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Effect of bisphenol A on morphology and apoptosis in the mammary gland of adult Wistar rats

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Abstract

Background: The environmental factors affect the health of human beings by direct and indirect exposure. One of the factors is bisphenol A (BPA) which is used in the manufacturing of epoxy resins and polycarbonate plastics. The study was conducted to evaluate the toxic effect of BPA on mammary gland in female Wistar rats by histological and immunohistochemistry process. Females were administered BPA orally (5, 50, 300, 600, and 800 mg BPA/kg bw/week) for 90 days. The control groups received olive oil only.

Result: BPA induced a decrease in the number of ducts and fibrous collagenous connective tissue and was increased in non-pregnant, but the number of ducts and connective tissue showed no significant changes in cesarean and post-term females. Immunohistochemical results showed a significant increase in apoptotic color in non-pregnant whereas, in cesarean and post-term, no significant color was observed.

Conclusion: The study concludes that BPA induced structural changes and affected the mammary gland.

Keywords: Bisphenol A, Histology, Immunohistochemistry, Mammary gland

Background

Bisphenol A is a chemical compound, commonly used in industries for the manufacturing of polycarbonate plastics, where BPA releases into food or beverages in contact with the plastics (ECB, 2008). It acts as endocrine disruptors (EDs) in animals, including human. EDs act like hormones in the endocrine system and disrupt the physiologic function of endogenous hormones and may lead to negative health effects (Valentino et al. 2013). In human, the BPA is absorbed rapidly following ingestion and converted to a number of metabolites in the liver, mainly BPA glucuronide (Fenichel, Chevalier, and Brucker-Davis, 2013). Studies with BPA showed the increased susceptibility to cancerous changes (Jenkins et al. 2009), effect on fertility and reproductive tract Al-Hiyasat, Darmani, and Elbetieha, 2002), oxidative toxicity (Kabuto, Amakawa, and Shishibori, 2004), neurotoxic effects (Le, Carlson, Chua, and Belcher, 2008), genotoxic effects (Karim and Husain, 2010), and other health problems (Fernandez et al. 2007).

The mammary gland is composed of the epithelium and the stroma. The epithelium develops into branching ductal (ductal and alveolar) that is surrounded by the complex stroma, the mammary fat pad, which contains adipose tissue, fibroblasts, blood vessels, and immune cells (Gjorevski and Nelson, 2011; Tiede and Kang, 2011). In a mature mammary gland, alveoli are rarely present in the quiescent glands that do not lactate. Their alveoli are fully developed only in the course of pregnancy and lactation. The secretory ducts of the mammary gland start to sprout during pregnancy. Alveoli and lobules form. The connective tissue recedes. At the height of lactation, differently shaped alveoli are formed in close proximity of each other, and secretory products are visible in some of the gland (Russo and Russo, 1987).

Studies of EDC like BPA effects in rodents indicate that multiple toxicants can alter mammary gland development and physiology, with or without changing other markers of puberty (Macias and Hinck, 2012; Watson and Khaled, 2008). EDCs can cause transient and persistent effects on mammary gland development

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depending on dose, exposure parameters, and whether exposure was during critical periods of gland growth or differentiation. Adverse effects from these abnormal developmental patterns include the presence of carcinogen-sensitive structures in greater numbers or for longer periods in the gland and inhibited functional differentiation leading to malnutrition or increased mortality of their offspring. Developmental toxicants of the mammary gland could lead to an increase in the incidence of mammary tumors if they alter circulating or tissue-localized hormone levels, gland receptor expression patterns, hormone transport, or metabolism that results in altered response to endogenous hormones or growth factors. Environmental disruptors of rodent mammary gland development must be identified for informed decisions in epidemiological studies aimed at identification of environmental factors contributing to breast cancer risk, altered breast development during puberty, or inability to produce sufficient breast milk (Fenton, 2006).

The role of hormone modulation on non-pregnant adult females show few ducts and surrounded by dense connective tissue but in late pregnancy and lactation, ducts become enlarged and connective tissue sheath surrounds each alveolus (Halperin, Dorfman, Fraunhoffer, and Vitullo, 2013). At late pregnancy, the marked expansion of alveolar part of the glandular tissue and minimal interlobular adipose connective tissue observed when the pattern of E-cadherin expression in the mammary gland is studied (Abunasef and El-Beshbishy, 2014). Moreover, intraluminal secretion and lactational changes such as secretory changes in epithelial cells and secretory product in the lumen were observed in neonatal genistein treatment group (Foster, Younglai, Boutross-Tadross, Hughes, and Wade, 2004). The carmine staining of mammary glands of mouse reveals the decreased density of ducts and tubules compared with control when exposed to alcohol in the gestation period (Amos-Kroohs et al. 2016). In addition, a decreased number of the alveolar lumen and reduced proliferation of ducts at lactation and did not affect virgin when Panx1 ablated form mammary gland (Stewart, Plante, Penuela, and Laird, 2016).

In non-pregnant, the strong positive E-cadherin reaction of the epithelial lining of mammary gland was observed and faint positive reaction in the alveolar part of the late pregnancy and at lactation (Abunasef and El-Beshbishy, 2014). The hormone modulation affects the mammary gland immune reaction. The mammary ductal system of non-pregnant females have a positive expression of prolactin but the immunoreactivity increases as pregnancy progress, in the epithelium and in stroma strong the reactions are visualized in lactating females (Halperin et al. 2013).

The mammary gland of BPA-treated group are showing relatively intense immunoreactions in the form of a brown color in both the epithelial cells of the mammary gland and connective tissue as compared to control group when stained with Ki-67, activated caspase-3, and ER-α in non-pregnant adult female rats (Ibrahim, Elbakry, and Bayomy, 2016). Similarly, increased apoptosis with elevated expression of caspase-3 was detected in the testis of mice pups exposed to BPA during pregnancy and lactation (Liu, Chen, Wang, Shen, and Zhao, 2013). Interstitial tissue was positive for annexin in virgin rats. However, the immunoreactivity of annexin became weaker in the epithelial cells of the mammary gland of lactating, but mammary epithelial cells became positive for annexin in pregnant females (Duangjai, Tomohiro, Shiro, and Mitsumori, 2010).

There is an increasing rate of breast cancer worldwide, and there are several environmental factors that have been implicated in this increase. This study aimed to investigate the influence one such factor, BPA, on the histological and immunohistological structure of the resting, late pregnancy, and lactating mammary gland of the adult female albino rat, and to explore its effect on epithelial cell proliferation and apoptosis status.

Materials and methods

Test material

Bisphenol A [2, 2-bis (4-hydroxyphenyl propane)] (purity 099.5%, CAS no. 80–05-7) was purchased from Sigma Aldrich and diluted with olive oil to obtain a final concentration of doses of groups.

Animals

Adult female Wistar rats, 5–6 months old, weighing 180–220 g, were used in the investigation. The animals bred in our laboratory and maintained in the Departmental Experimental Facility with light and dark (12 h/12 h) schedule in an individual cage. The temperature in the animal house during the study period was maintained at 23 ± 2 °C and relative humidity was ranged between 32 and 70%. Animals were fed with rat pellet diet (Ashirwad Industries Limited, Chandigarh) and free access to safe drinking water ad libitum in glass bottles. The animals were maintained under perfect veterinary supervision and in accordance with the guidelines of *Committee for the Purpose of Control and Supervision of Experiments on Animals* (CPCSEA, 2007).

Ethical approval

The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC), Department of Zoology, University of Rajasthan, Jaipur.

Experimental design

Animals were allocated into 6 groups, containing 10 animals each. Parallel control used for termination of

each phase. During treatment, all animals were allowed to mate and get pregnant depending on the possibilities of the outcome, namely, non-pregnant, cesarean and full-term of pregnancy.

Group I: Control (olive oil alone)
Group II: Oral administration of 5 mg BPA/kg bw/week
Group III: Oral administration of 50 mg BPA/kg bw/week
Group IV: Oral administration of 300 mg BPA/kg bw/week
Group V: Oral administration of 600 mg BPA/kg bw/week
Group VI: Oral administration of 800 mg BPA/kg bw/week

Autopsy schedule

Doses were given orally every 168 h for 13 weeks (91 days). During the treatment, only group I got pregnant and divided into cesarean and full-term subgroups whereas the rest of the group (III–VI) did not respond toward pregnancy and kept under non-pregnant subgroup. Necropsies were done at the end of the experiment and rats were sacrificed 24 h following the last given dose that is 91st day for non-pregnant. For cesarean and post-term females, necropsied of animals were done at the 20th day of gestation and after delivery of litters respectively.

Histopathology

The mammary gland was fixed in 4% paraformaldehyde, dehydrated in ethanol, cleared in Xylene, and embedded in paraffin wax. Five-micron-thick section was stained with hematoxylin and eosin for light microscopic observation.

AnnexinDetection of apoptosis by immunohistochemistry in mammary glands

Immunohistochemical staining of mammary gland tissue was done by Avidin-Biotin Complex (ABC) Staining Method (Hsu, Raine, and Fanger, 1981). After heat induction of formallinn fixed paraffin embedded tissues, annexin V antigen was retrieved. 2.5 μg/ml of primary antibody (anti-annexin A5 mouse Ab) was used for staining. After incubation with the primary antibody, slides were incubated with biotinylated secondary antibody (conjugated with anti-mouse and anti-rabbit horseradish peroxidase), followed by horseradish peroxidase-streptavidin and chromogen (diaminobenzene).

Results

There were no deaths recorded throughout the experimental period.

Phase I (non-pregnant)

The H&E-stained sections of non-pregnant rat mammary gland showed abundant fibrous collagenous tissue (*), and most of the ducts (arrow) were lined with a single layer of epithelial cells in control group (Fig. 1a). BPA-treated groups (50, 300, 600, and 800 mg/kg bw/week) showed a decrease in the number of ducts (arrow) that were lined with a single layer of epithelial cells and abundant fibrous collagenous connective tissue(*) as the dose increased (Fig. 1b–e).

Microscopic examination of immunostained sections showed a faint positive reaction in the epithelial linings of ducts (arrow) and fibrous connective tissue (*) in the control group (Fig. 1f). BPA-treated groups (50, 300, 600, and 800 mg/kg bw/week) showed dark positive brown reaction localized to all the borders of epithelial cell lining of ducts (arrow) and fibrous collagenous connective tissue (*) as the dose increased (Fig. 1g–j).

Phase II (cesarean)

The H&E-stained sections of cesarean rat mammary gland showed marked proliferation of the alveolar part of the lobules at the lesser extent of fibrous collagenous tissue in the control group (Fig. 2a). BPA-treated group (5 mg/kg bw/week) showed no significant changes in it (Fig. 2b).

Microscopic examination of immunostained sections showed a very faint positive reaction in the fibrous connective tissue and negative in alveolar parts in the control group (Fig. 2c). BPA-treated group (5 mg/kg bw/week) showed similar results with control (Fig. 2d).

Phase III (post-term)

The H&E-stained sections of cesarean rat mammary gland showed secretory epithelial cells of alveoli. A very thin fibrous collagenous tissue sheath surrounds each alveolus. The lumen of the alveoli is full of milk in the control group (Fig. 3a). BPA-treated group (5 mg/kg bw/week) showed no significant changes in it (Fig. 3b).

Microscopic examination of immunostained sections showed a very faint positive reaction in the fibrous collagenous tissue sheath surrounds each alveolus and negative reaction localized to the alveolar epithelial cell linings in the control group (Fig. 3c). BPA-treated group (5 mg/kg bw/week) showed similar results with control (Fig. 3d).

Discussion

The results presented herein underscore the consequences of BPA exposure in non-pregnant, cesarean, and post-term female rats, and the histological and immunological structure of the mammary gland was examined. In histological sections, BPA caused a decrease in number of the ducts, and an altered amount of dense coarse fibrous

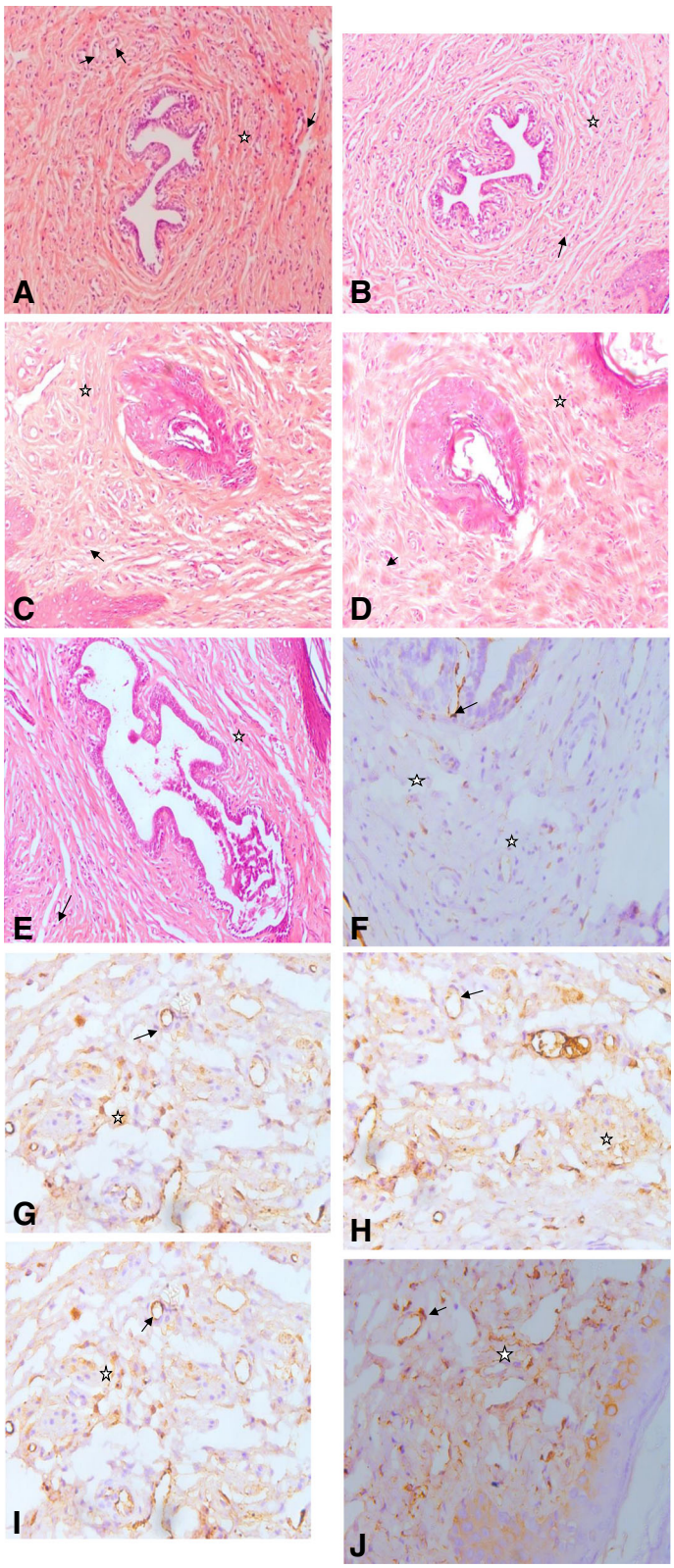

Fig. 1 (See legend on next page.)

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Fig. 1 a In mammary gland (bar = 100 μm), most of the ducts (arrow) were lined with a single layer of epithelial cells in control group and is surrounded by abundant fibrous collagenous tissue (*). H&E, × 200. **b** In 50 mg BPA-treated group (bar = 100 μm), a decrease in the number of ducts (arrow) that were lined with a single layer of epithelial cells and very abundant fibrous collagenous connective tissue (*). H&E, × 200. **c** In 300 mg BPA-treated group (bar = 100 μm), the number of ducts (arrow) that were lined with a single layer of epithelial cells are decreasing and fibrous collagenous connective tissue are very abundant (*). H&E, × 200. **d** In 600 mg BPA-treated group (bar = 100 μm), there were less number of ducts (arrow) that were lined with a single layer of epithelial cells and very abundant fibrous collagenous connective tissue (*). H&E, × 200. **e** In 800 mg BPA-treated group (bar = 100 μm), there were rare number of ducts (arrow) that were lined with a single layer of epithelial cells and very abundant fibrous collagenous connective tissue (*). H&E, × 200. **f** In mammary gland (bar = 50 μm), a faint positive reaction in the epithelial linings of the ducts (arrow) and fibrous connective tissue (*) in control group was observed, × 400. **g** In mammary gland of 50 mg BPA-treated (bar = 50 μm), dark positive brown reaction localized to all the borders of epithelial cell lining of ducts (arrow) and fibrous collagenous connective tissue (*), × 400. **h** In mammary gland of 50 mg BPA-treated (bar = 50 μm), dark positive brown reaction localized to all the borders of epithelial cell lining of ducts (arrow) and fibrous collagenous connective tissue (*), × 400. **i** In mammary gland of 600 mg BPA-treated (bar = 50 μm), very dark positive brown reaction localized to all the borders of epithelial cell lining of ducts (arrow) and fibrous collagenous connective tissue (*), × 400. **j** In mammary gland of 800 mg BPA-treated (bar = 50 μm), intense dark positive brown reaction localized to all the borders of epithelial cell lining of ducts (arrow) and fibrous collagenous connective tissue (*), × 400

collagenous connective tissue was formed around the epithelial structures of ducts in mammary gland of non-pregnant females, the number of ducts and epithelial structure and dense coarse fibrous collagenous connective tissue remained unaltered in both cesarean and post-term females of Wistar rats. These results were compared with some previous studies that examined on the mammary gland of different rodents.

Vandenberg et al. 2008 reported that the number of ducts decreases in mammary glands but the density of

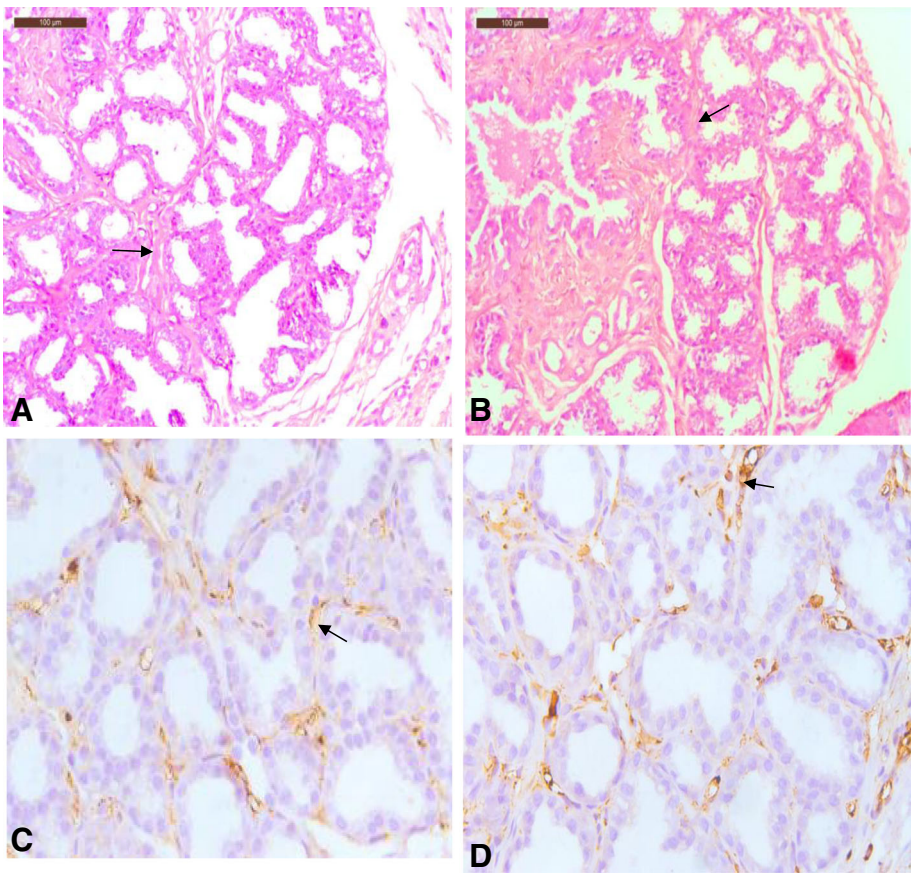

Fig. 2 a In mammary gland (bar = 100 μm), the stromal connective tissue further recedes (arrow) and the alveolar buds progressively increased in the control group. H&E, × 200. **b** In mammary gland of 5 mg treated (bar = 100 μm), the alveolar buds increased and connective tissue recedes (arrow). H&E, × 200. **c** The epithelial of ducts does not show positive staining, but stromal tissue (arrow) shows faint positive staining in control group of the mammary gland (bar = 50 μm), × 400. **d** The epithelial of ducts does not show positive staining, but stromal tissue (arrow) shows faint positive staining in 5 mg BPA-treated group of the mammary gland (bar = 50 μm), × 400

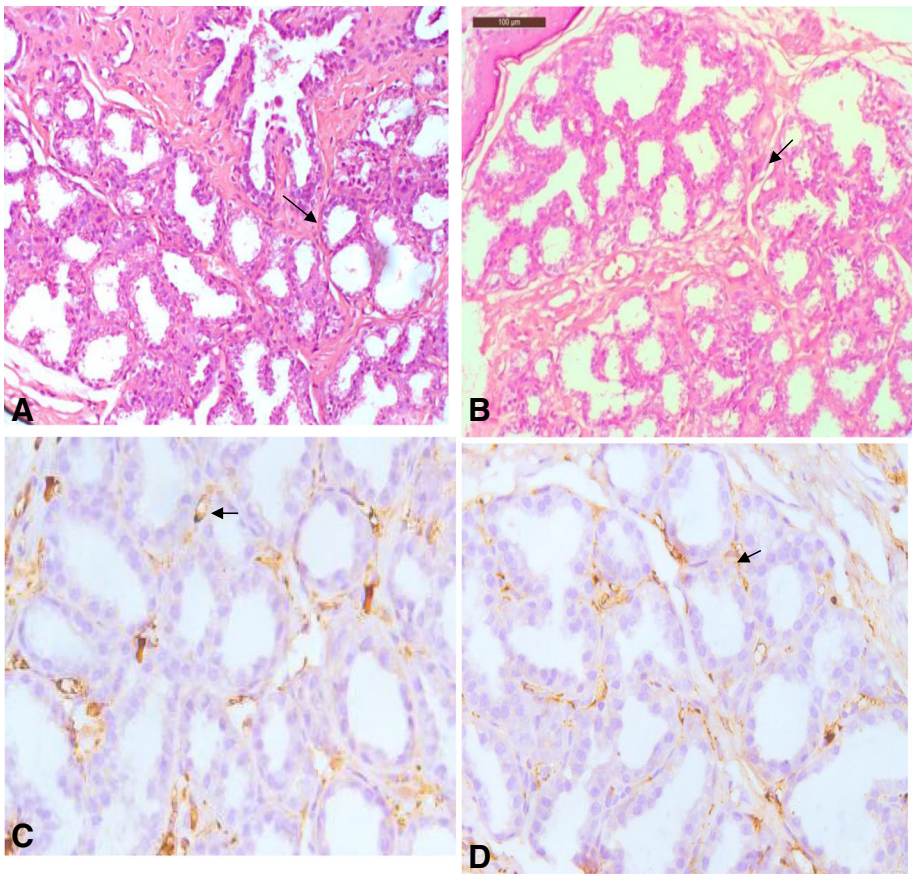

Fig. 3 **a** In mammary gland (bar = 100 μm), the stromal connective tissue further recedes (arrow) and the alveolar buds progressively increased in the control group. H&E, × 200. **b** In mammary gland (bar = 100 μm), the stromal connective tissue further recedes (arrow) and the alveolar buds progressively increased in 5 mg BPA-treated group. H&E, × 200. **c** The epithelial of ducts does not show positive staining but stromal tissue (arrow) shows faint positive staining in control group of the mammary gland (bar = 50 μm), × 400. **d** The epithelial of ducts does not show positive staining but stromal tissue (arrow) shows faint positive staining in 5 mg BPA-treated group of mammary gland (bar = 50 μm), × 400

collagen remains as such in BPA-treated mice (Vandenberg et al. 2008). BPA induced an increase in the number of the acini and ducts in the mammary gland of treated rats. The collagen fiber content was significantly increased in the connective tissue stroma separating the ducts (Ibrahim et al. 2016). However, perinatal exposure to 0.25 mg/kg BPA can induce increased ductal mammary growth in offsprings (Mandrup, Boberg, Isling, Christiansen, and Hass, 2016). The decreased density of collagen fibers in the stromal compartment was observed in the fetal mammary glands of BPA-exposed mice (Vandenberg et al. 2007; Wadia et al. 2013). BPA exposure has been shown to alter collagen expression within the mammary gland when exposure occurs in utero (Betancourt, Mobley, Russo, and Lamartiniere, 2010).

The immunoreactivity of annexin is examined in mammary epithelial cells. The BPA exposure affected the epithelial cells of the mammary gland and fibrous collagenous connective tissue in non-pregnant groups and showing the active expression of annexin became intense positive brown color as the dose increased whereas the mammary epithelial cells were not observed as intense positive color in cesarean and post-term.

The mammary gland of BPA-treated group showed relatively intense immunoreactions in the form of a brown color in both the epithelial cells of the mammary gland and connective tissue in non-pregnant adult females of rats (Ibrahim et al. 2016). The accumulation of BPA in human breast adipose tissue potentially induces an immunosuppressive response and disturbs the integrated interactions among epithelial cells and stromal cells, resulting in altered mammary epithelial phenotypes (Wang, Liu, and Liu, 2017). Adipocytes are the most abundant stromal cells, and they produce adipokines that induce mammary branching (Gjorevski and Nelson, 2011); the effects of BPA on adipocyte differentiation and maturation have been reported in animals (Vandenberg et al. 2007). Some authors have hypothesized that in the stroma, BPA exposure promoted maturation of the fat pad and altered the localization of collagen. Within the

epithelium, BPA exposure led to a decrease in cell size and delayed lumen formation. Because mammary gland development is dependent on reciprocal interactions between these compartments, the advanced maturation of the fat pad and changes in the extracellular matrix may be responsible for the altered growth, cell size, and lumen formation observed in the epithelium and may lead to apoptosis (Vandenberg et al. 2007).

The study implicates that these BPA-induced changes set up the stage for a prominent and permanent mammary gland lesion which, with uncontrollable exposure to that chemical could be related eventually to an increased incidence of breast cancer.

Conclusion

Bisphenol A caused a decrease in a number of the ducts and an altered amount of fibrous collagenous connective tissue in the mammary gland of non-pregnant females, the number of ducts and fibrous collagenous connective tissue remained unaltered in both cesarean and post-term females of Wistar rats. Therefore, it is important to raise public awareness about the potential toxicity of BPA as a plasticizer.

Abbreviations

BPA: Bisphenol A; ER-α: Estrogen receptor-α

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Availability of data and materials

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Authors' contributions

The authors worked upon the problem analyzed it in its various parameters, derived inferencesconclusion on the basis of those observations, and produced the manuscript. Both authors read and approved the final manuscript.

Ethics approval

The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC), Department of Zoology, University of Rajasthan, Jaipur.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

Abunasef, S. K., & El-Beshbishy, R. A. (2014). The histological changes of the female rat mammary gland during the fertile period with a special reference to E-cadherin expression. *The Egyptian Journal of Histology*, *37*, 45–55.

Al-Hiyasat, A. S., Darmani, H., & Elbetieha, A. M. (2002). Effects of bisphenol A on adult male mouse fertility. *European Journal of Oral Science*, *110*, 163–167.

Amos-Kroohs, R. M., Cheng, A. A., Clugston, R. D., Huang, T. N., Yen, C. L. E., Blaner, W. S., & Smith, S. M. (2016). Mammary gland structure and functional changes in mouse model of chronic gestational alcohol exposure. *The FASEB Journal*, *30*.

Betancourt, A. M., Mobley, J. A., Russo, J., & Lamartiniere, C. A. (2010). Proteomic analysis in mammary glands of rat offspring exposed in utero to bisphenol A. *Journal of Proteomics*, *73*, 1241–1253.

Guidelines on the regulation of scientific experiments of animals, Minister of Environment and forests department (animal Welfare Divison) Government of India, 2007

Duangjai, R., Tomohiro, Y., Shiro, K., & Mitsumori, K. (2010). Immunohistochemical localization of annexin A5 in the mammary gland of rats: up-regulation of expression by pup removal. *The Journal of Veterinary Medical Science*, *72*, 19–22.

ECB (2008). European Union risk assessment report draft: (bisphenol A). (CAS No. 80-0507; EWINECS No. 201-245-8).

Fenichel, P., Chevalier, N., & Brucker-Davis, F. (2013). Bisphenol A: anendocrine and metabolic disruptor. *AnnalesEndocrinologie*, *74*, 211–220.

Fenton, S. E. (2006). Endocrine disrupting compounds and mammary gland development: early exposure and later life consequences. *Endocrinology*, *147*, 18–24.

Fernandez, M. F., Arrebola, J. P., Taoufiki, J., Navalon, A., Ballesteros, O., Pulgar, R., … Olea, N. (2007). Bisphenol-A and chlorinated derivatives in adipose tissue of women. *Reproductive Toxicology*, *24*, 259–264.

Foster, W. G., Younglai, E. V., Boutross-Tadross, O., Hughes, C. L., & Wade, M. G. (2004). Mammary gland morphology in Sprague-Dawley rats following treatment with an organochlorine mixture *in utero* and neonatal genistein. *Toxicological Sciences*, *77*, 91–100.

Gjorevski, N., & Nelson, C. M. (2011). Integrated morphodynamic signaling of the mammary gland. *Nature Reviews Molecular Cell Biology*, *12*, 581–593.

Halperin, J., Dorfman, V. B., Fraunhoffer, N., & Vitullo, A. D. (2013). Estradiol, progesterone, and prolactin modulate mammary gland morphogenesis in adult female plains vizcacha (*Lagostomusmaximus*). *Journal of Molecular Histology*, *44*, 299–310.

Hsu, S. M., Raine, L., & Fanger, H. (1981). Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *Journal of Histochemistry & Cytochemistry*, *29*, 577–580.

Ibrahim, M. A. A., Elbakry, R. H., & Bayomy, N. A. (2016). Effect of bisphenol A on morphology, apoptosis and proliferation in the resting mammary gland of the adult albino rat. *International Journal of Experimental Pathology*, *97*, 27–36.

Jenkins, S., Raghuraman, N., Eltoum, I., Carpenter, M., Russo, J., & Lamartinere, C. (2009). Oral exposure to bisphenol A increase dimethylbenzanthracene-induced mammary cancer in rats. *Environmental Health Perspectives*, *117*, 910–915.

Kabuto, H., Amakawa, M., & Shishibori, T. (2004). Exposure to bisphenol A during embryonic fetal life and infancy increases oxidative injury and causes underdevelopment of the brain and testis in mice. *Life Science*, *74*, 2931–2940.

Karim, Z., & Husain, Q. (2010). Application of fly ash adsorbed peroxidase for the removal of bisphenol A in a batch process and the continuous reactor: assessment of genotoxicity of its product. *Food and Chemical Toxicology*, *48*, 3385–3390.

Le, H. H., Carlson, E. M., Chua, J. P., & Belcher, S. M. (2008). Bisphenol A is released from polycarbonate drinking bottles and mimics the neurotoxic actions of estrogen in developing cerebellar neurons. *Toxicology*, *17*, 149–156.

Liu, X. L., Chen, X. Y., Wang, Z. C., Shen, T., & Zhao, H. (2013). Effects of exposure to bisphenol A during pregnancy and lactation on the testicular morphology and caspase-3 protein expression of ICR pups. *Biomedical Reports*, *1*, 420–424.

Macias, H., & Hinck, L. (2012). Mammary gland development. *Wiley interdisciplinary Reviews-Developmental Biology*, *1*, 533–557.

Mandrup, K., Boberg, J., Isling, L. K., Christiansen, S., & Hass, U. (2016). Low-dose effects of bisphenol A on mammary gland development in rats. *Andrology*, *4*, 673–683.

Russo, J., & Russo, I. H. (1987). Development of the human mammary gland. In M. C. Neville, & C. W. Daniel (Eds.), *The mammary gland. Development, regulation and function*, (pp. 67–93). New York: Plenum Publishing.

Stewart, M. K. G., Plante, I., Penuela, S., & Laird, D. W. (2016). Loss of Panx1 impairs mammary gland development at lactation: Implications for breast tumorigenesis. *PLoS One, 11*, 1–23.

Tiede, B., & Kang, Y. (2011). From milk to malignancy: the role of mammary stem cells in development, pregnancy and breast cancer. *Cell Research*, *21*, 245–257.

Valentino, R., D'Esposito, V., Passaretti, F., Liotti, A., Cabaro, S., Longo, M., … Formisano, P. (2013). Bisphenol-A impairs insulin action and up-regulates inflammatory pathways inhuman subcutaneous adipocytes and 3T3-L1 cells. *PLoS One*, *8*, e82099.

Vandenberg, L. N., Maffini, M. V., Schaeberle, C. M., Ucci, A. A., Sonnenschein, C., Rubin, B. S., & Soto, A. M. (2008). Perinatal exposure to the xenoestrogen bisphenol-A induces mammary intraductal hyperplasias in adult CD-1 mice. *Reproductive Toxicology*, *26*, 210–219.

Vandenberg, L. N., Maffini, M. V., Wadia, P. R., Sonnenschein, C., Rubin, B. S., & Soto, A. M. (2007). Exposure to environmentally relevant doses of the xenoestrogen bisphenol-A alters the development of the fetal mouse mammary gland. *Endocrinology*, *148*, 116–127.

Wadia, P. R., Cabaton, N. J., Borrero, M. D., Rubin, B. S., Sonnenschein, C., Shioda, T., & Soto, A. M. (2013). Low-dose BPA exposure alters the mesenchymal and epithelial transcriptomes of the mouse fetal mammary gland. *PLoS One*, *8*, e63902.

Wang, Z., Liu, H., & Liu, S. (2017). Low-dose bisphenol A exposure: a seemingly instigating carcinogenic effect on breast cancer. *Advanced Science*, *4*, 1600248.

Watson, C. J., & Khaled, W. T. (2008). Mammary development in the embryo and adult: a journey of morphogenesis and commitment. *Development*, *135*, 995–1003.