CHAPTER IV

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Oral administration of paraben (p-hydroxy benzoic acid; 67.5 and 135 mg/kg body weight/day) for 30 days caused significant reduction in body weight as well as gain in body weight of mice. The decrease in body weight might be due to reduced feed intake as well as altered protein and energy metabolism. Butyl paraben in diet also produced a significant decrease in body weight in rats, dogs and mice with rapid loss of muscular control (ataxia) and deep depression of the central nervous system. In addition, growth rate was slower as compared to controls (Matthews et al., 1956). Rats fed a diet of paraben (2 or 8%) for up to 12 weeks resulted in many early deaths of females. In rats, paraben (504 mg/kg) for 12 weeks produced weight loss or decreased weight gain and death (Matthews et al., 1956). In eight-week-old mice orally given butyl paraben (0.15, 0.3, or 0.6%) in the diet for 102 weeks, a high incidence of amyloidosis affecting the spleen, liver, kidney, and/or adrenal gland was observed. At dose greater than 7500 mg/kg, all animals died within the first 2 weeks. At dosage of 900 mg/kg and less, body weight gain of treated mice was much lesser than that of controls (Inai et al., 1985). In a feeding study, ethyl paraben was administered orally to rat at dose levels of 0.2, 1 and 2% in diet for 25 weeks showed (Sado, 1973) significant decreases in body weights. In another dermal toxicity study in rats, the effects of daily exposure to a product formulation containing 0.7% methyl paraben and another containing 0.3% propyl paraben were studied for 13 weeks. In the treatment group, body weight gain was significantly
decreased (CTFA, 1981). Mortality was not observed in the present study. It could be due to smaller dose of paraben given to comparatively shorter duration.

Paraben treatment for 30 days caused an increase in absolute and relative weights of liver and kidney of mice. It might be due to accumulation of lipid in the liver. Biochemical analysis (Table 3.3, 3.8) revealed increased total lipid and cholesterol contents in liver and kidney of mice. Histopathological studies also revealed accumulation of lipids in liver and kidney (Plate E, L). Oishi (2001) also reported increase in absolute and relative weights of vital organs in rats.

Aqueous ginger extract alone treatment did not have any significant effect on morphological changes, change in body weight gain as well as absolute and relative weights of liver and kidney as compared to other controls. Administration of aqueous ginger extract along with paraben (Groups 6, 7) caused significant amelioration in morphological alterations, body weight as well as absolute and relative weights of liver and kidney as compared to paraben alone treated animals (Groups 4, 5). It could be due to antioxidative action of ginger extract. The antioxidant action of ginger has been proposed as one of the major possible mechanism for the protective actions against toxicity and lethality of radiation (Jagetia et al., 2003; Haksar et al., 2006). Ahmed et al. (2000) have shown that body weight gain was lower when the rats were treated with malathione; however ginger in diet tended to reduce the effect of malathione.

Histopathological studies revealed increase in sinusoids and hepatocellular necrosis in the liver of paraben-treated mice (Plate E). In kidney the histopathological studies revealed degenerative changes, increased vacuolization, disorganization of
glomerulus and increased space between the glomerulus and the capsule wall in paraben-treated mice (Plate L). In isolated rat hepatocytes, paraben caused cytotoxicity, cell injury and lysis (Sugihara et al., 1997; Nakagawa and Moldeus, 1998; Nakagawa et al., 1999). Paraben-induced cytotoxicity on red blood corpuscles was also reported (Table 3.17). These degenerative changes could be due to increased lipid peroxidation and reduced antioxidative capacity in liver and kidney of mice. Our present study also revealed that paraben causes lipid peroxidation in liver and kidney homogenates in in vitro studies (Table 3.5, 3.11). Peroxidation of membrane lipids initiated membrane integrity, cytotoxicity and cell lysis (Yamashoji and Isshiki, 1998, 2002; Nishizawa, 2006).

Histopathological studies also revealed fatty accumulation in liver of paraben-treated mice. Biochemical analysis in liver of paraben-treated mice also revealed significant increase in cholesterol and total lipid contents. Exact mechanism for significant rise in total lipid and cholesterol contents in liver is not clearly understood. It could be due to more biosynthesis of fat, its reduced utilization or both. Beynen and Geelen (1982) reported that paraben inhibits fatty acids esterification and oxidation by alteration in acetyl-CoA carboxylase in isolated rat liver cells. The inhibition of fatty acids esterification and oxidation leads to accumulation of fatty acids in liver.

Aqueous ginger extract treatment did not show any significant effect on histopathological changes in liver and kidney of mice as compared to controls. However, administration of aqueous ginger extract along with paraben caused amelioration in paraben-induced histopathological changes observed in liver (Plate E, F) and kidney (Plate M, N) of mice. It might be due to antioxidative effect of ginger. Results shown in Table 3.5 and 3.11 indicate significant reduction in lipid peroxidation in liver and kidney.
of mice treated with paraben and aqueous ginger extract. Ajith et al. (2007 b) reported that the hepatoprotective effect of aqueous 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. He also reported that Z. officinale extract significantly and dose dependently protected the nephrotoxicity induced by cisplatin (Ajith et al., 2007 a). The extract from the rhizome of Zingiber officinale could be useful in preventing chemically-induced acute liver injury caused by CCl4 and acetaminophen which was confirmed by histopathological examination of the liver (Yemitan and Izegbu, 2006).

Oral administration of paraben caused significant increase in cholesterol and lipid content in liver and kidney of mice. Aqueous ginger extract alone treatment did not have any significant effect on cholesterol and lipid content in liver of mice. However, aqueous ginger extract along with paraben treatment caused significant amelioration in paraben-induced alterations in cholesterol content in liver of mice. A study reported that the activity of hepatic cholesterol-7 alpha-hydroxylase, the rate-limiting enzyme of bile acid biosynthesis, was significantly elevated in ginger-treated animals suggesting that ginger can stimulate the conversion of cholesterol to bile acids, an important pathway of elimination of cholesterol from the body (Srinivasan and Sambaiah, 1991). At a dose of 500 mg/kg body weight, raw ginger was significantly effective in lowering cholesterol and triacylglycerol levels in the ginger-treated streptozotocin (STZ)-induced diabetic rats compared with the control diabetic rats (Al Amin et al., 2006). Treatment with a methanolic extract of dried rhizomes of ginger produced a significant reduction in fructose-induced elevation of lipid levels, and organ weights in rats (Kadnur and Goyal,
2005). Ginger reduces cholesterol and inhibits LDL oxidation (Fuhrman et al., 2000; Nicoll and Henein, 2007) and has hypolipidaemic effect in rats (Sharma et al., 1996; Ahmed and Sharma, 1997).

Oral administration of paraben for 30 days caused significant reduction in sugar content in liver and kidney (Table 3.3, 3.8). It might be due to increased glycogenolysis and decreased glycogenesis or both. It was found that p-hydroxy benzoic acid increased peripheral consumption of glucose in rat. Thus, hepatic glucose level and glycogen storage did not increase (Peungvicha et al., 1998). Parabens increase the absorption of ocularly applied insulin, and the absorbed insulin decreases serum glucose concentration in albino rabbits (Sasaki et al., 1995). This might be responsible for reduced glycogen content in liver of mice. Administration of ginger extract ameliorated the paraben-induced changes in sugar content. Anti-oxidative properties of ginger might be responsible for increase in sugar content in liver of mice.

Another reason for decrease in carbohydrate content might be due to alteration in enzyme activities that are responsible for the metabolism of carbohydrate. The free radical produced during lipid peroxidation was found to induce fragmentation of hyaluronic acid and glucose (Cornoveld et al., 1993). Reducing sugars like glucose, mannose and non-reducing sugars like deonysugars, hyaluronic acid reacts with hydroxyl radicals (Greenvald and May, 1980), causing destabilization of various connective tissues and organs where carbohydrate is mainly stored. Thus, reduction in carbohydrate content during paraben treatment in mice might be due to dysfunction of enzymes involved in carbohydrate metabolism or interaction of reactive oxygen species with reducing sugar.
Paraben treatment caused significant, dose-dependent reduction in DNA, RNA and protein contents in liver and kidney of mice (Table 3.4, 3.9). Deposition of paraben might decrease the synthesis of protein, amino acids, DNA and RNA contents. It has been reported that parabens inhibit nucleic acid synthesis via inhibition of many enzyme systems and also decreases protein content in microorganisms and embryonic mouse fibroblasts (Krauze and Fitak, 1971; Shiralkar et al., 1978). Krauze and Fitak (1971) reported reduced biosynthesis of both DNA and RNA in embryonic mouse fibroblast cultures when treated with methyl-, ethyl- and propyl-paraben. Nes and Eklund (1983) studied the effects of parabens, on both DNA and RNA synthesis in cells permeabilized by toluene in *Escherichia coli* and *Bacillus subtilis* were inhibited. Protein synthesis in cell free extracts (S-30 fraction) of *Bacillus subtilis* was even more sensitive to parabens than DNA and RNA synthesis. Darbre et al. (2004) reported detection of parabens in samples of human breast tumor tissue. Paraben intoxication blocks the DNA and sweat ducts which leads to formation of tumor and breast cancer. Deposition of paraben might decreases synthesis of proteins, amino acids and DNA and RNA.


Expression of eukaryotic genes is controlled by proteins that bind to specific regulatory sequences and modulate the activity of RNA polymerase. Activation domains of some transcription factors are rich in negatively charged residues. These domains are thought to stimulate transcription by interacting with basal transcription factors such as
TFIIB and TFIID, facilitating the assembly of a transcription complex on the promoter (Lodish et al., 1995; Cooper, 1997). On the other hand, histones are small proteins containing a proportion of basic amino acids that facilitate binding to the negatively charged DNA molecules and inhibit transcription (Lodish et al., 1995; Cooper, 1997).

Our results showed that aqueous ginger extract treatment along with paraben significantly mitigates paraben-induced changes in DNA, RNA and protein contents in the liver and kidney of mice. The amelioration in DNA, RNA and protein contents might be due to increased DNA synthesis and reduction of cytotoxicity and lipid peroxidation. Ginger oil has dominative protective effect on DNA damage induced by H₂O₂. Ginger oil might act as a scavenger of oxygen radical and might act as an antioxidant (Ping et al., 2003). Ginger given intragastrically showed a mean significant increase in DNA-p/min of 3.23 ± 1.02 (p<0.05) (Desai et al., 1990). Gingerol also exerts inhibitory effects on xanthine oxidase (Chang et al., 1994). Nirmala et al. (2007) quantitated benzopyrene-induced DNA damage in terms of comet ratios. Benzopyrene resulted in significant reduction in comet ratio in cell lines. Carcinogen plus ginger extract ameliorated the DNA damage in a dose-dependent manner with increase in levels of comet ratio, thus suggesting antigenotoxic potential of ginger which may be related to its antioxidant and antimutagenic potential.

Oral administration of paraben for 30 days caused significant increase in lipid peroxidation in liver and kidney of mice (Table 3.5, 3.11). Lipid peroxidation is regarded as one of the primary key events in cellular damage (Mead, 1976; Plaa and Witschi, 1976) and the relatationship between GSH levels, lipid peroxidation and cell lysis has been reported (Anundi et al., 1978). The increased lipid peroxidation in paraben-treated
animals is in conjunction with findings reported previously by Nishizawa et al. (2006) and Handa et al. (2006).

Lipid peroxidation can alter the fluidity of cell membranes, can damage to membrane structure and polymerize membrane components (Niki et al., 1991). The end products of lipid peroxidation can inhibit protein synthesis (Fraga et al., 1989) and react with nitrogenated bases of DNA (Park and Floyd, 1992). All amino acid residues of a protein molecule are potential targets of hydroxyl radicals. Under aerobic conditions, hydroxyl radical can cause fragmentation of peptide chain. Free radicals can cause damage in nucleic acid by attacking the nucleotide bases and deoxyribose sugars. For the double standard DNA, the deoxyribose moiety which is located externally in the helix is extremely susceptible to oxidation (Demple, 1990), which causes cross-linking of DNA strands and thus alter the transcription of genetic information. High concentration of oxygen free radicals also cause strand scission in DNA. Replication can be blocked when DNA polymerase encounters a lesion or when the enzyme misreads the modified genetic message on the strand and hence generates a faulty daughter strand (Murata et al., 1997).

Increase in lipid peroxidation could be due to significant reduction in enzymatic antioxidants, catalase, superoxide dismutase, and glutathione peroxidase activities as well as non enzymatic antioxidants glutathione and total ascorbic acid contents in the liver and kidney of paraben-treated mice as compared to controls. Superoxide dismutase protects cells from oxidative damage by breaking down a potentially hazardous free radical superoxide (O$_2^-$) to H$_2$O$_2$ and O$_2$. The H$_2$O$_2$ can then be decomposed enzymatically by catalase and glutathione peroxidase. Glutathione peroxidase not only decomposes H$_2$O$_2$ but can also interact with lipid peroxidation (Venkateshwaran et al., 1987). Thus
significant reduction in these enzymatic activities (Table 3.5, 3.11) could be responsible for increased lipid peroxidation observed in paraben-treated mice.

Glutathione content is significantly decreased after paraben treatment (Table 3). Glutathione has a beneficial effect by virtue of possessing –SH groups. It helps to protect biological membranes, which are readily susceptible to injury by peroxidation (Venkateshwaran et al., 1987). In normal and α-linolenic acid (LNA)-loaded cultured rat hepatocytes, butyl paraben-induced severe cell injury accompanied by a significant decrease in cellular levels of both glutathione (GSH) and protein-SH (Sugihara et al., 1997). In hepatocytes suspension, propyl paraben caused a concentration- and time-dependent decrease in the levels of ATP and GSH, which was accompanied by a loss of cell viability (Nakagawa and Moldeus, 1998).

Paraben treatment caused significant reduction in ascorbic acid contents in the liver and kidney of mice. During free radical scavenging action, ascorbic acid is transformed into L-dehydroascorbate (Breimer, 1990). Reduced glutathione is required for the conversion of L-dehydroascorbate back to ascorbate (Breimer, 1990). The fall in the level of reduced glutathione decreases conversion of L-dehydroascorbate to ascorbate and probably explains the lowered level of ascorbic acid in the paraben-treated animals.

Our in vitro studies revealed that addition of paraben to liver and kidney homogenates caused significant, persistent and concentration-dependent increase in \( \text{H}_2\text{O}_2 \)-induced TBARS suggesting that it causes lipid peroxidation.

Oral administration of aqueous ginger extract for 30 days along with paraben caused significant amelioration in paraben-induced lipid peroxidation by increasing the
antioxidative activity of the cells. Activity of enzymatic antioxidants (catalase, superoxide dismutase, and glutathione peroxidase) as well as non enzymatic antioxidants (glutathione and total ascorbic acid contents) were significantly increased in liver and kidney of paraben-treated mice.

Ginger is a potential activator of the Nrf2/ARE (Nuclear localization of the transcription factor, antioxidant response element) dependent detoxification pathway and also activates detoxification system and neutralize lipid peroxidation in hepatocyes (Nakamura et al., 2004). Ginger extract exhibited very strong antioxidant activity and suppress lipid oxidation (Shobana et al., 2000; Mansour and Khalil, 2000; Rehman et al., 2003; Chung et al., 2003). Ahmed et al., (2000) reported that ginger extract significantly lowered lipid peroxidation by maintaining the activities of the antioxidant enzymes-superoxide dismutase, catalase and glutathione peroxidase in rats. Simultaneous feeding with ginger (Zingiber Officinale Rosc) lowered the levels of TBARS and hydroperoxides formation and raised significantly activities of SOD, CAT and GSH levels in liver and kidney as compared to rats fed an unsupplemented normal or high fat diet respectively (Jeyakumar et al., 1999). Lipid peroxidation induced by FeSO₄-ascorbate was inhibited by 67, 72 and 87%, respectively in human erythrocytes by ginger (Sujatha and Srinivas, 1995). Ginger supplementation prevented carcinogenic nitrosamine-induced depletion of the antioxidant enzymes and the scavenger antioxidants glutathione and vitamins A, C and E and provided protection against free radical and reactive oxygen species-induced tissue lipid peroxidation in liver (Shanmugasundaram et al., 1994). Ginger increases GSH-Px and reduces LPO in blood of hyperlipidemic rats. Ginger could inhibit plasma lipid levels and scavenge radicals of rat body (Liu et al., 2003). Gingerol, the pungent
factor in ginger oleoresin, inhibited phospholipid peroxidation induced by the FeCl₃ ascorbate system (Aeschbach et al., 1994). Dehydrogingerone, a synthetic analogue of zingerone, showed inhibition of lipid peroxidation by acting as free radical scavenger (Rajkumar and Rao, 1993). Administration of ginger powder caused significant decreases in TBARS levels. This decrease in TBARS levels might be due to increased activity of glutathione peroxidase (GPX) in treated rats and hence cause inactivation of lipid peroxidation reactions (Aydin et al., 2001; Levy et al, 1999). Ginger significantly increased the superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione content while decrease of malondialdehyde (MDA) levels were estimated in the hepatic tissue (Mallikarjuna et al., 2007).

Oral administration of paraben for 30 days caused significant reduction in activities of succinic dehydrogenase (SDH) and ATPase in liver and kidney of mice. The effect was comparatively more pronounced in high dose paraben- treated group than that of low dose. Succinic dehydrogenase is a key enzyme in mitochondrial Kreb's cycle, which mainly concerned with the aerobic oxidation of acetyl CoA and the generation of ATP. Putilina and Eschanoko (1969) explained that among the Kreb's cycle dehydrogenases, SDH is a very important key enzyme than any other enzyme. Therefore, reduction in SDH activity clearly indicates reduction in aerobic metabolism.

Nakagawa and Moldeus (1998) have shown that in hepatocyte suspensions propyl paraben caused a concentration- and time-dependent decrease in the levels of ATP, which was accompanied by a loss of cell viability. The compound inhibited both NAD⁺- and FAD-linked respiration and, with succinate as substrate, acted as an
uncoupler of oxidative phosphorylation. The mitochondrial membrane potential was reduced in both the isolated organelles and in hepatocytes in situ.

It could be due to function of parabens causing cytotoxicity, and most likely through impairment of mitochondrial function and a consequent decrease in the cellular level of ATP. The insufficient supply of ATP, in turn, limits activities of all energy-requiring reactions and eventually leads to cell death.

Gottfried (1962) and Thompson and Moldeus (1988) have indicated that the phenolic hydroxy group is essential for the inhibition of mitochondrial respiration, while the alkyl group esterified to hydroxybenzoic acid enhances mitochondrial dysfunction by increasing the hydrophobicity of the molecule. Mitochondrial dysfunction and its consequences are common mechanisms of cytotoxicity caused by a wide range of chemicals and a number of pathological conditions (Redegeld et al., 1989; Nieminen et al., 1990; Kehrer et al., 1990).

Treatment with aqueous ginger extract along with paraben significantly ameliorates paraben-induced changes in SDH and ATPase in liver and kidney of mice (Table 3.6, 3.10). The ameliorative effect of aqueous ginger extract might be due to presence of large amount of polyphenols having antioxidative properties. Antioxidants have a property to protect all membrane lipids and unsaturated fatty acids against oxidative degradation (Dean and Cheeseman, 1987). Gingerol activated ATPase activity in skeletal and cardiac sarcoplasmic reticulum (Kobayashi et al., 1987). Both the o-methoxyphenol and hydrocarbon chain in the molecule of gingerol analogues are necessary for the activation of the Ca$^{2+}$-pumping ATPase activity of sarcoplasmic
reticulum (Ohizumi et al., 1996). The protective effect of the ginger extract on CCl₄ and acetaminophen-induced damage was confirmed by maintaining serum and liver marker enzymes like ALT, AST, ALP, LDH and SDH, thus of Zingiber officinale could be useful in preventing chemically-induced acute liver injury in rats (Yemitan and Izegbu, 2006).

The paraben treatment significantly increased in serum GOT and GPT activities in mice (Table 3.12). Significant increase in serum GOT and GPT activities indicates that paraben-induced damage to cytosol and also to mitochondria. These are the markers of liver damage. This coincides with our histological studies which revealed necrosis in liver and kidney (Plate E, L).

Aqueous ginger extract plus paraben treatment significantly ameliorated the changes in serum GOT and GPT activities. Aqueous extract of Z. officinale significantly protected the hepatotoxicity by significantly (p<0.01) decreasing the activities of SGPT and SGOT in the acetaminophen alone treated animals (Ajith et al., 2007b).

Paraben produced blebbing of the plasma membrane, which has been recognized by previous investigators as an early event in chemically-induced toxicity and hypoxic damage in hepatocytes (Kehrer et al., 1990). The formation of “blebs” may be causally related to depletion of ATP because the latter is necessary for polymerization of microtubules and microfilaments, which are involved in the interactions between the cytoskeleton and plasma membrane (Nicotera et al., 1994). It has also been suggested that the phenomenon can be caused by either a collapse of the mitochondrial membrane potential or an elevation of cytosolic free Ca²⁺ (Nicotera et al., 1994). The former can result from an impairment of electron transport and uncoupling. Collapse of the
mitochondrial membrane potential can lead, in turn, to the inability of mitochondria to take up calcium from the cytosol with a consequent rise in its level.

A decrease in cellular ATP levels would also adversely affect transport of calcium into the endoplasmic reticulum, which is an energy-requiring process. A rise in cytosolic free Ca\(^{2+}\)-induced by parabens has been observed (Sone \textit{et al.}, 1990) in smooth muscle from guinea pig ileum, although in this case it was reported to be the result of activation of a calcium channel.

If parabens do indeed open plasma membrane calcium channels and increase intracellular Ca\(^{2+}\), one may offer an alternative hypothesis that could explain, in part, mitochondrial dysfunction. It is possible that this compound, like some other chemicals induce a calcium-dependent increase in permeability of the mitochondrial inner membrane to small ions and molecules (a so-called "permeability-transition") which leads to the collapse of mitochondrial membrane potential and swelling of the organelles (Wallace \textit{et al.}, 1997; Niemenen \textit{et al.}, 1990, Gunter and pHeiffer, 1990, Imberti \textit{et al.}, 1993, Park \textit{et al.}, 1994). If this occurs, it could aggravate the damage caused by direct inhibition of electron transport. Methyl paraben causes release of calcium ions from storage sites of rats peritoneal mast cells (Fukugasako \textit{et al.}, 2003). Paraben release Ca\(^{2+}\) ions mediated by ruthenium red sensitive Ca\(^{2+}\) release channel, present in skeletal muscle terminal cisternae (Cavagna \textit{et al.}, 2000). p-hydroxy benzoic acid accelerated Ca\(^{2+}\) release rate from the sarcoplasmic reticulum (SR) in a dose-dependent manner.

Liver cells which are lethally injured by several toxins exhibit alterations in intracellular Ca\(^{2+}\) homeostasis after excessive accumulation of Ca\(^{2+}\) (Nicotera \textit{et al.},
1992). During hepatocellular necrosis, excessive intracellular Ca\(^{2+}\) is known to thrust the metabolism in an unmanageable disorder which leads to mitochondrial dysfunction, inhibition of enzymes and denaturation of structural proteins (Fagian et al., 1990). This coincides with our histological studies as well as enzyme levels (Table 3.6, 3.10) and protein content (Table 3.4, 3.9).

**Ameliorative effect of aqueous ginger extract on paraben-induced toxicity in human RBC (An *in vitro* study)**

The present results demonstrate that (0-150 μg/ml) cause destabilization of RBC membrane leading to influx of water into the cells thereby causing hemolysis (Table 3.17). However, exact mechanism of this action is not clearly understood. It might be due increase in lipid peroxidation and oxidative damage. Paraben has remarkable effects on impairment of sperm membrane function (Song et al., 1991). In human and rabbit erythrocytes *in vitro*, butyl paraben (0.02%) induced hemolysis of cells (CIR, 1984). Concurrent addition of paraben (150 μg/ml) and aqueous ginger extract (0-100 μg/ml) to RBC suspension caused significant reduction in rate of hemolysis which may be due to presence of antioxidants in the ginger extract. Incubation of cell lines with T-2 toxin and different antioxidants decreased significantly the cytotoxicity induced by T-2 toxin (Shokri et al., 2000). The antioxidant action of ginger has been proposed as one of the major possible mechanisms for the protective actions of the plant against cytotoxicity (Jagetia et al., 2003; Haksar et al., 2006). The antioxidant activity of ginger suggests that in addition to imparting flavor to the food, they possess potential health benefits by
inhibiting the lipid peroxidation (Shobana and Naidu, 2000; Masuda et al., 2004). We conclude that ginger aqueous extract significantly ameliorates paraben-induced hemolysis in RBC suspensions (Table 3.7, 3.18).

The predominant active component present in ginger is (6)-gingerol. Our studies also revealed the presence of (6)-gingerol in aqueous ginger extract (Fig. 3.39). (6)-gingerol, a pungent ingredient present in ginger possess potent antimitogenic and anticarcinogenic activities, which are often associated with their antioxidative and anti-inflammatory activities (Surh, 2002). Gingerol, a sesquiterpene compound, in tropical ginger *Zingiber officinale*, potentiated the gene expression of several Nrf2/antioxidant response element-dependent phase II enzyme genes, including gamma-glutamyl cysteine synthetase, glutathione peroxidase & hemeoxygenase-1 (Nakamura et al., 2004). Two new glucosides of (6)-gingerdial including 5-O-beta-D-glucopyranosyl-3-hydroxy-1-(4-hydroxy-3-methoxy-phenyl) decane were isolated from ginger and structures determined by HRFAB-MS and NMR analyses. It showed antioxidative activity in linoleic acid model system and DPPH radical-scavenging activity (Sekiwa et al., 2000). (6)-gingerol, an antioxidant from ginger was shown to protect HL-60 cells from oxidative stress induced cell death in promyelocytic leukemia cells, caused DNA fragmentation and inhibited Bcl-2 expression in cells (Wang et al., 2003). (6)-Gingerol, pungent phenolic compound present in ginger, inhibited nitric oxide (NO) production dose-dependently and reduced inducible NO synthase in lipopolysaccharide-stimulated J774.1 cells. It is also a protector against peroxynitrite-mediated damage (Ippoushi et al., 2003). (6)-gingerol inhibited phorbol/DMBA induced skin cancer and suppressed phorbol (TPA) induced epidermal ornithine decarboxylase activity and inflammation (Park et al., 1998).
Gingerols relaxed muscle and modulate the response to eicosanoids: increasing effect by PGF2 alpha, PGE2, PGI2-Na, and TRK-100, but suppressed the response to PGD2, U-46619, LTC4, LTD4, NA and PhE (Kimura et al., 1989). Bile secretion increased by ginger is attributed to (6)-gingerol and (10)-gingerol (Yamahara et al., 1985). (6)-gingerol, found to inhibit 12-O-tetradecanoylphorbol-13-acetate-induced cyclooxygenase-2 expression in mouse skin in vivo by blocking the p38 MAP kinase- nuclear factor-kappaB signaling pathway (Kim et al., 2005). Topical application of (6)-gingerol inhibited phorbol 12-myrist 13-acetate -induced cyclooxygenase -2 expression, suppressed NF-kappaB DNA binding activity in mouse skin and inhibited the phosphorylation of p38 mitogen-activated protein kinase (Kim et al., 2004). Thus the study unraveled the molecular mechanisms underlying antitumor promoting effects of (6)-gingerol in mouse skin in vivo.

Therefore (6)-Gingerol may be the key ingredient in ginger aqueous extract which effectively contributed towards ameliorating the paraben-induced toxicity in mice.