Bisphenol A, a xenoestrogen, is one of the chemicals produced in highest volume worldwide, commonly incorporated in many plastics. Present study was undertaken to evaluate bisphenol A – induced toxic effects on vital organs of mice (liver) with subchronic exposure. Toxic effects of bisphenol A on human erythrocytes and mice liver homogenates were also studied to get better understanding of the mechanism involved in bisphenol A – induced toxicity. Green tea has attracted significant attention recently, both in scientific and in consumer communities for its broad spectrum health benefits for a variety of disorders. Green tea has been used in the present study to mitigate bisphenol A exerted toxicity in various in vitro and in vivo conditions.

PART I: Evaluation of bisphenol A toxicity in liver of mice

In vivo study was designed to evaluate hepatic damage caused by subchronic administration of bisphenol A in mice. Summary of the bisphenol A – induced hepatotoxicity is as follows:

Bisphenol A treatment for 30 days caused dullness and lethargy in treated mice. However, no clinical signs were observed in untreated and vehicle control mice.

Oral administration of bisphenol A resulted in significant and dose – dependent reduction in body weight of the animals. No significant changes in the body weight were noted between different control groups (Group I and II). Contrary to that at the end of the treatment absolute and relative weight of the liver of bisphenol A- treated animals were found to increase principally due
to fat deposition, which was confirmed by histopathological examinations of the bisphenol A–
intoxicated liver.

Alterations in macromolecule contents of liver such as protein, glycogen, DNA, RNA, total lipid and cholesterol was found to be one of the prominent feature of bisphenol A toxaemia. Hepatic contents of protein, glycogen, DNA and RNA were found to reduce significantly and dose–dependently, whereas elevation in the contents of total lipid and cholesterol were noted with bisphenol A oral administration. No significant change was observed between untreated and vehicle control group.

There were no significant alterations found between untreated and vehicle control groups in the AST, ALT, ACP and ALP activities in liver of mice. Oral administration of BPA for 30 days caused significant and dose-dependent elevation in activities of AST, ALT, ACP and ALP in liver of mice as compared to vehicle control.

No significant changes were observed in the activities of SDH and ATPase in the liver of different control groups. However, oral administration of bisphenol A for 30 days resulted in significant and dose-dependent reduction in the activities of enzymes involved in energy production (SDH and ATPase) resulting in energy depleted state of the tissue.

Examination of oxidative stress markers revealed the central role of free radicals in bisphenol A–induced hepatotoxicity. In mice liver, no significant changes were found in lipid peroxidation, non-enzymatic (GSH and TAA) and enzymatic (CAT, SOD, GR, GPx and GST) antioxidants in between different control groups. Results revealed that oral administration of BPA for 30 days caused significant and dose–dependent increase in lipid peroxidation in liver of mice. This could be due to significant and dose-dependent reduction in non-enzymatic and enzymatic antioxidants as compared to vehicle control.

No significant alteration was found between different control groups in protein content as well as in AST, ALT, ACP and ALP activities in serum of mice. Oral administration of BPA for
30 days caused significant and dose-dependent depletion in protein content; however activities of AST, ALT, ACP and ALP were significantly elevated in serum of mice as compared to vehicle control. Increased activities of hepatic marker enzymes in serum indicate the presence of hepatic damage.

Oral administration of bisphenol A for 30 days caused severe fat deposition, intracellular vacuolization, necrosis and loss of histoarchitecture as compared to control in liver of mice. No significant changes were observed in all groups of control animals.

PART II: Phytochemical screening and analysis of antioxidative potential of green tea extract

Phytochemicals are the secondary metabolites present in minute concentrations in plants and principally responsible for the medicinal value of the same. Polyphenolic content of hydro-alcoholic green tea extract was qualitatively and quantitatively estimated in the present study. The extract showed presence of phenolics, flavonoids, tannins and ascorbic acid in considerably high amount indicating its good antioxidative potency as phytochemicals are known reductants. Results of the quantitative analysis showed presence of more phytochemicals in hydro-alcoholic extract.

Various chemical antioxidant assay systems were used to evaluate free radical scavenging activity of green tea extract. Hydro-alcoholic crude polyphenols extracted from green tea effectively scavenged superoxide, hydroxyl and nitrous oxide radicals in vitro conditions. Measurement of reducing ability indicates ability of a compound to reduce oxidative molecules and stabilize them. Results indicated that green tea extract can effectively reduce the oxidized compounds and chelate metal ions. Results of free radical scavenging assay as well as reducing ability showed that hydro-alcoholic extract of green tea was more potent than Liv. 52 which may be due to higher amount of phytochemicals present in extract.
PART III: Evaluation of bisphenol A toxicity on some in vitro and in silico models and its amelioration by green tea extract

Incubation of liver homogenates with various concentrations of bisphenol A under in vitro conditions resulted in increased lipid peroxidation accompanied by reduction in SOD and CAT activity. Alteration in enzymatic antioxidant system by bisphenol A – intoxication showed its ability to induce oxidative stress. This change in redox status of liver homogenate was significantly ameliorated by co- treatment with green tea extract. Reduction in lipid peroxidation was accompanied by increasing activities of CAT and SOD.

When liver homogenates were incubated with various concentrations of bisphenol A under in vitro conditions it resulted in reduced SDH and ATPase activities indicated depletion in energy metabolism. This indicates, the damage to mitochondria and energy generation in them is inevitably inhibited which contributes to the overall loss in the energy production. Co - treatment with different concentration of green tea extract along with the high concentration of BPA caused significant and dose – dependent increase in the activities of SDH and ATPase as compared to BPA alone treated group.

Effect of bisphenol A on human erythrocytes was also studied. Various concentrations of bisphenol A were found to induce damage in erythrocytes. The damage to the RBC membrane caused by bisphenol A finally led to rupturing of cells causing hemolysis to occur in a concentration – dependent manner. This hemolytic effect of bisphenol A was due to its ability to induce oxidative stress in human erythrocytes. To find out the exact mechanism of action of bisphenol A, we compared its effect with the standard oxidant, H$_2$O$_2$. The effect of BPA and H$_2$O$_2$ were found to be additive. For the confirmation, binding capacity of bisphenol A with erythrocyte proteins (hemoglobin, catalase and glutathione peroxidase) were inspected using molecular docking tool, which showed formation of many hydrogen bonds of BPA with the proteins. The present data clearly indicates that BPA cause oxidative stress in the similar way as
**PART IV: Ameliorative effect of green tea extract in liver of bisphenol A intoxicated mice**

Efficacy of green tea extract to reduce bisphenol A exerted hepatotoxicity was evaluated using three different doses of the hydro-alcoholic extract. Co-treatment of green tea extract with high dose of bisphenol A in mice resulted in significant mitigation of bisphenol A–induced hepatic changes which are as follows:

Co-treatment of green tea extract and bisphenol A significantly mitigated bisphenol A-induced clinical symptoms such as lethargy and dullness. No treatment related mortality was observed in any of the experimental group.

No significant changes in the body weight were noted between different control groups. Oral administration of BPA caused significant reduction in body weight of mice. Administration
of green tea extract along with BPA caused significant and dose-dependent amelioration in body weight, as compared to only BPA – treated groups.

Animals of different control groups showed non-significant differences in absolute and relative liver weights. However, oral administration of BPA for 30 days caused significant increase in absolute and relative liver weights in mice as compared to vehicle control. When green tea extract was administered orally along with BPA significant and dose-dependent mitigation in absolute and relative liver weights were observed as compared with animals of BPA alone treated groups.

Efficacy of green tea extract to reduce changes in macromolecule content – induced by bisphenol A was also evaluated. Significant elevation in protein, glycogen, DNA and RNA content was noted in green tea co-treated animals which was reduced in case of bisphenol A intoxication. Increases in total lipid and cholesterol contents were also successfully ameliorated by oral administration of plant extract along with bisphenol A. No significant change was observed in different control group (Group I, II and III).

Hepatoprotective potency of green tea extract was evaluated by studying biochemical examination of liver markers which were significantly altered in bisphenol A toxaemia. Treatment of green tea along with bisphenol A resulted in reduction in the activities of AST, ALT, ACP and ALP in liver as well as in serum indicating reduction in hepatic damage.

Protective effect of plant extract on mitochondrial function and energy metabolism was established by assaying the activities of SDH and ATPase in liver of mice. Bisphenol A – induced reduction in the activities of these enzymes were found to be mitigated by co-administration of green tea extract.

Green tea is known to possess strong antioxidative potency and was used to combat bisphenol A – induced oxidative stress in this study. Treatment of green tea extract in various doses resulted in significantly reduced levels of lipid peroxidation in bisphenol A – treated
animals. Levels of non-enzymatic antioxidants such as GSH and TAA were found to increase with co-treatment of green tea along with bisphenol A. Due to its free radical scavenging effect, green tea increased the activities of SOD, CAT, GR, GPx and GST which were all reduced in case of bisphenol A treatment.

Histopathological examination of green tea co-treated animals showed reduction in bisphenol A – induced fat deposition, vacuolization and restored back the normal morphology and integrity of hepatocytes.

The hepatoprotective index (H) for all in vivo parameters was calculated. The hepatoprotective activity of plant extracts was compared with the standard polyherbal drug Liv. 52. Results revealed that green tea possess more hepatoprotective activity than Liv. 52.

Green tea, an indigenous medicinal plant, is commonly used in therapeutic applications, clinical use and treatment of various diseases. We have tested efficacy of green tea extract up to 100 mg/kg bw in mice. It can be extrapolated to human dose using body surface area which is 8.10 mg/kg for 60 kg human being (Reagan-shaw et al., 2007). Previously it has been reported that administration of green tea at a daily dose of 800 mg is nontoxic (Chow et al., 2003). The present study scientifically proves hepatoprotective efficacy of plant and also it is even more hepatoprotective than Liv. 52. Therefore we recommend the daily use of green tea as prophylactic agent to overcome adverse effect of BPA as well as any other toxicants to which we are exposed in daily life.

The results of the above study showed toxic effects of bisphenol A in various biological systems and support usage of green tea for the management of this toxicity and reinforce the importance of ethanobotanical approach as a potential source of bioactive substances.
FUTURE PERSPECTIVES:

Present study was an attempt to establish a correlation between xenobiotic – induced toxicity and its remediation by traditionally used medicinal plant. As this thesis provides strong base of various biochemical and histopathological evidence for bisphenol A – induced toxicity (in vitro and in vivo) and protective effect of the plant extract against it, followings are the some of the future perspective for which the study can be extended:

1) Detailed and systematic investigation of mechanism of bisphenol A toxaemia in various in vitro and in vivo models by sophisticated analytical techniques and provide more scientific research base for the same.

2) Large scale evaluation of bisphenol A exposure and toxicities on human population supported by surveys and clinical trials.

3) Study of molecular mechanism and genes involved in toxicity can be designed to provide necessary information for the management of the adverse effects caused by bisphenol A.

4) Comparative potency evaluation of green tea extract in various chemical and animal models.

5) Isolation, purification and characterization of various active components possessing physiological actions from green tea extracts which can be tested further for the management of numerous diseases and disorders.

6) Interactive study of the green tea with bisphenol A on more precise and sophisticated instruments can be initiated.