

AMELIORATIVE EFFECT OF *EMBLICA OFFICINALIS* AQUEOUS EXTRACT ON OCHRATOXIN-INDUCED LIPID PEROXIDATION IN THE KIDNEY AND LIVER OF MICE

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Abstract

Objectives: The present study was an attempt at investigating whether the aqueous extract of *Emblica officinalis* may have an ameliorative effect on ochratoxin-induced lipid peroxidation in the kidney and liver of mice. **Methods:** Adult male albino mice were orally administered 50 µg (LD, low dose) and 100 µg (HD, high dose) of ochratoxin/0.2 ml of olive oil/animal/day for 45 days. **Results:** The results revealed a significant ($p < 0.05$), dose-dependent increase in lipid peroxidation as well as a decreased activity of enzymatic antioxidants (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione transferase) and non-enzymatic antioxidants (glutathione and total ascorbic acid) in both the organs, as compared to the findings for olive oil-treated control group. Administration of *Emblica officinalis* aqueous extract (2 mg/animal/day) and ochratoxin for a period of 45 days caused a significant amelioration in the ochratoxin-induced lipid peroxidation in mouse liver and kidney.

Key words:

Kidney, Liver, Antioxidants

INTRODUCTION

About 75–80% of the world's population depends on herbal medicine for primary health care, both in the developing and developed countries. Herbs are believed to be well compatible with the human body and to produce less side effects than the pharmaceuticals. Herbal medicine has already been mentioned in the Indian Ayurveda and Unani medicines, but the data on animal studies and efficacy of herbal preparations are not available and, therefore, these products cannot be placed either on the local, Indian, market, or on foreign markets. During the last two decades, scientists have been seeking for new plant products that possess antioxidant properties and may play a role in the prevention of various diseases associated with oxidative

stress, such as cancer, and cardiovascular, reproductive and neurodegenerative diseases.

Ochratoxin is a toxic secondary fungal metabolite produced by *Aspergillus ochraceus* and *Penicillium verdicatum* [1]. Human exposure to ochratoxin is a widespread problem in some of the European countries as well as in India. The contamination of various food products by this group of mycotoxins leads to ochratoxicosis both in humans and animals [2]. Ochratoxin A is primarily a nephrotoxic agent and has been classified as a possible human carcinogen (Group 2B) by the International Agency for Research on Cancer (IARC), Lyon, France. Ochratoxin A has been shown to induce renal adenomas and carcinomas in mice and rats (IARC, 1993). Ochratoxin exposure was found to be a putative cause of the Balkan endemic

Received: June 3, 2009. Accepted: October 26, 2009.

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nephropathy (BEN) [3]. The chemical was found to exert hepatotoxic, cytotoxic and carcinogenic effect on various laboratory animals and on humans [4].

Emblica officinalis (Amla), normally known as Indian gooseberry, has been used extensively in the ancient Indian Ayurveda as a potent rasayana i.e. a herbal formulation that helps attain longevity and rejuvenation [5]. It is a member of a small genus *Emblica* (family Euphorbiaceae) which is commonly found in India and South East Asia. The fruit is a major constituent of chyavanprash [6], a traditional Ayurvedic herbal jam also used for pickle preparation in India. *Emblica officinalis* is one of the myrobalans (plants that exhibit various therapeutic properties) commonly used in triphala (three fruits) preparations. Triphala is a traditional Ayurvedic herbal formulation which is considered an important rasayana in Ayurvedic medicine [7]. The rasayana medications are believed to promote health, immunity and longevity. *Emblica officinalis* extract has been shown to possess high antioxidative, anticancer, lipid-lowering, antisclerotic, hepatoprotective, and anti-HIV potentials [8–10]. *Emblica officinalis* is highly nutritious and it is an important dietary source of vitamin C, minerals and amino acids. In the edible fruit tissue, the protein concentration is three times as high as in the apple, and of ascorbic acid concentration, 160 times as high. *Emblica officinalis* contains a range of polyphenols, especially tannins, and other phenolic compounds. These include hydrolysable tannins (10–12%) with a molecular weight of less than 1000, including quercetin, emblicanin A, emblicanin B, punigluconin and pedunculagin [11]. They show cytoprotective activity and a possible inhibitory effect on carcinogenesis, mutagenesis and tumor genesis [12].

The present study was undertaken to evaluate the possible ameliorative effect of the aqueous extract of *Emblica officinalis* (amla) on ochratoxin-induced lipid peroxidation and its antioxidant defense mechanism in the kidney and liver of mice.

MATERIALS AND METHODS

All the chemicals used in the present study were of analytical grade; they were obtained from HiMedia Laboratories Pvt. Ltd, Mumbai.

Ochratoxin production and analysis

A pure toxigenic strain of *Aspergillus ochraceus* (ITCFF NO-1456) was obtained from the Indian Agricultural Research Institute, New Delhi, India, and was maintained on potato dextrose agar medium. It was grown on yeast extract sucrose (YES) medium at $28 \pm 2^\circ\text{C}$ for 10 days [13]. Fifty ml of YES liquid medium was taken in a 500 ml Erlenmeyer flask and sterilized at the pressure of 1.03 bar for 20 min. 0.5 ml of spore suspension, having 10^8 conidia/ml, which was prepared in sterilized distilled water from a 5-day-old culture of *Aspergillus ochraceus*, was used for inoculating the medium under aseptic conditions (Laminar flow). After 10 days of incubation at $28 \pm 2^\circ\text{C}$, the inoculated flasks were autoclaved and the contents of the flasks were filtered through Whatman filter paper No. 41. The culture filtrates were analyzed for ochratoxin. They were examined under ultraviolet (UV) light (at 360 nm) for fluorescence. The samples that gave bright green-yellow fluorescence were considered positive and processed for ochratoxin extraction. Aqueous culture filtrates with a positive BGYF (blue green yellow fluorescence) test were extracted twice with chloroform (1:2, v/v) in a separating funnel, and the lowermost chloroform layer was passed through the bed of anhydrous sodium sulphate (Na_2SO_4). The chloroform extract was evaporated to dryness, transferred and stored in vials for qualitative and quantitative analysis of ochratoxin. The TLC spotted with ochratoxin extract was developed in a solvent system comprising toluene: ethyl acetate: formic acid (50:40:10 v/v). Further chemical confirmation of ochratoxin was performed by spreading a developed chromatoplate with ammonia solution in the field [14]. Each spot was scraped separately, dissolved in methanol, and subjected to spectrophotometry at 332 nm, using UV spectrothermo Spectronic UV-1 Spectrophotometer [15].

Preparation of plant extract and the phytochemical analysis

Ripe fruits of *Emblica officinalis* were obtained from a local market, and species identification was confirmed by the Botany Department, School of Sciences, Gujarat University, Ahmedabad, India. The extract was prepared according to WHO protocol [16]. Shade dried pulp was ground

with mortar and pestle. 5 gm of powder was suspended in 100 ml distilled water and mixed repeatedly for 3 hours at 40°C. After cooling, the content was filtered successively through ordinary and Whatman filter paper No. 1. Both the fractions were dried and stored in dark bottles at 4°C. During the experiment, a known amount of dried extract was redissolved in water and used.

The tannin content in the crude extract of *Emblica officinalis* was determined by oxidation-reduction titration using indigo-caramine reagent [17]. A known amount of dry extract was boiled in distilled water and filtered. The filtrate was treated with indigo-caramine reagent. The solution was then titrated with 0.1 N potassium permanganate solution. The endpoint was the appearance of a yellowish green colour.

The flavonoid content was determined by the method of Chang et al. [18]. Plant extract in methanol was mixed separately with aluminum chloride and potassium acetate along with distilled water. The absorbance of the reaction mixture was measured at 415 nm.

Total ascorbic acid was estimated by the method of Chinnoy et al. [19]. The blue dye, dichlorophenol indophenol, is reduced to a colourless form on addition of ascorbic acid, where ascorbic acid reduces to dehydroascorbic acid. The buffered HPO₃ extract was treated with dichlorophenol indophenol dye solution. The absorbance was read at 520 nm.

Emblica officinalis aqueous extract was found to contain 3.4% ascorbic acid, 2.2% tannin and 0.7% flavonoid of dry weight.

Animal experiment

Young adult male inbred Swiss albino mice (*Mus musculus*), weighing approximately 30–33 g, were obtained from Zydyus Research Centre, Ahmedabad, India. The animals were provided with animal feed and water *ad libitum* and kept in 12 h:12 h light/dark cycles at 26±2°C. Animal feed was prepared as per the formulation obtained from the National Institute of Occupational Health, Ahmedabad, India, and was confirmed to be free of mycotoxins. Guidelines for Animal Care and Use in Scientific Research, 1991, published by the Indian National Science Academy, New Delhi, India, were followed.

Seventy animals were divided into seven groups and caged separately. Group 1 animals (untreated controls) were maintained without any treatment. Animals of Groups 2 and 3 received olive oil (0.2 ml/animal/day) and *Emblica officinalis* aqueous extract (2 mg/animal/day), respectively, for 45 days, and served as pre-treatment controls. Animals of groups 4 and 5 were orally administered ochratoxin at 50 µg (LD, low dose) and 100 µg (HD, high dose) in 0.2 ml olive oil/animal/day (1.5 and 3.0 mg/kg b.w./day) for 45 days. Group 6 and 7 animals were orally given ochratoxin, as described for groups 4 and 5, and aqueous extract of *Emblica officinalis* (2 mg/animal/day) for 45 days, Table 1.

Olive oil was obtained from Figaro, Madrid, Spain. Ochratoxin was dissolved in olive oil and was used as a vehicle in Group 2. Ochratoxin dose was based on WHO Environmental Health Criteria for ochratoxin and trichothecenes [20]. The dose of *Emblica officinalis* aqueous extract was based on earlier studies [21]. All the agents were administered orally for 45 days, using a feeding tube attached to a hypodermic syringe.

Biochemical analysis

On completion of the treatment, the animals were sacrificed by cervical dislocation. In all the control and treated groups of animals, the kidneys and livers were quickly isolated, blotted free of blood and utilized for biochemical analyses,

The 10% homogenate for lipid peroxidation (LPO) was prepared by homogenizing a known amount of tissue in phosphate-buffered saline (PBS). The LPO was assessed using the method of Ohkawa et al. (1979) which consists in quantifying the thiobarbituric acid reactive substances (TBARS) [22]. The homogenate for superoxide dismutase (SOD) was prepared by homogenizing a known amount of tissue in cold normal saline. SOD activity was measured by modified spectrophotometric method of Kakkar et al. (1984) [23]. For the preparation of glutathione peroxidase (GPX) and catalase (CAT) homogenate, a known amount of tissue was homogenized in 0.1% chilled digitonin. GPX and CAT activities were assayed using modified methods of Pagila and Valentine (1967), and Luck (1963),

Table 1. Study protocol

Study group	Treatment received	Days of treatment	Day of autopsy	No. of animals
1.	No treatment — untreated controls	45	46th	10
2.	Olive oil (0.2 ml/animal/day) — vehicle controls	45	46th	10
3.	<i>Emblica officinalis</i> aq. Extract (2 mg/animal/day) — antidote controls	45	46th	10
4.	Low dose (LD) ochratoxin (50 µg/0.2 ml olive oil/animal/day)	45	46th	10
5.	High dose (HD) ochratoxin (100 µg/0.2 ml olive oil/animal/day)	45	46th	10
6.	Low dose ochratoxin (50 µg/0.2 ml olive oil/animal/day) + <i>Emblica officinalis</i> aq. Extract (2 mg/animal/day)	45	46th	10
7.	High dose ochratoxin (100 µg/0.2 ml olive oil/animal/day) + <i>Emblica officinalis</i> aq. Extract (2 mg/animal/day)	45	46th	10

respectively [24,25]. The homogenate for glutathione reductase (GRX) determination was prepared by homogenizing a known amount of tissue in 5 ml of 1% bovine serum albumin, and for glutathione transferase (GST) analysis, a 10% tissue homogenate was prepared in phosphate buffer. GRX and GST activities were assessed according to Mavis and Stellwagen (1968), and Habig et al. (1967), respectively [26,27]. All the samples were analyzed for protein content by the method of Lowry et al. (1951) [28]. For reduced glutathione (GSH) analysis, a known amount of tissue was homogenized in 3 ml of 3% metaphosphoric acid and 1 ml distilled water saturate with salt solution. For the total ascorbic acid (TAA), the homogenate was prepared in 10 ml Norit reagent. GSH and TAA concentrations were determined according to Grunert and Philips (1951) and Roe and Kuether (1943), respectively [29,30].

Statistical analysis

The results are expressed as means \pm SD. The data were analyzed statistically using one-way analysis of variance (ANOVA) followed by the Tukey test. The level of significance was adopted at $p \leq 0.05$. The P values were compared between different study groups

RESULTS

Table 1 presents the study protocol for the experiment. Table 2 shows the effect of ochratoxin and ochratoxin with *Emblica officinalis* aqueous extract on lipid peroxidation

and antioxidant defense mechanism in mouse kidney. No significant changes were observed between the different control groups (Groups 1, 2, 3). In mice receiving ochratoxin (Groups 4, 5), a significant dose-dependent increase in lipid peroxidation, detected by measuring renal TBARS concentration, was found (LD: 3.55 ± 0.37 ; HD: 4.54 ± 0.85), as compared to the findings for the vehicle controls (Group 2).

Table 2 also shows the effect of ochratoxin and ochratoxin with the aqueous extract of *Emblica officinalis* on the non-enzymatic and enzymatic antioxidants in mouse kidney. No significant difference was noted between the different control groups (Groups 1, 2, 3). Ochratoxin treatment caused a significant, dose-dependent decrease in renal concentrations of glutathione (LD: 34.32 ± 2.15 ; HD: 23.81 ± 1.16) and total ascorbic acid (LD: 2.53 ± 0.22 ; HD: 2.24 ± 0.22) (Groups 4, 5), as compared to the findings for the vehicle controls (Group 2). The activity of enzymatic antioxidants, like catalase (LD: 26.82 ± 0.72 ; HD: 20.50 ± 1.26), superoxide dismutase (LD: 2.79 ± 0.06 ; HD: 1.97 ± 0.09), glutathione peroxidase (LD: 0.25 ± 0.03 ; HD: 0.13 ± 0.03), glutathione reductase (LD: 2.10 ± 1.04 ; HD: 1.83 ± 0.15) and glutathione transferase (LD: 37.22 ± 0.98 ; HD: 26.68 ± 0.69) in the kidney were significantly lower in ochratoxin-treated mice than in the vehicle controls (Group 2).

The treatment with aqueous extract of *Emblica officinalis* in Group 3 animals did not bring about any significant changes in the above mentioned parameters. However, oral administration of ochratoxin and *Emblica officinalis*

Table 2. Effect of aqueous extract of *Embllica officinalis* on ochratoxin-induced changes in lipid peroxidation and antioxidant defense mechanism in mouse kidney

Parameter	Study groups						
	1	2	3	4	5	6	7
Lipid peroxidation TBARS (nmoles MDA/mg protein/60 min)	2.59±0.03	2.60±0.03	2.61±0.06	3.55±0.37 ^{abcde}	4.54±0.85 ^{abcde}	2.69±0.03 ^{deg}	2.93±0.03 ^{abcde}
Total ascorbic acid concentration (mg/gm tissue weight)	3.28±0.25	3.44±0.34	3.49±0.18	2.53±0.22 ^{bcdef}	2.24±0.22 ^{abcde}	3.37±0.25 ^{deg}	2.61±0.12 ^{abcde}
Glutathione concentration (µg/100 mg tissue weight)	64.94±1.29	66.07±0.82	63.40±1.10	34.32±2.15 ^{abcde}	23.81±1.16 ^{abcde}	65.49±1.45 ^{deg}	55.76±1.54 ^{abcde}
Catalase activity (µmoles H ₂ O ₂ consumed/ mg protein/min)	31.44±1.26	32.72±1.26	32.70±1.39	26.82±0.72 ^{abcde}	20.50±1.26 ^{abcde}	31.56±1.26 ^{de}	31.54±1.70 ^{de}
Superoxide dismutase activity (units/mg protein)	3.39±0.15	3.34±0.15	3.39±0.12	2.79±0.06 ^{abcde}	1.97±0.09 ^{bcdef}	3.26±0.06 ^{deg}	2.66±0.06 ^{abcde}
Glutathione peroxidase activity (µmoles NADPH consumed/ mg protein/min)	0.39±0.06	0.38±0.09	0.38±0.09	0.25±0.03 ^{abcde}	0.13±0.03 ^{abcde}	0.37±0.09 ^{deg}	0.32±0.12 ^{abcde}
Glutathione reductase activity (nmoles NADPH consumed/mg protein/min)	2.64±0.31	2.64±0.31	2.66±0.79	2.10±1.04 ^{abcde}	1.83±0.15 ^{abcde}	2.50±0.25 ^{de}	2.28±0.34 ^{abcde}
Glutathione transferase activity (µmoles of CDNB-GSH conjugate formed/ mg protein/min)	46.59±1.54	47.00±1.10	46.36±0.85	37.22±0.98 ^{abcde}	26.68±0.69 ^{abcde}	46.88±0.79 ^{deg}	40.86±1.42 ^{abcde}

Group 1: Untreated controls (UC); Group 2: Vehicle controls (VC); Group 3: Antidote controls (AC); Group 4: Low dose ochratoxin (LD); Group 5: High dose ochratoxin (HD); Group 6: Low dose+Antidote (LD+A); Group 7: High dose+Antidote (HD+A)

Values are means ±SD; n = 10.

^a As compared to group 1; p < 0.05.

^b As compared to group 2; p < 0.05.

^c As compared to group 3; p < 0.05.

^d As compared to group 4; p < 0.05.

^e As compared to group 5; p < 0.05.

^f As compared to group 6; p < 0.05.

^g As compared to group 7; p < 0.05.

Table 3. Effect of aqueous extract of *Embllica officinalis* on ochratoxin-induced changes in lipid peroxidation and antioxidant defense mechanism in mouse liver

Parameter	Study groups						
	1	2	3	4	5	6	7
Lipid peroxidation TBARS (nmoles MDA/mg protein/60 min)	3.54±0.06	3.54±0.03	3.51±0.03	4.45±0.18 ^{abcd} fg	6.22±0.15 ^{abcd} fg	3.50±0.02 ^{de}	3.96±0.12 ^{de}
Total ascorbic acid concentration (mg/gm tissue weight)	5.89±0.28	5.84±0.31	5.93±0.31	3.53±0.25 ^{abcde} fg	2.61±0.09 ^{abcd} fg	5.70±0.25 ^{deg}	4.92±0.47 ^{abcde} fg
Glutathione concentration (µg/100 mg tissue weight)	74.63±1.77	75.16±1.51	76.37±1.51	34.18±1.51 ^{abcde} fg	42.45±1.21 ^{abcd} fg	74.05±1.13 ^{deg}	57.58±1.23 ^{abcde} fg
Catalase activity (µmoles H ₂ O ₂ consumed/mg protein/min)	43.91±2.90	44.19±1.89	45.00±1.89	28.73±2.02 ^{abcde} fg	18.83±3.19 ^{abcd} fg	43.49±2.62 ^{de}	34.52±2.43 ^{ede}
Superoxide dismutase activity (units/mg protein)	4.03±0.25	3.98±0.31	3.93±0.31	3.46±0.22 ^{abcde} fg	2.44±0.09 ^{abcd} fg	3.85±0.09 ^{deg}	3.38±0.22 ^{def}
Glutathione peroxidase activity (µmoles NADPH consumed/mg protein/min)	0.41±0.006	0.41±0.009	0.40±0.009	0.27±0.003 ^{abcde} fg	0.15±0.006 ^{abcd} fg	0.40±0.01 ^{deg}	0.38±0.003 ^{abcde} fg
Glutathione reductase activity (nmoles NADPH consumed/mg protein/min)	3.13±0.37	3.07±0.41	3.15±0.41	2.43±0.31 ^{abcde} fg	1.69±0.0.31 ^{abcd} fg	2.97±0.56 ^{deg}	2.60±0.25 ^{def}
Glutathione transferase activity (µmoles of CDNB-GSH conjugate formed/mg protein/min)	51.82±0.98	51.28±0.98	52.68±1.51	41.33±0.79 ^{abcde} fg	24.26±1.92 ^{abcd} fg	49.28±1.32 ^{deg}	42.24±1.29 ^{abcde} fg

Abbreviations as in Table 2

caused a significant amelioration of the ochratoxin-induced lipid peroxidation in the kidney of mice receiving ochratoxin and *Emblica officinalis* (Groups 6, 7) than in the animals treated with ochratoxin alone (Groups 4, 5).

Table 3 shows the effect of ochratoxin and ochratoxin with *Emblica officinalis* on lipid peroxidation and antioxidant defense mechanism in mouse liver. The results revealed no significant alterations in lipid peroxidation between the different control groups (Groups 1, 2, 3). The level of lipid peroxidation was significantly higher in ochratoxin-treated mice than in the vehicle controls (Groups 4, 5). The effect was dose-dependent (LD: 4.45 ± 0.18 ; HD: 6.22 ± 0.15).

Table 3 also shows the effect of ochratoxin and ochratoxin with *Emblica officinalis* on total ascorbic acid and glutathione concentrations as well as on the activity of catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase and glutathione transferase in mouse liver. The results revealed no significant alterations between the different control groups (Groups 1, 2, 3).

Total ascorbic acid and glutathione concentrations were significantly lower in ochratoxin-treated mice than in the vehicle controls (Group 2). The effect was dose-dependent (total ascorbic acid — LD: 3.53 ± 0.25 ; HD: 2.61 ± 0.09 and glutathione — LD: 34.18 ± 1.51 ; HD: 22.45 ± 1.21). The 45-day treatment with ochratoxin produced a significant, dose-dependent reduction in the activity of catalase (LD: 28.73 ± 2.02 ; HD: 18.83 ± 3.19), superoxide dismutase (LD: 3.46 ± 0.22 ; HD: 2.44 ± 0.09), glutathione peroxidase (LD: 0.27 ± 0.003 ; HD: 0.15 ± 0.006), glutathione reductase (LD: 2.43 ± 0.31 ; HD: 1.69 ± 0.031) and glutathione transferase (LD: 41.33 ± 0.79 ; HD: 24.26 ± 1.92) in mouse liver, as compared to the findings for the vehicle controls (Group 2). Thus, ochratoxin treatment caused a significant increase in lipid peroxidation by decreasing the antioxidant defense mechanism of the cell.

The treatment with aqueous extract of *Emblica officinalis* alone (Group 3) did not have any significant effect on lipid peroxidation in mouse liver. However, oral administration of the aqueous extract of *Emblica officinalis* and ochratoxin caused a significant amelioration of the ochratoxin-induced lipid peroxidation in mouse liver (Groups 6, 7).

DISCUSSION

The results shown in the tables clearly indicate a significant increase in lipid peroxidation (LPO) and a decrease in the activity of enzymatic and non-enzymatic antioxidants in the kidney (Table 2) and liver (Table 3) in ochratoxin-treated mice as compared to the vehicle controls (Group 2). Lipid peroxidation and oxidative damage is regarded as one of the primary causes of cellular damage. It is postulated that ochratoxin increases cell permeability to Ca^{2+} and enhances cellular concentration of Ca^{2+} . Besides, in the presence of prooxidants, ochratoxin uncouples oxidative phosphorylation [31]. This results in an increased leakage of electrons from the respiratory chain, thus producing oxygen and hence H_2O_2 . An increase in lipid peroxidation accompanied by the leakage of Ca^{2+} from Ca^{2+} loaded microsomes would produce further damage to the endoplasmic membrane [32]. Increased malondialdehyde (MDA) production, indicating LPO increase, was reported earlier *in vitro* [33]. Petrik et al. (2003) demonstrated that a very low concentration of ochratoxin was sufficient to induce apoptosis and oxidative damage to kidney cells in Wistar rats [34].

The intracellular glutathione status appears to be a sensitive indicator of the cell's overall condition and its ability to counteract a toxic challenge [35]. Decreased glutathione concentration may be due to a continuous attack of free radicals. The ability of ochratoxin to react with reduced glutathione (GSH) was reported by Dai et al. [36]. Ascorbic acid is transformed to L-dehydroascorbate during free radical scavenging. Our results indicating a significant reduction in glutathione and ascorbic acid concentrations corroborate with the findings of Atroshi et al. [37]. The decrease in the activity of non-enzymatic antioxidants in the cell increases the susceptibility to injury due to peroxidation.

The levels of enzymatic antioxidants (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione transferase) were significantly lower in ochratoxin-treated mice than in the vehicle controls (Group 2). Superoxide radicals (O_2^-) have been reported in several pathological disorders. A decrease in

SOD activity contributes to increasing the level of superoxide radicals, thus leading to increased oxidative stress which enhances early cell death. Further decrease in catalase activity would increase H_2O_2 concentration in the cell, leading to increased lipid peroxidation and oxidative stress. Glutathione reductase reduces the oxidized form of glutathione (GSSG) to GSH. A decrease in the activity of glutathione reductase would further decrease the concentration of ascorbic acid. Glutathione peroxidase reduces H_2O_2 to H_2O by oxidizing reduced glutathione. A decrease in glutathione peroxidase would increase the concentration of H_2O_2 , thus signaling an increase in further oxidative stress. Active oxygen species increase the level of glutathione transferase that metabolizes the toxic products of lipid peroxidation. A decrease in glutathione transferase concentration would increase the activity of active oxygen species. Our finding of the decreased activity of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione transferase corroborates with that of earlier findings [38–39]. In the case of ochratoxicosis, a decrease in the activity of these enzymes could be due to a number of factors. First of all, the toxin decreases the rate of protein biosynthesis by competitively inhibiting phenylalanine tRNA synthetase, and it decreases the production of these enzymes. Secondly, the toxin produces reactive oxygen species (ROS) that directly or indirectly interact with proteins (enzymes), DNA and RNA, and alter their activity, thus affecting the overall production of enzymes. A decrease in the DNA, RNA and protein ratios would affect the transcription and translation processes, ultimately having impact on the synthesis of various enzymes [40]. The oxidative stress and its involvement in the mechanism of ochratoxin-induced toxicity and oxidation of proteins have been reported earlier [41].

Oral administration of *Emblica officinalis* aqueous extract at the time of ochratoxin exposure significantly decreased the extent of lipid peroxidation. This may have been due to the presence of radical scavengers showing antioxidant property that have a capacity to decrease the formation of peroxides, hydroxyl and superoxide radicals. Our findings are consistent with those of Khopde et al. [42]. Vitamin C present in the fresh fruit extract maintains the first natural

antioxidant defense activity and acts as a powerful inhibitor of lipid peroxidation [43].

Oral administration of *Emblica officinalis* aqueous extract and ochratoxin also produced a significant increase in glutathione and ascorbic acid concentrations in mouse liver and kidney. This observation implies that the extract used in the present experiment has a potential to diminish ochratoxin-induced toxicity in all these major body organs. Increased concentration of glutathione and ascorbic acid decreases the cell's vulnerability to oxidant attack. Sharma et al. reported that the *Emblica officinalis* extract was found to increase the level of reduced glutathione thus showing an antimutagenic activity in mice exposed *in vivo* to cyclophosphamide [44]. Anilakumar et al. noted that the aqueous extract of *Emblica officinalis* increased the level of hepatic ascorbic acid and glutathione in rats [45]. Oral administration of the aqueous extract of *Emblica officinalis* and ochratoxin caused a significant increase in the activity of superoxide-dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione transferase (Groups 6, 7) compared to the findings for mice treated with ochratoxin alone (Groups 4, 5, 7). The extract of the *Emblica officinalis* fruit, where pyrogallol was an active component, was found to inhibit proliferation of four human tumour cell lines *in vitro* [46].

Increased activity of catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase and glutathione transferase was reported in gamma-radiated mice treated with *Emblica officinalis* [47]. The aqueous extract of *Emblica officinalis* was found to modulate the increase in the activity of antioxidant enzymes in cyclophosphamide-treated animals [48]. Emblicanin A (37%) and Emblicanin B (33%) enriched fraction of fresh juice of the *Emblica* fruit was found to exert antioxidant effect on ischaemic reperfusion-induced (IRI) oxidative stress in rat heart [49]. Emblicanin is a type of antioxidant found in *Emblica officinalis*. Emblicanin is different from most other antioxidants in that it is a cascading antioxidant that is completely free of pro-oxidation. Emblicanin A (one of the key compounds of Emblicanin) aggressively seeks and attacks free radicals. After it neutralizes a free radical, Emblicanin A is transformed into Emblicanin B, another

antioxidant. Emblicanin B in turn attacks free radicals and is transformed into emblicanin oligomers. This makes Emblicanin one of the best free radical scavenging antioxidants. Oral administration of *Emblica officinalis* to mice treated with dimethylbenzyl anthracene caused a significant increase in the activity of hepatic antioxidants, mainly glutathione, glutathione peroxidase, glutathione transferase and glutathione reductase [50]. Vitamin C has been found to be an important antioxidant present in the extract. *Emblica officinalis* fruit normally contains ascorbic acid (0.4 % w/w), and vitamin C accounts approximately for 45–70% of the antioxidant activity [51].

ACKNOWLEDGEMENT

The authors are thankful to the Department of Zoology, Gujarat University, Ahmedabad, Gujarat, India, for making it possible to conduct the experimental work and for providing the necessary facilities when required.

REFERENCES

- Pitt JI, Hocking AD. *Fungi and Food Spoilage*. Weimer: Aspen Publishers; 1997.
- Kuiper-Goodman T, Scott P.M. *Risk assessment of the mycotoxin, ochratoxin A*. Biomed Environ Sci 1989;2:179–248.
- IARC. Ochratoxin A. In: *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*. Lyon: IARC Press; 1993. p. 489–521.
- Verma RJ, Mathew S. *Histopathological alterations in the liver and kidney of rabbits during experimental toxicosis*. Indian J Environ Toxicol 1997;7:20–3.
- Satyavati GV, Raina MK, Sharma M. *Medicinal Plants of India*, New Delhi: Indian Council of Medical Research; 1976. p. 337.
- Scartezzini P, Antognoni F, Raggi MA, Poli F, Sabbiani C. *Vitamin C content and antioxidant activity of the fruit and of the Ayurvedic preparations of Emblica officinalis Gaertn.* J Ethnopharmacol 2006;104:113–8.
- Vani T, Rajani M, Sarkar S, Shishoo CJ. *Antioxidant property of the Ayurvedic formulation Triphala and its constituents*. Inter J Pharmacol 1997;35:313–7.
- Rajak S, Banerjee SK, Sood S, Dinda AK, Gupta YK, Gupta SK, et al. *Emblica officinalis causes myocardial adaptation and protects against oxidative stress in ischemic-reperfusion injury in rats*. Phytother Res 2004;18:54–60.
- Rao TP, Sakaguchi N, Juneja LR, Wado E, Yokozaroa T. *Amla (Emblica officinalis Gaertn.) extracts reduced oxidative stress in streptozotocin-induced diabetic rats*. J Med Food 2005;8:362–8.
- El-Mekkawy S, Mesclhmym R, Kusumoto IT, Kadota S, Hattori M, Namba T. *Inhibitory effects of Egyptian folk medicines on human immunodeficiency viruses (HIV) reverse transcriptase*. Chem Pharm Bull 1995;43:641–8.
- Ghosal S, Tripathi VK, Chauhan S. *Active constituents of Emblica officinalis. Part I. The chemistry and antioxidative effects of two new hydrolysable tannins, emblicanin A and B*. Indian J Chem Sec B 1996;35:941–8.
- Sai Ram M, Neetu D, Deepti R, Vandana M, Ilovazhagan G, Kumar D, et al. *Cytoprotective activity of Amla (Emblica officinalis) against chromium (VI)-induced oxidative injury in murine macrophages*. Phytother Res 2003;17:430–3.
- El-Shayeb NM, Mabrouk SS, Abdel-Fattah AM. *Production of ochratoxin by some Egyptian Aspergillus strain*. Zentralbl-Mikrobiol 1992;147:86–9.
- Vorster LJ. *A method for the analysis of cereals and groundnuts for three mycotoxins*. Analyst 1969;94:136–42.
- Van der Merwe KJ, Steyn PS, Fourie LO. *Mycotoxin. 11. The constitution of ochratoxin A, B and C metabolites of Aspergillus ochraceus Wilhelm*. J Chem Soc 1965;5:7083–8.
- WHO. *Protocol CG-06. Extraction and fractionation for biological and phytochemical studies*. APJF/IP 1001, A. Geneva: World Health Organization; 1983.
- Association of Official Analytical Chemists. *Official Method of Analysis*, Washington DC: AOAC; 1970.
- Chang C, Yang M, Wen H. *Estimation of total flavonoid content in propolis by two complementary colorimetric methods*. J Food Drug Anal 2002;10:178–82.
- Chinoy JJ, Singh YD, Gurumurthi K. *Colorimetric determination of ascorbic acid turnover in plants*. Ind J Plant Physiol 1976;22:122–30.
- WHO. *Selected mycotoxins: Ochratoxins, trichothecenes, ergot: Environmental Health Criteria No 105*. Geneva: WHO; 1990. p. 263.

21. Rajeshkumar NV, Pillai M, Kuttan R. *Induction of apoptosis in mouse and human carcinoma cell lines by Emblica officinalis polyphenols and its effect on chemical carcinogenesis.* Indian J Exp Biol 2003;22:201–12.
22. Ohkawa H, Ohishi N, Yagi K. *Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction.* Anal Biochem 1979;95:351–8.
23. Kakkar P, Das B, Viswanathan PN. *A modified spectrophotometric assay of superoxide dismutase.* Indian J Biochem Biophys 1984;21:130–2.
24. Pagila DE, Valentine WN. *Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase.* J Lab Clin Med 1967;70:158–69.
25. Luck H. *A spectrophotometric method for the estimation of catalase.* In: Bergmeyer HU, editor. *Methods of Enzymatic Analysis.* New York: Academic Press; 1963. p. 886–7.
26. Mavis RD, Stellwagen E. *Purification and subunit structure of glutathione reductase from bakers' yeast.* J Biol Chem 1968;243:809–14.
27. Habig WH, Pabst MJ, Jakoby WB. *Glutathione S-transferases. The first enzymatic step in mercapturic acid formation.* J Biol Chem 1974;249:7130–9.
28. Lowry DH, Rosenberg NJ, Farr AI, Randell RJ. *Protein measurements with folin-phenol reagent.* J Biol Chem 1951;193:265–75.
29. Grunert RR, Philips PH. *A modification of the nitroprusside method of analysis for glutathione.* Arch Biochem 1951;30:217–25.
30. Roe JH, Kuether CA. *The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenyl hydrazine derivatives of dehydroascorbic acid.* J Biol Chem 1943;147:399–407.
31. Hoehler D, Marquardt RR, Mcintosh AR, Xiao H. *Free radical generation as induced by ochratoxin A and its analogs in bacteria (Bacillus brevis).* J Biol Chem 1996;271:27388–94.
32. Verma RJ, Mathew S. *Intracellular calcium accumulation during ochratoxicosis.* Med Sci Res 1998;26:95–6.
33. Petrik J, Zanic-Grubisic T, Barisic K, Pepezynjak S, Radic B, Ferencic Z, et al. *Apoptosis and oxidative stress induced by ochratoxin A in rat kidney.* Arch Toxicol 2003;77:685–93.
34. Baudrimont I, Betbeder A, Creepy EE. *Reduction of the ochratoxin A induced cytotoxicity in Vero cells by aspartame.* Arch Toxicol 1997;71:290–8.
35. Kosomer NS, Kosomer EM. *The glutathione status of cells.* Int Review Cytology 1978;54:109–56.
36. Dai J, Park G, Wright MW, Adam M, Akman SA, Mandeville RA. *Detection and characterization of glutathione conjugate of ochratoxin A.* Chem Res Toxicol 2002;15:1581–8.
37. Atroshi F, Biese I, Saloniemi H, Alivehmas T, Saari S, Rizzo A, et al. *Significance of apoptosis and its relationship to antioxidants after ochratoxin A administration in mice.* J Pharm Sci 2000;3:281–91.
38. Meki AR, Hussein AA. *Melatonin reduces oxidative stress induced by ochratoxin A in rat liver and kidney.* Comp Biochem Physiol Toxicol Pharmacol 2001;130:305–15.
39. Soyoz M, Ozcelik N, Kilinc I, Altantas I. *The effects of ochratoxin A on lipid peroxidation and antioxidant enzymes: a protective role of melatonin.* Cell Biol Toxicol 2004;20:213–19.
40. Verma RJ, Chakraborty D. *Alterations in DNA, RNA and protein contents in the liver and kidney of mice treated with ochratoxin and their amelioration by Emblica officinalis extract.* Acta Pol Pharm 2008;65:3–9.
41. Verma RJ, Chakraborty D. *Emblica officinalis aqueous extract ameliorates ochratoxin-induced lipid peroxidation in the testis of mice.* Acta Pol Pharm 2008;65:187–94.
42. Khopde SM, Priyadaarshini IK, Mohan H, Satav JG, Gowandi VB, Yakhmi JV, et al. *Characterizing the antioxidant activity of amla (Phyllanthus emblica) extract.* Curr Sci 2001;81:185–90.
43. Sultana S, Ahmad S, Khan N, Jahangir T. *Effects of Emblica officinalis (Gaertn) on CCl₄-induced hepatic toxicity and DNA synthesis of Wistar rats.* Indian J Exp Biol 2005;43:430–6.
44. Haque R, Bin-Hafeez B, Ahmad I, Parvez S, Pandey S, Raisuddin S. *Protective effects of Emblica officinalis Gaertn. in cyclophosphamide-treated mice.* Human Exp Toxicol 2001;24:643–50.
45. Anilakumar KR, Nagaraj NS, Santhanam K. *Protective effects of amla on oxidative stress and toxicity in rats challenged with dimethylhydrazine.* Nutrition Res 2004;24:313–9.
46. Khan MTH. *Identification of pyrogallol as an antiproliferative compound present in extracts from the medicinal plant Emblica officinalis: Effects on in vitro cell growth of human tumor cell lines.* Int J Oncol 2002;21:187–92.
47. Hari Kumar KB, Sabu MC, Lima PS, Kuttan R. *Modulation of haematopoietic system and antioxidant enzyme by Emblica*

- officinalis Gaertn and its protective role against gamma radiation-induced damages in mice.* J Radiat Res 2004;45:549–55.
48. Sharma N, Trikha P, Athar M, Raisuddin S. *Inhibitory effect of Emblica officinalis on the in vivo clastogenicity of benzo (α) pyrene and cyclophosphamide in mice.* Human Exp Biol 2000;19:377–84.
49. Bhattacharya SK, Bhattacharya A, Sairam K, Ghosal S. *Effect of bioactive tannoid principles of Emblica officinalis on ischemia reperfusion-induced oxidative stress in rat heart.* Phytomed 2002;9:171–4.
50. Banu SM, Selvendiran K, Singh PV, Sakthisekheran D. *Protective effect of Emblica officinalis ethanolic extract against 7,12-dimethyl benzyl (α) anthracene (DMBA) induced genotoxicity in Swiss albino mice.* Hum Exp Toxicol 2004;23:527–31.
51. Scartezzini P, Antognoni F, Raggi MA, Poli F, Sabbiani C. *Vitamin C content and antioxidant activity of the fruit and of the Ayurvedic preparations of Emblica officinalis Gaertn.* J Ethnopharmacol 2006;104:113–8.