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ZINGIBER OFFICINALE ROSCOE CO-OPERATE ACETAMINOPHEN ACTION AND PREVENT HEPATOTOXICITY RESULTED IN ITS OVERDOSE IN EXPERIMENTAL RATS

Atef E. Abd El-baky† and El-Ibiari M. Hesham

ABSTRACT

Recently the controlling and treating diseases tend to prefer natural plants which provide drugs to maintain good health of human without undesirable side effects of xenobiotics. Zingiber officinale (Z. officinale) potentiate acetaminophen (AAP) action and have hepatoprotective effect against AAP overdose is the aim of this study. We utilized Z. officinale extract and APP in normal and inflamed experimental rats. The inflammation were induced by with formalin (one intraperitoneal (I.P) dose), hepatic toxicity induced by single oral high dose of APP (3 g/kg body weight). Also, we used single oral high dose of APP (1.5 g/kg body weight). Z. officinale extract was given as oral single dose (400 mg/kg) prior to AAP. After 24 h of Z. officinale extract and APP administration blood was collected directly from orbital sinus of each animal then animals were sacrificed using ether anesthesia; and their livers were collected. AAP were elevate serum transaminases (ALT, AST) and alkaline phosphatase (ALP) where this action protected by Z. officinale extract, while this extract was elevate antioxidant status in liver cells, as reduction of hepatic lipid peroxidation takes place. Prostaglandin and cyclooxigenase enzyme were enhanced by induction of inflammation while these decline after ingestion of Z. officinale extracts alone. This action was enhanced by of this extract and AAP in combination. The results of the present study concluded that Z. officinale have anti-inflammatory action and relive pain so, it was potentiate acetaminophen action. Also, it had hepatoprotective effect 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: Xenobiotics, Acetaminophen, Antioxidant, Zingiber officinale, Prostaglandin

INTRODUCTION

Acetaminophen is a widely used analgesic/antipyretic drug with low peripheral adverse effects, possibly related to its weak activity as inhibitor of peripheral cyclooxygenase (COX) and completely inhibition of prostaglandin E(2) [PGE(2)] synthesis (Greco et al., 2003).

Overdose of acetaminophen is a well-known cause of acute liver failure. therapeutic doses of acetaminophen is very unusual to from hepatotoxicity (Graham et al., 2005) and it can be taken in high doses safely (Richard et al., 2007; Graham et al., 2005; Tylenol 2006), even by patients with stable liver disease (Benson et al., 2005). Hepatotoxicity might develop following ingestion of >150 mg/kg over 8 hours or less (Tylenol 2006), but might occur with lower doses in patients at risk (Larson et al., 2005). The onset of analgesia is approximately 11 minutes after oral administration of paracetamol (Moller et al., 2005).

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 (Yuda et al., 1991). Despite the presence of strong antioxidant defense mechanism to counteract the oxygen free radical (OFR) and to minimize the plausible oxidative damage, OFR dependent damage to DNA and other biomolecules accumulate during the life time of organisms. Many organs are capable of metabolizing chemicals to more toxic reactive intermediates. Liver protects the body from potentially injurious substances 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. In spite of 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. Recent evidences suggest that reactive metabolite formation; glutathione depletion is one of the initiating 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 modern years. Herbs and spices are generally considered safe to be effective against various human ailments.

Biochemistry Department, Faculty of Pharmacy, Minia University, Egypt
†Corresponding author: atena2065@yahoo.com

Their medicinal use has been gradually increasing in developed countries. Zingiber officinale Roscoe, commonly known as ginger, is one of the commonly used spices 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; 2008). Irradiation of the animals resulted in a dose-dependent elevation in the lipid peroxidation and depletion of GSH on day 31 postirradiation; both effects were decreased when pretreatmented with Z. officinale extract (Jagetia et al., 2003). Ginger extracts showed selective anticancer activities (Leal et al., 2003) and may have potential in the treatment and prevention of ovarian cancer (Rhode et al., 2007).

Ginger extracts have been shown to inhibit joint swelling in an animal model of rheumatoid arthritis (Funk et al., 2009) and reduce knee pain in human subjects suffering from osteoarthritis (Altman and Marcussen 2001). This extract has a slightly effect in the treatment of nausea and vomiting of pregnancy (Portnoi et al., 2003; Vutyavanich et al. 2001).

Z. officinale extract improves insulin sensitivity (Goyal and Kadnur 2006), so it has great value in managing the effects of diabetic complications in human where it is effective in reversing the diabetic proteinuria that observed in the diabetic rats (Al-Amin et al., 2006). The extract also exhibit lipid lowering activity in diabetic rats (Bhandari et al., 2005) and cholesterol-lowering effect (Thomson et al., 2002).

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. In the 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).

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). The possible initiate action of acetaminophen by aqueous ethanol extract of Z. officinale and the expected mechanism of this extract in hepatic protection against acetaminophen overdose are the aim of this study.

MATERIALS AND METHODS

Materials:

Acetaminophen: It obtained from El Naser Pharmaceutical Chemicals Co. "ADWIC" Abu-Zaabal, (EGYPT).

Zingiber officinale extract:

Preparation of Zingiber officinale 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 1500xg 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 (Ajith et al., 2007).

Animals and Experimental Design: Sixty four adult male albino rats (180 ± 20) were used in this study and were purchased from the National Research Centre Cairo, Egypt. They were kept under constant experimental conditions with free access to food and water. They were left for one week for accommodation before starting the study. Animals were divided into two main groups and each group was divided into four subgroups (eight in each). During the period of experiment; animals were kept at 12 h light/12 h dark cycle.

Table 1.

Groups	Drug dose and administration route	Dosing reference
Group 1	Normal animals were divided into four subgroups (eight in each)	
Subgroup-1	Oral dose of saline vehicle only	
Subgroup-2	Single dose of aqueous extract of Z. officinale orally (400 mg/kg body weight)	Ajith et al., 2007
Subgroup-3	oral single high dose of acetaminophen (3 g/kg body weight)	Daly et al., 2008
Subgroup-4	oral single dose of aqueous extract of Z. officinale (400 mg/kg) prior to a high dose of acetaminophen (3 g/kg body weight)	
Group 2	Inflamed rats were divided into four subgroup (eight in each)	
Subgroup-A	S.C injection of single dose of formalin (50 µL) in to one hind paw of rats	Vaccarino and Couretn 1995, Okuda et al., 2001 and Gao et al, 2011
Subgroup-B	After one day of inflammation, oral single dose of Z. officinale extract (400 mg/kg body weight)	
Subgroup-C	After one day of inflammation, oral single low dose of acetaminophen (1.5 g/day)	Forget et al., 2011
Subgroup-D	After one day of inflammation, oral single dose of Z. officinale extract (400 mg/kg) with a low dose of acetaminophen (1.5 g/day).	
	The extract and AAP were administered by oral gavage.	

Animal Approval Committee

An approval was taken from the University committee resident in College of Medicine/Minia University. The groups were classified and dosed as follows (Table 1).

Blood Collection and Biochemical Analysis:

24 hours after acetaminophen and Z. officinale administration, blood was obtained from orbital sinus and allowed to coagulate and then centrifuged at 3000 *xg* for 15 min at 4°C. The separated sera were used for measurement of ALT, AST and ALP activities.

Determination of serum ALT and AST activity was done using a test reagent kit according to the method described by (Reitman and Frankel, 1957). Determination of serum ALP activity was carried out using a test reagent kit according to the method of (Kind and King 1954). In group II, determination of serum prostaglandin according to the method described by (Hamberg and Samuelsson 1971 & Ujihara et al., 1988) using ELISA Kit for Rat Prostaglandin (PGE_2), (Cat. No.: E0749Ra) (Uscn Life Science Inc. Wuhan) and cyclooxigenase enzyme according to the method described by (Zschocke and van Staden, 2000) using ELISA Kit for Rat cyclooxygenase-2 (COX-2**), (**Cat. No.: E0699Ra) (Uscn Life Science Inc. Wuhan).

Determination of hepatoprotective effect of Z. Officinale:

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 homogenizer at 20°C. The homogenate was centrifuged at 3000 *xg* 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) activities and the lipid peroxidation. 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). The level of lipid peroxidation was measured as malondialdehyde (MDA) (Ohkawa et al., 1979). All dry chemicals and solvents were obtained from Sigma Chemicals.

Pain induction and inflammation:

A subcutaneous (S.C) injection of 50 µL of formalin into one hind paw was used for induction of continuous pain and inflammation (Vaccarino and Couretn 1995, Okuda et al., 2001 and Gao et al, 2011).

Statistical Analysis

The data obtained were expressed as means (Mean ±SE), and analyzed using repeated measures of variance. The differences between the means were analyzed statistically with one-way analysis of variance (ANOVA) utilizing computerized statistical program (InStat).

RESULTS

Administration of Z. officinale extract (subgroup-2) had no effect on both serum activities of ALT, AST, ALP (Table 2) and had no effect on activities of hepatic SOD, CAT, GPx and MDA (Table 3) ($p < 0.001$) when compared to the normal animals (subgroup-1). Also, administration of this extract (subgroup-B) had no effect on both prostaglandin and cyclooxigenase enzyme (Table 4) ($p < 0.001$) as compared to the normal animals (subgroup-1). Single dose of AAP (subgroup-3) significantly elevated ALT, AST and ALP activities when compared to the normal animals (subgroup-1), while administration of Z. officinale extract one hour prior to AAP administration (subgroup-4) significantly protected the elevation of transaminases and ALP activities ($p < 0.001$) when compared to the control group (subgroup-1) table (2). Also, single dose of AAP (subgroup-3) significantly decreased hepatic SOD, CAT and GPx while MDA level was significantly elevated ($p < 0.001$) when compared to the normal animals (subgroup-1) and animals received Z. officinale extract (subgroup-2) (Table 3). Pretreated animals with Z. officinale extract one hour prior to AAP administration (subgroup-4) significantly elevated of hepatic SOD,

CAT and GPx while MDA level was decreased significantly ($p < 0.001$) when compared to the control group (subgroup-1) (Table 3).

Rats that received formalin (subgroup-A) were significantly elevated both prostaglandin and cyclooxigenase enzyme ($p < 0.001$) when compared to the normal animals (subgroup-1). The treated animals with Z. officinale extract alone (subgroup-B) and AAP alone (subgroup-C) significantly decreased prostaglandin and cyclooxigenase enzyme ($p < 0.001$) when compared to rats with inflammation (subgroup-A). The treated animals with Z. officinale extract & AAP in-combination significantly decreased prostaglandin and cyclooxigenase enzyme when compared to rats with inflammation (subgroup-A) ($p < 0.001$) and when compared to rats that treated with Z. officinale extract alone (subgroup-B) ($p < 0.05$) and AAP alone (subgroup-C) ($p < 0.01$) table (4).

Table 2. Effect of aqueous ethanol extract of Z. officinale on serum ALT, AST and ALP activities in rats with acute acetaminophen (AAP) administration.

Groups	ALT (IU/l)	AST (IU/l)	ALP (IU/l)
Subgroup-1	30.1±1.77	37.0±1.68	126.9±2.44
Subgroup-2	32.6±1.72	39.6±2.15	128.2±2.32
Subgroup-3	124.5±3.69*[a]	140.7±5.38*[a]	264.0±2.85*[a]
Subgroup-4	58.7±2.59[ab]	64.1±2.85[ab]	164.1±3.0[ab]

*Significantly different from normal group ($p < 0.001$).
[a]Significantly different from control-1 group ($p < 0.001$).
[b]Significantly different from control-2 group ($p < 0.001$).

Table 3. Effect of aqueous ethanol extract of Z. officinale on hepatic SOD, CAT, GPx and MDA activities in rats with acute acetaminophen (AAP) administration.

Groups	SOD (U/mg tissue)	CAT (U/mg tissue)	GPx (U/mg tissue)	MDA (nmol/g tissue)
Subgroup-1	25.4±0.92	57.9±1.59	26.4±1.23	60.6±2.2
Subgroup-2	25.9±0.71	56.5±1.67	28.4±0.96	61.2±2.63
Subgroup-3	14.2±0.90*[a]	27.7±1.59*[a]	15.2±1.14*[a]	110.6±4.47*[a]
Subgroup-4	22.8±1.17[b]	45.1±1.14[ab]	24.1±0.84[b]	72.5±2.31[b]

*Significantly different from normal group ($p < 0.001$).
[a]Significantly different from control-1 group ($p < 0.001$).
[b]Significantly different from control-2 group ($p < 0.001$).

Table 4. Effect of aqueous ethanol extract of Z. officinale and acetaminophen (AAP) administration on serum prostaglandin and cyclooxigenase enzyme.

Groups	Prostaglandin (ng/mL)	Cyclooxigenase enzyme (ng/mL)
Subgroup-1	0.10±0.015	12.7±0.68
Subgroup-A	0.74±0.036*	65.7±3.74*
Subgroup-B	0.18±0.014[#]	20.4±1.04[#]
Subgroup-C	0.21±0.017[#]	28.7±2.42[#]
Subgroup-D	0.098±0.007[#AB]	14.5±0.99[#B]

*Significantly different from normal group ($p < 0.001$).
[#]Significantly different from inflamed group ($p < 0.001$).
[A]Significantly different from ZO group ($p < 0.05$).
[B]Significantly different from AAP group ($p < 0.01$).

DISCUSSION

Acetaminophen (AAP) is a common analgesic and antipyretic drug that is used for the relief of fever, headaches, and other minor aches and pains. AAP is metabolized primarily in the liver, where most of it (60-90% of a therapeutic dose) is converted to inactive compounds by conjugation with sulfate and glucuronide, and then excreted by the kidneys. Only a small portion (5-10% of a therapeutic dose) is metabolized via the hepatic cytochrome P450 enzyme system; the toxic effects of paracetamol are due to a highly reactive intermediary metabolite N-acetyl-p-benzo-quinone imine (NAPQI) that is then irreversibly conjugated with the sulfhydryl groups of glutathione (Orecnius and Moldeus, 1984; Borne Ronald 1995).

Prostaglandins which are the compounds associated with pain and inflammation and the inhibition of prostaglandins (PGs) would management of painful and inflammatory conditions (Ojewole 2006). Administration of AAP resulted in the inhibition of prostaglandins synthesis and cyclooxygenase enzyme (COX-2). In agreement with the present study, AAP is considered to be the inhibition of cyclooxygenase (COX), and highly selective for COX-2 (Hinz et al., 2008). AAP should be considered to inhibit the production of PGs although the cause of its selectivity; analgesic and antipyretic effects with anti-inflammatory effects (Garry et al., 2001). AAP effectively reduces prostaglandin E_2 synthesis in brain macrophages by inhibiting enzymatic activity of cyclooxygenase (Anita et al., 2003).

Also, administration of Z. officinale extract resulted in the inhibition of PGs synthesis and cyclooxygenase enzyme, so it acts as anti-inflammatory effect. This results in confirm with (Hiroshi Shimoda et al., 2010), they were founding a potent suppressive effect of Z. officinale extract on acute and chronic inflammation (Thomson et al., 2002). (Shen et al., 2003) showed the inhibitory effects of Z. officinale extract on prostaglandins and NO production and this extract have been used to treat inflammation and have been reported to inhibit cyclooxygenase (COX-2) (Van Breemen et al., 2011).

The present study demonstrated that, co-administration of Z. officinale with AAP resulted in further inhibition of PG synthesis and cyclooxygenase enzyme. So it potentiates AAP action in managing more severe pain and inflammation with minimizing overall side-effects of AAP. This is in parallel with the other study (Borne Ronald 1995), who demonstrated that AAP is useful in managing more severe pain, allowing lower dosages of additional non-steroidal anti-inflammatory drugs (NSAID) or opioid analgesics to be used, so decrease its side-effects. Z. officinale extract possesses analgesic, anti-inflammatory and hypoglycaemic properties; and thus lend pharmacological support to folkloric, ethnomedical uses of ginger in the treatment and/or management of painful, arthritic inflammatory conditions, as well as in the management and/or control of type 2 diabetes mellitus (Ojewole 2006). Z. officinale extract could be used as a cholesterol-lowering, antithrombotic and anti-inflammatory agent (Thomson et al., 2002).
Administration of a single high dose of AAP significantly 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 dose of aqueous ethanol extract of Z. officinale could produce protection. Cytochrome 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 (Dahlin et al., 1984; Borne Ronald 1995).

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. Z. officinale was lowered lipid peroxidation in accordance with (Ahmed et al., 2008).
Treatment of Z. officinale plus AAP significantly enhanced the hepatic antioxidant activity including the hepatic GPx level compared to the AAP alone treated animals. The GPx present in the cells can catalyze the conversion of oxidized glutathione into reduced glutathione (GSH) that can function's as a reductant in the metabolism of hydrogen peroxide and various organic peroxides and so, partially explain the hepatoprotective mechanism of the Z. officinale. The depletion of GSH below a threshold value was associated with conversion of xanthine dehydrogenase to reversible xanthine oxidase, a superoxide radical generation reaction catalyzing enzyme (Cighetti et al., 1993).

Therefore the enhanced hepatic GPx and SOD activities in Z. officinale plus AAP treated group further support its hepatoprotective effect. The

elevated antioxidant status in the liver of Z. officinale plus AAP treated group is related to the decreased MDA level, could maintain the membrane integrity and prevented the leakage of hepatic enzymes to the serum. A diet containing naturally occurring compounds as Z. officinale is effective in exerting protective effects by modulating oxidative stress (Ahmed et al., 2008; Zhang et al., 2009).

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. Z. officinale extract have radical scavenging activity in vitro (Kikuzaki and Nakatani, 1993; Masuda et al., 2004). This direct radical scavenging activity might also be involved in the exhibited hepatoprotective activity. Hence hepatoprotective effect of Z. officinale demonstrated in this study may explore its nutraceutical role in human diet.

CONCLUSION

The results of the present study concluded that aqueous ethanol extract of Z. officinale would potentiate acetaminophen action and significantly prevented the AAP-induced acute hepatotoxicity by enhancing the hepatic antioxidant activity. So may be use Z. officinale extract with lowest dose of AAP to exert pharmacological action as analgesic/antipyretic and anti-inflammatory without any side effect of AAP overdose. However, further detailed studies are required to establish its clinical application.

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