Modulation of acute cadmium toxicity by *Emblica officinalis* fruit in rat*

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The efficacy of *Emblica officinalis* in modifying the acute cytotoxicity of cadmium in male rats was evaluated. Oral administration of *Emblica* fruit juice (500 mg/kg, b.w.) for 8 days followed by a single toxic dose of Cd as CdCl$_2$ (3 mg/kg, b.w. ip.), considerably reduced the mortality in rats as well as prevented to some extent the cadmium induced histopathological damage in testis, liver and kidneys. Biochemical investigation also revealed reduced levels of Cd induced serum glutamate oxaloacetate transaminase, glutamate pyruvate transaminase and gamma glutamyltranspeptidase. The enhanced levels of Cd and lipid peroxidation in liver, kidney, and testes and metallothionein and total sulphydryl in liver and kidney by Cd were significantly reduced by *Emblica* pretreatment. These results suggest cytoprotective potential of *Emblica* fruit in acute cadmium toxicity which could be due to its multiple role in biological system.

Cadmium (Cd) highly toxic to both human and animals, is ubiquitous due to its wide application in electroplating and galvanizing, as a colour pigment in paints, batteries, plastic and fertilizer industries, cigarette smoke, air pollution and most foods.$^{1,2}$ It has been classified as human carcinogen by International Agency for Research on Cancer.$^3$ Generally testis, liver and heart are most damaged following acute exposure to Cd$^{4,5}$ whereas renal toxicity predominates after chronic low level poisoning $^{6,7}$.

Although, high incidence of low level exposure to cadmium takes place, proper therapeutic intervention remains obscure. Most of the synthetic antidotes in the management of Cd toxicity have met with limited success due to their inherent toxicity, non specificity causing essential metal depletion and low acceptability by exposed subjects.$^8$

Free radical scavengers and antioxidants are useful in protecting Cd toxicity.$^9$ Fariss$^{10}$ has studied the cytoprotective property of a-tocopheryl succinate in Cd toxicity. Ascorbic acid, too acts as a pro-oxidant or an oxygen radical scavenger depending on an optimal threshold dose for effective antagonism by the chemical.$^{11}$

There are many reports of correlation between high consumption of fruit and vegetables or of some dietary antioxidants (vitamin C, carotenoids, vitamin E) and a relatively low incidence of several cancers$^{12}$.

Dietary antioxidants, in general, act by removing reactive oxygen species before they have a chance to cause damage to biological molecules.

Fruits of *Emblica officinalis* Gaertn. (Family Euphorbiaceae) commonly known as 'amla' or the Indian gooseberry has been extensively used in Indian Ayurvedic and Siddha system of traditional medicine for the treatment of wide spectrum of diseases. It is the constituent of several marketed herbal preparations such as Chyavanaprasha, Brahmi Rasayana, Haritaki, Triphala, Septilin etc. Some of the properties of the fruit experimentally proved include antifungal, antibacterial antidiabetic, antipyretic, antioxidant, anticlastogenic, hepatoprotective etc.$^{13,16}$

*Emblica* fruit contains tannins such as gallic acid, ellagic acid, albumin, crude cellulose, nicotinic acid, amino acids like glutamic acid, proline, aspartic acid, alanine and lysine, minerals, Cr, Zn, Fe and Cu apart from the highest amount of vitamin C in natural form. Some cytokine- like substances also identified are zeatin, r. riboside, r. nucleotide.

Despite its extensive medicinal use, limited knowledge is available regarding its role in heavy metal toxicity. Few studies have been carried out on its modifying effect against nickel, lead and aluminium elastogenicity in mice$^{13,14}$. However, its antioxidative and cytoprotective potential against Cd toxicity remains unexplored. Therefore, the purpose of this study was to delineate its role in acute Cd-induced hepatic, renal and testicular damage in rat.

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Materials and Methods

Chemicals
Cadmium chloride was obtained from Sigma (St. Louis, MO, USA). Fresh Emblica fruits procured from National Botanical Research Institute, Lucknow, were grated, weighed and crushed in pestle and mortar. The juice was pressed through cheese cloth and a 50% solution was prepared in double distilled water. Other chemicals were of analytical grade.

Animals and treatment
Forty eight healthy male albino rats of Druckrey strain of ITRC colony were used. For the first set of experiment, 24 animals were divided into four groups of six rats each. Emblica juice (500 mg/kg/5 ml) was fed orally to two groups of 6 rats each, daily for 8 days. On the last day, one hour after the Emblica dose, Cd as CdCl₂ (3 mg Cd/kg/ml in normal saline) was injected ip to one group and the other group of Emblica was injected saline. One set of 6 rats were administered Cd and the remaining 6 animals were injected saline and served as controls. The mortality pattern was observed over a period of 48 hrs. (The LD₅₀ of Cd is 3.55 mg/kg).

Another set of 24 rats were treated exactly as above and sacrificed after 6 hours of Cd treatment. Blood from jugular vein was collected for serum separation and the tissues (liver, kidney and testis) were dissected and cleaned free of extraneous material. A part of liver, half kidney and one testis were immediately put in 10% neutral buffered formalin for histopathological examination. A portion of liver, half kidney and a piece of testes were kept at -20°C for Cd estimation and a portion of all the three tissues was used for the assay of lipid peroxidation and total sulphhydryl content.

Assay of lipid peroxidation, total sulphhydryl (SH), serum enzymes, metallothionein (MT) and cadmium levels
A 20% homogenate of liver, kidney and testes was prepared in 1.15% KCl and a 10% homogenate in 0.02M EDTA under ice-cold conditions for lipid peroxidation and total sulphhydryl level, respectively, and estimated the very same day. A part of the homogenate in KCl was spun at 100,000 g (60 min., 0°C) and the supernatant fractions used for MT determination. Serum was refrigerated and used for biochemical parameters the next day.

The estimation of lipid peroxidation was monitored by malondialdehyde (MDA) formation by the method of Ohkawa et al.¹⁹ and total SH by Sedlak and Lindsay.²⁰ The clinical biochemical analysis in serum was performed on Autolab Autoanalyser of Boehringer Mannheim, Germany. Analysis was carried out at 37°C in serum samples according to the instructions of the manufacturer (Boehringer Mannheim, Application Sheets, 1995). Briefly, glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) activity were determined according to the International Federation of Clinical Chemistry Kinetic method, alkaline phosphatase (ALP) using AMP buffer and gamma glutamyl transpeptidase (GGT) activity according to the kinetic method of Szasz.

Metallothionein (MT) in liver, kidney and testes was estimated using Cd-Chelex assay with some modifications. Non radioactive Cd as CdCl₂ (1mM) was used for saturation of MT in the assay and the Cd content in the supernatant was measured on Varian Spectr AA 250 Plus Atomic Absorption Spectrophotometer. Wet acid digestion procedure for measuring Cd levels in liver, kidney and testes using HNO₃ and HClO₄ was carried out and the resultant solution was made up with deionized water. Cadmium was analysed on Varian AAS at 228.8 nm wave length.

Histological analysis of tissue damage
For histopathological assessment of hepatic, renal or testicular damage, tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin.

Statistical analysis
Significance of mean values of different parameters between the treatment groups were analysed using One Way analysis of variance, after ascertaining the homogeneity of variance between the treatments. Pair wise comparisons were done by calculating the least significant difference.

Results
A single intraperitoneal injection of 3 mg Cd/kg caused 66% mortality over a period of 48 hrs. However, Cd induced mortality was markedly reduced when the rats were pretreated with Emblica fruit juice (500 mg/kg, oral) for 8 days (Table 1).

On histopathological examination, Cd alone caused typical spectrum of testicular lesions in all the animals which comprised of edematous vasculitis and hemorrhage in stroma. The seminiferous tubules demonstrated
Table 1 — Effect of Emblica fruit on Cd induced mortality in rat

<table>
<thead>
<tr>
<th>Treatment * mg/kg</th>
<th>Mortality</th>
<th>No. Dead/Treated</th>
<th>Percentage</th>
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<tr>
<td>Cd</td>
<td>Emblica</td>
<td></td>
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<tr>
<td>0</td>
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<td>0/6</td>
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<td>500</td>
<td>0/6</td>
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<td>4/6</td>
<td>66</td>
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<tr>
<td>3</td>
<td>500</td>
<td>1/6</td>
<td>16</td>
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+Assessed 48 hr after Cd treatment.
*Rats were pretreatment with Emblica fruit juice for 8 days, followed by Cd 1 hr after Emblica on the last day.

Significant loss of spermatogenesis along with characteristic multinucleate structures prominent in the lumen. Pretreatment with Emblica showed some cytoprotective effect on Cd-induced testicular lesions except that the moderate edema developed in the interstitium with minimal hemorrhage and the spermatogenesis appeared less affected, whereas Emblica alone did not produce any histological damage in the testis (Fig.1 a,b,c).

In the hepatic tissue, Cd alone produced characteristic focal necrosis along with acute inflammatory cells. There was congestion at places and sinusoids were not patent. However, when the rats were pretreated with Emblica, Cd induced hepatic lesions were minimal as some of the hepatocytes at places still showed cloudy swelling and fatty changes. Emblica alone did not influence the histology of liver (Fig.2 a,b,c).

Regarding the renal tissue, Cd treatment caused widespread congestion in the cortical region along with prominent tubular necrosis, degeneration and sloughing of tubular epithelial cells of proximal and distal tubules. Emblica pretreatment, had a very marginal protective effect in kidney on the acute toxic effects of Cd as evinced by the persistence of moderate degree of congestion, degeneration and desquamation of tubular epithelium with occasional necrosis. The kidney from Emblica alone treated group resembled closely to that of saline control group (Fig.3a,b,c).

Among the biochemical changes, a marked increase in MDA formation in testis, followed by liver and kidney was observed in rats treated with Cd. Emblica pretreatment resulted in a significant decrease in Cd induced MDA. Rats treated with Emblica alone displayed a slight lowering in MDA formation in liver and kidney but not in testis (Table 2). The total sulfhydryl content of liver and kidney was elevated in Cd treated rats. However, on Emblica pretreatment these Cd induced values declined substantially (Table 2).

Fig. 1 — Microscopic evaluation of testicular tissue from cadmium chloride, Emblica pretreated and Emblica alone group, H & E×125. (a) Microscopic changes in rat testes 6 hr following administration of cadmium chloride (3 mg Cd/kg, ip). The seminiferous tubules demonstrated significant loss of spermatogenesis along with multinucleate structures prominent in the lumen. Interstitial tissues showed edema, hemorrhage and vascular thrombosis. (b) Section of testes from rats pretreated with Emblica (500 mg/kg, orally) for 8 days followed by Cd 1 hr after Emblica on the last day. The seminiferous tubules appeared normal, except that the edema persisted in the interstitium with minimal hemorrhage. (c) Section of testes from rats treated with Emblica alone. The seminiferous tubular cells and interstitial tissue were normal.
The activities of serum GOT, GPT and GGT significantly increased in rats treated with Cd as compared to the untreated group. The values of ALP remained unaltered. However, in the rats pretreated with Emblica followed by Cd, the increased levels of serum GOT, GPT and GGT was partially prevented (Fig. 4). Emblica in the present study caused slight induction of MT in liver and MT like protein in testes, similar to...
the induction of hepatic MT reported by ascorbic acid, but to a lesser degree. Cadmium induced MT in liver and kidney was reduced by 28% and 24% respectively by Emblica pretreatment (Table 3).

Since Emblica clearly ameliorated the toxicity of cadmium as assessed by lethality, pathological and biochemical changes, experiments were performed to determine if it was due to altered toxicokinetics of Cd. As compared to the untreated rats, increase in Cd was observed in liver followed by kidney and testis. Emblica pretreatment, however, had a marked effect on the distribution of Cd in these tissues. The uptake of Cd was reduced by 26, 33 and 48% in the hepatic, renal and testicular tissue, respectively (Table 3).

**Discussion**

The present findings based on the lethality pattern, histological changes in the target organs, Cd and MT levels and various biochemical alterations in serum and organs indicate that Emblica pretreatment moderated the acute cytotoxicity of Cd in rat. Several possibilities exist as to the mechanism by which Emblica...
afforded protection. Emblica pretreatment reduced the Cd levels in liver, kidney and testis. The ameliorative role of Emblica evidenced by a normalizing trend in morphological and serum biochemical picture strongly suggest that the Emblica may exert a stabilizing action on cell membranes. This in turn may affect the leakage of enzymes and other metabolic products into the blood circulation and may also reduce the uptake of Cd by liver, kidney and testis. The reduced levels of MT in liver and kidney in the Emblica pretreatment group also suggest reduced uptake of the metal in the two organs. The possibility of enhanced metal excretion cannot be ruled out. On the contrary, the MT like protein in the testis exhibiting an increase, propose a different mechanism of action with respect to the ameliorative role of Emblica.

The ameliorative potential of many vegetables and fruits is attributed to the combined effect of their constituents rather than to a single factor for e.g. the antimitagenic activity of spinach is due to chlorophyllin and ascorbic acid while in citrus fruits, to citric acid and ascorbic acid.

Since lipid peroxidation is considered an early and sensitive index of Cd exposure the lowering of Cd induced MDA formation by Emblica pretreatment in the present study could be attributed to the inherent antioxidant property of Emblica fruit. Higher efficacy of phyllanthus fruit extract rather than of ascorbic acid was reported in alleviating the elastogenic action of lead and aluminium in bone marrow cells of mice.

Some studies, especially in the case of testes, have suggested relationship between lipid peroxidation and Cd induced hemorrhagic necrosis. In fact, Cd induced testicular toxicity can be prevented by pretreatment with antioxidants such as ascorbic acid, α-tocopherol and methyl B12. This indicated that Cd induced lipid peroxidation plays a causative role in testicular lesions. The present study shows that Emblica pretreatment reduced the testicular MDA formation by 50% as well as the typical spectrum of testicular lesions induced by Cd. In contrast to the testes, some studies have indicated that lipid peroxidation does not seem to play a causative role in Cd induced liver lesions. The mechanism of Cd induced toxicity may differ in different tissues or tissue components. A modest increase in Cd induced MDA formation and total sulphydryl in liver and kidney were modulated on pretreatment with Emblica.

The protective effect of L-ascorbic acid in preventing Cd induced mortality in mice was related to MT induction in liver. Similar mechanism appears to be operative to some extent in the present study. Kidney, not being the major organ to be affected by a single acute dose of Cd, showed mild morphological alterations which still persisted with Emblica pretreatment.

The results of this study suggest that the Emblica fruit as a whole (comprising of vitamin C, tannins, cellulose, amino acids, minerals, metals, cytokine-like substances etc.) induces endogenous antioxidant defence system and reduces lipid peroxidation by Cd in target organs which can have important direct cytoprotective effects, specially in the event of oxidant stress induced injury by modifying its toxicokinetics and cell membrane integrity as well as stimulating MT synthesis. Further experiments are underway to study the therapeutic efficacy of Emblica in long term Cd intoxication in rat.

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