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Effects of leaf decoction from *Lophira lanceolata* Tiegh. Ex Keay (Ochnaceae) on arterial blood pressure and electrocardiogram in anesthetized rabbits

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Lophira lanceolata (Ochnaceae) is a plant used in traditional medicine to treat many illnesses such as headaches, dysentery, diarrhoea, cough, abdominal pains and cardiovascular diseases. Ethnobotanical surveys in the centre of Côte d'Ivoire revealed that this plant is employed in the treatment of hypertension by traditional healers. As no or few scientific works were undertaken on this side, the present study was aimed to assess the effects of a decoction of *Lophira lanceolata* on arterial blood pressure and electrocardiogram in rabbits. The recording of the arterial blood pressure was achieved by a mercury manometer kymograph of Ludwig while the registration of electrocardiogram was implemented by an electrocardiograph (CARDIOFAX ECG-6851K Nihon Kohden, Japan). The decoction of the fresh leaves of *Lophira lanceolata* (DALLA) induced a dose dependent hypotension in rabbits for doses ranging from 0.5 to 40 mg/kg body weight with an ED₅₀ (effective dose) of 8.67 mg/kg body weight. DALLA significantly decreased the transient hypertension caused by adrenaline at 5.10⁻³ mg/kg body weight. The hypotension induced by DALLA was significantly attenuated by atropine (a muscarinic cholinergic antagonist). On rabbit electrocardiogram, DALLA decreased the amplitudes of P, QRS and T waves, the duration of PQ interval and heart rate. However, the duration of QT interval increased. The phytochemical screening of the extract revealed the presence of sterols and polyterpenes, phenols, flavonoids, catechic tannins, alkaloids and saponins. These results suggested that DALLA contained cholinomimetic substances and also interacted with adrenoceptors. Some of the phytochemical compounds such as phenols, flavonoids, catechic tannins and alkaloids which are known for their antihypertensive effects may explain the use of this plant by traditional healers to treat hypertension.

Keyword: *Lophira lanceolata*, hypertension, electrocardiogram, phytochemical screening, cholinomimetic.

1. Introduction

Hypertension is the most common cardiovascular disease in sub-Saharan Africa [1, 2, 3]. With a prevalence of 20% in Côte d'Ivoire [4], hypertension is an important cause of morbidity and mortality. Alongside the range of antihypertensive drugs offered by modern

medicine, several medicinal herbal recipes are recommended by traditional medicine for the treatment of this pathology. In this context, during an ethnobotanical survey of plants used in the treatment of hypertension in Bouaké (Centre of Côte d'Ivoire), several traditional healers mentioned that the decoction of *Lophira*

lanceolata is used in beverage for the treatment of this pathology [5]. *Lophira lanceolata* is a tree of the wooded savannah. It often grows gregariously on fallow land at the edge of forests. It is found out from Senegal to Cameroon and Sudan and also in Côte d'Ivoire where the Baoulé (an ethny in the centre of Côte d'Ivoire) calls it « n'goïn yassoua » [6] in reference of an oil made from the seeds. It is a tree of 8 to 10 m tall, straight or twisted, with leaves alternate, clustered at the end of short straight branches, glabrous, bright and blade oblong-lanceolate. The bark surface is corky grey [7]. *Lophira lanceolata* is used in traditional medicine to treat several illnesses. The decoction of the fresh leaves is administered orally against headaches, dysentery, diarrhoea, cough, abdominal pains and cardiovascular diseases. It is also used on skin to cure wounds [7]. No or few scientific studies with *Lophira lanceolata* was mentioned about its use in the treatment of hypertension. Thus, this work was aimed to assess the effects of a decoction of *Lophira lanceolata* on arterial blood pressure and electrocardiogram in rabbit.

2. Material and methods

2.1 Animals

Rabbits (*Oryctolagus cuniculus*) weighing 2±0.1 kg were used. They were bred in Animal house of Animal Physiology, Pharmacology and Phytotherapy 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).

2.2 Plant material

Fresh leaves of *Lophira lanceolata* were collected locally from the savannah region of Bouaké in the centre of Côte d'Ivoire in August 2010. Taxonomical identification of the leaves was established by Professor Aké-Assi Laurent from the National floristic Centre of University of Felix Houphouët Boigny, Cocody- Abidjan, Côte d'Ivoire, voucher n° 9397, *Lophira lanceolata*

Tiegh. ex Keay in December 1966 of Côte d'Ivoire national herbarium [8].

2.3 Plant extraction

A decoction was made with one hundred grams (100 g) of fresh leaves of *Lophira lanceolata* in two liters (2 l) of distilled water for 45 minutes. The resulting solution was carefully filtered three times on cotton wool and on Whatman (n° 1) filter paper. The filtrate was dried in an oven at 60 °C. The powder obtained corresponded to the decoction of *Lophira lanceolata* (DALLA).

2.4 Direct arterial blood pressure measurement in rabbits

The method was as previously described by some authors [9, 10]. The rabbits were anaesthetized using ethyl urethane (40%) at a dose of 1 g/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.

2.5 Registration of the global electrical activity (ECG) of the rabbit

The method was as previously described by many authors [11, 12, 13, 14]. The electrocardiogram of the rabbit was recorded by the technique of external electrodes used in the human practices and adapted to the rabbit [11]. Briefly, the saphenous vein of the anesthetized rabbit by an intraperitoneal injection of 40% ethyl urethane (1 g/kg body weight) was intubated in order to administer plant extract [11]. The armpits of the anterior limbs and the groin of the posterior limbs were shaved and cleaned with 90% ethanol. After applying electrolytic dough, four electrodes were put and bound to the four sockets of the registration cable connected to the electrocardiograph (CARDIOFAX ECG-6851K, Nihon Kohden, Japan). The studied parameters (P, QRS, T waves, PQ, QT intervals and cardiac frequency) were recorded from the DIII derivation of the standards or bipolar Einthoven

derivations on thermo sensitive paper, at constant speed (25 mm/s). DALLA was dissolved in Mac Ewen solution of the following composition (mM): NaCl 130; KCl 2.5; CaCl₂ 2.4; NaH₂PO₄ 1.18; CO₃NaH 11.9; MgCl₂ 0.24; C₆H₁₂O₆ 2.2 with a pH adjusted to 7.4.

2.6 Phytochemical screening

The leaf decoction of *Lophira lanceolata* was screened for the presence of polyphenols, tannins, flavonoids, saponins, alkaloids, sterols and ployterpenes, and quinones. Detection of these constituents was carried out as described by certain authors [15].

2.7 Chemicals

Atropine and adrenaline were purchased from Prolabo (France).

2.8 Data analysis

All values were expressed as mean \pm standard

error on the mean ($m \pm sem$). 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) of the multiple tests of comparison of Tukey-Kramer. Differences between concentrations were considered statistically significant when $p < 0.05$.

3 Results

3.1 Dose response effect of DALLA on rabbit arterial blood pressure

The injection of increasing doses of DALLA ranging from 0.5 to 40 mg/kg b.w. in rabbits caused a significant ($p < 0.05$) dose-dependent hypotension. A fall of the normal blood pressure from $7.17 \pm 1.34\%$ to $38.66 \pm 1.85\%$ was recorded. The sigmoidal dose response curve permitted to determine an ED₅₀ value of 8.67 mg/kg b.w. (Figure 1).

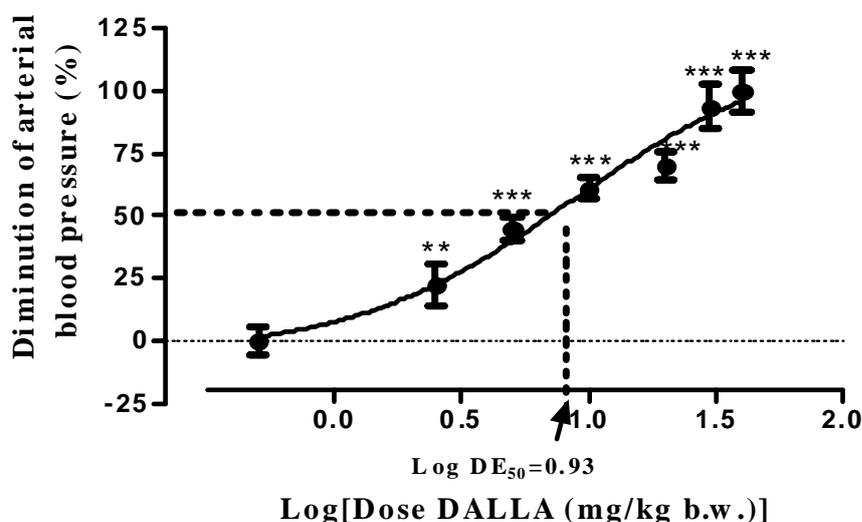


Fig 1: Dose response effect of DALLA on arterial blood pressure of rabbit

DALLA induced significant dose dependent decrease of arterial blood pressure in rabbit. The ED₅₀ determined graphically was 8.67 mg/kg b.w. ** $P < 0.01$; *** $P < 0.001$; $n = 5$.

3.2 Effect of DALLA on rabbit arterial blood pressure in presence of Atropine

To investigate a possible involvement of cholinceptors in the hypotension induced by DALLA, atropine, a muscarinic antagonist of cholinceptors, was used. Control value of hypotension induced by DALLA at 20 mg/kg

b.w. was $40.3 \pm 2.57\%$. Increasing doses of atropine ranging from 10^{-7} to 10^{-2} mg/kg b.w. significantly ($p < 0.05$) attenuated the hypotension elicited by DALLA at 20 mg/kg b.w. Indeed, the control value of hypotension was reduced and reached $34 \pm 3.31\%$ (Atr 10^{-7} mg/kg b.w.) to $23.1 \pm 2.22\%$ (Atr 10^{-2} mg/kg b.w.). The action of

Atropine on hypotension caused by DALLA is illustrated in Figure 2.

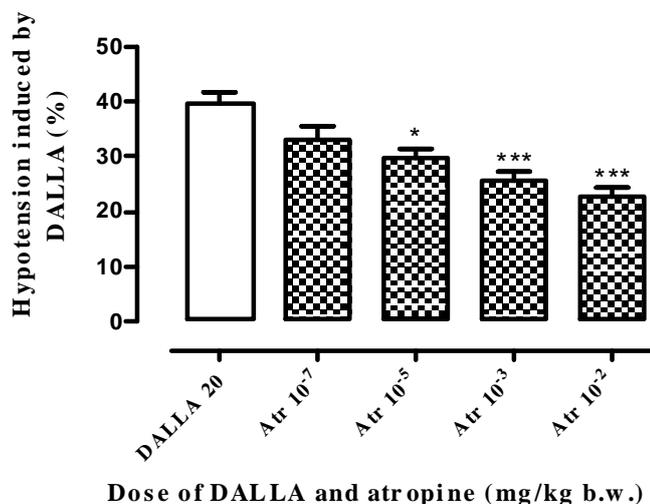


Fig 2: Effect of atropine on DALLA-induced hypotension in rabbit

The pretreatment of rabbit with increasing doses of atropine significantly reduced the hypotension caused by DALLA.

*P<0.05; ***P<0.001; n=4.

3.3 Effect of DALLA in presence of Adrenaline on rabbit arterial blood pressure

The intravenous administration of Adrenaline at 5 10⁻³ mg/kg b.w. increased normal blood pressure of rabbit from 64±1.77%. The injection of increasing doses of DALLA from 5 to 30

mg/kg b.w. significantly (p<0.05) diminished the hypertension induced by adrenaline at 5 10⁻³ mg/kg b.w. Control hypertension caused by Adrenaline decreased and attained 51.2±1.57% to 28.7±1.15% (Figure 3).

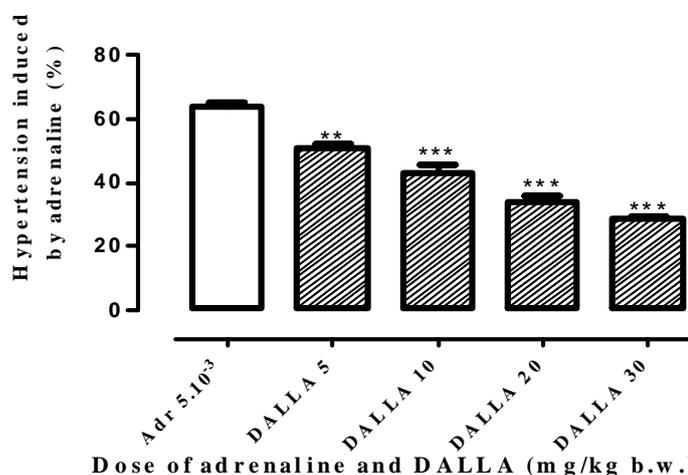


Fig 3: Effect of DALLA on adrenaline-induced hypertension in rabbit

DALLA significantly decreased the hypertension elicited by adrenaline in rabbit. **P<0.01; ***P<0.001; n=4.

3.4 Dose response effect of DALLA on rabbit electrocardiogram

As shown in Table 1, the dose response effect of DALLA was achieved on rabbit electrocardiogram. For doses ranging from 0.5 to

50 mg/kg b.w., DALLA influenced the different parameters of rabbit ECG. The normal ECG values recorded (control) significantly (p<0.05) decreased for P, T, and QRS waves. Indeed, P wave diminished from 99.1±3.87 to 71.5±4.36 μV

while T wave dropped from 124.2±15.16 to 50.05±0.5 μv. QRS complex control value (432±3.11 μv) was reduced and reached 337±2.63 μv. The cardiac frequency also decreased from 271±1 to 225±0.37 cycles/min. PQ interval was significantly (p<0.05) affected

and fell from 77.3±0.7 to 62±1.1 ms for doses of DALLA ranging from 0.5 to 50 mg/kg b.w. However, QT interval was augmented but not significantly (p>0.05) from 160.7±9.9 to 201±6.3 ms in the same range of doses.

Table 1: Dose response effect of DALLA on rabbit electrocardiogram

DALLA (mg/kg b.w.)	P wave (μV)	QRS complex (μV)	T wave (μV)	PQ Interval (ms)	QT Interval (ms)	Cardiac Frequency (Cycles/min)
0 (Control)	99.1±3.87	432±3.11	124.2±15.16	77.3±0.7	160.7±9.9	271±1
0.5	94±2.6	423±3.04	116.1±10.1	75.9±0.9	166.1±7.8	269±0.9
2.5	92.1±3.1	407±2.71	119.7±9.7	70.1±0.6	171.7±8.3	262.6±0.6
5	90.8±2.6	394±4.65	106.05±8	69.4±0.5	173.7±8.4	261.4±0.7
10	88.6±4	384±5.05	101.6±5.7	68.1±0.8	171.6±9.3	253.3±0.4**
20	85.8±2.5	374±4.85	83.95±6	65.4±0.7*	176±8.7	235±0.6**
30	82.5±2	360±3.57**	77.2±2.1**	63.4±0.8**	185.9±6.4	228.7±0.7***
40	77±3.35 **	348±2.65***	63.71±3.2***	62.8±0.9 **	189.4±7	225±0.4***
50	71.5±3.46 ***	337±2.63***	50.05±0.5***	62±1.1**	201±6.3	225±0.37***

P, T, QRS waves, PQ interval and cardiac frequency were significantly dropped by increasing doses of DALLA. QT interval was augmented but not significantly in presence of DALLA.

* P<0.05; **P < 0.01; ***P < 0.001; n=5.

3.5 Phytochemical screening of DALLA

Phytochemical screening of DALLA, as shown in Table 2, revealed the presence of sterols and

polyterpenes, polyphenols, flavonoids, catechin tannins, alkaloids and saponins.

Table 2: Phytochemical screening of DALLA

Constituents	Reagents	DALLA
Polyphenols	FeCl ₃ test	++
Tannins	Stiasny test	++
	FeCl ₃ test	-
Flavonoids	Cyanidine test	++
Quinones	Borntraëger test	-
Alkaloids	Bouchardât test	++
	Dragendorff test	++
Saponins	Frothing test	++
Sterols and polyterpenes	Liebermann test	++

- = absence, ++ = abundant

4. Discussion

The leaf decoction of *Lophira lanceolata* (Ochnaceae) induced a significant dose-dependent hypotension in rabbits for doses ranging from 0.5 to 40 mg/kg b.w. These results are similar to those obtained with plant extracts such as an ethanolic extract of *Teucrium polium* L. [16], an aqueous extract of *Bambusa vulgaris* [17], a chromatographic fraction from *Bidens pilosa* L. leaves [9], an extract of *Raphanus*

sativus [18]. Atropine pathway was hypothesized in the hypotension induced by DALLA. Thus, rabbits were pretreated with increasing doses of atropine (10⁻⁷-10⁻² mg/kg b.w.). Results exhibited significant inhibition of the hypotension induced by DALLA, suggesting that this extract contained cholinomimetic substances acting via muscarinic receptors. The same conclusion was highlighted by some authors on interaction of plant extracts with atropine. Indeed, some authors [18] showed

that the hypotension induced by an extract of *Raphanus sativus* in rats was inhibited by atropine. Other authors [19] reported that dried flowers 70% aqueous methanol extract from *Lavandula stoechas* produced a drop in blood pressure in anesthetized normotensive rats which was abolished by atropine. The presence of acetylcholine-like substances in the alcoholic extract of *Sesamum indicum* seeds was indicated by another group of researchers [20]. Besides, some studies [21] showed that the aqueous extract of *Desmodium styracifolium* caused hypotension which was mediated through cholinergic receptor stimulation in rats. The actions of acetylcholine on blood pressure are well known. According to two researchers [22], the intravenous injection of acetylcholine in humans or animals leads to an immediate and transient drop in blood pressure resulting from cardiac slowing and vasodilatation. The slowing of the heart is explained by cell hyperpolarization following the opening of potassium channels that are directly related to G proteins [23, 24]. The decrease in the force of contractions followed by hypotension is due to a reduction in Ca^{2+} entry caused by an inhibition of adenylate cyclase and also a reduction of Ca^{2+} release from sarcoplasmic stores [25]. Peripheral vasodilatation is secondary to the activation of a G protein-coupled to acetylcholine muscarinic receptors. This coupling leads to production of nitric oxide (NO) or a vasodilator substance called Endothelium Derived Hyperpolarizing Factor (EDHF), which has a relaxing effect on vascular smooth muscle [26, 27]. The antihypertensive effect of DALLA was assessed on adrenaline-induced hypertension in rabbit. Increasing doses of DALLA ranging from 5 to 30 mg/kg b.w. significantly diminished the transient high blood pressure produced by adrenaline at 5×10^{-3} mg/kg b.w. This result suggested interference of the extract with certain classes of adrenergic receptors or with the sympathetic system. It was probable that certain antihypertensive substances in DALLA could be opposed to effects of adrenaline. Similar results in the same experimental conditions were observed with *Morinda morindoides* [28], *Parkia biglobosa* [29] and *Bidens pilosa* [9]. These authors

showed that extracts from the plants cited above reduced adrenaline-induced hypertension in rabbits and stated a possible interaction with adrenoceptors. Some researchers [30] found out that the hydroalcoholic extract from *Solanum sisymbriifolium* root intravenous administration elicited antihypertensive effect in anesthetized hypertensive rats. Moreover, another group of scientists [31] also argued that the crude extract of *Viscum album* blocked noradrenaline-induced elevation of blood pressure in normotensive rats and suggested a possible involvement of sympathetic mechanism. However, further investigations on DALLA are necessary to elucidate the complete mechanism of the involvement of adrenoceptors and the sympathetic system.

DALLA was tested on rabbit ECG. Results revealed decreases of P, QRS and T waves, cardiac frequency and PQ interval. Therefore, the general outcome of the action on the global electrical activity on rabbit heart was inhibition. These effects could be due to the presence of cholinomimetic substances in DALLA which action was found to produce a decrease of cardiac activity. Similar findings were obtained with plant extracts such as an aqueous leaf extract of *Sesamum radiatum* [12], a methanol extract from the stem barks of *Erythrina senegalensis* [13] and a chromatographic fraction from the aqueous leaf extract of *Bidens pilosa* [14]. These authors suggested that the inhibitory effects of their extracts were attributable to acetylcholine-like actions resulting in a depressive effect on the sinus node and thus reducing global depolarization of other cardiac tissues. This corroborates what was observed with DALLA. QT interval increased slightly but not significantly. This is interesting because as stated by certain authors, a prolongation of QT interval could be source of arrhythmias [32, 33].

A phytochemical screening was implemented with DALLA and exhibited heterogeneity of chemical compounds composed of sterols and polyterpenes, polyphenols, flavonoids, saponins, catechin tannins and alkaloids. These substances may be responsible or involved in the pharmacological effects on the cardiovascular

system. Indeed, many workers acknowledged the beneficial effects of certain plant compounds on the cardiovascular system. Some [18, 34, 35] showed that some alkaloids, saponins, polyphenols and flavonoids could be beneficial for the cardiovascular system in experimental animals. According to another author [36], tannins, polyphenols and flavonoids present in the aqueous leaf extract of *Psidium guajava* were responsible for hypoglycemic and hypotensive effects of this plant. The positive effects of polyphenols on the cardiovascular system were largely argued by a group of scientific workers [37].

5. Conclusion

The effects of a leaf decoction of *Lophira lanceolata* (DALLA) were investigated on arterial blood pressure and electrocardiogram of rabbit. DALLA caused dose dependent hypotension due to cholinomimetic substances and interacted with adrenoceptors by reducing hypertension induced by adrenaline. The global electrical activity of the heart of rabbit was diminished by increasing doses of DALLA suggesting the action of cholinomimetic substances. The richness of the phytochemical compounds such as polyphenols and flavonoids present in DALLA could explain the effects of this extract on the cardiovascular system. These results could justify the use of this plant in traditional medicine to treat hypertension.

6. References

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