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Protective Role of Ascorbic Acid (Vitamin C) Against Hyperlipidemia and Enhanced Oxidizability of Low Density Lipoprotein in Young Smokers

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ABSTRACT

Smoking is an important risk factor for many diseases. Smoking has been strongly implicated as a risk factor for chronic obstructive pulmonary disease, cancer and atherosclerosis. Vitamin-C (Ascorbic acid), the most abundant and effective water soluble antioxidant in the biological fluid, is though to be important for protection against diseases and degenerated processes caused by oxidative stress. In this study we investigated the efficacy of antioxidant agent Vitamin-c or Ascorbic acid by analyzing all the parameters in plasma, TC, TG, TL, VLDL-C, LDL-C, HDL-C, HDL2-C, HDL3-C, TBAR, MDA and in-vitro oxidizability of LDL in absence or presence of Ascorbic acid. All the plasma lipid parameters, TC, TG, TL, VLDL-C, LDL-C, TBAR and MDA significantly increased in young smokers. Ascorbic acid significantly reduces the overall oxidative burden and effectively ameliorated the above altered parameters. Hence daily intake of Vitamin-C by smokers may be useful in the prevention and treatment of tobacco induced hyperlipidemia and atherosclerosis. In addition, daily intake of dietary Vitamin-c will efficacious and cost effective.

Key Words : Ascorbic acid, TBAR, Hyperlipidemia, oxidizability of LDL and Atherosclerosis.

INTRODUCTION

Smoking has been strongly implicated as a risk factor for chronic obstructive pulmonary disease, cancer and atherosclerosis [1]. Cigarette smoking contains a large number of oxidants leading to

the production of free radicals which results for oxidative damage to critical biologic substance [2]. Tobacco epidemic death toll is 100 million in the 20th century which is estimated to be one billion during the 21st century [3]. Currently 5.4 million deaths every year but by 2030 it may rise to 8 million deaths every year Cigarette smoking is a mixture of over 4000 chemicals containing bioactive substances[4]. 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 [5]. Further damage may be caused by the endogenous formation of oxidants which affect the inflammatory immune response [6]. 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 [7]. 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 [8] and contributes significantly to the appearance of endothelial dysfunction and to the alterations which induce arteriosclerosis [9]. Increase in lipid per oxidation products [10] particularly important for increase in LDL oxidation [11] accompanied by a decrease in HDL cholesterol level is reported [12]. Tobacco is associated in the active smoker with the occurrence of ischemic cardiopathy, acute myocardial infraction [13], sudden coronary death, arterial hypertension [14], atherosclerosis [15] and in passive smoker with increased prevalence of CVD [16]. Now a day's Hand smoking is a highly addictive habit involving pharmacological addictions to nicotine. 20% of overall deaths in the industrialized world die from diseases directly linked to smoking [17]. The biological effects of smoking include decrease in the level of ascorbic acid in serum [18]. Active smokers have more than 25% lowẽr circulating concentration of ascorbic acid, α and β carotene and cryptoxanthin. Nicotine is absorbed very rapidly and enters circulation stimulates directly the sympathetic nervous system, increasing the secretion of catecholamines produces vasoconstriction reduced oxygen tension, increased arterial blood pressure, contractability of myocardium and cardiac output [19]. 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 [20]. OxoLDL consists of a heterogenous numerical of modified lipid and protein molecules. 'Ascorbic' name comes from the property of Vit. C of preventing and curing scurvy. Primates including humans have lost the ability to synthesis Ascorbic acid and must obtain it in their food. These are water soluble vitamin. The effectiveness of Vit. C in LDL oxidation inhibition is evident from invitro studies [21]. Ascorbate prevents oxidative modification of LDL primarily by scavenging free radicals and other reactive species, thereby preventing them from interacting with LDL [22] and also by decreased binding of Cu2+ to LDL oed to dehydro- L- ascorbic acid and other decomposition products of ascorbate that react with specific amino acid residues on ApoB, high normally bind Cu2+ thus decreasing the affinity of these amino acid residues for the metal ion. Ascorbate can also prevent the pro-oxidation activity of α- tocopherol by reducing the tocopheroxyl radical to tocopherol, thereby acting as a co-antioxidant and inhibiting LDL oxidation [23] improved lag phase as observed when Vit. C as supplemented in patients with established CVD combined with Vit. E and β carotene [24]. Vit. C is able to scavange both Reactive Oxygen Species (ROS) and

Reactive Nitrogen Species (RNS). The physiological role of Vit. C stems from its very strong reducing power (high reductive potential) and its ability to be regenerated using intracellular reductants such as Glutathione, nicotinamide adenine dinucleotide ans nicotinamide adenine dinucleotide phosphate. In this study we investigated the efficacy of antioxidant agent Vit. C by analyzing all the parameters in plasma, VLDL-C, LDL-C, HDL-C, HDL2-C, HDL3-C, LDL-P, HDL-P, VLDL-P, TBAR, MDA and *invitro* oxidizability of LDL in absence and presence of Vit. C.

MATERIAL 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 [25], Carbon mono-oxide saturation [25], Plasma Triglycerides [26], Fractionation of plasma lipoproteins such as LDL [27], HDL and its subtractions HDL2 and HDL3 [28], Protein estimation [29], Plasma FRAP [30], LDL oxidation in presence and absence of Vit. C [31-32], ApoB Protein [33], Total Anti Oxidant Power (TAP) [34], Haemoglobin content[35]. Statistical analysis of data was done by employing two-tailed Student t- test as described by [36]. 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.

Collection of Blood and Plasma: The Overnight fasted blood from each smoker was collected in heparinized tubes, mixed gently by inversion 2-3 times and incubated at 4^{o} C for 2-3 hrs. Plasma was separated from the blood by centrifugation at 25000 rpm for 30 min, aliquoted and either stored at 4^{o} C or frozen at -20^{o} C for use in other experiments.

RESULT AND DISCUSSION

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.

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

Parameters	Non-smokers	Young-Smokers
Number	9	11
Male	5	11
Female	4	-
Age (yrs)	20.77±0.186*	23±0.27*
Weight (kg)	54.55±0.15*	65.55±0.15*
Height (cm)	167.66±0.19*	169.63±0.17*
Number of cigarattes/day	-	8±0.301*
Smoking history (yrs)	-	5±0.30*
Smoking Index**	-	22±0.213*
Nicotine (µg/ml)	2.18±0.07	5.26±0.08 (+141.28)[a]
CarbonMonooxide Saturation	1569.69±11.22	1705.42±12.23(+8.65)[a]

**Values are mean±S.D. from all groups of subject **Number of cigarettes per day ×smoking history. + (increase), - (decrease). Significantly different from nonsmokers [a]P>0.001.*

Measurement of Nicotine, Sco%, TC and Lipid Parameters: The results depicted in Table 1 and Fig. 1 indicate that increase in the levels of Nicotine and Carbon mono-oxide saturation occurred due to smoking of cigarette which also increases the risk of myocardial infraction in young population. Similarly TG, TC, VLDL-C, HDL-C, HDL2-C, HDL3-C, non HDL-C were significantly increased from the normal control value. The results are in accordance to the publications of National Institute of Drug Abuse Reports (2010) and Science Daily. Increased concentration of HDL-C and its subfractions has also been reported by [38]. Increase concentration of LDL-C has been proven by [39].

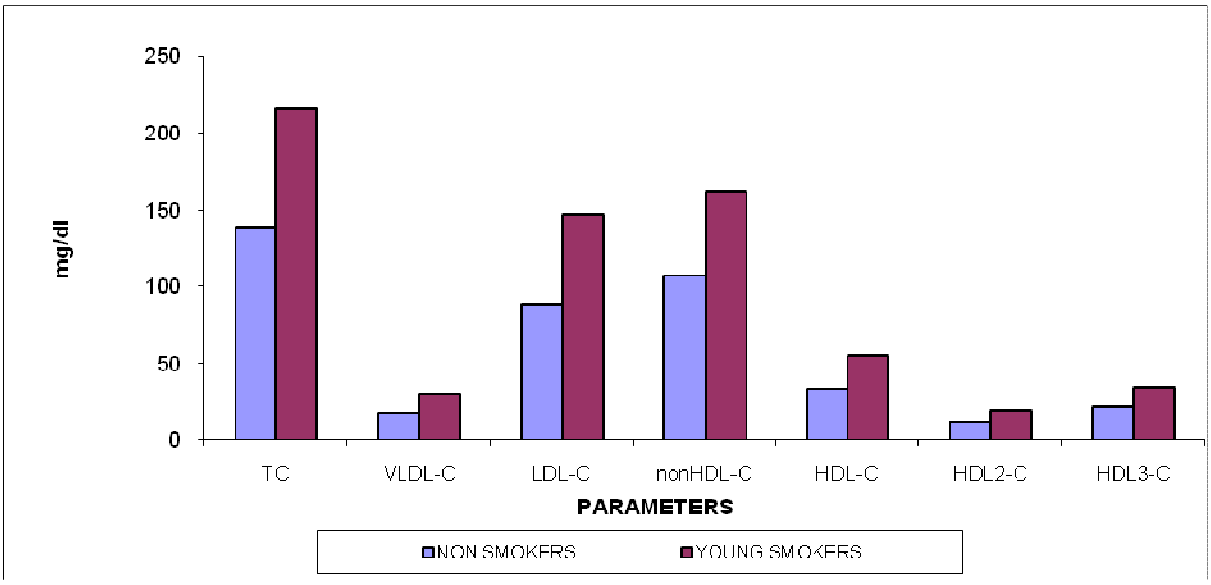

Fig.1 Average value of nicotine, Sco%, TC and Lipid Parameters.
TC (Total Cholesterol), VLDL-C (Very Low Density Lipoprotein, LDL (Low Density Lipoprotein), Non-HDL value= TC-HDL-C.

Estimation of protein of LDL, HDL & VLDL of non-smokers and young-smokers: The results depicted in Fig. 2 indicate that increase in the levels of VLDL-Protein, LDL-Protein but decrease in HDL-Protein for smokers in comparison to non smokers. Similar results were obtained through the work done by [39].and [40].

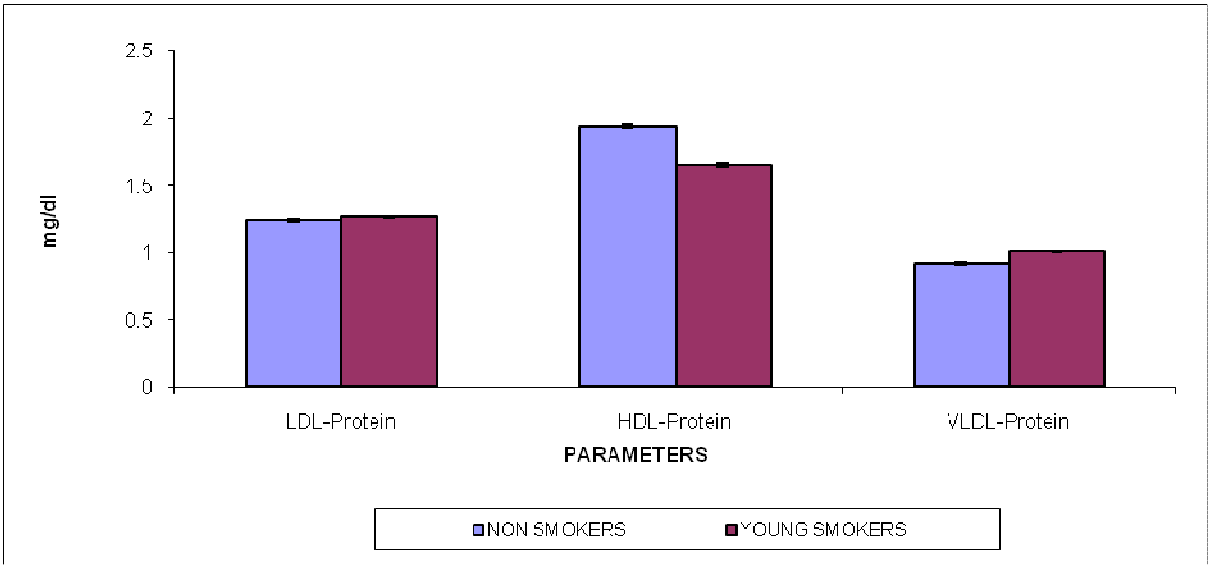

Fig.2 Estimation of protein of LDL, HDL & VLDL of non-smokers and young-smokers.

Measurement of the ratios of LDL-C/HDL-C, TC/LDL-C, HDL2-C/HDL3-C & TC/HDL-C:

The results depicted in Table 2 showed significant increase in the ratio of LDL-C/HDL-C and HDL2-C/HDL3-C while decrease in the ratios of TC/LDL-C and TC/HDL-C for young smokers compared to non smokers.

Table 2. Measurement of the ratio of LDL-C/HDL-C, TC/LDL-C, HDL2-C/HDL3-C and TC/HDL-C.

Parameters	Non-smokers	Young-Smokers
LDL-C/HDL-C	2.685±0.093*	2.755±0.005* (+2.6%)[b]
TC/LDL-C	1.565±0.010*	1.466±0.013* (-1.3%)[b]
HDL2-C/HDL3-C	0.505±0.004*	0.556±0.011* (+10.09%)[b]
TC/HDL-C	4.186±0.005*	3.872±0.006* (-7.50%)[b]

**Values are mean±S.D. from all groups of subject. + (increase), - (decrease). Significantly different from nonsmokers [b]P>0.05.*

Measurement of Total Antioxidant Power (TAP) in Ascorbic Acid at different concentration: The results depicted in Table 3 showed the antioxidant impact of Vitamin C. at different concentrations which significantly increased compared to basal value of 10µl/µg. Similar increase in TAP for increasing concentrations of Vit. C was reported by [37] and [38].

Table 3. Measurement of Total Antioxidant Poŵer (TAP) in Ascorbic Acid at different concentration.

Conc (µl/µg)	O.D. at 595nm	TAP (nm/mg)
10	0.307	0.8614 (basal level)
20	0.409	0.9035 (+4.88%)[b]
40	0.6512	1.032 (+19.80)[a]
60	0.752	1.110 (+29.09%)[a]
80	0.883	1.110 (+28.85%)[a]
100	1.186	1.115 (+29.4%)[a]

+ (increase), - (decrease). Significantly different from nonsmokers [a]P>0.001 and [b]P>0.05.

Measurement of Total Antioxidant Power (TAP) in lipid parameters: results depicted in Table 4 showed the antioxidant impact in LDL-C, HDL-C and HDL3-C levels increased in young smokers compared to non smokers while values of HDL2-C, VLDL-C were decreased from normal control value.

Table 4. Average value of TAP in lipid parameters. *Values are mean±S.D. from all groups of subject.

Lipoprotein	Non-smoker TAP (nm/mg)	Young-smoker TAP (nm/mg)
VLDL-C	1.025±0.009*	0.212±0.004* (-79.31%)[a]
LDL-C	0.2219±0.0005*	0.655±0.001* (+195.17%)[a]
HDL-C	1.4092±0.0002*	1.873±0.0008* (+32.92%)[a]
HDL2-C	0.8865±0.001*	0.628±0.011* (-29.15%)[a]
HDL3-C	0.535±0.009*	0.639±0.010* (+19.43%)[a]

+ (increase), - (decrease). Significantly different from nonsmokers [a]P>0.001.

***Invitro* impact of Ascorbic Acid on Total Antioxidant Power (TAP) in Plasma:** results depicted in Table 5 showed for *Invitro* impact of Vit. C on TAP in Plasma isolated from young smokers reduced from non smokers value due to excessive increase in free radicals in smokers which reduces the Ferric Reducing Ability od Plasma (FRAP). *Invitro* treatment of normal and smokers with Vit. C significantly increases with TAP of each group. Oxidative stress may be increased in smokers due to a higher production of Reactive Oxygen Species (ROS) such as hydrogen peroxide, superoxide radical, hydroxyl radical and /or deficiency in in the antioxidant defense system. Therefore as the balance between the ROS production and antioxidant defense loosens, the resultant oxidative stress through a series of events deregulates the cellular functions leading to various pathological damages. Our result showed a significant decrease value in total antioxidant status in plasma of young smokers and significant increase after treatment with Vitamin C results are similar to that predicted by [39-40].

Table 7. *Invitro* impact of Ascorbic Acid on Total Antioxidant Power (TAP) in Plasma.

Conc (µl)	Non-smoker TAP (nm/mg)		Young-smoker TAP (nm/mg)	
	Ŵithout Vit. C	Ŵith Vit. C	Ŵithout Vit. C	Ŵith Vit. C
10	0.8405±0.0004*	1.0028±0.001* (+19.30%)[a]	0.8243±0.001*	0.9215±0.004* (+11.79%)[a]
20	0.676±0.014*	0.699±0.017* (+3.4%)[b]	0.865±0.012*	0.9237±0.0007* (+6.78%)[b]

**Values are mean±S.D. from all groups of subject. + (increase), - (decrease). Significantly different from without treatment [a]P>0.001 and [b]P>0.05.*

***In vitro* antioxidant impact on Conjugated diene and Malondialdehyde formation in LDL Oxidation: :** results depicted in Table 6 shows the ex-viva Baseline Diene Conjugation level and Malondialdehyde values of young smokers to significantly increase in comparision to normal control values. After the Cu2+ mediated oxidation of LDL were seen to increase. *Invitro* LDL oxidation was carried out in the presence of Vit. C, which decreased the maximum amount of CD of LDL oxidation after 4 hrs incubation. These results indicate that Vit. C has strong anti-oxidative properties.

Table 6. *Invitro* antioxidant impact on conjugated diene and Malondialdehyde formation in LDL Oxidation.

Conc (µl)	Incubation at 37° C	CD formation (nmol/mg Protein)		MDA formation (nmol/mg Protein)	
		Non-smoker	Young -smoker	Non-smoker	Young -smoker
10	0 time	180.25±0.04*	246.19±0.05* (+36.58%) [a]	0.365±0.16*	0.3915±0.001* (+7.26%) [b]
10	0+CuSO4(4hrs)	270.32±0.07* (+49.47%)[a]	311.26±0.07* (+26.43%) [a]	0.8004±0.008* (+119.28%) [a]	0.9423±0.0006* (140.68%) [a]
10	CuSO4+Vit. C (4 hrs)	210.09±0.02* (-22.28%) [a]	270.45±0.09* (-13.11%) [a]	0.1436±0.001* (-82.05%) [a]	0.4326±0.0008* (-54.03%) [a]

**Value is mean±S.D. from all groups of subject. Values are obtained from LDL, isolated from pooled plasma in each group. LDL oxidation (10 µl) as carried out in the absence and presence of Vit. C (10 µl). + (increase), - (decrease). Significantly different from without treatment [a]P>0.001 and [b]P>0.05.*

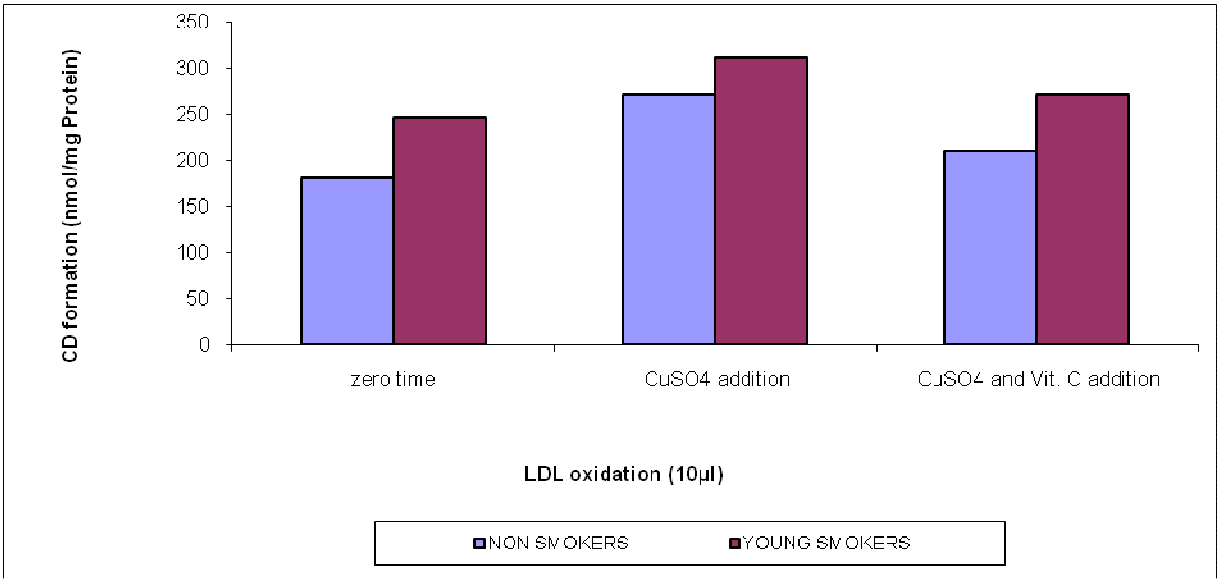

Fig. 3. *Invitro* antioxidant impact on Conjugated diene on LDL Oxidation

Ascorbic acid mediated multiple therapeutic benefits in the present study. Daily intake of Vit. C as dietary supplement by young/old/moderate or heavy smokers including passive smokers maybe useful in prevention and treatment of tobacco including hyperlipidemia and atherosclerosis. In addition, daily intake of Vit. C will be efficacious and cost effective.

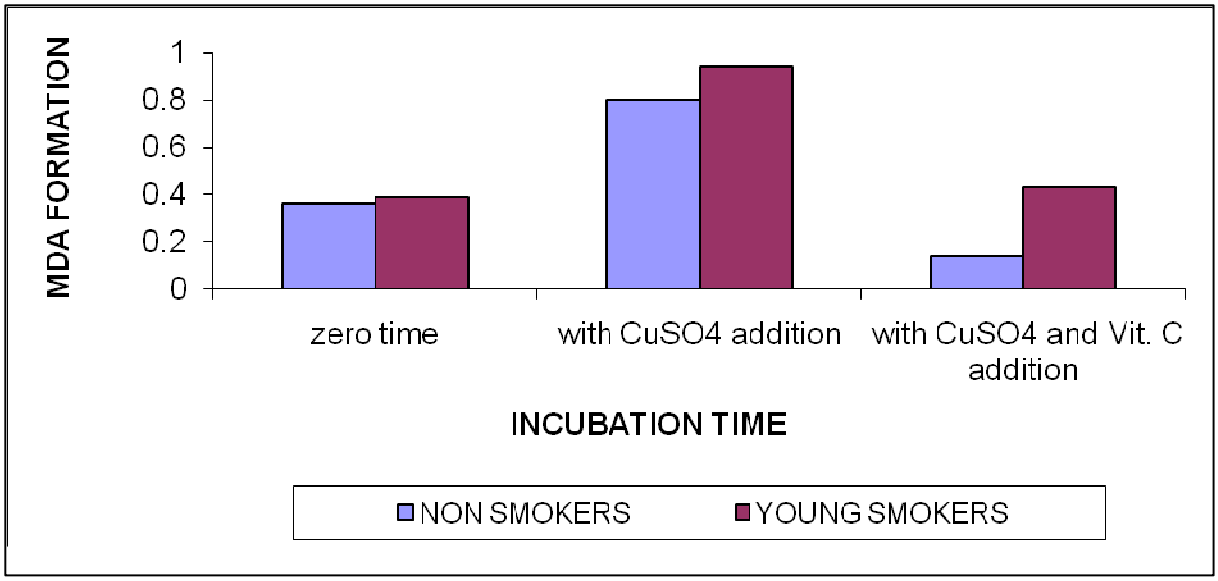

Fig. 4. Invitro antioxidant impact on Conjugated diene on LDL Oxidation

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