IJPT
1735-2657/07/62-177-184
IRANIAN JOURNAL OF PHARMACOLOGY & THERAPEUTICS

IJPT 6:177-184, 2007

RIDR

RESEARCH ARTICLE

Dose-Dependent Inhibitory Effect of Ferulic Acid, A Dietary Antioxidant on Nicotine-Induced Tissue Oxidative Stress in Experimental Rats

ADLURI RAM SUDHEER, MARIMUTHU SRINIVASAN, NAGARAJAN DEVIPRIYA and VENUGOPAL PADMANABHAN MENON

For author affiliations, see end of text.

Received November 20, 2006; Revised June 9, 2007; Accepted August 14, 2007

This paper is available online at *http://ijpt.iums.ac.ir*

ABSTRACT

The present study was aimed at elucidating the protective effect of ferulic acid (FA), a natural polyphenol, against nicotine-induced tissue damage, including damages to lung, liver and kidney of experimental rats. Female albino rats of Wistar stain were used for the experimental study. Lung toxicity was induced by subcutaneous injection of nicotine at a dose of 2.5 mg/kg body weight (5 days a week, for 22 weeks) and FA was given simultaneously by intragastric intubations for 22 weeks. To establish the most effective protective support, we have used three different doses of FA (10, 20 and 40 mg/kg body weight). The levels of lipid peroxidative indices viz., thiobarbituric acid reactive substances and hydroperoxides, nitric oxide and protein carbonyl content in lung, liver and kidney of nicotine-treated rats increased significantly in nicotine-treated rats when compared to control, which were brought down to near normal in FA-treated groups. The body weight gain of rats and endogenous antioxidant status viz., superoxide dismutase, catalase, glutathione peroxidase and reduced glutathione were found to be significantly decreased in lung, liver and kidney of nicotine-treated group, which were significantly increased in FA-administered groups. The dose 20 mg/kg body weight of FA was found to be more effective than the other two doses. Our data suggest that FA exerts its protective effect by modulating lipid peroxidation and augmenting antioxidant defense system in tissues.

Keywords: *Antioxidants, Ferulic acid, Llipid peroxidation, Nicotine*

Nicotine, an alkaloid composed of a pyridine and a pyrrolidine ring is found in the plant kingdom throughout a wide range of families [1, 2]. Tobacco abuse and nicotine replacement therapies are the main sources of human exposure to nicotine [3]. Oxidative stress in the cells or tissues refers to enhanced generation of reactive oxygen species (ROS) and/or depletion in antioxidant defense system causing an imbalance between pro-oxidants and antioxidants [4]. Nicotine is a potential oxidant and has been shown to induce free radical generation and lipid peroxidation, which cause severe damage to tissues [5]. In addition, nicotine has also been found to disturb the antioxidant defense mechanisms in rats fed a high fat diet [6].

Phenolic acids are naturally occurring polyphenolic compounds that are widely distributed in fruits, vegetables, whole grains and beverages such as red wine and tea. They attract special attention because they are consumed daily in considerable amounts and exhibit a wide variety of health-protective properties such as free radical scavenging, metal-chelation, modulation of enzymic activity and more recently, to effect signal transduction, activation of transcription factors and gene expression [7-10]. These facts have directed us to focus presently on investigating the antioxidant capability of ferulic acid (FA), a most abundant natural phenolic compound in fruits and grains, against nicotine-induced toxicity. Ferulic acid (3-methoxy-4-hydroxy cinnamic acid) is a phytochemical commonly found in fruits and vegetables such as tomatoes, sweet corn, and rice bran [11]. It arises from metabolism of phenylalanine and tyrosine by Shikimate pathway in plants. FA is a strong membrane antioxidant in humans and known to protect against cancer, cold, flu, influenza, skin aging and muscle wasting [12]. FA can be characterized as a natural antioxidant and a chemopreventive agent, as it has been reported to suppress experimental carcinogenesis in fore stomach, lungs, skin, tongue and colon [12].

Table 1. Changes in the levels of body weight gain

S. No	Groups	Weight gain (g)
1	Control	150.5 ± 13.45a
2	Nicotine	59.33 ± 6.28^{e}
3	Nicotine+ FA (10 mg/kg b.w)	85.33 ± 5.93^{d}
4	Nicotine+ FA (20 mg/kg b.w)	134.66 ± 4.38^{b}
5	Nicotine+ FA (40 mg/kg b.w)	102.32 ± 5.32^{c}

Values are mean ± S.D from 6 rats in each group.
The data were compared by ANOVA followed DMRT. Values that are not sharing the common superscript differ significantly at $p \leq 0.05$.

Table 2a. Changes in the levels of TBARS in lung, liver and kidney

S. No	Groups	Lung (U^{a})	Liver (U^{a})	Kidney (U^{a})
1	Control	0.63 ± 0.02^{e}	0.72 ± 0.02^{e}	0.45 ± 0.03^{c}
2	Nicotine	1.21± 0.02^{a}	1.13 ± 0.05^{a}	0.67 ± 0.03^{a}
3	Nicotine+ FA (10 mg/kg b.w)	0.96 ± 0.02^{b}	1.01 ± 0.05^{b}	0.62 ± 0.03^{ab}
4	Nicotine+ FA (20 mg/kg b.w)	0.72 ± 0.01^{d}	0.81 ± 0.04^{d}	0.49 ± 0.05^{c}
5	Nicotine+ FA (40 mg/kg b.w)	0.81 ± 0.03^{c}	0.93 ± 0.03^{c}	0.58 ± 0.01^{b}

Table 2b. Changes in the levels of HP in lung, liver and kidney

S. No	Groups	Lung (U^{b})	Liver (U^{b})	Kidney (U^{b})
1	Control	51.21 ± 1.29^{e}	40.19 ± 0.81^{e}	32.28 ± 1.53^{e}
2	Nicotine	79.78 ± 0.82^{a}	65.44 ± 1.25^{a}	47.59 ± 1.15^{a}
3	Nicotine+ FA (10 mg/kg b.w)	71.87 ± 1.05^{b}	60.76 ± 1.81^{b}	45.81 ± 1.47^{b}
4	Nicotine+ FA (20 mg/kg b.w)	54.46 ± 1.13^{d}	44.30 ± 2.34^{d}	35.27 ± 1.38^{d}
5	Nicotine+ FA (40 mg/kg b.w)	62.82 ± 1.18^{c}	52.73 ± 1.83^{c}	40.94 ± 1.07^{c}

(U^{a}) and (U^{b}) - mmole/100 g tissue
Values are mean ± S.D from 6 rats in each group.
The data were compared by ANOVA followed DMRT. Values that are not sharing the common superscript differ significantly at $p \leq 0.05$

FA is an effective scavenger of free radicals and it has been approved in certain countries as a food additive to prevent lipid peroxidation. Rukkumani et al. [13] have also reported that FA is an efficient hepatoprotective agent against alcohol and heated polyunsaturated fatty acid (PUFA)-induced liver damage.

Although the effect of FA on prooxidant/antioxidant status in circulation has been evaluated during nicotine-induced toxicity [14], this effect has been not evaluated on tissue oxidative stress. Hence, the present study was designed to examine the effect of FA on lipid peroxidation and antioxidant status during nicotine-induced toxicity in lung, liver and kidney of Wistar rats.

MATERIALS AND METHODS

Maintenance of animals

Thirty female albino rats, Wistar strain of body weight ranging from 145-165g, were bred in the central Animal House, Rajah Muthiah Medical College, Tamil Nadu, India. The animals were housed in polypropylene cages (47 × 34 × 18 cm) in an air-conditioned room with controlled temperature (25 ± 2°C) and automatic lighting (alternating 12 h periods of light and dark). The animals were fed on the standard pellet diet (Hindustan Lever Limited, Mumbai, India) and water was given ad libitum. The standard pellet diet comprised 21% protein, 5% lipids, 4% crude fiber, 8% ash, 1% calcium, 0.6% phosphorus, 3.4% glucose, 2% vitamins and 55% nitrogen free extract (carbohydrates). It produces a metabolisable energy of 3600 Kcal. The animals used in the present study were maintained in accordance with the guidelines of the National Institute of Nutrition, Indian Council for Medical Research, Hyderabad, India and approved by the Animal Ethical Committee, Annamalai University (Reg. No.160/1999/CPSEA) (Approval number: 360)

Chemicals

Nicotine, FA and other fine chemicals were obtained from Sigma Chemical Company, St Louis, USA. All the chemicals and reagents used were of analytical grade.

Experimental induction of nicotine toxicity

Nicotine (2.5mg/kg body weight) was dissolved in physiological saline and the pH was adjusted to 7.2 with

Table 3. Changes in the levels of NO in lung, liver and kidney

S. No	Groups	Lung (U^a)	Liver (U^a)	Kidney (U^a)
1	Control	6.54 ± 0.60^c	10.43 ± 0.40^e	4.95 ± 0.50^e
2	Nicotine	12.46 ± 1.13^a	22.04 ± 2.04^a	8.03 ± 0.19^a
3	Nicotine+ FA (10 mg/kg b.w)	10.95 ± 0.82^b	19.59 ± 0.89^b	7.43 ± 0.41^b
4	Nicotine+ FA (20 mg/kg b.w)	7.11 ± 0.64^c	12.59 ± 1.32^d	5.18 ± 0.33^d
5	Nicotine+ FA (40 mg/kg b.w)	9.93 ± 0.78^b	15.76 ± 1.27^c	6.67 ± 0.42^c

(Ua)- x 10-3 μM of nitrite/mg protein

Values are mean ± S.D from 6 rats in each group.

The data were compared by ANOVA followed DMRT. Values that are not sharing the common superscript differ significantly at $p \leq 0.05$.

Table 4. Changes in the levels of PCC in lung, liver and kidney

S. No	Groups	Lung (U^a)	Liver (U^a)	Kidney (U^a)
1	Control	3.98 ± 0.13^d	4.51 ± 0.41^e	2.34 ± 0.24^e
2	Nicotine	10.64 ± 0.61^a	12.99 ± 0.64^a	8.43 ± 0.51^a
3	Nicotine + FA (10 mg/kg b.w)	8.55 ± 0.75^b	10.54 ± 0.80^b	7.11 ± 0.17^b
4	Nicotine + FA (20 mg/kg b.w)	4.66 ± 0.49^d	5.50 ± 0.42^d	3.57 ± 0.37^d
5	Nicotine + FA (40 mg/kg b.w)	7.48 ± 0.51^c	7.65 ± 0.45^c	6.16 ± 0.19^c

(Ua)- nmol/mg protein

Values are mean ± S.D from 6 rats in each group.

The data were compared by ANOVA followed DMRT. Values that are not sharing the common superscript differ significantly at $p \leq 0.05$.

sterile 0.1N HCl to ensure the stability of the chemical. Nicotine was injected subcutaneously for 5 days a week for 22 weeks [15].

Treatment with ferulic acid

Ferulic acid was dissolved in water and administered to the rats through intragastric intubations at different doses - 10 mg/kg body weight, 20 mg/kg body weight, 40 mg/kg body weight daily for 22 weeks [14]. Most of the previous studies used 20 mg/kg body weight of FA against different pathological conditions, including alcohol and heated PUFA and CCL_4-induced toxicity in Wistar rats [16, 17]. So, we used this range of FA to fix the effective dose. Daily FA was administered immediately after administration of nicotine at 10.00 am.

Experimental design

The animals were randomized into five groups of six animals each.

Group 1: Control (Received 1 ml of physiological saline per day, subcutaneously)

Group 2: Nicotine (Received subcutaneously at the dose of 2.5 mg/kg b.w for 22 weeks)

Group 3: Nicotine + FA (10 mg/kg b. wt., Given orally for 22 weeks)

Group 4: Nicotine + FA (20 mg/kg b. wt., Given orally for 22 weeks)

Group 5: Nicotine + FA (40 mg/kg b. wt., Given orally for 22 weeks)

Preparation of tissue homogenate

At the end of the experimental period of 22 weeks, the rats were kept fasting overnight and sacrificed by cervical dislocation after anaesthetizing the animals with intramuscular injections of ketamine hydrochloride (30 mg/kg body wt.). The lung, liver and kidney were excised, blood was cleared off by several washings with phosphate buffered saline (1X PBS) and the tissues were immediately transferred to –80° freezer for storage until the analysis done. On the day of the analysis, each tissue was homogenized in 0.1 M Tris-HCl buffer (2 ml/100 mg tissue), pH 7.4. The homogenate was centrifuged at 3000 rpm for 5 min and the supernatant was used for the estimation of biochemical parameters. The total protein in the tissue extract was determined after trichloroacetic acid precipitation by the method of Lowry [18].

Biochemical investigations

The extent of lipid peroxidation (LPO) was determined by analysing the levels of thiobarbituric acid reactive substances (TBARS) as described by Niehaus and Samuelsson [19], hydroperoxides (HP) as depicted by Jiang et al. [20], and nitric oxide (NO) based on work done by Lepovire et al. [21]. The protein carbonyl content (PCC) was determined by the method of Levine et al. [22].

Endogenous antioxidant status was evaluated by estimating the levels of reduced glutathione (GSH) by the method of Ellman's [23], the activities of superoxide dismutase (SOD) by utilizing the technique of Kakkar et al. [24], catalase (CAT) activity by the method of Sinha [25] and the activity of glutathione peroxidase (GPx) by the method of Rotruck et al. [26].

Statistical analysis was performed by one-way analysis of variance (ANOVA) and the groups were compared by Duncan's Multiple Range Test (DMRT) using SPSS Software Package, version 11.0. Results were expressed as mean ± standard deviation in each

group. A difference with p value ≤0.05 was considered to be statistically significant.

RESULTS

Body weight gain

Table 1 gives the changes in body weight gain. There was a significant decrease in the body weight gain of nicotine-treated rats when compared to control rats. Treatment with FA significantly improved the weight gain when compared to nicotine group. Treatment with 20mg/kg b. wt. was found to be more effective than 10 and 40mg/kg b. wt.

Extent of lipid peroxidation

As Table 2a and 2b show, the levels of lipid peroxidative markers- TBARS and HP were significantly increased in lung, liver and kidney of nicotine-treated group when compared to control. FA treatment effectively brought back the TBARS and HP levels to near normal, further middle dose showed better effect.

Levels of nitric oxide

As illustrated in Table 3, the NO levels were elevated in lung, liver and kidney of nicotine-treated animals, when compared to control group. The levels of NO were significantly decreased in all three tissues of FA-treated animals when compared to nicotine-treated animals. The effect of FA on NO was more prominent at 20mg/kg b.wt. concentration.

Protein carbonyl content levels

Table 4 depicts the Protein carbonyl content **(**PCC) levels in lung, liver and kidney of control and experimental rats. PCC levels were significantly elevated in lung, liver and kidney of nicotine-treated rats when compared with control. FA treatments effectively brought back the PCC levels to near normal in all the tissues, but middle dose was more promising.

Endogenous antioxidant status

Table 5 demonstrates the activities of SOD (Tab. 5a), CAT (Tab. 5b) and GPx (Tab. 5c) and levels of GSH (Tab. 5d) in lung, liver and kidney of control and experimental rats. The activities of SOD, CAT and GPx and levels of GSH were significantly decreased in all three tissues of nicotine-treated rats when compared to control. Treatment of FA at different doses effectively enhanced the antioxidant status in lung, liver and kidney when compared to nicotine-treated rats, but middle was (20 mg/kg b. wt.) more effective than other two doses.

DISCUSSION

Nicotine is known to cause severe damage to the tissues including lung, liver and kidney characterized by inflammation and fibrosis. The generation of reactive oxygen species (ROS) has contributed to this injury, which could be partially prevented by antioxidants and free radical scavengers.

Effects of FA on the body weight gain

Nicotine, an active ingredient in tobacco is known to influence body weight. In our study, the initial body weight of rats was of 145-160g. Our results showed a significant decrease in weight gain of nicotine-treated rats when compared to control at the end of the experimental period. The weight loss during nicotine treatment may include one or more of the following reasons: (i) stimulation of the metabolic rate [27]; (ii) activation of lipoprotein lipase [28]; (iii) suppression of glycolysis [29]. Moreover, nicotine affects almost all organs of the body by its potent oxidant capacity, which in turn may reflect indirectly on the body weight gain [30].

Treatment with FA, especially 20 mg/kg body weight showed a significant improvement in body weight gain. This might be due to the antioxidant property of FA, which neutralizes free radicals, reduces the tissue damage and thus helps in the improvement of body weight. It has been reported that supplementation of FA in combination with weight training improves strength and increase the lean muscle mass [13].

Effects of FA on the lipid peroxidative index

The presence of oxidative stress in response to nicotine is supported by an increase in lipid peroxidation. In our study, we estimated the levels of lipid peroxidation in the lung, liver and kidney. The lung is primary organ exposed to cigarette smoke, the liver is the major metabolism site of nicotine, and the kidney is the organ that involves in the elimination of toxic nicotine metabolites. Nicotine increases the free radical production by various mechanisms. It has been reported that nicotine disrupts the mitochondrial respiratory chain leading to an increased generation of superoxide anions and hydrogen peroxide [31]. In addition free radicals might be generated through the increased activity of CYP450 enzymes during the intracellular metabolism of nicotine [32] leading to increased formation of lipid peroxidative products including TBARS and HP.

Administration of FA caused a significant decrease in the levels of TBARS and HP in the lung, liver and kidney of FA treated rats. It may be due to effective antioxidant property of FA. As shown in Figure 1, FA possesses distinct structural motifs that can possibly contribute to the antioxidant property of this compound. The presence of electron donating groups on the benzene ring [3-methoxy and more importantly 4-hydroxyl] of FA gives additional property for terminating free radical chain reaction. The next functionality – the carboxylic acid group in FA with adjacent unsaturated C–C double bond can provide additional attack sites for free radicals and thus prevent them from attacking the mem

Table 5a. Changes in the activities of SOD in lung, liver and kidney

S. No	Groups	Lung (U^a)	Liver (U^a)	Kidney (U^a)
1	Control	12.68 ± 0.74^a	9.38 ± 0.67^a	8.30 ± 0.40^a
2	Nicotine	6.27 ± 0.43^e	5.65 ± 0.35^d	4.47 ± 0.31^e
3	Nicotine+ FA (10 mg/kg b.w)	8.38 ± 0.64^d	6.64 ± 0.35^c	5.42 ± 0.42^d
4	Nicotine+ FA (20 mg/kg b.w)	11.68 ± 0.88^b	8.85 ± 0.68^a	7.50 ± 0.28^b
5	Nicotine+ FA (40 mg/kg b.w)	9.92 ± 0.65^c	7.31 ± 0.38^b	6.13 ± 0.25^c

Table 5b. Changes in the activities of CAT in lung, liver and kidney

S. No	Groups	Lung (U^b)	Liver (U^b)	Kidney (U^b)
1	Control	42.45 ± 1.58^a	75.38 ± 1.10^a	20.79 ± 1.23^a
2	Nicotine	24.80 ± 0.97^e	50.42 ± 1.56^e	10.33 ± 0.91^e
3	Nicotine+ FA (10 mg/kg b.w)	29.89 ± 1.30^d	55.33 ± 1.04^d	12.14 ± 0.97^d
4	Nicotine+ FA (20 mg/kg b.w)	40.20 ± 1.19^b	72.55 ± 1.43^b	18.36 ± 1.06^b
5	Nicotine+ FA (40 mg/kg b.w)	33.55 ± 1.14^c	63.27 ± 1.78^c	14.88 ± 1.03^c

Table 5c. Changes in the activities of GPx in lung, liver and kidney

S. No	Groups	Lung (U^c)	Liver (U^c)	Kidney (U^c)
1	Control	14.62 ± 0.56^a	10.52 ± 0.79^a	9.60 ± 0.32^a
2	Nicotine	8.66 ± 0.55^e	7.45 ± 0.31^e	5.62 ± 0.49^e
3	Nicotine+ FA (10 mg/kg b.w)	9.45 ± 0.36^d	9.05 ± 0.18^d	6.51 ± 0.39^d
4	Nicotine+ FA (20 mg/kg b.w)	12.65 ± 0.60^b	9.67 ± 0.38^b	8.55 ± 0.44^b
5	Nicotine+ FA (40 mg/kg b.w)	10.82 ± 0.40^c	8.16 ± 0.20^c	7.20 ± 0.28^c

Table 5d. Changes in the levels of GSH in lung, liver and kidney

S. No	Groups	Lung (U^d)	Liver (U^d)	Kidney (U^d)
1	Control	119.65 ± 7.26^a	131.88 ± 6.35^a	95.81 ± 4.39^a
2	Nicotine	71.35 ± 2.85^e	81.82 ± 4.77^e	65.46 ± 3.4^d
3	Nicotine+ FA (10 mg/kg b.w)	81.39 ± 4.50^d	92.89 ± 4.02^d	73.05 ± 2.96^c
4	Nicotine+ FA (20 mg/kg b.w)	108.20 ± 7.30^b	122.40 ± 5.68^b	91.96 ± 4.77^a
5	Nicotine+ FA (40 mg/kg b.w)	98.54 ± 4.43^c	103.59 ± 3.98^c	81.93 ± 4.24^b

(U^a) – enzyme required for 50% inhibition of nitroblue tetrazolium reduction/min/mg protein,
(U^b) - µmoles of hydrogen peroxide utilised/min/mg protein.
(U^c) - µmoles of GSH utilized/min/mg protein.
(U^d) – mg/100 g tissue
Values are mean ± S.D from 6 rats in each group.
The data were compared by ANOVA followed DMRT. Values that are not sharing the common superscript differ significantly at $p \leq 0.05$

brane. In addition, the carboxylic acid group also acts as an anchor of FA by which it binds to the lipid bilayer providing some protection against lipid peroxidation. The presence of electron donating substituents enhances the antioxidant properties of FA [33]. Despite the direct scavenging of ROS, FA can chelate the ferrous ion and decreases the formation of hydroxyl radical via inhibition of iron-dependent Fenton's reaction [34]. Thus FA effectively quenches the free radicals, prevents them from attacking the membrane and thus protects the membrane.

Effects of FA on nitric oxide

Nitric oxide, a highly reactive free radical is formed from L-arginine by nitric oxide synthase. NO in excess can cause organ damage either directly or by reacting with superoxide anion to yield peroxynitrate [35], a powerful oxidant and nitrosating agent, which may account for the increased toxicity in nicotine-treated rats. In our study, higher levels of nitric oxide were observed in nicotine treated rats. Nitric oxide is a Ca^{2+} calmodulin requiring enzyme. Ca^{2+} influx causes activation of nitric oxide synthase and thus produces high levels of nitric oxide [36] Rosecrans et al., [37] have reported that nicotine cholinergic receptors activation by nicotine leads to enhanced entry of Ca^{2+} into the cell. Thus, by increasing the influx of Ca^{2+}, nicotine elevates the level of nitric oxide.

FA in nicotine treated rats showed significantly reduced levels of NO in lung, liver and kidney. It might be due to (i) electron-donating methoxy group, which has a greater ability to quench the unpaired electron of

NO in its outer shell orbital and (ii) also undergoing nitration reaction due to the presence of an aromatic ring there by preventing the peroxynitrite-mediated nitration of amino acids [38]. Thus in our study, we found decreased levels of NO in FA treated group.

Effects of FA on protein carbonyl content

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are known to damage all types of biological molecules including proteins [39]. An increased level of protein carbonyl groups was observed in lung, liver and kidney during nicotine treatment. Nicotine can oxidize amino acids of proteins forming more number of carbonyl groups by oxidation process with the evidence of high levels of carbonyl groups. This damage may be because of increased production of ROS and RNS by nicotine. Previous reports have shown that ROS and RNS have capacity to oxidize proteins by forming more number of carbonyl groups [40]

Treatment with FA caused a significant reduction in the levels of protein carbonyl groups. FA has been reported to scavenge ROS and RNS [41, 42and 15] by which it might reduce the attack of ROS and RNS on amino acids and thus diminish the production of carbonyl groups.

Effects of FA on the endogenous antioxidant status

Humans have evolved a highly sophisticated and complex antioxidant protection system to protect the cells and organ systems of the body against ROS. To investigate the oxidative stress inducing action of nicotine, we have also quantified the degree of oxidative challenge by measuring both enzymatic and non-enzymatic antioxidants. The decreased activities of these enzymes in lung, liver and kidney reflect perturbations in normal oxidative mechanisms during nicotine ingestion. Husain *et al.*, [43] have also reported that chronic administration of ethanol and nicotine decreases the activities of GPx, SOD and CAT in the lung and kidney. Nicotine was reported to increase substantial amount of H_2O_2, which may be released into circulation and/or transferred to the kidney for detoxification [44]. The production of H_2O_2 by nicotine leads to oxidative damage to liver and kidney, which may be counteracted by SOD. The decreased activity of CAT is suggestive of enhanced synthesis of superoxide anion during the ingestion of nicotine since superoxide anion is a powerful inhibitor of CAT [44]. In our study, we found decreased activity of GPx in lung, liver and kidney during nicotine treatment. GPx scavenges and decomposes excess hydroperoxides, including H_2O_2, formed under oxidative stress. Previous reports have shown that chronic administration of ethanol and nicotine decreases the activities of GPx in lung and kidney [43]. Decreased GSH levels may be due to increased utilization of GSH to counteract the free radicals produced by nicotine. Previous reports have also suggested decreased level of GSH during nicotine induction in the tissues [45].

Administration of FA to nicotine treated rats increased the activities of SOD, CAT and GPx and levels of GSH in the in lung, liver and kidney. This may be due to the antioxidant sparing action of FA. Being an effective antioxidant, FA has been reported to scavenge free radicals [46]. Reports have shown that FA normalizes the malondialdehyde content and improves the antioxidant status in circulation [47]. Castelluccio et al., [46] have shown that antioxidant effect of FA is far greater than that of vitamin C, a well-proved antioxidant, against LDL-oxidation. Thus, in our study, FA by its antioxidant sparing action might have decreased the utilization of antioxidants and thus restored their activities in lung, liver and kidney.

Dose response protection of FA

In our study, the lower dosage 10mg/kg body weight was not effective, because its concentration might not be enough to compensate all free radicals generated by nicotine. The high dose 40 mg/kg body weight was not as effective as medium dose 20mg/kg body weight, because at higher concentration, FA might interact with some ligands in the system and thus might not be completely available for quenching free radicals [16]. Based upon our results, FA at the dosage of 20 mg/kg body wt. could be an effective candidate against nicotine-induced oxidative stress.

CONCLUSION

The present findings demonstrate that FA could exert its protective effects against nicotine-induced toxicity through scavenging of free radicals and reactive oxygen species, there by inhibition of oxidation of lipids and proteins, and also improvement of endogenous antioxidant status. The protective effect appears to be dose-dependent. However, the mechanisms and probable mode of action of FA need to be studied in great detail.

REFERENCES

1. Doolittle DJ, Winegar R, Lee CK, Caldwell WS, Hayes AW, deBethizy JD. The genotoxic potential of nicotine and its major metabolites. Mutat Res 1995;344:95-102.
2. Schievelbein H. Nicotine, resorption and fate. Pharm Ther 1982;18:233-48.
3. Doolittle DJ, Winegar R, Lee CK, Caldwell WS, Hayes AW, deBethizy JD. The genotoxic potential of nicotine and its major metabolites. Mutat Res 1995;344:95-102.
4. Schievelbein H. Nicotine, resorption and fate. Pharm Ther 1982;18:233-48.
5. Yildiz D, Ercal N, Armston DW. Nicotine enantiomers and oxidative stress. Toxicology 1998;130:155-65.
6. Yildiz D. Nicotine, its metabolism and an overview of its biological effects. Toxicon 2004;43:619-32.
7. Duthie GG, Duthie S, Kyle JAM. Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. Nutr Res Rev 2000;13:79-106.
8. Prior RL, Cao GH. Antioxidant phytochemicals in fruits and vegetables: diet and health implications. Hort Sci 2000;35:588-92.
9. Boyd-Kimball D, Sultana R, Poon HF, Mohmmad-Abdul H, Lynn BC, Klein JB, Butterfield DA. Gamma-glutamylcysteine ethyl ester protection of proteins from Abeta(1–42)-mediated oxidative stress in neuronal cell culture: a proteomics approach. J Neurosci Res 2005;79:707-13.

10. Masumune A, Satoh M, Kikuta K, Suzuki N, Satoh K, Shimosegawa T. Ellagic acid blocks activation of pancreatic stellate cells. Biochem pharmacol 2005;70:869-78
11. Paganga G. The polyphenolic content of fruit and vegetables and their antioxidant activities. What does a serving constitute? Free Radic Res 1999;30:153-62.
12. Murakami A, Nakamura Y, Koshimizu K, Takahashi D, Matsumoto K, Hagihara K, Taniguchi H, Nomura E, Hosoda E, Tsuno T, Maruta Y, Kim HW, Kawabata K, Ohigashi H. FA 15, A hydrophobic derivative of ferulic acid, suppresses inflammatory responses and skin tumor promotion: Comparison with ferulic acid. Cancer Lett 2002;180:121-9.
13. Rukkumani R, Aruna K, Varma PS, Menon VP. Influence of ferulic acid on circulatory prooxidant-antioxidant status during alcohol and PUFA induced toxicity. J Physiol Pharmacol 2004;55:551-61.
14. Sudheer AR, Kalpana C, Srinivasan M, Menon VP. Ferulic acid modulates altered lipid profiles and prooxidant/antioxidant status in circulation during nicotine-induced toxicity: A dose dependent study. Toxicol Mech Meth 2005;15:375-81.
15. Kalpana C, Menon VP. Protective effect of Curcumin on circulatory lipid peroxidation and antioxidant status during nicotine-induced toxicity. Toxicol Mech Meth 2004;14:339-43.
16. Rukkumani R, Aruna K, Suresh VP, Menon VP. Hepatoprotective role of ferulic acid: A dose dependent study. J Med Food 2004;7:456-61.
17. Srinivasan M, Rukkumani R, Sudheer AR, Menon VP. Ferulic acid, a natural protector against carbon tetra chloride-induced toxicity. Fun Clin Pharmacol 2005;19:491-6.
18. Lowry OH, Rosebrough MJ, Farr L, Randall RJ. Protein measurement with the folin-phenol reagent. J Biol Chem 1951;93:464-78.
19. Niehaus WG, Samuelsson B. Formation of malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. Eur J Biochem 1968;6:126-30.
20. Jiang ZY, Hunt JY, Wolff SP. Detection of lipid hydroperoxides using the 'fox method'. Anal Biochem 1992;202:384-9.
21. Lepovire M, Chenais B, Yapo A, Lemire G, Thelander L, Tenu JP. Alteration of ribonucleotide reductase activity following induction of nitrite generating pathway in adenocarcinoma cells. J Biol Chem 1990;6:126-30.
22. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG, Ahn BW, Shalteil S, Stadtman ER. Determination of carbonyl content in oxidatively modified proteins. Method Enzymol 1990;186:464-78.
23. Ellman GL. Tissue sulphydryl groups. Arch Biochem Biophys 1959;82:70-7.
24. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase (SOD). Indian J Biochem Biophys 1984;21:130-2.
25. Sinha KA. Colorimetric assay of catalase. Anal Biochem 1972;47:389-94.
26. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: Biochemical rates as a component of glutathione peroxidase. Science 1973; 179:588-90.
27. Frankish HM, Dryden S, Wang Q, Bing C, Mocfarlane IA, Willians G. Nicotine administration reduces neuropeptide Y and neuropeptide Y mRNA concentrations in the rat hypothalamus: NPY may mediate nicotine's effects on energy balance. Brain Res 1995;694:139-46.
28. Winders SE, Grunberg NE. Effects of nicotine on body weight food consumption and body composition n male rats. Life Sci 1990;46:1523-30.
29. Maritz GS. Maternal nicotine exposure and carbohydrate metabolism of fetal and neonatal lung tissue: response to nicotine with drawl. Respiration 1987;51:232-40.
30. Fornari A, Pedrazzi P, Lippi G, Picciotto MR, Zoli M, Zini I. Nicotine withdrawal increases body weight, neuropeptide Y and Agouti-related protein expression in the hypothalamus and decreases uncoupling protein-3 expression in the brown adipose tissue in high-fat fed mice. Neurosci Lett 2006. (In press)
31. Gvozdjakova A, Kucharska J, Gvozkjak J. Effects of smoking on the oxidative process of cardiomyocytes. Cardiology 1992;81:81-4.
32. Benowitz NL, Lake T, Keller KH, Lee BL. Prolonged absorption with development of tolerence to toxic effects after cutaneous exposure to nicotine. Clin Pharmacol Therapeutics 1987;42:119-220.
33. Kanski J, Aksenova M, Stoyanova A, Butterfield DA. Ferulic acid antioxidant protection against hydroxyl and peroxyl radical oxidation in synaptosomal and neuronal cell culture systems in vitro: structure-activity studies. J Nutr Biochem 2002;13:273-81.
34. Zhang Z, Wei T, Hou J, Li G, Yu S, Xin W. Ion- induced damage and apoptosis in cerebellar granule cells: attenuation by tetramethylpyrazine and ferulic acid. Eur J Pharmacol 2003;467:41-7.
35. Blough NV, Zafiriou N. Reaction of superoxide with nitrix oxide to form peroxynitrite in alkaline aqueous solution. Inorg Chem 1995;24:3502-5.
36. Pogun S, Demirgoren S, Taskiran D, Kanit L, Yilmaz O, Koylu EO, Balkan B, London ED. Nicotine modulates nitric oxide in brain. Euro Neuropsychopharmacol 2000;10:463–72.
37. Rosecrans JA, Karan LD. Neurobehavioral mechanisms of nicotine action: role in the initiation and maintenance of tobacco dependence. J Subst Abuse Treat 1993;10:161–70.
38. Pannala A.S., Razaq R., Halliwell B., Singh S., Rice-Evans C.A.: Inhibition of peroxinitrite dependent tyrosine nitration by hydroxycinnamates: nitration or electron donation? Free Radic Biol Med 1998;24:594-606.
39. Dalle-Donne I, Rossi R, Giustarini D, Milazani A, Colombo R. Protein carbonyl goups as biomarker of oxidative stress. Clin Chim Acta 2003;329:23-38.
40. Berlett BS, Stadman ER. Protein oxidation in aging, disease and oxidative stress. J Biol Chem 1997;272:20313-6.
41. Kanski J, Aksenova M, Stoyanova A, Butterfield DA. Ferulic acid antioxidant protection against hydroxyl and peroxyl radical oxidation in synaptosomal and neuronal cell culture systems in vitro: structure-activity studies. J Nutr Biochem 2002;13:273–81.
42. Kikuzaki H, Hisamoto M, Hirose K, Akiyama K, Taniguchi H. Antioxidant properties of ferulic acid and its related compounds. J Agric Food Chem 2002;50:2161–8.
43. Husain K, Scott BR, Reddy SK, Somani SM. Chronic ethanol and nicotine interaction on rat tissue antioxidant defense system. Alcohol 2002;25:89-97.
44. Ashakumary L, Vijayammal Pl. Additive effect of alcohol and nicotine on lipid peroxidation and antioxidant defense mechanism in rats. J Applied Toxicol 1996;16: 305-8.
45. Cantin AM, Hubbard RC, Crystal RG. Glutathione deficiency in the epithelial lining fluid of the lower respiratory tract in idiopathic pulmonary fibrosis. Am Rev Respir Dis 1989;139:370-2.
46. Castelluccio GP, Bolwell CG, Gerrish C, Rice-Evans CA. Differential distribution of ferulic acid to the major plasma constituents in relation to its potential as an antioxidant. Biochemistry 1996;316:691-4.
47. Zheng RL, Zhang H. Effects of ferulic acid on fertile and asthenozoospermic infertile human sperm motility, viability, lipid peroxidation, and cyclic nucleotides. Free Radic Biol Med 1997;22:581-6.

Current Author Addresses

Adluri Ram Sudheer, Ph. D. Research Scholar,Dept. of Biochemistry and Biotechnology, Annamalai University, India. E-mail: biosudheer99@yahoo.co.in (Corresponding author)

Marimuthu Srinivasan, Ph. D Research Scolar,Dept. of Biochemistry and Biotechnology, Annamalai University, India.

Nagarajan Devipriya, Ph.D Research Scholar,Dept. of Biochemistry and Biotechnology, Annamalai University, India.

561 Venugopal Padmanabhan Menon, Dean, Faculty of Science,Prof and
562 Head, Dept. of Biochemistry and Biotechnology, Annamalai Uni-
563 versity, India.