Article

Beneficial Effect Associated with Use of Watery and Alcoholic Extract of Garlic as a Supplement in Hyperlipidemic Guniea Pigs

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Abstract

Background: Garlic is naturally occurring sulphur containing dietary agent belong to *Allium* family. Members of this family i.e. Garlic, Onions are found to have beneficial effect on atherosclerosis and ischemic heart diseases in both experimental animals and in human beings. **Objective:** To compare the potency of garlic as antihyperlipidemic and antiplatelet and antithrombotic between its watery and alcoholic extract for their effects. **Methods:** 25 guinea pigs were fed cholesterol (0.5g/kg body weight) for an initial period of 4 weeks. Cholesterol was then discontinued and the animals were divided into 3 groups. Group-I (n=7) was fed with stock diet and was taken as control. Group-II (n=9) was given 1ml of watery extract and Group-III (n=9) was fed with 1ml of alcoholic extract of garlic with normal diet daily for 4 weeks. The garlic content of both the extract was 2 gm/ml by wt/vol. Fasting blood samples were collected at the end 4 weeks and finally at the end of the study i.e.8 weeks for estimation of serum cholesterol, serum triglyceride, LDC-C, HDL-C, VLDL-C,atherogenic index and platelet adhesiveness index. Bleeding time and clotting time were also detected in all the 3 groups.**Results:** In the present study, the watery and alcoholic extract of garlic shows significant hypolipidemic activity as they reduced significantly serum cholesterol, serum triglyceride, LDC-C, VLDL-C and atheriogenic index in hyperlipidemic guinea pigs ($p<0.01$) compared to control. The significant rise in HDL-C was observed in group II but not in group III animals. On comparison between two extracts, watery extract of garlic was more potent hypolipidemic agent, compared to alcoholic extract. Both extract significantly reduces platelet adhesiveness and bleeding time. But between two extract the watery extract was found more potent anti-thrombotic agent as it showed significant decrease in platelet adhesiveness ($P<0.01$). BT was significantly increased in group II compared to group I and III. **Conclusion:** It can be concluded that watery extract of *Allium Sativum* is more potent hypolipidemic and antiplatelet, and anti-thrombotic agent.

Key Words-Allium Sativum, Hyperlipidemia, platelet adhesiveness index, watery and alcoholic extracts.

J Bangladesh Soc Physiol. 2011 June; 6(1): 22-26
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http://www.banglajol.info/index.php/JBSP

Introduction

Garlic, Latin name: *Allium sativum, family*: liliaceace, is a unique plant, with a long history of its use as both food and medicine and highly valued as both. Garlic, known as 'Lahsun', is known for both its culinary and curative properties. Garlic and its preparations have been widely recognized as agents for prevention and treatment of cardiovascular and other metabolic diseases, including atherosclerosis, hyperlipidemia,

thrombosis, hypertension and diabetes. 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 and cancer, stimulation of immune function, enhanced detoxification of foreign compound, hepatoprotection, antimicrobial effect and antioxidant effect.[1]

Increased platelet aggregation plays a significant role in the aetiology of cardiovascular disease. Patient with coronary artery disease have been reported with defective fibrinolytic activity[2], increased serum cholesterol[3], decreased clotting time[4] and increased platelet aggregation.[3] Garlic is reported to prevent cardiovascular disease by multiple effects, one of which is the inhibition of platelet aggregation. This ability has been extensively investigated in vitro, however, in vivo studies are limited.[5] Short term as well as long term intake of garlic oil and raw garlic have been associated with increased fibrinolytic activity (FA). In 1975, Bordia first demonstrated that garlic oil increased FA after 3 hours of administration.[6] But the relative potency of its watery extract versus alcoholic extract has not been investigated.

Therefore, this study was under taken to discover the relative potency of these two extracts with respect to their anti-hyperlipidemic and anti-thrombotic effect.

Methods

This experimental study was conducted in 25 healthy guinea pigs of either sex with mean (±SD) body weight 510± 4.2gms with a duration of 8 weeks. These animals were randomly divided into three groups .Group-I (n=7), Group-II (n=9), Group-III (n=9). In all three groups experimental hyperlipidemia was induced by feeding cholesterol (500mg/kg body weight per day) orally, in 5ml of milk, for 4 weeks.

After inducing hyperlipidemia the Group-I was kept on normal standard diet and fed with 1 ml of normal saline for four weeks. Group-II and Group-III were also fed with standard normal diet supplemented with 1ml of emulsified watery and alcoholic extract of garlic respectively for 4 weeks.

The garlic content for both the extract was 2gm garlic/ml. The garlic extract was prepared by crushing about 200gm garlic in grinder and was kept in distilled water (for watery extract) and in ethyl alcohol (for alcoholic extract) in beaker at room temperature, packed in muslin cloth bags for overnight covered with Petridis. Then the solution was filtered and evaporated, to obtained 100ml of extract.

At the end of study , after supplementation of garlic extract for 4 weeks , fasting blood samples of all groups were collected carefully from the right ventricle of heart after anesthetizing the animal with pentobarbitone (35mg/kg/I.P).

These blood samples were then divided in two parts. One part is kept for serum separation which is used for estimation of serum cholesterol, triglyceride, LDL-C, HDL-C, VLDL-C and atherogenic index[7-10]. While the other portion of sample was poured in EDTA plastic vials which is used for the estimation of platelet count and for calculating the platelet adhesiveness index by Hellem's method[11]. Clotting time was assessed as per Wright's method and bleeding time by filter paper method by pricking the ear lobes[12]. All the animals received commercial diet (bran and fresh vegetables) and water adlib for

the total period of study. The results were analyzed statistically by ANOVA and Newman-Keul's procedure.

Results

Table I Illustrate the effect watery and alcoholic extract of garlic (*Allium sativum*) on hyperlipidemic guinea pigs. On comparison with group-I both watery and alcoholic extract of garlic produces significant ($p<0.05$) reduction in the serum cholesterol, triglyceride and LDL-C and VLDL-C. No Significant difference in HDL levels was observed in both types of extract of garlic. On comparison between watery and alcoholic extract of garlic, watery extract was more potent as it showed very significant fall in serum cholesterol, triglycerides, LDL and VLDL($p<0.01$) levels compared to group I and group III.

This result show's that *Allium sativum's* watery extract is much more effective in reducing serum lipids as compared to alcoholic extract.

Effect of two types of garlic extract on platelet adhesiveness index, clotting time and bleeding time is illustrated in Table II. In comparison to group I and group III watery extract of garlic (group II), showed significant decrease in platelet adhesiveness ($P<0.01$) while bleeding time was increased ($P<0.01$). The alcoholic extract did not produce significant changes in these parameters. Clotting time changes were found statistically nonsignificant ($P>0.05$)

Table I: Effect of Watery & Alcoholic extract of allium sativum on lipid profile of Hyperlipidemic guinea pigs(n=25)

Lipid profile parameters	Group-I normal saline feeding(n=7)	GroupII Watery extract feeding(n=9)	GroupIII alcoholic extract feeding(n=9)	ANOVA P value
Cholesterol mg/dl	94.49 ±8.71	48.57** ±10.75	71.77* ±4.11	p<0.01
Triglyceride mg/dl	91.84 ±5.86	56.10** ±6.13	75.12*±5.75	p<0.01
LDL-C mg/dl	64.70 ±7.70	24.35** ±10.33	45.30 * ±3.16	p<0.01
HDL-C mg/dl	11.43 ±0.98	13.00** ±1.22	11.34 ±1.33	p>0.01
VLDL-C mg/dl	18.37 ±1.17	11.22** ±1.23	15.05 * ±1.15	p<0.01
Atherogenic Index #	5.70±0.91	1.88±0.76	4.00± 0.52	p<0.001

Comparison between different groups is done by Newman-Keul's procedure
*P<.05 as compared to group I
**P<.05 as compared to group III and group I
Atherogenic Index= LDL-C/HDL-C ratio

Table II: Effect of watery & alcoholic extract of allium sativum on platelet adhessiveness, bleeding time and clotting time

Parameters	Group I normal saline feeding(n=7)	Group II watery extract feeding(n=9)	Group III alcoholic extract feeding(n=9)	ANOVAP value
Platelet Adhessiveness index (%)	25.33 ± 2.05	14.52 ± 0.902*	24.17 ± 2.56	P<0 .01
Bleeding time (in Seconds)	29.28 ± 4.5	37 ± 4.92**	34.44 ± 3.9	P<0.01
Clotting time (in Seconds)	41.04 ± 5.56	48.88 ± 4.86	43.88 ± 4.17	P>0.05

Comparison between different groups is done by Newman-Keul's procedure
*P<0.05 as compared to group I and group III
**P<0.05 as compared to group I

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 TG (induced by dietary fat), by decreasing them and increasing the HDL-C, fibrinolytic activity and clotting time[15] in patient with myocardial infarction and coronary artery disease[13-15]. Garlic has been reported to minimize the adverse effect of hyperlipidemia[16]. Bordia and verma as well as Jain have shown reversibility of cholesterol induced experimental atherosclerosis in rabbits by garlic[17-18]. Bordia, in his study found that low dose of garlic led to inhibition of platelet aggregation[19]. Sainani et al. has shown higher level of cholesterol, triglycerides, fibrinogen and shorter clotting time in Jain community who doesn't eat garlic[15].

Chutani and Bordia in their study showed that raw garlic and fried garlic significantly increased the fibrinolytic activity[20]. Jain in his study showed that there was increase in bleeding and clotting time in rabbits and patient with coronary artery disease, myocardial infarction after supplementation of garlic extract[21].

Garlic contains 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.[22] 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[23].

Garlic extracts and several garlic constituents have been reported to cause significant anti-platelet and antithrombotic actions both in vitro and in vivo. Anti-platelet constituents of garlic are reported as allicin, adenosine, and ajoene [24-25]. Ajoene a secondary degradation product of allicin, is the most active compound responsible for the antithrombotic activity[26]. Ajoene inhibits thromboxone synthesis through the inhibition of cyclooxygenese and lipoxy genase enzymes[24].

Yen et al. shows this inhibitory potency of individual water soluble and lipid soluble compound of garlic[23]. Among water soluble compounds s-allylcysteine, s-ethylcysteine and s-propylcysteine reduces cholesterol synthesis by 40-60%. Lipid soluble sulphur compounds like diallylsulphide, diallyldisulphide diallyl-trisulphide, dipropylsulphide and di-propyl disulphide inhibits cholesterol synthesis by 10-15%.

Conclusion

The present study concluded that watery extract of garlic is more potent hypolipidemic and antithrombotic agent as compared to alcoholic extract which was shown possessing less potent hypolipidemic activity.

In view of this study it can be recommended that watery extract of garlic should be encouraged for reduction or prevention of hyperlipidemia and thrombo-embolic phenomenon.

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