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Research Article

A MECHANISM BASED PHARMACOLOGICAL EVALUATION OF EFFICACY OF *ALLIUM SATIVUM* IN REGULATION OF DYSLIPIDEMIA AND OXIDATIVE STRESS IN HYPERLIPIDEMIC RATS

ATUL SHRIVASTAVA, UPMA CHATURVEDI, SHIV VARDAN SINGH, JITENDRA KUMAR SAXENA, GITIKA BHATIA*

***Senior Principal Scientist,Division of Biochemistry,CSIR-Central Drug Research Institute, Chhattar Manzil Palace,1 Mahatma Gandhi Marg, Lucknow (India) –226001, India , CDRI communication No: 8251 , E-mail:**gitikabhatia2011@gmail.com

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ABSTRACT

Aqueous extract of *Allium sativum* has been studied in hyperlipidemic Charles foster rats fed on high fat diet to find out possible mechanism responsible for its lipid lowering and antioxidant behavior. Plasma and hepatic lipid levels were found to be lowered by *A. sativum* (200mg/kg b.w.). *A. sativum* activates 1ecithin: cholesterol acyltransferase, which converts cholesterol into HDL. Enhanced activities of plasma and hepatic lipoprotein lipases cause reduction in levels of LDL & VLDL. *A. sativum* treatment caused increased synthesis of bile acids from cholesterol, results in enhanced excretion. *A. sativum* significantly reduces oxidative stress and normalizes the activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase in liver. Lipid peroxidation in plasma has also been found significantly decreased in treated animals. Moreover, the body weight of treated animals was found to be significantly lesser (47%) than untreated animals. These findings suggest that activation of LCAT as well as enhanced synthesis & excretion of bile acids are mainly responsible for reduction of cholesterol level and elevation of high density lipoprotein level.

Keywords: *Allium sativum*; dyslipidemia; oxidative stress; lecithin: cholesterol acyltransferase; antioxidant, lipid lowering.

INTRODUCTION

The involvement of dyslipidemia in development of macro-/microvascular complications viz., cardiovascular disease (CVD), cerebro-vascular disease and peripheral vascular disease, which account for more than 70% of deaths in individuals with diabetes mellitus[1]. When plasma cholesterol exceeds the level required, it results in the development of atherosclerosis and stroke[2]. The treatment of dyslipidemia reduces cardiovascular events[3]. The modern pharmacological therapy for hyperlipidemia is effective but associated with side effects leading to patient incompliance[4]. Moreover, the lipid lowering drugs viz. fibrates, statins and bile acid sequestraints do not possess antioxidant property[5]. 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, hepatoprotective and anticarcinogenic properties[6, 7]. Garlic and its extract have been shown to possess beneficial effects for prevention of cardiovascular diseases[8]. Our previous study showed that *A. sativum* possesses marked hypolipidemic activity at a dose of 200mg/kg body weight as well as potent antioxidant activity in enzymatic and non-enzymatic (*in vitro*) systems [9].

The present study was designed to investigate possible mechanism behind hypolipidemic behavior of *A. sativum* in hyperlipidemic Charles *foster* rats fed on high fat diet. The *in vivo* antioxidant potential was also studied.

MATERIALS & 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 18.7g of dried extract of *A. sativum* (ASE), which was used for *in vivo* studies.

Animals: Male adult rats of *Charles Foster* strain (100-150g) bred in the animal house of Central Drug Research Institute, Lucknow India. The animals were used after approval of Institutional Animal Ethics Committee.

Drugs and standards: All chemicals were procured from Sigma Chemical Company, St Luis, MO (USA).

Composition of high fat diet: Fat – 45%, Fructose – 17% and Cholesterol – 12.5g / 4057 Kcal.

Experiment design: The Charles Foster rats were randomly divided into five groups: group 1 – control (fed on normal diet), group 2 – HFD (fed on High fat diet), group 3 – HFD with ASE (200mg/kg b. w.) and group 4 – HFD with standard drug Gemfibrozil (50mg/kg b. w). The animals were fed with high fat diet for 30 days [10]. From the day 31 ASE and standard drug were given orally simultaneously fed with high fat diet for next 30 days. Weight of the animals was monitored on alternate days. At the end of treatment the animals were fasted for 18 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. The animals were sacrificed to collect liver tissue in ice cold glass tubes.

Biochemical analysis of plasma: The levels of total cholesterol (TC), triglycerides (TG), phospholipids (PL), free fatty acids (FFA), high density lipoproteins (HDL) and post heparin lipolytic activity (PHLA) & Plasma lecithin: cholesterol acyltransferase (LCAT) activity were estimated according to methods mentioned[11-17]. VLDL and LDL were calculated according to following formulas [18].

$$VLDL = TG/5$$

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

Biochemical analysis of liver: The excised liver tissue was rinsed with0.15m KCl and kept in -70°C till their use. The total lipolytic (LPL) and TG lipase (TGL) activities were estimated using standard methods[19]. The lipid extract of each homogenate was used for estimation of TC, TG and PL [11-13].

Measurement of faecal excretion of bile acids: Faeces of each group were collected daily from day 31 to day 60 of the experiment. The amount of cholic acid and deoxycholic acid excreted through faeces was estimated [20].

Antioxidant activity: Lipid peroxidation in plasma was measured by thiobarbituric acid reaction as TBARS[21]. Hepatic Super oxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), and Glutathione reductase (GRh) were estimated by methods reported earlier [22-25].

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 p >0.05 = ns = not significant).

RESULTS

Body weight: Animals fed on high fat diet (group 2) gained more weight (+1.9fold) than those fed on normal diet. Animals treated with ASE gained significantly lesser (-47%) than HFD group. (Figure 1a)

Lipid profile in plasma and liver: The chronic feeding with HFD caused a marked increased in plasma levels of TC (+2.1 fold), TG (+3.2 fold), PL (+1.9 fold), LDL (+4.0 fold) and VLDL (+3.18 fold) and decrease in HDL (-45.4%). These effects were shown to be reversed by the treatment with ASE by 18.5%, 10.5%, 16.9%, 43.6%, 4.3% and 51% respectively (Figure 1b). The increased levels of TC (+1.7fold), TG (+1.6fold) and PL (+1.5fold) in liver of HFD fed rats were observed to be lowered by 25, 24 and 46% *due to* their treatment with miglitol (Figure 1c).

Enzyme analysis: HFD feeding caused the inhibition of PHLA (40%) & LCAT (45%) in plasma and LPL activity (37%) & TGL activity (48%) in liver (Figure 2a). Treatment with ASE reactivated these lipolytic activities in plasma and liver of hyperlipidemic rats.

Faecal excretion of bile acids: Feeding with HFD caused a significant decrease in the faecal excretion of cholic acid (44%) and deoxycholic acid (60%) and these levels were shown to be recovered by the treatment with ASE by 34.7 & 41% respectively (Figure 2b).

Antioxidant potential: Due to the chronic feeding of HFD plasma levels of TBARS significantly increased (+2.7 fold) which was reduced by ASE (28%)(Figure 2c). The activities of SOD, Catalase, GPx and GRh were found to be significantly higher in HFD fed animals *due to* high oxidative stress than in those fed on normal diet. The treatment of ASE significantly normalized these effects (Figure 2d).

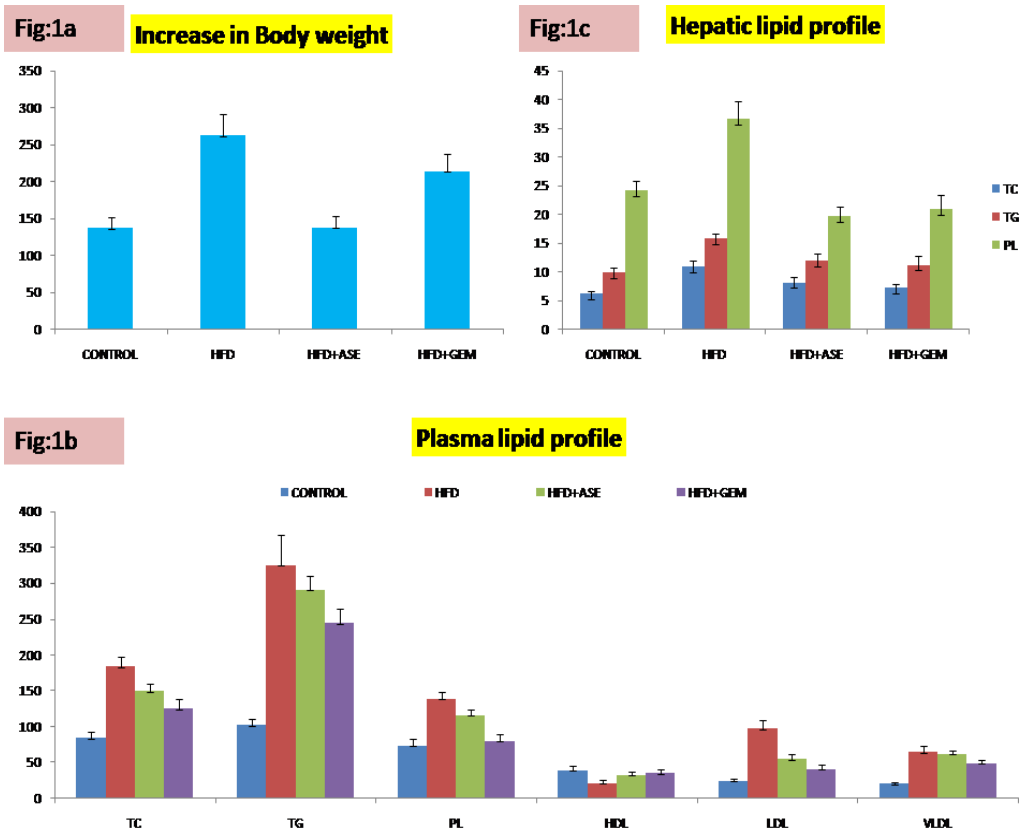

Figure1: Effect of A. sativum on body weight, lipid profile.

1a: Effect of ASE on body weight. Chronic treatment with ASE significantly controls gain in body weight of animals fed on high fat diet in comparison of untreated animals. 1b: Biochemical analysis of plasma of HFD induced hyperlipidemic animals. 1c: Hepatic lipid profile. Units, mg/dl for TC, TG, PL, HDL, LDL and VLDL.

DISCUSSION

High fat diet induces endothelial dysfunction, atherosclerosis and increases oxidative stress by increasing the expression of oxidation-sensitive genes[26, 27]. The present investigation shows that ASE significantly decreases levels of cholesterol in plasma and liver by increasing the activity of LCAT, which plays a key role in lipoprotein metabolism. LCAT converts free cholesterol into cholesteryl ester (a more hydrophobic form of cholesterol), which is then sequestered into the core of a lipoprotein particle, eventually making the newly synthesized HDL. Therefore activation of LCAT lowers cholesterol levels and increases levels of HDL. The activation of PHLA in plasma and hepatic LPL is responsible for a significant decrease in levels of LDL & VLDL. Elevation of TGL activity by ASE treatment causes significant decrease in levels of triglycerides. The increased faecal bile acid excretion is also linked with hypolipidemic activity of ASE. Cholic acid is synthesized in liver from cholesterol, therefore increased excretion of cholic acid results in decreased level of cholesterol in plasma and liver. Furthermore ASE treated animals gained significantly lesser (47.5%) body weight than untreated animals.

High fructose consumption has pro-oxidant effect. Fructose fed rats display oxidative stress, an imbalance between free radical production and antioxidant defense in many tissues[28-30]. The lipid peroxidation was analyzed by TBARS. The Thiobarbituric Acid (TBA) test is a very non-specific technique and is widely used as a marker [31]. ASE therapy lowered the levels of TBARS indicating decreases in lipid peroxidation. The elevated activities of antioxidant enzymes (SOD, Catalase, Glutathione Reductase and Glutathione peroxidase) indicate high levels of oxidative stress. ASE significantly normalized the activities of the antioxidant enzymes, as it reduces oxidative

stress as it possesses free radical scavenging property as well as inhibiting the generation of superoxide anion & hydroxyl free radicals [9].

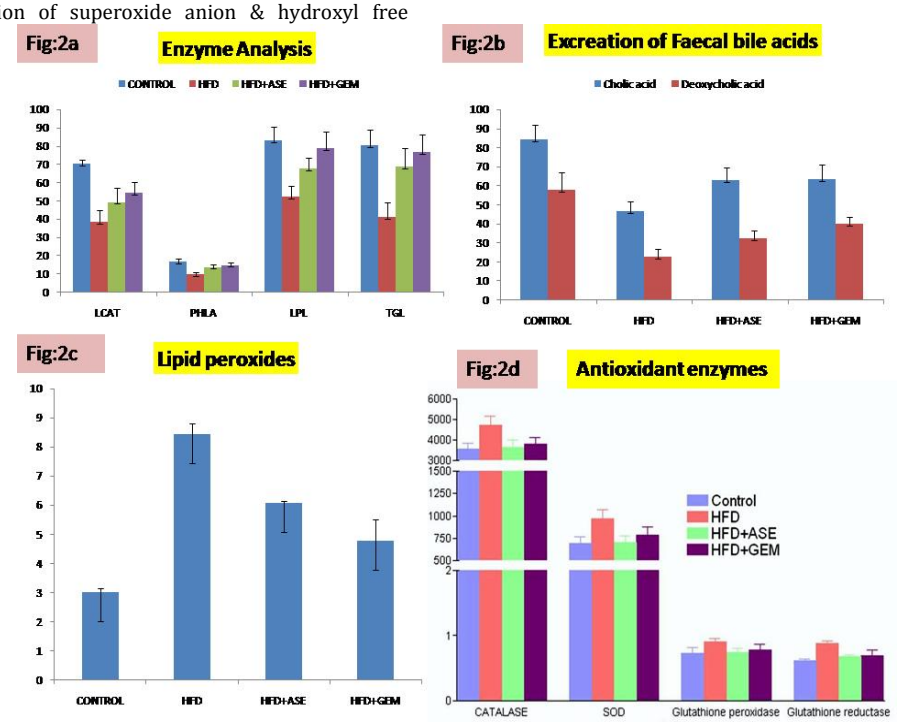

Figure 2: Effect of A. sativum on metabolic enzymes, bile acid excretion and oxidative stress

2a: Effect of ASE on lipolytic enzymes was studied. Protocol for experimentation and statistical analysis of data are given in Materials and methods. Units, n mol free fatty acid formed/h/ml for PHLA and n mol cholesterol released/h/L for LCAT, n mol free fatty acid formed/h/mg for LPL and TGL. 2b: Effect of ASE on bile acid excretion. 2c: Effect of ASE treatment was observed on lipid peroxidation as TBARS (unit, nmol malonaldehyde /ml) in plasma. 2d: Effect of ASE on antioxidant enzymes.

In conclusion, the hypolipidemic action of *A. sativum* is *due to* activation of 1ecithin: cholesterol acyltransferase, plasma and hepatic lipolytic enzymes, triglyceride lipase as well as enhanced synthesis and excretion of bile acids. *A. sativum* reduces oxidative stress and normalizes the activity of antioxidant enzymes by its property to inhibit generation of superoxide anion and hydroxyl free radical along with free radical scavenging behavior. Some of these affects were comparable to that of gemfibrozil. The present study reveals that *Allium sativum* is a better option for therapy of patients with life style diseases (*viz.* obesity, diabetes and cardiovascular diseases), as it is a natural product having both hypolipidemic and antioxidant properties.

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