

## Original Article

# Perillyl Alcohol Protects Against Fe-NTA-Induced Nephrotoxicity and Early Tumor Promotional Events in Rat Experimental Model

Tamanna Jahangir and Sarwat Sultana

Section of Chemoprevention and Nutrition Toxicology, Department of Medical Elementology and Toxicology, Jamia Hamdard (Hamdard University), Hamdard Nagar, New Delhi 110062, India

Plants have been widely used as protective agents against a wide variety of processes and compounds that damage tissues via free radical mechanisms. Perillyl alcohol (PA) is a naturally occurring monoterpene found in the essential oils of numerous species of plants including mints, cherries and celery seeds. This monocyclic monoterpene has shown antioxidant and therapeutic activity in various studies against various xenobiotics. In this study, we have analyzed the effects of PA against single intraperitoneal dose of ferric nitrilotriacetate (Fe-NTA) (9 mg iron per kg body weight)-induced nephrotoxicity and early tumor promotional events. The pretreatment of Fe-NTA-treated rats with 0.5% per kg body weight dose and 1% per kg body weight dose of PA for seven consecutive days significantly reversed the Fe-NTA-induced malondialdehyde formation, xanthine oxidase activity ( $P < 0.001$ ), ornithine decarboxylase activity ( $P < 0.001$ ) and  $^3\text{H}$ thymidine incorporation in renal DNA ( $P < 0.001$ ) with simultaneous significant depletion in serum toxicity markers blood urea nitrogen and creatinine ( $P < 0.001$ ). Significant restoration at both the doses was recorded in depleted renal glutathione content, and its dependent enzymes with prophylactic treatment of PA. Present results suggest that PA potentially attenuates against Fe-NTA-induced oxidative damage and tumor promotional events that preclude its development as a future drug to avert the free radical-induced toxicity.

**Keywords:** Fe-NTA – oxidative damage – perillyl alcohol – tumor promotion markers

## Introduction

Ferric nitrilotriacetate (Fe-NTA), an established nephrotoxicant (1), induces iron-dependent lipid peroxidation (LPO), leading to acute tubular necrosis (1,2). Nitrilotriacetic acid (NTA) is used as polyphosphate substitute in detergents in various countries (3); it forms water-soluble chelate complexes with metal cations at neutral pH (4). Intraperitoneal administration of Fe-NTA has shown to induce renal proximal tubular necrosis and renal adenocarcinoma (5). Several phytochemicals and micronutrients present in fruits and vegetables are often considered as cancer-chemopreventive agents. Perillyl alcohol (PA), a monoterpene is isolated from the essential oils

of lavender and several other plants (6,7). Monoterpenes are formed in the mevalonic acid pathway in plants, some aspects of cholesterol metabolism were thought to be involved in cancer growth and monoterpenes have been shown to interfere with animal cholesterol synthesis, thereby reducing cholesterol levels and hence suppression in tumor promotion (7). Monoterpenes increase levels of liver enzymes involved in detoxifying xenobiotics (8). Animal studies have demonstrated that monoterpenes inhibit the formation of chemically induced breast, colon, liver, skin and pancreatic tumors (9). A variety of mechanisms are proposed to explain PA's chemopreventive and chemotherapeutic effects. One such mechanism is that it promotes 'apoptosis', a self-destructing ability the cell has when its DNA is severely damaged. In cancer, these cells lack this self-destructing ability, resulting in abnormal cell growth (10–12). PA is one of the promising bioactive food components being investigated at the National Cancer Institute in prevention clinical trials to reduce breast

For reprints and all correspondence: Dr Sarwat Sultana, Department of Medical Elementology and Toxicology Faculty of Science, Jamia Hamdard (Hamdard University), Hamdard Nagar, New Delhi 110062, India. Tel: +91-11-26054685 extn: 5565/5566 and +91-11-26089688; Fax: +91-11-26059663; E-mail: sarwat786@rediffmail.com

© 2007 The Author(s).

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/2.0/uk/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

cancer risk (13). PA has also been used in various preventive experimental studies (14). In addition, *in vitro* data suggest its efficacy in treating neuroblastomas and leukemias (14). PA actively induces apoptosis in cancer cells without affecting normal cells and may revert tumor cells back to a differentiated state (15). It has been shown to induce mannose-6-phosphate/insulin-like growth factor II receptors, decrease ras protein prenylation, decrease ubiquinone synthesis, and induce Phase I and Phase II detoxification systems (16). Hence, this study is aimed to investigate the efficacy of PA in ameliorating the toxic effects via inhibiting oxidative damage and tumor promotion of Fe-NTA in a rat experimental model.

## Methods

### Chemicals

PA was purchased from ACROS Organics from NJ, USA. [<sup>14</sup>C]ornithine (specific activity 56 mCi mmol) and [<sup>3</sup>H]thymidine (specific activity 82 Ci mmol) were purchased from Amersham Corporation (UK). All other chemicals were of the highest purity and commercially available.

### Experimental Design

The treatment regimen for PA was based on the preliminary studies carried out in our laboratory. To study the biochemical and serological changes, 25 male Wistar rats (150–200 g) were divided into five groups with equal number of animals in each group. The study was approved with the Committee for the purpose of control and supervision of experimental animals (CPCSEA). Registration number and date of registration: 173/CPCSEA, January 28, 2000. CPCSEA guidelines were followed for animal handling and treatment.

Group I served as saline-treated control and was administered with saline (0.85%). Group II served as treated control and was administered Fe-NTA (9 mg Fe per kg body weight) only. Fe-NTA solution was prepared fresh immediately before its use by the method of Awai *et al.* (17). Groups III and IV were pretreated with PA orally at doses 0.5% per kg body weight and 1% per kg body weight for seven consecutive days followed by administration of Fe-NTA (9 mg Fe per kg body weight i.p.) on the 7th day. Group V was given higher dose (D<sub>2</sub>) of PA for seven consecutive days. All rats were sacrificed 12 h after toxicant administration. Blood was collected from para-orbital venous complexes and serum was separated and stored at 4°C for the estimation of blood urea nitrogen (BUN) and creatinine. Kidney tissue was processed for estimating renal ornithine decarboxylase (ODC) activity, reduced glutathione (GSH) content, microsomal LPO and other biochemical estimations.

For [<sup>3</sup>H]thymidine incorporation 25 male Wistar rats were divided into five groups, same treatment regimen was followed except all were given intraperitoneal [<sup>3</sup>H]thymidine (30 µCi

per 0.2 ml saline per animal) 2 h before sacrifice. Time of sacrifice was after 18 h of Fe-NTA (9 mg Fe per kg body weight i.p.) administration; kidney sections were quickly excised, rinsed with ice-cold saline, freed of extraneous material and processed for the quantification of [<sup>3</sup>H]thymidine incorporation into the renal DNA. Time of sacrifice for the DNA synthesis study was decided as per the preliminary studies carried in our laboratory (1).

### Post-Mitochondrial Supernatant and Microsome Preparation

Tissue processing and preparation of post-mitochondrial supernatant (PMS) were done as described by Athar and Iqbal (18). Kidneys were removed quickly, cleaned free of extraneous material and immediately perfused with ice-cold saline (0.85% sodium chloride). The kidneys were homogenized in chilled phosphate buffer (0.1 M, pH 7.4) containing KCl (1.17%) using a Potter Elvehjen homogenizer. The homogenate was filtered through muslin cloth, and was centrifuged at 800× *g* for 5 min at 4°C by Eltek Refrigerated Centrifuge (model RC 4100 D) to separate the nuclear debris. The aliquot so obtained was centrifuged at 12 000 r.p.m. for 20 min at 4°C to obtain PMS, which was used as a source of enzymes. A portion of the PMS was centrifuged for 60 min by ultracentrifuge (Beckman L7-55) at 34 000 r.p.m. at 4°C. The pellet was washed with phosphate buffer (0.1 M, pH 7.4) containing KCl (1.17%). All the biochemical estimations were completed within 24 h of animal sacrifice.

### Biochemical Estimations

#### *Reduced GSH estimated by the yellow color on a spectrophotometer*

Reduced GSH was determined by the method of Jollow *et al.* (19). One milliliter sample of PMS was precipitated with 1.0 ml of sulfosalicylic acid (4%). The samples were kept at 4°C for 1 h and then centrifuged at 1200× *g* for 20 min at 4°C. The assay mixture contained 0.1 ml filtered aliquot, 2.7 ml phosphate buffer (0.1 M, pH 7.4) and 0.2 ml 1,2-dithio-bisnitrobenzoic acid (DTNB) (100 mM) in a total volume of 3.0 ml. The yellow color developed was read at 412 nm on a spectrophotometer.

Glutathione peroxidase activity was assayed by the method of Mohandas *et al.* (20). The reaction mixture consisted of 1.49 ml phosphate buffer (0.1 M, pH 7.4), 0.1 ml EDTA (1 mM), 0.1 ml sodium azide (1 mM), 0.05 ml glutathione reductase (GR) (1 IU ml<sup>-1</sup>), 0.05 ml GSH (1 mM), 0.1 ml NADPH (0.2 mM), 0.01 ml H<sub>2</sub>O<sub>2</sub> (0.25 mM) and 0.1 ml 10% PMS in a total volume of 2 ml. The disappearance of NADPH at 340 nm was recorded at 25°C. Enzyme activity was calculated as nmol NADPH oxidized per min per mg protein using molar extinction coefficient of 6.22 × 10<sup>3</sup> M<sup>-1</sup> cm<sup>-1</sup>.

#### *Enzyme activity quantitated at 25°C by measuring disappearance of NADPH*

GR activity was determined by the method of Carlberg and Mannervik (21). The reaction mixture consisted of 1.65 ml phosphate buffer (0.1 M, pH 7.6), 0.1 ml EDTA (0.5 mM), 0.05 ml oxidized GSH (1 mM), 0.1 ml nicotinamide adenine dinucleotide phosphate reduced (NADPH) (0.1 mM) and 0.1 ml 10% PMS in a total volume of 2 ml. Enzyme activity was quantitated at 25°C by measuring disappearance of NADPH at 340 nm and was calculated as nmol NADPH oxidized per min per mg protein using molar extinction coefficient of  $6.22 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ .

#### *Glucose-6-phosphate dehydrogenase activity determined by changes in absorbance*

The activity of glucose-6-phosphate dehydrogenase was determined by the method of Zaheer *et al.* (22). The reaction mixture consisted of 0.3 ml Tris-HCl buffer (0.05 M, pH 7.6), 0.1 ml NADP (0.1 mM), 0.1 ml glucose-6-phosphate (0.8 mM), 0.1 ml MgCl<sub>2</sub> (8 mM), 0.3 ml PMS (10%) and 2.1 ml distilled water in a total volume of 3 ml. The changes in absorbance were recorded at 340 nm and enzyme activity was calculated as nmol NADP reduced per min per mg protein using a molar extinction coefficient of  $6.22 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ .

#### **Lipid Peroxidation**

The assay for microsomal LPO was done following the method of Wright *et al.* (23). The reaction mixture in a total volume of 1.0 ml contained 0.58 ml phosphate buffer (0.1 M, pH 7.4), 0.2 ml microsomes, 0.2 ml ascorbic acid (100 mM) and 0.02 ml ferric chloride (100 mM). The reaction mixture was incubated at 37°C in a shaking water bath for 1 h. The reaction was stopped by adding 1.0 ml 10% trichloroacetic acid (TCA). Following the addition of 1.0 ml 0.67% thiobarbituric acid (TBA), all tubes were placed in boiling water bath for 20 min and then shifted to crushed ice-bath before centrifuging at 2500× *g* for 10 min. The amount of malondialdehyde (MDA) formed in each of the samples was assessed by measuring optical density of the supernatant at 535 nm using spectrophotometer (Milton Roy 21 D) against a reagent blank. The results were expressed as nmol MDA formed per hour per gram tissue at 37°C using molar extinction coefficient of  $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ .

#### **Assay for Xanthine Oxidase Activity**

The activity of xanthine oxidase (XO) was assayed by the method of Stripe and Della Corte (24). The reaction mixture consisted of 0.2 ml PMS that was incubated for 5 min at 37°C with 0.8 ml phosphate buffer (0.1 M, pH 7.4). The reaction was started by adding 0.1 ml xanthine (9 mM) and kept at 37°C for 20 min. The reaction was terminated by adding of 0.5 ml ice-cold perchloric acid (PCA) (10% v/v). After 10 min, 2.4 ml of distilled water was added and centrifuged at 4000 r.p.m. for 10 min and µg uric acid formed per minute per mg protein was recorded at 290 nm.

#### **Catalase Activity**

Catalase activity was assayed by the method of Claiborne (25). The reaction mixture consisted of 1.95 ml phosphate buffer (0.1 M, pH 7.4), 1.0 ml hydrogen peroxide (0.019 M) and 0.05 ml 10% PMS in a final volume of 3 ml. Changes in absorbance were recorded at 240 nm. Catalase activity was calculated as nmol H<sub>2</sub>O<sub>2</sub> consumed per min per mg protein.

#### **Blood Urea Nitrogen**

Estimation of BUN was done by diacetyl monoxime method of Kanter (26). Protein-free filtrate was prepared by adding serum and equal amount of 10% TCA, then mixture was centrifuged at 2000 r.p.m. and supernatant was taken. To 0.5 ml of protein-free filtrate, were added 3.5 ml of distilled water, 0.8 ml diacetylmonoxime (2%) and 3.2 ml sulphuric acid-phosphoric acid reagent (reagent was prepared by mixing 150 ml 85% phosphoric acid with 140 ml water and 50 ml of concentrated sulphuric acid). The reaction mixture was placed in a boiling water bath for 30 min and then cooled. The absorbance was read at 480 nm.

#### **Creatinine**

Creatinine was estimated by the alkaline picrate method of Hare (27). Protein-free filtrate was prepared. To 1.0 ml serum were added, 1.0 ml sodium tungstate (5%), 1.0 ml sulfuric acid (0.6 N) and 1.0 ml distilled water. After mixing thoroughly, the mixture was centrifuged at 800× *g* for 5 min. The supernatant was added to a mixture containing 1.0 ml picric acid (1.05%) and 1.0 ml sodium hydroxide (0.75 N). The absorbance at 520 nm was read exactly after 20 min.

#### **Assay for ornithine decarboxylase activity**

ODC activity was determined using 0.4 ml renal 105 000 *g* supernatant fraction per assay tube by measuring release of <sup>14</sup>C<sub>2</sub> from [<sup>14</sup>C]ornithine by the method of O'Brien *et al.* (28). The kidneys were homogenized in Tris-HCl buffer (pH 7.5, 50 mM) containing EDTA (0.1 mM), pyridoxal phosphate (0.1 mM), phenylmethyl sulfonyl fluoride (PMSF) (1.0 mM), 2-mercaptoethanol (1.0 mM), dithiothreitol (0.1 mM) and Tween 80 (0.1%) at 4°C. In brief, the reaction mixture contained 400 µl cytosol and 0.095 ml co-factor mixture containing pyridoxal phosphate (0.32 mM), EDTA (0.4 mM), dithiothreitol (4.0 mM), ornithine (0.4 mM), Brig 35 (0.02%) and [<sup>14</sup>C]ornithine (0.05 µCi) in a total volume of 0.495 ml. After adding buffer and co-factor mixture to blank and other test tubes, the tubes were closed immediately with a rubber stopper containing 0.2 ml ethanolamine and methoxyethanol mixture in the central well and kept in a water bath at 37°C. After 1 h of incubation, the enzyme activity was arrested by injecting 1.0 ml citric acid solution (2.0 M) along the sides of glass tubes and the incubation was continued for 1 h to ensure complete absorption of <sup>14</sup>C<sub>2</sub>. Finally, the central well was transferred to a vial containing 2 ml ethanol and 10 ml toluene-based scintillation fluid was added. Radioactivity was

counted in a liquid scintillation counter (LKB Wallace-1410). ODC activity was expressed as pmol  $^{14}\text{CO}_2$  released per hour per mg protein.

### Renal DNA Synthesis

The isolation of renal DNA and assessment of incorporation of [ $^3\text{H}$ ]thymidine into DNA were carried out by the method of Smart *et al.* (29). The rat kidneys were quickly removed and cleaned free of extraneous material and homogenate (10% w/v) was prepared in ice-cold water. The precipitate thus obtained was washed with cold TCA (5%) and incubated with cold PCA (10%) at 4°C overnight. After this, incubation mixture was centrifuged and the precipitate was washed with cold PCA (5%). The precipitate was dissolved in warm PCA (10%), incubated in a boiling water bath for 30 min and filtered through Whatman 50 paper. The filtrate was used for [ $^3\text{H}$ ] counting in a liquid scintillation counter (LKB Wallace-1410) after adding scintillation fluid. The amount of DNA in the filtrate was estimated by the diphenylamine method of Giles and Myers (30). The amount of [ $^3\text{H}$ ]thymidine incorporated was expressed as d.p.m. per  $\mu\text{g}$  DNA. The protein concentration in all (both enzymatic and ODC) samples was determined by the method of Lowry *et al.* (31).

### Statistical Analysis

Differences between groups were analyzed using analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test. All data points are presented as the treatment groups mean  $\pm$  standard error of the mean (SEM).

## Results

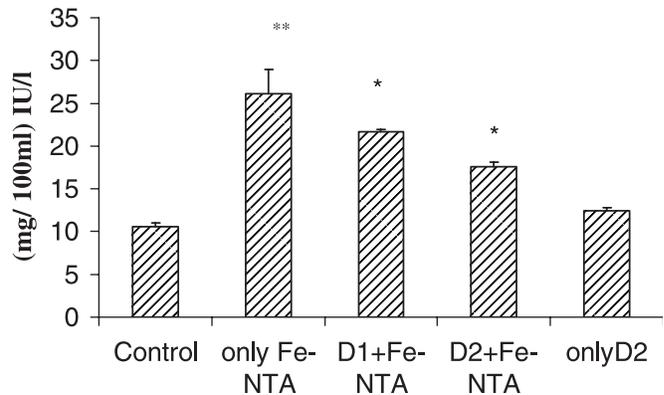
### PA Pretreatment Inhibits Renal Toxicity Markers BUN and Creatinine

The effect of prophylactic administration of PA on Fe-NTA mediated leakage of kidney marker enzymes in serum is shown in Figs 1 and 2. Only Fe-NTA treatment induced BUN by 224% and creatinine by 105% as compared with saline. Marked inhibition in BUN 42% at dose I and by 65% at dose II, creatinine level was inhibited by 37% at dose I and 44% at higher dose in PA-treated groups.

### Tumor Promotion Markers Significantly Inhibited by PA Pretreatment

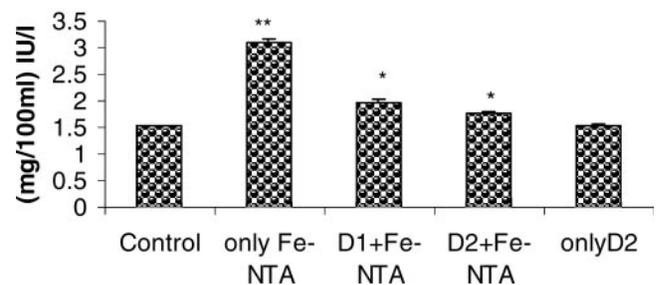
The results of treatment of PA on tumor promotion markers, namely ODC and renal DNA synthesis are presented in Figs 3 and 4. Administration of Fe-NTA resulted in increased rate of [ $^3\text{H}$ ]thymidine incorporation by 459% into renal DNA and increase by 165% in ODC activities, which are often considered as early markers of tumor promotion. The prophylactic treatment of rats with PA showed a marked simultaneous and significant inhibition of ODC activity by

### Consequence of pretreatment of PA on Blood Urea Nitrogen in serum

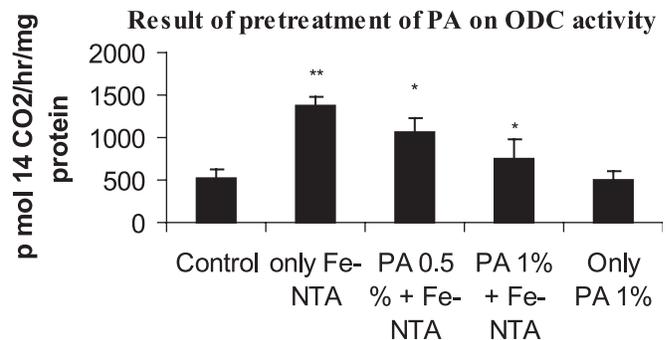


**Figure 1.** Results represent mean  $\pm$  SE of five animals per group. Results obtained are significantly different from saline-treated group (\*\* $P < 0.001$ ). Results obtained are significantly different from Fe-NTA-treated group (\* $P < 0.001$ ). PA, perillyl alcohol; D1 = 0.5%; D2 = 1%.

### Outcome of pretreatment of PA on Serum creatinine levels



**Figure 2.** Results represent mean  $\pm$  SE of five animals per group. Results obtained are significantly different from saline-treated group (\*\* $P < 0.001$ ). Results obtained are significantly different from Fe-NTA-treated group (\* $P < 0.001$ ). PA, perillyl alcohol; D1 = 0.5%; D2 = 1%.



**Figure 3.** Results represent mean  $\pm$  SE of five animals per group. Results obtained are significantly different from saline-treated group (\*\* $P < 0.001$ ). Results obtained are significantly different from Fe-NTA-treated group (\* $P < 0.001$ ).

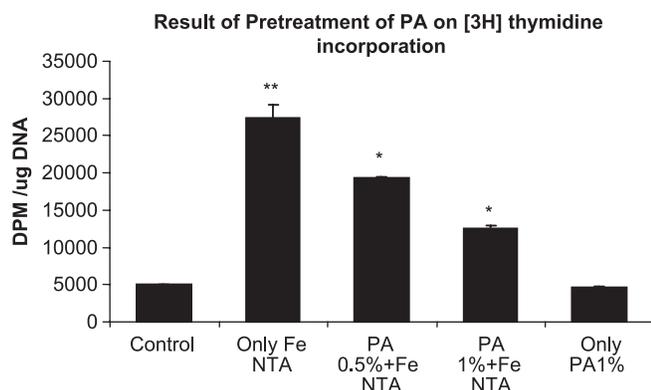
24% at low dose and 45% at higher dose. A marked suppressing effect by dose I 30% and 55% by dose II on the rate [ $^3\text{H}$ ]thymidine incorporation into renal DNA was recorded treated controls.

### XO Levels Enhanced by Prophylactic Treatment

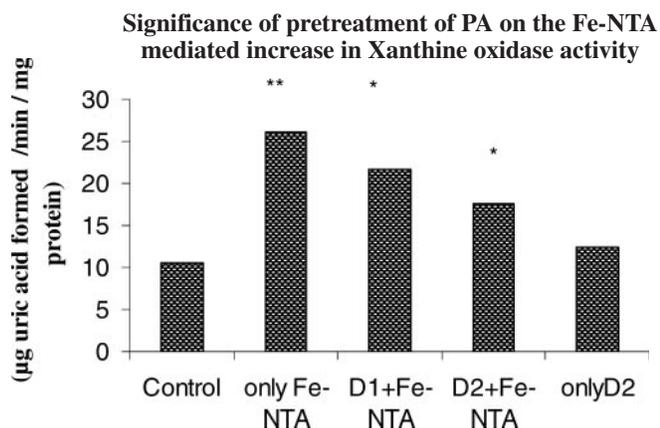
The results of prophylactic treatment of rats with PA against Fe-NTA-induced elevation in XO level and MDA formation is shown in Figs 5 and 6. There was enhancement of XO levels by 50% and MDA levels by 146% in only toxicant group as compared to saline control. Prophylactic administration of PA prior to Fe-NTA administration significantly decreased the MDA by 17% at lower dose and by 33% at higher dose and XO by 25% at dose I and 26% at dose II. However, PA alone group produced results near to saline control values.

### Renal GSH Content and Antioxidant Enzymes Restored by Pretreatment

Fe-NTA administration leads to 25% depletion of renal GSH, its metabolizing enzymes. There was marked suppression by 22% of GR enzyme following Fe-NTA administration shown in Table 1. Pretreatment with PA (0.5% and 1% per kg body

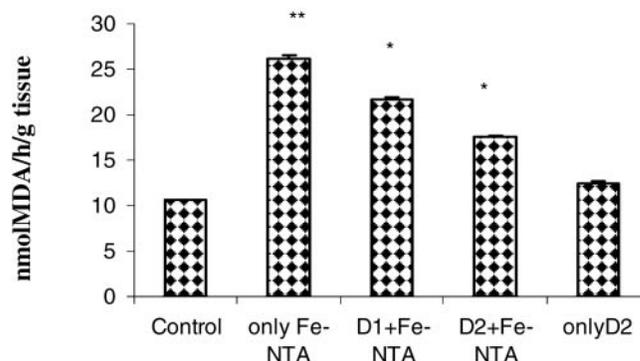


**Figure 4.** Results represent mean  $\pm$  SE of five animals per group. Results obtained are significantly different from saline-treated group (\*\* $P < 0.001$ ). Results obtained are significantly different from Fe-NTA-treated group (\* $P < 0.001$ ).



**Figure 5.** Results represent mean  $\pm$  SE of five animals per group. Results obtained are significantly different from saline-treated group (\*\* $P < 0.001$ ). Results obtained are significantly different from Fe-NTA-treated group (\* $P < 0.001$ ). PA, perillyl alcohol; D1 = 0.5%, D2 = 1%.

### Conclusion of pretreatment of PA on Lipidperoxidation



**Figure 6.** Results represent mean  $\pm$  SE of five animals per group. Results obtained are significantly different from saline-treated group (\*\* $P < 0.001$ ). Results obtained are significantly different from Fe-NTA-treated group (\* $P < 0.001$ ). PA, perillyl alcohol; D1 = 0.5%; D2 = 1%.

weight) restored renal GSH content by 23% at dose I and by 30% at dose II and GR by 20% at this low dose and by 25% at the higher dose. Antioxidant enzymes CAT, GPx and G6PD were depleted by 46, 23 and 50%, respectively as compared with the saline-treated control group. These antioxidant enzymes, namely CAT, GPx and G6PD, were significantly restored by 19, 7 and 34% at dose I and by 67.9, 25.9 and 69.3% at dose II as shown in Table 2.

### Discussion

Oxidative stress and inflammation are closely associated with tumor promotion (32). Iron deposition within kidney tissue is often related to generation of reactive oxygen species, leading to oxidative damage, LPO and concomitant increase in serum toxicity markers and depletion of renal GSH content (33). Previously, it has been reported that oxidative stress plays an important role in the pathogenesis of nephrotoxicity caused by Fe-NTA (34). Tumor promotion, followed by iron administration, possibly due to generation of free radicals in the tissue (35)  $\text{FeCl}_3$ ,  $\bullet\text{OH}$  formed by a reaction of Fe-NTA with  $\text{H}_2\text{O}_2$  *in vivo* might be involved in nephrotoxicity and renal carcinogenesis (36). Therefore, administration with Fe-NTA is a good model to induce oxidative stress producing  $\bullet\text{OH}$  to investigate antioxidant nature of PA. The PA is a naturally occurring anticancer compounds, which is effective against a variety of rodent organ-specific tumor models (15).

In animal studies, monoterpenes exert an anti-tumor efficacy and can prevent a wide range of tumors with low toxicity, namely mild gastrointestinal irritation (37). *In vitro*, at pharmacologically achievable levels, PA inhibited cell hyperproliferation and induced apoptosis in mammary and hepatic tumor cell lines (8,38). It is evident from present results that PA treatment prior to Fe-NTA administration leads to restoration of renal GSH and its dependent enzymes, namely GR, GPx and G6PD. The conjugation of electrophilic Phase I intermediates with GSH, for instance, frequently results in

**Table 1.** Outcome of pretreatment of PA on the Fe-NTA mediated depletion in glutathione content and glutathione reductase in kidney of Wistar rats

Treatment regimen per group	Reduced glutathione (nmol GSH per g tissue)	Glutathione reductase (nmol NADPH oxidized per min per mg protein)
Group I Saline-treated control	0.40 ± 0.003	135.5 ± 2.34
Group II Fe-NTA alone	0.30 ± 0.005 <sup>###</sup>	106.3 ± 4.43 <sup>###</sup>
Group III Fe-NTA + PA D1	0.37 ± 0.002 <sup>#</sup>	127.2 ± 0.76 <sup>#</sup>
Group IV Fe-NTA + PA D2	0.39 ± 0.001 <sup>#</sup>	132.2 ± 0.94 <sup>#</sup>
Group V Only PA D2	0.44 ± 0.002	137.15 ± 0.40

Results represent mean ± SE of five animals per group. Results obtained are significantly different from saline-treated group (<sup>###</sup>*P* < 0.001). Results obtained are significantly different from Fe-NTA-treated group (<sup>#</sup>*P* < 0.001). PA, perillyl alcohol; D1 = 0.5%; D2 = 1%.

**Table 2.** Results of pretreatment of PA on antioxidant enzymes like catalase, glutathione peroxidase, glucose-6-phosphate dehydrogenase on Fe-NTA administration in kidney of Wistar rats

Treatment regimen per group	Catalase (nmol H <sub>2</sub> O <sub>2</sub> consumed per min per mg protein)	Glutathione eroxidase (nmol NADPH oxidized per min per mg protein)	Glucose-6-phosphate dehydrogenase (nmol NADP reduced per min per mg protein)
Group I Saline-treated control	24.8 ± 6.32	88.05 ± 0.09	36.7 ± 0.79
Group II Fe-NTA alone	13.4 ± 1.44 <sup>###</sup>	68.05 ± 0.60 <sup>###</sup>	18.6 ± 0.71 <sup>###</sup>
Group III Fe-NTA + PA D1	15.9 ± 0.64 <sup>#</sup>	72.40 ± 0.55 <sup>#</sup>	24.8 ± 0.46 <sup>#</sup>
Group IV Fe-NTA + PA D2	22.5 ± 0.43 <sup>#</sup>	85.7 ± 0.60 <sup>#</sup>	31.5 ± 0.66 <sup>#</sup>
Group V Only PA D2	25.8 ± 1.05	89.38 ± 0.49	38.5 ± 1.05

Results represent mean ± SE of five animals per group. Results obtained are significantly different from saline-treated group (<sup>###</sup>*P* < 0.001). Results obtained are significantly different from Fe-NTA-treated group (<sup>#</sup>*P* < 0.001). PA, perillyl alcohol; D1 = 0.5%; D2 = 1%.

detoxification. Role of detoxification enzymes in modulation and degradation of electrophilic metabolites is well studied and documented (33).

PA pretreatment in rats diminishes Fe-NTA-induced malonaldehyde formation; with concomitant depletion in enhanced XO levels with simultaneous suppression of serum toxicity markers supports the efficacy of PA in improving renal armory. The mechanism of action of many chemopreventive agents involves alteration of the metabolic fate of carcinogens by modulating activities of either, or both, the Phase I and/or Phase II drug metabolizing enzymes. Induction of Phase II metabolizing/detoxification enzymes enhances carcinogen inactivation by facilitating the clearance of activated metabolites through conjugation with GSH and/or glucuronides (32,33). Phase II enzymes, namely GR, GPX, etc., which primarily catalyze conjugation reactions (32,39,40) were significantly induced in PA pretreated rats, gives ample evidence of its protective nature. Renal damage and toxicity has often been associated with boost in serum markers, namely BUN creatinine. Fe-NTA-treated rats showed elevation in BUN and serum creatinine. Pretreatment of rats with PA before Fe-NTA depleted the serum toxicity markers appreciably. Rats treated with PA had BUN and creatinine significantly lower than those receiving Fe-NTA. These results suggested that PA may protect against Fe-NTA-induced renal toxicity and might serve as a novel agent to limit renal injury.

Induction of ODC activity, and consequently rate of DNA synthesis, is often recognized as an immediate biomarker of tumor promotion in chemopreventive studies (32). Prophylactic treatment of PA significantly suppressed enhanced ODC

activity and [<sup>3</sup>H]thymidine incorporation in renal DNA. These data gives some insight that PA is a potent defensive measure against xenobiotics in which there is involvement of free radicals in toxicity induction. Present results suggest that oxidative damage *in vivo* due to Fe-NTA administration is checked to certain amount by PA pretreatment in rodent model.

## Acknowledgment

The authors are thankful to Hamdard National Foundation, New-Delhi, India, for providing the funds to carry out this work.

## References

- Khan N, Sultana S. Chemomodulatory effect of *Ficus racemosa* extract against chemically induced renal carcinogenesis and oxidative damage response in Wistar rats. *Life Sci* 2005;77:1194–210.
- Rahman A, Ahmed S, Vasenwala SM, Athar M. Glyceryl trinitrate, a nitric oxide donor, abrogates ferric nitrilotriacetate-induced oxidative stress and renal damage. *Arch Biochem Biophys* 2003;418:71–9.
- Anderson RL, Bishop WE, Campbell RL. A review of the environmental and mammalian toxicology of nitrilotriacetic acid. *Crit Rev Toxicol* 1985;15:1–102.
- Mottola HA. Nitrilotriacetic acid as a chelating agent; applications, toxicology and bioenvironmental impact. *Toxic Environ Chem Rev* 1974;2:99–161.
- Toyokuni S, Okada S, Hamazaki S, Minamiyama Y, Yamada Y, Liang P, et al. Combined histochemical and biochemical analysis of sex hormone dependence of ferric nitrilotriacetate-induced renal lipid peroxidation in ddY mice. *Cancer Res* 1990;50:5574–80.
- Gould MN. Cancer chemoprevention and therapy by monoterpenes. *Environ Health Perspect* 1997;105:977–9.
- Elson CE, Yu SG. The chemoprevention of cancer by mevalonate-derived constituents of fruits and vegetables. *J Nutr* 1994;124:607–14.

8. Crowell PL, Gould MN. Chemoprevention and therapy of cancer by d-limonene. *Crit Rev Oncog* 1994;5:1–22.
9. Elson CE. Suppression of mevalonate pathway activities by dietary isoprenoids: protective roles in cancer and cardiovascular disease. *J Nutr* 1995;125:1666S–72S.
10. Michael, Gould N. Cancer Chemoprevention and therapy by monoterpenes. *Environ Health Perspect* 1997;105:977–9.
11. Gomez-Contreras PC, Hernandez-Flores G, Ortiz-Lazareno PC, Del Toro-Arreola S, Delgado-Rizo V, Lerma-Diaz JM, et al. In vitro induction of apoptosis in U937 cells by perillyl alcohol with sensitization by pentoxifylline: increased BCL-2 and BAX protein expression. *Chemotherapy* 2006;52:308–15.
12. Greenwald P. Clinical trials in cancer prevention: current results and perspectives for the future. *J Nutr* 2004;134:3507S–12S13.
13. Crowell PL. Antitumor effect of limonene and perillyl alcohol against pancreatic and breast cancer. In: *American Institute for Cancer Research. Dietary Phytochemicals in Cancer Prevention and Treatment*, New York: Plenum Press, 1996.
14. Vigushin DM. Phase I and pharmacokinetic study of D-limonene in patients with advanced cancer. Cancer Research Campaign Phase I/II Clinical Trials Committee. *Cancer Chemother Pharmacol* 1998;42:111–7.
15. Reddy BS. Chemoprevention of colon carcinogenesis by dietary perillyl alcohol. *Cancer Res* 1997;57:420.
16. Belanger JT. Perillyl alcohol: applications in oncology. *Altern Med Rev* 1998;3:448–57.
17. Awai M, Narasaki M, Yamanoi Y, Seno S. Induction of diabetes in animals by parenteral administration of ferric nitrilotriacetate. A model of experimental hemochromatosis. *Am J Pathol* 1979;95:663–72.
18. Athar M, Iqbal M. Ferric nitrilotriacetate promotes N-diethylnitrosamine-induced renal tumorigenesis in the rat: implications for the involvement of oxidative stress. *Carcinogenesis* 1998;19:1133–9.
19. Jollow DJ, Mitchell JR, Zampaglione N, Gillette JR. Bromobenzene induced liver necrosis: protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic intermediate. *Pharmacology* 1974;11:151–69.
20. Mohandas J, Marshall JJ, Duggin GG, Horvath JS, Tiller D. Differential distribution of glutathione and glutathione related enzymes in rabbit kidney. *Cancer Res* 1984;44:5086–91.
21. Carlberg I, Mannervik B. Glutathione reductase levels in rat brain. *J Biol Chem* 1975;250:5475–80.
22. Zaheer N, Tiwari KK, Krishnan PS. Exposure and solubilization of hepatic mitochondrial shunt dehydrogenases. *Arch Biochem Biophys* 1965;109:646–8.
23. Wright JR, Colby HD, Miles PR. Cytosolic factors which affect microsomal lipid peroxidation in lung and liver. *Arch Biochem Biophys* 1981;206:296–304.
24. Stripe F, Dellacorte E. The regulation of rat liver xanthine oxidase. *J Biol Chem* 1969;244:3855–63.
25. Claiborne A. Catalase activity. In: Greenwald RA (ed). *CRC Handbook of Methods in Oxygen Radical Research*. Boca, Raton: CRC Press, 283–4.
26. Kanter MW. *Clinical Chemistry*, USA: The Bobber Merrill Company Inc., 80.
27. Hare RS. Endogenous creatinine in serum and urine. *Proc Soc Exp Biol Med* 1950;74:148.
28. O'Brien TG, Simsiman RC, Boutwell RK. Induction of the polyamine biosynthesis enzymes in mouse epidermis by tumor promoting agents. *Cancer Res* 1975;35:1662–70.
29. Smart RC, Huang MT, Conney AA. Sn 1,2, diacylglycerols mimic the effects of TPA *in vivo* by inducing biochemical changes associated with tumor promotion in mouse epidermis. *Carcinogenesis* 1986;7:1865–70.
30. Giles KW, Myers A. An improved diphenylamine method for the estimation of deoxyribonucleic acid. *Nature* 1965;206:63.
31. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the phenol reagent. *J Biol Chem* 1964;193:2370–8.
32. Khan N, Sultana S. Inhibition of potassium bromate-induced renal oxidative stress and hyperproliferative response by *Nymphaea alba* in Wistar rats. *J Enzyme Inhib Med Chem* 2005;20:275–83.
33. Khan N, Sharma S, Sultana S. Attenuation of potassium bromate-induced nephrotoxicity by coumarin (1,2-benzopyrone) in Wistar rats: chemoprevention against free radical-mediated renal oxidative stress and tumor promotion response. *Redox Rep* 2004;9:19–28.
34. Yamada M, Awai M, Okigaki T. Rapid *in vitro* transformation system for liver epithelial cells by iron chelate, Fe-NTA. *Cytotechnology* 1990;3:149–56.
35. Athar M, Khan WA, Mukhtar H. Effect of dietary tannic acid on epidermal, lung, and forestomach polycyclic aromatic hydrocarbon metabolism and tumorigenicity in Sencar mice. *Cancer Res* 1989;49:5784–8.
36. Umemura T, Sai K, Takagi A, Hasegawa R, Kurokawa Y. Oxidative DNA damage, lipid peroxidation and nephrotoxicity induced in the rat kidney after ferric nitrilotriacetate administration. *Cancer Lett* 1990;54:95–100.
37. Haag JD, Gould MN. Mammary carcinoma regression induced by perillyl alcohol, a hydroxylated analog of limonene. *Cancer Chemother Pharmacol* 1994;34:477–83.
38. Shi W, Gould MN. Induction of cytostasis in mammary carcinoma cells treated with the anticancer agent perillyl alcohol. *Carcinogenesis* 2002;23:131–42.
39. Padmavathi B, Rath PC, Rao AR, Singh RP. Roots of *Withania somnifera* inhibit forestomach and skin carcinogenesis in mice. *Evid Based Complement Alternat Med* 2005;2:99–105.
40. Han Y, Son SJ, Akhalaia M, Platonov A, Son HJ, Lee KH, et al. Modulation of radiation-induced disturbances of antioxidant defense systems by ginsan. *Evid Based Complement Alternat Med*. 2005;2:529–36.

Received October 11, 2006; accepted November 20, 2006



**Hindawi**  
Submit your manuscripts at  
<http://www.hindawi.com>

