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Alcohol-induced oxidative stress in rat liver microsomes: Protective effect of *Emblica officinalis*

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Abstract

The protective effect of *Emblica officinalis* fruit extract (EFE) against alcohol-induced oxidative damage in liver microsomes was investigated in rats. EFE (250 mg/kg b.wt/day) and alcohol (5 g/kg b.wt/day, 20%, w/v) were administered orally to animals for 60 days. Alcohol administration significantly increased lipid peroxidation, protein carbonyls with decreased sulfhydryl groups in microsomes, which were significantly restored to normal levels in EFE and alcohol co-administered rats. Alcohol administration also markedly decreased the levels of reduced glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) in the liver microsomes, which were prevented with EFE administration. Further, alcohol administration significantly increased the activities of cytochrome P-450, Na^+/K^+ and Mg^{2+} ATPases and also membrane fluidity. But, administration of EFE along with alcohol restored the all above enzyme activities and membrane fluidity to normal level. Thus, EFE showed protective effects against alcohol-induced oxidative damage by possibly reducing the rate of lipid peroxidation and restoring the various membrane bound and antioxidant enzyme activities to normal levels, and also by protecting the membrane integrity in rat liver microsomes. In conclusion, the polyphenolic compounds including flavonoid and tannoid compounds present in EFE might be playing a major role against alcohol-induced oxidative stress in rats.

Keywords: Alcohol; CYP-450; *Emblica officinalis*; Microsomes; Oxidative stress

1. Introduction

Liver is the principle organ responsible for alcohol metabolism and is more susceptible to alcohol-induced toxicity. Oxidative stress has been suggested as key factor capable of both initiating and sustaining the mechanisms of pathogenesis leading to alcohol liver disease (ALD) [1]. As the main source of reactive oxygen species (ROS), hepatic microsomes are susceptible to ROS attack, especially upon cytochrome P-450 2E1 (CYP-450) activation by ethanol [2]. ROS generated from ethanol metabolism can directly damage cell membranes by peroxidation of membrane polyunsaturated fatty acids. Increased lipid peroxidation changes the activities of various membrane bound enzymes. Especially ATPases are very sensitive to peroxidation reactions and membrane fluidity [3]. Chronic alcohol consumption also alters redox thiol status which might have biological relevance and contribute to the pathologies associated with several disease states. The free radicals generated during ethanol metabolism interact with proteins, lipids and DNA and thereby forming adducts [4]. Adduct formation may lead to interference with protein function, stimulation of fibrogenesis and induction of immune responses, which finally contributes to the progression of alcohol-induced liver diseases [5,6].

Alcohol-induced oxidative stress has been found mainly due to an increased production of oxygen free radicals and lower cellular antioxidant levels [7]. The endogenous antioxidant enzymes are responsible for the detoxification of deleterious oxygen radicals. Antioxidants play an important

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role to protect liver against alcohol-induced damage caused by reactive oxygen species [8]. Dietary phytochemicals are capable of removing free radicals. Among them, phenolic and polyphenolic compounds, such as flavonoids and tannins in edible fruits, exhibit potent antioxidant activities. Therefore, the possibility of preventing the onset of alcohol toxicity using herbal medicines has attracted considerable attention [9].

Literature mapping reveal that the fruit of *Emblica officinalis*, commonly known as amla, is a potential source with many therapeutic principles and has been used in more than hundred herbal formulations of Ayurveda, an Indian traditional medicine [10]. Studies showed that *Emblica* contains more than 20 hydrolyzable tannins [11]. The fruit contains flavonoids (*e.g.* kaempherol-3-β-O-D-glucoside, quercetin-3-O-β-glucoside), polyphenols (*e.g.* emblicanin A and B, punigluconin and pedunculagin) and also contains phyllantine and zeatin alkaloids, and a number of benzenoids including amlaic acid, corilagin, ellagic acid, 3-6-di-O-galloyl-glucose, ethyl gallate, 1,6-di-O-galloyl-β-D-glucose, 1-di-O-galloyl-β-D-glucose, putranjivain A, digallic acid, phyllemblic acid, emblicol, and alactaric acid and gallic acid [12–14]. *In vitro* and *in vivo* studies showed that EFE had strong antioxidant and radical scavenging activities against DPPH, $O_2^{\bullet -}$, $OH^{\bullet}$, and NO radicals [8]. Moreover, studies have shown that *Emblica* possess antidiabetic [15], hypolipidemic, anticancer [16], antiatherogenic [17], hepatoprotective [18,19] and neuroprotective properties [20]. Most of the reports suggest that these health effects could be attributed to the antioxidant activities of the EFE. Phenolic compounds, especially hydrolyzable tannins and flavonoids in combination with vitamin C, are considered to be the major antioxidants and bioactive components in EFE. Based on these considerations, we hypothesized that EFE may be a promising phytomedicine that could attenuate alcohol-induced oxidative stress in the liver microsomes.

2. Materials and methods

2.1. Chemicals

The chemicals used in the present study were procured from Sigma Chemical Co. (St. Louis, MO, USA) and SISCO Research Laboratories (Mumbai, India). Ethanol used for administration to rats was obtained by re-distillation. *E. officinalis* fruit extract dry powder (90.8% water soluble extractives including 49.5% tannins) was obtained from Chemiloids Ltd., Vijayawada, India (Manufacturers and exporters of herbal extracts).

2.2. Animals and experimental design

Two-month-old male albino Wistar rats, weighing about 120–140 g, were maintained in animal house with commercial pellet diet (Hindustan Lever Ltd., Bangalore, India) and tap water *ad libitum*. The animals were procured from Sri Venkateswara Enterprises, Bangalore, India, were divided into four groups of eight rats in each group. Group I control rats (C), which received glucose instead of alcohol (*i.e.* caloric equivalent to alcohol), group II (A), which received 20% (v/v) alcohol in water, group III rats (EFE), which received EFE alone in water, group IV rats (A + EFE), which received 20% (v/v) alcohol and then EFE after 8 h. Alcohol 5 g/kg b.wt/day and EFE 250 mg/kg b.wt/day was administered through stomach tube daily to each rat for 60 days. The dose of *Emblica* fruit extract was fixed based on earlier reports [8]. Food and water intake of all the animals was recorded daily and weight of rats was followed on alternate days. At the end of the experimental period, the rats in each group were fasted overnight and then sacrificed by cervical dislocation. Tissues were collected and processed immediately for further analysis. Institutional ethical committee approved this study.

2.3. Preparation of rat liver microsomes

Rat liver microsomes were prepared according to the method described previously [21]. Microsomes were prepared by centrifuging the post mitochondrial supernatant from the first 7000 × g centrifugation at 12,000 × g for 15 min. The pellet and the floating lipid were discarded, and the supernatant was centrifuged at 144,000 × g for 1 h for sedimentation of microsomes. All operations of preparation and subsequent fractionation were carried out at 4 °C.

2.4. Measurement of TBARS, protein carbonyls and sulfhydryl groups

Thiobarbituric acid reactive species (TBARS) were measured by the formation of malondialdehyde [22]. Protein carbonyls were determined as described previously [23]. Protein sulfhydryl groups were measured using Ellman's reagent [24].

2.5. Determination of cytochrome P-450 activity

The activity of the CYP-450 was assayed as described previously [25]. Briefly, microsomal suspensions (1.0 mg protein/ml) in phosphate buffer treated with a few grains of sodium dithionite and take it in the reference and sample cuvettes (or in the two halves of the split cuvette) and record the baseline from 400 to 510 nm. The contents of the sample cuvette were transferred to new tube and bubble gently with carbon monoxide (CO) for 60 s. Transfer back to the sample cuvette and record the difference spectrum from 400 to 510 nm again. The difference in absorbance between 450 and 490 nm can then be used for the calculation of CYP-450 content using the extinction coefficient difference ($\Delta E_{450–490\,nm}$ of 91 cm^{-1} mM^{-1}).