Effect of *Emblica officinalis* (Gaertn) on CCl₄ induced hepatic toxicity and DNA synthesis in Wistar rats

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A single dose of CCl₄ (1 ml/kg body weight, po in corn oil) increased the levels of SGOT (serum glutamate oxaloacetate transaminase), SGPT (serum glutamate pyruvate transaminase), LDH (lactate dehydrogenase), glutathione-S-transferase and depletion in reduced glutathione, glutathione peroxidase and glutathione reductase. It also caused enhancement in the levels of lipid peroxidation (LPO) and DNA synthesis. There was also pathological deterioration of hepatic tissue as evident from multi vacuolated hepatocytes containing fat globules around central vein. The pretreatment of *E. officinalis* for 7 consecutive days showed a profound pathological protection to liver cell as depicted by univacuolated hepatocytes. Pretreatment with *E. officinalis* at doses of 100 and 200 mg/kg body weight, prior to CCl₄ intoxication showed significant reduction in the levels of SGOT, SGPT, LDH, glutathione-S-transferase, LPO and DNA synthesis. There was also increase in reduced glutathione, glutathione peroxidase and glutathione reductase. The results suggest that *E. officinalis* inhibits hepatic toxicity in Wistar rats.

**Keywords:** CCl₄; *Emblica officinalis*; Hepatic toxicity.

Liver is a versatile organ of the body that regulates internal chemical environment. Liver injuries induced by various hepatotoxins have been recognized as a major toxicological problem for years. Carbon tetrachloride (CCl₄), an established hepatotoxicant for both animals and humans and has been used as solvent to coal tar, oil, resins and raw material of fluorocarbon for coolant and as fire extinguishing agent. CCl₄ is metabolized in the liver by cytochrome P₄₅₀ to trichloromethyl radical that reacts with GSH to form a GSH containing radical and causes various pathological and toxicological manifestations. Early events associated with the cytotoxicity of CCl₄ following activation includes lipid peroxidation and covalent binding to proteins and lipids followed immediately by diminished protein and lipid turnover and alterations in calcium homeostasis. CCl₄ has been shown to activate Kupffer cells by increasing intracellular Ca²⁺ concentration, causing release of harmful cytokines that contribute to the death of hepatocytes and oxidative stress. The cellular infiltration of activated neutrophilic leukocytes amplifies inflammatory response and cellular injury or death due to release of superoxide anions and other toxic mediators. Free radicals bind covalently to membrane proteins and lipids causing enzyme inactivation and promotion of lipid peroxidation as a result of interaction with membrane unsaturated fatty acids.

Regenerating liver, a model of synchronized cellular proliferation, provides an opportunity to study the factors that can suppress or intensify the manifestations involved in the regenerative process. Historically plants have been used as folk medicine against various types of diseases. Experimental work on several plants has been carried out to evaluate their efficacy against chemically induced toxicity. In the present study the efficacy of extract of fruits of *Emblica officinalis* (Gaertn) has been elucidated against experimental tissue injury, oxidative stress and proliferative response induced by hepatotoxic agent, CCl₄.

Phytochemical evaluation of the plant *E. officinalis* showed the presence of quercetin, ascorbic acid and ellagic acid. The major principle is quercetin. The polyphenolic components of higher plants reportedly act as antioxidant or as agents of other mechanisms contributing to anti carcinogenic action. Flavonoid
and other plant phenolics possess multiple biological activity including anticarcinogenic, anti-inflammatory and anti-allergic activity.\textsuperscript{14} Flavonoids are reported scavenger of superoxide radicals,\textsuperscript{15} peroxy radicals and inhibitor of lipidperoxidation.\textsuperscript{16} Quercetin has been shown to mediate the down regulation of mutant formation of mutagens is between nitrite and vitamin C, vitamin E, gallic acid, ferrulic acid and caffeic acid.\textsuperscript{19} The above information and present status of the dietary intervention prompted the present work on the evaluation of the efficacy of the fruit extract of \textit{Emblica officinalis} against CCl\textsubscript{4} induced hepatic toxicity and DNA synthesis.

### Materials and Methods

**Chemicals**—Oxidized and reduced glutathione (GSSG and GSH), NADPH, H\textsubscript{2}O\textsubscript{2}, dithionitrobenzene (DTNB), 1-chloro-2, 4-dinitrobenzene (CDNB), dinitrophenyl hydrazine (DNPH), glutathione reductase, reduced nicotinamide adenine dinucleotide (NADPH), ethylenediamine tetra acetic acid (EDTA), pyridoxal phosphate, phenyl methyl sulphonyl fluoride (PMSF), 2-mercaptoethanol, dithiothreitol, Tween 80, Brij 35, ethanolamine, methoxyethanol, citric acid, \(\gamma\)-glutamyl p-nitroanilide, diethyl nitrosamine, 2-acetylamino fluorine and quercetin were purchased from Sigma Chemical Co. (St. Louis, Mo. USA). \(^{1}H\) thymidine (specific activity 73.0 Ci/m mol) was purchased from Amersham Corporation, UK. All other chemicals and reagents used were of highest purity commercially available.

**Plant extraction**—Dried fruits of the plant \textit{E. officinalis} were procured from Jamia Hamdard campus and authenticated at source by Prof. M Iqbal, Department of Environmental Botany, Jamia Hamdard, New Delhi, India. Freshly collected fruits were shade-dried and coarsely powdered in a grinder. The extraction procedure was followed as described by Didry \textit{et al.}\textsuperscript{20} Powdered dried fruits (500 g) were extracted in round-bottomed flask with 2000 ml petroleum ether, benzene, ethyl acetate, acetone, methanol and double distilled water. The residues yielded from each solvent (14, 10, 16, 30, 42 and 22 g respectively) were stored at 4°C. The extracts were evaporated to dryness under reduced pressure in a rotatory evaporator (Buchi Rotavapor, Switzerland). The methanol fraction obtained (42 g) was suspended in water to prepare the required dilutions at the time of dosing.

**Animals**—Male albino Wistar rats (130-150 g) were obtained from Central Animal House of Hamdard University, New Delhi, India. They were housed in polypropylene cages in groups of six rats per cage and were kept in a room maintained at 25° ± 2°C with a 12:12 hr L:D cycle. They were given free access to standard laboratory feed. (Hindustan Lever Ltd., Bombay, India) and water ad libitum. The animals were sacrificed according to the guidelines of the current laws of CPCSEA (Ethical Committee for the purpose of control and supervision of experiments on animals), India.

### Experimental protocol

To study the biological, serological and pathological changes 24 rats were divided into 4 groups of 6 each. Group I served as saline treated control. Rats in Group II were given CCl\textsubscript{4} [1 ml/kg body weight, po, in corn oil (1:1)]. Groups III and IV animals were given \textit{E. officinalis} extract at doses of 100 and 200 mg/kg body weight, respectively for 7 consecutive days followed by CCl\textsubscript{4} intoxication on the seventh day. Animals in all the groups were sacrificed 24 hr after CCl\textsubscript{4} or saline administration. The doses of the plant extracts were selected after performing \textit{in vitro} assays like microtanal lipid peroxidation and cytochrome P\textsubscript{450}. Serum was separated and stored at 4°C for the estimation of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), lactate dehydrogenase (LDH). Tissue was processed for the estimation of glutathione (GSH) content, microsomal lipid peroxidation and activities of glutathione-S-transferase (GST), glutathione peroxidase (GPx) and glutathione reductase (GR). Sections of liver from each group were cut and preserved in buffered formalin for histopathological studies.

For \(^{1}H\) thymidine incorporation study, grouping of animals was same as described above. All the animals were given intraperitoneal \(^{1}H\) thymidine (25 
\textmu\textsubscript{ci} per 0.2 ml/saline/100 g of animal) 2 hr before killing. All animals were sacrificed 48 hr of CCl\textsubscript{4} intoxication and liver sections were quickly excised, rinsed with ice cold saline, freed of extraneous material and processed for the quantification of \(^{1}H\) thymidine incorporation into the hepatic DNA.
Post-mitochondrial supernatant (PMS) and microsome preparation—The samples were homogenized in chilled phosphate buffer (0.1 M, pH 7.4) using a Potter Elvehjen homogenizer. The homogenate was centrifuged at 3000 rpm for 5 min at 4°C by Eltek Refrigerated Centrifuge. The aliquot obtained was centrifuged at 12000 rpm for 20 min at 4°C to obtain post-mitochondrial supernatant (PMS). PMS was centrifuged for 60 min by ultracentrifuge at 34,000 rpm at 4°C. The pellet was washed with phosphate buffer (0.1 M, pH 7.4).

Biochemical estimations—Tissue processing and preparation of post mitochondrial supernatant (PMS) were done immediately after animal sacrifice. For all biochemical estimations, serum samples were utilized. All the biochemical estimations were completed within 24 hr of animal sacrifice. SGOT, SGPT, LDH, LPO, GST, GPx, GR were estimated as per standard procedures.

Protein estimation—Protein content in all samples was estimated by the method of Lowry et al. using bovine serum albumin as standard.

Statistical analysis—The level of significance between different groups is based on Dunnett's t test, followed by the ANOVA test.

Results and Discussion

The effect of prophylactic administration of the extract on CCl₄ mediated leakage of liver marker enzymes in serum is shown in Table 1. CCl₄ administration resulted in a significant rise in the levels of SGOT, SGPT and LDH by 278%, 349% and 155% of saline treated control respectively. Pretreatment with *E. officinalis* extract at higher dose showed marked alleviation of SGOT, SGPT and LDH by 94%, 127% and 31% respectively compared to CCl₄ treated group. Hepatoprotective effects of the extracts can be explained on the basis of decreased liver enzyme leakage in serum on pretreatment and significant recovery of the cell membrane towards stabilization as evident from pathological recovery. CCl₄ is metabolized in liver by cytochrome P₄₅₀ dependent electron transport chain system yielding trichloromethyl radical that in aerobic condition rapidly gets converted to its peroxyl radical. These radicals bind directly to lipids and proteins through covalent bonds and also interact with membrane phospholipids leading to promotion of lipid peroxidation. Apart from the studies of mechanism of CCl₄ induced injury, recent studies have shown the involvement of Kupffer cells, cytokines, and neutrophilic leucocytes. A recent study has also shown the protective effect of plant against CCl₄ induced hepatic damage.

The effect of pretreatment of extract on the GSH metabolizing enzymes GST, GPx and GR is shown in Table 2. The administration of CCl₄ resulted in significant (P<0.001) elevation of GST activity by 168% and a concomitant decrease in the activities of GPx and GR by 42% and 39% as compared with saline treated control group. The pretreatment of rats with *E. officinalis* resulted in marked down regulation of GST by 39% at higher dose of the plant. The concurrent alleviation in the GPx was observed on pretreatment of *E. officinalis* by 27% and of GR activity by 31% respectively compared to CCl₄ treated group.

Liver possesses growth ability after cellular loss in hepatotoxicant induced liver injury. Regenerating liver needs growth factors to ensure the proliferation of liver cells. The results of the present study suggest that the plant has the capacity to upregulate the expression of growth factors. The levels of growth factors such as IGF-I and IGF-II were also upregulated in the plant treated group.
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Liver provides a model to study the factors that modulate the process of regeneration. Acute hepatic necrosis on CCl₄ administration is followed by proliferative response that reaches peak at 48 hr, with scarce mitotic cells.

The effect of pretreatment of animals with crude extract of E. officinalis plants on CCl₄ induced GSH depletion, enhanced MDA formation and DNA synthesis is shown in Table 3. Pretreatment of rats with extract produced a marked inhibitory effect on GSH depletion with concomitant alleviation of MDA levels. E. officinalis showed a significant protection of GSH content by 30% and decrease in MDA formation by 111% as compared with CCl₄ treated group. Administration of CCl₄ resulted in significant (P<0.001) 263% increase in the rate of [³H] thymidine incorporation into hepatic DNA, which is a marker of DNA synthesis as compared with saline group. The pretreatment of rats with E. officinalis showed a marked (P<0.01) suppressing effect on the rate [³H] thymidine incorporation by 102% at higher dose of the plant as compared with CCl₄ treated group. Cellular thiol status is an important consideration for the study of hepatotoxicity, particularly in conditions of oxidative tissue injury35. GSH is a tripeptide (γ-glutamyl cysteinyl glycine) non-protein thiol abundant in liver that plays pivotal role in the detoxification processes including conjugation of reactive intermediates and maintenance of GSH redox cycle as antioxidant armory34. The enhancement of hepatotoxicity by GSH depletion has been noted during the metabolism of several compounds35. CCl₄ administration resulted in marked depletion of GSH content consequently increasing vulnerability of tissue to free radical attack causing lipid peroxidation. Protective agents have shown to exert their action against CCl₄ mediated lipid peroxidation either through decreased production of free radical derivatives or due to antioxidant activity of the agent itself36.

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>GST (nmol conjugate formed/min/mg protein)</th>
<th>GPx (nmol NADPH oxidized min/mg protein)</th>
<th>GR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>865.91 ± 34.63</td>
<td>210.56 ± 6.27</td>
<td>189.54 ± 4.92</td>
</tr>
<tr>
<td>CCl₄ alone</td>
<td>1454.78 ± 65.19</td>
<td>122.12 ± 6.42</td>
<td>115.65 ± 3.47</td>
</tr>
<tr>
<td>CCl₄ + EO (D1)</td>
<td>1271.88 ± 34.94</td>
<td>154.82 ± 7.93</td>
<td>155.2 ± 6.97</td>
</tr>
<tr>
<td>CCl₄ + EO (D2)</td>
<td>1096.22 ± 46.68</td>
<td>179.27 ± 6.21</td>
<td>174.92 ± 7.43</td>
</tr>
</tbody>
</table>

P values: *<0.01 (comparing to saline treated group)
$<0.001$ (comparing to CCl₄ treated group)
$<0.001$ (comparing to CCl₄ treated group)
EO=E. officinalis; D1= 100 mg/kg body weight of E. officinalis; D2= 200 mg/kg body weight of E. officinalis

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>GSH (nmol GSH/g tissue)</th>
<th>LPO (nmol MDA formed/h/g tissue)</th>
<th>DNA synthesis (dpm/mg DNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>0.62 ± 0.05</td>
<td>8.42 ± 0.8</td>
<td>31.3 ± 5.8</td>
</tr>
<tr>
<td>CCl₄ alone</td>
<td>0.52 ± 0.04</td>
<td>24.31 ± 1.0³</td>
<td>82.4 ± 12.9³</td>
</tr>
<tr>
<td>CCl₄ + EO (D1)</td>
<td>0.45 ± 0.05</td>
<td>18.1 ± 0.9³</td>
<td>65.9 ± 8.7³</td>
</tr>
<tr>
<td>CCl₄ + EO (D2)</td>
<td>0.52 ± 0.03</td>
<td>15.4 ± 1.2³</td>
<td>50.4 ± 6.5³</td>
</tr>
</tbody>
</table>

P values: *<0.01 (comparing to saline treated group)
$<0.001$ (comparing to CCl₄ treated group)
$<0.001$ (comparing to CCl₄ treated group)
EO=E. officinalis; D1= 100 mg/kg body weight of E. officinalis; D2= 200 mg/kg body weight of E. officinalis
pretreatment of animals with *E. officinalis* significantly ameliorated GSH content with concomitant decrease in lipid peroxidation. It could be suggested that antioxidant presence of several active constituents including quercetin, ascorbic acid and ellagic acid in *E. officinalis* may have counteracted the free radicals through effective scavenging and blocking the conjugation of reactive intermediates to GSH as evident from ameliorated GSH content and decreased MDA formation. Similarly, Vitamin E has been shown to suppress free radical mediated chain initiation and consequently protecting tissue from pathophysiological insult. Induction of GST activity has been correlated to increased GSH consumption and 4-hydroxynonenol (4-HNE) levels in tissue. The sudden elevation of hepatocytes growth factor (HGF) in plasma provides mitogenic signals that trigger DNA synthesis during liver regeneration. In the present, study CCl₄ administration resulted in enhancement in the rate of DNA synthesis when compared to normal group. Pretreatment of rats with *E. officinalis* extracts significantly suppressed the rate of [³H] thymidine incorporation into hepatic DNA in a dose dependent manner. The enhancement of DNA synthesis has been shown as a consequent step to the severe necrosis induced by hepatotoxins.

The protective effect of plant *E. officinalis* on the basis of histopathological findings is shown in Fig. 1. Figure 1a shows the normal pathology of liver section. Administration of CCl₄ resulted in pathological deterioration of hepatic tissue as evident multivacuolated hepatocytes containing fat globules showing fatty changes around central vein and large vacuolated cells with central nuclei at the periphery of lobule showing necrosis. (Fig. 1b). The pretreatment of animals with *E. officinalis* fruit extract showed a profound pathological protection to liver cells as depicted by univacuolated hepatocytes surrounding multivacuolated hepatocytes, mild infiltration and fatty changes around vascular channels (Fig. 1c, d). In summary, the present data suggest that pretreatment

![Fig. 1](https://example.com/fig1.jpg)

**Fig. 1**—Effect of pretreatment of rats with *E. officinalis* on CCl₄ induced pathological changes in rat liver. a-d: H & E: (a) saline only (x 50), (b) only CCl₄, (c) *E. officinalis* (D1) + CCl₄ and (d) *E. officinalis* (D2) + CCl₄; b-d x 250.
oxidative stress and lipid peroxidation induced by necrogenic dose of CCl₄ and also suppressed tissue injury and hepatotoxicant post necrotic regeneration process.

Acknowledgement
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