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Hepatoprotective activity of ellagic acid against carbon tetrachloride induced hepatotoxicity in rats*

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Administration of CCl_4 to normal rats and consequent oral feeding with ellagic acid (50 mg/kg) provided a significant protection against the biochemical alterations in serum and liver produced by CCl_4. *In vitro* experiments showed that liver microsomes from animals treated with ellagic acid and CCl_4, decreased lipid peroxidation compared to microsome prepared from rats exposed to CCl_4 alone.

Ellagic acid (EA), a naturally occurring polyphenol found in many plant products, is known to effectively protect against oxidative stress[1]. Carbon tetrachloride (CCl_4) has been extensively studied as a liver toxicant and its metabolites such as trichloromethyl radical (CCl_3^-) and trichloromethyl peroxy radical ($CCl_3O_2^-$) are involved in the pathogenesis of liver damage[2]. Feeding of EA increased the levels of reduced glutathione (GSH) and glutachione reductase (GSSG-reductase) in the liver of normal mice[3]. Present study has been undertaken to investigate the effect of oral administration of ellagic acid on CCl_4 induced lipid peroxidation in rats.

Adult male rats of Charles Foster strain weighing about 200 g, were maintained on pellet diet. The rats were divided into 3 groups namely Control, CCl_4 treated and CCl_4 + drug treated containing six animals in each. Two groups of rat were injected (ip) CCl_4 without any vehicle (0.7ml/kg) thrice a week for two weeks[4]. Of these two, one group received ellagic acid macerated with 2% aqueous gum acacia suspension and given orally by feeding canula once daily at a dose of 50 mg/kg for 15 days. Normal healthy rats, fed with same amount of gum acacia served as control. After completion of experimental regimen the rats were fasted overnight and samples of blood collected from retro-orbital plexus. The animals were sacrificed, and liver excised immediately. The blood was centrifuged and serum was assayed for alkaline phosphatase[5], glutamate oxaloacetate transminase (GOT)[6], and glutamate pyruvate transminase (GPT)[6], and bilirubin[7] by standard spectrophotometric methods. Liver was homogenized in Potter-Elvenhjem type homogenizer to a concentration of 10% with ice cold KCl (0.15 *M*) and centrifuged in cold for separation of mitochondrial, microsomal and cytosolic fraction[8]. The enzyme activities of glutathione-S-transferase (GST)[9], glutathione reductase (GSSG-reductase)[10], glutathione peroxidase ($GSHP_x$)[11] and the levels of reduced glutathione (GSH)[12] and oxidised glutathione (GSSG)[12] were assayed in cytosolic fraction. Microsomes were used for the assay of nonenzymic and enzymic lipid peroxidation *in vitro* as described earlier[13]. Superoxide dismutase (SOD)[14] activity was assayed in post mitochondrial supernatant while lipid peroxide[15] and glycogen[16] were estimated in total homogenate.

Student's t test was used for the statistical evaluation of results. The difference between mean was considered significant when $P< 0.05$.

The changes in the level of serum and liver biochemical markers of hepatic damage induced by CCl_4 administration in rats and their recovery by the treatment with EA are shown in Table 1. Increased activity of serum enzymes such as alkaline phosphatase, GOT, GPT and the level of bilirubin followed by depletion of hepatic GSH indicated a damage to liver cell membrane due to CCl_4 treatment. Administration of EA promoted the conversion of GSSG into GSH by the reactivation of hepatic GSSG-reductase enzyme in CCl_4 treated animals. The availability of a sufficient amount of GSH thus increased the detoxification of active metabolites of CCl_4 through the involvement of GST and $GSHP_x$. Treatment with EA is reported to increase the total hepatic GST activity via induction of GST-Ya gene and inhibit the xenobiotic metabolizing enzymes[17,18].

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Table 1—Serum and liver biochemical parameters in ellagic acid treated rats damaged with CCl_4
[Values are mean ± SD of 6 rats]

Parameters	Control	CCl_4 Treated	CCl_4+ellagic acid treated
Serum			
Alkaline-phosphatase[a]	42.09± 4.57	59.91 ±3.92***	49.67 ±1.69***
GOT[b]	67.74± 5.08	97.86 ±9.21***	83.05 ± 8.12*
GPT[b]	49.23±6.91	122.62±12.97***	99.4±6.63**
Bilirubin[c]	0.59±0.06	1.10±0.08***	0.85±0.67***
Liver			
GSH[d]	4.15±0.30	2.62±0.18**	3.00± 0.25*
GSSG[d]	0.220±0.06	0.378±0.03***	0.307±0.030***
GST[e]	2054±92.2	1257±57.2***	1667±51.3***
GSSG-reductase[f]	41.18±0.96	32.64±2.19*	39.67±3.82***
$GSHP_x$	337.16±34.4	223.18±14.2***	272.06±12.4***
Lipid peroxide[g]	326.43±30.15	443.84±43.46**	336.28±19.8***
SOD Activity[h]	2.90±0.25	1.86±0.06***	2.62±0.27***
Glycogen[i]	38.70±3.49	25.15±3.17**	28.50±3.17**

Units: [a]μmole p-nitrophenol/min/dl; [b]μmole sodium pyruvate/min/L; [c]mg/dl; [d] μmole/g; [e] nmole CDNB Complexed/min/mg/protein; [f] n mole NADPH oxidised/min/mg/protein; [g] nmole MDA/g; [h] unit/mg protein; [i] mg/g.
P values: * < 0.05; ** < 0.01; *** < 0.001

Liver microsomes of CCl_4 treated rats were more prone to peroxidation of their lipids (Table 2). Ellagic acid treatment made them less susceptible against peroxide damage. Ito *et al.*[19] and Cholbi *et al.*[20] have reported that hepatoprotective activity of EA and some polyphenols are apparently due to their antioxidative effects and results presented here are further supportive of this conclusion.

Table 2—Effect of ellagic acid on hepatic microsomal lipid peroxidation in CCl_4+ treated rats
[Values are mean ± SD of 6 rats]

In vitro parameters	Control	CCl_4	CCl_4 ellagic acid
None (without prooxidant added)	1.86±0.04	3.17±0.14	2.49±0.14
Fe^{+2}-ascorbate induced system (nonenzymic peroxidation)	3.09±0.18	4.31±0.39	3.38±0.24
NADPH Induced system (enzymic peroxidation)	3.29±0.20	5.26±0.24	3.73±0.45*

Unit: nmole MDA/mg protein.
P values: * <0.01; all other values in CCl_4 and treated group. *P*< 0.001.

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