Vol. 04, No. 02, pp. 71 –76 (2023)
ISSN: 2708-1818

JOURNAL OF LIFE AND BIO-SCIENCES RESEARCH

www.jlbsr.org

Morphological and Histological Impacts of Bisphenol A on Chicken Embryos

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Abstract

The aim of the current study was to investigate the morphological and histological effects of Bisphenol A on chicken Ross 308 during embryonic development A total of 150 eggs were randomly divided into five groups: negative control, positive control injected with con oil and treatment groups administered with different concentrations of Bisphenol A (0.005, 0.02 and 0.1 microliters/egg). The eggs were injected on the second day of incubation and the evaluation was conducted on days 10 and 21 of incubation. Our results showed different abnormal observations at both ages of incubation including skin redness, presence of hemorrhagic spots on the head and around the eyes, congenital anomalies in the upper and lower limbs, chick lethargy and inflammation, particularly in the caudal region. Additionally, there was a clear paralysis in a chicken aged 21 days. Microscopically, there were changes in the liver structure representing congestion in the central vein, mild focal intercellular changes in hepatocytes, few necrosis and atrophy of hepatic cords. At day 21, there was also dispersed hemorrhagic, inflammatory infiltration slight proliferation of Kupffer cells, fatty degeneration, irregularity of hepatocytes and hepatic cords as well as cloudy swelling.

Keywords: *Bisphenol A, liver, Ross 308, chicken embryos*

Received: May 25th, 2023/ Accepted: July 20th, 2023/Online: July 22nd, 2023

I. INTRODUCTION

Bisphenol A (BPA), also known as 2,2-bis (4-hydroxyphenyl), is a non-volatile toxic crystalline chemical compound. It has a molecular weight of 228.28 g/mol and is insoluble in water but soluble in fats, ether, and alcohol. BPA has a boiling point of 220°C, a melting point of 156°C and a flash point of 79.4°C (Sharma *et al*., 2019). This substance is widely used in the industrialization of polycarbonate plastics and epoxy resins (Xiao *et al*., 2020). BPA is also used in the production of thermal paper, medical devices and dental materials (Cimmino *et al*., 2020). Moreover, it is incorporated into various other industries, including safety equipment, motorcycle helmets, and sunglasses manufacturing due to its lightweight and optical clarity (Rogers, 2021). The global production of BPA has reached 15 billion pounds annually and some studies have reported that over 100 tons of BPA are released into the environment (Petrie *et al*., 2019). The production of millions of plastic materials in a year contributes to the presence and increase of BPA in our environment (Cohen *et al*., 2021). As a result, BPA can be found in water, air, soil and foods (Ribeiro *et al*., 2017; Petrie *et al*., 2019; Salamanca -Fernandez *et al*., 2021). This chemical material enters the body through ingestion (Lopes-Rocha *et al*., 2021) or by using hand creams and these are the main cause of making BPA more permeable and has deep absorption as well as not easily eliminated. Thus, some global companies have attempted to manufacture and use alternatives to BPA such as PBS, PBF and TMBPF, which are probably less toxic (Maffini and Canatsey, 2020; Harnett *et al*., 2021). However, due to its estrogen-like activity, BPA has attracted the interest of researchers for many years due to its negative impacts on the health of living organisms (Ma *et al*., 2019). A study conducted on mice has demonstrated the negative effects of BPA on fetal growth and vital body functions even at low doses, as it causes changes in proteins involved in the biosynthesis of fatty acids and cholesterol (Tonini *et*

doi:10.38094/jlbsr402108

al., 2021). Exposure to BPA increases the expression of the caspase-3 enzyme in the liver, which is a biological marker for programmed cell death due to the increase of oxidative stress. Histological changes have been observed in rats' liver such as focal necrosis with dilation.

II. MATERIALS AND METHODS

A. Experimental Design

A total of 150 eggs were divided into five secondary groups as follows: negative control group (1) which was untreated, positive control group (2) treated with corn oil, and experimental groups (3, 4, 5) treated with different concentrations of Bisphenol A. The treatment was administered on the 2^{nd} of incubation.

B. BPA media

BPA was purchased from Solarbio Beijing Japanese Company. BPA powder was dissolved in corn oil (Atay *et al*., 2020) then three concentrations (0.005, 0.02 and 0.1) were used for the current study depending on LC50. 100 µL of BPA was injected with an insulin needle on day 2^{nd} of incubation of central veins and congestion, hepatocellular vacuolation and irregularly shaped nuclei induced by BPA (Abdel-Rahman *et al*., 2018). In addition, BPA-induced structural alterations in the liver, characterized by programmed cell death, infiltration of white blood cells, cytoplasmic vacuolation, swelling of the rough endoplasmic reticulum, mitochondrial degeneration, nuclear enlargement, fat droplet deposition and elevated liver enzymes (Ahmed Zaki *et al*., 2021). BPA can stimulate mRNA and cause organ damage, leading to changes in the levels of enzymes that can cause health damage (Faheem *et al*., 2019). However, it is still less information about BPA effects on birds during development. The present study, therefore, focused used different concentrations of BPA to see whether it has toxicity on the development.

C. Preparation of tissue sections

Tissue sections were prepared after 10 and 21 days of incubation Embryos were anesthetized by chloroform. The liver was removed from embryos then liver tissues were prepared for microscopic examination. The sections were then processed following a series of steps, as described by (Suvarna *et al*., 2019). fixation with neutral formalin for 24-48 hours, rinsing with running water for 30 minutes, dehydration in ascending concentrations of ethyl alcohol (50%, 70%, 90%, and 100%) for 30 minutes each (except the last concentration, which was done for 2 hours), clearing with xylene for 30 minutes, infiltration and embedding using paraffin wax with a melting point of 54-56°C. The samples were placed in an oven at a temperature of 60°C. Trimming and sectioning were performed using a microtome rotary at a thickness of 5-7 micrometers to obtain longitudinally and transversely oriented ribbon sections. The wax was removed from the tissue sections by incubating them in a jar containing hot xylene in the oven. The sections were then stained with general histological stains (Hematoxylin and Eosin H&E) following the method described by (Culling *et al*., 1985). Sections were mounted with DPX medium and then checked using a compound light microscope with a camera. The magnification power of the images was determined using the lens magnification factor and the ocular lens magnification factor.

III. RESULTS

Morphologically, both control groups at day 10 of incubation revealed normal growth with normal head, normal eyes, beak and normal limbs (Figure 1 A and B). The treated groups with BPA exhibited a variety of changes depending on the concentration. Treatment of the embryos with 0.005 showed similar visual changes to the aforementioned concentration effects, including skin reddening, the presence of haemorrhagic spots around the head and eyes, retardation in the growth, and abnormal limbs with abnormal digits all compared to the control groups (Figure 1 C). At 0.02 embryos were characterized by distinct deformities in their shape, slow growth and severe haemorrhaging in various areas of the body (Figure 1 D). There was also thinning of the body cavity wall and abnormal limbs manifested from constriction bands in the fingers in comparison to control groups. The present study also revealed that the high concentration of BPA (0.1) caused high effects and abnormal embryos (Figure 1 E). In addition, at day 21 of incubation, the control groups also were normal compared to treated embryos (Figure 2 A and B). However, the experimental group with 0.005 embryos were physically weak with paralysis in some of them. Also, clear structural deformities observed in the toes of the hind limbs (Figure 2 C) embryos were then died after a short time of hatching. At 0.02 concentration, the embryos were weak and they needed assistance to be out of eggs. Those chicks exhibited several congenital deformities, including abdominal swelling and showed tumors in the stomach and intestines, internal bleeding, fatty degeneration and congestion in certain areas of the liver along with reduced size (Figures 2 D and E). Additionally, structural abnormalities were observed in the hind limbs and toes. The embryos in the experimental group 0.1 exhibited complete deformities and undeveloped embryos (Figure 2 F) suggesting the effectiveness of BPA increases with higher concentrations.

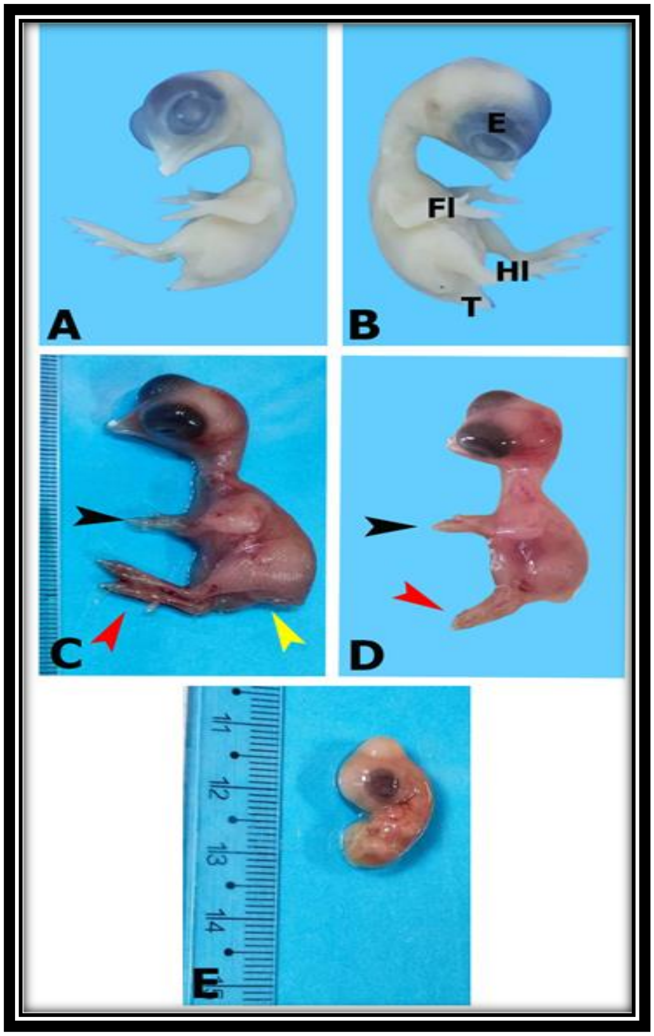

Figure 1. Lateral view of whole chicken embryos at day 10 of incubation, showing the negative control (A) and positive control (B) groups showing normal development in the body organs. (C), chicken embryo treated with (0.005) group.

There is redness of the skin, haemorrhagic patches observed in the head region, particularly under the eyes, impaired growth and abnormal hind limb (black arrowhead) with adhesion between them towards the fingers in the forelimb (red arrowhead). Mild swelling is present on the lower right limb and undeveloped tail (yellow arrowhead). D, showing a chicken embryo treated with (0.02) group, there is a slow growth compared to the control and other treatment groups. Clear deformities in the shape of the embryo and haemorrhage in various body regions, thinning of the body wall and abnormal limbs (black and red arrowheads). E, representing a chicken embryo treated with the (0.1) group, a complete deformity of the embryo is observed, including the protrusion and lack of development of the brain, resulting in growth cessation. E: eye, FL: forelimb, Hl: hind limb, T: tail (magnification in mm).

Histological examination of the embryos at day 10 of incubation showed normal structure in the liver of the control groups. The liver tissue consisted of sinusoids and the hepatic cords were regular and parallel to central vein (Figure 3 A and B). However, embryos treated with 0.005 (Figure 3 C and D) showed congestion in the central veins and sinusoids, along with small to moderate gaps in the hepatocytes beneath the liver capsule (mild vacuolar change).

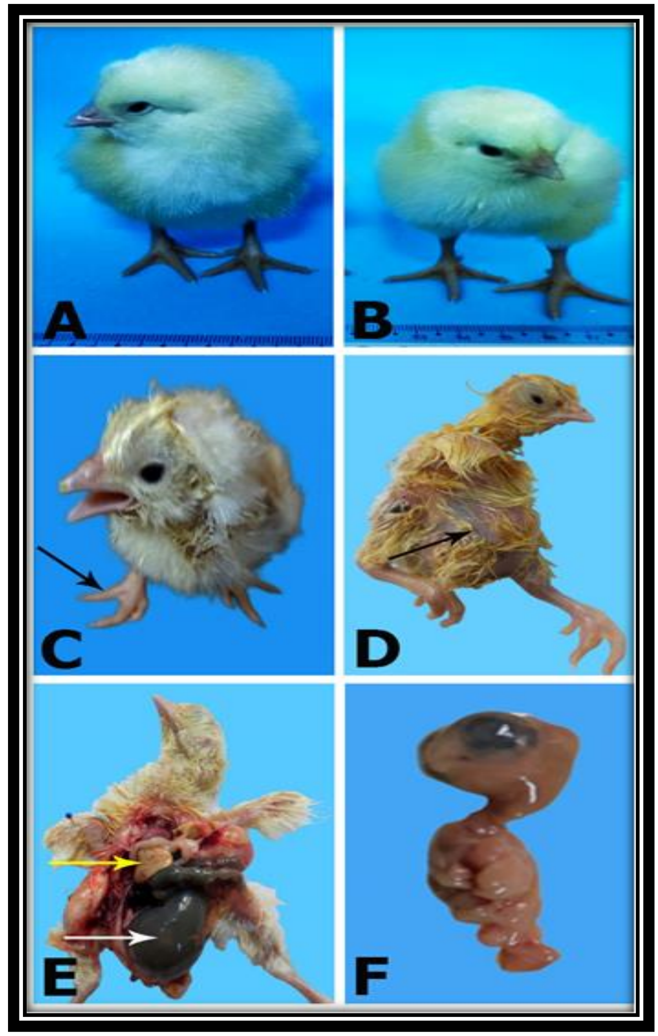

Figure 2. Chicken embryos at day 21 of incubation. (A) the negative control group, (B) the positive control group treated with corn oil, chickens are healthy without any deformities. (C) chicken treated with 0.005 showing weakness, reduced movement, staggering, paralysis and deformities in the toes of the hind limbs (black arrow). (D and E) chicken treated with 0.02 showing abdominal body (D- black arrow) and the dissected one (E) showing tumor in the stomach and intestines (white arrow), along with internal bleeding. The liver is small with fatty infiltration, and congestion in certain areas (yellow arrow). (F) representing a chicken treated with (0.1) abnormal growth is clear (scale in mm).

Furthermore, focal mild vacuolar degeneration of hepatocytes beneath the liver capsule was observed. In group 0.02, the embryos showed more pronounced vacuole degeneration of hepatocytes particularly beneath the liver capsule with thickening of the liver capsule (Figure 3 E). In experimental group 0.1, the embryos exhibited severe congestion of the hepatic sinusoids, moderate vacuolar change and cloudy swelling in other areas of the liver tissue

(Figure 3 F). There was evidence of programmed cell death (apoptosis) in hepatocytes, indicative of an inflammatory response in the tissue. Focal thickening of the liver capsule was also observed in some areas. In the histological examination of the liver on day 21 of incubation, the control group showed regular structure of the hepatic cords and clear visualization of the central vein (Figures 4 A and B).

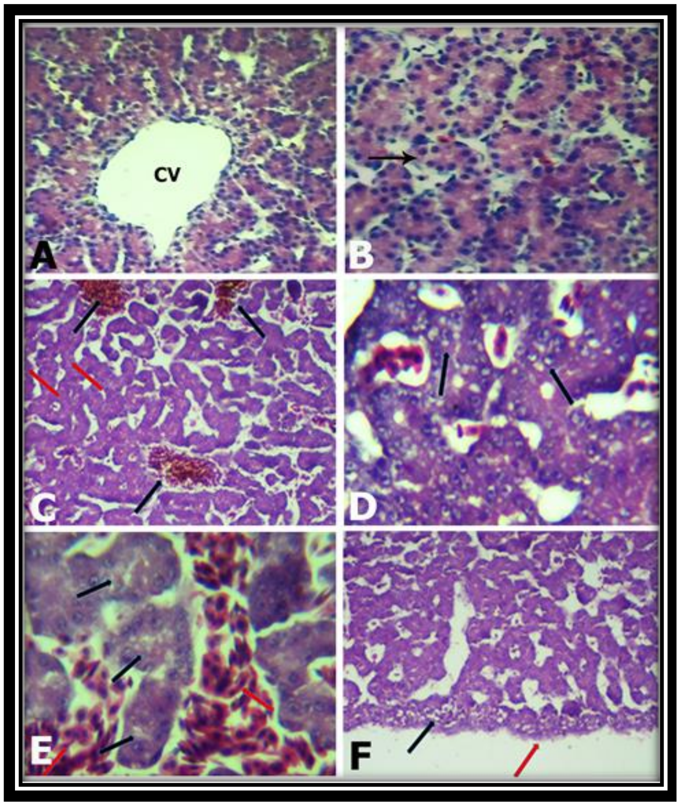

Figure 3. Liver histological sections in chicken embryos incubated for 10 days. (A), the negative control group (B) control group treated with corn oil. Normal structure with normal central vein and hepatocytes (black arrow) are shown. (C and D) the embryos treated with 0.005 reveal severe congestion of the central vein and blood capillaries (black arrow), as well as hepatocytes exhibiting mild focal necrosis (red arrow). Figure D also shows focal mild vacuolar degeneration of hepatocytes beneath the hepatic sinusoid (black arrow). (E), embryos treated with 0.02 reveals mild vacuolation with atrophy beneath the hepatic sinusoid and thickening of the hepatic sinusoid wall in some focal areas (red arrow). There is also focal vacuolar degeneration of hepatocytes, more pronounced beneath the hepatic sinusoid (black arrow). (F), embryos treated with 0.1 showed moderate focal vacuolation of hepatocytes (black arrow), severe congestion of hepatic capillaries (red arrow), marked cellular swelling of hepatocytes (cloudy swelling), and enlargement of the hepatic sinusoid. CV: central vein, (H and E staining, magnification power 400X).

In the experimental group 0.005, the embryos exhibited congestion in the central veins and hepatic sinusoids accompanied by cloudy swelling, necrosis and focal inflammatory infiltrates (Figure 4 C and D). There was also a slight proliferation of Kupffer cells beneath the hepatic sinusoids, scattered areas of haemorrhage, fatty degeneration, and irregularity of hepatocytes and hepatic cords. In the embryos of the 0.02 group, more severe fatty changes were observed along with scattered areas of haemorrhage, focal inflammatory infiltrates, dilatation of blood sinusoids, and irregularity of hepatic cords (Figure 4 E). Fatty changes were also present in the liver parenchyma. In the 0.1 group, embryos exhibited necrosis in multiple foci accompanied by severe congestion of hepatic sinusoids (Figure 4 F). Moreover, focal inflammatory infiltrates were present beneath the hepatic capsule, failure of hepatic cord formation along with hepatocyte atrophy. The liver parenchyma was also predominantly occupied by dense fatty tissue and haemorrhage. Thus, it can be concluded that BPA may have a toxic effect comparable to that of other hazardous chemicals.

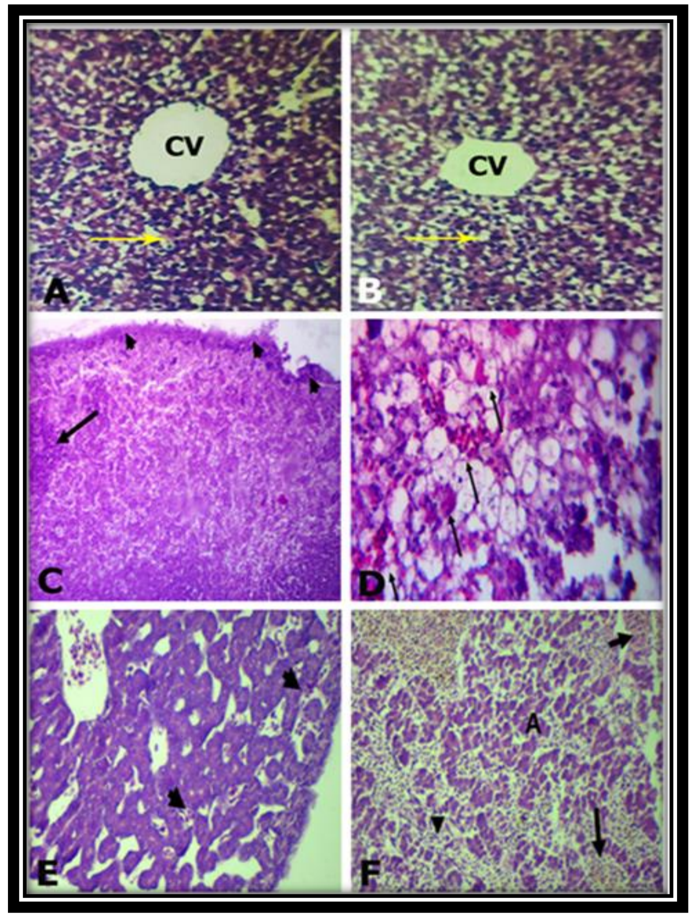

Figure 4. Liver histological sections in chicken embryos incubated for 21 days. (A), illustrates the histological composition of the negative control group and positive (B) control group. Normal blood capillaries and the central vein, normal structure of hepatic cells (yellow arrow). (C and D), liver structure in chicken embryos treated with 0.005 showing congestion of the central veins and blood capillaries, cellular swelling, and focal inflammatory infiltrates (black arrow). (E), liver structure in chicken embryos treated with 0.02 reveals the presence of severe fatty changes with scattered focal haemorrhagic areas and focal inflammatory infiltrates, dilation of blood capillaries, and irregularity of hepatic cords. (F), chicken treated with (0.1) reveals multiple areas of coagulated necrosis (arrowhead) accompanied by severe congestion of the hepatic sinusoids. Focal inflammatory infiltrates are observed beneath the hepatic sinusoid, and failure of hepatic cord formation with hepatocyte atrophy and haemorrhage are also present (arrow). CV: central vein, H and E staining, magnification 400X).

IV. Discussion

The current study focused on BPA as a chemical material that has a wide spread in our daily life. Results showed that this material has a potential effect on the morphology of chicken embryos at both ages (10 and 21 of incubation). It caused abnormal growth in addition to its effect on the liver structure. A study conducted on 21-day-old chicken embryos to investigate the toxicity of insecticides revealed similar effects of BPA including brain protrusion, absence of eye development, beak deformities, delayed formation of the body cavity wall, presence of organs outside the body cavity and the occurrence of haemorrhage in blood vessels surrounding the yolk sac (Taha and Mohammed, 2022). The current study suggests that the observed deformities in fetuses and chicks may be attributed to the complex programmed and highly specific process of growth (Najjar *et al*., 2022). This process requires cell differentiation to form organs and determine their natural functions. Therefore, any disruption in these processes can lead to harmful effects on tissues and organs, resulting in congenital malformations. The process of oxidation and reduction that occurs in cells during embryonic development controls DNA synthesis, enzyme activity, gene expression, signal transduction and the cell cycle. The oxidative stress can alter the mechanism of oxidation and reduction, which is responsible for many of the aforementioned cellular functions and growth. Thus, oxidative stress also plays a significant role in causing growth disorders (Suzuki *et al*., 1997; Arrigo, 1999; Shackelford *et al*., 2000). Some studies have revealed the toxic effects of BPA such as Faheem *et al*. (2019) who demonstrated that exposure of Catla catla fish to BPA leads to tissue alteration in liver cells, structural changes, oxidative stress, lipid accumulation, haemorrhage and necrosis. Similarly, Ateş and Hatipoğlu (2022) showed mononuclear cell infiltration, karyomegaly, oxidative changes and necrosis induced by mg/kg100 of BPA in mice. The study also revealed bile duct hamartoma (BDH), hepatocerebral dystrophy (HCD) and activation of Kupffer cells. According to Al-Jomily *et al*. (2022), exposure of quails to certain chemical toxins leads to liver discoloration, shrinkage, suppuration, cellular necrosis and oxidative stress in liver cells. BPA can alter the heart structure in mice females and their embryos (Al-Sultan *et al*., 2023). BPA affects nuclear protein receptors such as PPARγ, C/EBP, Nrf2, and HAND2 that are responsible for a range of biological effects including energy homeostasis, metabolism, cellular proliferation, fat formation, cell differentiation and immune response (Cimmino *et al*., 2020). PPARγ, in particular, plays a role in lipid and glucose metabolism in various tissues, including the liver (Somm *et al*., 2009). Hence, we can say that the morphological and histological changes documented in the current study on the livers of chicken embryos and offspring treated induced by BPA indicate high toxicity that results in alterations in liver structure. This organ is a vital organ involved in the production of various crucial substances in the body, including bile, which aids in fat decomposition, protein synthesis, which affects blood clotting, and the production of essential cholesterol for the synthesis of certain growth hormones. As previously mentioned, there is a possibility of BPA binding to hormone receptors, which could inhibit the production of these necessary substances for organism growth (Al-Mustafa and Al-Sultan., 2022).

V. Conclusion

To conclude, the present study showed that exposure to BPA even at low concentrations has a potentially toxic effect on chicken embryos which probably leads to a toxic impact on the human body and other specific organs including the liver. Thus, caution should be taken into account when dealing with or using BPA in the industries used in our daily life.

Acknowledgements

We would like to thank the University of Mosul and the College of Education for Girls for providing support in this work.

Conflict of Interest

The authors have declared that no conflict of interest exists.

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