

Full Length Research Paper

Effects of *Gmelina arborea*, Roxb (Verbenaceae) aqueous extract on arterial pressure of Wistar rats

Roland Lawson^{1,3}, Bonaventure Awede^{1*}, Razack Osseni¹, Fernand Gbaguidi², Joachim Gbenou² and Anatole Laleye³

¹Unité de Physiologie, Faculté des Sciences de la Santé, Université d'Abomey-Calavi, Cotonou, Benin.

²Laboratoire de Pharmacognosie et des Huiles Essentielles, Faculté des Sciences de la Santé - Faculté des Sciences et Techniques, Université d'Abomey-Calavi, Cotonou, Benin.

³Unité de Biologie Humaine, Faculté des Sciences de la Santé, Université d'Abomey-Calavi, Cotonou, Benin.

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In this study, the effect of *Gmelina arborea* aqueous extract, a medicinal plant used in the treatment of hypertension, on rat blood pressure was investigated. After harvest, leaves of *G. arborea* were dried, reduced in powder and used for aqueous extract preparation. The aqueous extract was administered at 500 mg/kg/day to Wistar rats, which has been made hypertensive by NG-Nitro-L-arginine methyl ester (L-NAME), treatment at 20 mg/kg/day. Alkaloid compounds, tannins, anthocyanins, leucoanthocyanins, mucilages and saponins were detected in *G. arborea* leaves. 7 and 14 days administration of L-NAME induced an increase of mean arterial blood pressure from 99±4 mmHg to respectively 155±2 and 179±10 mmHg. *G. arborea* leaves extract reduced these blood pressures to respectively 126±13 and 147±7 mmHg. These data confirm the antihypertensive effect of *G. arborea* leaves. Further investigations will analyze the active compounds and the molecular mechanisms underlying this pharmacological effect.

Key words: Hypertension, L-NAME, medicinal plant, *G. arborea*.

INTRODUCTION

Urbanisation and changes in life style are associated with an epidemiological transition in developing countries. In Sub-Saharan African population, this transition is characterized by the emergence of non-communicable diseases among which cardiovascular diseases constitute an important health problem. In this context, hypertension, the main risk factor of the cardiovascular diseases prevalence has been estimated in 2010 to 30.8% (Adeloye and Basquill, 2014). Its incidence

increases with age with minimal difference between males and females (Addo et al., 2007; Opie and Seedat, 2005). In Benin, a west-African country, during the past decades, hypertension prevalence has been characterized by a progressive rise to reach 27.9% in 2008 (Houinato et al., 2012).

Several factors make the therapeutic management of hypertension difficult. Many hypertensive subjects are not aware of their hypertensive status, and are often

*Corresponding author. E-mail: bonawed@yahoo.com. Tel: 00229 95104353

diagnosed when complications arise. The low living standard and the relatively high cost of hypertension treatment make access to medical care difficult. Thus, a significant proportion of hypertensive patients used medicinal plants for their treatment.

In West Africa, more than one hundred of medicinal plants used in the treatment of hypertension have been reported by ethnobotanical studies (Gbolade, 2012; Karou et al., 2011). One of those plants frequently used in folk medicine is *Gmelina arborea*. Except a previous work showing a transient reduction of rat blood pressure induced by an intravenous administration of *G. arborea* leaves extract (Wansi et al., 2009), no study on the effectiveness of this plant in the treatment of hypertension in human or in an animal model has been performed. The present work was therefore undertaken to study the effects of *G. arborea* leaves extract on blood pressure in hypertensive rats.

MATERIALS AND METHODS

Plant material and extraction

G. arborea leaves were used to prepare a standard decoction based on traditional medicine remedies. Fresh leaves from wild plants were collected from the southern region of Benin (Abomey-Calavi and Zê) and authenticated by the Herbarium National du Bénin (reference N°AA6337/HNB). The collected sample was dried under controlled temperature (20 to 25°C) and then powdered by a grinder and stored in an air-dry container at room temperature. The powder of *G. arborea* leaves was used to prepare the aqueous extract. The powder (50 g) is boiled for 10 min in distilled water (500 ml). The decoction was filtered and the extract was obtained after cooling of the preparation at room temperature and evaporation under reduced pressure at 70°C with a rotavapor. The extract was protected against light and humidity and stored at -20°C.

Phytochemical screening

Phytochemical screening to identify the major compounds of the extract was performed based on methods described by Houghton and Raman (1998). Mayer and Dragendorff tests are used for the identification of alkaloids. The powdered sample (5 g) was extracted with 25 ml of ammonia. The suspension was macerated 24 h and the filtrate was extracted with 5% HCl. The Mayer reagent was added to the aqueous phase. For the Dragendorff test, a maceration of 10 g of powdered sample in 50 ml H₂SO₄ (10%) was used. The Dragendorff reagent was added to the filtrate. The presence of tannins was detected by boiling 1g powdered sample in 10 ml distilled water, followed by addition of 1% FeCl₃ to the filtrate. Catechintannins identification was carried out using reagent of Stiasny. For gallic tannins, the study filtered the solution of catechin tannins identification and the filtrate was collected and saturated with sodium acetate.

The addition of 3 drops of FeCl₃ (1%) allowed to identify the presence of gallic tannins by the appearance of intense blue-black coloration. Flavonoids were revealed by the Shinoda test. 15 mg of dry extract was dissolved in 1 ml of a mixture of 50% ethanol/concentrated HCl (2:1 v/v). Magnesium turnings were added. Test for anthocyanins was carried out with a filtrate of boiled powdered sample (1 g in 10 ml distilled water) by adding drops of 1% HCl and 50% ammonia.

Test for leuco-anthocyanins was carried out with a filtrate of

boiled powdered sample (1 g in 10 ml distilled water) by adding v/v Shinoda reagent heated 15 min in a water bath at 90°C. Detection of anthracene-derivate was investigated by boiling 1 g of the powdered sample in 10 ml chloroform for 3 min at 90°C. Ammonia was added to the filtrate to reveal unbounded anthracene-derivate. Quinones were identified by extracting 2 g sample with 20 ml chloroform after adding 2 ml 5% HCl. The solution was filtered and 5 ml of 50% ammonia was added. Cyanogenic components were identified by dissolving 0.5 g powdered sample in 10 ml sterile water and the mixture was filtrated. Sodium picrate paper was added to the filtrate and heated to boil. The Fehling test was performed to detect the presence of reducing sugar. Saponins were detected on the basis of froth upon vigorous shaking. Mucilages are determined by observing the viscosity after addition of absolute ethanol. The detection of coumarins was carried out by extracting 0.5 g of sample in 10 ml ether.

The suspension was filtrated, evaporated and 2 ml distilled water were added. A fluorescence was observed in Ultraviolet (UV) light at 365 nm. For sterols and triterpenes, 1 g of leaves powder was macerated in 10 ml ethanol 70° for 30 min after this step, 10 ml of distilled water and 2 ml of plumbic acetate 10% was added. The residue after chloroform extraction was used for Liebermann Burchard reaction. A small amount of the residue was dissolved in dry chloroform (0.5 ml) in a test-tube and acetic-anhydride (0.5 ml) was added. A few drops of concentrated sulfuric acid were added along the side of the test-tube. Development of bluish color in the chloroform layer immediately turning to violet and finally to green indicates the presence of sterols whereas a deep pink color in the chloroform layer indicated the presence of triterpenes.

Animal experiment

Animals and Induction of hypertension

Twelve weeks-old male Wistar rats were used for this investigation. Rats were housed in cages and maintained in a light controlled environment (12:12 h light-dark cycle) and had unlimited access to food and water. Rats were made hypertensive by oral gavage administration of N(G)-Nitro-L-Arginine-Methyl Ester (L-NAME, Fluka), a non-selective nitric oxide synthase (NOS) inhibitor, at the dose of 20 mg/kg/day for 7 or 14 days.

Experimental groups and designs

The experiments were run during 14 days. All products administered to rats were dissolved in distilled water and given by oral gavage. According to the duration of L-NAME administration, two experimental designs, each of four (4) experimental groups, were tested (Figure 1).

Protocol 1:

1. Control group: rats of this group were orally administrated (gavage) with distilled water
2. L-NAME group: rats were treated with L-NAME at 20 mg/kg/day from day1 to day 7 and distilled water from day8 to day14
3. L-NAME-GA extract group: rats were treated with L-NAME at 20 mg/kg/day from day1 to day 7 and with *G. arborea* (GA) aqueous extract at 500 mg/kg/day from day8 to day14
4. L-NAME-Captopril group: rats were treated with L-NAME at 20 mg/kg/day from day1 to day 7 and with captopril, an angiotensin-converting enzyme inhibitor, given as reference treatment at 100 mg/kg/day from day8 to day14.

Protocol 2:

Experimental groups were similar to those of design 1 with the



Figure 1. Leaves of *G. arborea*.

difference that in L-NAME, L-NAME-GA extract and L-NAME-Captopril groups the duration of administration of L-NAME to rats was 14 days.

In each experimental group, there were five rats, except L-NAME-Captopril group of protocol 2 where there were 2 rats.

Blood pressure measurement

At the end of the treatment (day 15), rats were anesthetized by intra peritoneal injection of thiopental (40 mg/kg of body weight) and their blood pressure were measured by catheterization of carotid artery.

Statistical analysis

Results are expressed as means \pm SEM (standard error of mean). Statistical comparison between experimental groups was performed using an analysis of variance (ANOVA) followed by Dunnett's multiple comparison (GraphPad Prism V4, USA). A value of $p < 0.05$ was considered significant.

RESULTS

Phytochemical screening

Results of the phytochemical analysis of *G. arborea*

leaves are presented in Table 1. This screening indicated that amongst thirteen chemical groups investigated, six have been detected in *G. arborea* leaves. These chemical compounds are alkaloid compounds, tannins, anthocyanins, leucoanthocyanins, mucilages and saponins.

Plant extract

For standardization purpose, the study performed, before administration to rats, a series of three different extract preparations as described in materials. The mean yield of plant material after drying was 17.63 ± 0.07 %.

Effect of *G. arborea* extract on blood pressure

As shown in Figure 2, 7-days administration of L-NAME to rats at 20 mg/kg/day induced a rise in mean arterial blood pressure from 99 ± 4 mmHg to 155 ± 2 mmHg. Administration of *G. arborea* aqueous extract (500 mg/kg/day) to rats during 7 days (day 8 to day 14) following L-NAME treatment induced a reduction of mean arterial pressure to 126 ± 13 mmHg. The effect of captopril was a reduction of blood pressure to 129 ± 4 mmHg. When L-NAME was administrated to rats for 14 days

Table 1. Phytochemical analysis of *G. arborea* leaves.

Chemical families	Expression
Alkaloids	+
Tannins: 1. Catechin; 2. Gallic	++; +/-
Flavonoids	-
Anthocyanins	+/-
Leucoanthocyanins	+/-
Mucilage	++
Reducing sugars	-
Cyanogenic glycosides	-
Quinones	-
Saponins	++
Sterols and triterpenes	-
Anthracen glycosides	-
Coumarins	-

(+) detected, (++) abundantly detected, (+/-) weakly detected, (-) absent.

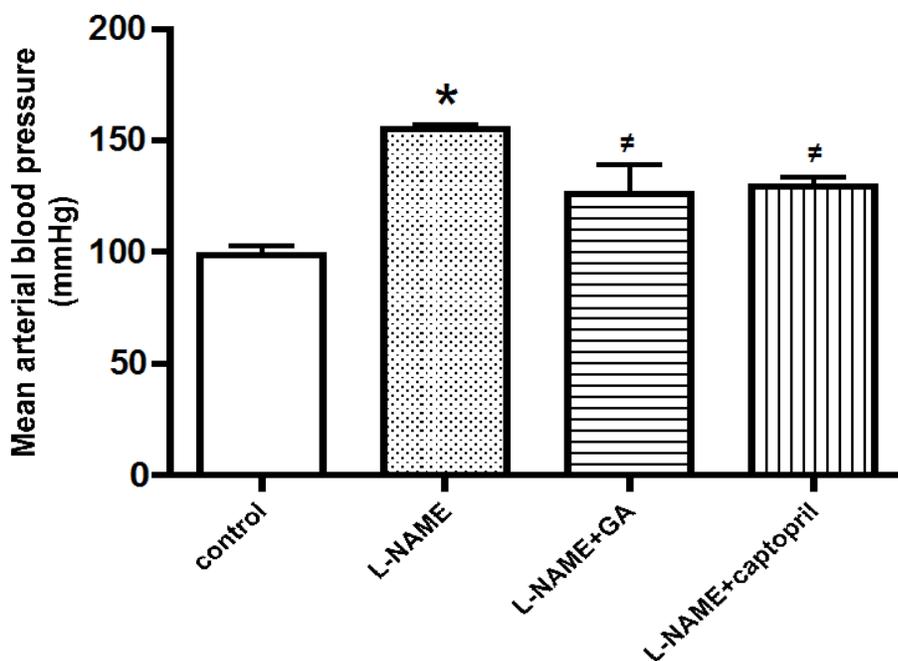


Figure 2. Effects of *G. arborea* leaves extract on blood pressure of rats made hypertensive by 7 days administration of L-NAME. Values are mean \pm SEM, n = 5 rats/group. * Significantly different from control value with $p < 0.05$; # Significantly different from L-NAME group value with $p < 0.05$.

(Figure 3), an increase of rat mean arterial pressure to 179 ± 10 mmHg was observed. *G. arborea* extract induced a significant reduction of blood pressure from 179 ± 10 mmHg to 147 ± 7 mmHg when administrated to rats treated by L-NAME for 14 days. Administration of captopril to rats, in this case, was without effect on blood pressure (176 ± 2 mmHg).

DISCUSSION

The main finding of this study is that 7-days administration of aqueous extract of *G. arborea* induced a significant reduction of blood pressure in hypertensive rats. Although *G. arborea* leaves use in the treatment of hypertension has been reported (Gbolade, 2012;

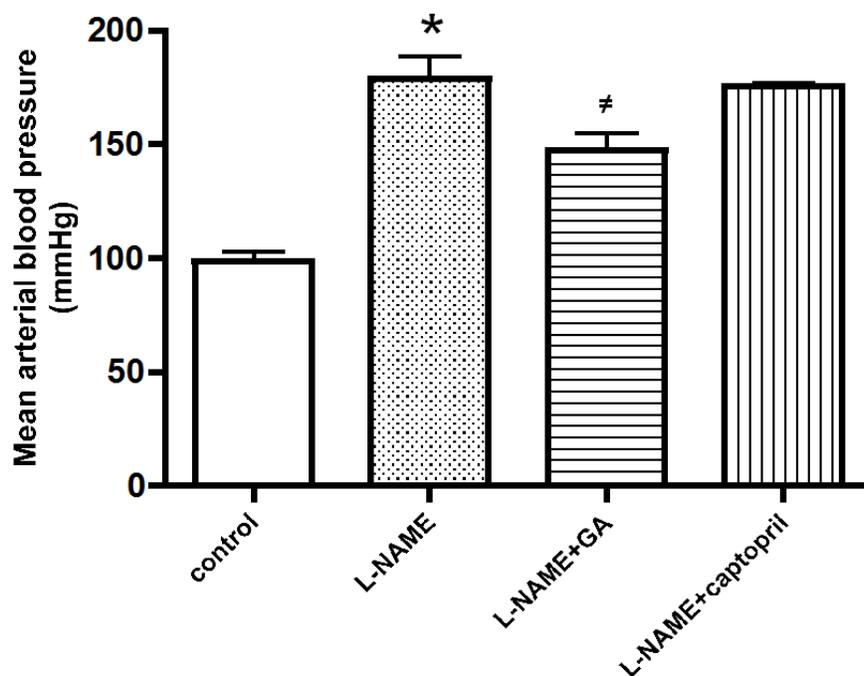


Figure 3. Effects of *G. arborea* leaves extract on blood pressure of rat made hypertensive by 14 days administration of L-NAME. Values are mean \pm SEM, n=5 rats/group except L-NAME-captopril group where n=2; *Significantly different from control value with $p < 0.05$ (ANOVA); # Significantly different from L-NAME group value with $p < 0.05$ (ANOVA).

N'guessan et al., 2009), this is the first study to assess the effect of oral administration of *Gmelina arborea* leaves extract on high blood pressure.

To investigate the effect of *G. arborea* extract on blood pressure, L-NAME-induced hypertension model has been used. In this model, inhibition of nitric oxide synthase (NOS) by L-NAME leads to a suppression of nitric oxide vasorelaxation activity. Although in rats, many authors have used administration of doses higher than 20 mg/kg/day (Santos de Araujo et al., 2013; Sung et al., 2013), in this study, 7-days treatment of rats at this dose was sufficient to induce a significant increase in blood pressure. These data are in agreement with those of Biancardi where significant increase of blood pressure was observed after 2 and 7 days L-NAME administration (Biancardi et al., 2007). Moreover the increased pressure induced by L-NAME in the present work was enough higher for at least 7 additional days from the end of the hypertension induction protocol when compared with control group.

G. arborea extract induced a significant reduction of blood pressure when administrated to rats following 7 days treatment with L-NAME or during the 7 last days of a 14-days treatment with L-NAME. In a previous study investigating the effect of aqueous extract of *G. arborea* on a salt-induced hypertension in rat, single dose intravenous administration of the extract induced a dose

dependent but transient decrease of rat blood pressure (Wansi et al., 2009). As the measurement of blood pressure 24 h after the last administration of L-NAME, the study data showed that repeated oral administration of *G. arborea* leaves extract could induce not only a transient, but also a sustained decrease of blood pressure. Further studies will be useful in determining the time course and reversibility of *G. arborea* effect on high blood pressure.

In contrast to *G. arborea* extract, Captopril failed to reduce significantly blood pressure when L-NAME is administrated to rat until day 14 although its effect was similar to that of the extract when administrated following the period of 7 days of hypertension induction. These data suggest that action mechanism of *G. arborea* extract is, in part, independent of rennin angiotensin aldosterone system. In view of the hypertension model used, active secondary metabolites detected (phytochemical screening) in *G. arborea* leaves could act directly or synergistically on vessels to induce vaso relaxation.

Conclusion

Indeed, *in vitro* study on rat aortic rings showed a vasorelaxation effect of *G. arborea*, which is, in part, NO dependent (Wansi et al., 2012). *G. arborea* extract could thus act by reversing the inhibiting effect of L-NAME on

NO synthase. In addition to its vasoactive activity, other possible mechanisms could be involved in the effect of *G. arborea*. Thus, diuretic activity of *G. arborea* fruit extracts has been reported (Nayak et al., 2013).

Amongst secondary metabolites detected in *G. arborea* leaves, alkaloids have been shown to induce hypotensive effect in wistar rat associated with either alteration of cardiac function (decreased heart rate and contractility) or decreased vascular contractility (Mugabo et al., 2012; Jadhar et al., 2013). Beneficial effects of polyphenol compounds on cardiovascular system have also been described.

Thus, endothelium dependent vasorelaxant effect of many anthocyanins has been reported (Andriambelosen et al., 1998; Bell and Gochenaur, 2006). It has also been shown that proanthocyanidins (condensed tannins) induced antihypertensive effect associated with endothelium dependent vasorelaxant activity (Kawakami et al., 2011; Furuuchi et al., 2012). The observed antihypertensive effect could thus result from the synergistic action of pharmacological properties of alkaloids, tannins and anthocyanins present in *G. arborea* leaves.

Conflicts of interest

Authors have not declared any conflict of interest.

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