
DRUG BIOCHEMISTRY

ALTERATIONS IN DNA, RNA AND PROTEIN CONTENTS IN LIVER AND KIDNEY OF MICE TREATED WITH OCHRATOXIN AND THEIR AMELIORATION BY *EMBLICA OFFICINALIS* AQUEOUS EXTRACT

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Abstract: The present investigation was an attempt to study ameliorative effect of *Embllica officinalis* aqueous extract on ochratoxin-induced toxicity on DNA, RNA and protein contents in liver and kidney of mice. Adult male albino mice were orally administered with 50 and 100 µg of ochratoxin/0.2 mL of olive oil/animal/day for 45 days. The results revealed significant ($p < 0.05$), dose-dependent decrease in the concentrations of DNA, RNA as well as total, acidic, basic and neutral protein contents in the liver and kidney of ochratoxin-treated mice. Administration of *Embllica officinalis* aqueous extract (2 mg/animal/day) for 45 days along with ochratoxin caused significant amelioration in the ochratoxin-induced reduction in DNA, RNA and protein contents in the liver and kidney of mice.

Keywords: DNA, *Embllica officinalis*, kidney, liver, ochratoxin, protein, RNA.

Mycotoxins are well known for causing variety of effects ranging from immediate toxic response to potential long-term carcinogenic and teratogenic effects in humans and other animals. Mycotoxins are produced in cereals either during pre- or post-harvest conditions by the growth of toxigenic fungi (1). The most significant mycotoxins are contaminants of agricultural commodities especially food and feed stuffs.

Among all mycotoxins identified so far, ochratoxins have received considerable attention because of its toxicity on kidneys along with other organs (2). Ochratoxins are secondary toxic fungal metabolites produced by *Aspergillus ochraceus* and *Penicillium verrucosum* (3). Consumption of ochratoxin contaminated food/feed stuffs cause ochratoxicosis in both human beings and animals (4). Ochratoxins were found to be a putative cause of Balkan endemic nephropathy (BEN). Also ochratoxin A 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 (5). Inhibition of protein synthesis, DNA and RNA damage are the major mechanisms involved in ochratoxin-induced cellular toxicity (6). Oxidative stress also play important role in ochratoxin-induced toxicity and carcinogenicity (7).

Embllica officinalis commonly known as amla belongs to family *Euphorbiaceae*, normally found in tropical countries such as India, Pakistan, Bangladesh, China, Sri Lanka and Malayan peninsula. *Embllica officinalis* fruit contains an array of bioactive compounds showing antioxidative property and is widely used in India as a traditional medicine (8), ayurvedic herbal formulation and also in Unani medicines.

Embllica officinalis is highly nutritious and is an important dietary source of vitamin C, minerals and amino acids. The fruit contains a range of polyphenols especially tannins and other phenolic compounds. They show effective result against oxidative damage along with cytoprotective activity (9). The present study was undertaken to evaluate the possible ameliorative effect of *Embllica officinalis* aqueous extract on ochratoxin-induced alterations in DNA, RNA as well as total, acidic, basic and neutral protein contents in liver and kidney of mice.

MATERIALS AND METHODS**Production and analysis of ochratoxin**

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

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medium. It was grown on yeast extract sucrose (YES) liquid medium at $28 \pm 2^\circ\text{C}$ for 10 days (10). Thereafter, the inoculated flasks were autoclaved and the content was filtered through Whatman filter paper No. 41. The culture filtrates 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 sulfate (Na_2SO_4). The chloroform extract was evaporated to dryness, transferred and stored in vials for qualitative and quantitative analysis of ochratoxin.

The quantification of the toxin was done by spotting 50 μL sample of ochratoxin on activated TLC coated with silica gel G (Hi-Media Laboratories Pvt. Ltd., Mumbai, India), developed in a solvent system consisting of toluene: ethyl acetate: formic acid (50:40:10 v/v/v) and the plates were observed under long-wave UV light (11). Chemical confirmation of ochratoxin was done by spraying a developed chromatoplate with ammonia solution (12). Each spot was scraped separately and dissolved in methanol and subjected to spectrophotometric measurement at 332 nm using UV Spectrothermo Spectronic UV-1 spectrophotometer (Thermo Spectronic, England) (13).

Preparation of plant extract

The ripe fruits of *Emblica officinalis* were obtained from local market and confirmed with the help of Botany Department, School of Sciences, Gujarat University, Ahmedabad, India. The extract was prepared according to WHO protocol CG-06 (14). Shade dried pulp was ground with mortar and pestle. 5 g of powder was suspended in 100 mL of distilled water and mixed repeatedly for 3 h at 40°C . After cooling, the content was filtered successively through ordinary and then through Whatman filter paper No.1, respectively. The supernatant was dried and stored in dark bottles in the cold condition at 4°C . During experiment, the known amount of dried extract was dissolved in water and used.

Phytochemical analysis

The qualitative analysis of extract for tannins and flavonoids was done by the method described by Daniel (15). To 2 mL of ethanolic extract, 10% ethanolic ferric chloride solution was added. Dark blue or greenish gray coloration indicated the presence of tannins in the *Emblica officinalis* extracts. For flavonoids, to 2 mL of ethanolic extract, a piece of magnesium ribbon and 1 mL of concentrated hydrochloric acid was added. Pink red colorations of the solution indicated the presence of flavonoids in the *Emblica officinalis* extracts.

The quantitative estimation for tannins, flavonoids and vitamin C were also done. The tannin contents in the crude extract of *Emblica officinalis* was determined by oxidation-reduction titration using indigo-caramine reagent (AOAC Guidelines) (16). The known amount of dry extract was boiled in distilled water and filtered. The filtrate was treated with indigo-caramine reagent. The solution was later on titrated with 0.1 M potassium permanganate solution. The end point was the appearance of yellowish green color.

The flavonoid content was determined by the method of Chang et al. (17). The plant extract in methanol was separately mixed 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 Chinoy et al. (18). The blue dye, dichlorophenol indophenol, is reduced to the colorless form on addition of ascorbic acid. The buffered HPO_3 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.

Experimental animals

Young adult inbred Swiss strain male albino mice (*Mus musculus*) weighing approximately 30-33 g were obtained from Zydus Research Centre, Ahmedabad, India. The animals were provided with animal feed and water *ad libitum* and maintained in 12 h light/dark cycles at $26 \pm 2^\circ\text{C}$. Animal feed was prepared as per the formulation given by the National Institute of Occupational Health, Ahmedabad, India and was confirmed to be free of mycotoxins. Guidelines for care and use of animals in scientific research, 1991 published by Indian National Science Academy, New Delhi, India, were followed. Permission for breeding and experiments on animals (reg. No. 167/1999/CPCSEA) was obtained from Government of India, Ministry of Social Justice and Environment, New Delhi, India.

Experimental design

Seventy animals were divided into seven groups and caged separately. Group-1 (untreated control) animals 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 pretreatment controls. Animals of groups 4 and 5 were orally administered with

ochratoxin 50 and 100 µg in 0.2 mL olive oil/animal/day (1.5 and 3.0 mg/kg body weight/day) for 45 days. Groups 6 and 7 animals were orally treated with ochratoxin as mentioned for groups 4 and 5 along with aqueous extract of *Emblica officinalis* (2 mg/animal/day) for 45 days.

Experimental feed

Olive oil was obtained from Figaro, Madrid, Spain. Ochratoxin was dissolved in olive oil, hence it was used as a vehicle in group 2. The dose of ochratoxin was based on the report of WHO working group (19). The dose of *Emblica officinalis* aqueous extract was based on earlier studies (20). All the treatments were given orally using a feeding tube attached to a hypodermic syringe.

Biochemical investigations

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

The estimation of DNA in the liver and kidney of control and all treated groups of animals were carried out by the method of Giles and Meyer (21). DNA in the supernatant reacts with diphenylamine to give a blue colored complex whose optical density is measured colorimetrically at 620 nm. The estimation of RNA in the liver and kidney of control and all treated groups of animals were carried out by the method of Mejboum (22). RNA in the supernatant reacts with orcinol reagent to give greenish color, which is measured colorimetrically at 670 nm. The acidic, basic and neutral proteins of liver and kidney of control and all the treated groups of mice were extracted separately by the method of Sharma and Malhotra (23). The known amounts of tissue samples were homogenized in 10% trichloroacetic acid (TCA) to remove nucleic acids. The tubes were centrifuged after incubation at 70°C for 20 min. The supernatant was discarded; to the residue 0.2 M HCl was added and incubated at 90°C for 30 min. After centrifugation, the supernatant was collected for acid soluble basic proteins (I). To the residue, 0.1 M NaOH was added and kept overnight at room temperature. Then the tubes were centrifuged and the supernatant was collected for base soluble acidic proteins (II). To the residue, distilled water was added and kept for 4 h. After centrifugation the supernatant was collected for neutral protein (III). The different protein fractions were estimated by the method of Lowry et al. (24) using bovine serum albumin as a standard.

The results were expressed as the means ± S.E.M. Percent changes between vehicle control and low dose and high dose (Groups 4, 5) ochratoxin-treated mice were calculated. In addition, percent changes between group 4 (low dose ochratoxin alone treated) and group 6 (low dose ochratoxin plus *Emblica officinalis*) as well as changes between group 5 (high dose ochratoxin alone treated) and group 7 (high dose ochratoxin plus *Emblica officinalis*) were also calculated by one way analysis of variance (ANOVA) followed by Tukey test. The level of significance was accepted at $p < 0.05$. The comparisons of p values between different groups were performed.

RESULTS

The results shown in Tables 1 and 2 revealed the effect of ochratoxin and ochratoxin plus *Emblica officinalis* aqueous extract treatment on DNA, RNA and protein contents in liver and kidney of mice. No significant difference in DNA, RNA, total, acidic, basic and neutral protein contents were observed between different control groups (Groups 1, 2, 3) in liver and kidney.

DNA and RNA contents were significantly decreased, as compared to vehicle control (Group 2), in the liver of ochratoxin-treated mice. The effect was dose-dependent (DNA : LD : -12.65%; HD : -51.68% and RNA : LD : -23.80%; HD : -49.74%) (Groups 4, 5). Oral administration of ochratoxin for 45 days caused, as compared with vehicle control (Group 2), significant, dose-dependent reduction in total (LD : -20.95%; HD : -50.66%), acidic (LD : -21.31%; HD : -47.59%), basic (LD : -13.43%; HD : -53.70%) and neutral protein (LD : -16.81%; HD : -55.26%) contents in liver of mice.

Ochratoxin treatment for 45 days caused, as compared with vehicle control (Group 2), significant, dose-dependent decrease in DNA (LD : -33.35%; HD : -62.06%) and RNA (LD : -60.19%; HD : -76.29%) contents in the kidney of mice (Groups 4, 5). A reduction in the RNA content was more than that of DNA in the kidney of ochratoxin-treated mice. Oral administration of ochratoxin for 45 days also caused significant, dose-dependent reductions in total (LD : -33.50%; HD : -63.95%), acidic (LD : -36.73%; HD : -67.70%), basic (LD : -23.51%; HD : -53.21%) and neutral protein (LD : -37.71%; HD : -67.88%) contents in kidney of mice (Groups 4, 5) as compared to vehicle control (Group 2).

Administration of aqueous extract of *Emblica officinalis* alone (Group 3) did not have any significant effects on DNA, RNA and protein contents in

Table 1. Effect of aqueous extract of *Embilica officinalis* on ochratoxin-in-induced changes in the DNA, RNA and different protein contents in the liver of mice.

Parameters	Experimental groups						
	1	2	3	4	5	6	7
DNA (μ moles/100 mg tissue weight)	575.11 \pm 5.02	574.92 \pm 4.39	572.20 \pm 3.64	502.20 \pm 3.64 ^{abceef}	277.84 \pm 6.41 ^{abdefg}	539.58 \pm 8.65 ^{deg}	496.38 \pm 4.08 ^{abceef}
RNA (μ moles/100 mg tissue weight)	660.70 \pm 7.52	662.78 \pm 6.23	663.85 \pm 5.02	505.07 \pm 6.04 ^{ef}	333.15 \pm 7.61 ^{abdefg}	640.00 \pm 2.31 ^{deg}	539.41 \pm 6.14 ^{abceef}
Total protein (mg/100 mg tissue weight)	23.84 \pm 0.79	23.88 \pm 0.95	23.60 \pm 0.98	19.24 \pm 0.57 ^{abceef}	11.63 \pm 0.44 ^{abdefg}	22.36 \pm 0.38 ^{de}	19.55 \pm 0.86 ^{abce}
Acidic protein (mg/100 mg tissue weight)	13.00 \pm 0.47	12.86 \pm 0.67	12.94 \pm 0.54	10.12 \pm 0.44 ^{abceef}	6.74 \pm 0.26 ^{abdefg}	12.07 \pm 0.16 ^{de}	10.26 \pm 0.72 ^{de}
Basic protein (mg/100 mg tissue weight)	5.99 \pm 0.25	5.96 \pm 0.36	5.99 \pm 0.24	5.16 \pm 0.18 ^{abce}	2.76 \pm 0.25 ^{abdefg}	5.69 \pm 0.25 ^{eg}	4.95 \pm 0.20 ^{abceef}
Neutral protein (mg/100 mg tissue weight)	4.85 \pm 0.27	4.76 \pm 0.28	4.67 \pm 0.25	3.96 \pm 0.07 ^{abceef}	2.13 \pm 0.05 ^{abdefg}	4.60 \pm 0.20 ^{deg}	4.34 \pm 0.20 ^{def}
DNA: RNA	0.87	0.86	0.86	0.99	0.83	0.84	0.92
DNA: protein	24.12	24.38	24.24	26.10	23.88	24.13	25.39
RNA: protein	27.71	28.10	28.12	26.25	28.64	28.62	27.59

Group 1: Untreated control (UC); Group 2: Vehicle control (VC); Group 3: Antidote control (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 the means \pm S.E.M.; n=10^aAs compared to group 1; p < 0.05^bAs compared to group 3; p < 0.05^cAs compared to group 4; p < 0.05^dAs compared to group 5; p < 0.05^eAs compared to group 7; p < 0.05^fAs compared to group 6; p < 0.05

Table 2. Effect of aqueous extract of *Embelica officinalis* on ochratoxin-in-induced changes in the DNA, RNA and different protein contents in the kidney of mice.

Parameters	Experimental groups						
	1	2	3	4	5	6	7
DNA (μ moles/100 mg tissue weight)	443.46 \pm 5.11	445.28 \pm 6.22	442.10 \pm 6.13	297.25 \pm 6.70 ^{abcdfg}	169.20 \pm 7.29 ^{abcdhfg}	407.14 \pm 8.01 ^{defg}	368.49 \pm 6.09 ^{abcdeh}
RNA (μ moles/100 mg tissue weight)	599.62 \pm 6.15	598.95 \pm 5.48	596.94 \pm 7.20	238.48 \pm 4.63 ^{abcdfg}	142.03 \pm 6.32 ^{abcdhfg}	538.70 \pm 6.09 ^{de}	491.12 \pm 6.90 ^{abcde}
Total protein (mg/100 mg tissue weight)	18.17 \pm 0.95	17.09 \pm 0.96	18.07 \pm 0.30	11.96 \pm 0.37 ^{abcdfg}	6.48 \pm 0.62 ^{abcdhfg}	17.00 \pm 0.31 ^{defg}	14.89 \pm 0.27 ^{def}
Acidic protein (mg/100 mg tissue weight)	9.88 \pm 0.41	9.72 \pm 0.48	9.74 \pm 0.20	6.15 \pm 0.27 ^{abcdfg}	3.14 \pm 0.39 ^{abcdhfg}	9.08 \pm 0.24 ^{de}	8.04 \pm 0.24 ^{de}
Basic protein (mg/100 mg tissue weight)	4.72 \pm 0.40	4.68 \pm 0.32	4.68 \pm 0.26	3.58 \pm 0.23 ^{abcdf}	2.19 \pm 0.35 ^{abcdhfg}	4.48 \pm 0.16 ^{de}	3.94 \pm 0.20 ^{abcde}
Neutral protein (mg/100 mg tissue weight)	3.57 \pm 0.31	3.58 \pm 0.28	3.65 \pm 0.17	2.23 \pm 0.19 ^{abcdfg}	1.15 \pm 1.01 ^{abcdhfg}	3.44 \pm 0.24 ^{de}	2.91 \pm 0.27 ^{abcde}
DNA: RNA	0.73	0.74	0.74	1.24	1.19	0.75	0.75
DNA: protein	24.40	24.87	24.46	24.85	26.11	23.94	24.74
RNA: protein	33.00	33.46	33.03	19.93	21.91	31.68	32.98

Group 1: Untreated control (UC); Group 2: Vehicle control (VC); Group 3: Antidote control (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 \pm S.E.M.; n=10^aAs compared to group 1: p < 0.05^bAs compared to group 2: p < 0.05^cAs compared to group 3: p < 0.05^dAs compared to group 4: p < 0.05^eAs compared to group 5: p < 0.05^fAs compared to group 6: p < 0.05^gAs compared to group 7: p < 0.05

liver and kidney of mice. However, treatment with aqueous extract of *Emblica officinalis* along with ochratoxin caused significant amelioration in DNA, RNA as well as different protein contents (Groups 6, 7), as compared to ochratoxin alone treated mice (Groups 4, 5), in both the organs. Amelioration was higher in the high dose ochratoxin plus extract-treated mice than that of respective low dose in all the above mentioned parameters.

Alterations in DNA : RNA, RNA : protein as well as DNA : protein ratios indicate changes in transcription and translation process in the liver and kidney of mice.

DISCUSSION

The present study clearly indicates that oral administration of ochratoxin for 45 days caused significant reduction in DNA and RNA contents in the liver and kidney of mice (Tables 1 and 2). It could be due to formation of DNA adducts (25). Many authors have reported the presence of high levels of DNA adducts in kidneys of male and liver of female mice (26, 27). Grosse et al. have reported that the amount of total DNA adduct level was increased by increasing ochratoxin concentration (28). Ochratoxin was also found to lower total mRNA concentration (29).

Our results also showed that *Emblica officinalis* aqueous extract treatment along with ochratoxin significantly mitigates ochratoxin-induced changes in DNA and RNA contents in the liver and kidney of mice. This might be due to an ability of *Emblica officinalis* aqueous extract to decrease adduct formation. The *Emblica officinalis* extract was found to inhibit DNA adduct formation induced by benzo- α -pyrene and aflatoxin B₁ (AFB₁) (30).

The ochratoxin treatment for 45 days caused significant dose-dependent reductions in total, acidic, basic and neutral protein contents in the liver and kidney of mice. A decrease in protein contents in various organs of different animals have been reported by various authors (31). Ochratoxin is known to act primarily on protein synthesis and inhibits the transcription process. The toxin shows competitive inhibition with phenylalanine and inhibits phenylalanine tRNA synthetase. Here aminoacylation and peptide synthesis is mainly inhibited and this is considered to be the primary factor responsible for the decline in the amount of protein (29). Ochratoxin impairs protein biosynthesis by forming adducts with DNA and RNA (26). The toxin was found to form adducts with protein and the association of ochratoxin with plasma protein is considered to be an impor-

tant mechanism of ochratoxin toxicity (32). The major protein present in the nucleus of cells of the major organs is histone protein, forming an essential constituent of chromatin. Histone plays an important role in the regulation of genetic expression. One important feature of histones is their high content of basic amino acids. A decrease in basic amino acid content in all these organs reflects a decrease in histone protein in them. Reactive oxygen species-induced lipid peroxidation in case of ochratoxicosis has been reported to disrupt the structural integrity of the protein and hence destroy them (33). This also involves inactivation of critical cellular enzymes, which are involved in protein synthesis.

Changes in DNA : RNA, RNA : protein and DNA : protein ratios indicate alterations in transcription and translation processes. A decrease in transcription and translation processes will affect overall concentration of structural and functional proteins (enzymes).

Oral administration of aqueous extract of *Emblica officinalis* along with ochratoxin (Groups 4, 5) for 45 days, caused, as compared with ochratoxin alone treated groups, a significant increase in acidic, basic, neutral and total protein contents in the liver and kidney of mice. This is mainly because of the presence of various antioxidants like vitamin C, flavonoids, tannoids and other polyphenols, which mainly inhibit lipid peroxidation in different organs. A decrease in reactive oxygen species (ROS) production will reduce the protein deprivation in all the animals (34). Another possible cause might be an ability of the extract to create nitrogen balance.

It is thus concluded that oral administration of *Emblica officinalis* aqueous extract along with ochratoxin significantly ameliorates ochratoxin-induced reduction in RNA, DNA and protein in the liver and kidney of mice.

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