Xenoestrogens are a group of many different synthetic and naturally occurring chemicals, foreign to the body but once ingested or absorbed can mimic the behavior of natural hormones (Pelekanou et al., 2011) and attach to estrogen receptor sites of human beings and animals (Chen et al., 2001). Xenoestrogens, also known as endocrine disrupting chemicals (EDCs), could exert estrogen dominance symptoms (EDC) characterized by excess estrogen resulting in alteration in sexual development and menstrual cycle, formation of breast tumors in females and reduce fertility, erectile dysfunction, testicular and prostate cancers, alterations in pituitary and thyroid glands and development of secondary sexual characteristics in males (Rogan et al., 2003). Burden of EDCs to humans 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.

Molecules defined as endocrine disruptors constitute an extremely heterogeneous group and include synthetic chemicals used as industrial solvents/lubricants and their by-products (polychlorinated biphenyls, polybrominated biphenyls, dioxins), plastics (bisphenol A), plasticisers (phthalates), pesticides [methoxychlor, chlorpyrifos, dichlorodiphenyl-trichloroethane (DDT)], fungicides (vinclozolin) and pharmaceutical agents (diethylstilbestrol) (Phillips and Foster, 2008). Natural chemicals found in human and animal food (phytoestrogens such as genistein, coumestrol, etc.) also acts as endocrine disruptors (Abaci et al., 2009). Many food contaminants (pesticides, heavy metals, bisphenol A) are such endocrine disruptors which are known to interfere with the endocrine system and can affect development and/or reproduction in many species even at low doses (Maffini et al., 2006). These substances, which are generally thought to have relatively low binding affinity to estrogen receptors (ERs), are widely consumed...
and are components of infant formula (Dickerson and Gore, 2007). Reports had proved cumulative toxicity of these chemicals as they get incorporated in food chain and readily absorbed and accumulate in fat tissues of body. From these phthalates and bisphenol A, are frequently used in daily life, are commonly found xenoestrogens. Phthalates used in plastic commodities, were found to possess estogenic activity and cause cancer, developmental and sex-hormonal abnormalities and can cause infertility (Fisher, 2004; Barrett, 2005). Bisphenol A (BPA) widely used in polycarbonated plastic items leaches out from the plastics into foods. It also exerts strong estogenic activity in various animal models (Hewitt and Korach, 2011). Research revealed that 2-5 parts per billion (ppb) of bisphenol A is enough to cause breast cancer cell proliferation. It is selected for evaluation because of its broad-spectrum of use indicating the potential for widespread human exposure.

**Bisphenol A**

The use of plastics has become one of the defining characteristic of modern life. But many of the plastic products people use everyday contain components that can prove harmful to human health and the environment. One such component is a chemical called bisphenol A (Figure 1.1). Bisphenol A is one of the most widely used synthetic chemical in the World and is major components of plastic articles. Since 1940, the chemical BPA has been used to make plastics. It is currently produced in enormous quantities throughout the World to manufacture polycarbonate plastic and a constituent of epoxy and polystyrene resins (Ranjit et al., 2010). Additionally, it has been estimated that every year over 100 tons of BPA are released into the atmosphere (Vandenberg et al., 2009). Before it is used as plastics, in 1930, scientists discovered BPA as an artificial estrogen and its estogenic effect was used to enhance the rapid growth of cattle and poultry to promote industry profit (Breast Cancer Fund, 2008 a, b). Acting as an endocrine disruptor, BPA may interfere with endocrine transduction mechanisms at very low
doses and the exposure to this contaminant has been correlated with a wide variety of adverse health effects in both males and females including birth defects, reproductive, developmental, metabolic, immune, and neurobehavioral disorders (Rochester, 2013).

**Physicochemical characteristics:**

Bisphenol A is colorless white solid (melting point is 158°C) crystals or flakes with a mild phenolic odour under ambient conditions that is produced by the acid or alkaline catalyzed condensation of phenol and epoxy resins (Table 1.1). It is a single hydrocarbon molecule that binds with other molecules to form polymers, such as polystyrene and polycarbonates (Le *et al.*, 2008). Bisphenol A is generally considered to be a moderately hydrophobic compound with a slight polarity due to two hydroxyl groups. It is soluble in acetic acid and very soluble in ethanol, benzene and diethyl ether (Lide, 2004). Bisphenol A is generally considered to be fairly soluble in water, with a solubility of 300 gm/m³ at 25°C. Bisphenol A has a relatively high boiling point (220°C) and low vapour pressure (5 x 10⁻⁶ Pa at 25°C).

Bisphenol A has measurable vapor pressures, aqueous solubilities and octanol – water partition coefficients are expected to partition some extent to all available environmental phases. Phenyl ring containing bisphenol A lacks functional ionic group making it highly lipophilic and easily detectable compounds in UV detectors.

**Why bisphenol A is ideal plasticizer?**

It has high impact strength, hardness, toughness and transparency. Bisphenol A is ideal plasticizer and acts as a shatter-resistant. It is light in weight. It has durability, high modulus of elasticity and high melting point. It is characterized by good electrical insulation properties and enhanced weather and radiation resistance. It is resistant to temperatures between about 40°C to 145°C and resistance to many acids and oils (Staples *et al.*, 1998).
Where is it in the environment?

As the use of this plastic products has strongly increased in the last years, BPA is today one of the highest volume chemical produced worldwide with a total of 2.8 million metric tons manufactured in 2002 that increased to 5.5 million metric tons in 2011 (Rochester, 2013). A large pool of data has shown that BPA is mainly used for the production of polycarbonate plastics, epoxy resins and non-polymer additives (Kuruto-Niwa et al., 2007; Grumetto et al., 2008).

Polycarbonate plastics can be found in construction materials for building, optical storage media such as CDs, DVDs, Blu-Ray and other discs as well as car parts (transparent plastic parts) such as reflectors. Cell phones, eyeglass lenses, computer housing, water boilers, medical and sports equipments as well as food containers are also manufactured with polycarbonate plastics. Further, it is used to make safety glass, parts for plugs, switches, electronic devices, coffee machines, autoclavable flasks, reusable drinking bottles, baby bottles and plastic cups (Goodson et al., 2004; Munguia-Lopez et al., 2005; Maragou et al., 2008).

Epoxy resins are predominantly used in the form of resin adhesives, coating resins, varnishes, beverage cans and food cans as inner coatings, metal jar lids, printed circuit boards in electronic products, adhesives, inner coating; for decontamination of drinking water containers, water tanks and pipes, food and beverage cans, syringes and powder coatings (Vandenberg et al., 2007; Vogel, 2009).

Bisphenol A is also used as non-polymer additives for coating thermal paper, color developers, carbonless copy paper, tickets, receipts, in the production and processing of polyvinylchloride, in brake fluids, flame retardant, dental fillings, sealing materials and prosthesis (Vandenberg et al., 2007; patisaul and Polaton, 2008).

Due to the broad spectrum of use of BPA to manufacture products used in many applications, it has been speculated that human exposures to BPA may be widespread and it has been postulated that these exposures may reach high levels (Vom Saal and Hughes, 2005;
Vandenberg et al., 2007). So BPA has been listed as one of chemicals subjected to reporting requirements under the section 313 of Emergency Planning and Community Right-to-know Act (EPCRA) also known as Tittle III of the Superfund Amendments and Reauthorization ACT of 1986 (SARA) (Bishop, 2000).

Both the US National Toxicology Program (NTP, 2008) and the European Union (EU) (European Union Risk Assessment Report, 2010) have recently released lengthy reports devoted to BPA which reference hundreds of studies on BPA’s potential hazard and exposure routes. Bisphenol A is released to the environment both accidentally and through permitted discharges (Staple et al., 1998) and its widespread distribution has been a major cause of concern to regulatory agencies and others (Safe, 2000). A 6-month Canadian review of 150 research studies resulted in findings contradictory to the FDA. The Canadian Health Minister announced that there is concern for newborn and infants and that Canada is the first country to declare BPA a health hazard and take steps to ban it from products (Schmidt, 2008). Very recent publications have reported that BPA exposure may lead to diseases in new born babies resulting in ban on the production of baby bottles in Canada and subsequent manufacture of BPA – free plastics (Prins et al., 2007; Webster, 2008; salian et al., 2009).

**Exposure routes:**

Bisphenol A is mainly used in the manufacture of plastics and food cans liners (Willhite et al., 2008). Hence, it becomes an integral part of the food chain (Vandenberg et al., 2010; Huang et al., 2011). Food is acknowledged to be a main source of exposure to BPA as a consequence of BPA migration from food containers (Fernandez et al., 2007). Studies have shown that exposure occurs because when BPA molecules are polymerized; they are linked by ester bonds that are subjected to hydrolysis, which is accelerated as temperature increases and in response to contact with acidic or basic substances. The consequence is that as polycarbonate products are repeatedly washed, or polycarbonate plastics or metal cans are exposed to heat and/or basic conditions,
significant leaching of BPA due to hydrolysis of the ester bond occurs and/or because of incomplete polymerization residual BPA may leach from the epoxy resin and has the potential to contaminate stored foods (Vom Saal and Hughes, 2005; Carlwile et al., 2009).

Bisphenol A migration can be affected by heating time, temperature, storage time and many other factors (Kang and Kondo, 2003; Munguia-lopez and Soto-Valdez et al., 2005). Kawamura et al. (2001) reported leaching of BPA from cans into water following heating at 120°C for 10 and 60 min were 64 and 87 ng/ml, respectively, for cans from company A and 99 and 166 ng/ml, respectively, for cans from company B. Yoshida et al. (2001) reported that the concentration of BPA in the solid portion of canned vegetables was higher than that in the aqueous portion. These investigators found that BPA leached from the cans into the aqueous portion and migrated to the solid portion during storage. BPA concentrations in canned beverages ranged from <0.02 µg/L to 8.10 µg/L (Geens et al., 2010).

A study of Arizono’s group reported that BPA concentrations eluted from new and used polycarbonate baby bottles were below 1.0–3.5 ppb and below 1.0–6.5 ppb, respectively, but were 10–28 ppb from used and scratched bottles. Similar results were obtained for new and old polycarbonate cages. Similarly, Brede et al. (2003) found that mean BPA levels in the first (new baby bottles), second (51 days of use) and third tests (169 days of use) were 0.2, 8.4 and 6.7 gm/dm, respectively. This means that BPA can migrate from plastics after washing and sterilization in alkaline solutions or in hot water. Many studies have also examined BPA levels leaching from epoxy resins lining cans to specific foods. Bisphenol A has been detected in canned pet foods, vegetables, fish and infant formula (kang and Kondo, 2002; Kuo and Ding, 2004; Munguia – Lopez et al., 2005).

Krishnan et al. (1993) has found that autoclaving cell culture media in polycarbonate flasks led to the release of an unknown estrogenic substance. Using NMR and mass spectrometry, it was determined that the flasks were leaching BPA. At that time, Krishnan et al. (1993)
speculated that these results could impact other scientific experiments using media autoclaved in polycarbonate flasks. Subsequent studies have examined leaching from polycarbonate baby bottles using a variety of methods including High performance liquid chromatography (HPLC), Liquid chromatography – Mass Spectrometry (LC–MS), and Gas chromatography – Mass Spectrometry (GC–MS). Alternatively, Brede et al. (2003) found that rounds in a dishwashing machine, boiling water and brushing led to significantly higher concentrations of BPA leaching into water. Based on these measured levels of leaching, average dietary exposure to BPA was estimated for infants from birth through 3 months of age, the period when infants consume exclusively liquid foods (Wong et al., 2005); are exposed to the highest levels of BPA (24 μg/kg bw/day).

Several resin-based monomers are used in dentistry as preventative sealants, adhesives and restorative materials. Since the 1960’s, BPA diglycidyl methacrylate has been used as a component of many dental restorative materials. Small quantities of unreacted monomers have been shown to leach from polymerized dental materials and the potential exists for either residual BPA carried over from the manufacture of these monomers or from biological breakdown of the leached monomers to BPA in vivo. In a study of 18 adults, Olea et al. (1996) applied approximately 50 mg total of sealant to 12 molars. Total saliva was collected continuously for 1 h before and 1 h after the application procedure. After the treatment, all samples were found to contain variable amounts of BPA, ranging from 3.3 to 30.0 μg/ml saliva.

Exposure from thermal paper can occur through oral exposure from receipt to fingers and then from fingers to mouth or food. A recent study by Biedermann et al. (2010) revealed that 11 out of 13 thermal printing papers collected in Switzerland contained between 8 and 17 mg BPA/gm paper with an average of 13.3 mg/gm. This means that the total mass of BPA on a receipt is 250–1000 times higher than the amount of BPA typically found in a can of food or baby formula, as well as the amount that leaches from a BPA- based plastic
baby bottle (Environmental Working Group, 2010). The hypothesis of dermal absorption of BPA after contact with thermal paper becomes more likely since Zalko et al. (2011) observed that viable skin efficiently absorbs BPA in short-term cultures.

Studies have also revealed that BPA can be measured in humans in serum, urine, amniotic fluid, follicular fluid, placental tissue, and umbilical cord blood. In some cases, the levels of total BPA (free and conjugated) in human blood and other fluids are higher than the concentrations that have been reported to stimulate a number of molecular endpoints in cell culture in vitro (Wetherill et al., 2007). Several studies have examined BPA levels in serum from pregnant women, umbilical cord blood, and fetal plasma (Yamada et al., 2002; Tan and Ali Mahd, 2003). The results from these studies indicate that BPA crosses the maternal-fetal placental barrier.

In one report (Ikezuki et al., 2002), the human maternal sera showed average BPA at 1.4–2.4 ng/ml concentrations, whereas the 15–18-week fetal amniotic fluid showed higher levels averaging 8.3 ng/ml. Serum BPA concentrations, detected using ELISA, were significantly higher in 11 healthy men compared to 14 healthy women (Takeuchi and Tsutsumi, 2002). Since 1999 (Sajiki et al., 1999), more than a dozen studies using a variety of different analytical techniques have measured unconjugated BPA concentrations in human serum at levels ranging from 0.2 to 20 ng/ml serum and exceeding 100 ng/gm in one study of placental tissue. These studies have examined blood from both men and women from several countries and at different ages. The techniques used to measure BPA in human serum have included GC–MS, HPLC and derivatization with different chemical agents followed by GC, and ELISA. A limited number of studies have examined BPA levels in other bodily fluids such as follicular fluid (Ikenzuki et al., 2002) and semen (Inoue et al., 2002). Bisphenol A levels measured in follicular fluid by ELISA showed an average of 2.0 ng/ml (Ikenzuki et al., 2002). Nevertheless, the detection of BPA in human follicular fluid is of particular concern because of the report that orally administered low-dose BPA in adult mice causes congression failure and aneuploidy in oocytes (Hunt et al., 2003).
Additional and important consideration for the health of the developing neonate is potential BPA exposure from breast milk. Because BPA is a somewhat lipophilic compound, it may partition into fat and breast milk. Using HPLC with fluorescence detector, Sun et al. (2004) found BPA in the breast milk of all 23 healthy women they examined, at a range of 0.28–0.97 ng/ml with a mean concentration of 0.61 ng/ml (Sun et al., 2004). Another study of interest reported BPA concentrations in human colostrum, breast milk produced within the first 3 days after giving birth (Kurutto-Niwa et al., 2007).

Braun et al. (2011a) found significantly higher urinary BPA levels in women who consumed at least once a day canned vegetables as compared to those who consumed no canned vegetables at all. Calafat et al. (2005) reported that BPA was detected in 95% of urine samples (≥0.1 ng/ml) collected from 394 adults with different ages, places of residence, or sex. Several studies suggested that high urinary BPA levels are positively correlated with the consumption of canned foods (Matsumoto et al., 2003). This study reported average levels of 1.63 and 1.12 ng/ml of total BPA in male and female urine respectively. Similar results were also obtained in a study of 90 young girls; BPA was detected in 94% of samples (Wolff et al., 2007). These results mean that the environment (aquatic environment, air and soil) can also be one of the important source of human BPA exposure.

Bisphenol A can be found in wastewater from factories that produce it because it is not completely removed during wastewater treatment. This wastewater containing BPA can be a source of contamination of the aquatic environment (Furhacker et al., 2000; Korner et al., 2000). Recently, high levels of BPA were identified in leachates from a waste landfill (Yamamoto et al., 2001; Filho et al., 2003). Yamamoto et al. (2001) reported that the levels of BPA in the leachates of a hazardous waste landfill ranged from 1.3 to 17,200 ng/ml. Bisphenol A in river waters can be degraded under aerobic conditions (Kang and Kondo, 2002a), but not under anaerobic conditions (Kang and Kondo, 2002b). Bisphenol A was detected in all river samples in concentrations
ranging from 500 pg/L to 16 ng/L; BPA levels in drinking water ranges from 300 pg/L to 2 ng/L. A recent study reported that levels of BPA in fish varied from 2 to 75 ng/gm dry weight (DW) in the liver and from 1 to 11 ng/gm dry weight in the muscle, but ranged from <0.01 to 0.33 ng/ml in the surface water. Interestingly, BPA can persist longer in seawater than in river water without any degradation (about 30 days) and the possibility of BPA contamination in a marine organism can be higher than that in a freshwater organism (Ying and Kookana, 2003; Kang and Kondo, 2005).

Air and dust levels of BPA serve as another source for human exposure. Because of large amounts of BPA produced annually, it is plausible that BPA enters air particles during production at plastics manufacturing plants. Rudel et al. (2001) has reported that the concentrations of BPA ranged from 2 to 208 ng/m\(^3\) in three of seven air samples. In a survey of 120 homes for the presence of endocrine disrupting chemicals, Rudel et al. (2003) found BPA present in 86% of house dust samples at concentration ranging from 0.2 to 17.6 \(\mu\)g/gm. Bisphenol A was detected in air samples with an average level of 0.51 ng/m\(^3\).

Bisphenol A released to ground or surface water can be absorbed to soil or sediments. In fact the levels of BPA in sediments are higher than those in surface waters (Bolz et al., 2001; Fromme et al., 2002). From a study using C\(^{14}\)-BPA, Fent et al. (2003) reported that the half-life for BPA in soil is less than 3 days. However, BPA contamination in soil can be positively correlated with human densities because of an increase in BPA pollution by human wastes such as domestic and/or industrial wastes (Kawahata et al., 2004).

**Pharmacokinetics:**

Bisphenol A is highly lipophilic in nature having high n-octanol/water coefficient, which allows its easy penetration and retention by fatty tissues (Doerge and Fisher, 2010). The pharmacokinetics of BPA has been studied extensively in mice, rats and primates, including humans (Willhite et al., 2008). The current studies revealed that BPA readily and efficiently gets
absorbed through skin and gastrointestinal tract (Kurebayashi et al., 2002; Volkel et al., 2002) and undergoes extensive first-pass metabolism in the gut wall (Inoue et al., 2003) and in the liver (Inoue et al., 2001; Pritchett et al., 2002). During the first pass of metabolism, biotransformation of BPA to BPA-glucuronide, a metabolic process called glucuronidation, is carried out by enzymes primarily present in liver.

Glucuronidation makes BPA more soluble in water and therefore easier to eliminate in the urine and also minimizing its ability to interact with biological processes in the body. It is the major pathway of bisphenol A biotransformation in primates and in rats. Unconjugated parent (commonly referred as free) BPA is converted to other metabolites primarily BPA sulfate.

In controlled studies in human, only BPA-glucuronide was identified as a metabolite of BPA present in urine or blood (Volkel et al., 2002); however, the presence of BPA-sulfate has been reported in human urine samples (Ye et al., 2005) and the potential for formation of BPA-sulfate has been shown with in vitro studies on human hepatocytes (Pritchett et al., 2002). However BPA-glucuronide is also the major BPA metabolite formed in human hepatocytes (Pritchett et al., 2002; Kuester and Sipes, 2007), and sufficiently unique and stable to serve as a biomarker to assess BPA exposure. Formation of BPA conjugates is considered as a deactivation reaction, since both BPA-glucuronide and bisphenol A-sulfate are devoid of estrogenic activity (Matthews et al., 2001; Shimizu et al., 2002) whereas BPA has a low affinity to estrogen receptor.

There are evidences from laboratories rodents that very young animals metabolize BPA less efficiently than adult animals (Miyakoda et al., 2000; Matsumoto et al., 2002; Domoradzki et al., 2004). Neonatal rats have higher circulating concentration of free BPA in their blood compared to older animals given an equal exposure, presumably due to an underdeveloped ability to glucuronidate early in life (Domoradzki et al., 2004). However, a number of the enzymes involved in metabolizing BPA to BPA-sulfate in humans are known and have been shown to be active in fetal and neonatal life (Hines, 2008).
Elimination of BPA and its metabolites was examined in Sprague Dawley rats that were gavaged with BPA and C\textsuperscript{14} - BPA at 10 mg/kg bw (Domoradzki et al., 2003). Most of the radioactivity (65-78\%) was eliminated in feces. Elimination in urine accounted for 14-22\% of the dose, and considerable variability for urinary elimination among animals was evident by the large standard deviations, which were 50\% of means. The authors stated that BPA glucuronide represented 62-70\% of radioactivity in urine and BPA represented 19-23\% of radioactivity in urine. In feces, 83-89\% of radioactivity was represented by BPA and 2-3\% was represented by BPA glucuronide.

**Toxicological evaluation:**

Bisphenol A is part of xenoestrogenic organic endocrine-disrupting chemicals (EDC), which represent a class of industrial man-made substances able to bind estrogen receptors (ER) to increase estrogen-dependent gene transcription (D’cruz et al., 2012). In 1980s, the lowest-observable - adverse effect-level (LOAEL) for BPA was determined at 50 mg/kg bw/day, and the Environmental Protection Agency (EPA) calculated a “reference dose” of 50 mg/kg bw. Since that time, abundant scientific evidence have suggested that BPA can interfere with the endocrine signaling pathways at doses below the calculated safe dose after fetal, neonatal, perinatal and adult exposure.

Acting as an endocrine disruptor, BPA may interfere with endocrine transduction mechanisms at very low doses and the exposure to this contaminant has been correlated with a wide variety of adverse health effects in both males and females including birth defects, reproductive, developmental, metabolic, immune, and neurobehavioral disorders (Rochester, 2013). Epidemiological studies have highlighted the correlation between the increased levels of BPA in the environment and the incidence of hormone-related cancers including breast, prostate, ovarian and endometrium malignancies (Keri et al., 2007). Since 1993 several studies have
reported that BPA has estrogenic activity both in vitro and in vivo, hence the estrogen-like action was connected with its adverse effects on human health and the onset of hormone-dependent tumors.

Besides, BPA is able to bind the membrane-bound form of ER (mER) and the G protein coupled estrogen receptor GPR30/GPER (Alonso-Magdalena et al., 2012). In particular, the estrogen-like activity of BPA has been recently shown to occur through G-protein estrogen receptor (GPER) in both normal and neoplastic cells (Bouskine et al., 2009; Pupo et al., 2012). Although the action of BPA in cancer mainly mimics estrogen-like mechanisms, it may also act via the androgen receptor, the thyroid receptor, the peroxisome proliferator-activated receptor-γ and other endocrine signaling pathways (Vandenberg et al., 2013). Many studies have investigated the long-term impact of early BPA exposure during stages of tissue organization on the male (Watanabe et al. 2003; Prins et al. 2007) and the female reproductive tracts (Suzuki et al. 2002; Vandenberg et al. 2007), and behavior (Della Seta et al. 2005; Fujimoto et al. 2006).

**Human data:**

Li et al. (2010a) examined the effect of occupational BPA exposure on male reproductive function. The workers (n = 164) were exposed to mean air levels of 0.006 mg/m$^3$ of BPA, the highest levels being in packaging operations (geometric mean 0.016 mg/m$^3$), and their sexual function was evaluated using a standardized male sexual function inventory. Bisphenol A exposed workers reported higher levels of reduced sexual desire, erectile or ejaculation difficulty, and reduced satisfaction with their sex life. When sexual function among these workers was correlated with urinary BPA levels, a significant correlation between urinary BPA levels and self-reported sexual dysfunction was seen (Li, et al. 2010b). In their third study, Li et al. (2011) reported a statistically significant association between increasing urinary BPA levels and
decreasing sperm concentration, total sperm count, sperm vitality and motility among 218 men working in these same factories.

Cha et al. (2008) reported decreased testosterone levels and increased luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels among the 25 epoxy resin painters with increased urinary BPA levels (2.61 μg/gm creatinine vs. 1.38 μg/gm creatinine in controls).

Regarding developmental effects, Braun et al. (2009, 2011b) examined the relationship between gestational BPA exposure (measured as serial urinary BPA samples) and neurobehavioral effect in infants. An association between BPA levels and externalizing behaviour (aggression, hyperactivity) among 2-year old girl was noted. At the age of 3, the girls showed a more anxious and depressed behaviour and poorer emotional control and inhibition. An association between BPA exposure and lower birth weight, small for gestational age (SGA) infants and disturbed adipogenesis has also been suggested (Chou et al. 2011) but increased birth weight has been observed by Wolff et al. (2008).

Animal data:

Aikawa et al. (2004) reported that injection of BPA (50 μg/animal, about 15-20 mg/kg bw/day) for the first 5 days after birth resulted in a decrease in the percentage of moving sperm and an increase in the incidence of malformed sperm in the epididymides of mice at 10 weeks of age. Al-Hiyasat et al. (2002) reported that administration of 25 and 100 μg/kg bw/day doses of BPA to male Swiss mice resulted in a significant decrease in daily sperm production as well as a decrease in the number of sperm per mg epididymis, which was associated with a significantly lower proportion of pregnancies in the females. Salian et al. (2009) reported a significant increase in post implantation loss, decrease in litter size and decrease in sperm count and motility in the offspring of female Holzman strain rats dosed at 1.2 and 2.4 μg/kg bw of BPA by gavage.

Richter et al. (2007) investigated in vivo effects of low doses of BPA in laboratory rodents. In male rodents, BPA exposure leads to effects in brain, reproductive system and to
metabolic processes and in female rodents, it causes effects in immune system and also reproductive system. In many experimental studies, BPA has been shown to affect many reproductive indices, such as ovary, uterus and vaginal weights (indicating estrogenic action); egg shape; fertilization rate; number of live-born neonates per liter; distance between the genital pore and the anus in newborns (indicating the degree of masculinization or feminization of external genitalia); time of vaginal opening (indicating the start of female puberty); and onset of the estrous cycle (indicating sexual maturity) (Maffini et al., 2006; Richter et al., 2007; Cabaton et al., 2011; Nah et al., 2011).

Higher concentration of BPA administered to gestating dams (50-600 mg/kg bw/day) reduced body weight in growing Sprague-Dawley rat and CD-1 mouse pups (Tyl et al., 2002, 2008). Kim et al. (2001) investigated possible adverse effects in pregnancy and embryo - fetal development after oral maternal BPA exposure (0, 100, 300 and 1000 mg BPA/kg bw) to Sprague-Dawley rats. In the high and mid doses group, female rats showed an increased pregnancy failure, reduced maternal body weights, expansion and congestion of stomach and intestines, decreased body weights of male and female fetuses, increased fetal death, early resorption, reduced anogenital distance in males and reduced ossification. This study supports that prenatal BPA exposure has effects on the developmental toxicity in rats (Kim et al., 2001; Chapin et al., 2008).

Newbold et al. (2009) investigated effects of BPA in pregnant CD-1 mice that were treated with BPA on days 9 to 16 of gestation with concentrations between 0.1 - 1000 µg/kg bw/day. The reproductive tissues of their offspring were evaluated at the age of 18 months. The authors reported a significant increase of ovarian cysts in the 1 µg/kg BPA - treated group. They observed an increase in progressive proliferative lesions after BPA treatment. Negative effects on fertility and spermatogenesis of male rodent offsprings were shown after maternal BPA exposure
of environmentally relevant BPA doses. Perinatal BPA exposure showed effects on the male germ line. This was manifested as impairments in the fertility of the male offspring (Salian et al., 2011).

Male Long-Evans rats were fed 2.4 μg/kg bw/day dose of BPA from postnatal day (PND) 21-35. Bisphenol A suppressed serum LH (by 60%), testosterone (by 35%) and estradiol (by 30%). Treatment of Leydig cells (the cells in the testes that secrete testosterone) in culture with a 2.3 pg/ml (0.01 nM) dose of BPA resulted in a 25% decrease in testosterone synthesis associated with decreasing in the androgen-synthesizing enzymes 17α-hydroxylase and C17-20 lyase. Aromatase activity also decreased (Akingbemi et al., 2004).

Higher levels of urinary BPA have been correlated with cardiovascular diseases (Lang et al., 2008) and may be associated with an increased risk for miscarriages with abnormal embryonic karyotype (Sugiura-ogasawara et al., 2005). In utero exposure to environmentally relevant doses of BPA – induced morphological and functional alterations in the reproductive tract in mice that were revealed during adulthood. These findings included decreased weight of the vagina and endometrial lamina propria, increased proliferative activity of epithelial cells in the endometrial glands and increased expression of estrogen receptor α (ERα) and progesterone receptor (PR) in the luminal epithelium of the endometrium and subepithelial stroma (Markey et al., 2005).

Exposure to estrogens throughout life is the main known risk factor for breast cancer. The development of the mouse mammary gland is affected by perinatal exposure to environmentally relevant levels of BPA (Markey et al., 2001). Perinatal exposure to BPA decreases DNA synthesis in mammary gland epithelial cells at 10 days of age. By 6 months of age, the mammary glands showed a dramatic expansion of the ductal network with a significant increase in terminal ducts and alveolar structures relative to the control. The appearance of the mammary glands of these virgin mice resembled that of early pregnancy (Markey et al., 2001). In addition, mice and rats exposed perinatally to BPA showed altered patterns of estrous cyclicity in adulthood (Markey et al., 2003). Low dose effects of bisphenol A also determined that prenatal exposure in mice leads
to an enlarged prostate (Nagel et al., 1997). Also, in juvenile rats exposed to 25 or 250 µg/kg prenatally, the prostate showed altered development of the prostatic stroma, decreased AR expression and decreased secretion of prostatic acid phosphatase (Ramos et al., 2001). Wetherill and colleagues et al., (2002) first noted that BPA was able to alter the fate of prostate-cancer cell lines in vitro.

The marginal increases in leukemias in male and female rats, along with increases in the combined incidence of lymphomas and leukemias in male mice, suggest that BPA may be associated with increased cancers of the hematopoietic system. The fact that BPA is able to induce effects on cell cycle and apoptosis in acute myeloid leukemia (AML) model indicates that BPA actions can go beyond the endocrine interference. 3-hydroxybisphenol A (3-OHBPA) is a biological metabolite of BPA and has a high cytotoxicity (Nakagawa and Suzuki, 2001). The cytotoxicity becomes higher by oxidation of 3-OHBPA to its ortho-quinone. Bisphenol A-o-quinone has been reported to form adducts with DNA in vivo (Atkinson and Roy, 1995a) and in vitro (Atkinson and Roy, 1995b). Quinones are highly redox active molecules with their semiquinone radicles, leading to formation of ROS, including superoxide, hydrogen peroxide and hydroxyl radical (Bolton et al., 2000). Production of ROS could cause DNA oxidation and strand breaks, leading to a high cytotoxicity. The convincing demonstration of an environmental adverse effect was found in the laboratory accident whereby elution of BPA from plastic cages makes many abnormal oocytes possessing meiotic aneuploidy in female mice (Cohen, 2003). This effect resulting in the production of chromosomally abnormal eggs, was verified by the studies using female or pregnant mice (Susiarjo et al., 2007).

Bisphenol A and its brominated and chlorinated derivatives were the first environmental chemical shown to bind to the thyroid hormone receptor (TR) (Zoeller et al., 2005). It was demonstrated by Meerts et al. in 2001 that polybrominated BPA compounds have estrogenic activities and are able to bind to the ERα. Moriyama et al. (2002) who utilized rat liver nuclei that
expresses both the TRα and TRβ, showed that BPA binds to the TR and act as an antagonist on this receptor.

The majority of studies on bisphenol A have focused on their endocrine disrupting and potential adverse effects on the developing reproductive system. However, in comparison to reproductive organs, there is insufficient data available on the effect of bisphenol A on the vital organs of mammals. The liver is the major organ for the metabolism and detoxification of xenobiotics, including BPA (Knaak and Sullivan, 1966). It is also involved in most of the biochemical pathways for growth, fighting against diseases, nutrient supply, energy provision, secretion of bile, storage of vitamins and reproduction (Ahsan et al., 2009). Therefore, the liver could be largely exposed to BPA, and could be susceptible to lower doses, than other organs (Moon et al., 2012).

There are some reports indicating that high doses of BPA altered liver weight in mice and rats (Tyl et al., 2002; Tyl et al., 2008) and decreased the viability of rat hepatocytes (Nakagawa and Tayama, 2000). Kupffer cells (KCs) are the hepatic macrophages residing in the lumen of the liver sinusoids. On activation, KCs release various cytokines and play important role in the pathogenesis of various liver diseases (Wu et al., 2010). Kupffer cells have been implicated as the source of the inflammatory response, because they are known to produce proinflammatory cytokines, such as interleukin (IL)-1β and IL-6 when activated (Kopf et al., 2010). Increased levels of proinflammatory cytokines disturb the homeostasis of oxidants/anti-oxidants and DNA repair enzymes, all of which appear to be involved in BPA-associated inflammatory processes (Yongvanit et al., 2012). The production of these mediators leads to a second phase of liver injury, including endothelial cell adhesion molecules that mediate the adhesion and transmigration of neutrophils from the vascular space into the hepatic parenchyma (Colletti et al., 1996). These accumulations release oxidants and proteases that directly injure hepatocytes and vascular endothelial cells (Jaeschke and Smith, 1997).
Adult male ICR mice were injected i.p. for 5 days with 25 or 50 mg/kg bw/day BPA and tissues were examined 6 h after the last injection. Thiobarbituric acid reactive substances (TBARS) were measured as a lipid peroxidation indicator. Also glutathione content as well as activities of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and catalase was also studied. Oxidative stress reduces tissue levels of glutathione. There was a dose-related decrease in liver catalase, superoxide dismutase, glutathione peroxidase activities at 50 mg/kg bw/day bisphenol A suggesting that BPA produces an overproduction of hydrogen peroxide in the liver (Kabuto et al., 2003). Mathur et al. (2003) reported that BPA induces oxidative stress in the liver of rats by decreasing antioxidant enzymes. It has been reported that absorption of large amount of bisphenol A through skin causes extensive damage to liver, kidney and other vital organs in humans resulting in carcinogenesis (Suarez et al., 2000).

Oxidative stress results from an imbalance between radical generating and radical scavenging systems that is increased free radical production or reduced activity of antioxidant defence or both these phenomena. It has been estimated that the average person has around 10000-20000 free radicals attacking each body cell each day (Volko et al., 2006). Reactive oxygen species are dangerous, however when present in excess and can attack biological molecules such as lipids, proteins, enzymes, DNA and RNA leading to cell or tissue injury associated with degenerative diseases (Valentao et al., 2002). Oxidative stress has been implicated in various pathological conditions involving cardiovascular disease, cancer, neurological disorders, diabetes, reperfusion, aging and other diseases (Jenner, 2003; Dall-Donne et al., 2006). Although the mammalian body has certain defense mechanisms to combat and reduce oxidative damage induced by free radicals, a number of antioxidants are in charge to remove excessive ROS and to maintain a physiological redox balance (Halliwell and Gutteridge, 1985).

Epidemiological evidence indicates that the consumption of foodstuffs containing antioxidant phytonutrients - notably flavonoids and other polyphenolics - is advantageous for our
health (Pulido et al., 2000). Flavonoids and other plant phenolics, such as phenolic acids, tannins, lignans and lignin, are especially common in the leaves, flowering tissues, and woody parts of plants. The antioxidant activity of these phenolics is mainly due to their redox properties, which allow them to act as reducing agents or hydrogen-atom donors. Thus, natural antioxidants function as free-radical scavengers and chain breakers, complexers of pro-oxidant metal ions and quenchers of singlet-oxygen formation (Pratt, 1992).

The use of herbs and herbal teas as medicines has played an important role nearly in every culture on earth. Tea infusions are consumed by two thirds of the world’s population. Tea is for the most part of the world, simply considered a tasteful drink, but the scientific community has recently rediscovered the therapeutic potential of this beverage (Luczaj and Skrzydlewska, 2005). According to its processing, tea can be classified into four major forms, namely green, black, oolong tea and white tea. Green tea is prepared in such a way as to preclude the oxidation of green leaf polyphenols. During black tea production, oxidation is promoted so that most of these substances are oxidized. Oolong tea is a partially oxidized product. White tea is made from new growth buds and young leaves that have been steamed to inactivate polyphenol oxidation and then dried (Sharangi, 2009).

**Green tea:**

Tea is the nature’s treasure to the mankind. It is next to water as the most consumed beverage in the world (Gomikawa et al., 2008). China is credited for introducing green tea to the world. According to Chinese legend, in 2737 BC the Emperor Sheng Nung (the ‘Divine Healer’) discovered the healing power of tea leaves and passed this knowledge on to his subjects (Hara, 2001). Green tea is derived from the leaves of the plant *Camellia sinensis* (L.) Kuntze, which is an angiosperm dicot plant (Figure 1.2). Green tea is a member of the Theaceae family. It is an evergreen shrub with white flowers and is indigenous to Asia, but commercially grown in Africa,
Sri Lanka, Malaysia and Indonesia (Hicks, 2001). It is estimated that about 2.5 million tons of tea leaves are produced each year throughout the world, with 20% produced as green tea, which is mainly consumed in Asia, some parts of North Africa, the United States, and Europe (Chacko et al., 2010).

**Classification:**

- **Kingdom:** Plantae
- **Subkingdom:** Tracheobionta
- **Division:** Magnoliophyta
- **Superdivision:** Spermatophyta
- **Class:** Magnoliopsida
- **Subclass:** Dilleniidae
- **Order:** Theales
- **Family:** Theaceae
- **Genus:** Camellia L.
- **Species:** *Camellia sinensis* (L.) Kuntze

**Traditional uses:**

Green tea contains high levels of polyphenols which may have a number of positive health effects in the prevention of lifestyle-related diseases (Klaus et al., 2005). The regular consumption of green tea has long held value among traditional medical practices of promoting overall health (Sasazuki et al., 2004). Green tea is traditionally used as a stimulant, diuretic, to treat headaches, aching body parts, astringent, to improve health, treating flatulence, regulating body temperature, blood sugar, promoting digestion, improve mental processes and to improve life expectancy (Makay and Blumberg, 2002). The effectiveness of green tea in treating any type
of diarrhea and typhoid has been known in Asia since ancient times (Lu et al., 2003; Wu et al., 2003). In addition to all of these reported properties which have helped the recognition of green tea as a functional food by some authors (Ferrari and Torres, 2003), green tea is also currently used in the preparation of a variety of foods, pharmaceutical preparations dentifrices and cosmetics (Arburjai and Natsheh, 2003).

**Phytochemical constituents:**

The chemical composition of green tea is complex - proteins (15-20% dry weight), whose enzymes constitute an important fraction; amino acids (1-4% dry weight) such as theonine or 5-N-ethyl glutamine, glutamic acid, tryptophan, glycine, serine, aspartic acid, tyrosine, valine, leucine, threonine, arginine, and lysine; as well as carbohydrates (5-7% dry weight) such as cellulose, pectins, glucose, fructose, sucrose (Hou et al., 2005). The other compounds in green tea with interest for human health are fluoride, caffeine, minerals, trace elements like chromium and manganese (Powell et al., 1998; Cabrero et al., 2003; Hope et al., 2006). It contains trace amounts of lipids (linoleic acid, alpha linoleic acid), sterols (stigmasterol), vitamins (A, B, C, E, K), xanthine bases (caffeine, theophylline), pigments (chlorophyll, carotenoids), and volatile compounds (aldehyde, alcohol, esters, lactones, hydrocarbons) (Graham, 1992).

The active constituents in green tea extract are powerful antioxidants called polyphenols. It contains 20 times more powerful antioxidant activity than vitamin C and E (Tang et al., 2010; Prochazkova et al., 2011). Tea is reported to contain nearly 4000 bioactive compounds of which one third is contributed by polyphenols (Tariq et al., 2010). Among the polyphenols in tea, is a family of compounds called the flavonoids. Flavonoids are the basic phenolic compounds in green tea responsible for antioxidant activities such as neutralization of free radicals that are formed in the process of metabolism (Horzic et al., 2009). These flavonoids contain substances called catechins. Major catechins present in green tea are epicatechin (EC), epigallocatechin gallate
EGCG), epigallocatechins (EGC) and epicatechin gallate (ECG) (Figure 1.3). It also contains theoflavin, theoflavin-3-gallate, theoflavin-3, 3-bigallate as major part of biologically active substances.

The content of catechins present in green tea depends on how the leaves are processed before drying. A certain grade of fermentation and heating of tea leaves during the manufacturing process can result in polymerization of monopolyphenolic compounds such as the catechins, leading to conformational changes and thus modifying its properties. Other factors influencing catechins content are the geographical location and growing conditions (soil, climate, agricultural practices and fertilizers), the type of the infusion (eg. amount of the product used, brew time, temperature) (Hakim et al., 2000; Wu and Wei, 2002).

Wu and Wei in 2002 indicated that a cup of green tea (2.5 gm of green tea leaves/200 ml of water) may contain 90 mg of EGCG (Wu and Wei, 2002). Green tea is said to contain over four times the concentration of antioxidant catechins than black tea; about 70 mg catechins per 100 ml compared to 15 mg per 100 ml of black tea.

Epigallocatechin gallate is the most common polyphenol found in green tea, making up to 10% of its dry weight, comprising 60-70% of its total catechins. Many of green teas health promoting abilities are attributed to EGCG. Epigallocatechin gallate and other catechins exhibit strong antioxidant activities due to their single electron reducing potential. Free radicals are harmful, and reactive molecules are made unstable by this unpaired electron. They are involved in treatment of diseases from blood clots to cancer (Bonoli et al., 2003).

Epigallocatechin gallate (EGCG) is the single most studied catechins in relation to health contributing potential (Carmen et al., 2006). Epicatechin gallate (ECG) has poor levels of direct activity and cause severe disruption in the process of cell division in Methicillin resistant Staphylococcus aureus (MRSA) (Miller and Shah, 1999). Epicatechin (EC) may improve blood flow and has potential for cardiac health. Epigallo catechin (EGC), one of green tea polyphenols,
has been shown to inhibit growth of cancer cells. However, its mechanism of action is poorly known epigallocatechins strongly inhibit the growth of breast cancer cell lines (MCF-7 and MDA-MB-321) but not that of normal breast epithelial cells (David et al., 2002). Green tea extracts containing polyphenols have biological activities including modulation of key signal transduction pathways; however the possible significance of these activities in inhibition of carcinogenesis in vivo depends on the bioavailability of polyphenols (Xu et al., 2011).

The yellow color in green tea infusion is mainly determined by the water soluble flavonols (1.3 to 1.5% of the tea leaves dry weight), which include kaempferol, quercetin, isoquercetin, myricetin, myricitrin, rutin, kaempferitrin, etc and flavones (0.02% of the tea leaves in dry weight) which include apigenin, isovitexin, vitexin, saponarin, vicenin-2, etc as well as their glycosides; besides the water soluble anthocyanins (Chaturvedula and prakash, 2011). Other polyphenols present in green tea are flavanols and their glycosides, as chlorogenic acid, coumarylquinic acid. Amino acid degradation is involved in the biogenesis of the tea aroma (Balentine, 1997). Chlorophyll, carotenoids, lipids and volatile compounds are not major constituents in a tea brew but they also play an important role in the development of the aroma (Hara et al., 1995).

Metal analysis of green tea reveals that it is rich source of mineral elements which are essential for health like zinc, manganese, iron, magnesium, silver, copper, titanium, aluminium, bromium, sodium, potassium as well as nickel, chromium and phosphorus (Anna et al., 2005). These metal ions promote the antioxidant property of green tea. The concentration of non-toxic metals like Ag, Na, Cr in green tea lies within the acceptable daily intake (Arifa and Rabia, 2011).
**Pharmacological properties:**

Green tea not only captures the taste, aroma and color of spring, but delivers its qualities along with the highest concentration of beneficial phytonutrients and the least caffeine of all the teas. Its secret lies with its rich source of catechins (polyphenols) which possess powerful antioxidant property. Green tea has gained lot of importance in the field of phytochemistry because of its various pharmacological and biological activities such as antibacterial and antiviral as well as anticarcinogenic and antimutagenic properties (Figure 1.4) (Archana and Jayanthi, 2011).

Scavenging effect of lipid free-radicals (one antioxidant property) of polyphenols in green tea extracts can be clearly observed in experiments (Li and Jiang, 2010). The ability of GTP in green tea extracts to eliminate lipid-derived free radicals is noticeably stronger (almost 50 times) than that of *Ginkgo biloba* extracts (Li and Jiang, 2010). Further investigations indicate that the boosting level of SOD and GPx may account for the inhibitory effect of green tea catechins (GTC) against lipid peroxidation (rancidification) (Monobe *et al*., 2010). *In vivo* studies showed that green tea catechins increase total plasma antioxidant activity (Yokozawa *et al*., 2002; Skrzydlewska *et al*., 2002a).

Intake of green tea extracts also increases the activity of SOD in serum and the expression of catalase in the aorta; these enzymes are implicated in cellular protection against reactive oxygen species (Skrzydlewska *et al*., 2002a; Negishi *et al*., 2004). This action is combined with direct action on oxygen species by a decrease in the nitric oxide plasma concentration (Yokozawa *et al*., 1999). Malondialdehyde, a marker of oxidative stress, also decreases after green tea intake (Yokozawa *et al*., 2002). Since catechins can act as antioxidants *in vitro*, they might prevent the oxidation of other antioxidants, such as vitamin E. The higher antioxidant activity of green tea makes it more beneficial in protecting the body from oxidative damage due to free radicals. It is appeared that these antioxidants slow or halt the initiation of cancer, heart
disease, suppresses immune function and accelerated aging (Hamilton-Miller, 2001).

Epigallocatechin gallate is the most potent one and has also been found to outperform vitamin C and β carotene 10 times in scavenging the allyl peroxyl radical. However, at the same time evidences in a study suggests a reverse correlation between the amount of phenolic compound in green tea and its antioxidant potentials i.e., the quantity of these phenolic compounds is not always correlated with its quality (Armoskaite et al., 2011). Sano et al. (1995) reported the inhibitory effects of green tea leaves against tert-butyl hydroperoxide-induced lipid peroxidation, and a similar antioxidant effect on the kidney was observed after oral administration of the major tea polyphenol EGCG. Green tea catechins have the potential to affect absorption and metabolism of ions because flavonoids interact with a variety of metal ions (Mira et al., 2002).

Green tea extract exhibited a significant protective action on liver evident by a reduction in elevation levels of serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) are in tamoxifen, azathioprine and streptozotocin - induced hepatotoxicity (EI-Beshbishy, 2005; EI-Beshbishy et al., 2010; Abolfathi et al., 2012). The long-term consumption of green tea has been reported to greatly reduce the risk of liver injury (Jin et al., 2008). Previous reports shown that green tea consumption has protective and beneficial effects in cirrhotic conditions caused by the administration of CCl₄ in experimental animals (Shahid et al., 2013). The hepatoprotective effect of green tea is due to its potential antioxidant effects and oxidative stress reducing ability. The antioxidant and prooxidant activities are performed by scavenging ROS via enzymatic and non-enzymatic reactions by polyphenolic compounds in cells (Pyo et al., 2004; Kandemir et al., 2009).

Green tea increases the biliary flow and bile, helps to eliminate the bile salts, fats and toxins from the body. Green tea enhanced the synthesis of total protein and albumin which accelerates the regeneration process and the protection of liver cells. Studies using animal models
show that green tea catechins provide some protection against degenerative diseases. Some studies indicated that green tea has an antiproliferative activity on hepatoma cells and a hypolipidemic activity in hepatoma-treated rats, as well as the prevention of hepatotoxicity (Vanessa and Gary, 2004).

In the last ten years, cancer preventive effect of green tea have been widely supported by epidemiological, cell culture, animal and clinical studies. Extracts of green tea and green tea polyphenols have exhibited inhibitory effects against the formation and development of tumors at different organ sites in animals. These include animal models for skin, lung, oral cavity, oesophagus, stomach, intestine, colon, liver, pancreas, bladder, mammary gland, and prostate cancers (Yang et al., 2010). Green tea can inhibit tumorigenesis during the initiation, promotion and progression stages in animal models of carcinogenesis (Lambert et al., 2010). Green tea contains higher concentrations of monomeric polyphenols, which affect numerous intracellular signalling pathways involved in prostate cancer (CaP) and breast cancer development. The majority of *in vitro* cell culture, *in vivo* animal, and clinical intervention studies provided strong evidences supporting a chemopreventive effect of green tea extract (Henning et al., 2011; Wu and Butler, 2011). Yuan (2011) studied that consumption of green tea, DNA damage caused by smoking was decreased, cell growth was inhibited and found that it can block the cigarette-induced increase in sister chromatid exchange frequency.

Several humans and animal based studies suggested that green tea and its flavonoids block thromboxane and appears to be their natural inhibitor which significantly reduces the risk of stroke and coronary heart disease and also have anti-diabetic effects (Wu et al., 2004; Iso et al., 2006; Balasuriya and Rupasinghe, 2011). Green tea has recently become the latest weapon in fighting over weight conditions. Green tea catechins enhance exercise - induced abdominal fat loss in overweight and obese adults (Maki et al., 2009). The consumption of green tea extract associated with a significant reduction in total and low density lipoprotein (LDL) cholesterol
levels and also reduced adipocytes differentiations, proliferation and lipogenesis (Wolfram et al., 2006; Kim et al., 2011).

Leaves extract of green tea indicates the presence of potent antibacterial activity. The green tea polyphenols have been found to be inhibitory against *Escherichia coli, Enterococcus faecalis, Salmonella typhi, Staphylococcus aureus* and *Pseudomonas sp.* (Archana and Jayanthi, 2011). In a similar study, antibacterial activity of the water and ethanolic extracts of green tea was found against *Streptococcus mutans* and *Lactobacillus acidophilus* (Arifa and Rabia, 2011). Research analysis show that green tea also blocks viral attachment and entry into cells. In a preliminary study, AIDS/HIV prevention research has shown that green antioxidant catechins especially EGCG have anti-HIV activity in each step of the HIV life cycle (Yamaguchi et al., 2002). Effects of green tea catechins and theanine are effective in preventing influenza as well as against the *Herpes simplex* virus (Matsamoto et al., 2011). Weber et al. (2003) observed that adenovirus infection is inhibited *in vitro* by green tea catechins. Recent studies demonstrated beneficial effects of green tea in inflammatory allergy. It has been studied that green tea has immunoregulatory effects on human IgE responses *in vitro*. It suppresses the B cells production of IgE without inducing apoptosis (Ehab et al., 2010).

Green tea consumption has also been associated with increased bone mineral density, and it has been identified as an independent factor protecting against the risk of hip fractures; this effect was considered independent of smoking status, hormone replacement therapy, coffee drinking, and the addition of milk to tea (Muraki et al., 2003). Green tea strengthens the immune system action because it protects it against oxidants and radicals. Recent studies suggested that GTPs might protect against Parkinson's and Alzheimer's diseases and other neurodegenerative diseases (Reznichenko et al., 2010; Okello et al., 2011). Studies have demonstrated GTP neuroprotectant activity in cell cultures and animal models, such as the prevention of neurotoxin-
induced cell injury (Pan et al., 2003). The renal failure is also a condition where green tea has shown to have protective effect (Mowafy et al., 2011).

Among oral diseases like dental caries, periodontal disease, and tooth loss, dental caries is a multifactorial infectious disease in which nutrition, microbiological infection, and host response play important roles. *Streptococcus mutans* is mainly responsible for causing dental caries. Green tea has been proved to have anti-*Streptococcus mutans* activity (Naderi et al., 2011). It has been found that routine intake of green tea may also help in fighting against these oral diseases. It promotes healthy teeth and gums. Green tea “catechins” are among a number of antioxidants such as vitamin C, vitamin E, lutein, and zeaxanthin thought capable of protecting the eye (Chu et al. 2010). Yang et al. (2007) concluded that the administration of EGCG increased the cell count and the cell activity after UV irradiation in cultured human retinal pigment epithelial cells. In an animal trial it was found that green tea may protect against the formation of cataracts (Gupta et al., 2009). Green tea may protect against age related macular degeneration and glaucoma (Zhang and Osborne, 2006).

Green tea is effective in the area of skin care, particularly in alleviating the symptoms of acne and eczema. The *in vitro* and *in vivo* animal and human studies have suggested that GTP are photo protective in nature, and can be used as pharmacological agents for the prevention of solar UVB light-induced skin disorders including photo aging, melanoma and non-melanoma skin cancers (Katiyar, 2003; Lee et al., 2004). So far, the benefit of green tea is known only to the body. But, green tea polyphenols are only recently understood as positive factors in hair growth and follicle health (Patil et al., 2010). They possess some of the mechanisms of action as including inhibition of apoptosis (programmed cell death), radioprotection of follicle cells, profound antioxidant activity, and potential follicular inhibition of TGF-beta (Charles et al., 2008).
Liv. 52

Traditional medicines are effective in certain disorders and are based on experience in the use of plant products in amelioration of common diseases. Liv. 52 is an indigenous multiherbal formulation, which contain extracts of seven herbs and widely used in various hepatic disorders (Poli et al., 1985; Dhumal et al., 1989). Liv. 52 contains time tested ayurvedic herbs and minerals that protect and heal the liver and has been subjected to over 300 clinical trials and research studies to validate its efficacy. It detoxifies and protects liver cells from harmful toxins and supports the liver’s ability to regenerate itself. Liv. 52 aids in the effective metabolism of drugs by maintaining levels of cytochrome P450. It also helps to regulate the liver enzymes and prevents fat from infiltrating the liver. Liv. 52 facilitate rapid elimination of acetaldehyde, the toxic intermediate of alcohol metabolism and detoxify liver cells. A number of research article have been published in its favor including experimental as well as clinical studies (Mandal and Roy, 1983; Dange, 2010).

Liv. 52 has been reported to have protective effects in paracetamol, anticancer drugs, antibiotics, oral contraceptives, alcohol, allyl alcohol and carbon tetra chloride. Liv. 52 enhances the activity of microsomal drug metabolizing enzymes. Further studies revealed that Liv. 52 protects mice liver against cadmium intoxicification (Bardhan et al., 1985; Rathor and Saraswat, 1986). Goel and Dhawan (1991) have reported efficacy of Liv. 52 in preventing the structural integrity of liver. The oral administration of Liv. 52 reported to prevent liver from carbon tetrachloride (Kataria and Singh, 1997). Hepatoprotective and anti-inflammatory effects of some of the individual ingredients of Liv. 52 is reported in literature (De Silva et al., 2003). The antioxidant effect and resultant hepatoprotective ability of Liv. 52 may be attributed to flavonoids, α and β – carotenes, Vitamin A and C present in the multiherbal preparation (Chopra and Singh, 1994), which explains its ability to reduce the levels of LPO and restore the antioxidant status.
SCOPE OF THE THESIS:

- This thesis embodies the study regarding the toxicity of bisphenol A in liver of mice. Activities of liver marker enzymes, the extent of lipid peroxidation, alteration in enzymatic and non-enzymatic antioxidant, changes in levels of important constituents like protein, DNA and RNA and other relevant biochemical parameters as well as histopathological examinations were taken onto consideration in this study.

- As medicinal herb green tea, having long history of herbal usage, is selected as an antidote to mitigate bisphenol A – induced toxicities. The antioxidative potential of the plant extract has been ascertained using superoxide dismutase, nitrous oxide, hydroxyl radical and reducing power scavenging assay.

- The *in vitro* hepatoprotective potential of green tea extracts has been analysed against bisphenol A – induced toxicity and also showed *in silico* interaction with the proteins of erythrocytes.

- The mitigatory effects of the plant extracts on bisphenol A – induced toxicity were determined biochemically and histopathologically. The hepatoprotective activity of the plant extracts was compared with the standard polyherbal drug Liv. 52.

- The liver protecting property of all the tested compounds has been compared on the basis of their hepatoprotective index (H).

- The results were statistically analysed and relevance of the present results are discussed with reference to recent development.
AIMS AND OBJECTIVES

The aims and objectives of the present study was to evaluate:

(a) *In vivo* bisphenol A toxicity in liver of mice

(b) Phytochemical analysis of green tea extract

(c) Bisphenol A toxicity in some *in vitro* and *in silico* models and its amelioration

(d) Possible *in vivo* mitigatory effects of green tea extract against bisphenol A – induced toxicity.

In a nutshell, the present study was an attempt to understand the toxicity of bisphenol A on vital organ (Liver) and its possible amelioration by green tea extract.
Hypotheses tested

The basic questions which led to the commencement of this piece of research was revolving around the toxicity of bisphenol A on the vital organ (Liver). The following hypotheses were tested in this proposed work which was based primarily on null hypothesis.

(a) Bisphenol A does not have toxicity in mice
(b) Green tea may not possess significant antioxidative property
(c) Treatment of green tea extract may not ameliorate bisphenol A – induced toxicity \textit{in vitro}
(d) Bisphenol A does not have any \textit{in silico} interaction with hemoglobin, catalase, and glutathione peroxidase
(e) Therapeutic treatment of green tea extract \textit{in vivo} will not be effective against bisphenol A toxicity