ORIGINAL ARTICLE

Aged Black Garlic Extract Improves Blood Cell Counts & Blood Lipids in Wistar Albino Rats

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ABSTRACT

Objective: The study was conducted to evaluate effects of aged black garlic extracts (ABG) on blood cell counts and blood lipids in Wistar albino rat model.

Study Design: Experimental study

Place and Duration: Isra University Hyderabad from November 2013 to May 2014.

Material and Methods: Sixty adult albino rats were divided into four groups; Group I. controls received 0.9% isotonic saline, Group II. Received garlic extract orally (200 mg/kg), Group III. Received garlic extract orally (300 mg/kg) for 30 days. Blood samples were collected for hematological analysis and plasma lipids. The data was analyzed on SPSS 21.0. (IBM incorporation, USA) using one-way ANOV. Post Hoc Duncan testing was used for multiple comparisons for variables which showed significant F ratio and p-value. A p-value of =0.05 was taken statistically significant.

Results: Complete blood cell counts and blood lipids showed significant differences between the controls, and experimental garlic groups at different doses (p=0.001). The hemoglobin, packed cell volume, red and white cell counts, and platelets were raised in garlic extract groups particularly in high doses of 300mg/kg. The differential white blood cell counts also revealed significant differences. The blood lipids showed a reduction in total cholesterol, LDL-c and a rise in HDL-c levels in ABG groups. (p=0.001).

Conclusion: The garlic extract influences hematological parameters in albino rats and reduces plasma lipids fractions which may be protective against cardiovascular disease.

Key words: Aged black garlic, Blood cells, LDL, HDL, VLDL

INTRODUCTION

Botanically the garlic is known as allium sativum. Garlic is a well known spice and herbal medicine for the prevention and treatment of a variety of diseases ranging from infections to heart diseases.[1] It is reported that garlic shows antimicrobial activity.[2] Garlic is considered as a plant with antibiotic, anticancer, antioxidant, immunomodulatory, anti-inflammatory, hypoglycemic and cardiovascular protecting effects.[3] It is also reported that garlic has the tendency of lowering serum and liver cholesterol.[4] In previous studies the positive effects of garlic supplements have shown significant differences on plasma lipids as reported.[5] Previous studies have demonstrated that lowering plasma total cholesterol (TC), low density lipoprotein cholesterol (LDLC) and increasing high density lipoprotein cholesterol (HDLC) are beneficial in preventing risk of cardiovascular disease (CVD).[6] There are divergent views on the effect of garlic on plasma cholesterol concentration and coronary heart disease. Studies have reported the ability of garlic to reduce plasma cholesterol in humans.[7,8] Evidence on the role of garlic in lowering plasma cholesterol in developed populations abounds.[7,8] Several studies have also reported the antihypertensive effects of garlic.[8,9] Garlic extracts have been used in the treatment of a wide range of disorders in the past.[10] Caro et al[11]

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Demonstrated that garlic oil is active against fatty change of the liver. Allicin, diallyl disulfide-oxide, an active ingredient released from garlic is a systemic vasodilator.[12] Also reported was garlic containing preparation which showed significant decrease in diastolic blood pressure in severely hypertensive patients.[13] Ether extracts of garlic and partially purified distilled extracts of garlic have been reported to inhibit human platelet aggregation in vitro.[14] Treatment with garlic extracts was found to improve the activation of natural killer cells, the function of T-lymphocytes and the level of interleukin-2.[15] Also in vitro and in vivo studies showed that aged garlic extracts stimulate immune functions.[16] Many studies have been reported on effects of garlic on various organs in rat model: however, scanty information is available regarding effects of garlic on hematological parameters. The present study aimed to investigate the possible effects of aqueous extract of aged black garlic (ABG) on hematological parameters and blood lipid levels in experimental albino rat model.

MATERIAL AND METHODS:

An experimental study was conducted at the animal house of Isra University in albino rat model November 2013 to May 2014. Sixty male albino rats weighing 200-250 grams were included in the study. Sick animals and animals weighing more or less than mentioned in inclusion criteria were excluded from study. Animals were housed in animal house at an optimal room temperature with 55-60% humidity and exposed to 12 hour light-dark cycles. The chaw and clean water were provided ad-libitum.

- **Animals and experimental design**

At the end of experimental period, 12-hour-fasted rats were anesthetized during the post absorptive period. Blood was drawn from the vena cava into heparin tubes centrifuged and stored at 4°C; the plasma was stored in a freezer for later assays. The rats were given garlic in different doses for 30 days.

The rats were divided into three groups;

Group I. Control Group (n=20) Rats received 0.9% isotonic saline orally throughout the experimental period.

Group II. Experimental Group (n=20) Rats were given aqueous garlic extract, administered orally at a dose of 200 mg/5ml of distilled water per kilogram of body weight.

Group III. Experimental Group (n=20) Rats were given aqueous garlic extract, administered orally at a dose of 300 mg/5ml of distilled water per kilogram of body weight.

- **Garlic extract preparation**

The aqueous garlic extract was prepared by dissolving garlic in pure water at a quantity of 200mg and 300mg. The aqueous garlic extract was administered orally at a dose of 200 and 300 mg/5ml of distilled water per kilogram of body weight.

- **Blood sample:** The blood samples were collected from vena cava into EDTA tubes centrifuged and stored at 4°C. Blood was stained with Leishman`s stain for blood counts and peripheral smear study. Plasma was separated for determination of blood lipids.
- **Lipid estimation:** Plasma triglyceride (TG), total cholesterol (TC), low density lipoprotein (LDL) and high-density lipoprotein-cholesterol (HDL-C) were enzymatically determined using immunoassay kits (Asia Pharmaceuticals, Seoul) and an enzyme-linked immunosorbent assay reader (Pharmacia Biotech, Cambridge, United Kingdom) according to the manufacturer's protocol. Plasma low-density lipoprotein-cholesterol (LDL-C) was calculated using the Friedewald's equation.

The data was analyzed on SPSS 21.0. (IBM incorporation, USA) using one-way ANOV. Post Hoc Duncan testing was used for multiple comparisons for variables which showed significant F ratio and p-value. A p-value of =0.05 was taken statistically significant.

RESULTS:

The present study observes major differences in the complete blood cell counts and blood lipid profile among controls and aged black garlic extract experimental rats. Significant differences were observed in the packed cell volume (PCV), hemoglobin, RBC count, white blood cells and differential white cell counts as shown in table-1. Statistically a significant differences were noted for multiple comparisons (p=0.001).

Similarly significant differences were observed in the lipid profile of controls and aged black garlic extract experimental rats. The most significant differences in lipid profile was observed at high doses of ABG at 300 mg (p=0.0001) as shown in table-2.

Table-1. Hematological Parameters in Different Animal Groups

Parameter	Group I (Controls)	Group II (*AGB 200mg)	Group III (AGB 300mg)
Red blood cells ($x10^3 \mu L^{-1}$)	4.29	4.68	4.81
Hemoglobin (gdl^{-1})	13.1	15.1	15.5
Packed cell volume (%)	40.1	42.3	44.9
White blood cells (μL^{-1})	6710	6970	8789
Neutrophils (%)	70.1	73	86
Monocytes (%)	1.8	2.1	3.5
Lymphocytes (%)	34	27.5	18.42
Eosinophils (%)	1.0	1.1	1.5
Basophils (μL^{-1})	0.1	0.02	0.18
Platelets ($x10^3 \mu L^{-1}$)	4.3	4.2	4.85

* Aged black garlic extract

Table-2. Blood Lipid Profile in Different Animal Groups

Plasma Lipids (mgdl-1)	Group I (Controls)	Group II (*AGB 200mg)	Group III (AGB 300mg)
Triglycerides (mg/dl)	112	143	99
Total cholesterol (mg/dl)	136	138	112
LDL (mg/dl)	43	36	29
VLDL (mg/dl)	41	39	38
HDL (mg/dl)	40	43	46

* Aged black garlic extract

DISCUSSION:

The hemoglobin, packed cell volume, red blood cell counts, white blood cells counts and platelets revealed statistically significant differences between controls and garlic extract groups. The study showed that rats given higher doses of garlic had a preponderance of neutrophil over lymphocyte (table-1). Also, a higher dose of 300mg/day garlic extract was observed to cause a significant increase in hematological counts and indices, and reductions in blood lipid fractions (table-2). These data therefore support the earlier reports by Sumiyoshi[15] and Oluwole et al[10] that garlic extracts stimulate immune functions. This observation may partly explain the role of garlic in activating the natural killer cells, the function of T-lymphocytes and the level of interleukin-2 as reported previously.[14]

In the present study, within group analysis showed a significant decrease in plasma total lipids in the experimental groups fed on high garlic extract. There were however no significant changes in the plasma TG, HDLC and LDLC when compared groups II and III with controls. Reduced plasma TC, and LDLC and increased mean plasma HDLC were obtained in rats fed on high garlic amounts (300mg/dl). Earlier studies[7,9] showed that garlic when administered raw caused significant reduction in total cholesterol in subjects with raised plasma cholesterol. The groups that had garlic incorporated into the diet at high doses of 300 mg/dl had the highest mean plasma HDLC

Level. One proposed mechanism of cholesterols reduction is through the inhibition of HMG-Co A reductase by garlic, the rate-limiting enzyme that mediates the first step in cholesterol biosynthesis. On the other hand the plasma LDLC was significantly decreased in the groups of animals who were fed high garlic extract. This perhaps supports the ability of garlic to reduce LDL cholesterol concentration. Evidence from available studies showed that garlic can affect vasculature by improving aortic elasticity as well as retardation of atherosclerosis progression[8,9] perhaps through increase excretion of LDL-cholesterol. As evident from this study, garlic also decreases plasma triglyceride level in the rats fed on garlic containing diets, most likely through the stimulation of lipase. Available reports from a similar study indicated that garlic is a potential stimulant of lipase.[16,17] The results of present study suggest that garlic has hypolipidemic effect. Available report shows that garlic consumption is beneficial in the prevention of cardiovascular disease.[18,19] The garlic extract in high doses increases hematological parameters and reduces bad cholesterol of plasma lipids as observed in present study.

CONCLUSION:

The aged black garlic extract improves blood cell counts, increases high density lipoprotein and reduces low density lipoproteins. The aged black garlic may be used for cardiovascular disease prevention and treatment. Further studies are warranted to authenticate the results of present study.

REFERNCES:

1. Elagib HAA, El-Amin WIA, Elamin KM, Malik HEE. Effect of Dietary Garlic (Allium sativum) Supplementation as Feed Additive on Broiler Performance and Blood Profile. J Anim Sci Adv. 2013;3(2):58-64.
2. Xiaonan Lu, Barbara A R, Dong-Hyun K, Jamie M FJ, Aston DE, Michael E K. Infrared and Raman Spectroscopic Studies of the Antimicrobial Effects of Garlic Concentrates & Diallyl Constituents on Foodborne Pathogens. Anal Chem. 2011;83(11):4137-46.
3. Silagy C, Neil A: Garlic as a lipid lowering agenta meta analysis. Coll JR Physicians Lond.1994;28:39-45.
4. Yasser M F, Reem Y. Hepato-cardiac disorders. World J Hepatol. 2014; 6(1): 41-54.
5. Tekeli A, Celik L, Kutlu HR, Gorgulu M. Effect of dietary supplemental plant extracts on performance, carcass characteristics, digestive system development, intestinal microflora & some blood parameters of broiler chicks. Proceedings of 12th European Poultry Conference, Verona, Italy. 2006:307-8.
6. Ebesunun MO, Popoola OO, Agbedana EO, OLisekodiaka JM, Onuegbu JA, Onyeagala AA. The effects of garlic on plasma lipis and lipoproteins in rats fed on high cholesterol enriched diet. Biokemisri. 2007;19 (2):53-8.
7. Jain AK, Vegas R, Gotzkaowska S M, Mahon FG. Can garlic reduce levels of serum lipids? A controlled clinical study. Am J. Med.1993;94:632-5
8. Rotzch WJ, Azneim F. Postprandial lipemia under treatment with Allium sativum controlled double blind study in healthy volunteers with reduced HDL2- cholesterols. Arzneim Forsch. 1992;42:1223-7.
9. Ried K, Frank OR,Stocks NP. Aged garlic extract reduces blood pressure in hypertensives: a doseresponse trial. Eur J Clin Nutr. 2013;67(1):64-70.
10. Oluwole FS. Effects of garlic on some hematological and biochemical parameters. Afr J Biomed Res.2001;4 (3):139-41.
11. Caro AA, Adlong LW, Crocker SJ, Gardner MW, Luikart EF, Gron LU. Effect of garlic-derived organosulfur compounds on mitochondrial function and integrity in isolated mouse livermitochondria. Toxicol Lett. 2012;214(2):166-74.
12. Premkumar LS. Transient Receptor Potential Channels as Targets for Phytochemicals. ACS Chem Neurosci. 2014;5(11):1117-30.
13. Ried K, Fakler P. Potential of garlic (Allium sativum) in lowering high blood pressure:

Mechanisms of action and clinical relevance. Integr Blood Press Control. 2014;7:71-82.
14. Tang Z, Sheng Z, Liu S, Jian X, Suin K, Yan M. Preventing function of garlic on experimental oral pre-cancer and its effect on natural killer cells. Bulletin of Human Medical University.1997;22:312-46.
15. Sumiyoshi H. New pharmacological activities of garlic and its constituents (Review). Folia Pharmacological Japonica.1997;110(1):93-7.
16. Warshafsky S, Russel SK, Steven LS. Effect of garlic on total serum cholesterol. Annals Intern Med.1993;119:599-605.
17. Mader SH. Treatment of hyperlipidemia with garlic powder tablets. Arneim Forsch 1990; 40:1111-6.
18. Lau BH, Adetumbi SMA, Sachez A. Allium sativum (Garlic) and atherosclerosis. A review. Nutr Res.1983;3:119-28.
19. Stevinson C, Gridley DS, Fittler E. Garlic for treating hypercholesterolemia; a mental analysis of randomized clinical trial. Annals Intern Med.2000;133:420-9.