The Study of Antihyperlipidemic Effect of Allium Sativum in Rats Induced With Hyperlipidemia Using Fat Rich Diet.

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Abstract

The atherosclerosis is characterized by elevated levels of cholesterol. The underlined cause of most of the coronary cardiovascular diseases is elevated lipid level also known as hyperlipidemia. There are some synthetic antihyperlipidemc drugs are available in market but the exhibit numerous of adverse effect which are undesired. Currently, lots of efforts have been made for development of herbal formulation which exhibit lesser side effect compared to synthetic drugs. The objective of this work is to evaluate the phytoconstituent profile and antihyperlipidemic activity of allium sativum on rat fed with fat rich diet. The phytochemical analysis was carried out by performing the various test established in literature. The activity was evaluated by determining triglycerides, cholesterol and HDL-cholesterol of rats treated with standard and test substance. The phytochemical screening of garlic extract revealed the presence of saponin, terpenoid, flavonoids, volatile oil, amino acid and protein and cardiac glycosides. The extract is found to exhibit antihyperlipidemic activity at dose of 200 mg/kg. The significant drop in levels of total cholesterol triglycrides and HDL-cholesterol was observed. Thus this work demonstrated that the extract of Allium sativum has antihyperlipidemic effect on rat fed with fat rich diet.

Keywords: Allium sativum, Antihyperlipidemic, phytoconstituents, hypolipidemic

Introduction

Hyperlipidemia is primarily characterized by elevation plasma levels of the different lipids and lipoprotein, which is a major risk factor for heart attack, coronary artery syndrome, stroke, atherosclerosis, myocardial infarction and pancreatitis[1,2]. These lipids include cholesterol, cholesterol esters, phospholipids, and triglycerides. Hyperlipidemia is associated with elevation of any or all lipid profile or lipoproteins in the blood[3]. Lipids are circulated in blood as large 'lipoproteins' and have been reported as the most common reason of death in developed as well as developing countries.

There are many synthetic hypolipidemic drugs like statins are available in the market, but they associated with various adverse effects like hyperuricemia, diarrhoea, myositis, hepatotoxicity, etc.[1] The herbal agents exhibit lesser side effects and they have significant compatibility leads improving patients tolerance for long term use. Natural products originated from herbs are a vital source of medicine used for centuries to treat different disorders[4]. The much importance is given to investigation of agents exhibiting hypolipidemic activity from plant sources[5]. Thus we have used garlic (allium sativum) for this work.

Allium sativum is a member of the Lillaceae family, along with onions, chives, and shallots[6]. It is known to exhibit various effects that can be attributed to decreased risk factors for heart diseases, decrease in risk of cancer risk, antioxidant activity, antimicrobial activity[7,8]. Raw garlic buds, dried powder or aquous extracts can be used for variety of applications. Allicin (allyl 2-propenethiosulfinate or diallyl thiosulfinate) is the major chemical constituent present in the aqueous extract of garlic or raw garlic. During chopping and crushing, activation of allinase enzyme take place which leads to produce allicin from alliin. It also contains 1 -propenyl allyl thiosulfonate, allyl methyl thiosulfonate, ajoene and glutamyl-S-alkyl- L-cysteine2. Garlic is known to reduce blood pressure[9,10], antioxidant[11], inhibition of platelet aggregation[12,13] and reduced blood glucose[10]. The antioxidant activity[14,15] anticancer activity[16,17] antimicrobial activity[18] is also reported in garlic.

In this work, we have evaluated anti-hyperlipidemic activity of aqueous extract from raw buds of allium sativum against fat rich diet induced hyperlipidemia in rats.

Materials and Methods

Plant materials

The raw buds of Allium sativum was collected from local market of Malkapur, Maharashtra, India in the month of December, 2019. The plant material was authenticated by department of Pharmacognosy, DRG College of Pharmacy, Malkapur.

The peel of buds was removed, and crushed using mortor and pestle. The crushed buds of Allium sativum were used for the preparation of extract.

Chemicals

All the analytical grade chemicals used in this study were obtained from Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), Empire Industries (Ankleshwar, India), ACS chemicals (Ahmedabad, India) , Alpha Chemica (Mumbai, India). Cholesterol and cholic acid was obtained from Pat impex (Vadodara). All the chemicals used in this study were of analytical grade.

Preparation of extract

About 20 g of raw buds of Allium sativum were weight and their peel was removed. The buds are then crushed using mortor and pestle. The 100 ml of sterile distilled water was added in it. This mixture was stirred on water bath shaker at 40-50 °C for about 12 to 14 hours. This mixture was then filtered through filter paper (Whatman No 1). The filtrate was used for preliminary analysis of phytoconstituents.

Phytochemical Screening of Extract

The extract of Allium sativum was subjected to preliminary screening for determination of phytoconstituents. The alkaloids and flavonoids were estimated by using the method described by Harborne (1973) and Edeoga et al. (2005)[19].The estimation of saponins and tannins were done using the method described by Farnsworth (1966) [19].Glycosides were estimated by using the method described by Haborne (1973). Similarly, steroids, terpenoids were estimated using the Siddiqui and Ali (1997)[20]. Carbohydrate were estimated using Benetic's test, phenol by Ferric chloride test, protein were estimated by Lowry's method (Siddiqui and Ali, 1997)[20]

Animals

All the animal experiment were performed in DRG College of Pharmacy, Laboratory of Pharmacology approved by the Institutional Animal Ethics Committee (IAEC), by Ministry of Environment and Forests, Government of India, New Delhi, India. The albino male rats (Wistar albino rats) weighing about 170-260 g were used for the study. They were group (n= 6) under a standard 12 h light/dark cycle under constant conditions of temperature and humidity (25±2 °C, 50-60%). Rats were received with standard rodent chow and water ad libitum. Rats were habituated to environmental lab conditions for 7 days prior to the experiments. For every set of experiment, different group of rats (n=6) were used.

Evaluation of cute toxicity

The acute oral toxicity was carried out according to the guidelines of Organization for Economic Co-operation and Development (OECD) [21]. Animals were kept on fast by oral administration of water, and extract Allium sativum (50, 100, 150, 200, 300 mg/kg/day) was for 4 days of six groups of rats. In order to evaluate antihyperlipidemic effect, animals were observed for any kind of behavioral changes or mortality.

Conditioning of hyperlipidemia in rats

The hyperlipidemia was induced in rats by feeding fat rich diet for 15 days. The major contents of fat rich are dalda (25%), cholesterol (3%), cholic acid (1%), and coconut oil (6%). The level of serum lipoproteins and lipids were measured and noted to confirm hyperlipidemia.

Design of Experiment

Group I: Normal (vehicle alone)

Group II: Hyperlipidemic rats treated with vehicle alone

Group III: Rats with Hyperlipidemia treated with aqueous extract of A. sativum (100mg/kg, p.o.) Group IV: Rats with Hyperlipidemia treated with llium. sativum (100mg/kg, p.o.) (200mg/kg, p.o.) Group V: Rats with Hyperlipidemia treated with orlistat (control) (60 mg/kg/day p.o.)

The rats were treated with the test extracts for 14 days. The rats were kept on fast on 15th day and using mild ether anesthesia, the blood sample was collected by retro orbital sinus puncture. The centrifugation of sample was done for 10 minutes at 2000 rpm. The serum samples are collected and used for various biochemical investigations. The content of serum triglycerides, total cholesterol and HDL-cholesterol were determined by using commercial kits. [22, 23]

Statistical analysis was carried out by using one way ANOVA and Dunnett's test. Significance value is accepted at $p \leq 0.05$. The data is represented as mean ± standard deviation.

Results and Discussions

The phytochemical screening of garlic extract revealed the presence of saponins, terpenoids, flavonoids, volatile oils, amino acids and proteins and cardiac glycosides.

Phytochemical screening of aqueous extract of Allium sativum was performed as shown in table 1.

High concentration of the compound (+++), Moderate concentration of the compound (++), Low concentration of the compound (+), Absence of the compound (-)

Table 1. Phytochemical screening of aqueous extract of Allium sativu

Sr. No	Inference	Result
1.	**Saponin:** Foam will remain for 10 mints, presence of saponin	+++
2.	**Tannin:** Blue or green colour indicates presence of tannin	–
3.	**Phenol:** Dark blue colour indicates presence of phenol	–
4.	**Alkaloids:** Orange indicates presence of alkaloids	–
5.	**Terpenoid:** Brown colour ring formation at the junction of two liquid indicates the presences of terpenoid	++
6.	**Flavonoids:** Intense yellow colour appears Then yellow colour disappears.	+
7.	**Amino acid and protein:** Violet colour indicates presence of protein.	+++
8.	**Carbohydrates:** Red precipitate indicates the presence of carbohydrate	–
9.	**Volatile oil:** White precipitate indicates presence of volatile oil.	+++
10.	**Glycosides:** Brick red precipitate indicates presence of glycosides	–
11.	**Hydrolysable tannin:** Formation of emulsion precipitate indicates the presence of hydrolysable tannin	–
12.	**Cardiac glycosides:** Formation of three layer of different color indicates the presence. Upper layer: Green color Middle layer: Brown color Lower layer: Violet.	+++

The antihyperlipidemic activity of the aqueous extract Allium sativum in hyperlipidemia induced rats is given in table 2 and figure 1. For normal and experimental animals, the levels of serum total cholesterol (TC), triglycerides (TG) and serum high density lipoprotein (HDL) were determined. The activity levels of serum total cholesterol (TC) and triglycerides (TG) were found to be elevated in group II animals as compared to that of normal groups. The serum level of serum high density lipoproteins (HDL) were depleted in the rat fed with fat rich diet. The activity levels of serum total cholesterol (TC) and triglycerides (TG) were significantly decreased in group III, IV and V animals compared to that of normal groups. The HDL level was also found to be increased in the group III, IV and V.

Table 2. The antihyperlipidemic activity of Allium sativum in hyperlipidemiemia induced rats

Group	Drug	Dose	Total Body Weight (g)	
			Onset of experiment	Post experiment
I	Normal 1	Normal saline sol	170.10 ± 7.01	190 ± 7.25
II	Control 1	Fat rich diet	180.05 ± 7.50	256 ± 7.65
III	*Allium sativum*	100 mg/kg p.o	205.01 ± 7.40	199 ± 8.12
IV	*Allium sativum*	200 mg/kg p.o	198.05 ± 8.12	190 ± 7.23
V	Orlistat	60 mg/kg p.o	200.25 ± 7.51	180 ± 7.54

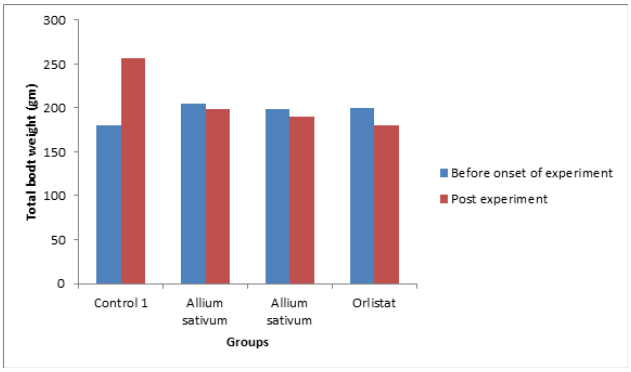

Figure 1. Antihyperlipidemic effect of extract of Allium sativum on body weight in hyperlipidemia induced rat

Table 3. Effect of Allium sativum on total cholesterol, triglycerides and high density lipoproteins levels (mg/dL) in hyperlipidemia induced rat

Treatment	Dose	Total cholesterol (mg/dL)	Triglyce rides (mg/dL)	High density lipoproteins (mg/dL)
Normal 1	Normal saline	**82.00 ± 5.00**	**84.00 ± 4.50**	**38.00 ± 4.00**
Control 1	Fat rich diet	**145.10 ± 5.00**	**138.12 ± 5.00**	**28.00 ± 4.61**
Allium sativum	100 mg/kg p.o	**102.22 ± 5.65**	**99 ± 4.12**	**31.42 ± 4.56**
Allium sativum	200 mg/kg p.o	**97.15 ± 5.18**	**86.12 ± 4.54**	**32.87 ± 4.67**
Orlistat	60 mg/kg p.o	**84.44 ± 5.64**	**85.20 ± 4.74**	**35.10 ± 4.22**

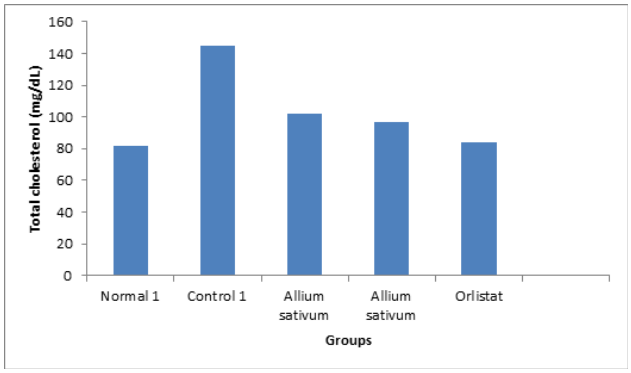

Figure 2. Effect of Allium sativum on total cholesterol levels (mg/dL) in hyperlipidemia induced rat

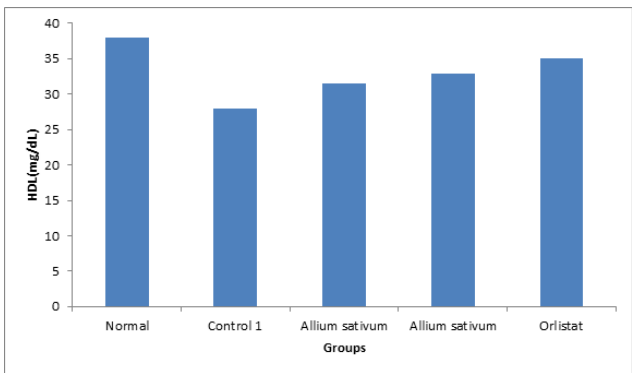

Figure 3. Effect of Allium sativum on high density lipoproteins levels (mg/dL) in hyperlipidemia induced rat

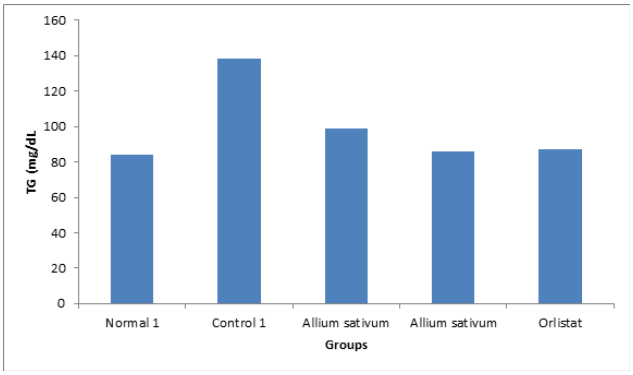

Figure 4. Effect of Allium sativum on triglycerides (mg/dL) in hyperlipidemia induced rat

Allium sativum is a traditional medicinal agent having various biological activities and pharmacological function including lowering serum lipid level. In the present work, rats were fed with diets rich in fats such as cholesterol causes elevation in the level of triglycerides and cholesterol. From this study, it is found that oral administration aqueous extract of Allium sativum helps in reducing the total cholesterol levels in plasma after 15 days of administration.

Conclusion

Based on above results we can conclude that the aqueous extract of Allium sativum exhibited a significant hypolipidemic activity. There was increase in weight and mesenteric fat of rats fed with fat rich diet. The decrease in the elevated levels of the fat was observed in the groups of animals treated with aqueous extract of Allium sativum. This effect can be utilized for lowering the serum lipid content in order to reduce risk of atherosclerosis and other coronary cardiovascular disorders.

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