Saudi Journal of Biological Sciences 29 (2022) 1543–1549

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Maternal and developmental toxicity of Bisphenol-A in SWR/J mice

Khalid Mashay Al-Anazi [a], Mohammed Jabr Aljuaid [a], Mohammad Abul Farah [a,*], Ahmed Hossam Mahmoud [a], Abdulmohsen I. Algefare [b], M. Ajmal Ali [c], Faisal M. Abou-Tarboush [a]

[a] Department of Zoology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia
[b] Department of Biological Sciences, Faculty of Science, King Faisal University, Hofuf, Al-Ahsa 31982, Saudi Arabia
[c] Department of Botany and Microbiology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia

ARTICLE INFO

Article history:
Received 18 September 2021
Revised 29 October 2021
Accepted 4 November 2021
Available online 12 November 2021

Keywords:
Bisphenol-A
Congenital malformation
Foetal resorption
Developmental toxicity
Teratogenic

ABSTRACT

Bisphenol-A (BPA), an organic compound with two phenol functional groups, is a widely used industrial plasticizer with known estrogenic properties. It is used in the manufacture of epoxy resins and polycarbonate plastics. This study was designed to evaluate and assess the possible toxicity arising from the oral administration of BPA to pregnant mice. Pregnant SWR/J mice (15 mice/group) were administrated oral doses of BPA (125, 250 and 500 mg/kg/day) over the course of five-day intervals during gestation (D1-5, D6-10 and D11-15), while control groups received only corn oil. The results indicated that BPA was associated with a reduction in the body weight of the pregnant mice from around 2–3 days after administration until the end of gestation. The greatest effects were evident when the BPA was given during the later stages of pregnancy, and with higher doses. They also showed marked reduction in food intake and, to a lesser extent, in water intake. Furthermore, doses of BPA induced a reduction in implantation sites, lower foetal body weight and increased mortality rates. Abortion and foetal resorption rates were not affected by BPA administration, however. The above findings were concluded by discussing the possible mechanisms involved in producing these effects.

1. Introduction

Bisphenol-A (BPA) is one of the most widely used chemicals due to its strength, flexibility and low density. It was first discovered by the Russian scientist Dianin in 1891 by intensifying acetone with two phenol equivalents where the reaction is stimulated by a strong acid. The process was commercialized in 1957 (Omer et al., 2016). It is used as a monomer in the manufacture of polycarbonate plastics and as a component of epoxy resins used in many consumer products. BPA is used extensively in food packaging, tooth fillings, medical equipment, toys, electronics, plastic household utensils, water containers and papers used in faxes, printers and sales receipts (Vandenberg et al., 2009; Ranjit et al., 2010; Tiwari et al., 2012; Kharrazian, 2014). Annually, about 100 tons of this compound leaks into the environment (Kim et al., 2001; Kharrazian, 2014), raising growing concern about its access to living ecosystems and the potential for multiple adverse effects on human health and animals (Takahashi and Oishi, 2003; Salian et al., 2009; Tiwari et al., 2012; Dobrzyńska and Radzikowska, 2013).

Despite the large number of reproductive and structural studies that have recorded the effects of this compound on experimental animals (Song et al., 2002; Richter et al., 2007), there is limited information about the effects of BPA on the embryos and foetuses of the animals. Studies on rodents have yielded conflicting results with respect to the effects of bisphenol exposure, which are affected by the species and strains of the animal tested, as well as the dose, means of administration, timing and duration of BPA treatment (Steinmetz et al., 1998; Pottenger et al., 2000). Hardin et al. (1981) indicated that this compound has multiple adverse effects on rat embryos, and Kim et al. (2001) indicated its administration to pregnant rats led to pregnancy failure, loss of many embryos before and after implantation, and significant delay in embryonic formation, but did not induce any congenital defects in embryos. The results of the study by Tachibana et al. (2007), meanwhile, have shown that this compound leads to functional defects in the placentas and increases foetal absorption and mor-

* Corresponding author at: Department of Zoology, College of Science, King Saud University, Riyadh 11451, Saudi Arabia.
E-mail address: mfarah@ksu.edu.sa (M. Abul Farah).
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.11.014
1319-562X/

tality of new-born infants. On the other hand, Morrissey et al. (1987) reported that the treatment of pregnant rats with BPA did not induce foetal toxicity or morphological defects in the treated mothers. Fischer 344 rats appear to be particularly sensitive to bisphenol. For example, while these animals showed a two- to three-fold increase in uterine weight after three days of treatment with 0.3 mg/kg BPA subcutaneously, the same dose did not have any effect on uterine weight in female Sprague-Dawley rats (Steinmetz et al., 1998).

In view of the increasing concern about the harmful effects of BPA on humans and animals, and the lack of studies in this area, the current research aimed to study the toxic, teratogenic and the growth suppressing effects of this compound on the embryos and foetuses of SWR/J strain of mice. Pregnant mice were treated orally with various doses of BPA at different stages of their gestation, followed by the observations made in terms of congenital malformations, mortality rates and body weight changes. Possible mechanisms responsible for the adverse effects of BPA are discussed.

2. Materials and method

2.1. Experimental animals

In this study, male and female laboratory mice of the pure strain SWR/J were obtained from the Animal House, Faculty of Pharmacy, King Saud University, Riyadh. The mice were aged between 8 and 10 weeks and weighed between 25.61 and 27.49 g, and were raised and maintained in a special room at the Department of Zoology, Faculty of Science, King Saud University, under standard conditions and procedures with a temperature of 22 ± 1 °C, a relative humidity of 45 ± 5%, and a light/dark cycle of 10/14 h. Large cages (25 × 24 × 15 cm) were used for the breeding and delivery, and small cages (25 × 17.5 × 14 cm) for running the experiment. All the cages were lined with an appropriate amount of clean sawdust and were washed twice a week with a water and antiseptic solution in order to prevent the growth of bacteria, fungi and other microorganisms.

The food (General Corporation of Grain Silos and Flour Mills in Riyadh) and water (in clean glass bottles) were provided continuously *ad libitum*. The mice were numbered for close follow up. A rectangular paper card (12.5 × 7.5 cm) was placed in an appropriate piece of metal that was attached to one side of each cage to allow the daily recording of data such as the animal's age and weight, and the intervention dosage(s) (Al-Enazy and Abou-Tarboush, 2001).

The mice were killed on the 17th day of their pregnancy using the cervical dislocation procedure. This was performed by placing the mouse on the cage lid and holding the tail using the left hand and placing the end of the scalpel horizontally on the neck area directly behind the head. The neck area and the immediate torso of the tail until the cervical vertebrae were dislocated.

2.2. Experimental studies

Groups of five virgin female mice of the SWR/J pure strain were placed with one male in each cage for mating, with the females being monitored daily for the presence of a vaginal plug as a sign of fertilization. The pregnant females were transferred into separate clean cages at the day on which the vaginal plug was detected (D_0 of pregnancy). A total of 120 pregnant females were selected to study the effects of BPA on embryos and foetuses. All the animals (120) were distributed equally into four experimental groups. Group I (control) was divided equally into three subgroups each treated with 0.5 ml of corn oil through oral administration using the gauge needle during a different 5-day interval, day 1 to 5 of pregnancy (D1-D5) for the first subgroup, day 6 to 10 (D6-D10) for the second subgroup, or day 11 to 15 (D11-D15) for the third subgroup. Group II was also divided equally into three equal subgroups with these animals being infused orally during D1-D5 of pregnancy with either 125 mg/kg, 250 mg/kg or 500 mg/kg BPA. These sub-lethal doses were selected on the basis of oral LD_{50} reported previously in mouse (Staples et al, 1998). The treatments for Group III and Group IV were the same as for Group II but during D6-D10 and D11-D15 of pregnancy, respectively.

In the embryo and foetus study, the pregnant females were monitored daily to observe any toxic effects arising from the administration of BPA. Weights, mortality, activity and any general symptoms of sickness were recorded. Water and food intake were also recorded every 24 h in the middle of the daylight period for a random selection of five mothers from each subgroup. For this, each of the selected pregnant females was placed in a cage alone and estimated food intake was the difference between the weight of the food provided and the weight of that remaining. The base of the cage was lined with cardboard to facilitate the collection and weighing of the spilled food, although this did not exceed 0.1 g/day.

On the seventeenth day of pregnancy, the pregnant females were killed using the method of cervical dislocation and dissected. Uterine horns were separated and stretched using standard methods (Abu Tarboush and Bahrawi, 1998). The number of intact and absorbed embryos and foetuses were recorded, along with their location in the uterus of each female. Then the uterus was opened with sterile scissors and the number of live and dead foetuses was recorded. The spontaneous movement and reddish body colour and/or the movement generated by the gentle pressure of the forceps on the neck of the foetuses was the criteria used to distinguish between live and dead foetuses. Then, the foetuses were collected from the uterine horns of each female, dried on special drying sheets and then weighed using a precision electronic balance (AC-1200D, Denver Instrument Company, USA). Each foetus was then examined with a dissecting microscope (Dissecting Microscopy, Wild Hebrugg, Switzerland), and classified as either normal or abnormal (Al-Enazy and Abou-Tarboush, 2001).

2.3. Statistical analysis

The Graph Pad in Stat software was used in the analysis of the data obtained for the resorptions, where the Chi-square value for the 2 × 2 Contingency Table (X^2) was used for the real numbers obtained, and T-tests were used to analyse the significant differences between mean scores of the groups treated with BPA and the corresponding control groups (Sokal and Rohlf, 1981).

3. Results

3.1. The toxic effects of BPA on the pregnant mice

Tables 1–3 show that about 2–3 days after the administration of BPA the normal increase in body weight of the pregnant mice started to slow down significantly, with this continuing until the end of gestation. The greatest effects compared to the control groups were noticed when the BPA was given during the later stages of pregnancy and with the 250 or 500 mg/kg doses. Tables 1-3 also indicate that treatment with any of the doses of BPA at any given day of pregnancy did not lead to any statistically significant differences compared to the control group in the percentages of the total number of females who aborted, had fully absorbed their embryos, showed any clear signs of maternal poisoning, or died during the testing period.

Table 1
Effect of various doses of BPA administered to pregnant SWR/J mice on days 1–5 of gestation on the mice weights, rates of abortion and full absorption of embryos.

Dose (mg/kg)	No. of dams used	Body weights of dams in gram (Mean±S.E.)					Percentage of aborted females	Percentage of females with fully resorbed embryos
		D0	D1	D3	D5	D17		
Control	**15**	27.88±0.63	28.33±0.70	28.81±0.67	29.32±0.63	49.17±1.21	0.00	0.00
125	**15**	26.71±0.90	28.25±0.74	28.71±0.76	29.68±0.76	46.85±1.04	6.67	0.00
250	**15**	28.27±0.73	26.87±0.70	27.21±0.81	28.14±0.86	43.84±0.72**	6.67	6.67
500	**15**	28.80±0.90	29.09±0.94	28.78±0.88	26.33±0.77*	42.13±1.17**	13.33	6.67

* The differences are statistically significant compared to the control group at p <0.05.
** The differences are statistically significant compared to the control group at p <0.01.

Table 2
Effect of various doses of BPA administered to pregnant SWR/J mice on days 6–10 of gestation on the mice weights, rates of abortion and full absorption of embryos.

Dose (mg/kg)	No. of dams used	Body weights of dams in gram (Mean±S.E.)					Percentage of aborted females	Percentage of females with fully resorbed embryos
		D0	D6	D8	D10	D17		
Control	**15**	28.51±0.65	33.05±0.82	34.25±0.53	36.47±0.61	51.67±1.00	0.00	0.00
125	**15**	28.01±0.74	30.57±0.89	32.51±0.65	32.73±0.76	46.70±0.76*	6.67	0.00
250	**15**	28.00±0.43	30.69±0.49	29.57±0.44**	30.36±0.70**	44.33±1.01**	6.67	0.00
500	**15**	26.35±0.79	29.99±0.60	28.92±0.51**	29.77±0.94**	39.42±2.06**	13.33	6.67

* The differences are statistically significant compared to the control group at p <0.05.
** The differences are statistically significant compared to the control group at p <0.01.

Table 3
Effect of various doses of BPA administered to pregnant SWR/J mice on days 11–15 of gestation on the mice weights, rates of abortion and full absorption of embryos.

Dose (mg/kg)	No. of dams used	Body weights of dams in gram (Mean±S.E.)					Percentage of aborted females	Percentage of females with fully resorbed embryos
		D0	D11	D13	D15	17		
Control	15	27.79±0.65	38.46±0.61	42.83±0.79	45.97±1.43	49.50±1.57	0.00	0.00
125	**15**	26.70±0.70	35.43±0.68	39.02±0.93	41.33±1.20	41.70±1.56**	6.67	0.00
250	**15**	27.41±0.70	36.54±0.83	38.61±0.89	38.17±0.96**	41.43±0.91**	6.67	0.00
500	**15**	27.99±0.81	36.95±0.92	36.09±0.96**	38.11±1.00**	41.27±0.90**	13.33	6.67

* The differences are statistically significant compared to the control group at p <0.05.
** The differences are statistically significant compared to the control group at p <0.01.

The results also showed that, compared to the control group, the daily water consumption of the treated pregnant mice did decrease significantly ($p > 0.05$), except for the dose of 500 mg/kg of BPA given between the 11th and 15th day of gestation (Table 4). Table 4 also indicates, however, that although administration of 125 mg/kg BPA did not affect their food consumption during any of the three stages of gestation studied, both the 250 and the 500 mg/kg doses were associated with a significant decrease ($p < 0.05$) in the daily food intake at all the stages compared to the pregnant control mice.

3.2. The toxic effects of BPA on the embryos and foetuses of treated mothers

The data in Tables 5–7 indicate that the lower dose (125 mg/kg) of BPA, given at any stages of pregnancy of the mice, did not have a significant effect on the number of implantation sites, the number of live or dead embryos or the body weights of the live foetuses, compared to the control, but that the higher doses did have a significant negative effect on these parameters. On the other hand, the percentages of congenital malformations on day 17 of gestation were significantly ($P < 0.05$) increased when dams were administered 250 and 500 mg/kg of BPA during days 1–5, 6–10 and 11–15 of their pregnancy, compared to the control group. Figs. 1–4 showed that the defects included haematoma in the tails and backside, defects in the ribs, exencephaly and two types of tail abnormalities (reduced tail length and kinking or curling of the tail).

4. Discussion

The present study was conducted in order to evaluate the toxicity of BPA (0, 125, 250, or 500 mg/kg), given orally at different

Table 4
Effect of various doses of BPA on the daily water intake of SWR/J mice administrated on days 1–5, 6–10 and 11–15 of pregnancy.

Dose(mg/kg)	No. of Pregnant mice	Amount of water consumed / pregnant female mean (ml/day) ± standard error			Amount of food consumed / pregnant female mean (gm/day) ± standard error		
		D11-15	D6-10	D1-5	D11-15	D6-10	D1-5
Control	**5**	6.3±0.099	5.8±0.099	5.60±0.12	11.3±0.28	11.7±0.099	10.81±0.10
125	**5**	6.1±0.099	6.1±0.098	5.49±0.12	9.8±0.21	10.2±0.12	11.1±0.11
250	**5**	5.7±0.098	5.7±0.091	5.44±0.23	6.2±0.26*	6.8±0.11*	6.7±0.10*
500	**5**	6.7±0.099	5.4±0.12	3.18±0.31*	6.1±0.26*	6.8±00.09*	6.3±0.11*

*The differences were statistically significant compared to the control group at the level (p <0.05).

Table 5
Effect of different doses of BPA given during the days 0–5 of pregnancy of SWR/J mice on the mice embryos and foetuses, obtained on day 17 of gestation.

Dosage (mg/kg)	Number of dams used	Number of implantation sites/female (Mean ± SE)	Number of live foetuses/female (Mean ± SE)	#Non-live embryos/female (%)	Live foetal body weight in g/dam (Mean ± SE))
Control	15	12.27 ± 0.57	11.87 ± 0.58	(3.26)	1.15 ± 0.03
125	15	10.40 ± 0.69	9.33 ± 0.77	(10.82)	1.08 ± 0.03
250	15	9.27 ± 0.42**	7.60 ± 0.58**	(17.99)**	0.90 ± 0.03*
500	15	8.60 ± 0.48**	6.33 ± 0.68**	(26.39)**	0.85 ± 0.02**

Non-living embryos include dead and absorbed embryos and aborted sites.
* The differences are statistically significant compared to the control group at $p < 0.05$.
** The differences are statistically significant compared to the control group at $p < 0.01$.

Table 6
Effect of different doses of BPA given during the days 6–10 of pregnancy of SWR/J mice on the mice embryos and foetuses, obtained on day 17 of gestation.

Dosage (mg/kg)	Number of dams used	Number of implantation sites/dam (Mean ± SE)	Number of live foetuses/dam (Mean ± SE)	#Non-live embryos/dam (%)	Live foetal body weight in g/dam (Mean ± SE)
Control	15	12.13 ± 0.66	11.74 ± 0.64	(3.29)	1.17 ± 0.03
125	15	10.40 ± 0.69	9.33 ± 0.77	(9.94)	1.14 ± 0.03
250	15	6.53 ± 0.56	8.13 ± 0.69**	(15.38) **	0.90 ± 0.03*
500	15	9.00 ± 0.56**	7.00 ± 0.74**	(22.22) **	0.84 ± 0.07**

Non-living embryos include dead and absorbed embryos and aborted sites.
* The differences are statistically significant compared to the control group at $p < 0.05$.
** The differences are statistically significant compared to the control group at $p < 0.01$.

Table 7
Effect of different doses of BPA given during the days 11–15 of pregnancy of SWR/J mice on the mice embryos and foetuses, obtained on day 17 of gestation.

Dosage (mg/kg)	Number of dams used	Number of implantation sites//dam (Mean ± SE))	Number of live foetuses / dam (Mean ± SE)	#Non-live embryos/dam (%)	Live foetal body weight in g/dam (Mean ± SE)
Control	15	12.33 ± 0.61	11.66 ± 0.62	(3.78)	1.11 ± 0.03
125	15	10.27 ± 0.79	9.55 ± 0.79	(5.84)	1.09 ± 0.02
250	15	10.13 ± 0.50	8.33 ± 0.46*	(7.89)	1.07 ± 0.03
500	15	9.89 ± 0.78	8.27 ± 0.75*	(12.24)	0.95 ± 0.04

Non-living embryos include dead and absorbed embryos and aborted sites.
* The differences are statistically significant compared to the control group at $p < 0.05$.

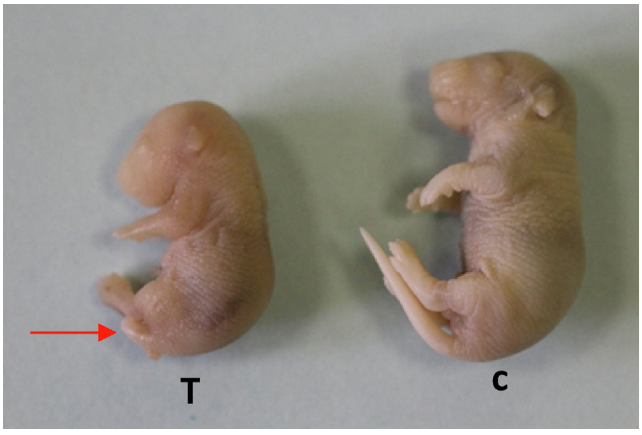

Fig. 1. Foetus showing reduced tail length obtained on day 17 from dams treated (T) with 250 mg/kg Bisphenol -A compared to the untreated control (C).

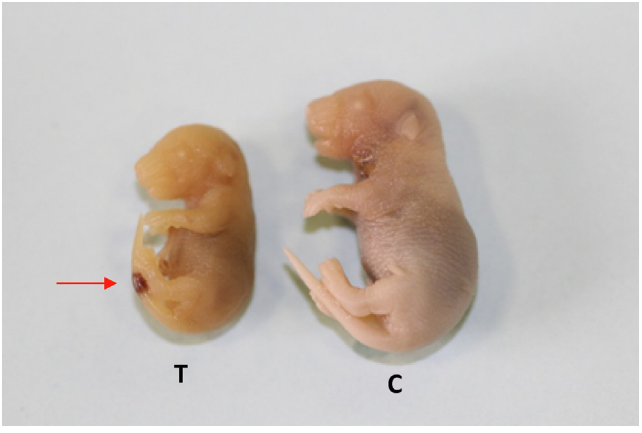

Fig. 2. Foetus showing hematoma in the tail obtained on day 17 from dams treated (T) with 500 mg/kg Bisphenol -A compared to the control (C).

stages of gestation, on treated mothers and their embryos and foetuses. The pregnant mothers were dissected on day 17 of gestation, and uterine horns were removed in order to allow the study of embryos and foetuses. The embryos were weighed, examined, and classified as normal or abnormal.

4.1. Effect of BPA on treated mothers

The results of this part of the current research showed that there was a significant decrease in the mean body weights of all the pregnant females treated with BPA, compared to the control

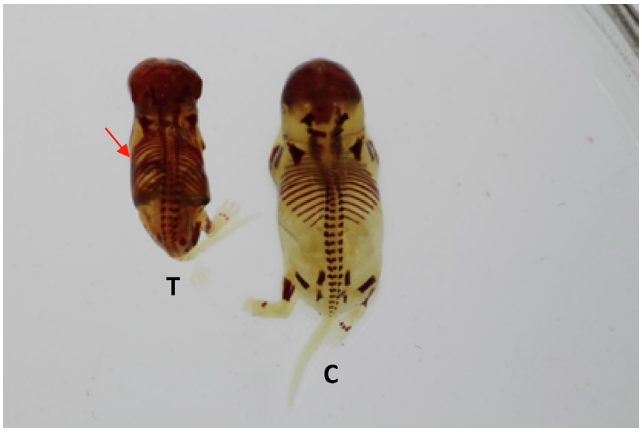

Fig. 3. Dorsal view of foetal skeleton showing defects in ribs (stained with alizarin red) obtained on day 17 from dams treated (T) with 250 mg/kg Bisphenol -A compared to the control (C).

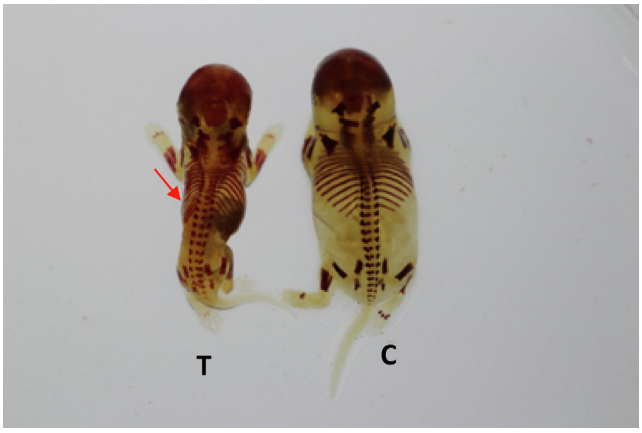

Fig. 4. Dorsal view of foetal skeleton showing defects in ribs (stained with alizarin red) obtained on day 17 from dams treated (T) with 500 mg/kg Bisphenol -A compared to the control (C).

groups, especially by the 17th day of pregnancy, except when the lowest dose of 125 mg/kg was given early in the gestation period. This effect may be attributed to an increased incidence of resorbed embryos and dead foetuses, especially at 250 and 500 mg/kg doses of BPA. This effect may also be attributed to BPA causing a loss of appetite resulting from toxicity and ulcers in the digestive tracts of treated mice (Al-Enazy and Abou-Tarboush, 2001; Preethi et al., 2014). This is suggested also by the results indicating low body mass and decreased water and food intake at the higher doses of bisphenol.

The above finding is consistent with earlier studies indicating that exposure to BPA may influence appetite. For example, Sharf-El Deen et al. (2015) revealed that pregnant rats treated with BPA (300 and 600 mg/kg/day) from the sixth up to the 15th and 19th days of gestation exhibited a marked reduction in food intake as well as in body weight. Long Evans rats showed a significant increase in uterine weight at 200 mg/kg BW/d for three days, but the injection of 100 mg/kg BW by the same method was ineffective (Laws et al., 2000), while premature Alpk/AP rats showed a strong response to uterine tension at a dose of 400 mg/kg. On the other hand, Dhagga and Srivastava (2017) pointed out that giving female rats low doses of 5 and 50 mg/kg per week orally for a period of three months, using olive oil as a solvent for bisphenol, did not lead to any statistically significant effect on the body weight of the mothers, nor to any other signs of toxicity. This dose related effects are comparable to the results of the low and high doses used in the present investigation.

Several studies have indicated that BPA interferes with the regulation of hormones in both male and female mammals, and affects the axis of the reproductive glands and pituitary gland at all levels (Patisaul et al., 2006; Xi et al., 2011). Isidori et al. (2005) indicated that the decrease in sex hormones of pregnant women treated with BPA leads to a statistically significant reversal in the gain acquired in the body weight during pregnancy, way by decreasing the mass of the bones and muscles. Also, Benachour and Aris (2009) showed that BPA increased the level of tumour necrosis factor (TNF) and induced necrosis and apoptosis in the studied cells and concluded that BPA may cause intrauterine growth restriction, preterm delivery and miscarriage in humans.

4.2. Effect of BPA on embryos and foetuses of treated mothers

The results of this part of the present study showed a statistically significant decrease, compared to the mice in the control groups, in the mean number of implantation sites and live embryos, and a significant increase in mortality rates of foetuses in mothers given 250 and 500 mg/kg BPA during days 1–5, 6–10 and 11–15 of gestation. The body weight of live foetuses decreased significantly with both doses given at the three studied intervals, except when 250 mg/kg of BPA was given during days 11–15. Sharf-El Deen et al. (2015) study revealed that BPA doses induced a reduction in implantation sites and foetal body weight, and an increase in both mortality and resorption rates. The lower doses of orally administered BPA in Dhagga and Srivastava (2017) study, however, led to no statistically significant effect on implantation, corpus luteum or absorption, weight and size of foetuses or skeletal structure in the treated groups compared with the control. Again, the doses used in Dhagga and Srivastava (2017) study may be comparable to the 125 mg/kg dose used in the present study, and those used in Sharf-El Deen et al. (2015) study are comparable to the high doses. The dose level, duration and time of exposure to BPA, as well the species, could be factors in these discrepancies.

The occurrence of BPA in free and conjugated forms has been validated in the blood of pregnant women, with the highest concentrations being measured in the placenta and developing foetus (Schönfelder et al., 2002). This accumulation can lead to birth defects, as shown in animal studies (Lee et al., 2013). The passage of BPA across the placenta has also been reported, and it has been found in the maternal serum, amniotic fluid and cord serum at birth, as well as in the placenta (Yamada et al., 2002; Padmanabhan et al., 2008). These results suggest that BPA is able to cross the placenta in small amounts during the organismic phase, although higher doses can still lead to sufficient crossing the placenta to cause the death of embryos, especially during the later stages of gestation. Days 11–15 of pregnancy are considered to be the late stage of the organismic phase and thus foetuses remain sensitive to the toxic and deforming effect of BPA. Tsutsumi et al. (2002) studied the levels of BPA in biological fluids such as maternal plasma, foetal plasma, placental tissue, amniotic fluid and umbilical cord blood, with results suggesting that BPA can cross the choroid barrier easily and, while many studies have ruled out the hypothesis of excessive foetal exposure to BPA in the later stages of pregnancy, the possibility of such exposure in the early stages of growth cannot be ruled out because of incomplete growth. Infant systems are not able to metabolize BPA through the process of "Glucuronidation", as in adults, and the maternal rate of hepatic glucuronidation is slightly lower during pregnancy. Therefore, associated or non-associated BPA does not accumulate during the mother's pregnancy because the half-life of BPA in the healthy adult body is less than six hours and is com-

pletely discharged from the body within 24 h, mainly through urine in humans. In rodents, it is released mainly through the bile and faeces and to a lesser extent in the urine (Tsukioka et al., 2004; Chapin et al., 2008). Mahalingaiah et al. (2008) reported a 26% increase in the concentration of BPA in urine among pregnant women compared with their pre-pregnancy levels. This may explain the weakness of its foetal toxicity. BPA can be deconjugated via β-glucuronidase, which is present at high concentrations in liver, kidney, intestine and placenta, however, increasing the potential reactivation of BPA induced effects (Ginsberg and Rice, 2009).

The current research also agrees with some of the results of Lee et al. (2013), which indicated that oral dosage of BPA during pregnancy leads to an increase in mortality rate and to lower levels of follicle-stimulating hormone (FSH), Luteinizing hormone (LH), oestradiol, testosterone, Bcl-2 protein, and expression of the gene StAR in rats foetuses, and that the long-term exposure to bisphenol affects the reproductive system growth. In contrast, Ma et al. (2017) indicated that BPA increased the concentration of oestradiol in the foetuses of the treated pregnant mice. The persistence of pregnancy in mothers depends mainly on the precise functioning of the hormonal system, especially during the first 6–10 days of pregnancy, and these toxic and lethal effects on the embryos are fully consistent with the period when the corpus luteum is almost completely dependent on the expression of luteinizing hormone (Al-Enazy and Abou-Tarboush, 2001; Balakrishnan et al., 2010).

The mutagenic effect of BPA may contribute to all the effects observed in the embryos and foetuses seen in the current study. Shuang et al. (2017) analysis of protein expression revealed a decrease in Bcl-2 levels and increased Bax levels due to exposure of spermatocytes and granulosa cells to BPA, and argues that this plays a role in stimulating apoptosis, suggesting that BPA affects the reproductive system and embryos across the programmed apoptosis pathway associated with mitochondria and that this may also explain the toxicity of BPA. Exposure to BPA stimulates oxidative stress, which can be another possible mechanism for genetic reproductive toxicity (Tiwari and Vanage, 2017). In addition, sub-toxic doses of BPA have the potential to act to inhibit post-translational modification of protein in the Golgi apparatus by inhibiting Ca2 + ATPases activity, and the metabolic output of BPA, BPA-quinone, was able to form covalent linkages with DNA (Atkinson and Roy, 1995).

The results of the current research are consistent with the results of several other studies that have been studied with non-bisphenol compounds (Al-Enazy and Abou-Tarboush, 2001; Al-Zahrany, 2006) and BPA (Tyl et al., 2002; Alonso-Magdalena et al., 2010; Ma et al., 2017). However, the exact causes of the differences between the current results and some other studies are not clear, the common factors involved in the differing results may be attributed to the fact that the studies are varied *in vivo* versus *in vitro* and the ways of BPA administration. The relative bioavailability of bisphenol was lower when given orally after birth than either subcutaneous or peritoneal and therefore was less effective (Pottenger et al., 2000). Also, the doses, timing and length of exposure were varied, and variation in animal strain was an influential factor in study results. Furthermore, these combined factors and other factors mentioned above may be the cause of this effect of BPA, so there is an urgent need for other similar studies to verify those observable effects of BPA and identify potential factors responsible for them. Nevertheless, this study is one of the few reports that have detailed follow-up data on mortality rates, toxic and mutagenic effects, and mean birth weights for female laboratory mice given BPA orally, and as a good warning indicator of the potential adverse effects of BPA on individuals exposed to it.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors would like to extend their sincere appreciation to the Researchers Supporting Project number (RSP-2021/154), King Saud University, Riyadh, Saudi Arabia.

References

Abu Tarboush, F., Bahrawi, A., 1998. A practical student guide for the dissection of laboratory rats and their study with commensal and wild rodents and their relation to humans and ways to combat them. Al-Shorouq Press, Riyadh, p. 28PP7..

Al-Enazy, K.M., Abou-Tarboush, F.M., 2001. Effects of cyclophosphamide on mice offsprings maternally treated with the drug, a follow-up study. J. Egy. Ger. Soc. Zoo. 36 (B), 27–42.

Alonso-Magdalena, P., Vieira, E., Soriano, S., Menes, L., Burks, D., Quesada, I., Nadal, A., 2010. Bisphenol A exposure during pregnancy disrupts glucose homeostasis in mothers and adult male offspring. Environ. Health. Persp. 118 (9), 1243–1250.

Al-Zahrany, H.M., 2006. Toxic, teratogenic and cytogenetic effects of neemix-4.5 on fetuses and offspring of SWR/J mice. M.Sc. Thesis, 2006; King Saud University, Riyadh, pp 130.

Atkinson, A., Roy, D., 1995. In vivo DNA adduct formation by bisphenol A. Env. Mol. Mutagen. 26 (1), 60–66.

Balakrishnan, B., Henare, K., Thorstensen, E.B., Ponnampalam, A.P., Mitchell, M.D., 2010. Transfer of bisphenol A across the human placenta. Am. J. Obst. Gyneco. 202(4), 393-e1-393e7.

Benachour, N., Aris, A., 2009. Toxic effects of low doses of Bisphenol-A on human placental cells. Toxicol. App. Pharm. 241 (3), 322–328.

Chapin, R.E., Adams, J., Boekelheide, K., Gray, L.E., Hayward, S.W., Lees, P.S.J., McIntyre, B.S., Portier, K.M., Schnorr, T.M., Selevan, S.G., Vandenbergh, J.G., Woskie, S.R., 2008. NTP-CERHR expert panel report on the reproductive and developmental toxicity of bisphenol A. Birth Def. Res. Part B: Dev. Rep. Tox. 83 (3), 157–395.

Dhagga, N., Srivastava, S., 2017. A study on developmental toxicity of bisphenol A (BPA) in maternal exposed Wistar rats. Int. J. Pharm. Sci. Res. 45 (2), 82–85.

Dobrzyńska, M.M., Radzikowska, J., 2013. Genotoxicity and reproductive toxicity of bisphenol A and X-ray/bisphenol A combination in male mice. Drug. Chem. Tox. 36 (1), 19–26.

Ginsberg, G., Rice, D.C., 2009. Does rapid metabolism ensure negligible risk from bisphenol A? Environ. Health Persp. 117 (11), 1639–1643.

Hardin, B.D., Bond, G.P., Sikov, M.R., Andrew, F.D., Beliles, R.P., Niemeier, R.W., 1981. Testing of selected workplace chemicals for teratogenic potential. Scan. J. Work Environ. Health 7 (4), 66–75.

Isidori, A.M., Giannetta, E., Greco, E.A., Gianfrilli, D., Bonifacio, V., Isidori, A., Lenzi, A., Fabbri, A., 2005. Effects of testosterone on body composition, bone metabolism and serum lipid profile in middle-aged men: A meta-analysis. Clin. Endocrin. 63 (3), 280–293.

Kharrazian, D., 2014. The potential roles of bisphenol A (BPA) pathogenesis in autoimmunity. Autoimm. Dis. 2014, Article ID 743616.

Kim, J.-C., Shin, H.-C., Cha, S.-W., Koh, W.-S., Chung, M.-K., Han, S.-S., 2001. Evaluation of developmental toxicity in rats exposed to the environmental estrogen bisphenol A during pregnancy. Life Sci. 69 (22), 2611–2625.

Laws, S.C., Carey, S.A., Ferrell, J.M., Bodman, G.J., Cooper, R.L., 2000. Estrogenic activity of octylphenol, nonylphenol, bisphenol A and methoxychlor in rats. Toxicol. Sci. 54 (1), 154–167.

Lee, S.G., Kim, J.Y., Chung, J.-Y., Kim, Y.-J., Park, J.-E., Oh, S., Yoon, Y.-D., Yoo, K.S., Yoo, Y.H., Kim, J.-M., 2013. Bisphenol A exposure during adulthood causes augmentation of follicular atresia and luteal regression by decreasing 17β-estradiol synthesis via downregulation of aromatase in rat ovary. Environ. Health Persp. 121 (6), 663–669.

Ma, S., Shi, W., Wang, X., Song, P., Zhong, X., 2017. Bisphenol-A Exposure during Pregnancy Alters the Mortality and Levels of Reproductive Hormones and Genes in Offspring Mice. BioMed Res. Int. 2017, 1–8.

Mahalingaiah, S., Meeker, J.D., Pearson, K.R., Calafat, A.M., Ye, X., Petrozza, J., Hauser, R., 2008. Temporal variability and predictors of urinary bisphenol A concentrations in men and women. Environ. Health Persp. 116 (2), 173–178.

Morrissey, R.E., George, J.D., Price, C.J., Tyl, R.W., Marr, M.C., Kimmel, C.A., 1987. The developmental toxicity of bisphenol A in rats and mice, Fund. App. Toxicol. 8(4), 571–582.

Leena, O., Hassn, A., Abdalla, E., 2016. Determination of bisphenol A in exposed bottled water samples to direct sun light using multi walled carbon nanotubes as solid phase extraction sorbent. J. Environ. Chem. Ecotox. 8 (7), 51–57.

Padmanabhan, V., Siefert, K., Ransom, S., Johnson, T., Pinkerton, J., Anderson, L., Tao, L., Kannan, K., 2008. Maternal bisphenol-A levels at delivery: a looming problem? J. Perinatol. 28 (4), 258–263.
Patisaul, H., Fortino, A., Polston, E., 2006. Neonatal genistein or bisphenol-A exposure alters sexual differentiation of the AVPV. Neurotox Terato 28 (1), 111–118.
Pottenger, L.H., Domoradzki, J.Y., Markham, D.A., Hansen, S.C., Cagen, S.Z., Waechter Jr, J.M., 2000. The relative bioavailability and metabolism of bisphenol A in rats is dependent upon the route of administration. Toxicol. Sci. 54 (1), 3–18.
Preethi, S., Lebonah, D., Sreedevi, B., Chandrasekhar, K., Sarojamma, B.J., Pramoda, K., 2014. Toxicity Studies of Bisphenol-A Induced Sprague Dawley Rats. Int. J. Pharm. Pharmac. Sci 2 (1), 11–19.
Ranjit, N., Siefert, K., Padmanabhan, V., 2010. Bisphenol-A and disparities in birth outcomes: a review and directions for future research. J. Perinatol. 30 (1), 2–9.
Richter, C.A., Birnbaum, L.S., Farabollini, F., Newbold, R.R., Rubin, B.S., Talsness, C.E., Vandenbergh, J.G., Walser-Kuntz, D.R., vom Saal, F.S., 2007. In vivo effects of bisphenol A in laboratory rodent studies. Rep. Toxicol. 24 (2), 199–224.
Salian, S., Doshi, T., Vanage, G., 2009. Perinatal exposure of rats to Bisphenol A affects the fertility of male offspring. Life Sci. 85 (21-22), 742–752.
Schönfelder, G., Wittfoht, W., Hopp, H., Talsness, C.E., Paul, M., Chahoud, I., 2002. Parent bisphenol A accumulation in the human maternal-fetal-placental unit. Environ. Health Persp. 110 (11), a703–a707.
Sharf-El Deen, O., Bakry, S., Ali, A.S.W., 2015. Teratogenicity of Bisphenol-A (BPA) in Pregnant RAT. Sci. AEJTS 7 (4), 229–238.
Shuang, M.A., Wanyu, S., Xiaodan, W., Pengyan, S., Xiuhui, Z., 2017. Bisphenol A exposure during pregnancy alters the mortality and levels of reproductive hormones and genes in offspring mice. Biomed Res. Int. 2017, 3585809.
Sokal, R.R., Rohlf, F.J., 1981. "Biometry: The Principles and Practice of Statitstics in Biological Research". W. H. Freeman and Company, San Francisco, p. pp859..
Song, K.H., Lee, K., Choi, H.S., 2002. Endocrine disrupter bisphenol a induces orphan nuclear receptor Nur77 gene expression and steroidogenesis in mouse testicular Leydig cells. Endocrinology 143 (6), 2208–2215.
Staples, C.A., Dome, P.B., Klecka, G.M., Oblock, S.T., Harris, L.R., 1998. A review of the environmental fate, effects, and exposures of bisphenol A. Chemosphere 36 (10), 2149–2173.
Steinmetz, R., Mitchner, N.A., Grant, A., Allen, D.L., Bigsby, R.M., Ben-Jonathan, N., 1998. The xenoestrogen bisphenol A induces growth, differentiation, and c-fos gene expression in the female reproductive tract. Endocrinology 139 (6), 2741–2747.
Tachibana, T., Wakimoto, Y., Nakamuta, N., Phichitraslip, T., Wakitani, S., Kusakabe, K., Kiso, Y., 2007. Effects of bisphenol A (BPA) on placentation and survival of the neonates in mice. J. Rep. Dev. 53 (3), 509–514.
Takahashi, O., Oishi, S., 2003. Testicular toxicity of dietarily or parenterally administered bisphenol A in rats and mice. Food Chem. Toxicol. 41 (7), 1035–1044.
Tiwari, D., Vanage, G., 2017. Bisphenol A induces oxidative stress in bone marrow cells, lymphocytes, and reproductive organs of holtzman rats. Int. J. Toxicol. 36 (2), 142–152.
Tiwari, D., Kamble, J., Chilgunde, S., Patil, P., Maru, G., Kawle, D., Vanage, G., 2012. Clastogenic and mutagenic effects of bisphenol A: an endocrine disruptor. Mut. Res/Gen. Toxicol. Env. Mut. 743 (1), 83–90.
Tsukioka, T., Terasawa, J.I., Sato, S., Hatayama, Y., Makino, T., Nakazawa, H., 2004. Development of analytical method for determining trace amounts of BPA in urine samples and estimation of exposure to BPA. J. Environ. Chem. 14 (1), 57–63.
Tsutsumi, O., Ikezuki, Y., Takai, Y., Kamei, Y., Taketani, Y., 2002. Determination of bisphenol A concentrations in human biological fluids reveals significant early prenatal exposure. Human Rep. 17 (11), 2839–2841.
Tyl, R.W., Myers, C.B., Marr, M.C., 2002. Three-generation reproductive toxicity study of dietary bisphenol A in CD Sprague-Dawley rats. Toxicol. Sci. 68 (1), 121–146.
Vandenberg, L.N., Maffini, M.V., Sonnenschein, C., Rubin, B.S., Soto, A.M., 2009. Bisphenol-A and the great divide: a review of controversies in the field of endocrine disruption. Endocrine Rev. 30 (1), 75–95.
Xi, W., Lee, C.K.F., Yeung, W.S.B., Giesy, J.P., Wong, M.H., Zhang, X., Wong, C.K., 2011. Effect of perinatal and postnatal bisphenol A exposure to the regulatory circuits at the hypothalamus–pituitary–gonadal axis of CD-1 mice. Rep. Toxicol. 31 (4), 409–417.
Yamada, H., Furuta, I., Kato, E.H., Kataoka, S., Usuki, Y., Kobashi, G., Sata, F., Kishi, R., Fujimoto, S., 2002. Maternal serum and amniotic fluid bisphenol A concentrations in the early second trimester. Rep. Toxicol. 16 (6), 735–739.