Full Length Research Paper

Low blood pressure and cardio-protective impacts of Garlic (Allium sativum) and Ginger (Zingiber officinale) extracts in experimental animals

Aishatu Mohammed[1]*, Aminu Ibrahim[2] and Ali Atiku[3]

[1,2,3]Department of Anatomy and Physiology, Faculty of Medicine, Kaduna State University, Kaduna , Nigeria.

This study evaluated the blood pressure effects and cardio-protective function of ginger and garlic extracts in laboratory animals. Wistar rats of both sexes were assigned into the following groups: Group I: Served as control + distilled water; Group II: 20 mg/kg of garlic; Group III: 40 mg/kg of garlic; Group IV: 20 mg/kg of ginger; Group V: 40 mg/kg of ginger; Group VI: garlic 10 mg and ginger 10 mg/kg; Group VII: garlic 20 mg and ginger 20 mg/kg. Animals were euthanized after four weeks of treatment. Blood samples were collected and serum separated for lipid profile assay. The effect of extracts on perfused rabbit heart was also investigated. Moreover, the effect of extract on blood pressure was tested in anaesthetized cats. The results obtained in the study showed a statistically significant decrease (p<0.05) of serum total cholesterol (TC), triglyceride and low density lipoprotein-cholestero (LDL-C) levels at all single and combined doses of the extract when compared with the control group. The extract single and combined doses significantly increased (p<0.05) serum high density lipoprotein-cholesterol (HDL-C) level in the treated animals when compared with the control group. The results showed a significantly decreased rate and force of contraction at the combined doses when compared with the baseline line control level (p<0.05). The study also revealed that the administration of only garlic at dose of 0.1 and 1 mg/ml produced a significant decrease (p<0.05) in the diastolic blood pressure. There was a statistically significant decrease (p<0.05) in diastolic blood pressure in all single doses of garlic as well as the combined doses of garlic and ginger extract administered. The result obtained showed that administration of only single doses of ginger produced a significant decrease in p < 0.05. Administration of single doses of garlic (0.1 and 1 mg/ml) produced significant reduction (P < 0.05) in mean arterial pressure (MAP) when compared with control group. In conclusion, the findings suggest that the extract as well as its combination improved lipid profile and may have a cardio-protective effect.

Key Words: Garlic, ginger, isolated perfuse heart, total cholesterol, triglyceride, low-density lipoprotein, blood pressure.

INTRODUCTION

Garlic (*Allium sativum*) and ginger (*Zingiber officinale*) are widely consumed spices in food and drink form. Several

studies have been done on these plants separately especially on the heart and blood pressure. But there is no record of their combined effects. However, these plants are often consumed in combined form as food spices. Human and animal studies have substantiated that garlic and ginger lowers serum cholesterol and triglycerides and increases the amount of high-density lipoproteins (HDL) (Brankovic et al., 2011). Garlic is a popular supplement well-perceived as a healthy choice among people looking to increase cardiovascular wellness. Approximately 4% of all cardiovascular disease patients who use herbal supplements take garlic (Hendenreich et al., 2011). Known risk factors for cardio-vascular disease include inflammation, high cholesterol, high homocysteine, high blood pressure, diabetes and vascular dementia (Ginter and Simko, 2010). Garlic reduces cholesterol synthesis by inhibiting 3-hydroxy-3-methylglutaryl-CoA. Garlic has been shown to inhibit low-density lipoproteins (LDL) oxidation, platelet aggregation, arterial plaque formation, decrease homocysteine, lower blood pressure, and improve micro circulation (Lai et al., 2012). *In vitro* studies by Benavides et al. (2007) have confirmed the vasoactive ability of garlic's sulfur compounds whereby red blood cells convert garlic's organic polysulfides into hydrogen sulfide, a known endo-genous cardio-protective vascular cell signaling molecule. In traditional Chinese medicine, ginger is used to improve the flow of body fluids. A Japanese study showed that active constituents in ginger reduced the blood pressure and decreased cardiac workload (Rehman et al., 2011). Several reports, mainly from rat studies, have suggested that ginger exerts many direct and indirect effects on blood pressure and heart rate (Yang et al., 2011). In Guinea pig paired atria, the crude extract exhibited a cardio-depressant activity on the rate and force of spontaneous contractions (Yu et al., 2011). In this study, we compared the blood pressure effects and cardio-protective function of ginger and garlic extracts separately to the combination of both extracts in laboratory animals.

MATERIALS AND METHODS

Management of experimental animals

Strains of albino Wistar rats of both sexes between the ages of 12 and 16 weeks' old and weighing between 150 and 200 g were procured from the Animal House of the Department of Pharmacology Animal House of Ahmadu Bello University, Zaria. The animals were kept in well aerated laboratory cages in the Animal House of the Department of Human Physiology, Ahmadu Bello University, Zaria. They were allowed to acclimatize to the laboratory environment for a period of two weeks before the commencement of the experiment. The rats were given access to standard animal feeds and drinking water a*d libitum* during the

*Corresponding author. E-mail: Aishamohammed@gmail.com

acclimatization period.

Collection and preparation of plant extract

The fresh plant of garlic bulbs and dried rhizome ginger were purchased from Samaru market in Zaria, 11° 10´ N, 07° 38' E Nigeria. The plant was taken to the herbarium unit of the Department of Biological Sciences where the plant was identified by the taxonomist Mallam Musa Mohammed with the voucher numbers 050913 and 0250913, and was deposited. They were dried under shade and then ground into fine powder using laboratory mortar and pestle. The powder (150 g) of garlic and (208 g) of ginger was macerated in cold water at room temperature for 24 h at light/dark cycle of 12:12 h. This was then filtered using a filter paper (Whatmann size no. 1) and the filtrate was evaporated to dryness on water bath at 40°C to a dry residue of 24 g of garlic and 34 g of ginger. The powder was kept in an air-tight bottle until it was reconstituted for administration.

Experimental design

Thirty five Wistar rats were used. Rats were allocated into 7 groups and treated as follows:

Group 1: Served as control and were administered with 1 ml of distilled water orally.
Group 2: Received 20 mg/kg body weight of garlic orally
Group 3: Received 40 mg/kg body weight of garlic orally
Group 4: Administered with received 20 mg/kg body weight of ginger orally.
Group 5: Received 40 mg/kg body weight of ginger orally.
Group 6: Received garlic 10 mg and ginger 10 mg/kg body weight orally
Group 7: Received garlic 20 mg and ginger 20 mg/kg body weight orally

Collection and preparation of sera samples for lipid profile

Animals were treated with extracts orally as mentioned earlier. Four weeks after the treatment period, all animals were sacrificed after mild anaesthesia with chloroform. Blood samples were drawn from the heart of each anaesthetized animal from all groups by cardiac puncture. Blood samples (5 ml) were collected in ethylenediaminetetraacetic acid (EDTA) bottles for the determination of haematological parameters. Other blood samples (5 ml) were collected in plain tubes and were allowed to clot, and the serum was separated by centrifugation using Denley BS400 centrifuge (England) at 1,968 × g for 10 minutes. The serum collected was used to determine lipid profile assay.

Determination of lipid profile

Lipid profile was determined spectrophotometrically, using enzymatic colometric assay kits (Randox Laboratories Limited kits, United Kingdom) as the following.

Determination of serum total cholesterol

The serum concentration of total cholesterol was quantified after

enzymatic hydrolysis and oxidation of the sample as described by Stein (1987). The value of total cholesterol present in the serum was expressed in mg/dl.

Total cholesterol concentration = A sample/A standard × 195.0 mg/dl.

Determination of serum triglyceride

The serum triglyceride concentration was determined after enzymatic hydrolysis of the sample with lipases as described by Tietz (1990). Briefly, 1000 μl of the reagent was added to each of the sample and standard. This was incubated for 10 min at 20 to 25°C after mixing, and the absorbance of the sample (A_{sample}) and standard ($A_{standard}$) were measured against the reagent blank within 30 min at 546 nm. The concentration of triglyceride present in the serum was expressed in mg/dl.

Triglyceride concentration = A sample/A standard > 196.0 mg/dl.

Determination of serum high-density lipoprotein cholesterol

The serum level of HDL-C was measured by the method of Wacnic and Albers (1978). Low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL) and chylomicron fractions in the sample were precipitated quantitatively by addition of phosphotungstic acid in the presence of magnesium ions. The mixture was allowed to stand for 10 min at room temperature (37°C), and centrifuged for 10 min at 1,957 × g. The supernatant

represented the HDL-C fraction. The cholesterol concentration in the HDL fraction, which remained in the supernatant, was determined. The value of HDL-C was expressed in mg/dl.

Determination of serum low-density lipoprotein cholesterol

The serum level of LDL-C was measured according to the protocol of Friedewald et al. (1972) using the relationship as follows:

LDL-C = TC-TGL/5 + HDL-C

where LDL-C is low-density lipoprotein cholesterol, TC is total cholesterol, TGL is triglyceride and HDL-c is high-density lipoprotein. The value was expressed in mg/dl.

Determination of effect of extract on perfused rabbit heart

The effects of various doses of the extract on the perfused rabbit heart were recorded after the baseline was established. One dose was given at a time, beginning from a very low dose of 1 mg/ml, then other doses were also tested. Each effect was washed off 2 min before the next. First, the effect of *A. sativum* was studied, and then the effect of *Z. officinale* and finally their combined effects were studied.

Determination of the effect of extract on blood pressure of the cat (*in vivo* studies)

Four cats were used for this study. They were anaesthetized by injection of 40 mg/kg of pento barbbitone sodium (stock

concentration 60 mg/ml) intraperitioneally. They were cannulated as the following.

Their carotid arteries and vagus nerve (in the neck) were exposed by dissection. Two short ligatures were placed wider than the carotid arteries and the peripheral one was tied tightly.
1) The carotid artery was temporarily occluded centrally with a clip. A cannula was inserted into the artery, and then connected to a pressure transducer coupled to a recording microdynamometer.
2) Heparin normal saline was injected to prevent clothing.
3) The record of the blood pressure began immediately after the occlusion.
4) The left femoral vein was exposed, tied off with a peripheral ligature.

A 2 ml syringe, containing normal saline for flushing of injected drugs was attached. The various tests drugs were administered beginning from a very low dose of 1 mg/ml to a maximum dose of 10 mg/ml. The effect of different doses of the extract on the blood pressure was recorded. The volume of extract used for each concentration was 0.2, 0.4, 0.8 and 1.0 ml. No additional dose was given until the preparation had fully recovered to the former control level or a new control level. Each effect was flushed by administering 2 ml of 0.9% normal saline. This procedure was done for *A. sativum* and *Z. officinale* separately, and then in combined form.

Statistical analysis

Data were expressed as mean ± standard error of mean (SEM). The data obtained were statistically analyzed using one-way analysis of variance (ANOVA) with Tukey's multiple comparison post hoc tests to determine the level of significance between control and experimental groups. Values of P < 0.05 were considered as significant (Duncan et al., 1977).

RESULTS

There was a significant (P<0.05) reduction of serum total cholesterol, triglyceride and low-density lipoprotein levels at all single and combined doses of the extract administered to the animals, when compared to the control group. The extract at single and combined doses significantly (P<0.05) elevated serum high-density lipoprotein level in the treated animals when compared with the control group (Table 1).

The study assessed the effect of garlic and ginger on blood pressure parameters in cats. The study also revealed that the administration of only garlic at dose of 0.1 and 1 mg/ml produced a significant decrease (p<0.05) in the systolic blood pressure when compared with normal control group. On the other hand, there was a statistically significant decrease (p<0.05) in diastolic blood pressure in all single doses of garlic as well as the combined doses of garlic and ginger extract administered when compared with the control group. The result obtained also showed that administration of only single doses of ginger produced a significantly reduced (p<0.05) pulse pressure when compared with normal control group. In regards to mean arterial blood pressure, administration of single doses of garlic (0.1 and 1 mg/ml) produced a significant reduction in mean arterial pressure when compared with the control group (Table 2).

Table 1. Effects of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) (single and combined) on serum lipid profile in Wistar rats (mg/dl) (n = 5).

Treatment	Serum total cholesterol (mg/dl)	Serum triglyceride (mg/dl)	Serum low- density lipoprotein (mg/dl)	Serum high-density lipoprotein (mg/dl)
Normal + Distilled water	106.97±2.41[a]	86.14±4.15[a]	64.16±4.23[a]	21.57±1.91[a]
Garlic 20 mg/kg body weight	62.49±4.09[b]	48.09±5.96[b]	22.63±2.24[b]	31.64±3.40[b]
Garlic 40 mg/kg body weight	72.64±4.65[b]	49.54±4.31[b]	19.84±1.77[b]	42.89±4.27[b]
Ginger 20mg/kg body weight	59.2.4±4.09[b]	27.66±2.63[b]	11.81±1.13[b]	41.97±3.56[b]
Ginger 40 mg/kg body weight	58.17±5.83[b]	31.24±5.67[b]	15.06±1.44[b]	37.40±3.51[b]
Garlic 10 mg + Ginger 10 mg/kg body weight	51.27±5.29[b]	30.00±6.45[b]	13.49±9.28[b]	31.79±2.54[b]
Garlic 20 mg + Ginger 20 mg/kg body weight	81.86±12.35[b]	27.20±7.16[b]	13.69±2.28[b]	62.73±12.23[b]

[a,b]Values of P < 0.05 with different superscript significantly different when compared with control group (Normal + Distilled water).

Table 2. Effects of garlic *(Allium sativum)* and ginger (*Zingiber officinale*) on blood pressure parameters in cats (single and combine).

Dose concentration (mg/ml)	SBP (mm/Hg)	DBP (mm/Hg)	Pulse pressure (mm/Hg)	MABP (mm/Hg)
Normal saline	75.75±0.85[a]	64.3±1.09[a]	11.95±0.82[a]	68.11±0.84
Garlic (0.1)	54.3±1.32[b]	34.5±1.55[b]	19.8±0.25[ns]	41.1±1.46[a]
Garlic (1)	54.0±0.41[b]	35.8±0.48[b]	18.3±0.25[ns]	41.8±0.44[a]
Garlic (10)	55.8±1.12[a]	36.8±0.95[b]	19.0±0.71[ns]	43.1±0.96[ns]
Garlic (20)	59.0±2.48[a]	42.8±0.63[b]	22.8±0.75[ns]	48.8±2.53[ns]
Ginger (0.1)	63.5±2.50[a]	51.5±3.50[a]	12.0±1.00[b]	55.5±3.20[ns]
Ginger (1)	66.2±3.13[a]	56.0±2.97[a]	10.2±0.31[b]	59.4±3.10[ns]
Ginger (10)	56.5±3.52[a]	45.0±4.71[a]	11.5±1.55[b]	48.6±4.45[ns]
Ginger (20)	46.6±0.93[b]	38.8±0.80[b]	7.8±0.73[b]	41.4±0.76[a]
Garlic + Ginger (0.1l)	60.5±0.65[a]	38.8±2.59[b]	21.8±1.88[a]	45.9±1.68[ns]
Garlic + Ginger (1)	59.0±2.48[b]	36.3±2.59[b]	22.75±0.75[ns]	43.8±2.53[ns]
Garlic + Ginger (10)	62.8±1.75[b]	44.5±2.63[b]	18.25±1.11[ns]	50.6±2.32[ns]
Garlic + Ginger (20)	63.5±2.75[b]	43.0±2.79[b]	20.50±0.28[ns]	49.8±2.78[ns]

[a,b]Values of with different superscript are significantly (P<0.05) different when compared to control group (Normal saline + distilled water).

DISCUSSION

Diseases mainly affecting the heart or blood vessels are primarily termed as cardiovascular diseases (CVDs). Today, CVDs constitute one of the major causes of mortality and have become a major health hazard all over the world since they account for more than 30% of the global deaths every year (American Heart Association medical/scientific statement, 1994; Thomas, 2011). Hyperlipidaemia is an important risk factor of coronary artery diseases which often lead to myocardial infarction and heart failure (NCEP, 1993; Ghotto et al., 2005). The present study investigated the cardio-protective activity of garlic and ginger extract and their combination in laboratory animals. Management of plasma cholesterol levels continues to be a cardinal issue in cardiovascular disease prevention, as hypercholesterolemia plays a crucial role in pathogenesis of atherosclerosis and related heart diseases (Ginte and Simko, 2011). The results showed that there was a statistically significant (P<0.05) decrease of serum total cholesterol concentration in the animals administered both single and combined doses of aqueous garlic and ginger extract when compared with the control group. Allicin has been proposed as the active compound produced by garlic which is responsible for its hypocholesterolemic effect (Ginte and Simko, 2011). Garlic and ginger have been reported to modify lipid metabolism by inhibiting cellular cholesterol biosynthesis, increasing bile acid biosynthesis to eliminate cholesterol from the body and increasing fecal cholesterol excretion, therefore, cholesterol synthesis is reduced by up to 75% (Singh and Poter, 2006; Matsuda et al., 2009). The cholesterol lowering effects of garlic has also been attributed not only to its organosulfur constituents but also to a

variety of steroidal saponins present in garlic extract (Ramaa et al., 2006). There was also significantly decreased serum triglyceride and low density lipoprotein levels at all single and combined doses of the aqueous garlic and ginger administered to animals when compared with the control group. However, the extract at single and combined doses significantly elevated (p<0.05) serum high density lipoprotein level in the treated animals when compared with the control group. These findings are in agreement with Ali et al. (2000) who suggested that administration of garlic to rats is effective in decreasing total cholesterol and triglycerides significantly. The mechanism for triglycerides lowering effect of garlic is not well understood. However, Yeh and Yeh (1994) demonstrated that the rate of acetate incorporation into fatty acid was reduced in hepatic cell culture treated with garlic extract. Thus, the triglycerides lowering effect of garlic may somehow be due to the inhibition of fatty acids synthesis. Elshater et al. (2009) revealed that post treatment with ginger extract for 6 weeks to diabetic rats produced significant reduction in the levels of plasma cholesterol, triglycerides and LDL-cholesterol and significant elevation in the HDL-cholesterol when compared with diabetic group. In another study, Alizadeh et al. (2008) investigated the effect of 45 days ginger capsules on the lipid levels in patients with hyperlipidemia and indicated that ginger has a significant lipid lowering effect when compared with placebo. The reduction in LDL-c level by garlic may be due to allicin, an active compound produced by garlic which reduces the production and release of LDL-c by the liver and promote LDL receptors activity in the liver cells, which helps the liver to clear the circulating LDL-c (Holzgartner et al., 1992). Brousseau et al. (2004) reported that the increase in HDL-c level is usually attributed to allicin, which significantly altered the distribution of cholesterol among HDL and LDL subclasses. Hyperlipidemia is the underlying pathophysiology of the number one killer, atherosclerotic coronary artery heart disease (Elrokh et al., 2010). The present study also revealed that administration of the extract especially at the combined doses produced a reduction on the rate and force of contraction when compared with the baseline line control level. The interaction between the extract and the standard drug (adrenaline) revealed that the extract completely blocked the action of adrenaline when co-administered, hence reducing both the rate and force of contraction. This suggests that the extracts may be acting via β_1-adrenergic receptor located on the myocardium. The blockade of β_1 receptors has been shown to cause negative inotropic and chronotropic effect which in turn bring about decrease in cardiac work and cardiac output (Katzung, 2007). These effects are useful in the treatment of cardiac infarction, cardiac arrhythmias and angina pectoris. Recent *in vitro* studies by Benavides et al. (2007) have confirmed the vasoactive ability of garlic's sulfur compounds whereby red blood cells convert garlic's organic polysulphides into hydrogen sulfide, a known endogenous cardio-protective vascular cell signaling molecule. Ghayur and Gilani (2005) reported that the crude extract of ginger induced a dose-dependent decrease in the arterial blood pressure of anesthetized rats and in guinea pig paired atria, the crude extract exhibited a cardio-depressant activity on the rate and force of spontaneous contractions. The study also revealed that the administration of only garlic at dose concentration of 0.1 and 1 mg/ml produced a significant decrease (p<0.05) in the systolic blood pressure when compared with normal control group. Similarly, there was a statistically significant decrease (p<0.05) diastolic blood pressure in all single doses of garlic as well as the combined doses of garlic and ginger extract administered when compared with the control group. The result obtained also showed that administration of only single doses of ginger produced a significant decrease (p<0.05) pulse pressure and mean arterial blood pressure when compared with the normal control group. Nitric oxide (NO) is an important mediator of blood pressure (BP) homeostasis. It has been reported that pharmacologically reducing the bioavailability of NO can lead to hypertension in normal rats (Ribeiro et al., 1992). In our present study, garlic may appear to exert blood pressure reducing activity by modulating the activity of several mechanisms that are vital in blood pressure homeostasis, which include the prostaglandin system (Al-Qattan et al., 2003), rennin-angiotensin system (Sharifi et al., 2003), and renal tubular transport mechanisms (Al-Qattan et al., 2003). Another possible mechanism by which garlic might reduce blood pressure is the direct and indirect vasodilatory actions of NO (Kim-Park and Ku, 2000; Gouvea et al., 2003). Garlic was reported to contain arginine and enhance the synthesis of NO (Kim et al., 2001).

Conclusion

Conclusively, the findings suggest that the extract as well as its combination improves lipid profile in the animals and the cardio-protective effect of garlic and ginger was alongside its negative ionotropic and chronotropic effect.

Conflict of interest

The authors of this research work declare that there is no conflict of interest concerning the publication of this manuscript.

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