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Hypolipidemic effects and Antioxidant Activity of Tocotrienols on Cigarette Smoke Exposed Rats

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ABSTRACT

Cigarette smoking is one of the major causes of mortality involving respiratory and cardiovascular illness in developing countries. Cigarette smoking is known to contain abundant of free radicals, which is able to cause tissue oxidative damage at various levels and to the alterations which induce the arteriosclerotic process. The body has the ability to produce hepatic antioxidants such as Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (Gpx) and Glutathione reductase (Gred). When there are excessive free radicals generated due to smoking, the available tissue antioxidants may become depleted, leading to oxidative stress. In this study we investigate the efficacy of antioxidant and hypolipidemic agent Tocotrienols by analyzing all the parameters in plasma lipoprotein lipids, TL, TC, TG, VLDL-C, LDL-C, HDL-C, as well as hepatic antioxidant enzymes (SOD, CAT, Gpx and Gred), as investigated in smoke exposed control rats. All the plasma lipids parameters as well as hepatic antioxidant enzymes were significantly increased/decreased in smoke exposed control rats. After 4 weeks administration of Tocotrienols, significantly restore the above altered parameters. In conclusion, Tocotrienols may be useful in the prevention and treatment of tobacco-induced dyslipidemia/hyperlipidemia and atherosclerosis.

Keywords: Tocotrienols, Cardiovascular illness, Dyslipidemia, Hyperlipidemia, Hepatic antioxidant enzymes

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INTRODUCTION

Cigarette smoking is 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.

It has been demonstrated that one of the prominent risk factors for increased lipid peroxidation is smoking [1]. 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 [2]. The smoke is able to cause tissue oxidative damage at various levels [3] and contributes significantly to the appearance of endothelial dysfunctions [4] and to the alterations which induce the arteriosclerotic process [5]. As a matter of fact, one can observe in smokers an increase of lipid peroxidation products [6]. The body has the ability to produce endogenous hepatic antioxidants such as Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (Gpx) and Glutathione reductase (Gred). In the instance where there are excessive free radicals, the available tissue antioxidants may become depleted, leading to oxidative damage as in the case of cigarette smoke. The various roles of hepatic enzymatic antioxidants (SOD, CAT, Gpx and Gred) and non-enzymatic antioxidants (Vitamin C, Vitamin E, carotenoids, lipoic acid and others) in the protection against oxidative stress can be found in a numerous reviews and original papers [7, 8, 9]. Compounds, which possess antioxidant properties, have the potential to decrease oxidative stress and thus may protect against smoking-induced pathology. Compounds that have been investigated are lipoic acid, taurine, ubiquinone, selenium, garlic, ginkgo biloba and polyphenols. Other compounds, which have been widely tried from their antioxidant properties, are vitamins such as alpha-tocopherol [10], and ascorbic acid. 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 like ascorbic acid, Vitamin E, carotenoids, polyphenols and other low molecular weight compounds such as α-lipoic acid. 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. 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. Palm oil is different from other plant and animal oils in that it contains 50% saturated fatty acids, 40% unsaturated fatty acids, and 10% polyunsaturated fatty acids. In this study, we investigate the efficacy of antioxidant, anti-atherogenic and hypolipidemic agent Tocotrienols by analyzing all the parameters in plasma, TC, VLDL-C, LDL-C, HDL-C (HDL_2-C, HDL_3-C), Hepatic TG, TC, CD, LHPO, MDA and antioxidant enzymes (CAT, SOD, Gpx, Gred).

MATERIAL 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: Fractionation of Plasma lipoprotein such as LDL [11], HDL and its fractions-HDL_2, HDL_3 [12], Plasma FRAP [13], determination of triglyceride and total cholesterol in liver homogenate [14], activities of antioxidant enzymes such as Catalase [15], Superoxide dismutase [16], Glutathione peroxidase [17] and Glutathione reductase [18] in liver homogenate were measured by following known procedures.

Experimental Design: The experimental study was approved by the Dolphin Institute of Biomedical and Natural Science, 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, Smoke exposed control rats (SC); six rats in this group were administered 1.0 ml saline/rat/day through gastric intubation for 4 weeks, Smoke exposed Tocotrienols treated rats (S-T_3T); six rats in this group were given 6.0 mg Tocotrienols/rat/day through gastric intubation for 4 weeks

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 Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. Six rats in S-T_3T group were given 6.0 mg Tocotrienols/rat/day, through gastric intubation 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.

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.

Statistical evaluation: This was done by employing two-tailed Student t-test as described by Bennet and Franklin [19]. P value less than 0.02 were considered significant.

RESULTS

Impact of Tocotrienols on average body weight in each group of rats: As seen in **Table 1**, the average body weight (g) of N-C, S-C, S-T_3T was 171g, 173g and 178g, whereas, the average body weight of N-C, S-C, S-T_3T rats showed a significant gain of 31%, 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 N-C rats.

Average body weight/rat (g)		
Group	Before treatment	After Treatment
N-C	171.25±2.13*	225.21±9.12 (+31.51%)
S-C	173.12±4.11*	189.52±8.41 (+09.47%)[b]
S-T_3T	178.21±4.72*	243.41±9.13 (+36.59%)[a]

TABLE-1.Average body weight in each group of rats before and after 4 weeks of Tocotrienols treatment,
**Values are mean ±SD from 6 rats in each group. N-C, normal control; S-C, smoke control; S-T_3T fed 6 mg Tocotrienols/rat/day for 4 weeks, Significantly different from N-C at [b]p<0.001, Significantly different from S-C at [a]p<0.001.*

Impacts of Tocotrienols on Plasma Total Lipids (TL), Triglyceride (TG) and Total Cholesterol (TC) after 4 weeks of treatment: As seen in **Fig. 1**, all the plasma lipids parameters were significantly increased in smoke control (S-C) rats, when compared to N-C values. Total lipids (TL), triglycerides (TG) and total cholesterol (TC) significantly increased from 390, 59, and 90 mg/dl in N-C to 526, 114, and 150 mg/dl, respectively, in S-C group. After 4 weeks of Tocotrienols treatment, levels of TL, TG, and TC were significantly decreased by 11 %, 38 %, and 24 %, respectively, when compared to corresponding N-C values.

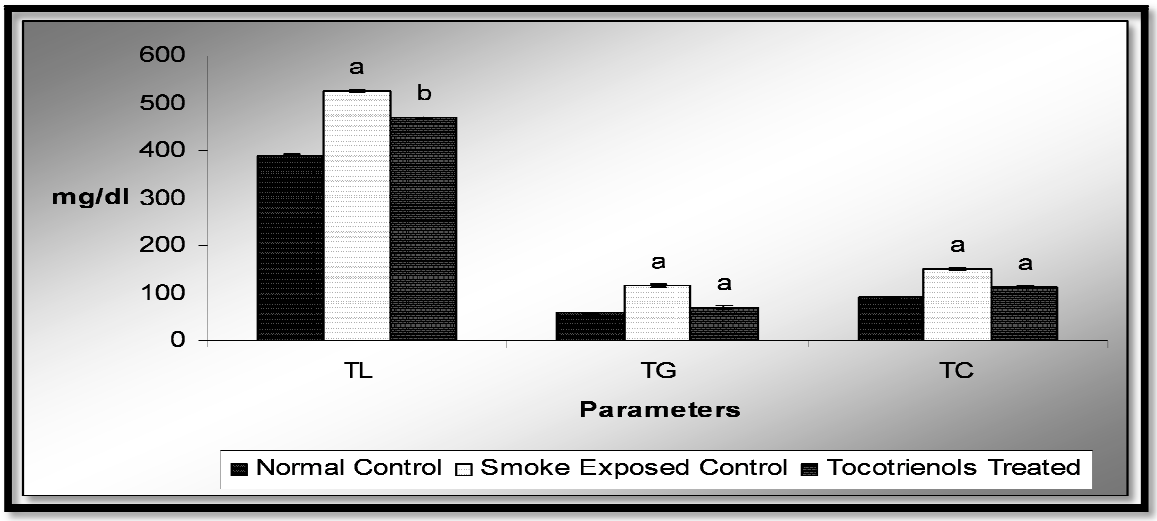

Fig. 1 Impacts of Tocotrienols on plasma Total lipid (TL), Triglyceride (TG) and Total Cholesterol (TC) in cigarette smoke exposed rats after 4 weeks of tocotrienols treatment
Values are mean (mg/dl) ±SD from pooled plasma of 6 rats in each group. N-C, normal control; S-C, smoke control; S-T_3T fed 6 mg Tocotrienols/rat/day for 4 weeks. Significantly different from N-C at [a]p<0.001. Significantly different from S-C at [a]p<0.001and [b]p<0.005

Impacts of Tocotrienols on Plasma Lipoprotein fractions and ratio of LDL-C/HDL-C and HDL-C/TC: As seen in **Fig. 2**, depicts that VLDL-C, LDL-C and non-HDL-cholesterol (non-HDL-C) levels were significantly increased from 12, 53 and 64 mg/dl in N-C to 23 mg/dl (83%), 114 mg/dl (113 %) and 136 mg/dl (112 %) respectively, in S-C. After 4 weeks of Tocotrienols treatment, VLDL-C, LDL-C and non-HDL-C levels showed a significant reduction 32%, 42% and 40%, respectively, in S-T_3T. Whereas HDL-C, HDL_2-C and HDL_3-C levels were decreased from 24, 8 and 15 mg/dl in N-C to 18 mg/dl (24%), 6 mg/dl (21%) and 14 mg/dl (5%), respectively, in S-C 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 80%, 120% and 43%, respectively, when

compared to corresponding values in S-C. These results demonstrate that Tocotrienols is effective in reducing VLDL-C and LDL-C levels. On the other hand, in comparison to S-C values, treatment of smoke exposed rats with Tocotrienols mediated a significantly higher increase in HDL-C, HDL_2-C and HDL_3-C concentration.

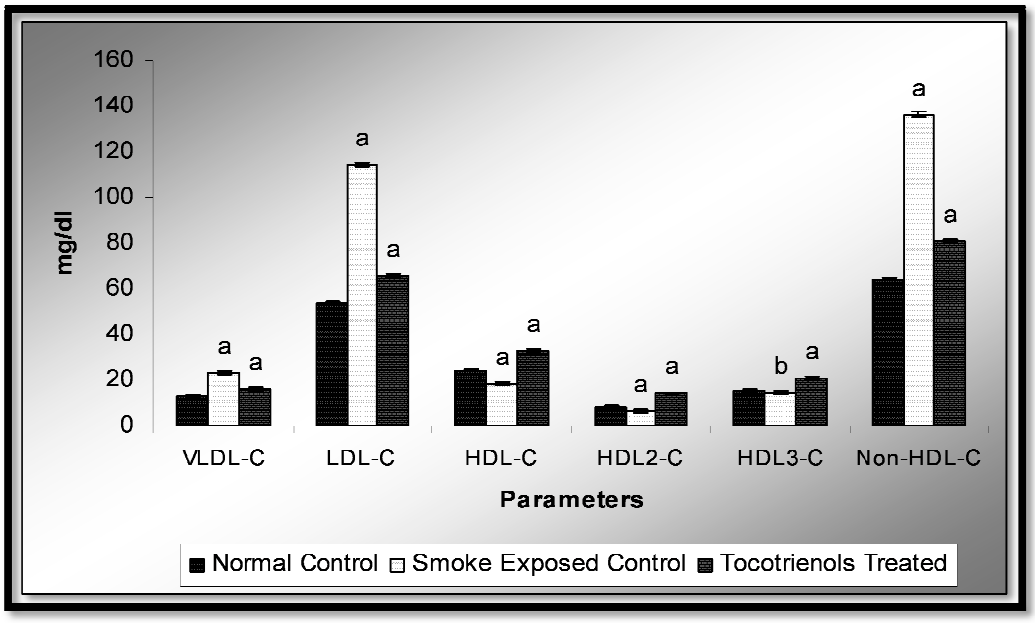

Fig. 2 Impacts of Tocotrienols on Plasma VLDL-C, LDL-C, HDL-C, HDL2-C, HDL3-C and Non-HDL-C after 4 weeks of treatment

Values are mean (mg/dl) ±SD from pooled plasma of 6 rats in each group. N-C, normal control; S-C, smoke control; S-T_3T fed 6 mg Tocotrienols/rat/day for 4 weeks. Significantly different from N-C at $^{a}p<0.001$ and $^{b}p<0.02$. Significantly different from S-C at $^{a}p<0.001$.

On the other hand, in **Table 2**, LDL-C/HDL-C ratio was significantly increased from 3.10 in N-C to 7.85 (153%) in S-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 4.10 in S-T_3T, which is close to normal control value. HDL-C/TC ratio was significantly decreased from 0.345 in N-C to 0.210 (39%) in S-C group. Tocotrienols 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.

Parameters	N-C	S-C	S-T_3T
LDL-C/HDL-C	$3.10\pm0.092^{**}$	$7.85\pm0.243^{**}$ (+153.23 %)^{a}	$4.10\pm0.079^{**}$ (-47.77 %)^{a}
HDL-C/TC	$0.345\pm0.022^{**}$	$0.210\pm0.015^{**}$ (-39.13 %)^{a}	$0.315\pm0.025^{**}$ (+50.00 %)^{a}

Table 2 Impacts of Tocotrienols on the ratio of LDL-C/HDL-C and HDL-C/TC in cigarette smoke exposed rats after 4 weeks of tocotrienols treatment. 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; S-C, smoke control; S-T_3T fed 6 mg Tocotrienols/rat/day for 4 weeks. Significantly different from N-C at $^{a}p<0.001$. Significantly different from S-C at $^{a}p<0.001$.

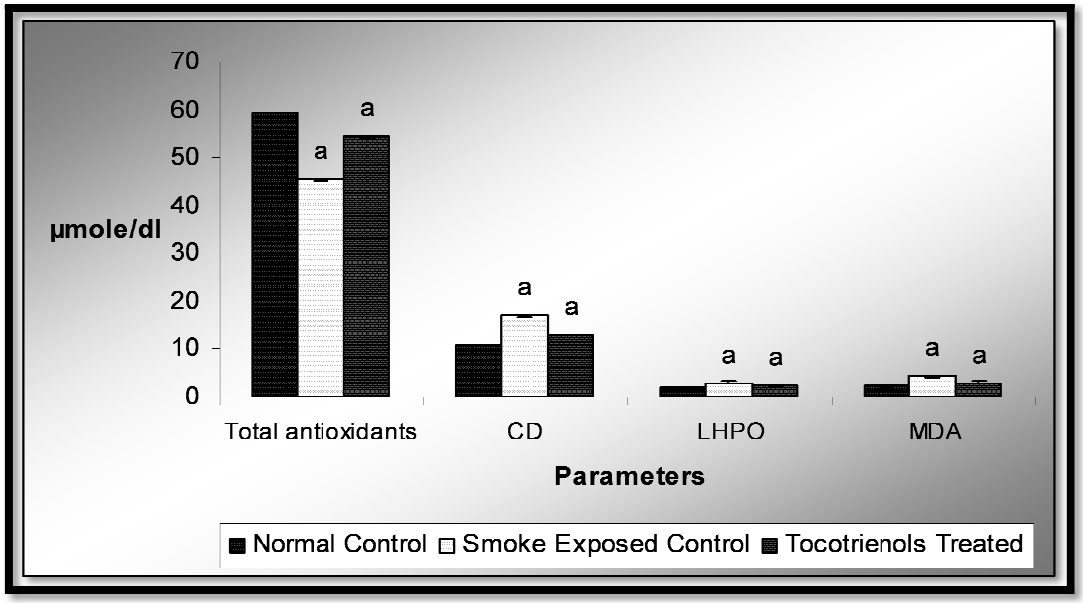

Fig. 3 Impacts of Tocotrienols on Plasma Total antioxidants, Conjugated diene (CD), Lipid hydroperoxide (LHPO) and Malondialdehyde (MDA) contents in cigarette smoke exposed rats after 4 weeks of treatment.
Values are mean (µmole/dl) ± SD from pooled plasma of 6 rats in each group. N-C, normal control; S-C, smoke control; S-T_3T fed 6 mg Tocotrienols/rat/day for 4 weeks. Significantly different from N-C at $^a p<0.001$. Significantly different from S-C at $^a p<0.001$.

Impacts of Tocotrienols on plasma total antioxidants and lipid peroxidation products:
Fig. 3 depicts the antioxidant impact of Tocotrienols on plasma concentrations of total antioxidants, conjugated diene (CD), lipid hydroperoxide (LHPO) and malondialdehyde (MDA) in smoke exposed rats. In S-C rats, plasma total antioxidants level was reduced from a control value of 59 to 45 (23%) µmole/dl. Treatment of S-T_3T rats with Tocotrienols for 4 weeks resulted in a significant increase of total antioxidants levels by 19% when compared to S-C value. The oxidative stress induced in S-C rats significantly enhanced plasma lipid peroxidation products, such CD, LHPO and MDA. Formation of CD, LHPO and MDA in plasma was increased from 11.10, 1.90 and 2.25 µmole/dl in N-C to 16.95 (52%), 3.05 (60%) and 4.35 (93%) µmole/dl, respectively, in S-C. After Tocotrienols treatment, in S-T_3T, a significant decrease of 22%, 26% and 31% was seen in the formation of CD, LHPO and MDA, respectively, when compared to corresponding values in S-C rats. These results demonstrate that in S-C rats, due to increase in oxidative stress, total antioxidants level was decreased, whereas, concentration of plasma CD, LHPO and MDA were significantly increased. Tocotrienols treatment significantly restored the total antioxidants level and blocked the increase in plasma CD, LHPO and MDA to a level close to corresponding normal values.

Tocotrienols effects 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 smoke control rats (S-C) by 32% and 91% respectively, when compared to corresponding values in N-C. Feeding of Tocotrienols to smoke exposed rats for 4 weeks was associated with a significant decline in liver TG and TC levels by 15% and 36% respectively, in S-T_3T. On the other hand, formation of

conjugated diene (CD), lipid hydroperoxide (LHPO) and malondialdehyde (MDA) in liver of smoke control (S-C) rats were significantly increased by 28%, 51% and 64%, respectively. Feeding of Tocotrienols to S-T_3T rats for 4 weeks, was associated with a significant decline in the formation of liver CD, LHPO and MDA by 35 %, 27 % and 33 %, respectively, when compared to corresponding values in S-C group. These results demonstrate that increased levels of TG, TC, CD, LHPO and MDA in liver of smoke exposed rats were significantly reduced after 4 weeks of Tocotrienols treatment.

Parameter	NC	SC	S-T_3T
Triglycerides*	0.59±0.002	0.78±0.014(+32.20%)[a]	0.66±0.21(-15.39%)[a]
Total cholesterol*	2.10±0.051	4.01±0.030(+90.95%)[a]	2.55±0.029(-36.41%)[a]
Conjugated diene**	6.42±0.024	8.25±0.015(+28.51%)[a]	5.33±0.026(-35.40%)[a]
Lipid Hydroperoxide**	0.93±0.002	1.41±0.001(+51.61%)[a]	1.03±0.003(-26.95%)[a]
MDA**	2.50±0.012	4.10±0.080(+64.00%)[a]	2.73±0.068(-33.41%)[a]

Table 3 Impact of Tocotrienols on triglycerides, total cholesterol and various lipid peroxidation products in the Liver homogenate after 4 weeks treatment of cigarette smoke exposed rats.

**Values are mean (mg/100 mg protein) ± SD from homogenate of pooled liver of 6 rats in each group. **Values are mean (nmole/mg protein) ± SD from homogenate of pooled liver of 6 rats in each group. N-C, normal control; S-C, smoke control; S-T_3T, fed 6 mg Tocotrienols/rat/day for 4 weeks, Significantly different from N-C at [a]p<0.001 and [b]p<0.01, Significantly different from S-C at [a]p<0.001.*

Impacts of Tocotrienols on the various antioxidant enzymes activities in the liver homogenate: Catalase (CAT) activity in liver was significantly decreased from a value of 3.69 unit in N-C to 2.38 (35%) in S-C, respectively as seen in **Table 4**. Administration of Tocotrienols to smoke exposed tocotrienols treated rats (S-T_3T) resulted in a significant increase in liver CAT activities by 3.21 (34%) unit, respectively. However, in comparison to corresponding tissue values of normal control rats (N-C), the decline in hepatic Superoxide dismutase (SOD) activity of smoke control(S-C) rats was 26%. Treatment of Tocotrienols to smoke exposed tocotrienols treated (S-T_3T) rats resulted in a significant increase in hepatic SOD activity by 30%, respectively from normal value. As seen in Fig. 4, in smoke exposed rats, Glutathione peroxidase (Gpx) activity in liver was significantly increased from a value of 56 units in N-C to 68 (20%) units, in S-C rats. As evident, after 4 weeks of treatment with Tocotrienols, Gpx activity in liver was significantly decreased by 23%, when compared to corresponding tissue values in S-C group. On the other hand, in smoke exposed rats, the enzymatic activities of hepatic Glutathione reductase (Gred) was decreased significantly by 29%, when compared to corresponding values of N-C rats. Feeding of Tocotrienols to smoke exposed rats significantly blocked the decrease in hepatic Gred activities and increased them to a similar value of 31%, Compared to corresponding values of Gred activities in N-C. Administration of Tocotrienols 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 smoke exposed rats. However, Tocotrienols treatment to smoke exposed rats substantially quenches these free radicals (ROS), thus positively normalizing the above enzyme levels.

Group	Catalase[†]	Superoxide dismutase[††]	Glutathione peroxidase[β]	Glutathione reductase[‡]
N-C	3.69±0.139[*]	0.71±0.004	56.92±0.104[*]	10.17±0.204
S-C	2.38±0.210[*](-35.50 %)[a]	0.52±0.010(-26.76 %)[a]	67.61±1.35[*](+18.78%)[a]	7.22±0.239(-29.01%)[a]
S-T_3T	3.50±0.118[*](+47.06 %)[a]	0.68±0.004(+30.77 %)[a]	53.20±1.53[*](-21.31%)[a]	9.75±0.161(+35.04%)[a]

Table 4 Impacts of Tocotrienols on liver Catalase, Superoxide dismutase, Glutathione peroxidase and Glutathione reductase activities in cigarette smoke exposed 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. [β]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. [*]Values are mean ± SD from homogenate or PMS fraction of pooled liver of 6 rats in each group, N-C, normal control; S-C, Smoke exposed control and S-T_3T, fed 6 mg Tocotrienol/rat/day for 4 weeks. Significantly different from N-C at [a]$p<0.001$. Significantly different from S-C at [a]$p<0.001$

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. The cigarette smoke induced extensive proatherogenic changes that occurred in young smokers, were reflected on a variety of parameters, such as, plasma and lipoprotein lipids including cholesterol and plasma lipid peroxidation products, plasma total antioxidants. Treatment of smoke exposed rats with tocotrienols (Tocotrienols 6mg/day) 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. Recent experimental and clinical data support the hypothesis that cigarette smoke exposure increases oxidative stress as a potential mechanism for initiating cardiovascular dysfunction [20]. Our results indicate a modest and significant increase in plasma total lipid (35%), TG (93%), and TC (66%) in smoker control (S-C) rats. The increase in plasma TG levels is apparently due to an increase in VLDL-C (83%) 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. Therefore, tocotrienols may exert their cholesterol lowering effect in cigarette smoke exposed rats in a similar manner as previously reported for hyperlipidemic animals [21, 22] and humans [23, 24]. 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 [21, 25]. 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 [21], 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 [25]. 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* [25]. 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 [25]. 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 [26]. 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 [27]. Tocotrienols effectively blocked the increase in the above lipid parameters and reversed them to 11%, 38%, 24% 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 (83%, 113%, 112 % respectively) in smoke control rats. After 4 weeks of tocotrienols treatment, values decreases to 32%, 43%, 40% respectively in compared to smoke control rats. In contrast to atherogenic LDL, cholesterol associated with anti-atherogenic HDL was significantly lower (24%) 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 80% of HDL-C value as compared to smoke control(S-C) rats. 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 [28]. The ratio of 3.10 was increased to a much higher LDL-C/HDL-C ratio value of 7.85 in smoke control (S-C) rats. Tocotrienols treatment of smoke exposed rats significantly reduced this ratio to a normal value of 4.10. Similarly, in normal control (N-C) rats, HDL-C/TC ratio of 0.345 was observed. This ratio of 0.345 in N-C rats was significantly reduced to a ratio value of 0.210 in smoke control rats, which was significantly increased to near normal ratio of 0.315 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. 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 malondialdehyde (which measure the degradation phase of lipid peroxidation) in plasma are significantly increased in smoke control (S-C) 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. Bloomer [29] 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. During the course of oxidative stress, oxygen-derived free radicals such as superoxide anion ($O^{\cdot}_2$) are known to be generated in the cells. This $O^{\cdot}_2$ forms H_2O_2 by dismutation, which finally undergoes an iron-catalyzed reaction to from cytotoxic $OH^{\cdot}$. The breakdown of membrane phospholipids and lipid peroxidation, demonstrated in many diseases, is known to be mediated by free radicals. The

significantly increased concentrations of conjugated diene, lipid hydroperoxide and MDA in liver (**Table 3**), is apparently due to enhanced lipid peroxidation in cigarette smoke stressed rats. These results are consistent with earlier reports indicating increased lipid peroxidation products in tissues of rats exposed to cigarette smoke either for 30 days [30] or 90 days [31]. Cigarette smoke-induced oxidative stress is associated with an impairment of the antioxidant defense system along with an increase in the generation of lipid peroxidation products. This finding provides a perfect correlation between lipid peroxidation products and decreased activities of CAT and SOD, which play an important role in scavenging the toxic intermediate products of incomplete lipid peroxidation. A decrease in the activity of these enzymes, as seen in liver of S-C rats (**Table 4**), can lead to the excessive availability of superoxides and peroxyl radicals, which in turn generate hydroxyl radicals, resulting in the initiation and propagation of more lipid peroxidation products [32]. A similar decrease in hepatic CAT and SOD activities, in rats exposed to cigarette smoke for 90 days, has previously been reported [31]. Tocotrienols feeding of S-C rats significantly reduced the lipid peroxidation products, and increased the CAT and SOD activities in liver, and reversed these parameters to near normal levels. These results indicate a potent free radical scavenging property of tocotrienols. It is well known that glutathione (GSH) acts as a reducing agent and plays a vital role in detoxification. It provides antioxidant protection in the aqueous phase of cellular systems [33], its antioxidant activity is through the thiol group of its cysteine residue. Like ascorbic acid, another important water soluble antioxidant, GSH can directly reduce a number of ROS and is oxidized to GSSG in the process. Liver is viewed as a glutathione-generating site, which supplies the kidney and intestine with other constituents for glutathione resynthesis [33]. Intra-hepatic glutathione is reported to afford protection against liver dysfunction by at least two ways: (i) as a substrate for glutathione peroxidase (Gpx), GSH serves to reduce large variety of hydroperoxides before they attack unsaturated lipids or convert already formed lipid hydroperoxide to the corresponding hydroxyl compounds; (ii) As a substrate of glutathione-S-transferase (GST), it enables the liver to detoxify foreign compounds or other metabolites and to excrete the products, preferably in to bile. Our results show a decline in Gred (**Table 4**), and an increased GPx activity in liver of S-C rats. The decrease in tissue GSH levels in cigarette smoke stressed rats may be due to the effect of declined Gred activity and possibily reduced NADPH supply [34, 35]. Gpx is also a scavenging enzyme, but an increase in its activity in tissues of S-C rats may further reduce the GSH content. In addition, an increased Gpx activity represents a compensatory mechanism to degrade H_2O_2. Thus, during oxidative stress, depletion of GSH, which is of clinical importance in tissue injury, mediated a significant impact on the antioxidant poise of liver cells. Our results are in agreement with an earlier report showing a reduction in Gred activity but an increase in Gpx activity in liver of cigarette smoke exposed rats for 90 days [31]. However, in their study hepatic GSH content was significantly increased, in smoke exposed rats, which is in contradiction to our results. Treatment of cigarette smoke stressed rats with Tocotrienols for 4 weeks, significantly restored the altered tissue activities of SOD, catalase, Gpx, Gred and GST including total, free and protein bound-SH contents of glutathione, to near normal control values, indicating an almost total alleviation of oxidative damage by these antioxidants. Elevated lipid peroxidation products formed by cigarette smoke exposure may generate a tissue antioxidant/oxidant imbalance that could represent a crucial link between cigarette smoke and atherosclerosis. Administration of either 6.0 mg Tocotrienols/rat/day to S-C rats significantly rectifies this imbalance, even though the exact mechanism(s) remain obscure. Tocotrienols mediate a near normalization of peroxide levels and scavenging enzyme activities as well as GSH in liver, of

cigarette smoke exposed rats, indicating a strong anti-lipid/lipoprotein peroxidative effect of these hypolipidemic agents. Based on these results in cigarette smoke exposed rats showing that supplementation of Tocotrienols for 4 weeks significantly improved the integrity of erythrocytes membrane as seen by improved protection against lipid peroxidation as well as reversal of enzymatic activities of CAT, SOD, Gpx and Gred to near normal levels (**Table 4**). 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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