HISTOPATHOGENICITY IN LUNG AND LIVER OF CIGARETTE SMOKE EXPOSED, TOBACCO AND NICOTINE INDUCED MICE

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ABSTRACT

Introduction: Tobacco consumption on prolong may cause cellular damage in liver and lung. This study aimed to observe histopathological changes and oxidative stress on liver and lung in cigarette smoke exposure, nicotine and tobacco induced mice.

Materials: Total 30 male mice were randomly selected, each group contain six mice were as following: controls (Group I), cigarette smoke exposed (Group II), nicotine induced (group III), Tobacco induced (Group IV) and cigarette smoke exposed plus Tobacco induced (group V) for 45 days each time for 30 minutes. Mice were sacrificed and subjected to histopathological observation and biochemical study for oxidative stress.

Results: Present study, Histopathological findings of liver showed lymphatic infiltration of hepatocytes, few hepatocytes undergoing mitosis, inflamed kupffer cell. Lung showed lymphatic nodules around bronchus with red cell mass and emphysema with damaged air sacs. Liver and lung show presence of mucus secretion and undergoing fibrosis. MDA level in liver and lung were high ($p<0.0001$) in Group II 528.85±134 and 331.2±97.61; Group III 667.74±156.92 and 392.6±63.58, Group IV 435.36±101.76 and 300.7±58.69; and Group V 269.76±138.88 and 173.6±66.78 µmol/mg respectively in compared to control group.

Conclusion: Present study concluded that cigarette smoke exposure, nicotine and tobacco induced mice undergo histopathogenicity of lung and liver with increased MDA level.

Keywords: Reactive oxygen species, oxidative damage and histopathogenicity

INTRODUCTION

Tobacco is used in different forms as cigarette, bidi, pan, surti etc in India and it is well documented that tobacco are responsible to cause oxidative stress[1, 2, 3]. Lung is the first and the most frequent vital organ to counter cigarette smoke contents and metabolize in tobacco users. Liver is another organ where the most of the cigarette metabolites undergoes metabolism of metabolites of tobacco is an important organ for elimination toxic metabolites from the body [4, 5]. Nicotine is absorbed through the lungs during consumption of tobacco and is rapidly metabolized in the liver[6].

Cigarette smoking (CS) yields chemical substances with cytotoxic potentials[7]. These chemicals created by smoking induce oxidative stress of hepatocytes associated with lipid peroxidation[6]. CS is a complex mixture of at least 4000 different carcinogens that includes nicotine, nitrosamine, polycyclic aromatic hydrocarbons, aromatic amines, unsaturated aldehydes (e.g. crotonaldehyde) and some phenolic compounds which mediate tumor initiation and promotion[8].

Long-term exposure to tobacco smoke causes permanent inflammation. Nicotine through smoking, induced an inflammatory response in the lung and plays a role in pathogenesis of obstructive pulmonary diseases[9, 10]. Apoptosis is strongly induced in alveolar epithelium exposed to smoking[11]. Nicotine induces risk of developing hepatocellular carcinoma, chronic liver diseases and finally hepatocellular carcinoma and varying degrees of fibrosis in liver.

Aim of the present study to observe the histopathogenicity and lipid peroxidation in lung and liver induced by cigarette smoke exposure and different forms of tobacco in *swiss* albino mice.

MATERIALS AND METHODS

The present study was conducted in the Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005, Uttar Pradesh, India. Adult Swiss albino mice with an average weight and age about 20-30 grams and 60-90 days old respectively were used in this study. For using these animals, the consent with reference Dean/2015/CAEC/1433 was taken from institutional ethical committee of Banaras Hindu University, Varanasi.

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Procurement and Acclimatization of Animals

Swiss albino mice were procured from the animal house of Department of Anatomy, Institute of Medical Sciences, Banaras Hindu University. They were reared in polypropylene cage (25x 20x15 cm) under standard laboratory condition (25º ± 5º C, 12 hr L/D cycle, 55± 5 Relative Humidity) 2 weeks proper acclimatization. Dry rice bran was used as a bedding material. They were fed pelleted diet obtained from animal feed supply centre, Varanasi and tap water ad libitum.

Cigarette Smoking System

Smoking system apparatus contains three chambers for creation of environment of cigarette smoke exposure to experimental mice.15x10x10 cm sized air generator contains exhaustion fan one of its surface, which was for production of oxygenated air for ignition of the cigarette. 20x10x10cm sized smoke chamber was meant for ignition and production of smoke, which was connected by the air pipe to the air generator. The produced cigarette smoke was expelled to the inhalation chamber. Cigarette smoke was accumulated in 50x25x20cm sized inhalation chamber was connected to the smoke chamber.

Preparation and Induction of Smokeless Tobacco Extract

An aqueous extract of tobacco (BHR-5) commercially available tobacco (BHR-5) (pan bahar ltd., Delhi, India) was finely powdered using a mortar and pestle.10gms of the powdered tobacco were dissolved in 30 ml of distilled water and incubated at 37 °C for 30 min with thorough shaking. The dissolved contents were filtered through 125 mm filter paper (Whatman) to remove larger-sized materials, and again through a 0.22 µm filter (Corning) to sterilize the recovered products. The recovered sterile solution was stored at 4° C. The 255 µl sterile solution was mix in 345 µl distilled water, pH tested, and 100 µl of diluted solution administered orally angulated blunt tuberculin syringe of each mouse for 45 days.

Nicotine treatment

(−) Nicotine hydrogen tatrate salt (Sigma Chemical Co., St. Louis, MO) was dissolved in

0.9 % physiological (sterile) saline and passed through a 0.45 µm filter for intraperitoneal (IP) injection and delivered in an injection volume of 0.5 mg/ kg of body weight for 45 days. The pH of drug solutions was adjusted to ~7.4.

Experimental design

Thirty male mice were taken in the present study and randomly assigned into five groups. Each group contained six mice were as following; Group I: Control (given equivalent amount of distilled water), Group II: Cigarette smoke exposed (CSE) (four cigarettes twice a day for one hour each time thirty minutes), Group III: nicotine induced (0.5 mg/kg body weight), Group IV: Tobacco induced (dose mentioned above), and Group V: CSE plus Tobacco induced (dose mentioned above).

Sacrifice of the mice

Treated and control mice of each group were sacrificed by cervical dislocation at the end of the experiment (46th day morning). The liver and lung were autopsied weighed and subjected for histological observation and biochemical study.

Tissue preparation for histological study

Immediately after cervical dislocation, abdomen and thorax was exteriorized by mid line incision. Adequate quantity of liver and lung tissues were obtained, thus obtained organs were fixed in 10% formaldehyde for minimum seven days. After fixation, organs were weighed and taken for tissue processing in ascending grades of alcohol (70%, 90%, 95%, absolute I and absolute II) followed by xylene I and xylene II. 6 µ thicknesses was sectioned from the tissue block and mounted on glass slides. Stained by Haematoxylin and eosin (H&E), periodic acid Schiff (PAS) and Mallory Trichrome (MT) stains were done. By using NIKON ECLIPSE E200 camera, the histopathological changes in mice liver and lung tissue were examined.

Tissue collection and biochemical assay

Immediately after cervical dislocation, abdomen and thorax was exteriorized by midline incision. Adequate quantity of liver and lung tissues were autopsied, kept in phosphate buffer solution (PBS) at -20° C and10% (w/v) tissues were homogenated in ice cold PBS (0.1 M, pH 7.4). The homogenate was centrifuged and the resulting supernatant was used for MDA level. All chemicals and reagents were obtained from Sigma Chemical Co (St Louis, MO, USA).

Malondialdehyde (MDA) was estimated by thiobarbituric acid reactive substances (TBARS) test protocol from [12] at 532 nm by ELICO- SL-104 double beam UL-UV Spectrophotometer.

Statistical analysis

Obtained data were entered in excel sheet of MS-2007. Statistical analysis was carried out by using Graph pad Prism version 6 for Windows. The results were reported as MEAN ± SD. Statistically analysis by using one-way ANOVA and Bonferroni Multiple Comparison (BMC) test were used to compare between control, cigarette smoke exposure, nicotine and tobacco induced groups where value $p<0.05$ was considered statistically significant.

RESULTS

Liver and Lung weight

The weight of liver and lung were gradually reduced in nicotine induced group (Group III) found to be 0.81±0.06 and 0.15± 0.01gm respectively in compared to other groups, whereas weight of organs were reduced in combination of cigarette smoke exposed plus tobacco induced group (Group V) found to be 1.01±0.17 and 0.14±0.04 gm respectively in compared to control group (Group I) (Table 1).

Table 1: Liver and Lung weight in various groups of mice at the end of experiment

Organ	Group I(gm)	Group II(gm)	Group III(gm)	Group IV(gm)	Group V(gm)
Liver	1.21±0.08	1.17±0.11	0.81±0.06	1.01±0.21	1.01±0.17
Lung	0.31± 0.07	0.23± 0.04	0.15± 0.01	0.25± 0.03	0.14±0.04

Histological feature of liver

Histological feature of control group of liver showed normal hepatocytes, hepatic sinusoids, kupffer cells and central vein (CV), in all treated group of mice liver showed the density of fat accumulation in hepatocytes, few hepatocyte undergoing mitosis, distorted hepatic sinusoid, inflamed kupffer cells and hematoma congestion in central vein; it was also observed lymphatic infiltration of hepatic lobule (Fig 1). PAS stain shows PAS negative, MT stain shows abundant number of fibres around hepatocytes (Fig.1).

Histological feature of lung

Bronchus of control mice showed normal histomorphic structure with respiratory epithelium and few mucin accumulated on it. Muscular layer showed considerable amount of smooth muscle. In treated group of mice bronchus showed hyperplasia lined by shedded respiratory epithelium. Lymphatic nodule showed considerable increase around bronchus. Abundantly, mucin accumulation on the shedded epithelial lining and red cell mass were observed and also observed blood congestion in vessels (Fig. 2). Parenchyma of control mice showed normal parenchyma with uniform alveoli, in treated group of mice lung showed emphysematous lung parenchyma undergone severe histopathogenicity. In all treated group, alveoli sac dilated and bronchial filled with mucus, Inter alveolar septum thickened, red cell mass and pneumocytes get inflamed and lymphatic infiltration (Fig. 2). PAS stain shows mucin secretion in lung parenchyma, MT stain shows abundant number of fibres in interalveolar septum (Fig. 2).

Malondialdehyde level

MDA level of liver and lung were significantly higher in nicotine induced group (Group III) found to be 667.74±156.92 and 392.6 ±63.58 in compared to other groups, however combination with cigarette smoke exposed plus tobacco induced group (Group V) was found to be 269.76±138.88 and 173.6 ±66.78 in compared to control group (Table. 1).

On comparison of MDA level in various groups by one-way ANOVA, Bonferroni multiple comparison (BMC) test in control group (Group I) showed, insignificant ($p>0.05$) where as Group III was significantly higher ($p<0.0001$) in compared to other groups, however, Group V was significantly increased ($p<0.0001$) in Group V in compared to control group (Group I) (Fig. 1).

DISCUSSION

Present study had taken the different forms of tobacco such as cigarette smoke, tobacco extracts obtained from gutkha, and nicotine solution in mice experiment which may translate to the human consumption significance of different forms of tobacco in India since according to national family health survey II, 1998-99, 37% population of 15 yrs and above and this prevalence had been increased to 14.6% in 2009[13, 14]. The present study showed the significantly affected in gross change in organ weight, biochemical changes and pathogenicity in the organs.

Present study showed that the relatively low weight of organs such as liver and lung in treated groups in compared to control which could be caused by several factors. Vascular changes due to nicotine and carbon monoxide could limit delivery

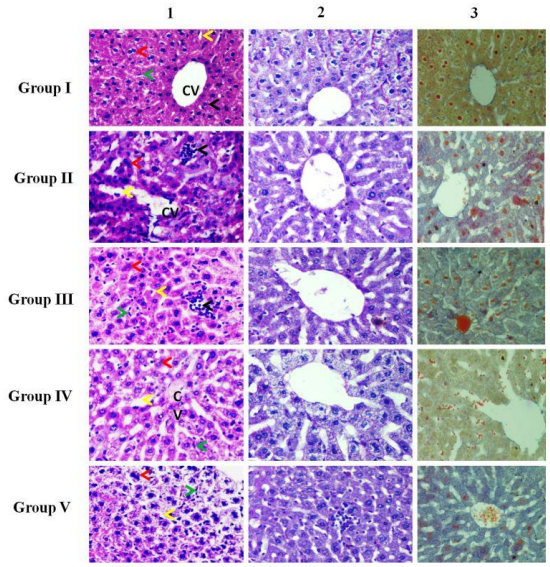

Fig.1 Groups I (Control) showing H&E stain 1, PAS stain 2, MT stain 3, Group II, III (CSE, nicotine respectively) showing congestion central vein (CV), lymphatic infiltration of hepatocytes (black arrow), distorted hepatic sinusoid (yellow arrow), inflamed kupffer cell (green arrow) 1, PAS stain showing pas negative material 2, MT stain showing presence of around hepatocytes 3; Group IV, V (tobacco, CSE plus tobacco respectively) showing Enlarged and distorted hepatic sinusoid (yellow arrow), congestion central vein (CV), mitosed hepatocyte (red arrow), inflamed kupffer cell (green arrow) 1, PAS stain showing pas negative material 2, MT stain showing abundant number of fibres around hepatocytes 3.

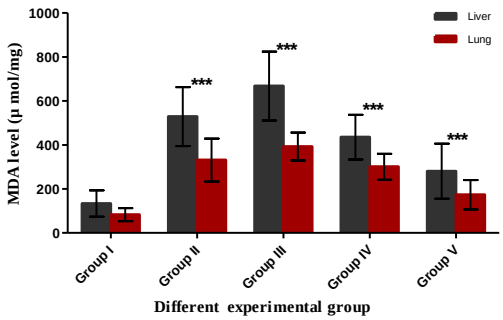

Fig 3: MDA level of liver and lung of treated mice shows significance difference.
***** p……<0.0001**

release from the adrenal glands and nerve cells due to the exposure of tobacco can cause vasoconstriction[15,16,17]. Insulin signaling pathways stimulation in the brain is required to uptake glucose synthesis in the liver[18]. Dysfunction of insulin in the brain would cause a decrease in glucose level since glucose cannot be absorbed and stored as glucose in the cigarette smoke exposed adult mice due to which in the present study, the liver and lung weight had been significantly reduced in group II, III, IV and V in compared to group I .

In present study we also evaluate the microscopic structure of liver and lung. In group II, III, IV and V lung showed hyperplasia respiratory epithelium around Bronchus. Lymphatic nodule

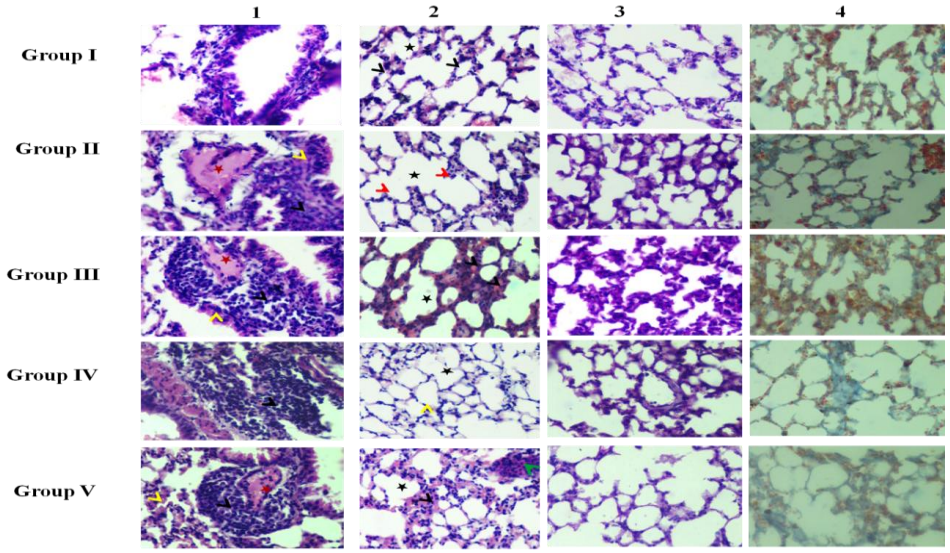

Fig 2: Group I control showing H&E stain bronchus 1, parenchyma with alveoli 2, PAS stain 3, MT stain 4, Group II, III, IV, V (CSE, nicotine, tobacco, CSE plus tobacco respectively) Bronchus showing multiple lymphatic nodules around bronchus (black arrow), red cell mass (yellow arrow), hematoma in blood vessels (*) with inflamed respiratory epithelium 1; parenchyma with alveoli showing inflammatory cells (green arrow), dilated antrum (*), emphysema with damaged air sacs (red arrow), red cell mass (black arrow), thin inter alveolar septum (yellow arrow) 2; PAS stain showing mucin secretion in alveoli 3; MT stain showing more number of fibres present interalveolar septum 4;

of nutrients to liver. Similarly, catecholamine showed considerably around bronchus.

Abundantly, mucin accumulation on the epithelial lining was observed fibrosis within thicken wall. There was presence of lymphatic infiltration entirely around bronchus. MT stain showed minimal presence of fibre in parenchyma of the lung.

Liver in group II, III, IV and V showed red cell mass in hepatic lobule, distorted portal vein, congestion of vein and some sinusoid were highly appreciated. Density of vaculation in some hepatocyte, large area of hepatic lobule occupied by vacoular degeneration, and accumulation of fatty in hepatocyte were observed. The necrosis and apoptosis of hepatocyte and markedly presence of monocular infiltration around central vein were observed. Similar studies were observed by other researchers[19, 20, 21, 22].

Studies in the cigarette smoke reported that occurrence of oxidative toxicity arises because of increase in production of free radicals, reducing agents and decrease antioxidant capabilities consequences the oxidative and tissues damage[23]. Liver is an important organ of storage, detoxification, metabolism and excretion of many metabolites so they are particularly vulnerable to oxidative damage.

Present study, cigarette smoke expose to mice induced the biochemical impairment increasing the highest MDA level. This activity showed that reactive oxygen species such as hydroxyl, peroxyl contained in cigarette smoke via lipid peroxidation leading to cause an imbalance between cellular pro-oxidant and antioxidant levels leads to the oxidative stress resulting in tissue damage in liver and lung[24]. In correlation with the alterations in enzymes activities, present data demonstrated an induction of inflammatory changes in tissues due to the effect of cigarette smoke, nicotine and tobacco induced to mice.

CONCLUSION

Present study concluded that physiological alteration, histopathogenicity in organ like lung and liver, and biochemical alteration in rise in lipid peroxidation by MDA are inter related which may translate to such affects the human on consumption since India is one of the highest consumption of tobacco products in the form of smoke, tobacco extracts as surti, gutkha, paan and nicotine solution as injection in addiction.

Conflict of interest: none

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