Original Article

ASE on Lipoproteins and Blood Indices

Effects of Allium Sativum Extract (ASE) on Blood Lipoproteins and Blood Indices in Wistar Albino Rat Model

Kashif Rasheed Shaikh[1], Alina Saqib[2] and Umair Ali Soomro[3]

ABSTRACT

Objective: To study the effects of Allium sativum extract (ASE) on blood lipoproteins and blood indices in Wistar albino rat model.

Study design: Experimental study

Place and Duration of Study: This study was conducted at the Animal House, Isra University Hyderabad from September 2014 to June 2015.

Materials and Methods: 80 albino rats were divided into 4 groups; Group 1- Controls (Placebo 0.9% isotonic saline), Group 2- ASE 100 mg/kg, Group 3- ASE 200 mg/kg and Group 4- ASE 300 mg/kg were given orally for 30 days. Blood sample was collected by cardiac puncture. Statistical analysis was performed on SPSS 22.0 by one way ANOVA and post Hoc Duncan test at 95% confidence interval.

Results: Triglycerides, total cholesterol, LDLc and HDLc showed statistically significant differences among groups (p =0.0001). High dose fed ASE showed significant reductions in TAG, TC and LDLc and a rise in HDLc. Also the blood indices showed improvement in ASE treated rats (p=0.001).

Conclusion: The Allium sativum extract reduces triglycerides, total cholesterol and low density lipoproteins and increases high density lipoprotein (HDLc). Blood indices were also improved in high dose rats.

Key Words: Allium sativum, Blood lipoproteins, Blood indices, Rats

Citation of article: Shaikh KR, Saqib A, Soomro UA. Effects of Allium Sativum Extract (ASE) on Blood Lipoproteins and Blood Indices in Wistar Albino Rat Model. Med Forum 2016;27(7):34-37.

INTRODUCTION

Allium sativum is a popular spice herb used in food cooking. It is commonly known as Garlic. Its use in herbal medicine has been reported for a number of disorders ranging from the infections to cardiac disorders. It is used for both prevention and treatment of diseases.[1-5] A previous study reported anti-microbial activity of Allium sativum extract (ASE).[2] ASE has shown promising results as an antibiotic antioxidant, anti hyperglycemic agent, anti inflammatory, anti cancer and anti atherosclerosis agent. It use for cardiovascular disease is confidently reported.[3,4] ASE is reported to reduce the blood total cholesterol and also the liver cholesterol.[4,5] A review of published literature shows the ASE supplements reduces the blood lipoproteins and has anti hyperlipidemic effect.[5-7]

Allium sativum is reported to protect against cardiovascular disease by lowering Triglycerides (TAG), total cholesterol (TC), Low density lipoprotein-cholesterol (LDLc) and increasing high density lipoprotein-cholesterol (HDLc). A reduction in bad cholesterol (LDLc) and a rise in good cholesterol (HDLc) is of clinical importance as it prevents the atherosclerosis, coronary artery disease (CAD) and other vascular disorders (CVD).[8,9] The effect of Allium sativum extract (ASE) on the plasma lipoproteins shows diverging views of different researchers as regards a reduction in CAD. Recently published research has shown ASE protects against CAD by reducing blood cholesterol in human beings.[10-12] Sufficient scientific evidence is available from research in developed countries, while the research from developing countries like Pakistan is lacking.[11,13] Previous studies had reported anti-hypertensive effects of ASE.[13-14] ASE has been used for a variety of ailments since centuries back.[15-16]

Sang et al[17] concluded that the ASE oil prevented against the fatty liver in a previous study. ASE active ingredient is known as the "allicin". Allicin is biochemically a diallyl-disulfide-oxide compound reported to cause vasodilation.[18] Another previous study reported a reduction in diastolic blood pressure (DBP) in uncontrolled hypertension subjects.[19] Inhibition of human platelet aggregation has been reported in an in-vitro study using Allium sativum ether extract.[20]

1. Department of Pharmacology, Muhammad Medical College, Mirpurkhas, Sindh
2. Department of Anatomy, Indus Medical College, Tando Muhammad Khan, Sindh
3. Department of Pathology, Isra University, Hyderabad, Sindh

Correspondence: Dr Kashif Rasheed Shaikh,
Assistant Professor, Department of Pharmacology
Muhammad Medical College, Mirpurkhas, Sindh
Contact No: 0333-7103324
Email: drkashifshaikh@hotmail.com

Received: April 23, 2016; Accepted: May 30, 2016

Allium sativum extract enhances the immune cell activity. A previous study reported it increase the NK (natural killer) and T cell activity. An increase in interleukin-2 (IL-2) has also been noted.[21] Immune stimulating effects of ASE have been reported in both in-vitro and in-vivo studies.[22]

Various studies have been conducted to evaluate different biological effects of ASE in animal models, but none has ever evaluated the effects on blood lipoproteins and blood parameters with raw Allium sativum extract. As the cardiovascular disorders are on rise due to increasing diabetes mellitus, sedentary life style, stress, etc, it is worth to search into common remedies which should be easily available and inexpensive. The present study reports on beneficial effects of Allium sativum extract (ASE) on blood lipoproteins and blood indices in experimental rat model.

MATERIALS AND METHODS

The present experimental study took place at the animal house of Faculty of Medicine and Allied Medical Sciences (FMAMS) Isra University. Duration was from September 2014 to June 2015. 80 albino rats of Wistar strain were selected according to criteria of inclusion and exclusion. Rats of 200- 280 grams of either gender were enrolled for the study purpose. Rats of weight more or less, sick rats, and rats not feeding well were excluded form study protocol. Animal housing was in accordance to NIH guidelines. Room temperature, 55-60 % humidity and 12/12 dark light cycles were ensured. Ventilation, fresh water availability and chow were made available on priority basis.

Controls and experimental rats were divided into four groups;

- **Group 1.** Control Group (n=20) Placebo (0.9% isotonic saline) given orally.
- **Group 2. Experimental Group** (n=20) Allium sativum extract (ASE) orally- 100 mg/5ml/kg b.w.
- **Group 3. Experimental Group** (n=20) Allium sativum extract (ASE) orally- 200 mg/5ml/kg b.w.
- **Group 4. Experimental Group** (n=20) Allium sativum extract (ASE) orally- 300 mg/5ml/kg b.w.
- **Allium sativum extract (ASE) preparation**

Fresh Allium sativum was purchased. They were dissolved in pure water. Three containers were marked for preparation of ASE at quantity of 100mg, 200mg and 300mg by calculation, so that the final concentration was 100, 200 and 300 mg/5ml. Experimental rats were given ASE at dose of 100, 200 and 300 mg/5ml/kg b.w. ASE was given for 30 days duration.

- **Animal euthanasia and Experimental protocol**

12 hour fasting animals were given GA (general anesthesia) and were sacrificed by cervical dislocation (CD) after thirty days. Blood sampling was performed by cardiac puncture (24G B.D Disposable syringe). Blood was taken into heparinized test tubes. Blood was centrifuged and stored at 4°C. Sera were separated by centrifugation at 300xs for 10 minutes. Samples were stored in deep refrigerators if assays were performed late.

- **Complete blood counts (CBC)**

CBC was performed on automated Hemato-analyzers (Sysmex KX 21).

- **Blood lipoprotein estimation**

TAG, TC and HDLc were estimated enzymatically using assay kits (Asia Pharmaceuticals, Seoul) and an enzyme-linked immuno-sorbent assay reader (Pharmacia-Biotech, Cambridge, UK). Friedewald's formula was used for the estimation of LDLc.

- **Data analysis**

Statistical analysis was performed on SPSS 22.0. Numerical data variables were compared by one-way ANOVA. Post Hoc Duncan test was used for difference between groups. Data was analyzed at 95% CI (P-value ≤ 0.05) of significance.

RESULTS

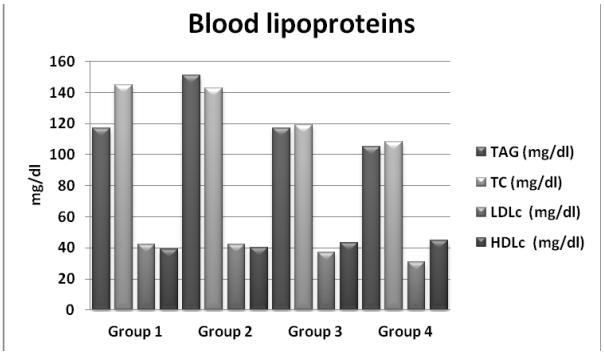
The experimental study evaluated the blood lipoproteins and blood indices in experimental albino rat model. Triglycerides (TAG), total cholesterol (TC), LDLc and HDLc showed statistically significant differences among controls and experimental groups (p =0.0001) as shown in table 1. Significant reductions in TAG, TC and LDLc were noted in high dose Allium sativum treated group.

Table No. 1: Blood lipoproteins and blood indices in different animal groups

	Group 1	Group 2	Group 3	Group 4
TAG (mg/dl)	117.92	151.5	117.8	105.18
Total cholesterol (TC) (mg/dl)	145	143.6	119.78	108.6
LDLc (mg/dl)	42.8	42.1	37.5	31.98
HDLc (mg/dl)	39.4	40.4	43.5	45.75
Hemoglobin (g/dl)	14.2	14.1	14.9	15.1
Packed cell volume (%)	42.7	42.9	44.1	46.1
Red blood cells ($x10^3/\mu L$)	3.89	3.99	4.39	4.93
White blood cells (/µL)	9711	7350	8871	9321
Neutrophils (%)	61	65	67	81
Lymphocytes (%)	39	31.1	33.2	28.41
Monocytes (%)	2.12	2.34	2.56	3.11
Basophils (/µL)	0.3	0.21	0.12	0.11
Eosinophils (%)	1.0	1.1	1.14	1.27
Basophils (/µL)	0.3	0.21	0.12	0.11
Blood Platelets ($x10^3/\mu L$)	4.25	4.23	4.19	5.18

Good cholesterol- the HDLc was elevated in Allium sativum treated animals compared to controls. Similarly, improvement was noted in the hemoglobin,

packed cell volume, red blood cell counts, white blood cell counts and white blood differential cell counts ($p \leq 0.02$) as shown in table 1. Bar graph 1 shows the differences of various blood lipoproteins in controls and experimental rats.



Graph No.1: Blood lipoproteins and blood indices in animal groups

DISCUSSION

The present experimental study evaluated the lipoproteins and blood indices in Wistar albino rat model. To the best of knowledge, it is the first being reported on the effects of ASE on the blood lipoproteins and blood indices. In the present study TAG, TC, LDLc, and HDLc showed statistically significant differences between controls and experimental groups ($p = 0.0001$). High dose fed ASE showed significant reductions in TAG, TC and LDLc and a rise in HDLc. Good cholesterol (HDLc) is a finding of clinical importance for the patients. As the present study is a pre-clinical experimental it needs confirmation in clinical trials. However, it is noted that the ASE oil is already being prescribed in clinical practice. The need is to evaluate the underlying mechanisms and active ingredient of Allium sativum. It is the first study which reported on the effects of ASE on blood indices. Hemoglobin, PCV and RBC were increased in Allium sativum treated rats. Similarly the WBC and WBC-differential cell counts ($p \leq 0.02$) revealed statistically significant differences. Highly significant reductions of lipoproteins, an increase in HDLc (good cholesterol) and increase in blood indices were prominent at high of ASE. The reduction in lipoprotein levels are consistent with previous studies[21,22] Previous studies had reported immune boosting effects of ASE in rat studies with a improve NK cells, T-cells and IL-2.[20,22] The findings support the present study as the blood indices were significantly improved in high dose ASE treated rats.

Previous studies[11,12] had reported raw garlic extract reduced blood total cholesterol in subjects with hypercholesterolemia. The findings are in keeping with the present study findings. Previous research[23-25] had reported that the ASE might inhibit the HMG-CoA reductase enzyme which is main regulatory enzyme of cholesterol biosynthesis in liver.

Blood bad cholesterol (LDLc) was reduced and good cholesterol (HDLc) are the worth findings of the present study. ASE has blood lipoprotein modulating effects has been proved in the present study and are supported by previous studies.[11-14] This may be clinically important for inhibiting the initiation and progression of atheroma plaque formation in patients. How the ASE reduces bad cholesterol and increases good cholesterol, it is before time to propose any mechanisms, but the most probable mechanism lies within the liver. TAGs were also reduced by ASE in present study which is in keeping with previous studies.[22,23] Previous studies[22,23] proposed that the ASE probably stimulates the hormone sensitive lipase and mobilizes blood triglycerides. However, it is not more than just a speculation. Evidence based results of present study and available reports of previous literature[24,25] shows the ASE is of potential use for hyperlipidemia and improving blood indices.

CONCLUSION

The Allium sativum extract reduces triglycerides, total cholesterol and low density lipoproteins (LDLc) and increases high density lipoprotein (HDLc). Good cholesterol (HDLc) was increased in Allium sativum treated animals. Similarly, an improvement was noted in the hemoglobin, packed cell volume, red blood cell counts, white blood cell counts and white blood differential cell counts. Further studies are recommended.

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Siddiqui NA, Asadullah M. Primary hyperlipidemic patients; differences in lipid profile with original product of simvastatin and herbal product of allium sativum. Professional Med J 2016; 23(5):564-570.
2. Kavita Y, Lal MK, Urmila C, Meghshyam S. Garlic (Allium sativum): A Natural Remedy for Metabolic Syndrome. Sch Acad J Biosci 2016; 4(3A):199-202.
3. Lachhiramka P, Patil S. Cholesterol lowering property of garlic (Allium sativum) on patients with hypercholesterolemia. Int J Med Sci Public Health 2015; 5:1-3.
4. Zeng T, Guo FF, Zhang CL, Song FY, Zhao XL, Xie KQ. A meta-analysis of randomized, double-blind, placebo controlled trials for the effects of garlic on serum lipid profile. J Sci food Agri 2012; 92(9):1892-1902.
5. 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.
6. Tekeli A, Kutlu HR, Celik L, Var I, Yurdakul E, Avcı A. The use of Propolis as an alternative to antibiotic growth promoters in broiler diets. Proceedings of 23rd World's Poultry Congress Brisbane, Australia 2008;482-3.
7. Reid K, Toben C, Fakler P. Effects of garlic on serum lipids: an updated meta-analysis. Nutr Rev 2013;71(5):282-99.
8. Majewski M. Allium sativum: facts and myths regarding human health. Natl Inst Pub Health 2014;65(1):1–8.
9. 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.
10. Kim MJ, Kim HK. Effect of garlic on high fat induced obesity. Acta Biologica Hungarica 2011; 62(3):244–54.
11. Ebrahimi T, Behdad B, Abbasi MA, Rabati RG, Fayyaz AF, Behnod V, et al.. High doses of garlic extract significantly attenuated the ratio of serum LDL to HDL level in rat-fed with hyper-cholesterolemia diet Diagnostic Pathol 2015; 10:74.
12. Ogunlesi OO, Oladele OA, Aina OO, Esan OO. Effects of dietary garlic (Allium sativum) meal on skin thickness and fat deposition in commercial broiler chickens. Bulgarian J Vet Med 2016;1:1-8.
13. Douaouya L, Bouzerna N. Effects of garlic (Allium sativum) on biochemical parameters and histopathology of Pancreas of Alloxan induced diateic rats. Int J Pharm Pharm Sci 2016; 8 (6):202-206.
14. Balasenthil S, Arivazhagan S, Nagini S. Garlic enhances circulatory antioxidants during 7, 12-dimethylbenz anthracene-induced hamster buccal pouch carcinogenesis. J Ethnopharmacol 2000; 72: 429–33.
15. Oluwole FS. Effects of garlic on some hematological and biochemical parameters. Afr J Biomed Res 2001; 4 (3): 139-41.
16. Alan DK, Nossaman BD, Ibrahim IN, Feng CJ, Mc-Namara DB, Agarwal KC, et al. Analysis of responses of allicin, a compound from garlic, in the pulmonary vascular bed of the cat and in the rat. Euro J Pharmacol 1995; 276:21-6.
17. Sang GK, Nam SY, Chung HC, Hongand SY, Jung KH. Enhanced effectiveness of dimethyl-4,41[1]-dimethoxy–5,6,5[1],6[1],-dimethylene dioxybiphenyl –2,2[1]–dicarboxylate in combination with garlic oil against experimental hepatic injury in rats and mice. J Pharm Pharmacol 1995; 47: 678-82.
18. McMahon FG, Vargas R. Can garlic lower blood pressure? A pilot study. Pharmacotheraphy 1993; 13(4):406.
19. Apitz-Castro R, Cabrera S, Cruz MR, Ledezma E, Jain MK. Effects of garlic extracts and of these pure components isolated from it on human platelet aggregation, arachidonate metabolism, release reaction and platelet ultrastructure. Thromb Res 1993; 32:155.
20. 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 Med Univ 1997; 22:312-46.
21. Sumiyoshi H. New pharmacological activities of garlic and its constituents (Review). Folia Pharmacological Japonica 1997;110(1):93-7.
22. Warshafsky S, Russel SK, Steven LS. Effect of garlic on total serum cholesterol. Annals Intern Med 1993;119:599-605.
23. Mader SH. Treatment of hyperlipidemia with garlic powder tablets. Arneim Forsch 1990; 40:1111-6.
24. Lau BH, Adetumbi SMA, Sachez A. Allium sativum (Garlic) and atherosclerosis. A review. Nutr Res 1983;3:119- 28
25. Stevinson C, Gridley DS, Fittler E. Garlic for treating hypercholesterolemia; a mental analysis of randomized clinical trial. Annals Intern Med 2000; 133: 420-29.