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RESEARCH ARTICLE

ANTIHYPERLIPIDAEMIC ACTIVITY OF ALLIUM SATIVUMLEAVES METHANOLIC EXTRACTS OF TRITON WR -1339 INDUCED HYPERLIPIDAEMIC RATS

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ABSTRACT

Hyperlipidaemia is the greatest risk factor of coronary heart disease. Currently available hypolipidemic drugs have been associated with number of side effects. Herbal treatment for hyperlipidaemia has no side effects and is relatively cheap and locally available.Literature claims that Saponins are able to reduce hyperlipidemia. Based on high content of saponin in herbal plants, Garlic (*Allium sativum L.*) was selected and focus on the anti-hyperlipidemic activity of ethanolic seed extract of *Allium sativum L*against triton-WR1339 induced hyperlipidemia in rats. Hyperlipidemia was induced in Wistar rats by Intraperitoneal (I.P) injections of Triton WR-1339 at a dose of 400 mg/kg body weight. *Allium sativum L* was administered orally at a dose of 200 mg/kg to triton WR-1339 induced hyperlipidaemic rats. After administration of *Allium sativum L* shows a significant decrease in the levels of cholesterol, phospholipids, triglycerides, LDL, VLDL and significant increase in the level of HDL in serum and liver tissues against triton induced hyperlipidaemic in rats. Therefore it effectively suppressed the triton induced hyperlipidemia in rats, suggesting the potential protective role in Coronary heart disease.

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INTRODUCTION

Hyperlipidemia mainly increased level of cholesterol or low-density lipoprotein cholesterol (LDL-C) contributes significantly to the manifestation and development of atherosclerosis and coronary heart diseases (CHDs). Cardiovascular diseases, including atherosclerosis, are the most common causes of mortality and morbidity worldwide[1]. Approximately 12 million people die of cardiovascular disease each year worldwide. Although several factors such as diet high in saturated fats and cholesterol, age, family history, hypertension, and lifestyle play a significant role in causing heart failure, the high level of cholesterol, particularly LDL-C is mainly responsible for the onset of coronary heart diseases [2]. The lowering of lipids and cholesterol levels by drug or dietary interventions could reduce the risk of coronary heart diseases. The known lipid-lowering drugs (fibrates, statins, bile acid sequestrants, etc.) regulate the lipid metabolism by different mechanisms, but they also have many side effects[3]. Therefore, the development of lipid lowering drugs from natural sources is the best option and is in great demand. Medicinal plants continue to provide valuable therapeutic agents, both in modern medicine and in traditional systems. Plants and many plant derived preparations have longbeen used as traditional remedies and in folklore medicine for the treatment of hyperlipidemia in many parts of the world.

There are many plants and their products that have been reputedly and repeatedly used in Indian traditional system of medicine**.** Recently, the searches for appropriate antihyperlipidemic agents have been again focused on plants because of less toxicity, easy availability and easy absorption in the body that may be better treatment than currently used drugs[4].Plants that were once considered of no value are now being investigated, evaluated and developed in to drugs with no side effects. One of such plant is, *Allium sativum L,* commonly known as vellulli button weed' belongs to the family Liliaceaeand is widely distributed throughout the world as a useful medicinal plant[5]. The seeds of plants as confection are cooling demulcent and given in diarrhea and dysentery. Seeds have been recommended as a substitute for coffee. Bulbs are crushed in to paste and taken orally to treat stomach problems[6]. According to some studies, *Allium sativum L* has also anti-hypertensive activity[7]. The plant has been extensively studied for its phytochemical composition and a large number of active ingredients such as, Allicin, Alliin, Diallyl di sulfide, Allyl methyl sulfide, Allixin, Ajoene. Recently, pharmacological studies have shown that, *Allium sativum L* leaves exhibit antidiabetic properties in rats[8]. Hence, in the present study, the ethanolic extract of, *Allium sativum L* leaves was investigated for Antihyperlipidemic activity in triton - WR 1339 induced hyperlipidaemic rats.

MATERIALS AND METHODS

Collection and authentication of plant material and extraction: The leaves of *Allium sativum L* were collected from Vangapally. The plant was identified by the voucher specimen was deposited at the Herbarium of Botany department, SLNS Degree college of Arts and Science, Yadadri Bhongir, Telangana, India. The dried leaves were made into fine powder with an auto-mix blender and were kept separately in an airtight container until use. The powder was exhaustively extracted with methanol in the ratio of 1:5 for 24hr by using soxhlet apparatus. The extract was completely evaporated to dryness using rotary flash. This extract was dissolved in 5% carboxy methyl cellulose (CMC) solution and used in the study.

Test for carbohydrates

Molisch's test: The test is positive with soluble, as well as, insoluble carbohydrates. It consists of treating the compounds with alpha napthol and concentrated sulphuric acid which gives purple colour ring at the junctions of two layer.

Reduction of Fehling's solution: To the extract, equal quantity of Fehling's solutions A and B is added. After heating, brick red precipitate is obtained.

Test for amino acids

Million's Test: To the extract add 2 ml of million's reagent, white precipitate indicates presents of amino acid.

Ninhydrin Test: To the extract add ninhydrin solution, boil, violet colour indicates presence of amino acid.

Test for Alkaloid: The qualitative chemical tests used for detection of alkaloids are dependent on the characters of alkaloids to give precipitates as salts of organic acids or with compounds of heavy metals, likemercury, gold, platinum, etc. The different reagents used are Mayer's reagent (potassium mercuric iodide solution) giving cream coloured precipitate. Dragendorff's reagent (potassium bismuth iodide solution) giving reddish brown precipitate. Wagner's reagent (iodine-potassium iodide solution) yielding reddish brown precipitate. Some alkaloids also give yellow colouredprecipitates with picric acid called as Hanger's reagent and picrolonic acid. Individual alkaloid gives colour or precipitate with certain specific reagent.

Dragendorff's Test: To the extract, add few drops of dragendorff's reagent. Orange brown precipitate is formed.

Mayer's Test: To the extract, add few drops of Mayer's reagent give cream colour precipitate.

Hager's Test: To the extract, add few drops of Hager's reagent give yellow precipitate.

Wagner's Test: To the extract, add few drops of Wagner's reagent give reddish brown precipitate.

Test for Glycosides

Keller kiliani Test: The test consists of extract with 10 ml 70% alcohol for 2 to 3 minutes. The extract is filtered to the filtrate is added, 5 ml water and 0.5 ml strong solution of lead acetate. Shake well and separate the filtrate. The clear filtrate is treated with equal volume of chloroform and evaporated to yield the extractive. The extractive is dissolved in glacial acetic acid and after cooling, 2 drops ferric chloride solution is added to it. These contents are transfer to a test tube containing 2 ml concentrated sulphuric acid. A reddish brown layer acquiring bluish-green colour after standing is observed.

Legal Test: The extract is dissolved in pyridine, sodium nitro prusside solution is added to it and made alkaline-pink or red colour is produced.

Baljet Test: To the extract add sodium picrate solution is added. It shows yellow to orange colour.

Test for cardiac Glycosides

Kedde's Test: To the extract add chloroform, evaporate to dryness. Add 1 drop of 90% alcohol and 2 drops of 2% 3.5 Di nitro benzoic acid in 90% alcohol. Make alkaline with20% sodium hydroxide solution, purple colour is produced. The colour reaction with 3, 5 Di nitro benzoic acid depends on the presence of alpha, beta unsaturated lactones in the aglycone.

Keller-kiliani Test (Test for deoxy sugars): Add chloroform to the extract and evaporate it to dryness. Add 0.4 ml of glacial acetic acid containing trace amount of ferric chloride. Transfer to a small test tube; add carefully 0.5 ml of concentrated sulphuric acid by the side of the test tube. Acetic acid layer shows blue colour.

Raymonds Test: Treat the extract with hot methanolic alkali, violet colour is produced.

Legal's Test: Treat the extract with pyridine and alkaline sodium nitro prusside solution, blood red colour appears.

Baljet Test: Treat the extract with picric acid or sodium picrate, orange colour is formed.

Test for Tannins

Ferric chloride Test: A small amount of extract treat with ferric chloride solution, blue colour appears if hydrolysable tannins are present and green colour appears if condensed tannins are present.

Phenazone Test: To the 5 ml of extract add 0.5 gram of sodium acid phosphate then warm it and filter. To the filtrate add 2% Phenazone solution, precipitate is formed which is often bulky coloured.

Gelatin Test: To the extract add 1% gelatin solution containing 10% sodium chloride. Precipitate is formed.

Test for Saponins

Foam Test: Take 2 ml of extract with methanol in a test tube. To it add small amount of water, shake well, stable froth (foam) is formed.

Haemolysis Test: Add 0.2 ml of extract (prepared in 1% normal saline) to 0.2 ml of blood in normal saline and mix well. Centrifuge and note the red supernatant compare with control tube containing 0.2 ml of 10% blood in normal saline diluted with 0.2 ml of normal saline.

Animals: Eight week old adult male albino rats of Wisterstrain, weighing approximately 150 to 200 g, were acclimatized for 7 days at room temperature (22±2ºC) and humidity of 45-64% in a 12-hour light/dark cycle in a room under hygienic condition[9]. They were given access to water and a commercial diet *ad libitum*. The experiments were carried out in the Department of Pharmacology, Swami Vivekananda Institute of Pharmaceutical Sciences, Vangapally, as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India, and approved by the Institutional Animal Ethical Committee (IAEC).

Chemicals: Triton WR-1339 (A non-ionic detergent, Isooctyl polyoxymethylene phenol) was obtained from Sigma Chemicals Co,Hyderabad.

Triton WR-1339 Induced of Hyperlipidemia: Administration of triton WR-1339 to normal rats in the dose of 200 mg/kg caused significant increase in lipid profile such as TG,TC ($p<0.001$) as compared to normal control rats. pretreatment of aqueous and methanolic extracts in the dose of 100mg/kg b.w and 200mg/kg b.w p.o significantly modified the increased level of TG TC ($p<0.001$). Treatment with standard drug (atorvastatin 0.4 mg/kg) significantly decreased the level of TG, TC ($p<0.001$).pretreatment of aqueous (AQ)100 and 200 mg/kg b.w methanolic extract (MeOH) 100 and 200 mg/kg b.w decreased the level of TG,TC,($p<0.001$) which was increased doe to triton administration, where as pretreatment with petroleum ether(PEMA),chloroform(CHLMA, ethyl acetate (EAMA) extracts did not modify the triton induced hyperlipidaemia. however pre-treatment with *Allium Sativum* l extracts have shown significant increase in HDL compared to triton induced hyperlipidaemic group.

High fat diet Induced of Hyperlipidaemia: Chronic administration of HFD to normal animals causes significant rise ($p<0.001$) in the level of TG,TC as compared to normal control rats, whereas lower levels of HDL compared to NPD-fed rats.TC/HDL and LDL/HDL ratios were significantly elevated. simultaneous administration of aqueous and methanolic extracts in the dose of 100 mg/kg b.w and 200mg/kg b.w p.o showed significantly decreased the TG, TC ($p<0.001$)as compared to HFD treated rats (groups0.treatment with reference drugs (atovastratin 0.4mg/kg)also decreased the level of TG,TC ($p<0.001$)as compared to HFD treatment group .moreover atovastratin also significantly increased HDL level. Animals fed with HFD, showed significant increase in body weight as compared to normal (NPD) treated groups. Chronic administration of aqueous and alcoholic extracts of MA at the dose of 100 and 200 mg/kg significantly reduced the increased body weight due to HFD administration compared to HFD fed control group**.**

Histopathological observations:

- In normal control group showing normal architecture; with no inflammation, no fibrosis, no fatty changes and necrosis.
- Hyperlipidaemic group showing; altered architecture. Hepatocytes focally show fatty vacuoles, focal lymphocytic infiltration around bile ductless. Nucleus is pushed by fatty vacuoles to one side with no fibrosis, inflammation or necrosis. This feature favors fatty changes in the liver with a fatty filtration and granular degeneration.
- There was no alterations found in the liver histology for the group treated with standard drug atovastratin showing negligible cytoplasmic fatty filtration and granular degeneration
- Group treated with *Allium sativum* aqueous extracts showing mild cytoplasmic fatty infiltration and mild granular degeneration.
- Group treated with *Allium sativum* methanolic extracts showing mild cytoplasmic fatty infiltration and mild granular degeneration

Fig. 1. Histological changes in liver tissue in (N) rat

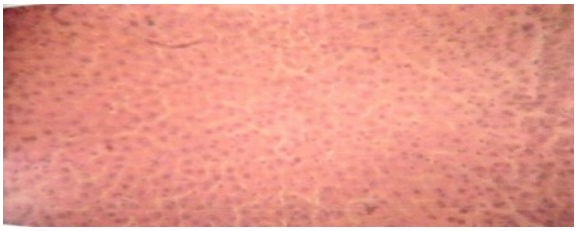

Fig. 2. Histological changes in liver tissue in triton induced rat

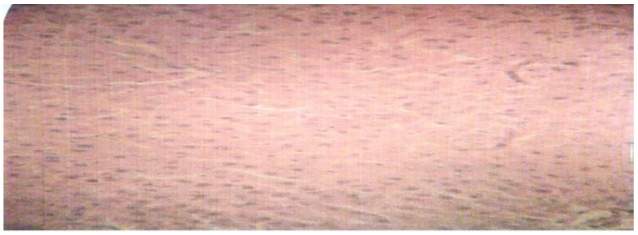

Fig.3. Histological changes in liver tissue in (N) rat

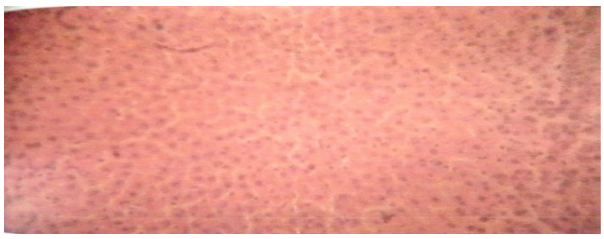

Fig.4. Histological changes in liver tissue in A S A Q rat

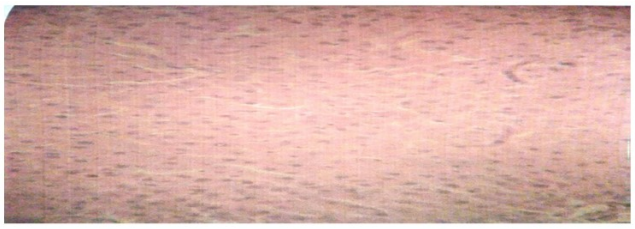

Fig. 5. Histological changes in liver tissue in A S C E rat

Fig. 6. Histological changes in liver tissue in A S E E rat

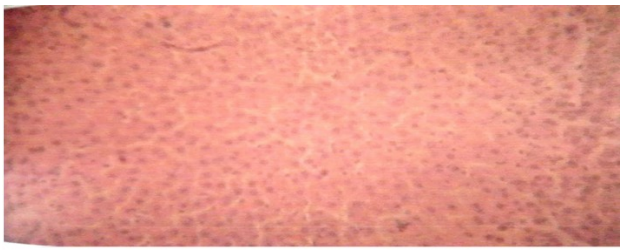

Fig. 7. Histological changes in liver tissue in Standardrat.

Table 1. Preliminary phytochemical screening of *Allium sativum L*

Ingredients	ACE
Alkaloids	+
Amino Acids	-
Flavonoids	+
Glycosides	+
Gums and Mucilage	-
Proteins	-
Reducing Sugars	+
Starch	+
Steroids	-
Tannins	+
Terpenoids	-

(+Presence, -Absence)

Statistical Analysis: Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) using SPSS software package 9.05. Results were expressed as mean ± SD from 8 rats in each group. *P* values <0.05were considered as significant.

RESULTS

The results were discussed under the following headings. Lipid profile in serum and liver tissues. Table 2 shows the levels of cholesterol and phospholipids were found to be significantly increased in serum and liver tissues of triton induced rats when compared to control rats. These levels were found to be significantly reduced in hyperlipidaemic rats treated with methanolic leaves extract of *Allium sativum L*.

In triton induced rats the levels of triglycerides, LDL and VLDL were significantly higher in serum and liver tissue when compared to those in control rats, while the HDL levels were significantly decreased when compared to control rats. After the treatment with ethanolic leaves extract of *Allium sativum L* at the doses 200 mg/kg in the Triton induced rats, a significant reduction in LDL, VLDL and significant increase in HDL were observed when compared to control rats were shown in the Table 2. Table: 2.antihyperlipidaemic activity of methanolic extracts ofAllium sativum leaves on triton wr -1339 induced Hyperlipidaemia in rats

DISCUSSION

Hyperlipidaemia is associated with heart disease, which is the leading cause of death in the world.

Table 2.

Group	T C	TG	HDL	LDL	VLDL
Control	74.66±4.50	84.66±6.77	35.16±2.8	23.66±5.5	15±1.41
H C	174.83±2.31	165.83±8.03	28±3.46	105.166±7.1	31.33±1.63
A S M E	154±6.5**	117.83±5.5**	30.5±2.8	96.5±5.16	23.33±1.63
A S A Q	143.5±2.42**	114.16±8.10**	34.5±3.08	88±4.51	23.16±2.31
A S C E	136.5±3.44**	112±5.5**	35.16±2.85	79.16±4.70	23.5±1.04
A S E E	98.1±5.15***	88.16±7.96***	40.33±4.17	48.33±6.28	24±1.41
Standard	90.83±2.8	89.5±4.08***	42.16±3.48	45.66±6.80	15.16±2.31

***P<0.0001. **P<0.001

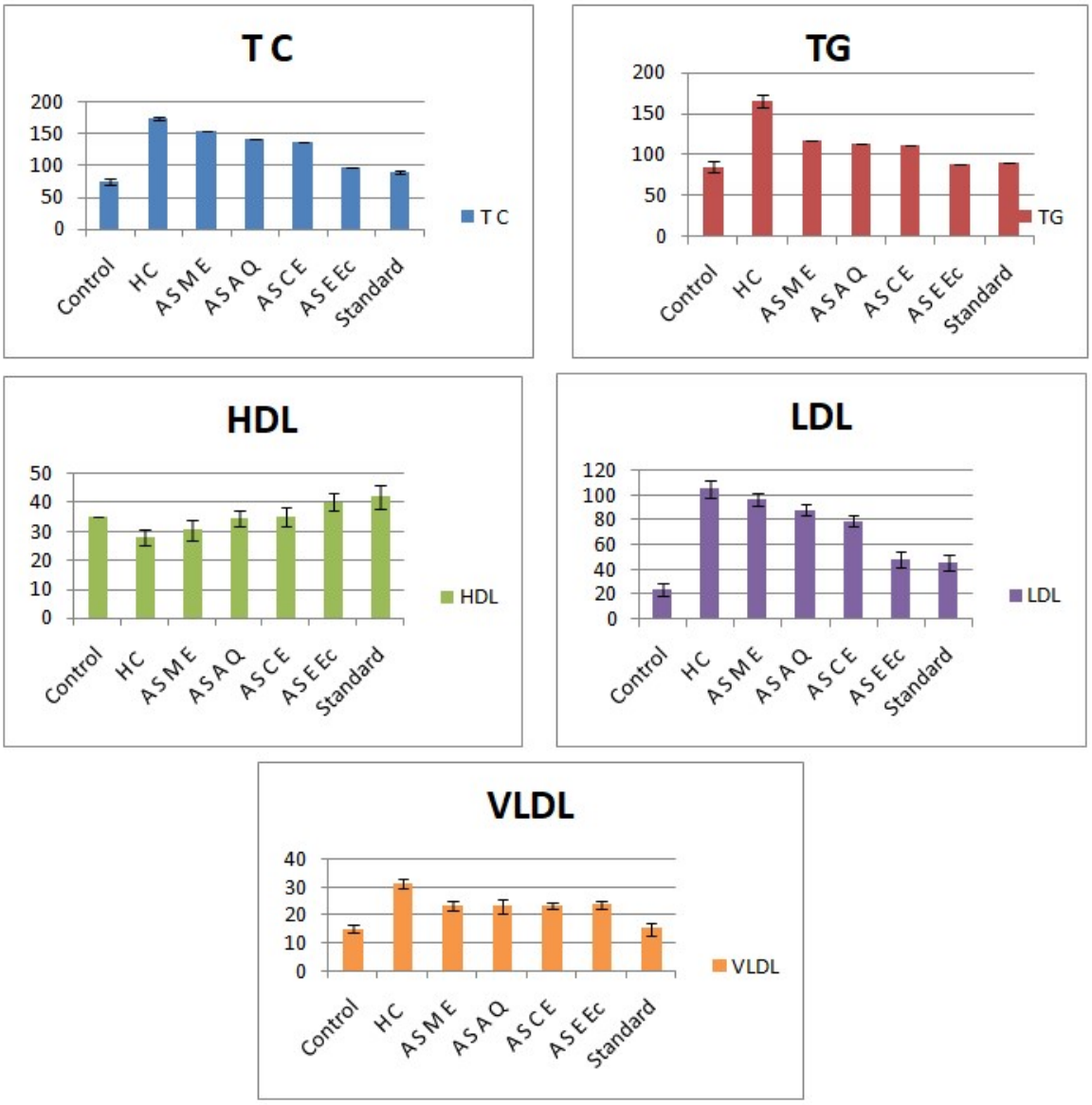

Fig. 8. Antihyperlipidemic activity of methanolic extracts of*Allium sativum* leaves on triton wr -1339 induced Hyperlipidaemia in rats (HDL, LDL, VLDL, TC, TG)

The lowering of the levels of harmful lipids to satisfactory values has been confirmed byseveral experimental animal and interventional studies indicating lowered morbidity and mortality in coronary heart diseases. Several studies reveal that an increase in HDL cholesterol and decrease in cholesterol, LDL cholesterol and triglycerides is associated with a decrease in the risk of ischemic heart diseases[10]. Triton WR-1339 has been widely used to block clearance of triglyceride rich lipoproteins to induce acute hyperlipidemia in several animals[11].The present investigation shows that all triton induced rats displayed hyperlipidemia as shown by their elevated levels of serum and liver cholesterol, triglycerides, phospholipids and the reduction in the HDL level. Most of the antihyperlipidemic drugs are causing significant reduction in both total cholesterol and increase in HDL cholesterol levels[12]. This model is widely used for a number of different aims[13].particularly, in rats it has been used for screening natural or chemical hypolipidemic drugs[14]. Infact, flavonoids, steroids, saponins and anthocyanin, a heterogeneous group of ubiquitous plant polyphenols have exhibited a variety of pharmacological activities including the anti atherogenesis.

The plant steroids reduce the absorption of cholesterol and thus increase fecal excretion of cholesterol[15]. Lupeol, a triterpenoid was reported for its cardioprotective activity against experimental hyper cholesterolemia[15]. Interestingly, the results of the present study show that ethanolic extract of *Allium sativum L* leaves reduces the level of cholesterol, triglycerides, phospholipids and increase the level of HDL, which may probably be due to the presence of steroids, flavonoids and triterpinoids[16.]Demonstrated that a parenteral administration of a dose of TritonWR-1339 to adult rats induced hyperlipidaemia. The large increase in cholesterol and triglycerides due to Triton WR-1339 injection results mostly from an increase of VLDL secretion by the liver accompanied by a strong reduction of VLDL and LDL catabolism[17]. The reduction of total cholesterol by the *Allium sativum L* extract was associated with a decrease of its LDL fraction, which is the target of several hypolipidemic drugs. This result suggests that cholesterol lowering activity of the herb extract can be result from the rapid catabolism of LDL cholesterol through its hepatic receptors for final elimination in the form of bile acids as demonstrated[18]. The *Allium sativum* markedly lowers the levels of serum and liver tissue of VLDL and LDL due to the presence of large number of active ingredients in seeds of plant such as Borreline, Ursolic acid and Isorhmnatin. In conclusion, the present study demonstrated that the ethanolic extract of *Allium sativum* leaves exhibited maximum efficacy in lowering the elevated lipid levels.

CONCLUSION

The values of AI in hyperlipidemic and garlic treated groups were showed in figure 1. The ratio was significantly increased in Triton induced hyperlipidemic rats compared with normal group and these elevated ratios were returned to near normal levels in groups of rats treated with methanolic extracts of garlic and atorvastatin. The rise in AI in hyperlipidemic rats enhances the probability of cardiovascular pathogenesis and endothelial dysfunction. A significant decrease in AI value was observed in herbal supplemented animals, suggests the atheroprotective / cardio protective potential of this herb. In conclusion, all the fractions obtained from the methanolic extracts of Allium sativum significantly reduced the Triton-X-100 induced hyperlipidemia in rats.

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