ABOUT JPAP

Journal of Physiology and Pathophysiology (JPAP) is published monthly (one volume per year) by Academic Journals.

Journal of Physiology and Pathophysiology (JPAP) is an open access journal that provides rapid publication (monthly) of articles in all areas of the subject such as effect of different sitting posture on pulmonary function, study of anti-plasmodial and toxicological effects of methanolic bark extract of Chrysophyllum albidum, serum testosterone and morphology of the testes in wistar rats following chronic garlic feeding etc.

The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in JPAP are peer-reviewed.

Contact Us

Editorial Office:       jpap@academicjournals.org
Help Desk:             helpdesk@academicjournals.org
Website:               http://www.academicjournals.org/journal/JPAP

Submit manuscript online http://ms.academicjournals.me/
Editor
Dr. Kazi Mirajul Hoque
Scientist and Ramalingaswami Fellow, Molecular Pathophysiology Division, National Institute of Cholera and Enteric Diseases, P-33, CIT Road, Scheme-XM, Beliaghata, Kolkata-700010, INDIA...

Editorial Board Members
Dr. Yan-Qiu YU
Department of Pathophysiology, Sri Ramachandra Deemed University, College of Basic Medical Sciences, China Medical University, 92. Beier street, Heping District, Shenyang, 110001, P. R. China.

Dr. Wanzala Wycliffe
P.O. Box 11177-Tom Mboya-00400, Nairobi, Kenya

Venkateswara Rao Gogineni
Research Scientist, University of Illinois College of Medicine at Peoria, Department of Cancer Biology and Pharmacology, One Illini Drive, Peoria, IL 61605, USA

Editorial Team
Dr. Ajibola, Abdulwahid Olabisi Onabanjo University, Ago-Iwoye, Nigeria.

Dr. Mostafa Azimzadeh
Dep. of Life Science Engineering, Faculty of New Sciences and Technologies, University of Tehran, Tehran, Iran.

Dr. Birendra Nath Mallick
School of Life Sciences, Jawaharlal Nehru University, New Delhi - 110 067, India.

Dr. Ben-zhi CAI
Department of Pharmacology, Harbin medical university, Harbin, 150081, China.
Dr. Linda R. Chambliss  
4440 North Dromedary Rd  
Panjab University,  
Phoenix, Arizona 85018,  
USA.

Dr. Kazi Mirajul Hoque  
3501 St. Paul Street, APT# 819, Baltimore, MD-21218,  
USA.

Dr. Rajesh Chandra Sharma  
301 Sai Mahal Co- Hsg. Society,  
Sector -3, plot no. 40 New Panvel, Navi Mumbai, Maharashtra-410205,  
India.

Dr. Tao Lianyuan  
RM 5209, QingNianGongYu, XinKai Road 80#, Dong Dan,  
Dong Cheng  
District, Beijing 100005,  
China.

Dr. De-Yu Chen,  
Shantou University Medical College (SUMC),  
22 Xinling Road, Shantou, Guangdong, P. R. China,  
515041,  
China.

Dr. Ayman El-Meghawry El-Kenawy Ali,  
22 El-Galaa St., El-Mansoura, Egypt.

Dr. Karunakaran Ganesh Kumar,  
Department of Metabolism and Aging, The Scripps Research Institute,  
130 Scripps Way, Jupiter, FL-33458,  
USA.

Dr. Farouk M. F. El-Sabban,  
Department of Family Sciences, College for Women,  
Kuwait University, P. O. Box 5969, Safat 13060  
State of Kuwait.

Dr. Majid Hassanpour-Ezatti,  
Dept. of Biology, Science School, Shahed Univ. Tehran,  
Iran.

Dr. Hedef Dhafir El-Yassin,  
College of Medicine, University of Baghdad,  
Baghdad, Bab-Almuadum, Baghdad,  
Iraq.

Dr. Irawan Satriotomo,  
Dept. Comparative Bioscience, University of Wisconsin  
2015 linden Dr., Madison, WI 53706,  
USA.

Dr. Jingdong Li,  
Institute of Cardiovascular Disease, Union Hospital,  
Tongji Medical College, Huazhong University of Science and Technology  
Wuhan, Hubei Province, 430022,  
P.R.of China.
ARTICLE

Research Article

Effects of *Gmelina arborea*, Roxb (Verbenaceae) aqueous extract on arterial pressure of Wistar rats
Roland Lawson, Bonaventure Awede, Razack Osseni, Fernand Gbaguidi, Joachim Gbenou and Anatole Laleyé
Full Length Research Paper

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

Roland Lawson¹,³, Bonaventure Awede¹*, Razack Osseni¹, Fernand Gbaguidi², Joachim Gbenou² and Anatole Laley³

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.

Receive 31 May, 2015; Accepted 10 December, 2015

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 administrated 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

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License.
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 (Gbólade, 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 Zé) and authenticated by the Herbier National du Bénin (reference N°A66337/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 extract (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 were 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 1 g 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 anthraquinone-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 unbound anthraquinone-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 picate paper was added to the filtrate and heated to boil. The FeHing test was performed to detect the presence of reducing sugar. Saponins were detected on the basis of froth upon vigorous shaking. Muclages 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. Afuorescence 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 minu 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-NNAME, 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-NNAME 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-NNAME group: rats were treated with L-NNAME at 20 mg/kg/day from day1 to day 7 and distilled water from day8 to day14
3. L-NNAME-GA extract group: rats were treated with L-NNAME 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-NNAME-Captopril group: rats were treated with L-NNAME 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
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 thiopenthal (40 mg/kg of body weight) and their blood pressure were measured by catheterization of carotid artery.

Statistical analysis
Results are expressed as means ± 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.

<table>
<thead>
<tr>
<th>Chemical families</th>
<th>Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Tannins: 1. Catechin; 2. Gallic</td>
<td>++; +/-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>+/-</td>
</tr>
<tr>
<td>Leucoanthocyanins</td>
<td>+/-</td>
</tr>
<tr>
<td>Mucilage</td>
<td>++</td>
</tr>
<tr>
<td>Reducing sugars</td>
<td>-</td>
</tr>
<tr>
<td>Cyanogenic glycosides</td>
<td>-</td>
</tr>
<tr>
<td>Quinones</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>++</td>
</tr>
<tr>
<td>Sterols and triterpenes</td>
<td>-</td>
</tr>
<tr>
<td>Anthracen glycosides</td>
<td>-</td>
</tr>
<tr>
<td>Coumarins</td>
<td>-</td>
</tr>
</tbody>
</table>

(+)+ 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 ± 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 ± 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 (Andriambeloson 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 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.

**REFERENCES**


Journal of Physiology and Pathophysiology

Related Journals Published by Academic Journals

- Journal of Physiotherapy and Occupational Therapy
- Journal of Parasitology and Vector Biology
- Clinical Reviews and Opinions
- Journal of Clinical Pathology and Forensic Medicine
- Journal of Infectious Diseases and Immunity
- International Journal of Nutrition and Metabolism
- Journal of Dentistry and Oral Hygiene