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Full Length Research Paper

Effect of *Zingiber Officinale* on paracetamol-induced genotoxicity in male rats

Salah S. H.[1]*, Abdouh S.[1], Hodaf Boolesand[1] and Abdel Rahim E. A.[2]

[1]Department of Cell Biology, National Research Center, Dokki, Egypt.
[2]Department of Biochemistry, Faculty of Agriculture, Cairo University, Giza, Egypt.

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The mutagenic effects of paracetamol (acetaminophen) in male rat using *in vivo* mutagenicity tests, chromosomal aberrations of somatic and germ cells, molecular assay and biochemical were studied. Paracetamol genotoxicity on normal divided cells has been reported. The data obtained showed that the ability of paracetamol to bind and interact with genetic material lead to changes in chromosomal behavior and structure during mitosis. The significant increase in chromosomal aberration, the changes in the number, position and intensity of bands, liver and renal damages induced by paracetamol may be attributed to the fact that paracetamol can induce genotoxicity through DNA damage. Paracetamol also stimulated AST and ALT activity, these stimulations indicated liver cell necrosis. Paracetamol-induced acute renal damage by the elevations in blood urea, uric acid and creatinine levels. Paracetamol showed abnormal values of protein profile in blood. The treatments with ginger presented hepatoprotective effect, also ginger can protect against oxidative kidneys tissue damage that reduced lipid peroxiation in liver and kidneys. The possible mechanism by which ginger exhibited significant protection against paracetamol- induced genotoxicity and hepatotoxicity may be due to its antioxidant effect. It may also be responsible for the hepatoprotective activity and attainment of normal frequencies of chromosomal aberration in ginger-treated rats. Thus, the present study indicated that the genotoxicity products at low concentration and for long time treatment showed the hazard of paracetamol addiction on human's life.

Key words: *Zingiber officinale*, paracetamol, chromosomal aberrations, genotoxicity.

INTRODUCTION

Environmental toxins and the toxicity of chemical materials start at molecular biological structure of the cells which needs long time before it appears. The human continuous exposure to chemicals directly or indirectly leads to their accumulation in the body and causes different genetic diseases, such as cancer and congenital malformation (Wengsie and Meclean, 1999; Meyer and Kulkarni, 2001). Analgesic drugs are commonly consumed to reduce or prevent pain and soreness encountered after completion of unaccustomed exercises (Trappe et al., 2002). Paracetamol is widely used as analgesic-antipyretic drug. Although it is considered as a safe drug, its overdose produces hepatic necrosis and renal failure due to increases in lipid peroxide levels and depletion of glutathione (Abraham, 2005). *In vitro*, it causes congenital malformation in embryo (Ying and Yi, 2000).

However, the mechanism of analgesic action of paracetamol is less clear. Genotoxic effects of paracetamol induction on DNA and inhibition of replicative as well as repair synthesis on nucleic acids have been reported in rodents by Hongslo et al. (1994). It also inhibits the activity of ribonucleotide reductase, which leads to decrease in protein biosynthesis (Abraham, 2005). The genotoxicity effect of paracetamol was recorded in many *in vivo* test systems as it changed the chromosome structure in lymphocytes of human volunteers after intake of 3 g by intravenous (i.v.) injections (Kocisova and Sram, 1990).

Meanwhile, antioxidant supplementation has been

*Corresponding author. E-mail: ahmedamm33@yahoo.com.

Table 1. ISSR primer codes and sequences used for ISSR-PCR and their annealing temperature.

Primer set	Primer code	Primer sequence (5 →3)	Annealing T (°C)
1	814	5'(CT) b TG 3'	44
2	17898b	5'(CA) 6 GT 3'	48
3	HB 11	5'(GT) 6 CC 3'	48
4	HB 12	5'(CAC) 3 GC 3'	48
5	HB 14	5' (CTC) 3 GC 3'	52

advised as the therapeutic adjuvant in the treatment. Ginger (*Zingiber officinale* Roscoe) has been cultivated for medicinal and culinary purposes for at least two millennia. It contains a host of compounds which include acids resins, vitamin C compounds (folic acid, inositol, choline and pantothenic acid), gingerol, sesquiterpene, vitamin B3 and B6, volatile oils and bio-trace elements [calcium (Ca), magnesium (Mg), phosphorus (P) and potassium (K)] (Arfeen, 2000; Ernst and Pittler, 2000; Nicoll and Henein, 2009). Ginger's high antioxidant value has proved highly effective with its ability to scavenge a number of free radicals and protect cell membrane lipid from oxidation in a dose-dependent manner. Studies on rat have showed that ginger significantly lowered lipid peroxidation and raised the levels of antioxidant enzymes, together with blood glutathione (Ahmed et al., 2000; Amin and Hamza, 2006).

It has been reported that ginger or its extracts present some pharmacological activities, including anti-inflammation (Penna et al., 2003), antiemesis (Sharma and Gupta, 1998), analgesic effect (Young et al., 2005), anti-tumor (Katiyar et al., 1996), anti-oxidation (Masuda et al., 2004), anti-platelet, anti-rhinoviral, anti-hepatotoxicity and anti-arthritic effects (18). Ginger also relieves the symptoms of nausea and vomiting associated with motion sickness, surgery and pregnancy (Giloin and Rahman, 2005; Sakr, 2007). In this study, we investigated the effect of ginger as antioxidant protective against paracetamol toxicity using cytogenetic, molecular genetic assay and biochemical studies.

MATERIALS AND METHODS

Animals and chemicals

Six-week old male rats (100 ± 5 g.) were used in the present study. The animals were obtained from the animal house colony, National Research Center, Dokki, Giza, Egypt. They were caged in groups and kept under normal laboratory and nutritional conditions throughout the experimental period. Paracetamol (acetaminophen) was dissolved in distilled water immediately before used. A dose of 5 mg/100 g body weight (bw) was administered by gavage in a volume of 0.5 ml/100 g bw. Tropical ginger (*Z. officinale* Roscoe) was administered orally, at a dose of 5mg/kg bw as a protective against paracetamol toxicity.

Experimental design and methods

Rats were divided into five groups. Control animals (group 1) received an equal volume of distilled water. The second group of animals received a single daily dose of paracetamol for 2 weeks. Group three received a paracetamol dose for 2 weeks then received a dose of ginger daily for 4 weeks. The 4th group was administered by gavage ginger for 4 weeks then a dose of paracetamol daily for 2 weeks. Animals_in the last group were administered orally with a dose of paracetamol concurrently with a dose of daily ginger for 6 weeks.

Cytogenetic studies

Animals were injected intraperitoneally (ip.) with 2 mM colchicine, 0.5 ml/100 g at 2 h before sacrifice. The rats were sacrificed 24 h after the last administration of paracetamol or ginger based on the retention of the drugs in tissues (Taylor, 1978). Bone marrow preparations for the analysis of chromosomal aberrations in metaphase cells were obtained as described by Yosida et al. (1977), while spermatogonial metaphases were prepared from the tests according to Brewen and Preston (1978). Only well-spread metaphases with 42 ± 1 chromosomes were analyzed. About 50 metaphases per animal were screened to determine the structural chromosomal aberrations per cell. Polyploid metaphases were considered in numerical aberrations.

Molecular genetic assay

DNA was extracted from the tested animals according to the method described by Sharma et al. (2000). The concentration of DNA and its relative purity were determined using a spectro-photometer based on absorbance at 260 and 280 nm, respectively. The integrity of extracted genomic DNA was checked by electrophoresis in 0.8% agarose gel using DNA molecular weight marker (Eurblio, Paris, France). Inter-simple sequence repeat (ISSR) analysis was performed using five different primers listed in Table 1. The primers contained different di- and tri-nucleotide repeat motifs in order to achieve genome coverage as wide as possible.

The polymerase chain reaction (PCR) solution (25 µL total volumes) contained 0.5 units of Taq DNA polymerase (Pharmacia®), 1× reaction buffer, 2.5 mM $MgCl_2$, 0.2 µM primer, 200 µM of each dNTP, and up to 30 ng of genomic DNA. PCR amplifications were performed with the following conditions: 94°C for 2 min, 35 cycles of: 94°C for 30 s, annealing temperature for each primer, 72°C for 1 min; 72°C for 20 min and 4°C soak step. For each primer, the annealing temperature was chosen after different trials with different temperatures (Table 1) in order to maximize the information obtained from the patterns, that is-maximum amplification, minimum smear on gels (from non-specific amplification), and well-resolved bands. To exclude PCR artifacts and verify responsibility of results, negative controls and replicates were included in each PCR amplification.

For ISSR marker profiling, PCR products were subjected to electrophoresis on 1.5% agarose gels containing 0.05% ethidium bromide. The electrophoretic patterns of the PCR products were

recorded digitally using a Gel-Doc 2000 image analysis system (Bio-Rad) according to the instruction of manufactory. The polymorphic and monomorphic bands for ISSR analysis were scored on the basis of the band mobility, clear bands were scored using Gel-Doc (Bio-Rad) Gel analysis program as (1) for presence and (0) for absence in a binary data form, the unclear unidentified bands were excluded. Amplicon sizes were estimated using 1 kb DNA ladder standard (Fermentas, Germany).

Biochemical analysis

Blood samples were collected in clean, dry and sterile centrifuge tubes. Serum was separated after leaving the blood to clot and then centrifuged at 3000 rpm for 10 min. Serum samples were kept in clean, dry and sterile glass vials for liver and kidneys function determination. Liver function aspartate transaminase (AST) and alanine transaminase (ALT) activities were determined following the method of Reitman and Frankel (1957). As for the kidneys function, uric acid was determined according to the method of Schirmeister et al. (1964); urea was determined following the method of Patton and Crouch (1977); creatinine was determined following the method of Young et al. (1975). Protein was determined according to the method of Peters (1968) and albumin was determined by the method of Doumas and Giggs (1971). As for the lipid profile, the triglycerides were determined by the method of Fassatil and Prencipe (1982), while cholesterol was determined by the method of Richmond (1973).

Chromosomal analysis

The antimutagenic potential of ginger against paracetamol genotoxicity was evaluated using *in vivo* assays of rat bone marrow and spermatogonial cells. A dose of 5 mg/kg of ginger was tested in combination with paracetamol concurrently or as pre and post treatments compared with the negative control.

Statistical analysis

The differences in chromosomal aberrations analysis among different groups were statistically analyzed using one-way analysis of variance test (ANOVA) as referred to Gupta (1995), with its respective P value. P value of ≤ 0.05 was considered to be significant.

RESULTS

Cytogenetic results

The antimutagenic potential of ginger against paracetamol genotoxicity was evaluated using *in vivo* assays of rat bone marrow and spermatogonial cells. A dose of 5 mg/kg .of ginger was tested in combination with paracetamol concurrently or as pre and post treatments compared with the negative control. Results of bone marrow chromosomal analysis are summarized and presented in Table 2. Paracetamol treatment induced highly significant increases of all types of chromosomal aberrations compared to the control ($p \leq 0.01$).The most frequent aberrations were chromatid deletions, followed by chromatid breaks and fragments. Paracetamol also increased the frequencies of chromatid and chromosomal gaps. In post treatment, administration of 5 mg/kg of ginger before treatment with paracetamol showed no significant differences in the frequency of chromosomal aberration caused by paracetamol. While in pre-treatment, administration of ginger for long time after treatment with paracetamol caused a marked decrease in the aberrant mitosis ($p \leq 0.05$).

Spermatogonial metaphases also showed that oral administration of paracetamol significantly increased the frequencies of individual and total chromosomal aberrations when compared with the control. This increase was found to be statistically significant at $p \leq 0.01$ (Table 3). Also, chromatid deletions were the most frequent aberration followed by breaks, centromeric attenuation and gaps. Ginger significantly decreased the frequencies of chromosomal aberrations induced by pre-treatment of paracetamol at $p \leq 0.05$. Spermatogonial cells showed slightly less effect than bone marrow cells. Ginger also decreased significantly the number of cells with numerical aberrations in both types of examined cells. In general, the results obtained showed that all treatments of ginger were capable of reducing the frequency of paracetamol-induced chromosomal aberrations and indicated that ginger exhibited antioxidant activities.

Molecular genetic results

ISSR analysis was aimed at verifying genetic effects of ginger on genetic material. It was performed on DNA extracted from liver of animals after treatment with paracetamol and ginger in comparison with the control. Data were considered for 5 primers (Figures 1 and 2). Five anchor primers (814, 19878b, HB11, HB12 and HB 14) were used in the present study to analyze the genetic variation among the tested groups (Table 1). The molecular size of amplified bands ranged from 75 to 2000-bp (primers 814, 19878b, HB11 and HB14) and from 200 to 2000-bp (primer HB12). Each primer generated between 3 (primers HB11 and HB14) and 6 (primer 814) bands, with an average 3 bands per primer. The five primers developed a total of 95 bands in which 66 of them were polymorphic (91% polymorphism) as shown in Table 4. Also, the results indicated that all the primers produced 100% polymorphic bands except two primers (814 and HB14), which showed monomorphic bands.

Animals in treated groups had disappeared or appeared bands compared with animals in control group (Table 5). In control group, 21 ISSR bands were obtained compared to 14, 23, 19 and 18 bands for groups 2, 3, 4 and 5, respectively. These results revealed that the number of lost bands was obviously higher than the number of new bands in the animals treated groups compared with control group. Animals treated with paracetamol (group 2) showed the highest lost bands (the total bands was 14 compared to 21 for control group), which could be an obvious indication for the high genotoxic effect of paracetamol due to losses of alleles compared with

Table 2. Frequencies of different chromosomal aberrations induced in male bone marrow cells of all experimental groups.

Group	No. of animals	Structural chromosomal aberrations (M±SE)						Numerical chromosomal aberration (M±SE)			
		Chr. gap	Cht. gap	Break	Deletion	Fragment	Total	Hyperploidy	Hypoploidy	Polyploidy	Total
Control	6	0.0 ± 0.0^{a}	0.500±0.302^{a}	0.0 ±0.0^{a}	0.333±0.293^{a}	0. ± 0.0^{a}	0.833±0.404^{a}	0.0 ± 0.0	1.000 ±0.427^{a}	0.167±0.260^{a}	1.166±0.441^{a}
Paracetamol	6	1.167±0.261^{b}	3.333±0.369^{c}	1.833±0.261^{d}	5.667±0.600^{d}	1.167±0.354^{c}	13.000±0.718^{d}	0.0 ± 0.0	2.667 ±0.368^{b}	1.667±0.449^{b}	4.333±0.572^{c}
Paracet. then Ginger	6	0.167±0.260^{a}	1.833±0.354^{b}	0.500±0.302^{ab}	1.833±0.261^{b}	0.333±0.293^{ab}	4.666±0.415^{b}	0.0 ± 0.0	1.167 ±0.354^{a}	0.833±0.404^{ab}	2.000±0.459^{ab}
Ginger then Paracet.	6	0.500±0.373^{a}	3.167±0.354^{c}	1.167 ±0.354^{c}	3.333±0.369^{c}	0.833±0.354^{bc}	9.000±0.562^{c}	0.0 ± 0.0	2.000±0.386^{ab}	1.500±0.302^{b}	3.500±0.302^{bc}
Paracet + Ginger	6	0.333±0.293^{a}	2.667±0.415^{bc}	1.000 ± 0.0^{bc}	2.667±0.415^{bc}	0.833±0.354^{bc}	7.500±0.558^{c}	0.0 ± 0.0	1.667±0.293^{ab}	1.000±0.325^{ab}	2.667±0.369^{ab}

Different letters (a, b, c, d) in the same column are significantly different ($p \leq 0.05$). Paracet = paracetamol; Chr. = chromosome; Cht. = chromatid.

Table 3. Frequencies of different chromosomal aberrations induced in male spermatogonial cells of all experimental groups.

Group	No. of animals	Structural chromosomal aberrations (M±SE)					Numerical chromosomal aberration (M±SE)			
		Gap	Break	Deletion	Centromeric attenuation	Total	Hyperploidy	Hypoploidy	Polyploidy	Total
Control	6	0.66 ± 0.368^{a}	0.167 ±0.260^{a}	0.833±0.354^{a}	0.167 ± 0.260^{a}	1.833 ±0.404^{a}	0.000 ± 0.000^{a}	1.000 ± 0.324^{a}	0.333 ± 0.293^{a}	1.333 ± 0.293^{a}
Paracet	6	3.667 ±0.540^{c}	1.833 ±0.261^{b}	3.000±0.386^{c}	2.333 ± 0.369^{c}	10.833±0.495^{d}	1.667 ± 0.449^{c}	2.333 ± 0.501^{b}	3.167 ± 0.261^{d}	7.167 ± 0.536^{c}
Paracet then Ginger	6	1.667±0.369^{ab}	1.000±0.427^{ab}	1.500±0.302^{ab}	0.660 ±0.369^{ab}	4.833 ±0.536^{b}	0.500 ± 0.302^{ab}	1.500 ± 0.302^{ab}	0.833 ± 0.354^{ab}	2.833 ± 0.441^{b}
Ginger then Paracet	6	2.333 ±0.293^{b}	1.667 ±0.293^{b}	2.500±0.418^{bc}	1.167 ± 0.261^{b}	7.667 ± 0.449^{c}	1.000 ± 0.325^{bc}	1.667 ± 0.477^{ab}	1.667 ± 0.293^{c}	4.333 ± 0.522^{b}
Paracet + Ginger	6	1.833±0.354^{ab}	1.333 ±0.369^{b}	2.000±0.427^{bc}	0.833 ±0.354^{ab}	6.000±0.678^{bc}	0.667 ± 0.293^{ab}	1.333 ± 0.293^{ab}	1.167 ± 0.354^{bc}	3.167 ± 0.354^{b}

Different letters (a, b, c, d) in the same column are significantly different ($p \leq 0.05$). Paracet = paracetamol.

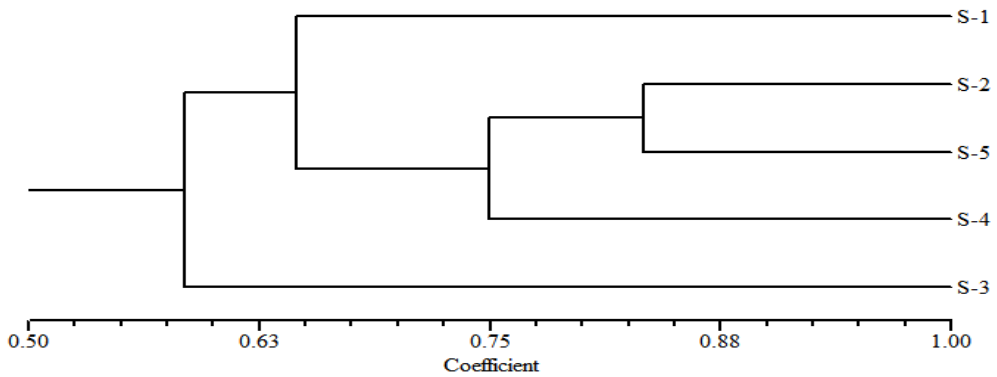

Figure 1. Dendrogram demonstrating the relationships among treated mice and control group.

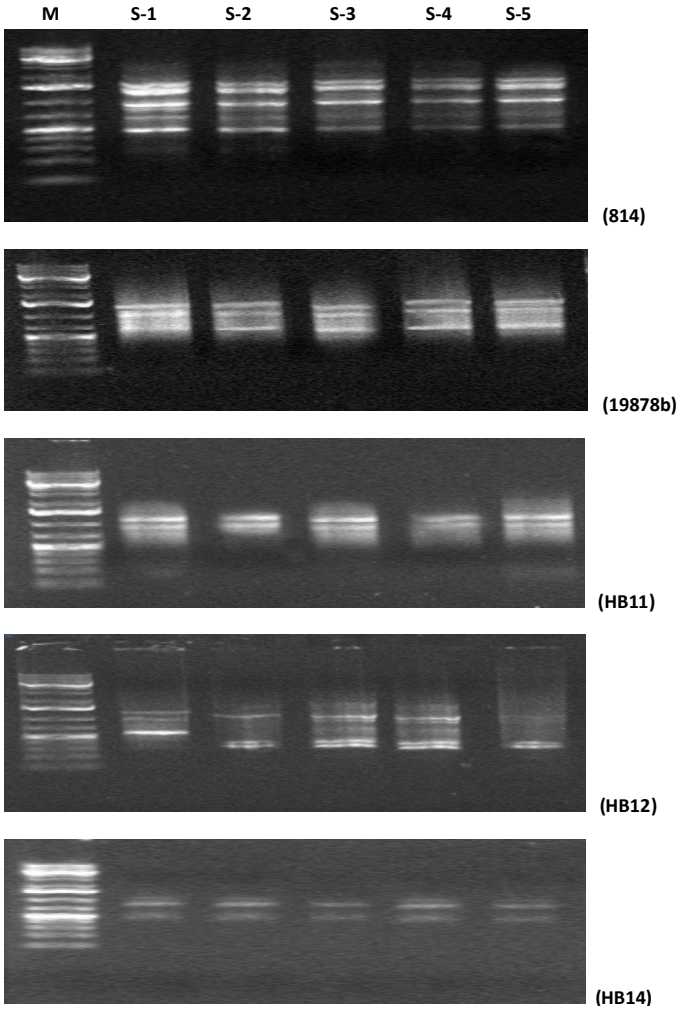

Figure 2. PCR products of genomic DNA from tested mice with five ISSR primers.

groups treated with paracetamol and ginger [groups 4 (19 bands) and 5 (18 bands)]. The animals in group 3 treated with paracetamol dose for 2 weeks and dose of ginger daily for 4 weeks after paracetamol treatment, showed increases in bands number (23 bands) compared with control. These results suggested that the animals treated with ginger and received paracetamol or the animals treated with paracetamol- ginger had less genotoxic effect than those treated with paracetamol only.

Biochemical results

The effect of ginger as antioxidant against the paracetamol harmful effects in male albino rat metabolism such as liver and kidneys functions, as well as lipid and protein profiles were statistically analyzed and illustrated in Table 6. The results showed that paracetamol induced stimulated AST and ALT activity as the paracetamol induction caused liver damage. The three treatments with ginger into paracetamol-induced animals were characterized by alleviation and normalization in the both transaminases activity. The influences of present treatments on kidneys function of paracetamol-induced animals was done by determination of blood uric acid, urea and creatinine which were statistically analyzed. These data showed that paracetamol induction caused significant increases in uric acid, urea and creatinine of blood compared with control. The treatments with ginger showed significant improvement in the three parameters of kidneys function.

The present result in Table 6 also showed that the levels of plasma total soluble protein and albumin were significantly decreased in paracetamol treated rats compared to plasma globulin levels. These abnormal values of protein profile were readjusted and improved by ginger ingestion. The three ginger treatments had the same trend. Table 6 also showed significant increase in total cholesterol, triglycerides and LDL-C in paracetamol treated rats, but decreased level of HDL-C in plasma male albino rats under the same condition. Hence, the treatments of ginger against paracetamol toxicity exhibited different influences on plasma lipid profile; the ginger significantly improved and alleviated the drastic status of paracetamol.

DISCUSSION

Paracetamol (acetaminophen) has been used as an analgesic and antipyretic for many years, with toxicity first noted in the 1960s. Afterward, the incidence of poisoning has increased in overdose. Paracetamol is now the most common drug in self-poisoning, with a high rate of morbidity and mortality (Sheen et al., 2002). Paracetamol mainly causes liver injury and failure (Larson et al., 2005; Ryder and Beckingham, 2001). Damages to the liver or hepatotoxicity, results not from paracetamol itself, but from one of its metabolites, N-acetyl-p-benzoquinone imine (NAPQI). NAPQI depletes the liver's natural antioxidant glutathione and directly damages cells in the liver, leading to liver failure. It remains in its toxic form in the liver and reacts with cellular membrane molecules resulting in widespread hepatocyte damage and death, leading to acute hepatic necrosis (Larson et al., 2005; Ryder and Beckingham, 2001). Paracetamol toxicity can result from normal use; this may be due to individual differences in the expression and activity of certain enzyme in one of the metabolic pathways that handle paracetamol (Vuppalanchi et al., 2007). The liver is also the major site for xenobiotic metabolism, and various chemicals can lead to the formation of active metabolites with toxic effects. The high concentration exposure and metabolic activity make the liver one of the primary targets for various types of chemical-induced toxicity.

Ginger as a good antioxidant agent significantly

Table 4. Number of obtained bands using ISSR analysis in treated groups compared with the control group.

Analysis type	Marker type	Marker name	Total band number	Mobility Range (bp)	Number of bands				
					Group 1	Group 2	Group 3	Group 4	Group5
ISSR	1	814	14	500 - 1000	6	4	5	4	5
	2	19878b	14	550 - 950	5	3	5	3	4
	3	HB 11	13	650 - 950	3	2	4	3	4
	4	HB 12	14	300 - 950	4	3	6	6	3
	5	HB 14	14	500 - 750	3	2	3	3	2

Table 5. Detected polymorphism for ISSR marker in the five tested groups.

Marker no.	Marker	Monomorphic	Polymorphic	Total	Polymorphism (%)
1	814	4	10	14	71
2	19878b	0	14	14	100
3	HB 11	0	13	13	100
4	HB 12	0	14	14	100
5	HB 14	2	12	14	86

Table 6. Effect of ginger as antioxidant on functions of liver and kidneys as well as profiles of protein and lipid in paracetamol intoxicated rats.

Treatment	Liver function				Kidneys function					
	AST		ALT		Creatine		Urea		Uric acid	
	U/L	%	U/L	%	Mg/dl	%	Mg/dl	%	Mg/dl	%
Normal control	73.10 ± 4.21^{b}	100	35.10 ± 2.16^{d}	100	0.72 ± 0.04^{b}	100	46.16 ± 3.01^{c}	100	3.67 ± 1.66^{b}	100
Paracetamol control	155.02 ± 8.18^{a}	212	72.22 ± 4.21^{a}	206	1.61 ± 0.91^{a}	224	100.0 ± 6.17^{a}	217	6.21 ± 3.41^{a}	169
Ginger control	70.61 ± 3.91^{b}	97	32.11 ± 2.00^{d}	91	0.68 ± 0.41^{b}	94	43.11 ± 2.91^{c}	94	3.29 ± 1.67^{b}	90
Paracetamol (2 weeks) then Ginger (4 weeks)	85.12 ± 5.16^{b}	116	44.12 ± 2.91^{c}	126	0.82 ± 0.51^{b}	114	69.13 ± 4.00^{b}	150	4.02 ± 2.21^{b}	110
Ginger (4 weeks) then Paracetamol (2 weeks)	88.00 ± 5.10^{b}	120	50.24 ± 3.21^{b}	143	0.80 ± 0.42^{b}	111	71.11 ± 4.11^{b}	154	4.20 ± 2.61^{b}	114
Ginger and Paracetamol (6 weeks)	79.02 ± 4.11^{b}	108	36.17 ± 1.99^{d}	103	0.76 ± 0.41^{b}	106	55.02 ± 3.00^{c}	119	3.31 ± 1.81^{b}	90
LSD at 5%	23.01		6.00		0.51		13.21		1.76	

Table 6. Contd.

Treatment	Protein profile						Lipid profile							
	T. Protein		Albumin		Globulin		Cholesterol		Triglyceride		HDL		LDL	
	g/dl	%	g/dl	%	g/dl	%	Mg/dl	%	Mg/dl	%	Mg/dl	%	Mg/dl	%
Normal control	7.01 ± 3.67[a]	100	4.61 ± 2.71[a]	100	2.40 ± 1.31[a]	100	71.02 ± 3.97[b]	100	76.11±4.12[b]	100	33.21 ± 2.71[a]	100	25.03 ±1.61[c]	100
Paracetamol control	4.61 ± 3.00[b]	66	2.59 ± 1.88[b]	56	2.02 ± 1.11[a]	84	50.01 ± 8.18[a]	211	162.02±9.00[a]	213	21.06 ± 1.67[b]	64	48.21 ±2.91[a]	193
Ginger control	7.21 ± 4.11[a]	103	4.79 ± 2.98[a]	104	2.42 ± 1.33[a]	101	70.92 ± 4.11[b]	100	71.69±3.92[b]	94	34.00 ± 2.01[a]	102	24.01 ±1.31[c]	96
Paracetamol (2 weeks) then Ginger (4 weeks)	5.32 ± 3.00[b]	76	3.00 ± 1.71[b]	65	2.32 ± 1.12[a]	97	65.99 ± 3.95[b]	93	80.24±4.21[b]	105	28.66 ± 1.91[a]	80	28.10 ±1.51[c]	112
Ginger (4 weeks) then Paracetamol (2 weeks)	5.19 ± 2.91[b]	74	3.21 ± 1.81[b]	70	1.98 ± 1.00[a]	83	141.11 ±8.19[a]	199	150.01±8.17[a]	197	30.22 ± 1.51[a]	91	35.09 ±1.61[b]	124
Ginger and Paracetamol (6 weeks)	6.66 ± 3.81[a]	95	4.30 ± 2.71[a]	93	2.36 ± 1.41[a]	98	76.16 ± 4.12[b]	107	77.09±4.11[b]	101	31.11 ± 1.61[a]	94	27.61 ±1.41[c]	110
LSD at 5%	1.11		1.00		0.62		20.11		21.26		6.09		5.66	

Values are presented as % relative to control. Each value represented the mean of 6 rats (mean SD). Means in the same column followed by the same letter are not significantly different at $P < 0.05$.

lowers plasma total cholesterol, triglycerides and LDL-C but raises HDL-C as effectively as conventional hypolipidemic drugs (Thomson et al., 2002). It was also found that ginger acted on liver to reduce cholesterol biosynthesis and may stimulate cholesterol's conversion to bile acids and increase lipid fractions facecal excretion (Verma et al., 2004). Weidner and Sigwart (2000) reported that ginger is generally considered a safe herbal medicine. Although ginger has been proposed as a safe and effective alternative to conventional anti-emetic drugs (Boone and Shields, 2005), it has few and insignificant adverse side effects (Ali et al., 2008).

In the present study, the protective effect of ginger on paracetamol toxicity was investigated in male albino rats. The results indicated that paracetamol is a genotoxic drug in rats' bone marrow and spermatogonial cells as it increases chromosomal aberrations significantly. The genotoxic action of paracetamol in somatic cells has already been reported for mice bone marrow as chromosomal aberrations (CA) and sister chromatid exchange (SCE) (Giri et al., 1992). Treatment with paracetamol also induced SCE and CA and inhibits replicative DNA synthesis in mouse mammary tumor cell line (Hongslo et al., 1990). The significant increase in CA induced by paracetamol may be attributed to the fact that paracetamol can induce genotoxicity through DNA damage. In addition, paracetamol also reacts directly with DNA before chromatin condensation and causes the clastogenic abnormality such as bridges in ana-telophase, chromosome and chromatic break and micronuclei in all concentration (Morakinyo et al., 2010). Paracetamol reduces DNA synthesis by a specific inhibition of ribonucleotide reductase, including SCE and CA as breaks and chromatic exchanges (Hongslo et al., 1990). The genotoxicity effect of paracetamol was also recorded in many *in vitro* test systems such as congenital malformation in embryo, inhibition of DNA synthesis and increase of SCE in hamster cell (Ying and Yi, 2000), Hantson et al. (1996) stated that the therapeutic use of paracetamol could represent a genotoxic hazard to man and attributed the contradiction between the cytogenetic results in man to differences in sensitivity between persons or interference with other environment condition (Garbulli and Bashasha, 2008).

The results of the present study showed a high frequency of CA in the 4th group that received ginger first for 4 weeks then paracetamol for 2 weeks. This can be explained by Kirkland et al. (1992) who stated that taking a maximum daily dose of paracetamol exhibited significantly elevated frequencies of chromatid breaks 24 h. Afterward, the levels returned to normal between 3 and 7 days later. Furthermore, the results of spermatogonial metaphases showed that oral administration of paracetamol significantly increased the frequencies of individuals and total chromosomal aberrations when compared with the control. The increase was found to be statistically significant at $P \leq 0.01$ (Table 3). Extensive research has been carried out in the National Institute of Nutrition (NIN) on some important spices like ginger, garlic, onion, etc., for their genome protective effects. Ginger and garlic have demonstrated their antimutagenic effect through being used *in vivo* rat model (Polasa et al., 2006). The natural antioxidants can protect DNA and other molecules from cell damage induced by oxidation and can improve sperm quality and

increase reproductive efficiency of men (Yang et al., 2006). Ginger was also found to possess a protective effect against DNA damage induced by H_2O_2 and it enhanced sperm healthy parameters in rats (Khaki et al., 2009). The cytogenetic results proved that co-administration of paracetamol and ginger clearly reduced the CA in both somatic and germ cells towards normal. The results also showed the capabilities of ginger to interfere with the mutagenic mechanism of paracetamol and reflected its activity to enhance DNA repair system.

The molecular study results of cluster and genetic diversity analysis was performed based on the changes of the ISSR bands in the pattern. The changes in ISSR pattern between control group and treated groups were obvious, including the changes in the number, the position and the intensity of the bands. The differences in the number of tested ISSR primers bands among control and groups 3, 4 and 5 were less compared with group 2 (paracetamol treated animals) (Table 5). Primer HB11 showed clear variation among treated groups which proved that this primer was specific in our experimental studies compared with other tested primers. The appearance of new fragments may be attributed to some sites becoming accessible to the primer after point mutations and/or large rearrangements occur in genomic DNA (Williams et al. 1990), while the disappearance of bands possibly resulted from the presence of DNA photoproducts (pyrimidine dimmers), which can act to block or reduce DNA polymerization in the PCR reactions (Nelson et al., 1996). The decrease in band intensity was considered as the result of the loss of some alleles (Weinberg, 1991; Peinado et al., 1992). Previous studies proved that changes in DNA fingerprint could reflect DNA alteration in genome from single base changes (point mutations) to complex chromosomal rearrangements (Atienzar et al., 1999, 2002). The results of molecular assay, therefore, confirmed the results obtained by cytogenetic and biochemical analysis.

The results of biochemical analysis (Table 6) showed that paracetamol induction stimulated AST and ALT activity. These stimulations indicated liver cell necrosis and the magnitude of increase correlated with the extent necrosis (Porchezhian and Ansari, 2005; Shyamal et al., 2006). The three treatments with ginger in paracetamol-induced animals were characterized by alleviation and normalization in the both transaminases activity. The paracetamol induction caused liver damage; the present hepatotoxicant was metabolized by sulfation and glucuronidation to reactive metabolites and then activated by cytochrome-P-450 system to produce liver injury (Shyamal et al., 2006). Ginger treatments presented hepatoprotective effect in liver function enzymes against paracetamol influences. The treatments with ginger produced significant improvement in the three parameters of kidneys function.

Paracetamol-induced acute renal damage is induced by the elevations in blood urea, uric acid and, urea and creatinine levels and occurrence of tubular necrosis histologically (Abraham, 2005). The mechanisms of acute toxic effects to the animal kidney have not been well defined. Renal toxic metabolite of paracetamol was formed by *in situ* metabolic activation. The kidney is thought to form a toxic metabolite only when it is glutathione depleted. The animals studied indicated that when the kidney is overwhelmed with paracetamol, its oxidation via the P-450 system results in tubular damage (Mitic-Zlatkovic et al., 1998; Abraham, 2005). The regular intake of ginger through diet can protect against oxidative kidney tissue damage that reduces lipid peroxidation in liver and kidneys, and inhibits the protein oxidative products in liver. This indicated the antioxidant potential of ginger when consumed naturally through diet (Kota et al., 2008).

Furthermore, animals treated with paracetamol showed abnormal values of protein profile, which were readjusted and improved by ginger ingestion. Trappe et al. (2002) reported that paracetamol suppresses the protein synthesis response in tissue after eccentric resistance exercise. Thus, paracetamol may work through a common mechanism to influence protein. Hongslo et al. (1994) found that paracetamol induced inhibited DNA synthesis in mice tissue (liver, spleen, intestine, bone marrow and kidneys). The over dose of paracetamol increased the level of DNA breaks and inhabitation of replicative and repair synthesis of DNA. The antioxidant effects of ginger in liver and kidney tissues of ginger-fed rats suggest that the antioxidative status of host tissue can be enhanced by consuming ginger. Inhibition of protein oxidative products reinforces the hypothesis that daily ginger ingestion can result in improved antioxidant status (Kota et al., 2008).

In conclusion, the possible mechanism by which ginger exhibited significant protection against paracetamol-induced genotoxicity and hepatoxicity may be due to its antioxidant effect and its constituents like vitamins C, B3, B6, volatile oils and bio-trace elements. It may also be responsible for the hepatoprotective activity and attainment of normal frequencies of CA in ginger-treated rats.

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