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ANTIDYSLIPIDEMIC AND ANTIOXIDANT ACTIVITY OF MEDICINAL PLANTS IN RAT MODEL OF HYPERLIPIDEMIA

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ABSTRACT: The antidyslipidemic and antioxidant activities of *Allium sativum* (Garlic) and *Aloe vera* (Ghee- kuwar) extracts have been studied in two models of hyperlipidemia, triton and cholesterol rich high fat diet induced hyperlipidemia. *A. sativum*, *A. vera* and Gemfibrogil were macerated with 0.2% aqueous gum acacia and the suspension was fed orally to rats of group III, IV and V at a dose of 400mg, 1g/Kg, and 50 mg/Kg (b.w.p.o.) respectively with triton. Serum lipids were found to be lowered by *Allium sativum* and *Aloe-vera* in triton induced hyperlipidemia. On the other hand chronic feeding of these extracts to animals in cholesterol rich high fat diet induced hyperlipidemia for 30 days caused lowering in lipid and protein levels of β lipoproteins followed by an increase in α lipoproteins. The results of present study demonstrate that hypolipidemic activity of these extracts mediate through inhibition of hepatic lipid synthesis, increased faecal bile acid excretion and induced plasma LCAT activity in rats. Furthermore these extracts (100 and 200 µg/ml) inhibited the *in vitro* generation of superoxide anions and hydroxyl radicals in both enzymatic and non enzymatic systems.

INTRODUCTION: Dyslipipoproteinemia is an independent risk factor for the development of coronary artery diseases, myocardial infarction, and hypertension in hyperlipidemic patients [1].

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Clinically diabetic patients are characterized by marked increase in blood glucose level followed by normal or mild hyperlipidemia.

Elevated level in low density lipoprotein (LDL) along with triglyceride especially in very low density lipoprotein (VLDL) and cholesterol in low density lipoprotein with free radicals and oxidative stress mediated formation of modified LDL is recognized as a leading cause of development of Atherosclerosis and coronary heart disease in

diabetes mellitus [2]. Furthermore hyperlipidemia may also induce abnormalities like oxidation of lipids, formation of ketone bodies as well as resistance to insulin in muscle and liver cells in diabetic patients. Treatment of hyperlipidemia with available lipid lowering drugs: fibrats and bile acid sequestrants are not free from many side effects such as myositis, gastrointestinal upset along with elevated hepatic renal function tests [3].

Allium Sativum (Lat.), (Eng: Garlic, Urdu: '*Lahsan*') is widely distributed in all parts of the world and used not only as spice but also as a popular remedy for prevention and treatment of a variety of diseases like rheumatism, dermatitis, abdominal disorders and diabetes mellitus. 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, cancer and stimulation of immune functions, enhanced detoxification of foreign compound, hepatoprotective, antimicrobial effect and antioxidant effect.[4] Garlic is reported to prevent cardiovascular disease by multiple effects, one of which is the decrease total cholesterol and triglycerides [5], LDLc, VLDLc, while increase HDLc [6] and suppression of the cholesterol biosynthesis [7]. Studies prior to 1995 consistently concluded hypolipidemic action of garlic [8, 9]. However, studies after 1995 using enteric-coated preparation of raw garlic did not manifest any hypolipidemic effect [10–12].

These paradoxical observation warrant a systemic study to resolve the controversy. It is a remarkable plant, which has multiple beneficial effects such as antimicrobial, antithrombotic, hypolipidemic, antiarthritic, hypoglycemic and antitumor activity. Additionally, garlic has known hypoglycemic properties, which have been demonstrated in alloxan induced diabetic rats and rabbits. The extract of garlic and its component, S-allylcysteine sulfoxide, significantly decreased blood glucose concentration. Its activity appears to be in part due to stimulation of insulin secretion from β-cell in the pancreas [13].

Aloes are members of the Liliaceae family and are mostly succulent with a whorl of elongated, pointed leaves. Taxonomists now refer to Aloe barb and enosis as *A. vera*. In an evaluation of children with diabetes mellitus (type1) from Turkey and Germany, aloe Vera was one of the most commonly consumed herbal medicines used by 12.9% and 7.3% of the patients respectively[14]. Therefore on the basis of above facts there is an urgent need to develop a drug possessing antidiabetic together with lipid lowering and antioxidant activities for which natural products are the best options.

This is because natural products are easily available, cost effective and safe because they are more harmonious with biological systems. The natural products from medicinal plants like Allium cepa (onion, piyaz), *Ficus bengalensis* (Banyan tree; Bargad), *Gymnema Sylvestra* (Gurmar) *Pterocarpus morsupium* (Vijayasar), *Momordica charantia* (Bitter guards; Karela) *Trigonella foenum graecum* (Fenugreek ; methi) *Allium sativum* (Lehsun; garlic) *Eugenia jambolana* (Black plum: Jamun), *Azadiracta indica* (Margosa: neem), *Galega officinalis* etc have been used as hypoglycemic agent. However these effects on dyslipidemia are not well known.

Only Metformin from *Galega officinalis* is the only ethical drug approved for treatment of diabetes also possesses some antidyslipidemic activity[15]. No other drug or natural agent has been shown to generate this activity [16]. Asian Ginseng is commonly used in traditional Chinese medicine to treat diabetes. It has been shown to enhance the release of insulin from the pancreas and to increase the number of insulin receptor [17]. It has also a direct blood glucose lowering effect. The current therapies used for controlling hyperlipidemia;

fibrates, stains and bile acid sequestraints are almost inefficient to regulate lipid metabolism. Furthermore, these drugs also cause a number of serious adverse effects in patients. Currently available treatment for hyperlipidemia in modern medicine, fibrats, statins or bile acids sequestraints and their combinations do not regulate lipid metabolism up to a appreciable mark, also have several adverse effects in patients [18]. *A. vera*, a member of Liliaceae family is widely used as a traditional remedy of a variety of ailments over a long period of time however the biochemical details of its action on physiological/ pathophysiological functions in body have not been systematically investigated. Previous experimental results revealed the blood glucose lowering property of ethanolic extract of *A. vera* gel in streptozotocin (STZ) induced diabetic rats [19].

The *A. sativum* and *A. vera* supplementation also reduced significant aortic plaque deposits in cholesterol fed rabbits [18]. Though pharmacological actions of *A. sativum* and *A. vera* have been well documented and shown it to possess potent antioxidant and antidiabetic activities. However its lipid lowering and ROS scavenging action have not been well studied. Therefore present study was undertaken to evaluate the antidyslipidemic and antioxidant activities of the *A. sativum* and *A. vera* plant extracts. The results obtained with these extracts were compared with gemfibrozil a known hypolipidemic drug.

MATERIALS AND METHODS:

Plant Material: *A. sativum* and *Aloe-vera* were collected from local area of Lucknow and identified taxonomically by the division of Botany, Central Drug Research Institute, Lucknow India. 200 gm bulbs of *A. sativum* were cut into small pieces and extracted with absolute ethyl alcohol. The alcohol content was evaporated to dryness. The final yield of 20.0 gm of crude extract (concentrate) was added with 50 ml of triple distilled water and was used for *in vivo* and *in vitro* studies. A dose of 400 mg/Kg was administered to rats orally, daily for 15 days. The aqueous extract of *A. vera* was prepared by boiling 50 gms of the plant leaf gel with 100 ml of water for 10 min. After cooling to room temperature, the extract was filtered and stored in refrigerator until used. The dose (1g/Kg, b.w.) was administered orally daily for 30 days[18].

Preparation of the cholesterol-rich high-fat diet:

Deoxycholic acid (5 g) was mixed thoroughly with 700 g of powdered rat chow diet supplied by Ashirvad Industries, Chandigarh, India. Simultaneously cholesterol (5g) was dissolved in 300 g warm coconut oil. This oil solution of cholesterol was added slowly into the powdered mixture to obtain a soft homogenous cake. This cholesterol-rich high-fat diet (HFD) was molded into pellets of about 3 g each[19].

Animals:

In vivo experiments were conducted as per guidelines provided by Animal Ethics Committee of Central Drug Research Institute, Lucknow, India. Male adult rats of Charles Foster strain (100-150g) bred in animal house of the Institute were used. The animals were housed in polypropylene cages and kept in uniform hygienic conditions, temperature 25-26 °C, relative humidity 50-60% and 12/12 h light/dark cycle (light from 8:00 a m to 8:00 p m) and provided with standard rat pellet diet and water ad libitum [20].

Lipid lowering activity in hyperlipidemic rats:

The rats were divided into five groups consisting of six rats in each group: Group I (normal control) group II (triton treated hyperlipidemic), Group III (Triton + *A. sativum),* Group IV (Triton plus *A.vera*) and Group V (Triton + gemfibrozil).

In the acute experiments hyperlipidemia in rats of group II to IV was induced by administration of triton WR -1339 (Sigma Chemical company, St. Lovis, MO USA) at a dose of 400mg/Kg b.w. by intraperitoneal injection. *A. sativum*, *A. vera* and Gemfibrogil were macerated with 0.2% aqueous gum acacia and the suspension was fed orally to rats of group III, IV and V at a dose of 400mg, 1g/Kg, and 50 mg/Kg (b.w.p.o.) respectively with triton. In another induced set of experimental rats, hyperlipidemia was induced in group II- V by feeding with HFD to animals (for 15 days. These extracts of of *A. sativum* (400mg/Kg), *A. vera* (1gm/Kg) and gemfibrozil (500mg/Kg) were administered orally once daily for 30 days. Control animals received the same amount of normal saline

or ground nut oil. In another identical set of experimental rats, hyperlipidemia was produced in group II by feeding the animals with HFD for 15 days. At the end of the experiments rats were fasted overnight. On the next day animals were anaesthetized with thiopentone (50mg/kg). Blood was withdrawn from retro-orbital plexus, kept at 20°C for 15 min and centrifuged at 2500x g for 20 min. The animals were sacrificed and their livers were excised promptly. Faeces were collected throughout the experimental period from all the groups. The cholic acid and deoxychlic acid content in the faeces was estimated [20].

Biochemical analysis:

Plasma lecithin cholesterol acetyl transferase (LCAT) activity was measured [21] and post heparin lipolytic activity (PHLA) was assayed [22]. Serum was fractionated into very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) by polyanionic precipitation[23]. Serum lipids were analyzed for their total cholesterol (TC) phospholipids (PL), triglyceride (TG), protein and Apoprotein by standard procedures reported earlier[24]. Liver homogenate (10%, w/v) prepared in 0.1 M Tris HCl buffer (pH 8.1) was centrifuged at 2500 rpm for 10 min. The supernatant was used for lipoprotein lipase activity [25].

Assessment of free radical scavenging activity:

Superoxide anions (O^{2-}) were generated enzymatically by xanthine oxidase (0.04 units) and nitroblue tetrazolium (320µM) in absence or presence of test compounds in 100 mM phosphate buffer (pH 8.2). Fractions were well sonicated in phosphate buffer before use. The reaction mixture was incubated at 37°C. After 30 min the reaction was stopped by adding 0.5 ml glacial acetic acid and the amount of formazone formed was measured at 560 nm on a spectrophotometer [26]. Percentage inhibition was calculated taking absorption coefficient of formazone as 7.2 x 103 M-1 cm-1. In another set of experiment, effect of test compounds on generation of hydroxyl radicals (OH) was also studied by nonezymic reactants. Briefly OH were generated in a non-enzymic system comprised of deoxyribose (2.8 mM), $FeSO4.7H_2O$ (2 mM), Sodium ascorbate (2.0 mM) and H_2O_2 (2.8 mM) in 50 mM KH_2PO4 buffer, pH 7.4 to a final volume of 2.5 ml. The above reaction mixtures in the absence or presence of the test extracts were incubated at 37^0C for 90 minutes. Reference tubes and reagent blanks were also run simultaneously [27]. Malondialdehyde (MDA) content in both experimental and reference tubes were estimated spectrophotometrically by thiobarbuteric acid method [28].

Statistical analysis:

The statistical analysis of variance (ANOVA-New men's student't' test) was performed by comparison of values for control, hyperlipidemic and hyperlipidemic with drug treated (*A.vera*, *A. sativum* and gemfibrogyl) groups. Similarly Streptozotocin induced diabetic group was compared with control and diabetic group with drug treatment. All hypothesis testing were two tailed $p<0.05$ was considered statistically significant and the results were expressed as mean±SD. The statistical analysis was carried out by 14 graph pad JN STAT 3.0 software. Similarly the generation of oxygen free radicals with two concentrations of *A.sativum* and *A.vera* extracts were compared with that of their formation without addition of extracts in reaction mixture. The values were tested for significance at a $p< 0.05$ [29].

RESULTS:

Effect of *A. sativum* and *A. vera* extract in triton induced hyperlipidemia:

The acute administration of triton WR-1339 induced marked increase in serum level of TC (+3.18F), PL (+2.97F), TG (+2.87F) and protein (+1.59F). Treatment with *A. sativum* and *A. vera* extract caused reversal in the levels of TC (22&26%), PL (24&28%), TG (22&27%) and protein (19&24%) respectively (**Table 1**). The lipid lowering activity of these extracts in the hyperlipidemic rats were comparatively less to that of Gemfibrozil.

Effect of *A. sativum*, *A. vera* and Gemfibrozil on lipid composition in serum lipoproteins and hepatic lipids

The data in **Table 2** shows that administration of HFD in rats increased their serum levels of TC (+2.35 F), PL (+2.46F), TG (+2.47F) and protein (+1.38F) respectively. Feeding with the extract of *A. sativum* and *A. vera* decreased the levels of TC

(22&28%), PL (21 & 28%), TG (24 &26%) and protein (18, 19%) respectively in HFD treated animals. The analysis of hyperlipidemic serum showed a marked increase in the level of lipids and apoproteins constituting β-lipoproteins and these effects were pronounced for VLDL-TG (2.24F) and LDL-TC (+4.84F).

Treatment with *A. sativum* and *A. vera* significantly reduced these levels of VLDL TG (22&26%) as well as LDL-TC (21&25%), PL (16&25%), TG (26&28%) and apo LDL (21&23%) respectively in hyperlipidemic rats. At the same time the decreased levels of HDL and apo-HDL in these animals were partially recovered (**Table 2**). The increased level of TC (+1.5 F), PL (+1.55 F), TG (+1.52 F), protein (+1.44 F) in livers of HFD fed rats were observed to be lowered by their treatment with the extracts (**Table 2**). The standard Gemfibrogil treatment was more effective than these natural drugs.

TABLE 1: EFFECT OF *A. SATIVUM, A. VERA* AND GEMFIBROZIL ON SERUM LIPIDS IN TRITON INDUCED HYPERLIPIDEMIA

Group of animals	Total cholesterol [a]	Phospholipids[a]	Triglyceride [a]	Protein[b]
Control (GrI)	88.74±5.91	84.12±6.29	81.30±5.30	5.64±0.23
Triton treated (GrII)	282.37±26.54*** (+3.18F)	250.40±20.17*** (+2.97F)	231.62±18.17*** (+2.87F)	8.97±0.31 (+1.59F)
Triton + *A. sativum* (GrIII)	220.44±16.37** (–22)	190.10±12.88*** (–24)	180.20±14.10** (–22)	7.23±0.44* (–19)
Triton + *A. vera* (GrIV)	208.11±13.77*** (–26)	180.00±14.17*** (–28)	168.28±12.17*** (–27)	6.84±0.37*** (–24)
Triton + Gemfibrozil (standard drug) (Gr V)	185.53±12.14*** (–34)	165.33±11.77*** (–33)	150.28±14.00*** (–35)	6.00±0.20*** (–33)

Units a: mg/dl; b: g/dL. Values are mean±SD of six animals Triton group compared with control and triton plus *A. sativum, A. veva* and gemfibrozil treated groups. values in the parentheses are % change. ***p<0.001; **p<0.01; *p<0.05

TABLE 2: EFFECT OF *A. SATIVUM, A. VERA* AND GEMFIBROZIL ON HFD INDUCED HYPERLIPEMIC RATS

Parameter	Control (GrI)	HFD (GrII)	HFD+*A. sativum* (GrIII)	HFD+*A. vera* (GrIV)	HFD+ Gemfibrozil (Gr V)
A. Serum					
Total cholesterol[a]	82.77±6.38	194.69±18.65 *** (+2.35F)	151.22±13.12 ** (–22)	140.14±12.00 *** (–28)	127.33±18.88 *** (–34)
Phospholipid[a]	81.24±10.08	200.33±14.00 *** (+2.46F)	158.27±12.14 ** (–21)	144.00±11.11 *** (–28)	132.26±10.14 *** (–33)
Triglyceride[a]	80.20±6.23	198.23±13.77 *** (+2.47F)	150.12±12.10 *** (–24)	146.30±13.00*** (–26)	132.66±10.20 *** (–33)
Protein[b]	5.99±0.61	8.28±0.57*** (+1.38F)	6.80±0.32* (–18)	6.69±0.17* (–19)	6.40±0.30 *** (–23)
B. VLDL					
Total cholesterol[a]	8.32±0.41	32.43±2.12 *** (+3.89F)	25.09±1.62** (–22)	23.99±1.77 *** (–26)	20.80±1.00 *** (–35)
Phospholipid[a]	14.87±0.31	30.18±1.24*** (+2.02F)	26.12±1.80** (–20)	23.33±1.00 *** (–23)	22.17±0.82*** (–31)
Triglyceride[a]	38.69±1.27	86.77±5.12 *** (+2.24F)	65.01±2.82*** (–25)	63.17±4.28 *** (–25)	60.66±4.00 *** (–30)
Apoprotein[a]	6.30±0.50	12.12±1.90*** (+1.92F)	9.75±0.64** (–20)	9.40±0.37 ** (–22)	9.00±0.62 *** (–25)
C. LDL					
Total cholesterol[a]	13.23±0.88	64.16±5.72 *** (+4.84F)	50.29±3.67** (–21)	47.88±3.00 *** (–25)	46.10±2.14 *** (–28)
Phospholipid[a]	12.14±0.47	43.36±3.36 *** (+3.57F)	36.41±2.73* (–16)	32.12±2.10 *** (–25)	30.83±2.70 *** (–28)
Triglyceride[a]	15.12±0.17	36.62±2.68 *** (+1.58F)	27.12±2.12*** (–26)	26.17±1.77 *** (–28)	25.78±1.66*** (–30)
Apoprotein[a]	17.56±1.00	28.62±1.88 (+1.62F)	22.50±1.33** (–21)	22.00±1.00 *** (–23)	20.37±1.00*** (–28)
D. HDL					

Total cholesterol[a]	45.38±2.71	38.14±2.80* (–16)	44.28±4.00* (+14)	44.80±4.00* (+15)	45.00±4.10* (+15)
Phospholipid[a]	37.41±2.61	28.61±2.14 *** (–23)	32.83±2.66* (+13)	34.00±2.73* (+16)	35.66±3.12** (+20)
Triglyceride[a]	15.14±1.10	12.13±0.94 ** (–20)	14.09±1.14* (+14)	14.10±1.00* (+14)	14.27±1.18* (+15)
Apoprotein[b]	168.20±13.50	120.35±14.40 *** (–28)	140.80±7.50* (+15)	142.77±10.37* (+16)	144.22±13.00 * (+17)
E. Plasma					
LCAT activity[c]	67.59±3.94	37.77±2.66 *** (–44)	48.39±2.42** (+22)	50.44±5.00 *** (+25)	52.88±5.11 *** (+29)
PHLA[d]	17.66±1.06	10.38±0.70 *** (–41)	13.72±0.64*** (+24)	14.00±1.00 *** (+26)	14.77±1.10*** (+30)

Units a: mg/dl; b: g/dL; c: nmol cholesterol released/hr /L plasma; d : n mol free fatty acid formed /hr/mL plasma. Values are mean±SD from 6 animals. HFD compared with control and HFD.plus *A. sativum, A. vera* and gemfibrozil treated groups. values in the parentheses are % change. ***p<0.001; **p<0.01; *p<0.05

Effect of *A. sativum* and *A.vera* on Lipolytic Enzymes and Faecal excretion of bile acids:

HFD *A. sativum* and *A.vera* feeding to rats caused the inhibition of plasma LCAT (–44%) and PHLA (–41%) respectively (**Table 3**) and total lipolytic activity (–45%) in liver (**Table 3**). Treatment with *A. sativum*, *A. vera* and gemifibrozil partially reactivated these lipolytic activities in plasma and livers of hyperlipidemic rats. *A. sativum* and *A.vera* Feeding with HFD caused a significant decrease in the faecal excretion of cholic acid (–41%) and deoxycholic acid (–56%) and these levels were shown to be recovered by the treatment with *A. sativum* (+18&20%), *A. vera* (+38&41%) and gemifibrozil (23&45%) respectively in HFD fed animals (**Table 3**).

TABLE 3: EFFECT OF *A. SATIVUM, A. VERA* EXTRACT AND GEMFIBROZIL ON HEPATIC BIOCHEMICAL PARAMETERS AND FAECAL BILE ACID EXCRETION IN HFD INDUCED HYPERLIPIDEMIC RATS

Parameters	Control (GrI)	HFD (GrII)	HFD+*A. sativum* (GrIII)	HFD+*A. vera* (GrIV)	HFD+ Genfibrozil (GrV)
A. Liver					
LPL activity[a]	130.37±8.84	71.23±3.42 *** (–45)	81.81±6.12* (+13)	83.94±7.00 * (+15)	88.95±5.02 ** (+20)
Total cholesterol[b]	6.62±0.14	10.04±0.32 *** (+1.51F)	8.32±0.10* (–16)	8.00±0.37 ** (–20)	7.32±0.27 *** (–27)
Phospholipid[b]	23.33±2.00	36.12±1.87 *** (+1.55F)	28.78±2.00** (–20)	26.39±2.12 *** (–26)	25.00±1.88 *** (–30)
Triglyceride[b]	10.34±0.70	15.72±0.88 *** (+1.52F)	12.22±1.10** (–22)	11.80±1.00 *** (–24)	11.00±0.77 *** (–30)
Protein[b]	150.30±12.50	217.50±15.0 *** (+1.44F)	180.01±10.39 * (–17)	173.39±16.00 ** (–20)	160.12±13.19*** (–26)
B. Faecal bile acid					
Cholic acid[c]	81.47±4.87	47.63±3.12 *** (–41)	58.12±3.10 ** (+18)	59.20±5.00 ** (+20)	61.99±3.77 *** (+23)
Deoxycholic acid[c]	53.66±3.12	23.41±1.77 *** (–56)	38.29±3.00 *** (+38)	40.29±3.12 *** (+41)	43.27±4.00 *** (45)

Units: a: n mol free fatty acid formed/h/mg protein, b: mg/g; c/ µg/g. Values are mean±SD of six animals. HFD groups compared with control and HFD plus *A. sativum, A. vera* and gemfibrozil treated groups were compared with HFD. ***p<0.001; **p<0.01; *p<0.05

Effect of *A. sativum* and *A. vera* extract on generation of super oxide anions:

The data in **Table 4** showed that enzymic oxidation of xanthine to uric acid (A) as well as the generation of O_2^- anions in xanthine–xanthine oxidase system, as measured by reduction of NBT to Formazone (B) were inhibited to varying extents by *A. sativum* and *A. vera* extract in a concentration dependent manner and this effect was maximum by 32 and 46 % respectively at 200 µg/ml of *A. sativum* and *A. vera* extract. The extract also trapped the O_2^- anions generated by non enzymic system of NADH – Phenozine–Methosulphate and was responsible for reduction of NBT in the

reaction mixture. The effect was dose dependent and was highest by 35%, 50% at 200 µg/ml of *A. sativum* and *A. vera* extract respectively.

Effect of *A. sativum* and *A. vera* extract on generation of hydroxyl radicals:

The data in **Table 4** also showed that *A. sativum* and *A. vera* extract inhibited the formation of OH^- by enzymic system of hypoxanthine–xanthine oxidase and Fe^{++}. Addition of extract (50-500 µg) inhibited the OH^- mediated formation of 2, 3 dihydroxybenzoate in concentration dependant manner which was 50% at 500 µg/ml of test extract. Furthermore, this preparation , when added with reaction mixture containing Fe^{2+} –Sodium ascorbate- H_2O_2 employed for nonenzymic generation of OH^- inhibited fragmentation of deoxyribose into MDA and this effect was maximum by 38 and 51 % at peak concentration 200 µg/ml of *A. sativum* and *A. vera* extract respectively.

TABLE 4: EFFECT OF *A. SATIVUM* AND *A. VERA* EXTRACT ON GENERATION OF OXYGEN FREE RADICALS *IN VITRO*.

Concentration of extract	Generation of O_2- anions			Generation of OH· Radicals	
	Enzymic System		Non enzymic System (NADH-PMS-NBT-System)[b]	Enzymic System (Sodium Salicylate-FeSO4 HypoXn-XnOD-System)[c]	Non enzymic System ($FeSO_4$– EDTA-H_2O_2-Sodium ascorbate-Deoxy ribose-System)[d]
	(Xn-XnOD-System)[a]	(Xn-XnOD-NBT-System)[b]			
None	45.42± 1.47	112.87 ± 23.70	323.98 ± 17.93	543.89 ± 43.86	28.12 ± 2.19
Concentration of *A. sativum* extract 100µg/ml	37.21 * ± 1.12 (-18)	90.69 * ± 8.47 (-12)	229.91 ± 7.49** (-29)	500.69 ± 14.93* (-11)	21.00 ± 1.97** (-25)
Concentration of *A. sativum* extract 200µg/ml	33.34 ** ± 0.78 (-27)	77.11 ** ± 3.86 (-32)	210.80** ± 12.83 (-35)	410.98** ± 24.67 (-24)	17.44** ± 0.78 (-38)
Concentration of *A. Vera* extract 100µg/ml	29.78** ± 0.76 (-34)	65.65** ± 4.79 (-42)	180.94** ± 4.97 (-44)	359.99** ± 18.32 (-34)	14.98** ± 1.34 (-47)
Concentration of *A. Vera* extract 200µg/ml	23.24** ± 0.36 (-49)	60.44** ± 2.64 (-46)	160.74** ± 11.81 (-50)	318.69** ± 18.53 (-41)	13.77** ± 2.21 (-51)

Values are mean ± SD of four separate observations. The systems added with *A. sativum* and *A. vera* extract were compared with those without adding *A. sativum* and *A. vera* extract separately. *p<0.05, ** p<0.001. Units: a; n mol uric acid formed/min, b; n mol formazon formed/min, c; n mol 2, 3 dihydroxy benzoate formed/hr, d; n mol Malondialdehyde formed/hr.

DISCUSSION: Triton WR-1339 acts as a surfactant and suppresses the action of lipases to block the uptake of lipoproteins from circulation by extra hepatic tissues, resulting in increased blood lipid concentration [20]. The present study show that *A. sativum* and *A. vera* possess antidyslipidemic and antioxidant activities altogether.

In the present study, *A. sativum* and *A. vera* were tested for their anti-dyslipidemic and antioxidant activities in two models of hyperlipidemia, triton and cholesterol rich high fat diet induced hyperlipidemia. Lipases play a significant role in lipoprotein metabolism and decreased lipoprotein lipases activities are main cause of atherosclerosis [30]. However, treatment with *A. sativum* and *A. vera* reversed these effects. *A. sativum* and *A. vera* could increased the level of HDL by increasing the activity of LCAT, which might contribute to the regulation of blood lipids. LCAT play a key role in lipoprotein metabolism and most of the lipoprotein changes are the outcome of primary abnormality owing to the diseases related with lipid metabolism [31]. *A. sativum* and *A. vera* extract enhanced the excretion of bile acids through feces and this contributed to regress the cholestesteosis in liver damage.

The dyslipidemia and oxidative stress are the important etiologic factors implicated in the development of variety of complications. To overcome these ailments, as drug having multifold properties such as lipid lowering and anti-oxidant activities together is in great demand. *A. sativum*, *A. vera* and gemfibrozil caused a significant decrease in the serum level of lipids in triton

induced hyperlipidemic rats. The present investigation with HFD fed hyperlipidemic animals shows that *A. sativum* and *A. vera* could increase the level of HDL by increasing the activity of LCAT, which plays a key role in lipoprotein metabolism. The stimulation of lipolytic activity in liver and the increase in the level of blood HDL-TC followed by the decrease of β-lipoprotein-lipids and the decrease in hepatic lipid levels by these extracts are of great utility for regressing atherosclerosis. The extract of *A. sativum* and *A. vera* and gemfibrozil caused significant decrease in the plasma levels of serum lipids in streptozotocin induced hyperglycemic rats. Treatment of hyperlipidemia with available lipid lowering drugs fibrates,statins and bile acid sequestrants are not free from many side effects such as myositis, arthralgias gastrointestinal upset along with elevated hepatic and renal function tests [32].

In general oxidative damage takes place in LDL of plasma by the hydroxyl radicals (OH) generated by the metal ions present in the serum due to alterations in their oxidation states. It has been observed that oxidative damaged LDL are relatively more atherogenic than the native LDL. Currently several drugs being used for dyslipidemia intervene by lowering cholesterol (LDL and total cholesterol) or by lowering triglyceride in plasma. These extracts may also enhance the synthesis of LDL apoprotein (ApoB) as well as receptor protein to accelerate protein decreased the rate of hepatic lipid synthesis and inhibition of oxidative modifications of LDL may regulate the cholesterol level in the body. It is increasing evidence that it involves the regeneration of islet β-cells by neutralization of cytotoxic free radicals. The abnormal high concentration of serum lipids in diabetes is mainly due to the increase in the mobilization of free fatty acids from the peripheral depots, since insulin deficiency promotes the hormone sensitive lipases [33].

The lipid lowering activity of *A. sativum* and *A. vera* might be due to inhibition of hepatic cholesterol biosynthesis, activation of tissue lipases and these beneficial effects may be due the bioactive compounds like typical alkaloids and S-allylcysteine sulfoxide present in *A. sativum* and *A. vera*.

CONCLUSION: It is concluded that *A. sativum* as well as *A. vera* has the regulatory effect on the lipolytic activities of plasma and liver in hyperlipidemic conditions and they also possess the power of regulating the faecal excretion of bile acids. Treatment with these test compounds also caused reversal in the levels of total cholesterol, phospholipids, triglycerides and free fatty acids in dyslipoproteinemia. The outcomes of the present study suggest that the extracts of these two plants can contribute their potential as antidyslipidemic and antioxidant drugs to the world of natural products in the field of dyslipoproteinemia. It should be pointed out here that plant derived natural compounds have established a proven platform for developing new drug synthesis with fewer side effects. Our study validates a strong antioxidant and hypolipidemic activities of *A. sativum* and *A. vera* in hyperlipidemic rats.

CONFLICT OF INTEREST STATEMENT: The authors declare that they have no conflict of interest.

ETHICAL APPROVAL: This article does not contain any studies with human participants performed by any of the authors. The study was approved by the Institutional Animal Ethics Committee of Central Drug Research Institute and was carried out in accordance with the current guidelines set by Organization for Economic Co-operation and Development (OECD), received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India for the care of laboratory animals

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