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Research Article

# Antidyslipidemic, Antiatherogenic and Antioxidant Activity of Allium Sativum in Charles Foster Rats.

Shrivastava A., Chaturvedi U., Sonkar R., Saxena J. K., Khanna A. K., Bhatia G.*

*Biochemistry Division, Central Drug Research Institute, Lucknow India*

**Abstract**

The aim of present study was to provide the pharmacological basis for the medical use of *Allium sativum* in diabetes and dyslipidemia using the aqueous extract of its bulb, to prevent atherosclerosis and other cardiovascular diseases. The lipid lowering activity of *Allium sativum* has been studied in triton treated and high fat diet fed hyperlipemic C.F. rats (*in vivo*). It has also been for antioxidant activity *in vitro*. Serum lipids were lowered by feeding *Allium sativum* (200 & 400 mg/kg, b.w.) in triton WR-1339 induced hyperlipemic rats. Chronic feeding of the extract at doses in animals simultaneously fed with high fat diet for 30 days caused lowering in the lipids and apolipoprotein levels of very low density lipoprotein and low density lipoprotein as well as atherogenic index. The *A. sativum in vitro* inhibited the generation of superoxide anion and hydroxyl radicals in both enzymatic and non-enzymatic systems. The results of the present study demonstrate the lipid lowering and antioxidant activities in extract of *A. sativum*, which could help in prevention of cardiovascular diseases, particularly atherosclerosis.

**Key words:** Diabetic-Dyslipidemia, Atherosclerosis, Lipid lowering, Antioxidant, *Allium sativum*.

## Introduction

Dyslipidemia is one of the major modifiable risk factor for cardiovascular diseases including atherosclerosis [1]. Furthermore, disorders of lipid metabolism are associated with increased oxidative stress and overproduction of oxygen free radicals [2]. Free radicals are implicated in etiology of several lifestyle-related diseases such as atherosclerosis, stroke, diabetes, and

*Author for Correspondence
Email: cdri.atul@gmail.com

cancer [3]. The treatment of dyslipidemia reduces cardiovascular events [4]. The modern pharmacological therapy for hyperlipidemia is effective but associated with side effects leading to patient incompliance [5]. Moreover, the lipid lowering drugs viz. fibrates, statins and bile acid sequestraints do not possess antioxidant property [6]. Therefore, a drug having dual property of antidyslipidemic and antioxidant activities from natural products is the most preferred option. *Allium sativum (A. sativum; Liliaceace)*, commonly called Garlic is used as a spice and medicinal herb. *A. sativum* has been reported to possess immunomodulatory, hepatoprotactive and anticarcinogenic properties [7, 8]. Garlic and its extract have been shown to possess beneficial effects for prevention of cardiovascular diseases [9]. Therefore, the antidyslipidemic and antioxidant activities of *A. sativum* triton induced and HFD fed hyperlipidemic C.F. rats and the *in vitro* effect on reactive free radicals was carried out.

*Abbreviations:* C.F. Charles Foster; ASE – *Allium sativum* extract; TG –Triglycerides; TC – Total Cholesterol; TP – Total Protein; FFA – Free Fatty Acids; HDL – High density lipoprotein; LDL – low density lipoprotein; VLDL – Very low density lipoprotein; HFD – High Fat Diet

## Materials and methods

Plant Material:

*A. sativum* was collected from Lucknow and identified taxonomically by the Division of Botany, Central Drug Research Institute, Lucknow, India. The bulbs were dried in shade and grounded into fine powder. The powder (1Kg) was extracted thrice with triple distilled water for 8 hours in a percolator at room temperature and the fraction were pooled and concentrated by rotavapour. The vacuum drying concentrated fraction yielded 23.5g of dried extract of *A. sativum* (ASE), which was used for *in vivo* and *in vitro* studies.

Drugs and Standards:

Triton WR-1339 and standard drug Niacin along with other chemicals were procured from Sigma Chemical Company, St Luis, MO, USA.

Animals:

Male adult rats of *Charles Foster* stain (100-150g) bred in the animal house of the institute were used after approval of Animal Ethics Committee.

Triton-induced hyperlipidemia:

The animals were divided into five groups, group 1 – control, group 2 – triton treated, group 3 – ASE (200mg/kg b. w.), group 4 – ASE (400mg/kg b. w.) and group 5 – standard drug Niacin (100mg/kg b. w.). Each group contained six animals which were kept in controlled

conditions of temperature (25-26 °C), relative humidity (60-80%) and 12/12h light/dark cycle (light from 8:00 am to 8:00pm) and provided with standard pellet diet (Lipton India Ltd) and water *ad libitum.* Hyperlipidemia in rats was induced by a single dose of triton WR-1339 (400mg/kg b. w.) intraperitoneally [10]. After dosing, the rats were fasted for 18h and then anaesthetized with sodium pentothal solution (50mg/kg *i.p.*) prepared in normal saline. Blood was withdrawn from retro-orbital sinus using glass capillary in EDTA coated tubes (3mg/ml blood). The blood was centrifuged at 2500Xg for 10min at 4°C and plasma was separated, which was used for biochemical analysis.

High Fat Diet-induced Hyperlipidemia:

The Charles Foster rats were randomly divided into five groups. All groups (except control) were injected a single dose of streptozotocin (*i.v.*) to induce type-II diabetes mellitus. The ASE was given orally, simultaneously fed with high fat diet for 30 days [11]. At the end of treatment the animals were fasted for 24 hours, the blood of anesthetized animals was collected by cardiac puncture in EDTA coated glass tubes and centrifuged at 2500Xg for 10min in Sigma 3-30k centrifuge to obtain plasma.

Blood glucose and Glucose tolerance test:

Blood glucose was estimated by one-touch electronic glucometer and the oral glucose tolerance test was performed as reported by Vand & Karr [12].

Estimation of Lipid profile and total protein level:

The levels of serum cholesterol, triglycerides, phospholipids, free fatty acids, high density lipoproteins and total protein were estimated according to methods mentioned [13-18]. VLDL, LDL and atherogenic index were calculated according to following formulas [19]:

$$VLDL = TG/5$$

$$LDL = TC - HDL - TG/5$$

$$\text{Atherogenic index} = (TC - HDL)/HDL$$

Estimation of Antioxidant Activity:

Generation of Superoxide anions ($O_2^-$) was maesured in an enzymatic system composed of Xanthine (0.122mg/ml), Xanthine oxidase (12µl/ml) and nitrobluetetrazolium (NBT) (0.74mg/ml) with or without addition of ASE (50-400µg) [20]. The amount of formazone formed as a result of reduction of NBT by $O_2^-$ was measured at 560 nm on spectrophotometer. The system employed for non-enzymatic generation of $O_2^-$ comprised of phenazine methosulphate (PMS) (0.28mg/ml), NADH (1.65mg/ml) and NBT (1.286mg/ml) in the absence or presence of ASE (50-400µg). After the incubation the amount of formazone formed was measured as above [21]. Generation of Hydroxyl free radicals and effect of ASE

on the formation of hydroxyl free radicals ($OH^{\bullet}$) was measured in an enzymatic system composed of hypoxanthine (0.4mM), $FeSO_4 \cdot 7H_2O$ ($Fe^{+2}$) (0.mM), sodium salicylate (5mM) and xanthine oxidase (0.07 units), 3,4-dihydroxybenzoate formed by $OH^{\bullet}$ mediated hydroxylation of salicylate was measured as reported by Richmond *et al* [22]. In another set of experiment $OH^{\bullet}$ were generated non-enzymatically by $FeSO_4$ (0.276mg/ml), sodium ascorbate (1.9mg/ml), $H_2O_2$ (5%) and deoxyribose (0.94mg/ml) in absence or presence of ASE (50-400µg), and malondialdehyde produced was measured [23].

Statistical analysis:

All groups were compared by one way analysis of variance (ANOVA) & the significance of mean difference between different groups was done by Tukey's post hoc test. A two tailed (α=2) probability $p<0.05$ was considered statistically significant ($p < 0.05$ = *, $p < 0.01$ = **, $p < 0.001$ = *** and ns = not significant).

**Results**

*Effect of A. sativum extract on triton-induced hyperlipidemia:*

Administration of triton caused a marked increase in serum cholesterol (5.2 folds), triglyceride (4.6 folds), phospholipids (3.9 folds) and total protein (3 folds) in treated C.F. rats as compared to control animals. The treatment of rats with ASE (200 & 400mg/kg b. w., orally) caused significant reversal of above effects [figure: 1].

*Effect of A. sativum extract on high fat diet-induced dyslipidemia:*

The HFD fed rats showed an increase in TC (2.4 folds), TG (4.9 folds), PL (1.8folds), FFA (3.65folds), TP (2.43folds), plasma glucose (3.37folds), LDL (4.6 folds) and atherogenic index (9.1 folds). The HDL showed 57% decrease as compared to control. The oral administration of ASE (200 & 400mg/kg) caused decrease in TC (18 & 24%), TG (14 & 24%), PL (14 & 23.8%), FFA (24 & 27%), TP (20 & 29%), glucose (19 & 28%), LDL (49 & 54%) and atherogenic index (52 & 62%) respectively. The treatment caused 34-46% increase

*Antioxidant Activity:*

Enzymatic generation of $O_2^-$ anions in xanthine-xanthine oxidase system was inhibited to varying extent by ASE exhibiting 47% decrease at 400µg concentration. The ASE also inhibited the $O_2^-$ anions generation non-enzymatic by 66.3% at 400µg concentration. ASE caused 43 & 52% inhibition in the formation of $OH^{\bullet}$ by enzymatic system and non-enzymatic system at 400µg concentration respectively [figure: 3b].

**Figure: 1**

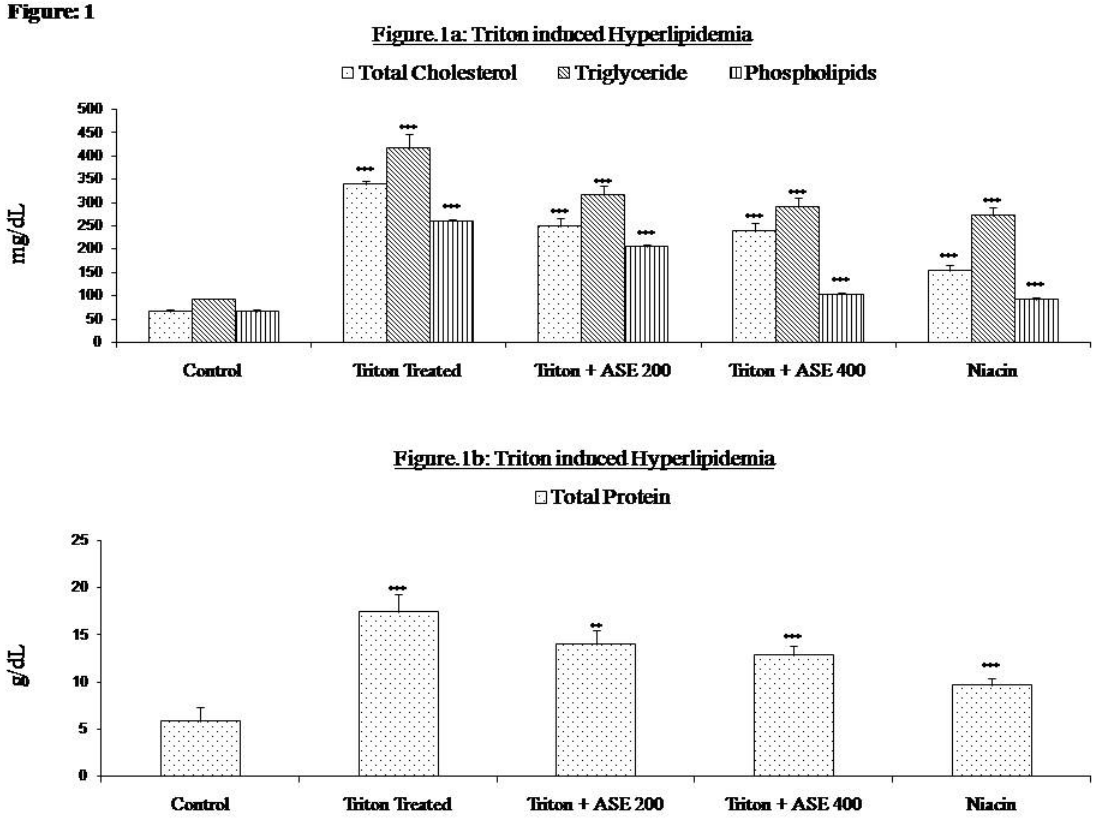


Figure1: Effect of ASE on total cholesterol, triglyceride, phospholipids and total protein in triton induced hyperlipidemia. Protocol for experimentation and statistical analysis of data are given in Materials and methods. Control group was compared with Triton treated; ASE treated with Triton treated. $p < 0.05$ was considered statistically significant. $p < 0.05$ ($^{*}$), $p < 0.01$ ($^{**}$) and $p < 0.001$ ($^{***}$). in HDL levels [figure: 2].

The oral administration of glucose (3g/kg b. w.) caused a marked increase in postprandial plasma levels of blood glucose of the rats from 0min-120min.The treatments with ASE showed 30-32% improvement in oral glucose tolerance [figure: 3a].

## Discussion

In present study two different models viz., triton WR-1339 and high fat diet induced hyperlipidemia were used to evaluate the possible effects of *A. sativum* extract. Triton WR-1339 (tyloxapol) is a non-ionic surfactant being widely used to explore possible mechanism of lipid lowering drugs [10]. Triton causes drastic increase in serum TG and TC levels *due to* increase in 3-hydroxy, 3-methyl-glutaryl CoA (HMG-CoA) reductase activity and by inhibition of lipoprotein lipase responsible for hydrolysis of plasma lipids [24, 25]. In fasting condition the only source of serum lipid is by endogenous production. Significant inhibition of increase in serum lipid levels by ASE treatment in this model might be *due to* inhibition of

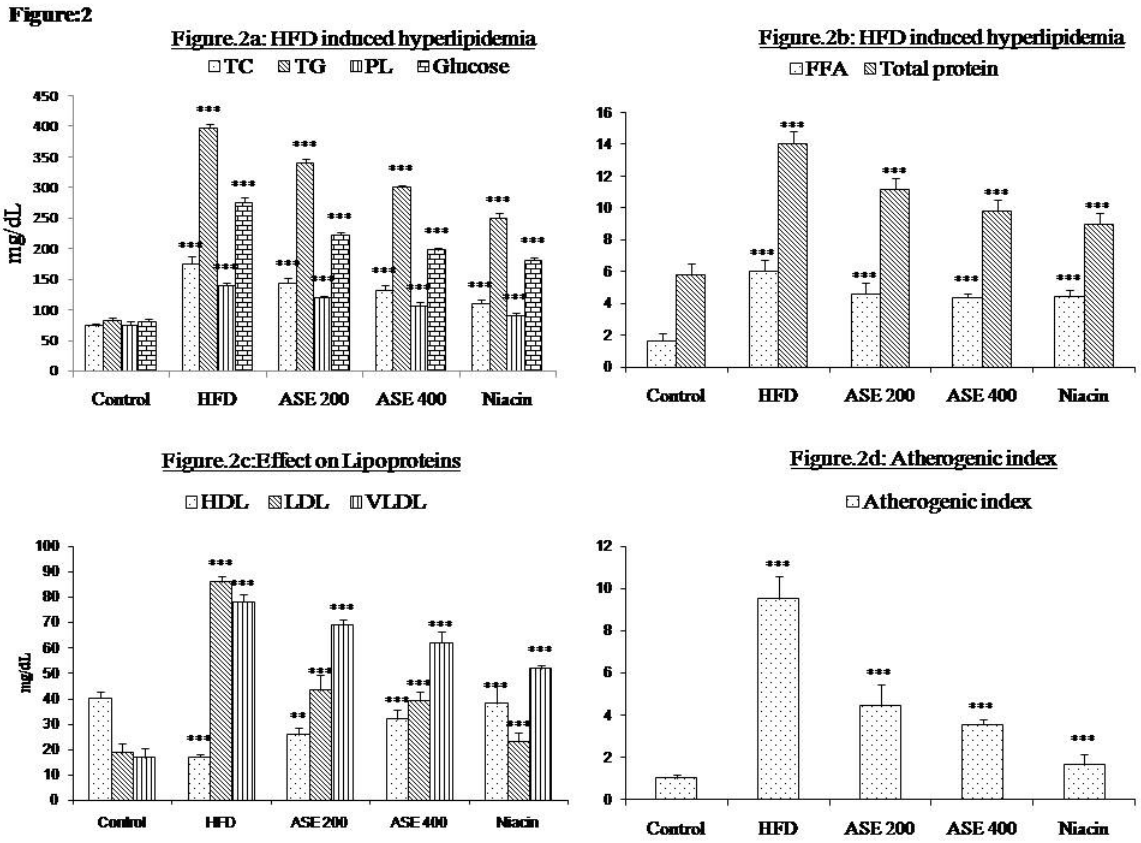


Figure2: Effect of ASE on HFD induced hyperlipidemia. Protocol for experimentation and statistical analysis of data are given in Materials and methods. Control group was compared with HFD fed; ASE treated with HFD rats. $p < 0.05$ was considered statistically significant. $p < 0.05$ ($^{*}$), $p < 0.01$ ($^{**}$) and $p < 0.001$ ($^{***}$).

HMG-CoA reductase. This enzyme plays a key role in controlling lipid levels in plasma and other tissues. High fat diet induces endothelial dysfunction, atherosclerosis [26] and increases oxidative stress by increasing the expression of oxidation-sensitive genes, such as Elk-1 and pCREB [27]. HFD containing cholic acid increases TC, LDL and decreases HDL by enhancing intestinal absorption and secretion of cholesterol likewise decreasing its catabolism [28]. Treatment with ASE caused a significant decrease in mean serum TC, TG, LDL in triton treated and HFD induced hyperlipidemia and increase in HDL levels. The ASE also caused significant reduction in atherogenic index, which is considered a better indicator of coronary heart disease risk than individual lipoprotein concentration [19]. Streptozotocin, an antibiotic produced by *Streptomyces achromogenes* var *streptozoticus*, is particularly toxic to β-cells of pancreas and inhibits the insulin production [29]. The administration of streptozotocin caused increased mobilization of free fatty acids from peripheral deposits, since insulin inhibits hormone-sensitive lipase [30]. The decrease in free fatty acid levels by ASE indicates that it inhibited the hormone-sensitive lipase. The results of our study showed that the ASE possesses the antioxidant property as it inhibited the *in vitro* generation of $O_2^-$

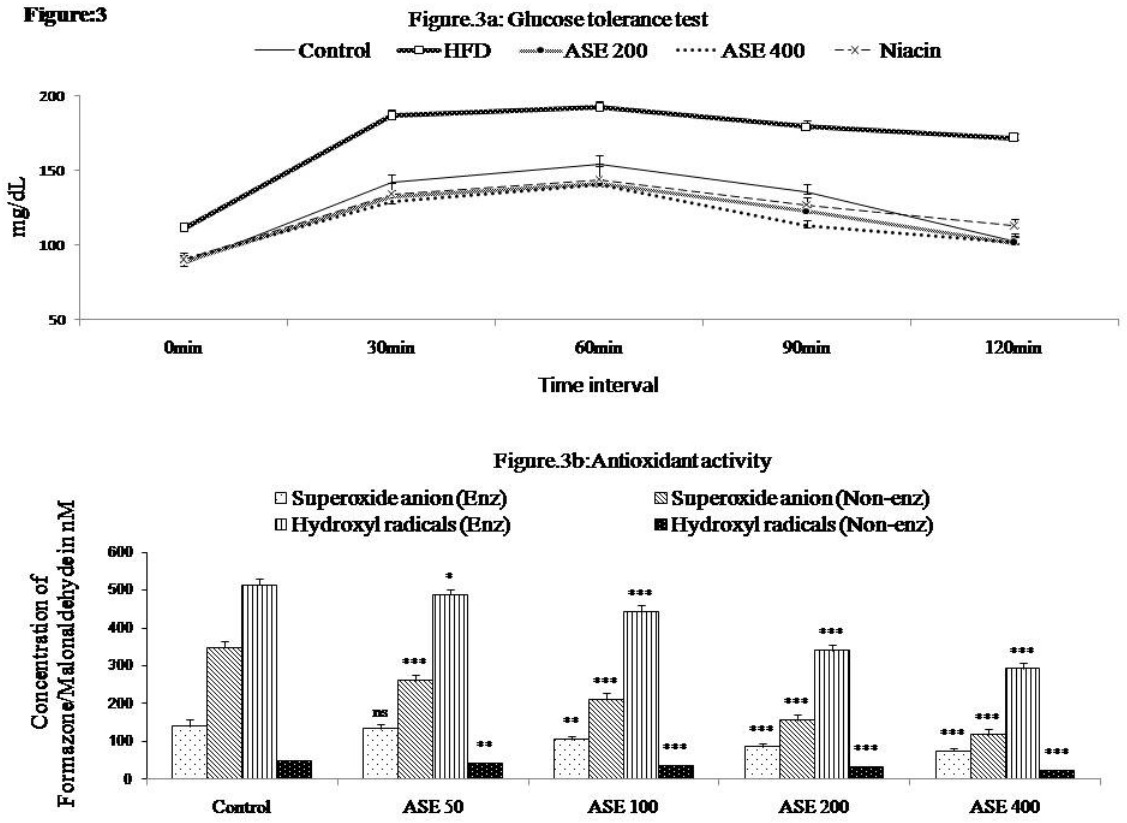


Figure3: *a*: Oral glucose tolerance test. Control group was compared with HFD fed; ASE treated with HFD rats. *b*: Antioxidant test. Effect of ASE on superoxide anion and hydroxyl free radicals generation. Experimental group compared with reference group. $p < 0.05$ was considered statistically significant. $p < 0.05$ ($^{*}$), $p < 0.01$ ($^{**}$) and $p < 0.001$ ($^{***}$).

and $OH^{\bullet}$ free radicals in both enzymatic and non-enzymatic systems. The oxidative stress due to HFD increases the oxidation of LDL resulting in development of atherosclerosis. Due to strong antioxidant property, *A. sativum* prevents the oxidation of LDL. The antidyslipidemic activity of *A. sativum* can be correlated to its inhibitory effect on lipid metabolism viz. biosynthesis, absorption and secretion. The antidiabetic, antioxidant and antidyslipidemic activities of *A. sativum* might be useful in reducing the development of diabetes and cardiovascular diseases.

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