ORIGINAL ARTICLE

EFFECT OF *ALLIUM SATIVUM* ON EXPERIMENTALLY INDUCED HYPERLIPIDEMIA IN GUINEA PIGS

Prema Ram Choudhary, Jaidev Singh Shekhawat*, Megh Shyam Sharma, Jyotsna Dashora*****

Department of Physiology, *Anatomy, CU Shah Medical College, Surendranagar, Gujarat, **Department of Physiology, RNT Medical College, Udaipur, Rajasthan, ***Department of Physiology, MMIMSR, Mullana Ambala, Haryana, India

Background: Garlic (*Allium sativum*) is naturally occurring sulphur containing dietary agent belongs to family liliaceace. Members of this family like garlic and onions are found to have beneficial effect on atherosclerosis and ischemic heart diseases in experimental animals and humans. The objective of this study was to find out the effect of *Allium sativum* on experimentally induced hyperlipidemia in guinea pigs. **Methods:** Twenty-five guinea pigs were fed cholesterol (0.5 g/Kg body weight/day) for an initial period of 4 weeks. Cholesterol was then discontinued and the animals were divided into 3 groups. Group-I (control, n=7) was now fed normal diet with 1 ml normal saline. Group-II (n=9) was given 1 ml of aqueous extract with normal diet, and Group-III (n=9) was fed normal diet with 1 ml of alcoholic extract of garlic daily for 4 weeks. The garlic contents of both extracts were 2 g/ml. Fasting blood samples were collected at the end of 4 weeks after induced hyperlipidemia and finally at the end of the study (i.e., 8 weeks) for estimation of total serum cholesterol, serum triglyceride, HDLc, LDLc, VLDLc and atherogenic index was calculated in all 3 groups. **Results:** The aqueous and alcoholic extracts of garlic showed a significant hypolipidemic activity as they reduced significantly serum cholesterol, serum triglyceride, LDLc, VLDLc and atheriogenic index in hyperlipidemic guinea pigs ($p<0.001$) as compared to control group. The significant rise in HDLc was observed in group II but not in group I and III animals. On comparison between the two extracts, aqueous extract of garlic was found to be more potent hypolipidemic agent than to the alcoholic extract. **Conclusion:** Both extracts have hypolipidemic activity but aqueous extract of *Allium sativum* is more potent than alcoholic extract.

Keywords: *Allium sativum*, alcoholic extract, aqueous extract, lipid profile, hyperlipidemia

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INTRODUCTION

Allium Sativum (Lat.), (Eng: Garlic, Urdu: '*Lahsan*') is widely distributed in all parts of the world and used not only as spice but also as a popular remedy for prevention and treatment of a variety of diseases like rheumatism, dermatitis, abdominal disorders and diabetes mellitus. Effect of garlic in cardiovascular diseases was more encouraging in experimental studies, which prompted several clinical trials. Dietary factors play a key role in the development of various human diseases, including cardiovascular disease. Garlic has attracted particular attention of modern medicine because of its widespread health use around the world, and the cherished belief that it helps in maintaining good health warding off illnesses and providing more vigor.

To date, many favorable experimental and clinical effects of garlic preparations, including garlic extract, have been reported. These biological responses have been largely attributed to reduction of risk factors for cardiovascular diseases, cancer and stimulation of immune functions, enhanced detoxification of foreign compound, hepatoprotective, antimicrobial effect and antioxidant effect.[1] Garlic is reported to prevent cardiovascular disease by multiple effects, one of which is the decrease total cholesterol and triglycerides[2], LDLc, VLDLc, while increase HDLc[3] and suppression of the cholesterol biosynthesis[4]. Studies prior to 1995 consistently concluded hypolipidemic action of garlic.[5,6] However, studies after 1995 using enteric-coated preparation of raw garlic did not manifest any hypolipidemic effect.[7–9] These paradoxical observation warrant a systemic study to resolve the controversy. The present study was undertaken to examine the effect and relative potency of *Allium sativum* preparations (aqueous and alcoholic extracts) on induced hyperlipidemia in guinea pigs.

MATERIAL AND METHODS

Twenty-five, healthy guinea pigs of either sex, 2–4 month old, obtained from the animal house of the Dr. SN Medical College, Jodhpur (Rajasthan), India, weighing 460–582 g. The animals were housed in standard environmental conditions. Animals were fed standard pelleted feed (manufactured by Lipton Ltd. India) containing 17% protein, 11% fat, 47% carbohydrate, 2.5% minerals, 4.5% fibre, 11.5% water, and green vegetables, and given filtered water in bowl *ad libitum*. All the animals were taken care of and maintained as per guidelines of the CPCSEA.

All 25 guinea pigs were fed cholesterol (Sigma, USA) (0.5 g/Kg body weight/day) orally, in 5 ml of milk, for an initial period of 4 weeks. Cholesterol was then discontinued after inducing hyperlipidemia and animals were randomly allocated into three groups. Group-I (control, n=7) was fed normal standard diet with 1 ml of normal saline for four weeks. Group-II (n=9) and Group-III (n=9) were also fed standard diet with 1 ml of emulsified aqueous and alcoholic extract of garlic respectively for 4 weeks. The garlic content was 2 gm garlic/ml in each extract.

The garlic extract was prepared by crushing about 200 gm garlic in grinder and was kept in distilled water (for aqueous extract) and in ethyl alcohol (for alcoholic extract) in beaker at room temperature, packed in muslin cloth bags and transferred to soxhlet extractor for completion of 6 cycles. Then the solution was filtered and evaporated, to obtained 100 ml of extract.

At the end of 4 weeks after induced hyperlipidemia and end of study (i.e., 8 weeks), after supplementation of garlic extracts for 4 weeks, fasting blood samples of all groups were collected from the right ventricle of heart after anaesthetising the animal with pentobarbitone (35 mg/Kg/IP).

The blood samples were kept at room temperature for separation of serum, used for estimation of serum total cholesterol (CHOD/POD method), triglyceride (GPO method), LDLc, HDLc (modified PGEME method), VLDLc and atherogenic index.[10,11] All parameters were estimated by using semi-autoanalyser (Transasia, ERBA Chem-5 Plus, Transasia Bio-Medicals Ltd.), and diagnostic kits by the same manufacturer. The results were analysed statistically applying Student's *t*-test.

RESULTS

In hyperlipidemic guinea pigs cholesterol level was 114±20 mg/dl. A significant decrease was observed in total serum cholesterol levels in all groups compared to hyperlipidemic guinea pigs ($p<0.001$), after lapse of four weeks. Similar results were also obtained in the levels of triacylglycerol ($p<0.001$) and LDLc ($p<0.001$) and VLDLc ($p<0.001$) when group I, II & III were compared with hyperlipidemic guinea pigs.

In hyperlipidemic guinea pigs HDLc level was 11±1. There was a non-significant difference ($p>0.05$) observed when group I was compared to hyperlipidemic guinea pigs and group III compared to group I where as significant increase ($p<0.001$) when group II was compared with hyperlipidemic guinea pigs.

Atherogenic index was also calculated to find out the decreased risk of atherosclerosis by diet with supplementation of garlic preparations from LDLc/ HDLc ratio and TC/HDLc ratio ($p<0.001$). Results indicate that LDLc/HDLc ratios were significantly decreases in all three groups as compared to hyperlipidemic guinea pigs. Similar trend was also observed in TC/HDLc ratio ($p<0.001$).

There were significant decreases ($p<0.001$) in the total serum cholesterol, triacylglycerol, and LDLc, VLDLc, and atherogenic index when group II was compared with group III. On comparison between aqueous and alcoholic extracts of garlic, aqueous extract was found more potent compared to alcoholic extract as it showed very significant fall ($p<0.001$) in serum cholesterol, triglycerides, LDLc, and VLDLc levels compared to group I and group III, and decreased atherogenic index by lowering LDLc and VLDLc and rising HDLc. (Table-1 and 2).

Table-1: Effect of garlic extracts on lipid profile of hyperlipidemic guinea pigs at the end of the study (Mean±SD)

Lipid profile	HL-Group (n=25)	Group-I (n=7)	*p**	Group-II (n=9)	*p***	Group-III (n=9)	*p****
TC (mg/dl)	114±20	94±8	<0.05	48±10	<0.001	71±4	<0.001
TG (mg/dl)	102±8	91±5	<0.01	56±6	<0.001	75±5	<0.001
LDL_{-C} (mg/dl)	83±18	64±7	<0.01	24±10	<0.001	45±3	<0.001
HDL_{-C}(mg/dl)	11±1	11±0.98	>0.05	13±1	<0.01	11±1	>0.05
$VLDL_{-C}$ (mg/dl)	20±1	18±1	<0.001	11±1	<0.001	15±1	<0.001
LDL/HDL	10±1.4	8.3±1	<0.01	3.74±0.75	<0.001	6.3±0.65	<0.001
TC/HDL	7.4±1.39	5.7±0.91	<0.01	1.8±0.76	<0.001	4.0±0.52	<0.001

*When Group-I compared to hyperlipidemic group, **when Group-II compared to Group-I, ***when Group-III compared to Group-I

Table-2: Comparison of lipid profile in Group-II and Group-III at the end of the study (Mean±SD)

Lipid profile	Aqueous garlic extract	Alcoholic garlic extract	*p*
TC (mg/dl)	48±10	71±4	<0.001
TG (mg/dl)	56±6	75±5	<0.001
LDL_{-C} (mg/dl)	24±10	45±3	<0.001
HDL_{-C}(mg/dl)	13±1	11±1	<0.001
$VLDL_{-C}$ (mg/dl)	11±1	15±1	<0.001
LDL/HDL	3.74±0.75	6.3±0.65	<0.001
TC/HDL	1.8±0.76	4.0±0.52	<0.001

DISCUSSION

There are many herbs which are used as hypolipidemic agents. Garlic is one these herbs that has protective and curative effect against the increase in serum cholesterol and triacyl glycerol (induced by dietary fat), by decreasing them and increasing the HDLc, fibrinolytic activity and clotting time in patient with myocardial infarction and coronary artery disease.[12,13] Garlic has been reported to minimize the adverse effect of

hyperlipidemia.[13] Bordia *et al*[14] have shown reversibility of cholesterol induced experimental atherosclerosis in rabbits by garlic. Garlic has sulphur-containing compound allin, which is converted to an active ingredient 'allicin' when garlic bulb is crushed. This compound has inhibitory effect upon the key enzymes involved in cholesterol biosynthesis, such as HMG CoA reductase.[15] Hypocholesterolemic effect of garlic is exerted by decreasing hepatic cholestrol biosynthesis, whereas the triglyceride lowering effect appears to be due to the inhibition of fatty acid synthesis by mallic enzymes, fatty acid synthase and glucose-6-phosphate dehydrogenase.[16] Among water soluble compounds s-allylcysteine, s-ethylcysteine and s-propylcysteine reduces cholesterol synthesis by 40–60%. Lipid soluble sulphur compounds like diallylsulphide, diallyldisulphide diallyltrisulphide, dipropylsulphide and di-propyldisulphide inhibits cholesterol synthesis by 10–15%.[16]

In the present study, there was significant decrease in total cholesterol, triglycerides, LDLc, VLDLc, and atherogenic index in the three groups at the end of study but more significant decreases in all lipid profile parameters, except HDLc, in the Group-II compared to control group-I and group-III. Our results also indicate that garlic has hypolipidemic activity and decreases the atherogenic index, hence protects from the cardiovascular disorders. The changes in lipid profile observed in the present study are in accordance with the previous studies.[1,5,6] reporting that garlic extract or garlic oil significantly reduce total cholesterol, triglyceride, LDLc, VLDLc, and significantly increase HDLc.

CONCLUSION

Both aqueous and alcoholic extracts of *Allium sativum* have hypolipidemic activity but aqueous extract is more potent than alcoholic extract and it rapidly decreases the bad cholesterol in blood and increases the good cholesterol, hence decreases the risk of atherosclerosis. It is recommended that garlic or aqueous extract of garlic should be encouraged in food being the cheapest way to reduce hyperlipidemia and prevent coronary heart disease.

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Address for Correspondence:

Prema Ram Choudhary, Assistant Professor, Department of Physiology, CU Shah Medical College, Dudhrej Road, Surendranagar, Gujarat, India. **Tel:** +91-9558258818

Email: prema5252@gmail.com