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EMBLICA OFFICINALIS AQUEOUS EXTRACT AMELIORATES OCHRATOXIN-INDUCED LIPID PEROXIDATION IN THE TESTIS OF MICE

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Abstract: The present study was undertaken to assess the ameliorative effect of *Emblica officinalis* aqueous extract on ochratoxin-induced lipid peroxidation in the testis of mice. Adult male albino mice were orally administered with 50 and 100 µg of ochratoxin (Groups 4, 5) in 0.2 mL olive oil/animal/day for 45 days. The results revealed a significant increase in LPO (lipid peroxidation) in the testis of mice treated with ochratoxin compared to that of vehicle control (Group 2). The levels of non-enzymatic antioxidants: GSH (glutathione) and TAA (total ascorbic acid) as well as enzymatic antioxidants: SOD (superoxide dismutase), CAT (catalase), GPX (glutathione peroxidase), GRX (glutathione reductase) and GST (glutathione transferase) were significantly decreased in the testis of ochratoxin-treated mice. Oral administration of *Emblica officinalis* aqueous extract (2 mg/animal/day) along with ochratoxin (Groups 6, 7) for 45 days, caused, significant, amelioration in ochratoxin-induced LPO by increasing the contents of non-enzymatic (GSH and TAA) and activities of enzymatic (SOD, CAT, GPX, GRX and GST) antioxidants in the testis of mice as compared with those given ochratoxin alone animals (Groups 4, 5). Thus, oral administration of *Emblica officinalis* aqueous extract along with ochratoxin significantly ameliorates ochratoxin-induced lipid peroxidation in the testis of mice.

Keywords: *Emblica officinalis*, LPO (lipid peroxidation), ochratoxin, testis.

Ochratoxins are secondary toxic fungal metabolites produced by *Aspergillus ochraceus* (1). The presence of ochratoxins in staple foods, beverages, milk and milk products have been reported from various parts of the world (2). Experimental studies have shown that ochratoxins are nephrotoxic, hepatotoxic, carcinogenic, teratogenic, cytotoxic and immunotoxic in nature (3). Male reproductive health has been deteriorated in many countries during the last few decades. A number of toxins in environment have been suspected to affect reproductive system in male, and ochratoxin is one of them.

Ochratoxin A is a reproductive toxicant and prolong intake of it may bring abnormalities in the morphology of sperm cells (4). The presence of ochratoxin-DNA adducts in the testis of rats indicates spermatotoxic effect of ochratoxin (5). LPO and oxidative stress are also believed to play an important role in ochratoxin-induced toxicity and carcinogenicity (6). Oxidative stress can cause mutagenecity, cytotoxicity and stimulate changes in gene expression in the development of cancer (7).

Emblica officinalis normally known as amla, has been used extensively in ancient Indian Ayurveda. It is a member of small genus *Emblica* (family Euphorbiacae) which is commonly found in India and South-East Asian countries (8). The fruit contains an array of bioactive components mainly quercetin, phyllambic compound, gallic acid, tannins, flavonoids, pectin and vitamin C (9). These are the major group of phenolics exhibiting marked pharmacological activity (10). Several studies have shown that *Emblica officinalis* possesses antioxidative activity. This may be because of the presence of high concentration of flavonoids, tannoids, and vitamin C which show effective results against oxidative damage (11). *Emblica officinalis* extract also have been shown to possess powerful antidiabetic, lipid-lowering, antisclerotic, hepatoprotective and anticancer activities (12).

The present study was undertaken to evaluate the possible ameliorative effect of *Emblica officinalis* aqueous extract on ochratoxin-induced lipid peroxidation and its antioxidative defense mechanism in the testis of mice.

MATERIALS AND METHODS

Production and analysis of ochratoxin, preparation of plant extract, characterization of experi-

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mental animals and their division (7 groups) and experimental protocol were the same as described earlier (13).

Biochemical investigations

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

The 10% homogenate for LPO was prepared by homogenizing known amount of tissue in phosphate buffered saline (PBS). The LPO was estimated in the testis of control and all the treated groups of mice by the method of Ohkawa et al. by quantifying thiobarbituric acid reactive substances (TBARS) (14). The homogenate for SOD was prepared by homogenization of known amount of tissue in cold normal saline. The activity of SOD in the testis of control and all the treated groups of mice was measured by the modified spectrophotometric method of Kakkar et al. (15). For the preparation of GPX and CAT homogenate, known amount of tissue was homogenized in 0.1% chilled digitonin. The activities of GPX and CAT in the testis of control and all the treated groups of mice were assayed by the modified method of Pagila and Valentine (16) and Luck (17), respectively. The homogenate for GRX was prepared by homogenization of known amount of tissue in 5 mL of 1% bovine serum albumin and for GST 10% of tissue homogenate was prepared in phosphate buffer, respectively. The activities of GRX and GST in the testis of control and all the treated groups of mice were assayed by the method of Mavis and Stellwagen (18) and Habig et al. (19), respectively. For GSH, known amount of tissue was homogenized in 3 mL of 3% metaphosphoric acid and 1 mL of distilled water saturated with salt solution. For the TAA, homogenate was prepared in 10 mL of Norit reagent. The concentration of GSH and TAA were estimated in the testis of control and all the treated groups of mice by the method of Grunert and Philips (20) and Roe and Kuether (21), respectively. All the samples were analyzed for protein content by the method of Lowry et al. (22).

The results were expressed as the means ± S.E.M. Percent changes between vehicle control and low dose or high dose (Groups 4, 5) ochratoxin-treated mice were calculated. Additionally, 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

Table 1. Experimental protocol.

Experimental groups	Treatments	Days of treatment	Day of autopsy	No. of animals
1.	Untreated control	45	46th	10
2.	Vehicle control-olive oil (0.2 mL/animal/day)	45	46th	10
3.	Antidote control-*Emblica officinalis* aq. extr. (2 mg /animal/day)	45	46th	10
4.	Low dose ochratoxin (50 µg/0.2 mL olive oil/animal/day) treated	45	46th	10
5.	High dose ochratoxin (100 µg/0.2 mL olive oil/animal/day) treated	45	46th	10
6.	Low dose ochratoxin (50 µg/0.2 mL olive oil/animal/day) + *Emblica officinalis* aq. extract (2 mg/animal/day) treated	45	46th	10
7.	High dose ochratoxin (100 µg/0.2 mL olive oil/animal/day) + *Emblica officinalis* aq. extract (2 mg/animal/day) treated	45	46th	10

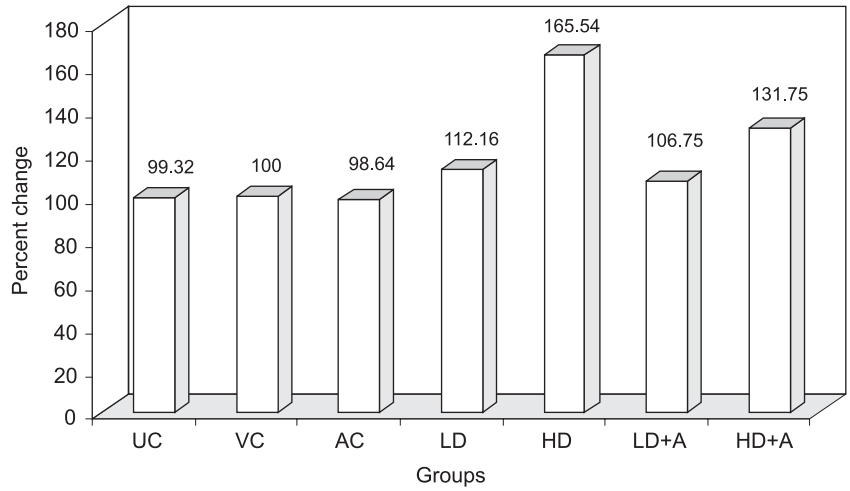

Figure 1. Percent change in lipid peroxidation from vehicle control in testis.
In figures 1–7: UC – Untreated control; VC – Vehicle control; AC – Antidote control; LD – Low dose; HD – High dose; LD + A – Low dose + Antidote; HD + A – High dose + Antidote

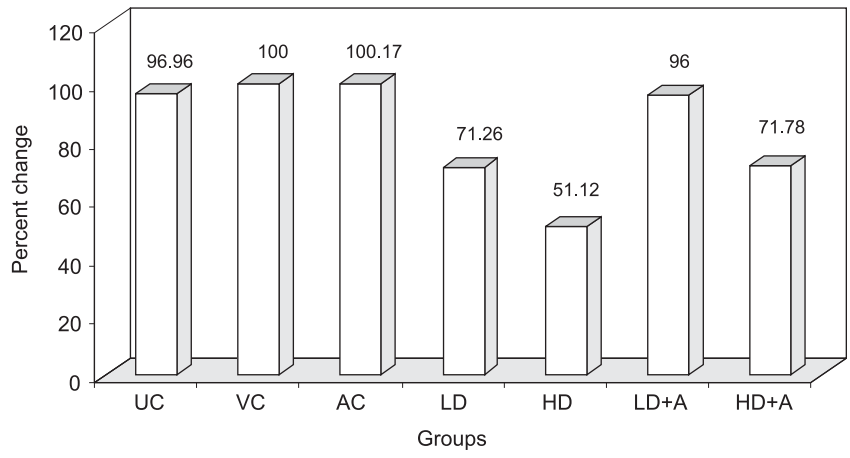

Figure 2. Percent change in the activity of catalase from vehicle control in testis.

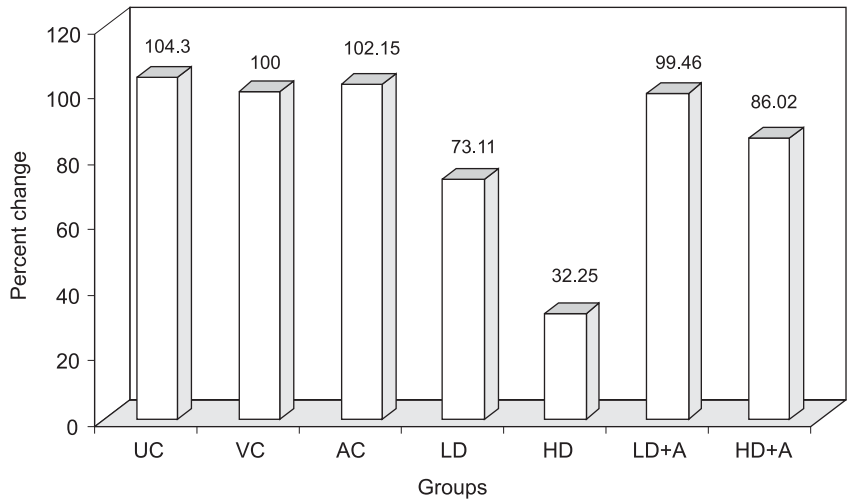

Figure 3. Percent change in the activity of superoxide dismutase from vehicle control in testis.

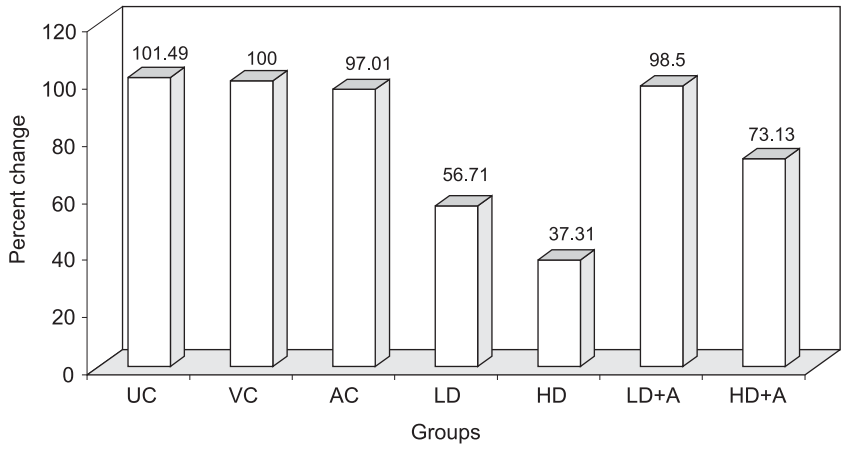

Figure 4. Percent change in the activity of glutathione peroxidase from vehicle control in testis.

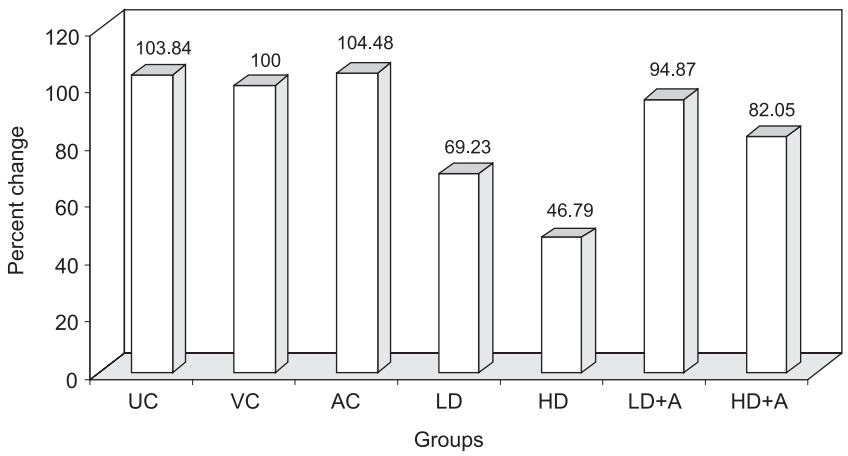

Figure 5. Percent change in the activity of glutathione peroxidase from vehicle control in testis.

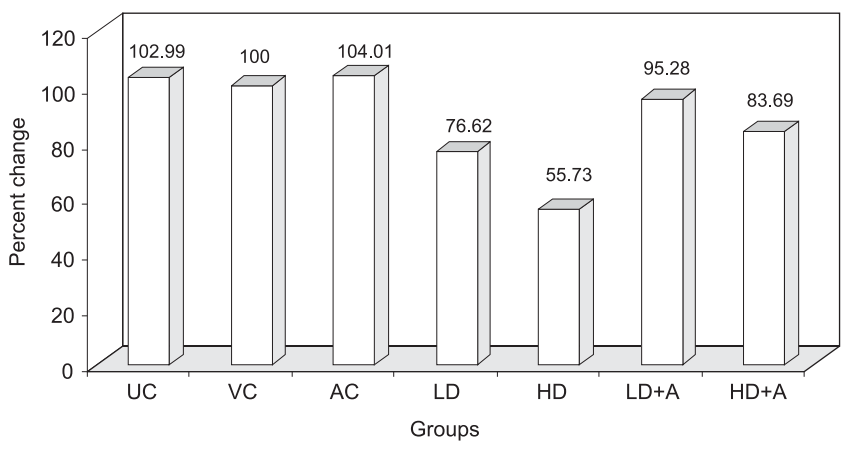

Figure 6. Percent change in the activity of glutathione transferase from vehicle control in testis.

Table 2. Effect of aqueous extract of *Emblica officinalis* on ochratoxin-induced changes in the lipid peroxidation and antioxidative defense mechanism in the testis of mice.

Parameters	Experimental groups						
	1	2	3	4	5	6	7
Lipid peroxidation (nmoles MDA/ mg protein/ 60 min)	1.47 ± 0.01	1.48 ± 0.02	1.46 ± 0.07	2.45 ± 0.05^{abcefg}	3.15 ± 0.02^{abcdfg}	1.58 ± 0.09^{de}	1.95 ± 0.01^{de}
Total ascorbic acid (mg/g tissue weight)	3.93 ± 0.10	4.10 ± 0.07	4.31 ± 0.06	3.29 ± 0.08^{abcef}	1.94 ± 0.08^{abcdfg}	3.98 ± 0.02^{ceg}	3.22 ± 0.06^{abfe}
Glutathione (µg/ 100 mg tissue weight)	47.84± 0.33	46.40 ± 0.39	46.03 ± 0.44	26.85 ± 0.33^{abcefg}	17.37 ± 0.37^{abcdfg}	46.09 ± 0.35^{deg}	37.46 ± 0.32^{abcdef}
Catalase activity (µmoles H_2O_2 consumed/ mg protein/ min)	22.34 ± 0.39	23.04 ± 0.31	23.08 ± 0.35	16.42 ± 0.24^{abcef}	11.78 ± 0.35^{abcdfg}	22.12 ± 0.48^{deg}	16.54 ± 0.24^{abcef}
Superoxide dismutase activity (unit S/ mg protein)	1.94 ± 0.03	1.86 ± 0.03	1.90 ± 0.02	1.36 ± 0.02^{abcefg}	0.60 ± 0.03^{abcdfg}	1.85 ± 0.04^{deg}	1.60 ± 0.02^{abcdef}
Glutathione peroxidase activity (µmoles NADPH consumed/ mg protein/ min)	0.68 ± 0.03	0.67 ± 0.02	0.65 ± 0.02	0.38 ± 0.01^{abcefg}	0.25 ± 0.01^{abcdfg}	0.66 ± 0.05 ^{deg}	0.49 ± 0.05^{abcdef}
Glutathione reductase activity (nmoles NADPH consumed/ mg protein/ min)	1.62 ± 0.07	1.56 ± 0.08	1.63 ± 0.05	1.08 ± 0.05^{abcefg}	0.73 ± 0.07^{abcdfg}	1.48 ± 0.06^{deg}	1.28 ± 0.06^{abcdef}
Glutathione transferase activity (µmoles of CDNB-GSH conjugate formed/ mg protein/ min)	16.17 ± 0.35	15.70 ± 0.35	16.33 ± 0.74	12.03 ± 0.28^{abcefg}	8.75 ± 0.25^{abcdfg}	14.96 ± 0.36^{acdeg}	13.14 ± 0.23^{abcdef}

Group 1: Untreated control; Group 2: Vehicle control; Group 3: Antidote control; Group 4: Low dose ochratoxin; Group 5: High dose ochratoxin;
Group 6: LD+ Antidote; Group 7: HD+ Antidote. (Values are the mean ± S.E.M.; n = 10)

[a]As compared to group 1: $p < 0.05$ [c]As compared to group 3: $p < 0.05$ [e]As compared to group 5: $p < 0.05$ [g]As compared to group 7: $p < 0.05$
[b]As compared to group 2: $p < 0.05$ [d]As compared to group 4: $p < 0.05$ [f]As compared to group 6: $p < 0.05$

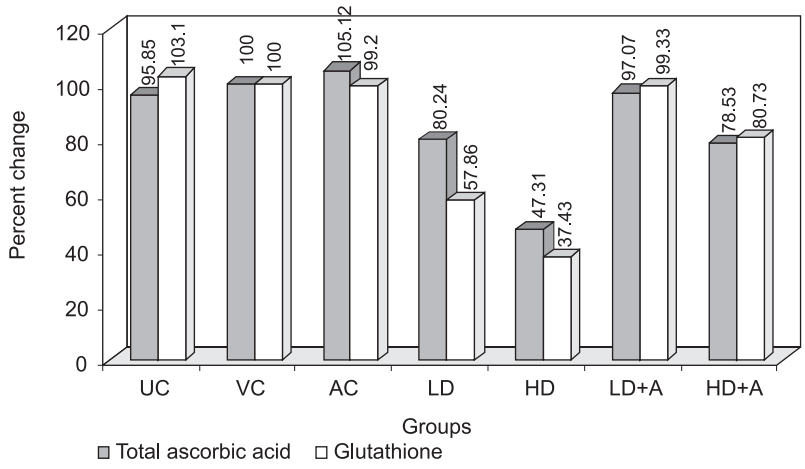

Figure 7. Percent changes in non-enzymatic antioxidants from vehicle control in testis.

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 with $p \leq 0.05$. Comparisons of p values between different groups were performed.

RESULTS

The results shown in Table 2 revealed the effect of ochratoxin and ochratoxin plus *Emblica officinalis* aqueous extract on LPO and in the activities of CAT, SOD, GPX, GRX, GST along with the concentration of GSH and TAA in the testis of mice. Also percent changes from vehicle control are shown in Figures 1 – 7. The results revealed no significant alterations between different control groups (Groups 1-3) in all the above mentioned parameters.

The level of LPO was significantly higher in ochratoxin-treated mice than that of the vehicle control (Group 2). Percent change in case of lipid peroxidation for low dose was 12.16% and for high dose was 65.54%, than that of vehicle control (Figure 1). A decrease in the activities of various enzymatic antioxidants: CAT (LD : -30.71%; HD : -56.59%; Figure 2), SOD (LD : -26.89%; HD : -67.75%; Figure 3), GPX (LD : -43.29%; HD : -62.69%; Figure 4), GRX (LD : -30.77%; HD : -53.21%; Figure 5), GST (LD : -23.38%; HD : -44.27%; Figure 6) as well as in the content of various non-enzymatic antioxidants: GSH (LD : -42.14%; HD : -62.57%; Figure 7) and TAA (LD : -19.76%; HD : -52.69%; Figure 7) in comparison to the vehicle control (Group 2) were observed in the testis of mice. In case of CAT, SOD, GRX and GST the effect was dose dependent.

However, the treatment with aqueous extract of *Emblica officinalis* along with ochratoxin caused a significant decrease in LPO and significant increase in the activities of CAT, SOD, GPX, GRX, GST along with the concentration of GSH and TAA (Groups 6, 7), than ochratoxin alone treated animals (Groups 4, 5). The amelioration was higher in case of LPO in high dose ochratoxin plus extract treated mice than that of low dose. In case of enzymatic antioxidants, the amelioration was higher in case of high dose ochratoxin plus extract treated mice in the activities of SOD, GRX and GST than that of their respective low doses, whereas, in case of CAT and GPX amelioration was higher in low dose than that of the respective high dose. The amelioration was less in low dose ochratoxin plus extract treated mice in case of GSH and TAA than that of respective high dose.

DISCUSSION

The results clearly indicate a significant increase in LPO in the testis of ochratoxin-treated mice as compared to vehicle control. Oxidative stress is considered to be involved in the mechanism of ochratoxin-induced toxicity. An increase in malondialdehyde (MDA) production, indicating a rise in LPO have been reported under *in vitro* and *in vivo* conditions (23). The studies have demonstrated that very low concentration of ochratoxin was sufficient to induce apoptosis and oxidative damage to kidney cells in Wistar rats (24). Free radicals oxidative stress may lead to various diseases in humans which can be serious when there is an imbalance between oxidative stress and antioxidant defense system. Oxidative stress can lead to various disorders

including infertility in animals (25). The levels of enzymatic antioxidants (CAT, SOD, GPX, GRX and GST) were significantly lower in ochratoxin-treated mice than in control. Superoxide radicals ($O_2^{-\bullet}$) have been reported in several pathological disorders and susceptible for elevated oxidative stress. A decrease in SOD activity will increase the level of superoxide radicals, leading to an increase in oxidative stress enhancing early cell death, probably by apoptotic mechanisms. A decrease in CAT activity will increase the concentration of H_2O_2 in the cell leading to an increase in LPO and oxidative stress. A decrease in the activity of GRX will further decrease the concentration of ascorbic acid. A decrease in GSH and GPX will increase the concentration of H_2O_2 signaling by further increasing the oxidative stress. A decrease in GST will increase the concentration of active oxygen species. Our finding of decrease in the activities of CAT, SOD, GPX, GRX and GST corroborates with that of previous studies (26). An increase in LPO and a decrease in SOD in the kidneys of rats in case of ochratoxicosis have been reported earlier (30). Various authors have reported decreased activities of SOD, CAT and GPX in case of ochratoxicosis (27). Testicular CAT is an important and useful Sertoli cell marker, because there is a good correlation between testicular CAT activity and relative numbers of Sertoli cells. A decrease in CAT activity will decrease the numbers of Sertoli cells affecting spermatogenesis directly or indirectly (28). So decline in CAT activity in the testis may leads to infertility in ochratoxin-treated mice.

A decrease in the activities of these enzymes in case of ochratoxicosis could be due to a decrease in the rate of protein biosynthesis as ochratoxin competitively inhibits phenylalanine tRNA synthetase and alters transcription processes. Secondarily, the toxin produces reactive oxygen species (ROS) that directly or indirectly interact with enzyme protein and alter their activity. Oxidative stress and its involvement in the mechanism of ochratoxin-induced toxicity along with oxidation of proteins have been reported (29).

Oral administration of ochratoxin for 45 days caused significant decrease in the levels of non-enzymatic antioxidants mainly GSH and TAA in testis of mice in comparison to the vehicle control. Intracellular GSH status appears to be a sensitive indicator of cell's overall health and its ability to toxic challenges. Being tripeptide in nature, a decrease in GSH content probably is the result of continuous attack of free radicals on it (30). The ability of ochratoxin to react with reduced GSH has been reported earlier. Various authors have reported a decrease in GSH as primary effect in case of ochratoxin-induced toxicity (31).

Ascorbic acid is transformed to L-dehydroascorbate during free radical scavenging (32). Reduced GSH is required for the conversion of L-dehydroascorbate to ascorbate. A decrease in the concentration of GSH will reduce the conversion process and slowly the ascorbic acid concentration will decrease. Thus significant decrease in GSH level will further aggravate the toxic effects of ochratoxin. A decrease in the non-enzymatic antioxidants in cells will increase the susceptibility to injury by peroxidation.

Oral administration of aqueous extract of *Emblica officinalis* along with ochratoxin for 45 days caused a decrease in LPO and an increase in the activities of CAT, SOD, GPX, GRX, GST along with an increase in GSH and TAA contents. This is mainly because of the presence of various antioxidants principally flavonoids, tannoids and other polyphenols concomitantly with vitamin C that will inhibit LPO. Vitamin C present in the fresh fruit extract maintains first natural antioxidant defense and acts as a powerful inhibitor of LPO (33). Antioxidizing activity of *Emblica officinalis* has been reported earlier (34).

An increase in the activities of enzymatic antioxidants has been reported in gamma-radiated mice treated with *Emblica officinalis* (35). Aqueous extract of *Emblica officinalis* was found to modulate an increase in antioxidant enzymes in cyclophosphamide treated animals (36). Emblicannin A (37%) and Emblicannin B (33%) enriched fraction of fresh juice of Emblica fruit was found to have antioxidant activity against ischemic reperfusion (IRI) induced oxidative stress in rat heart (37). Oral administration of *Emblica officinalis* to dimethylbenzyl anthracene treated mice caused significant increase in liver antioxidants mainly GSH, GRX, GPX and GST (38). An increase in the concentration of GSH and TAA decreases vulnerability to oxidant attack. This might be due to the presence of radical scavengers, having ability to decrease peroxide, hydroxyl and superoxide radicals formation.

It is thus concluded that oral administration of *Emblica officinalis* aqueous extract along with ochratoxin significantly ameliorates ochratoxin-induced LPO in the testis of mice.

REFERENCES

1. Krogh P.: Mycotoxic porcine nephropathy: A possible model for Balkan endemic nephropa-

thy, in : Puhlev, A., Ed., Endemic Nephropathy, p. 266, Publishing House of the Bulgarian Academy of Science, Sofia 1974.
2. Krogh P.: Food Chem. Toxicol. 30, 213 (1992).
3. Kuiper-Goodman T., Scott P. M.: Biomed. Environ. Sci. 2, 179 (1989).
4. Bose S., Sinha S.P.: Food Chem. Toxicol. 32, 533 (1994).
5. Kamp G.H., Eisenbrand G., Schlatter J., Wurth K., Janjowski C.: Toxicology 206, 413 (2005).
6. Rahimtula A.D., Bereziat J.C., Bussachini-Griot V., Bartsch H.: Biochem. Pharmacol. 3, 4469 (1988).
7. Ames B.N., Gold L.S., Willet W.C.: Proc. Natl. Acad. Sci. USA 92, 52 (1996).
8. Satyavati G.V., Raina M. K., Sharma M.: Indian Council of Medical Research. 1, 337 (1976).
9. Bhattacharya A., Chaterjee A., Ghosal S., Bhattacharya S.K. Indian J. Exp. Biol. 37, 676 (1999).
10. Chopra R.N., Nayar S.L., Chopra I.C.: Council of Scientific Research, 265 (1965).
11. Haque R., Bin-Hafez B., Ahmad I., Parvez S., Pandey S., Raisuddin S.: Hum. Exp. Toxicol. 20, 643 (2001).
12. Gulati A.K., Agarwal S., Agarwal S.S.: Indian J. Exp. Biol. 33, 261 (1995).
13. Verma R. Chakraborty D.: Acta Pol. Pharm. Drug Res. 65, 3 (2008).
14. Ohkawa H., Ohishi N., Yagi K.: Anal. Biochem. 95, 351, (1979).
15. Kakkar P., Das B., Viswanathan P. N.: Indian J. Biochem. Biophys. 21, 130 (1984).
16. Pagila D.E., Valentine W.N.: J. Lab. Clin. Med. 70, 158 (1967).
17. Luck H.: A spectrophotometric method for the estimation of catalase, In: Methods of Enzymatic Analysis, Bergmeyer H.U. Ed., p. 886, Academic Press, New York 1963.
18. Mavis R.D., Stellwager.: J. Biol. Chem. 243, 809 (1968).
19. Habig W.H., Pabst M.J., Jakoby W.B.: J. Biol. Chem. 249, 7130 (1974).
20. Grunert R.R., Philips P.H.: Arch. Biochem. 30, 217 (1951).
21. Roe J.H., Kuether C.A.: J. Biol. Chem. 147, 399 (1943).
22. Lowry D.H., Rosenberg N.J., Farr A.I., Randell R.J.: J. Biol. Chem. 193, 265 (1951).
23. Baudrimont I., Betbeder A., Creepy E.E.: Arch. Toxicol. 71, 290 (1997).
24. Soyoz M., Ozcelik N., Kilinc I., Altantas I.: Cell. Biol. Toxicol. 20, 213 (2004).
25. Cheeseman K.H., Slater T.S.: Brit. Med. Bull. 49, 481 (1993).
26. Meki A.R., Hussein A.A.: Comp. Biochem. Physiol. Toxicol. Pharmacol. 130, 305 (2001).
27. Ozcelik N., Soyoz M., Kilinc I.: J. Appl. Toxicol. 24, 211 (2004).
28. Ihrig T.J., Renston R.H., Renston J.P., Gondos B.: J. Reprod. Fertil. 39, 105 (1974).
29. Domijan A.M., Rudes K., Peraica M.: Arh. Hig. Rada Toksikol. 56, 311 (2005).
30. Duke R.C., Ocius D.M., Young J.D.E.: Sci. Am. 12, 79 (1996).
31. Hong J.T., Park K.L., Han S.Y., Park K.S., Kim H.S., Oh S.D., Park H.J., Lee R.D., Jang S.J.: Yakhal Hoeji 42, 336 (1998).
32. Breimer L.H.: Mol. Carcinogenesis 3, 188 (1990).
33. Jeena K.J., Kuttan R.: J. Clin. Biochem. Nutr. 19, 63 (1995).
34. Khopde S.M., Priyadaarshini I.K., Mohan H. et al.: Curr. Sci. 81, 185 (2001).
35. Hari K., Sabu M.C., Lima P.S.: J. Radiat. Res. 456, 549 (2004).
36. Haque R., Bin-Hafeez B., Ahmad I., Parvez S., Pandey S., Raisuddin S.: Hum. Exp. Toxicol. 24, 643 (2001).
37. Bhattacharya S.K., Bhattacharya A., Sairam K., Ghosal S.: Phytomedicine 9, 171 (2002).
38. Banu S.M., Selvindiran K., Singh P.V., Sakthisekeran D.: Hum. Exp. Toxicol. 23, 527 (2004).

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