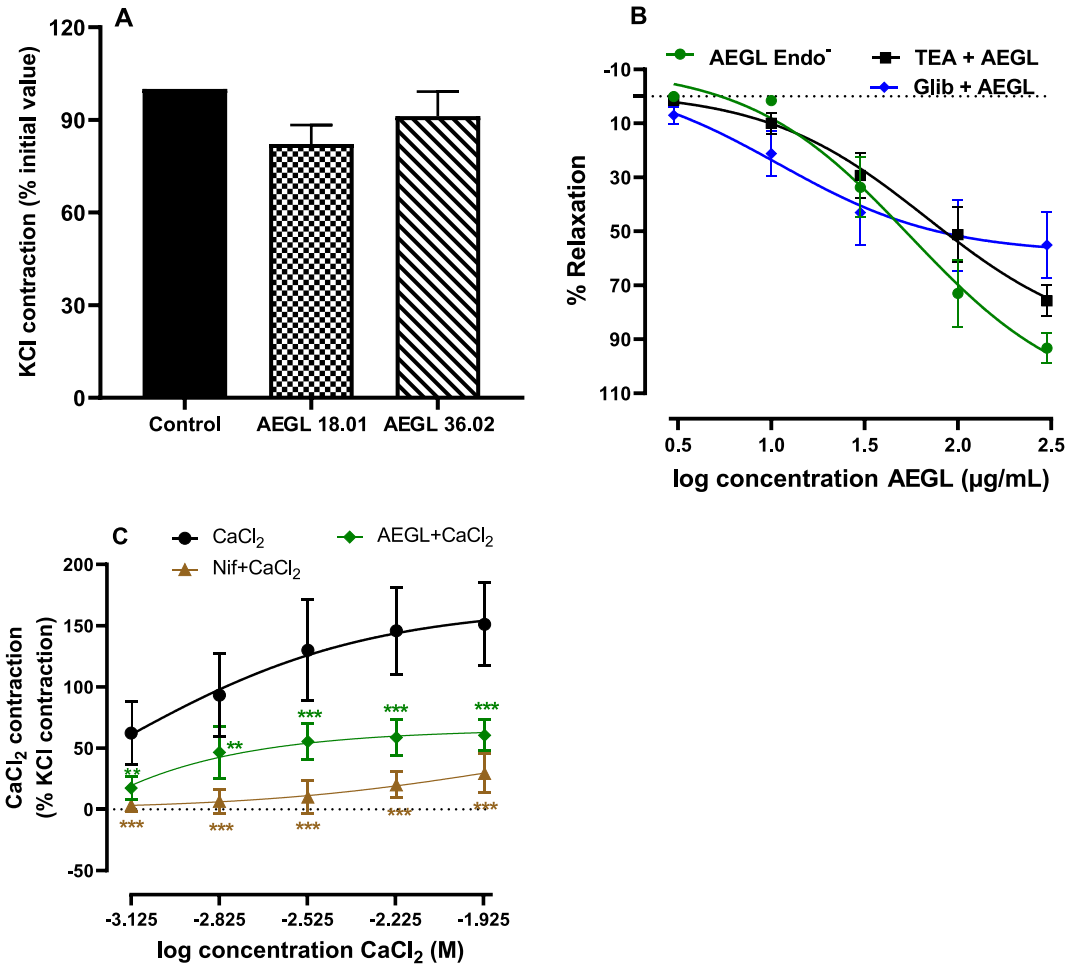

Fig. 6. Effect of *Garcinia lucida* extract (18.01 and 36.02 µg/mL) on potassium channels (A) and calcium influx (C), and effect of tetraethylammonium and glibenclamide on the vasorelaxant effect of *Garcinia lucida* (B). Each point represents the mean ± SEM of five different experiments. Data were analyzed using ANOVA two-way with Bonferroni post-test. $*p < 0.05$, $**p < 0.01$ and $***p < 0.001$, significantly different compared to the effect of the control. AEGL: aqueous extract of *Garcinia lucida;* TEA: Tetraethylammonium (5×10^{-6} M). Glib: glibenclamide (10×10^{-6} M), Nif: nifedipine (10^{-5} M).

5. Conclusion

In light of what precedes, it can be concluded that the aqueous extract from the stem bark of *G. lucida* possesses an endothelium-dependent and endothelium-independent vasorelaxant effect that accounts for its antihypertensive effect. The endothelium-dependent vasorelaxation is mediated by the activation of the NO/sGC/cGMP pathway. The vasorelaxant effect of *G. lucida* could be attributed to the presence of GB1 and GB2. The stem bark aqueous extract of *G. lucida* lacks a diuretic effect.

Data availability

Data will be made available on request

CRediT authorship contribution statement

Elvine Pami Nguelefack-Mbuyo: Conceptualization, Data curation, Formal analysis, Funding acquisition, Project administration, Software, Writing – original draft, Methodology, Resources, Visualization. **Christelle Stéphanie Sonfack:** Formal analysis, Investigation, Resources, Writing – original draft, Visualization. **Christian Kuété Fofié:** Formal analysis, Investigation. **Chamberlin Fodem:** Investigation. **Magloire Kanyou Ndjenda II:** Investigation. **Alain Bertrand Dongmo:** Resources, Writing – review & editing. **Télesphore Benoît Nguelefack:** Conceptualization, Data curation, Formal analysis, Methodology, Resources, Supervision, Validation, Writing – review & editing.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used Grammarly free writing AI assistance to improve language style for readability. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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List of abbreviations

AEGL	aqueous extract of *Garcinia lucida*
AHT	Arterial hypertension
cGMP	cyclic guanosine monophosphate
GFR	glomerular filtration rate
LC-MS	liquid chromatography–mass spectrometry
L-NAME	Nω-nitro-L-arginine methyl ester
NE	norepinephrine
NO	nitric oxide
PKG	protein kinase G
sGC	soluble guanylate cyclase
TEA	tetraethylammonium

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e21896.

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