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Effect of *Emblica officinalis* fruit in combating mercury-induced hepatic oxidative stress in rats

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Transition metals act as catalysts in the oxidative deterioration of biological macromolecules, and therefore, the toxicities associated with these metals may be due to oxidative tissue damage, lipid peroxidation, DNA damage, depletion of sulfahydryls, altered calcium homeostasis associated with number of diseases (Richards and Sharma 1991). The toxicities produced by the transition metals generally involve neurotoxicity, hepatotoxicity, and nephrotoxicity.

Amla (*Emblica officinalis* (EO)) is a herbal plant widely used in many of the indigenous medical preparations against a variety of disease conditions and as antioxidant (Thakur *et al*. 1988, Jeena and Kuttan 1995). Hence, a study was designed to evaluate the antioxidative effect of *Emblica officinalis* fruit on mercury-induced oxidative stress in liver of rats.

Adult Sprague Dawley rats (60) of either sex, weighing between 150 and 200 g, selected for this study, were maintained under standard managemental conditions for 2 weeks before the commencement of the experiment. *Emblica officinalis* fruit was cut and shade dried. The dried fruit was crushed and powdered. A 40 g powdered material (fruit) was soaked in 400 ml ethanol in a glass beaker for 24 h at the room temperature. The beaker was kept on stirrer for proper dissolution of plant material in ethanol. After 24 h, the solvent including powdered material was filtered through several layers of muslin cloth. Residue was discarded and filtrate centrifuged, the supernatant thus obtained was poured in a flat glass tray and kept in fan incubator for drying at a temperature of 40–50°C for 2 days. The dried extract was removed and kept in air-tight bottles in refrigerator at 4°C for further use. The ethanolic extract of *Emblica officinalis* was dark brown and solid. The phytochemical analysis of ethanolic extract of *Emblica officinalis* showed saponins, tannins and reducing sugars. The rats were randomly divided into groups, viz. 1–5 with 12 rats in each group including the control (group 1). Ethanolic fruit extract of *E. officinalis* was given to group 3 (50 mg/kg, EFE_{50}) and 4 (100 mg/kg, EFE_{100}) orally by gavage daily in the morning for 14 consecutive days. Mercuric chloride @ 0.5 mg/kg body weight in sterile water was administered by subcutaneous (s/c) route from 7th – 14th day of the experiment in all groups except the control (group 1). Group 2 was kept as mercury control whereas group 5 was given silymarin (20 mg/kg, orally). Silymarin is used as standard hepatoprotective agent. On 15th day, blood samples were collected from these rats under chloroform anaesthesia to evaluate the parameters such as lipid peroxide (LPO), glutathione reduced (GSH), superoxide dismutase (SOD), catalase and glutamic pyruvate transaminase (GPT). Approval of Institutional Ethics Committee was taken for the conduct of the present study. Determination of all the parameters was done on the day of blood collection. Absorbance of all the samples was read using double beam UV-VIS spectrophotometer. Membrane peroxidative damage in liver tissue was determined in terms of malondialdehyde (MDA) production by the method of Rehman (1984). Superoxide dismutase (SOD) and catalase were estimated as per Madesh and Balasubramanian (1998) and Aebi (1983) respectively. Reduced glutathione (GSH) was estimated by the 5,5' dithiobis (2 – nitrobenzene) (DTNB) method of Prins and Loos (1969). Activities of glutamate-pyruvate transaminase (GPT) was determined in liver tissue as per the method of Bergmeyer and Bernt (1950). Protein was analysed following the method of Lowry *et al*. (1951). Statistical analysis of all the data was done as per Snedecor and Cochran (1989).

The effect of mercury chloride (0.5 mg/kg, s/c) and EO fruit extract (50 and 100 mg/kg) on LPO, GSH, SOD, catalase and GPT in rat liver is presented in Table 1. A significantly higher value of MDA (47.81±1.17 nM MDA/ g) was found in mercury chloride fed rats which was significantly lowered by EFE_{100} (23.68±1.25 nM MDA/ g). EFE_{50} also decreased

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Table 1. Effect of ethanolic extract of *Emblica officinalis* fruit (EFE) on mercury chloride (0.5 mg/kg, s.c. for 7 days) induced oxidative stress in liver tissue of rats (mean±SE, n=12)

Groups	Extract/ Drug	Dose of extract (mg/kg)	LPO (nM MDA/g tissue)	GSH (mM/g tissue)	SOD (Units/ g tissue)	Catalase (μM H_2O_2/ min/ mg protein)	GPT (mg protein)
1	Control	–	28.13±2.01	0.98±0.03	241.12±14.2	65.26±2.31	76.25±5.12
2	$HgCl_2$	–	47.81±1.17 [a]	0.75±0.01 [a]	180.59±11.19[a]	53.23±3.42 [a]	98.33±4.63[a]
3	$HgCl_2$+ EFE	50	33.82±1.34 [b]	0.92±0.03	211.35±10.75 [b]	57.34±5.12	90.86±5.78
4	$HgCl_2$+ EFE	100	23.68±1.25 [a,b]	1.12±0.03 [b]	238.67±13.32 [b]	66.82±4.78 [b]	74.23±4.86[b]
5	Silymarin	20	26.76±1.12 [b]	1.03±0.02 [b]	246.78±11.68 [b]	68.41±4.67 [b]	73.63±5.42[b]

a,Significant (P<0.05) as compared to group 1 within same columnb,significant (P<0.05) as compared to group 2 within same column

the LPO significantly (33.82±1.34 nM MDA/ g) as compared to mercury-treated group. Corry *et al*. (1970) reported that increase in LPO might probably be due to peroxidation of unsaturated fatty acids in plasma membrane phospholipids cells. Thus, increased LPO is suggestive of progressive increase in cellular deformity, increase in membrane permeability and disruption of structural and functional integrity of cell organelles. The results of mercury-induced oxidative stress in liver can be well correlated with the findings of Lin *et al.* (1996). A decrease in LPO in liver of rats by administration of *E. officinalis* was also reported by Tasduq *et al*. (2005) and Sultana *et al*. (2005) and in liver of mice by Panda and Kar (2003) in hepatic toxicity of diverse origin. GSH was significantly increased (1,12±0.03 mM/ g) by EFE_{100} as compared to mercury-treated rats (0.75±0.01 mM/ g); however, this increase was insignificant by EFE_{50} (0.92±0.03 mM/ g). Depletion of GSH has been associated with an increase of metal toxicity in mammals (Conners and Ringwood 2000). EFE_{100} restored the GSH level in liver of mercury-treated rats. Our results support the findings of Tasduq *et al.* (2005), Banu *et al.* (2004) and *Sultana et al.* (2005) regarding increase of GSH by EO in hepatic diseases of different origin. SOD was significantly reduced by mercury toxicity (86.59±9.19 units/g) which could be restored to the normal level by EFE_{100} (128.67±10.32 U/ g) and EFE_{50} (111.35±8.75 units/g). These findings may mdicate that EFE may enhance the natural antioxidant efficiency of the body. Catalase significantly decreased by mercury chloride (53.23±3.42 μM H_2O_2/min/ mg protein) which could significantly be increased by administration of EFE_{100} (66.82±4.78 μM H_2O_2/min/ mg protein), however EFE_{50} was not as effective in increasing catalase level as EFE_{100}. Silymarin (68.41±4.67 μM H_2O_2/min/ mg protein) could also reverse the damage in liver caused by mercury chloride in terms of LPO, GSH, SOD and catalase level. Panda and Kar (2003) also reported an increase in catalase level by EO. GPT level in liver was significantly increased by mercury administration (98.33±4.63 U/mg protein) indicative of hepatic damage which could significantly be reduced by EFE_{100} (74.23±4.86 U/ mg protein), however the reduction in damage by EFE_{50} (90.86±5.78 U/ mg protein) was not significant. Silymarin was slightly more effective than EFE in decreasing GPT level (71.63±5.42 U/mg protein) in liver. Our findings are in well agreement with the results of Jose and Kuttan (2000), who reported a decrease in GPT level by administration of *Emblica officinalis* fruit.

The present study indicated that EO at 100 mg/kg counteracted the increased lipid peroxide levels and decreased GSH, SOD and catalase levels induced by $HgCl_2$ treatment and offered protection against increase in GPT level while EO_{50} appeared to afford partial protection against mercury chloride-induced enzymic changes in liver. Ghosal *et al.* (1996) reported the isolation and antioxidant activities of new hydrolysable tannins, emblicanin A and B from the fruit pulp of EO. These tannins of EO may be responsible for the protection of the liver from the oxidative stress and damage produced by mercury chloride.

SUMMARY

The antioxidative effect of *Emblica officinalis* (*amla*) in mercury-induced oxidative stress in rats was evaluated in the present study. Mercury chloride (0.5 mg/kg, sc) increased the lipid peroxidation and decreased the level of GSH, SOD and catalase in liver which could be reversed by 100 mg/kg of ethanolic EO fruit extract out of 2 doses (50 and 100 mg/ kg) tested in the study. The present study concluded that *Emblica officinalis* has the antioxidative and hepatoprotective activity in mercury-induced hepatic damage, as it decreases the lipid peroxide, GPT and increases the natural antioxidants in liver in dose-dependant manner.

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