Antiarrythmic And Anti-Hyperlipidaemic Potentials Of Aqueous Garlic Extract In Hypercholesterolaemic Rats

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Citation

H Nwanjo, G Oze. *Antiarrythmic And Anti-Hyperlipidaemic Potentials Of Aqueous Garlic Extract In Hypercholesterolaemic Rats*. The Internet Journal of Nutrition and Wellness. 2006 Volume 3 Number 2.

Abstract

The antiarrythmic and anti-hyperlipidaemic potentials of aqueous garlic extract in hypercholesterolaemic rats were investigated in this study. The lethality studies showed that the aqueous extract of the plant had an LD50 value of 625.08mg/kg rat intraperitioneally. The weight, heart rate and lipid parameters were measured after 6 weeks. There was an increase in weights in hypercholesterolaemic rats ($P<0.05$) when compared with the control group, which then significantly reduced in hypercholesterolemic rats treated with aqueous garlic extract. The study also showed that 100 and 200 mg/kg of the extract reduced the heart rate, which was increased in hypercholosterolaeminc group. There was a significant increase ($P<0.05$) in serum total cholesterol, LDL-cholesterol and triacylglycerol levels in hypercholesterolaemic rats when compared with the control. These were brought to near normal when treated with 100 and 200 mg/kg body weight of the aqueous garlic extract and this action was dose dependent. Garlic extracts are reported to inhibit 3-hydroxy-3-methoxy-butyryl-CoA reductase. This could help to explain the decrease in serum lipid indices. The normalization of atherogenic risk predictor indices and the restoration of heart rate by the two doses of the extract confirm the beneficial use of the aqueous garlic extract in traditional medicine practice to manage hypertension and some cardiovascular related problems.

INTRODUCTION

An elevated plasma level of cholesterol is linked to the development of atherosclerosis and Ischaemic conditions. Many studies indicate that lowering serum cholesterol may prevent, control or even reverse atherosclerosis and coronary heart disease (Ononogbu and Emole, 1998). In fact, it is almost accepted that atherosclerosis is a disorder of lipid transport and metabolism. Apart from the lipid from the diet source, the body in turn manufactures its own cholesterol. Inefficient clearance of excess cholesterol for reasons that are largely genetic (Kritchersky 1970), results in accumulation of cholesterol in the blood, and deposition of lipid in the inner layer of arterial walls. Studies have however shown that increased high density lipoprotein cholesterol (HDL-C) appears to prevent the development of atherosclerosis while reduced level are associated with increased risk for coronary artery diseases (Mendez et. al., 1975). Furthermore, high blood lipid levels, particularly total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) are usually related to promoting atherosclerotic syndrome. Hence interventions that lower these lipids in the blood can prevent the progression of the disease processes. Epidemiological studies have shown that diet rich in fruits, spices and vegetables are associated with a low risk of cardiovascular disease. Heart rate which is a term used to describe the frequency of the cardiac cycle is also affected in the course of the disease. Some vegetables, including garlic are known to amelorate the condition.

Garlic acquired a reputation in the folkore of many culture over centuries as a formidable prophylactic and therapeutic medicinal agent. During the earliest Olympics in Greece, garlic was fed to the athletes for increasing stamina (Moves, 1996; Lawson, 1998). The Indian ancient medical text (Charaka-Sambila) recommends garlic for the treatment of heart disease and arthritis. Garlic attracted the attention of modern medicine because of its widespread health use in different parts of the world. There is a common belief in folkloric medicine that it helps in maintaining good health, warding off illnesses and as a stimulant tonic.

Recent animal and clinical studies showed that garlic extract: (i) reduce the risk factors for cardiovascular diseases and cancer, (ii) stimulate immune function (iii) enhance detoxification of xenobiotics (iv) confer hepatoprotection (v) exhibit antimicrobial effects and (vi) antioxidant properties (Banerjee and Maulik, 2002).

Allicin (9-allyl- 2-propenethiosulfonate) is the principal bioactive compound and is produced from allin (S-allyl cysteine sulphoxide, present in intact garlic) by allinase enzyme when garlic is chopped or crushed.

Since the utility and the beneficial use of garlic and its preparations have been widely recognized and it has been alleged that traditional healers in the management of hypertension and some cardiovascular related problems use the extract of the plant. It is therefore our intention in this study to carry out scientific investigation on antiarrythmic and anti-hyperlipidaemic potentials of aqueous garlic extract in hypercholesterolaemic rats

MATERIALS AND METHOD

PLANT MATERIALS

Fresh garlic bulbs were purchased from a local market in Owerri metropolis in September 2005. They were identified and authenticated by Dr. S. C. Okeke (Head of Department of Plant Science and Biotechnology, Imo State University, Owerri). The voucher samples are kept in the University herbarium for references.

PREPARATION OF EXTRACT

The bulbs were cut into pieces and oven dried at 60OC. The dried bulb was ground to powder (500g). The ground material was sieved through 1mm sieve. A measured weight of 100g of the resulting fine powder was soaked in 200mls of distilled water and the suspension left to stand for 24hrs with intermittent shaking. The material was then filtered and filtrate extracted with a rotary evaporator to obtain a dry filtrate, and the solid residue referred to as extract. Appropriate concentrations of the extract were made in distilled water for the experiment.

ACUTE TOXICITY TEST

The acute toxicity of the extract was tested using 36 albino rats randomly assigned to 6 groups of 6 rats each. Each group was injected intraperitoneally with one of the following doses, 100, 200, 400, 800, 1600 mg/kg of the aqueous extract. The control group was injected with isotonic saline. The maximum volume given to all groups was 0.5 ml. The groups were returned to their home cages, and provided with food and water ad libitum. After 24 hr, the mortality in each cage was assessed. The percentages mortalities were converted to probit units (probability unit) using a standard probit table and plotted against the log10 of the doss of the extract. Regression lines were fitted by the least squares method and confidence limits for the LD50 values were calculated by the method of Litchfield and Wilcoxon (1949).

ANIMALS

Young adult albino rats weighing between 180-210g both males and females maintained at room temperature in the animal house of the College of Medicine and Health Sciences, Imo State University, Owerri, were used for this study. The animals were fed with normal commercial rat chow (product of Pfizer Nigeria Ltd) and they had free access to water.

EXPERIMENTAL DESIGN

Thirty rats included for this study were randomly assigned into 5 groups of 6 animals each.

GROUP A: Rats in this group served as negative control. They were fed with normal commercial rat chow and had free access to water throughout the period of the experiment.

GROUP B: These rats served as positive control and were fed with normal commercial diet supplemented with cholesterol and groundnut oil at 1%, for period of the experimental.

GROUP C: Rats in this group were also fed with hypercholesterolaemic diet for 3 weeks and subsequently with normal diet and 100mg/kg of aqueous garlic extract for another 3 weeks.

GROUP D: These rats were also fed with hypercholesterolaemic diet for 3 weeks and subsequently with normal diet and 200mg/kg of aqueous garlic extract for another 3 weeks.

ANALYTICAL PROCEDURE

Twenty-four hours after the last treatment given all the rats were weighed and quickly sacrificed under chloroform anesthesia and the heart rate counted with a stopwatch. Then, with a sterile syringe and needle, 5ml blood was collected from each animal by cardiac puncture and transferred into a centrifuge tube and allowed to clot before centrifuging using Wisperfuge Model 1384 centrifuge (Tamson, Holland) for 5 min and the resulting supernatant used for the lipid profiles.

In every sample collected, serum total cholesterol was estimated using established enzymatic methods of Allain et al (1974) with the Randox cholesterol kit (Randox, England). HDL-cholesterol was isolated by HDL-C precipitant method (Lopes-Vitrella et al. 1977). Triacylglycerole was assayed enzymatically as described by Tietz (1990), while LDL-cholesterol was calculated with a Friedwald formular (Friedwald, 1972).

RESULTS

The acute toxicity studies showed that the extract from the aqueous garlic extract produced an LD50 of 625.08mg/kg rat, I.P. The high dosed recipients were immobile and were lying on their abdomen. There was a significant increase in the weight of hypercholesterolaemic rats (Group B, C) at (P<0.05 which then significantly reduced in hypercholesterolaemic rats treated with various concentrations of aqueous garlic extract. (Table 1)

Figure 1

Table 1: Mean Weight Changes (g) in Rats Treated with Hyperlipidaemic Diets.

Group	Initial wt	Final wt	wt gain	%wt gain
A	190.15±8.63	190.2±8.6	0.05	0.03
B	188.3±6.5	200.0±9.1	**11.7	6.2
C	168.5±11.9	192.0±8.1	*3.52	1.9
D	168.5±11.9	192.0±8.1	*3.52	2.1

**: Significantly increased when compared with other groups (P<0.05)

* Significantly increased when compared with control (group A) (P<0.05)

Table 2 summarizes the lipid profiles of different experimental groups of rats and the control group. It was observed that there was a significant increase (P<0.05) in serum total cholesterol, LDL-Cholesterol, triacylglycerol and decrease in HDL-C levels in groups of rats fed with normal feed diet supplemented with cholesterol and groundnut oil at 1%. These were brought to near normal when treated with 100 and 200 mg/kg of the aqueous garlic extract and that this action was dose dependant.

Figure 2

Table 2: Serum lipid levels of different experimental groups of rats and control.

Group		HDL-C	LDL-C	
TC (mg/dl)	TG (mg/dl)	(mg/dl)	(mg/dl)	
A	77.1 ± 5.06	35.02 ± 6.41	44.08 ± 6.89	25.00 ± 4.47
B	112.75 ± 6.88*	80.50 ± 3.56*	32.50 ± 3.94*	74.06 ± 3.42*
C	74.00 ± 9.63	35.21 ± 6.84	46.63 ± 3.68	25.30 ± 2.28
D	68.00 ± 12.13	32.96 ± 5.29	48.32 ± 3.33	18.01 ± 2.60

*Significantly different from normal controls in group A (P<0.05)

In table 3, the atherogenic risk predictor indices HDL-C/TC and LDL-C/HDL-C of the both the experimental and control groups were calculated. It was observed that Group B (hypercholesterolemia group) were atherogenic and undesirable which was also normalize to desirable level in groups treated with 100 and 200 mg/kg body weight of the extracts.

Figure 3

Table 3: Atherogenic risk predictor indices in experimental animals and control.

Stage	A	B	D	E
HDL-C/TC	0.57 ± 0.06	0.29 ± 0.03	0.63 ± 0.03	0.71 ± 0.07
LDL-C/HDL-C	0.58 ± 0.22	2.28 ± 0.41	0.54 ± 0.03	0.37 ± 0.02

Values of HDL-C/TC ratio ≥ 0.3 are antiatherogenic or desirable

Values of HDL-C/TC ratio < 0.3 are atherogenic or undesirable.

Values of LDL-C/HDL-C ratio ≤ 2.3 are antiatherogenic or desirable

Table 4 also showed that 100 and 200 mg/kg of the extract reduced the heart rate, which was increased in hypercholosterolaeminc group (Group B)

Figure 4

Table 4: Heart rate in the various experimental and control groups

Group	Heart Rate	% Heart Rate
A	89.0 ± 2.5	-
B	120.5 ± 16.10*	35%
C	90.3 ± 2.7	25.2%
D	87.6 ± 2.7	26.2%

*Significantly different from normal controls in group A (P<0.05)

DISCUSSIONS

Artherosclerosis with subsequent manifestations of cardiomyopathies is one of the major causes of morbidity and mortality in the world. Various studies indicate that high serum levels of cholesterol are strongly related to coronary atherosclerosis and increased risk of coronary heart disease. Clinical studies in human have shown that lowering levels of serum cholesterol (especially LDL-C) with diet or drugs decreases the incidence of coronary heart disease (Gotto et al, 1990). The earlier investigations of experimentally induced atherosclerosis were done in rabbit or rat models by feeding with milk, meat and egg yolk. In 1970, Kritcherslay established that the atherosclerosis – causing component of the diet was cholesterol.

It has previously been shown in laboratory and clinical studies that aqueous extract of garlic lowers serum cholesterol (Ali and thon, 1995).

The result from the present investigations confirmed that 200mg/kg garlic extract significantly reduce to serum TC, LDL-C and TG levels after 6 weeks of treatments with hyperlipidemic diets ($P<0.05$). However the serum HDL-C was also significantly increased during the period ($P<0.05$) (table 2). These findings agree with the work of Chi et al., 1982. The heart rate of the animals positively correlated with the changes in serum lipid profiles (tables 2 and 4): the abnormal rate of 103 15 per second in the hyperlipidemic rats were corrected to 75 3.9 beats per minutes in the rats which received 100 and 200 mg/kg garlic extract respectively. There seems to be few studies relating heart rate to the level of serum lipids.

Calculation of the risk predictor indices HDL-C/TC and LDL-C/HDL-C showed that hyperolesterteroemic rets (group B) are 0.29 ± 0.03 and 2.28 ± 0.41respectively indicating that the values are atherogenic and undesirable. This was returned to desirable ratio in groups treated with 100 and 200 mg/kg of aqueous garlic extract. This shows that the aqueous garlic extract has the potential to reduce the risk of development of heart diseases since high HDL-C/TC and low LDL-C/HDL-C ratios have been shown to be beneficial and is indicative of a lower risk of coronary heart diseases A similar observation was made by Heinle and Betz (1994) and Ali and Thomson (1995).

Merat and Fallalizadeh (1996) shows that garlic extract inhibits 3-OH-3-me-Glutaryl-CoA (HMG-CoA) reductase which is a rate limiting enzyme in cholesterol biocynthesis. This may likely be the explanation for the significant reduction in serum lipids by the garlic extract (Chi et al, 1982). It has also been shown to depress the hepatic activities of other lipogenic, cholesterogenic enzymes such as malic enzymes, fatty acid synthase, glucose-6-phosphate dehydrogenase (Yu-Yan and Liu, 2001). Thus, the triglyceride-lowering effect of garlic may be due to the inhibition of fatty acid synthesis. Further, LDL-c isolated form human subjects given aqueous garlic extract was found to be more resistant to oxidation. Suppressed LDL-c oxidation of lipids may therefore be one of the mechanisms for the possible antioxidant benefits of garlic extract especially in atherosclerotic cases (Banerjee and Maulik, 2002)

These actions may partly explain the beneficial use of garlic extract in managing ischaemic heart disease related conditions in folk medicine. These properties can further explain the outcome of the present study in which the garlic extract concomitantly increased the atherogenic indices and lowered the tachycardic syndrome in the hyperlipidaemic rats. Further studies may be required to confirm the validity or otherwise of these results.

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