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Effect of Lovastatin on Lipoprotein Lipid Peroxidation and Antioxidant Status in Inflammation Induced Hyperlipidemic Rats

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ABSTRACT

Cardiovascular diseases (CVD) are the major contributor to the global burden of disease. Coronary heart disease, cerebrovascular disease, hypertension, artery disease, rheumatic heart disease, congenital heart disease and failure of heart came under the category of CVD. Epidemiological studies have suggested a link between atherosclerosis, infection and inflammation. Atherosclerosis is a multifaceted disease process with several different well defined risks factors, such as hypercholesterolemia, hypertension and diabetes. In this study we investigate the efficacy of hypolipidemic, anti-atherogenic and antioxidant agent Lovastatin by analyzing all the parameters in plasma, Total lipid, TC, TG, VLDL-C, LDL-C, non-HDL-C, MDA and Hepatic TG, TC as well as hepatic antioxidant enzymes (Catalase, Superoxide dismutase, Glutathione peroxidase and Glutathione reductase). All the plasma lipids parameters as well as hepatic antioxidant enzymes level were significantly increased/decreased in inflammation induced hyperlipidemic (IIH-C) rats. After 4-weeks administration of Lovastatin significantly restore the above altered parameters. In conclusion, Lovastatin may be useful in the prevention and treatment of inflammation induced hyperlipidemia, CVD and atherosclerosis.

Keywords: CVD, Hypercholesterolemia, Hypolipidemic, Inflammation, Atherosclerosis Lovastatin and Hepatic antioxidant enzymes**.**

INTRODUCTION

Cardiovascular diseases (CVD) are the major contributor to the global burden of disease. (CVD) is the number one cause of death worldwide [1, 2, 3] and is projected to remain the leading cause of death. Hence, this disease greatly contributes to the rising costs of health care in the world. It

is a major public-health challenge, especially in low and middle income countries, where 80 % of these deaths occur [4]. Coronary heart disease, cerebrovascular disease, hypertension, artery disease, rheumatic heart disease, congenital heart disease and failure of heart came under the category of CVD. It is widely recognized that atherosclerosis is an inflammatory disorder. Moreover, recent epidemiological studies have strongly suggested that disorders that lead to systemic inflammation increases the risk of developing CVD. Atherosclerosis is a multifaceted disease process with several different well defined risks factors, such as hypercholesterolemia, hypertension and diabetes. Hyperlipidemia refers to increased levels of lipids (fats) in the blood, including cholesterol and triglycerides. Approximately two-thirds of the blood cholesterol is found in the LDL fraction and higher LDL cholesterol concentrations have been associated with an increased incidence of coronary artery disease. Infection and inflammation induce the systemic host response known as acute phase response (APR), and produce many abnormalities that could increase the risk of developing atherosclerosis. In animal models, Infection and inflammation are produced by administration of turpentine or croton oil (acute localized sterile inflammation). Each of these stimuli is a well-characterized inducer of APR. [5, 6, 7, 8, 9] APR represents the initial line of defense against injury as well as bacterial and parasitic infections. The APR has been shown to reduce the expression of certain conjugation enzymes as well as antioxidant enzymes. Cytokines, secreted from macrophages in response to infection and inflammation, have been implicated in the suppression of activity of these enzymes in addition to their role in oxidative stress. These enzymes are important in defending the body against free radicals as well as toxic substances by converting them to a form that can be readily excreted. Therefore, any changes in these enzymes could be potentially detrimental to the host by altering these defense mechanisms. Normal cellular metabolism involves the production of ROS [10], low levels of ROS are vital for proper cell functioning, while excessive *in vivo* generation of these products can adversely affect cell functioning [11, 12]. The body has the ability to produce endogenous antioxidants such as Superoxide dismutase, Catalase and Glutathione peroxidase. Under normal circumstances, there is a balance between these endogenous antioxidants and the production of free radicals in the body. It has been reported that endotoxin injection caused renal tissue damage and decreased the SOD, Gpx and CAT activities compared to control rats [13, 14]. A significant decrease in hepatic GSH as well as in enzymatic activities of SOD, CAT, Gpx and GST were observed in d-galactosamine/lipopolysaccharide-intoxicated rats as compared with the levels of normal control rats [15]. Oxidative modification of lipoproteins is believed to play a central role in the pathogenesis of atherosclerosis [16, 17] because plasma contains several antioxidants [18] and lipoproteins with oxidative damage have been isolated from atherosclerotic lesions,[16, 17]enzymatic antioxidant defenses include Superoxide dismutase (SOD), Glutathione peroxidase (GPx), Catalase (CAT). To protect the cells and organ systems of the body against reactive oxygen species, humans have evolved a highly complex antioxidant protection system. These include antioxidant enzymes that catalyze free radical quenching reactions, and diet-derived antioxidants. Lipid-lowering drugs such as statins have also been shown to antagonize inflammation [4, 19, and 20] they lower cholesterol by inhibiting the enzyme HMG-CoA reductase, which is the rate-limiting enzyme of the mevalonate pathway of cholesterol synthesis. Inhibition of this enzyme in the liver results in decreased cholesterol synthesis as well as increased synthesis of LDL receptors, resulting in an increased clearance of low-density lipoprotein (LDL) from the bloodstream. In this study we investigate the efficacy of anti-atherogenic hypolipidemic and antioxidant agent Lovastatin by analyzing all the parameters in plasma TC, VLDL-C, LDL-C, HDL-C, and its sub fraction HDL2-C and HDL3-C, MDA, Hepatic TG, TC and antioxidant enzymes (Catalase, Superoxide dismutase, Glutathione peroxidase and Glutathione reductase).

EXPERIMENTAL SECTION

Chemicals:-1- Chloro 2, 4-Dinitrobenzene was purchased from Central drug house, Pvt. Ltd. (India). All other chemicals used for this study were of analytical grade and obtained from HIMEDIA (India), Sisco (India), Ashirwad (India), Sigma-Aldrich (USA), Miles (USA), Acros (USA) and Lovastatin drug was supplied as a gift from Saimira Innoform Pvt. Ltd. Chennai, India.

Estimation: Fractionation of Plasma lipoprotein such as LDL[21], HDL and its fractions-HDL_2, $HDL_{3[}$22], Plasma FRAP[23], determination of triglyceride and total cholesterol in liver homogenate[24], activities of antioxidant enzymes such as Catalase[25], Superoxide dismutase[26], Glutathione peroxidase [27] and Glutathione reductase [28] in liver homogenate were measured by following known procedures.

Experimental Design: The experimental study was approved by the Dolphin Institute of Biomedical and Natural Sciences, Dehradun, Uttarakhand, where the study was conducted. Healthy male albino rats, weighing about 150-180 g were purchased from Indian Veterinary Research Institute (IVRI), Bareilly (India), were maintained to animal house environmental condition prior to the experiment. For the present study, animals were divided into following 3 groups:

Normal Control (NC); six rats were given 1.0 ml saline/rat/day through gastric intubation for 4 weeks, inflammation induced hyperlipidemic Control (IIH-C) rats; six rats in this group were administered 1.0 ml saline/rat/day through gastric intubation for 4 weeks, Inflammation induced hyperlipidemic Lovastatin treated rats (IIH-LT); six rats in this group were given 1.0 mg Lovastatin/rat/day through gastric intubation for 4 weeks.

Diet/Drug Administration:-The rats were given pelleted rat chow. Maintenance and treatment of all the animals was done in accordance with the principles of Institutional Animal Ethics Committee constituted as per the directions of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. Six rats in IIH-LT group were given 1.0 mg Lovastatin/rat/day, through gastric intubation for 4 weeks.

Induction of Inflammation: - Inflammation was induced in IIH-C and IIH-LT group by the subcutaneous injection of turpentine (0.5ml/rat) in the dorsolumbar region and left for five hours.

Collection of Blood and Plasma: For the estimation of different parameters, overnight fasted rats in each group were anaesthetized and blood drawn from cardiac puncture, and were collected in heparinised tube. Plasma was separated from blood by centrifugation at 2500 rpm for 30 min.

Total cholesterol and triglyceride estimation in liver homogenate:- Liver were excised and chilled in ice cold saline. Weight of all liver was taken only after drying the tissue. The volume of each homogenate was recorded and centrifuged at 1000 rpm for 10 min at 4^0C. After centrifugation, a portion of each homogenate from liver thus obtained was used for the estimation of total cholesterol and triglyceride content in it.

Statical evaluation: This was done by employing two-tailed student t-test as describe by Bennet and Franklin (1967). P values less than 0.02 were considered significant.

RESULT

Effects of Lovastatin on average body weight in each group of rats:-Table-1 depicts the average body weight (g) of N-C, IIH-C, IIH-LT was 165g, 170g and 176g, whereas, the average body weight of N-C, IIH-C, IIH-LT rats showed a significant gain of 34%, 13% and 42% respectively after 4 weeks of treatment. These results demonstrate that in inflammation induced hyperlipidemic lovastatin treated (IIH-LT) rats the gain in body weight after 4 weeks were significantly higher than N-C rats.

Table-1 Average body weight in each group of rats before and after 4 weeks of Lovastatin treatment

Average body weight/rat (g)		
Group	Before treatment	After Treatment
N-C	165.52±2.13*	223.25±12.23 (+34.87%)
IIH-C	170.53±4.11*	194.05±9.45 (+13.79%)[b]
IIH-LT	176.23±4.72*	250.44±11.15 (+42.10%)[a]

**Values are mean ± SD from 6 rats in each group. N-C normal Control; IIH-C inflammation induced hyperlipidemic control rats; IIH-LT fed 1mg Lovastatin/rats/day for 4 weeks, Significantly different from N-C at [b]p<0.001. Significantly different from IIH-C at [a]p<0.001*

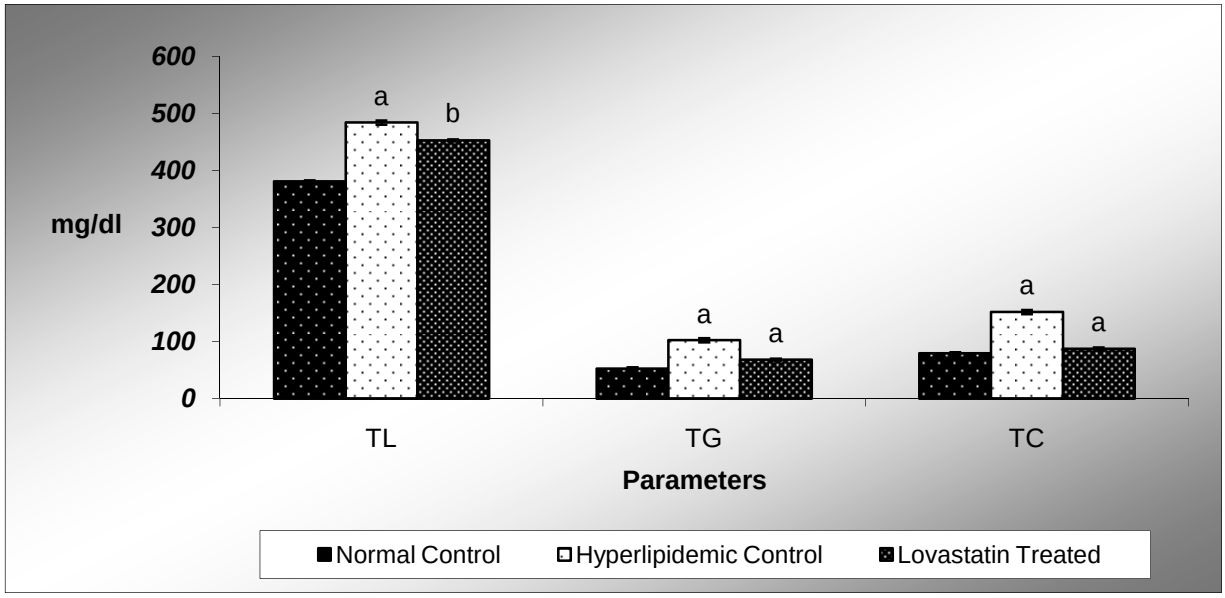

Fig.1 Impact of Lovastatin on, plasma Total lipid (TL), Triglycerides (TG) and Total Cholesterol (TC) in inflammation induced hyperlipidemic rats after 4 weeks of treatment.

**Values are mean (mg/dl) ± SD from pooled plasma of 6 rats in each group. N-C, normal control; IIH-C, Inflammation induced hyperlipidemic control rats; IIH-LT fed 1 mg Lovastatin/rat/day for 4 weeks. Significantly different from N-C at [a]p < 0.001, Significantly different from IIH-C at [a]p<0.001 and [b]p<0.05.*

Effects of Lovastatin on plasma total lipid (TL), triglycerides (TG) and total cholesterol (TC) in inflammation induced hyperlipidemic rats after 4 weeks of treatment: As seen in Fig 1, all the plasma lipids parameters were significantly increased in Inflammation induced hyperlipidemic (IIH-C) rats, when compared to N-C values. Total lipids (TL), triglycerides (TG) and total cholesterol (TC) significantly increased from 380, 52, and 79 mg/dl in N-C to 483, 102,

and 151 mg/dl, respectively, in IIH-C group. After 4 weeks of Lovastatin treatment, levels of TL, TG, and TC were significantly decreased by 6 %, 33 %, and 42 %, respectively, when compared to corresponding N-C values. These results demonstrate that 4-week treatment of inflammation induced hyperlipidemic rats with 1.0 mg Lovastatin mediated a significant reduction in above lipid parameters.

Effects of Lovastatin on plasma lipoprotein fraction and the ratios of LDL-C/HDL-C and HDL-C/TC: As seen in Fig 2, plasma VLDL-C, LDL-C and non-HDL-cholesterol (non-HDL-C) levels were significantly increased from 9 mg/dl, 52 mg/dl and 63 mg/dl in N-C to 20 mg/dl (112%), 110 mg/dl (111 %) and 125 mg/dl (98 %) respectively in IIH-C. After 4 weeks of Lovastatin treatment, both VLDL-C, LDL-C and non-HDL-C levels showed a significant reduction 39 %, 49 % and 44 %, respectively, in IIH-LT. Whereas HDL-C, HDL_2-C and HDL_3-C levels were decreased from 19, 6 and 10 mg/dl in IIH-C to 16 mg/dl (15 %), 4 mg/dl (34 %) and 9 mg/dl (5 %), respectively, in IIH-C values. After 4 weeks of Lovastatin treatment (IIH-LT) HDL-C, HDL_2-C and HDL_3-C levels showed a significant increase of 54 %, 122 % and 40 %, respectively, when compared to corresponding values in IIH-C. These results demonstrate that Lovastatin is effective in reducing VLDL-C and LDL-C levels. On the other hand, in comparison to IIH-C values, treatment of Inflammation induced hyperlipidemic rats with Lovastatin mediated a significantly higher increase in HDL-C, HDL_2-C and HDL_3-C concentration.

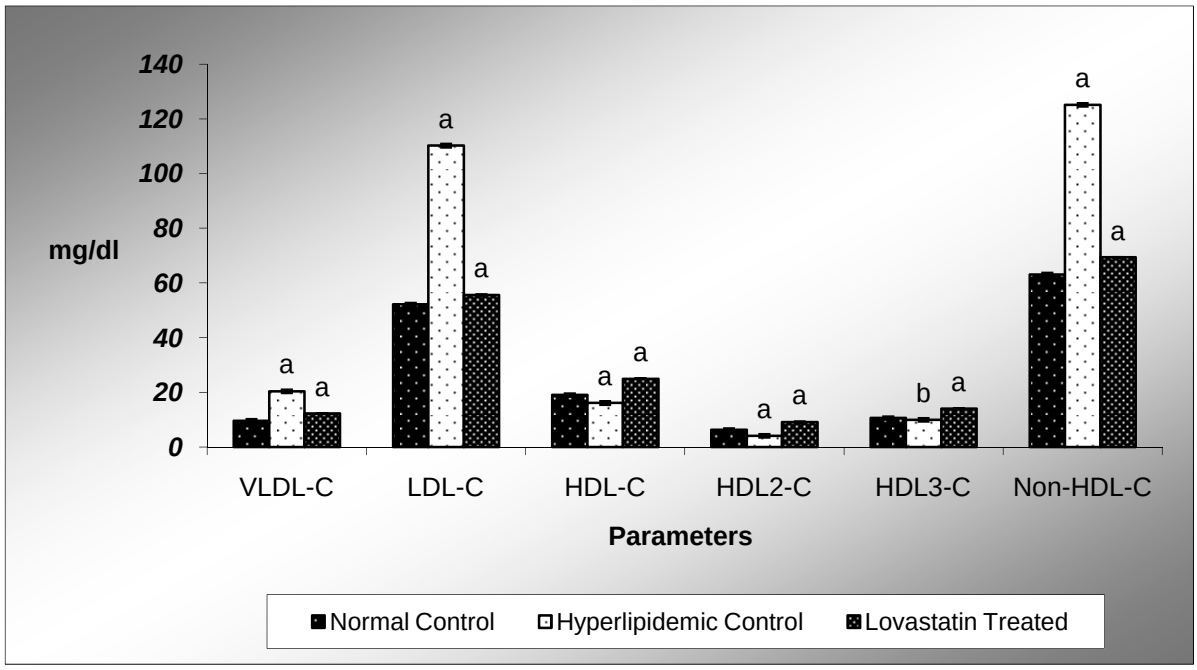

Fig 2 Impacts of Lovastatin on plasma VLDL-C, LDL-C, HDL-C, HDL2-C, HDL3-C and Non-HDL-C in inflammation induced hyperlipidemic rats after 4 weeks of treatment

Values are mean (mg/dl) ±SD from pooled plasma of 6 rats in each group, N-C, normal control; IIH-C, Inflammation induced hyperlipidemic control rats; IIH-LT fed 1 mg Lovastatin/rat/day for 4 weeks, Significantly different from N-C at $^{a}p<0.001$ and $^{b}p<0.02$, Significantly different from IIH-C at $^{a}p<0.001$.

On the other hand, LDL-C/HDL-C and HDL-C/TC ratios were calculated from the data presented in Table 2 and 3. LDL-C/HDL-C ratio was significantly increased from 2.73 in N-C to 6.83 (150 %) in IIH-C group, when compared to ratio in N-C. After 4 weeks of treatment, the increase in LDL-C/HDL-C ratio was significantly prevented and decreased to 2.22 in IIH-LT, which is close to normal control value. HDL-C/TC ratio was significantly decreased from 0.240 in N-C to 0.106 (55 %) in IIH-C group, as seen in Table 2. Lovastatin treatment to these rats

significantly prevented the increase in HDL-C/TC ratios and fully restored them to a ratio value similar to N-C.

Table 2 Impacts of Lovastatin on the ratio of LDL-C/HDL-C, HDL-C/TC, in inflammation induced hyperlipidemic rats after 4 weeks of treatment.

Parameters	N-C	IIH-C	IIH-LT
LDL-C/HDL-C	2.73±0.023**	6.83±0.043** (+150.18 %)[a]	2.22±0.011** (-67.49 %)[a]
HDL-C/TC	0.240±0.027**	0.106±0.018** (-55.83 %)[b]	0.285±0.019** (+62.80 %)[a]

For the calculation of ratio, data have been taken from fig 1 and 2.
Values are mean (mg/dl) ± SD from pooled plasma of 6 rats in each group, N-C, normal control; IIH-C, Inflammation induced hyperlipidemic control rats; IIH-LT fed 1 mg Lovastatin/rat/day for 4 weeks, Significantly different from N-C at [a]p<0.001, Significantly different from IIH-C at [a]p<0.001.

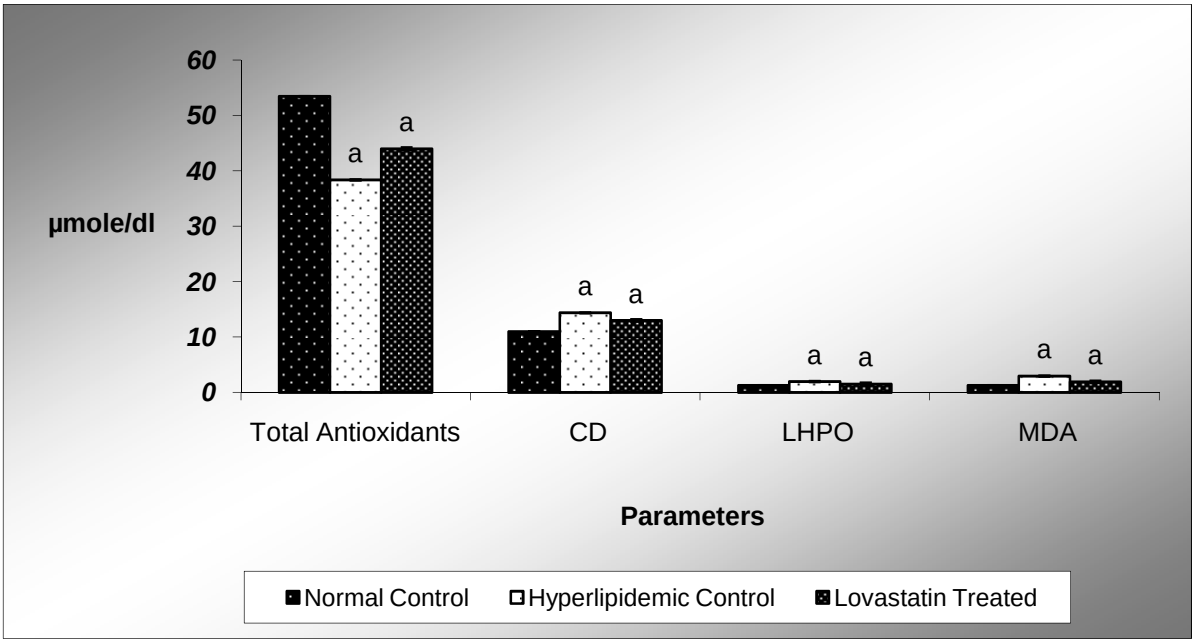

Fig.3 Impact of Lovastatin on plasma total antioxidant, Conjugated diene (CD), Lipid hydroperoxide (LHPO) and Malondialdehyde (MDA) contents in inflammation induced hyperlipidemic rats after 4 weeks of treatment

Values are mean (µmole/dl) ± SD from pooled plasma of 6 rats in each group. N-C normal Control; IIH-C inflammation induced hyperlipidemic control rats; IIH-LT feed 1mg Lovastatin /rats/day for 4 weeks. Significantly different from N-C at [a] p< 0.001.Significantly different from IIH-C at [a]p<0.001.

Effects of Lovastatin on plasma total antioxidants and lipid peroxidation products:-Fig-3 depicts the antioxidant impact of lovastatin on plasma concentrations of total antioxidants, conjugated diene (CD), lipid hydroperoxide (LHPO) and malondialdehyde (MDA) in inflammation induced hyperlipidemic rats. In IIH-C rats, plasma total antioxidants level was reduced from a control value of 53 to 38 (27%) µmole/dl. Treatment of IIH-LT rats with Lovastatin for 4 weeks resulted in a significant increase of total antioxidants levels by 13 % when compared to IIH-C value. The oxidative stress induced in IIH-C rats significantly enhanced plasma lipid peroxidation products, such as conjugated diene, lipid hydroperoxide and MDA. Formation of conjugated diene, lipid hydroperoxide and MDA in plasma was increased from 10.97, 1.26 and 1.29 in N-C to 14.38 (31 %), 1.98 (57 %) and 2.96 (129 %) µmole/dl,

respectively, in IIH-C. After Lovastatin treatment, in IIH-LT, a significant decrease of 9 %, 22 %and 35 % was seen in the formation of conjugated diene, lipid hydroperoxide and MDA, respectively, when compared to corresponding values in IIH-C rats. These results demonstrate that in IIH-C rats, due to increase in oxidative stress, total antioxidants level was decreased, whereas, concentration of plasma conjugated diene, lipid hydroperoxide and MDA were significantly increased. Tocotrienols treatment significantly restored the total antioxidants level and blocked the increase in plasma conjugated diene, lipid hydroperoxide and MDA to a level close to corresponding normal values.

Lovastatin effect on Triglycerides (TG), Total Cholesterol (TC) and various Lipid peroxidation products in the Liver homogenate:-As seen in Table 3, hepatic levels of triglyceride (TG) and total cholesterol (TC) were significantly increased in inflammation induced hyperlipidemic control rats (IIH-C) by 38 % and 113 % respectively, when compared to corresponding values in N-C. Feeding of lovastatin inflammation induced rats for 4 weeks was associated with a significant decline in liver TG and TC levels by 9 % and 29% respectively, in IIH-LT. On the other hand, formation of conjugated diene, lipid hydroperoxide and MDA in liver of inflammation induced hyperlipidemic (IIH-C) rats was significantly increased by 47%, 34 % and 42 %, respectively. Feeding of lovastatin to IIH-LT rats for 4 weeks, was associated with a significant decline in the formation of liver conjugated diene, lipid hydroperoxide and MDA by 26 %, 21 % and 20 % respectively, when compared to corresponding values in IIH-C group. These results demonstrate that increased levels of TG, TC, conjugated diene, lipid hydroperoxide and MDA in liver of inflammation induced hyperlipidemic rats were significantly reduced after 4 weeks of lovastatin treatment.

Table 3 impact of Lovastatin on triglycerides, total cholesterol and various lipid peroxidation products in the Liver homogenate after 4 weeks treatment of inflammation induced hyperlipidemic rats.

Parameter	NC	IIH-C	IIH-LT
Triglycerides*****	0.493±0.001*	0.685±0.006* (+38.94%)^{a}	0.623±0.003* (-9.05%)^{a}
Total cholesterol*****	1.34±0.034	2.86±0.018 (+113.43%)^{a}	2.02±0.011 (-29.37%)^{a}
Conjugated diene******	4.97±0.020*	7.32±0.020* (+47.28%)^{a}	5.35±0.005* (-26.91%)^{a}
Lipid Hydroperoxide******	0.952±0.001	1.280±0.002 (+34.45%)^{a}	1.002±0.018 (-21.71%)^{a}
MDA******	2.25±0.013	3.20±0.083 (+42.22%)^{a}	2.55±0.011 (-20.31%)^{d}

*******Values are mean mg/100mg protein ± SD from homogenate of pooled liver of 6 rats in each group.* ******** *Values are mean µmole/dl ± SD from homogenate of pooled Liver of 6 rats in each group. N-C normal control; IIH-C inflammation induced hyperlipidemic control rats; IIH-LT feed 1 mg of Lovastatin/rats/day for 4 weeks, significantly different from N-C at $^{a}p<0.001$ and $^{b}p<0.001$, significantly different from IIH-C at $^{a}p<0.001$.*

Effects of Lovastatin on the various antioxidant enzymes activities in the liver homogenate:- As seen in Table 4, Catalase (CAT) activity in liver was significantly decreased from a value of 3.07 unit in N-C to 2.30 (37 %) in IIH-C, respectively. Administration of Lovastatin to inflammation induced hyperlipidemic lovastatin treated rats (IIH-LT) resulted in a significant increase in liver CAT activities by 3.20 (39 %) unit, respectively. However, in comparison to corresponding tissue values of normal control rats (N-C), the decline in hepatic Superoxide dismutase (SOD) activity of inflammation induced hyperlipidemic (IIH-C) rats was 26 %.Treatment of lovastatin to inflammation induced hyperlipidemic lovastatin treated (IIH-

LT) rats resulted in a significant increase in hepatic SOD activity by 22 %, respectively from normal value. In inflammation induced rats, Glutathione peroxidase (Gpx) activity in liver was significantly increased from a value of 48 units in N-C to 61 (27 %) units, in IIH-C rats. As evident, after 4 weeks of treatment with lovastatin, Gpx activity in liver was significantly decreased by 32 %, when compared to corresponding tissue values in IIH-C group. On the other hand, in smoke exposed rats, the enzymatic activities of hepatic Glutathione reductase (Gred) was decreased significantly by 37 %, when compared to corresponding values of N-C rats. Feeding of lovastatin to inflammation induced hyperlipidemic rats significantly blocked the decrease in hepatic Gred activities and increased them to a similar value of 36 %, Compared to corresponding values of Gred activities in N-C. Administration of lovastatin to smoke exposed rats significantly prevented the decrease in Gred activity and increased to a level, which is similar to normal value. In summary, hepatic CAT, SOD, Gpx and Gred enzymes, which constitute a mutually supportive team of defense against ROS, are significantly decreased in inflammation, induced hyperlipidemic rats. However, lovastatin treatment to inflammation induced hyperlipidemic rats substantially quenches these free radicals (ROS), thus positively normalizing the above enzyme levels.

Table 4 Impact of Lovastatin on Liver Catalase, Superoxide dismutase, Glutathione peroxidase and Glutathione reductase activities in inflammation induced hyperlipidemic rats after 4 weeks of treatment †One unit(U/mg protein) of enzyme activity is defined as the µmoles of H_2O_2 decomposed/min/mg protein. ††One unit (U/mg protein) of enzyme activity is defined as the amount of enzyme required to inhibit O.D. at 560 nm of chromogen production by 50%in one minute. $^{\beta}$One unit (U/mg protein) of enzyme activity is defined as nmole oxidized Glutathione formed /min/mg homogenate protein. ‡One unit (U/mg protein) of enzyme activity is defined as nmole NADPH oxidized/min/mg PMS protein.

Group	Catalase†	Superoxide dismutase††	Glutathione peroxidase$^{\beta}$	Glutathione Reductase‡
N-C	3.70±0.123*	0.755±0.003	48.49±1.02*	8.45±0.198
IIH-C	2.30±0.201* (-37.83 %)^{a}	0.552±0.002 (-26.88%)^{a}	61.56±1.41* (+27.08%)^{a}	5.26±0.231 (-37.75%)^{a}
IIH-LT	3.20±0.013* (+39.13 %)^{a}	0.665±0.005 (+22.47 %)^{a}	41.38±1.74* (-32.78%)^{a}	7.18±0.199 (+36.50%)^{a}

***Values are mean ± SD from homogenate or PMS fraction of pooled liver of 6 rats in each group, N-C, normal control; IIH-C inflammation induced hyperlipidemic control rats and IIH-LT fed 1 mg Lovastatin/rats/day for 4 weeks. Significantly different from N-C at $^{a}p<0.001$. Significantly different from IIH-C at $^{a}p<0.001$.*

DISCUSSION

Several epidemiological studies suggest a link between infection/inflammation and atherosclerosis. The present study demonstrates the extensive proatherogenic changes that occurred as a part of the host response to turpentine (acute localized sterile inflammation) administration, on a variety of parameters, like, plasma and lipoprotein lipids in plasma, liver lipid peroxidation products, liver and plasma total antioxidant. Pretreatment of stressed rats with Lovastatin significantly reduced the overall oxidative burden and effectively ameliorated the above altered parameters, thus, indicating a potent atheroprotective effect of Lovastatin. The change in lipids and lipoproteins are similar to those proposed to promote atherogenesis, they may initiate or aggravate atherosclerosis if the course of infection or inflammation is prolonged [30, 31]. Our results demonstrate a significant increase in plasma total lipids (27%), TG (94%) and TC (90%) in turpentine (IIH-C) stressed rats. A similar increase in serum TG in LPS treated hamsters or rats were previously reported [5]. In another report an increase in plasma TG level was seen during inflammation, induced by turpentine oil in pigs [32]. The increase in plasma TG

levels is apparently due to an increase in VLDL (112%) which can be the result of either increased VLDL production or decreased VLDL clearance. Similar to plasma lipids, VLDL-C, LDL-C and atherogenic non-HDL-C levels were also increased in stressed animals, indicating that the increase in plasma TC is apparently due to increase in VLDL-C and LDL-C concentrations. On the other hand, a low decrease of 15 % in plasma antiatherogenic HDL-C level of IIH-C rats was seen. Similar to plasma TG and TC in liver was also significantly increased in IIH-C rats. Low density lipoproteins are composed of distinct subclasses that differ in size, density, chemical composition, and their association with cardiovascular disease [33, 34, and 35]. It has been established that LDL-C/HDL-C and HDL-C/TC ratios are good predictors for the presence and severity of CAD [36]. LDL-C/HDL-C and HDL-C/TC ratios were calculated from the data presented in fig1 and fig2. LDL-C/HDL-C ratio was significantly increased from 2.73 in N-C to 6.83 (150 %) in IIH-C group, when compared to ratio in N-C. After 4 weeks of treatment, the increase in LDL-C/HDL-C ratio was significantly prevented and decreased to 2.22 in IIH-LT, which is close to normal control value. On the other hand, HDL-C/TC ratio was significantly decreased from 0.240 in N-C to 0.106 (55 %) in IIH-C group. Lovastatin treatment to these rats significantly prevented the increase in HDL-C/TC ratios and fully restored them to a ratio value of 0.285 which is close to N-C. In addition, the ratios related to HDL-C in Lovastatin treated rats were positively modulated and restored similar to normal control value, indicating normalization of cholesterol levels associated with the above lipoproteins Oxidative modification of lipoproteins is believed to play a central role in the pathogenesis of atherosclerosis [16, 17] Similar to plasma TG and TC in liver was also significantly increased in IIH-C rats. Therefore, tocotrienols may exert their cholesterol lowering effect in inflammation /infection induced hyperlipidemic rats in a similar manner as previously reported for hyperlipidemic animals [37] and humans [38, 39]. Mechanism wise, as previously shown in HepG2 cells, as well as in normolipidemic and hyperlipidemic rats, tocotrienols reduce cholesterol synthesis by suppressing HMG-CoA reductase activity, which in turn is reduced by a decline in its protein mass [37, 40] The decline in protein mass may be achieved by inhibition of HMG-CoA reductase synthesis and/or enhanced degradation. Consistent with *in vivo* results in rats [37], γ-tocotrienol has been shown to mediate the suppression of enzymatic activity and protein mass of HMG-CoA reductase in HepG2 cells through decreased synthesis (57 % of control) and enhanced degradation (2.4-fold versus control) of the enzyme [40]. In addition, γ-tocotrienol was shown to up regulate LDL receptor in mammalian cells and may be implicated in part for the reduction of apoB-lipoprotein *in vivo* [40]. Thus, tocotrienols reduce cholesterol formation in mammalian cells by suppressing HMG-CoA reductase activity through two actions: decreasing the efficiency of translation of HMG-CoA reductase mRNA and increasing the controlled degradation of HMG-CoA reductase protein, post-transcriptionally [40]. In addition, another report indicates that γ-tocotrienol influences apoB secretion by both co translational and posttranslational processes involving a decreased rate of apoB translocation and accelerated degradation of apoB in HepG2 cells. This activity correlated with a decrease in free and esterified cholesterol [41]. Taken together, the information indicates an association between the suppression of hepatic cholesterol synthesis and apoB secretion, and the observed lowering of apoB and LDL-C levels in animal and human models [41]. Our data show that systemic oxidation of lipid/lipoprotein particles occurs as a part of the host response to infection and inflammation. Conjugated diene (which measure the initial phase of lipid peroxidation), lipid hydroperoxide (intermediate product of lipid peroxidation) and MDA (which measure the degradation phase of lipid peroxidation) in plasma and liver are significantly increased in rats after, turpentine administration. The increase in plasma lipid peroxidation products is associated with a significant decline in total antioxidants capacity of plasma. Administration of turpentine (acute localized sterile inflammation) generally leads to fulminant release of reactive oxygen species (ROS) [42, 43]. Our results indicate a significant decrease in plasma lipid peroxidation

products with a concomitant and significant increase in plasma total antioxidants in IIH-C group, pretreated with 1.0 mg Lovastatin/day for 28 days before turpentine injection. The acute phase response represents the initial line of defense against injury as well as bacterial and parasitic infections. Turpentine which elicits the APR has been shown to reduce the expression of certain conjugation enzymes as well as antioxidant enzymes. Cytokines, secreted from macrophages in response to infection and inflammation, have been implicated in the suppression of activity of these enzymes in addition to their role in oxidative stress. These enzymes are important in defending the body against free radicals as well as toxic substances by converting them to a form that can be readily excreted. Therefore, any changes in these enzymes could be potentially detrimental to the host by altering these defense mechanisms. Normal cellular metabolism involves the production of reactive oxygen species (ROS) [10], low levels of ROS are vital for proper cell functioning, while excessive *in vivo* generation of these products can adversely affect cell functioning [11, 12] Malondialdehyde (MDA) is one of the final products of lipid peroxidation in human cells, and an increase in ROS causes overproduction of MDA, which is considered a surrogate marker of oxidative stress [44, 45]. The major intracellular antioxidant enzyme, superoxide dismutase (SOD), specifically converts superoxide radicals to hydrogen peroxide, [46] and catalase (CAT) as well as glutathione peroxidase (Gpx) detoxifies hydrogen peroxide to water [47]. Gpx protects against free radical injury by reducing the peroxide concentration via a glutathione dependent reduction process, thereby reducing the amount of peroxides available to produce cellular damage. Reduced glutathione is a major intracellular non-enzymatic antioxidant. It has many biological functions, including maintenance of membrane protein and lipoprotein SH groups in the reduced form, the oxidation of which can otherwise cause altered cellular structure and function. Glutathione cycle operates in the erythrocytes for the disposal of H_2O_2 generated in the cell supplementing the function of catalase. GSH and H_2O_2 are twin substrates for glutathione peroxidase. GSH is formed from its oxidized form, GSSG by the enzyme glutathione reductase (Gred), which requires NADPH as a cofactor. Therefore, as the balance between free radical production and antioxidant defenses is lost, the resultant oxidative stress through a series of events deregulates the cellular functions leading to various pathological conditions. An antioxidant compound might contribute partial or total alleviation of such damage. An impaired ROS scavenging function has been linked to the decreased activity of enzymatic and nonenzymatic scavengers of free radicals. Our results demonstrate that, catalase activity in liver was significantly decreased from a value of 3.70 unit in N-C to 2.30 (37 %) in IIH-C, respectively. Administration of Lovastatin to Inflammation induced hyperlipidemic Lovastatin treated rats (IIH-LT) resulted in a significant increase in liver catalase activities by 3.20 (39 %) unit, respectively. However, in comparison to corresponding tissue values of normal control rats (N-C), the decline in hepatic SOD activity of Inflammation induced hyperlipidemic (IIH-C) rats was 26 %. Treatment of Lovastatin to Inflammation induced hyperlipidemic Lovastatin treated (IIHC-LT) rats resulted in a significant increase in hepatic SOD activity by 22 %, respectively from normal value. These results are consistent showing a decrease in CAT activity and an increase in Gpx activity in LPS treated rats [48]. Lovastatin given 28 days before the onset of infection and inflammation significantly improved the integrity of erythrocytes membrane as shown by improved protection against lipid peroxidation as well as reversal of SOD, CAT, Gpx, and Gred to near normal level. The above results, which represent an initial demonstration, indicate that the host response to infection and inflammation induces several changes: hyperlipidemia, enhanced lipid peroxidation in plasma and tissues, with depletion in plasma total antioxidants, and overall weakening of antioxidant defense system. LDL particles with a greater tendency to become oxidized might thus be more likely to participate in proatherogenic events. Increased oxidation of LDL density subfractions that occurs during infection and inflammation could be one of the mechanisms that may promote atherosclerosis in patients with chronic infection and inflammatory diseases. Oral pretreatment of rats with

Lovastatin for 28 days significantly prevented the turpentine induced adverse effects and ameliorated the levels of all the evaluated parameters. Our results strongly suggest that the alleviation of inflammatory conditions is due to potent lipid lowering and free radical scavenging properties of Lovastatin and, thus, can be useful in the therapy of systemic inflammatory process which might induce atherosclerosis. Based on these findings, the antiinflammatory potential of Lovastatin looks promising and more comprehensive studies should be undertaken to determine their actual mode of action. In conclusion, considering the strong hypolipidemic/atheroprotective and antioxidant, and possibly anti-inflammatory actions of Lovastatin, intake of Lovastatin may be useful in the prevention and treatment of infection/inflammation induced hyperlipidemia and atherosclerosis.

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