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The protective effects of Curcumin and Caffeic acid alone or in combination on Nicotine-induced lung injury in rats

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Abstract

The present study was performed to explore the protective effects ofcaffeic acid (20 mg/kg.bw) and curcumin (50mg/k.g.b.w.) on nicotine-induced lung injury alone and in combination.Their effect was compared to N-acetylcysteine (500mg/k.g.b.w.) as known modulator of oxidative stress. Nicotine treatment (0.6mg/kg/day, i.p, for 21 consecutivedays) resulted in a significantincrease ($p<0.05$) in plasmaalanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C) and well as plasma and lung thiobarbituric acid reactive substances (TBARS), nitric oxide(NO) and tumor necroses factor- (TNF-) concomitant with significant decline in non-enzymatic antioxidant like reduced glutathione(GSH) and in enzymatic antioxidants like catalase (CAT) andsuperoxide dismutase (SOD) as well as high density lipoprotein cholesterol (HDL-C). Furthermore, nicotine treatment caused severe injury indicated bythe histopathological examination of lung tissue compared to normal control group. Oral treatment with caffeic acid alone orcurcuminalone or in combination as well as N-acetylcysteine alone prevented the elevation in plasma ALT, AST, LDH,TC, TG, LDL-C, NO, TNF- and TBARSlevels concomitant with an increments in the HDL-C, reduced glutathione GSH and antioxidant enzymes (CAT and SOD) and amelioration inhistopathological changes and injury induced by nicotine. Lung protection was prominent in curcuminand N-acetylcysteine alone more than caffeic acid alone or caffeic acid and curcuminin combination. Moreover, curcumin has the potential to be used in a combination therapy with caffeic acid, with decreasing the therapeutic dose of caffeic acid and therefore its side-effects.

Keywords: Nicotine, lung, curcumin, caffeic acid, N-acetylcysteine, oxidative stress biomarkers

Introduction

Nicotine, a major toxic component of cigarette smoking, has long been recognized to result in oxidative stress by inducing the generation of reactive oxygen species [1]. Nicotine, found in tobacco(Nicotianatobacum), is a natural alkaloid and an agent that weakens the immune system. Nicotine and metabolites increase lipid peroxidation and also affect the activities of antioxidant enzymes, thus, causing oxidative damage [2]. Nicotine increased oxidative stress results from excess generation of reactive chemical species called free radicals from a number of sources and/or from decreased enzymic and nonenzymic antioxidant defenses[3].Oxygen and oxygen-derived free radicals are very important mediators of cell and tissue injury [3& 4]. Free radicals are atoms or molecules that have one or more unpaired electrons in their outer orbital, which increase reactivity of the chemical derivatives extraordinarily [5]. As a consequence, these radicals interact with molecules such as DNA, RNA and several proteins in their surroundings [2-6]. The interaction usually causes a decrease or even loss of function of these molecules. Although many different radicals occur in our body, the most effective ones are related to oxygen [5, 6].Natural antioxidants arebest obtained from plants. Thesephytonutrients serve as potential therapeuticagents against a wide variety of environmentalstresses and pathological conditions.Curcumin is a β-diketone compound which contains two ferulic acid molecules linked via a methylene bridge at the carbon atoms of the carbonyl groups [7]. Various curcumin-related phenols (*Curcuminoids*) have also been found in edible plants, especially *Zingiberaceae* plants [8-10]. Extracts of rhizomes of turmeric have been widely used in Indian medicine and they are considered to be efficacious in the treatment of liver disorders and certain pyrogenic infections [11]. Curcumin exhibits anti-inflammatory [12], hepatoprotective [13] and inhibits of tumor initiation by various

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carcinogens [14,15]. Caffeic acid (3,4-dihydroxycinnamic acid) is present in many plants and occurs in diet as part of fruits, tea, coffee and wine[16].*In vivo* and *in vitro* studies have reported that caffeic acid showed antioxidant, free radical scavenging, antitumor andanti-inflammatory[17-19].In addition; N-acetylcysteine is also widely used as an antiangiogenic [20], antifibrotic [21], neuroprotective [22], renoprotective [23],antioxidant [24] and as a chelating agent in the treatment of heavy metal poisoning [25]. N-acetylcysteine effectiveness is primarily attributed to its ability to reduce extracellular cystine to cysteine, and as a source of sulfhydryl groups [26]. The aim of this study was to elucidate the role of oxidative stress in nicotine-induced lung injury.In addition, studying the possible protective effects of different antioxidants ascurcumin and caffeic acid alone and in combinationon nicotine-induced lung injury and compare their effects with N-acetylcysteine; known modulator of oxidative stress.

Materials and Methods

Chemicals

Nicotine, Curcumin, caffeic acid and N-acetylcysteine were purchased from Sigma, USA.

Rats
This experiment was conducted in accordance with guidelines established by the Animal Care and use Committee of Faculty of Pharmacy, October 6University, Egypt. Adult rats weighing around180 ± 20gms were purchased from National Cancer Institute, Cairo University , Egypt. They were individually housed in cages in an air-conditioned room with a temperature of 22 ± 2ºC, a relative humidity of 60%, and an 8:00 to 20:00 light cycle. During the acclimatization period, each animal was raised on a regular diet *adlibitum*.

Experimental design

The animals enrolled in the present study were divided into 6 groups , each group consists of 8 animals, two controls groups and four treatment groups:
Group (1): Control negative (0.9% saline, 3ml/kg.b.w., orally).
Group (2): Positive control (nicotine 0.6 mg/kg.b.w. suspended in 1ml 0.9% saline) was given I.Pdaily for 21 days) [27].
Group (3): Nicotine 0.6 mg/kg.b.w./day(I.P.)+ caffeic acid (20mg/kg.bw, orally) daily for 21 days [28].
Group (4): Nicotine 0.6 mg/kg.b.w.(I.P.)+ curcumin (50mg/k.g.b.w., orally)daily for 21 days [29].
Group (5):Nicotine 0.6 mg/kg.b.w.(I.P.)+ caffeic acid (20mg/kg.bw orally) + curcumin (50mg/k.g.b.w.) daily for 21 days.
Group (6):Nicotine 0.6 mg/kg.b.w.+N-acetylcysteine (500mg/k.g.b.w., orally) daily for 21 days [30].
At the end of the experiment, rats of each group were sacrificed by cervical decapitation.

Blood samples

Blood samples were collected at the end of experimental period in dry, clean, and screw capped tubes. Blood was divided into two parts; first part used for GSH [31], SOD [32] and CAT [33] estimation, Also, the second part was centrifugated at 2500r.p.m for 15 minutes. Plasma was separated by automatic pipette and received in dry sterile samples tube and kept in a deep freeze at -20oC until used for subsequent biochemical analysis ALT [34], AST [34], LDH [35],TC [36], TG [37], HDL-C[38], LDL-C [39], NO[40], TNF- [41] and TBARS [42].

Tissue specimen (lung tissue)

The abdomen and chest were opened and the lung specimen was quickly removed and opened gently using a scrapper, cleaned by rinsing with ice-cold isotonic saline to remove any blood cells, clots, then blotted between 2 filter papers and quickly stored in a deep freezer at (-20 C) for subsequent biochemical estimation of lung GSH [31], SOD [32], CAT [33] and TBARS [42]. Lung protein and blood hemoglobin (Hb) were estimated by the method of Lowry et al[43] and Jain [44], respectively.

Histological assessment

lungs from rats of different groups were fixed in 10% neutral formalin solution, dehydrated in graded alcohol and embedded in paraffin. Fine sections obtained were mounted on glass slides and counter-stained with Hematoxylin and Eosin (H&E) for light microscopic analyses according to the method of Bancroft and Steven [45]. The slides were coded and examined by a histopathologist who was ignorant about the treatment groups after which photographs were taken.

Statistical analysis

The obtained data were statistically analyzed and using the statistical package for social science (SPSS, 13.0 software, 2003) [46], for obtaining mean and standard deviation and error. The data were analyzed using one-way ANOVA to determine the statistical significance of differences among groups. Duncan's test was used for making a multiple comparisons among the groups for testing the inter-grouping homogeneity.
Results
Table 1 showed that I.P. administration of nicotine (0.6mg/kg.b.w) resulted in a significant increase in plasma ALT, AST and LDH compared to the normal control group ($p< 0.05$). Supplementation of caffeic acid (20 mg/kg.bw.) and curcumin (50 mg/k.g.b.w.) alone and in combination and N-acetylcysteine (500mg/k.g.b.w.) resulted in a significant decrease in plasma ALT, AST and ALP compared to the group that received nicotine($p< 0.05$). Effect of curcuminand N-

acetylcysteine alonewas more than caffeic acid alone orcaffeic acid and curcumin in combination.

Table 2showed that I.P. administration of nicotine (0.6 mg/kg.b.w) resulted in a significant increase in plasma total cholesterol (TC), triglycerides (TG) and LDL-C as well as a significant decrease in plasma HDL-C compared to the normal control group ($p < 0.05$). Oral supplementation of caffeic acid (20 mg/kg.bw.) , curcumin (50 mg/k.g.b.w.) alone or in combination and N-acetylcysteine (500mg/k.g.b.w.) resulted in a significant decrease in plasma total cholesterol (TC), triglycerides (TG) and LDL-C as well as a significant increase in plasma HDL-C compared to the group that received nicotine ($p < 0.05$).

Table 3showed that I.P. administration of nicotine (0.6 mg/kg.b.w) resulted in a significant decrease in blood reduced glutathione (GSH) and activities of superoxide dismutase (SOD) and catalase (CAT) as well as a significant increase in plasma TBARs compared to the normal control group ($p < 0.05$). Supplementation of caffeic acid (20 mg/kg.bw.) and curcumin (50 mg/k.g.b.w.) alone and in combination and N-acetylcysteine (500mg/k.g.b.w.) resulted in a significant increase in blood GSH, SOD and CAT as well as a significant decrease in plasma TBARs compared to the group that received nicotine ($p < 0.05$).

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Table 5 showed that administration of nicotine (0.6 mg/kg.b.w., I.P.) resulted in a significant increase in plasma and lung nitrous oxide (NO) and tumor necrosis factor- (TNF-) compared to the normal control group ($p < 0.05$). Administration of caffeic acid (20 mg/kg.bw.) and curcumin (50 mg/k.g.b.w.) alone and in combination and N-acetylcysteine (500 mg/k.g.b.w.) to rats resulted in a significant decrease in plasma and lung nitrous oxide (NO) and tumor necrosis factor- (TNF-) compared to the group that received nicotine ($p < 0.05$).

Table 1: Activity of alanine transaminase (ALT), aspartate transaminase (AST), lactate dehydrogenase (LDH) of normal and experimental groups of rats.

No.	Groups	ALT (U/L)	AST (U/L)	LDH (U/L)
(I)	Normal 0.9% saline	35.47 ± 2.36	17.60 ± 3.10	110.96 ± 6.44
(II)	Positive control: Nicotine (0.6mg/Kg.b.w.)	83.11 ± 4.50^{*a}	52.80 ±4.67^{*a}	225.08 ±11.39^{*a}
(III)	Nicotine (0.6mg/Kg.b.w.) + Caffeic acid (20mg/Kg.b.w.)	61.48 ±3.08^{*ab}	44.23 ±5.70^{*ab}	146.00 ±8.50^{*ab}
(IV)	Nicotine (0.6mg/Kg.b.w.)+ Curcumin (50mg/Kg.b.w.)	40.83 ±5.24^{*bc}	19.40 ±4.64^{*bc}	110.22 ±12.80^{*bc}
(V)	Nicotine (0.6mg/Kg.b.w.)+ Caffeic acid (20mg/Kg.b.w.) + Curcumin (50mg/Kg.b.w.)	57.33 ± 4.20^{*bcd}	26.15 ± 2.98^{*abcd}	128.07 ±11.40^{*bcd}
(VI)	Nicotine (0.6mg/Kg.b.w.) + N-acetylcysteine(500mg/Kg.b.w.)	38.60 ±2.68 ^{*bce}	20.59 ±3.26^{bcde}	109.25 ± 9.80^{bce}

Values are given as mean ± SD for groups of eight animals each. * Significantly different at *p< 0.05*. a: significant from normal control; b: significant from nicotine (0.6mg/Kg.b.w.) supplement group; c: significant from caffeic acid (20mg/Kg.b.w.); d: significant from curcumin (50mg/Kg.b.w.). e: significant from caffeic acid+ curcumin group of rats.

Table 2: Level of plasma total cholesterol (TC), triglycerides (TG), HDL-C and LDL-C of normal and experimental groups of rats.

No.	Groups	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
(I)	Normal 0.9% saline	129.06 ± 7.87	105.38 ± 11.24	42.25 ± 4.03	65.71 ± 6.11
(II)	Positive control: Nicotine (0.6mg/Kg.b.w.)	212.38 ± 8.56*a	162.80 ±5.73 *a	25.47 ±3.66*a	154.4 ±9.80*a
(III)	Nicotine (0.6mg/Kg.b.w.) + Caffeic acid (20mg/Kg.b.w.)	163.46 ±9.97*ab	129.29 ±5.40*ab	30.29 ±2.60 *ab	107.67 ±11.25*ab
(IV)	Nicotine (0.6mg/Kg.b.w.)+ Curcumin (50mg/Kg.b.w.)	133.60 ±12.04*bc	106.74 ±5.24*bc	39.06 ±4.58*bc	73.24 ±6.48*bc
(V)	Nicotine (0.6mg/Kg.b.w.)+ Caffeic acid (20mg/Kg.b.w.) + Curcumin (50mg/Kg.b.w.)	145.87 ± 9.98*abcd	115.59 ± 5.07*abcd	34.80 ±6.68*abcd	87.96 ±9.16*abcd
(VI)	Nicotine (0.6mg/Kg.b.w.) + N-acetylcysteine(500mg/Kg.b.w.)	131.25 ±11.69*bce	102.65 ±6.20*bce	38.17 ± 3.65*bce	72.58 ± 5.00*abc

Values are given as mean ± SD for groups of eight animals each. * Significantly different at *p< 0.05*. a: significant from normal control; b: significant from nicotine (0.6mg/Kg.b.w.) supplement group; c: significant from caffeic acid (20mg/Kg.b.w.); d: significant from curcumin (50mg/Kg.b.w.); e: significant from caffeic acid+ curcumin (50mg/Kg.b.w.) group of rats. LDL-C (mg/dl) = TC-HDL-[TG / 5].

Table 3: Level of blood reduced glutathione (GSH) and activities of superoxide dismutase (SOD), catalase (CAT) and plasma Thiobarbaturic acid reactive substances (TBARs) in normal and experimental groups of rats.

No.	Groups	GSH (mg %)	SOD (U/g Hb)	CAT (U/g Hb)	TBARs (mmol/dL)
(I)	Normal 0.9% saline	34.64 ± 4.09	14.38 ± 0.34	3.68 ± 0.55	4.23 ± 0.41
(II)	Positive control: Nicotine (0.6mg/Kg.b.w.)	12. 59 ± 3.84*a	6.70 ± 0.29*a	1.65 ± 0.43*a	8.05 ± 1.29*a
(III)	Nicotine (0.6mg/Kg.b.w.) + Caffeic acid (20mg/Kg.b.w.)	19.88 ±1.49*ab	13.15 ±0.34*ab	2.75 ±0.24*ab	5.80 ±0.79*b
(IV)	Nicotine (0.6mg/Kg.b.w.)+ Curcumin (50mg/Kg.b.w.)	30.19 ±4.23*bc	14.08 ±0.21*b	3.88 ±0.44*bc	4.15 ±0.44*bc
(V)	Nicotine (0.6mg/Kg.b.w.)+ Caffeic acid (20mg/Kg.b.w.) + Curcumin (50mg/Kg.b.w.)	23.27 ± 2.23*abcd	10.30 ± 0.14*bd	3.00 ± 0.45*abc	4.62 ± 0.45*bd
(VI)	Nicotine (0.6mg/Kg.b.w.) + N-acetylcysteine(500mg/Kg.b.w.)	33.40 ±2.74*bce	13.96 ±0.43*bce	3.76 ±0.25*bce	4.03 ±0.60*bce

Values are given as mean ± SD for groups of eight animals each. * Significantly different from normal group at *p< 0.05*. a: significant from normal control; b: significant from nicotine (0.6mg/Kg.b.w.) supplement group; c: significant from caffeic acid (20mg/Kg.b.w.); d: significant from curcumin (50mg/Kg.b.w.); e: significant from caffeic acid+ curcumin (50mg/Kg.b.w.) group of rats.

Table 4: Level of lung reduced glutathione (GSH) and activities of superoxide dismutase (SOD), catalase (CAT) and Thiobarbaturic acid reactive substance (TBARs) in normal and experimental groups of rats.

No.	Groups	GSH (µg/mg protein)	SOD	CAT	TBARs (mmol/g tissue)
(I)	Normal 0.9% saline	113.58 ± 6.98	15.69 ± 2.38	48.37 ± 4.50	1.17 ± 0.28
(II)	Positive control: Nicotine (0.6mg/Kg.b.w.)	66.38 ± 3.36*a	8.86 ± 1.20*a	26.80 ± 3.15*a	2.84 ± 0.35*a
(III)	Nicotine (0.6mg/Kg.b.w.) + Caffeic acid (20mg/Kg.b.w.)	85.25 ±8.27*ab	11.45 ±0.88*b	31.35 ±3.10*ab	1.80 ±0.21ab
(IV)	Nicotine (0.6mg/Kg.b.w.)+ Curcumin (50mg/Kg.b.w.)	106.63 ±5.72*bc	15.37 ±2.05*bc	42.68 ±3.03*bc	1.03 ±0.19*bc
(V)	Nicotine (0.6mg/Kg.b.w.)+ Caffeic acid (20mg/Kg.b.w.) + Curcumin (50mg/Kg.b.w.)	95.88 ± 8.63*bc	10.52 ± 0.99*abcd	36.08 ± 5.95*bcd	1.66 ± 0.13*abcd
(VI)	Nicotine (0.6mg/Kg.b.w.) + N-acetylcysteine(500mg/Kg.b.w.)	109.75 ±4.43*bce	14.68 +2.21*bce	45.26 +4.16*bce	1.15 +0.49*bce

Values are given as mean ± SD for groups of eight animals each. * Significantly different from at $p< 0.05$.SOD; one unit of enzyme activity was taken as the enzyme reaction, which gave 50% inhibition of pyrogallol autoxidation in one minute/mg protein; CAT;µmol of H_2O_2 consumed/min/mg protein; a: significant from normal control; b: significant from nicotine (0.6mg/Kg.b.w.) supplement group; c: significant from caffeic acid (20mg/Kg.b.w.); d: significant from curcumin (50mg/Kg.b.w.); e: significant from caffeic acid+ curcumin (50mg/Kg.b.w.) group of rats.

Table 5: Levels of plasma and lung nitrous oxide (NO) and tumor necroses factor- (TNF-) of normal and experimental groups of rats

No.	Groups	NO		TNF-	
		Plasma (umol/ml)	Lung (u mol/gtissue)	Plasma pg/ml	Lung (U/g tissue)
(I)	Normal 0.9% saline	15.86 ± 2.98	50.06 ± 8.47	225.35 ± 11.42	20.76 ± 3.96
(II)	Positive control: Nicotine (0.6mg/Kg.b.w.)	37.05 ± 3.94*a	92.74 ± 8.93*a	298.82 ±17.33*a	46.38 ± 5.20*a
(III)	Nicotine (0.6mg/Kg.b.w.) + Caffeic acid (20mg/Kg.b.w.)	25.49 ±3.74*ab	78.55 ±4.98*ab	252.28 ±4.15*ab	31.56 ±3.85*ab
(IV)	Nicotine (0.6mg/Kg.b.w.)+ Curcumin (50mg/Kg.b.w.)	17.27 ±2.97*bc	49.06 ±3.41*bc	216.08 ±13.72*bc	18.46 ±3.59*bc
(V)	Nicotine (0.6mg/Kg.b.w.)+ Caffeic acid (20mg/Kg.b.w.) + Curcumin (50mg/Kg.b.w.)	22.55 ± 3.43*abcd	60.93 ±4.86*bcd	244.63 ± 7.91*bcd	27.90 ±2.49*abcd
(VI)	Nicotine (0.6mg/Kg.b.w.) + N-acetylcysteine(500mg/Kg.b.w.)	16.67± 2.90*bce	44.48 ± 5.90*bce	222.39 ± 9.42*bce	20.22 ± 3.83*bce

Values are given as mean ± SD for groups of eight animals each. * Significantly different at $p< 0.05$. a: significant from normal control; b: significant from nicotine (0.6mg/Kg.b.w.) supplement group; c: significant from caffeic acid (20mg/Kg.b.w.); d: significant from curcumin (50mg/Kg.b.w.); e: significant from caffeic acid+ curcumin (50mg/Kg.b.w.) group of rats.

Histopathology examination of the lung tissues

Group (1) control negative group showed micrograph of lung section is normal the bronchial veolar unit parenchyma, is normal (Figure 1A). Group 2 (control positive; rats treated with nicotine (0.6 mg/kg.b.w.) showed partially collapsed lung tissue with severe congestion and dense inflammation. (Figure 1B). Group (3); rats treated with nicotine (0.6 mg/kg.b.w.) + caffeic acid (20mg/Kg.b.w.)showed refilling of the alveoli, marked decreased congestion and inflammation (Figure 1C). Group (4); rats treated with nicotine (0.6mg/Kg.b.w.)+ curcumin (50mg/Kg.b.w.) showed refilling of the alveoli, mild decreased congestion and inflammation. (Figure 1D). Group 5; rats treated with nicotine (0.6mg/Kg.b.w.)+ curcumin (50mg/Kg.b.w.) +caffeic acid (20mg/Kg.b.w.)showed

refilling of the alveoli with compensatory emphysema (Figure 1E). Group (6); rats treated with nicotine (0.6 mg/kg.b.w.) + N-acetylcysteine(500mg/Kg.b.w.) showed improvement with refilling of the alveoli, marked decreased of congestion and inflammation with reparative type II pneumocytes hyperplasia.

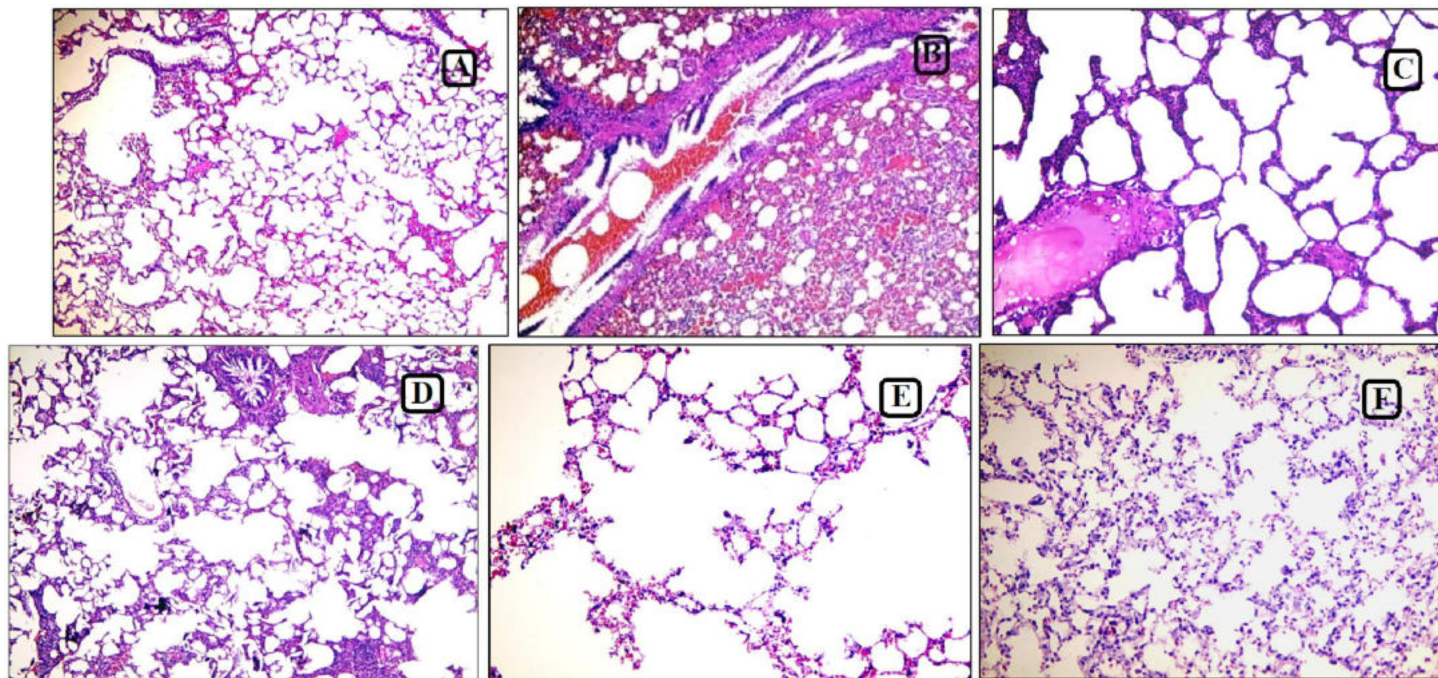

Figure (1A-F): Sections in the lungs of groupsat 400 magnification:

Figure (1A): Negative control of rat lung. **Figure (1B):** Positive control (nicotine, 0.6mg/Kg.b.w) rat lung with inflammation. **Figure (1C):** nicotine (o.6 mg/Kg.b.w.) + caffeic acid (20mg/Kg.b.w), lung tissue showed mild inflammation. **Figure (1D):** nicotine (0.6 mg/Kg.b.w) + curcumin (50 mg/Kg.b.w), the lung tissue showed refilling of the alveoli. **Figure (1E):** nicotine (0.6 mg/Kg.b.w) + curcumin (50 mg/Kg.b.w) + caffeic acid (20mg/Kg.b.w), the lung showed moderate decrease inflammation. **Figure (1F):** nicotine (50mg/Kg.b.w) + N-acetylcysteine (500mg/Kg.b.w.) showed improvement with refilling of the alveoli.

Discussion

Nicotine, the major component of cigarette smoke, plays an important role in the development of lung complications. Early-stage disease can be treated with curative intent although the risk for relapse is notoriously high. Unfortunately, the majority of lung cancer patients present at an advanced stage. Despite an initial response to treatment, most of these late stage patients will eventually progress on standard therapy and die from their disease. Despite the complex nature of lung cancer biology, its molecular underpinnings are becoming increasingly clear[47& 48]. Nicotine is considered a prototype polycyclic aromatic hydrocarbon (PAH), classic DNA damaging agent and carcinogen. Antioxidants are the first source of protection of the body against free radicals and other oxidants, being the compounds that attack the formation of radical species within cells. The group of antioxidants inside the organism is known as the total antioxidant state (TAS)[49].The antioxidant protection of human cells includes enzyme mediated and non-enzymatic defense mechanisms. Superoxide dismutase (SOD), catalase (CAT) and glutathione-peroxidase (Gpx) are the most important antioxidant enzymes. SOD catalyses' the reaction of superoxide anion to hydrogen peroxide (H_2O_2); in turn, CAT converts H_2O_2 into water and oxygen. The affinity of CAT for H_2O_2 is relatively low, therefore, some H_2O_2 remains in the cell. GSH-px is capable of detoxifying the remaining H_2O_2[50].The present study showed that oral administration of nicotine (0.6 mg/kg.b.w.) resulted in a significant increase in plasma ALT, AST and LDH. Some investigators claimed ALT, AST and LDH increased by cigarette smoking [51]which agreed with our results. Liver enzymes were strongly influenced by smoking, consistent with other studies [52, 53] concerning osteoporosis have documented increased serum ALT, AST and LDH levels in current smokers, as a mainly marker of the liver and bones turnover and also present in the kidneys and leukocytes count [54].

The present study showed that oral administration of curcumin (50 mg/kg.b.w.) resulted in a significant decrease in plasma ALT, AST

and LDH. Curcumin, an antioxidant and anticarcinogenic substance, was reported to have a protective effect against liver damage [55]. The protective action of curcumin against adverse effects of toxicants had also been reported [56]. Curcumin could exert antioxidative effects either directly as a chemical antioxidant due to its ability to scavenge reactive oxygen and nitrogen free radicals or by modulating cellular defenses which themselves exert antioxidant effects [57, 58].

Oral administration of caffeic acid at the present dose(20mg/kg b.w) attenuated the nicotine induced elevation of the plasma levels of these marker enzymes indicating that caffeic acid may have the capacity to provide protection to the rat hepatic tissue. This hepato-protection might have been exerted through its membrane stabilizing and antilipoperoxidative property. Pari and Prasath [59] have already shown the membrane stabilizing and anti-lipoperoxidative effects of caffeic acid in protectingcell membrane against peroxidative damage. Furthermore, N-acetylcysteine (500mg/Kg.b.w.) treatment ameliorated hepatic injury in terms of the AST, ALT and LDH levels against nicotine treated rats. The reason may be due to N-acetylcysteine with its antioxidant effects could prevent nicotine-induced oxidative damage. This evidence of liver damage are consistent with the previous findings by Santra et al [60] who mentioned that administration of N-acetylcysteine stimulates the synthesis of glutathione, which exhibited therapeutic effects on nicotine-induced liver fibrosis in rats.

The present study showed that administration of nicotine resulted in a significant increase in plasma total cholesterol, triglycerides and LDL-cholesterol as well as significant decrease in HDL-cholesterol compared to the normal control group which are in agreement with the earlier studies [61, 62].The increased free fatty acids in tissues of nicotine treated rats may serve as the substrate for lipid peroxidation. In the present study the cholesterol level was elevated in the nicotine-treated animals. The prevalence of hypercholesterolemia and triglyceridemia has been reported in heavy smokers [63]. This increased level of cholesterol is attributed to the increased activity of 3-hydroxy-3-methyl-glutaryl CoA reductase (HMG-CoA reductase) and increased incorporation of labelled acetate into cholesterol [64]. In curcumin treated group the level of cholesterol is decreased probably due to the increase in the CYP7A1 gene expression which is a rate limiting enzyme in the biosynthesis of bile acid from cholesterol as suggested by Kim and Kim[65]. Curcumin showed an obvious hypocholesterolemic effect that could be due to an effect on total cholesterol absorption [66], degradation or elimination [67]. Triglyceride lowering effect is probably due to multiple inductions of fatty acid catabolism [68].The influence of Caffeic acid at the TG, TC and LDL-cholesterol might be attributed to Caffeic acid decrease triglyceride creation by decrease the countenance of both HMG-CoA reductase and glycerol three phosphate acyltransferase in the liver by arrangement of adenosine mono phosphate activated kinase [69]. While that increase of HDL-cholesterol, in all study groups treated with caffeic acid might be indicated that doses of caffeic acid may have beneficial effect on HDL-cholesterol, by its effect as anti-lipid oxidation and suggested that caffeic acid lead to diminish oxygen species, and thus reduces DNA from impairment, which could be important in the regulation of liver function, this result agreement with result recorded by Codrington, et al [70]. N-acetylcysteine has been shown to reduce cholesterol levels in plasma of rats[71]. Krieger et al. [72] mentioned a slight reduction in plasma lipid fractions by means of N-acetylcysteine supplementation in hypercholesterolemic LDL receptor. Putative mechanisms accounting for the lipid-lowering effects of N-acetylcysteine might be related to its antioxidant properties. N-acetylcysteine in our study led to reduced plasma LDL-C. The maintenance of the normal structure of lipoprotein receptors is indispensable for their function, improving the cellular uptake of plasma lipids from the blood. Reactive oxygen species produced during oxidative stress react with lipoproteins to produce oxidation states, diminishing the cellular uptake of lipids from the blood [73-75]. Thus the antioxidant action of N-acetylcysteine might contribute to elevated cellular lipid uptake, resulting in the decrease of serum cholesterol levels. According to Lin and Yin [76], the lipid lowering action of N-acetylcysteine in mice consuming a high fat diet is attributed partially to the suppression of mRNA expression of three lipogenic-related enzymes (malic enzyme, fatty acid synthase and 3-hydroxy-3-methylglutaryl coenzyme A reductase).

The present study showed that oral administration of nicotine (0.6 mg/kg.b.w) resulted in a significant increase in plasma and lung TBARs as well as significant decrease in blood and lung SOD, GSH and CAT compared to the normal control group. Nicotine is a highly addictive alkaloid induced oxidative stress both *in vivo* andin *vitro* [77]. It has been reported that the nicotine disrupts the mitochondrial respiratory chain leading to an increase generations of super oxide ions and hydrogen peroxide[78]. Superoxide anion and hydrogen peroxide are the main sources of the nicotine induced free radical generation and depletion of the cellular antioxidant [79]. Glutathione being an important cellular reductant involves in protection against free radicals, peroxides and toxic compounds [80]. Therefore depletion of GSH not only impairs cell defense against toxic compounds but also results in enhanced oxidative stress and tissue damage [81, 82]. Our observation shows that nicotine treatment more significantly ($p<0.05$) depletes GSH, SOD and CAT level of blood and lung of both dietary groups indicating higher level of tissue damage. Curcumin reduces the effect in nicotine treated group due to its high antioxidant capacity [83].The supplementation of caffeic acid is found to be effective in decreasing the lipid peroxidation, lipidhydroperoxides and conjugated diene levels inthe hepatic tissue. Lipid peroxidation, which is self perpetuating, terminates when chain breaking antioxidants acts in any step of thechain. This indicates that caffeic acid has antilipoperoxidative and anti-oxidative properties. The above effect, of caffeic acid is favored by the presence of two hydroxyl groups attached to its main ring that may produce a site for chelation. In this context, caffeic acid has already been shown to chelate the free radicals[84].N-

acetylcysteine treatment enhancement the levels of antioxidant enzymes SOD and CAT beside GSH content and ameliorated the levels of MDA in plasma and lung as compared to nicotine group. These results may be due to the antioxidant scavenging properties of N-acetylcysteine to remove the ROS like OH; O and H_2O_2 radicals which librated from nicotine treatment. This result was coincide with Gurer et al [85] who reported that, N-Acetylcysteine is a thiolcontaining antioxidant that has been used to reduce various conditions of oxidative stress. Its antioxidant action is attributed to GSH synthesis; therefore maintaining intracellular GSH levels and scavenging reactive oxygen species (ROS) [86].

The present study showed that oral administration of nicotine (0.6 mg/kg.b.w) resulted in a significant increase in plasma and lung NO and TNF- compared to the normal control group.

Curcumin is a potent "scavenger" of the superoxide radical, a free radical that initiates potentially harmful oxidative processes such as lipid peroxidation [87]. Through in Curcumin also increases survival of cells exposed *in vitro* to the enzyme hypoxanthine/xanthine oxidase, which stimulates superoxide and hydrogen peroxide production. Curcumin demonstrates several other *in vitro* effects linked to free radical scavenging. Moreover, curcumin has also been shown to quench reactive oxygen species and scavenge superoxide anion radicals and hydroxyl radicals and strongly inhibits nitric oxide (NO) production by down-regulating inducible nitric oxide synthase gene expression[88, 89].Furthermore, caffeic acid has been reported to up-regulate the expression of Nrf-2 gene and downregulate the expression of NF-kB gene by which it can reduce the generation of free radicals via the augmentation of cellularanti-oxidative machinery in cells [90]. Also, In the current study, N-acetylcysteine significantly attenuated NO and TNF- -induced reduction of the activities of SOD and GSH, two major endogenous antioxidants, and attenuated TNF- -induced increase in the lipid peroxidation product malondialdehyde. This may represent a major mechanism by which N-acetylcysteine attenuates TNF- -induced cell toxicity [91, 92].

Finally, histopathological examination of lung showed severe congestion and dense inflammation in nicotine-treated rats (Figure 1B). Comparing the beneficial effect of caffeic acid and curcumin alone and in combination as well as N-acetylcysteinewith that of nicotine-induced lung toxicity. Results indicated that curcumin, caffeic acid and N-acetylcysteine showed refilling of the alveoli, decreased congestion and inflammation(fig. 1C-F).

In conclusion, the present study showed that caffeic acid and curcumin alone and in combination as well as N-acetylcysteinehas a powerful antioxidant and lung protective activity against nicotine induced lungtoxicity. These effects could be due to membrane protective action of tested compounds by scavenging the free radicals and its antioxidant action.

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