HYPOTENSIVE EFFECTS OF A BUTANOL ACTIVE FRACTION FROM LEAVES OF *BLIGHIA UNIJUGATA* BAK. (SAPINDACEAE) ON ARTERIAL BLOOD PRESSURE OF RABBIT

*N’dia Kouadio Frédéric¹, Kouakou Kouakou Léandre¹, Bléyéré Nahounou Mathieu¹*, Yapo Angoué Paul¹, Ehilé Ehouan Etienne¹

¹Laboratory of Animal Physiology, Pharmacology and Pharmacopoeia of UFR-SN, University of Nangui Abrogoua, Côte d’Ivoire.

ABSTRACT

*Blighia unijugata* is a plant used in traditional to treat many pains and diseases such as fever, headaches, kidney pain and stiffness, dizziness and high blood pressure. No or few studies were achieved on its effects on cardiovascular system. So, the present study was aimed to evaluate the effects of a butanol fraction from the leaves of *Blighia unijugata* (BFBu) on the arterial blood pressure of rabbit and to determine its mechanism of action. The recording of the arterial blood pressure was achieved by a mercury manometer kymograph of Ludwig. BFBu induced dose dependent hypotension for doses ranging from 0.5 to 50 mg/kg b.w. This hypotension was reduced by increasing doses of atropine (5x10⁻⁸-5x10⁻⁵ mg/kg b.w.) and propranolol (5x10⁻⁷-5x10⁻² mg/kg b.w.). BFBu lowered the transient hypertension caused by adrenaline at 5x10⁻³ mg/kg b.w. These findings suggested the presence of cholinomimetic and β-adrenoceptors agonist-like substances in the extract and the possible interaction of compounds on the sympathetic system. These effects could justify the use of this plant in traditional medicine to treat hypertension.

Keywords: *Blighia unijugata*, arterial blood pressure, cholinomimetic, β-adrenoceptors agonist-like substances.
INTRODUCTION
Among the non transmissible chronic diseases, hypertension is the most dramatic and the most common cardiovascular disease in sub-Saharan Africa. It is one of the major causes of disability, morbidity and mortality among populations. In Côte d’Ivoire, the prevalence is estimated to 20%. Several antihypertensive drugs offered by modern medicine are used by populations. However, in the developing countries, nearly 80% of the population resorts to traditional medicine for healthcare. \textit{Blighia unijugata} is among the great source of plants used. It is a forest species widespread in tropical Africa, from Sierra Leone to Cameroon. It is found in Côte d’Ivoire in the secondary formations in reforestation. \cite{7,8,9} \textit{Blighia unijugata} is a shrub with 6 to 9 m tall, but sometimes up to 30 m tall, with a dense crown. The bark is gray or brown, generally smooth, fine, sometimes with warts. The leaves are reddish when young and become shiny green when adult. \cite{7,10} Many traditional indications of this plant have been reported. \textit{Blighia unijugata} is used as a vegetable and also in the treatment of fever, nausea and vomiting, leprosy, eyes aches, coughing, headaches, rheumatism, kidney pain and stiffness, dizziness and high blood pressure. \cite{7,9} Ethanol extracts of roots, stem barks and leaves showed antibacterial activity, especially against \textit{Staphylococcus aureus}. \cite{11} The study of the toxicity of oils extracted from seeds and aril of \textit{Blighia unijugata} on hematological and biochemical parameters and histopathological analysis in rats revealed that these oils induced no signs of clinical toxicity. \cite{12} The presence of steroids, saponins and tannins was shown in all these extracts. \cite{12} No or few scientific study has been mentioned on the cardiovascular system to confirm the use of this plant in the treatment of hypertension. The goal of this work was to study the effects of a butanol fraction from leaves of \textit{Blighia unijugata} on arterial blood pressure of rabbits and to elucidate the possible mechanisms of action.

MATERIAL AND METHODS
Animals
Rabbits (\textit{Oryctolagus cuniculus}) weighing 2 ± 0.2 kg were used. They were bred in Animal house of Animal Physiology, Pharmacology and Pharmacopoeia of the University of Nangui Abrogoua (Former University of Abobo-Adjamé, Abidjan, Côte d’Ivoire) according to the principles for the care and use of laboratory animals of the Ethical Committee of the University (Nangui Abrogoua, Abidjan, Côte d’Ivoire).
Plant material
Fresh leaves of *Blighia unijugata* (Sapindaceae) were collected in Abidjan (Côte d'Ivoire) in June 2009. Taxonomical identification of those leaves was established by Professor Aké-Assi Laurent from the National Floristic Centre of University of Felix Houphouet Boigny, Cocody- Abidjan, Côte d'Ivoire, voucher n°165 of Côte d'Ivoire national herbarium.

Plant extraction
Fresh leaves of *Blighia unijugata* were dried under shade and powdered with an electric grinder (Culatti, France). The extraction process was implemented according to the methods described by some authors.\[13\] \[14\] \[15\] One hundred grams (100g) of the leaves powder were macerated for 48 hours in 2l of ethanol 96% under magnetic shaking. The resulting solution was filtered (Whatman n°1) and concentrated under reduced pressure using a rotary evaporator (Büchi R110, type MKE 6540/2) at a temperature of 60°C. After drying in an oven at 45°C for 48 hours, 17.3 g of ethanol extract were obtained. Ten grams (10 g) of ethanol extract were dissolved in 200 ml of boiling distilled water. The mixture was homogenized under magnetic shaking for 15 minutes at 27 ± 2°C. The aqueous solution obtained was then exhausted for 10 minutes at 27 ± 2°C with 200 ml of hexane to give two phases after decantation: a residual aqueous phase and an organic phase. The residual aqueous phase was again treated for 10 minutes at 27 ± 2°C with 200 ml of chloroform to give two phases: an organic phase and a residual aqueous phase. The same operation was continued by successively treating the residual aqueous phase with ethyl acetate and then with butanol. Each of these organic phases and the final residual aqueous phase obtained were concentrated under reduced pressure at 60°C using a rotary evaporator as described above. Organic fractions obtained from these partitions were hexane fraction (0.6 g), chloroform fraction (1.1 g), ethyl acetate fraction (1.6 g), and butanol fraction (3.18 g) and the aqueous fraction was the final residual aqueous fraction (3.12 g).

Direct arterial blood pressure measurement in rabbits
The method was as previously described by by some authors.\[16\] \[17\] \[18\] \[19\] The rabbits were anaesthetized using ethyl urethane (40%) at a dose of 1g/kg b.w. The saphenous vein was cannulated with heparinized polyvinyl tubing for intravenous injection of the extract and drugs. The left common carotid artery was cannulated and connected to a mercury manometer kymograph of Ludwig. Thus, the variations of the carotid blood pressure were transmitted to the mercury and recorded by a stylet on paper. The different fractions from
leaves of *Blighia unijugata* were tested at 0.5, 2.5, 5, 10, 20, 30, 40 and 50 mg/kg b.w. Atropine was used at $5 \times 10^{-8}$, $5 \times 10^{-6}$, $5 \times 10^{-4}$, and $5 \times 10^{-2}$ mg/kg b.w. Propranolol was employed at $5 \times 10^{-7}$, $5 \times 10^{-5}$, $5 \times 10^{-3}$ and $5 \times 10^{-2}$ mg/kg b.w. Adrenaline was used at $5 \times 10^{-3}$ mg/kg b.w. The fractions and the chemicals were dissolved in Mac Ewen solution of the following composition (mM): NaCl 130; KCl 2.5; CaCl$_2$ 2.4; NaH$_2$PO$_4$ 1.18; CO$_3$NaH 11.9; MgCl$_2$ 0.24; C$_6$H$_{12}$O$_6$ 2.2 with a pH adjusted to 7.4.

**Chemicals**

Atropine and adrenaline were purchased from Prolabo (France) and propranolol from AstraZeneca (France).

**Data analysis**

All values were expressed as mean ± standard error on the mean (m ± sem). Statistical analysis and graphics were carried out using the software GraphPad Prism 5.01 (San Diego California, USA). The significance of the differences observed between the doses was achieved by analysis of variances (ANOVA) followed by Tukey test. The differences between the doses were considered statistically significant when $p<0.05$.

**RESULTS**

**Effects of ethanol extract from *Blighia unijugata* and its different fractions on arterial blood pressure of rabbit**

The results of the change in blood pressure obtained after intravenous injections of increasing doses of ethanol extract or fractions from *Blighia unijugata* leaves (0.5, 2.5, 5, 10, 20, 30, 40 and 50 mg/kg b.w.) on arterial blood pressure of rabbits are shown in Table 1. All these extracts and fractions caused drops in blood pressure. The ethanol extract and its hexane, chloroform, ethyl acetate and aqueous fractions of *Blighia unijugata* caused respective falls of 35.4 ± 1.4%, 7.3 ± 1.11 %, 6.52 ± 0.55 %, 17.7 ± 0.59% and 25.2 ± 1.24 % of the blood pressure as compared to baselines blood pressure of rabbits. However, these effects were less important and statistically different ($p < 0.01$, $n = 5$) than that of the butanol fraction which induced sustained hypotension of 43.3 ± 1.41%. Therefore, the butanol fraction (BFBu) was retained for the rest of this study.
Table 1: Effects of ethanol extract and its fractions from *Blighia unijugata* Bak. leaves on rabbit arterial blood pressure

<table>
<thead>
<tr>
<th>Extract/Fractions</th>
<th>Doses (mg/kg b.w.)</th>
<th>0.5</th>
<th>2.5</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ETE</td>
<td>± 2.55</td>
<td>10.5</td>
<td>15.4</td>
<td>20.1</td>
<td>24.4</td>
<td>30.1</td>
<td>33.3</td>
<td>35.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>± 0.41</td>
<td>2.00</td>
<td>4.75</td>
<td>6.00</td>
<td>6.25</td>
<td>7.3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>± 0.25</td>
<td>0.25</td>
<td>2.28</td>
<td>3.59</td>
<td>5.10</td>
<td>5.97</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>± 0.025</td>
<td>0.39</td>
<td>6.95</td>
<td>9.17</td>
<td>10.9</td>
<td>12.9</td>
<td>17.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>± 0.052</td>
<td>1.16</td>
<td>1.35</td>
<td>1.16</td>
<td>1.35</td>
<td>0.96</td>
<td>1.27</td>
<td>1.40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>± 0.52</td>
<td>1.74</td>
<td>2.38</td>
<td>1.20</td>
<td>1.5</td>
<td>1.25</td>
<td>1.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>± 0.33</td>
<td>0.79</td>
<td>0.66</td>
<td>0.64</td>
<td>0.84</td>
<td>1.31</td>
<td>1.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>± 0.33</td>
<td>1.16</td>
<td>1.35</td>
<td>1.16</td>
<td>1.35</td>
<td>0.96</td>
<td>1.27</td>
<td>1.40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>± 0.52</td>
<td>1.74</td>
<td>2.38</td>
<td>1.20</td>
<td>1.5</td>
<td>1.25</td>
<td>1.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>± 0.33</td>
<td>0.79</td>
<td>0.66</td>
<td>0.64</td>
<td>0.84</td>
<td>1.31</td>
<td>1.24</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**ETE**: Ethanol extract; **Hex.**: Hexane extract; **Chl.**: Chloroform extract; **e.a.**: Ethyl acetate extract; **But.**: Butanol extract; **Aq.**: Aqueous extract

**Dose response effect of BFBu on arterial blood pressure of rabbit**

A typical recording of the dose response effect of BFBu is shown in Figure 1. For doses ranging from 0.5 to 50 mg/kg b.w., BFBu significantly (p<0.001) and dose dependently impaired arterial blood pressure of rabbits from 11.60 ± 1.74% to 43.30 ± 1.41% as compared to the reference values of rabbits blood pressure in rabbits. The effective dose 50% (ED<sub>50</sub>) of BFBu determined graphically was 3.45 mg/kg b.w. (Figure 2).
Figure 1: Dose response effects of BFBu on rabbit arterial blood pressure

Control recording with Mac Ewen (M.E.). (A1); Effect of BFBu at 0.5 (A2); 2.5 (A3); 5 (A4); 10 (A5); 20 (A6); 30 (A7); 40 (A8) and 50 (A9) mg/kg b.w.

The arrows indicate the moment of administration of the substances.

Figure 2: Decrease of arterial blood pressure of rabbit induced by BFBu

***P < 0.001; n=5.
3-Interaction BFBu-Atropine on arterial blood pressure of rabbit
The effects of BFBu in the presence of increasing doses of atropine (5x10^{-8} - 5x10^{-2} mg/kg b.w.) were achieved on the arterial blood pressure of rabbits. A control value of the effect of BFBu at 20 mg/kg b.w. indicated a decrease of 34.90 ± 1.70% of blood pressure. This hypotension was gradually reduced by prior injection of atropine. Indeed, the decrease of blood pressure recorded varied from 30.40 ± 0.79% to 15.10 ± 0.90 % as compared to baseline blood pressure. However, this inhibition was partial because a residual hypotension remained even at higher doses of atropine. The effect of atropine was significant (p < 0.001) on BFBu-induced hypotension (Figure 3).

![Figure 3: Hypotension induced by BFBu in presence of atropine](image)

* P<0.05; ***P<0.001; n=5

4-Interaction BFBu-propranolol on arterial blood pressure of rabbit
Propranolol, a non specific blocker of β-adrenergic receptors, was used in order to study the involvement of β-adrenergic agonists in the hypotension induced by BFBu. At 20 mg/kg b.w., BFBu caused a control hypotension of 43.90 ± 3.37%. Propranolol, for doses ranging from 5x10^{-7} to 5x10^{-2} mg/kg b.w. significantly p < 0.001 and partially inhibited the hypotension triggered by BFBu at 20 mg/kg b.w. So, the hypotension caused by BFBu dropped and reached 19.50 ± 1.99% in the presence of propranolol at 5x10^{-2} mg/kg b.w. (Figure 4).
5-Effect of BFBu in presence of adrenaline on rabbit arterial blood pressure

The intravenous injection of adrenaline at $5 \times 10^{-3}$ mg/kg b.w. elicited a transient increase of 69.60 ± 4.16 % of rabbit blood pressure. The administration of increasing doses of BFBu in a range of doses from 2.5 to 20 mg/kg b.w. was found to reduce dose dependently and significantly $p < 0.001$ the transient hypertension induced by adrenaline. Control hypertension caused by Adrenaline fell and attained 55.60 ± 3.87 to 17.60 ± 1.30% (Figure 5).

DISCUSSION

Successive fractionation of ethanol extract from the leaves of *Blighia unijugata* with five solvents produced five fractions. The ethanol extract and its fractions were tested on rabbit
blood pressure. The results revealed that the butanol fraction of *Blighia unijugata* (BFBu) was the most active. This difference between the effects of these extracts could be due to the separation of the major chemical constituents present in the ethanol extract of this plant. The study of the pharmacological effects of BFBu on the arterial blood pressure of rabbits showed that the butanol fraction of this plant induced dose-dependent and significant hypotension for doses ranging from 0.5 to 50 mg/kg b.w. These effects were similar to those obtained in the same experimental conditions with plant extracts such as a chromatographic fraction from *Bidens pilosa* leaves,\(^{17}\)\(^{18}\) the aqueous extract of *Bambusa vulgaris* and the aqueous extract from the leaves of *Lophira lanceolata*.\(^{20}\)\(^{19}\) These authors showed that aqueous extracts of these plants caused dose-dependent hypotension in rabbits. In addition, the hypotensive effects induced by BFBu were also similar to those well known of acetylcholine on blood pressure as described by a researcher.\(^{21}\) Therefore it was hypothesized that cholinomimetic substances could be present in the butanol extract. To verify this suggestion, the effects of BFBu were evaluated in presence of atropine, a muscarinic cholinceptors antagonist. The results exhibited a partial and significant inhibition of the hypotension elicited by BFBu in presence of atropine. So it was clear that BFBu contained cholinomimetic substances which used acetylcholine pathway to generate hypotension as shown by many authors with different plant extracts like a water extract of *Hibiscus sabdariffa* and an alcoholic extract of *Sesamum indicum* seeds.\(^{22}\)\(^{23}\) The actions of acetylcholine on blood pressure are well-known. The injection of acetylcholine leads to a transient hypotension due to cardioinhibition and vasodilatation and are largely described by many authors.\(^{24}\)\(^{25}\)

The partial inhibition of hypotension induced by BFBu in the presence of atropine suggested that this extract could possess other substances capable of reducing blood pressure. In that extent, the β-adrenergic agonists’ pathway was investigated. In presence of propranolol, a β-adrenoceptor antagonist, the hypotensive effects induced by BFBu were partially and significantly reduced, suggesting the presence of β-adrenoceptors agonist substances in this extract. These agonist-like substances could act on β2-adrenoceptors to produce a relaxant action on the vascular smooth muscles and therefore lower blood pressure. These findings were similar to those obtained with an ethanol extract from *Pavetta crassipes* leaves and an active chromatographic fraction of *Bidens pilosa*.\(^{26}\)\(^{18}\) These authors suggested the presence of β-adrenoceptors agonist agents in these plants. Moreover, some authors suggested the presence of agonist-like agents that would act on β2-adrenoceptors to trigger a relaxant action on the vascular smooth muscles, since propranolol, a beta-blocker was able to block the
action of the crude aqueous leaf extract of *Viscum album* on arterial blood pressure of rats.\[^{27}\] It is also known that the intravenous injection of isoprenaline, a non-specific agonist of $\beta_1$ and $\beta_2$-adrenoceptors in the anesthetized rat causes hypotension which is reduced by propranolol.\[^{28}\] Indeed, hypotension induced by $\beta$-adrenoceptors agonist agents largely depends on their vasodilator effect. These substances act on the $\beta_2$-adrenoceptors to produce a relaxing effect on vascular smooth muscle that propranolol would be able to block.\[^{29}\]

The antihypertensive effect of BFBu was assessed in presence of adrenaline. The results indicated that BFBu significantly reduced the transient hypertension induced by adrenaline, suggesting interference of BFBu with certain classes of adrenergic receptors and the sympathetic system. Similar findings were reported by some authors who reported that the antihypertensive action of *Morinda morindoides*, *Parkia biglobosa*, *Bidens pilosa* and *Lophira lanceolata* could involve interaction of these plant extracts with the sympathetic system.\[^{30\text{[31][17][19]}\]

**CONCLUSION**

The pharmacological effects of a butanol fraction from leaves of *Blighia unijugata* (BFBu) on the arterial blood pressure of rabbits showed that this fraction induced hypotension and antihypertensive effects probably due to cholinomimetic and $\beta$-adrenoceptors agonist substances. These results could justify the use of this plant in traditional medicine to treat hypertension.

**REFERENCES**


19. Kouakou KL, Bléyéré NM, Oussou NJB, Konan BA, Amonkan KA, Abo KJC, Yapo AP, Ehilé EE. Effects of leaf decoction from Lophira lanceolata Tiegh. Ex Keay (Ochnaceae)


