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Research Paper

Antihypertensive activity of Wakouba, a salt extracted from *Elaeis guineensis*, on blood pressure of rabbit

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Abstract

Wakouba,* a salt extracted from the fronds of *Elaeis guineensis* Jacq. oroil palm tree, is traditionally used by the people originating from the Western-Centre and South-western regions of Côte d'Ivoire for the treatment of hypertension. The purpose of this study was to evaluate the antihypertensive effects of this salt on blood pressure of rabbit. To do this, we used direct measurement of Ludwig. A mercury manometer or Ludwig gauge comprising of a recording cylinder connected to a catheter toward the heart direction that was used to incubate the rabbit carotid artery. Different concentrations of salt extract of *Elaeis guineensis* (*Wakouba*) was injected into the rabbit through the catheter and blood pressure changes was recorded on the smoked paper wrapped around the cylinder. The study of the effects of *Wakouba* on the changes in blood pressure and hypertension induced by adrenaline in rabbits revealed that *Wakouba* at concentrations ranging from 4.16 to 25 mg/kgbw causes dose-dependent hypotension similar to that of acetylcholine (ACh) a well-known hypotension inducer. This Hypotension is totally suppressed by atropine at concentration of 2 10^{-1}mg/kgbw. *Wakouba* thus contain muscarinic cholinergic compounds. Moreover, the comparative study on *Wakouba* and tenordate (nifedipine and atenolol), a commercially available antihypertensive substance, performed on an hypertensive rabbit showed that *Wakouba* at dose of 12mg/kgbw completely suppressed the hypertension induced by adrenaline. *Wakouba* have an hypotensive and antihypertensive effects on blood pressure of rabbit similar to those of tenordate Therefore *Wakouba* would have the same mechanism of action like that of tenordate thus a β blocker. These results justify its use in traditional medicine as an antihypertensive.

Keywords: *Wakouba*, Hypotension, Hypertension, Antihypertensive, Tenordate

Introduction

Considered in the past to be a typical overweight disease in advanced countries, hypertension could not appear as a priority in the health program of the developing countries, where priority is given to infectious and parasitic diseases that cause nearly one in two deaths 50% mortality[1].But the reality is different today. Indeed, the prevalence of hypertension in 2005 was estimated at 976 million worldwide, including 639 million in developing countries[2]. This prevalence , is very high in Africa, in Ghana 13.78% , 16.6% to 17% in Nigeria , 13.1% in Côte d'Ivoire , 5% to 15% in Senegal , 23.7% in Sahelian zone in Mali, 43.6% in urban areas in Guinea[3-6]. Cardiovascular diseases such as coronary heart disease, stroke and heart failure are favored by hypertension[7]. The treatment of hypertension is very difficult for majority of patients who are often financially handicapped and who cannot use modern drugs often very expensive. Therefore these patients use traditional medicine for their treatment. In addition, according to the resolution n° AFR/RC50/R3 drawn at the 31/08/2000, the

World Health Organization encourages all countries to develop regional strategies for the promotion and use of traditional medicine in health care. Moved by this resolution, our team like other researchers around the world is engaged in the research and enhancement of utilization of natural substances in cosmetic and therapeutic uses. Ethnobotanical surveys of traditional healers have led to the identification of several plants used in traditional treatment of hypertension. One of the investigated plants, namely *Elaeis guineensis Jacq* known as oil palm tree, seems highly effective. The salt extracted from *E. guineensis Jacq.* is traditionally used against the high blood pressure by the people from the south-western region in Côte d'Ivoire, especially the *Godie* people. The current study aims to probe the physiological activity of this salt during the hypertensive trouble.

Materials and Methods

Animal material

Rabbits (*Orictolaguscuniculus,* Leporidae) species, weighing 2±1.7 to 2.5±1.5 kg and obtained from Bingerville poultry farms, have been used. These rabbits were acclimated for three weeks in the animal unit of the faulty of Biosciences to harmonize their normal physiological state before any experimentation.

Chemicals

In this study we have used as chemicals substances the ethyl urethane (SIGMA SWITZERLAND), sodium chloride (Merck, ALLMANGNE), heparin (SANOFIE-Synthelabo, France), distilled water, potassium chloride, Adrenaline (used as control hypertension enhancer salt), and the tenordate.

Experimental instruments

Arterial blood pressure (BP) of rabbit was measured according to the method of Ludwig mercury manometer. The Ludwig mercury manometer has a " U " tube composed of two branches containing mercury (Hg). One of the branches has a plate floating on the surface of the mercury. On the floating plate is a metal rod on top of which is attached listing stylus. It records changes in BP on the smoky cylinder driven by a motor. The other branch has two leads. The lower lead is connected to a catheter through a flexible polyvinyl tube. These set up is filled with heparinized solution. This catheter is used to intubate the carotid artery. The upper lead has a syringe attached through a flexible polyvinyl tube. This set is also filled with heparinized solution and allows for high pressure.

Intubation of the saphenous vein and carotid artery

Rabbit asleep is placed supine on a roasting dissection of twine raised its forelegs. The posterior portion is facing the side and kept stable against the fence. The saphenous vein is stripped and hemisection is practiced at its lower portion. A catheter filled with heparinized NaCl solution was introduced to be regularly injected into the vein to prevent blood from coagulating. Carotid intubated towards the heart using a cannula filled with heparinized NaCl solution and connected to LUDWIG gauge. Different concentrations of *Wakouba* a salt from *Elaeis guineensis (Jacq)* were injected into rabbit through the catheter and the blood pressure was recorded on the smoked paper wrapped around a cylinder.

Place and period of study

The experiments were performed between November 2009 and February 2010in the laboratory of animal physiology and the laboratory of biochemical pharmacodynamy located at the Félix Houphouet-Boigny University, Abidjan, Côte d'Ivoire.

Processing of results and records

The smoky papers on which results were recorded were imbibed in cellulose varnish. Recorded readings were scanned and cleaned using Microsoft Paint software. The experiments were performed in triplicate and statistically valued at 5% significance level. The curves were plotted from these recorded results with GraphPad software (GP prism 4, Microsoft Sendiegie, California, USA), statistical data were collected using Statistica software. Results are expressed as mean ± standard error obtained from separate experiments.

Results and Discussion

Comparison of the dose- response effects of *Wakouba* and ACh on blood pressure

Figure 1 shows the effect of *Wakouba* on the arterial blood pressure (BP) of rabbit. The administration into rabbits increasing doses of *Wakouba* (4.16, 8.33, 12.50, 20, 83 and 25 mg/kg BW) causes a dose-dependent and progressive decrease of blood pressure or hypotension. This hypotension is

reversible at low doses and irreversible at high doses (25 mg/kg.bw) Hypotension values obtained compared to the normal blood pressure of rabbit (102±4 mmHg) are : 10±2.5, 20±3, 40±1.9, 50±3.1, and 60±2.6 mmHg, relying in respective decreases percentages of 9.8%, 31.37%, 41.11%, 45%, and 49% (Figure 1 A). The Ach doses between $1.4*10^{-5}$ and $1.4*10^{-2}$ mg/kgbw induce a dose-dependent hypotension varying from 115±3mmHg (normal blood pressure) to 49±2 mmHg, a percentage decrease of 0 to 63.20% (Figure 1 B). The figures 2C and 2D drawn from respective *Wakouba* and Acetylcholine allow the determination of the effective doses for 50% (ED_{50}). The ED_{50} value of *Wakouba* is 8.85 mg/kg bw while the ED_{50} of Ach is $2.5\ 10^{-1}$ mg/kg bw.

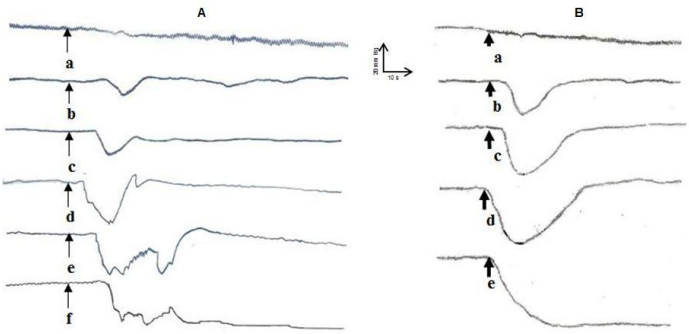

Figure 1: Dose-effect responses of *Wakouba* (A) and Acetylcholine (B) on arterial blood pressure of rabbits

At the stage ''a'', only NaCl 0.009 mg/mL is used. For Wakouba, the doses are: b = 4.12 mg/kg bw, c = 8.33 mg/kg bw, d= 12.50 mg/kg bw, e = 20.83 mg/kg bw, f = 25 mg/kg bw, administered as shown by the respective arrows. For Acetylcholine, the doses are: b = $2.4*10^{-5}$mg/kg bw, c = $2.4*10^{-4}$mg/kg bw, d = $2.4*10^{-3}$ mg/kg bw, e = $2.4*10^{-2}$ mg/kg bw, administered as shown by the respective arrows. The effective dose 50 % (ED_{50}) is $2.5\ 10^{-1}$ mg/kg bw, considered at 5% significance level for triplicate experiments. The Ach induces dose dependent hypotension.

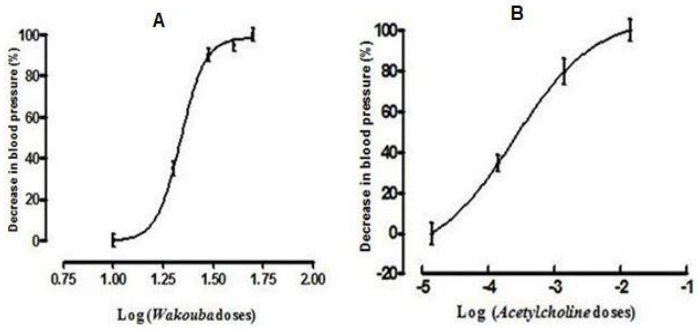

Figure 2: Dose-effect responses of *Wakouba* (A) and acetylcholine (B) on arterial blood pressure of rabbits expressed as decrease percentage from their normal blood pressure (115±3 mmHg)

The $ED_{50}$is $2.5.10^{-1}$ mg/kg bw.

Antagonistic effects from Atropine–*Wakouba* and Atropine-Acetylcholine interactions

Wakouba at dose of 12.50 mg/kg body weight (bw), causes control hypotension of 36.6±1 mmHg, a percentage decrease of 32.67% with respect to the normal blood pressure of 112±2 mmHg. Atropine (ATR) at doses between $3*10^{-5}$ mg/kg bw and $3*10^{-2}$ mg/kg bw, gradually decreases this hypotension until total suppression at $2*10^{-2}$ mg/kg bw (Figure 3A). Similarly hypotension (52±2.3 mm Hg) induced by Ach at dose of $2.4*10^{-3}$ mg/kg bw is progressively suppressed by ATR to values ranging between 48±2.66 and 0±2.5 mmHg for ATR doses between $1,28.10^{-5}$ and $1,28.10^{-2}$ mg/kg bw.A percentages decrease ranging from 7.7 to 100% with respect to the reference blood pressure (52 mmHg). The obtained values allowed us to draw the histograms of Figure 4A and Figure 4B.

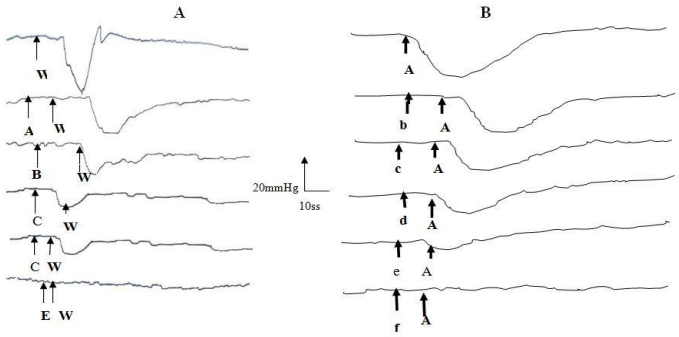

Figure 3A: Atropine (ATR)-Wakouba interaction effect on arterial blood pressure of rabbits Wakouba(W) at 20 mg/kg bw, added to ATR at doses of $2*10^{-5}$mg/kg bw (A), $2*10^{-4}$mg/kg bw (B), $2*10^{-3}$ mg/kg bw (C), $2*10^{-2}$mg/kg bw (D), and $2*10^{-1}$mg/kg bw (E)
Figure 3B: Hypotension values induced by Ach in the presence of Atropine (ATR). Ach (A)at $1.28*10^{-3}$ mg/kg bw, added to ATR at doses of $1.28*10^{-5}$ mg/kg bw (b), $1.28*10^{-4}$ mg/kg bw (c), $1.28*10^{-3}$ mg/kg bw (d), $1.28*10^{-2}$ mg/kg bw (e), $1.28*10^{-1}$ mg/kg bw (f)

Arrows indicate the moment of injection of different substances. Atropine reduced significantly hypotension induced by Ach at lower doses until total elimination of this hypotension at high doses.

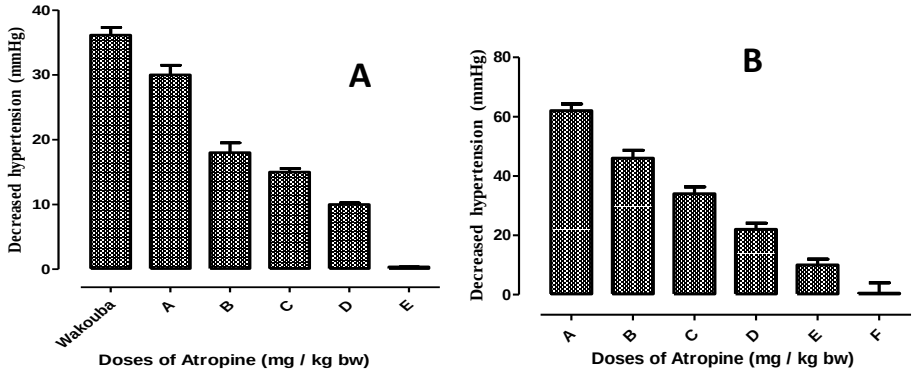

Figure 4A: Rate of decrease in the hypotension induced by *Wakouba*at the presence of increasing doses of Atropine
Figure 4B: **Rate of decrease in the hypertension induced by Acetylcholine at the presence of increasing doses of Atropine**

Antagonists effects of Adrenaline-*Wakouba and* Adrenaline-tenordate interactions
The antagonists effects resulting from both interactions are drawn in figures 5 and 6. The adrenaline administered at $3*10^{-2}$ mg/kg bw induced initial hypertension of 38±1.2mmHg. In the presence of increasing doses of *Wakouba* (4 to 20 mg/kgbw), the induced high blood pressure gradually decreases still complete removal at 12 mg/kg bw. However, beyond 12 mg/kg bw, the *Wakouba* salt led to significant hypotension from the rats.

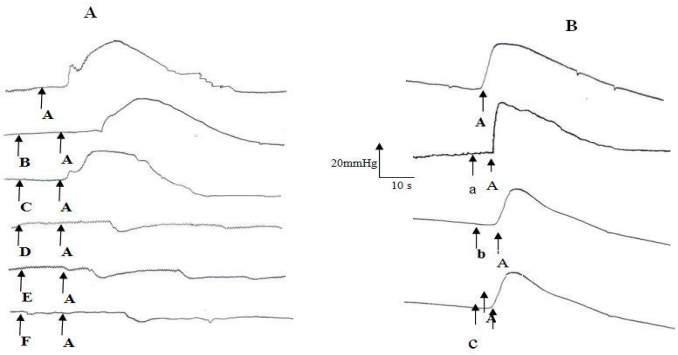

Figure 5: Adrenaline-Wakouba (A) and Adrenaline-Tenordate (B) interactions effects on the arterial blood pressure of rabbits

The hypertension values observed at each dose is as follows: 38±1.2mm Hg, 34±2mm Hg, 14±1.5 mm Hg, 12±1.2 mm Hg, and 10±1mmHg. With the tenordate, increasing doses (0.85 mg/kg bw, 1.71 mg/kg bw, 3.42 mg/kg bw, and 6.85 mg/kg bw) gradually dropped the hypertension enhanced by the initial Adrenaline dose ($3*10^{-2}$ mg/kg bw) and led to complete removal at the dose of 3.42 mg/kg bw. A partial hypertension followed by a profound hypotension is observed when tenordate is used at doses above 3.42 mg/kg bw.

For ADR-WAK, the Adrenaline (A) at $3*10^{-2}$ mg/kg bw is added with Wakouba (W) at doses of 4 mg/kg bw (b),8 mg/kg bw (c), 12 mg/kg bw (d), 16 mg/kg bw (e), and 20 mg/kg bw (f). For ADR-TEN, the Adrenaline (A)at $3*10^{-2}$ mg/kg bw is added with Tenordate at 0.85mg/kg bw (a), 1.71 mg/kg bw (b), and 3.42 mg/kg bw (c).Triplicate experiments.

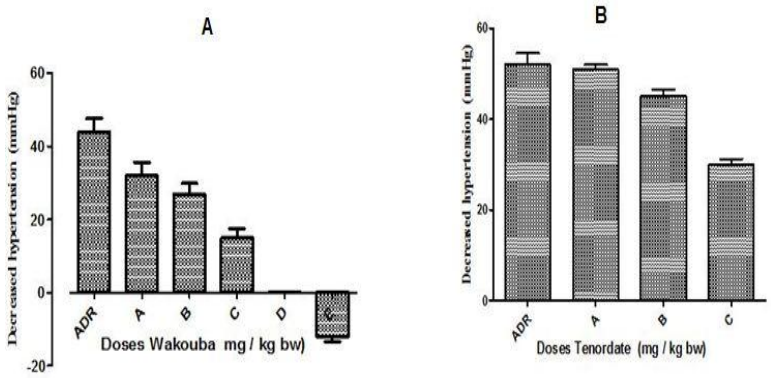

Figure 6: **Antagonistic effects resulting from Adrenaline-Wakouba (A) and Adrenaline-Tenordate (B) interactions on the arterial blood pressure of rabbits**

The more the Wakouba (or tenordate) doses are higher the more the hypertension induced by the Adrenaline at $3*10^{-2}$ mg/kg bw is deeply (or partially) suppressed. Experiments achieved in triplicate.

Analysis of the results of our study showed that *Wakouba* causes at low doses reversible hypotension and at high doses irreversible hypotension. Our results are in agreement with those obtained by [8] with *Stephaniatetrandra*[9] with *Mareeya micrantha*[10] with total protein and total extract dichloro - methane Ethanol of *Morinda morindoides*. Hypotensive activity cause by *Wakouba* is similar to that obtained with acetylcholine (ACh),a reference hypotensive substance. These results are in agreement with those of many authors. Indeed, according to [11, 12]administration of ACh into rabbit induced hypotension resulting from systemic vasodilation [13]showed that changes in arterial blood pressure is mainly dependent on two factors namely the cardiac activity on one hand and the vascular resistance on the other . Once secreted into the body acetylcholine (ACh) causes peripheral vasodilation subsequent to stimulation of M2 receptors coupled with G protein membrane and the release of a vasodilating substance called endothelium derived relaxing factor EDRF or nitric oxide (NO)[14-17].In the heart , ACh binds to muscarinic cholinergic cardiac receptors causing a slowing of the heart beat and cell hyperpolarization by opening of the potassium channels directly linked to G proteins[18,19]**.** This leads to a reduction of calcium entry due to the inhibition of adenylcyclase and a reduction of the release of calcium from the sarcoplasmic reticulum. Then a decrease in the force of heart contractions then follows and subsequently hypotension[20].

Wakouba is a substance of plant origin which exercises its hypotensive effect in the same mechanism as that of acetylcholine (ACh). *Wakouba* could react either in the vessels to cause vasodilation, or in the heart by binding to muscarinic cholinergic receptors to cause the observed effect. This hypothesis is confirmed by the results of the study of the interaction Atropine-*Wakouba* (Atropine (ATR) is a specific antagonist of muscarinic cholinergic receptors). Our results showed that in the presence of ATR, hypotension induced by *Wakouba* is completely removed. Our results are consistent with those[10].with the total extract of dichloromethane -ethanol, suggesting that *Wakouba* may contain muscarinic cholinergic compounds. These compounds would bind to the muscarinic cholinergic receptors in the heart to produce the observed effect. The anti-hypertensive activity of *Wakouba* was compared to that of Tenordate, reference antihypertensive substance commercially available in the market. The antihypertensive ténordate is used in the treatment of hypertension. This is a capsule composed of Atenolol (50mg) aβ blocker and nifedipine(20mg) a calcium channel blocker(CCB). The

β -blockers generally exercise their hypotensive effect by reducing the activity of the catecholamine in the heart, and decreasing the secretion of renin, which decreases cardiac output and leads to hypotension. In the presence of a single dose of adrenaline (ADR) which causes a control hypertension, Tenordate at the doses used, partially removed the control hypertension (Figure 3A) , unlike *Wakouba* which completely remove the control hypertension induced by the Adr (Figure 3C). *Wakouba* may contain calcium channel blockers and β -blockers compounds. Wakouba is a hypotensive and antihypertensive substance like the Tenordate. These results confirm the use of *Wakouba* in traditional medicine for the treatment of hypertension.

Conclusion

Wakouba induces a dose-dependent hypotension in rabbit reversible at low doses and irreversible at high doses. This hypotension identical to that induced by acetylcholine (ACh), is completely suppressed in the presence of atropine. *Wakouba* like Tenordate (ATENOLOL + NIFEDIPINE) completely suppresses hypertension induced by Adr. These hypotensive and antihypertensive properties of Wakouba confirms it uses in Ivorian traditional medicine as an antihypertensive substance.

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