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EFFECT OF BISPHENOL- A ON ANTIOXIDANT ENZYMES, REPRODUCTIVE HORMONES AND APOPTOTIC CHANGES IN PLACETAL AND UTERINE TISSUES OF ALBINO RAT

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ABSTRACT

Bisphenol-A is a widely used industrial plasticizer with known estrogenic properties. It is omnipresent in the environment and widely distributed and unavoidable. It accumulates in pregnant adult females and its continued exposure during gestation is likely to have an impact on the development of the fetus. Bisphenol- A was administered orally from 8th day of gestation to 15th day in a dose of (50 mg and 500mg/kg, b.wt) during the same period of gestation. The aim of this study was to evaluate the anti oxidant enzyme analysis, reproductive hormones and apoptotical changes in placenta and uterine tissues. In this study, we observed imbalance of antioxidant enzymes in serum, altered the expression levels of reproductive hormones, changes in apoptotic proteins that is proapoptotic protein caspase-3 was significantly increased in placental and uterine tissues of BPA treated rats.

KEYWORDS: **Bisphenol-A, Reproductive hormones, Antioxidant enzymes, Apoptosis, Placenta, Uterus.**

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INTRODUCTION

Humans are widely exposed to chemicals that mimic the actions of endogenous hormones, thereby affecting endocrine function. Low doses of these chemicals can affect endocrine function in many species[1]. Bisphenol-A (BPA) is used in the manufacture of polycarbonate plastics, epoxy resins, and dental sealants and is an estrogenic endocrine disruptor affecting fertility and reproduction[2]. Exposure to BPA occurs as it leaches from polycarbonate bottles, epoxy resin coating the interior of food cans, and dental sealants[3]. BPA is virtually ubiquitous in the environment and food supply, 95% of human urine samples contain BPA[4]. Perinatal exposure has been associated with female reproductive tract abnormalities[5]. These reproductive effects are likely mediated by BPA's estrogenic activity. Antioxidants are scavengers by preventing cell and tissue damage that could lead to cellular damage and disease[6]. Regardless of the presence of this antioxidant system, an over or unbalanced production of reactive oxygen species (ROS) due to contact with chemicals may resulted in a number of clinical disorders. BPA can cause oxidative stress by disturbing the redox status in cells and disrupt the balance of the oxidant and anti-oxidant system. In contrast, imbalances between ROS production and antioxidant systems induce oxidative stress that negatively impacts reproductive processes. High levels of ROS during embryonic, fetal and placental development are a feature of pregnancy. BPA also decreases the levels of the antioxidant enzymes in rats7. Chitra et al[8] observed significant decrease in SOD and glutathione peroxidase and increase in the levels of lipid peroxidation in epididymal sperm of BPA treated rats. Hormones are natural substances that control the development of all embryos and fetus. Exposure to supra-normal levels of such substances some time can alter the development of embryo, growth of young one and also reproductive potential of adult. Leutinizing hormone, (LH) Follicle stimulating hormone (FSH), Estrogen hormone and progesterone hormones are an important for successful pregnancy. BPA alters the hormonal path way, as it is known mainly an endocrine disruptor and estrogen receptor agonist. BPA has adverse effects on oocytes, leading to increased rates of aneuploidy in mouse embryos; this may in part explain the observed incidence of pregnancy loss in women exposed to BPA[9]. BPA also has cytotoxic actions on various cells and tissues through multiple signalling pathways[10]. For example, BPA at low doses induced apoptosis and up regulation of Bax, caspase-3 and down regulation of Bcl-2 in murine ovarian granulosa cells, indicating that the intrinsic apoptotic pathway is involved[11]. Apoptosis is a process of programmed cell death and involved in various physiological and pathological events[12]. Caspases are a group of cysteine proteases within cells and their activation cascade plays an important role following induction of apoptosis. Caspase-3, as a main final common executor of apoptosis, is responsible for the cleavage of the key cellular proteins, leading to typical morphological changes observed in cells undergoing apoptosis[13]. Apoptosis hall marks include inter nucleosomal DNA fragmentation, chromatin condensation, protease and endonuclease activation, membrane blebbing and cell shrinkage reported by Thornberry et al[14]. Placental development is a key event for the success of pregnancy. So abnormalities in this event may lead to embryo developmental defects and death[15]. Adequate placental growth and function are fundamental to the well being, growth, and development of the fetus throughout gestation. A complex interplay among fetal, placental, and maternal hormones regulates the rate of uterine growth[16], and the apoptosis, or physiological cell death, as a potentially important determinant of placental size and function. Thus, Smith et al[17] demonstrated that placental apoptosis is elevated in human pregnancies complicated by intrauterine growth retardation and placental apoptosis also increases over the course of normal human pregnancy. Development of an animal model to study the link between feto-placental growth and placental apoptosis is thus important, and so the present study investigated the extent of apoptosis in the rat placenta and uterus of BPA treated pregnant rats. In this study BPA administration to rats from Day 8th to 15th day of gestation caused antioxidant enzyme imbalance, hormonal changes and stimulated an increase in apoptotic markers in the placenta and uterus. In this study, we want to evaluate the role of BPA on antioxidant enzymes, we examined the effect of BPA on reproductive hormones and in addition we examined the effect of BPA on the expression profile of proapoptotic protein (Caspase-3) by western blot analysis.

MATERIALS AND METHODS

Maintenance of Experimental Animals

Healthy rats of Wistar strain were purchased from authorized vendor (M/S Raghavendra Enterprises, Bangalore, India). All rats were housed in polypropylene cages (18" 10"x 8") lined with sterilized paddy husk, and provided filtered tap water and rat food ad libitum in an air-conditioned environment (25±2°C) with a 12-h light and 12-in dark cycle. The experiments were carried out in accordance with the guidelines of the committee for the purpose of control and supervision on experiments on animals.

Experimental Protocol

Female Wistar rats three months old, weighing 250g to 300 g were used for the experiment .The status of estrous cycle stages were determined every morning between 7:00 am by collecting of vaginal secretion with a plastic pipette filled with 10 µL of normal saline (NaCl 0.9%) by inserting the tip into the rat vagina. One drop of vaginal fluid was placed on glass slides the unstained material was observed under a light microscope, without the use of the condenser lens, with 10 and 40 x objective lense. Two females of pro-estrous stage were paired with a male overnight and the next morning, males were removed and females were assessed for the presence of sperm in the vaginal flush. Animals with positive sperm in the flushes are designated as day 1 of gestation. Six pregnant rats were used in each experimental group.

Treatment

BPA was given orally on 8th to 15th day of gestation period (total 8 days) of female albino rats. After that

animals were sacrificed and took the serum and selected tissues were isolated for further investigations.

Estimation of antioxidant enzymes in rat serum after administration of BPA

Serum samples was used for the assay of antioxidant enzymes namely catalase, glutathione peroxidase, superoxide dismutase and lipid peroxidation.

Malondialdehyde contents (MDA) assay

0.5mL sample dilution was removed to the test tube, and 2 ml of 0.6% TBA solution was added. TBA solution was dissolved by a small amount of 1 mol/L sodium hyroxide, and then diluted to the concentration by 10% trichloroacetic acid. The test tube was sealed by plastic wrap with a little hole. After in the water bath boiling for 15 minutes, the test tube was cooled in the water, and then centrifuged at 10,000 rpm for 10 minutes. The absorbance value of the supernatant was measured at 450 nm, 532 nm and 600 nm of wavelength. The formula C = 6.45 × (D532 − D600) − 0.56 × D450 was used to calculated the concentration of MDA.

Estimation of reproductive hormones (Luteinizing hormone (LH), Follicles-stimulating hormone (FSH), Estrogen and Progesterone) in rat serum after administration of BPA

Estimation of serum Luteinizing hormone (LH), Follicles-stimulating hormone (FSH), Estrogen and Progesterone levels followed by RIA method

Western blot analysis

Placental and uterine tissues was homogenized in a lysis buffer (50 mM Tris-HCL, 150 mM Nacl , 0.1%SDS,100ug/ml PMSF,1ug/ml Aprorinin, 1% NP-40) and centrifuged at 4 o C for 10 min at 12000 X g. Protein homogenate of tissue containing 40µg protein denatured in a gel loading buffer (100 mM Tris-HCL (pH6.8), 200 mM DTT, 4% SDS, 0.2% Bromophenol blue, 20% glycerol) at 100o C for 3 min and loaded on a various appropriate concentrations of SDS-PAGE: 12% for Bcl-2 and 15% for caspase-3. Gels containing the SDS-PAGE separated proteins were equilibrated in transfer buffer (25 mM Tris, pH 8.3, 190 mM glycine, 0.05% SDS, and 20% methanol) and were electro transferred to nitrocellulose membranes. Blocking was performing with TTBS buffer (20 mM Tris, pH 7.4, 150 mM NaCl, and 0.05% Tween 20) containing 5% nonfat dry milk for 1 h. Then the transferred membrane was incubated with primary antibodies Bcl-2 (catalog no. SC-1004) and caspase-3 (catalog no.SC-11024) for the detection of apoptotic and antiapoptotic proteins. After exposure to horseradish peroxidise conjugated anti-rabbit IgG (caspase-3 and Bcl-2) secondary antibodies (diluted 2000-fold to 5000-fold) for 1 h, blots were washed and developed by enhanced chemiluminescence. Each blot was stripped with 100 mM glycine, pH 2.3, and was reprobed with β-tubulin to normalize for any variations incorporated in Protein loading. Densities of each protein of interest were expressed as a ratio to that of β-tubulin.

Statistical analysis

The mean, standard deviation (SD), percent change and one way analysis of variance (ANOVA) (Steel and Torrle) were performed using the Statistical Package for Social Sciences (SPSS) Package programming techniques on “Intel Core 2 Duo Processor” personnel computer. Probability values less than 0.05 were considered significant.

RESULTS

Effect of BPA on antioxidant enzymes (Super oxide dismutase, Catalase, Glutathione peroxidise, Lipid peroxidise) in rat serum

In the present study, super oxide dismutase, catalase, glutathione peroxidise activities in serum of BPA treated rats (lower dose or 50mg/kg.b.w/day and higher dose or 500mg/kg.b.w/day) showed significant variations ($P<0.05$), (Fig-1), when compared to control group. On treatment with BPA rats, showed significantly decreased super oxide dismutase, catalase, glutathione peroxidise activities, the gradual decreased super oxide dismutase, catalase, glutathione peroxidise activities were found in the serum of higher dose followed by lower dose BPA treated groups. Thus it is revealed that the above enzyme activities were decreased highly in higher dose of BPA treated rats when compared with control group. In the present study, the level of malondialdehyde activity in serum of BPA treated rats (lower dose and higher dose) showed significant variations ($P<0.05$), (Fig- 1), when compared to control group. On treatment with BPA rats, showed significant elevated levels of malondialdehyde activity, the gradual increased malondialdehyde activity was found in the serum of higher dose, lower dose BPA treated groups. Thus the above observations revealed that highly increased malondialdehyde activity in higher dose BPA treated group when compared to control group.

Table 1

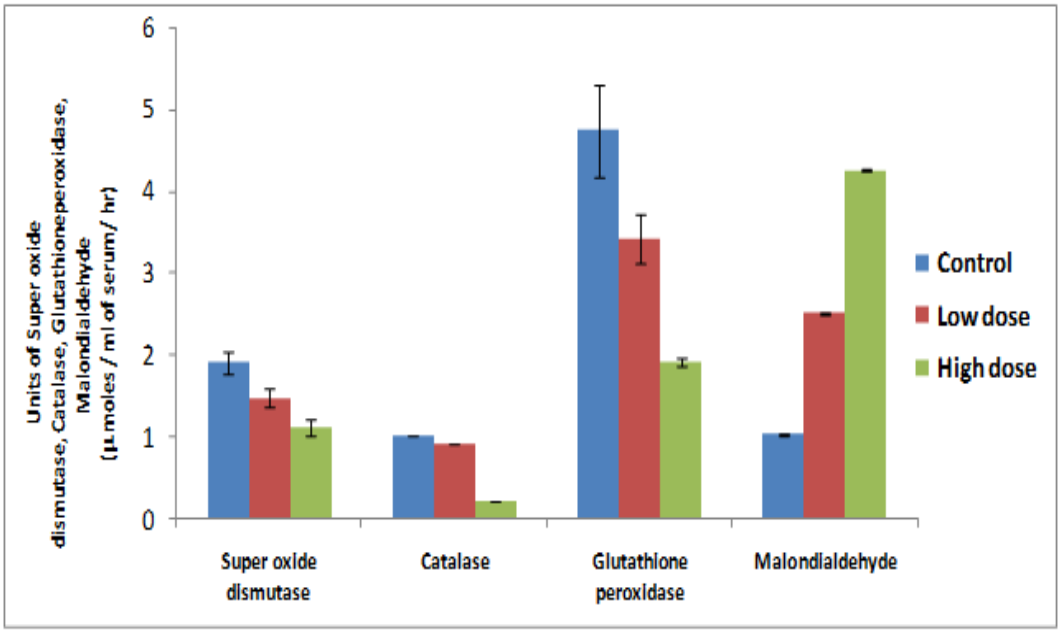
Changes in antioxidant enzyme levels (μ moles / ml of serum/ hr) of BPA treated rats and controls. Values in parentheses indicate percent change over control

Name of the Antioxidant enzymes	Control	Low dose (50 mg/kg/ body wt))	High dose (500 mg/kg/ body wt))
Super oxide dismutase Mean SD PC	1.914 ± 0.132	1.482 ± 0.105 (22.50)	1.125 ± 0.097 (41.20)
Catalase Mean SD PC	1.015 ± 0.003	0.927 ± 0.002 (8.60)	0.216 ± 0.001 (78.70)
Glutathione Peroxidase Mean SD PC	4.749 ± 0.559	3.428 ± 0.296 (27.80)	1.924 ± 0.039 (59.40)
Malondialdehyde Mean SD PC	1.040 ± 0.015	2.522 ± 0.014 (42.50)	4.254 ± 0.012 (88.60)

All the values are mean ± SD of six individual observations
SD: Standard deviation
PC: Percent change over control

Figure 1

Superoxide dismutase, Catalase, Glutathione peroxidase, Malondialdehyde activity Levels in different serum samples of albino rats exposed to BPA



Effect of BPA on reproductive hormones (LH, FSH, Estrogen, Progesterone) in rat serum:

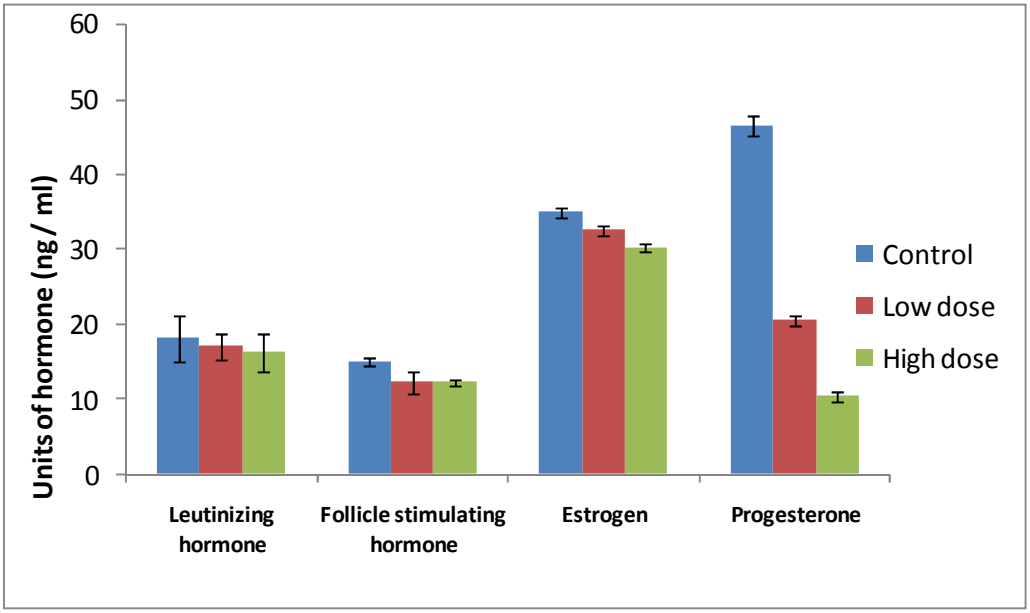
Table 2
Changes in hormone levels (ng / ml of serum) of BPA treated rats and controls. Values in parentheses indicate percent change over control

Name of the hormones	Control	Low dose (50 mg/kg/ body wt)	High dose (500 mg/kg/ body wt)
Leutinizing hormone			
Mean	18.10 ± 3.01	17.00 ± 1.80 (6.00)	16.23 ± 2.50 (10.30)
SD			
PC			
Follicle stimulating hormone			
Mean	14.92 ± 0.51	12.17 ± 1.54 (18.40)	12.14 ± 0.46 (18.60)
SD			
PC			
Estrogen (E_2)			
Mean	34.93 ± 0.71	32.46 ± 0.71 (7.00)	30.15 ± 0.58 (13.60)
SD			
PC			
Progesterone			
Mean	46.44 ± 1.31	20.42 ± 0.66 (24.80)	10.34 ± 0.67 (66.20)
SD			
PC			

All the values are mean ± SD of six individual observations
SD : Standard deviation
PC : Percent change over control

In the present investigation, statistical analysis revealed that there was significant variation (P<0.05) in the activity levels of LH, FSH, estrogen and progesterone in serum of lower and higher dose of BPA treated rats. The experimental rats exposed to BPA showed statically significant (P<0.05) decrease in LH, FSH, estrogen and progesterone activity levels in lower and higher dose than in control group respectively. The decrease in LH, FSH, estrogen and progesterone activity levels were dose concentration dependent in BPA treated rats. The gradual decrease was observed in BPA treated rats when compared to control rats.

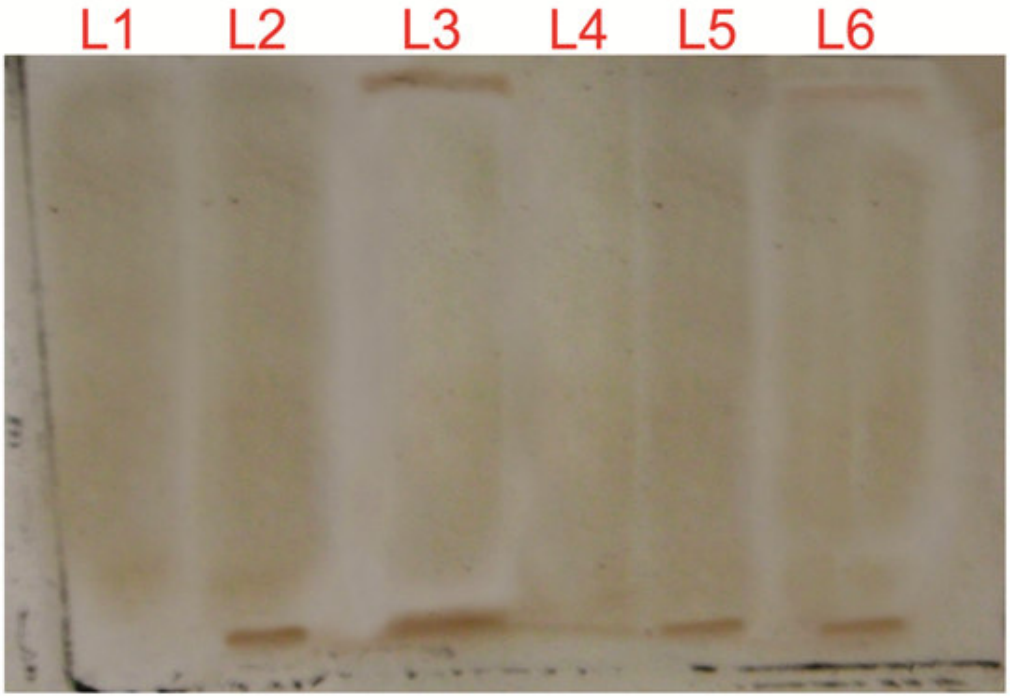
Figure 2
Leutinizing hormone, Follicle stimulating hormone, Estrogen, Progesterone activity levels in different serum samples of albino rats exposed to BPA



Effect of BPA on expression of proapoptotic protein caspase-3 in placenta and uterus:

Various degrees of active caspase-3 expression in the uterus and placental tissues of all groups (BPA treated and untreated control rats) were detected by Western blotting analysis (Fig-3). Image and statistical analyses showed that there was a significant difference in relative expression levels of active caspase-3 between the control and 50 and 500 mg BPA/kg/day. In the 50 and 500 mg BPA/kg/day rat groups, expression levels of active caspase-3 were increased when compared to corresponding controls. Moreover, expression of active caspase-3 was significantly increased with the increasing BPA dose and normal level of expression in untreated rats.

Figure 3
Westernblot analysis for expression of active caspase-3 protein in placenta and uterus of BPA treated and untreated control rats on day 15 of gestation



L1: *Control uterus, L2: Lower dose uterus, L3: Higher dose uterus, L4: Control placenta, L5: Lower dose placenta, L6: Higher dose placenta*

Figure 3.1
Westernblot analysis; cleaved caspase-3 was expressed in lower dose (50 mg/kg) and highest cleaved caspase-3 protein was expressed in higher dose BPA (500 mg/kg) treated group compared with the control. Standard is β-actin

CU: Control uterus, LU: Lower dose uterus, HU: Higher dose uterus, CP: Control placenta, LP: Lower dose placenta, HP: Higher dose placenta.

DISCUSSION

The results of the present study revealed that, serum antioxidant capacity was significantly decreased in BPA treated groups, normal level of antioxidant capacity was seen in control group. The activities of antioxidant enzymes SOD, CAT, and GPx were decreased significantly, while the levels of H_2O_2 generation and lipid peroxidation increased in the animals treated with BPA. Similar results were reported by Bindhumol et al[18], Chitra et al[19] and Kabuto et al[20]. Enzymatic scavengers like SOD, CAT and GPx protect the cell system from deleterious effects of ROS. The present data revealed that BPA caused marked oxidative impact as evidenced by the increase in lipid peroxidation (MDA level) as well as the decrease in antioxidants activities including SOD, CAT and GPx compared to their activities in control group. This might reflect the oxidative damage and inhibitory effect of BPA on antioxidants in serum. In this study, increased MDA concentration due to lipid peroxidation and decreased levels of antioxidant enzymes, resulting in oxidative cell damage. SOD is the first enzyme to respond against oxygen radicals (McCord and Fridovich, 1969) and is the one that offers the greatest response to oxidative stress (Winston and Di Giulio, 1991). SOD protects cells from oxidative stress and damage by catalyzing the conversion of $O_2^{\bullet-}$ to H_2O_2, a more stable reactive oxygen speices.

Catalase catalyzes the dismutation of the superoxide anion (O_2^-) into hydrogen peroxide (H_2O_2) which then converts hydrogen peroxide to water, in this manner, providing protection against reactive oxygen species. Hence, this enzyme protects cells from highly reactive hydroxyl radical (OH), derived from H_2O_2[21]. GPx, a seleno-enzyme that neutralizes ROS such as organic and hydrogen peroxides (Mates, 2000) activity in BPA treated groups showed a significantly decreased activity compared to control. CAT and GPx activities are fundamental to remove hydrogen peroxide from cytoplasm. In theory, reduced enzymatic activity implies that some ROS are not being quenched, thus predisposing cells to oxidative stress. The low GPx activity might be due to a direct BPA inhibition of enzyme synthesis or due to increased generation of hydroperoxide which may have inhibited the enzyme activity. Increased generation of hydroperoxide is capable of causing further cell damage and decreases the GPx capacity. Lipid peroxidation is often the first enzyme analyzed for proving the involvement of free radical damage. Thus, the presence of MDA is considered as an indicator of free-radical damage through lipid peroxidation. In the current study, the levels of serum MDA, a marker for lipid peroxidation, significantly increased in BPA treated groups and decreases the antioxidant capacity compared to control group, this result is in agreement with the previous results of Karafakioglu and Aslan[22]. Collectively, our experience shows that BPA may lead to induce toxic response of oxidative system throughout embryonic period. Therefore embryo protective pathways are disturbed by oxidative stress were able to inecrease the risk of free radicals generates from metabolites of BPA. Bindhumol et al., reported that treatment with BPA induced oxidative stress in various tissues of rodent by decreasing antioxidant enzymes and increasing hydrogen peroxide and lipid peroxidation. Bisphenol-A is a xenoestrogen and endocrine disruptor that is thought to act as a weak estrogen by binding to the estrogen receptor with low affinity. During critical periods of embryonic development, the hormonal milieu is crucial for the correct organization of neuroendocrine circuits that coordinate sex-specific physiology so, the altered expression levels of hormones at the hypothalamus and pituitary levels may be the cause and the consequence of the changes in gonadal steroidogenesis and sex hormone production[23]. The results of the present study revealed that, BPA induced a significant decrease in the LH, FSH, estrogen and progesterone levels in treated groups when compared to control group (Fig-2). The decrease of serum LH level in females could be resulted from BPA-induced reduction in Luteinizing hormone releasing hormone (LHRH) biosynthesis in the hypothalamus or/ and from direct effect of BPA on LH secretion from pituitary due to decrease stimulation of gonadotropes by GnRH (Gonadotrope releasing hormone) as a result of impairing inositol system. Knobil et al[24] pointed out that the decrease serum LH level by BPA may be due to the consequence of the increase in GnRH pulse frequency, leading to desensitization of the pituitary. Additionally, decrease of serum LH might cause by estrogenic action of BPA at the level of the HPG axis, mimicking the estrogenic suppression of LH, FSH secretion, while it inhibited LH, FSH secretion, suggesting that the effect of BPA on gonadotropes is specific to the mechanism that underlies the control of LH, FSH secretion. This selectivity of BPA on gonadotropin secretion may be due to, that the estrogen regulates LH mRNA more robust than that of FSH as mentioned by Furuta, et al[25]. It is evident in the present study that the decrease in the LH, FSH levels are associated with GnRH pulse frequency and estrogenic action of BPA in treated groups. Then BPA can alter the levels of progesterone receptor expression within the mediobasal hypothalamus. These induced changes in neural systems that could impact upon FSH and LH release. Gharravi et al[26] noticed significant decrease in serum level of LH in BPA treated rats daily intraperitoneally at 50 µg/kg/b. wt for 12 days. The decrease of serum estrogen level in the present study may be resulted from interference of BPA with developmental mechanisms of local regulatory circuits of hypothalamus and pituitary which occur during gestational period and seems to be a critical 'exposure window' for BPA to affect reproductive neural circuits in hypothalami of treated rats. Adewale[27] reported that, BPA produces its effects by interfering with one or both of the primary forms of the estrogen receptor (ER; formerly known as ERα and ERβ) within the hypothalamic-pituitary-gonadal (HPG) axis in rats. BPA is recognized as a potential environmental estrogenic chemical which can interfere with endogenous estrogen[28]. Estrogen (E2) plays an important role in regulating many physiological functions and in the development of diverse organs in vivo[29]. Estrogen is thought to be of essence in development of reproductive organs, bone, liver and cardiovascular systems[30]. In humans, estrogen exerts reproductive function and regulates the development of secondary sexual characteristics[31]. The estrogenicity of BPA, it could presumably disturb or mimic estrogen action, which is necessary for the normal maintenance of female reproduction and hormonal balance during adulthood. To date, many animal studies have focused on prenatal BPA exposure because animals are very sensitive to exogenous chemicals during these periods[32]. BPA exposure has also been associated with other endometrial defects, Pregnancy loss, uterus and placental growth restriction, endometriosis, and endometrial hyperplasia can each be driven by alteration of progesterone action[33]. BPA is a selective estrogen receptor modulator and antagonizing estrogens (E2's) ability to mediate progesterone action and alter activity levels in this study. Progesterone is produced in the ovaries and placenta and is required for the maintenance of pregnancy. The progesterone receptor (PR) mediates the physiologic effects of progesterone and has two isoforms, PR-A and PR-B, differentiated by the addition of 165 amino acids in the N terminus of PR-B[34]. An inadequate response to progesterone due to changes in PR gene expression can to lead to infertility, pregnancy loss etc. Estradiol normally maintains PR expression, xenoestrogen endocrine disrupting compound such as BPA typically mimic estrogen, acting as weak estrogen receptor agonists. Here we hypothesized that BPA acts as a selective endocrine disruptor with the ability to antagonize estrogen action on the expression of progesterone. In this study, we examined the effect of

BPA on the expression profile of apoptotic protein (proapoptotic protein caspase-3) in placenta and uterine tissues by western blot analysis. These results were in agreement with Benachour and Aris[35] and Rajareddy et al[36]. Apoptosis is a physiological process that is highly orchestrated at the molecular level by the activation of an aspartate-specific cysteine protease (caspase) cascade. The present study of results revealed that, an increase in apoptosis, thereby strongly inhibiting catalase activity, resulting in an increase in hydrogen peroxide, and that the oxidative stress elicited by excess H_2O_2 might activate caspase-3, which induces DNA fragmentation, leading to apoptosis. BPA administration has been shown to enhance oxidative stress and lipid peroxidation promoting apoptosis in treated tissues and disrupt cell-to-cell communication, because BPA can act as a pro-oxidant induces the formation of reactive oxygen species[37] are generated the free radicals. Free radicals are cytotoxic agents that lead to significant oxidative damage after causing covalently bind to oxidize cellular macromolecules such as DNA, protein and lipid lead to cell death. Similar to our results, Hassan et al[38] demonstrated that 50 mg/kg of BPA generate ROS, but reduce antioxidant content and activity (glutathione, superoxide dismutase, glutathione peroxidise). Then BPA induces over production of hydrogen peroxide, it is easily converted to hydroxy radicals who causes oxidative stress in cells leads to cell death and raises apoptosis in treated tissues. The induction of apoptosis is correlated with the activation of caspases, which have proteolytic activity and are able to cleave proteins to result in cell shrinkage, chromatin condensation and DNA fragmentation. We observed the significant increases in the activity of caspase-3 in BPA-treated rats when compared to the corresponding control group, suggesting that apoptosis is caspase dependent. Caspase-3, a key molecular marker of apoptotic signaling, is activated by either mitochondria dependent or death receptor dependent apoptotic pathways. Previous researches reported that BPA induced mitochondria dysfunction in the isolated rat hepatocytes[39] and human HepG2 cell[40], which led us to study the role of mitochondria played in liver cell apoptosis induced by BPA. Consistent with the change of caspase-3 activity and in placenta and uterine tissues of BPA-treated rats indicated an involvement of a caspase dependent apoptotic pathway of apoptosis. It is evident from our study that expression of the caspase dependent apoptotic pathway could indirectly explain the observed effects of BPA on placenta and uterine tissues of BPA-treated rats. Rajareddy et al., observed that the activation of the caspase-dependent apoptotic pathways in neonatal exposure to BPA rat ovary. Apoptosis first appears in the 32- to 64-cell embryo and can be demonstrated during the whole embryogenesis, when it plays an essential role in virtually all of the stages of development necessary to produce a normally developed newborn[41]. Then pathophysiology of pregnancy complications, such as intrauterine growth retardation, gestational hypertension, and preeclampsia caused by apoptotic changes. The present study has shown that BPA administration enhanced the apoptotic process in placental and uterine tissues.

CONCLUSION

Our studies, shown a significant lowering in the SOD, CAT, GPx and increased MDA, thereby inducing oxidative stress of BPA which was postulated to be associated with placental and uterine growth restriction. Uterine proliferation and differentiation during the pre implantation period in pregnancy have been well characterized, and these processes are dependent on LH, FSH, estrogen & progesterone. Progesterone is produced in the ovaries and placenta and is required for the maintenance of pregnancy. These hormones are necessary and sufficient to achieve endometrial development and to support pregnancy. In this study we concluded that, interaction between BPA and hormone receptors may take place during fetal development stage and causes the toxicological effects at a critical stage of reproduction and development (uterus & placental growth) in female rats. The present study has shown that BPA administration enhanced the apoptotic process and causes fetoplacental growth restriction by activation of caspase dependent pathway of apoptosis, these findings suggest that, BPA is toxic to placental and uterine growth in treated rats. In overall conclusion, this study also, sends several warnings to the mankind to exert more efforts at national and international levels to informing the world community about the deleterious effects of BPA as one of the endocrine disruptors agents not only on the pregnant dams but also on their offspring and should be make rules and precautions from the contamination and ingestion of such compounds to save ourselves and our offspring from the serious harmful effects.

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CONFLICT OF INTEREST

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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