Protective effect of *Andrographis paniculata* Nees and Vitamin-C in nicotine-induced oxidative stress in liver and kidney

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**ABSTRACT**

Nicotine, a major toxic component of cigarette smoke has been identified as a major risk factor for different diseases. In the present study, we evaluated the protective effects of aqueous extract of *Andrographis paniculata* (AE-AP) and Vitamin C on lipid peroxidation and antioxidants status against nicotine treated liver and kidney toxicity. A group of male Wistar rats were induced by subcutaneous injection of vehicle, nicotine (2mg/kg body weight/day), nicotine plus AE-AP250 (250 mg/kg body weight/day), nicotine plus AE-AP500 (500 mg/kg body weight/day) and nicotine plus Vitamin C (5 mg/kg body weight/day) for the period of 15 days. Measurement of biochemical marker enzyme lactate dehydrogenase (LDH), lipid peroxidation (MDA), conjugated dienes and antioxidants were used to monitor the antiperoxidative effects of AE-AP and Vitamin C. The decreased biochemical marker enzyme as well as increased lipid peroxides and conjugated dienes in liver and kidney of nicotine treated rats was accompanied by a significant decrease in the levels of glutathione (GSH and GSSG) and the activities of glutathione peroxidase (GSH-Px), glutathione reductase (GR), glutathione-S-transferase (G-S-T), superoxide dismutase (SOD) and catalase (CAT). The results of the present study suggest that the administration of AE-AP and Vitamin C significantly supplement the biochemical marker enzyme, lipid peroxidation and enhanced the antioxidant status.

**Keywords:** *Andrographis paniculata*, Vitamin C, Nicotine, Oxidative stress, Tissue toxicity.

**INTRODUCTION**

Nicotine, a pharmacologically active substance in tobacco, is generally regarded to be a primary risk factor for the development of cardiovascular disorders, pulmonary disease and lung cancer [1]. Nicotine has been reported to induce oxidative stress both in vivo and in vitro [2]. Oxidative stress arises when there is an imbalance between oxygen free radical (OFR) formation and scavenging by antioxidants. Increased OFR production has been directly linked to oxidation of cellular macromolecules, which may cause lung injury or induce a variety of cellular responses through the generation of secondary metabolite reactive species [3]. Nicotine induces alteration of hepato – renal phosphates activities [4]. 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 [5].

Multiple studies have shown a protective effect of vitamin C on lung function. Increased vitamin C intake is associated with decreased chronic obstructive pulmonary disease in adult smokers [6] and increased expiratory flows in adults [7]. Conversely, decreased vitamin C intake has been associated with adult-onset wheezing [8] and lower forced expiratory volumes (FEVs) in children [9]. Maritz and van Wyk [10] have demonstrated that vitamin C protects against some of the effects of nicotine on lung development in a rat model.
Medicinal plants and their active principles have received greater attention as potential antiperoxidative agent [11]. *Andrographis paniculata* Nees, an important herbal drug has been widely used for centuries as an indigenous medicine. *Andrographis paniculata*, commonly known as 'Kalmegh', is a well-known drug in the Ayurvedic system of medicine. It has been reported that *Andrographis paniculata* has a broad range of pharmacological activities such as analgesic, antipyretic, antiallergic [12, 13] and choleretic [14]. Herbal products are known to exert their protective effects by scavenging free radicals and modulating carcinogen detoxification and antioxidant defense system.

The present study aimed to investigate the antiperoxidative role of different doses of AE-AP and Vitamin C in nicotine induced oxidative damage in liver and kidney.

**MATERIALS AND METHODS**

**Chemicals:** Nicotine tartrate and other fine chemicals were purchased from Sigma Chemical Company, USA. Salt and vitamin mixtures were purchased from Merck, Germany. All other chemicals and reagents were purchased from Sisco Research Laboratory Pvt Ltd (SRL), India, and were of analytical grade.

**Animals and diet:** Adult male albino rats (n=60) of Wistar strain of body weight 100-120 g were obtained. They were maintained in accordance with the guidelines of the rule of Institutional Animal Ethics Committee of Vidyasagar University, Midnapore, and were housed in polypropylene cages and fed standard pellet diet (Hindusthan Lever Ltd, India) for 1 week and water *ad libitum*. Animals were divided into five groups and each group consisting 12 animals.

**Collection, Identification and Preservation of Plant material:** Fresh plant part was collected from the campus of IIT, Kharagpur, West Bengal, India. The taxonomic identity of this plant was determined by the expertise of the Department of Botany of our University. Specimen was labeled, numbered and noted with date of collection. Plant part was rinsed with sterilized distilled water, air dried and stored in airtight bottle at 4°C for further use.

**Preparation of aqueous extract:** Clean dry plant sample was collected in a cotton bag. The material was grinded to fine powder with the help of mixer grinder. Then this powdered material was used for the preparation aqueous extract. 2 gm of powdered material was mixed with 20 ml of sterile distilled water and kept on a rotary shaker for 12 hours at 38°C. Thereafter, it was filtered with the help of Whatman No. 1 filter paper. The filtrate was then centrifuged at 2000 rpm for 10 min. Then the supernatant was collected and stored at 4°C for further use [15].

**Mode of treatment:** Animals were divided into five groups of almost equal average body weight of twelve animals each. The animals of four groups were induced by subcutaneous injection with nicotine tartrate (dissolved in 0.9% physiological saline) at a dose of 2 mg / kg body weight per day for 15 days. The animals of three of the nicotine treated groups serving as the supplemented groups injected AE-AP250 (250 mg/kg body weight/day), AE-AP500 (500 mg/kg body weight/day) and Vitamin C (5 mg/kg body weight/day) daily at an interval of six hours after injection of nicotine tartrate for a period of 15 days. The animals of the remaining group received only the vehicle (0.9% physiological saline), served as control.

**Animals sacrifice and sample preparation:** After completion of drug treatment the animals were fasted overnight prior to sacrifice by the use of anesthesia. The intact liver and kidney were dissected out and adhering blood and tissue fluid were blotted dry weighted and kept at -20°C prior to homogenization and analysis.

**Analytical methods:** Lipid peroxidation was measured according to the method of Ohkawa et al. [16]. Malondialdehyde (MDA) was determined from the absorbance of the pink coloured product (TBARS) of thiobarbituric acid-MDA reaction, at 530 nm. The reaction of MDA with TBA has been widely adopted as a sensitive method of lipid peroxidation in animal tissues. Conjugated dienes were measured according to the method of Slater [17]. Lipids were extracted with chloroform methanol and the lipid residue was dissolved in cyclohexane and absorbance of the formed hydroperoxide is noted at 233 nm in spectrophotometer. The activity of lactate dehydrogenase (LDH) was measured by the method of Young et al. [18].

GSH (reduced glutathione) was measured according to the method of Griffith [19]. GSSG was also assayed after derivatization of GSH with 2 vinylpyridine. GSSG (oxidized glutathione) was measured by the method of Griffith [19].

Catalase activity was determined at room temperature by using a slightly modified version of Aebi [20]. The molar extinction coefficient of
43.6Mcm−1 was used to determine CAT activity. One unit of activity is equal to the millimoles of H₂O₂ degraded per minute per milligram of protein. SOD activity was estimated by measuring the percentage inhibition of the pyrogallol auto-oxidation by SOD according to the method Marklund & Marklund [21].

The rate of oxidation of reduced glutathione (GSH) by H₂O₂ as catalyzed by the glutathione peroxidase (GSH-Px) present in the homogenate is assayed for the measurement of enzyme activity. Glutathione peroxidase activity was measured according to method of Pagila and Valentine [22]. The activity of glutathione reductase was measured by the method of Miwa [23]. Glutathione S-transferase activity was measured according to the method of Habig et al. [24].

Total protein of plasma and tissues was estimated according to the method of Lowry et al. [25]. Substances contain two or more peptide form a purple complex with copper salts in alkaline solution. In this method, the final colour developed is a result of (i) reactions of peptides with copper ion in alkali and (ii) reduction of phosphomolybdic-phosphotungstic reagent by the tyrosine and tryptophane present in the samples containing protein.

**Statistical Analysis** : The data were expressed as mean ± S.E.M. Comparisons of the means of control, nicotine, nicotine with AE-AP250, nicotine with AE-AP500 and nicotine with Vitamin C group were made by two-way ANOVA with multiple comparison ‘t’-test, P<0.05 as a limit of significance.

**RESULTS**

The level of MDA was significantly increased in liver and kidney by 75.1 % and 32.2%, respectively, as compared to the control group (Figure-1). Supplementation with AE-AP500, AE-AP500 and Vitamin C showed significant diminution of MDA content in liver by 22.85%, 31.42% and 32.85%, and in kidney by 12.82%, 16.67% and 20.51%, respectively, as compared to nicotine treated group (Figure-1). On the other hand, the levels of conjugated dienes were significantly increased in liver and kidney by 73.22% and 221.55%, respectively, when compared with control group (Figure-2). Significantly decreased levels of conjugated dienes were seen after supplementation with AE-AP500, AE-AP500 and Vitamin C in liver by 22.73%, 35.08% and 48.54% and in kidney by 27.37%, 40.40% and 60.81%, respectively, when it compared with nicotine treated groups of animals (Figure-2).

The activity of biochemical marker enzyme LDH was significantly lower in nicotine treated rats compared with control in liver and kidney by 15.48%, 37.55% and 49.62% and in kidney by 20.07%, 31.10% and 34.57%, respectively, in comparison to nicotine treated animals (Figure-3).
The activities of SOD and CAT were significantly reduced in liver by 64.42% and 55.04%, and in kidney by 67.19% and 50.02%, respectively, in response to nicotine treatment when compared with control group (Figure 4 & 5). AE-AP500, AE-AP500 and Vitamin C supplementation increased the activity of SOD in liver by 60.37%, 99.98% and 111.32%, and in kidney by 70.03%, 116.93% and 147.74%, respectively, in comparison to nicotine administered animals (Figure-4). But the supplementation with AE-AP500, AE-AP500 and Vitamin C showed significant elevation of GSH content in liver by 41.66%, 53.24% and 87.74%, and in kidney by 5.12% (not significantly), 23.26% and 35.03%, respectively, as compared to nicotine treated group (Figure-6). Significantly increased level of GSSG was seen after supplementation with AE-AP500, AE-AP500 and Vitamin C in liver by 59.98%, 70.05% and 79.8% and in kidney by 18.75%, 56.25% and 81.25%, respectively, in comparison to nicotine administered animals (Figure-7).
and Vitamin C in nicotine treated rats. Data represents mean ± SE, N=12. \( a \) \( P < 0.05 \) compared to control, \( b \) \( P < 0.05 \) compared to nicotine.

The activities of GSH-Px, GR and G-S-T were significantly decreased in liver by 63.30%, 57.37% and 37.69%, and in kidney by 53.40%, 47.90% and 36.05%, respectively, in response to nicotine treatment when compared with control group (Figure 8, 9 & 10). AE-AP500, AE-AP500 and Vitamin C supplementation increased the activity of GSH-Px in liver by 71.13%, 88.05% and 119.18%, and in kidney by 23.51%, 62.25% and 88.76%, respectively, in comparison to nicotine administered animals (Figure 8). But the supplementation with AE-AP500, AE-AP500 and Vitamin C the activity of GR elevated in liver by 27.96%, 60.02% and 91.31%, and in kidney by 24.16%, 64.37% and 70.36%, respectively, when compared with nicotine treated animals (Figure 9). On the other hand, significantly increased activity of G-S-T were seen after supplementation with AE-AP500, AE-AP500 and Vitamin C in liver by 16.81%, 29.17% and 35.04% and in kidney by 3.13% (not significant), 18.98% and 24.87%, respectively, in comparison to nicotine administered animals (Figure 10).

**DISCUSSION**

Enhanced lipid peroxidation and conjugated diene associated with antioxidant depletion in liver and kidney is a characteristic observation in nicotine treated rats (Figure 1&2). Nicotine, a potent carcinogen, used in the present study has been reported to be oxidizing into its metabolite cotinine mainly in liver and to a significant extent in lung and kidney and plays a key role in the pathogenesis of tissues [26, 27]. Previous studies have shown that endothelial and epithelial membrane damage, increased vascular permeability, membrane lipid peroxidation and the influx of polymorphonuclear
leucocytes to the site of lung injury in nicotine treated rats are the hallmark of inflammation [28]. 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 C5a thus generating oxygen free radical [29]. Further, nicotine disrupts the mitochondrial respiratory chain leading to the increased generation of superoxide anions and hydrogen peroxide [30]. Chronic nicotine administration also induces cytochrome P<sub>450</sub> type (CYP1A1 in lung) as well as generates free radicals and exerts oxidative tissue injury [31]. Thus, the decreased activity of marker enzyme LDH (Figure-3) and increased the level of MDA and conjugate diene in liver and kidney of nicotine treated rats in the present study may be due to excessive generation of free radicals by nicotine.

The excessive generation of oxygen free radicals can be prevented or scavenged by host antioxidant defense mechanism. Ineffective scavenging of free radicals due to depletion of antioxidants plays a crucial role in cell injury [32]. Previous studies have suggested that superoxide anion and hydrogen peroxide are the main source of nicotine-induced free radicals depleting the cellular antioxidant [33]. GSH plays a crucial role in protecting the liver and kidney from oxidative stress by detoxifying exogenous toxicants and quenching reactive oxygen species (ROS). High concentration of GSH is found in cells as the major antioxidants defense, especially in regulating the extent and duration of oxidative ‘burst’ [34]. GPx has a well-established role in protecting cells against oxidative injury. GPx utilizes GSH as a substrate to catalyses the reduction of organic hydroperoxides and hydrogen peroxide [35]. Therefore the excess H<sub>2</sub>O<sub>2</sub> and lipid peroxides generated during nicotine ingestion are efficiently scavenged by GPx activity. The depression of this enzyme activity reflects perturbations in normal oxidative mechanisms during nicotine ingestion. On the other hand, it was found that GR activity inducing the production of GSH from GSSG. There are alternative functions for GSH in cellular metabolism independent of its antioxidant properties. GSH also participates in the detoxification of xenobiotics as a substrate for the enzyme glutathione-S-transferase. Superoxide dismutase is the antioxidant enzyme that catalyses the dismutation of the highly reactive superoxide anion to O<sub>2</sub> and to the less reactive species H<sub>2</sub>O<sub>2</sub> [36]. Numerous studies have shown the importance of SOD in protecting cells against oxidative stress [37]. Thus decrease in the activity of SOD (Figure-4) observed in the present study could be due to a feedback inhibition or oxidative inactivation of enzyme protein due to excess ROS generation. Catalase, which acts as preventative antioxidant plays an important role in protection against the deleterious effects of lipid peroxidation [38]. The inhibition of CAT activity (Figure-5) is suggestive of enhanced synthesis of superoxide anion during the ingestion of nicotine since superoxide anion is a powerful inhibitor of catalase [39]. Previous reports have shown the decreased activity of SOD and CAT in the tissues of nicotine treated rats [40]. Husain et al. [26] have reported that chronic administration of ethanol and nicotine decreased the level of GSH and activities of GPx, SOD and CAT in the lung and kidney. In the present study, it was found that the level of GSH and GSSG are decreased significantly (Figure-6&7). On the other hand, the activities of GPx, GR and G-S-T are also significantly decreased (Figure-8,9&10). This depletion of GSH, GSSG, GPx, GR, G-S-T, SOD and CAT in liver and kidney of nicotine treated rats may be due to enhanced utilization during detoxification of nicotine.

Administration of aqueous extract of Andrographis paniculata (AE-AP) at the dose of 250 and 500 mg/kg body weight/day and also Vitamin C significantly enhanced the antioxidant status in liver and kidney of nicotine treated rats and protect cells against the damaging effects. It is reported that vitamin C can completely prevent lipid peroxidation in human plasma exposed to cigarette smoke [41]. Recently, it indicates that comparatively large doses of vitamin C may protect the smokers from cigarette smoke–induced oxidative damage and associated degenerative diseases [42]. Helen and Vijayammal [43] reported that an intake of a mega dose of vitamin C can protect the liver from oxidant damage caused by cigarette. Andrographis paniculata treatment prevents bhc induced increase in the activity of enzyme ð - glutamyl transcriptase, glutathione-S-transferase and lipid peroxidation. It has protective effects on oxidative stress by increasing activity of antioxidant enzyme and decreases lipid peroxidation [44]. These results indicate antioxidant action of Andrographis paniculata. In our laboratory, we have demonstrated that andrographolide is having hepato-renal protective activity against ethanol-induced toxicity [45]. Andrographolide were tested for a protective effect against liver toxicity produced in mice by giving them carbon tetrachloride [46]. In another study, andrographolide was shown to produce a significant increase in bite flow [14]. Biochemical and histological evidences indicate that andrographolide was hepatoprotective against galactosamine or paracetamol induce rats [47]. The
current study evaluates the antioxidant and antinflammatory activity of the plant *Andrographis paniculata*, and the findings describe that the extract posses significant antioxidant and antinflammatory property [48].

In our present study, it was found that the Vitamin C, AE-AP250 and AE-AP500 exert its protective effect against nicotine-induced oxidative stress in liver and kidney by modulating the extent of lipid peroxidation and augmenting antioxidant defense system. It was found that Vitamin C and AE-

AP500 are more protective than the AE-AP250 against nicotine-induced oxidative stress. So, it may be concluded that the aqueous extract of *Andrographis paniculata* and Vitamin C at a specific dose and duration may act as a preventive agent against nicotine induced oxidative stress in liver and kidney.

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