DISCUSSION

Concerning the migration of BPA from the polycarbonate plastic containers and canned food products into the food and drinks, it has been clear from the results of this study revealing the BPA leaching in all the samples. Regardless of the age of the container, whether the bottles were newly purchased or were previously consumed by the consumers, detectable amount of BPA migrated from the polycarbonate feeding bottles. The feeding bottles were purchased of different brands and included both Indian made and imported. The lowest amount of BPA was detected in sample FB3 with BPA concentration of 52.54 mg/L while the lowest concentration was found in sample FB4 of 2.8 mg/L. The amount of BPA migration in feeding bottles by adding hot water into them revealed in this study ranged from 2.8 to 52.54 mg/L. These values are considerably higher than the values reported by Shrinithiviahshini et al. in the feeding bottles which revealed average BPA concentration of 17-19 ng/ml in different three brands of feeding bottles all leaching significant levels of BPA (189). Similar results were also obtained by Maragou et al. in which they tested leaching of BPA from the feeding bottles in to water samples at concentration of 14 ng/ml (190). The BPA migration was also studied by Biedermann-Brem et al. considering different scenarios of using the bottles which includes heating, washing in dishwashers and alkali treatment resulting in releasing significant levels of BPA in into the samples (191). Similar migration experiments were also performed by Brede et al. with both newly purchased and previously used polycarbonate bottles thus revealing a significant increase in migration of BPA leaching due to continuous use (192). This could be explicated by the fact that prolonged usage may weaken the polymer, hence resulting in polymer degradation due to continuous usage of the polycarbonate container.

Although, the BPA concentration observed in samples of the present study is below the maximum acceptable or reference dose of “0.05 mg/kg of the body weight/day” (50µg/kg body weight/day), established by the United States Environmental Protection Agency (USEPA) and European Union (EU), however, the observed values could not be considered as safe, as the daily intake of liquid is far more than the samples taken for this study. It has been observed that the daily intake of milk by an infant of about 4.5 kg of body weight is approximately 700 ml, whereas the samples taken for this were about of 100 ml.
Apart from the polycarbonate plastic containers, BPA has been detected in canned products as well. BPA is being used in the inner protective epoxy resin coating of the cans in order to prevent food from coming in contact with the reactive material of cans. However, many studies have reported that BPA can potentially leach out from the protective coating in to the food and drinks, thus exposing the consumers towards this harmful chemical. In this study, two canned products were tested for BPA migration into the foods and drinks and it was found that both the canned BPA when analyzed by HPLC technique and the migration levels ranged from 0.067 to 0.09 mg/L.

Similar study was conducted by Yoshida and co-workers which have revealed by high performance liquid chromatography that BPA migrates in to the canned fruits and vegetables (193). The limits of quantification were 10 ng/g for the solid portion and 5 ng/mL for the aqueous portion, respectively. The maximum level of BPA was found to be 11µg/can which was detected mainly in the solid portion of the canned food product. A study was conducted by Errico et al in 16 samples of canned tomatoes of six commercial brands, marketed in Italy, and BPA migration was tested from the epoxy lining of the cans (194). All the tomato samples demonstrated BPA migration at concentration of 0.4µg/kg, while a substantial increase in migration levels was observed when the samples were exposed to a slight higher temperature or the damage done to the cans while denting.

A previous study done by Goodson et al determined the percentage level of BPA migrated into the food and drinks by the comparing the BPA levels in both the epoxy coating of the can and in food and drinks as well (195). It was found that 80-100% of the total BPA present in the epoxy lining of the can had migrated to foods straight after the can was processed by pilot plant filling with food or simulant, sealing and sterilization. This indicated that most of the migration had occurred during the processing of the can and there was hardly any change in the level of BPA by extended storage (up to 9 months) or can damage by denting. The data reported in this paper strongly suggest that some foods preserved in lacquer-coated cans acquire estrogenic activity.

Brotons et al conducted several experiments to check the biological activity that may be related to estrogenic substances such as phytoestrogens or estrogenic pesticides confined in vegetables before they are canned or plastic monomers or additives used in the manufacture of food containers (38). There were no phytoestrogens and organochlorine pesticides present in the foods
packed neither they were in fresh vegetables. However, when the cans in which the vegetables were packed were filled with water and autoclaved, BPA was detected in the autoclaved water and in the preserved liquid of the vegetables as well, thus proving the BPA migration into the canned food products.

Many other studies were conducted in different parts of the world where the researchers tested the migration of BPA in various foods with different concentrations which includes ham with BPA levels of 422ng/g (195), 842 ng/g in sauces (196), 102.7ng/g in fish (197), and 95.3 ng/g in vegetables (193).

Hence, all these studies done by various researchers in different parts of the world have revealed that BPA migration occurs from the polycarbonate plastic containers and canned food products thus exposing the human population towards this endocrine disrupting chemical. A large number of populations depend mainly on these types of containers for their daily storage of food and drinks. People of all age groups are getting exposed to this type of chemical and its harmful effects as well. The infants and children are continuously getting exposed to this chemical through baby feeding bottles. These form the most vulnerable population as their organs are at a developing stage, hence, the endocrine disruptors could make a long term effect causing hormonal balance disorders at later stage in life.

Even though, researchers have revealed weak estrogenic properties of BPA, however, the hormone disrupting effects of the chemical by influencing enzyme activity or receptor expression through non-genomic pathways and antagonizing the effects of hormones including estrogens, androgens and thyroid hormones (198).The effect of BPA exposure in neonate rats was also studied and it was demonstrated that the BPA exposure during organ development a and continuing through puberty produce effects resulting in obstinate alterations of the affected organs in various strains of mice (19, 200; 201; 202; 203; 204; 205; 206; 207, 208).

The survey conducted from 2012 to 2015 in districts of Haryana and Punjab, India revealed the level of BPA exposure through consumption of food and drinks from plastic containers, dependency on plastics and health conditions of participants. In the questionnaire, the main focus was on exposure of BPA and dependency on plastic in population. According to the result of survey it was observed that urban population was more dependent upon plastics in daily practice
while rural population considerably has less exposure to plastics. The exposure route to plastic is mainly through food and water which could be from water bottles, microwave bowls, plastic cups, canned food and beverages. More than 90% of the urban population depends on the polycarbonate containers for food and water storage. It indicates that urban population is more exposed to plastic or BPA as compared to rural population. The rate of use of canned food products in urban population is also much higher than the rural area. More than 60% of the urban population use canned food products while the percentage is less than 20 in the rural. In rural area mostly used plastic or second hand plastic were used for daily practice. More than 7% of the women population is suffering from PCOS in those districts while the rate is comparatively higher in Punjab as compared to Haryana. The rate is similar in developed countries where 5-10% of the population has been affected by PCOS (209, 210). The developing countries with increasing urbanization and adoption of modern lifestyle are also experiencing increase in the number of PCOS females as well (211, 212, 213). Moreover, it was also observed that the females suffering from PCOS and other hormonal disorders do not have any family history of such hormonal imbalance disorders which signifies that the lifestyle, food and environment are paying a major role in causing these disorders.

When considering obesity factor in the participants it was found that 53% of the PCOS females were overweight or fall under the category of obese while 26% of the healthy non-PCOS females were overweight or obese. The role of obesity is also an influential factor in exaggerating the PCOS with 40-60% of the PCOS women being obese (49). In this study, the total subjects in both the groups were categorized into five age groups which includes; 15-20, 21-25, 26-30, 31-35, 36-40 and 41-46. According to the survey result 21 to 30 year aged females fell under the most exposed category towards BPA sources and these includes approximately 30% of the total female population. Apart from the polycarbonate containers and cans, the population also gets exposed to BPA through dental sealants or composites which leach out at low levels directly into the body directly (214). In the survey conducted, it was found that 17% of the total PCOS females had their dental filling done within the time span of 6 months to five years while 6% of the healthy females had their dental fillings done.

The PCOS is concerned with the hormonal imbalance of various hormones leading to several disorders to it. A comparative study was done to correlate the hormone levels of PCOS women
with respect to the healthy females. The hormones associated with PCOS includes thyroid hormones (T3, T4 and TSH), prolactin, LH, FSH, testosterone etc. The thyroid stimulating hormone was found to be slightly elevated in case of PCOS women with average value of 3.73μIU/ml as compared to healthy females which was noted as 3.3μIU/ml in this study. Similar results were also observed by Du and Li (2013) in which they found higher levels of TSH among PCOS women as compared to non-PCOS women (215). Another correlation was also found between the hyperthyroidism and disrupted menstrual cycles and ovulation, thus leading PCOS (216). Elevated levels of LH were also observed in case of PCOS women which were rather less in case of healthy females. Moreover, the ratio of LH/FSH was also noticed to be increased in PCOS women with value of 1.44 while it was slightly less in case of healthy females as 1.13. Significant higher levels of LH were also observed in PCOS women as compared to non-PCOS women by Huang et al 2010. Corresponding to the higher LH levels in PCOS women, the ratio of LH/FSH was also found to be higher in PCOS women (215). The above change in the LH/FSH ratio is enough to interrupt ovulation, hence causing disturbed menstrual cycles and even leading to infertility. Another hormone playing a major role in PCOS development and the symptoms associated with it is free testosterone which has been observed to higher in case of PCOS women as compared to the non-PCOS counterparts. The free testosterone levels were found to be 30.9ng/dl in PCOS women, while levels were significantly lower in non-PCOS women with the values of 28.45ng/dl in this study. In a study conducted by Huang et al, the researchers observed significant differences in the free testosterone values with 5.1 pg/ml in case of PCOS women while the value was as low as 2.9pg/ml in non-PCOS counterparts (217). Free testosterone is believed to be more sensitive examination for excess androgens which can lead symptoms of hirsutism and disturbed menstrual cycles. Thus, it is clear that a significant variation in hormone levels exists which can exaggerate the ovulation process and thus leading to PCOS. Moreover, the adolescents are becoming more and more prone to hormone imbalance disorders due to the ever increasing chemical burden of the endocrine disrupting chemicals.

Further investigation was done in the study to determine the correlation between the BPA levels in PCOS women and their non-PCOS counterparts. It was observed that the blood BPA levels were significantly higher in case of PCOS women as compared to non-PCOS subjects. The BPA levels found in the present study ranged from 1.92 μg/L to 6.38 μg/L among the PCOS women while the levels found in non-PCOS women ranged from 0.97 μg/L to 4.47 μg/L in non-PCOS
women. A similar study was also done by Kandaraki et al 2011 in which the researchers revealed significant higher levels of BPA among PCOS women with levels of 1.05 ng/ml while the BPA levels observed in non-PCOS women were 0.72 ng/ml (105). PCOS, as categorized by enduring anovulation and hyperandrogenism according to NIH criteria, establishes the most common endocrine disorder of women of reproductive age (218). Even though, the pathogenesis of this disorder is not clear till now, however, the role of environmental factors including the endocrine disruptors is being anticipated for the development of PCOS (219). Although very limited data is available on the humans regarding the elevated BPA levels and PCOS, however, recent studies on animals have clearly demonstrated the association between BPA and PCOS. Fernandez and co-workers (2010) studied the exposure of high doses of BPA exposure and the development of PCOS in female Sprague-Dawley rats by altering the hypothalamic–pituitary–gonadal axis during the period of brain sexual differentiation (188).

Hence, it is clear that humans get exposed to BPA during the developmental stages of life. The BPA leeching through the feeding bottles is the main source of exposure towards BPA in infants and children. The exposure at these stages of life could make a long lasting impact with various disorders at later stages in life. He results of the study have proved that the adults as well are at increased exposure towards BPA through polycarbonate plastic containers, canned food products and other packed food. This could be directly correlated with the rise in the number of the hormonal disorders among the women and men as well. Another most noticeable effect of BPA is that the women with BPA levels had elevated levels of thyroid as well LH/FSH ratio and testosterone levels as compared to the non-PCOS women. This provides strong evidence the BPA could be responsible for the hormonal imbalance in PCOS women which could trigger the development of PCOS in women of reproductive age.