CHAPTER - V
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Butylparaben is the xenoestrogen human population been chronically exposed to for decades. Present study was undertaken to evaluate butylparaben-induced toxic effects on vital organ of mice (liver) with sub chronic exposure. Toxic effects of butylparaben on human erythrocytes and mice liver homogenate were also studied to get better understanding of the mechanism involved in butylparaben-induced toxicity. *O. sanctum* is known Indian culinary herb having broad spectrum of therapeutic application and long history of clinical usage. *O. sanctum* extracts were used in the study to mitigate butylparaben exerted toxicity in various *in vitro* and *in vivo* conditions.

Phytochemicals are the secondary metabolites present in plants in minute concentrations and principally responsible for the medicinal value of the same. Various polyphenol content of aqueous and alcoholic extracts were quantitatively estimated in the present study. Both 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 aqueous extract than in alcoholic extract. Various chemical antioxidant assay systems were used to evaluate free radical scavenging activity of *O. sanctum* extracts. Aqueous and alcoholic crude polyphenols extracted from *O. sanctum* leaves effectively scavenged superoxide, hydroxyl, nitrous oxide and DPPH radicals in *in vitro* conditions. Reducing ability and Fe$^{+2}$ chelating activity indicates ability of the compound to reduce oxidatively rendered molecules and stabilize them as well as terminate the chain of deleterious reactions of peroxide formation by chelating metal ions need for

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them. Results indicated that both the extracts of *O. sanctum* can effectively reduce the oxidized compounds and chelate metal ions. Ferric reducing antioxidant power is also an important assay for the measurement of total antioxidative potency of a compound which was found to be higher in aqueous extract than in alcoholic. Results of free radical scavenging assay as well as reducing ability and chelating activity showed that aqueous extract was more potent than alcoholic may be due to higher amount of phytochemicals present in aqueous extract. Acute oral toxicity study of *O. sanctum* was performed and results stated that the plant possess very high LD$_{50}$ value and was not toxic at the dose 2000 mg/kg bw/animal indicating its potential use as medicinal drug.

Incubation of liver homogenate with various concentrations of butylparaben under *in vitro* conditions resulted in high elevation in lipid peroxidation accompanied by reduction in GSH content as well as SOD and CAT activity. Disturbance in the enzymatic and non-enzymatic antioxidant system by butylparaben – intoxication showed its ability to induce oxidative stress. This change in redox status of liver homogenate was significantly ameliorated with *O. sanctum* extracts (aqueous and alcoholic) cotreatment. Reduction in lipid peroxidation by plant extract resulted in increased content of GSH and SOD and CAT activity.

Butylparaben effect on human erythrocyte morphology, hemolysis and plasma MDA level were studied. Various concentrations of butylparaben were found to induce morphological alterations in RBC membrane resulting in formation of echinocyte and ghost membrane formation. The damage to the RBC membrane caused by butylparaben finally led to rupturing of cell causing hemolysis to occur in a concentration-dependent manner. This haemolytic effect of butylparaben was due to its ability to induce oxidative stress in human erythrocytes as treatment of whole blood with varying concentration of butylparaben resulted
in significant increase in plasma MDA level. Cotreatment of aqueous extract of *O. sanctum* extract resulted in concentration-dependent decrease in plasma MDA level majorly due to its polyphenols acting as antioxidants. *O. sanctum* extract significantly reduced morphological alterations and reduced formation of echinocytes and ghost membrane in a concentration-dependent manner. In the same manner due to its membrane protecting antioxidants *O. sanctum* extract reduced butylparaben-induced hemolysis in human erythrocytes.

*In vivo* study was designed to evaluate hepatic damage caused by subacute administration of butylparaben in mice. Summary of the butylparaben-induced hepatotoxicity is as follows:

1) Oral administration of all three doses of butylparaben resulted in significant body weight reduction of the animals. Contrary to that at the end of the treatment absolute and relative weight of the liver of butylparaben-treated animals was found to increase principally due to fat deposition, which was confirmed by histopathological examination of the butylparaben-intoxicated liver. Histopathological study also showed presence of structurally rendered hepatocytes as well as loss of normal architecture and compactness of liver with butylparaben treatment.

2) Alteration 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 butylparaben 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 was noted with butylparaben oral administration.
3) Biochemical examination of hepatic markers revealed dose-dependent and significant increase of the same in liver as well as serum characterizing liver toxicity caused by butylparaben. Activities of ALT, AST, ACP and ALP were found to elevate in liver and serum of butylparaben-treated animals. In the same fashion activities of LDH and $\gamma$-GT were also increased in the liver and serum of butylparaben-intoxicated animals indicating presence of hepatic damage.

4) Butylparaben administration for 30 days resulted in significantly reduced activities of enzymes involved in energy production (SDH and ATPase) resulting in energy depleted state of the tissue.

5) Examination of oxidative stress markers revealed free radical generation playing central role in butylparaben-induced hepatotoxicity as oral administration of it significantly altered enzymatic and non-enzymatic antioxidant systems of liver. Level of lipid peroxidation was increased dose-dependently in butylparaben-intoxicated animals as compared to control resulting in reduction in GSH and TAA contents of liver. Reduction in the activities of SOD, CAT, GPx, GR and GST – enzymes involved in scavenging of free radicals were found to reduce significantly with butylparaben treatment.

Efficacy of *O. sanctum* to reduce butylparaben exerted hepatotoxicity was evaluated using three different doses of the aqueous extract of the plant. Cotreatment of *O. sanctum* with butylparaben in mice resulted in significant mitigation of butylparaben-induced hepatic changes which are as follows:

1) Treatment of 100, 200 and 300 mg/kg bw of *O. sanctum* extract along with butylparaben reduced changes in body weight as well as absolute and relative weights of liver. Reduction in body weight by butylparaben was significantly ameliorated by *O. sanctum*
treatment in a dose-dependent manner. All three dose of *O. sanctum* reduced absolute and relative weights of liver which was significantly increased with butylparaben-treatment. Histopathological examination of *O. sanctum* cotreated animals showed reduction in butylparaben-induced fat deposition and restored back the normal morphology and integrity of hepatocytes.

2) Efficacy of plant extract to reduce changes in macromolecule contents - induced by butylparaben was also evaluated. Significant elevation in protein, glycogen, DNA and RNA content was noted in *O. sanctum* cotreated animals which was reduced in case of butylparaben intoxication. Increase in total lipid and cholesterol contents was also successfully ameliorated by oral administration of plant extract along with butylparaben.

3) Hepatoprotective potency of *O. sanctum* extract was evaluated by studying biochemical examination of liver markers which were significantly altered in butylparaben toxaemia. Treatment of *O. sanctum* along with butylparaben resulted in reduction in the activities of ALT, AST, ACP and ALP in liver as well as serum indicating reduction in hepatic damage. In the same manner activities of LDH and γ-GT were also found to reduce with *O. sanctum* cotreatment.

4) 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. Butylparaben-induced reduction in the activities of these enzymes were found to be mitigated by coadministration of *O. sanctum*.

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

*O. sanctum* is reported to contain wide variety of pharmacologically active phytochemicals. Luteolin is one of the flavonoid present in species of *Ocimum* possessing curative and preventive potency against numerous disorders. Quantification of luteolin from the *O. sanctum* aqueous extract was done using HPLC method and found to be in considerably high amount in contrary to the earlier reported studies. The interactive study between luteolin (antioxidant) and butylparaben (oxidant) was performed and results indicated that both the compounds chemically react with each other which could be the reason for amelioration of butylparaben – induced toxicity. The results of the above study showed toxic effects of butylparaben in various biological systems and support usage of *O sanctum* 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 toxicities and its remediation by traditionally used medicinal plant. As this thesis provides strong base of various biochemical and histopathological evidence for butylparaben – induced toxicity (*in vivo* and *in vitro*) and protective effect of the plant extract against it following are the some of the future perspective for which the study can be extended:
1) Detailed and systematic investigation of mechanism of butylparaben 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 butylparaben 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 butylparaben.

4) Comparative potency evaluation of various extracts of *O. sanctum* on various chemical and animal models.

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

6) Interactive study of the active constituents of *O. sanctum* with butylparaben on more precise and sophisticated instruments can be initiated.