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

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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.

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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

Table 1
Effect of ethanol extract of *Z. officinale* (ZO) on serum GOT, GPT and ALP activities in rats with acute acetaminophen (AAP) administration

Groups	Treatment (mg/kg)	SGPT (IU/l)	SGOT (IU/l)	ALP (IU/l)
Normal	Vehicle	34.5 ± 3.5	71.3 ± 10.9	138.2 ± 3.1
Control (AAP)	3000	$112.8 \pm 17.2^{*}$	$133.3 \pm 11.1^{*}$	$274.3 \pm 44.2^{*}$
ZO + AAP	200	$87.2 \pm 3.5^{**}$	$107.3 \pm 9.4^{**}$	$205.2 \pm 13.4^{**}$
ZO + AAP	400	$74.9 \pm 13.0^{**}$	$87.0 \pm 9.8^{**}$	$167.0 \pm 9.9^{**}$

Values are mean ± S.D, $n = 6$ animals.
* $p < 0.01$ (Dunnett's *t*-test) significantly different from normal group.
** $p < 0.01$ (Dunnett's *t*-test) significantly different from control group.

ALP activities. The activities of SGPT and SGOT in the high dose *Z. officinale* plus AAP treated group were 74.9 ± 13.0 and 87.0 ± 9.8 IU/l, respectively. Similarly the activity of ALP was significantly ($p < 0.01$) decreased in the *Z. officinale* plus AAP treated group (167.0 ± 9.9 IU/l) than the control group (274.3 ± 44.2 IU/l).

Activities of hepatic SOD, CAT and GPx were provided in the Table 2. SOD and GPx activities were significantly ($p < 0.01$) enhanced only in the high dose of *Z. officinale* plus AAP treated group. However the hepatic CAT activity was found to be non-significant when compared to the control group. Moreover, the activities of CAT and GPx and level of GSH (Fig. 1) in the 200 mg/kg *Z. officinale* plus acetaminophen treated group were non-significantly differ from the control group. Hepatic MDA level was significantly ($p < 0.01$) elevated in the control group (196.0 ± 9.9 nmol/g tissue) than the normal animals (125.0 ± 6.5 nmol/g tissue) (Fig. 2). Treatment of *Z. officinale* prior to AAP significantly prevented the elevation of MDA. Further the activity of GST was enhanced and normalized in the *Z. officinale* (400 mg/kg) plus AAP treated animals (Fig. 3). The activities of GST in the

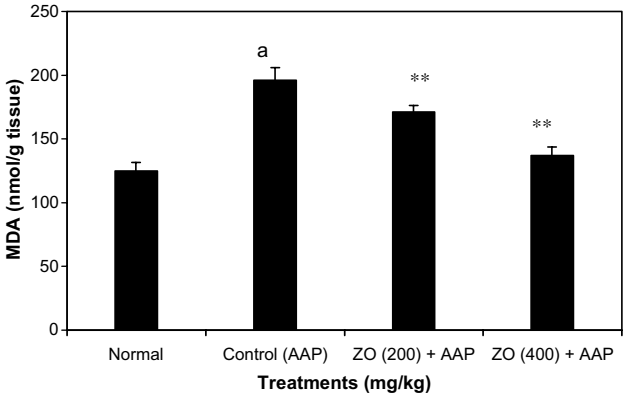

Fig. 2. Effect of ethanol extract *Z. officinale* (ZO) on hepatic MDA level in rat treated with acetaminophen (AAP). Values are mean ± S.D, $n = 6$ animals. $^{*}p < 0.01$ (Dunnett's *t*-test) significantly different from control group. $^{a}p < 0.01$ (Dunnett's *t*-test) significantly different from normal group.

Table 2
Effect of ethanol extract of *Z. officinale* (ZO) on hepatic SOD, CAT and GPx activities in rats with acute acetaminophen (AAP) administration

Groups	Treatment (mg/kg)	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)
Normal	Vehicle	19.30 ± 1.30	52.43 ± 3.38	25.34 ± 0.28
Control (AAP)	3000	14.16 ± 1.94^{b}	37.75 ± 7.43^{a}	20.20 ± 0.86^{a}
ZO + AAP	200	$20.11 \pm 4.27^{*}$	43.65 ± 1.35^{NS}	18.02 ± 2.04^{NS}
ZO + AAP	400	$19.79 \pm 3.77^{**}$	40.25 ± 5.23^{NS}	$24.26 \pm 3.00^{**}$

Values are mean ± S.D, $n = 6$ animals.
$^{**}p < 0.01$, $^{*}p < 0.05$ (Dunnett's *t*-test) significantly and NS non-significantly different from control group.
$^{a}p < 0.01$ and $^{b}p < 0.05$ (Dunnett's *t*-test) significantly different from normal group.

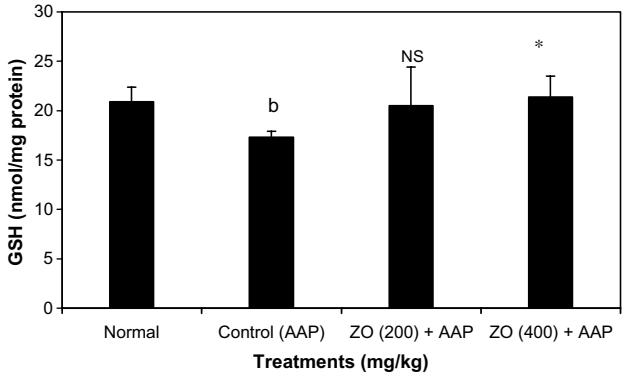

Fig. 1. Effect of ethanol extract *Z. officinale* (ZO) on hepatic GSH level in rat treated with acetaminophen (AAP). Values are mean ± S.D, $n = 6$ animals. $^{*}p < 0.05$ (Dunnett's *t*-test) significantly and NS non-significantly different from control group. $^{b}p < 0.05$ (Dunnett's *t*-test) significantly different from normal group.

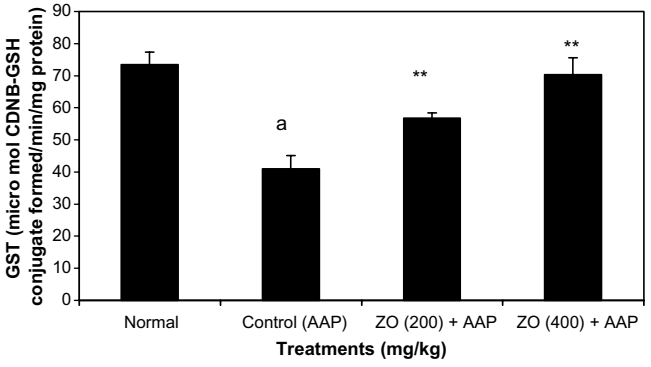

Fig. 3. Effect of ethanol extract *Z. officinale* (ZO) on the activities of renal GST in rat treated with acetaminophen (AAP). Values are mean ± S.D, $n = 6$ animals. $^{*}p < 0.01$ (Dunnett's *t*-test) significantly different from control group. $^{a}p < 0.01$ (Dunnett's *t*-test) significantly different from normal group.

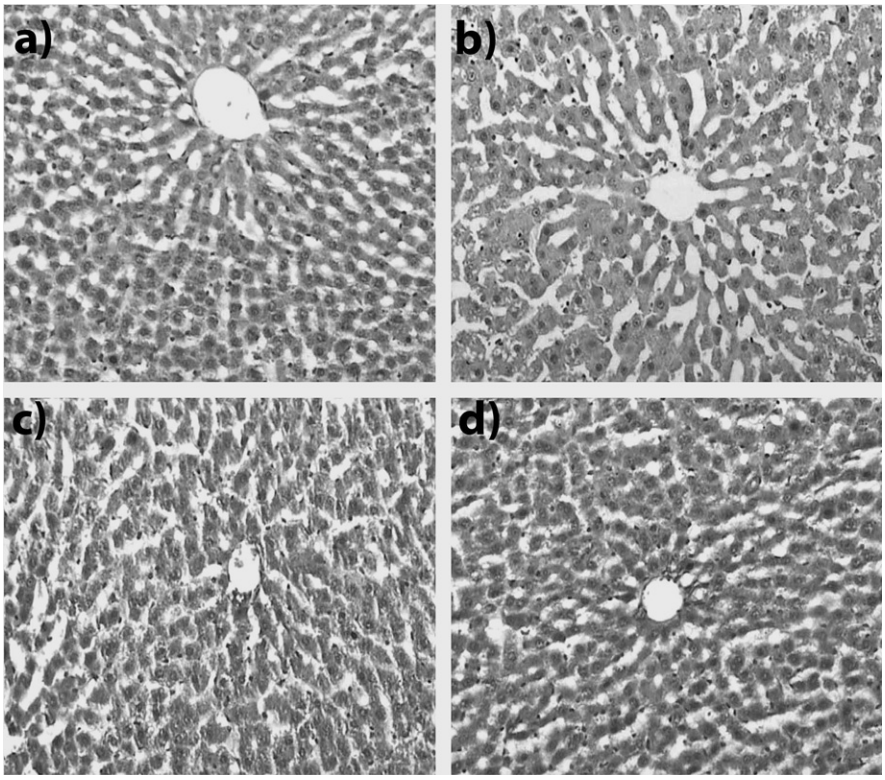

Fig. 4. Hepatoprotective effect of *Z. officinale* (ZO) against acetaminophen (AAP)-induced acute hepatotoxicity in rats. Liver sections were stained with H&E: (a) normal; (b) AAP; (c) ZO (200 mg/kg body wt) + AAP and (d) ZO (400 mg/kg body wt) + AAP. Magnification ×20.

normal and *Z. officinale* (400 mg/kg) plus AAP treated groups were 73.4 ± 3.4 and 70.0 ± 5.3 μmol CDNB-GSH conjugate formed/min/mg protein, respectively.

Histopathological analysis of the acetaminophen alone treated animal showed severe centrilobular necrosis, fatty infiltration and lymphocytes infiltration (Fig. 4). The findings were significantly decreased in the *Z. officinale* plus acetaminophen treated groups.

4. Discussion

Administration of a single high dose of AAP significantly ($p < 0.01$) elevated the serum transaminase and ALP activities compared to the normal animals. This indicated necrosis of hepatocytes that results in the leakage of transaminases and the elevation of serum ALP from a possible cholistasis. The significantly decreased serum transaminases and ALP activities in the *Z. officinale* administered groups prior to AAP demonstrated its hepatoprotective effect. However, a single high dose of aqueous ethanol extract of *Z. officinale* could produce only a partial protection. Hence, more prophylactic doses of extract of *Z. officinale* are required to render a complete protection.

Cytochrome P-450 (P-450) enzymes are the major catalysts involved in the metabolism of drugs. AAP is mainly metabolized by cytochrome P-450 to form an electrophilic metabolite, *N*-acetyl-*p*-benzoquinonimine, which is primarily inactivated by conjugation with glutathione (Orecnius and Moldeus, 1984; Dahlin et al., 1984). A large number of the metabolites produced by AAP are found to generate superoxide anion and other free radicals in the biological systems (Vries, 1984). However, at a higher dose of AAP, intermediate metabolites accumulate and cause liver damage. Depletion of glutathione beyond certain critical level can lead to oxidative stress and development of overt hepatotoxicity (Mitchell et al., 1973).

The reduced hepatic antioxidant status is related to oxidative stress and elevation of lipid peroxidation that resulted in the leakage of hepatic enzymes to serum in the AAP alone treated animals. Treatment of *Z. officinale* at 400 mg/kg plus AAP significantly enhanced the hepatic antioxidant activity including the hepatic GSH level compared to the AAP alone treated animals. The elevated hepatic reduced GSH level could partially explain the hepatoprotective mechanism of the *Z. officinale* at 400 mg/kg dose. Reduced GSH can function as a reductant in the metabolism of hydrogen peroxide and various organic peroxides. The GPx present in the cells can catalyze this reaction. Cighetti et al. (1993) reported that depletion of GSH below a threshold value was associated with a significant conversion of xanthine dehydrogenase to reversible xanthine oxidase, a superoxide radical generation reaction-catalyzing enzyme. Therefore the enhanced hepatic GPx and SOD activities in the high dose *Z. officinale* plus AAP treated group further support its hepatoprotective effect. The elevated antioxidant status in the liver of *Z. officinale* (400 mg/kg) plus AAP treated group is related to the decreased MDA level, could maintain the membrane integ-

rity and prevented the leakage of hepatic enzymes to serum. The histopathological analysis of liver section indicates a moderate centrilobular necrosis, fatty infiltration and lymphocytic infiltration in the high dose *Z. officinale* plus AAP treated animals with respect to the AAP alone treated animals.

Non-Se glutathione peroxidase activity was found to be decreased in the AAP treated animals that are associated with the increased steady-state level of hydrogen peroxide and hydroperoxides (Arnaiz et al., 1995). The decreased hepatic GST activity of the AAP alone treated animals in the present study could support the enhanced lipid peroxidation. Administration of *Z. officinale* plus AAP significantly and dose dependently elevated the hepatic GST activity and protected the liver toxicity. The enhanced GST activity, a phase II enzyme, can also explain the increased detoxification of the reactive metabolites generated from the AAP metabolism in the liver of *Z. officinale* treated animals.

Low dose of *Z. officinale* used in this study did not prevent the AAP-induced decline of hepatic activity of CAT, GPx and GSH level. However, this dose was effective to render hepatoprotection as evident from the decreased activities of serum transaminases; ALP and hepatic MDA level when compared to AAP alone treated animals. Further hepatic SOD and GST activities were significantly enhanced. Hence the protection at low dose of *Z. officinale* may be partially related to the significantly elevated hepatic GST and SOD activities. Reports had shown that silimarin, a 3-oxyflavone with antioxidant properties protects against AAP-induced liver damage, even at low levels of reduced GSH, possibly by scavenging the superoxide anion and alkoxyl radicals (Murel et al., 1992).

Most of the reactive metabolites generated from the drug metabolisms including the AAP metabolism are found to be highly electrophilic that can attack the cellular macromolecules. Previous studies have also demonstrated the significant *in vitro* radical scavenging activity of the *Z. officinale* extract (Kikuzaki and Nakatani, 1993; Masuda et al., 2004). This direct radical scavenging activity might also be involved in the exhibited hepatoprotective activity. In the fresh ginger rhizome, the gingerols (polyphenols) were identified as the major active components (Masuda et al., 2004). The volatile oil (2–3%) of ginger consists of mainly mono and sesquiterpenes; camphene, beta-phellandrene, curcumene (Kikuzaki et al., 1991). The protection from hepatic diseases in experimental animals had been achieved by administrating the chemopreventive agents that modulate the metabolic processing of xenobiotics include phenolic antioxidants, indoles, isothiocyanates, coumarins, flavanones, allyl sulfides, etc. (Kensler, 1997). The presence of antioxidant compounds in the *Z. officinale* extract might possibly be related to the exhibited hepatoprotective activity.

According to glossary produced by American Diabetics and Association, neutriceutics are substances considered as food or a part of it that offers health or medicinal benefits, including prevention and treatment of diseases (Bloch and Thomson, 1995). Some of the natural products find their use not as pharmaceuticals (real medicine) but as a novel class of dietary supplements or nutraceuticals that fall well into the concept of functional foods. Moreover ginger has been listed in ''Generally Recognised as Safe'' (GRAS) document of the US FDA. A dose of 0.5–1.0 g of ginger powder ingested 2–3 times for periods ranging from 3 months to 2.5 years did not cause any adverse effects (Langner et al., 1998). Hence hepatoprotective effect of *Z. officinale* demonstrated in this study may explore its nutraceutical role in human diet.

The results of the present study concluded that aqueous ethanol extract of *Z. officinale* significantly and dose dependently prevented the AAP-induced acute hepatotoxicity by enhancing the hepatic antioxidant activity. However, further detailed studies are required to establish its clinical application.

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