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# Protective effect of ellagic acid on t-butyl hydroperoxide induced lipid peroxidation in isolated rat hepatocytes*

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Ellagic acid, a plant polyphenol, showed protective effect on isolated rat hepatocytes against destruction due to lipid peroxide formation induced by t-butyl hydroperoxide *in vitro*. Ellagic acid inhibited the generation of superoxide anions and hydroxyl radicals both in enzymic and non enzymic systems, thus providing protection against oxidative damage.

Lipid peroxidation results in severe damage to cellular membranes, and organelles in biochemical systems[1]. Ellagic acid (EA) is a naturally occurring polyphenol which is present in certain food items and is known to bring about effective protection against oxidative stress[2]. In the present study inhibitory effect of EA has been evaluated on generation of superoxide anions ($O^{\cdot}_2$) and hydroxyl radicals ($OH^{\cdot}$) and consequent formation of lipid peroxide induced by tert-butyl hydroperoxide (t-butyl-OOH) with isolated rat hepatocytes as *in vitro* model.

Male adult rats of Charles Foster strain (200-250g) were used for isolation of hepatocytes by collagenase as described by Seglen[3] with some modifications[4]. These hepatocytes were suspended in buffer containing NaCl 0.83%, KCl 0.05%, HEPES 0.24%; *p*H 7.4 and viable cells identified using trypan blue solution (0.02% w/v) in methanol. A portion of cell suspension was precipitated with 10% TCA and precipitate assayed for protein. Remaining hepatocytes suspension was then diluted with same buffer to a final concentration of 10 mg protein/ml. The reaction mixture containing 0.1 ml hepatocytes and 10 µl of 100-fold diluted t-butyl-OOH (70% v/v aqu soln) in absence or presence of EA in a final volume of 1.5 ml in above HEPES buffer, was incubated at 37°C for 4 hr. The viability of incubated hepatocytes was determined using trypan blue dye. The reaction mixture was delipidated with $CHCl_3$: $CH_3OH$ (2:1) mixture and the delipidated fraction assayed for lipid peroxide[5].

The effect of EA on generation of $O^{\cdot}_2$ in both nonenzymic and enzymic systems was investigated[6]. Hydroxyl radicals generated enzymically[7] in the absence or presence of varying concentrations of EA, was evaluated as 2, 3-dihydroxy benzoate formed by $OH^{\cdot}$ mediated hydroxylation of salicylate which was estimated spectrophotometrically. In another set of experiment $OH^{\cdot}$ generated nonenzymatically[7] was assayed for MDA[5].

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

Normal hepatocytes when incubated alone, exhibited a slight change in viability and lipid peroxide content. However, when these hepatocytes were incubated in presence of t-butyl-OOH, the level of lipid peroxide in reaction mixture significantly increased followed by a decrease in the viability of the cells (Table 1). In presence of EA lipid peroxidation was significantly suppressed with an increase in the

Table 1—Effect of ellagic acid on lipid peroxidation in rat hepatocytes induced by t-butyl hydroperoxide
[Values are mean ± SD of 4 separate observations]

| Ellagic acid (µmole) | Unincubated lipid peroxides (nmole MDA/mg mg protein) | Cell viability (%) | Incubated lipid peroxides (nmole MDA/mg protein) | Cell viability (%) |
|---|---|---|---|---|
| 0.00 | 1.35±0.08 | 94± 8 | 2.33±0.20[b] | 80±7[a] |
| 0.10 | 1.35 ± 0.06 | 94± 6 | 2.16±0.18[b] | 81± 8[a] |
| 0.15 | 1.34±0.12 | 94±6 | 1.98±0.20[b] | 82± 8[a] |
| 0.20 | 1.32 ± 0.10 | 95±8 | 1.78 ± 0.15[b] | 86 ± 9[NS] |
| 0.25 | 1.31±0.09 | 95 ± 9 | 1.70±0.13[b] | 87 ± 8[NS] |

*P* values: [a]< 0.05; [b]< 0.01; [NS]non significant

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Table 2—Effect of ellagic acid on generation of superoxide anions *in vitro*
[Values are mean±SD of 4 separate observations]

| Ellagic acid (µmole) | NADH-PMS NBT system system (nmoles formazone/ min) | Xanthine-Xan.oxidase system (n moles uric acid/ min) | Xanthine-Xan oxidase NBT system (nmoles formazone/ min) |
|---|---|---|---|
| 0.00 | 129.3±14.8 | 21.12±1.16 | 190.3±6.2 |
| 0.01 | 112.5±9.5 | 17.38±1.97[a] | 169.7±10.6[NS] |
| 0.05 | 103.5±7.3[a] | 13.19±0.24[b] | 158.9±9.9[a] |
| 0.10 | 64.1±3.9[c] | 10.57±0.70[c] | 133.3±13.0[b] |
| 0.15 | 37.3±2.7[c] | 7.85±0.18[c] | 85.87±8.1[c] |
| 0.20 | 25.0±0.6[c] | 6.24±0.24[c] | 60.76±2.4[c] |
| 0.25 | 5.4±0.8[c] | 4.38±0.55[c] | 37.2±3.8[c] |

*P* values: [a]< 0.05; [b]< 0.01; [c]< 0.001; [NS]non significant

Table 3—Effect of ellagic acid on generation of hydroxyl radical *in vitro*
[Values are mean±SD of 4 separate observations]

| Ellagic acid (µmole) | $Fe^{+2}$-sodium ascorbate $H_2O_2$system (n mole MDA/hr) | Hypoxanthine-Xan oxidase+$Fe^{+2}$ system (nmole 2,3 dihydroxy benzoate/hr) |
|---|---|---|
| 0.00 | 23.75±1.96 | 593.2±11.6 |
| 0.01 | 16.79±2.13[b] | 316.5±42.5[NS] |
| 0.05 | 3.99±0.21[c] | 462.3±26.7[a] |
| 0.10 | 2.76±0.30[c] | 346.7±25.6[b] |
| 0.15 | 2.73±0.28[c] | 265.3±26.0[c] |
| 0.20 | 2.62±0.25[c] | 231.0±14.4[c] |
| 0.25 | 2.49±0.10[c] | 223.0±16.2[c] |

*P* values: [a]< 0.05; [b]< 0.01; [c]< 0.001; NS = non significant

number of viable hepatocytes in concentration dependent manner (Table 1). Tertery butyl-OOH is a redox active compound which produces direct oxidative effects on biological materials to initiate the reaction of lipid peroxidation[8]. EA has been reported to suppress the formation of lipid peroxide in many other systems *in vivo*[9,10] and *in vitro*[11,12]. EA potentially inhibited the generation of $O^{\cdot}_2$ and $OH^{\cdot}$ in both enzymic and nonenzymic systems *in vitro* probably by its metal chelating property thus providing protection against lipid peroxidation (Table 2 and 3). In conclusion, ellagic acid possess the properties of a potent free radical scavenger which suggests its usefulness as a drug for antioxidant therapy.

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