

Ginger (*Zingiber Officinale*) Potentiate Paracetamol Induced Chronic Hepatotoxicity in Rats

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ABSTRACT

Paracetamol, the most commonly sold over-the-counter antipyretic analgesic, is generally considered harmless at therapeutic doses. However, paracetamol overdose causes severe and sometimes fatal hepatic damage in humans and experimental animals. This study was undertaken to examine the effects of *Zingiber Officinale* (ginger) powder on paracetamol induced hepatotoxicity in rats. Rats were given ginger 1% orally in the diet 7 days before induction of hepatotoxicity of paracetamol (1g/kg bwt) orally for 21 days. Paracetamol induced severe liver damage as assessed by increased serum liver marker enzymes, hypoalbuminemia with hyperglobulinemia. Paracetamol induced hepatic lipid peroxidation with reduction in reduced glutathione and antioxidant enzymes. Also, paracetamol caused changes in serum lipid profile. Unfortunately, away from general notion, ginger did not protect paracetamol induced hepatic injuries but potentiate the toxic effects of paracetamol on liver as evident by highly increase in serum globulin fractions with decreased serum albumin, increased serum activities of AST, LDH, ALP and GGT, reduced hepatic activities of antioxidant enzymes (GST, GR and GPX) and reduced glutathione level. In conclusion, ginger fails to protect rats against paracetamol induced chronic hepatotoxicity but enhance its adverse effects on liver.

Key words: Paracetamol, Ginger, Hepatotoxicity, Glutathione, Antioxidant.

INTRODUCTION

Liver disease is a serious medical problem. Some of the liver injuries are caused by the use and abuse of drugs. Conventional and/or synthetic drugs such as steroids, vaccines, antivirals, and other medications can cause serious side effects, even toxic effects on the liver, especially when used for prolonged periods of time¹.

Paracetamol, a widely used over-the-counter (OTC) analgesic and antipyretic, is one of the best known experimental models of hepatotoxicity². It is safe at therapeutic doses but causes a fatal hepatic necrosis and hepatic failure in overdose³. It was found that induction of CYP2E1, CYP1A2, CYP3A4, depletion of intracellular GSH, and oxidative stress are the major mechanisms involved in the pathogenesis of paracetamol induced liver injury⁴.

Paracetamol at therapeutic doses is rapidly metabolized in the liver principally through glucuronidation and sulfation and only a small portion is oxidized by cytochrome P-450 2E1 to generate a highly reactive and cytotoxic intermediate, *N*-acetyl *-P*- benzoquinoneimine (NAPQI), which is quickly conjugated by hepatic glutathione to yield a harmless water soluble product, mercapturic acid⁵. When paracetamol is dosed at higher dose levels in animals or humans, metabolism of paracetamol through glucuronidation and sulfation is saturated and NAPQI is synthesized in enough amounts to cause acute hepatotoxicity⁶.

Many research efforts are directed to the discovery and development of agents, which might protect cells from oxidative reactions with potential antioxidant and hepatoprotective effects⁷. The most popular antioxidant for paracetamol hepatotoxicity is *N*-acetyl-L-cysteine (NAC)⁸.

There is a global trend towards the use of traditional herbal preparations for the treatment of liver diseases. The list of hepatoprotective biologically active compounds (BAC) in the scientific literature is quite long, but only some of them have enough strong effects to combat different types of liver damage. Ginger (*Zingiber officinale*) is commonly used as food spice in India and other Asian and African countries. In many traditional Chinese, Ayurvedic and Unani herbal medicines ginger had been recommended for the treatment of catarrh, rheumatism, nervous diseases, gingivitis, toothache, asthma, stroke, constipation and diabetes for centuries⁹. Several recent studies reported the protective effects of ginger extracts against alcohol induced toxicity¹⁰, bromobenzene induced hepatotoxicity¹¹, fenitrothion or lead induced developmental toxicity¹², fungicide induced liver toxicity¹³ and ethionine-induced toxicity¹⁴.

Based on these data, the present study aims to trace the antioxidant and hepatoprotective effects of ginger (*Zingiber officinale*) powder on paracetamol induced chronic hepatotoxicity in rats.

MATERIAL AND METHODS

Table 1 : Effect of ginger and/or paracetamol on serum protein patterns of rats

	Total protein	Albumin	Alpha-1 globulin	Alpha-2 globulin	Beta globulin	Gamma globulin
Control	6.28 ± 0.07 ^c	4.12 ± 0.13 ^b	0.10 ± 0.01 ^d	0.85 ± 0.02 ^d	0.61 ± 0.03 ^d	0.44 ± 0.04 ^c
Paracetamol	5.80 ± 0.17 ^d	3.12 ± 0.11 ^d	0.14 ± 0.00 ^c	0.98 ± 0.02 ^c	0.74 ± 0.02 ^c	0.62 ± 0.04 ^b
Ginger 1%	7.96 ± 0.16 ^a	4.90 ± 0.07 ^a	0.16 ± 0.01 ^b	1.09 ± 0.02 ^b	0.86 ± 0.06 ^b	0.66 ± 0.05 ^{ab}
Paracetamol + Ginger 1%	7.30 ± 0.10 ^b	3.50 ± 0.07 ^c	0.20 ± 0.00 ^a	1.45 ± 0.03 ^a	1.13 ± 0.01 ^a	0.76 ± 0.03 ^a

Values are expressed as mean ± SE, The value with different letter within the same column significantly differ at $P < 0.05$

Chemicals and medicinal plant: Cumme hydroperoxide, 1-chloro-2, 4-dinitrobenzene (CDNB), 5-5-dithiobis-2-nitrobenzoic acid (DTNB) were obtained from (Sigma chemical Co. St., Louis, MO, USA). Thiobarbituric acid (TBA) and reduced glutathione (GSH) were obtained from Fluka Chemical Co. Trichloroacetic acid (TCA) and tris base were obtained from Merk Chemical Co. Paracetamol was provided from El-Nile Pharmaceutical Company (Cairo, Egypt). All the reagents used were of analytical grade.

The fresh rhizomes of ginger was locally purchased from local market, identified and authenticated by botanists in the department of Botany, Faculty of Agriculture, Alexandria University, Egypt, then grinded to fine powder to be supplied to rats in the commercial basal diet.

Animals and experimental design: Forty adult male albino rats weighing 200 ± 20 g (obtained from medical research institute, Alexandria University, Egypt) were used in this study. They were fed standard diet pellets and allowed food and water ad libitum for an acclimation period of two weeks. The animals were maintained in a strictly controlled temperature ($18 \pm 1^\circ\text{C}$). Humidity was kept at 50% and the lighting cycle was 14 hr light and 10 hr dark with adequate ventilation. Animals were handled with human care in accordance with the National Institutes of Health guidelines. The rats were randomly divided into four groups each consisting of ten animals as the following design: the first group fed on basal diet and distilled water ad libitum and kept as control group. The 2nd group termed as paracetamol treated group in which the rats were treated orally with paracetamol at dose of 1 g/kg body weight for three weeks (4 times per week) for induction of chronic hepatotoxicity. The 3rd group is ginger treated group, the rats fed with 1% ginger powder containing diet for four weeks and water ad libitum. The 4th group termed as ginger and paracetamol group, the rats fed with 1% ginger powder containing diet for one week followed by three weeks oral administration of paracetamol at dose 1 g/kg body weight (4 times per week).

Preparation of blood Samples: At the end of experiment, blood samples were withdrawn from the retro-orbital vein of each rat and each sample was collected into clean tubes. The blood samples were allowed to coagulate and then centrifuged at 3000 rpm for 5 min. The separated sera were kept at -20°C until used for the estimation of serum activity of ALT, AST, ALP, GGT and LDH, total protein, albumin and globulin fraction levels and lipid profile (total cholesterol, triglycerides, HDL-c, LDL-c and VLDL-c).

Preparation of liver sample: The rats were sacrificed by cervical dislocation, and then the livers were rapidly

removed. A part of each liver was weighed and homogenized, using glass homogenizer with ice-cooled saline to prepare 25% W/V homogenate. The homogenate was divided into two aliquots. The first one was deproteinized with ice-cooled 12% trichloroacetic acid and the obtained supernatant, after centrifugation at 1000 xg was used for the estimation of reduced glutathione (GSH) content. The second aliquot was centrifuged at 1000 xg and the resultant supernatant was used for estimation of glutathione peroxidase (GPx), glutathione-S-transferase (GST) and glutathione reductase (GR) activities and level of malondialdehyde (MDA).

Biochemical blood analysis: Hepatic injury was assessed using the serum levels of AST, ALT, LDH, ALP, and GGT. Cholesterol, triglyceride, and HDL-c levels were determined using automated enzyme analyzers (Biochemical analyzer AE-600N, ERMA-INC-Japan) and commercial diagnostic kits.

The protein was separated according to their respective electrical charges at pH 8.8 on a cellulose acetate plate using both the electrophoretic and electroendosmotic forces. After the proteins were separated, the plate was placed in a solution of sulfosalicylic acid and Ponceau S stain to stain the protein bands. The relative percents and absolute values for each band were automatically calculated by the densitometer using 525 nm filter and the narrow slit with computer accessories according to Alper¹⁵.

Oxidative stress and antioxidants: Tissue lipid peroxides (LP) level was determined as thiobarbituric acid-reactive substances, measured as malondialdehyde (MDA)¹⁶. GSH level in liver homogenate were estimated by spectrophotometer according to the method of¹⁷. Liver glutathione peroxidase (GPx) activity was determined using reduced glutathione and cumme hydroperoxide as substrate by the modified method of¹⁸. Glutathione reductase (GR) and glutathione-S-transferase (GST) activities were measured according to the method of^{19,20}, respectively.

STATISTICAL ANALYSIS

Data were analyzed using the SPSS package. Results are expressed as mean ± SEM with the experiment repeated at least three times. Statistical evaluations were done using the analysis of variance (ANOVA). A p value of < 0.05 was considered significant.

RESULTS

The data represented in table (1) showed that administration of paracetamol significantly decreased

Table 2: Effect of ginger and/or paracetamol on liver enzyme markers of rats

	ALT (U/L)	AST (U/L)	LDH (U/L)	ALP (U/L)	GGT (U/L)
Control	55.02 ± 3.12 ^b	185.66 ± 10.07 ^b	271.92 ± 20.37 ^b	1205.91 ± 141.93 ^a	3.30 ± 0.41 ^b
Paracetamol	119.54 ± 37.57 ^a	293.44 ± 56.82 ^a	487.07 ± 97.47 ^a	1306.95 ± 65.41 ^a	5.69 ± 0.48 ^b
Ginger 1%	36.71 ± 2.79 ^c	136.78 ± 5.71 ^c	163.27 ± 25.53 ^c	785.95 ± 150.43 ^b	2.00 ± 0.32 ^b
Paracetamol + Ginger 1%	49.80 ± 2.17 ^b	211.37 ± 20.64 ^b	465.12 ± 97.65 ^a	1399.65 ± 92.26 ^a	10.54 ± 2.82 ^a

Values are expressed as mean ± SE, The value with different letter within the same column significantly differ at P < 0.05

Table 3: Effect of ginger and/or paracetamol on serum lipid profile of rats

	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL-c (mg/dl)	LDL-c (mg/dl)	VLDL-c (mg/dl)
Control	69.08 ± 2.42 ^b	49.90 ± 3.07 ^b	53.34 ± 1.81 ^b	5.76 ± 0.47 ^c	9.98 ± 0.61 ^b
Paracetamol	63.36 ± 2.60 ^b	36.86 ± 1.08 ^c	44.54 ± 2.01 ^c	11.25 ± 1.61 ^b	7.37 ± 0.22 ^c
Ginger 1%	80.94 ± 2.37 ^a	33.22 ± 0.75 ^c	67.84 ± 1.47 ^a	7.06 ± 0.69 ^c	6.84 ± 0.27 ^c
Paracetamol + Ginger 1%	65.46 ± 1.79 ^b	76.04 ± 5.38 ^a	30.44 ± 1.03 ^d	23.01 ± 3.73 ^a	15.21 ± 1.08 ^a

Values are expressed as mean ± SE, The value with different letter within the same column significantly differ at P < 0.05

Table 4: Effect of ginger and/or paracetamol on oxidative stress and antioxidants

	MDA (nmole/g tissue)	GST (µmole/min/g tissue)	GPX (U/g tissue)	GR (U/g tissue)	GSH (µmole/g tissue)
Control	96.73 ± 8.56 ^c	232.17 ± 12.57 ^a	132.12 ± 5.84 ^a	1058.18 ± 21.37 ^b	41.51 ± 2.35 ^b
Paracetamol	407.18 ± 38.70 ^a	274.53 ± 23.89 ^a	104.33 ± 4.24 ^b	1252.78 ± 49.28 ^a	14.22 ± 1.57 ^c
Ginger 1%	80.35 ± 5.41 ^c	248.60 ± 11.31 ^a	134.76 ± 3.34 ^a	1086.76 ± 9.05 ^b	60.50 ± 1.03 ^a
Paracetamol + Ginger 1%	256.24 ± 13.68 ^b	223.51 ± 13.70 ^a	96.01 ± 6.28 ^b	1066.01 ± 69.08 ^b	17.24 ± 1.35 ^c

Values are expressed as mean ± SE, The value with different letter within the same column significantly differ at P < 0.05

serum total protein and albumin levels while serum prealbumin, alpha-1, alpha-2, beta- and gamma-globulins levels were significantly increased as compared to control group. Feeding ginger 1% significantly increased serum total protein, albumin, prealbumin, alpha-1, alpha-2, beta- and gamma-globulins levels when compared to control or paracetamol groups. Administration of ginger 1% together with paracetamol significantly increased serum total protein, albumin and prealbumin levels as compared to paracetamol group meanwhile; serum alpha-1, alpha-2, beta- and gamma-globulins levels were significantly also increased when compared to control, paracetamol or ginger groups.

Table (2) revealed that oral ingestion of paracetamol at 1000 mg/kg b.wt significantly increased serum aminotransferases (ALT and AST) and lactate dehydrogenase (LDH) enzymatic activities but not significantly increased serum alkaline phosphatase (ALP) and gamma glutamyl transferase (GGT) activities when compared to control group. Administration of ginger 1% significantly decreased serum enzymatic activities of ALT, AST, LDH and ALP activities, while not significantly

decreased serum GGT activity as compared to control group. Administration of ginger 1% together with paracetamol significantly decreased serum ALT and AST activities, not significantly decreased serum LDH activity, not significantly increased serum ALP activity, while significantly increased serum GGT activity as compared paracetamol group.

Table (3) showed that administration of paracetamol at dose of 1000 mg/kg b.wt not significantly decreased serum total cholesterol level, significantly decreased serum triglycerides; HDL-c and VLDL-c levels while significantly increased serum LDL-c level as compared to control group. Feeding ginger 1% significantly increased serum total cholesterol and HDL-c levels, significantly decreased serum triglycerides and VLDL-c levels when compared to control group. Administration of ginger and paracetamol significantly increased serum triglycerides, LDL-c and VLDL-c levels while significantly decreased serum HDL-c level with no difference in serum total cholesterol level when compared to control or paracetamol group.

The data represented in table (4) revealed that paracetamol at dose 1000 mg/kg b.wt significantly increased hepatic MDA level and glutathione reductase (GR) enzymatic activity with non significant increase in glutathione transferase (GST) activity while glutathione peroxidase (GPX) enzymatic activity and level of reduced glutathione (GSH) were significantly decreased when compared to control group. Feeding ginger 1% significantly decreased level of MDA and increased hepatic GSH content with no statistical difference in antioxidant enzymatic activities (GST, GPX and GR) as compared to control. Administration of ginger and paracetamol together significantly increased hepatic MDA content as compared to control group but significantly decreased as compared to paracetamol group. The level of GSH was significantly decreased when compared to control group, GR enzymatic activity was significantly decreased as compared to paracetamol group, GPX enzymatic activity was significantly decreased when compared to control group with no statistical difference in GST enzymatic activity as compared to control or paracetamol groups.

DISCUSSION

The liver is a major target organ for toxicity of xenobiotics and drugs, because most orally ingested xenobiotics and drugs pass through the liver, and some chemicals are metabolized into toxic intermediates in the liver²¹. Paracetamol, when used at high doses, could cause acute liver injury most probably via formation of N-acetyl-p-benzoquinoneimine, a toxic metabolite, by cytochrome P4502E1 (CYP2E1). N-acetyl-p-benzoquinoneimine is usually inactivated by hepatic glutathione, but it, when produced excessively, covalently binds to centriobular hepatic proteins, contributing to hepatic toxicity^{21,22,23}.

In the assessment of liver damage by paracetamol, the determination of enzyme activities such as ALT and AST is largely used. In the present study, the increase in serum activities of ALT, AST, LDH, ALP and GGT in paracetamol treated rats had been attributed to the damaged structural integrity of the liver, because these are normally located in the cytoplasm, mitochondria or microsomes and are released into the circulation after cellular damage²⁴ or due to alterations in the permeability of cell membrane and increased synthesis or decreased catabolism of aminotransferases²⁵. Our results were in accordance with those of²⁶ who found that the serum levels of both ALT and AST were elevated almost fourfold in paracetamol treated group in comparison with the control group. Also, Kanchana and Sadiq,²⁷ mentioned that oral administration of 400 mg/kg paracetamol in rats increased serum activities of ALT, AST, LDH, ALP and GGT. Additionally, histological findings showed that paracetamol administration to rats revealed a remarkable centriobular (zone III) necrosis, cytoplasmic changes, and sinusoidal narrowing around the central vein, and it has also been reported in some other studies that paracetamol intoxication can result in severe hepatic damage characterized by hemorrhagic centriobular necrosis in both humans and animals^{28,29}. Moreover, chronic administration of paracetamol statistically decreased

serum albumin and increased serum globulin fractions which were evident for chronic hepatic necrosis. Albumin is decreased in chronic liver disease and is generally accompanied by an increase in the α and β globulins as a result of production of IgG and IgM³⁰. The present results were in harmony with³¹ who revealed that paracetamol induced toxic injury of rat hepatocytes as assessed by significant decrease in albumin level and increase LDH leakage.

An observable significant improvement in the activities of ALT, AST, LDH, ALP and GGT enzymes was recorded in ginger supplemented groups. Hepatoprotective effects of ginger based on biochemical and/or histopathological assessment due to its antioxidant effect³². Ginger products exert their antioxidant effect by quenching free radicals due to the effect of polyphenol compounds (6-gingerols and its derivatives³³. Unfortunately, co-administration of ginger with paracetamol potentiate its toxic effects on liver as consistent by statistically increased serum globulin fractions and decreased serum albumin level indicating severe hepatic necrosis which may be develop to fibrosis, also exhaustion of hepatic cytoplasmic enzymes as ALT more evident for severe hepatic injury. These effects can be explained as ginger may increase gastrointestinal absorption of paracetamol because some active components of ginger were reported to stimulate digestion, absorption, relieve constipation and flatulence by increasing muscular activity in the digestive tract³⁴. Also, intraduodenal administration of dried ginger 150 mg/kg containing [6]-shogaol 2 mg/kg increased intestinal blood flow³⁵, or induction of drug metabolizing enzymes as cytochrome P-450 which increase the formation of toxic metabolites of paracetamol. This observation was confirmed by³⁶ who revealed an increased mRNA level of CYP2B1 in the liver of rats after treatment of ginger.

It is established that covalent binding of N-acetyl-P-benzoquinoneimine, an oxidation product of paracetamol, with Sulfhydryl groups of protein result in cell necrosis and lipid peroxidation in the liver³⁷. In addition, NAPQI can increase the formation of superoxide anion, hydroxyl radical and hydrogen peroxide, nitric oxide and peroxynitrite respectively. Excess levels of these species can attack biological molecules such as DNA, protein and phospholipids which leads to lipid peroxidation, nitration of tyrosine and depletion of antioxidant enzymes that further results in oxidative stress³⁸. In experimental toxicology, paracetamol induced liver injury is used as a model of hepatotoxicity both in vitro and in vivo. The basic mechanism of paracetamol toxicity in the liver is well known and is related to the covalent binding of its reactive metabolite N-acetyl-p-benzoquinoneimine (NAPQI) to sulfhydryl groups of GSH and various thiol containing proteins and their subsequent oxidation⁴. Thus GSH depletion is considered one of the main biochemical markers for paracetamol caused hepatotoxicity. Furthermore, the depletion of GSH causes the endogenous reactive oxygen species (ROS) to bind to cellular macromolecules leading to initiation of processes of lipid peroxidation, membrane breakdown, and cell death³⁹.

In the present study, paracetamol administration was accompanied by increased lipid peroxidation, depletion in GSH stores and reduced GPx activity in the liver. It has been generally accepted that P450-dependent bioactivation of paracetamol is the main cause for potentially fulminant hepatic necrosis upon administration or intake of lethal doses of paracetamol^{15,40}. NAPQI is initially detoxified by conjugation with reduced GSH to form mercapturic acid⁴¹. Under conditions of NAPQI formation following toxic paracetamol doses, GSH concentrations become very low in the centrilobular cells^{42,43} which could account for the observed depletion in liver GSH stores.

GPx plays a critical role in maintaining balance in the redox status of animals under acute oxidative stress and protects against chemically-induced oxidative destruction of lipids and proteins⁴⁴. Consequently, it could be consumed during this process which would explain the observed reduced GPx activity in the paracetamol-treated group.

Feeding ginger 1% in diet reduced hepatic lipid peroxidation, maintained antioxidant enzymes within normal levels and increased level of reduced glutathione. It could be due to antioxidative properties of ginger extracts. Gingeriol and shogaol and other chemicals in ginger inhibit prostaglandine and leukotriene biosynthesis through suppression of 5-lipoxygenase synthetase⁴⁵. Also, feeding ginger to rats modulates the antioxidant enzymes in a manner that favors the lowering of lipid peroxidation and a possible adaptive mechanism to counteract oxidative stress situation⁴⁶. Co-administration of ginger with paracetamol had negative effects on hepatic antioxidant enzymes and reduced glutathione which may be attributed to the interaction between herb and drug.

Concerning serum lipid profile, oral administration of paracetamol decreased serum triglyceride, HDL-c and VLDL-c levels with no significant change in serum total cholesterol level but increased serum LDL-c level. Paracetamol seems to cause impairment in lipoprotein metabolism and also alterations in cholesterol metabolism⁴⁷. The results didn't come into agreement with the result of⁴⁸ in that paracetamol at 2 g/kg has enhanced the cholesterol level and reduced the serum levels of HDL. Also, this result disagree with⁴⁹ who represented that paracetamol treated animals showed an elevation in the concentrations of total lipids, cholesterol, triglycerides and serum LDL-cholesterol with depletion in the levels of serum HDL-cholesterol and tissue phospholipids. The discrepancy in these results may be attributed to the differences in paracetamol doses and duration of experiment. Administration of ginger 1% significantly increased serum total cholesterol and HDL-c levels, decreased serum triglyceride and VLDL-c levels with no significant changes in serum LDL-c level. Ginger exhibit a hypolipidemic activity which was in agreement with⁵⁰ who revealed that ethanolic extract of ginger produced significant decrease in serum total cholesterol and triglycerides levels and increased HDL-cholesterol level as compared to diabetic rats, and the extract exhibit a significant lipid lowering activity and protect the tissues from lipid peroxidation. The increase in serum total

cholesterol observed in our study attributed to increased serum HDL-c content. Administration of ginger together with paracetamol statistically increased serum triglyceride, LDL-c and VLDL-c levels and decreased level of serum HDL-c promoting accumulation of lipids in liver which may explained by the metabolic interaction between ginger components and paracetamol.

In conclusion, paracetamol most notably caused hepatic toxicity as indicated by increased serum liver enzymatic activities, decreased albumin and increased globulin fractions, induction of oxidative stress and depletion of antioxidant. Unfortunately, ginger potentiates the toxic effects of paracetamol on liver indicating an interaction between them. Further experimental studies are necessary to explain such interaction.

Conflict of interest: The authors declare that there are no conflicts of interest.

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