CHAPTER - I

INTRODUCTION
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Physiological estrogens (estradiol, estrone and estriol) - female sex hormones are synthesised and secreted by ovary under the influence of follicle stimulating hormone (FSH), of pituitary which is, in term under the control of hypothalamic gonadotrophin releasing hormone (GnRH). Estrogens most importantly estradiol plays central role in development of sexual and reproductive organ development in females (Watanabe et al., 2003). Xenoestrogens are the chemical compound, foreign to the body but once ingested or absorbed can mimic estrogenic activity in the biological system (Chen et al., 2001). Human population is largely exposed to variety of natural and synthetic xenoestrogens. Naturally occurring xenoestrogens may come from food and plant sources (soya, flaxseeds) which are also known as phytoestrogens, Whereas Human exposure to synthetic/chemical estrogens comes from pesticides, plastics, cosmetics, industrial wastes, personal care products, paints and various solvents (Eubanks, 2004).

Xenoestrogens, also known as endocrine disrupting chemicals (EDCs) could exert estrogen dominance symptoms (EDS) characterised by excess estrogen resulting in alteration in sexual development and menstrual cycle, formation of breast tumors in females and development of secondary sexual characteristics in males(Harrison et al., 1995; Wolfgang et al., 2001, Rogan et al., 2003). Burden of EDCs to human is increasing day by day as reports had been published stating that more than 70,000 registered chemicals in United States exerts estrogenic effect in human and experimental animals. Accumulation of xenobiotics in human body leads to variety of abnormalities such as altered reproductive functions, weight gain, constipation, depression and fluid retention once it crosses the detoxification limit of liver.
Health consequences of estrogen dominance symptoms:

- Infertility
- Decreased sperm count
- Breast cancer
- Endometriosis
- Prostate cancer
- Ovarian cancer
- Asthma
- Allergies
- Obesity

COMMONLY FOUND XENOESTROGENS:

**Organochlorides** are group of compounds having chlorine bonded to carbon atom. Pesticides (e.g. Dichloro-diphenyl-trichloroethane DDT), PVC plastics, detergents, petrochemicals such as polychlorinated biphenyls (PCBs) belong to this class of xenobiotics (Bolz et al., 2001; Gagne et al., 2001; Rogers-Gray et al., 2000; Kolpin et al., 2002; Murk et al., 2002). Reports had proved cumulative toxicity of these chemicals as they gets incorporated in food chain and readily absorbed and accumulate in fat tissues of body. Continuous exposure of a woman to xenoestrogens could be the lead reason for the development of breast tumors. Studies showed that organochlorides are complete carcinogen by nature which can initiate and promote tumor growth. **Bisphenol** A widely used in polychlorinated plastic items was found to exert estrogenic activity in various animal models (Hewitt and Korach, 2011). Research revealed that 2-5 parts per billion of bisphenol is enough to cause breast cancer cell proliferation. **Phthalates** - chemicals used in cosmetics
Figure 1.1: Chemical structure of estradiol (physiological estrogen) and some xenoestrogen
and plastic commodities were also found to possess estrogenic activity and cause cancer, developmental and sex-hormone abnormalities (including decreased testosterone and sperm levels and malformed sex organs) in infants, and can affect fertility (Legler et al., 2001, Fisher 2004, Barrett 2005, Swan et al., 2005). Parabens constitute class of commonly used preservatives. For many years, parabens were considered to have low systemic toxicity and safe for usage in various products due to its antimicrobial activity. In contrast to the earlier reports recent research is emerging showing its endocrine disrupting ability in human and experimental animals.

PARABENS

Parabens were discovered and used in cosmetics, foods and drug preservative way back in 1920’s and since then have been among the most researched and studied compound. Synthetic methyl, ethyl, propyl and butylparaben, commonly used paraben esters, are synthesized from benzoic acid and were considered effective and economical. It is estimated that 99% of cosmetics and personal care products contains one or the other form of paraben. These molecules are used in over 22,000 cosmetics as preservatives in concentrations up to 0.8% (mixtures of parabens or up to 0.4% (single paraben). Parabens are included in the list of endocrine disrupting chemicals (EDC) mimicking activity of natural estrogen once absorbed in the body (Byford et al., 2002; Inui et al., 2003; Kang et al., 2003). These EDCs interfere with normal hormonal function and metabolism (Pederson et al., 2002). Estrogenicity of various parabens varies depending upon chemical structure and alkyl chain length of the ester (Darbre and Harvey, 2008).
Figure 1.2: General structure of commonly used parabens
**Chemical structure and types:**

Benzoic acid, the simplest aromatic carboxylic acid containing carboxyl group bonded directly to benzene ring, is a white, crystalline organic compound. Its aqueous solution is weakly acidic. Benzoic acid is converted to its salts and esters for the use of preservative application in foods, drugs and personal care products. Sodium benzoate, sodium salt of benzoic acid, is used preferably as one of the principal anti-microbial preservative used in foods and beverages (but it's concentration is limited usually not exceeding 0.1% because it is poisonous), as it is about 200 times more soluble than benzoic acid. Sodium benzoate is also used in medications, anti-fermentation additives and tableting lubricant for pharmaceuticals. The industrial applications are as a corrosion inhibitor, as additive to automotive engine antifreeze coolants and in other waterborne systems, as nucleating agents for polyolefin, as a dye intermediate, as a stabilizer in photographic processing and as a catalyst. Wide range of benzoic esters are used as solvents, dying carrier, disinfectant additives, penetrating agents pesticides and manufacturing other compounds.

Benzoic acid derivatives substituted by hydroxy group or ether containing oxygen atom have active bacteriostatic and fragrant properties. They are typically used in pharmaceutical and perfumery industry. Esters of hydroxybenzoic acid commonly known as parabens are recognized as antiseptics. Figure 1 shows general structure of parabens containing six-member carbon ring with hydroxyl group (-OH) at one side of the ring and a side chain called an alkyl ester on the opposite side of the ring. They have various prefixes that refer to the length of the alkyl ester group that branches off of the acid. These include methyl, ethyl, benzyl, propyl and butyl and are shown in figure 2. Parabens, substituted at 1,4 position, are widely used as preservatives in food and pharmaceuticals. When combining two or more parabens, their antimicrobial performance is enhanced due to a synergistic effect.
<table>
<thead>
<tr>
<th></th>
<th>Methylparaben</th>
<th>Ethylparaben</th>
<th>Propylparaben</th>
<th>Butylparaben</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water 15° C</td>
<td>0.16</td>
<td>0.07</td>
<td>0.023</td>
<td>0.015</td>
</tr>
<tr>
<td>Water 25° C</td>
<td>0.25</td>
<td>0.12</td>
<td>0.04</td>
<td>0.15</td>
</tr>
<tr>
<td>Water 80° C</td>
<td>3.2</td>
<td>0.86</td>
<td>0.45</td>
<td>0.15</td>
</tr>
<tr>
<td>Ethanol 10%</td>
<td>0.5</td>
<td>-</td>
<td>0.1</td>
<td>-</td>
</tr>
<tr>
<td>Ethanol 50%</td>
<td>18</td>
<td>-</td>
<td>18</td>
<td>-</td>
</tr>
<tr>
<td>Ethanol 100%</td>
<td>52</td>
<td>72</td>
<td>95</td>
<td>200</td>
</tr>
<tr>
<td>Propylene Glycol 100%</td>
<td>22</td>
<td>22</td>
<td>26</td>
<td>-</td>
</tr>
<tr>
<td>Propylene Glycol 50%</td>
<td>2.7</td>
<td>-</td>
<td>0.9</td>
<td>-</td>
</tr>
<tr>
<td>Glycerine</td>
<td>1.7</td>
<td>0.6</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>Peanut oil</td>
<td>1.0</td>
<td>-</td>
<td>1.4</td>
<td>-</td>
</tr>
</tbody>
</table>
Physiochemical characteristics:

In pure form, parabens are generally small colorless crystals or crystalline powders. Parabens are odorless and tasteless chemical compounds. As the alkyl chain of paraben increases aqueous solubility of the compound reduces. Due to this long chain parabens are lipophilic in nature having high water/octanol coefficient. Generally, parabens are stable in air and are resistant to hydrolysis in water (hot and cold) and in acidic solutions. An increase in alkyl chain length of parabens increases the resistance to hydrolysis. Appreciable hydrolysis occurs at pH above 7.

Phenyl ring containing paraben lacks functional ionic group making it highly lipophilic and easily detectable compounds in UV detectors. All parabens have low water solubility, but are dissolved in most systems at temperatures above 60°C. Most of the parabens are freely soluble in alcohol, acetone, ether and other organic solvents.

Uses:

Why parabens are ideal preservatives:

- They possess broad spectrum antimicrobial activity
- They are both bactericidal and fungicidal
- They are stable and effective over a wide pH range, can withstand temperatures up to 100°C, and are biodegradable.
- They are highly compatible with other compounds.

Parabens in cosmetics

Lipophilicity of parabens makes them most suitable preservative for formulation of various oil based cosmetic products. Parabens are having history of more than 80 years of usage as cosmetic preservatives. Generally parabens used in cosmetics are in combination with other compounds as it is well suited for the preservation. In most cosmetics, parabens
Graph 1
Distribution of preservatives in cosmetic products in 1995

Graph 2
Distribution of preservatives in cosmetic products in 2006

Figure 1.3 Paraben usage as cosmetic preservative.
Source: www.aesthetictrends.com | January - February 2010
are used at very low levels ranging from 0.01 to 0.3% but as cosmetics are intended to use on the daily basis the exposure of parabens to human is generally exceeding this limit. Parabens - routinely used in cosmetics are butyl-, propyl- and methyl paraben (Berke et al., 1982; Jackson, 1992). These parabens are used in nearly all types of cosmetics, with reported use in over 13,200 formulations (Elder, 1984). Parabens formulate well because they have no perceptible odor or taste, they do not produce discoloration, they are practically pH neutral and they do not cause hardening (Subert et al., 2007). Parabens are most active against molds and yeasts, and have been successfully used in cosmetics for long period of time (Weber, 1993). Because of their stability at high temperature, products containing parabens can be safely autoclaved without significant loss of antimicrobial activity, as a result of hydrolysis (Sznitowska et al., 2002). Preferential usage of various esters of parabens as cosmetic preservative is found in following order by Rastogi et al. (1995) and Pouillot et al. (2006): Methyl-, > ethyl-, > propyl-, > butyl- > and benzyl-paraben

Parabens in Food

Parabens have been approved by the United States Food and Drug Administration as preservatives in commercial products (Prusakiewicz et al., 2007). The major uses of the parabens in the food industry are cakes, pastries, pie-crusts, icings, toppings and fillings (0.03–0.06% of a 3:1 in ratio methyl and propyl parabens); soft drinks (0.03–0.05% of a 2:1 ratio of methyl and propyl parabens); creams and pastes (0.1% of a combination of parabens); jams, jellies and preserves (0.07% of a 2:1 ratio of methyl and propyl parabens); olives and pickles (0.1% of a combination of parabens); and syrups (0.07%). Among the parabens, methyl and propyl parabens are the most extensively used in food and are considered safe under FDA regulation when used with a limit of 0.1% for each. They are used in processed vegetables, baked goods, fats and oils, seasonings, sugar substitutes, and frozen dairy
Table 1.2: Urinary levels of parabens (ng/ml) in 100 US residents:

<table>
<thead>
<tr>
<th>Paraben</th>
<th>Percent detection</th>
<th>50th percentile</th>
<th>75th percentile</th>
<th>90th percentile</th>
<th>95th percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl</td>
<td>99</td>
<td>43.9</td>
<td>180</td>
<td>412</td>
<td>680</td>
</tr>
<tr>
<td>Ethyl</td>
<td>58</td>
<td>1.0</td>
<td>6.9</td>
<td>25.1</td>
<td>47.5</td>
</tr>
<tr>
<td>Propyl</td>
<td>96</td>
<td>9.1</td>
<td>49.2</td>
<td>144</td>
<td>279</td>
</tr>
<tr>
<td>Butyl</td>
<td>69</td>
<td>0.5</td>
<td>3.3</td>
<td>14.5</td>
<td>29.5</td>
</tr>
</tbody>
</table>

products. Their usage in fruit juices, pickles, sauces and soft drinks is regulated between 450 - 2000 ppm concentrations (Daniel, 1986). To determine the specific foods in which parabens were used and at what levels, a representative cross-section of food manufacturers was surveyed (FDA, 1973).

**Parabens in Pharmaceuticals**

Parabens were first used in pharmaceutical products in 1924. Since then, they have been incorporated as preservatives in a variety of pharmaceutical formulations. Among the parabens, propyl paraben is one of the most effective fungicide used in pharmaceutical preparations. A variety of drug formulations including suppositories, anesthetics, pills, syrups, weight gaining solutions, injectable solutions and contraceptives are known to contain parabens as preservatives. Combinations of parabens are more active than individual parabens as drug preservatives (Boehm and Maddox, 1972; Charnock and Finsrud, 2007). The Ophthalmic Drug Panel of the US Food and Drug Administration (FDA) has determined that methyl and propyl paraben, if used alone and at concentrations effective against microorganisms, are not suitable as preservatives in OTC ophthalmic products, because they are irritating to the eyes.

**Safety assessments and Human exposure:**

Parabens were first approved for use in cosmetic products in 1984 when the Cosmetic Ingredient Review (CIR) determined they were safe. Safety assessment of parabens were further evaluated and found to be safe at following level (CIR, 2010):

- Butylparaben: Up to 0.4% if used alone
- Ethylparaben: Up to 0.4% if used alone
- Methylparaben: Up to 0.4% if used alone
- **Propylparaben**: Up to 0.4% if used alone
- **Paraben mixes**: Up to 0.8%

In spite of its usage in permissible limit parabens possess human health risk due to its wide spread and long term usage. Parabens have been approved by the United States Food and Drug Administration as preservatives in commercial products (Prusakiewicz et al., 2007). According to the U.S. Food and Drug Association, the average amount of parabens in cosmetics is 0.01% to 0.3% (Parabens. U.S. Department of Health and Human Services. http://www.fda.gov/cosmetics/productandingredientsafety/selectedcosmeticingredients/ucm128042.htm. Accessed September 23, 2010). Studies conducted by the Centers for Disease Control and Prevention (CDC) did find methylparaben, ethylparaben, propylparaben, and butylparaben in human urine samples, indicating exposure despite the very low levels in products (Ye et al., 2006; Ye et al. 2007). The source of exposure was assumed to be topical application of personal care products, as studies have shown that parabens can be absorbed through the skin (Janjua 2007). Although parabens do penetrate the dermis, metabolism of parabens takes place within the skin, which likely will result in less than 1% unmetabolized parabens available for absorption in the body (Caon et al., 2010).

In 2009 the Danish National Food Institute published a risk assessment for parabens, including more recent studies showing endocrine disrupting properties of parabens in animals, and also studies on uptake and metabolism of parabens in humans (Boberg et al., 2010). This risk assessment concluded that the oestrogenic burden from paraben exposure may exceed the amount of natural oestrogen in children, and that for propylparaben the safety margin between possible levels in humans and effect concentrations in young animals is very small. In December 2010 the Scientific Committee on Consumer Products (SCCP) of the European Union published an updated opinion on parabens. They concluded that there is not enough data to perform risk assessments for propylparaben and butylparaben in humans, and
Table 1.3. Levels of parabens measured in human tissues (Darbre et al., 2008)

<table>
<thead>
<tr>
<th></th>
<th>Human breast tissue mean ng g⁻¹ tumour n = 20</th>
<th>Human urine (USA) median ng ml⁻¹ n = 100</th>
<th>Human serum mean peak level (3 h) ng ml⁻¹ n-butyl applied as topical cream n = 26</th>
<th>Human urine mean level mg 24 h⁻¹ n-butyl applied as topical cream n = 26</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methylparaben</td>
<td>12.8</td>
<td>43.9</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethylparaben</td>
<td>2.0</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Propylparaben</td>
<td>2.6</td>
<td>9.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Butylparaben</td>
<td>2.3</td>
<td>0.5</td>
<td>0.135</td>
<td>2.6</td>
</tr>
</tbody>
</table>
meanwhile the maximum concentration of these parabens in consumer products should be lowered from 0.8% to 0.19%. In March 2011 Denmark prohibited propylparaben and butylparaben in products for use by children younger than three years of age. Of the 72,609 samples tested, Ishiwata et al. (1997) reported detection of parabens in 903 out of 9876 food products (9.1%). The detection rate and average concentration in all foods in which these are permitted for use were 28.6% and 8.8% of the limits, respectively. The highest detection rate among the foods permitted for use was soy sauce (49.8%) and the average concentration in all tested soy sauce samples was also the highest, 0.0342 g/kg as p-hydroxybenzoic acid. This concentration corresponded to 13.7% of the permissible limit. The use of parabens is permitted under the European Union Directive No. 95/2/ECon food additives other than colors and sweeteners. Use of parabens (methyl-, ethyl-, propyl-, butyl-, and benzyl-; also includes isobutyl- and isopropyl-paraben) in cosmetic products as a preservative with a maximum concentration of 0.8% (w/w), calculated as p-hydroxybenzoic acid, is permitted by the Danish and EEC regulations (Howlett, 1992). The particular parabens were found in breast tumor in relative concentrations that closely parallel their use in the synthesis of cosmetic products (Rastogi et al., 1995). The maximum concentration limit set for any single ester was 0.4%. Some researchers have reported presence of p-hydroxybenzoic acid (parent acid of paraben) in blood and milk samples of all the patients suffering from breast cancer (Nakazawa et al., 1999; Cashman and Warshaw, 2005).
BUTYLPARABEN

Butylparaben ([C\textsubscript{11}H\textsubscript{14}O\textsubscript{3}]; mol. wt. = 194.25) is butyl ester of p-hydroxybenzoic acid, made by esterifying 1-butanol with parent acid of paraben.

**Physiochemical characteristic:**

Appearance: White crystalline powder

Color: Colorless

Odor: Odorless

Melting point: 68-69° C

Solubility: Water (slightly soluble), acetone, alcohol, ether, propylene glycol

Octanol-water partition coefficient (log K\textsubscript{OW}): 3.57

**Exposure:**

Products used by adults that potentially expose large areas of the body surface to butylparaben that were listed in CIR (1984) include:

- Fragrance powders and men's talcum
- Bath oils, tablets, and salts
- Face, body, and hand skin care preparations
- Moisturizing skin care preparations
- Suntan oils/lotions
- Arthritis analgesic creams with methylsulfonylmethane

Butylparaben is generally used as a preservative along with other parabens preferably with methylparaben. Exposure of a 60-kg woman using multiple product categories (bath products, colognes and toilet waters, powders, hairsprays, shampoos, tonics and other hair
grooming aids, blushers, foundations, lipstick, makeup bases, bath soaps and detergents, deodorants, skin cleansers, depilatories, face preparations, body moisturizers, skin fresheners, and sun products) was estimated by multiplying estimated product exposure in milligrams per kilogram per day by the reported maximum butylparaben concentration in the product category. This resulted in a total composite butylparaben exposure of 0.307-1.02 mg/ kg bw/day.

Based on Cross and Roberts (2000), it is reasonable to assume that approximately 40% of butylparaben could be dermally absorbed, which results in a butylparaben dose of approximately 0.12-0.41 mg/ kg/day. It is important to note that this estimate assumes that an individual uses every product in the above list every day (i.e., worst-case assumption). The calculated butylparaben dose of 0.12-0.41 mg/kg/day also assumes that dermal esterases do not further reduce or completely metabolize this dose to p-hydroxybenzoic acid (PHBA). In the following sections, potential risks from parabens as a consequence of their possible weak estrogenic activity are assessed by comparing the paraben doses required to produce an in vivo effect with total paraben exposure levels (i.e., 0.12-0.41 mg/ kg/day) and contrasting these levels and their estrogenic potency with amounts of estrogen known to produce adverse effects. In the 1983 National Institute for Occupational Safety and Health (NIOSH) National Occupational Exposure Survey (NOES), an estimated 24,427 employees were exposed to butylparaben in 704 facilities; of the total, 18,593 were females (RTECS, 2003). Maximum values for butylparaben in health drinks, tonic drinks, and cold formulas were 106.8, 120.1, and 184.6 μg/mL (549.8, 618.3, and 950.3 μM), respectively, which exceeded the regulatory limit of 100 μg/mL for preservatives (Lin et al., 2000). Butylparaben is regulated by the U.S. Environmental Protection Agency (EPA) under the Toxic Substances Control Act (TSCA) and the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). It is permitted as a
Table 1.4. Estimated butylparaben exposure

<table>
<thead>
<tr>
<th>Products containing butylparaben</th>
<th>µg/day</th>
<th>Route</th>
</tr>
</thead>
<tbody>
<tr>
<td>Personal care products, adult</td>
<td>14,590</td>
<td>Dermal</td>
</tr>
<tr>
<td>Pharmaceuticals/OTC, adult</td>
<td>1 00</td>
<td>Oral</td>
</tr>
<tr>
<td>Pharmaceuticals/OTC, child</td>
<td>13,500</td>
<td>Oral</td>
</tr>
<tr>
<td>Soil (dust) ingestion, pica</td>
<td>39.2</td>
<td>Oral</td>
</tr>
<tr>
<td>Soil (dust) ingestion, hand-to-mouth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child</td>
<td>0.392</td>
<td>Oral</td>
</tr>
<tr>
<td>Adult</td>
<td>0.196</td>
<td>Oral</td>
</tr>
<tr>
<td>Soil adherence to skin (child)</td>
<td>0.431</td>
<td>Dermal</td>
</tr>
<tr>
<td>Indoor air</td>
<td></td>
<td></td>
</tr>
<tr>
<td>child</td>
<td>0.032</td>
<td>Inhalation</td>
</tr>
<tr>
<td>adult male</td>
<td>0.048</td>
<td>Inhalation</td>
</tr>
<tr>
<td>adult female</td>
<td>0.036</td>
<td>Inhalation</td>
</tr>
</tbody>
</table>
synthetic flavoring substance and adjuvant and is to be used in the minimum quantity required to produce its intended effect.

**Pharmacokinetics:**

Orally administered butylparaben gets rapidly and passively absorbed from gastrointestinal tract and hydrolyzed to more polar compounds by liver and kidney carboxylesterases. The major metabolites of parabens are \( p \)-hydroxybenzoic acid (pHBA), \( p \)-hydroxyhippuric acid, \( p \)-hydroxybenzoyl glucuronide, and \( p \)-carboxyphenylsulfate. Biotransformation of parabens mainly includes hydrolysis of the ester bond and glucuronidation reactions performed by human UDP-glucuronosyltransferase (UGT) isoforms located in liver. In humans, liver microsomal and cytosolic fractions are involved in butylparaben hydrolysis and the rate of hydrolysis (at 100 \( \mu \)M) by microsomes was 31±4 nM/min/mg. Metabolism of butylparaben includes formation of glucuronides and sulfate conjugates which gets excreted in urine. Dermally applied butylparaben is hydrolysed by skin carboxylesterases isoform hCE2 to 4-hydroxybenzoic acid. Loperamide, a specific inhibitor of human carboxylesterase-2 inhibited butylparaben hydrolysis by more than 90% in human skin indicating involvement of esterase in metabolism. Study on human epidermis showed butylparaben retention by them. Rapid hydrolysis of parabens was measured with human liver microsomes depending on the alkyl chain length, with \( t^{1/2} \) varying from 22 min for methylparaben to 87 min for butylparaben. In conclusion, the parabens were readily metabolized in human liver through esterase hydrolysis and glucuronidation by several UGT isoforms (Abbas et al., 2010)

Butylparaben has also been studied in an in vivo absorption experiment in Wistar rats. After 4 h, almost 50% of the 14\(^{\text{C}}\)-labeled administered dose was recovered in the urine, and
10% was recovered in the skin (Yamashita et al., 1994). Similarly, in an in vitro study with butylparaben, 88% of the applied dose was recovered at the end of the skin diffusion experiments (Koyama et al., 1994). Intravenous and oral administration of butylparaben to dogs resulted in recovery of 48 and 40% of the paraben conjugates in urine sample whereas intravenous administration of higher dose of butylparaben had resulted in recovery of pure esters from brain, spleen and pancreas and metabolites were detected in liver and kidney (Jones et al., 1956; Cited by JECFA. 2001).

**TOXICOLOGICAL EVALUATION:**

A variety of *in vitro* and *in vivo* studies have focused on the biological and toxicological effects of butylparaben, a commonly used preservative.

**Reproductive system:**

Parabens are known to exert a weak estrogenic activity, with butylparaben showing the most potent activity among methyl-, ethyl- and propyl esters in *in vitro* recombinant yeast assay and in *in vivo* uterotrophic assay. Animals fed with 0.01%, 0.1% and 1.0% of butylparaben in diet showed decrease weight of epididymis significantly and dose-dependently upon eight weeks treatment. Reduction in sperm count (58.2%) and cauda epididymal sperm reservoir was also noted with butylparaben treatment (Oshi et al., 2001; Oshi et al., 2001). Decrease in testosterone level was also observed in animals of same study due to exposure of postweaning mammal to butylparaben indicating its adverse effect on male reproduction. Weak estrogenic activity of butylparaben was reported in some *in vitro* screening tests, such as ligand binding to the estrogen receptor, regulation of CAT gene expression, and proliferation of MCF-7 cells. Result of a competitive binding assay using rat ER stated that butylparaben can effectively compete with [3H] estradiol with an affinity
approximately five orders of magnitude less than diethylstilbestrol (DES) under in vitro conditions (Routledge et al. 1998). In a recombinant yeast assay, cells transfected with the human estrogen receptor (ERα) gene together with expression plasmids (containing estrogen-responsive elements) were incubated in medium containing butylparaben to determine their estrogenic activity in comparison with estrogen (17β-estradiol) (Miller et al., 2001) and showed positive results. Studies have reported increase in uterine weight with subcutaneous administration of butylparaben at 600 mg/kg bw/day in immature rat. In addition, modest increases in uterus wet and dry weights were observed when immature rats were orally administered doses ranging from 800 to 1,200 mg/kg BW/day. Kang et al. (2002) investigated the effects of maternal exposure of butylparaben during gestation and lactation periods on the development of the reproductive organs of the F1 offspring. At post-natal day 49, weights of testes, seminal vesicles and prostate glands were significantly decreased in rats exposed to 100 mg/ kg bw of butylparaben. Butylparaben administration did not affect the weights of female reproductive organs in pups but in male animals, the sperm count and the sperm motility in the epididymis were significantly decreased. Subcutaneous administration of butylparaben (100 or 200 mg/kg bw [0.515 or 1.03 mmol/kg bw) in pregnant rats from gestation day 6 to postnatal day 20 decreased the proportion of pups born alive and the proportion of pups that survived up to the weaning period.

**Breast cancer:**

Some estrogens are known to drive the growth of tumors; however the estrogenic activity and mutagenic activity of estrogens are not the same, with the latter dependent on free radical chemistry and not estrogen receptor activity.

Average levels of 20 nanograms/gm of parabens have been detected in a small sample of 20 breast tumors. Also, the lipophilic properties of these chemicals allow them to accumulate in fatty tissues of human and animals (Stellman et al., 1998; Turusov et al.,
Butylparaben was found to stimulate cell proliferation, as reported by Darbre et al. (2002) in MCF7 cell lines. These findings, along with the demonstrated ability of some parabens to partially mimic estrogen, a hormone known to play a role in the development of breast cancers, have led some scientists to conclude that the presence of parabens may be associated with the occurrence of breast cancer, and to call for investigation into whether or not a causal link exists. The lead researcher of the UK study, molecular biologist Philippa Darbre, reported that the ester-bearing form of the parabens found in the tumors indicate that they came from something applied to the skin, such as an underarm deodorant, cream or body spray, and stated that the results helped to explain why up to 60% of all breast tumors are found in just one-fifth of the breast - the upper-outer quadrant, nearest the underarm. In human breast cancer tissue, butylparaben represented 11% of the total paraben content (Darbre et al., 2004).

**Acute toxicity:**

Acute toxicity study of butylparaben on mice, rats and dogs were reported to have a low order of acute toxicity; the main effect was an acute myocardial depression accompanied by hypotension that was transient in nature. In mice, rapid onset of ataxia, paralysis, and deep depression, which was similar to anesthesia, were seen. With nonfatal doses, recovery generally occurred within 30 min; with fatal doses, death usually occurred within an hour (Matthews et al., 1956). Intraperitoneal (i.p.) injection of butylparaben (230 mg/kg [1.18 mmol/kg]) resulted in lacrimation in the eyes of mice (Tsuzi, 1956). Butylparaben (5%) was a mild irritant when applied to the skin of guinea pigs for 48 hours (Danish EPA, 2001). In rabbits, a product formulation containing 0.2% butylparaben was nonirritating, although the primary irritation index indicated moderate irritation. Slight increase in mortality of butylparaben intoxicated animals was observed. In another study, a product with 0.3%
butylparaben applied for three days to the back of rabbits produced mild irritation with erythema and/or edema (CIR, 1984).

**Short-term and Subchronic Exposure:**

Feeding of food pellets containing butylparaben (0.6, 1.25, 2.5, 5, or 10%) to mice for six weeks resulted in animal death within the first two weeks in two highest doses treatment. Except 0.6% dose all the other doses produced significant atrophy of lymphoid tissue in the spleen, thymus, and lymph nodes were observed. Additionally, multifocal degeneration and necrosis were seen in the liver parenchyma (Inai et al., 1985).

Results of subchronic exposure study of butylparaben on rats showed high mortality. Rats fed a diet of butylparaben (2 or 8%) for up to 12 weeks resulted in 100% mortality before the end of the treatment period in males given the high dose. Females also had many early deaths. High - dose of butylparaben (8%) caused significant reduction in body weights of the animals (Matthews et al., 1956). A diet of 4% butylparaben for nine days acted entirely on the prefundic region of the forestomach epithelium adjacent to the fundic mucosa (Rodrigues et al., 1986).

**Chronic Exposure:**

Result of chronic study revealed development of high incidence of amyloidosis affecting the spleen, liver, kidney, and/or adrenal gland in mice orally fed with butylparaben (0.15, 0.3 and 0.6%). Moreover tumors at various sites including the hematopoietic system, lung and soft tissue were also observed. These occurred in 45 and 27% of males and females, respectively, that survived for >78 weeks or died with tumors during the experimental period (Inai et al., 1985).
Cytotoxicity

Butylparaben was found to exert cytotoxic effect in isolated rat hepatocytes. Treatment of butylparaben (2 mM [0.4 mg/mL]) caused 88% cell death, almost total loss of adenosine triphosphate (ATP), and ~60% reductions in the total adenine nucleotide pool and mitochondrial membrane potential of hepatocytes culture. Butylparaben (0.05, 0.10, or 0.25 mM [9.8, 19, or 49 µg/mL]) intoxication in rat mitochondria increased the rate of state 4 oxygen consumption and inhibited the rate of state 3 oxidation (Nakagawa and Moldeus, 1998; Nakagawa et al., 1999). In normal and α-linolenic acid (LNA)-loaded cultured rat hepatocytes, butylparaben-induced severe cell injury accompanied by a significant decrease in cellular levels of both glutathione (GSH) and protein-SH in absence of lipid peroxidation (Sugihara et al., 1997). Yamashoji and Isshiki, (1998, 2002) showed cytotoxic effect of butylparaben on animal cells using menadione-catalyzed hydrogen peroxide production assay.

Hematological effect:

Butylparaben strongly inhibited agonist-induced thromboxane (TXB2) synthesis irreversibly in vitro and inhibited or suppressed platelet function (Yamazaki et al., 1998). In mitogen stimulated peripheral human lymphocytes, butylparaben was the most potent of the parabens tested with C1- to C4-alkyl ester groups in inhibiting release of lysosomal enzymes (45-50% inhibition at 0.06 mM) (Bairati et al., 1994). In human and rabbit erythrocytes in vitro, butylparaben (0.02%) induced hemolysis in 6 and 12% of the cells, respectively (CIR, 1984).
OCIMUM SANCTUM

_Ocimum sanctum_ L. (also known as _Ocimum tenuiflorum_, Tulsi) has been used for thousands of years in Ayurveda for its diverse healing properties. A member of the mint family, _Ocimum sanctum_, is a small annual shrub native to tropical and sub-tropical regions. The bushy branches of the plant bear toothed leaves and flowers that are purple or crimson. Known as Holy Basil in India, it is considered a sacred plant, especially among Hindus. It is grown in close proximity to the house to purify the air and for its spiritual powers to open the heart and mind and bring love, compassion and clarity.

*Plant profile:*

There are many varieties of basil as:

Sweet basil - used in Italian food

Lemon basil - Lemon basil has a strong lemony smell and flavour different from other varieties as it contains a chemical called citral

Holy basil - _tulsi_, a revered home-grown plant in India and Nepal

African Blue - African blue basil has a strong camphor smell because it has camphor and camphene

Holy Thai basil -

Pinyin - Chinese basil

*Classification:*

Kingdom: Plantae

Order: Lamiales

Family: Lamiaceae

Genus: Ocimum
Figure 1.4: Aerial parts of plant *Ocimum sanctum*
Figure 1.5: Herbarium sample of *Ocimum sanctum*
Species: *O sanctum* / *O. tenuiflorum*

Common name is **sacred basil, holy basil.**

Among the plants known for medicinal value, the plants of genus *Ocimum* belonging to family Lamiaceae are very important for their therapeutic potentials. *Ocimum sanctum* L (Tulsi), *O. gratissimum* (Ram Tulsi), *O. canum* (Dulal Tulsi), *O. basilicum* (Ban Tulsi), *O. kilimandschricum*, *O. americanum*, *O. camphora* and *O. micranthum* are examples of known species of genus *Ocimum* that grow in different parts of the world and are known to have medicinal properties (Pattanayak et al., 2010).

**Nutrition Value**

Different parts of the plants (leaves, stem, root) are traditionally used for therapeutic purpose and as they are found to possess high nutritional status. *O. sanctum* contains vitamin C and A, and minerals like calcium, zinc and iron, as well as chlorophyll and many other phytonutrients (Pattanayak et al., 2010). It also enhances the efficient digestion, absorption and use of nutrients from food and herbs.

**Content/100 gm**

- Protein: 30 Kcal, 4.2 g;
- Fat: 0.5 g;
- Carbohydrate 2.3 g;
- Calcium: 25 mg;
- Phosphorus 287 mg;
- Iron: 15.1 mg and
- Vitamin C 25 mg
Figure 1.6: Chemical structure of some of the active components present in O. sanctum
Phytochemical Constituents

*O. sanctum* revealed the presence of tannins, basil camphor, flavonoid (like luteolin, orientin, vicenin) and five fatty acids – stearic, palmitic, oleic, linoleic, linolenic acids. Basil is a good source of beta carotene and calcium. It also contains volatile oil (1% including eugenol, linalool, estragol, methyl chavicol, methyl cinnamate, cileole and other terpenes), triterpene; urolic acid, zinc, manganese.

Various extracts of *O sanctum* are found to contain variable amounts of phytochemicals and protective effect of plant is generally denoted by the synergistic effect of these plant extracts because of that traditionally whole plant had been used for therapeutic purpose rather than an individual active component. *O. sanctum* contains large number of chemically active secondary metabolites. The leaf contains various volatile oils such as eugenol (1-hydroxy-2-methoxy-4-allylbenzene, euginal (also called eugenic acid), urosolic acid (2, 3, 4, 5, 6, 6a, 7, 8, 8a, 10, 11, 12, 13, 14b-tetradecahydro-1H-picene-4a-carboxylic acid, carvacrol (5-isopropyl-2-methylphenol), linalool (3,7-dimethylocta-1,6-dien-3-ol, limatrol, caryophyllene (4,11,11-trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene), methyl carvicol (also called estragol: 1-allyl-4-methoxybenzene while the seed volatile oil have fatty acids and sitosterol; The sugars are composed of xylose and polysaccharides. Although Tulsi is known as a general vitalizer and increases physical endurance, it contains no caffeine or other stimulants. The stem and leaves of holy basil contain a variety of constituents that may have biological activity, including saponins, flavonoids, triterpenoids, and tannins. In addition, the following phenolic actives have been identified, which also exhibit antioxidant and antiinflammatory activities, Rosmarinic acid ((2R)-2-[[((2E)-3-(3,4-Dihydroxyphenyl)-1-oxo-2-propenyl]oxy]-3-(3,4-dihydroxyphenyl) propanoic acid, a p i g e n i n (5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran- 4-one, c i r s i m a r i t i n (5,4'-dihydroxy-6,7 dimethoxyflavone), isothymusin (6,7-dimethoxy-5, 8, 4'-trihydroxyflavone) and isothymonin.
Two water-soluble flavonoids: orientin (8-C-beta-glucopyranosyl-3',4', 5, 7-tetrahydroxyflav-2-en-3-one) and vicenin (6-C-beta-D-xylopyranosyl-8-C-beta-D-glucopyranosyl apigenin), have shown to provide protection against radiation-induced chromosomal damage in human blood lymphocytes (Devi et al., 2000; Kelm et al., 2000; Jaggi et al., 2003; Shishodia et al., 2003). Luteolin one of active flavonoid isolated from *O. sanctum* species id great antioxidant and possesses numerous pharmaceutical properties (Xu et al., 2009).

**Luteolin:**

Pure luteolin [C_{15}H_{10}O_{6}] is yellow crystalline powder belonging to the class of flavonoids specifically flavone. Luteolin is one of the most potent flavonoid present in leaves of various herbs and spices having excellent antioxidative properties. Plants of *Ocimum* species also contain significant amount of luteolin in them. Free radical scavenging activity of luteolin allows it to act as an agent preventing inflammation, promoting carbohydrate metabolism, and modulating immune system (Jang et al., 2008). These characteristics of luteolin are also believed to play an important role in the prevention of cancer. Multiple research experiments describe luteolin as a biochemical agent that can dramatically reduce inflammation and the symptoms of septic shock. It has been reported to be non-mutagenic, antimutagenic, antitumorigenic and anti-inflammatory-allergic and has been recognized as a hydroxyl radical scavenger and an inhibitor of protein kinase C (Byun et al., 2010). Study revealed that preadministration of luteolin reduced the frequency of micronucleated reticulocytes (MNRETS) in mouse peripheral blood cells, suppressed Q-ray-induced lipid peroxidation in mouse bone marrow and spleen.
PHARMACOLOGICAL PROPERTIES:

*O. sanctum* phytochemicals extracted by various methodologies are potent source of antioxidants and found to protect against deleterious effects of stress (Arya *et al*., 2006). Aqueous extract of *O. sanctum* L. inhibit the hypercholesterolemia-induced erythrocyte lipid peroxidation activity in a dose-dependent manner in male albino rabbits. Oral feeding also provides significant liver and aortic tissue protection from hypercholesterolemia-induced peroxidative damage (Halim and Mukhopadhy, 2006). The effect of methanolic extract of *O. sanctum* L. leaves in cerebral reperfusion injury as well as long-term hypoperfusion was studied by Yanpallewar *et al*. (2004). This antioxidative effect is due to presence of phenolic compounds, viz., cirsinilineol, cirsimaritin, isothymusin, apigenin and rosmarinic acid, orientin, vicenin, luteolin and appreciable quantities of eugenol from OS extract of fresh leaves and stems possessed good antioxidant activity (Nair *et al*., 1982; Vrinda and Uma Devi, 2001).

Administration of *O. sanctum* L. seed oil (0.8 gm/kg body weight/day) for four weeks, in cholesterol-fed (100 mg/kg body weight/day) rabbits significantly decreases serum cholesterol, triacylglycerol and LDL + VLDL cholesterol as compared to untreated cholesterol-fed group suggesting the hypocholesterolemic activity of *O. sanctum* L. Fresh leaves of *O. sanctum* L. mixed in 1g and 2g in 100 g of diet given for four weeks brought about significant changes in the lipid of normal albino rabbits (Sarkar *et al*., 1994; Trevisan *et al*., 2006). This resulted in significant lowering in serum total cholesterol, triglyceride, phospholipids and LDL-cholesterol level and significant increase in the HDL-cholesterol and total fecal sterol contents. Singh *et al*. (2005) in his study suggested that higher content of linoleic acid in *O. sanctum* L. fixed oil could contribute towards its antibacterial activity. The oil show good antibacterial activity against *Staphylococcus aureus*, *Bacillus pumius* and *Pseudomonas aeruginosa*, where *S. aureus* was the most sensitive organism.
Ethanolic extract of *O. sanctum* L. significantly decreases the blood glucose, glycosylated hemoglobin and urea with a concomitant increase in glycogen, hemoglobin and protein in streptozotocin-induced diabetic rats (Narendhirakannan et al., 2006). This extract also resulted in an increase in insulin and peptide levels and glucose tolerance. The constituents of *O. sanctum* leaf extracts have stimulatory effects (Hannan et al., 2006) on physiological pathways of insulin secretion, which may underlie its reported antidiabetic action. Grovel et al. (2005) suggested that treatment with *O. sanctum* L. extract for 30 days to normal rats fed with fructose for 30 days significantly lowered serum glucose level in comparison with control group. However, *O. sanctum* extract has no significant effect on hyperinsulinemia. *O. sanctum* L. may potentially regulate corticosteroid-induced diabetic mellitus. In another study the effect of *O. sanctum* L. on three important enzymes of carbohydrate metabolism [glucokinase (gk), hexokinase (hk) and phosphofructokinase (PFK)] along with glycogen content of insulin-dependent (skeletal muscle and liver) and insulin-independent tissues (kidneys and brain) was studied by Vats et al. (2004) in streptozotocin (STZ, 65 mg/kg)-induced model of diabetes for 30 days in rats. Administration of *O. sanctum* L. extracts 200 mg/kg for 30 days lead to decrease in plasma glucose levels by approximately 9.06 and 24.4% on 15th and 30th day. *O. sanctum* L. significantly decreased renal but not liver weight (expressed as % of body weight) *O. sanctum* L. glycogen content in any tissue; also *O. sanctum* L. partially corrected the activity of glucokinase (gk), hexokinase (hk) and phosphofructokinase (PFK) distributed in the diabetic control. Tulsi (*O. sanctum* L.) leaf powder (Rai et al., 1997) was fed at the 1% level in normal and diabetic rats for a period of one month and the result indicated a significant reduction in fasting blood sugar urogenic acid, total amino acids level. This observation indicates the hypoglycemic effect of *O. sanctum* L. in diabetic rats. It was also reported that oral administration of alcoholic extract of leaves of *O. sanctum* L. led to marked lowering of blood sugar level in normal, glucose-fed
hyperglycemic and streptozotocin-induced diabetic rats (Chattopadhyay, 1993). Furthermore, the extract potentiates the action of exogenous insulin in normal rats. The activity of the extract was 91.55 and 70.43% of that of Tolbutamide in normal and diabetic rats, respectively.

Fresh leaf paste (topically), aqueous and ethanolic extract (orally) were evaluated for their chemopreventive activity against 7,12-dimethylbenzaanthracene (DMBA) - induced (0.5%) hamster buccal pouch carcinogenesis (Karthikeyan et al., 1999). Incidence of papillomas and squamous cell carcinomas were significantly reduced and increased the survival rate in the topically applied leaf paste and orally administered extracts to animals. Histopathological observation made on the mucosa confirmed the profound effect of the orally administered aqueous extract than other. Prashar et al. (1998) in their study reported that O. sanctum L. leaf extract blocks or suppresses the events associated with chemical carcinogenesis by inhibiting metabolic activation of the carcinogen. In this study, primary cultures of rat hepatocytes were treated with 0-500 mg of O. sanctum L. extract for 24 h and then with 7,12-dimethyland benz[a]anthracene (DMBA, 10 or 50 mg) for 18 h. Cells were then harvested and their DNA was isolated and analyzed by 32p post-labeling. A significant reduction in the levels of DMBA/DNA adducts was observed in all cultures pretreated with O. sanctum L. extract. Hepatocytes that were treated with the highest dose of extract (500 mg) showed a maximum reduction of 93% in the mean values of DMBA/DNA adducts. This suggests the inhibition of metabolic activation of carcinogen. The chemopreventive activity of seed oil of O. sanctum was evaluated against subsequently injected 20-methylcholanthrene-induced fibrosarcoma tumors in the thigh region of Swiss albino mice. Supplementation of maximal-tolerated dose (100 ml/kg body wt.) of the oil significantly reduced 20-methylcholanthrene-induced tumor incidence and tumor volume (Prakash and Gupta, 2000). The enhanced survival rate and delay in tumor incidence was observed in seed
oil supplemented mice. Liver enzymatic, non-enzymatic antioxidants and lipid peroxidation end product, malondialdehyde level were significantly modulated with oil treatment as compared to untreated 20-methylcholthrene injected mice. The chemopreventive efficacy of 100 mg/kg seed oil was comparable to that of 80 mg/kg vita.-E.

O. sanctum extract was found to protect against ethanol induced ulceration in rats. The aqueous extract of O. sanctum significantly reduces the incidence and severity of ulceration in ethanol induce ulcer model. Ethanol induces ulcers by the reduction of gastric mucosal blood flow and mucus production in the gastric lumen, a decrease in endogenous glutathione and prostaglandins levels and increase of ischemia, gastric vascular permeability, generation of free radicals, and production of leukotrienes. It had been found that oxygen-derived free radicals are implicated in the mechanism of acute and chronic ulceration and scavenging these free radicals can play an appreciable role in healing these ulcers. Ethanol induced generation of free radicals elevate the lipid peroxide level and reduces the cysteine, which is required for glutathione synthesis, thereby depleting glutathione levels. Reduced glutathione is found in high concentration in gastric mucosa of rats and humans. Glutathione is important for the maintenance of mucosal integrity and depletion of glutathione from the gastric mucosa induces macroscopic mucosal ulceration.

Radio-protective effect of aqueous extract of O. sanctum (40 mg/kg, for 15 days) in mice exposed to high doses (3.7 MBq) of oral 131 iodine was investigated by studying the organ weights, lipid peroxidation and antioxidant defense enzyme in various target organs like liver, kidney, salivary glands and stomach at 24 h after exposure. Pretreatment with O sanctum in radiiodine-exposed group showed significant reduction in lipid peroxidation in both kidney and salivary glands. In liver, reduced glutathione (GSH) levels showed significant reduction after radiation exposure while pretreatment with O. sanctum exhibited
less depletion in GSH level even after 131 iodine exposure. However, no such changes were observed in the stomach. The results indicate the possibility of using aqueous extract of *O. sanctum* for ameliorating 131 iodine induced damage to the salivary glands. Two polysaccharides isolated from *O. sanctum* could prevent oxidative damage to liposomal lipids and plasmid DNA induced by various oxidants such as iron, AAPH and gamma radiation. Vrinda et al. reported that two water-soluble flavonoids, Orientin (Ot) and Vicenin (Vc), isolated from the leaves of *O. sanctum* L. provide significant protection against radiation lethality and chromosomal aberration in vivo (Vrinda and Devi, 2001). In order to select the most effective drug concentration, fresh whole blood was exposed to 4 Gy of cobalt-60 gamma radiation with *O. sanctum* without a 30 min pretreatment with 6.25, 12.5, 15, 17.5 and 20 micron of Ot/Vc in micronucleus test. Radiation significantly increased the micronucleus (MN) frequently. Pretreatment with either Ot or Vc at all concentration-dependent manner, with optimum effect at 17.5 mm. The effect of aqueous extract of leaves of *O. sanctum* against radiation lethality and chromosome damage was studied by radiation-induced lipid peroxidation in liver (Devi and Ganasoundari, 1999). Adult Swiss mice were injected with 10 mg/kg of gamma radiation 30 min after last injection. Glutathione (GSH) and the antioxidant enzymes glutathione transferase (GST), reductase (GSRx), peroxidase (GSPx) and superoxide dismutase (SOD) as well as lipid peroxide (LPx) activity were estimated in the liver at 15 min, 30 min, 1, 2, 4 and 8 h post-treatment. Aqueous extract itself increased the GSH and enzymes significantly above normal level, whereas radiation significantly reduced all the values and significantly increased the lipid peroxidation rate, reaching a maximum value at 2 h after exposure (3.5 times of control). Aqueous extract significantly reduced the lipid peroxidation and accelerated recovery to normal levels. In a comparative study of radioprotection by ocimum flavonoids and synthetic aminothiol protectors in mouse showed Ocimum flavonoids as promising human radiation protectant.
(Devi et al., 1998). In this study, adult Swiss mice were injected intraperitoneally with 50 mg/kg body weight of Orientin (OT) or vicenin (Vc) 20 mg/kg body weight of 2-mercaptopropionyl glycine (MPG) 150 mg/kg body weight of WR2721 and exposed to whole body irradiation of 2 Gy gamma radiation 30 min later. After 24 hours, chromosomal aberrations were studied in the bone marrow of the femur by routine metaphase preparation after colchicines treatment. Pretreatment with all the protective compounds resulted in a significant reduction in the percentage of aberrant metaphases. Vicenin produced the maximum reduction in per cent aberrant cells while MPG was the least effective; OT and WR-2721 showed an almost similar effect. Ganasoundari et al. (1998) investigated the radio-protective effect of the leaf extract of *O. sanctum* in combination with WR-2721 (WR) on mouse bone marrow. Adult Swiss mice were injected intraperitoneally with *O. sanctum* (10 mg/kg for five consecutive days) alone or 100-400 mg/kg WR (Single dose) *O. sanctum* combination of the two and whole body was exposed to 4.5Gy gamma irradiation (RT). Metaphase plates were prepared from femur bone marrow on days 1, 2, 7 and 14 post-treatment and chromosomal aberrations were scored. Pretreatment with *O. sanctum* or WR individually resulted in a significant decrease in aberrant cells as well as different types of aberrations. The combination of the two further enhanced this effect; resulting in a two-fold increase in the protection factors (PF = 6.68) compared to 400 mg/kg WR alone.

*O. sanctum* extracts are known hepatoprotectors and found to ameliorate various hepatotoxin (paracetamol, CCl4 and hydrogen peroxide) induced injuries. Leaf extract of *O sanctum* reduced morphological alteration in HepG2 cell line and also increased cell viability in ethanol intoxicated hepatocytes. Both aqueous extract and steam distilled oil of *O. sanctum* were found to reduce elevated enzyme levels such as Aspartate amino transferase (AST) and Alanine amino transferase (ALT) in isolated hepatocytes culture also showed marked
reduction in fatty degeneration of rat liver on histopathological examination (Chattopadhay et al., 1992) Administration of combination of *O. sanctum* aqueous leaf extract and gentamicin, significantly prevented rise in levels of serum creatinine and blood urea when compared to the gentamicin only treated group in rats. Leaves and seeds of *O. sanctum* plant have been reported to reduce blood and urinary uric acid level in albino rabbits and also possessed diuretic property (Muglikar et al., 2004). *O. sanctum* leaf was also found to protect against cardiac damage by reducing levels of lipid peroxidation and strengthening antioxidative defense system. *O. sanctum* leaf extract prevents stress induced dendritic deficiency in hippocampal neurons in albino rats. In Ayurvedic medicine, *O sanctum* has therapeutic potential either alone or in combination with other plants in various clinical conditions like eye disorders (glaucoma, cataract, & chronic conjunctivitis), catalepsy, snake and scorpion bites. Samson et al. (2007) showed protective effect of *O. sanctum* extract on noise-induced oxidative stress in various regions of brain. The protective effect was majorly due to the polyphenols of *O. sanctum* extract possessing antioxidative effect.

**Significance of the present work:**

The principle objective of the present work is to evaluate subchronic toxicity in mice liver and provide more in depth scientific database on butylpraben exerted toxicity on vital organ. As Indian herb *O. sanctum* is having long history of medicinal usage is selected as an antidote to mitigate butylparaben induced toxicities. Though it’s known pharmacological potencies standardization of various extracts of the herb as well as detailed investigation of their phytochemical constituents as well as antioxidative potency majorly responsible for its therapeutic potency needs to be evaluated. Present work provides good information about hepatoprotective efficacy of *O. sanctum* extract.
against butylparaben toxicity which can be used further for pharmaceutical preparation. Interactive property of plant phytochemicals are one of the potent mechanism for remediation of various toxicities induced by xenobiotics. An attempt was made to evaluate interaction between Luteolin (one of the active component of *O. sanctum* extract) and butylparaben under *in vitro* conditions to provide the necessary base for the mechanism involved in protective effect of plant extract.