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ORIGINAL RESEARCH ARTICLE

Monotonic Dose Effect of Bisphenol-A, an Estrogenic Endocrine Disruptor, on Estrogen Synthesis in Female Sprague-Dawley Rats

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Abstract Bisphenol-A (BPA) is a ubiquitous environmental chemical that produces adverse effect on reproduction system due to its potent estrogenic endocrine disruptive activity. The present study was aimed to investigate the monotonic dose effect of BPA on estrogen synthesis in female Sprague-Dawley rats. For this purpose, we administered three different doses of BPA (10, 50, 100 µg/kg bw/day) into rats and analyzed various biochemical, hormonal, molecular and histological parameters. 10 µg BPA treated rats showed significantly decreased levels of phase I detoxification agents (CYP450, Cyt-b5). Overexpression of eNOS with decreased expression of StAR and steroidogenic enzymes (CYP11A1, aromatase) indicate decreased production of estrogen. Increased levels of serum gonadotropins (FSH, LH) and decreased levels of estradiol suggest mimetic action of BPA and its feedback inhibition. Increased body weight, lipid profile status of 10 µg BPA treated rats and histological analysis of ovary and mammary tissue support the study. Overall, our results suggest that BPA exerts its estrogen mimetic effects in a monotonic manner.

Keywords Bisphenol-A · Estrogen · Ovary · Mammary gland

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Abbreviation

BPA	Bisphenol-A
TC	Total cholesterol
TG	Triglycerides
HDL-C	High density lipoprotein-cholesterol
TBARS	Thiobarbituric acid substances
GSH	Reduced glutathione
StAR	Steroidogenic acute regulatory protein
eNOS	Endothelial nitric oxide synthase
CYP11A1	Cytochrome P450 monooxygenase

Introduction

In recent years, the scientific knowledge about endocrine-disrupting chemicals (EDCs) has been increasing rapidly. EDCs are a global and ubiquitous problem and exposure to them occurs at home, in the office, on the farm, in the air we breathe, the food we eat and the water we drink. Of the hundreds of thousands of manufactured chemicals, it is estimated that about 1000 may have endocrine-acting properties [1]. EDCs are defined as an exogenous (xenobiotic) chemical, or mixture of chemicals, that interferes with any aspect of hormone action [2]. The endocrine system is one of the body's major interfaces with the environment, and play key roles in determining the quality of life, and many hormones are absolutely essential for survival. By interfering with the body's endocrine systems, EDC exposure can therefore perturbs many functions. EDCs have effects at extremely low doses (ppt to ppb range) to regulate bodily functions [3].

Bisphenol-A (BPA) is a well-known EDC and ubiquitous estrogenic chemical used for the manufacture of polycarbonate plastics such as water bottles, sports equipments, medical and dental devices, dental sealants, household electronics, eyeglass lens, thermal papers, etc. Epoxy resins containing BPA are used as coating PVC water pipe walls, coatings of all food and beverage cans. Worldwide, over 6 billion pounds of BPA used by the industries each year out of which over 100 tons are released into the environment [4].

BPA is a synthetic estrogen that interfere the estrogenic hormone system even exposures at trace amounts increasing the risk for developing breast cancer, prostate cancer, infertility, early puberty and other metabolic complications [5, 6]. Several in vivo studies have revealed the role of BPA on developmental alterations in reproductive organs [7, 8] as well as hormone signaling [9, 10]. Many in vitro studies have also stated that BPA has the ability to induce neoplastic transformation in human epithelial cells [11]. Many studies suggest that BPA promotes histological disruption in the reproductive tract [12, 13], estrous cycle modulation [14], diminished reproductive capacity [15], and altered hormone levels [9].

BPA induces excessive generation of reactive oxygen species (ROS) which exerts oxidative stress in the ovaries and mammary glands [16]. Increased nitrite level in oxidative stress enhances nitric oxide (NO) synthesis by overexpression of endothelial nitric oxide (eNOS) which affects estrogen production in granulose cells. eNOS plays an important role in the female reproduction including steroidogenesis, fertility and regulation of estrous cycle [17, 18]. Overexpression of eNOS downregulates the expression of StAR and steroidogenic enzymes such as CYP11A1 and CYP19.

The Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) reported that 50 μg/kg/day dose of BPA was considered as safe dose for human with reference to toxicity [19]. Since 2015, the European Environmental Safety Agency (EFSA) has revised the tolerable daily intake (TDI) to 4 μg/kg bw/day [20]. The adverse effects of BPA can be monotonic (no dose dependent) or non-monotonic dose response (NMDR) and most findings show that low doses of BPA (at μg level) actively interfere endocrine functions.

The present study hypothesized to investigate the monotonic dose effect of BPA on estrogen synthesis in female Sprague-Dawley rats after chronic administration of three different doses of BPA (10, 50, 100 μg/kg bw/day) by analyzing various biochemical, hormonal, molecular and histological parameters.

Materials and Methods

Chemicals

BPA (99.9% purity) was purchased from Sigma Aldrich chemicals Pvt. Ltd., Bangalore, India. Monoclonal antibodies for eNOS, StAR, CYP11A1, aromatase and β-actin were procured from Santa Cruz Biotechnology, USA. ELISA kits for TG, TC and HDL were purchased from Agappe diagnostics, Ernakulam, India. All the other chemicals used were of analytical grade.

Animal Model

Six to seven weeks old female Sprague-Dawley rats (weighed 100–120 g) were purchased from National Institute of Nutrition, Hyderabad, India and maintained in the Central Animal House, Annamalai University. The rats were maintained under a controlled condition of temperature (24 ± 2 °C) humidity (50 ± 10%), 12 h light/dark cycle feed and water provided ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee set up by the Committee for the Control and Supervision of Experimental Animal (CPCSEA Approval no: 1028).

Experimental Design

Total numbers of 24 rats were randomly divided into 4 groups each containing 6 rats. Group 1 animals served as control receiving just 1 ml of corn oil, whereas rats in groups 2–4 received BPA at doses of 10, 50, and 100 μg/kg bw (dissolved in 1 ml of corn oil), respectively. BPA was administered to rats orally daily using a gavages needle for a period of 12 weeks. At the end of 12th week, rats were kept fasting overnight and sacrificed by cervical decapitation. Blood, liver, ovary and mammary tissues were collected, processed, and stored for further experiments.

Biochemical Analysis

Excised mammary and ovary tissues were rinsed in ice-cold saline and a known amount of the tissue was homogenized in 0.1 M Tris–HCl buffer (pH 7.4) at 4 °C using a Teflon pestle. The homogenate was then centrifuged at 3000 g for 10 min and the supernatant was collected and used for analyzing various biochemical parameters. Microsomes from the liver and mammary tissues were isolated by the method of Hanioka et al. [21] and microsomal protein content was estimated by the method of Lowry et al. [22] and used for estimation of CYP450 and Cyt-b5 levels by the method of Omura and Sato [23]. The

concentration of serum TBARS was estimated by the method of Yagi [24]. The concentration of TBARS in the ovary and mammary tissues was estimated by the method of Ohkawa et al. [25]. Lipid extraction from serum was done by the method of Folch et al. [26]. SOD and CAT activities in the liver and mammary tissue were determined by the method of Kakkar et al. and Sinha [27, 28], respectively. GPx activity in the liver and mammary tissues was determined by the method of Rotruck et al. [29]. GSH in the liver and mammary tissues was determined by the method of Beutler and Kelley [30].

Lipid Profile

TC from serum was estimated by using the kit method of Zlatkis et al. [31]. TG in serum was measured by the method of Foster and Dunn [32]. HDL-C in serum was estimated by the method of Burnstein et al. [33] and the cholesterol content was determined by the method of Zlatkis et al. [31] LDL and VLDL-C levels were evaluated by the method of Friedwald et al. [34].

$$\text{LDL-C} = \text{TC} - (\text{HDL-C} + \text{VLDL-C}),\ \text{VLDL-C} = \text{TG}/5.$$

Analysis of Hormone Levels

Serum estradiol, progesterone, gonadotraphic hormones such as FSH and LH were measured using ELISA kits (BIOTRON DIOGNOSTICS INC, Hemet California, USA) according to manufacturer's instructions.

Histopathological Analysis

For histopathological analysis, the ovary and mammary tissues were sliced and immersed in 10% formalin solution for fixation, dehydrated with graded ethanol and then embedded in paraffin. Sections of 3–5 μm in thickness were cut and stained with haematoxylin and eosin and the slides were observed under the microscope.

Western Blotting

The ovary tissues were homogenized in ice-cold RIPA buffer and the homogenate was centrifuged at 12,000 rpm for 15 min at 4 °C. The supernatant was collected and the protein concentration was measured by the method of Lowry et al. [22]. The supernatant was denatured and a sample containing 50 μg of protein was loaded and separated using 10% SDS polyacrylamide gel electrophoresis. The separated proteins were transferred into PVDF membrane. The membranes were incubated with the blocking buffer containing 5% BSA (Bovine Serum Albumin) for 2 h to reduce non-specific binding sites and then incubated with the specific primary antibodies for overnight at 4 °C. After washing TBST thrice, the membranes were incubated with their corresponding secondary antibodies for 2 h at room temperature. Membranes were washed with TBST thrice and the protein bands were visualized using enhanced chemiluminescence method (GenScript ECL kit, USA). Bands were scanned using a scanner and quantified by ImageJ, a public domain Java image processing software.

Statistical Analysis

Statistical analysis was performed using SPSS 16 (SPSS, Inc., Chicago) statistical package. The data are expressed as mean ± standard deviation (SD). One way analysis of variance (ANOVA) followed by Duncan Multiple Range Test (DMRT) comparison method was used to correlate the difference between the variables. Data are considered statistically significant if p values are less than 0.05.

Results

Effect of BPA on Body Weight of Control and Experimental Rats

Table 1 shows the initial and final body weight of control and experimental rats. The final body weight of 10 μg BPA treated rats was significantly ($p < 0.05$) increased when compared to control rats, while the body weight of 50 and 100 μg BPA treated rats was significantly ($p < 0.05$) decreased when compared to control rats and 10 μg BPA treated rats and also significantly differ each other.

Effect of BPA on Antioxidant Status in the Liver and Mammary Tissues of Control and Experimental Rats

Tables 2 and 3 show the levels of antioxidants in the liver and mammary tissues of control and experimental

Table 1 Effect of BPA on body weight of control and experimental rats

Group	Initial (g)	Final (g)
I. Control	125.25 ± 8.12	192.21 ± 15.25[a]
II. BPA (10 μg)	132.51 ± 10.62	212.52 ± 17.01[b]
III. BPA (50 μg)	128.21 ± 8.52	172.35 ± 13.85[c]
IV. BPA (100 μg)	131.40 ± 11.67	155.62 ± 12.75[d]

Values are expressed as mean ± SD for six animals in each group

Values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT)

Table 2 Effect of BPA on antioxidant status in liver of control and experimental rats

Group	SOD	CAT	GPX	GSH
I. Control	8.95 ± 0.65[a]	72.04 ± 3.5[a]	9.32 ± 0.71[a]	53.36 ± 4.10[a]
II. BPA (10 μg)	7.92 ± 0.65[b]	63.05 ± 4.65[b]	8.35 ± 0.69[b]	44.05 ± 3.72[b]
III. BPA (50 μg)	7.54 ± 0.64[c]	61.20 ± 4.02[c]	8.00 ± 0.65[c]	42.12 ± 3.50[c]
IV. BPA (100 μg)	6.70 ± 0.59[d]	58.30 ± 3.95[d]	6.84 ± 0.59[d]	35.54 ± 2.95[d]

Values are given as mean SD from six rats in each group

The activities of enzymes are expressed as follows: *SOD* one unit of activity is 50% inhibition of nitrobluetetrazolium reduction in per min/mg protein, *CAT* μmoles of H_2O_2 consumed/min, GP_X μg of glutathione consumed per min/mg protein, *GSH* μg/mg of protein

Values not sharing a common superscript letter (a–d) differ significantly at $p < 0.05$ (DMRT)

Table 3 Effect of BPA on antioxidant status in the mammary tissue of control and experimental rats

Group	SOD	CAT	GPX	GSH
I. Control	14.97 ± 1.15[a]	51.25 ± 4.25[a]	14.32 ± 1.14[a]	14.62 ± 1.32[a]
II. BPA (10 μg)	12.65 ± 0.98[b]	46.01 ± 3.95[b]	12.76 ± 1.18[b]	12.21 ± 1.31[b]
III. BPA (50 μg)	11.55 ± 1.01[c]	43.45 ± 3.65[c]	12.26 ± 1.20[c]	12.73 ± 1.15[c]
IV. BPA (100 μg)	9.6 ± 0.74[d]	39.22 ± 2.95[d]	11.08 ± 1.01[d]	11.48 ± 1.09[d]

Values are expressed as mean ± SD for six animals in each group

The activities of enzymes are expressed as follows: *SOD* one unit of activity is 50% inhibition of nitrobluetetrazolium reduction in per min/mg protein, *CAT* μmoles of H_2O_2 consumed/min, GP_X μg of glutathione consumed per min/mg protein, *GSH* μg/mg of protein

Values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT)

Table 4 Effect of BPA on serum estrogen, progesterone, FSH and LH in control and experimental rats

Groups	Estrogen (pg/mL)	Progesterone (ng/mL)	FSH (mIU/mL)	LH (mIU/mL)
I. Control	127.50 ± 8.52[a]	18.52 ± 1.01[a]	1.98 ± 0.09[a]	9.89 ± 0.79[a]
II. BPA (10 μg)	115.52 ± 6.85[b]	16.21 ± 0.89[b]	3.10 ± 0.18[b]	13.20 ± 0.99[b]
III. BPA (50 μg)	120.23 ± 7.23[a]	17.65 ± 1.0[a]	2.50 ± 0.15[c]	11.10 ± 0.94[a]
IV. BPA (100 μg)	123.40 ± 7.90[a]	17.95 ± 1.09[a]	2.41 ± 0.12[c]	10.23 ± 0.90[a]

Values are expressed as mean ± SD for six animals in each group

Values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT)

rats. The activities of SOD, CAT and GPx and the level of GSH were significantly ($p < 0.05$) decreased in all BPA treated rats compared to control rats and also significant changes were also observed between each groups.

Effect of BPA on Estradiol, Progesterone and Gonadotrophic Hormones in Control and Experimental Rats

Table 4 shows the effect of BPA on serum estradiol, progesterone and gonadotrophic hormones (FSH and LH) in the control and experimental rats. Serum estradiol and progesterone levels were significantly ($p < 0.05$) decreased in 10 μg BPA (group II) treated rats compared to all other groups, whereas the levels of FSH and LH were significantly ($p < 0.05$) increased in 10 μg BPA (group II) treated rats compared to all other groups.

Effect of BPA on Lipid Profile in Control and Experimental Rats

Table 5 shows the status of serum lipid profile in control and experimental rats. The levels of TC, TG, LDL and VLDL were significantly ($p < 0.05$) increased with a slight decrease in the level of HDL in 10 μg BPA (group II) treated rats compared to control rats (group I). The levels of TC is significantly ($p < 0.05$) were increased in 50 and 100 μg BPA treated rats when compared with control rats and significantly differ when compared to 10 μg BPA treated rats, whereas HDL level was significantly decreased among 50 and 100 μg BPA treated rats as well as control rats (group I).

Table 5 Effect of BPA on serum lipid profile of control and experimental rats

Groups	TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
I. Control	104.86 ± 7.61^{a}	128.60 ± 7.21^{a}	32.12 ± 1.23^{a}	47.14 ± 2.63^{a}	25.60 ± 2.74^{a}
II. BPA (10 μg)	117.31 ± 8.60^{b}	148.21 ± 11.21^{b}	31.16 ± 1.80^{a}	56.95 ± 3.20^{b}	35.22 ± 2.20^{b}
III. BPA (50 μg)	110.51 ± 9.19^{c}	131.10 ± 8.86^{a}	28.35 ± 1.2^{b}	51.05 ± 2.60^{a}	27.08 ± 2.80^{a}
IV. BPA (100 μg)	109.24 ± 8.36^{c}	130.30 ± 8.20^{a}	25.08 ± 0.96^{c}	50.20 ± 3.25^{a}	27.80 ± 3.21^{a}

Values are expressed as mean ± SD for six animals in each group

Values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT)

Table 6 Effect of BPA on TBARS in the serum, mammary and ovary tissues of control and experimental rats

Groups	Serum (mM/dL)	Mammary tissue (mM/100 g wet tissue)	Ovary (nmole/mg protein)
I. Control	2.31 ± 0.14^{a}	1.67 ± 0.10^{a}	15.50 ± 1.18^{a}
II. BPA (10 μg)	2.38 ± 0.14^{a}	1.71 ± 0.12^{a}	21.51 ± 1.65^{b}
III. BPA (50 μg)	2.87 ± 0.19^{b}	2.15 ± 0.18^{b}	19.20 ± 1.46^{c}
IV. BPA (100 μg)	3.23 ± 0.23^{c}	2.56 ± 0.21^{c}	17.21 ± 1.32^{a}

Values are expressed as mean ± SD for six animals in each group

Values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT)

Table 7 Effect of BPA on phase I detoxification agents in the liver and mammary tissues of control and experimental rats

Group	Liver		Mammary tissue	
	Cytochrome P450	Cytochrome b5	Cytochrome P450	Cytochrome b5
I. Control	0.89 ± 0.07^{a}	0.59 ± 0.04^{a}	0.98 ± 0.13^{a}	0.68 ± 0.05^{a}
II. BPA (10 μg)	0.65 ± 0.05^{b}	0.46 ± 0.06^{b}	0.70 ± 0.09^{b}	0.55 ± 0.05^{b}
III. BPA (50 μg)	0.68 ± 0.11^{c}	0.50 ± 0.08^{c}	0.73 ± 0.11^{c}	0.63 ± 0.08^{c}
IV. BPA (100 μg)	0.72 ± 0.19^{c}	0.52 ± 0.18^{c}	0.74 ± 0.19^{c}	0.65 ± 0.14^{c}

Values are expressed as mean ± SD for six animals in each group

CYP450 nmoles/mg microsomal protein, *Cyt-b5* nmoles/mg microsomal protein

Values not sharing a common superscript differ significantly at $p < 0.05$ (DMRT)

Effect of BPA on Lipid Peroxidation in Control and Experimental Rats

Table 6 shows the level of TBARS in the serum, mammary and ovary tissues of the control and experimental rats. The level of TBARS in serum and mammary tissue was significantly ($p < 0.05$) increased in 50 and 100 μg BPA treated rats compared to control and 10 μg BPA treated rats. However, TBARS levels in the ovary tissues were significantly ($p < 0.05$) increased in 10 μg BPA treated rats when compared to control and 100 μg BPA treated rats.

Effect of BPA A on Phase 1 Detoxification Agents in Liver and Mammary Tissues of Control and Experimental Rats

Table 7 shows the levels of CYP450 and Cyt-b5 in the liver and mammary tissues of control and experimental rats. The levels of CYP450 and Cyt-b5 were significantly ($p < 0.05$) decreased in the liver and mammary tissues of all three doses of BPA but a more pronounced effect was observed in 10 μg BPA treated rats when compared to control rats. However, there was no significant difference between 50 and 100 μg BPA treated rats.

Histopathological Changes in Mammary Tissues of Control and Experimental Rats

Figure 1 shows the histopathological analysis of the mammary tissues of control and experimental rats. The control rats showed a normal architecture of mammary tissue, while BPA 10 μg treated rats showed hyperplasia of mammary epithelial cells. Rats treated with BPA 50 and 100 μg showed mild hyperplasia of epithelial cells with abnormal architecture of mammary tissues.

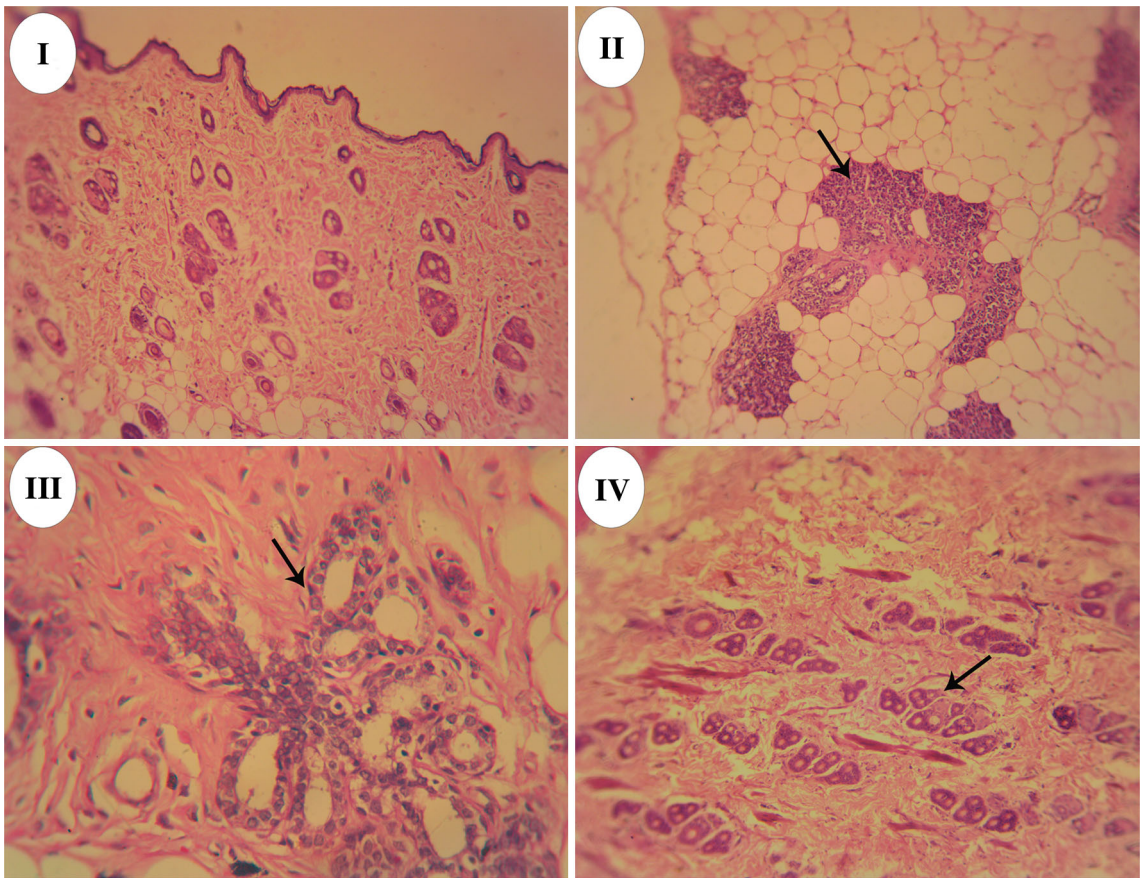

Fig. 1 Histopathological analysis of the mammary tissues of control and experimental rats. The control rats showed normal architecture of mammary tissues (**I**). Low dose BPA treated rats showed higher percentage of hyperplasic mammary epithelial cells (**II**). Medium dose BPA treated rats showed mild to moderate hyperplasic cells (**III**). High dose BPA treated rats showed mild hyperplasic of epithelial cells with abnormal architecture of mammary tissues (**IV**)

Histopathological Changes in Ovary of Control and Experimental Rats

Figure 2 shows the histopathological analysis of the ovary tissues of control and experimental rats. The control rats showed a normal architecture of mammary tissue, while BPA 10 µg treated rats showed decreased number of antral follicles. Rats treated with BPA 50 and 100 µg showed increased number of antral follicles.

Western Blot Analysis of eNOS, StAR, CYP11A1, Aromatase (CYP19) in Ovary of Control and Experimental Rats

Figure 3 shows western blot analysis of eNOS, StAR, CYP11A1, aromatase (CYP19) in the ovary of control and experimental rats. Overexpression of eNOS and downregulation expression of StAR, CYP11A1 and aromatase were observed in rats treated with 10 µg BPA compared to control rats. Treatment with BPA of 50 and 100 µg downregulated the expression of eNOS with overexpression of StAR, CYP11A1 and aromatase compared to 10 µg BPA treated rats.

Discussion

Globally, the prevalence and extensive use of BPA-containing products is a real threat to human beings [4]. BPA leaching occurs at high temperature, acidic or basic conditions in food and drink containers [35]. National Toxicology Panel (NTP) recommends avoiding microwaving food in plastic containers, using dishwasher, for cleaning plastics or using harsh detergents.

BPA acts as a highly potent estradiol (E2) mimetic and also disrupts the action of estradiol [36]. Estrogen plays a vital role in food intake and energy metabolism which contribute overall inhibitory effects on adipose in adulthood [37]. Prenatal exposure of BPA at low doses (1 mg/L) induces hyperinsulinemia, mild insulin resistance, glucose intolerance, obesity and hyperlipidemia in mice. BPA reduces adipocyte numbers but increases adipocyte volume

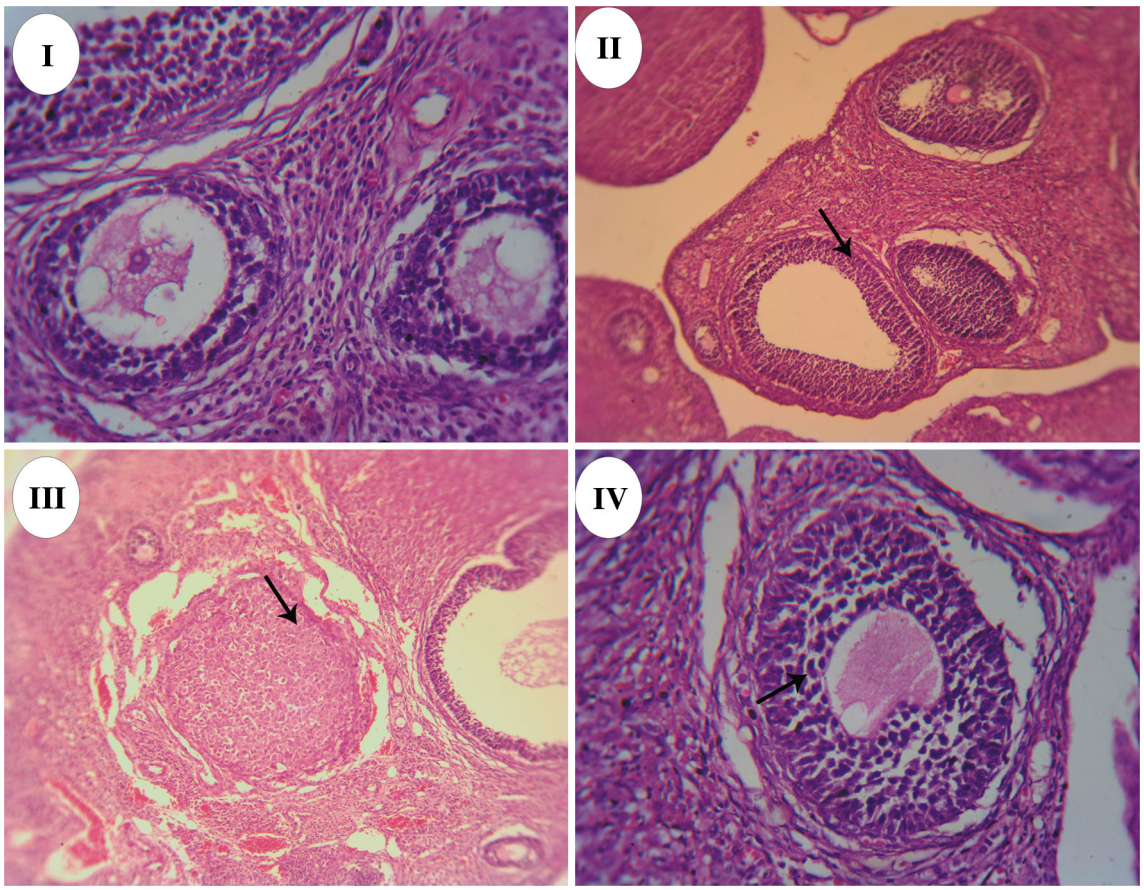

Fig. 2 Histopathological analysis of the ovary of control and experimental rats. The control rats showed normal oocytes and antral follicles of ovary tissues (**I**). Low dose BPA treated rats showed a decreased level of antral follicles compared to other two doses (**II**). Medium and high dose BPA treated rats showed increased level of antral follicle compare to low dose treated rats (**III** and **IV**)

with increase in body weight [38]. The changes in body-weight might be due to estrogenic action of BPA in the non-reproductive tissues particularly in adipose tissue. BPA disrupts the regulation of vital genes involved in lipid metabolism, mainly in cholesterol biosynthesis, associated with hypercholesterolemia, hyperglycemia and glucose intolerance in adult mice [39]. In this study, we observed an increased body weight, serum total cholesterol and triglycerides in 10 μg BPA treated rats which may be due to increased adipocyte volume and deposition of triglycerides. The above mentioned reports are in line with our results.

BPA activity depends on cell types and is influenced by a number of intrinsic and extrinsic factors. BPA exerts multiple effects on different cell types and non-single dose response at the cellular and intracellular levels at relevant doses. Exogenous estrogen modulates physiologically active hormonal system. Estrogenic chemicals induce cell proliferation at lower concentration and inhibit at higher concentration in MCF-7 cells [40]. The receptor mediated action of BPA increases initially and then decreases as the dose increases which are due to the property of receptor saturation i.e. no further increase in number of occupied receptors as a function of increase in dose once all the receptors are occupied [41]. In this study, we observed reduced level of estrogen and increased levels of gonadotropic hormones such as FSH and LH in 10 μg BPA treated rats. This may be antagonizing action of BPA which leads to feedback suppression of hypothalamic gonadotropic hormones. Further, the results are strengthened by histopathological observation of ovary and its decreased antral follicles may be linked with impaired estrogen production by theca cells.

BPA also inhibits the synthesis of estrogen via altering the enzymes of steroidogenesis. The process of steroidogenesis depends on StAR protein and the activities of enzymes such as eNOS, CYP11A1 and aromatase. Integral expression of eNOS is known to modulate estrogen production, fertility, regulation of estrous cycle in rat granulosa cells [17, 42]. Some Studies have also suggested that NO suppresses the expression and activity of StAR and CYP11A1 [43, 44]. Previous in vitro studies reported that elevated level of NO also concealed leydig cell function via inhibition of CYP11A1 [45, 46]. Reduced expression of StAR protein leads to a reduced transfer of cholesterol which impairs steroidogenic enzymes leading to cause

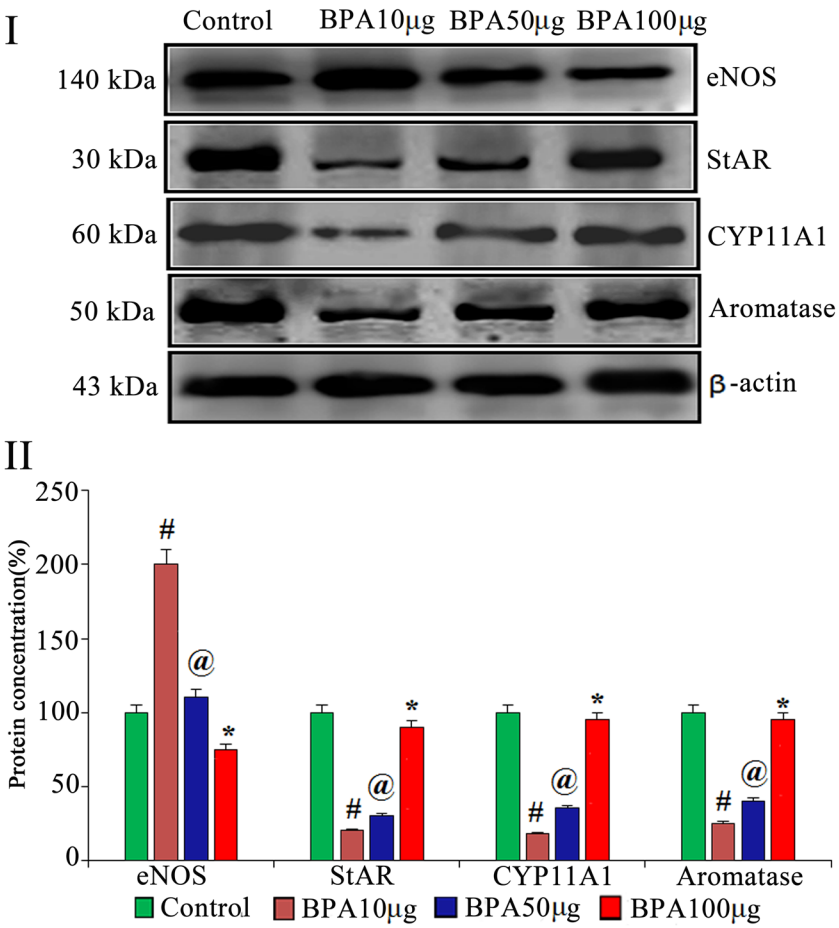

Fig. 3 Western blot analysis. **I** Representative western blot analysis of proteins. Protein samples (100 mg/lane) resolved in SDS-PAGE were probed with corresponding antibodies. β-actin was used as loading control. II Densitometric analysis of protein bands. The protein expression from control was designated as 100% in the graph. Values are given as mean ± SD for groups of six rats in each. Values not sharing a common superscript (hash, asterisk) differ significantly with each other ($p < 0.05$; DMRT)

decline the aromatase activity resulting in reduction of estrogen in BPA exposed female rats. Peretz et al. [47] revealed that different doses BPA (44 and 440 mM) inhibit growth of follicle and hormonal production through altering steroidogenic enzymes. Chronic exposure of BPA oral doses 0.001–0.1 mg/kg bw in adult female rats causes augmentation of follicular atresia and luteal regression by decreasing estradiol production through the inhibition of aromatase in ovary [48].

BPA can promote tissue damage via excessive generation of free radicals [49]. Previous studies reported that BPA promotes the inhibition of mitochondrial membrane complex I activity which results further increase in oxidative stress [50]. In this study, the antioxidant depletion in ovary leads to elevated level of TBARS which in turn induces over of expression eNOS. The eNOS prevents StAR transport from outer to inner mitochondria membrane which inhibits CYP11A1 leading to down regulation of aromatase enzyme. Our results are in corroborating with the study of Lee et al. [48]. Liver and mammary microsomal detoxification agents such as Cyp-450 and Cyp-b5 are decreased more in low dose BPA treated rats in this study indicates its inhibitory effect on cytochrome P450 s family enzymes [51]. Previous studies reported that rats treated with BPA and chlorotriazines inactivate the function of liver CYP2C11 [52].

Conclusion

Bisphenol A is well-known environmental xenobiotic chemical that interferes with estrogen hormone. Chronic BPA exposure to rats at three microgram level doses, significant results such as inhibition of steroidogenic enzymes and decreased level of estrogen and increased level of serum gonadotropins (FSH, LH) were observed in 10 μg BPA treated rats. Antioxidant and lipid peroxidation status reveals dose dependent action. Hence, the endocrine disruptive action of BPA is not dose dependent and at this dose it exerts estrogenic activity than toxicity. Further study about the mechanism of action is warranted to understand the signaling pathways.

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Compliance with Ethical Standards

Conflicts of interest All authors declare they have no conflicts of interest.

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