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Effect of Dietary Tocotrienols on Antioxidant Status and Low Density Lipoprotein Oxidation in Rats, Exposed to Cigarette Smoke

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ABSTRACT

Cigarette smoking is the one of the major causes of mortality and morbidity involving respiratory and cardiovascular illness in developing and developed countries. Smoking has been strongly implicated as a risk factor for chronic obstructive pulmonary disease, cancer and atherosclerosis. In this study we deal with the multiple therapeutic benefits of Tocotrienols dissolved in palm oil. Various parameters, such as blood carbonmonoxide saturation, blood nicotine and various lipid profiles, total antioxidant power, conjugated diene formation, malondialdehyde content and *ex vivo* and Cu^{++} mediated *in vitro* LDL oxidation, in addition to body weight were taken to consideration and were analyzed after 4 week administration of tocotrienols (6 mg mL^{-1}) to smoke exposed rats useful in the prevention and treatment of tobacco-induced dyslipidemia/hyperlipidemia and atherosclerosis. As a conclusion, daily use of dietary tocotrienols will be efficacious, cost effective and a good source of vitamin-E.

Key words: LDL oxidation, atherosclerosis, hyperlipidemia, dyslipidemia, tocotrienols

INTRODUCTION

Cigarette smoking is one of the major causes of mortality and morbidity involving respiratory and cardiovascular illness in developing and developed countries. The burning of tobacco at temperature of 830-900 degree centigrade, leads to the production of about 5000 already identified toxic substances. In addition, during cigarette smoking a considerable amount of free radicals are also liberated, estimated as 10^{14} and 10^{15} free radicals/puff in the tar and gas phases (Church and Pryor, 1985). Cholesterol is an amphipathic lipid and as such is an essential structural component of membranes and of the outer layer of plasma lipoproteins. Cholesterogenesis mostly occurs in the liver, which also regulates the level of circulating plasma cholesterol and serum lipoproteins. Increase in cholesterol is achieved by activation of HMG-CoA reductase and cholesteryl ester hydrolase activities as well as induction in synthesis of LDL receptors in order to receive cholesterol from non-hepatic tissues by receptor mediated endocytosis. There are six major classes of human plasma lipoproteins (Kamisah *et al*., 2005) these include chylomicrons, Very Low Density Lipoproteins (VLDL), Intermediate Density Lipoprotein (IDL), Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL) and lipoprotein (a) [Lp (a)] (Gofman *et al*., 1954;

Berg *et al.*, 1974). These lipoproteins are distinguished on the basis of their lipid content, ultracentrifugation size, electrophoretic mobility and surface proteins. These lipoproteins transport dietary cholesterol and TG from the intestine to the liver and peripheral tissues. High blood cholesterol which results from the overproduction and/or underutilization of LDL is known to be caused by two metabolic irregularities: (1) the genetic disease familial hypercholesterolemia; (2) the consumption of high cholesterol diet. Oxidative damage to cholesterol component of the Low-Density Lipoprotein (LDL) leads to oxidized LDL by a series of consecutive events. This induces endothelial dysfunction which promotes inflammation during atherosclerosis. Oxidized LDL acts as a trigger to initiate endothelial inflammation leading to atherosclerosis and vascular thrombosis (heart attack and stroke). Modified LDLs are produced during chemical modification that LDLs undergo after synthesis. Modifications take place in either plasma or in the inner layer of the artery and pertain to either the lipid or the protein fraction, induced by hydrolytic or proteolytic enzymes, O, OH or O^2 radicals or other non-enzymatic mechanisms, modifications concern the production of lipoprotein-autoantibody complexes. Oxidation takes place when naturally occurring antioxidant agents such as vitamin E and **β**-carotenes that normally inhibit LDL oxidation do not occur. The term antioxidant refers to any molecule capable of stabilizing or deactivation of free radicals before they attack cells. The tocotrienols isomers (**α**-, **β**-, γ and **δ**) are naturally occurring analogues of tocopherol isomers (vitamin E) found mainly in cereal grains and palm oil. Tocotrienols have been shown to have an intrinsic hypochlolesterolemic activity in animals and humans. The cholesterol lowering effect of tocotrienols was attributed mainly to their down regulation of HMG-CoA reductase the rate-limiting enzyme of the cholesterol biosynthetic pathway. Palm oil represents one of the most abundant natural sources of tocotrienols. The distribution of vitamin E in palm oil is 30% tocopherols and 70% tocotrienols.

On the other hand tocotrienols have been shown as anti-osteoporotic and antioxidant (Achuba, 2005) properties (Nazrun *et al.*, 2010, 2011). Osteoporosis is a metabolic bone disease affecting both men and women especially postmenopausal women. Osteoporosis has been associated with oxidative stress and therefore, the protective effects of antioxidants such as vitamin E were studied. Lately, there has been a growing interest in tocotrienol, a potent vitamin E with anti-cholesterol (Onyesom *et al.*, 2007), anti-cancer, anti-lipid peroxidation (Nur Azlina *et al.*, 2005) and perhaps anti-osteoporotic properties (Aktifanus *et al.*, 2012). We have thus investigated the hypolipidemic coupled with antioxidant impact of tocomin on base line levels of *ex vivo* diene conjugation and lag phase time of *in vitro* Cu^{++}-induced oxidation of LDL.

MATERIALS AND METHODS

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 tocotrienols drug as well as RBD palm olein were supplied as a gift from CAROTECH BHD, Chemor, Malaysia.

Estimation: Plasma triglyceride (Trinder, 1969), Plasma Cholesterol, LDL and HDL (Annino and Giese, 1976), Plasma VLDL-C (Friedwald *et al.*, 1972), Fractionation of Plasma lipoprotein such as LDL (Wieland and Seidel, 1983), HDL and its fractions-HDL_2, HDL_3 (Patsch *et al.*, 1989), Blood Nicotine and plasma Carbonmonoxide saturation (Varley and Goueenlock, 1976), Plasma FRAP (Benzie and Strain, 1996), *ex vivo* and *in vitro* Cu^{++}-mediated LDL oxidation (Esterbauer *et al.*, 1989, 1992) were measured using standard kits by following known procedures.

Experimental design: 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: NC (Normal Control), SC (Smoke Control), S-T_3T (Smoke exposed Tocotrienols Treated).

Diet/drug/exposure to cigarette smoke: The rats were given pelleted rat chow. Exposure to cigarette smoke was done in morning and evening by keeping two rats in bottomless metallic container (10×11×16 inch). 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 J.N. Medical College, Aligarh Muslim University, India. Six rats in S-T_3T group were given 6.0 mg tocotrienols/rat/day, through gastric intubations for 4 weeks.

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.

Statistical evaluation: Statistical analysis of data was done by employing two-tailed Student t-test as described by Bennet and Franklin (1967). The p-value less than 0.02 were considered significant.

RESULT

Impacts of tocotrienols on average body weight in each group of rats: Table 1 depicts the average body weight (g) of NC, SC, S-T_3T was 165, 166 and 173 g whereas, the average body weight of NC, SC, S-T_3T rats showed a significant gain of 30, 09 and 36%, respectively after 4 weeks of treatment. These results demonstrate that in smoke exposed tocotrienols treated rats (S-T_3T) the gain in body weight after 4 weeks was significantly higher than NC and SC rats.

Impacts of tocotrienols on blood carbon monoxide saturation and blood nicotine after 4 weeks of treatment: Table 2, indicated the blood carbon monoxide saturation

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

	Average body weight/rat (g)	
Group	Before treatment	After treatment
NC	165.23±2.13*	215.11±9.12* (30.19)
SC	166.22±4.11*	182.63±8.41* (09.87)[b]
S-T_3T	173.21±4.72*	236.33±9.13* (36.44)[a]

NC: Normal control, SC: Smoke control; S-T_3T: Smoked exposed tocotrienols treated (fed 6 mg tocotrienols/rat/day for 4 weeks). Values are mention as Mean±SD from 6 rats in each group, [a]Significantly different from SC at p<0.001, [b]Significantly different from NC at p<0.005. Values in bracets are percentages

Table 2: Impacts of tocotrienols on blood carbonmonoxide saturation and nicotine in cigarette smoke exposed rats

Group	Carbon monoxide saturation (SCO%)	Nicotine (μg mL^{-1})
NC	6.81±0.032*	1.26±0.033*
SC	9.32±0.231* (36.86)[a]	4.92±0.035 (290.48)[a]
S-T_3T	7.59±0.018* (18.56)[a]	1.56±0.038 (68.29)[a]

NC: Normal control, SC: Smoke control; S-T_3T: Smoked exposed tocotrienols treated (fed 6 mg tocotrienols/rat/day for 4 weeks). Values are mention as Mean±SD from 6 rats in each group, [a]Significantly different from SC at p<0.001, [b]Significantly different from NC at p<0.005. Values in bracets are percentages

(carboxyhemoglobin) and blood nicotine. Blood carbon monoxide saturation and blood nicotine levels were increased from 6.8 (SCO%) and 1.3 μg mL^{-1} in NC to 9.3% SCO (36%) and 4.9 μg mL^{-1} (290%), respectively, in SC rats. After 4 weeks of tocotrienols treatment blood carbon monoxide saturation and blood nicotine levels showed a significant reduction of 18 and 68% in S-T_3T, respectively, in comparison to values in SC rats.

Effects of tocotrienols on plasma total lipid, triglycerides and total cholesterol in cigarette smoke exposed rats after 4 weeks of treatment: In Fig. 1, all the plasma lipids parameters were significantly increased in SC rats, when compared to NC values. Total Lipids (TL), triglycerides (TG) and Total Cholesterol (TC) significantly increased from 380, 52 and 83 mg dL^{-1} in NC to 513, 103 and 142 mg dL^{-1}, respectively, in SC group. After 4 weeks of tocotrienols treatment, levels of TL, TG and TC were significantly decreased by 9.5, 38 and 25%, respectively, when compared to corresponding NC values. These results demonstrate that 4 week treatment of smoke exposed rats with 6 mg tocotrienols mediated a significant reduction in above lipid parameters.

Effects of tocotrienols on the plasma lipoprotein lipids and on the ratio of LDL-C/HDL-C and HDL-C/TC after 4 weeks of tocotrienols treatment: Figure 2, the plasma VLDL-C, LDL-C and non-HDL-cholesterol (non-HDL-C) levels were significantly increased from 10, 50 and 61 mg dL^{-1} in NC to 21 mg dL^{-1} (101%), 112 mg dL^{-1} (121%) and 132 mg dL^{-1} (115%), respectively, in SC. After 4 weeks of tocotrienols treatment, both VLDL-C, LDL-C and non-HDL-C levels showed a significant reduction 38, 44 and 43%, respectively, in S-T_3T. Whereas, HDL-C, HDL_2-C and HDL_3-C levels were decreased from 21, 6 and 13 mg dL^{-1} in NC to 16 mg dL^{-1} (23%), 4 mg dL^{-1} (33%) and 12 mg dL^{-1} (7%), respectively, in SC values. After 4 weeks of tocotrienols treatment (S-T_3T) HDL-C, HDL_2-C and HDL_3-C levels showed a significant increase of 87, 177 and 51%, respectively, when compared to corresponding values in SC. These results demonstrate that Tocotrienols is effective in reducing VLDL-C and LDL-C levels. On the other hand, in comparison to SC values, treatment of smoke exposed rats with tocotrienols mediated a significantly higher increase in HDL-C, HDL_2-C and HDL_3-C concentration. On the other hand, LDL-C/HDL-C and HDL-C/TC ratios were calculated from the data presented in Fig. 1 and 2. LDL-C/HDL-C ratio was

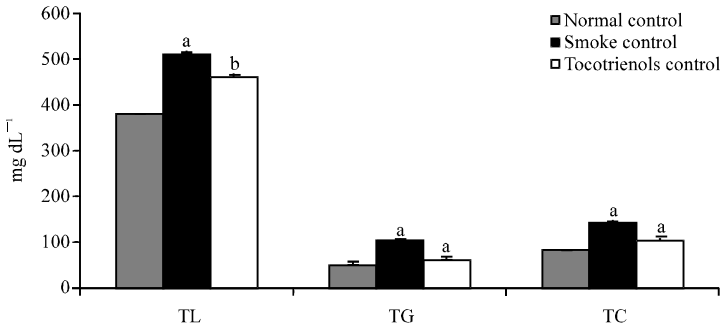

Fig. 1: Effects of tocotrienols on plasma total lipid (TL), triglycerides (TG) and total cholesterol (TC) in cigarette smoke exposed rats. Values are mean±SD from pooled plasma of 6 rats in each group. [a]Significantly different from NC at $p<0.001$, [b]Significantly different from SC at [b]$p<0.05$

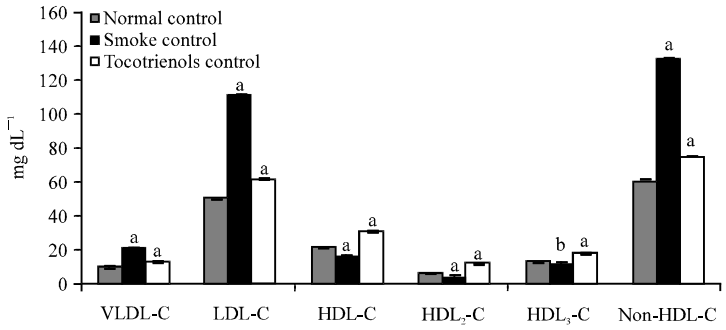

Fig. 2: Effects of tocotrienols on plasma VLDL-C, LDL-C, HDL-C, HDL_2-C and HDL_3-C sub fractions and non HDL-C, in cigarette smoke exposed rats. Values are Mean±SD from pooled plasma of 6 rats in each group. [a]Significantly different from NC at $p<0.001$ and $p<0.05$, [b]Significantly different from SC at $p<0.05$

Table 3: Effects of tocotrienols on the ratio of LDL-C/HDL-C, HDL-C/TC, in cigarette smoke exposed rats after 4 weeks of treatment

Parameters	NC	SC	S-T_3T
LDL-C/HDL-C	2.34±0.092	6.79±0.243 (+190.17)[a]	2.00±0.079 (-70.54)[a]
HDL-C/TC	0.258±0.022	0.116±0.015 (-55.04)[a]	0.293±0.025 (+152.59)[a]

[*]Values are Mean±SD (TC, LDL-C and HDL-C values are taken from Fig. 1, 2). NC: Normal control; SC: Smoke control; S-T_3T fed 6 mg Tocotrienols/rat/day for 4 weeks, [a]Significantly different from NC and SC at $p<0.001$, values inside brackets are percentages

significantly increased from 2.34 in NC to 6.79 (190%) in SC group, when compared to ratio in NC. After 4 weeks of treatment, the increase in LDL-C/HDL-C ratio was significantly prevented and decreased to 2.00 in S-T_3T which is close to normal control value. HDL-C/TC ratio was significantly decreased from 0.258 in NC to 0.116 (55%) in SC group as shown in Table 3. Tocotrienols treatment to these rats significantly prevented the increase in HDL-C/TC ratios and fully restored them to a ratio value similar to NC.

Impacts of tocotrienols on plasma total antioxidants and lipid peroxidation products: Figure 3 depicts the antioxidant impact of tocotrienols on plasma concentrations of total antioxidants, conjugated diene, lipid hydroperoxide and MDA in smoke exposed rats. In SC rats, plasma total antioxidants level was reduced from a control value of 54 to 39 (27%) μmole dL^{-1}. Treatment of S-T_3T rats with tocotrienols for 4 weeks resulted in a significant increase of total antioxidants levels by 22% when compared to SC value. The oxidative stress induced in SC 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 9.22, 1.18 and 1.73 in NC to 14.23 (54%), 2.21 (87%) and 3.92 (126%) μmole dL^{-1}, respectively, in SC. After tocotrienols treatment, in S-T_3T, a significant decrease of 23, 22 and 37% was seen in the formation of conjugated diene, lipid hydroperoxide and MDA, respectively, when compared to corresponding values in SC rats. These results demonstrate that in SC rats, due to increase in oxidative stress, total antioxidants level was decreased, whereas, concentration of plasma conjugated diene, lipid hydroperoxide and MDA were significantly

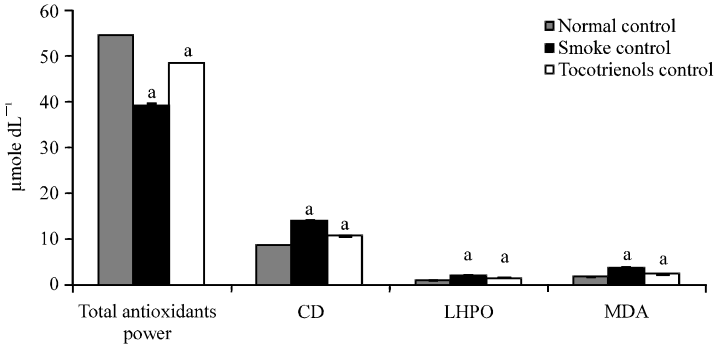

Fig. 3: Antioxidant impacts of tocotrienols on plasma total antioxidants, conjugated diene, lipid hydroperoxide and malondialdehyde contents in cigarette smoke exposed rats. Values are (µmole dL^{-1}) Mean±SD from pooled plasma of 6 rats in each group. [a]Significantly different from NC and SC at $p<0.001$

Table 4: *Ex vivo* and copper-mediated *in vitro* oxidation of LDL, conjugated diene formation, lag phase and total MDA release in cigarette smoke exposed rats

	LDL-oxidation				
	Conjugated diene formation*			MDA content#	
Group	Basal	Maximal^	Lag phase$ (min)	Basal	Maximal^
NC	175.25	1041.51	92	+4.87±0.73	14.25±0.96
SC	251.82 (+43.69%)†	1439.49 (+38.22)Ω	60 (- 34.78%)¶	+7.13±0.15[a] (+46.41%)‡	23.69±1.22[a] (+66.25)Ω
ST_3T	202.61 (-19.54%)††	1015.81 (-29.43)α	79 (+31.67%)§	+5.17±0.62[a] (-27.49%)‡‡	18.56±1.65[a] (-21.65)α

+Values are Mean±SD from pooled plasma of 6 rats in each group. *Conjugated diene values are expressed as nmole malondialdehyde equivalents/mg protein. Basal conjugated diene values represent the status of oxidized LDL *in vivo*. $The lag phase defined as the interval between the intercept of the tangent of the slope of the curve with the time expressed in minutes. ^Maximal *in vitro* oxidation of LDL was achieved after 12 h of incubation with $CuSO_4$ in each group, †Percent increase with respect to basal value in NC, ††Percent decrease with respect to basal value in SC, ¶Percent decrease with respect to lag phase value in NC, §Percent increase with respect to lag phase value in SC, ¶Percent increase with respect to maximal value in NC, αPercent decrease with respect to maximal value in SC, Significantly different from NC at [a]$p<0.001$

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.

Impacts of tocotrienols on the *ex vivo* and *in vitro* Cu^{++} mediated LDL Oxidation, conjugated diene formation, lag phase and total MDA release: Table 4, depicts the *ex vivo* base line diene conjugation (BDC) levels of LDL in SC rats was increased by 43%, in comparison to the corresponding NC values. Feeding of tocotrienols to SC rats partially blocked the *in vivo* oxidation of LDL and reduced their BDC levels by 19%, in comparison to the corresponding SC values. As expected, the lag phase time of LDL oxidation was reduced from 92 min in NC to 60 min in SC. Treatment of S-T_3T rats with tocotrienols restored the lag phase time of LDL oxidation to 79 min (31%). On the other hand, the *ex vivo* base line levels of MDA in LDL was significantly increased by 46% in SC rats, when compared to corresponding values in NC rats. After tocotrienols

treatment significantly blocked the *in vivo* increase in the formation of MDA of LDL in smoke exposed rats and reduced their levels by 27%, in comparison to SC rats. Similarly maximal *in vitro* oxidation of LDL was achieved after 12 h of incubation with $CuSO_4$ in each group. The CD and MDA formation were significantly increased when compared to NC values, after 4 weeks of T_3 treatment, both values are significantly blocked.

DISCUSSION

Cigarette smoking is firmly established as a primary risk factor for atherosclerotic cardiovascular disease. Increased oxidative stress is one of the principal mechanisms by which it may exert its pathological influence. This study is the first to examine the effect of dietary tocotrienols supplementation on overall proatherogenic actions of cigarette smoke. The cigarette smoke induced extensive proatherogenic changes, that occurred in young smokers, were reflected on a variety of parameters, such as, blood nicotine, carboxyhemoglobin, plasma and lipoprotein lipids including cholesterol and plasma lipid peroxidation products including *ex vivo* and *in vitro* oxidizability of LDL, plasma total antioxidants; malondialdehyde (MDA) release. Treatment of smoke exposed rats with tocotrienols (Tocotrienols 6 mg day^{-1}) for 4 weeks, significantly reduced the overall oxidative burden and effectively ameliorated the above altered parameters, thus, indicating a strong hypolipidemic/anti-atherogenic and antioxidant effect of tocotrienols. Several studies show that in addition to increase in oxidative stress, certain other compounds of cigarette smoke, such as nicotine and Carbon Monoxide (CO) play a role in atherogenesis. Nicotine alone acutely increases endothelial dysfunction by means of impaired vascular reactivity (Neunteufl *et al*., 2002). It may lead to increased platelet adhesiveness (Hawkins, 1972). Carbon monoxide constitutes 4% of cigarette smoke and directly leads to high levels of carboxyhemoglobin. Through sustained exposure to high levels of CO, chronic hypoxia ensues, leading to increased exercise-induced ischemia, ventricular dysfunction with CAD (Allred *et al*., 1989). In addition, in one cross-sectional study in Britain, carboxyhemoglobin levels appeared to be better predictor of atherosclerotic disease than smoking histories (Hammond *et al*., 1976). Consistent with earlier reports (Jarvis *et al*., 1987), our results show a 290% increase in blood nicotine and 36% increase in carboxyhemoglobin in SC rats. Four week Tocotrienols treatment of S-T_3T rats caused a significant reduction in nicotine and carboxyhemoglobin levels to 18 and 68%, respectively as compared to SC rats. These results indicate a strong protective effect of tocotrienols which may help lower the risk of myocardial infarction in smoke exposed rats. Present results indicate a modest and significant increase in plasma total lipid (34%), TG (97%) and TC (70%) in SC rats. The increase in plasma TG levels is apparently due to an increase in VLDL-C (101%) which can be the result of either increased VLDL production or decreased VLDL clearance. It is possible that massive free radical load in smoke control rats may stimulate VLDL production by increasing adipose tissue lipolysis, increasing hepatic *de novo* fatty acid synthesis and decreasing hepatic fatty acid oxidation, all of which provide fatty acid substrate for esterification into TG and assembly into VLDL particles in the liver. Tocotrienols effectively blocked the increase in the above lipid parameters and reversed them to 9, 38 and 25% level similar to their respective normal control values. As expected, plasma levels of VLDL-C, LDL-C and atherogenic non-HDL-C were significantly increased (101, 121, 115%, respectively) in smoke control rats. After 4 weeks of tocotrienols treatment, values decreases to 38, 44 and 43%, respectively in compared to smoke control rats. In contrast to atherogenic LDL, cholesterol associated with anti-atherogenic HDL was significantly lower (23%) in smoke control rats as compared to normal control rats. Tocotrienols treatment of smoke exposed rats blocked the

reduction in HDL-C and restored to 87% of HDL-C value as compared to SC rats. Therefore, tocotrienols may exert their cholesterol lowering effect in dyslipidemic smokers and hyperlipidemic rats exposed with cigarette smoke in a similar manner as previously reported for hyperlipidemic animals (Minhajuddin *et al.*, 1999; Beg *et al.*, 2000) and humans (Qureshi *et al.*, 1991, 1995). 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 (Minhajuddin *et al.*, 1999; Parker *et al.*, 1993). 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 (Minhajuddin *et al.*, 1999), 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 (Parker *et al.*, 1993). In addition, γ-tocotrienol was shown to upregulate LDL receptor in mammalian cells and may be implicated in part for the reduction of apoB-lipoprotein *in vivo* (Parker *et al.*, 1993). 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, posttranscriptionally (Parker *et al.*, 1993). In addition, another report indicates that γ-tocotrienol influences apoB secretion by both cotranslational 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 (Theriault *et al.*, 1999). 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 (Theriault *et al.*, 1999). However, elucidation of precise *in vivo* mechanism(s) of tocom in-mediated inhibition of HMG-CoA reductase at molecular level remains to be investigated. It has previously been established that LDL-C/HDL-C and HDL-C/TC ratios are good predictors for the presence and severity of CAD (Drexel *et al.*, 1992). The ratio of 2.34 was increased to a much higher LDL-C/HDL-C ratio value of 6.79 in smoke control(SC) rats. Tocotrienols treatment of smoke exposed rats significantly reduced this ratio to a normal value of 2.00. Similarly, in normal control (NC) rats, HDL-C/TC ratio of 0.258 was observed. This ratio of 0.258 in NC rats was significantly reduced to a ratio value of 0.116 in smoke control rats which was significantly increased to near normal ratio of 0.293 after Tocotrienols treatment. These results which represent an initial demonstration, indicate that treatment of smoke exposed rats with tocotrienols for 4 weeks effectively ameliorated all the lipid parameters including highly atherogenic LDL. Oxidative modification of lipoproteins is believed to play a central role in the pathogenesis of atherosclerosis (Berliner and Suzuki, 1996; Steinberg, 1997). Because plasma contains several antioxidants (Frei, 1995) and lipoproteins with oxidative damage have been isolated from atherosclerotic lesions (Berliner and Suzuki, 1996; Steinberg, 1997), lipoprotein oxidation generally is considered to occur in the vessel wall. Although lipid oxidation in the vessel wall is thought to occur as a result of a local deficiency of endogenous antioxidants or an excess of free metal ions, only limited data support these hypothesis. Research has shown that human atherosclerotic plaques contain massive amounts of lipid peroxidation products, despite the presence of large quantities of α-tocopherol (vitamin E) and ascorbate (Suarna *et al.*, 1995). Therefore, it is unclear whether oxidized lipoproteins originate in the arterial wall or are produced in the circulation and then enter the intimal space. Our data show

that due to sustained free radical load in smokers, oxidation of lipid/lipoprotein particles is considerably enhanced. 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 are significantly increased in SC rats. The increase in plasma lipid peroxidation products is associated with a significant decline in plasma total antioxidants. The former suggests increased production of oxidants while later indicates diminished antioxidant defense. Both the changes indicate an existence of profound oxidative stress. These results are consistent with the well known pro oxidant effect of cigarette smoke. Recently, Bloomer (2007) has shown that young novice smokers have a lower plasma antioxidant capacity and exhibited a greater degree of lipid peroxidation compared to nonsmokers. Our results indicate a significant decrease in plasma lipid peroxidation products with a concomitant and significant increase in plasma total antioxidants in tocotrienols treated rats. Therefore, cigarette smoke induced oxidative stress was not only attenuated but significantly reversed after Tocotrienols treatment. Plasma MDA content in SC rats was significantly increased by 140%. After 4 week of tocotrienols treatment, the plasma MDA content significantly decreased by 53%. The treatment of smoke exposed rats with tocotrienols effectively blocked the *in vivo* as well as *in vitro* susceptibility of plasma to lipid peroxidation and significantly reduced MDA levels. Based on these results, its seems possible that oxygen radicals formed over and above the detoxifying capacity of plasma can cause peroxidative breakdown of phospholipid fatty acids and accumulation of MDA and hence membrane damage. Severe hyperlipidemia in SC rats was associated with an increase in LDLwhich is shown to be more prone to oxidation than and hence more pro-atherogenic (De Graaf *et al*., 1991; Tribble *et al*., 1992; Dejager *et al*., 1993; Chait *et al*., 1993; Tribble *et al*., 1994). Present results demonstrate that in SC rats, *in vivo* oxidizability of LDL, measured as *ex vivo* base line diene conjugation (BDC), was increased (43%), while lag phase of its Cu^{++}-induced oxidation was reduced to 60 min, in comparison to NC rats. This difference in the oxidizability of LDL of normal rats is in agreement with previous reports indicating an inherently reduced concentration of antioxidants and free cholesterol, increased amount of more oxidizable polyunsaturated fatty acids including preformed hydro peroxides in LDL (De Graaf *et al*., 1991; Thomas *et al*., 1994; Sevanian *et al*., 1996; Tribble *et al*., 2001). After 4 weeks of tocotrienols treatment to S-T_3T rats, the lag phase values of LDL were restored to 31%, as compared to smoke control rats, indicating a better anti-oxidative effect of tocotrienols. In conclusion, based on tocotrienols mediated multiple therapeutic benefits, described in the present study, administration of tocotrienols to smoke exposed rats may be useful in the prevention and treatment of tobacco-induced dyslipidemia/hyperlipidemia and atherosclerosis. In addition, daily use of dietary tocotrienols will be efficacious, cost effective and a good source of vitamin E.

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