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Antihypertensive properties of *Allium sativum* (garlic) on normotensive and two kidney one clip hypertensive rats

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Summary: *Allium sativum* (garlic) is reported to act as an antihypertensive amidst an inconsistency of evidence. In this study, we investigated the cardiovascular effects of aqueous garlic extracts (AGE) on normotensive and hypertensive rats using the two-kidney one-clip (2K1C) model. Mean arterial blood pressure (MAP) and heart rate (HR) were measured in normotensive and 2K1C rat models anesthetized with thiopentone sodium (50 mg/kg body weight i.p.) through the left common carotid artery connected to a recording apparatus. The jugular vein was cannulated for administration of drugs. Intravenous injection of AGE (5-20 mg/kg) caused a significant ($p<0.05$) decrease in both MAP and HR in a dose-dependent manner in both the normotensive and 2K1C models, with more effects on normotensive than 2K1C rat model. The dose of 20mg/kg of AGE significantly ($p<0.05$) reduced systolic (16.7 ± 2.0%), diastolic (26.7 ± 5.2%), MAP (23.1 ± 3.6%) and HR (38.4 ± 4.3%) in normotensive rats. In 2K1C group, it significantly ($p<0.05$) reduced systolic (22.2 ± 2.1 %), diastolic (30.6 ± 3.2%), MAP (28.2 ± 3.1%) and HR (45.2 ± 3.5%) from basal levels. Pulse pressure was significantly ($P<0.05$) elevated (33.3 ±5.1%) in the 2K1C group. Pretreatment of the animals with muscarinic receptor antagonist, atropine (2 mg/kg, i.v.), did not affect the hypotensive and the negative chronotropic activities of the extract. AGE caused a decrease in blood pressure and bradycardia by direct mechanism not involving the cholinergic pathway in both normotensive and 2K1C rats, suggesting a likely involvement of peripheral mechanism for hypotension.

Keywords*:* Blood pressure, Garlic, Renal hypertension, 2K-1C, Heart rate, Bradycardia

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INTRODUCTION

In recent years, studies on the beneficial effects of extracts of *Allium sativum* (garlic) in the treatment of chronic diseases such as diabetes and hypertension have been carried out (Nwokocha *et al*., 2011). Garlic is claimed to have both prophylactic and curative properties in various conditions such as microbial infection, thrombosis, hypertension, hyperglycemia, hyperlipidemia, cancer, and thrombosis, (Pedraza-Chaverri *et al*., 1998; Ashraf *et al* 2005; Pittler and Ernst, 2007). The contents of the extract include sulfur active principles mainly in the form of cysteine derivatives such as s-alkyl cysteine and sulfaxides, which decompose into a variety of thiosulfinates and polysulfides by the action of an enzyme allinase on extraction (Augusti, 1996; Kaye *et al*., 2000; Batirel *et al* 2002).

Aqueous extract of garlic prevents platelet aggregation (Zbinden and Seiler, 2002) and oxidative stress (Vazquez-Prieto *et al.*, 2010). The cardioprotective effects of garlic are related to its antiatherogenic properties, decreased cholesterol and improvement in vascular function (Melzig *et al.*, 1995; Vazquez-Prieto *et al.*, 2010). Garlic extract has also been reported to decrease serum glucose and systolic blood pressure (Kiesewetter *et al.*, 1991) and pulmonary vascular resistance (Fallon *et al.*, 1998; Batirel *et al*, 2002).

The mechanisms for the blood pressure lowering effects of garlic (Foushee *et al*, 1982; Al-Qattan *et al*., 1999; Reinhart *et al*., 2008) will include vaso-relaxation mediated through H_2S production liberated from alliin and the enzyme alliinase (Benavides *et al.*, 2007), modulation of the production and function of both endothelium-derived relaxing and constricting

factors, (Fallon *et al*., 1998) and beta-adrenoceptor blocking action (Martín *et al*., 1992) Other proposed mechanisms include hyperpolarization by opening K^+ channels and closure of the L-type Ca^{2+} channels (Pedraza-Chaverri *et al*., 1998; Siegel *et al*., 1999), reduction in angiotensin converting enzyme (ACE) and angiotensin II activity (Hosseini *et al*., 2007; Castro *et al*., 2010), garlic as such seems to affect all vascular parameters.

But some researchers have reported insufficient data and evidence to recommend the use of garlic for hypertension management (Silagy & Neil, 1994; Ginter & Simko, 2010; Sobenin *et al*., 2010). The present study therefore investigated the comparative effect of garlic using *in vivo* techniques on blood pressure parameters in 2K1C hypertensive and normal rats. This is against the background that 2K1C hypertension model causes and sustains an elevated blood pressure through the increase in the renin-angiotensin activity (Nakata *et al*., 1987; Wilcox *et al.*, 1996) resulting in increased total peripheral resistance and development of renal hypertensive complications.

MATERIALS AND METHODS

Animals and experimental design

Male Wistar rats weighing between 150 - 180 g aged 5 - 7 weeks were obtained and kept at the Animal House of the Department of Pharmacology and Toxicology, University of Benin, Nigeria for this study. During the entire treatment period animals were kept at room temperature of 28 ± 2^{o}C with 12 h light/dark cycle. The rats were divided into two groups of control and experimental, fed with standard rat chow (Bendel Feeds and Flour Mill Ltd, Ewu, Nigeria) and water *ad libitum*. The experiments were conducted according to international protocols for the use of animals in experimental studies and the study was approved by the Faculty Ethics Committee.

Two-kidney, one- clip model

The animals were anesthetized with thiopentone sodium (50 mg/kg body weight) intraperitoneally (i.p.). The right renal artery was isolated through a flank incision, and a silver clip (0.12 mm internal gap) was placed on the renal artery. Sham-operated group underwent the same surgical procedure except for placement of the renal artery clip served as controls. This was done in an aseptic environment after which the animals were allowed to recover for two weeks (Guan *et al*., 1992; Navar *et al*., 1998) before the commencement of blood pressure experiments.

Preparation of garlic extract

Aqueous garlic extract was prepared from locally available garlic cloves bought from market within the city. The garlic cloves were peeled cut into small pieces and 50 g was homogenized in 75 ml of cold sterile 0.9% NaCl. The homogenized mixture was filtered thrice. The filtrate was centrifuged at 2000 rpm for 10 min and the clear supernatant was made up to 100 ml with normal saline. The concentration of this garlic preparation was considered to be 500 mg/ml on the basis of weight of the starting material (50 g/100 ml).

Measurements of blood pressure and heart rate

All animals were anaesthetized by injection of thiopentone sodium (50 mg/kg i.p.) and the trachea was cannulated to facilitate normal breathing. The left jugular vein was cannulated for the administration of test drugs. A cannula was also placed on the right carotid artery. The arterial cannula was then connected to a Statham p23 pressure transducer which was in turn connected to an Ugo Basile (Italy) unirecorder model 7050. To prevent clotting of blood, the arterial cannula was filled with 50 units' of heparinized saline.

An equilibration time of 30 min was allowed before blood pressure measurements were started. Heart rates were determined by increasing the chat speed from 10mm/min to 10mm/sec. The effects of bolus doses of aqueous garlic extract (5, 10, 15 and 20 mg/kg body weight) were recorded after the blood pressure returned to normal. To characterize pharmacologically the response of garlic on MAP, bolus injections of atropine (2 mg/kg), were first given followed by garlic extract (20 mg/kg) after 10minutes.

Statistical analysis

Results were expressed as mean ± SEM. Blood pressure changes were expressed as percentages of baseline (pre-injection) values. Statistical analyses were performed by use of Student's *t* test using GraphPad Prism version 5.0 for Windows (GraphPad Software, San Diego, Ca, USA) and $p<0.05$ was considered statistically significant.

RESULTS

Effect of garlic on blood pressure and heart rate of hypertensive rats

The results on the effects of intravenous infusion of garlic extract on blood pressure are presented in Fig. 1 and Table 1. Aqueous garlic extract caused a dose dependent reduction in the systolic pressure (SP),

Table 1.
Effect of garlic on blood pressure and heart rate of hypertensive rats

	Systolic (mmHg)	**Diastolic** (mmHg)	**Pulse pressure** (mmHg)	**MAP** (mmHg)	**Heart rate** (beats/min)
Pre- infusion	180 ±3	150 ±3	30 ±3	160 ±3	406 ±6
After infusion (20mg/ml)	150 ±5*	110 ±6*	40 ±5#	123 ±5*	250 ±5*
% reduction or increase	16.7	26.7	33.3#	23.1	38.4

MAP= Mean Arterial Pressure, * = P<0.05 compared with pre-infusion of extract, # = p<0.05 compared with pre-infusion of extract, n = 6.

Table 2.
Effect of garlic on blood pressure and heart rate of normotensive rats

	Systolic (mmHg)	**Diastolic** (mmHg)	**Pulse pressure** (mmHg)	**MAP** (mmHg)	**Heart rate** (beats/min)
Pre-infusion	90 ±4	72 ±5	18 ±5	78 ±5	378 ±5
After infusion (20mg/ml)	70 ±5*	50 ±4*	20 ±5#	56 ±5*	207 ±5*
% reduction or increase*	22.2	30.6	11.1	28.2	45.2

* = p<0.05 compared with pre-infusion of extract, # = P<0.05 compared with pre-infusion of extract, n = 6.

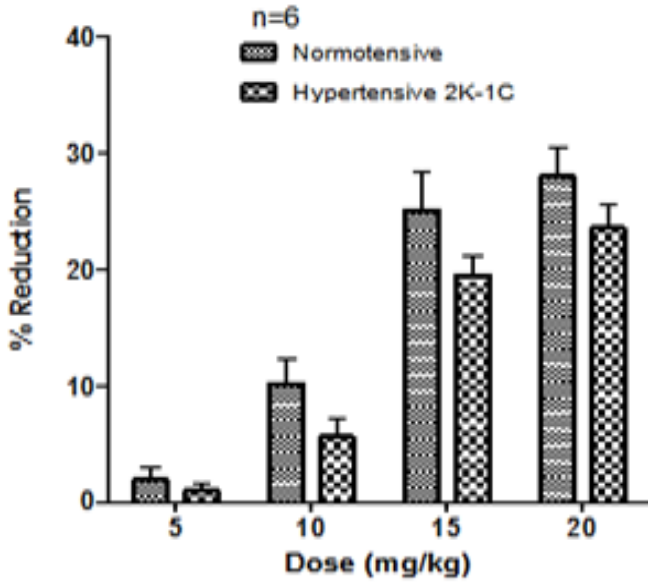

Figure 1.
Effect of graded dose of garlic extract on mean arterial pressure in rats

diastolic pressure (DP), mean arterial pressure (MAP) and heart rate (HR) of the animals.

The dose of 20 mg/kg significantly (p<0.05) decreased systolic blood pressure from 180±3 to 150±3 mmHg. The diastolic blood pressure was also significantly (p<0.05) reduced from 150±3 to 110±6 mmHg. Mean arterial pressure (MAP) was reduced from 160±3 to 123±3 mmHg. Heart rate was also significantly (p<0.05) reduced from 406±6 to 250±5 beats/min, but pulse pressure increased from 30±3 to 40±5. The bolus infusion of the extract therefore caused a 16.7% reduction in systolic blood pressure, 26.7% reduction in diastolic blood pressure, 23.1% reduction in MAP and 38.4% reduction in heart rate in hypertensive rats.

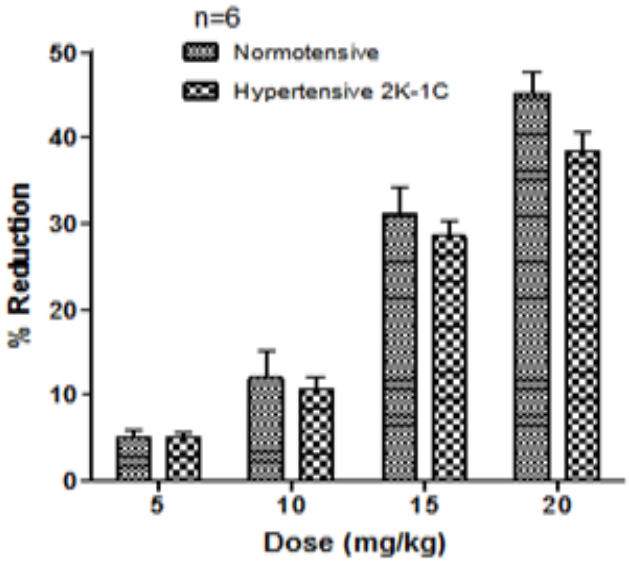

Figure 2.
Effect of graded dose of garlic extract on heart rate in rats

Effect of garlic on blood pressure and heart rate of normotensive rats

The results on the effects of intravenous infusion of garlic extract on blood pressure are presented in Table 2. A bolus infusion of 20 mg/kg significantly (p<0.05) decreased systolic blood pressure from 90±4 to 70±5 mmHg. The diastolic blood pressure was also significantly (p<0.05) reduced from 72±5 to 50±4 mmHg. Mean arterial pressure (MAP) was significantly (P<0.05) reduced from 78±5 to 56±5 mmHg. Heart rate was also significantly (p<0.05)

reduced from 378±5 to 207±5 beats/min., but pulse pressure increased from 18±5 to 20±5 (Fig. 2).

The bolus infusion of garlic aqueous extract therefore caused a 22.2% reduction in systolic blood pressure, 30.6% reduction in diastolic blood pressure, 28.2% reduction in MAP and 45.2% reduction in heart rate in normotensive rats.

Increasing doses of aqueous garlic produced a dose dependent decrease in the systolic pressure (SP), diastolic pressure (DP), mean arterial pressure (MAP) and heart rate (HR) of the animal. Bolus injections of 2 mg/kg atropine failed to block the drop in MAP produced by 20 mg/kg of aqueous garlic extract (results not presented), when infusion was repeated after a 30 min washout period, the rate and contractile responses were of a lower magnitude and animals went into bradycardia with higher doses.

DISCUSSION

The present study has demonstrated that intravenous infusion of garlic extract reduces blood pressure and heart rates in both hypertensive and normotensive rats. The study also provides evidence that garlic reduces blood pressure in a dose dependent manner by a mechanism not involving acetylcholine.

Various mechanisms for antihypertensive effect of garlic have been reported to include vasorelaxation through H_2S production (Benavides *et al.,* 2007), inhibition of angiotensin-converting enzyme *in vitro* (Rietz *et al*., 1995), endothelium-derived relaxing and constricting factors, (Sendl *et al*., 1992; Fallon *et al*., 1998), and beta-adrenoceptor blocking action (Martín *et al*., 1992). As a part of safety evaluation, this study was performed to verify the effects of garlic in hemodynamic parameters, particularly in relation to its effects on blood pressure and heart rate. The results showed that garlic at higher doses (15 and 20 mg/kg) induced marked hypotension and bradycardia when injected intravenously whereas at a lower doses (5 and 10 mg/kg) it produced only a slight and insignificant fall in MAP.

Part of the inhibitory actions of garlic on the blood pressure could be explained by myocardial mechanisms as blood pressure is known to be influenced by changes in cardiac contractility when total peripheral vascular resistance remains fairly unchanged. It is likely that the negative chronotropic effect of aqueous garlic extract is due to other mechanisms such as direct effect on the heart and blood vessels. Janssen *et al*, (2000) had reported that MAP and heart rates are influenced by cholinergic function, although they blood vessels contain muscarinic receptors, M_2 receptors in the heart mediate reduced heart rate but more profoundly affected by adrenergic beta-1 stimulation. The hypotensive action of garlic was unaffected in animals treated with atropine, an anticholinergic agent, indicating lack of cholinergic influence.

Our observation is inconsistent with findings of another study that reported no effect of garlic on normotensive rats (Al-Qattan *et al.*, 2006) as we observed a reduction in systolic, diastolic and heart rate values in both the normotensive and hypertensive models. Al-Qattan *et al*, (2006) also reported that blood pressure and heart rates were reduced significantly in garlic treated 2K1C hypertensive models, but our observation was that the effects were similar in both groups of animals, we even observed a reduced heart rate, systolic and diastolic values for the normotensive models which were not statistically significant. The only parameter elevated in the 2K1C hypertensive models was the pulse pressure.

The inconsistence in our findings with that of others may be because the garlic extracts were given intraperitoneally as against our protocol of infusion which was intravenous. As such, the discrepancy may be said to be due to experimental design and the duration of garlic administration since garlic was given i.p. for two weeks before the BP measurements, further studies may throw more light on this.

Our findings on increased pulse pressure in the hypertensive models could be attributed to the absence or reduction of pliability of the blood vessels and its increased vasoconstriction ability as reported in this model. This further shows that the effects of garlic are more pronounced in normotensive than in hypertensive conditions. The effects reportedly noticed over time in hypertensives treated with garlic can be due to an improvement of the pliability of the artery which decreases pulse pressure (Harauma and Moriguchi, 2006).

However, there is a possibility that garlic may act through other mechanisms especially the inhibition of the renin – angiotensin system which plays a significant role in 2K1C renovascular hypertension since garlic has been shown to exert some blood pressure lowering effect by reduction in angiotensin converting enzyme activity (Hosseini *et al.,* 2007; Castro *et al.*, 2010), and generally increasing the effects of vasodilators at same time decreasing those of the constrictors.

Our results showed that garlic extract caused a reduction in blood pressure and heart rates in both normotensive and hypertensive rat models. Wilcox *et al* (1996) had reported that several potent vasoconstrictors such as angiotensin II, 8-iso-prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), and thromboxane-A_2 are elevated in hypertensive models. Also, a functional alteration in vascular smooth muscle of resistance vessels from 2K1C rats was reported by Cauvin and Pegram (1983), these may have contributed to the

development of elevated total peripheral resistance in this model of hypertension and our observations that the effect of the extract was greater in normotensive than hypertensive rats.

In conclusion the findings of the present study indicate that aqueous garlic extract reduces blood pressure and heart rate in both hypertensive and normotensive rats in a dose-dependent manner by mechanisms that may not involve the cholinergic pathway.

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