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PHYSIOLOGICAL AND TERATOGENIC EFFECTS OF BISPHENOL-A (BPA) ON PREGNANT RATS

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ABSTRACT

Bisphenol A is an industrial chemical with wide spread uses all over the world. It can cross placenta and resulted in embryo and feto- toxicity and other adverse effects in pregnant animal and their offspring. This study was carried out on sixty pregnant rat divided into three groups control one (GI) administered with corn oil from day 6 to day 15 of pregnancy GII administrated orally with 25 g m /kg body weight BPA daily from day 6 to days 15 of pregnancy GIII administered BPA at dose of 50 g m /kg BW at the same protocol of GII. At 15 and 17 days of pregnancy 10 rats from each group were sacrificed and blood of rat was collected for serum collection and kept frozen for biochemical assay . All feoti were collected and counted from each dam and half of them saved in 10% formaldehyde for histopathological examination, Other half kept in ethyl alcohol for skeletal malformation study . The result revealed, reduction in fetal size due to BPA dosing with accompanying of skeletal malformation. Moreover, BPA administration could elevate estrogen level and decrease progesterone level in GII and GIII , compared with control group. In addition, liver enzymes activities were significantly increased in GII and GIII while total antioxidant capacity was significantly reduced. Histopathological examination of fetal tissues revealed pathological lesions in liver،lung and kidney of groups dosing BPA. From the previous results it could be concluded that, exposure of pregnant rat to BPA by a dose of 25 or 50 mg /kg BW resulted in strong abnormality in all biochemical parameters, malformation and damage in liver, kidney, lung, and hearts of fetus .

INTRODUCTION

Bisphenol-A (BPA) is a compound, enters in manufacturing polycarbonate plastics (PC), it is also an intermediate in epoxy resins preparations. Bisphenol-A is considered a one of the highest production volumes worldwide. BPA is widely used in food storage containers, food-contact paper, metal food cans, and bottles of baby, thermal papers, dental materials, and personal care products. BPA leaks into the environment throughout all procedures of fabrication, transporting, processing, and waste-disposal and its products. Furthermore, leaking of BPA into environment happens through all products containing it **(Baluka, and Rumbeiha, 2016 & Wang, et al, 2017)**

BPA is a xenoestrogen, exhibiting estrogen mimicking, hormone like actions, which increase bad feelings regarding its rightness in some human used especially food containers. Recently, many countries examined its safety that provoked trends to PC products pulling out. So that we can see U.S. Food and Drug Administration (FDA) and European Union and Canada terminated authorization of BPA using in baby bottles and food containers. **(FDA, 2014).**

BPA was detected in cord serum collected during second-trimester of pregnancy, suggesting that fetuses were exposed to BPA ***(Gerona et al., 2013)***. ***Schonfelder et al.***

(2002) reported that, BPA has been detected in the amniotic fluid, fetal plasma, fetal tissue and found that BPA was higher in male fetuses than female fetuses.

The prenatal exposure to BPA induced changes in the function of hypothalamic-pituitary gonadal axis, mammary development, as well as sex-specific behaviors in the offspring **(Vandenberg *et al.,* 2010).** In the same respect, **Markey et al. (2002)** stated that continued exposure to BPA during the gestation period have an impact on the development of the fetus and leads to intra-uterine growth retardation.

Lee et al. (2014) found that prenatal exposure to BPA affected birth weight especially between male neonates. **Weinberger et al. (2014)** showed that exposure of pregnant mothers to BPA associated with reduced gestation by 1.1 weeks, impair fetal growth and decreased birth weight. In rodents, it has been shown that BPA can cross the placenta and bind a - fetoprotein with decreased affinity to estradiol; this resulted in enhancement of its bioavailability during neonatal development **(Takahashi and Oishi, 2000).** The adverse effects of BPA could be reported to influences promoting many alterations in estrogen target organs involving brain, ovary mammary gland, and uterus among perinatally BPA-exposed females.

It BPA can interrupt oxidative homeostasis, including mitochondrial activity (***Ooe et al., 2005***), modulation of antioxidant enzymes and raise thio-barbituric acid reactive substances in organs of animals exposed embryonic route (***Lejonklou et al., 2017)***.

Ke, et al., (2016) , ***Peretz et al. (2010***), ***Jiang et al.(2014***) and ***Taha et al, (2016)*** reported that, BPA exposure could modulate lipid profile with up–regulated hepatic lipid metabolism and genes involved in lipogenesis pathway. Similarly, ***Hassan et al. (2012***) reported hepatotoxic effect in rats exposed to BPA with a significant increases in serum activition of liver enzymes and bilirubin level . Moreover, ***Geetharathan and Josthna (2016)*** revealed that PBA had kidney toxic effects that elevated creatinine level in exposed group. In the same respect, ***Badawi et al, (2013)*** and ***Hijazi et al,(2015)*** recognize morphometric changes histopathological finding of liver and kidney of BPA exposed animal . aim of the present article was to through some lights on the effect of BPA exposure by different doses on the pregnant rat as well as its effects on the their fetuses during gestation period.

MATERIAL AND METHODS

Experimental animals:

Sixty (60) adult female albino rats weighing 180±20 gm and aged about 4 months old, and ten (10) adult male albino rats weighing 250 -300 gm aged about 5 months, were obtained from Animals Experimental Unit, Department of Anatomy , Faculty of Medicine, Mansoura University. The animals were housed in plastic cages with wood shavings as bedding and kept under controlled condition (23± 1°C ,12 h light and 12 h dark cycle). Rats were fed on standard laboratory pelleted diet and water adlibitum.

Determination of zero day of pregnancy:

Determination of zero day of pregnancy was carried out through daily vaginal smear examination. The female proved to be in estrous (cornified cells) was caged with a fertile male overnight. In the next morning vaginal smear carried out. Female positive sperms was an indication of zero day of pregnancy **(Barcellona et al., 1977).**

Experimental design:

Thirty six (36) mature pregnant female rats were randomly separated into 6 groups 6 each. Rats were fed by BPA according to the following table.

Samples collection:

At day 15 and 17 all groups were subjected to anesthesia by diethyl ether and fresh blood was immediately collected from retro-orbital plexus by using micro-capillary tube 18-20 gauge pore size 1.5-2 inch length**.** Blood kept under room condition for coagulation and serum separated for biochemical assay.

Foeti and Specimen from foeti were collected and kept in 10% neutral buffered formalin. Size fetuses are determined macroscopically and whole fetus was subjected to skeletal examination .

Biochemical analysis:

Liver Function: Serum activities of ALT and AST as well as ALP were evaluated in both control and BPA- treated groups **(*Tietz, 1986*).**

Serum total proteins and albumin **(*Dumas and Biggs, 1972*)** were measured in both groups while the total globulins were calculated by subtracting serum albumin value from the value of serum total protein to obtain the albumin-globulin (A/G) ratio.

Estimation of Blood Glucose: Glucose level was estimated according to **Trinder (1969).**

Serum Lipid Profile: The serum total cholesterol, triglyceride levels , HDL-C were estimated by enzymatic colourimetric reaction .Low-density lipoprotein cholesterol (LDL-C) was calculated by computation, according to the methods described by ***Friedewald* et al., (*1972)*** .

Kidney Function: Urea and creatinine were measured (***Tabacco et al., 1979***) and **Fabiny and Ertingshausen, (1971),** respectively.

Total antioxidant capacity

Total antioxidant capacity (TAC) was measured spectrophoto-meterically following slandered methods using commercial available kits (Biodiagnostic, Cairo, Egypt).

Histopathological technique .

Specimens from foeti were fixed in 20 % neutral buffered formalin then subjected to routin histopathological technique (***Banchroft et al., 1996***)

Statistical Analysis:

Data were subjected to statistical analysis using statistical software program (SPSS for Windows, version 20, USA). Means and standard error for each variable were estimated. Differences between means of different groups at different time of collection were carried out using two way ANOVA with Scheffe multiple comparison tests. Dissimilar superscript letters in the same column or raw show a significance ($P<0.05$).

RESULTS

It was noticed that, estradiol level was significantly increased in group II when compared with group I and control at 17 day of gestation period, while at 15-day pregnancy BPA does not induce variation between groups. In the same respect, estradiol level was

significantly increased at 17 day when compared to 15 day pregnancy only in group II.

Concerning progesterone levels, the obtained data revealed that, progesterone level was significantly decreased in BPA treated groups when compared with the control rat either at 15 or 17 day at pregnancy. In regard to time, progesterone level showed a significant increase in 17 day when compared to 15day pregnancy only in group I.

It was reported that, cholesterol level was significantly increased in group II when compared with control and group I at 15 day pregnancy. In the same manner, at 17 day at pregnancy, cholesterol level significantly increased in BPA treated groups when compared to control one .

Cholesterol level was significantly increased at day 17 pregnancy than 15 day only in group I. Levels of TG was significantly increased in group I I either at 15 or 17 day pregnancy when compared with that group I or control group. However , at 15 day at pregnancy , group I did not show a significant variation with the control but at 17 day it significantly increased when compared to control one .TG level was significantly increased at 17 day than 15 day pregnancy in group I. Similar data was reported in group II. It was reported that , administration of pregnant rat with BPA significantly decreased HDL-C level at 17 day at pregnancy. On the other hand, progress of gestation could reduce level of HDL-C either in control or group I. The obtained results showed that, ‹LDL-C level was significantly increased in group II when compared with control and group I at 15 day pregnancy. similar data were recorded at 17 day when compared group I and group II to controls one. In the same respect, BPA dosing to pregnant rat significantly increased LDL-_C in groups I and II at 17 day pregnancy when compared to 15 day pregnancy.

It was reported that, levels were significantly reduced at 15 day pregnancy in group II when compared with group I and control rat. Similar data were obtained at 17 day pregnancy however level parallel decreased with the dose of BPA. The obtained results did not show any significant variation between 15 and 17 day pregnancy.

There was a significant increase in ALT activity of group I when compared with control or group II at 15 day pregnancy. Similar data was reported at 17 day however the most significant increases were recorded in group II. It was noticed that, AST activity was significantly increased in BPA treated rat than control one. The level was significantly increased parallel to BPA dose at 15 day. Similar data obtained at 17-day pregnancy. The time of pregnancy did not affect activity of AST either in control or BPA treated groups significantly. The obtained data did not show significant variation in creatinine levels either for groups or pregnancy days .

Table (1): Experiment design for prenatal toxicity of BPA.

Group	No. of Pregnant rats	Treatment	Dose. µgm /kg B.wt	Period of administration pregnancy interval (day)	Sacrificing
I	6	Corn oil	Oil	6^{th} to 15^{th}	15^{th} and 17^{th}
II	6	BPA	25	6^{th} to 15^{th}	15^{th} and 17^{th}
III	6	BPA	50	6^{th} to 15^{th}	15^{th} and 17^{th}

Table (2): Effects of BPA on serum levels of estradiol (pg/ml) and progesterone (ng/ml) in pregnant rat

	Estradiol pg/ ml			
	Control	**Group I**	**Group II**	**P. value**
15 day	43.66± 2.14	48.1±2.65	43.00±1.35A	0.25
17 day	42.6± 1.58a	48.4±2.49a	58.4±1.32bB	0.003
p. value	0.83	0.95	0.01	
	Progesterone ng/ ml			
	Control	Group I	Group II	P. value
15 day	27.2±0.61aA	16.7 ±3.40b	6.5±1.09c	0.001
17 day	33.73±1.63aB	5.33±0.40b	10.00±1.33c	0.001
p. value	0.03	0.06	0.21	

Means carry the different small letters in the same raw are significant at P< 0.05
Means carry the different Capital letters in the same column are significant at P< 0.05 .

Table (3): Effects of BPA on serum levels of lipid profile in pregnant rat

	Cholesterol			
	Control	**Group I**	**Group II**	**P. value**
15 day	83.33±4.37a	79.00±3.21Aa	100.00±2.08b	0.01
17 day	80.66± 1.76a	111.00±5.50bB	121.00±8.50b	0.007
p. value	0.30	0.02	0.13	
	TG			
	Control	Group I	Group II	P. value
15 day	40.00±1.15a	60.66±7.53Ba	60.00±3.05bA	0.03
17 day	56.66± 3.84a	76.00±5.29bB	100.00±2.64cB	0.001
p. value	0.07	0.02	0.02	
	HDL-C			
	Control	Group I	Group II	P. value
15 day	56 .33±2.96A	50.00±0.75	49.00±2.08A	0.09
17 day	51.66±2.76aB	48.00±1.52a	34.00±1.52bB	0.002
p. value	0.007	0.18	0.03	
	LDL-C			
	Control	Group I	Group II	P. value
15 day	20.00±1.38a	16.86±2.36aA	39.00±2.60bA	0.002
17 day	16.66± 1.65a	47.80±6.01bB	67.00±8.32bB	0.003
p. value	0.38	0.03	0.03	

Means carry the different small letters in the same raw for each variable are significant at P< 0.05
Means carry the different Capital letters in the same column for each variable are significant at P< 0.05

Table (4): Effects of BPA on serum levels and activities of total antioxidant capacity (mg/dl) , ALT (U/L), AST (U/L) and creatinine (mg/dl) in pregnant rat

	TAC			
	Control	Group I	Group II	P. value
15 day	0.53±0.009a	0.47±0.03ab	0.41±0.02b	0.02
17 day	0.57± 0.012a	0.49±0.012b	0.40±0.02c	0.001
p. value	0.15	0.65	0.80	
	ALT			
	Control	Group I	Group II	P. value
15 day	36.33 ±2.40aA	53.00±2.64b	45.00±5.12Aa	0.04
17 day	46.33± 3.52aB	55.66±3.52a	78.00±7.21bB	0.01
p. value	0.03	0.20	0.004	
	AST			
	Control	Group I	Group II	P. value
15 day	123.33±5.45a	177.00±3.21b	215.00±13.86c	0.001
17 day	149.33±3.52a	185.00±4.72a	231.00±5.13b	0.001
p. value	0.07	0.37	0.41	
	Creatinine			
	Control	Group I	Group II	P. value
15 day	0.60±0.11	0.7±0.15	0.40±0.05	0.25
17 day	0.5 ± 0.05	0.60±0.1	0.50±0.05	0.57
p. value	0.2	0.72	0.22	

Means carry the different small letters in the same raw for each variable are significant at P< 0.05
Means carry the different Capital letters in the same column for each variable are significant at P< 0.05

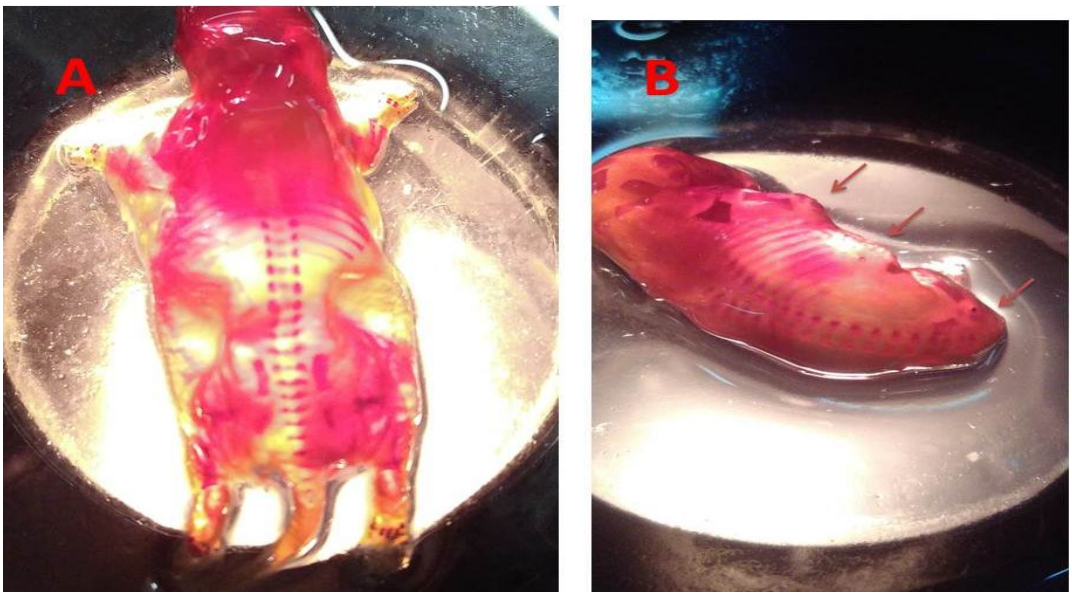

Figure 1 : Photograph of a dorsal view of fetus of control pregnant rat at 17 day gestation (A) showing normal fore limb and hind limb and tail and photograph of a lateral view of fetus of group I of pregnant rat at 17 day gestation (B) showing abnormal skull parts , fore limb , hind limb and tail bone (red arrows).

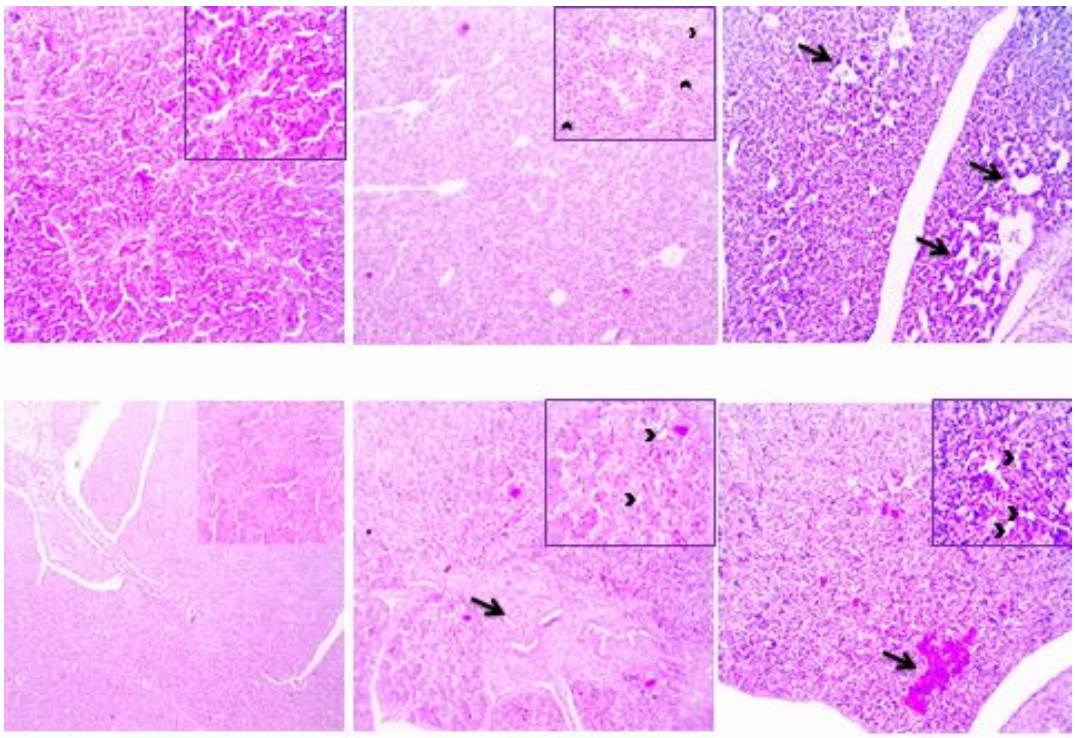

Figure 2: Liver tissue of revealed at 15 days of gestation normal appearance in control group (A), microvacuoles in hepatacytes (as pointed to in insert by arrowheads) in group 2 (B) and dilated hepatic blood vessels (arrows) in group 3 (C). (D-F): Liver of embryos at 17 days of gestation shows normal appearance in control group (D), portal fibrosis (arrow) with presence of microvacuoles in hepatacytes (as pointed to in insert by arrowheads) in group 2 (E), focal area of hemorrhage (arrow) with presence of macrovacuoles in hepatocytes (as pointed to in insert by arrowheads) in group 3 (F). Insert: is high magnification of figures. (A- F, H&E X: 100, insert X: 200).

DISCUSSION

Bisphenol A is a synthetic chemical that is used as a monomer to manufacture polycarbonate plastics, as well as an intermediate in the synthesis of epoxy resins. It was reported to be decay in the preserved foods and life applicant products that represent hazards for human consumption (***Wang, et al, 2017***). Serum estradiol level was significantly increased in group II when compared with group I and control at 17th day of gestation period while at 15th day pregnancy BPA does not induce variation between groups. In the same respect, estradiol level was significantly increased at 17 day when compared to 15-day pregnancy only in group II.

On the other hand, progesterone level was significantly decreased in BPA treated groups when compared with the control rat either at 15 or at 17 days of pregnancy. While progesterone level showed a significant increase in 17 day comparing to 15-day pregnancy only in group I.

The study of ***Taha et al,*** (***2016***) reported that there was a highly significant increase in FSH, progesterone, prolactin and estradiol levels accompanied with a significant decrease in LH level in the female treated group (G1) in compare with the control group.

In rodent studies, perinatal (***Xi et al. 2011***) low-dose BPA-exposure resulted in elevate serum estradiol (E) levels. Prenatal studies on rodents stated that Sprague-Dawley rats and one of pregnant ICR mice, low-dose BPA increased progesterone levels (***Fernández et al. 2010; Tan et al. 2013***). In these four studies, the doses used were lower than those in studies reporting BPA effects, indicating that BPA doses less than 20 mg/kg BW may not increase hormone production in animal models. On opposite side, another study (***Berger and Hancock, 2008***) found that although BPA did not alter estradiol levels, low-dose BPA decreased progesterone levels in adult mice during early pregnancy.

Regarding the lipid profile, our data came in a harmony with those recorded by ***Jiang et al.*** (***2014***) who found that Wister rats treated with BPA up–regulated hepatic lipid metabolism and up-regulated genes involved in lipogenesis pathway.

Our results coincide with those reported by ***Taha et al,*** (***2016***) who stated that total lipids, cholesterol، triglyceride and LDL-C values were significantly increased in BPA-treated group, while a significant decrease in HDL- C value was recorded between the control group and the BPA- treated group.

The present data disagree with the those of ***Ortiz-Villanueva et al.(2017)*** who observed that the serum cholesterol level was statistically decreased ($P<0.05$) in serum of BPA treated groups when compared to control one. A significant decrease in cholesterol level ($P<0.05$) was observed in serum of BPA treated groups compared to control group. Therefore, in the current study, serum cholesterol level was drastically decreased in serum of high dose BPA treated group. Significant effect on total antioxidant capacity(TAC) in BPA dose – dependent manner regardless the day of pregnancy, as TAC in GII reduced TAC level remarkably comparing to GI or control group , and both experimental group showed lower level of TAC. The day (15th and 17th day) of pregnancy showed insignificant differences in TAC levelsat day 15^{th} and 17^{th} .

The obtained result at BPA, which induce a significant increase in oxidative/nitrusative stress, while is accompanied by marked alterations in TAC (***Fridovich, 1997***). TAC in the present study decreased after BPA administration. Due to BPA caused induction of free radicals in the hepatic tissue, in consequence, it leads to disruption in the antioxidant defense system.

The current findings came in agree with findings obtained by ***Eid et al.,(2015)*** who reported that levels of total antioxidant capacity in BPA (0.5 and 50 mg/kg b.w.) were significantly ($p < 0.001$) decreased compared to the control in the three different pubertal periods. Moreover, BPA50 compared to BPA0.5 showed a significant decrease in levels of antioxidant capacity in all estimated periods.

The obtained results revealed a significant increase in GPT activity in and shower PBA treated groups compared activily with control, significant increases at 17^{th} day when compared to 15^{th} day pregnancy. Also BPA Affects serum activity of AST in pregnant rat. AST significantly increased in BPA treated Groups (BPA 50 > BPA 25) than control one. However, the day of pregnancy did not significantly affect activity of AST either in control or BPA treated groups. The significant change in the activities of ALT and AST showed the toxic effect of BPA. Elevated activity of serum enzymes are indicators of cellular leakage and loss of functional integrity of the cell membrane in liver. These are of major importance in assessing and monitoring functional status of liver. Thus, their increase in serum may give information on organ dysfunction. It has been reported that ALT activity is an important index to measure the

degree of cell membrane damage, while AST is an indicator of mitochondrial damage since it contains 80% of this enzyme . The high levels of ALT and ALP are attributed to damage have been previously reported considering the BPA toxicity (***Hall, 2015***).

This study coincide with those reported by ***Taha et al, (2016***) Who found increase in activities of liver function ALT and AST activities increase in BPA group exhibited highly significantly increased when compared to the corresponding control values.

The obtained data did not show a significant variation in creatinine levels either for groups or pregnancy days .

Study of ***Taha et al., (2016)*** established that kidney function parameters as creatinine, urea, were also highly significant in the BPA-treated group, as the serum creatinine level in BPA-treated group was remarkably higher than in control group.

Also, the study of ***Geetharathan and Josthna (2016)*** revealed that PBA had kidney toxic effects as both treated BPA groups (25mg/kg/d and 50mg/kg/d)showed elevated levels of creatinine then those in the control group.

Figure 2: hematoxylin –eosin histopathological lesions of Liver shows of embryo at 15 days of gestation normal appearance in control group along the experiment period, while microvacuoles in hepatacytes in treated group showed dilated hepatic blood vessels, portal fibrosis with presence of microvacuoles in hepatacytes, and focal area of hemorrhage with presence of macrovacuoles in hepatocytes.

These results explained that exposure to BPA progressively increased the intensity of these degenerative changes such as increased dilatation and congestion of sinusoids and central vein and hydropic degeneration with focal array of hepatic cords. The results are in agreement with ***Abdel Hameed (2004)*** who described the vacuolation of hepatocytes as ballooning degeneration and interpreted it as a kind of cellular defensive mechanism against injurious substances.

Moreover , results of ***Badawi et al, (2013***) agreement with results of the present study as it demonstrated that liver of treated with BPA in different doses and periods showed dilated and congested central veins, dilated sinusoids together with increased proliferation of von kupffer cells and lymphocytic infiltration, thickening and hyalinization of some portal veins with haemolysed blood cells in the blood vessels and mild edema and mild inflammatory areas in periportal zone.

CONCLUSIONS

Bisphenol A (BPA) can be considered as an extremely harmful substance although its production and uses in many life fields are tremendously increased.

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الملخص العربي

بيسيفينول أ هو مادة كيميائية صناعية ذات انتشار واسع تستخدم في جميع أنحاء العالم

ويمكن للبيسيفينول أ أن يعبر من المشيمة إلى الجنين وفيتو-سمية والآثار الضارة الأخرى في الحيوان الحامل والأجنة. وقد أجريت هذه الدراسة على ستين من الجرذان الحوامل مقسمة إلى ثلاث مجموعات حيث تخلط مع زيت الذرة وتجرع من يوم ٦ إلى يوم ١٥ من الحمل عن طريق الفم للمجموعة الأولى المجموعة الضابطة لم تجرع، المجموعة الثانية جرعت بـ ٢٥ غم / كغ يوميًا، والمجموعة الثالثة بجرعة ٥٠ غم / كغ. في ١٥ و١٧ يومًا من الحمل تم أخذ ١٠ جرذان من كل مجموعة وتم جمع الدم من الجرذان لجمع المصل وأبقيت مجمدة لفحص الكيمياء الحيوية. تم جمع الأعضاء منهم وحفظها في الفورمالين ١٠٪ لفحص الأنسجة، والنصف الآخر يُحفظ في الكحول الإيثيلي لدراسة تشوه الهيكل العظمي. وكشفت النتيجة عن انخفاض في حجم الجنين بسبب جرعات البيسيفينول أ مع تشوه الهيكل العظمي. وعلاوة على ذلك، يمكن للبيسيفينول أ رفع مستوى هرمون الاستروجين وانخفاض مستوى هرمون البروجسترون في مؤشر جيي وجيي. مقارنة مع مجموعة الكنترول. وبالإضافة إلى ذلك، زادت أنشطة انزيمات الكبد بشكل ملحوظ في مؤشر جيي وجيي بينما انخفضت القدرة المضادة للأكسدة الكلية بشكل ملحوظ. كشف الفحص النسيجي للأنسجة الجنينية الآفات المرضية في الكبد والرئة والكلى من مجموعات الجرعات البيسيفينول أ.