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IMPACT OF CURCUMIN ON NICOTINE-INDUCED LUNG CHANGE: HISTOPATHOLOGICAL, BIOCHEMICAL AND IMMUNOLOGICAL STUDY

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Abstract:-Background: Nicotine, major toxic component of cigarette smoke, is considered to be the main risk factor in the development of pulmonary diseases Purpose: The aim of the present study was to investigate the morphological, biochemical and immunological changes in albino rats following intraperitoneal injection of nicotine. The possible protective effect of curcumin was also evaluated. Material and Methods: The study included three groups, the first served as control. The second was injected with nicotine (2.5mg/ kg body weigh) for eight weeks. The third group was injected with nicotine as the second group in addition to curcumin (80mg/kg body weight) for also eight weeks. Results: Injection with nicotine resulted in replacement of air sacs in lung tissue by connective tissue full of large number of inflammatory cells and red cells. Respiratory bronchioles were disrupted and their lumen was closed with acidophilic material. Biochemically increased Malondialdehyde (MA) and nitric oxide (NO) and decreased reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (Ca) were documented. Immunologically, nicotine was an important immuno-suppressive constituent of cigarette smoke. The previous results were improved in curcumine treated group indicating its protective role against the oxidative stress .Conclusion: We concluded that, consumption of curcumine to smokers can protect against lung hazards.

Keywords: Nicotine, curcumin, nicotine induced lung changes.

INTRODUCTION

Nicotine, major toxic component of cigarette smoke, is considered to be the main risk factor in the development of pulmonary diseases .This toxicity has been reported to be due to its oxidative stress both in vivo and *in vitro* (Suleyman *et al*., 2002). Many authors believe in structural and functional modifications due to chemical alterations resulting from excessive free radical production and/or low antioxidant defense. In addition, it was found that inhalation or injection of nicotine alters the immunological function including innate and adaptive immune responses (Ogna *et a*l., 2012). Curcumin as a medicinal plant was discovered by some researchers to have a wide range of pharmacological effects such as antioxidant, antitumor, anti-inflammatory and hepatoprotective activities (Ellman, 1959).

So, this study was developed to evaluate the structural changes induced by nicotine histopathologically. Moreover, the enhanced lipid peroxidation and depletion of antioxidant was examined biochemically as well. Constructive referential evidence to its effect on the immune system was considered. The protective effect of curcumin on lung damage, induced by nicotine, was also evaluated.

MATERIALS AND METHODS

Twenty five albino rats with an average weight of 150-200 gm were randomly divided into 3 groups. Group 1: consisted of 5 rats and served as control. Group 2: consisted of 10 rats and injected with nicotine (2.5 mg/kg body weight) intraperitoneal for 8 weeks

Group 3:-consisted of 10 rats and administrated with curcumin (80mg kg body weight) by stomach tube simultaneously with

nicotine injection. The rats were housed in stainless-steal cages and fed with standard rat chow and tap water and libitum in the animal house of the research institute of ophthalmology.

HISTOPATHOLOGICAL METHODS

The rats of all groups were sacrificed after 8 weeks. Paraffin-embedded sections were prepared. Sections from lung tissue were immediately fixed in 10% formalin and then dehydrated in ascending grades of ethanol. This was followed by clearing in 2 changes of xylene for 30 minutes each .Tissues were then impregnated with paraplast (3 changes) at 58°C for 3 hr and then embedded in paraplast. Sections 4µm thick, were prepared with a microtome and stained with hematoxylin and eosin and examined by light microscopy.

BIOCHEMICAL METHODS

Preparation of hemolysate for biochemical analysis:

Blood was drawn from each rat, serum was separated, and a hemolysate was prepared, and samples were stored at -70ºC until analysis.

Preparation of tissue homogenate for biochemical analysis:

Lung tissue samples were removed, cleared of blood and immediately transferred to ice-cold containers containing 0.9% sodium chloride for various estimations. A known amount of tissue was weighed and homogenized in appropriate buffer (10%) for the estimation of various biochemical parameters. Protein content was measured according to Lowry et al. (1951).

Biochemical analysis of lung and blood samples:

Analysis of antioxidant enzymes:

Catalase (CAT) activity was determined by the method of Sinha (1972). In this test, dichromatic acetic acid is reduced to chromic acetate when heated in the presence of hydrogen peroxide (H2O2), with the formation of perchloric acid as an unstable intermediate. Superoxide dismutase (SOD) activity was determined by the method of Marklund and Marklund (1974). In this test, the degree of inhibition of pyrogallol auto-oxidation by the supernatant of the lung homogenate and/or hemolysate was measured.

Estimation of reduced glutathione:

Reduced glutathione (GSH) was determined according to the method of Ellman (1959).

Estimation of nitric oxide:

Nitric oxide was estimated by using the Griess method (Lepovire et al., 1990).

Estimation of malondialdehyde:

The extent of lipid peroxidation was determined by the method of Ohkawa et al. (1979).

Immunological methods:

The level of CD4 in serum was estimated by Eliza (Enzymed linked immunoassay). CD4 (cytokine 4) is carried by T cells in blood (T cell antigen receptor). Nicotine injected to the level animal induced changes in T cell responsiveness.

Statistical analysis

Data from biochemical investigation were analyzed using analysis of variance (ANOVA). The results were considered statistically significant if the $p < 0.05$.

RESULTS

Histopathological results:

Group I (control): The lung tissue was formed of alveoli lined by simple squamous epithelium. The bronchioles had patent lumen lined by columnar ciliated epithelium and surrounded by elastic fibers. The interalveolar septa were thin and composed of capillaries and a small amount of connective tissue (Fig. 1). Group II (subjected to nicotine for eight weeks): The alveolar spaces were replaced by connective tissue full with huge number of lymphocytes and red blood cells. The respiratory bronchioles were disrupted and their lumen was closed by acidophilic material (Fig. 2). Severe disruptions of alveolar septa were seen with widening of alveolar spaces. Lymphocytes and dust cells were numerous in the septa. There was also a thick-walled blood capillary with narrow lumen, full of red blood cells (Fig. 3). Group III (curcumin treated): The interalveolar septa were nearly free of inflammatory cells and bronchioles were intact (Fig. 4).

Biochemical results:

Table1: Activities of SOD and CAT in blood and lung samples.

Groups	SOD		CAT	
	In blood U/g Hb	In lung (U/mg Protein)	In blood U/g Hb	In lung (U/mgProtein
Control	2494± 79	12.34±0.95	128.4±2.5	41.83±0.65
Nicotine after 8 weeks	1471±150[a]	7.3±0.65[a]	86.2±1.22[a]	30.5±2.2[a]
Nicotine+curcumin after 8 weeks	2389±59[b]	11.65±0.7[b]	125±5.3[b]	40.91±2.1[b]

Values are mean ± SD; n=6.
a, Significant difference ($p<0.05$) when compared with 'control' group
b, Significant difference ($p<0.05$) when compared with group II

Table 2: Level of reduced glutathione and malondialdehyde in samples of blood and lung.

Groups	GSH		MDA	
	In blood (mg/dleryth)	In lung (mg/g tissue)	In blood (nmol/ml)	In lung (nmol/gmtissue)
Control	60.2±3.6	4.09±0.74	3.47±0.19	6.5±0.3
Nicotine After 8weeks	32.9±5.2 [a]	1.95±0.13[a]	5.99±0.15 [a]	12.6±0.7 [a]
Nicotine + curcumin After 8weeks	58.2±2.2 [b]	3.59±0.21[b]	4.08±0.05 [b]	6.9±0.3 [b]

Values are mean ± SD; n=6.
a, Significant difference ($p<0.05$) when compared with 'control' group.
b, Significant difference ($p<0.05$) when compared with group II.

Table 3: Level of nitric oxide in samples of blood and lung

Groups	NO (µmol/L) In blood	NO (µmol/mg protein) in lung
Control	36.38±7.15	7.81±0.03
Nicotine After 8weeks	50.90±8.53 [a]	11.96±0.05 [a]
Nicotine + curcumin After 8weeks	37.56±2.1 [b]	8.36±0.02 [b]

Values are mean ± SD; n=6.

a, Significant difference ($p<0.05$) when compared with 'control' group .
b, Significant difference ($p<0.05$) when compared with group II.

Activities of SOD and CAT in the hemolysate and lung tissue of control and experimental animals in each group are shown in Table (1). The activity of SOD and catalase were significantly decreased in nicotine-treated rats when compared to control. Oral administration of curcumin to nicotine-treated rats significantly increased the activity of SOD and catalase when compared with animals treated with nicotine alone. The levels of GSH, MDA and the level of NO in the hemolysate and lung tissue of control and experimental animals in each group are shown in Tables (2 and 3). The levels of MDA and NO were significantly elevated in animals treated with nicotine when compared to controls. Administration of curcumin to nicotine-treated animals significantly decreased the level of MDA and NO when compared with nicotine treated animals. On the other hand, the level of reduced glutathione was decreased in the hemolysate and lung tissue of nicotine-treated rats as compared with control. Supplementation of curcumin to nicotine-treated rats significantly elevated the levels of GSH

Immunological results:

Table 4: The level of CD4 (cytoke 4) in serum, T level of serum sample after injection of nicotine to the animal (mice) and its level after treatment of the same animals by curcumin.

No.	Sample	CD4 in
1	Nicotine injection	400/ml
2	Nicotine injection	450/ml
3	Treated sample	450/ml
4	curcumin	500/ml
control		400-1200/ml

In the first two samples, slight decrease was observed in the level of CD4/ml while after treatment in the other 2 samples, slight rise in CD4 level was seen to some extent (Nicotine appears as an immunosuppresor).

DISCUSSION

Nicotine had been proposed to be a major environmental risk factor for a variety of human diseases particularly the lung. Kalpana *et al*. (2007) proved that lipid peroxidation increased in nicotine-treated cases due to oxidative stress. The present study displayed histopathological changes due to lipid peroxidation as a result of oxidative stress. They were in the form of destruction of alveolar septa, moreover interstitial inflammatory reaction and thickening of the wall of the blood vessels were observed.

In agreement with the present results, Ogna *et al*. (2012) described interstitial lung changes in smokers that were characterized histopathologically by bronchiolocentric accumulation of pigmented alveolar macrophages, chronic inflammatory cells and fibrosis. Oxidative stress had been proved also biochemically in our study. Evidences had indicated that free radicals or ROS (reactive oxygen species) such as β-hydroxy ethyl radical, superoxide radical, hydroxy radical, peroxy radical and hydrogen peroxide, were implicated in nicotine-induced oxidative tissue injury (Hsu et al., 2006). Nicotine, a potential carcinogen, used in the present study had been reported to be oxidized into its metabolite cotinine, formal - dehyde and 4- (methylnitrosamino)-1-(3-pyridyl)-1-butanone mainly in the liver and to a significant extent in the lung and kidney, and plays a key role in the pathogenesis of tissues (Dicke *et al*., 2005).

Mostly, nicotine undergoes 5'-hydroxylation induced by CYP2A6 to form cotinine and related metabolites, including formaldehyde giving rise to ROS (Yamazaki et al., 1999). Thus, excessive generation of ROS as a consequence of induction of cytochrome CYP2A6 by nicotine plays a major role in the development of lipid peroxidation (LPO) and formation of lipid peroxidative end products. These findings support the elevation of lipid peroxidation in the lung and plasma in nicotine-treated rats. NO plays an important role in a diverse ranges of physiological processes. NO reacts with the superoxide anion to generate peroxynitrite, which is a selective oxidant, and nitrating agent that interacts with numerous biological molecules, there by damaging them (Choi et al., 2006).

In the present study, it was found that an increased level of NO in the lung and plasma of nicotine-treated rats. Various studies have shown that NO synthesis was high in tumor tissue and in plasma, which can be related to an alteration in the oxidant-antioxidant potential (Alderton *et al*., 2001). Thus, a higher level of NO in the tissues of nicotine-treated rats could be due to the high production of free radicals by the toxic metabolites released during metabolism of nicotine.

There was inverse correlation between nicotine-induced LPO and antioxidant status. ROS generated in tissues are normally scavenged by enzymatic and non-enzymatic antioxidants. Antioxidant defense system protects the aerobic organism from the deleterious effects of reactive oxygen metabolites. Previous studies had suggested that superoxide anion and hydrogen peroxide were the main source of nicotine-induced free radical production depleting the cellular antioxidants

(Kalpana and Menon, 2004).

Glutathione was a crucial component of the antioxidant defense mechanism and it functions as a direct reactive free radical scavenger (Romao *et al*., 2006).The decreased level of tissue GSH in nicotine-treated rats of the present study may be due to enhanced utilization during detoxification of nicotine. CAT and SOD, plays an important role in the protection against the deleterious effects of LPO (Ray and Husain, 2002). Depletion in the activities of SOD and CAT in nicotine-treated rats may be due to decreased synthesis of enzymes or oxidative inactivation of the enzyme protein.

In the present study, administration of curcumin significantly reduced the increase of MDA, NO and enhanced the antioxidant status in the lung, and plasma of nicotine-treated rats. The significant reduction in the levels of LPO products confirms that curcumin could effectively protect against the free radicals. Curcumin by scavenging or neutralizing free radicals, interacting with oxidative cascade, quenching oxygen, inhibiting oxidative enzymes like cytochrome P450, and by chelating metal ions like Fe+2, inhibits peroxidation of membrane lipids and maintains cell membrane integrity and their function (Lee *et al*., 2013).

Curcumin had been reported to reduce nitrite formation during NO oxidation. The inhibitory effect of curcumin on the oxidation of NO to nitrite was due to its known sequestration of the reactive intermediate nitrogen dioxide systems (Balasubramanyam *et al*., 2003).This suggests that administration of curcumin prevents the degradation of NO mediated by nicotine induced oxygen free radicals.

The antioxidant protective role of curcumine was evidenced in this study histopathologically by improvement of the alveolar wall thickness as well as regression of inflammatory reaction. Moreover, administration of curcumin to nicotine-treated rats enhanced the GSH level and increased the activities of SOD and CAT in the lung, and plasma. A previous study has shown that curcumin is a potent inducer of detoxifying enzymes and thereby prevents the toxicity induced by chemical carcinogens (Rukkumani *et al*., 2004).

Curcumin is recognized to prevent paraquat-induced lung toxicity and to exert protective effects on cell membranes (Venkatesan, 2000). Having polyphenolic structure and β-diketone functional groups, curcumin is a stronger antioxidant inhibitor of lipid peroxidation than other flavonoids, which have a single phenolic hydroxyl group (Phan *et al*., 2001).

Thus, curcumin exerts its protective effect against nicotine-induced toxicity by modulating the extent of lipid peroxidation and augmenting antioxidant defense system (Kalpana *et al*., 2007). The results of the present study suggest that curcumin can be used as a dietary supplement, especially by people who smoke, in order to prevent nicotine-induced oxidative stress.

The inflammatory reaction encountered histopathologically was emphasizes by the immunological results. The later showed that CD4 decreased in nicotine-treated group and hence the antigen proliferates and aggravates the inflammatory reaction. Meanwhile, curcumin increased CD4, and the inflammatory was ameliorated .This indicated that nicotine had a hazardous effect on immunosuppressive constituent of cigarette smoke.

Curcumin has been considered quite safe and elicited much better anti-inflammatory response than placebo did (Ajay et al., 2008). No reported side effects of curcumin therapy were known .It is safe and effective .We concluded that, consumption of curcumin to smokers can protect against these lung hazards toxicity.

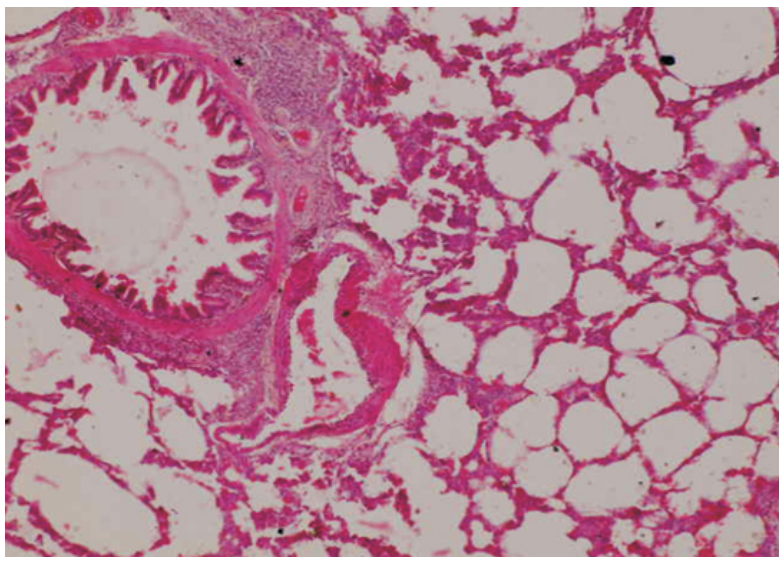

Fig. 1: Light micrograph of control rat lung tissue (Group I) composed of alveoli lined by simple squamous epithelium, interalveolar septa showing blood capillaries. Note respiratory bronchioles surrounded by elastic fibers and their lumen lined by columnar ciliated epithelium,note also no cartilage support nor submucosal gland in small bronchioles (H&E x 400).

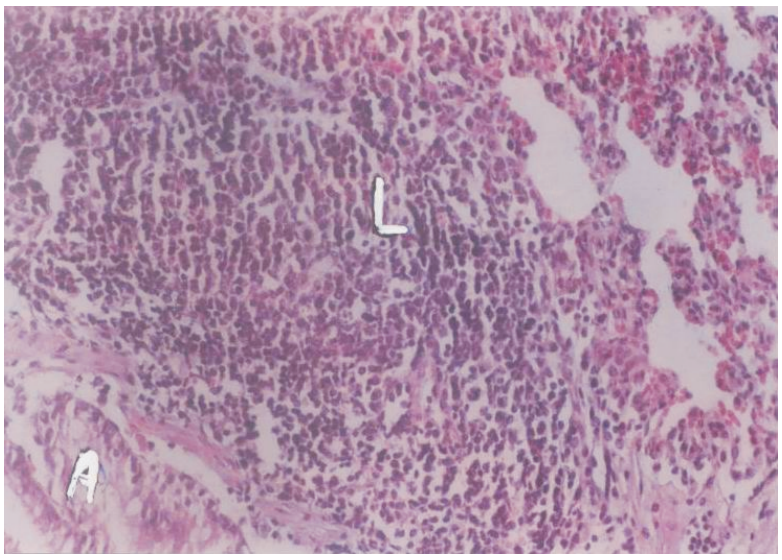

Fig. 2: Light micrograph of rat lung tissue subjected to nicotine for eight weeks: (group II). The respiratory bronchioles are disrupted and their lumen is closed by acidophilic material (A). The alveolar spaces are studded with huge number of lymphocytes forming lymphoid aggregates and red blood cells (H&E x400).

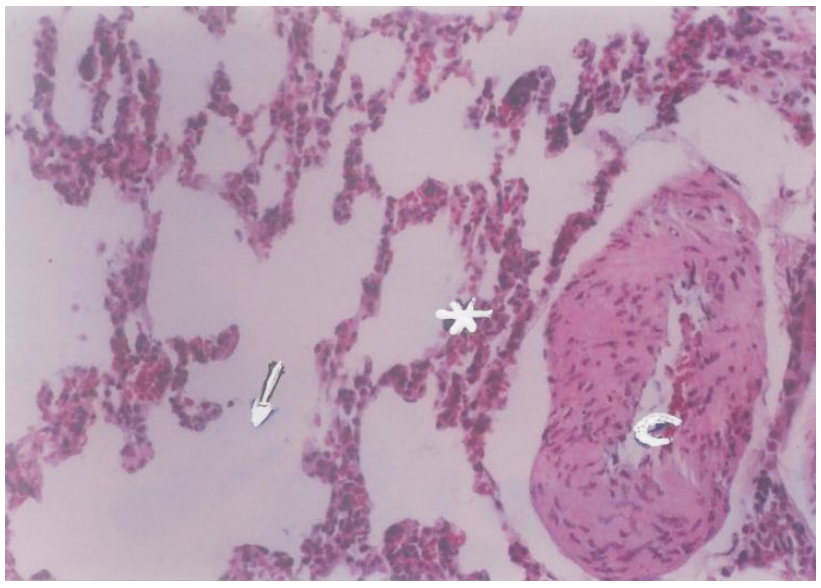

Fig. 3: Light micrograph of rat lung tissue (Group II) showing disruption of interalveolar septum (arrow) with widening of alveolar spaces, increased inflammatory cells mainly lymphocytes and dust cells are seen(*) and a blood capillary(c) with very thick wall and narrow lumen full of red cells (H&E x400).

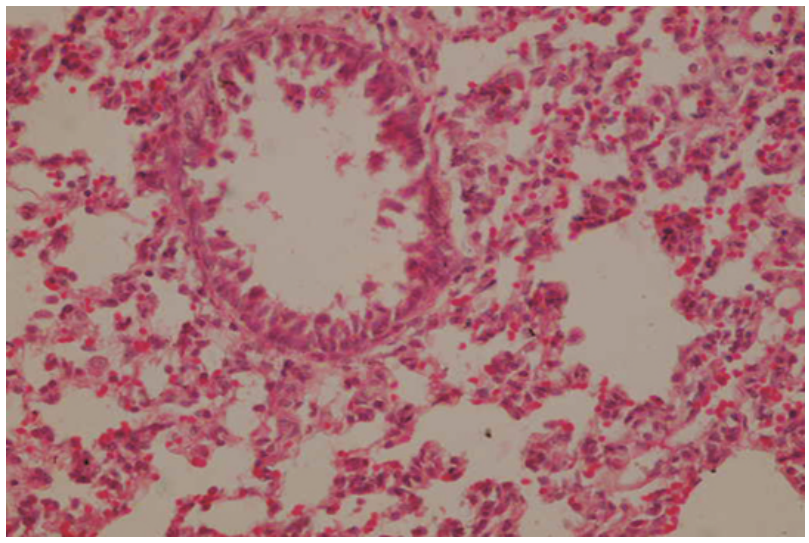

Fig. 4: Light micrograph of rat lung tissue (Group III) after curcumin treatment. It shows intact respiratory bronchioles. The alveolar septa and spaces are nearly free of inflammatory cells (H&E x400).

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