CHAPTER - IV

DISCUSSION

Results of in vivo evaluation of bisphenol A – toxicity stated that oral administration of bisphenol A for 30 days caused a significant, dose – dependent reduction in body weight of mice (Table 3.1). The decrease in body weight might be due to the reduction in feed consumption. The diminution in feed intake paralleled well with the decrease in body weight. A significant decline in body weight gain with reduction in feed intake in adult female rats after administration of 4.5 mg/kg bw/day of BPA for 15 days has been reported by Nunez et al. (2001). Effect of dietary BPA was evaluated in a mice two-generation study at 0, 0.018, 0.18, 1.8, 30, 300 or 3500 ppm (0, 0.003, 0.03, 0.3, 5, 50, or 600 mg BPA/kg bw/day) in 28 animals per sex per group. Bisphenol A at 3500 ppm reduced body weight and increased kidney and liver weights (Tyl et al., 2008). Tyl et al. (2002) have reported a significant decrease in body weight gain and increase in relative organ weights in weaning and adult rats. Many previous reports have confirmed the reduction in body weight after exposure of BPA. (Honma et al., 2002; Negishi et al., 2003; Alonso-Magdalena et al., 2010; Nakamura et al., 2011)

Treatment with bisphenol A caused a significant increase in absolute and relative liver weights of mice (Table 3.2). The marked increase in liver weight could be due to increased accumulation of lipid and cholesterol contents in liver (Table 3.3). Histopathological investigations in the present study also revealed fatty accumulation in liver of bisphenol A - treated mice (Plate E). An increase in liver weight was observed in adult male CD-1 mice when exposed to 3500 ppm of bisphenol A (Tyl et al., 2008).

Oral administration of bisphenol A caused significant decrease in glycogen content in liver of mice (Table 3.3). The primary function of liver is to store the energy in the form of glycogen.
So the reduced glycogen content observed in BPA – treated mice could be due to decreased biosynthesis and/or increased degradation of glycogen content, which shows the excess requirement of energy in liver. Jayashree et al. (2013) showed that BPA treatment in adult male albino rat decreased glycogen content in liver. Bisphenol A alters glucose metabolism and glucose uptake pathways leading to decreased glycogen content in liver.

Thirty days treatment of bisphenol A resulted in elevation of hepatic total lipid and cholesterol contents in mice (Table 3.3). Ronn et al. (2013) showed that increase in liver fat by BPA might be due to de novo lipid synthesis, decreased degradation or increase transport of cholesterol esters to the liver. Several animals studies showed that over stimulation of the estrogen receptor – alpha (ERα) in pancreatic β – cells by BPA produced excessive insulin signaling in the liver (Alonso – Magdalena et al., 2006, 2008). Bisphenol A considered as a potential “Obesogen” has been shown to stimulate the pathways involved in lipogenesis mainly through direct interaction with nuclear receptors (Grun., 2010; Casals-casas and Desvergne., 2011; Riu et al., 2011).

Masuno et al. (2005) have reported that when 3L3- L1 cells treated with bisphenol A shows increased levels of lipoprotein lipase and adipocyte – specific fatty acid binding protein mRNAs, confirming that BPA is able to accelerate the terminal differentiation of 3T3-L1 cells into adipocytes. Prenatal exposure to BPA has been shown to increase expression of lipogenic genes and adipocyte size in rodents (Somm et al., 2009). Studies on isolated cells have shown BPA to induce production of proinflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor- alpha (TNF – α) (Yamashita et al., 2005) and to induce expression of adipogenic transcription factors (Phrakonkham et al., 2008), including peroxisome proliferator-activated receptor - gamma (PPAR – γ) activation (Kwintkiewicz et al., 2010). Xia et al. (2014) explained that BPA intoxicated rats exhibited a greater accumulation of lipids in the liver and significantly elevated hepatic triglyceride (TG) at 27 weeks. A recent in vitro study reported that BPA –
induced lipid accumulation in HepG2 (human hepatocellular liver carcinoma cell line) cells by disturbing mitochondrial function (Huc et al., 2012). Another study done by Marmugi et al. (2012) reported that oral exposure of adult male mice to low doses of BPA increased hepatic mRNA and protein expression related to lipid biosynthesis and an activation of lipogenesis and cholesterol biosynthesis as the major mechanism involved, potentially associated with an inhibition of fatty acid oxidation. Wada et al. (2007) also confirmed that BPA has capacity to stimulate the accumulation of triacylglycerol in 3L3-L1 preadipocytes.

The present study revealed significant reduction in DNA, RNA and protein contents in liver of bisphenol A – treated animals (Table 3.3). This might be due to BPA-DNA adduct formation. Many authors confirmed the ability of BPA to form DNA adduct in acellular system (Edmonds et al., 2004; Qiu et al., 2004), in cell cultures (De flora et al., 2011; Izzotti et al., 2009) and in the liver of both rats (Atkinson and Roy, 1995a, 1995b) and mice (Izzotti et al., 2009) treated in vivo. Further, Izzotti et al. (2009, 2010) demonstrated that administration of BPA with drinking water results in the formation of DNA adducts and proteome alterations in the mammary tissue of mice. Bisphenol A exposure results in impaired chromosome synapsis, altered meiotic DNA double strand break repair progression, subsequent activation of the ATL-1 and CHK-1 DNA damage checkpoint kinase and increased CEP-1/P53 – dependent germ cell apoptosis (Allard and Colaiacovo, 2010).

Further, experiments in mice have demonstrated that BPA exposure can cause altered DNA methylation patterns in cell signaling genes, suggesting that BPA exert its effects through epigenetic mechanisms (Ho et al., 2006; Dolinoy et al., 2007; Yaoi et al., 2008). Both in humans and in experimental animals, BPA is metabolized to its glucuronide and hydroxylated derivatives, which finally converted to o-quinine (Edmonds et al., 2004). This oxidation of catechols to semiquinones and quinones is a mechanism of tumor initiation for endogenous and synthetic estrogens (Cavalieri and Rogan, 2006). This interaction might prevent RNA polymerase
transcribing the DNA and can inhibit the formation of mRNA. A failure in mRNA formation can result in an inhibition of protein synthesis which may be considered to be the cause of the liver cell necrosis (Korkmaz et al., 2010). Castro et al., (2013) have shown that administration of BPA to adult rats is associated with a decrease in mRNA and protein levels of both 5αR1 and 5αR2 isozymes. Hanioka et al. (1998) reported that BPA - induced significant reduction in protein content in rat liver.

Hassan et al. (2012) demonstrated that bisphenol A elicits depletion of antioxidant defense system by inducing oxidative stress ultimately led to hepatotoxicity. Oxidative stress takes place due to unregulated production of free radicals. Reactive oxygen species (ROS) are cytotoxic agents that lead to oxidative damage after causing covalent binding to oxidize cellular macromolecules such as nucleic acid bases, proteins and lipids thereby leading to cell death (Perry et al., 2001; Barnham et al., 2004). Thus, in addition to DNA adduct formation, oxidative stress could be another reason for alterations produced by BPA in DNA, RNA and protein contents. DNA lesions are constantly being produced in living cells by the deleterious actions of both endogenous and environmental DNA damaging agents (Kruman, 2004).

Oxidative DNA lesions include the oxidation of nucleotide bases, modifications to the sugar moiety of DNA, which may result in base-loss abasic (apurinic/apyrimidinic) sites and/or strand break (single and double strand breakers), DNA-DNA intra-strand adducts and DNA-protein cross-links, all of which are cytotoxic and some can be mutagenic (Powell et al., 2005; Volko et al., 2006; Hazara et al., 2007). Iso et al. (2007) reported that BPA - induced DNA strand breaks in ER-positive MCF-7 cells. Bisphenol A - induced micronucleus formation and structural chromosome aberrations in bone marrow of rats as well as DNA damage in lymphocytes (Tiwari et al., 2012). Free radical removes electrons from lipid membrane, leading to lipid peroxidation. It can induce the peptide chain’s fracture and cross-linking of amino acids in enzyme molecule. As a
result, the enzyme activity is renewed, lost or changed the structure of cell membranes are changed and destroyed (Ke et al., 2013).

The liver plays an important role in metabolism of xenobiotics and to remove toxic substance from the portal circulation, placing it in the first line and prone to be attacked by foreign materials (Ung et al., 2011; Kuriakose and Kurup, 2011). Acute injury to hepatocytes alters their transport function and membrane permeability, leading to leakage of marker enzymes from the cells (Yang et al., 2008). Bisphenol A treatment had significantly altered activities of enzymes in liver (Table 3.4) as well as liver marker enzymes in serum (Table 3.7) indicative of hepatocellular membrane damage and necrosis. Disruption of membrane integrity could be due to bisphenol A - induced oxidative stress causing either leaking out of the content or denaturing of enzyme structure resulting in reduced activities of the enzymes.

Elevated levels of liver marker enzymes such as ALT, AST, ACP and ALP are indicators of cellular leakage and loss of functional integrity of the cell membrane in liver (Drotman and Lawhorn, 1978). These are of major importance in assessing and monitoring functional status of liver. It has been reported that ALT activity, present in liver cytosol is an important index to measure the degree of cell membrane damage (Giboney, 2005), while AST is an indicator of mitochondrial damage since it contains 80% of this enzyme. When the liver hepatocytes are damaged, these enzymes are released into the blood where the significant increase in ALT and AST activities indicates the damage to the cytosol and also to mitochondria (Mathuria and Verma, 2008).

Many authors reported significant increase in ALT and AST activities in serum of rats treated with 25 mg/kg bw/day BPA (Korkmaz et al., 2010; Mourad and Khadrawy, 2012). Ghorpade et al. (2000) reported increased ALT and AST activities in liver and muscle of freshwater fish. Barse et al. (2007) and Mapuskar et al. (2007) also reported elevation of ALT and AST enzyme activities in mice. Yamasaki et al. (2002a, b) reported an increase in AST activity in
males treated with BPA ≥200 mg/kg bw/day. Various hepatocellular researches on humans as well as animal models had indicated great correlation between stress and increase in ALT and AST levels (Icen et al., 2005). Therefore, it could be suggested that oxidative stress induced by BPA may mediate the disturbance in hepatic function which is reflected by the present increase in ALT and AST.

Acid phosphatase (ACP) is a marker enzyme for the lysosomal integrity (Collins and Lewis, 1971). The present study revealed significant dose – dependent increase in ACP activity in liver tissue (Table 3.4) as well as in serum (Table 3.7) of BPA – treated mice. It might be due to the consequence of damage to lysosomal integrity in the liver. The significant elevation seen in the ACP activity after toxin administration may be attributed to increase in cellular degeneration and other pathological liver injury (Verma et al., 2004). This also suggested high tissue catabolism and cellular autophagya which are possible sequences leading to tissue damage (Abharam and Welfred, 2000). Many investigators have reported increase in serum ACP activity in BPA – treated animals (Ghorpade et al., 2000; Pereira et al., 2006; and Mapuskar et al., 2007).

Alkaline phosphatase (ALP) activity significantly and dose – dependently increased in liver tissue (Table 3.4) as well as in serum (Table 3.7) of BPA – treated mice. Alkaline phosphatase is a marker enzyme for plasma membrane and endoplasmic reticulum (Shahjahan et al., 2004) and is often employed to assess the integrity of plasma membrane (Akanji et al., 1993). Lipophilic BPA interact with plasma membrane and thereby could have led to increase in ALP activity. The increase in ALP activities in serum may be attributed to either de novo synthesis of the enzyme molecules or loss of other proteins from tissues (Umezawa and Hooper, 1982). Marmugi et al. (2011) suggested that BPA may influence de novo fatty acid synthesis thereby contributing to hepatic steatosis. Yamasaki et al. (2002a, b) reported increase in ALP activity in rat males treated with 600 mg/kg bw/day of BPA. Such increase in ALP activities can constitute a
threat to the life of cells that are dependent on a variety of phosphate esters for their vital process since there may be indiscriminate hydrolysis of phosphate esters of the tissues.

Oral administration of bisphenol A to mice for 30 days had significantly altered the energy status of hepatocytes. Succinate dehydrogenase (SDH) is a key enzyme in the mitochondrial Krebs cycle, which is mainly concerned with the aerobic oxidation of acetyl co A and generation of ATP. Putilina and Eschanko (1969) explained that among the Krebs cycle dehydrogenases, SDH is more active enzyme; therefore reduction in SDH activity clearly indicates reduction in aerobic metabolism, which might be result of reduced oxygen transport to tissues. Bisphenol A treatment resulted in drastic reduction in SDH activity which could be due to structural and functional disorganization of the mitochondrial assembly (Table 3.5). A recent in vitro study demonstrated that BPA had a direct effect on mitochondria.

A detailed study by Nakagawa and Tayama (2000) explained the relationship between the metabolism and the cytotoxic effects of BPA in freshly isolated rat hepatocytes and isolated hepatic mitochondria. The incubation of hepatocytes with BPA (0.25 – 1.0 mM) elicited a concentration - and time - dependent cell death, accompanied by losses of intracellular ATP and total adenine nucleotide pools. Bisphenol A at a low – toxic level (0.25-1.0 mM) in the hepatocytes suspensions was rapidly converted to its major conjugate, BPA- glucuronide and other minor products without marked loss of cell viability, although at a toxic level (0.5 mM), more than 65% of the compound presented in an unaltered form 2 h after the incubation.

The addition of BPA to isolated hepatic mitochondria caused a concentration (0-0.5 mM) – dependent increase in the rate of state 4 oxygen consumption in the presence of an FAD – linked substrate (succinate), indicating an uncoupling effect, whereas the rate of state 3 oxygen consumption was inhibited by BPA. Further, the addition of BPA (0.25 mM) reduced state 3 respiration with NAD+ - linked substrates (pyruvate plus malate) and/or with the FAD – linked substrate, whereas state 3 respiration with ascorbate plus tetramethyl-/> - phenylenediamine
(cytochrome oxidase-linked respiration) was not significantly affected by BPA. These results indicate that the onset of cytotoxicity caused by BPA may depend on the intracellular energy status and that mitochondria are important targets of the compound. Result of present investigation showed also significant reduction in ATPase activity in BPA intoxicated mice. Nakagawa and Tayama (2000) also showed BPA - induced mitochondrial dysfunction in human HepG2 cells by reduction in ATP levels and loss of total adenine nucleotide pool ultimately inducing cytotoxicity leading cell death.

Nakagawa and Tayama (2000) revealed that the phenolic hydroxyl group is essential for the inhibition of mitochondrial respiration while the elongation of the hydrocarbon bridge enhances mitochondrial dysfunction by increasing the hydrophobicity of their molecules. The toxicity caused by the inhibition of ATP synthesis may be related to the concentration of unmetabolised free BPA remaining in the cell suspensions which also contain phenolic hydroxyl group. The result of the present study shows that mitochondria are an important target for the BPA. Treatment of HepG2 cells with BPA for 2 hr leads to a deteriorated mitochondrial architecture. Many authors have also reported that BPA - induced impairment of mitochondrial functions by decreasing the activity of antioxidant enzymes or by inducing lipid peroxidation (Bindhumol et al., 2003; Ooe et al., 2005). When mitochondria are damaged, energy generation in them is inevitably inhibited which contributes to the overall loss in the energy production (Guo et al., 2005). Reduction in ATPase activity in liver suggests reduced utilization of ATP produced in the cell. Thus, reduced aerobic oxidation and ATP generation could be responsible for the reduction in ATPase activity.

Bisphenol A has been shown to be decomposed to many kinds of metabolites including bisphenol A radical by a reaction with radical oxygen (Sajiki et al., 2001). Bisphenol A was previously reported to induce oxidative damage in several tissues (Aydogan et al., 2010; Korkmaz et al., 2010; Korkmaz et al., 2011). The findings of the present study clearly indicate the
involvement of oxidative stress caused by reactive oxygen species generation in BPA - induced hepatotoxicity.

Measurement of MDA levels in the tissue is a marker of lipid peroxidation, which is among the chief mechanism of cell damage. Oral administration of BPA for 30 days has resulted in significant elevation in MDA levels (Table 3.6) which could be due to overproduction of reactive oxygen species and/or suppression of antioxidant enzyme activities resulting in the altered redox potential of cell causing lipid peroxidation and hence suggesting a considerable hepatocytic oxidative stress. Several study reported the occurrence of oxidative toxicity after BPA exposure in rats and mice (Gang and Han, 2006). Kabuto et al. (2004) attributed the underdevelopment of the kidney, brain and testes to the increased levels of TBARS in BPA exposed mice. Bisphenol A exposure caused oxidative stress by disturbing the balance between reactive oxygen species and antioxidant defense system in kidney, brain and testes of rats (Aydogan et al., 2010; Korkmaz et al., 2011, Chen et al., 2012) and mice administered BPA throughout the embryonic/fetal life and during infancy (Kabuto et al., 2004). This could be due to lipophilic nature of BPA which might allow its easy assimilation in lipid portion of the hepatocytes membrane and initiating chain reactions of lipid peroxidation (Doerge and Fisher, 2010).

Several studies indicated that BPA exerts potent oxidant activity in various tissues via reactive oxygen species production which results in inhibition of antioxidant enzymes, increased hydrogen peroxide and lipid peroxidation products (Obata and Kubota, 2000; Bindhumol et al., 2003; Chitra et al., 2003a, b; Kabuto et al., 2003,). Reduced glutathione (GSH) and ascorbic acid are important endogenous free radical scavenger and non – enzymatic antioxidants. The level of GSH and TAA were significantly reduced in BPA – treated animals (Table 3.6). This reduction might be due to excessively produced free radicals which crosses the scavenging potency of these
antioxidants. Reduced glutathione (GSH) is widely distributed in cells. Reduced glutathione (GSH) is an intracellular reductant and plays major role in catalysis, metabolism and transport.

Indeed, GSH depletion increases the sensitivity of cells to various aggressions and also has several metabolic effects, for example, a decrease in the rate of gluconeogenesis or an increase in glycogenolysis. This concept of a GSH threshold for drug detoxification was discussed by Jollow, (1980). GSH plays a key role in protecting cell against free radicals and electrophiles and this could be due to the nucleophilicity of thiol (-SH) group and due to the high reaction rate of thiol with free radicals. Among the protectors of oxidative stress and damage, cellular thiols are important, mainly GSH plays a major role. Thus GSH belongs to the second line of antioxidant defence which is the most abundant non-protein thiol synthesized in vivo and serves as a scavenger of different free radicals (Ray and Husian, 2002). Glutathione depletion also promotes oxidative stress, with a cascade of effects thereby affecting functional and structural integrity of cell and organelle membrane (Masella et al., 2005). The increased TBARS level and decreased GSH and TAA concentration indicate an increased generation of reactive oxygen species, which cause lipid peroxidation in the liver (Nandi et al., 2005).

Ascorbic acid is a most powerful antioxidant under physiological conditions. Hydrogen peroxide was converted into water by ascorbic acid via ascorbate peroxidase reaction (Noctor and Foyer, 1998). It is well established that GSH in blood keeps up the cellular levels of the active forms of vitamin C and vitamin E by neutralizing the free radicals. During free radical scavenging action, ascorbic acid is transformed into L-dehydroascorbate. Reduced glutathione is required for the conversion of L - dehydroascorbate back to ascorbate (Breimer, 1990). Resulting fall in the level of GSH decrease the conversion of L - dehydroascorbate and probably explains the lowered level of reduced ascorbic acid in BPA – treated mice. When there is reduction in the GSH content the cellular levels of vitamin C and vitamin E is lowered, indicating that all are closely interlinked to each other (Winkler, 1992). Our results are consistent with previous reports showing decreased
GSH and TAA concentration in the liver in BPA administered rats (Korkmaz et al., 2010). Earlier reports have also shown changes in these parameters in liver diseases (Goodfellow and Waugh, 2009).

Liver contains enzymatic antioxidants such as SOD, catalase, GPx, and GST which constitutes first line of defence against ROS – induced damage (Kale, 2007). Oral administration of bisphenol A for 30 days significantly reduced the activities of enzymes of antioxidant system (Table 3.6). A decrease in SOD activity contributes to increasing the level of superoxide radicals, thus leading to increased oxidative stress which enhances early cell death. Further decrease in catalase activity would increase $\text{H}_2\text{O}_2$ concentration in the cell, leading to increased lipid peroxidation and oxidative stress (Anderson et al., 1997). Glutathione reductase contributes to the regeneration of GSH, so the suppressed activity of GR could be the reason for the reduction of GSH content. The significantly reduced activities of these enzymes might be due to BPA - induced protein oxidation.

The interaction of LPO products with enzyme molecules leads to the exclusive modification of histidine residue and generation of protein – protein cross linked derivatives causing reduction in enzyme activity (Kwon et al., 2000). The destruction and degradation of phospholipids hydroperoxides are carried out by GPx and this suggested being a pathway of cytoprotection against the deleterious effects of phospholipids hydroperoxides. The decreased GPx activity in bisphenol A – intoxicated mice, leads to an increased cytotoxicity. Superoxide dismutase, CAT and GPx constitute a mutually supportive team of antioxidant enzymes which provide a defense system against ROS (Viswanatha swami et al., 2010). The decreased activities of GPx, CAT and SOD in liver homogenates of bisphenol A intoxicated mice, may be due to oxidative stress induced inactivation and/or exhaustion.

Bindhumol et al. (2003) suggested that the reduction in the activity of catalase may reflect the inability of liver mitochondria and microsomes to eliminate hydrogen peroxide produced after
exposure to BPA. Also it was reported that, the decreased GPx activity leads to $H_2O_2$ accumulation in the liver which in turns inactivates SOD (Kakkar et al., 1977; Godin et al., 1988). Glutathione –S - transferase is an important enzyme involved in conjugation reaction catalyzing the detoxification of a variety of endogenous and exogenous compounds. The decrease in GSH level during bisphenol A intoxication may be due to the combined effect of inhibited GR and reduced NADPH supply. Hepatic lipid peroxides formation can be initiated when oxidative stress overcomes antioxidant defenses (Sun et al., 1999).

Obata and Kubota (2000) reported that BPA increased hydroxyl radical formation in the rat striatum in an examination using in vivo microdialysis. Wu et al. (2011) showed significant decrease in the level of GSH and SOD in BPA - treated rats; this decrease indicated liver tissue damage. Similarly, others demonstrated that BPA generates ROS that cause oxidative damage in the brain, reproductive tract and kidney of rats (Korkmaz et al., 2010, Aydogan et al., 2010). Although BPA monoglucuronide is the major metabolite of BPA, it was postulated that BPA, in part, is metabolized to hydroxylated BPA with further formation of bisphenol-o-quinone via bisphenol semiquinone by peroxidase or hepatic microsomal cytochrome P450 activation systems (Knaak and Sulivan, 1966; Atkinson and Roy, 1995a). Oxidative damage might be induced by free radical generation via metabolic redox cycling between quinine and hydroquinone forms of BPA.

Histopathological studies revealed increase in sinusoidal lumen as well as increased vacuolization, fatty infiltration and hepatocellular necrosis in the liver of bisphenol A – treated mice (Plate E). Previous studies have reported that BPA cause cell infiltration and necrosis (Boshra and Moustafa, 2011; Mourad and Khadrawy, 2012), vacuolated hepatocytes (Roy et al., 2011) and liver damage (Hassan et al., 2012). Nakagawa and Tayama (2000), reported that in isolated rat hepatocytes bisphenol A caused cytotoxicity, cell injury and lysis. These degenerative changes could be due to increase in lipid peroxidation and reduced antioxidative capacity in liver
of mice. Korkmaz et al. (2011) reported necrotic lesions, congestion and mononuclear cell infiltration in the kidney of rats treated with BPA. Also Korkmaz et al. (2010) reported hepatic necrosis and congestion in liver of rats exposed to BPA because of ROS induction and disruption in the balance between ROS and antioxidant defense system. Moreover the study of Bindhumol et al. (2003) revealed that low doses of BPA generate ROS by decreasing the activities of antioxidant enzymes and increasing LPO thereby causing oxidative stress in liver of rats. Our present study also revealed that bisphenol A treatment caused increase lipid peroxidation in liver homogenates in vitro (Table 3.9).

Overproduction of reactive oxygen species is considered as a major cause of molecular injury and is implicated in the pathogenesis of several human diseases and age-related degenerative processes (Volko et al., 2007). Hence herbal drugs/herbal preparations containing such phytoconstituents especially antioxidants are gaining importance in the prevention and treatment of various organ toxicities due to xenobiotic/environment challenges (Larson et al., 1988). Health promoting effects of plants are primarily denoted by the presence of bioactive phytochemicals acting as potent antioxidants having nutritional and pharmacological properties (Cai et al., 2004; Han et al., 2007). Among the numerous naturally occurring antioxidants; phenols, flavonoids, tannins and ascorbic acid are most effective one to exert desired physiological effects in human body.

In the present study qualitative assessment of phytochemicals of green tea extract showed presence of phenols, flavonoids, tannins and ascorbic acids which were in accordance with the various reported studies (Jain et al., 2011). Atouí et al. (2005) showed that green tea contains more polyphenols than black tea. Flavonoids and many other phenolic compounds of plant origin have been reported as scavengers of ROS and viewed as promising therapeutic drugs for free radical pathologies (Chang et al., 2007). Polyphenols demonstrated diverse biological activities attributed to their general free radical trapping capacity and reduce or prevent some of damage
they can do to the body (Hawkins, 2007; Chopade, 2008). In the present study quantitative estimation of crude polyphenols from green tea extract revealed presence of significantly high amount of phytochemicals principally responsible for its protective effect (Table 3.8).

In the present study quantitative estimation of crude polyphenols from hydro-alcoholic extract of green tea revealed presence of significantly high amount of phytochemicals principally responsible for its protective effect (Table 3.8). Hepatoprotective activity present in this plant may be due to the activities of one or a combination of some of the classes of compounds present. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Osawa, 1994).

Free radicals of different forms are constantly generated for specific metabolic requirement and quenched by an efficient antioxidant network in the body. When the generation of these species exceeds the levels of antioxidant mechanism, it leads to oxidative damage of tissues and biomolecules, eventually leading to disease conditions, especially degenerative diseases (Gutteridge, 1995). Reactive oxygen species (ROS), including the free radicals superoxide anion ($\text{O}_2^-$), hydroxyl radicals ($\cdot\text{OH}$) and singlet oxygen ($\text{^1O}_2$) and non-free radical species, such as hydrogen peroxide ($\text{H}_2\text{O}_2$), are various forms of activated oxygen resulting from oxidative biological reactions or exogenous factors (Cerutti, 1991).

*In vitro* assessment of antioxidative properties of various plant extract using chemical models provides biochemical basis for the *in vivo* ethnopharmacology. Green tea extract was found to be potent scavenger of superoxide radical, hydroxyl radical, nitrous oxide and reducing power (Figure 3.11, 3.12, 3.13 and 3.14). Crepsy and Williamson (2004) reported that green tea extract displays antioxidants and free radical scavenging property. These scavenging properties are generally due to high reducing capacity of the polyphenols acting as primary antioxidants (Odabasoglu *et al*., 2004). Metal ions play central role in reactive oxygen species generation as
they can change the state from reduced to oxidized causing removal of electron from various biomolecules (Jomova and Volko, 2011). Druzynska et al. (2007) indicated that green tea extract possessed higher metal chealting activity.

Several studies reported direct correlation between the antioxidant activity and polyphenolic content of the plant extracts (Juliani and Sinnon, 2002; Kiselova et al., 2006), which was also seen in present investigation (Table 3.8). Li et al. (2009) had reported the existence of similar linear correlation between reducing power and total phenolic content. Previous studies reported that green tea extract scavenges superoxide radical (Bing et al., 2007), hydroxyl radical (Bors et al., 2009), and NO radical (Kelly et al., 2001). Table 3.8 showed presence of correlation between total phenolic content and antioxidant effect of hydro-alcoholic extract of green tea extract.

Xenobiotics – induced prolonged and unregulated free radical production could be the root cause of numerous degenerative disorders (Murugan et al., 2008). The widespread consumption of bisphenol A containing products has raised concerns among scientists and regulatory agencies that human exposure to bisphenol A may have adverse effects on different vital organs. In the present in vitro study, the recorded significant increased in lipid peroxidation and decreased SOD and CAT activities in the liver homogenates treated with different concentrations (50-250 μg/ml) of bisphenol A, reflects a state of increased oxidative stress in liver cells (Table 3.9).

In this study, bisphenol A increased ROS production in a dose-dependent manner, as assessed by the measurement of MDA concentration, an intermediary product of lipid peroxidation, which provides evidence of oxidative injury, occurred to the cells and indicates failure of antioxidative defense system (Table 3.9). Accumulation of MDA can cause membrane depolarization, induce enzyme inhibition, modulate transport of protein and ultimately renders membrane integrity (Sen et al., 2006), which is in accordance with the findings of a previous study that found MDA levels in the tissue increased after BPA administration (Kabuto, 2003).
Moreover, the study of Bindhumol et al. (2003) revealed that low doses of BPA generate reactive oxygen species by decreasing the activities of antioxidant enzymes and increasing lipid peroxidation thereby causing oxidative stress in liver of rats.

Addition of various concentrations (50-250 μg/ml) of bisphenol A reduced activities of antioxidant enzymes such as SOD and CAT significantly in liver homogenates (Table 3.9) which could be either due to inactivation/denaturation of enzymes or excessively produced reactive oxygen species. These enzymes are involved in the direct elimination of reactive oxygen metabolites, which probably is one of the most effective defenses of the living body against diseases (Ray et al., 2000). Our findings of the decreased activity of SOD and CAT for all selected concentrations of BPA corroborates with that of earlier findings by Chitra et al. (2003b) and Sangai et al. (2012). This indicates that H$_2$O$_2$ was most probably present in high levels, since CAT is involved predominantly in the detoxification of high H$_2$O$_2$ levels (Hermes-Lima, 2004). Reduction in the activity of CAT and SOD may reflect inability of liver metabolism to eliminate H$_2$O$_2$ produced after exposure to BPA.

When liver homogenates were treated with different concentrations (50-250 μg/ml) of BPA, it caused significant reduction in activities of SDH and ATPase because mitochondria are a target of bisphenol A at organelle level. A recent in vitro study reported that BPA – induced lipid accumulation in HepG$_2$ cells by disturbing mitochondrial function (Huc et al., 2012). One more study done by Moon et al. (2012) also indicated that BPA can induce hepatic damage by inducing mitochondrial dysfunction.

When liver homogenates were treated with different concentrations (10-50 μg/ml) of green tea extract along with high concentration (250 μg/ml) of bisphenol A, it resulted in significantly reduced levels of lipid peroxidation, which could be due to free radical scavenging effect of green tea polyphenols and degradation of lipid hydro peroxides which causes reactive aldehyde formation. Scavenging effect of lipid free radicals of polyphenols in green tea extracts can be
clearly observed in experiments (Li and Jiang, 2010). The ability of green tea polyphenols in green tea extracts to eliminate lipid derived free radicals is noticeably stronger (almost 50 times) than that of *Ginko biloba* extracts (Li and Jiang, 2010).

Different concentrations (10-50 μg/ml) of green tea extract when added to liver homogenates containing high concentration (250 μg/ml) of bisphenol A which caused significant reduction in oxidative stress as evidenced by a significant reduction in lipid peroxidation and increase in SOD and CAT activities which were restored close to corresponding control values. Green tea increases the body’s endogenous antioxidants to reduce oxidative damage. Rats given green tea extract orally exhibited increased levels of endogenous antioxidants such as SOD and CAT (Skrzydlewska et al., 2002a). Furthermore, green tea can directly prevent the levels of α-tocopherol and β-carotene, from being depleted by lipid oxidation through 2, 2’ Azobis (2-amido propane) dihydrochloride (AAPH) (Lotito and Fraga, 2000). These animal studies were backed up by clinical evidence which showed increased endogenous antioxidants after green tea administration. In a crossover clinical trial conducted by Young *et al.* (2002), in which green tea extract was administered, plasma antioxidant activity increased, which subsequently decreased oxidative damage.

Different concentration (10-50 μg/ml) of green tea extract when added to high concentration (250 μg/ml) of bisphenol A containing homogenates, caused significant (p<0.05), dose-dependent increase in the activity of SDH and ATPase, enzymes of energy metabolism. This could be due to its ability to restore oxidative phosphorylation capacity, promote mitochondrial biogenesis and to protect the thiol group from oxidative damage. Weinreb *et al.* (2008) reported that green tea increases the protein levels of the β subunit of ATP synthase. Other study has found that EGCG is able to decrease oxidative stress – mediated mitochondrial deterioration in aged brain (Srividhya *et al.*, 2009).
Findings of another *in vitro* study indicated that addition of H₂O₂ to the erythrocytes suspension caused significant (p<0.05) and dose-dependent increase in hemolysis (Table 3.11). Aslan *et al.* (2000) has also reported increased osmotic fragility of erythrocyte suspensions treated with H₂O₂. Interaction of H₂O₂ with iron of heme results in the generation of more potent hydroxyl radicals (Halliwell and Gutteridge, 1986; Van Der Berg *et al.*, 1992), causing increased lipid peroxidation by attacking membrane polyunsaturated fatty acids. This is a known mechanism of action of H₂O₂ causing oxidative stress.

Being lipophilic in nature (Doerge and Fisher, 2010), BPA may get stabilized in the hydrophobic plasma membrane of the cell and may initiate peroxide formation. It may cross the lipid membrane and bind to iron of hemoglobin resulting in dissociation of hemoglobin subunits and release iron which itself acts as pro-oxidant and produce high amount of hydroxyl radicals. Generated hydroxyl radicals may disturb the lipid membrane and initiate lipid peroxidation making the cell osmotically more fragile and ultimately causing hemolysis.

Earlier, it has been shown that bisphenol A - induced oxidative stress in different tissues in rat (Bindhumol *et al.*, 2003; Rashid *et al.*, 2009) and induced cellular apoptosis in hepatocytes (Asahi *et al.*, 2010). Moon *et al.* (2012) indicated that bisphenol A can induce hepatic damage and mitochondrial dysfunction by increasing oxidative stress in the liver. Sajiki (2003) confirmed that bisphenol A binds to ferric heme of red blood corpuscle and Obata and Kubota (2000), reported that bisphenol A increased hydroxyl radical formation in the rat striatum in a study using *in vivo* microdialysis. Another study done by Verma and Sangai (2009) reported significant and concentration-dependent rise in lipid peroxidation in liver and kidney homogenates treated with bisphenol A in *in-vitro* experiments, which supports our study.

Combinational studies (BPA and H₂O₂, Table 3.13 and 3.14) have revealed that addition of BPA along with H₂O₂ accelerates hemolysis which could be due to additive effect of both the
compounds. The above hypothesis was clearly proven as the hemolysis induced by bisphenol A and H$_2$O$_2$ were highly correlated (Figure 3.21, 3.22).

Addition of green tea extract along with BPA in RBC suspension, significantly (p<0.05) reduced BPA - induced hemolysis. Another study of Verma and Sangai (2009), have reported ameliorative effect of black tea extract (0-200 µg/ml) on bisphenol A - induced cytotoxicity. Maximal retardation in hemolysis was observed with 200 µg/ml concentration in case of black tea extract, which was 50 µg/ml concentration in green tea extract. It clearly indicates that green tea extract is comparatively more potent than the black tea extract. Polyphenols found in green tea show 20 times more powerful antioxidant activity than vitamin C (Chopde et al., 2008). Several studies (Asnani and Verma, 2006; Verma et al., 2006; Verma and Chakraborty, 2007) have reported environmental contaminants - induced cytotoxic effects on erythrocytes and its amelioration by use of natural antioxidants.

Green tea extract contains catechins, well known for their antioxidative property which reverses the elevation of lipid peroxidation, stabilizes plasma membrane of erythrocytes thereby reducing hemolysis (Guo et al., 1996). Catechins can also chelate metal ions such as iron (III) to form inactive complexes and prevent the generation of potentially damaging free radicals (Yang et al., 2002). Green tea extract is considered as potent scavengers of reactive oxygen species such as superoxide, hydrogen peroxide, hydroxyl radicals and nitric oxide produced by various chemicals (Schroeder et al., 2003). In a recent study, supplementation of green tea extract attenuates cyclosporine A – induced oxidative stress in rats (Mohamadin et al., 2005). Ostrowska et al. (2004) revealed that green tea may protect liver and brain cells against oxidative stress induced by ethanol intoxication.

Mathur et al. (2003) explained that bisphenol A elicit depletion of antioxidant defense system and induces oxidative stress. In another study done by Hassan et al. (2012), it was reported that bisphenol A induces hepatotoxicity through oxidative stress and ultimately decreasing the
antioxidant enzymes. Catalase and glutathione peroxidase are important enzymes of antioxidant defense systems, which protect tissue against oxidative stress induced by reactive oxygen species. Both these enzymes catalyze the hydrolysis of $H_2O_2$ into water and oxygen molecule to prevent the tissue injury.

We propose that bisphenol A increases hemolysis by increasing the formation of hydroxyl radicals which may directly or indirectly (through the inhibition of glutathione peroxidase and catalase) may induce lipid peroxidation. For confirmation of this hypothesis we have performed molecular docking of bisphenol A with hemoglobin, catalase and glutathione peroxidase. Molecular docking approach typically uses an energy-based scoring function to identify the energetically most favorable ligand conformation with the bound target. Binding energies of the protein-ligand interactions are important to describe how fit the ligand binds to the target macromolecule.

The general hypothesis is that lower energy scores represent better protein-ligand bindings compared to higher energy values. Hence, molecular docking can be formulated as an optimization problem, where the task is to find the ligand-binding mode with the lowest energy docking simulations of bisphenol A against proteins [hemoglobin (PDB id-2HHB), catalase (PDB id-1QQW) and glutathione peroxidase (PDB id-2f8A)] were evaluated based on the binding compatibility [docked energy (kcal/mol)] with the receptor (Fig. 4, 5 and 6). On the basis of the result we conclude that the binding affinity of bisphenol A is higher with the hemoglobin (-79.5491 kcal/mol) compared to the catalase (-36.9179 kcal/mol) and glutathione peroxidase (-71.8106 kcal/mol) as shown in the result. The docking results revealed that there were no van der Waals, hydrophobic and electrostatic interactions existed but only hydrogen bond played a major role in the binding of bisphenol A against hemoglobin, catalase and glutathione peroxidase. We sought to determine and explore the mechanism of bisphenol A by binding more efficiently
with heme than the CAT and GPx, produce hydroxyl radicals like H$_2$O$_2$ and induce lipid peroxidation.

Medicinal values of the plants depend on the bioactive constituents exerting desirable physiological action in humans. Antioxidative and hepatoprotective effect of green tea extracts are principally denoted by the phytochemicals acting as reductants and free radical scavenger. Traditionally green tea is being used as a whole herb in its natural form. For better understanding of mechanisms involved in toxicity as well as preventive effect can be well interpreted by interaction study with BPA chemically. Results showed decrease in absorbance of both the compounds within period of 240 min indicating presence of some chemical interactions between them which could be the reason for the protective effect of the extract.

Green tea extract treatment alone did not have any significant effect on body weight of mice as compared to vehicle control. Table 3.16 shows that co-administration of BPA along with green tea extract resulted in significant amelioration in body weight of the animals. This protective effect could be due to reduced oxidative stress in green tea extract treated animals resulting in normalization in metabolism. Many researchers reported that green tea extract have strong antioxidative properties and reduced oxidative stress (Ounjaijean et al., 2008; Yang et al., 2009). Heikal et al. (2013) shows that co-administration of green tea extract attenuated body weight reduction of cyromazine and chlorpyrifos intoxicated male rats. One more report shows significant ameliorates in body weight of rats by administration of green tea extract (Babu et al., 2006).

Bisphenol A treatment had significantly increased the absolute and relative weight of mice liver. Administration of green tea extract in three different doses significantly combated these changes with maximum reduction at 100 mg/kg bw/day dosage (Table 3.17). This effect could be due to lipid lowering effect of the plant material. Jigisha et al., (2012) showed that supplementation of green tea leaf powder resulted in significant lowering of total lipid, cholesterol
and triglycerides. This protective effect of fatty liver and cholesterosis were found to mitigate with plant extract treatment for 30 days.

Table 3.18 shows the effect of green tea extract on biomolecule contents. High dose of BPA has significantly reduced glycogen content (Table 3.3) in mice liver. Oral administration of green tea extract to the BPA – treated animals resulted in increase in protein and glycogen content of the animals. Bin Dajem et al. (2011) reported that green tea could ameliorate changes in the liver of mice and restore glycogen content which was greatly reduced in the female S. mansoni – infected mice. Swamy et al. (2011) also reported that green tea administration increased glycogen level in liver and muscle cells and reduced lipid peroxidation. Hypoglycemic effect of green tea extract has been reported by numerous scientists in various animal models (Roghani and Baluchnejadmojarad, 2010; Kumar et al., 2011). Blood sugar lowering effects of green tea extract could be due to decrease metabolism of glycogen ultimately increasing the glycogen storage in hepatic tissue.

Bisphenol A treatment for 30 days had significantly increased hepatic lipid content resulted in fatty infiltration of the liver and also increased hepatic cholesterol content (Table 3.3). Green tea extract when orally given to bisphenol A – treated animals in three different doses found to reduce this hyperlipidemic effect. Many studies have shown that green tea extract inhibits fatty acid synthase (Tian et al., 2004), increases lipid metabolism through the intervention in the process of adipogenesis and lipolysis (Kim et al., 2009). Lee et al. (2009) demonstrated in an in vitro study that EGCG modulates the increase in lipolysis by directly increasing the gene expression of Hormone sensitive lipase (HSL), demonstrating its important role in lipid metabolism.

Green tea catechins inhibit gastric lipase and pancreatic lipase (Chantre and Lairon, 2002), which is involved in lipid digestion. This inhibition is due to reduction in lipolysis of long chain triglycerides (Juhel et al., 2000) and interference with the emulsification, digestion and micellar –
solublization of lipids that support intestinal absorption of dietary lipids (Wang et al., 2006; Kool and Noh, 2007). This suggests that the reduced lipid emulsification and digestibility may be responsible for lowering intestinal absorption of dietary lipids, including triglyceride, cholesterol and other lipophilic compounds (Loest et al., 2002).

Green tea also lowers plasma cholesterol by increasing fecal bile acids and cholesterol excretion (Yang and Koo, 2000). This apparent decrease in cholesterol absorption and bile acid reabsorption by green tea should have lead to a reduction in liver cholesterol concentrations. Evidence from animal studies clearly indicates that green tea or its catechins lower the blood level of cholesterol in cholesterol – fed rats (Muramatsu et al., 1986; Yang and Koo, 1997), mice (Suzuki et al., 1998) and hamsters as well as the plasma levels of triglyceride in hamsters fed a high fat diet (Chan et al., 1999). One more study done by Anandh babu et al. (2006) showed that administration of green tea extract reduced cholesterol, triglyceride, free fatty acid and LDL-c levels, and increase HDL – c levels in the serum of diabetic rats. In addition, green tea extract decreased cholesterol, triglyceride, free fatty acid levels and lipoprotein lipase activity in the myocardium of diabetic rats. Also they reported that these beneficial effects of green tea extract are described to its antihyperglycemic and hypolipidemic activity.

Bisphenol A treatment for 30 days resulted in significant reduction in nucleic acid and protein contents in the liver (Table 3.3). Co - treatment of green tea extract/Liv. 52 significantly mitigated these changes majorly due to its antioxidative potency. Antioxidants can interfere with xenobiotic metabolizing enzymes, block activated mutagens/carcinogens, modulate DNA, RNA repair and even regulate gene expression. Green tea has been reported to decrease chromosome aberrations in bone marrow cells (Ekram and abdella, 2005) and reduced DNA damage (Glei and Pool-Zobel, 2006). Ostrowska (2004), showed that green tea reduce the morphological and biochemical alterations induced by a variety of hepatotoxicants. Hsu et al. (2007) demonstrated the role of green tea in reducing psoriasiform lesions in the flaky skin mouse model.
Camouse et al. (2008) showed that green tea extract could prevent simulated solar radiation - induced oxidative damage to DNA and Langerhans cells that may lead to immune suppression and carcinogenesis. Hakim et al. (2003) and Tang et al. (2008) reported that green tea decreased the biomarkers of DNA oxidative damage after receiving carcinogenic or oxidative damage. Green tea inhibited chromosomal damages induced by cell phone waves (Zahedifar and Baharara, 2013) as well as ultraviolet radiation (Katiyar et al., 2007). Green tea prevents UV – induced immune suppression through increased levels of IL-12 and as IL -12 and exhibited antitumor activity and the ability to repair UVB – induced DNA damage (Chen et al., 2004).

Green tea causes destruction of free radicals, prevention of DNA damage, micronucleus formation, chromosome injuries and inhibition of cell proliferation, gene transcription changes, changes in protein folding or by blocking certain enzymes to be effective in reduction of chromosomal damages induced by cell phone. Green tea has a strong inhibitory effect on deletion mutations in mitochondrial DNA of human blood lymphocytes (Iwai et al., 2006). Decreased DNA oxidative damage in human lymphocytes has been observed by Kim (2009) and Erba et al. (2005). Kada et al., (1982) reported that green tea extract has a bio-antimutagenic activity, and it improves the fidelity of DNA replication and also can exerts its anticancer effects through modulation of DNA methylation.

Oxidative RNA damage is also a feature in xenobiotic-induced toxicities suggesting that RNA oxidation may actively contribute to the onset or to the development of disease (Nunomura et al., 2006). Green tea also increased the mRNA levels of srebf2 and Insig1, which are positively regulated by SREBP2. Green tea polyphenols modulate BCI-2 protein expression that is important factor in signal transduction mediating the protective effect against MC-LR-induced apoptosis of hepatic cells (Xu et al., 2007). Restored levels of RNA and DNA by green tea extract normalise the process of transcription and translation resulting in elevation of protein content. Protein
content elevating effect of green tea extract could be due to stored energy status of the tissue resulting in sparing of protein for building muscle blocks.

Hepatotoxicity of BPA was evaluated by estimating the activities of various liver marker enzymes in tissue and serum. Activities of AST, ALT, ACP and ALP were found to increase with BPA treatment in liver as well as serum of mice as indicated in Table 3.4 and 3.7. Green tea extract is reported to possess antihepatotoxic effects and showed protection against various hepatotoxins (EI – Beshbishy, 2005; William et al., 2012). Our finding suggests that administration of all three doses of green tea extract significantly restored back the levels of liver marker enzymes (Table 3.19 and 3.23). In addition, ALP is membrane bound enzyme and its alteration is likely to affect the membrane permeability and produce derangement in the transport of metabolites (Mehana et al., 2012). Green tea enhanced antioxidative abilities in liver and protects liver cells and liver cell membrane (Ostrowska et al., 2004).

All enzymes come to normal serum values following green tea extract treatment which may be due to prevention of intracellular enzyme leakage resulting from cell membrane stability or cellular regeneration (Thabrew and Joice, 1987). Abolfathi et al., (2012) reported that green tea extract decreased the level of hepatic enzymes in serum of diabetic rats. Sinha et al. (2013) also reported that green tea restores the normal activity of liver marker enzymes (ACP, ALP, AST and ALT) and prevent the liver injury as indicated by the levels of enzymes status. Many authors also showed that green tea has capacity to normalize the levels of liver marker enzymes, which was elevated by various hepatotoxicants (Kumar et al., 2010; Bin Dajem et al., 2011; Heikal et al., 2013). One more study demonstrated that injection of green tea extract decreased the level of serum aminotransferases, alkaline phosphatase and increased the production of serum protein including albumin (Shekarforoush et al., 2014). In another study carried out by Khorsandi et al. (2010), it was revealed that green tea extract mitigates severe poisoning of liver due to
acetaminophen, improves liver necrosis and decreased liver marker enzymes, which agrees with the results of this study.

The BPA provoked significant loss of liver SDH and ATPase enzyme activities were subdued by the treatment with green tea extract in a dose–dependent manner (Table 3.20) indicating restoration of energy status of the cell and mitochondrial re–organization. Green tea extract contains active component EGCG, which has been extensively studied for its anticarcinogenic (Kanwar et al., 2012) and anti-inflammatory (Wu et al., 2012) effects and is a mitochondrial–targeted molecule displaying a selective antiapoptotic effect against inducers of mitochondrial oxidative stress in a variety of neuronal cell types (Schroeder et al., 2009).

EGCG has been found to prevent mitochondrial deterioration in aged rat brain (Srividhya et al., 2009), reduce cerebral amyloidosis (Rezai-Zadeh et al., 2005) and correct amyloid–induced mitochondrial dysfunction in a transgenic mice model of Alzheimer disease (Dragicevic et al., 2011). Valentine et al. (2013) shows that green tea treatment (EGCG) renews the capacity to produce energy by restoring the impaired activities of complex I, complex II (SDH), ATP synthase and overall rate of mitochondrial ATP synthesis.

Valentine et al. (2013) also indicate that EGCG protects mitochondrial architecture. ECGC by stimulating cAMP signaling pathways is able to restore oxidative phosphorylation capacity and promote mitochondrial biogenesis. In some animal models, EGCG enhances mitochondrial function that reduces oxidative stress in alcoholic fatty liver or diet induced obesity (Jimenez-lopez and Cederbaum, 2004; Klaus et al., 2005). Another study done by Dragicevic et al. (2011) suggest that green tea act as an antioxidant and protects mitochondrial function.

Normalized metabolism of protein, carbohydrate and lipid as well as free radical scavenging effects of plant improves integrity and oxidative phosphorylation in mitochondria which was highly disturbed in case of energy deficient state-induced by BPA. Also green tea was more effective in restoring the lipid peroxidation and antioxidant enzymes. Aedenosine
triphosphatase is the membrane bound and “-SH” group containing enzymes (Gubdjorson et al., 1983) and lipid dependent. Reduction in the activity of these enzymes might be due to enhanced lipid peroxidation by free radicals. Here treatment with green tea increased the activities of Na⁺/K⁺ ATPase, Ca²⁺ ATPase and Mg²⁺ ATPase, could be due to the ability of green tea extract to protect the –SH group from oxidative damage through the inhibition of peroxidation of membrane lipids.

Hepatoprotective effect of green tea extract is principally due to its antioxidative potency. Bisphenol A treatment in mice elevated levels of lipid peroxidation in liver (Table 3.6). Green tea doses significantly reduced levels of lipid peroxidation (Table 3.21) in bisphenol A intoxicated mice, which could be due to free radical scavenging effect of green tea polyphenols as it was well correlated in our in-vitro studies. Green tea extract inhibits lipid peroxidation in vitro systems, in experimental animals and in humans (Awad et al., 1998; Park et al., 2002).

Green tea can decrease lipid peroxidation marker in the liver, serum and brain including lipid hydroperoxides, 4-hydroxynonenal and malondialdehyde in rats (Skrzydlewska et al., 2002b). Hirano-Ohmori et al. (2005) and Nagao et al. (2005) reported that consumption of green tea decreased serum MDA modified LDL concentration in humans. Coimbra et al. (2006) also demonstrated that administration of green tea decreased the MDA level. Antiperoxidative effect of green tea were reported on various stress models by numerous researchers (Levites et al., 2002; Chen et al., 2003; Saffari and Sadrzadeh, 2004).

Content of non-enzymatic antioxidants of the hepatocytes were found to increase with cotreatment of green tea extract (Table 3.21). The effect was dose – dependent and significant. Glutathione is an important non-enzymatic antioxidant defense required to maintain the normal redox state of cells and to counteract deleterious effects of oxidative stress. Our result showed significant (p<0.05) decrease in GSH contents in animals exposed to BPA was restored back by free radical scavenging and sulfhydral (thiol) group protecting effects of green tea extract, which
indicated the potential of green tea extract to counteract the oxidative damage induced by BPA and to reinforce the antioxidant defense in normal condition. Ascorbic acid content also found increase due to proton donating effect of green tea extract sparing body’s natural antioxidants from getting oxidized. Increased level of glutathione could be the reason for reduction in lipid peroxidation as in the presence of GSH, lipid peroxidations are converted to less toxic alcohol derivatives rather than MDA (Manevich et al., 2002). Green tea extract – induced increase in non-enzymatic antioxidants had been reported by EI – Beshbishy et al. (2010).

Another study indicated that, the dietary intake of moderate amount of green tea in human subjects can improve plasma antioxidant status, as demonstrated by increase in TAA levels and decrease in peroxide levels (Erba et al., 2005). It was demonstrated that, plasma TAA increased after few hours from the intake of single dose of green tea (Sung et al., 2000). Results of current study revealed that green tea extract reversed the elevation of lipid peroxidation and increase GSH liver content. Hence, it is possible that the mechanism of hepatoprotection of green tea extract may be attributed to epicatechins (present in the green tea extract) that scavenge a wide range of free radicals including the most active hydroxyl radicals which may initiate lipid peroxidation. It prevents the loss of lipophilic antioxidants α – tocopherol by repairing eocophenyl radicals and protection of the hydrophilic antioxidant ascorbate. Therefore, it may decrease the concentration of lipid free radicals (Skryzdlewksa et al., 2002b). Moreover, it was reported previously that it chelates metal ions especially iron and copper which in turn inhibit generation of hydroxyl radicals and degradation of lipid hydroperoxides (Azram et al., 2004).

Activities of enzymatic antioxidants (SOD, CAT, GPx, GR and GST) were found to reduce with bisphenol A treatment for 30 days, which could be due to increased production of free radicals characterized by increased MDA contents. These enzymes are known to scavenge free radicals such as superoxide, hydroxyl and hydrogen peroxide, thus preventing damage caused by oxidative stress to the tissues (Shukla et al., 2004). Green tea administration significantly blocked
the oxidative stress as evidenced by a significant increase in hepatic enzymatic, non–enzymatic antioxidants and reduced lipid peroxidation to the levels close to corresponding control values.

The present study shows that green tea significantly increases activities of enzymatic antioxidants (SOD, CAT, GR, GPx and GST) in bisphenol A intoxicated mice (Table 3.22). In recent study, supplementation of green tea attenuates cyclosporine A – induced oxidative stress in rats (Mohamadin et al., 2005) and also reducing the formation of carcinogens in the body (Yang et al., 2001). It was found that green tea intake increase the activity of liver antioxidant enzymes as glutathione peroxidase (GPx) and oxidized glutathione (GSSG), as well as reduced glutathione (GSH) and improves the total antioxidant activity (TAA) (Sies et al., 1996). The level of thiol groups plays vital role in maintaining structural and functional integrity of membranous and enzymatic proteins (Tapple, 1973). This thiol sparing effect of green tea extract could be due to proton donating effect of extract, resulting in elevation of reduced glutathione and ascorbic acid levels ultimately maintaining enzyme protein structure and active site configuration (Sethi et al., 2007). Green tea extract considered as potent scavengers of reactive oxygen species, such as superoxide, hydrogen peroxide, hydroxyl radicals and nitric oxide produced by various chemicals (Schroeder et al., 2003). This could be the reason for green tea extract induced increase in enzymatic antioxidants. Antioxidative effect of green tea extract was also responsible for antimituation (Okuda et al., 1984) and reduces the risk of colorectal cancer (Brown, 1999). By blocking oxidative damage through lipid peroxidation and protein oxidation, green tea extract prevent the loss of membrane permeability and dysfunction of cellular proteins and decrease the endogenous level of hydroxyl radical and increase the level of enzymatic antioxidants (Seven et al., 2004). One more study reported that administration of green tea extract increase the level of enzymatic and non-enzymatic antioxidants (Sies et al., 1996).

Green tea treatment did not show any significant effect on histopathological changes in liver of mice as compared to untreated and vehicle control. However, co-treatment of green tea
along with bisphenol A caused alleviation in BPA caused alterations in liver (plate J) of mice. This might be due to blocking oxidative damage through lipid peroxidation and its antioxidative properties. Heikal et al. (2013) reported that green tea ameliorates alteration in histopathological changes attributed to the antioxidant capacity of green tea that attenuates the lipid peroxidation and liver antioxidant enzymes capacity which in turn restore the integrity of the cell membrane and improve the disturbance in permeability. In agreement with the results obtained in this study, Ostrowska et al. (2004), stated that the administration of green tea to ethanol – intoxicated rats, resulted in the normalization of lipid peroxidation as well as GSH and GPx activity in liver. Bitar and Laham (2013) reported that green tea administration significantly reduced severe ulceration and necrosis. Pretreatment with low dose of green tea extract in murine intestinal epithelial cells (IEC -18) protected against necrotic cell death by peroxynitrite and hydrogen peroxide (Fiorucci et al., 1999).

Tea polyphenols showed a protective role against liver injury in many animal models of liver diseases, liver fibrosis and hepatic ischemia-reperfusion injury (Zhong et al., 2002; Chen et al., 2002). Another study done by Abolfathi et al. (2012) reported that green tea extract improves histopathological changes induced by streptozotocin intoxicated diabetic rats. Many authors also reported that green tea has antioxidative capacity to protect against necrotic condition (Buetler et al., 2002; Xu et al., 2007). Green tea also slows the release of tumor necrosis kappa – β function, which is critical for tumor growth. Also similar results were reported by Shalan et al. (2005), Badiei et al. (2006), Khan et al. (2008) and Shaima et al. (2009) demonstrated that treatment with epigallocatechin gallate (the major flavonoid component of green tea) significantly protects the liver after ischemia/reperfusion possibly by reducing hepatic fat content, increasing hepatic energy status and functioning as an antioxidant.

Liv. 52, used as a reference standard drug in present study, is a well-known hepatoprotective herbal formulation used in the treatment of liver diseases, evidenced by various
experimental and clinical studies. Various animal experiments using different chemical toxicants have demonstrated the hepatoprotective effect of Liv. 52 (Mandal et al., 2000; Huseini et al., 2005; Sapakal et al., 2008; Girish et al., 2009). Goel et al. (1999) have studied influence of Liv. 52 on carbon tetrachloride – induced hepatotoxicity. Further, one more study revealed that Liv. 52 protects mice liver against cadmium intoxification (Rathore and Saraswat, 1986). Hepatoprotective and anti-inflammatory effects of some of the individual ingredients of Liv.52 are reported in literature (De Silva et al., 2003). Kataria and Singh (1997), have reported that oral administration of Liv. 52 to CCl₄ – treated rats involved growth. They have reported that Liv. 52 have protective effect on hepatic enzymes altered by CCl₄ hepatotoxicity.

Mitra et al. (2008), have tested the molecular mechanism underlying the hepatoprotective effect of Liv. 52 against alcohol – induced liver damage in HepG2 cells. The antioxidative and cytoprotective activities of Liv. 52 were explored in HepG2 in vitro model against t-BHP – induced liver toxicity and oxidative damage (Vidyashankar et al., 2009). However, Green tea extract is found to be more effective than the Liv. 52 according to the present study results.

Keeping in mind the result of the present study as well as aforementioned discussions, the null hypothesis proposed was rejected and alternative hypothesis is accepted as mentioned below.

(a) Bisphenol A has toxicity in mice
(b) Green tea possess significant antioxidant property
(c) Green tea ameliorates BPA – induced toxicity in vitro
(d) Bisphenol A has in silico interaction with hemoglobin, catalase and glutathione peroxidase
(e) Green tea mitigates BPA – induced in vivo toxicity