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MODULATORY EFFECTS OF CURCUMIN ON LIPID PEROXIDATION AND ANTIOXIDANT STATUS DURING NICOTINE-INDUCED TOXICITY

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Nicotine, a pharmacologically active substance in tobacco, has been identified as a major risk factor for lung diseases. In the present study, we evaluated the protective effects of curcumin on tissue lipid peroxidation and antioxidants in nicotine-treated Wistar rats. Lung toxicity was induced by subcutaneous injection of nicotine at a dose of 2.5 mg/kg (5 days a week, for 22 weeks). Curcumin (80 mg/kg) was given simultaneously by intragastric intubation for 22 weeks. The enhanced level of tissue lipid peroxides in nicotine-treated rats was accompanied by a significant decrease in the levels of ascorbic acid, vitamin E, reduced glutathione, glutathione peroxidase, superoxide dismutase and catalase. Administration of curcumin significantly lowered the level of lipid peroxidation and enhanced the antioxidant status. The results of the present study suggest that curcumin exerts its protective effect against nicotine-induced lung toxicity by modulating the extent of lipid peroxidation and augmenting antioxidant defense system.

Key words: *antioxidant, curcumin, lipid peroxidation, lung toxicity, nicotine*

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Abbreviations: CAT – *catalase,* DMSO – *dimethyl sulfoxide,* GPx – *glutathione peroxidase,* GSH – *reduced glutathione,* SOD – *superoxide dismutase,* TBARS – *thiobarbituric acid reactive substances*

INTRODUCTION

Nicotine, a major toxic component of cigarette smoke, is generally regarded to be a primary risk factor in the development of cardiovascular disorders, pulmonary disease and lung cancer [16]. Nicotine has been reported to induce oxidative stress both *in vivo* and *in vitro* [32]. Oxidative stress occurs when there is excessive free radical production and/or low antioxidant defense, and results in chemical alterations of biomolecules causing structural and functional modification [6]. The mechanisms of free radical generation by nicotine are not clear. However, it has been reported that nicotine disrupts the mitochondrial respiratory chain leading to an increased generation of superoxide anions and hydrogen peroxide [36]. Previous reports from our laboratory have shown enhanced lipid peroxidation and depletion of antioxidants by nicotine during experimental lung toxicity [18].

Medicinal plants and their active principles have received great attention as potential antiperoxidative agent [19]. Curcumin, an important constituent of turmeric (*Curcuma longa L.),* has been widely used for centuries as an indigenous medicine [8]. Curcumin exhibits a wide range of pharmacological effects such as antioxidant, antitumor, anti-inflammatory and hepatoprotective activities [2]. Previous studies from our laboratory have shown the protective effects of curcumin against 1,2-dimethylhydrazine-induced colon cancer, and alcohol – as well as carbon tetrachloride-induced hepatotoxicity [1, 7, 28].

Plant products are known to exert their protective effects by scavenging free radicals and modulating carcinogen detoxification and antioxidant defense system. The assay of lipid peroxidation and antioxidants in the liver, lung and kidney of nicotine-treated animals has evolved as a reliable method for screening protective agents. Earlier reports have shown the importance of tissue biomarkers to monitoring of the protective effect of plant products against nicotine-induced oxidative stress [11]. The present study was undertaken to evaluate the protective effect of curcumin on tissue oxidant – antioxidant status during nicotine-induced lung toxicity in Wistar rats.

MATERIALS and METHODS

Animals

Male Wistar rats (120–140 g) were obtained from the Central Animal House, Department of Medicine, Annamalai University, Tamil Nadu, India. The animals were housed six to a polypropylene cage and provided with food and water *ad libitum*. The animals were maintained under standard conditions of temperature and humidity with an alternating 12 h light/dark cycles. Animals were fed standard pellet diet (Agro Corporation Private Ltd., Bangalore, India) and maintained in accordance with the guidelines of the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, India, and approved by the ethical committee of Annamalai University.

Chemicals

Nicotine, curcumin (65–70% purity) and other fine chemicals were obtained from Sigma Chemical Company, St. Louis, USA. All other chemicals and reagents used were of analytical grade.

Treatment schedule

The animals were randomized into experimental and control groups and divided into four groups of six animals each. Rats in group 1 served as control. Group 2 animals received subcutaneous injection of nicotine at 2.5 mg/kg (in physiological saline), 5 days a week for 22 weeks [5]. The dilution was done in such a way that 1 ml of physiological saline contained the required dose of nicotine. Rats in group 3 were administered nicotine as in group 2 as well as curcumin (80 mg/kg) in 1 ml of 5% dimethyl sulfoxide (DMSO) daily using an intragastric tube for 22 weeks [7]. Rats in group 4 received curcumin alone as in group 3. Simultaneously, animals in group 1 received 1 ml of 5% DMSO in physiological saline.

The experiment was terminated at the end of 22 weeks and all animals were sacrificed by cervical dislocation after an overnight fast. Tissues (liver, lung and kidney) were removed, cleared off blood and immediately transferred to ice-cold containers containing 0.9% sodium chloride for various estimations.

Table 1. Levels of TBARS and hydroperoxides in the liver, lung and kidney (mean ± SD; n = 6)

Group	Treatment	TBARS nmol/100 g			Hydroperoxides nmol/100 g		
		Liver	Lung	Kidney	Liver	Lung	Kidney
1.	Control	0.72 ± 0.02[c]	0.66 ± 0.05[c]	0.42 ± 0.01[c]	41.05 ± 2.51[c]	52.16 ± 3.13[c]	33.27 ± 2.65[c]
2.	Nicotine	1.12 ± 0.05[a]	1.22 ± 0.05[a]	0.61 ± 0.03[a]	63.61 ± 3.64[a]	72.17 ± 3.90[a]	45.35 ± 3.52[a]
3.	Nicotine + curcumin	0.88 ± 0.04[b]	0.79 ± 0.04[b]	0.49 ± 0.01[b]	52.78 ± 3.19[b]	60.54 ± 3.88[b]	38.82 ± 1.28[b]
4.	Curcumin	0.70 ± 0.04[d]	0.60 ± 0.03[d]	0.39 ± 0.01[d]	35.05 ± 2.86[d]	44.61 ± 2.20[d]	30.59 ± 1.42[c]

Values not sharing a common superscript letter (a, b, c and d) differ significantly at $p < 0.05$ (Duncan's multiple range test)

Table 2. Levels of GSH and the activity of glutathione peroxidase in the liver, lung and kidney (mean ± SD; n = 6)

Group	Treatment	GSH mg/100 g			GPx mol of GSH utilized/min/mg of protein		
		Liver	Lung	Kidney	Liver	Lung	Kidney
1.	Control	126.87 ± 10.40[a]	113.07 ± 8.74[b]	95.32 ± 6.73[a]	11.33 ± 0.64[a]	13.01 ± 0.92[a]	9.56 ± 0.07[b]
2.	Nicotine	83.10 ± 7.36[c]	74.26 ± 5.34[d]	64.97 ± 5.46[c]	8.55 ± 0.31[c]	8.39 ± 0.67[c]	6.83 ± 0.05[d]
3.	Nicotine + curcumin	97.48 ± 4.48[b]	94.70 ± 6.68[c]	83.06 ± 7.83[b]	10.15 ± 0.59[b]	11.27 ± 0.97[b]	8.08 ± 0.04 [c]
4.	Curcumin	135.98 ± 7.32[a]	123.74 ± 6.30[a]	103.90 ± 8.64[a]	11.57 ± 0.65[a]	13.19 ± 0.65[a]	10.03 ± 0.08[a]

Values not sharing a common superscript letter (a, b, c and d) differ significantly at $p < 0.05$ (Duncan's multiple range test)

Biochemical investigation

Lipid peroxidation as evidenced by the formation of thiobarbituric acid reactive substance (TBARS) was measured by the method of Ohkawa et al. [22] and hydroperoxides were assayed by the method of Jiang et al. [15]. Tissue ascorbic acid was determined by the method of Roe and Kuether [26] and vitamin E level was measured by the method of Baker et al. [3]. Total reduced glutathione (GSH) was assayed by the method of Ellman (this method measures non-protein sulfhydryl concentration inclusive of GSH; however, 80–90% of non-protein sulfhydryl content of the cell represents free endogenous GSH) [9]. Glutathione peroxidase (GPx) activity was determined by the method of Rotruck et al. [27]. Superoxide dismutase (SOD) was estimated by the method of Kakkar et al. [17] and catalase (CAT) by the method of Sinha [31]. Protein was determined by the method of Lowry et al. [20].

Preparation of tissue homogenate

Tissue samples (lung, liver and kidney) were weighed and homogenized using 5 ml of appropriate buffer for concerned parameter (TBARS and hydroperoxides – 0.025 M Tris-HCl buffer, pH 7.5; GSH and GPx – 0.4 M phosphate buffer, pH 7.0; SOD – 0.025 M sodium pyrophosphate, buffer pH 8.3; CAT – 0.01 M phosphate buffer, pH 7.0). The homogenate was centrifuged for 5 min and the supernatant was used for the biochemical estimations.

Statistical analysis

Data from biochemical investigation were analyzed using analysis of variance (ANOVA) and the group means were compared by Duncan's Multiple Range Test (DMRT). The results were considered statistically significant if the $p < 0.05$.

RESULTS

The extent of lipid peroxidation in the liver, lung and kidney of control and experimental animals in each group is shown in Table 1. In nicotine-treated rats (group 2), the levels of TBARS were significantly increased by 35.71, 45.90 and 31.41% and hydroperoxides by 35.46, 27.72 and 26.63% in the liver, lung and kidney, respectively, as compared with control (group 1). Administration of curcumin to nicotine-treated rats (group 3) significantly decreased the levels of TBARS by 21.42, 35.24 and 19.67% and hydroperoxides by 17.02, 16.11 and 14.39% in the liver,

Table 3. Activities of SOD and CAT in the liver, lung and kidney (mean ± SD; n = 6)

Group	Treatment	SOD Enzymes required for 50% inhibition of NBT reduction/min/mg of protein			CAT µmol of H_2O_2 utilized/min/mg of protein		
		Liver	Lung	Kidney	Liver	Lung	Kidney
1.	Control	9.75 ± 0.60[b]	12.34 ± 0.56[b]	8.08 ± 0.09[b]	74.75 ± 4.05[b]	44.67 ± 3.66[a]	20.09 ± 0.89[b]
2.	Nicotine	6.43 ± 0.48[d]	7.29 ± 0.55[d]	5.02 ± 0.09[d]	56.56 ± 3.98[d]	30.58 ± 2.20[c]	12.27 ± 1.07[c]
3.	Nicotine + curcumin	8.75 ± 0.46[c]	10.72 ± 0.72[c]	7.11 ± 0.04[c]	65.91 ± 3.94[c]	38.03 ± 2.50[b]	18.53 ± 1.94[b]
4.	Curcumin	10.93 ± 0.65[a]	13.28 ± 0.58[a]	8.43 ± 0.05[a]	81.68 ± 5.12[a]	46.40 ± 2.52[a]	22.22 ± 1.74[a]

Values not sharing a common superscript letter (a, b, c and d) differ significantly at $p < 0.05$ (Duncan's multiple range test)

lung and kidney when compared with animals treated with nicotine alone (group 2).

Table 2 shows the levels of GSH and activity of GPx in the liver, lung and kidney of control and experimental animals in each group. The levels of GSH was decreased by 34.49, 34.33 and 31.84% and the activity of GPx was decreased by 24.53, 35.51 and 28.55% in the liver, lung and kidney, respectively, of nicotine-treated rats (group 2) as compared with control. Supplementation of curcumin to nicotine-treated rats (group 3) significantly elevated the levels of GSH by 14.47, 21.58 and 21.77% and GPx activity by 15.67, 25.55 and 15.47% in the liver, lung and kidney, respectively, when compared with animals treated with nicotine alone (group 2).

Activities of SOD and CAT in the liver, lung and kidney of control and experimental animal in each group are shown in Table 3. The activity of SOD was significantly decreased by 34.05, 40.92 and 37.87% and catalase by 24.33, 31.54 and 38.92%, respectively, in the liver, lung and kidney of nicotine-treated rats (group 2) when compared to control (group 1). Oral administration of curcumin to nicotine-treated rats (group 3) significantly increased the activity of SOD by 26.51, 31.99 and 29.39% and CAT by 14.18, 19.58 and 33.78% in the liver, lung and kidney when compared with animals treated with nicotine alone (group 2).

Table 4. Levels of vitamin E and vitamin C in the liver (mean ± SD; n = 6)

Group	Treatment	Vitamin E mg/100g	Vitamin C mg/100g
1.	Control	0.74 ± 0.01[b]	1.24 ± 0.03[b]
2.	Nicotine	0.54 ± 0.00[d]	0.83 ± 0.02[d]
3.	Nicotine + Curcumin	0.69 ± 0.01[c]	1.18 ± 0.02[c]
4.	Curcumin	0.76 ± 0.01[a]	1.27 ± 0.03[a]

Values not sharing a common superscript letter (a, b, c and d) differ significantly at $p < 0.05$ (Duncan's multiple range test)

Table 4 indicates the levels of vitamins C and E in the liver of control and experimental animals in each group. The levels of vitamins C and E were significantly decreased by 27.07% and 33.06%, respectively, in the liver of nicotine-treated rats (group 2) as compared with control. Administration of curcumin to nicotine-treated rats (group 3) significantly elevated the levels of vitamins C and E by 21.73% and 29.66%, respectively, in the liver when compared with rats treated with nicotine alone (group 2).

DISCUSSION

Enhanced lipid peroxidation associated with depletion of antioxidants in the liver, lung and kidney is a characteristic observation in nicotine-treated rats. Nicotine, a potent carcinogen, used in the present study, has been reported to be oxidized into its metabolite cotinine mainly in the liver and to a significant extent in the lung and kidney. Cotinine was shown to play a key role in the pathogenesis of tissue injury [13]. The mechanism of free radical generation by nicotine is not clear. However, it has been reported that nicotine is chemotactic for polymorphonuclear (PMN) leucocytes and enhances the responsiveness of PMN leucocytes to activated complement C_{5a}, thus generating oxygen free radical [37]. Further, nicotine disrupts the mitochondrial respiratory chain leading to the increased generation of superoxide anions and hydrogen peroxide [12]. Chronic nicotine administration also induces cytochrome P450 (CYP2A6 in liver and

CYP1A1 in lung) as well as generates free radicals and exerts oxidative tissue injury [14, 35]. Thus, higher levels of lipid peroxidation products TBARS and hydroperoxides in the tissue of nicotine-treated rats in the present study may be due to excessive generation of free radicals by nicotine.

The generation of oxygen free radicals can be prevented or scavenged by host antioxidant defence mechanism. Previous studies have suggested that superoxide anion and hydrogen peroxide are the main source of nicotine-induced free radicals depleting the cellular antioxidants [34]. Glutathione, an important cellular reductant, offers protection against free radicals, peroxides and toxic compounds [21]. The decreased level of tissue GSH in nicotine-treated rats may be due to the enhanced utilization during detoxification of nicotine. GPx and CAT, which act as preventive antioxidants, and SOD, a chain breaking antioxidant, play an important role in protection against the deleterious effects of lipid peroxidation [25]. Depletion of the activities of SOD, CAT and GPx in the liver, lung and kidney of nicotine-treated rats may be due to the increased utilization of these antioxidants to counter lipid peroxidation. Vitamin E, the major lipophilic antioxidant, and ascorbic acid, an essential water-soluble antioxidant, play a vital role in preventing the oxidative stress [10]. Increased lipid peroxidation in the liver of nicotine-treated rats was associated with the decreased vitamin C and E levels and this can, therefore, be related to insufficient antioxidant potential.

Administration of curcumin reversed the changes induced by nicotine supporting the hypothesis that plant products are effective antioxidative agents. 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 [4, 24]. Thus, curcumin may stabilize the cell membrane and significantly reduce the extent of lipid peroxidation in the liver, lung and kidney.

Curcumin significantly enhanced the antioxidant status in the liver, lung and kidney of nicotine-treated rats. Previous study has reported that curcumin is a potent inducer of detoxifying enzymes and thereby prevents the toxicity induced by a chemical carcinogen [30]. Curcumin has been reported to protect hepatocytes against alcohol- and PUFA-induced liver toxicity [28]. Curcumin is recognized to prevent paraquat-induced lung toxicity and to exert protective effects on cell membranes [33]. 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 [23]. Effective antioxidant property of curcumin decreases the utilization of vitamins C and E in the liver and thus maintains their levels [29]. Thus, curcumin exerts its protective effect against nicotine-induced toxicity by modulating the extent of lipid peroxidation and augmenting antioxidant defense system. 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.

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