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Emblica officinalis reverses thioacetamide-induced oxidative stress and early promotional events of primary hepatocarcinogenesis

Sarwat Sultana, Salahuddin Ahmed, Sonia Sharma and Tamanna Jahangir

Abstract

Emblica officinalis is widely used in Indian medicine for the treatment of various diseases. In the present study, it was found that fruits of *E. officinalis* inhibit thioacetamide-induced oxidative stress and hyper-proliferation in rat liver. The administration of a single necrotic dose of thioacetamide (6.6 mM kg^{-1}) resulted in a significant ($P < 0.001$) increase in serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and γ-glutamyl transpeptidase (GGT) levels compared with saline-treated control values. Thioacetamide caused hepatic glutathione (GSH) depletion and a concomitant increase in malanodialdehyde (MDA) content. It also resulted in an increase ($P < 0.001$) in the activity of glutathione-*S*-transferase (GST), glutathione reductase (GR), glucose 6-phosphate dehydrogenase (G6PD) and a decrease in glutathione peroxidase (GPx) activity ($P < 0.001$). Hepatic ornithine decarboxylase activity and thymidine incorporation in DNA were increased by thioacetamide administration. Prophylactic treatment with *E. officinalis* for 7 consecutive days before thioacetamide administration inhibited SGOT, SGPT and GGT release in serum compared with treated control values. It also modulated the hepatic GSH content and MDA formation. The plant extract caused a marked reduction in levels of GSH content and simultaneous inhibition of MDA formation. *E. officinalis* also caused a reduction in the activity of GST, GR and G6PD. GPx activity was increased after treatment with the plant extract at doses of 100 mg kg^{-1} and 200 mg kg^{-1}. Prophylactic treatment with the plant caused a significant down-regulation of ornithine decarboxylase activity ($P < 0.001$) and profound inhibition in the rate of DNA synthesis ($P < 0.001$). In conclusion, the acute effects of thioacetamide in rat liver can be prevented by pre-treatment with *E. officinalis* extract.

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

Sarwat Sultana, Salahuddin Ahmed, Sonia Sharma, Tamanna Jahangir

Correspondence: Sarwat Sultana, Department of Medical Elementology and Toxicology, Faculty of Science, Jamia Hamdard (Hamdard University), Hamdard Nagar, New Delhi 110062, India.
E-mail: sarwat786@rediffmail.com

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Introduction

Thioacetamide, used as a fungicide (Childs & Siegler 1946), is a potent hepatotoxin and carcinogen (Fitzhugh & Nelson 1948). It induces acute liver injury that is characterized by perivenous necrosis (Diez-Fernandez et al 1996; Sanz et al 1998). Thioacetamide triggers hepatic injury through the production of thioacetamide-*S*-oxide, a reactive metabolite formed as a result of bioactivation by the flavin adenine dinucleotide monoxygenase system. Free radicals generated by this oxidation pathway cause glutathione (GSH) depletion, leading to oxidative stress and lipid peroxidation (More et al 1990; Sanz et al 1998), an increase in cytosolic calcium (Diaz-Fernandez et al 1996) and enhanced DNA synthesis (Diaz-Fernandez et al 1998). It also exerts its toxic effect on kidney and thymus (Barker & Smuckler 1974). Acute thioacetamide toxicity has been shown to stimulate DNA, RNA and protein synthesis (Dashti et al 1987). Previous studies have shown that necrosis occurs within 24 h of thioacetamide intoxication, followed by a synchronous proliferative response starting immediately and reaching a peak within 48 h (Diez-Fernandez et al 1996; Sanz et al 1998).

Several studies have demonstrated that some dietary factors have a protective effect against preneoplastic liver foci and carcinogenesis in various organs (Mullauer et al 1996). Asian populations that consume a traditional diet are at a lower risk of cancer (breast, prostrate, colon) and coronary heart diseases than Western populations (Kolonel 1988). Increasing scientific investigation into indigenous herbal medicines gives ample evidence of the reliance on medicinal plants as a source of drugs (Bannerman 1980; Huang 1984).

Although crude drugs are not universally accepted, scientific evaluation of traditional medicine has revealed their efficacy in certain diseases, thus forming a valuable source of therapeutic agents for modern medicine (Hikino 1991; Lamartimere et al 1998). Screening of crude extracts of edible plants has revealed the mechanism of several compounds acting together in an additive, synergistic or antagonistic manner to prevent necrotic changes (Koshimizu et al 1988).

Emblica officinalis is used as a major constituent of various Indian herbal formulations for liver protective activity (Gulati et al 1995). Plant fruits are a rich source of vitamin C and are found to be effective as hypolipidaemic and anti-atheroscelerotic agents (Mathur et al 1996), and are reported to reduce serum aortic and hepatic cholesterol in rabbits (Thakur 1985). Fruits possess antimutagenic and anticarcinogenic properties, owing to the combined presence of β-carotene (Jeena et al 1996), ascorbic acid (Counseil & Hornig 1981) and chlorophylin (Ong et al 1986). Quercetin, a flavonoid present in fruits, has been proved to inhibit CCl_4-induced cytotoxicity in isolated hepatocytes (Anon et al 1992). Quercetin has also been shown to modulate several biochemical events associated with tumour promotion, such as alteration of protein kinase C activity (Gschwendt et al 1983) and interaction with calmodulin (Nishino et al 1984). Dietary supplementation of fruit extract to mice significantly reduced the clastogenic effect of 3,4-benzo(a)pyrene (Nandi et al 1997).

Phytochemical investigation of the plant revealed the presence of various constituents, including tannins (Brahmachari & Gupta 1958), triglylglucose (Damodaram & Nair 1936), flavonoid (Khanna et al 1982) and ellagic acid (Brahmachari & Gupta 1958). In the present study, we elucidated the suppressive properties of the 50% ethanolic extract of *E. offcinalis* fruit against thioacetamide-induced liver injury, oxidative stress and proliferative response in rat liver.

Materials and Methods

Chemicals

Oxidized and reduced GSH, NADPH, H_2O_2, dithionitrobenzene, 1-chloro-2,4-dinitrobenzene (CDNB), dinitrophenyl hydrazine, glutathione reductase (GR), diethylnitrosamine and 2-acetylaminofluorene were purchased from Sigma Chemical Co. (St Louis, MO, USA). [^{3}H]Thymidine (specific activity 73.0 Ci mmol^{-1}) was purchased from Amersham Corporation (Little Chalfont, Bucks, UK). All other chemicals and reagents used were of the highest purity commercially available.

Plant extraction

Dried fruits of the plant *E. officinalis* were procured from Jamia Hamdard campus and authenticated at source by Professor M. Iqbal (Department of Environmental Botany, Jamia Hamdard, New Delhi, India). Freshly collected fruits were shade-dried and coarsely powdered in a grinder. Powdered dried fruits (500 g) were extracted in a round-bottomed flask with 2000 mL petroleum ether (60–80°C) and then repeatedly with methanol using a Soxhlet apparatus. The methanol extract was recovered and evaporated to dryness by distillation under reduced pressure in a rotatory evaporator (Buchi Rotavapour; Buchi, Flawil, Switzerland). The concentrated methanol fraction obtained (42 g) was dissolved in aqueous suspension in the required amounts at the time of dosing.

Experimental protocol

For the study of biochemical and serological parameters, 24 male Wistar rats were divided into four groups. Group 1 served as a saline-treated control. Group 2 served as a treatment control and was given a single necrotic dose of thioacetamide (6.6 mM kg^{-1}, i.p.) freshly dissolved in normal saline (0.9%). Pre-treatment of Groups 3 and 4 with *E. officinalis* at doses of 100 and 200 mg kg^{-1}, respectively, was done for 7 consecutive days. On Day 7, a single necrotic dose of thioacetamide (6.6 mM kg^{-1}, i.p.) was given.

All the animals were killed after 24 h of intoxication with thioacetamide. Serum was separated for the determination of serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and γ-glutamyl transpeptidase (GGT) activity. Liver sections were removed, washed and rinsed with cold saline and processed for enzyme determination.

For ornithine decarboxylase (ODC) inhibition studies, 24 male Wistar rats were divided into four equal groups. The treatment protocol was the same as described above except that all animals were killed after 48 h of intoxication with thioacetamide. Liver sections were quickly excised, rinsed and processed for ODC activity.

For the [^{3}H]thymidine incorporation study, a similar experimental protocol was followed except that all the animals were administered [^{3}H]thymidine (24 μCi/0.2 mL saline/100 g bodyweight, i.p.) 2 h before being killed. Liver sections were quickly excised, rinsed and processed for quantification of thymidine incorporation in hepatic DNA.

Biochemical estimations

Tissue processing and preparation of post-mitochondrial supernatant (PMS) were done immediately after the animals were killed. Serum samples were used for all biochemical estimations, which were completed within 24 h of the animals being killed.

Determination of SGOT and SGPT

SGOT and SGPT were determined by the method of Reitman & Frankel (1957). Each substrate (0.5 mL) (2 mM α-ketoglutarate and either 200 mM L-alanine or L-aspartate) was incubated for 5 min at 37°C in a water bath. Serum (0.1 mL) was then added and the volume was adjusted to 1.0 mL with sodium phosphate buffer. The reaction mixture was incubated for exactly 30 min and 60 min for SGPT and SGOT, respectively. Then, 0.5 mL of dinitrophenyl hydrazine (1 mM) was added to the

reaction mixture and left for another 30 min at room temperature. Finally, the colour was developed by the addition of 5.0 mL of NaOH (0.4 M) and the product was read at 505 nm.

GGT

The GGT activity was determined by the method of Orlowski & Meister (1973) using γ-glutamyl *p*-nitroanilide as a substrate. The reaction mixture in a total volume of 0.1 mL contained 0.2 mL serum, which was incubated with 0.8 mL of the substrate mixture (containing 4 mM γ-glutamyl *p*-nitroanilide, 40 mM glycine and 11 mM $MgCl_2$ in 185 mM Tris-HCl buffer, pH 8.25) at 37°C. At 10 min after initiation of the reaction, 1.0 mL of trichloroacetic acid (TCA) (25%) was added and mixed to terminate the reaction. The solution was centrifuged and the supernatant fraction was read at 405 nm. The enzyme activity was calculated as nmol *p*-nitroaniline formed min^{-1} (mg protein)$^{-1}$ using a molar extinction coefficient of *p*-nitroaniline as $1.74 \times 10^3\ M^{-1}\ cm^{-1}$.

Estimation of lipid peroxidation

The assay of microsomal lipid peroxidation was done according to the method of Wright et al (1981). The reaction mixture consisted of 0.58 mL phosphate buffer (0.1 M, pH 7.4), 0.2 mL microsomes, 0.2 mL ascorbic acid (1 mM) and 0.02 mL ferric chloride (100 mM) in a total volume of 1 mL. The mixture was incubated at 37°C in a shaking water bath for 1 h. Then, 1 mL 10% TCA and 1 mL 0.67% thiobarbituric acid were added. All the tubes were placed in a boiling-water bath for 20 min. The tubes were placed in an ice bath and then centrifuged at 2500 *g* for 10 min. The amount of malanodialdehyde (MDA) formed in each of the samples was assayed by measuring the optical density of the supernatant at 535 nm. The results were expressed as nmol MDA formed h^{-1} (g tissue)$^{-1}$ at 37°C using a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

Estimation of GSH

GSH was determined by the method of Jollow et al (1974). A 1.0-mL 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 0.4 mL supernatant, 2.6 mL sodium phosphate buffer (0.1 M, pH 7.4) and 0.2 mL dithionitrobenzene (100 mM) in a total volume of 3.0 mL. A yellow colour developed and was read immediately at 412 nm on a spectrophotometer.

Glutathione-*S*-transferase (GST) activity

GST activity was estimated by the method of Habig et al (1974). The reaction mixture consisted of 1.425 mL sodium phosphate buffer (0.1 M, pH 7.4), 0.2 mL reduced GSH (1 mM), 0.025 mL CDNB (1 mM) and 0.3 mL PMS (10% w/v) in a total volume of 2.0 mL. Changes in absorbance were recorded at 340 nm and enzyme activity was calculated as nmol CDNB conjugate formed min^{-1} (mg protein)$^{-1}$ using a molar coefficient of $9.6 \times 10^3\ M^{-1}\ cm^{-1}$.

GR activity

GR activity was assayed by the method of Carlberg & Mannervick (1975). The assay system consisted of 0.1 M sodium phosphate buffer (pH 7.4), 0.5 mM ethylene diamine tetra acetic acid (EDTA), 1 mM oxidized GSH, 0.1 mM NADPH and PMS (10% w/v) in a total volume of 2.0 mL. The enzyme activity was measured at 340 nm and calculated using a molar extinction coefficient of $6.22 \times 10^3\ M^{-1}\ cm^{-1}$.

Glutathione peroxidase (GPx) activity

GPx activity was assayed by the method of Mohandas et al (1984). The assay mixture consisted of 0.1 M sodium phosphate buffer (pH 7.4), 1 mM EDTA, 0.2 mM NADPH, 1 mM sodium azide, 1 IU mL^{-1} glutathione reductase, 0.25 mM H_2O_2, and PMS (10% w/v) in a total volume of 2.0 mL. The activity was recorded at 340 nm and calculated using a molar extinction coefficient of $6.22 \times 10^3\ M^{-1}\ cm^{-1}$.

Glucose 6-phosphate dehydrogenase (G6PD) activity

G6PD activity was assayed by the method of Zaheer et al (1965). The reaction mixture in a total volume of 3.0 mL 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 $MgCl_2$ (8 mM), 0.3 mL of PMS (10%) and 2.1 mL distilled water. Changes in absorbance were recorded at 340 nm and the enzyme activity was calculated as nmol NADP reduced min^{-1} (mg protein)$^{-1}$ using a molar extinction coefficient of $6.22 \times 10^3\ M^{-1}\ cm^{-1}$.

ODC activity

ODC activity was determined by the method of O'Brien et al (1975). ODC activity was determined using 0.4 mL hepatic 105 000-*g* supernatant fraction per assay tube by measuring the release of CO_2 from DL-[^{14}C]ornithine. The liver was homogenized in Tris-HCI buffer (pH 7.5, 50 mM) containing EDTA (0.4 mM), pyridoxal phosphate (0.32 mM), phenylmethylsulfonyl fluoride (0.1 mM), 2-mercaptoethanol (1.0 mM), dithiothreitol (4.0 mM) and Tween 80 (0.1%) at 4°C using a Polytron homogenizer (PT 3000, Kinematica AG, Littau, Switzerland). In brief, the reaction mixture contained 400 μL of enzymes 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), Brij 35 (0.02%) and DL-[^{14}C]ornithine (0.05 μCi) in a total volume of 0.495 mL. After adding buffer and co-factor mixture to blank and other tubes, the tubes were closed immediately with rubber stoppers, containing 0.2 mL ethanolamine and methoxyethanol (2:1) 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 the glass tubes and the reaction was continued for 1 h to ensure complete absorption of CO_2. Finally, the central well was transferred to a vial containing 2.0 mL ethanol and 10.0 mL toluene-based scintillation fluid. Radioactivity was counted in a liquid scintillation counter (LKB Wallac-1410 Michigan, USA). ODC activity was expressed as pmol CO_2 released h^{-1} (mg protein)$^{-1}$.

Hepatic DNA synthesis

The isolation of hepatic DNA and incorporation of [^{3}H]thymidine in DNA was done by the method of Smart et al (1986). Liver was quickly removed, cleaned free of extraneous material, and homogenate (10% w/v) was prepared in ice-cold water. The precipitate obtained was washed with cold TCA (5%) and incubated with cold perchloric acid (10%) at 4°C overnight. After incubation, the precipitate was centrifuged and washed with cold perchloric acid (5%). The precipitate was dissolved in warm perchloric acid (10%), followed by incubation in a boiling-water bath for 30 min, and filtered though a Whatman 50 filter. The filtrate was used for [^{3}H]thymidine counting in a liquid scintillation counter (LKB-Wallac-1410) by adding the scintillation fluid. The amount of DNA in the filtrate was estimated by the diphenylamine method of Giles & Myers (1965). The amount of [^{3}H]thymidine incorporated was expressed as dpm (μg DNA)$^{-1}$.

Protein estimation

Protein content in all samples was estimated by the method of Lowry et al (1951) using bovine serum albumin as standard.

Statistical analysis

The level of significance between different groups was based on analysis of variance followed by Dunnett's *t*-test.

Results

Table 1 shows the effect of pre-treatment of rats with *E. officinalis* on thioacetamide-induced liver damage markers. The administration of a single necrotic dose (6.6 mM kg^{-1}, i.p.) of thioacetamide resulted in a significant ($P < 0.001$) increase in the levels of SGOT, SGPT and GGT by 365%, 346% and 234% of saline-treated control values. Pre-treatment with the *E. officinalis* extract for 7 consecutive days before thioacetamide intoxication at two different doses significantly ($P < 0.001$) depleted the enzyme leakage into the serum in a dose-dependent manner. *E. officinalis* inhibited SGOT, SGPT and GGT release in serum by 143%, 172% and 60% of treated control values.

Table 2 shows the effect of pre-treatment with *E. officinalis* on thioacetamide-induced modulation of GSH content and MDA formation in rat liver. Thioacetamide administration caused a significant ($P < 0.001$) progressive decrease of GSH content by 243% of the control value, with a concomitant increase in MDA formation to 270% of saline-treated control values. Pre-treatment with *E. officinalis* caused a marked recovery in levels of hepatic GSH content, with simultaneous inhibition of MDA formation. *E. officinalis* caused a significant ($P < 0.001$) improvement in GSH content (44.4% and 42.7%). A marked decrease in MDA formation of 114% was found after pre-treatment with *E. officinalis* ($P < 0.001$).

Table 2 Effect of pre-treatment of the extract of *Emblica officinalis* on thioacetamide-induced modulation of glutathione (GSH) content and malanodialdehyde (MDA) formation in rat liver

Group	GSH (mmol (g tissue)$^{-1}$)	MDA (nmol MDA h^{-1} (g tissue)$^{-1}$)
Saline	0.56 ± 0.06	10.39 ± 0.42
Thioacetamide	$0.24 \pm 0.03^{\#}$	$28.08 \pm 1.26^{\#\#}$
Thioacetamide + *E. officinalis* (100 mg kg^{-1})	0.36 ± 0.03*	20.41 ± 0.94**
Thioacetamide + *E. officinalis* (200 mg kg^{-1})	0.48 ± 0.04**	16.22 ± 0.63***

Results represent mean ± s.e. of six animals per group. $^{\#}P < 0.001$, $^{\#\#}P < 0.001$, significantly different compared with the saline-treated group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, significantly different compared with the thioacetamide-treated group.

Table 1 Effect of pre-treatment with the extract of *Emblica officinalis* on thioacetamide-induced release of liver damage markers in serum

Group	SGOT (IU L^{-1})	SGPT (IU L^{-1})	GGT (nmol *p*-nitroanilide formed min^{-1} (mg protein)$^{-1}$)
Saline	16.30 ± 0.67	22.32 ± 0.89	423.46 ± 18.62
Thioacetamide	$56.39 \pm 1.61^{\#\#}$	$81.47 \pm 2.40^{\#\#}$	$989.36 \pm 32.48^{\#\#}$
Thioacetamide + *E. officinalis* (100 mg kg^{-1})	44.42 ± 1.21***	68.46 ± 1.05***	790.09 ± 38.73**
Thioacetamide + *E. officinalis* (200 mg kg^{-1})	28.30 ± 1.13***	49.62 ± 0.97***	693.17 ± 38.73***

SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase; GGT, γ-glutamyl transpeptidase. Results represent mean ± s.e. of six animals per group. $^{\#\#}P < 0.001$, significantly different compared with the saline-treated group. **$P < 0.01$, ***$P < 0.001$, significantly different compared with the thioacetamide-treated group.

Table 3 Effect of pre-treatment of the extract of *Emblica officinalis* on glutathione metabolizing enzymes against thioacetamide-induced oxidative stress in rat liver

Group	GST (nmol conjugate formed min^{-1} $(mg\ protein)^{-1}$)	GPx (nmol NADPH oxidized min^{-1} $(mg\ protein)^{-1}$)	GR (nmol NADPH oxidized min^{-1} $(mg\ protein)^{-1}$)	G6PD (nmol NADP reduced min^{-1} $(mg\ protein)^{-1}$)
Saline	906.7 ± 35.9	258.5 ± 12.9	230.6 ± 8.9	178.2 ± 9.9
Thioacetamide	$1468.8 \pm 62.6^{\#\#}$	$124.1 \pm 10.5^{\#\#}$	$426.5 \pm 16.1^{\#\#}$	$368.6 \pm 19.4^{\#\#}$
Thioacetamide + *E. officinalis* ($100\ mg\ kg^{-1}$)	1262.3 ± 52.5*	173.1 ± 10.5*	382.9 ± 12.8	312.9 ± 11.4*
Thioacetamide + *E. officinalis* ($200\ mg\ kg^{-1}$)	1108.5 ± 42.3**	228.8 ± 6.7***	304.4 ± 16.3**	273.1 ± 14.3**

GST, glutathione-*S*-transferase; GPx, glutathione peroxidase; GR, glutathione reductase; G6PD, glucose 6-phosphate dehydrogenase. Results represent mean ± s.e. of six animals per group. $^{\#\#}P < 0.001$, significantly different compared with the saline-treated group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, significantly different compared with the thioacetamide-treated group.

Table 3 shows the effect of pre-treatment with *E. officinalis* on thioacetamide-induced modulation of enzymes involved in the GSH redox cycle. Thioacetamide administration resulted in a significant ($P < 0.001$) increase in the activity of GST, GR and G6PD by 162%, 185% and 207%, respectively, with a concomitant decrease in GPx activity by 52% of the saline-treated control. *E. officinalis* pre-treatment suppressed the enhanced GST, GR and G6PD activity by 40%, 53% and 54%, and recovery of GPx activity by 40% compared with treated control values.

Figure 1A shows the effect of pre-treatment of animals with *E. officinalis* extract on thioacetamide-induced ODC activity. Thioacetamide administration resulted in a significant induction ($P < 0.001$) of hepatic ODC to 341% of saline-treated control rats. Pre-treatment with *E. officinalis* extract caused a significant ($P < 0.001$) down-regulation of hepatic ODC by 128%. Figure 1B shows the effect of pre-treatment of rats with *E. officinalis* extract on [^{3}H]thymidine incorporation into hepatic DNA. Intraperitoneal administration of thioacetamide significantly ($P < 0.001$) enhanced [^{3}H]thymidine incorporation into hepatic DNA, suggesting tissue regeneration after injury. Pre-treatment of rats with *E. officinalis* resulted in a profound reduction in the rate of DNA synthesis. The maximum inhibition of 165% was found by *E. officinalis* compared with treated control groups. The results indicate that pre-treatment with *E. officinalis* extract potentially inhibits thioacetamide-induced oxidative stress and proliferation in rat liver.

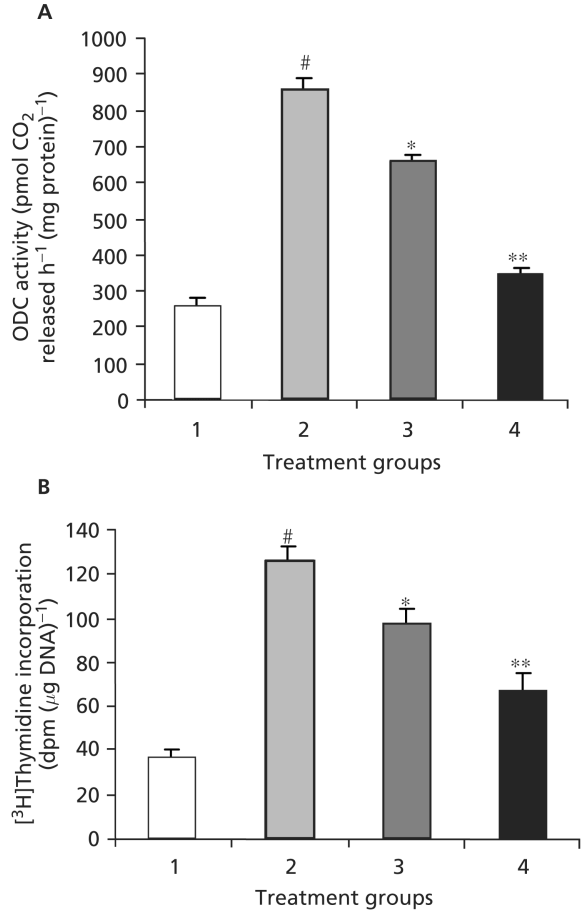

Figure 1 Efficacy of the extract of *Emblica officinalis* against thioacetamide-induced ornithine decarboxylase activity (A) and [^{3}H]thymidine incorporation (B) in rat liver. Group 1: saline only; Group 2: thioacetamide only; Group 3: *E. officinalis* ($100\ mg\ kg^{-1}$) + thioacetamide; Group 4: *E. officinalis* ($200\ mg\ kg^{-1}$) + thioacetamide. Each value represents the mean ± s.e. of six rats. $^{\#}P < 0.001$, significantly different values in Group 2 compared with Group 1. $^{*}P < 0.01$, $^{**}P < 0.001$, significantly different values in *E. officinalis* pre-treated Groups 3 and 4 compared with Group 2.

Discussion

Thioacetamide acts though the generation of free radicals that attack nucleophilic targets in the cell and membrane phospholipids, thereby leading to disruption of membrane viability and integrity. It mediates injury through microsomal oxidation in the mechanisms leading to cell death

(Cascales et al 1992; Mehendale et al 1994; Sanz et al 1998).

In the present study, pre-treatment with the fruit extract of *E. officinalis* caused a significant reduction in liver enzyme leakage in serum. The plant has been shown to possess antioxidant and anti-inflammatory activities, which may have counteracted the free radicals generated through metabolic activation by directly scavenging and neutralizing these radicals at target sites and thus decreasing the extent of necrosis and lipid peroxidation, as evident from the significantly lowered levels of damage marker enzymes such as SGOT, SGPT and GGT compared with the thioacetamide-treated group. *E. officinalis* fruit has been shown to possess antioxidant and antimutagenic activities (Jeena et al 1999). Pre-treatment with *E. officinalis* significantly down-regulated GST, GR and G6PD activities. GST is suggested to catalyse the conjugation of electrophiles generated as a result of thioacetamide intoxication with GSH to render them soluble and excretable. It can be suggested that pre-treatment with *E. officinalis* modulated the activation of thioacetamide and had protective potential by preventing GSH depletion and elevation of lipid peroxidation dose dependently. Thus, regulation of GSH content and bioavailability might have favoured the ameliorative effect of pre-treatment on the activity of enzymes involved in the GSH redox cycle. The GSH cycle plays an important role against oxidative stress by maintaining the thioacetamide redox balance of cells by the GPx/GR system coupled to the NADPH/$NADP^+$ pair (Kera et al 1987). In addition to inducing peroxidation of membrane phospholipids in cells, free radicals oxidize NADPH through cyclic reduction, leading to its depletion. This may cause cell death as a result of disturbed vital physiological and biochemical functions and impaired oxidative defences (Melchiorri et al 1996). Pre-treatment with *E. officinalis* extract may have afforded protection to nucleophilic sites to competitively outclass electrophilic moieties, thus preventing GSH depletion, oxidation of NADPH and down-regulating GSH generating enzymes, GR and G6PD, with concomitant recovery of the GSH utilizing enzyme cascade. It also restored the activity of GPx in a dose-dependent manner, which indicates neutralization of hydrogen peroxide.

Mammalian liver has been shown to possess growth ability after cellular loss in hepatotoxin-induced tissue injury (Mendel 1965; Steer 1995). The destruction of cells in perivenous necrosis by thioacetamide is followed immediately by a proliferative response, which reaches a maximum within 48 h of intoxication (Diez-Fernandez 1993). Thioacetamide administration resulted in a significant ($P < 0.001$) induction of hepatic ODC and [^{3}H]thymidine uptake into hepatic DNA. Pre-treatment of rats with *E. officinalis* before thioacetamide intoxication significantly inhibited ODC activity and the rate of [^{3}H]thymidine incorporation in hepatic DNA.

The phytochemicals present in the crude plant such as tannins, flavonoids and ellagic acid have been shown to be of particular significance in combating undesirable events associated with diseases such as cancer (Das et al 1987; Tanaka et al 1993; Birt et al 2001). A potential explanation for the protective action is that pre-treatment with the plant extract, by one or more mechanisms, lessens the acute hepatic injury induced by thioacetamide, thus preventing the extent of post-necrotic hepatic cellular regeneration. It can be concluded that treatment with the plant extract before thioacetamide administration markedly reduced the severity of liver injury by decreasing oxidative stress and lipid peroxidation, and consequently the post-necrotic hepatocellular regeneration by suppressing ODC activity and rate of [^{3}H]thymidine incorporation as a marker of DNA synthesis.

In conclusion, the acute effects of thioacetamide in rat liver can be prevented by pre-treatment with *E. officinalis* extract. Since thioacetamide induces biochemical parameters of toxicity that are also produced by many chronic/carcinogenic doses of a promoter, we suggest that *E. officinalis* extract may also be of some relevance in delaying the carcinogenesis process. We propose that *E. officinalis* can be used as a chemopreventive agent against tissue injury and as an indigenous source of drug in a number of inflammatory disorders and enhanced proliferative conditions.

References

Anon, M. T., Ubeda, A., Alcaraz, M. J. (1992) Protective effects of phenolic compounds on CCl_4-induced toxicity in isolated rat hepatocytes. *Z. Naturforsch.* [C] **47**: 275–279

Bannerman, R. H. (1980) Organization and cooperation for the development of studies on plants used in traditional medicine: some guidelines for research and studies. *J. Ethnopharmacol.* **2**: 189–192

Barker, E. A., Smuckler, E. A. (1974) Nonhepatic thioacetamide injury. II. The morphologic features of proximal renal tubular injury. *Am. J. Pathol.* **74**: 575–590

Birt, D. F., Hendrich, S., Wang, W. (2001) Dietary agents in cancer prevention: flavonoids and isoflavonoids. Dietary agents in cancer prevention: flavonoids and isoflavonoids. *Pharmacol. Ther.* **90**: 157–177

Brahmachari, H. D., Gupta, V. S. (1958) Studies on *Phyllanthus emblica* (AmLa) Part III. Role of tannins in stabilizing the ascorbic acid of the fruit. *Indian J. Appl. Chem.* **21**: 173–174

Carlberg, I., Mannervik, B. (1975) Glutathione reductase levels in rat brain. *J. Biol. Chem.* **250**: 5475–5480

Cascales, M., Martin-Sanz, P., Alvarez, A., Sanchez, M., Diez-Fernandez, C., Bosca, L. (1992) Isoenzymes of carbohydrate metabolism in primary cultures of hepatocytes from thioacetamide-induced rat liver necrosis. Responses to growth factors. *Hepatology* **16**: 232–240

Childs, J. F. L., Siegler, E. A. (1946) Controlling orange decay. *Ind. Eng. Chem. Res.* **38**: 82

Counseil, J. N., Hornig, D. H. (1981) *Vitamin C: Ascorbic acid.* Applied Science Publishers, New Jersey

Damodaram, M., Nair, K. R. (1936) A tannin from Indian goose (*Phyllanthus emblica*) with protective action on ascorbic acid. *Biochem. J.* **30**: 1014–1020

Das, M., Mukhtar, H., Bik, D. P., Bickers, D. R. (1987) Inhibition of epidermal xenobiotic metabolism in SENCAR mice by naturally occurring plant phenols. *Cancer Res.* **47**: 760–766

Dashti, H., Jeppsonn, B., Hagerstrand, I., Hultberg, B., Srinivas, U., Abdulla, M., Jeolson, B., Bengark, S. (1987) Early biochemical and histological changes in rats exposed to a single injection of thioacetamide. *Pharmacol. Toxicol.* **60**: 171–174

Diez-Fernandez, C. (1993) Relationship between genomic DNA ploidy and parameters of liver damage during necrosis and regeneration induced by thioacetamide. *Hematology* **18**: 912–918

Diez-Fernandez, C., Sanz, N., Cascales, M. (1996) Intracellular calcium concentration impairment in hepatocytes from thioacetamide-treated rats. Implications for the activity of Ca^{2+}-dependent enzymes. *J. Hepatol.* **24**: 460–467

Diez-Fernandez, C., Sanz, N., Alvarez, A. M., Zaragova, A., Cascales, M. (1998) Influence of aminoguanidine on parameters of liver injury and regeneration induced in rats by a necrogenic dose of thioacetamide. *Br. J. Pharmacol.* **125**: 102–108

Fitzhugh, O. G., Nelson, A. A. (1948) Liver tumors in rats fed thiourea or thioacetamide. *Science* **108**: 626–628

Giles, K. W., Myers, A. (1965) An improved diphenylamine method for the estimation of deoxyribonucleic acid. *Nature* **206**: 93

Gschwendt, M., Horn, F., Kittstein, W., Marks, F. (1983) Inhibition of the calcium- and phospholipid-dependent protein kinase activity from mouse brain cytosol by quercetin. *Biochem. Biophys. Res. Commun.* **117**: 444–447

Gulati, R. K., Agarwal, S., Agarwal, S. S. (1995) Hepatoprotective studies on *Phyllanthus emblica* Linn. and quercetin. *Indian J. Exp. Biol.* **33**: 261–268

Habig, W. H., Pubst, M. J., Jokoby, W. B. (1974) GST the first enzymatic step in mercapturic acid formation. *J. Biol. Chem.* **249**: 7130–7139

Hikino, H. (1991) Traditional remedies and modern assessment: the case of ginseng. In: Wijesekera, R. O. B. (ed.) *The Medicinal plant industry*. CRC Press, Boca Raton, pp 149–166

Huang, L. (1984) Drug research based upon the leads of the Chinese traditional medicine. In: Krogsgaard-Larsen, P., Christensen, S. B., Kofod, H. (eds) *Natural products and drug development*. Munksgaard, Copenhagen, p. 94

Jeena, K. J., Kuttan, G., George, J., Kuttan, R. (1996) Antimutagenic and anticarcinogenic activity of *Emblica officinalis* Gaertn. *J. Clin. Biochem. Nutr.* **22**: 171–176

Jeena, K. J., Joy, K. L., Kuttan, K. (1999) Effect of *Emblica officinalis*, *Phyllanthus amarus* and *Picrorrhiza kurroa* on N-nitrosodiethylamine induced hepatocarcinogenesis. *Cancer Lett.* **136**: 11–16

Jollow, D. J., Mitchell, J. R., Zampaglione, N., Gillette, J. R. (1974) Bromobenzene induced liver necrosis: protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic intermediate. *Pharmacology* **11**: 151–169

Kera, Y., Sippel, H. W., Pentilla, K. E., Lindros, K. O. (1987) Acinar distribution of glutathione-dependent detoxifying enzymes. Low glutathione peroxidase activity in perivenous hepatocytes. *Biochem. Pharmacol.* **36**: 2003–2006

Khanna, P., Tapania, R., Jain, S. C. (1982) Flavonoids from *Emblica officinalis* Gaertn. tissue cultures. *Indian J. Bot.* **5**: 43–44

Kolonel, L. N. (1988) Variability in diet and its relation to risk in ethnic and migrant groups. *Basic Life Sci.* **43**: 129–135

Koshimizu, K., Ohigashi, H., Tokuda, H., Kondo, A., Yamaguchi, K. (1988) Screening of edible plants against possible anti-tumor promoting activity. *Cancer Lett.* **39**: 247–257

Lamartimere, C. A., Murrill, W. B., Manzalillo, P. A., Zhangt, J. X., Barnes, S., Zhang, X., Wei, H., Brown, N. M. (1998) Genistein alters the ontogeny of mammary gland development and protects against chemically-induced mammary cancer in rats. *Proc. Soc. Exp. Biol. Med.* **217**: 358–364

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265–275

Mathur, R., Sharma, A., Dixit, V. P., Verma, M. (1996) Hypolipidaemic effect of fruit juice of *Emblica officinalis* in cholesterol-fed rabbits. *J. Ethnopharmacol.* **50**: 61–68

Mehendale, H. M., Roth, R. A., Gandolfa, A. J., Klauning, J. E., Lemasters, J. J., Curtis, R. L. (1994) Novel mechanisms in chemical induced hepatotoxicity. *FASEB J.* **8**: 1285–1295

Melchiorri, D., Reiter, R. J., Sewerynek, E., Hara, M., Chen, L., Nistico, G. (1996) Paraquat toxicity and oxidative damage. *Biochem. Pharmacol.* **51**: 1095–1099

Mendel, G. A. (1965) Studies on iron absorption. 3. Increased iron absorption during liver regeneration induced by partial hepatectomy. *J. Lab. Clin. Med.* **66**: 627–640

Mohandas, J., Marshall, J. J., Duggin, G. G., Horvath, J. S., Tiller, D. (1984) Differential distribution of glutathione and glutathione related enzymes in rabbit kidney. *Cancer Res.* **44**: 5086–5091

More, Y., Ninomiya, M., Fujiki, H. (1990) Present status of research on cancer chemoprevention in Japan. *Jpn. J. Clin. Oncol.* **20**: 219–224

Mullauer, L., Grasl-Kraupp, B., Bursch, W., Schulte-Hermann, R. (1996) Transforming growth factor beta 1-induced cell death in preneoplastic foci of rat liver and sensitization by the antiestrogen tamoxifen. *Hepatology* **23**: 840–847

Nandi, P., Talukedar, E., Sharma, A. (1997) Dietary chemoprevention of clastogenic effects of 3,4-benzo (a) pyrene by *Emblica officinalis* Gaertn. fruit extract. *Br. J. Cancer* **76**: 279–283

Nishino, H., Naitoh, E., Iwashima, A., Umezawa, K. (1984) Quercetin interacts with calmodulin, a calcium regulatory protein. *Experientia* **40**: 184–185

O'Brien, T. G., Simsiman, R. C., Boutwell, R. K. (1975) Induction of the polyamine-biosynthetic enzymes in mouse epidermis by tumor promoting agents. *Cancer Res.* **35**: 1662–1670

Ong, T., Whong, W. Z., Stewart, J., Brockman, H. E. (1986) Chlorophyllin: a potent antimutagen against environmental and dietary complex mixture. *Mutat. Res.* **173**: 111–115

Orlowski, M., Meister, A. (1973) γ-Glutamyl cyclotransferase distribution, isozymic forms and specificity. *J. Biol. Chem.* **248**: 2836–2844

Reitman, S., Frankel, S. A. (1957) Colorimetric method for the determination of serum oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.* **28**: 56–63

Sanz, N., Diez-Fernandez, C., Fernandez-Simon, L., Alvarez, A. M., Cascales, M. (1998) Necrogenic and regenerative responses of liver of newly weaned rats against a sublethal dose of thioacetamide. *Biochem. Biophys. Acta* **1384**: 66–78

Smart, R., Huang, M. T., Conney, A. H. (1986) Sn1, 1,2,diacylglycerols mimic the effects of TPA in vivo by inducing biochemical changes associated with tumor promotion in mouse epidermis. *Carcinogenesis* **7**: 1865–1870

Steer, C. J. (1995) Liver regeneration. *FASEB J.* **9**: 1396–1400

Tanaka, T., Kojima, T., Kawamori, T., Yoshimi, N., Mori, H. (1993) Chemoprevention of diethylnitrosamine-induced hepatocarcinogenesis by a simple phenolic acid protocatechuic acid in rats. *Cancer Lett.* **53**: 2775–2779

Thakur, C. P. (1985) *Emblica officinalis* reduces serum, aortic and hepatic cholesterol in rabbits. *Experientia* **41**: 423–424

Wright, J. R., Colby, H. D., Miles, P. R. (1981) Cytosolic factors which affect microsomal lipid peroxidation in lung and liver. *Arch. Biochem. Biophys.* **206**: 296–304

Zaheer, N., Tiwari, K. K., Krishnan, P. S. (1965) Exposure and solubilization of hepatic mitochondrial shunt dehydrogenases. *Arch. Biochem. Biophys.* **109**: 646–648