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Hypotensive effect of *Baissea axillaris* (Apocynaceae) leaves in normotensive rabbits

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Abstract

Baissea axillaris leaves are used in ethnomedicine to reduce elevated blood pressure. The effect of the aqueous extract of the leaves (obtained by decoction method) on blood pressure was tested on normotensive rabbits intravenously (i.v.) at doses of 2.5, 5, 10, and 20 mg/kg. The probable mechanism of action of the extract was examined by prior administration of atropine (0.5mg/kg i.v.) or promethazine (0.25mg/kg i.v.) followed by administration of the water extract . The effects of chloroform fraction was tested at tested at 0.625,1.25, 2.5mg/kg while the n-butanol fraction was tested at 2.5,5,10, 25, 50,and 100mg/kg dose points. The aqueous extract showed a dose-dependent reduction in blood pressure which was found to be significantly blocked by prior administration of atropine or promethazine. The chloroform fraction of the extract showed higher significant blood pressure lowering (hypotensive) activity than both the extract and n-butanol fraction. The leaves of *B. axillaris* have hypotensive action on blood pressure as claimed by ethnomedical practitioners.

Keywords: Hypotensive effect; *Baissea axillaris;* Normotensive rabbits

Introduction

High blood pressure is a major risk factor for the development of cardiovascular complications including stroke, congestive heart failure, coronary heart disease, periphery vascular disease, and renal failure (Lloyd-Jones *et al*, 2000). Information about high blood pressure, its determinations or risk factors and effective methods of controlling it have become important because of its relatively high prevalence in Nigeria where about 11% of entire population was reported to have the disease condition (Mabadeje, 2002).

There are quite a number of synthetic drugs with proven and established potency in reducing elevated blood pressure. The use of such drugs suffers some demerits due to side effects (some of which are life threatening), high costs, unavailability and precarious supply of the drugs particularly in rural areas where health care facilities for management of hypertension are almost non-existent. These reasons are responsible for widespread use of medicinal plants in combating many ailments and disease conditions including non-communicable ones like high blood pressure. Among the plants reported to have

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the potential of reducing high blood pressure are *Allium sativum* (Silagy and Neil, 1994); *A. cepa*, *Crataegeus monogyna* (Duke, 1997); *Hibiscus sabdariffa* flower (Haji Faraji and Haji Tarkhani, 1999) and *Musanga cecropioides* (Kamanyi *et al.*, 1996).

Traditional medical practitioners in some ethnic groups like Urhobos (in the Niger Delta region of Nigeria) usually prescribe oral administration of decoction of herbs like *Baissea axillaris* to treat high blood pressure. As there are no scientific reports on the ethnomedicinal importance of *B. axillaris*, in reducing blood pressure, this present work was carried out to examine the claimed use of this plant as well as the mechanism(s) of action involved using normotensive rabbits .

Experimental

Collection and preparation of plant material. *B. axillaris* is a perennial climber characterized by its milky sap. The leaves are 3-6 cm long and 1.5-2 cm wide. They are ovate in shape, alternately arranged on the tender stem. They have entire margin, pubescent surface with pinnate veination. After proper authentication by Dr. Idu (Taxonomist) Department of Botany, Faculty of Science, University of Benin) *B. axillaris* leaves were collected in August, 2003 at the Dentistry Quarters, University of Benin Teaching Hospital, Benin City. The leaves were dried in an electric oven maintained at 40°C for three days followed by grinding to powder using an electric mill. The powder was kept in airtight containers until required.

Phytochemical screening. Qualitative tests for the presence of plant secondary metabolites such as alkaloids, tannins, flavonoids and saponin glycosides were carried out on the leaf powder using standard procedures. (Trease and Evans, 1989).

Extraction and partitioning About 1.3kg of the powdered leaves was step-wisely extracted by decoction method for 30 minutes. After cooling and filtration, the water extract was concentrated under vacuum to a semi- solid residue which was kept in a refrigerator until needed. About 45g of the extract obtained above was re-dissolved in 400 ml distilled water and partitioned in succession with chloroform and n-butanol using a separating funnel. Each organic solvent fraction and the residual aqueous fraction was concentrated under vacuum and kept in refrigerator at 4°C.

Preparation of animals. Adult male rabbits (1.3–2.0 kg) were purchased and maintained in the Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy University of Benin, four (4) weeks before use . They had free access to normal rabbit pellets (Livestock feeds) and water *ad libitum*.

Drugs and chemicals used. These include Promethazine, Pentobarbitone Sodium (Sigma Chemicals), Atropine (Indus Pharma), Heparin (Pan Pharma).

Measurement of blood pressure. The animals were anaesthetized using Pentobarbitone sodium (40mg/kg i.p.). The marginal ear vein was cannulated for administration of the water extract, its organic solvent fractions and drugs. The carotid artery was cannulated and connected via a Bentley Physiological pressure transducer to a two-channel Ugo Basile recorder (Gemini 7070) for recording blood pressure and heart rates. The water extract was administered I.V. at doses of 2.5, 5, 10 and 20 mg/kg. The chloroform fraction (dissolved in 10% dimethylsulphoxide-DMSO) was administered at doses of 0.625, 1.25, and 2.5 mg/kg while the n-butanol fraction was tested at doses of 2.5, 5, 10, 50 and 100mg/kg. The residual aqueous fraction obtained after partitioning was also tested at 10, 20, 30 and 40mg/kg.

Effects of atropine and promethazine on the hypotensive effect of the water extract. Atropine (0.5 mg/kg) was administered (I.V.)

followed by administration of the various doses of the water extract as stated above. Also, promethazine (0.25mg/kg) was administered followed by administration of water extract. Each of the animal experiments was carried out in triplicate while the level of response significance between the doses were tested using Analysis of Variance (ANOVA) and *Students t-Test* where applicable.

Approval for the use of animals in this work was obtained from the Ethical Committee on the use of animals for experiments of the Faculty of Pharmacy, University of Benin.

Results

Phytochemical screening of *B. axillaris* leaves revealed the presence of tannins, saponins and flavonoids with no trace of anthraquinone glycosides and alkaloids.

The water extract of the leaves exhibited a dose- dependent reduction in rabbit blood pressure. A fall in the mean arterial pressure of 12.9+ 0.58 mmHg observed after administering 2.5mg/kg of the water extract was further decreased by 26.6 $\pm$ 1.8 and 34.43 $\pm$ 0.59 mmHg at doses of 10 and 20mgkg respectively. The variation in the doses was observed to be significant at $P<0.01$ using ANOVA.

Prior administration of atropine (0.5mg/kg) followed by 2.5 – 20mg/kg doses of water extract significantly reduced hypotensive effect compared to the water extract at each dose ($P<0.01$, t-test). Similar results were observed with promethazine (0.25mg/kg) administered before 2.5 – 20mg/kg water extract. However, the antagonistic effect of the promethazine was a little more pronounced when compared to that of atropine (fig. 1).

The chloroform fraction at a dose of 0.625mg/kg produced a fall in mean arterial pressure of 17.03 $\pm$ 2.02 mmHg. This was further decreased to 30.77 $\pm$ 0.73 and 31.37 $\pm$ 0.33 with doses of 1.25 and 2.5mg/kg respectively (fig.2). At a dose point of 2.5mg/kg the n-butanol fraction elicited a reduction in mean arterial measure of 5.95 $\pm$ 1.10mmgHg while a reduction of 12.78$\pm$2.4mmgHg was observed after administration of 10mg/kg. Higher doses of 25, 50 and 100mg/kg reduced the blood pressures by 17.68, 30.67 and 35.7mmHg respectively (fig 3). The residual aqueous fraction showed no hypotensive effect even at 40mg/kg (data not shown).

Discussion

The therapeutic effects of medicinal plants are usually due to the various secondary metabolites they contain. The leaves of *B. axillaries* were shown to contain saponins, flavonoids and tannins. More work is being carried out to ascertain the particular group of constituents that is responsible for the higher hypotensive effects shown by the chloroform fraction above than that of the water extract and the n-butanol fraction.

This study has established the claimed use of *B. axillaris* in reducing blood pressure and the effect was observed to be dose related. The water extract appears to be a more potent hypotensive drug than *Musanga cecropioides* stem bark earlier reported from our laboratory (Ayinde *et al.*, 2003). Unlike in the case of the stem bark of *M. cecropioides,* the effect of the water extract of *B axillaris* in reducing blood pressure was observed to be blocked by prior administration of either atropine or promethazine. These observations imply that the hypotensive effect of the plant appears to be due to stimulation of muscarinic receptors (which normally cause endothelium - derived -relaxing - factor mediated vasodilatation), an effect blocked by atropine, an antimuscarinic agent. The hypotensive effect also appears to be accompanied by the release of histamine, which was blocked by promethazine.

Fig.1: Hypotensive effects of water extract of *B.axillaris* in rabbits.

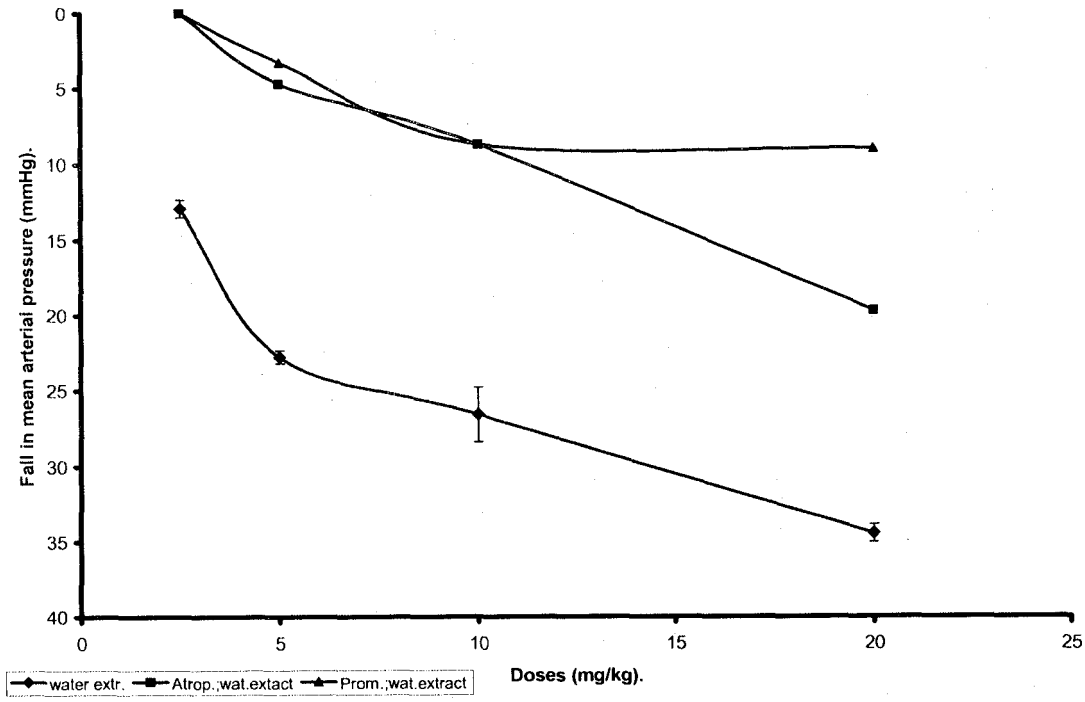
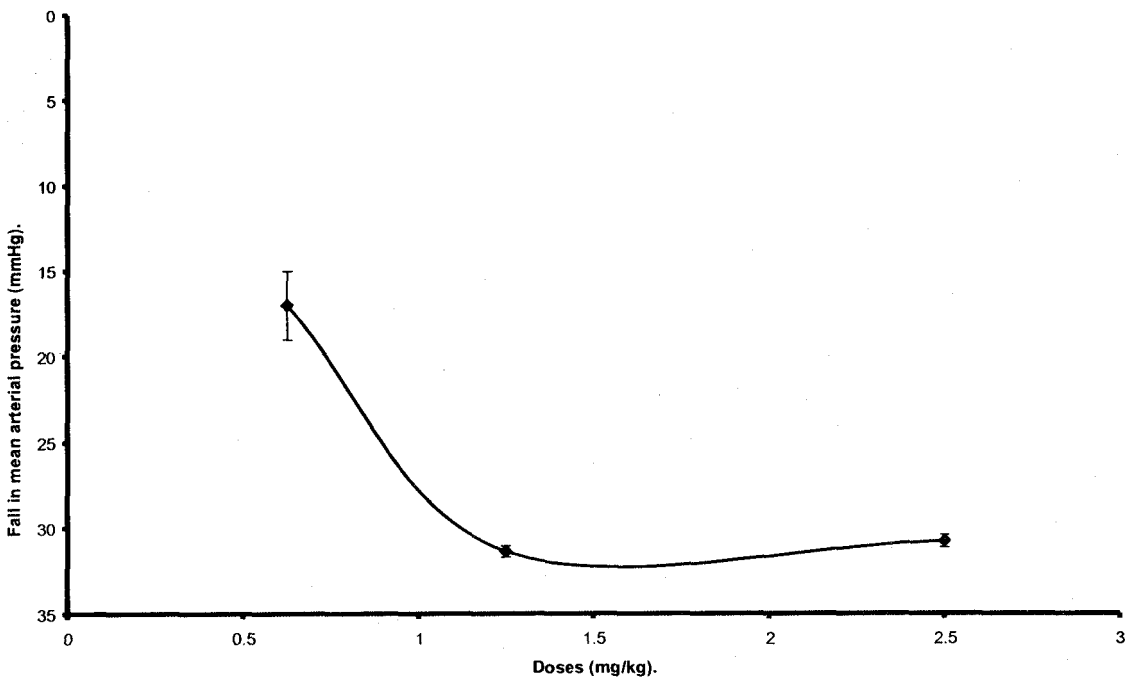


Fig.2 : Hypotensive effects of chloroform fraction of *B.axillaris* water extract.



Conclusion

In conclusion, this study has corroborated the traditional use of *B. axillaris* leaves as a hypotensive drug. The activity appears to be due to the constituents of the chloroform fraction. The reduction in blood pressure is either through the stimulation of muscarinic receptors in the heart or is associated with the release of histamine.

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