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Der Pharmacia Sinica, 2013, 4(3):14-21

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Invitro antioxidant impact of tocotrienols on lipoprotien lipid peroxidation in young smokers

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ABSTRACT

Smoking is responsible for the death of about 3.5 million subjects every year, 10,000 deaths per day. Smoking is a major risk factor for cancer, heart and lung diseases. The smoke is able to cause tissue oxidative damage at various levels. Tocotrienols are the primary form of Vitamin E. The identification of γ-tocotrienol as a choliesterogenesis inhibitory factor derived from barley represents a landmark early discovery highlighting the unique significance of tocotrienols in health and diseases. In this study we investigated the efficacy of antioxidative power of tocotrienols by analyzing all the parameters in plasma, VLDL-C, LDL-C, HDL-C, HDL2-C, HDL3-C, LDL-Protein, HDL-Protein, VLDL-Protein, MDA, CD and in vitro oxidizability of LDL in absence and presence of Tocotrienols.

Keywords: Tocotrienols, LDL, total antioxidant power, MDA, CD

INTRODUCTION

The World population statistic estimates 6.8 billion tobacco smokers in the world which means 20% of world's population [1]. The total number of tobacco smokers in the world is expected to rise to 1.6 billion during 2020s. [2]. At present, tobacco use causes death of 3.5 to 4 million people globally, which is expected to increase to about 10 million during 2020s. Developing countries need to be concerned because 7 million of these deaths would be occurring in these areas, mainly due to increasing trends of tobacco use [3]. Cigarette smoking is one of the major causes of mortality and morbidity involving respiratory and cardiovascular illness. Smoking has been strongly implicated as a risk factor for chronic obstructive pulmonary diseases, cancer and atherosclerosis[4]. Cigarette smoking is a mixture of over 4000 chemicals containing bioactive substances[5]. One puff of a cigarette exposes the smoker to more than 1015 free radicals and other oxidants and additional free radicals and oxidants are found in the tar of a cigarette [6]. Further damage may be caused by the endogenous formation of oxidants which affect the inflammatory immune response [7]. The burning of tobacco at temperature of 830-900° C leads to the production of about 5000 already identified toxic substances of heterogeneous mixture containing gaseous phase like CO, Nitrogen Oxides, Nitrosamine etc and solid phase which are products of pyrolysis like nicotine, phenols, aromatic polycyclic hydrocarbons in addition to free radicals [8]. The solid phase contains relatively stable free radicals while gaseous one contains small free radicals of oxygen, carbon and sulphur, high concentration of nitric acid and aldehyde moieties. The smoke is able to cause tissue oxidative damage at various levels [9] and contributes significantly to the appearance of endothelial dysfunction and to the alterations which induce arteriosclerosis [10].

Increase in lipid per oxidation products [11] particularly important for increase in LDL oxidation [12] accompanied by a decrease in HDL cholesterol level is reported [13]. Tobacco is associated in the active smoker with the occurrence of ischemic cardiopathy, acute myocardial infraction [14], sudden coronary death, arterial hypertension [15], atherosclerosis [16] and in passive smoker with increased prevalence of CVD [17]. The evidence supporting the hypothesis that LDL is the major atherogenic lipoprotein comes from epidemological studies, clinical trials, studies in lab animals, heritable hypercholesterolemia, pathological investigation; LDL oxidation takes place hen naturally occuring antioxidant agents such as Vit. E, β carotenes, Vit. C that normally inhibits LDL oxidation does not occur. Tocotrienols are the primary form of Vitamin E in the seed endosperm of most monocots including important cereal grains such as heat, rice and barley. Palm oil contains significant quantities of tocotrienol. [19]. Tocotrienol as first reported by Pennock and Whittle in 1964 isolated from rubber. The identification of γ-tocotrienol as a choliesterogenesis inhibitory factor derived from barley represents a landmark early discovery highlighting the unique significance of tocotrienols in health and diseases[19].In this study we investigated the efficacy of antioxidant agent , tocotrienols by analyzing all the parameters in plasma, TC, VLDL-C, LDL-C, HDL-C, HDL2-C, HDL3-C, LDL-P, HDL-P, VLDL-P,TBARS,MDA and *in vitro* oxidizability of LDL in absence and presence of tocotrienols.

MATERIALS AND METHODS

CHEMICALS: All the chemicals used in the study were of analytical grade and procured from standard suppliers like Himedia, Sigma Lab. Pvt. Etc. All the Glasswares used were of Borosil Company and plastic wares from MS Tarson and Himedia. Micropipette from Eppendorf Company, India. Instruments used during study were Electronic balance, pH meter, centrifuge, spectrophotometer, incubatory rotatory shaker, soxhlet apparatus, autoclave, deep freezer, refrigerator, magnetic stirrer, hot air oven, water bath etc.

ESTIMATION: Determination of Nicotine [20], Carbon mono-oxide saturation [21], Plasma Triglycerides [22], Fractionation of plasma lipoproteins such as LDL [23], HDL and its subtractions HDL2 and HDL3 [24], Protein estimation [25], Plasma FRAP [26], LDL oxidation in presence and absence of Vit. C [27-28], Lipid peroxidation [29], ThioBarbituric Acid reactive substances (TBARS) in LDL [30], Total Anti Oxidant Power (TAP) [31], TBARS in RBC [32]. Statistical analysis of data was done by employing two-tailed Student t- test as described by [33]. P value less than 0.02 were considered significant.

EXPERIMENTAL DESIGN: The research was carried out at the Department of Biotechnology and Pharmaceutical Chemistry, Uttaranchal College of Science and Technology, Dehradun. Healthy young male smoker and non smoker (control) were recruited from the college. All the subjects where ethnically homogenous with similar nutritional habits, free from alcohol consumption and were drinking maximum 3-2 cups of tea a day, had no vitamin intake 3 months before the initiation of the study.

RESULTS

MEASUREMENT OF PHYSIOLOGICAL PARAMETER OF NON-SMOKERS AND YOUNG SMOKERS: The results for the physiological parameters of age, height, weight, Number of cigarettes/day, Smoking history, Smoking index are shown in Table 1, do not have any significant difference. This depicts that they do not have any significant role in deciphering the smokers from the normal healthy persons.

MEASUREMENT OF NICOTINE, CARBON MONO-OXIDE SATURATION AND LIPID PROFILE OF NON-SMOKERS AND NOVICE SMOKERS: As shown in Table 2, the results show a modest and significant increase in blood nicotine and carbon mono-oxide. Plasma TC, VLDL-C, LDL-C, HDL-C, HDL2-C, HDL3-C and Non HDL-C levels in smokers as also observed to increase in smokers compared to non smokers as seen in Fig. 1. This maybe due to markedly increased production of oxidant and significantly diminished antioxidant defence. The results are in accordance to the reports of Science Daily. Similar results have been produced for increase in HDL-C and its subfractions HDL_2-C; HDL_3-C conc. [34**]** Increase conc of VLDL-C, LDL-C and TC has been also reported [35**]**.

Table 1. Measurement of Physiological parameter of Non-smokers and Young smokers

Parameters	Non-smokers	Young-Smokers
Number	9	11
Male	5	11
Female	4	-
Age (yrs)	19.77±0.186*	22±0.27*
Weight (kg)	52.55±0.15*	63.55±0.15*
Height (cm)	165.66±0.19*	167.63±0.17*
Number of cigarattes/day	-	7±0.301*
Smoking history (yrs)	-	7±0.30*
Smoking Index**	-	21±0.213*

**Values are mean±S.D. from all groups of subject **Number of cigarettes per day ×smoking history*

Table 2.Measurement of Average value of nicotine, Sco%, TC and Lipid Parameters

Parameters	Non-smokers	Young-Smokers
Nicotine (µg/ml)	2.10±0.03*	5.19±0.07* (+147.14%)
Carbon monooxide saturation (Sco%)	1667.63±0.01*	1801.42±0.006* (+8.022%)
TC (mg/dl)	139.97±0.008*	217.002±0.24* (+55.31%)
VLDL-C	19.126±0.008*	24.61±0.15* (+24.0%)
LDL-C	88.61±0.11*	148.002±0.024* (67.04%)
Non-HDL-C	105.75±0.11*	160.74±0.066* (+51.89%)
HDL-C	33.235±0.001*	55.28±0.03* (+66.33%)
HDL_2-C	11.009±0.0001*	19.18±0.012* (+74.22%)
HDL_3-C	22.145±0.007*	34.28±0.042* (+54.79%)

**Value are mean±S.D. from all groups of subject. TC(Total Cholesterol), VLDL-C(Very Lo Density Lipoprotein, LDL (Lo Density Lipoprotein), Non-HDL value= TC-HDL-C*

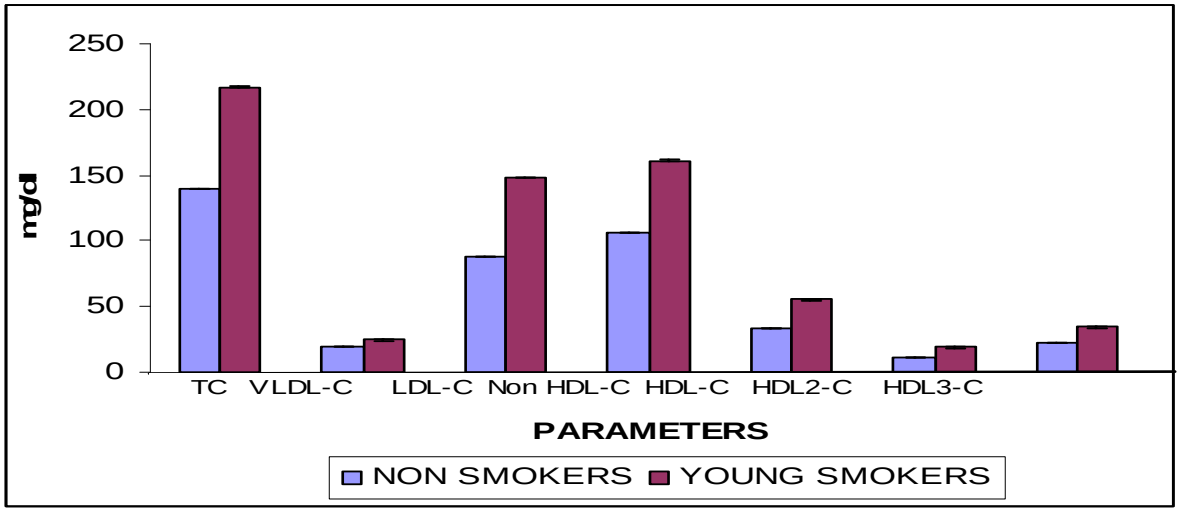

Fig. 1 Comparision of Lipid profile of Non smoker and Smoker

MEASUREMENT OF LIPID PROTEINS: As shown in Fig. 2, VLDL-Protein significantly increased while LDL-Protein and HDL-Protein significantly decreased for young smokers compared to control value. Similar results have been reported [36**]**.

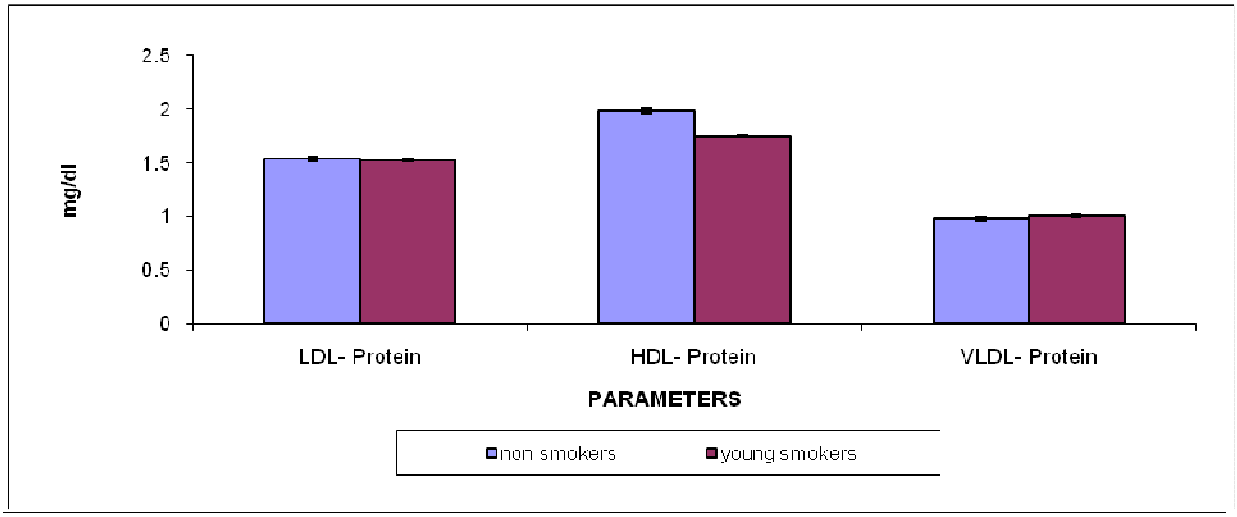

Fig. 2 Measurement of Lipid Proteins

ESTIMATION OF RATIOS OF LDL-C/HDL-C, LDL-C/TC, HDL_2-C/HDL_3-C and TC/HDL-C: Table 3 depicts the ratio of TC/HDL-C to decrease while LDL -C/TC,LDL-C/HDL-C and HDL_2-C/HDL_3-C to show increase.

Table 3. Average value of ratios of different parameters

Parameters	Non-smokers	Young-Smokers
LDL-C/HDL-C	2.588±0.024*	2.624±0.07* (+1.39%)
LDL-C/TC	0.632±0.011*	0.682±0.020* (+7.91%)
HDL_2-C/HDL_3-C	0.521±0.008*	0.583±0.018* (+11.9%)
TC/HDL-C	4.089±0.02*	3.848±0.14* (-5.89%)

**Value are mean±S.D. from all groups of subject*

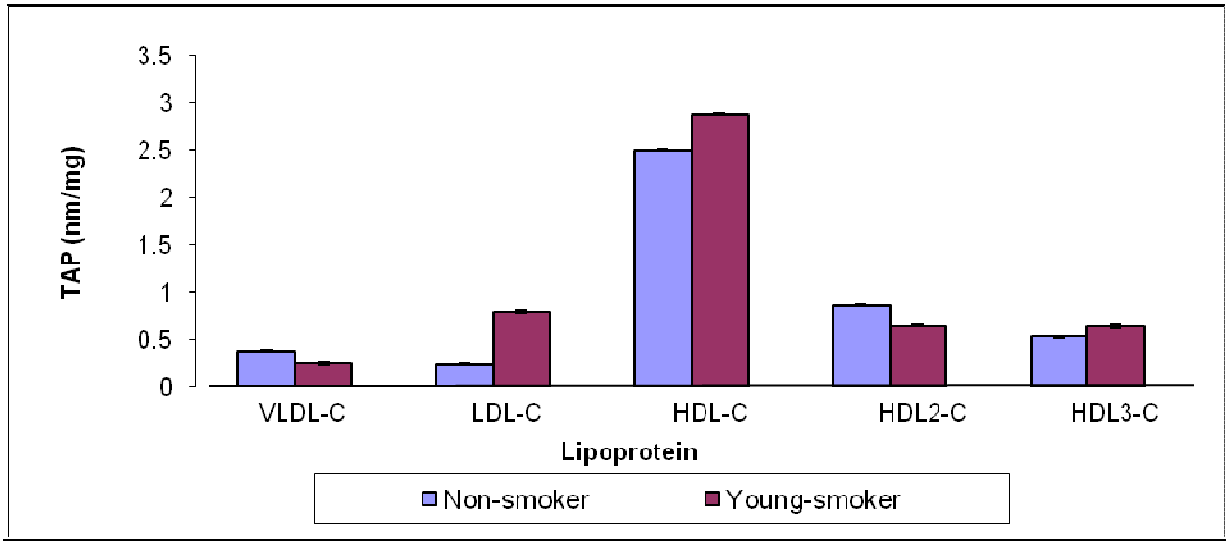

Fig. 3 TAP measurement in lipids

MEASUREMENT OF TAP IN LIPID PROFILE: Fig. 3 shows decrease in the total antioxidant power of LDL-C, HDL-C, HDL_2-C, HDL_3-C and VLDL-C for smokers compared to non smokers.

MEASUREMENT OF *INVITRO* IMPACT OF ASCORBIC ACID ON TOTAL ANTIOXIDANT POWER (TAP) IN PLASMA: As shown in Table 4, Fig. 4, due to excessive increase in free radicals in smokers,plasma total antioxidant level (FRAP) was reduced from a normal control. Invitro treatment of non smokers and smokers with T3 significantly increased with total antioxidant power of each group. As indicated above oxidative stress maybe increased in smokers due to a higher production of reactive oxygen species (ROS) such as superoxide radical, hydroxyl radical, hydrogen pweroxide or deficiency in the antioxidant defence system.Therefore,as te balance between ROS production and antioxidant defence is loosed,the resultant oxidative stress through a series of events deregulates the cellularfunction leading to various pathological conditions.As the antioxidant compound might contribute to total antioxidant of such damage,our TAP in the pl,asma of young smokers significantly decreased which significantly increased after treatment with tocotrienols.

Table 4. *Invitro* impact of Ascorbic Acid on Total Antioxidant Power (TAP) in Plasma

Conc (µl)	Non-smoker TAP (nm/mg)		Young-smoker TAP (nm/mg)	
	Without Vit. C	With Vit. C	Without Vit. C	With Vit. C
10	0.8803±0.0006*	1.394±0.002* (+19.30%)	0.503 ±0.004*	1.008±0.006* (+11.79%)
20	0.781±0.018*	0.945±0.01* (+3.4%)	0.894±0.014*	0.989±0.014* (+6.78%)

**Value are mean±S.D. from all groups of subject*

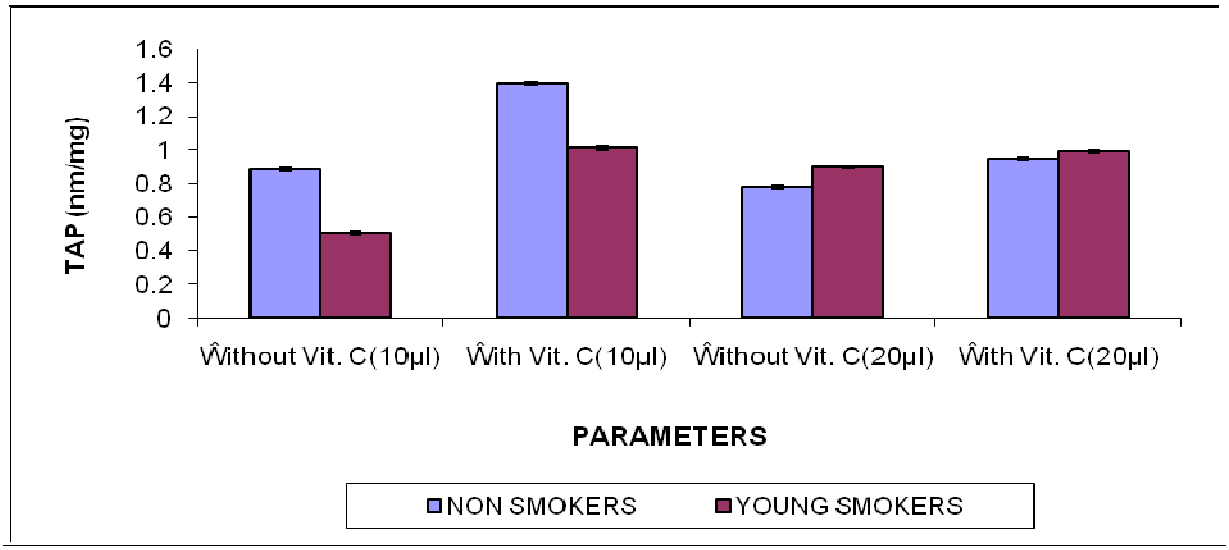

Fig. 4

MEASUREMENT OF LDL OXIDATION: The exvivo basal values of CD (Conjugated Diene) and MDA (MalonDiAldehyde) of LDL Oxidation in young smokers to significantly increase in comparison to non smokers value as seen in Fig. 5 and Fig. 6 respectively. These results indicate a strong antioxidative property of tocotrienol which could be a good source of natural antioxidant.

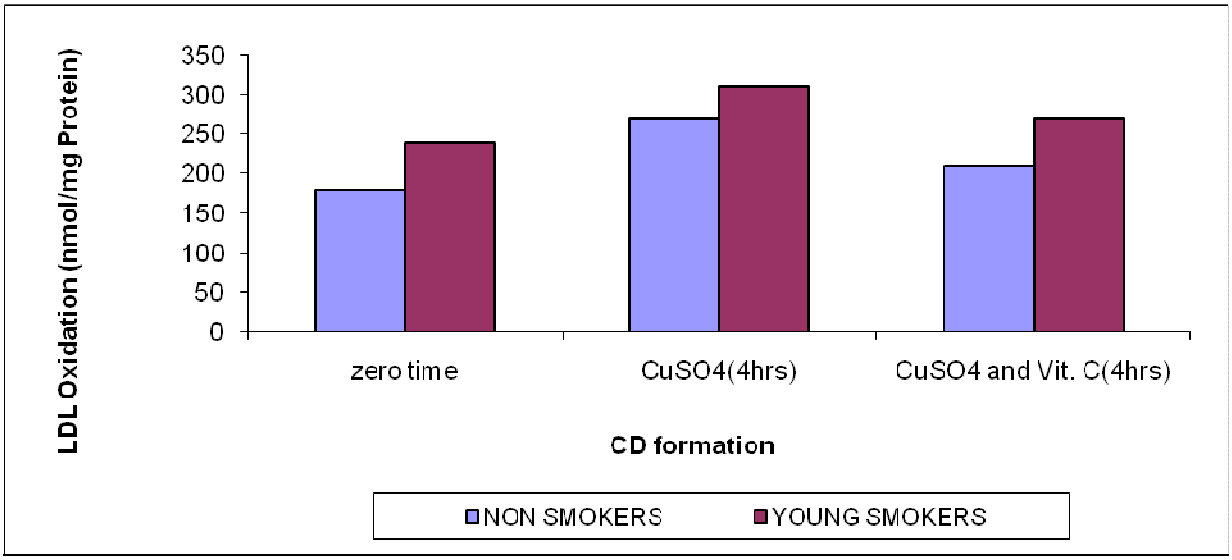

Fig. 5 *In vitro* antioxidant impact on conjugated diene formation in LDL Oxidation

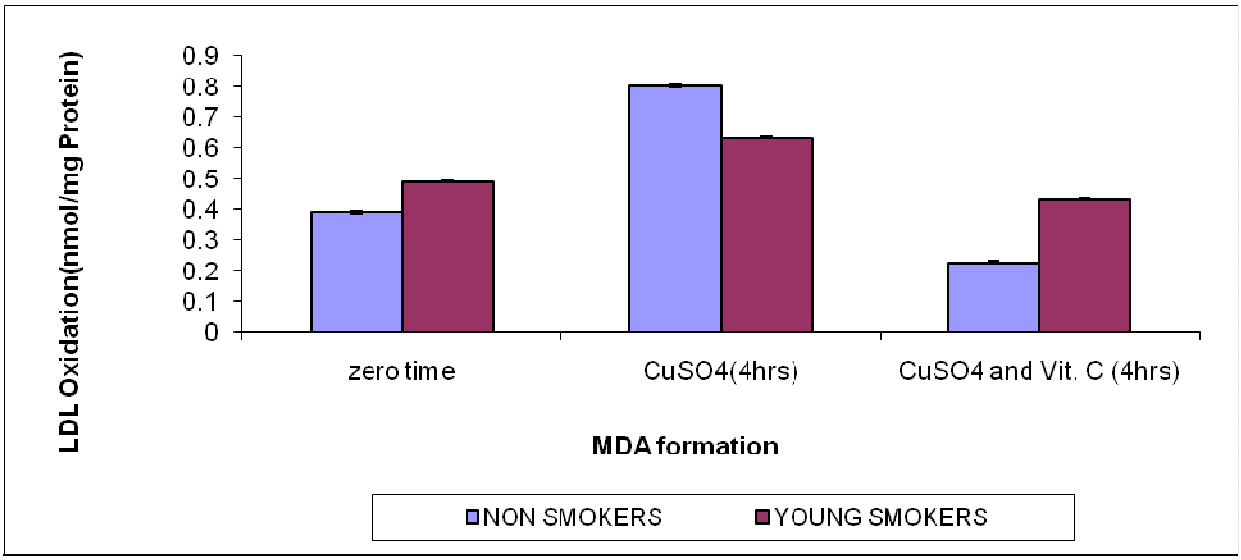

Fig. 6 *In vitro* antioxidant impact on conjugated diene and Malondialdehyde formation in LDL Oxidation

DISCUSSION

The results indicate a strong protective effect of dietary tocotrienols which may help lower the risk of myocardial infarction in hypercholestrolemic rats. Our results indicate a modest and significant increase in plasma total lipid, TG, TC and free fatty acids (FFA) in hypercholestrolemic rats. The increase in plasma TG levels is apparently due to an increase in VLDL which can be the result of either increased VLDL production or decreased VLDL clearance. It is possible that massive free radical load in hypercholestrolemic 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 as well as increase in plasma FFA. Therefore, tocotrienols may exert their cholesterol lowering effect in hyperlipidemic rats in a similar manner as previously reported for hyperlipidemic animals [37] and humans. 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, 38]. 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, 44-50], 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 and enhanced degradation (2.4-fold versus control) of the enzyme [38]. 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 [38]. 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 [38]. 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 [39]. 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.Oxidative modification of lipoproteins is believed to play a central role in the pathogenesis of atherosclerosis [40].Because plasma contains several antioxidants [41,42] and lipoproteins with oxidative damage have been isolated from atherosclerotic lesions [40], 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 hypotheses. 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 [43]. 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 high cholesterol diet in hypercholestrolemic rats, 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 hypercholestrolemic 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.

CONCLUSION

Daily intake of tocotrienols as dietary supplement by novice/young/old/moderate or heavy smokers as well as chronic smokers including passive smokers maybe useful in the prevention and treatment of tobacco including hyperlipidemia and atherosclerosis.it will be efficacious and cost effective which is a good source of Vit. C.

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