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Zingiber officinale Roscoe prevents acetaminophen-induced acute hepatotoxicity by enhancing hepatic antioxidant status

T.A. Ajith *, U. Hema, M.S. Aswathy

Department of Biochemistry, Amala Institute of Medical Sciences, Amala Nagar, Thrissur, Kerala 680 555, India

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Abstract

A large number of xenobiotics are reported to be potentially hepatotoxic. Free radicals generated from the xenobiotic metabolism can induce lesions of the liver and react with the basic cellular constituents – proteins, lipids, RNA and DNA. Hepatoprotective activity of aqueous ethanol extract of *Zingiber officinale* was evaluated against single dose of acetaminophen-induced (3 g/kg, p.o.) acute hepatotoxicity in rat. Aqueous extract of *Z. officinale* significantly protected the hepatotoxicity as evident from the activities of serum transaminase and alkaline phosphatase (ALP). Serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT) and ALP activities were significantly ($p < 0.01$) elevated in the acetaminophen alone treated animals. Antioxidant status in liver such as activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase and glutathione-S-transferase (GST), a phase II enzyme, and levels of reduced glutathione (GSH) were declined significantly ($p < 0.01$) in the acetaminophen alone treated animals (control group). Hepatic lipid peroxidation was enhanced significantly ($p < 0.01$) in the control group. Administration of single dose of aqueous extract of *Z. officinale* (200 and 400 mg/kg, p.o.) prior to acetaminophen significantly declines the activities of serum transaminases and ALP. Further the hepatic antioxidant status was enhanced in the *Z. officinale* plus acetaminophen treated group than the control group. The results of the present study concluded that the hepatoprotective effect of aqueous ethanol extract of *Z. officinale* against acetaminophen-induced acute toxicity is mediated either by preventing the decline of hepatic antioxidant status or due to its direct radical scavenging capacity.

Keywords: Antioxidant; Free radicals; Hepatotoxicity; *Zingiber officinale*

1. Introduction

Considerable attention has been focused on the involvement of oxygen free radical (OFR) in various diseases during the last decade. Active oxygen molecules such as superoxide and hydroxyl radicals have been demonstrated to play important role in the inflammation process produced by ethanol, carbon tetrachloride or carrageenan (Yoshikawa et al., 1983; Halliwell and Parihar, 1984; Yuda et al., 1991). Despite the presence of strong antioxidant defense mechanism to counteract the OFR and to minimize the plausible oxidative damage, OFR dependent damage to DNA and other biomolecules accumulate during the lifetime of organisms. Many organs are capable of metabolizing chemicals to toxic reactive intermediates. Liver protects the body from potentially injurious substances (endotoxins) absorbed from the intestinal tract, as well as the toxic by-products of metabolisms. Metabolic activation of the chemicals by phase I enzymes of the drug metabolizing system produces electrophilic reactants, which can interact with nucleophilic group in the macromolecules including DNA. A large number of xenobiotics are reported to be potentially hepatotoxic.

Acetaminophen is a safe, effective and widely used analgesic–antipyretic drug. However, an overdose can induce severe hepatotoxicity in experimental animals and humans (Thomas, 1993). Despite the substantial efforts in the past, the mechanisms of acetaminophen (paracetamol)-induced liver cell injury are still incompletely understood.

* Corresponding author. Fax: +91 487 2307 969.
E-mail address: taajith@rediffmail.com (T.A. Ajith).

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Recent evidences suggest that reactive metabolite formation; glutathione depletion is some of the initiating events for the toxicity (Jaeschke et al., 2006). Natural products and their active principles as sources for new drug discovery and treatment of diseases have attracted attention in recent years. Herbs and spices are generally considered safe and proved to be effective against various human ailments. Their medicinal use has been gradually increasing in developed countries. *Zingiber officinale* Roscoe, commonly known as ginger, is one of the commonly used spices in India and around the world. It is an indispensable component of curry, belongs to Zingiberaceae family. We had recently reported the nephroprotective activity of aqueous ethanol extract of *Z. officinale* against cisplatin-induced acute renal toxicity in mice (Ajith et al., 2007). Since a high dose acetaminophen-induced hepatotoxicity was resulted from the generation of free radicals during its metabolism at liver, the possible protection by aqueous ethanol extract of *Z. officinale*, was evaluated and the results are presented in this paper.

2. Materials and methods

2.1. Animals

Female Spraque Dawly rat 160 ± 20 g were purchased from Small Animal Breeding Centre, Kerala Agricultural University, Mannuthy, Thrissur, Kerala, India and were kept for a week under environmentally controlled conditions with free access to standard food and water ad libitum. Animals were handled according to the rules and regulations of Institutional Animal Ethics committee (IAEC), Amala Cancer Research Centre, Amala Nagar, Thrissur, Kerala, India.

2.2. Chemicals

Pyridine (C_5H_5N), ethanol (C_2H_5OH), *n*-butanol ($CH_3(CH_2)_3OH$), disodium hydrogen phosphate (Na_2HPO_4), hydrogen peroxide (H_2O_2), dihydrogen potassium phosphate anhydrous (KH_2PO_4) and thiobarbituric acid were purchased from Merck, India Ltd., Mumbai, India. Sodium azide (NaN_3), reduced glutathione (GSH), 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB), nitroblue tetrazolium (NBT) and riboflavin were purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Acetaminophen (paracetamol) (Calpol; Welcome Pharmaceuticals Ltd., Mumbai, India) was purchased from Amala Cancer Hospital Pharmacy, Amala Nagar, Thrissur, Kerala, India.

All other chemicals and reagents used were analytical reagent grade.

2.3. Preparation of the extract

Rhizome of *Z. officinalis* was purchased from the local market. The rhizome (500 g) were cut into small pieces and homogenized in a kitchen mixer using 50% ethanol (v/v). The homogenate was kept on water bath at 70–80 °C for 10–15 h with intermittent shaking. The homogenate was centrifuged at 1500*g* for 10 min and the supernatant was collected. Solvent in the pooled supernatant was completely evaporated at low temperature using a water bath. The residue was designated as ethanol extract (6.5 g). The extract was pre-solubilised in distilled water for the *in vivo* studies.

2.4. Determination of hepatoprotective effect of Z. officinale

Animals were divided into four groups of six animals each. Group I treated with vehicle (distilled water) was kept as normal. Group II treated with a single dose of acetaminophen (AAP) (3 g/kg body wt, p.o.) was kept as control. Group III and IV were treated with extract of *Z. officinale* 200 and 400 mg/kg body wt plus AAP. The extract was administered by oral gavage 1 h before AAP administration. The animals were sacrificed 24 h after the administration of acetaminophen using ether anesthesia; blood was collected directly from the heart of each animal. Serum was separated for the estimation of the activities of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and alkaline phosphatase (ALP). Liver was dissected out for the determination of antioxidant status.

Serum SGOT, SGPT and ALP were determined by kinetic method using the kit of Agappae Diagnostic Ltd., India in a double beam spectrophotometer (Elico SL-164, Elico Limited, Hyderabad, India). The transaminases activities were determined as change in absorbance/min at 340 nm. Serum ALP activity was determined from the rate of release of paranitrophenol at 405 nm.

Livers were excised, washed thoroughly in ice-cold saline to remove the blood. They were then gently blotted between the folds of a filter paper and weighed in an analytical balance. Ten percent of homogenate was prepared in 0.05 M phosphate buffer (pH 7) using a polytron homogeniser at 20 °C. The homogenate was centrifuged at 3000*g* for 20 min to remove the cell debris, unbroken cells, nuclei, erythrocytes and mitochondria. The supernatant was used for the estimation of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) activities and the levels of reduced glutathione (GSH), lipid peroxidation, and total protein. SOD activity was determined from the ability of the tissue homogenate to scavenge the superoxide anion generated from the photo-illumination of riboflavin according to the method of Mc Cord and Fridovich (1969). Tissue CAT activity was determined from the rate of decomposition of H_2O_2 (Beers and Sizer, 1952). GPx activity was determined by measuring the decrease in GSH content after incubating the sample in the presence of H_2O_2 and NaN_3 (Hafemann et al., 1974). Reduced GSH was determined according to the method of Moron et al. (1979) based on the formation of a yellow colored complex with DTNB. GST activity was determined from the rate of increase in conjugate formation between reduced glutathione and CDNB (Habig et al., 1974). The level of lipid peroxidation was measured as malondialdehyde (MDA), a thiobarbituric acid reacting substance (TBARS), using 1′1′3′3′-tetramethoxypropane as standard (Ohkawa et al., 1979). Protein content in the tissue was determined (Lowry et al., 1951) using bovine serum albumin (BSA) as the standard.

2.5. Histopathological examination

Portions of the liver were fixed in 10% formalin and then embedded in paraffin. Microtome sections 5 μm thickness were prepared from each liver samples and stained with hematoxilin–eosin. The sections were examined for the pathological findings of hepatotoxicity such as centrilobular necrosis, fatty infiltration, fibrosis, lymphocyte infiltration, etc.

2.6. Statistical analysis

All data were represented as mean ± SD. Significant difference between the mean values were statistically analyzed using one-way analysis of variance (ANOVA) using InStat Graphpad software. The control group (AAP alone treated group) and the extract plus AAP treated groups were further analyzed by Dunnett's *t*-test. *P* values less than 0.05 were considered as significant.

3. Results

Serum activities of transaminases, SGPT and SGOT, and ALP were given in Table 1. Single dose of AAP significantly elevated SGPT and SGOT activities when compared to the normal animals. Treatment of aqueous ethanol extract of *Z. officinale* 1 h prior to AAP administration significantly protected the elevation of transaminases and