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Original Article

Alterations of haemodynamic parameters in spontaneously hypertensive rats by *Aristolochia ringens* Vahl. (Aristolochiaceae)

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ABSTRACT

Aristolochia ringens Vahl. (Aristolochiaceae (AR); *馬兜鈴 mǎ dōu líng)* is used traditionally in Nigeria for the management of various disorders including oedema. Preliminary investigation revealed its modulatory effect on the cardiovascular system. This study was aimed at investigating the effect of the aqueous root extract of *A. ringens* (AR) on haemodynamic parameters of spontaneously hypertensive rats (SHRs). The effect of oral subacute (21 days) and intravenous acute exposure of SHRs to the extract were assessed using tail cuff and carotid artery canulation methods respectively. In the latter, the effect of chloroform, butanol and aqueous fractions of AR were also evaluated. The extract significantly reduced systolic and diastolic blood pressures in SHRs, with peak reductions of 20.3% and 26.7% respectively at 50 mg/kg by the 21st day of oral subacute exposure. Upon intravenous exposure, AR (50 mg/kg) reduced systolic and diastolic blood pressure by as much as 53.4 ± 2.2 and 49.2 ± 2.8 mmHg respectively. A dose-dependent reduction in heart rate, significant at 25 and 50 mg/kg was also observed. Hexamethonium (20 mg/kg) and atropine (1 mg/kg) inhibited the extract's reduction of systolic blood pressure, diastolic blood pressure and heart rate significantly. The extract's butanol fraction produced the greatest systolic and diastolic blood pressures reduction of 67.0 ± 3.8 and 68.4 mmHg respectively at 25 mg/kg and heart rate reduction of 40 ± 7 beats per minute at 50 mg/kg. HPLC analysis revealed the presence of 4-hydroxybenzoic acid and quercetin in AR. The extract's alterations of haemodynamic parameters in this study show that it has hypotensive effect on spontaneously hypertensive rats.

1. Introduction

Hypertension, a condition in which the arteries have persistently elevated blood pressure, is a prevalent risk factor for cardiovascular diseases; affecting over 1 billion people worldwide.[1] A continuous relationship between blood pressure and cardiovascular risks, renal disease and mortality has been reported. This relationship particularly holds more for systolic than for diastolic blood pressure.[2] There is a doubling of the risk of stroke and ischaemic heart disease mortality for every 20/10 mmHg increase in blood pressure over the level 115/75 mmHg.[3] In 2000, it was estimated that 25% of the world's adult population were hypertensive, and predicted that this would rise to 29% by 2025. By the age of 60, more than one-half of adults in most regions of the world will be hypertensive.[3]

Two forms of hypertension, namely primary and secondary hypertension, have been described. The more common form is primary hypertension also known as essential or idiopathic hypertension. It accounts for 90–95% of all cases of hypertension. In

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spite of the immense progress in understanding of its pathophysiology and availability of various efficacious management approaches, essential hypertension is a major risk factor for cardiovascular disorders. Hypertension increases the risk of cardiovascular diseases for millions of people worldwide. In the past decade, age-related incidence of stroke, renal and heart failure has reportedly risen. This observation is partly due to inadequate control of blood pressure in the hypertensive population.[4] It has been reported that less than 25% of treated hypertensive patients achieve target blood pressure.[5] Studies have demonstrated that although antihypertensives are very beneficial therapeutically, they are associated with certain limitations, the outcome of which however is influenced by risk factors such as patient's age and pre-existing co-morbid conditions.[6] The need for more effective and better tolerated antihypertensive therapies cannot be over-emphasized. Medicinal plants are very important sources of potential novel antihypertensives. Indeed, the antihypertensive effects of some of these plants have been validated and others disproved.

Aristolochia ringens is one of the over 500 species of the Aristolochiaceae family of medicinal plants. Historically, *Aristolochia* species of plants have long been in use in traditional medicine practice. They have also been very much featured in numerous chemical and pharmacological research.[7] However, there have been reports of the potential toxicity of plants of the Aristolochiaceae family due to the presence of aristolochic acids, which has resulted in the ban of products containing *Aristolochia* species or aristolochic acids in several countries of the world. Despite this fact, studies in some quarters have shown the immense potential benefits of *Aristolochia* species owing to the presence of other phytochemicals present in them. Kuo et al.[8] reported the neuroprotective, antispasmodic, antiaddictive and antimycobacterial effects of other *Aristolochia* plants' phytochemicals (other than aristolochic acids). Some other classes of phytochemicals isolated from the species include lignans, terpenoids, steroids, coumarins and flavonoids among others.

A. ringens is a commonly used species in Nigeria. It is known as "Akogun" (Yoruba language) in Southwestern Nigeria. It is used for the management of oedema, worm infestation, gastrointestinal and inflammatory disorders as well as several other ailments.[9] Studies have also revealed its antidiarrhoeal,[10] anti-inflammatory[11] and anticancer activities.[12] Although *A. ringens* contains aristolochic acid II, sesquiterpenes and monoterpenes have also been isolated from the plant.[13] Wu et al.[14] reported the presence of specific monoterpenoids (e.g. limonene) and sesquiterpenoids (e.g. caryophyllanes). Its composition of magnesium, phosphorus, calcium, iron, zinc, copper and other elements have also been reported.[15] Clearly, *A. ringens* contains other useful phytochemicals that could be harnessed as lead compounds for drug development in the management of various disorders including primary hypertension. A pilot study carried out in our laboratory revealed that it reduces inotropic and chronotropic effects of an isolated rabbit heart and also induces vasomodulatory activity on isolated rat aortic ring. This study was aimed at investigating some effects of the aqueous root extract of *A. ringens* (AR) on haemodynamic parameters of essential hypertension as modelled by spontaneously hypertensive rats.

2. Materials and methods

2.1. Plant collection and identification

The root of *A. ringens* collected from a local market in Mushin, Lagos, Nigeria was identified and authenticated by Mr. T.K. Odewo, a taxonomist of the Department of Botany and Microbiology, University of Lagos, Nigeria where a herbarium specimen was deposited with voucher number LUH 4061.

2.2. Plant extraction

Air dried root (100 g) was macerated in 1000 ml of distilled water and placed in a refrigerator at 4 °C for 5 days. It was then filtered using cotton wool and filter paper, and the filtrate dried in an oven (Gallenhamp®, England) at 40 °C. The percentage yield of the aqueous root extract of *A. ringens* (AR) obtained was 4.9% (w/w). For some of the experiments, the extract was further subjected to liquid-liquid partitioning to obtain chloroform, butanol and aqueous fractions of the extract.

2.3. Experimental animals

Adult male spontaneously hypertensive rats (180–320 g) were obtained from the Animal Research Unit and Service Centre (ARASC) of Universiti Sains Malaysia, Penang, Malaysia. The animals were allowed to acclimatize for a week, housed under standard environmental conditions in standard plastic cages and maintained under a 12 h light and 12 h dark cycle. They were fed with normal commercial rat chow (Gold Coin Feed Mills, Sdn Bhd, Malaysia) and water *ad libitum*. Animal handling and all procedures on animals were carried out in accordance with the guidelines of the Animal Care and Use Committee of the Universiti Sains Malaysia.

2.4. Chemicals

The chemicals used in this study include dimethyl sulfoxide, acetone, petroleum ether, butanol, chloroform, hexamethonium, atropine, Folin-Ciocalteu reagent, sodium bicarbonate, gallic acid, quercetin, 4 hydroxybenzoic acid, 2,2-diphenyl-1-picrylhydrazyl, Acetic acid (Sigma Aldrich, Malaysia), distilled water (Cardiovascular and Renal Units Laboratory, USM, Malaysia).

2.5. Effect of chronic administration of AR on blood pressure and heart rate

Four groups of 5 spontaneously hypertensive rats (SHRs) each were orally administered AR (25 and 50 mg/kg), enalapril (3 mg/kg) and vehicle (distilled water at 5 ml/kg) for 21 days. Blood pressure and heart rate were measured using the tail cuff method with CODA® non-invasive blood pressure monitor (Kent Scientific Co-operation, USA) on days 0, 7, 14 and 21. On these days, 24-hour urine samples were also obtained by placing each rat singly in metabolic cages for 24 h.[16] The volume of urine collected was measured using measuring cylinder; and the urine Na^+ and K^+ concentrations were determined using a flame photometer (Jenway, Uk).

2.6. Effect of acute exposure to AR on blood pressure and heart rate

This was done using a modification of the methods described by Shah and Gilani.[17] Spontaneously hypertensive rats fasted for 12 h, were anaesthetized with pentobarbitone (60 mg/kg, i.p.). Immediately following the anaesthesia, tracheotomy was performed using an endotracheal cannula (PP 240, Portex Ltd, Kent, UK) to maintain a free flow of air through the trachea. The left jugular vein was then catheterized using PP 50 tubing (Portex Ltd, Kent, UK) to allow the infusion of supplementary anaesthesia, AR and its vehicle and fractions. For the measurement of blood pressure and heart rate, the right carotid artery was cannulated with PP 50 tubing

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