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Efficacy of vitamin E against cigarette smoke induced alterations in pulmonary tissue, serum enzymes and lipid profile in albino rats

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Abstract : *The present study was designed to evaluate the protective role of vitamin E(50mg/kg bwt) against adverse effects of cigarette smoke (60 and 120 days) on lung tissue , serum enzyme activity and lipid profile in male albino rats. Reduction in the histopathological changes like emphysema, pulmonary edema, debris, capillary permeability and thick epithelium have been seen after supplementation of vitamin E in comparison to cigarette smoke exposed rats. Decrease in serum enzyme activity viz. SGPT ($P<0.05$), SGOT ($P>0.05$) and SALP ($P<0.05$) and serum lipid profile - serum cholesterol ($P>0.05$), serum triglycerides ($P<0.05$), LDL ($P>0.05$) and VLDL ($P<0.05$) with corresponding increase in HDL level ($P>0.05$) have also reported after pre-exposure supplementation of vitamin E in comparison to cigarette smoke exposed rats. Vitamin E is able to mitigate the toxic effects of cigarette smoke on albino rats.*

Key words: Cigarette smoke, Albino rat, Lung histopathology, SGPT, SGOT, SALP, Serum lipid profile, Antioxidant vitamin E

Introduction

Cigarette smoking is a world-wide major cause of preventable morbidity and mortality. Smoking yields chemical substances with cytotoxic potentials. Cigarette smoke is a complex mixture of chemical containing more than 4000 different constituents which are harmful for the health. It generates many toxic and carcinogenic compounds, such as nicotine, nitrogen oxides, carbon monoxide, hydrogen cyanide and free radicals (Hoffmann *et al*., 2001). The World Health Organization (WHO) predicts that tobacco deaths in India may exceed 1.5 million annually by 2020. By 2030, if current trends continues, smoking will kill more than 9 million people annually (Yanbaeva *et al*., 2007).Cigarette smoke contains approximately 10^{17} oxidant molecules per puff that can cause damage to lipids, proteins, DNA, carbohydrates and other biomolecules (Wei *et al*., 2001). The lungs are an essential organ of respiration and fuel us with oxygen, which pass into the blood stream, where It is rushed off to the tissue and organs that require it to function.

Blood lipids are well known factors associated with the development of cardiovascular diseases. Fat build up more easily on arterial wall damage by cigarette smoke and alters the level of lipoprotein (Neki, 2002). Plasma lipoprotein abnormalities are said to be the underlying major risk factors for the common occurrence of atherosclerotic vascular diseases (Shai *et al*., 2004). Increased production of free radicals in gas phase and tar phase of cigarette smoke produces oxidative stress and can result in the oxidation of lipids, consequently perturbs the antioxidant defense system (Ambrose and Barua, 2004).

Antioxidants are capable of stabilizing, deactivating or scavenging free radicals before

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they attack on cells (Sapakal *et al*., 2008). Vitamin E is an effective antioxidant and free radical scavenger which can help in reducing the cigarette smoke induced toxicity. It is a primary liposoluble antioxidant (Shaikh *et al*., 1999).Therefore, the aim of the present study is to investigate a possible protective role of vitamin E on serum enzymes and blood lipid profile with lung histopathology in male albino rats.

Materials and Methods

The inbred colony of healthy and adult male albino rats (150-200g) were kept in polypropylene cages in standard laboratory conditions of temperature 25±0.5^0 C, relative humidity 60±5% and photoperiod 12h/day. They were fed on pellet diet (Golden feed, New Delhi, India) and water *ad libitum*.

The experimental rats were grouped into six sets of five rats each- control set I and II were exposed to ambient air for 60 and 120 days, experimental set III and IV exposed to cigarette smoke for 1 hour/day for 60 and 120 days and experimental set V and VI exposed to cigarette smoke with pre-exposure supplementation of vitamin E (50 mg/kg b.wt) for 60 and 120 days.

Experimental rats were exposed in smoke chamber (Precision Instruments, Varanasi) with their cages. The rats were subjected to the whole body exposure of six Capstan filtered cigarette (69 mm), ITC Limited, Kolkata. Evion drops (Tocoferyl acetate) from Merck Company, Aurangabad, was used as an antioxidant vitamin E (50 mg/kg b.wt.) and administered in rats by gavaging. All experiments were carried out as per the guidelines of institutional ethical committee.

After the stipulated exposure period, rats were dissected and lung tissues of control and experimental rats were taken out to assess the histopathological study. Lung tissues were fixed in 10% formalin and blocked with paraffin. and paraffin slices of 5 micron thickness were prepared and stained with Haematoxylene and eosin. The slide preparations were observed microscopically and photomicrographs were taken.

Blood samples were collected from the ventricle of heart in sterilized plain centrifuge tubes and were centrifuged at 2500 rpm for 30 minutes. Serum was separated for the estimation of enzyme activity and lipid profile in control and experimental sets. Enzymatic activities were measured as kinetic reaction using IFCC method. The absorbance of reaction was determined at 340 nm by spectrophotometer.

Serum cholesterol was estimated by Roeschalu *et al*. (1974), serum triglycerides by Schettler and Nussel (1975) method, HDL by Wybenga and Pileggi (1970) method, LDL and VLDL were calculated by Friedwald *et al*. (1972) methods. The data were expressed as Mean±S.Em. They were signified by using 't' test. Statistical calculation was carried out by using one way ANOVA, KpKy plot (version 3.0).

Results and Discussion

Histopathological changes observed in lung tissue of cigarette smoke exposed rats in comparison to control rats are emphysema, pulmonary edema, debris, thick epithelium and capillary permeability after 60 days but these changes are more pronounced after 120 days cigarette smoke exposure [Plate-I (a) and (b) and Plate-II (a) and (b)]. After 60 days supplementation of vitamin E, a reduction in the areas of emphysema and pulmonary edema have been seen. Epithelium thickness and capillary permeability are observed at some places, while after 120 days supplementation of vitamin E, thickness of epithelium, and capillary permeability almost disappear and recovery in the areas of emphysema and edematous conditions [Plate- III (a) and (b)].

Data obtained for serum enzyme and serum lipid profile in control and experimental rats after 60 and 120 days are shown in Table 1 and Table 2. A significant increase in SGPT, SGOT and SALP in cigarette cmoke exposed rats in comparison to control rats after 60 and 120 days. Enzymatic activity of SGPT, SGOT and SALP are decreased after 60 and 120 days pre-exposure supplementation of vitamin E (Table 1and 2). An increase in serum triglyceride , LDL

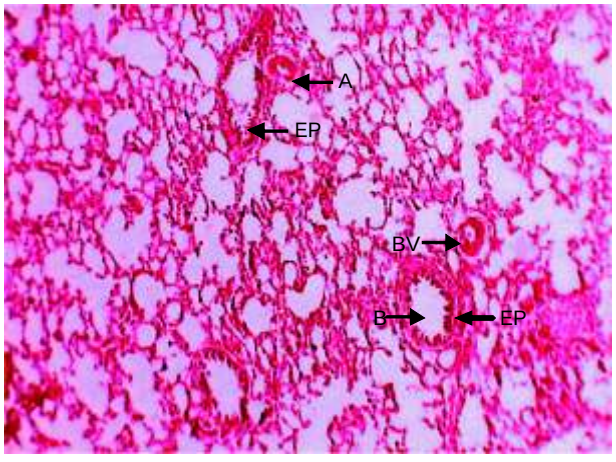

PLATE-I(a)

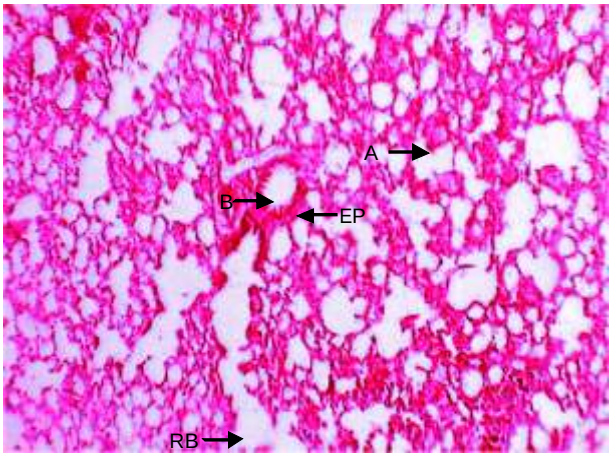

PLATE-II (b)

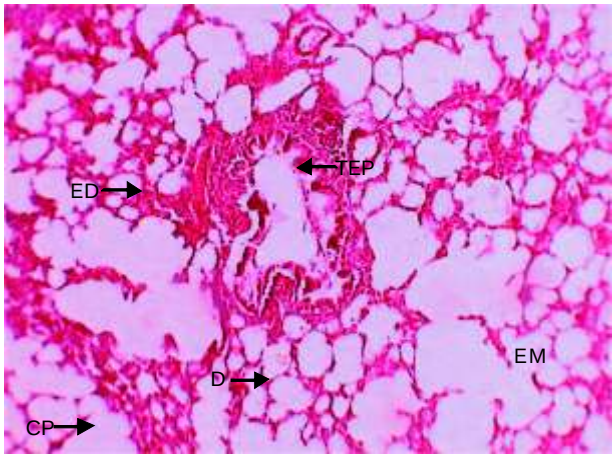

PLATE –II (a)

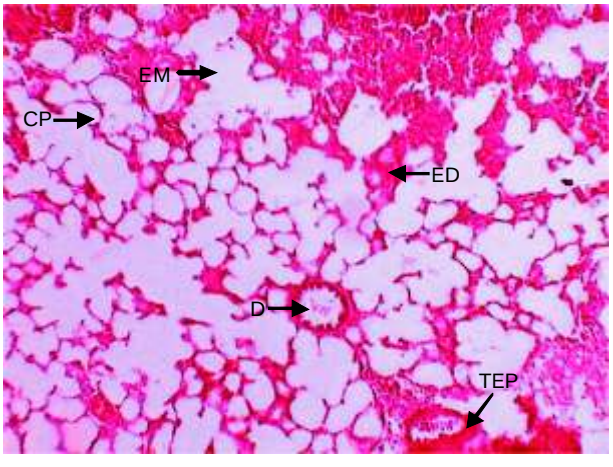

PLATE-II (b)

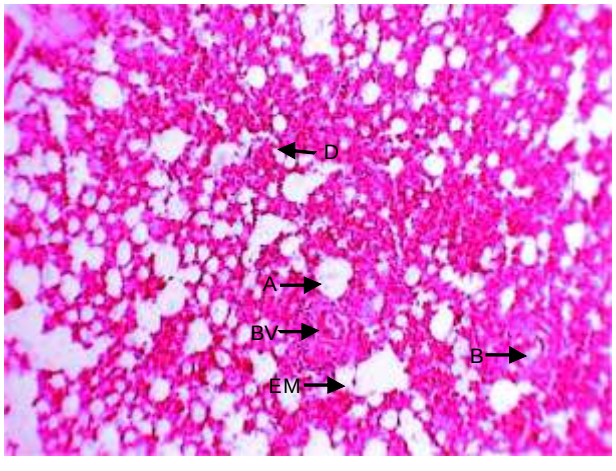

PLATE –III (a)

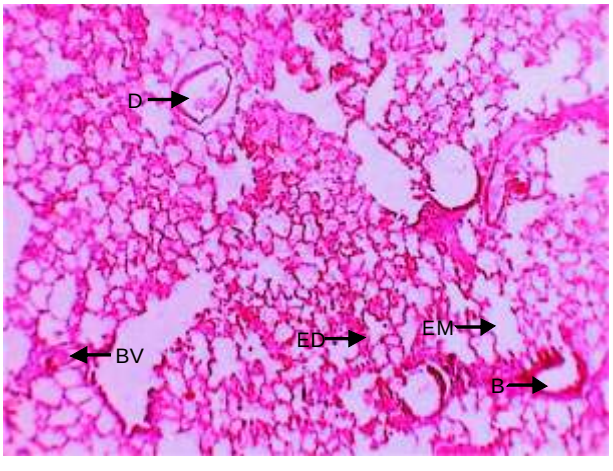

PLATE-III (b)

Plate- I (a) and (b) showing section of lung of control rats,Plate- II (a) and (b) Experimental rats after 60 and 120 days cigarette smoke exposure and Plate- III (a) and (b) Experimental rats after 60 &120 days cigarette smoke exposure with vitamin E (X 400)

[EP- Epithelium, A- Alveoli, B- Bronchiole, BV- Blood vessel, RB- Respiratory bronchiole, C- Capillary, TEP- Thick epithelium, D- debris, ED- Edema, EM- Emphysema, CP- Capillary permeability]

Table 1. Effect of cigarette smoke and vitamin E on serum enzyme activity and lipid profile after 60 days

parameters	Set I (5) Ambient air	Set III (5) Cigarette smoke exposure	Set V (5) Cigarette smoke exposure+vitamin E
	Mean±S.Em.	Mean±S.Em.	Mean±S.Em.
SGPT (U/L)	54.4±4.05	79.17±4.20**	54.04±7.01**
SGOT (U/L)	168.09±2.36	177.25±1.77*	173.97±3.62*
SALP (U/L)	167.6±2.49	196.3±4.36***	189.72±1.94*
Cholesterol (mg/dl)	83.32±0.77	86.85±1.82*	82.80±0.84*
Triglyceride(mg/dl)	88.55±1.75	94.17±1.54*	85.79±1.15**
HDL (mg/dl)	32.47±0.97	25.22±1.50***	31.25±0.70*
LDL (mg/dl)	32.74±1.41	37.78±1.56*	34.48±1.47*
VLDL (mg/dl)	17.70±0.35	18.83±0.30*	17.15±0.23**

Table 2. Effect of cigarette smoke and vitamin E on serum enzyme activity and lipid profile after 120 days

Parameters	Set II (5) Ambient air	Set IV (5) Cigarette smoke exposure	Set VI (5) Cigarette smoke exposure+vitamin E
	Mean±S.Em.	Mean ±S.Em.	Mean±S.Em.
SGPT (U/L)	53.1 ±4.89	72.95±5.71*	51.8±8.62*
SGOT(U/L)	170.97±4.16	180.13±3.36*	178.23±1.95*
SALP (U/L)	168.50±4.64	199.5±3.15***	192.09±2.37*
Cholesterol (mg/dl)	84.07±1.68	91.84±1.63*	84.21±1.58**
Triglyceride (mg/dl)	88.94±1.90	98.75±2.02**	87.41±2.32**
HDL (mg/dl)	30.75±1.01	23.69±1.001***	30.20±0.73***
LDL (mg/dl)	35.76±1.81	40.47±2.61*	36.88±2.12*
VLDL (mg/dl)	17.75±0.367	19.74±0.40**	17.48±0.46**

(5) = Number of albino rats
S.Em. = Standard Error of Mean
Non–significant (P>0.05)
*Significant (P<0.05)
**Highly significant ((P<0.01)
***Very highly significant (P<0.001)

and VLDL with corresponding decrease in HDL level have been observed after 60 and 120 days exposure to cigarette smoke, while after vitamin E supplementation, the serum lipid profile viz. serum cholesterol, serum triglyceride, serum LDL and serum VLDL were decreased with corresponding increase in serum HDL level after 60 and 120 days.

Cigarette smoke contains a large number of chemicals include polycyclic compounds, phenols, benzopyerene, carbon monoxide, formaldehyde and oxides of nitrogen, nitrosamines etc. It also contains various oxidants such as oxygen free radicals and volatile aldehydes which are probably the major cause of damage to biomolecules (Yeh *et al*., 2008). Cigarette smoke immediately increases markers of oxidative stress. It leads to the uptake of many hazardous compounds. Such compounds or their metabolites may be electrophilic and thereby able to react with biological macromolecules or they may give rise to oxidative stress by radical chain reaction (Vaart *et al*., 2004). According to Hackett *et al*. (2003), oxidants are the major mechanism of smoking inducing airways damage. Free radicals existing in cigarette smoke are proven to cause cellular damage in respiratory system diseases (Hobson *et al*., 1991).

Lung is a target organ for oxidative stress. Smoke particles are inhaled into the lung with greater velocity. This forced inhalation of the smoke aerosol opens the alveoli widely and facilitates the rapid saturation with nicotine. The secondary and tertiary bronchi of the lung and the alveolar region also lack the defense present in the major bronchi. Therefore, peripheral bronchi are less resistant to the toxic and carcinogenic constituents of cigarette smoke (Wynder and Muscat, 1995).

Inflammation due to cigarette smoke inhalation leads to progressive destruction of the muscles of the bronchiolar wall (Haschek and Rousseaux, 1991). Alveolar epithelial cells and inter-alveolar septa were markedly thick and their blood capillaries were congested due to long duration of cigarette smoke exposure. Besides this, many toxic substances especially ROS (reactive oxygen species) can damage cellular constituents (Marnett *et al*., 2003). Cigarette smoke also induces oxidative stress by stimulating NADPH oxidase and decreasing antioxidant defenses (Agarwal, 2005). The smoke was too irritating to inhale deeply. Carcinogens were deposited on the epithelium at the branches of central bronchi (Wynder and Hoffmann, 1994). Cigarette smoke known to stimulate the alveolar macrophages to release excessive level of free radicals, which are believed to play an important role in the development of emphysema and inflammatory diseases (Saskia *et al*.,2010). When cells die or are damaged due to inflammation, the enzymes leak out causing blood levels of these enzymes to rise. It is frequently used as an indication of tissue oxidative stress as a result of free radical attack on the cell membrane, leading to increased membrane permeability and cellular damage (Pannuru *et al*., 2009). Elevated level of SGPT, SGOT and alkaline phosphatase can be measured as an indicator of cell damage (Pant, 1992). The elevation of these enzymes in serum reflects some hepatic disorders because carcinogens in cigarettes are mainly through the lungs, into the blood and to the liver. Nicotine has a considerable influence on increasing the lipid levels in blood, resulting in creased lipid peroxidation. Lipid peroxidation determines membrane dysfunctions, inactivates membrane sites of the receptors and enzymes, increase the membrane permeability (Halliwell and Chirico, 1993). Free radicals generates lipid peroxidation resulting in lipidic radicals which react with oxygen in aerobic cells resulting in peroxyl radicals. The peroxyl radicals start up a reactions chain through which the polyunsaturated fatty acids are transferred in lipidic hydroperoxides. Reactive oxygen species are capable of initiating and promoting oxidative damage in the form of lipid peroxidation (Pop *et al*., 2008). Increased oxidative stress and generation of free radicals can results in the modification of LDL to oxidized

LDL that could lead to atherosclerosis lesions (Kharb and Singh, 2000).Cigarette smoke also decreases the activity of paraxonase, an enzyme that protects against LDL oxidation (Nishio and Watanabe,1997).Serum level of HDL is generally lower in smokers as compared to nonsmokers(Akbari *et al*.,2000).The elevated level of cholesterol and triglycerides may be due to cadmium, a metallic element present in the cigarette smoke which affects the metabolism of lipid in rat liver and heart. Increase in lipid peroxidation in tissue has been implicated in cadmium induced organ damage and dysfunction (Yalin *et al.,* 2006). The prevalence of hypercholesterolemia and triglycerides in heavy smokers have also been reported by Afira (2010). In the present study the changes in lipid profile were more increased after 120 days . According to Imamura *et al*.(2000) smoking alters the lipid profile and these changes are related to the duration and amount of smoking. An increased amount and duration causes more of dyslipidemia (Kshitish *et al*., 2010).

In the present study, after vitamin E supplementation, there is an improvement in the pulmonary tissue injury and serum enzyme profile after 60 and 120 days. Vitamin E has a capacity to destroy free radicals generated as a part of the oxidation reaction in the human body or by exogenous agents. Vitamin E is considered one of the most important dietary antioxidant in biological system due to its association with cell membrane and its ability to act directly on ROS preventing peroxidation . It is a membrane stabilizer and regulator of membrane fluidity (Ohyashiki *et al*.,1998). It has an effective role in maintaining cell structure against oxidative stress through blocking the chain reaction and stabilizes the cell membrane, maintaining its permeability and integrity (Shi *et al*., 1999). Cigarette smoking produces oxidant mediated changes in the lung which is important to the pathogenesis of emphysema so vitamin E may constitute an important component of the lung's defense against oxidant injury. It reduces lipid per oxidation and inhibits per oxidation of membrane lipid by scavenging lipid peroxyl radicals (Sendhilvadivu and Rajeswari, 2011).). It plays a protective role in preventing atherogenic modification of LDL (Francene and Alan,1998). In conclusion, the present study shows that supplementation of antioxidant vitamin E provide a wide scope in reducing the toxic effects of cigarette smoke in rats.

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