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Hepatoprotective activity of *Emblica officinalis* and Chyavanaprash

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Abstract

Hepatoprotective activity of *Emblica officinalis* (EO) and Chyavanaprash (CHY) extracts were studied using carbon tetrachloride (CCl_4) induced liver injury model in rats. EO and CHY extracts were found to inhibit the hepatotoxicity produced by acute and chronic CCl_4 administration as seen from the decreased levels of serum and liver lipid peroxides (LPO), glutamate-pyruvate transaminase (GPT), and alkaline phosphatase (ALP). Chronic CCl_4 administration was also found to produce liver fibrosis as seen from the increased levels of collagen-hydroxyproline and pathological analysis. EO and CHY extracts were found to reduce these elevated levels significantly, indicating that the extract could inhibit the induction of fibrosis in rats.

Keywords: Chyavanaprash; *Emblica officinalis*; Hepatoprotection; Herbal drugs

1. Introduction

Hepatic fibrosis is a common condition in which major amounts of liver parenchyma cells are replaced by fibrous connective tissue. Experimentally, hepatic fibrosis has been shown to be produced by the administration of CCl_4, thioacetamide, paracetamol, etc. Reactive oxygen free radicals have been known to produce tissue injury through covalent binding and lipid peroxidation. Lipid peroxidation per se has been shown to augment fibrosis as seen from increased collagen synthesis (Geesin et al., 1990). Scavenging of free radicals by antioxidants could reduce the fibrosis process in the tissues (Thresiamma and Kuttan, 1996).

Emblica officinalis Gaertn. (syn. *Phyllanthus emblica* L.); Euphorbiaceae (EO) is a herbal plant widely used in many of the indigenous medical preparations against a variety of disease conditions (Tiwari et al., 1968; Thakur et al., 1988). It is a major ingredient in Chyavanaprash (CHY), which is used as a health tonic (Ojha et al., 1975). Aqueous extracts of EO and CHY were found to inhibit the formation of superoxides, hydroxyl radicals in addition to lipid peroxidation in vitro (Jeena and Kuttan, 1995) and were found to be anticarcinagenic and antimutagenic (Jeena et al., 1997). EO has been used in several liver protecting drugs (Antarkar et al., 1980; Rao et al., 1993;

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Gulati et al., 1995) in indigenous medicines. In this study, we report on the protective effect of EO and CHY extract against hepatotoxin CCl_4 especially to determine the effect on lipid peroxidation and fibrosis.

2. Materials and methods

Thiobarbituric acid (TBA) and carbontetrachloride (CCl_4) were obtained from E. Merck (India) Ltd., Mumbai. L-hydroxyproline (Calbiochem, USA) was kindly donated by Dr K.A. Balasubramaniam, Wellcome Research Unit, Vellore.

Fruits of *Emblica officinalis* (EO) (a common berry in India) were purchased locally and Chyavanaprash (CHY) from 'Vaidyaratnam Oushadhasala', Ollur, India. Fresh fruit pulp of EO (50 g) was homogenized with 500 ml of water at room temperature. Supernatent was clarified by centrifugation and lyophilized. The lyophyllized powder was used for all the experiments. CHY extract was prepared by reconstituting the preparation in water at desired concentration.

2.1. Induction of acute hepatotaxicity by carbontetrachloride

Male Wistar rats weighing 150 g were divided into four groups containing six animals per group. Group I was kept as a control group. Groups II, III and IV received 0.25 ml of CCl_4 in liquid paraffin (1:1) per 100 g b.wt. intraperitoneally (i.p.) (Nishigaki et al., 1992). Group II acted as an untreated control. Group III was treated with 500 mg/kg b.wt. of EO extract and group IV was treated with 1 g/kg b.wt. of CHY extract. Drug treatment was started three days prior to CCl_4 administration and continued till the end of the experiment. After 48 h, animals were sacrificed by chloroform anaesthesia. Blood was collected by heart puncture and the serum was separated. The liver was immediately removed, a small piece was fixed in 10% formalin and pathological changes were analysed.

2.2. Determination of effect of EO and CHY on CCl_4 induced liver fibrosis

Male Wistar rats weighing 80–100 g were divided into five groups containing six animals per group. Group I was treated as a control group. Animals in groups II–V received 0.15 ml of CCl_4 in liquid paraffin (1:7 by volume) per 100 g b.wt. by i.p., three times weekly for a total of 20 doses (Rojkind, 1973). Group II received CCl_4 alone. Group III and IV were treated with 50 mg and 250 mg/kg b.wt. of EO extract and group V was treated with 2.5 g/kg b.wt.of CHY. EO and CHY were given orally starting from three days prior to CCl_4 administration and continued till the end of the experiment. Animals were sacrificed 24–48 h after the last dose using chloroform anaesthesia. Blood and liver were collected immediately, serum was separated and the tissue was kept frozen till the completion of the experiment. Part of the liver was fixed in 10% formalin. Sections of 3–5 μm were stained with haematoxylin-eosin and were analyzed by a pathologist.

2.3. Biochemical parameters

The following biochemical parameters were analysed to check the hepatoprotective activity of EO and CHY by the methods given below. Lipid peroxide levels in serum and liver were estimated using thiobarbituric acid (Ohkawa et al., 1979; Yagi, 1984). Protein was analysed following the method of Lowry et al. (1951) Activities of glutamate-pyruvate transaminase (GPT) (Bergmeyer and Bernt, 1980) and alkaline phosphatase (ALP) (King and Armstrong, 1980) were determined in both serum and liver tissue. Liver hydroxyproline was estimated by chloramine T oxidation and further reaction with *p*-dimethylaminobenzaldehyde (Kivirikko et al., 1967).

2.4. Statistical analysis

All the values are expressed $\pm$ standard deviation. The statistical difference was analysed by Student's *t*-test and significance was calculated as the *P* value and *P* values of less than 0.05 were regarded as significant.

Table 1
Effect of EO and CHY on serum and tissue lipid peroxides of rats treated with CCl_4 (acute toxicity)[a]

Treatment	Lipid peroxide	
	Serum (nmols/ml)	Liver (nmols per mg protein)
Normal	1.9 ± 0.2	1.7 ± 0.2
CCl_4 alone	3.4 ± 0.8	4.3 ± 0.4
CCl_4 + EO (500 mg)	1.9 ± 0.2*	2.0 ± 0.3**
CCl_4 + CHY (1 g)	2.3 ± 0.3*	3.1 ± 0.2**

[a] CCl_4: liquid paraffin (1:1) (0.25 ml/100 g b.wt) was given to rats i.p. EO (500 mg/kg b.wt.) and CHY (1 g/kg b.wt.) were administered orally.
* $P < 0.025$.
** $P < 0.001$ relative to CCl_4 treatment.

3. Results

3.1. Effect of EO and CHY extracts on acute CCl_4 toxicity

The effect of EO and CHY on acute CCl_4 toxicity are shown in Table 1. Acute CCl_4 administration increased serum lipidperoxide to 3.4 ± 0.8 nmols/ml as compared to normal value which was 1.9 ± 0.2 nmols/ml. Administration of EO (500 mg/kg b.wt.) and CHY (1 g/kg b.wt.) significantly reduced these elevated levels to 1.9 and 2.3 nmols/ml respectively. Acute CCl_4 administration increased liver lipid peroxide from the normal value of 1.7 ± 0.2 nmols/mg protein to 4.3 ± 0.4 nmols/mg protein. EO and CHY reduced these levels to 2.0 and 3.1 nmols/mg protein respectively. Similarly, serum and tissue levels of GPT and ALP were significantly increased by acute CCl_4 administration (Table 2). Serum GPT was increased to 1619 U/ml compared to the normal value of 277 U/ml, which was reduced significantly to 1219 and 1446 U/ml by the treatment of EO and CHY respectively. Tissue GPT was increased to 843 U/mg protein compared to the normal value of 783 U/mg protein, which was significantly reduced by CHY treatment. Serum and tissue ALP levels were increased to 52 KA/100 ml and 25.0 KA/mg protein $\times 10^{-3}$ respectively, compared to the normal value of 14 and 5.2. Administration of EO reduced these levels significantly.

Histopathological analysis showed diffused vacuolation of parenchyma cells in CCl_4 treated animals.The vacuoles were of different sizes and occasionally appeared as confluent areas. Acute cell swelling and individual cell necrosis were also present. These changes were minimized by EO and CHY treatment.

3.2. Effect of EO and CHY on chronic CCl_4 induced toxicity

Effect of EO and CHY on serum and tissue lipid peroxide levels in rats induced by chronic administration of CCl_4 is shown in Table 3. CCl_4 alone treated animals had increased serum lipid peroxide to 3.6 nmols/ml, compared to normal (1.9 nmols/ml) EO (250 mg/kg b.wt) and CHY (2.5 g/kg b.wt.) significantly reduced these levels.

Table 2
Effect of EO and CHY an serum and tissue levels of GPT and ALP of rats treated with CCl_4 (acute toxicity)[a]

Treatment	GPT		ALP	
	Serum (U/ml)	Liver (U/mg protein)	Serum (KA/100 ml)	Liver (KA/mg protein $\times 10^{-3}$)
Normal	277 ± 32	783 ± 15	14 ± 1	5.2 ± 2
CCl_4 alone	1619 ± 42	843 ± 6	52 ± 18	25 ± 4
CCl_4 + EO (500 mg)	1219 ± 28**	826 ± 27	27 ± 13*	17 ± 3*
CCl_4 + CHY (1 g)	1446 ± 14**	773 ± 10**	34 ± 11	15 ± 2**

[a] Experimental protocol is as given in Table 1.
* $P < 0.025$.
** $P < 0.001$.

Table 3
Effect of EO and CHY on serum and tissue levels of lipid peroxide of rats treated with CCl_4 (chronic toxicity)[a]

Treatment	Lipid peroxide	
	Serum (nmols/ml)	Liver (nmols/mg protein)
Normal	1.9 ± 0.2	1.7 ± 0.2
CCl_4 alone	3.6 ± 0.3	5.8 ± 1.0
CCl_4 + EO (50 mg)	3.4 ± 0.2	5.0 ± 1.2
CCl_4 + EO (250 mg)	2.3 ± 0.6**	4.2 ± 0.6*
CCl_4 + CHY (2.5 g)	1.8 ± 0.2**	4.9 ± 0.5

[a] CCl_4: liquid paraffin (1:7) (0.15 ml/100 g b.wt) was given to rats i.p., three times weekly for a total of 20 doses. EO (50 mg and 250 mg/kg b.wt.) and CHY (2.5 g/kg b.wt.) extracts were administered orally.
* $P<0.01$.
** $P<0.001$.

Tissue lipid peroxide was increased to 5.8 nmols/mg protein compared to the normal value of 1.7. Administration of EO (250 mg/kg b.wt.) significantly reduced these levels. Chronic administration of CCl_4 increased serum GPT and ALP levels significantly (Table 4). EO (250 mg/kg b.wt.) and CHY (2.5g/kg b.wt.) treatment significantly reduced these levels, while administration of 50 mg/kg b.wt. of EO reduced serum GPT but did not have any effect in reducing serum ALP. Increased tissue levels of GPT and ALP by chronic CCl_4 administration were significantly reduced by EO (250 mg/kg) b.wt. CHY (2.5 g/kg b.wt.) treatment significantly reduced tissue ALP and did not reduce tissue GPT.

Table 5
Effect of EO and CHY on liver collagen hydroxyproline of rats treated with CCl_4 (chronic toxicity)[a]

Treatment	Hydroxyproline (nmols/mg protein)
Normal	0.6 ± 0.3
CCl_4 alone	4.6 ± 0.2
CCl_4 + EO (50 mg)	5.0 ± 0.7
CCl_4 + EO (250 mg)	3.8 ± 0.2*
CCl_4 + CHY (2.5 g)	4.4 ± 0.2

[a] Experimental protocol is as given in Table 3.
* $P<0.001$.

Chronic administration of CCl_4 increased liver hydroxyproline levels significantly to 4.6 nmols/mg protein compared to the normal value of 0.6 (Table 5). EO 250 mg/kg b.wt. significantly decreased CCl_4 induced hydroxyproline levels.

Histopathological analysis are in good agreement with biochemical changes. Chronic CCl_4 treatment caused severe changes in the liver. Proliferation of fibroblasts replaced the hepatic parenchyma cells in focal areas. Large vacuolar spaces could be seen in the hepatocytes, and only the nucleus was retained in such cells. Focal dilated blood vessels and proliferation of few bile ducts were also observed. Extensive fat change

Table 4
Effect of EO and CHY on serum and tissue levels of GPT and ALP of rats treated with CCl_4 (chronic toxicity)[a]

Treatment	GPT		ALP	
	Serum (U/ml)	Liver (U/mg protein)	Serum (KA/100 ml)	Liver (KA/mg protein) $\times 10^{-3}$
Normal	277 ± 32	783 ± 15	13.8 ± 1.4	5.2 ± 0.3
CCl_4 alone	2521 ± 126	895 ± 57	41.2 ± 12.5	17.0 ± 0.2
CCl_4 + EO (50 mg)	1430 ± 235***	833 ± 41	34.1 ± 5.7	16.0 ± 0.5
CCl_4 + EO (250 mg)	742 ± 97***	794 ± 82*	15.8 ± 6.0**	12.0 ± 0.2**
CCl_4 + CHY (2.5 g)	802 ± 51*	860 ± 21	22.3 ± 3.1**	12.0 ± 0.4***

[a] Experimental protocol is as given in Table 3.
* $P<0.05$.
** $P<0.01$.
*** $P<0.001$.

and centrilobular necrosis were also observed. EO and CHY reduced these effects and caused only hydropic degeneration in the centrilobular region.

4. Discussion

The results of the present study demonstrate that the various biochemical changes produced in the liver and serum by acute and chronic CCl_4 toxicity were reversed or prevented by administration of EO and CHY. The toxicities produced by certain hepatotoxins have been postulated to be due to the formation of chemically reactive metabolic products. Free radical mediated reactions are involved in the inflammatory response and can contribute to liver necrosis (Gressner, 1991).

Aqueous extracts of EO and CHY were found to be potent antioxidants in vitro (Jeena and Kuttan, 1995). Lipid peroxidative processes and related aldehydic end products could be involved in mediating chronic poisoning and thus be able to affect biological phenomena during the development of chronic liver damage leading to fibrosis and eventually cirrhosis (Esterbauer, 1985). Acute and chronic CCl_4 administration increased lipid peroxide levels in serum and liver tissue. It has been reported that lipid peroxidation could stimulate collagen synthesis by fibroblasts (Slater, 1984) and hence the hydroxyproline levels. Pathological analysis supports the hepatoprotective activity of EO and CHY extracts.

The present study indicated that EO and CHY counteracted the increased lipid peroxide levels induced by acute CCl_4 treatment and offered partial protection against increase in GPT and ALP levels. There was also a similar tendency after chronic CCl_4 treatment and serum GPT and ALP were, in most instances, found to be significantly reduced. While higher doses of EO appears to afford partial protection against CCl_4-induced enzyme changes and liver hydroxyproline, lower doses were without any significant effect. CHY, in addition, was without effect.

EO is a constituent of various liver tonics use against acute viral hepatitis and other liver disorders (Antarkar et al., 1980; Handa et al., 1986). Antioxidants such as vitamin E ellagic acid (Thresiamma and Kuttan, 1996) and curcumin (Nishigaki et al., 1992) have been reported to protect liver injury and fibrosis induced by hepatotoxins. Recently Ghosal et al. (1996) have reported the isolation and antioxidant activities of new hydrolysable tannins, Emblicanin A and B from its fruit pulp. The hepatoprotective effect of EO and CHY extracts were related mostly to their reported antioxidant properties (Jeena and Kuttan, 1995). Protection against oxidative damage, liver necrosis and collagen deposition could be obtained by EO and CHY.

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