Changes In Serum Lipid Profiles And Heart Rate In Rats Treated With Aqueous Garlic Extract

H Nwanjo, G Oze

Citation

H Nwanjo, G Oze. *Changes In Serum Lipid Profiles And Heart Rate In Rats Treated With Aqueous Garlic Extract*. The Internet Journal of Nutrition and Wellness. 2006 Volume 4 Number 1.

Abstract

The serum lipid profiles and heart rate in rats treated with aqueous garlic extract were estimated in 24 Wistar albino rats. The rats were divided into four groups of 6 rats each. Group A received water and standard feeds for 6 weeks. Group B was fed with hyperlipidaemic diets for 6 weeks, while groups C and D had the hyperlipidaemic diets for 3 weeks and in addition administered 100 and 200 mg/kg aqueous garlic extract for another 3 weeks respectively. The body weights of the animals were simultaneously estimated. The results showed that the rats in all the groups gained weight: 0.03% for group A (negative Control), 6.2% for group B (positive Control), and 1.9 and 2.1% for groups C and D respectively. At the 6th week, the serum levels of Tc, Tg and LDL-c decreased significantly ($P<0.05$). but the HDL-c value increased significantly ($P<0.05$) too. The atherogenic risk predictor ratios also increased. The changes in groups C and D followed a dose-related pattern. Garlic extracts are reported to inhibit 3-hydroxy-3-methoxy-butyryl-CoA reduetase. This could help to explain the decrease in serum lipid indices. The increase in aterogenic risk ratio confirms the beneficial use of the aqueous garlic extract in traditional medicine practice to manage hypertension and some cardiovascular related problems. The restoration of heart rate by the two doses of the extract tend to validate the hypolipidaemic and antiarrythmic potentials of the garlic extract.

INTRODUCTION

An elevated plasma level of cholesterol is linked to the development of atherosclerosis and Ischemic conditions. Many studies indicate that lowering serum cholesterol may prevent, control or even reverse atherosclerosis and coronary heart disease (Ononogbu and Emole,1999). 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 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 ameliorate the condition.

Garlic acquired a reputation in the folklore 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 (Moyers, 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).

Garlic extracts have been shown to possess hypocholesteronemic property in clinical and experimental studies (Jain, 1997) and fresh garlic extracts have been found to lower blood pressure in spontaneously hypertensive rats, elicit depressant effect on the cardiovascular system and also exhibit anti-oxidant properties (Riggs et al, 1997).

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 of garlic and its preparations have been widely recognized in the prevention of cardiovascular and some other metabolic diseases such as atherosclerosis, hypercholesteronemia, thrombosis, hypertension and diabetes; it is our intention to investigate the changes in lipid profile and heart rate due to administration of aqueous garlic on hypercholesterolemic albino rats.

MATERIALS AND METHODS

Fresh garlic bulbs were purchased from a local market in Owerri metropolis. The botanical identification of the plant was done in the department of plant biology and biotechnology, Imo State University, Owerri Nigeria by Dr. C. Okeke. All chemicals and reagents used in the study were of analytical grades purchased from British Drug House (UK). The albino rats were obtained from the Animal House of College of Medicine and Health Sciences, Imo State University, Owerri. Laboratory bred albino rats of both sexes weighing between 150g to 200g, maintained under standard laboratory conditions of 12 hours light/dark cycle were used for the experiment. Commercial feeds (product of Pfizer) and water were provided ad libitum.

The bulbs were cut into pieces and oven dried at 60 ^{O} C. The dried bulbs were 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.

Two different concentrations of the extract were prepared (100mg/ml and 200mg/ml) using distilled water and the extract given according to the body weight of the animals.

Fifty albino rats of both sexes weighing 150-200mg were randomly divided into five groups with ten rats in each group and housed in stainless steel cages labeled A-E. The animals had access to water and feed (product of Pfizer Nigeria Ltd.) ad libitium for seven days to acclimatize before commencement of treatment. The method of Mendez et al (1975) was adapted. Rat in group A served as negative control and were allowed free access to water and normal commercial rat chow for the period (6 weeks) of the experiment. Rats in group B served as positive control and were fed with standard diet supplement, along with 1% cholesterol and 1% groundnut oil, for the period of the experiment. Group B represented the hyperlipidamic rats.

Rat in groups C and D were administered 100 and 200mg/kg of aq.garlic extract, followed by the hyperlipidaemic diets as in group B for 6 weeks. Treatment was administratered once daily by oral compulsion. Animals were fasted for 24 hours after last drug administration and feeding and sacrificed under chloroform anesthesia.

The total cholesterol, high-density lipoprotein (HDL), and triglycerides were determined using the following methods:

Cholesterol reacts with acetic anhydride in the presence of glacial acetic acid and concentrated sulphuric acid to form green coloured complex. Intensity of the colour is proportional to the cholesterol concentration and can be measured at 540nm wavelength spectrophotometrically. In the presence of phosphotungstic acid and magnesium chloride, LDL, VIDL Chylomicrons are precipitated. Centrifugation leaves only the HDL in the supernatant. Cholesterol in the HDL fraction can be tested by the usual method.

The serum lipids are extracted by isopropanol, which also precipitates serum proteins. The interfering phospholipids containing glycerol as integral part are removed by absorption on alumina. The action of metaperiodate converts glycerol into glyceraldehydes, which forms a yellow coloured complex with acetyl acetone. The intensity of the coloured complex is measured at 410nm wavelength calorimetrically.

STATISTICAL ANALYSIS

All values were expressed as mean± SD. The statistical analysis was carried out using Duncan multiple values test, students t– test to detect differences in the mean values of test and control. Test with ($P<0.05$) were considered significantly different.

RESULTS

The effect of normal and hyperlipidaemic diets, along with

100 and 200mg/kg of aqueous garlic extract on body weight of the Wistar albino rats is shown on table 1. A significant weight gain was observed in the hyperlipidaemic rats compared with the rats on normal diet. The weight gain was however reduced in groups fed with the diet and the extracts.

Table 2 compares the lipid levels in the experimental animals at 3 rd and 6 th week of the experiment. There were marginal changes in the serum concentration of the lipids at the end of the 3 rd week. But at the end of the 6 th week, there were significant decreases (P<0.05) in the serum lipid levels, except for HDL-c where the case was reversed.

Table 3 shows the atherogemic risk indices at the 3 rd and 6 th weeks. The 100 and 200mg/kg aq garlic extract caused significant (P<0.05), dose-dependent rise in the ratio at the end of the sixth week. No such observation was made for the 3 rd week where the ratio remained relatively unchanged.

In table 4 the heart rate of 103± 15.0 seen in the hyperlipidemic rats returned to the base-line by the 6 th weeks in the 100 and 200mg/kg aq. garlic treated rats (25.2 and 26% reduction respectively).

Figure 1

Table 1: Mean Weight (g) Changes In Rats Treated With Hyperlipidaemic Diets (mean ± SD) (n = 10).

Group	Initial wt	Final wt	wt gain	%wt gain
NC (Negative Control)	190.15±8.63	190.2±8.6	0.05	0.03
PC (Positive Control) Diet+1% C'rol+1% Gnut oil.	188.3±6.5	200.0±9.1	11.7	6.2
T_1 100mg/kg Extract+Diet+1% C'rol+1% Gnut oil	168.5±11.9	192.0±8.1	3.52	1.9
T_2 200mg/kg Ext.+Diet+1% C'rol+1%Gnut oil	168.5±11.9	192.0±8.1	3.52	2.1

Key
Nc = Negative Control   Pc = Positive Control
T_1 = Test-1 (100mg/kg) T_2 = Test-2 (200mg/kg)

Figure 2

Table 2: Mean serum lipid levels in hyperlipidaemic rats after 3 and 6 weeks of treatment with aq. Garlic extract

(Mean ± SD) (n = 10)

Group	Tc (mg/dl)	Tg (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)
Nc	69.11±6.21 (77.1±5.06)	34.02±6.36 (35.02±6.41)	43.08±6.20 (44.08±6.89)	24.01±2.95 (25.00±4.47)
Pc	110.65±8.27 (112.75±6.88)	66.26±5.60 (80.50±3.56)	31.34±2.04 (32.50±3.94)	72.50±3.75 (74.06±3.42)
T_1	109.80±16.15 (74.00±9.63)	68.25±4.60 (35.21±6.84)	30.94±2.98 (46.63±3.68)	71.99±4.78 (25.30±2.28)
T_2	112.14±19.88 (68.00±12.13)	70.43±3.73 (32.96±5.29)	31.56±1.91 (48.32±3.33)	73.34±3.56 (18.01±2.60)

Legend: Values in parentheses represent serum levels of lipids after the 6th week, and the values outside the parentheses for the 3rd week of aq. garlic extract treatment.
Key
Nc = Negative Control, T_1 = Test (100mg/kg)
Pc = Positive Control, T_2 = Test (200mg/kg)

Figure 3

Table 3: Mean atherogemic risk predictor indices in experimental animals at the 3rd and 6th week of treament with aq. garlic extract. (mean ± sd) (n = 10) (HDL-C/LDL-C)

Group	3rd Week	6th Week
Nc	1.79	1.76
Pc	0.43	0.44
T_1	0.43	2.68
T_2	0.45	2.84

Figure 4

Table 4: Effect of aq. garlic extract on heart rate.

	Heart Rate		
Group	3rd Wk	6th Wk	%
Nc	88±4.7	89±2.5	-
Pc	103±15.0	120.5±16.10	35%
T_1	75±3.9	90.1.3±2.7	25.2%
T_2	74±6.8	871.6±2.7	26.2%

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 aq. extract of garlic lowers serum cholesterol (Ali and thon, 1995).

The result from the present investigations showed that 200mg/kg garlic extract significantly reduced the 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. Moreover, the ratio of atherogenic risk predictor indices increased in the test groups after the sixth week (2.84 T_1 and 2.68 T_2) when compared with the positive control (0.44pc). A similar observation was made by Heinle and Betz (1994) and Ali and Thomson (1995).

Merat and Fallalizadeh (1996) showed 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)

Garlic extracts have been observed to possess hypotensive action, anti-platelet function and activation of the endogenous antioxidants, superoxide dismutase and catalase in laboratory rats (Banerjee et al, 2002).

These actions may partly explain the beneficial use of garlic extract in managing ischemic 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 hyperlipidamic rats. Further studies may be required to confirm the validity or otherwise of these results.

References

r-0. Ali M. and Thomson M. (1995). Consumption of garlic Clove a day could be beneficial in preventing thrombosis. Prostaglandins Leukot Essential fatty acids 53-211-212
r-1. Baneerjee S.K. and Maulik S.K. (2002). Effect of garlic On cardiovascular disorders. A review, Nutritional Journal 1:4.
r-2. Bannerjee S.K. Maulik M, Mancahanda S.C., Dinda A.K. Gupta S.K., Maulik S.K (2002). Dose dependent induction of endogenous antioxidants of garlic. Life science 70: 1509-1518.
r-3. Chi M.S., Koh E.T and Steriart T.J. (1982) Effect of Garlic on lipid metabolism in rats fed cholesterol or lard. Journal of Nutrition, 122:241-248.
r-4. Guyton A.C. (1980): Lipids and lipid metabolism; In Medical Physiology W.B., Sanders company Lodon. 857-859.
r-5. Gotto, A.M., Lorassa, J.C., and Hunringhake, D. (1990). The cholesterol. Facts Circulation 81:1721-1730.
r-6. Heinle, H. and Betz, E. (991). Effect of dietary garlic Supplementation in a Rat model of atherosclerosis. Arzenmitleforschung. 44:614-617.
r-7. Jain, R.C. (1997). Onion and garlic in experimental Cholesterol induced atherosclerosis. Indian journal of medical research 8: 1509-1515.
r-8. Kritchersky, S. (1970). The role of cholesterol as vehicle in Experiment atherosclerosis. American Journal of Clinical Nutrition 23: 1105-1110.
r-9. Lawson L.D. (1998): Garlic a review of its medicinal effects and indicated active compounds. In phytomedicine of Europe. Chemistry and Biological activity series 691 (Lawson L.D.R. Editions). American chemical society. Washington. 176-208
r-10. Lerner, D.Z, and Hulley, S.B. (1994). Does eating garlic lower cholesterol? Annals of Internal medicine. 120:969-973.
r-11. Mendez, E.Franlein, J., Gahegan, B.H. (1975). Simple manual method for determination of serum triglycerides chemistry. 21:760-770.
r-12. Merat, A., and Fallalizadeh, M. (1996). Effect of garlic on some blood lipids and HMGCoA reductase activity. Iran Journal of medical sciences. 21: (3&4) 146-151.
r-13. Moyer s. (1996). Garlic in health. History and world Cuisine stun coast press St. Petersburg. F.I. 1-36.
r-14. Nwanjo, H.U. (2003). Studies of plasma lipids and lipid peroxide in-patient with ischemic heart disease. Ph.D Thesis, UNN pp 78-98.
r-15. Nwanjo, H.U. and Ojiako, O.A. (2003). Effects of Aqueous garlic extract on atherogenic risk predictor indics in

wistar Albino rats.
r-16. Ononugbu, I.C. Emole, I. (1999). The effect of garlic on rats plasma
r-17. Riggs. D. R., Deltawen, J.I. and Lamn, D.L. (1997) Garlic treatment for murine ransitional cell. Carcinoma 79:10 74 - 87 cholesterol. Atherosclerosis 31:101-104.
r-18. Yu-Yan Yeh, Liu, L.: (2001) cholesterol-lowering effect of garlic extracts and organ sulfur compounds.: Human and animal studies. Journal of Nutrition, 131:9895-9935.
r-19. Zak. B (1957). Determination of total, free, esterified cholesterol, - using reaction with ferric chloride and sulpuric acid. American Journal of Clinical pathology. 27:583-589.

Author Information

H. U. Nwanjo
College of Medicine and Health Sciences, Imo State University

G. O. Oze
College of Medicine and Health Sciences, Imo State University