INTRODUCTION

1.1 General Introduction

Every year in India thousands of chemicals are manufactured and imported which increases the chemical load on to environment. Humans are continuously getting exposed to these types of chemicals through various routes and sources. The ever increasing chemical burden pose a severe threat to the endocrine system of human by interfering with endocrine system and interfering normal growth and development (1). The chemicals which destroy the normal function of endocrine system by interfering with synthesis, secretion or action of the hormones and thus producing adverse developmental, reproductive, neurological and immune effects are known as endocrine disruptors (EDs). These chemicals have ability to mimic or block endogenous hormone and altering the function of hormones. Endocrine disruptors including pesticides, fungicides, industrial components and by-products of industrial process and chemicals used in plastic manufacturing which act as a cancer causing agents by manipulating the cellular signal pathways. EDs are hormonally active agents that are persistent in food chain and are being inhaled by humans from food and environment (2, 3).

ED compounds are common chemicals which are used in variety of consumer products like plastic containers, inner lining of cans and silver foil; therefore exposure of Endocrine Disrupting Chemicals (EDCs) among human population is widespread. The major sources by which humans get exposed to EDCs include air inhalation, absorption by skin and ingestion of contaminated food and liquid is most common pathway (4). Transgenerational route is another pathway of EDCs exposure in which exposure of mother to EDCs before producing offspring can result in exposing the offspring to these chemicals. Bisphenol A (BPA) is one such chemical which is produced in large quantities every year especially for use in epoxy resins and polycarbonate material. In a study it is revealed that BPA, Polycarbonate Biphenyls (PCBs) and phthalates have been detected in more than 90% of the population in their blood samples. These results show that exposure to these chemicals among general population is widespread and ever rising in the population of every age group.

In early 1940 it was discovered that some chemicals like Dichlorodiphenyltrichloroethane (DDT) could cause reproductive and endocrine toxicity. Research conducted by Patisaual reveals the adverse effects of DDT on target species includes feminized male embryo, weakened eggshells, declined in birth rate of bird species and reducing nesting behavior among birds (5). After this
study in 1992 USA declared ban on use of DDT, however the use of DDT continued worldwide. In 1930, diethylstilbestrol (DES) was the first choice for prevention of miscarriage but after some time the research showed that this synthetic estrogen could affect adversely on reproductive health of female. Herbst et al., identified relation between DES and development of a very rare type of clear cell adenocarcinoma (CCA) of vagina and cervix in female offspring born to mother who took DES which lead to the ban on this synthetic estrogen (6). A lot of research was published indicating the results of exposure of DES in female offspring have been associated with breast cancer in older women (7), increased risk of CCA with age (8), natural menopause at younger age (9), early uterine leiomyomata diagnosis (10) and irregular menstrual cycle (11). PCBs, which are another class of EDCs, show both estrogenic and antiestrogenic properties depending upon the congener. In another study, phthalates have shown the potential to alter the antiandrogenic mechanism while BPA mimic estrogenic properties (12). PCBs, BPA and phthalates are most extensively studied EDCs and have demonstrated endocrine disrupting properties. The analysis of risk of reproductive toxicity in association with exposure to the selected classes of EDCs is the prime focus of the research.

1.2 Bisphenol A Background

BPA was firstly synthesized by the Russian Scientist A.P. Dianin in 1891. BPA is an organic compound and its estrogenic properties were discovered when researchers studied the BPA feeding experiment on ovariectomized rats. At this time researchers started thinking that BPA could be an option for estrogen replacement (13). Vandenberg et al have demonstrated the estrogenic properties of BPA having 10,000 to 100,000 time’s weaker affinity for traditional estrogen receptor than estradiol (14). The structural similarity has been shown in Fig. 1.1 and Fig. 1.2 for both BPA and estradiol respectively. Due its low affinity towards estrogen receptors, pharmaceutical industries chose DES over BPA to prevent the premature delivery and miscarriage (15). DES is now well known for harmful effects of endocrine disruption and development of rare clear cell carcinoma in offspring of DES treated mothers.

Figure 1.1 Structure of BPA
After being rejected as synthetic estrogen BPA did not remain unutilized; its usefulness as a building block for plastic to provide strength was soon identified. BPA consists of two phenol groups that act as monomer base for synthesis of polycarbonate plastics. Polycarbonate plastics (PC) have wide properties like transparency, thermo stability, shatter resistant and light in weight. BPA also act as a linker in manufacturing of epoxy resins, compounds that have corrosion resistance, heat resistance and flexibility characters. BPA is also used as a component of dental composites (16, 17), medical devices (18) and employed as colour development in thermal receipts (19).

As the demand for polycarbonate based plastic and epoxy resins in many consumer product increases, it directly affected the production of BPA with rise in its production up to 6 billion tones in a year which makes it the world's highest production volume chemical (20, 21). Due to excessive use in consumer products, BPA could be found everywhere like in house hold dust and air (22), in waste water or contaminated water (23, 14, 24). BPA has been also detected in variety of paper products (19, 25, 26) and recycled paper contains ten times more concentration of BPA than virgin paper (26).

BPA exposure in human population is ubiquitous due to so many contributing sources. Calafat and co-workers have shown in their study that the American population is highly exposed to BPA and 92.6 % of American population presented with BPA present in their urine (27). Research has proved that diet is the main contributory factor for high BPA exposure (28, 29, 30).
1.3 Sources of BPA Exposure

In 1960's FDA (Food and drug Administration) approved BPA for food packaging (31). In the food chain, BPA comes from mainly polycarbonated plastic containers and epoxy resins which are used for coating of container. Traditional food containers, infant formula container and beverages containers are coated with epoxy resins from the inside mainly to prevent the food from coming in contact with the metal coating. The can coating is required

1. To prevent metal can from corrosion
2. To protect food from acquiring a metallic taste and food colour and texture.
3. To withstand the stress of food processing including can distortion and high temperature sterilization (32, 33).

In July 2012 FDA banned the use of BPA in manufacturing of polycarbonate baby bottles and sippy cups (31). BPA is the main constituent of plastic serving dishes, plastic serving utensils, storage food containers and reusable water bottles. The concern of using BPA in food packaging material arose from its intensity to leach into the food product. Krishnan conducted an experiment in which he accidentally discovered the leaching of BPA from polycarbonate flask in experimental media (34). During early stage of trials they believed that yeast culture was showing estrogenic activity via competing with estradiol for estrogen receptor. Upon deeper investigation, they identified that it was not yeast culture, BPA was the substance which migrated into media during sterilization of media, acting as estrogen and competing with estradiol. This study confirmed the leaching of BPA from polycarbonate plastics and also demonstrated the estrogenic activity of BPA (34). A lot of studies were conducted after 1993 which proved the migration of BPA into products during variation in physical condition of storage like variation in temperature, age of plastic and boiling conditions (35). Besides polycarbonate material, studies revealed that Polyvinyl Chloride (PVC) made product is also not safe and leaching BPA from PVC material is higher than the other food contact materials (36).

McNeal et al 2000 have studied the migration of BPA molecules from resins into the food (37). Broton documented that BPA leaches from epoxy resin lining of canned food into products. In this study aqueous products were analyzed and as well as autoclaved the same cans containing water as product. The results showed that these liquids have hormonal activity attributable to BPA (38).
1.4 Effects of BPA Exposure to human beings

As mentioned above, the route of exposure of BPA to human is ubiquitous and health effects are limited. Researchers found the association between BPA level in blood and variety of conditions in women including obesity, endometrial hyperplasia, miscarriage, polycystic ovary syndrome (PCOS) and abnormal karyotype. Two different studies correlate the level of BPA in serum with PCOS. In these studies the researchers revealed that the level of BPA in PCOS patients is higher as compared to healthy women (39, 40). In another study negative correlation was found between BPA and FSH among men. In the same study epoxy resin worker also exposed with organic solvent due to cross sectional design of study it was unable to determine whether the BPA affects androgen level or androgen level affects metabolism of BPA in body (41). Three different studies relate the chromosomal abnormality with the exposure of BPA. One research shown that women having higher maternal serum BPA carrying fetuses with chromosomal abnormality as compared to women carrying fetuses with normal karyotype (42). Moreover, the average BPA level is more than three times higher in 45 women with a background of three or more consecutive first trimester miscarriage as compared to 32 nonparous women without any fertility problem. This study showed the relation between serum BPA level and recurrent miscarriage (43). Additionally 35 women included in this study that then became pregnant having lower BPA subsequently had successful pregnancy as compared to those who miscarried again. However it is an important factor that the distribution of exposure among the women with recurrent miscarriage was highly skewed and only few women highly exposed to BPA while others has median exposure in two groups. Finally it was positively found that sister chromatid exchange is associated with urinary BPA level in peripheral lymphocyte of adults (44). Although above studies provide enough preliminary data on potential health risks, but this research had lot of limitations like small sample size, concentration of BPA in serum, limited detail of subject selection criteria and cross sectional design of study. With this limitation in the design of study, it is difficult to make conclusions based on the epidemiology of potential health risk of BPA among women. These studies provide a strong base to identify whether BPA level in human being is directly involved in health risk or altered BPA metabolism is secondary pathway responsible for health defects.
1.5 Basic Function of Endocrine System

The endocrine system is the collection of glands that release out hormones and regulates the development and function of essentially all cells, tissues and organs (45, 46). Endocrine system controls various vital processes such as reproduction, growth, development and metabolism via regulation of hormones. The endocrine system also plays an important role in maintaining homeostasis under stress, such as infection, trauma, emotional stress, starvation, dehydration, haemorrhage, extreme conditions like temperature and cold etc. Disturbance or confusion in endocrine function leads to dysregulation of process leading to wide spectrum of endocrine based disorders such as goitre, diabetic conditions, growth inhibition, certain types of cancer and reproductive problems. Major endocrine glands and tissue are illustrated in figure:

The hormones are released from endocrine glands and tissues into the bloodstream to travel to target tissues hence controlling the endocrine system. There are many different hormones grouped mainly into four groups:
1. Steroids, estrogen and androgen
2. Biogenic amines such as thyroid hormones (T3 and T4)
3. Peptides and proteins such as insulin and oxytocin
4. Eicosanoids such prostaglandins (46)

The endocrine system produces hormones, which binds to specific receptor present on cell surface and start cascade of signaling event with in cell like kinase activation and phosphorylation of protein which lead to specific biological response of the cell. The series of events leads activation of transcription and activation of specific gene. Hydrophobic hormone like estrogen and androgen has ability to cross cell membrane and interact with intracellular receptors. These receptors are ligand regulated factors and initiate transcription of gene on the basis of ligand regulated transcription factors (45).

A single hormone have ability to bind with different types of receptor and activate different signal pathway, for instance, estrogen have two different receptors, alpha and beta as well as via receptor on cell membrane (47). The response of a single hormone may differ in tissue depending upon the type of receptor that is expressed. Responsiveness of tissue to hormone
signaling depends upon the up and down regulation of hormone receptors. Positive and negative regulation loops in endocrine glands and tissue maintains hormonal balance in tissues (45). For example corticotrophin releasing hormone (CRH) released by hypothalamus under emotional stress that stimulates pituitary gland and exert signal to release adenocorticotropic hormone (ACTH). Cortisol, adrenaline and noradrenaline are released by adrenal cortex in response of ACTH. Blood pressure, immune system, metabolism and wide range of functions are regulated by binding of hormone receptors in different tissues. The level of adrenaline, cortisol and noradrenaline are regularly monitored by hypothalamus and when the level of these hormones increases in the blood, negative regulation is initiated (45, 48). Some important actions of hormonal system related to the issue of EDCs are

1. Hormone expression depends upon the binding affinity for the receptor present on different tissue and action of hormone start after binding.
2. When the concentration is below receptor saturation, maximum effect is generated.
3. Low concentration initiates hormone action.
4. Binding affinity of hormone towards receptors varying.
5. A single hormone play different role at different life stages.
6. Hormone can act as agonist, antagonist or in synergistic fashion.

1.6 Mechanism of Endocrine Disruptors

A group of researchers have defined the endocrine disruption as a collection of different mechanism which alters the normal function of healthy endocrine system i.e. binding to receptor, hormone metabolism and transportation and product formation (49, 50, 51). Some EDCs have ability to mimic the characters of endogenous hormones and act as hormone receptor agonist, means they can bind to receptor and activate transcription of endocrine responsive genes. However the pattern of result on molecular level event differs from each other because of their potency and complexity (48). The lack of information regarding mechanism of action of endocrine disruptors leads to adverse health effects. EDC have been known to affect the cellular hormonal pathway in various possible ways and maximum data is obtainable with respect to the nuclear receptor (NR) family. The NRs represent a family of structurally related transcription factors. These NRs are being involved in performing the basic and vital functions in mammals
including fetal development, homeostasis, metabolism, reproduction and response to xenobiotic compounds. The hormone receptors of this family are the steroid hormone receptors ERs, androgen receptor (AR), progesterone receptors (PR), glucocorticoid receptor (GR), and mineralocorticoid receptor as well as the thyroid hormone receptors (TRs). The EDCs have the ability to bind directly to these receptors either as agonists or antagonists, thus augmenting or obstructing the hormonal effects respectively. Moreover, other mechanisms are also present by which the EDC an affect the endocrine system which includes the AhR which is ligand-activated transcription factor and a significant regulator of the cellular response to xenobiotic exposure. Belonging to the family of the bHLH-PAS proteins, it is expressed ubiquitously and its activation is sturdily triggered by the xenobiotic compounds such as BPA, PCBs, polychlorinated dibenzodioxins, dibenzofurans, polycyclic aromatic hydrocarbons (PAHs), benzo[a]pyrene (BaP) etc. (52, 53, 54).

Dysregulation of the hormone metabolism is the main mechanism by which the EDCs have been interfering with the endocrine system. Hormone accessibility is reliant on various processes which include biosynthesis of hormone, transportation of hormone to target tissues and hormone binding protein levels. All these process are believed to be affected by EDCs by interfering with their mechanisms. EDCs are described to affect particularly the steroid hormone catabolism, since most of the xenobiotic metabolizing enzymes are engaged in both the mechanisms. For instance, the entire main targets genes of AhR, including the P450 enzymes CYP1A2, CYP3A4, CYP1A1, and CYP1B1, are accountable for the hydroxylation of 17-b estradiol (55). Upon exposure of xenobiotic compounds, enzyme activation takes place which further lead to negotiated hormone signaling by augmented hormone catabolism. Recent research shows that CYP19B (aromatase) which is responsible for converting testosterone to estradiol is a direct AhR target gene for the xenobiotic compounds, hence, EDCs can activate AhR leading to degradation of steroid hormones.

1.7 Complex toxicity of Endocrine disruptors

Toxicity caused by EDCs are very complex and a challenge in front of researchers to develop new technique for testing toxicity and health risk assessment related to EDCs.
1. EDCs have multiple mechanism of action like single type of EDC has both estrogenic and antiandrogenic property. The mechanism of EDC on different tissue are different because hormone receptor expressed in different tissue may have different mechanism of action.

2. Selection of animal model for toxicity testing of EDCs are different because there is a lot of difference in sensitivity between a species e.g. different strains of rats have insensitivity towards estrogen (56, 57).

3. The effects of EDCs appeared after long time and to a subsequent generation. For administration of DES to pregnant in between 1940 to 1970 to prevent miscarriage leads to complication in daughters of DES treated mother with vaginal cancer (58).

4. Dose of EDC have a big issue of debate. Internationally dose related response of EDC is not clear.

1.8 Low dose effects

As mentioned above, a low concentration of endogenous hormone and variation may cause a biological response (46). EDCs act in the same manner as endogenous hormone did and increasing the response of already ongoing biological response by binding to more hormone receptors. Studies prove that a very low dose of EDC both in vitro and in vivo can cause side effects at developmental stages of life (59, 60).

Low dose definition is not available and is still a debatable topic at an International level. In literature low dose definition is used with different meanings e.g.

1. Low concentration as compared to standard level defined by regulatory of NOAEL
2. Concentration below health guidance value.

Based on primary research many experts claim that a threshold value cannot be defined for EDCs (48, 60, 61). Beronius and Hanberg 2013 discussed and strongly argumented on first the interaction, gene transcription activation and deactivation (62). However it has been proposed that threshold exist for higher points such as behaviour, reproduction and growth (63).
1.9 Different effects at low and high dose

Endogenous hormones can display variation in effects at low and high dose and known as non-monotonic dose response relationship because they have receptors binding nature (49). A U or inverted U-shape and bi-phasic curve results due to non-monotonicity which refers to dose response curve changes at change in dose concentration.

In several studies EDCs showed non-monotonic response curve in *in vitro* and *in vivo* models such as pesticides, PCBs, dioxins and phthalates (60, 64, 65, 67). Tamoxifen is the best and characterized example of non-monotonicity. It is reported that low dose or below therapeutic value causes cell proliferation in estrogen dependent cells (60, 68). Plotkin et al., 1978 have shown in their experiment that a growth of breast tumors identified in first week of therapy after drug administration before therapeutic dose concentration reached (69).

Non monotonicity can be explained with many examples such as

1. Competitive mechanism of estradiol e.g. low concentration of estradiol can initiate cell proliferation whereas high concentration causes cytotoxicity.
2. Down regulation of receptors due to over stimulation of hormone results in decline in response at high concentration.
3. High and low concentration of a receptor ligand activate different gene.
4. Selection of receptor depends upon the concentration like at low concentration compound binds on membrane receptor while at high concentration it binds to intracellular receptor.
5. Low dose of EDCs cannot compete for hormone receptor but at high concentration of EDC out-competes the hormone and binds with receptor.

1.10 Sensitive window of exposure

Endogenous hormones have variety of different function and exert various effects at various stages of life (46, 48). At early stages of life endocrine system involved in development of tissue and at young stage it regulates functions of tissue. EDC exposure at the fetal stage can cause deleterious effect that manifested at later stage of life (49). The exposure time has differential effect in case of EDC toxicity which was shown by research that developing organism is more
sensitive to EDC than adult. The roles of estrogen, androgen and thyroid hormones plays critical role in fetal development.

1.10.1 Estrogen

Estrogen is the main hormone of female and plays critical role in development, in male estrogen receptors are also present (45). Estrogen is also responsible for development of secondary characteristics in female (46). Estradiol, a form of estrogen involved in development of brain includes promoting and preventing synaptogenesis (formation of synapses) (70). Sexual dimorphic behaviour in animals and sexual differentiation in brain are developed by estrogen (71). The dimorphic behaviour of sexual orientation is not studied well. Vom Saal et al. 1997 studied the effect of ethinyl estradiol, DES and BPA at development stages and found out the side effects on reproductive organs (72). Exposure of EDC at development stage can cause problems with anxiety and cancer and behavioural changes (58, 73, 74).

1.10.2 Androgens

Testosterone and dihydrotestosterone are very important androgens as they drive and development of reproductive system of male. They also help in development of secondary male characteristics and eternal genitalia (46). McCarthy 2010 mentioned that androgens important for development of dimorphic areas in brain and behaviour (75, 76).

1.10.3 Thyroid hormone

(T4) plays critical role in normal development of brain in fetus (45; 77). Target genes of brain maturation such as myelination and cell differentiation are transcripted by receptor of fetal brain aspects bind to thyroid hormone. Inadequate supply of thyroid hormone, deficiency of iodine and disturbance in enzyme production for metabolism of thyroid hormone may result in neurological disorders like mental retardation in children (77).

1.11 BPA risk characterization

To characterize the risk of BPA exposure on children more research should be conducted. Till the date research data is not enough for risk characterization on children. Yamano et al., 2008 conducted a longitudinal study on school children of Japan (78). 100 school children from grade
1 to 6 were included in the study. The results show that as class grade increased the BPA level in urine decreases i.e. 100% in first grade, 97% in third grade and 86% in 6th grade. The decline of level of BPA in sample may be due to ban on use of plastic or consumption of canned food. In the school polycarbonate plastic serving dishes were replaced with polyethylene terephthalate which may be another reason of decline in urinary BPA level. In a study conducted in North Carolina, 9 school children aged between 2-5 years were included of two day care Centre to know the route of exposure of BPA. Sampling period was 48 hours and samples of floor dust, indoor outdoor air, diet samples and hand wipes collected. Activities of each student was monitored carefully and recorded in time activity diaries. Samples were collected from home and daycare Centre. It was concluded that BPA exposure in children was very low and primary source of exposure was diet (79).

In 2006 Wilson expanded his prior study; further he included 257 preschool children to determine the route of exposure in young children. Samples were taken from their day care Centre and homes for 48 hours. Samples include food, beverages, soil, hand swipes and urine collection. Indoor and outdoor air samples were also collected to know environmental role in exposure. 50% samples of indoor air, hand wipes, food and beverages were contaminated with BPA. After the study it was concluded that dietary ingestion is responsible for 90% exposure in children and 1% from air inhalation (22).

To understand the metabolism of BPA, a study was conducted on 5 families of San Francisco. These families were monitored closely during 8 days period of study. They monitored before, during and after meal of three times a day. First two days BPA level measured in their urine when they were consuming normal diet. Then samples were collected for three days when the families consuming BPA free diet. After three days families were told to return on their normal diet and samples of urine were again collected. The results of the study exhibited that BPA level declined to 66% in the families when they are feeding with BPA free diet. But the BPA level in the urine comes to pre-intervention level of study when they returned to normal diet. Study concluded that main exposure of BPA is diet and it is not possible to eliminate from food system. 99% of exposure of BPA to population depends upon the diet system they consume. Milk also acts as a main source of BPA in dietary system because when BPA leaches from PVC pipes during milk processing, it comes into the milk (80).
Carwile et al., 2011 showed that BPA level in urine increased 1221% in participant when they consume canned soup. The BPA level found low in participant who consumes fresh soup (81). Above studies clearly indicate that exposure route of BPA is mainly dietary and elimination of BPA from food system is not an easy task.

1.12 Poly-cystic ovary syndrome

PCOS is characterized by ovary dysfunction, polycystic ovary and hyperandrogenism. These descriptions lead to numerous symptoms with systemic as well as organ specific disorders. Obesity and cardiovascular factors are associated with PCOS and are more prevalent among females now days. The pathophysiology and long term effect of PCOS has been an important point of reason to prevent health problems associated with PCOS patients.

Research reveals that approximately 6-10% women in their reproductive years are affected from PCOS. Many studies have been conducted to characterize the PCOS and still many etiological mechanisms are not well understood. All the studies have been conducted on animal models that have contributed to our basic knowledge about complexity of syndrome and etiology.

1.13 Clinical features and Diagnostic criteria

Abnormal or absence of menstruation known as amenorrhea and polycystic ovary associated with hormonal imbalance such as increased androgen level. Stein and Leventhal in 1935 documented and explained these factors in a group of women (82). A plethora of references have been documented citing the polycystic ovarian morphology and their association with clinical endocrine abnormalities. The symptoms reflecting the reproductive elements of PCOS includes irregular menstruation (oligomenorrhoea) and difficult to conceive (83). PCOS is diagnosed by the presence of 12 antral follicles of size between 2 to 9 mm in diameter in fallopian tube and likely to be enlarged in volume (84). Ovaries of PCOS patients are polyfollicular rather than polycystic with extra follicles in ovary periphery and thick stromal section. There are many other physiological and biochemical symptoms that appeared in PCOS patients. The biochemical feature includes increased level of androgen leading to hirsutism and acne in PCOS patients (83). It was observed that PCOS patients have elevated levels of LH (Luteinizing hormone) and ratio
of LH to FSH (Follicle stimulating Hormone) (85, 86). Sometimes it has been observed that most of females suffering from PCOS are obese but not always, this condition gets worsened when it combines with metabolic disorder (83, 87). In obese condition hyperandrogenism initiated the accumulation of central fat leading to risk of cardiovascular diseases, type II diabetes and insulin resistant. It is reported that women with PCOS have increased serum insulin prior to and after administration of glucose (88, 89). Body mass of index does not affect insulin sensitivity in PCOS patient. The presence of dark pigments on skin (Acanthosis nigricans) is a marker of insulin resistant and clinical feature of PCOS (90, 91)

There are wide ranges of clinical features including physiochemical and biochemical available but standardized criteria to diagnose PCOS syndrome is still absent (92, 93). National Institute of Health (NIA) provides guidelines to diagnose PCOS including chronic anovulation with biochemical and clinical features which were sufficient to diagnose PCOS (94). In 2003 the Rotterdam added one more criterion that includes presence of polycystic ovary in to NIA guidelines (95). This resulted in more complexity and heterogeneity in PCOS. The different features according to Rotterdam that help in diagnosis of PCOS are listed in Table 1.1.

**Table 1.1 Rotterdam criteria for PCOS diagnosis**

<table>
<thead>
<tr>
<th>PCOS Traits</th>
<th>Mild PCOS</th>
<th>Ovulatory PCOS</th>
<th>Severe PCOS</th>
<th>Anovulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCOS</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Androgen</td>
<td>Increased</td>
<td>Highly increased</td>
<td>Highly increased</td>
<td>Highly increased</td>
</tr>
<tr>
<td>Ovulation</td>
<td>Irregular</td>
<td>Normal</td>
<td>Irregular</td>
<td>Irregular</td>
</tr>
<tr>
<td>Insulin</td>
<td>Normal</td>
<td>Increased</td>
<td>Highly increased</td>
<td>Highly increased</td>
</tr>
</tbody>
</table>

It was observed in a study that according to Rotterdam criteria only 10% women have severe PCOS and 10% will have only symptoms without PCOS (96). If Rotterdam criteria considered diagnosing PCOS, the prevalence of PCOS among women increased subsequently (97). In Western countries, obesity is related to increased level of glucose in blood (diabetes) and obesity is also related with anovulation, so Rotterdam criteria for PCOS makes it more complex (98). In a study it was proved that when PCOS patients reduce their diets and do exercise daily, it helps
in reinstigation of normal ovarian cycle (99-101). It is believed that PCOS is common in insulin resistant and metabolic disordered population (102). Researchers found that Hispanic Americans are more susceptible to PCOS as compared to white or black Americans (103). The prevalence of PCOS is found very high in women of Indian sub-continent (104). The data related to population of PCOS patient in Indian continent is not available.

1. **14 Aims and Objectives**

The aim of the study is to evaluate the risk assessment of BPA in the Indian population mainly concerning the women suffering from PCOS. The study mainly focused on the determination of BPA levels in PCOS and healthy females. BPA is an endocrine disrupter and is employed in the manufacture of wide range of consumer products. The exposure to BPA is chronic and widespread in people of all ages. It is also suspected that BPA is present in detectable levels in the Indian population. Moreover, it was suspected that there might be differences in the BPA levels among PCOS women and healthy subjects and in the hormone levels as well. There is no such data available among the Indian regarding the BPA levels in PCOS women and the healthy females and this is the first attempt to correlate the BPA levels among the two groups. The present investigation has been planned with the following objectives.

1. Determination of migration of BPA in polycarbonate plastic containers and cans.

2. Survey and involvement of PCOS patients regarding their dependency on plastic containers and packed food and to study variation in their hormone levels.

3. Determination of BPA levels in blood samples of few PCOS patients and healthy females.

4. Determination of parallelism between the hormone imbalance after exposure to BPA sources and its adverse effects in human health.

**Significance of study:**

BPA is present in most of the polycarbonate products and leaches from these containers into the foods and drinks. Infants and children are at most risk for the BPA exposure. Since, BPA has been banned in most of the countries for its use in baby feeding bottles and toys, however, in
India, most of the population is unaware of the BPA exposure and its consequences, and hence, its use is ubiquitous among the Indian population. Moreover, the cases of PCOS are rising day by day especially among the young and teenage girls of reproductive age group. BPA also interferes with the hormonal system of the body, and thus causing endocrine disturbances in the body and other metabolic disorders including obesity, diabetes, fertility issues, and many type of cancers as well. There is no data available regarding the relationship between BBPA and PCOS among the Indian females; hence the study was conducted to determine the BPA levels in PCOS as well as healthy females and the association between the BPA levels and hormonal imbalance among the two groups.