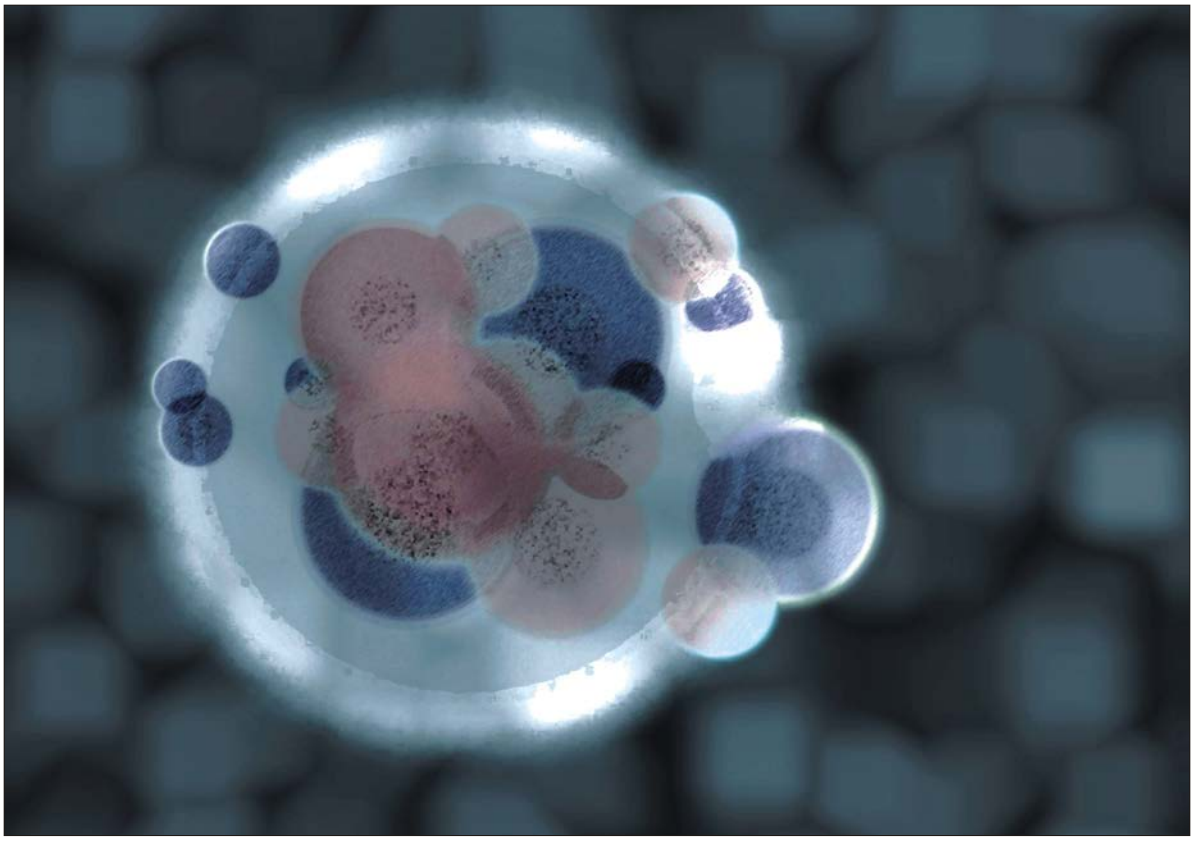

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Effects of Water Crude Leaf Extract of *Sclerocarya birrea* (A. Rich) Hochts (Anacardiaceae) on Normotensive Rat Blood Pressure

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Abstract: The present study was done to evaluate the hypotensive properties and the mechanisms action of the leaf aqueous extract in anaesthetized normotensive rats through a direct blood pressure measurement. The results showed that the extract caused a dose dependent fall in mean arterial pressure. Bilateral vagotomy did not abolished the hypotension induced by *S. birrea*. Pretreatment of rats with atropine, piperoxan and practolol did not abolished hypotension and suggests that muscarinic, α_2 and β_2 receptors are not involved in the induction of hypotension. These results suggest that the hypotensive effect of the extract is likely to be mediated through other factors like nitric oxide release or intracellular calcium decrease.

Key words: *Sclerocarya birrea*, anacardiaceae, normotensive, rat, blood pressure

INTRODUCTION

In Africa, the health care constituted a major problem for many rural peoples. They have not access to pharmaceutical products for economic, social and cultural reasons. A large part of them used medicinal plants to treat many diseases and affections. *Sclerocarya birrea* is a medicinal plant widely used and sold in some local markets by traditional practitioners. According to Guinko (1984) a bark decoction is used as anticough; the leaves, the pulp of fruit and the mistletoe were used to treat hypertension and some affections.

According to Pousset (1989) and Nacoulma-Ouédraogo (1996) the leaves and the bark were used as antihyperglycemic agent. This led us to study the antihypertensive effect of the plant. Indeed, according to Schorderet (1992), 15% of western population were affected by hypertension. Monkam-Mbouendé (1989) reported that hypertension affected 30 to 40% of western population. According to Toé (1992) the prevalence of hypertension in Burkina Faso (statistics from 1989 to 1991) was 57.14% of cardiovascular diseases. The present study was prompted by the claim of some traditional healers that decoctions and extracts of *S. birrea* leaves are effective remedies in the management or treatment of hypertension. The present study was, therefore to examine the hypotensive effect of *S. birrea* leaf aqueous extract in experimental animals in a view to justify the ethnomedical uses of the plant in the treatment of hypertension.

MATERIALS AND METHODS

Plant collection: Fresh leaves of *Sclerocarya birrea* were collected from Gampéla (Burkina Faso, west Africa) in July 2001. The plant was identified by Professor Millogo-Rasolodimby, Department of Botany, University of Ouagadougou. A voucher species has been deposited in this Department.

Preparation of the extract: The leaves were shade dried and powdered. Six grams of powder were first macerated in deionized water with shaking for 24 h at room temperature and then boiled for 10 min to mimic the traditional preparation methods. After cooling, the resulting extract was filtered with whatman No. 2 and evaporated 1.20 g of final extract yielded was obtained and used for this study.

Animals: Male Wistar rats (130-174 g) were used. All of them were obtained from IFFA-CREDO (France). The animals were kept at 22±2°C and submitted to a 12 h light/dark cycle with free access to food and water. Twelve hours before experimentation, the food was withdrawn, but water remained available *ad libitum*.

The animals were anaesthesized with ethyl carbamate (40%) at dose of 1 g kg^{-1} intraperitoneally (i.p.). The penis vein and the left carotid artery were exhibited and cannulated with catheters PE-$_{10}$ and PE-$_{50}$ respectively toward the heart. These catheters were filled with heparin saline solution (125 IU mL^{-1}) for further injection to prevent intravascular blood clotting.

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All animal procedures were strictly within national laws and guidelines.

Experimental protocol: The left carotid artery cannulated, the blood pressure from the carotid was recorded using a Elcomatic EM 750 SER No. 2203 transducer connected to a blood pressure amplifier unit (FC 137 Palmer Bioscience). The amplifier was then connected to an oscillograph (Palmer Bioscience 400 MD 4R pistes). After a 30 min equilibration period, the baseline blood pressure was recorded before samples were injected at doses indicated. The interval between injections was usually 15 min after the blood pressure has returned to control baseline.

Physiological solutions: All the solutions were injected through the penis vein cannulated for this effect.

The following substances were used:

Acetylcholine chloride 10^{-7} to 10^{-3} mg mL^{-1}
Atropine sulfate 10^{-5} to 10^{-4} mg mL^{-1}
Piperoxan HCl (α_2 blocker)
Practolol (β_2 blocker)
NaCl 0.9%.

All these substances were obtained from Sigma (Sigma Chemical Company, USA). All drug solution was freshly prepared.

Statistical analysis: Results were expressed as mean±SEM of six observations. Students t-test was used to test for significant difference between the means and $p<0.05$ was considered as significance.

RESULTS

The results showed here corresponded to many experiments with n = 6 on each test. The volume of different physiological solutions injected was 0.1 mL. The recordings corresponded 1 cm for 10 sec.

Effect of Acetylcholine (Ach) on anaesthesized normotensive rats: Intravenous administration of 0.1 mL of NaCl (0.9%) did not cause significant change in the blood pressure. However, intravenous injection of Ach decreased the blood pressure in dose-dependent manner. The hypotensive effect lasted at 5 to 20 sec at low doses (10^{-7} to 10^{-5} mg mL^{-1}) but was more than 40 sec at high doses (10^{-3} mg mL^{-1}) (Fig. 1).

Effect of *Sclerocarya birrea* on anaesthesized normotensive rats: Hypotensive effect was observed on anaesthesized rats at doses from 10^{-7} to 10^{-5} mg mL^{-1}, in a dose-dependent manner (Fig. 2). At high doses (10^{-3} mg mL^{-1} and more), some animals showed difficulties to breath, followed by death.

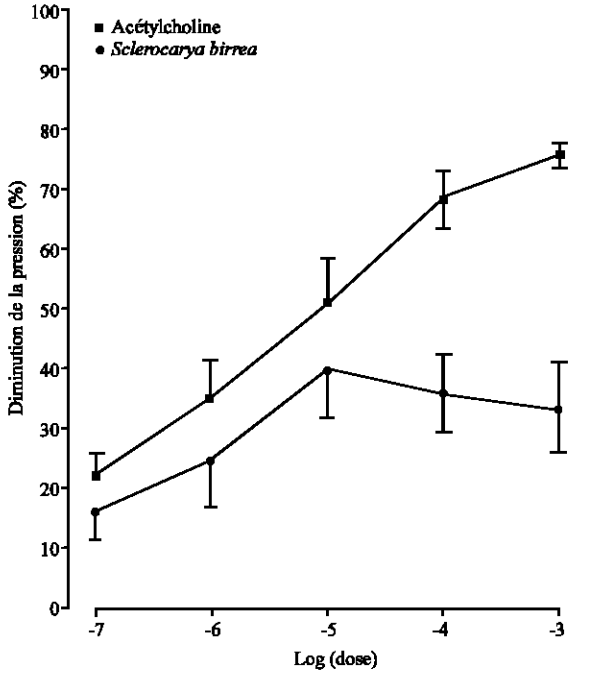

Fig. 1: Percentage decrease of blood pressure by acetylcholine and *Sclerocarya birrea* at different doses

Interactions *Sclerocarya birrea*-reference drugs: To attempt to indicate at least one of the mechanisms underlying the plant action, interactions were done with some reference drugs.

Interaction atropine -*Sclerocarya birrea*: Administration of Atropine (10^{-4} mg mL^{-1}) one minute before *S. birrea* injection did not abolished hypotension induced by the plant leaf extract (data not shown). This result seems indicate that muscarinic receptors were not involved in this phenomenon.

Interaction piperoxan -*Sclerocarya birrea*: When piperoxan (10^{-3} mg mL^{-1}) a α_2 blocker was injected one minute before administration of *S. birrea*, hypotension induced by the plant leaf extract was not abolished.

Interaction practolol -*Sclerocarya birrea*: Administration of practolol, a β_2 blocker one minute before injection of *S. birrea* did not impeded hypotension.

These two last interactions showed that the cardiac system seemed to be not involved.

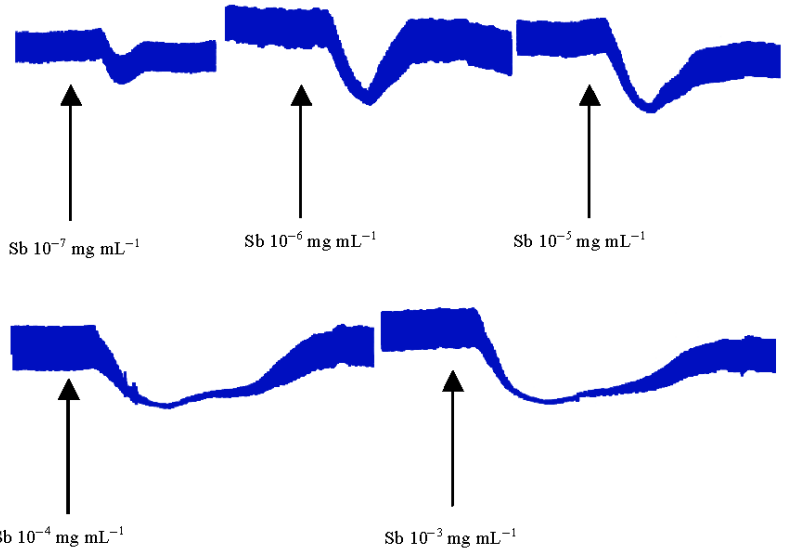

Fig. 2: Effects of *Sclerocarya birrea* on blood pressure at different doses (Sb = *Sclearcarya birrea*)

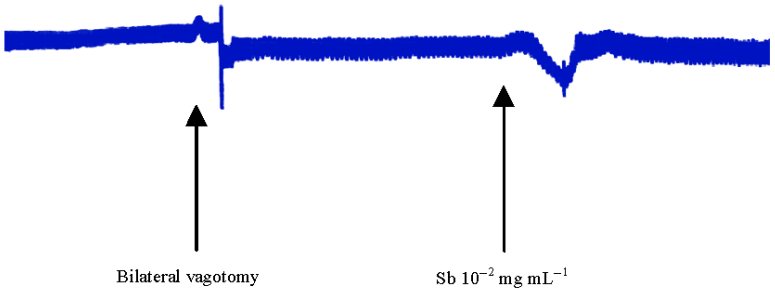

Fig. 3: Effects of *Sclerocarya birrea* on blood pressure after bilateral vagotomy

Bilateral vagotomy: Bilateral vagotomy did not abolished the *S. birrea* dose - hypotensive response but attenuated the magnitude of *S. birrea* induced fall in the blood pressure (Fig. 3).

DISCUSSION

It has been shown a long time ago that regulation of blood pressure was complex and involved many factors. The present study showed the hypotensive effect produced by water crude extract of *Sclerocarya birrea* in anaesthesized rats in dose dependent manner. At low doses (10^{-7} to 10^{-5} mg mL^{-1}) hypotension induced by *S. birrea* is similar to that induced by acetylcholine activity but at high doses (10^{-3} mg mL^{-1}), the effects of these substances are significative; the plant leaf extract is less active than acetylcholine (Fig. 1).

It has been reported that antihypertensive agents lower blood pressure by interfering with any of the blood pressure regulatory mechanisms (Cowley, 1984). Parasympathetic stimulation results in bradycardia and hypotension due the inhibitory effects of the cholinergic neurotransmitter, acetylcholine, on cardiac and vascular smooth muscle (Ajagbonna *et al*., 2001). Then, pharmacological agents that augment Ach action at neuroeffector sites will lower the blood pressure (Adeneye *et al*., 2006). Present results are similar to that obtained for the fresh leaves aqueous extract of *Musanga cecropioides* (Dongmo *et al*., 2002) and the aqueous stem bark extract of *Musanga cecropioides* (Adeneye *et al*., 2006) in rats.

As previously shown, hypotension induced by *S. birrea* seemed to be similar to that induced by Acetylcholine. Therefore, interaction has been done with

Atropine sulfate to research muscarinic receptors implication. The present study showed that these receptors were not involved in the action of the plant leaf extract. Other factors could be involved in induction of hypotension by *S. birrea* crude extract.

Some antihypertensive factors reduced the sympathic nervous system activities by stimulating structures which inhibited sympathic tonus. Such stimulation could be done through adrenergic receptors (Kanagy, 2005; O'Connell *et al*., 2006). The use of piperoxan and practolol, the well known α_2- and β_2 blockers, respectively (Schmitt, 1980; Cohen, 1986) was to research if hypotension induced by *Sclerocarya birrea* crude extract depended on the stimulation of these receptors. The present results showed that the extract did not exerted its effect through α_2- and β_2 adrenergic receptors.

Bilateral vagotomy was done to verify the involvement of parasympathic system in the mechanism action of *Sclerocarya birrea* leaf extract. The results reported here show that hypotension induced by *S. birrea* does not implicate cholinergic system as hypotension is not abolished after bilateral vagotomy. These results are in agreement with those of atropine sulfate and with those of Ojewole (2006) on stem bark aqueous extract of *S. birrea* in rats.

With regards to these results, it could be suggested that *Sclerocarya birrea* leaf extract could inhibited renin angiotensin aldosterone which decreased the release or the inhibition of Angiotensin Converting Enzyme (ACE) and accordingly increased bradykinin which decreases blood pressure (Pieri, 1992; Hansen *et al*., 1995). Indeed, Duncan *et al*. (1999) reported that *Sclerocarya birrea* leaf extract inhibited ACE at 68%.

It could be also suggested that the plant leaf extract can induce the release of endothelium derived relaxing factor (EDRF)/nitric oxide (NO) from endothelial cells. NO released from endothelium activates guanylate cyclase in arterial smooth muscle, which stimulates cyclic GMP production and then relaxes the muscle cells (Baillet and Nortier, 1992; Yao and Huang, 2000).

The last hypothesis is that *S. birrea* leaf extract could decrease the internal calcium concentration, since calcium ions are responsible for the contraction of smooth muscle and since internal calcium increase has been demonstrated to be involved in contraction of arterial tissue (Itoh *et al*., 1982) like in other muscular systems.

CONCLUSIONS

The present study shows that *Sclerocarya birrea* leaf extract induced hypotension in dose dependent manner in normotensive rats. These results seem justify its traditional use in the management of several diseases including hypertension. It is not yet known which of the phytochemicals are responsible for the observed pharmacological effects. Further investigations will be done leading to isolation of active compounds responsible of these effects.

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