The Effects of Bisphenol A of Polycarbonate Plastics on Various Blood and Fertility Parameters, along with Histological Changes in Male Albino Rats

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ABSTRACT

Background: Bisphenol A (BPA) is an essential monomer used in the manufacturing of polymers. They can bind to oestrogen receptors as agonists/antagonists, causing adverse effects on metabolic and endocrine system function. The current study aims to investigate the toxic effects of bisphenol A at various concentrations on male albino rats.

Methods: Twenty male albino rats were equally divided into four groups [Gp1, control; Gp2, 25 mg/kg b.wt. of BPA; Gp3, 50 mg/kg b.wt. of BPA; Gp4, 100 mg/kg b.wt. of BPA], all groups were treated orally for 35 days.

Result: Through the results, significant ($P \leq 0.01$) reductions were observed in the levels of sexual hormones (Testosterone (tt), Follicle-Stimulating Hormone (FSH) and Luteinizing (Hormone (LH) and Estrogen, for all groups dosed with BPA. Our results showed a significant ($P \leq 0.01$) decrease in the count of sperm, viability and sperm motility, for all groups dosed with BPA in contrast to the group under control. Bisphenol A at different doses leads to a significant ($P \leq 0.01$) increase in liver enzymes (AST and ALT) for all treated groups in contrast to the group under control, with various histological changes in the liver, including multiple severe fibrosis of the portal area and multiple sever portal amyloidosis and congestion with fibro portal extension to neighboring areas.

Key words: Bisphenol A, Estrogen, Follicle-Stimulating Hormone, liver enzyme, Polycarbonate plastics, Sperm count, Testosterone.

INTRODUCTION

Bisphenols (Bisphenol A, B, C, S, F, AF) are phenolic organic compounds, typically employed in the synthesis of epoxy resins and polycarbonate plastics, while primarily utilised to solidify plastic and is prone to leaking into meals and beverages (Kim *et al*., 2020). Bisphenol A is the most widely used and studied of the bisphenols. Bisphenol A {BPA: $C_{15}H_{16}O_2$} is a white crystal chemical that is produced industrially through phenol and acetone condensation; its production is expected to reach 10 million metric tonnes worldwide in 2022 (Abraham and Chakraborty, 2020). When looking for synthetic oestrogens in the 1930s, researchers investigated BPA, which A.P. Dianin had first synthesised in 1891 (Dodds, 1936). BPA is regarded as one of the environmental pollutants that is frequently employed during polycarbonate plastics manufacturing, including polyvinyl chloride (PVC), water pipes, baby feeding bottles, food packaging and epoxy resin (the coating found inside food cans made of metal), as well as a non-polymer additive used with other plastics such as cigarette filters, tableware and dental fillers (Hoekstra and Simoneau, 2013; Huang *et al*., 2012 ; Hameed *et al*., 2024). BPA exposure occurs in humans through various methods, but oral exposure is the most prevalent. Under extreme temperatures and acidity or alkalinity conditions, BPA could be hydrolysed, resulting in leakage into foods and beverage containers (Welshons *et al*., 2006; Hasan *et al*., 2024).

BPA belongs to a family of compounds known as “hormone disruptors” or “endocrine disruptors”, which can alter the body’s chemical messenger system (Elshaer *et al*., 2013; Hasan *et al*., 2024). Given the increased frequency of reproductive disorders in recent decades, concerns have been raised about the role of substances known as endocrine disruptors (EDs), which can modify or impair the function of the endocrine system (Diamanti-Kandarakis *et al*., 2009; Hasan *et al*., 2024). Because bisphenols can produce reactive oxygen species (ROS) as a result of reduced antioxidant enzyme levels, they can cause oxidative stress and have toxic effects (Hassan *et al*., 2012). Moreover, bisphenols have effects that vary with age, gender and dose. Breast milk, placental tissue, urine, serum and umbilical cord blood all contain higher-

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than-normal levels of bisphenol (Lee *et al*., 2018; Ezz *et al*., 2023). Since bisphenol is known to be harmful even at low concentrations, wherefore an evaluation of their impact on human health is required.

The US Environmental Protection Agency (EPA) is required to carry out endocrine-active chemical testing and screening programmes under the 1996 Food Quality Protection Act, which the Safe Drinking Water Act amended. Chemicals that disrupt the hormones include plasticizers, industrial compounds, surfactants, biocides and bisphenol-A (Shelby *et al*., 2012; Alankooshi *et al*., 2023). The European Food Safety Authority (EFSA) published a global re-evaluation of BPA exposure and toxicity in 2015 based on new data and methodologies, to lower the tolerable daily intake (TDI) of BPA from 50 (0.05 mg/kg bw/day) to 4 µg/kg bw/day (Cwiek-Ludwicka, 2015).

Since the liver is responsible for preserving homeostasis throughout the body, an important metabolic organ. It performs several vital tasks, including metabolism, xenobiotic detoxification, glycogen storage and the synthesis of proteins, cholesterol and bile (Grijalva and Vakili, 2013). This study investigated the harmful effects of BPA on some fertility hormones with semen parameters and some liver enzyme parameters and the histological alterations of the liver at three different concentrations of BPA by using Swiss albino rats as an animal model to elucidate the effect of sub-chronic exposure of BPA.

MATERIALS AND METHODS

Chemicals

Through the OMA company (Iraq), Bisphenol A (BPA, CAS No.: 80-05-7, >99% pure) was acquired from Sigma-Aldrich, USA. To determine the final concentration of BPA doses for each group, BPA was dissolved in corn oil due to its low water solubility (Shareef *et al*., 2006).

Animals

We acquired twenty male Swiss albino rats from the Ministry of Health in Iraq's Central Public Health Laboratory. Rats grow quickly during infancy and reach sexual maturity at the age of roughly six weeks (Sengupta, 2013). Therefore, rat exposure began at age between 5 and 6 weeks (weighing 125±5 g) and continued for eight weeks, that is, until they reached the age of 13 weeks or more. The temperature (26°C±2°C) and lighting (12-hour cycle) were maintained under standard conditions. The animals were given a regular diet and unlimited access to water. Rats were given seven days to acclimatise before being exposed. According to the guidelines for animal care, every animal received humane treatment.

Experimental design

The Scientific Committee of Mustansiriya University's College of Science examined and approved every method utilized in this study to ensure that it adhered to the moral standards for animal welfare. A total of twenty male albino rats were randomized, subdivided into four groups, each group has five rats and given a uniform orally volume 1 ml of BPA or corn oil once daily for 35 days; the animal's groups were classified as following:

Group 1 (Gp1, control): Rats were received corn oil once daily. (just for one time or 35 days).

Group 2 (Gp2): was given orally 25 mg/kg b.wt. of BPA once daily for 35 days.

Group 3 (Gp3): was given orally 50 mg/kg b.wt. of BPA once daily for 35 days.

Group 4 (Gp4): was given orally 100 mg/kg b.wt. of BPA once daily for 35 days.

The animals were sedated with ether at the end of the experiment and blood samples were taken through heart puncture.

The present study was carried out at Polymer Research Unit, College of Science, Al-Mustansiriyah University, Iraq. This study began in March 2020 and the practical part was completed in April 2022. The theoretical part and writing of the research began and the research was finally completed in April 2024.

Hormonal assay {Enzyme-Linked Immunosorbent Assay (ELISA)}

Samples were separated for measurement of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T) and Estrogen (E) concentrations, as well as measured aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes levels by using Enzyme-Linked Immunosorbent Assay (ELISA) kits provided by (Monobid Inc. lake forest CA 92630, USA).

Epididymal sperms analysis

In 2 millilitres of RPMI 1640 Medium, the caudal epididymis was minced to measure sperm motility and counts. Under a cover slip, one drop of a uniformly mixed sample was added to Neubauer's counting chamber. Both motile and immotile sperm were counted per unit area to determine quantitative motility, which was expressed as an index. Using a standard protocol, epididymal counts were determined and reported as millions/mL of suspension. According to Raji *et al*. (2003), 10 µl of eosin-nigrosine (Merck, Germany) was thoroughly mixed with 50 µl of freshly prepared epididymal sperm solution to determine the viability of the sperm.

Histological studies

Animals were slaughtered soon following the collection of blood samples and their liver was promptly removed and preserved for histological analysis in 10% formalin. The tissue was embedded in paraffin wax after being cleaned with xylol and dehydrated using an alcoholic series. Sections were examined under a light microscope after being stained with eosin and hematoxylin (Bancroft and Gamble, 2008).

Statistical analysis

The SAS (2018) software was utilized to ascertain the impact of distinct groups on the study parameters. In this

study, the T-test and the least significant difference (LSD) were utilized to compare means significantly.

RESULTS AND DISCUSSION

Effects of BPA on fertility hormones

Table 1 demonstrated a significant (P≤0.01) reduction in the level of FSH in treated groups with 25, 50 and 100 mg/kg b.wt of BPA (3.04±0.05, 2.63±0.12 and 1.61±0.25) µIU/ml, respectively, in contrast to the control group (3.65 ±0.32 µIU/ml). Furthermore, the findings revealed a significant (P≤0.01) reduction in the level of LH in treated groups with 25, 50 and 100 mg\kg b.wt of BPA (2.24±0.03, 2.13±0.04 and 1.45±0.21) µIU/ml, respectively, in contrast to the control group (2.53±0.09 µIU/ml). However, there was no significant difference between the group that was treated with 25 mg/kg b.wt. of BPA and the group treated with 50 mg/kg b.wt. of BPA.

Also, at the same Table 1, testosterone showed an obvious and significant (P≤0.01) decrease in all bisphenol A treatments (1.98 ±0.04, 1.63±0.14 and 0.983±0.04) ng/ml, respectively, in contrast to the control group (2.41±0.19) ng/ml. As for the estrogen hormone, it showed a significant (P≤0.01) difference according to the treatments. A significant (P≤0.01) reduction was observed in the treated control groups with 25 and 50 mg/kg b.wt of BPA (42.98 ±0.54 and 39.90±0.35) pg/ml, respectively, in contrast to the control group (46.20±1.00) pg/ml, while it was observed a significant (P≤0.01) elevation in the group receiving treatment with 100 mg/kg b.wt of BPA (49.46±0.84) pg/ml as compared with the control group.

Effects of BPA on semen parameters

The results in Table 2 showed that giving oral 25, 50 and 100 mg/kg b.wt. of BPA for 35 days to adult male rats caused a significant (P≤0.01) reduction in the count of epididymal sperm (81.72±0.35, 79.02±0.66 and 72.12±0.62)×10^6/ml, respectively, as compared with that of the control group (83.81±0.52)×10^6/ml.

As well, for sperm motility, significant (P≤0.01) reductions were seen in each treated groups that were given orally 25, 50 and 100 mg/kg b.wt. of BPA daily for 35 days (89.12±0.23, 83.23±0.22 and 78.88±0.32)%, respectively, as compared with that of the control group (93.28±1.15)%.

As for sperm viability, the adult male rats given 50 and 100 mg/kg b.wt. of BPA showed a significant (P≤0.01) decrease (87.62±0.46 and 87.40±0. 54)%, respectively, as compared with the control group (89.12±0.14)%. Nevertheless, no significant differences in the viability of sperm were observed in rats given 25 mg/kg b.wt. of BPA (88.63±0.57%), as compared with the control group (89.12±0.14%).

Effects of BPA on liver enzymes

ALT enzyme level significantly (P≤0.01) increased, according to the verified results in Table 3. in the treated groups with 25, 50 and 100 mg/kg b.wt. of BPA (28.50±0.07, 33.76±0.12 and 39.36±0.24) IU/L respectively after 35 days of treatment compared to the control group (23.36±0.12) IU/L. The results in the same table also showed a significant (P≤0.01) increase in the AST enzyme in the treated group with 25, 50 and 100 mg/kg b.wt. of BPA (30.40±0.14, 37.31 ±0.16 and 42.79±0.17) IU/L respectively

Table 1: The impact of BPA on adult male rats' serum levels of FSH, LH, Testosterone and Estrogen.

Parameters / Groups	Mean±SE			
	FSH (µIU/ml)	LH (µIU/ml)	Testosterone (ng/ml)	Estrogen (pg/ml)
G1: Control	3.65±0.32a	2.53±0.09a	2.41±0.19a	46.20±1.00b
G2: 25 mg/kg b.wt. of BPA	3.04±0.05b	2.24±0.03b	1.98±0.04b	42.98±0.54c
G3: 50 mg/kg b.wt. of BPA	2.63±0.12c	2.13±0.04b	1.63±0.14c	39.90±0.35d
G4: 100 mg/kg b.wt. of BPA	1.61±0.25d	1.45±0.21c	0.983±0.04d	49.46±0.84a
LSD value	0.405**	0.225**	0.235**	1.376**
P-value	0.0001	0.0001	0.0001	0.0001

Significant differences existed between the means that had different letters in the same column. **(P≤0.01).

Table 2: The impact of BPA on adult male rats' semen analysis (Mean±SE).

Parameters / Groups	Mean±SE		
	Sperm count (*10^6/ml)	Sperm viability (%)	Sperm motility (%)
G1: Control	83.81±0.52a	89.12±0.14a	93.28±1.15a
G2: 25 mg/kg b.wt. of BPA	81.72±0.35b	88.63±0.57a	89.12±0.23b
G3: 50 mg/kg b.wt. of BPA	79.02±0.66c	87.62±0.46b	83.23±0.22c
G4: 100 mg/kg b.wt. of BPA	72.12±0.62d	87.40±0.54b	78.88±0.32d
LSD value	1.041**	0.869**	1.164**
P-value	0.0001	0.0054	0.0001

Significant differences existed between the means that had different letters in the same column. **(P≤0.01).

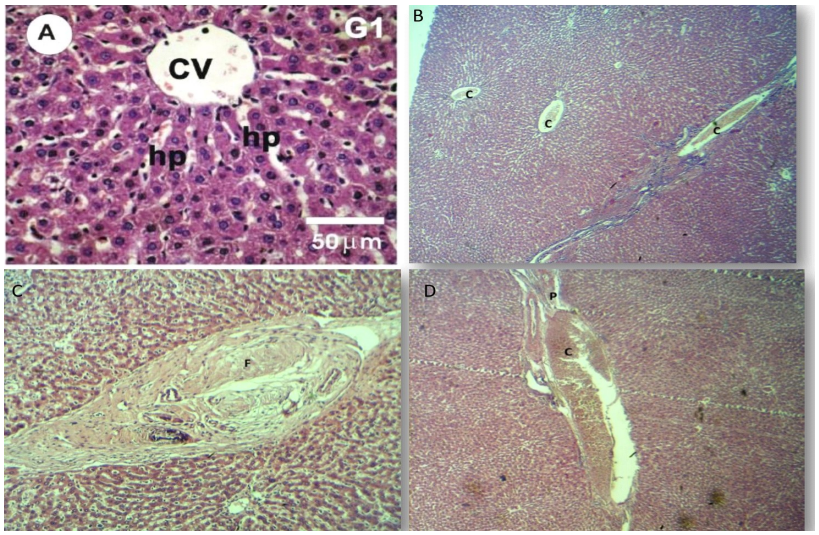
after the passage of 35 days compared to the enzyme level for the control group (25.16±0.03) IU/L.

The histopathological changes of liver

photomicrographs of rat liver sections stained with hematoxylin and eosin from various experimental groups. Hepatocytes in control (G1) rat liver sections had a normal structure (hp) (Fig 1 A). The histopathological examination of the liver for rats administrated orally 25 mg/kg b.wt. of BPA was restricted as general central venous congestion and sinusoidal congestion and most hepatocytes were normal (Fig 1 B). The results of histological changes for rats administrated orally 50 mg/kg b.wt. of BPA were multiple severe fibrosis of the portal area (Fig 1 C) and the other section showed oedema and congestion with fibrosis portal extension to neighboring areas and amyloid deposition. Finally, as for the concentration of 100 mg/kg b.wt. of BPA, the effect and the changes were very clear. The liver section showed multiple severe portal amyloidosis and congestion with fibro portal extension to neighboring areas (Fig 1D).

The structural resemblance of bisphenol A to 17-β estradiol makes it one of the most extensively researched endocrine-disrupting chemicals. Its ability to bind as an agonist or an antagonist to estrogen receptors cause negative effects on the metabolism and endocrine system (Mukhopadhyay *et al*., 2022). Under high temperatures and basic or acidic conditions, plastic products can release bisphenol A (BPA) into the air, water and food (Welshons *et al*. 2006; Hasan *et al*., 2024). Therefore, BPA exposure to humans is inevitable in day-to-day living.

Iraqis rely on drinking water that is bottled in plastic bottles, whether at home or in public places, in addition to using plastic for food packaging. Iraq is renowned for its scorching summers when highs surpass 55°C. Furthermore, the government always alerts the public when the UV radiation index rises above 10. In addition to this extreme weather, there is inadequate



A: Rat liver sections in control (G1) revealed normal structure of hepatocytes (hp). B: Cross section of liver for orally 25 mg/kg b.wt. of BPA group shows central venous congestion (C) (H and E 100X). C: Cross section of liver for orally 50 mg/kg b.wt. of BPA group shows fibrosis of portal area (HandE 100X). D: Cross section of liver for orally 100 mg/kg b.wt. of BPA group shows portal area (P) dilation and congestion (C) (H and E 40X).

Fig 1: Photomicrographs of rat liver sections in the different experimental groups stained with Haematoxylin and Eosin.

Table 3: Effect of BPA on ALT and AST in adult male rats (Mean±SE).

Parameters / Group	Means±SE	
	ALT (IU/L)	AST (IU/L)
G1: Control	23.36±0.12d	25.16±0.03d
G2: 25 mg/kg b.wt. of BPA	28.50±0.07c	30.40±0.14c
G3: 50 mg/kg b.wt. of BPA	33.76±0.12b	37.31±0.16b
G4: 100 mg/kg b.wt. of BPA	39.36±0.24a	42.79±0.17a
L.S.D.	0.494**	0.451**
P-value	0.0001	0.0001

Significant differences existed between the means that had different letters in the same column. **(P≤0.01).

storage of water bottles composed of polycarbonate or polyethene plastic. Instead of being kept in good condition, these bottles are kept outside of stores in direct sunlight. Because of the inherent possibility of exposure to BPA resulting from all these factors, research on the substance's effects at various concentrations and an emphasis on its detrimental effects are necessary.

As shown in Table 1, our results indicate that administration of BPA at a concentration 25, 50 and 100 mg/kg b.wt. for 35 days leads to a significant ($P \leq 0.01$) decrease in male fertility hormones (FSH, LH, Testosterone and Estrogen) when comparison with the control group and these results agreed with (Lü *et al*., 2024; Hasan *et al*., 2024; Ezz *et al*., 2023; Alankooshi *et al*., 2023) and from the observed results can be attributed to the potential harmful effects of BPA, according to (Cariati *et al*., 2019; Matuszczak *et al*., 2019; Pivonello *et al*., 2022; Ryu *et al*., 2023; Hasan *et al*., 2023; Hameed *et al*., 2023). As well as this study also comes in agreement with (Elashal *et al*., 2024; Hasan *et al*., 2022; Hasan *et al*., 2021) who gave the same concentration of BPA at a dose of 50/ mg/kg body weight and proved that giving BPA led to decreased serum levels of FSH, LH, testosterone, GSH concentration, total sperm count, motility and vitality. By contrast, a significant ($P \leq 0.01$) increase of Estrogen in rats treated with 100 mg\kg b.wt of BPA was observed as compared with the same control group, this could be due to that BPA induces oxidative stress and activates inflammatory signals (Cho *et al*., 2018; Hasan *et al*., 2024).

As shown in Table 2, the results revealed that administering 25, 50 and 100 mg/kg b.wt. of BPA through 35 days, led to significantly ($P \leq 0.01$) decreased sperm count, motility and vitality compared with the control group, these findings come matched with Barbagallo *et al*. (2020) and Ullah *et al*. (2019). The influence of BPA on oxidative stress on testicular function and ability has been established according to (Liu *et al*., 2022; ElGendy *et al*., 2020). These results can be ascribed to the potential adverse effects of BPA According to (Gules *et al*., 2019; Campos *et al*., 2019). In mice tests, exposure to BPA causes a decrease in sperm motility and counts as well as an increase in ROS and lipid peroxidation (Kaur *et al*., 2017).

As shown in Table 3, ALT and AST levels were significantly ($P \leq 0.01$) increased in rats orally administrated BPA at 25, 50 and 100 mg/kg b.wt. for 35 days, these findings are consistent with prior research that has shown the efficacy of BPA in increasing oxidative damage to hormones and liver enzymes caused by BPA according to Lv *et al*., (2019); Hameed *et al*. (2023). As well as these results match with those of Elswefy *et al*. (2016); Hassan *et al*. (2012). This could be explained by either the hyperactivity of the liver or oxidative damage to the liver caused by BPA that releases hepatic enzymes into the blood (Kourouma *et al*., 2015). It seems that in a normal state, corn oil preserves and enhances liver structure and function. Nevertheless, the groups that were given BPA dissolved in corn oil did not experience these positive effects. The liver enzymes of animals that ingest BPA may become less active (Paul *et al*., 2024; Hasan *et al*., 2022). This could be because the chemical affects the liver cells' metabolic processes and inhibits the activity of the enzymes that cause these processes, damaging the liver cells and causing enzymes to leak out of the cells and into the blood (Abdulhameed *et al*., 2022; Hasan *et al*., 2023; Hasan *et al*., 2021).

The liver is more susceptible to BPA doses than other organs because it is the organ that metabolizes BPA through glucuronic acid conjugation (Moon *et al*. 2012). Conversely, Fig 1 depicts the liver of the rat groups treated with BPA 25, 50 and 100 mg/kg b.wt. Following 35 days of treatment, the liver displayed a variety of histological changes, including multiple severe portal amyloidosis and congestion with fibro portal extension to neighboring areas. This agrees with Elswefy *et al*., (2016) who gave the same concentration of 50 mg/kg body weight/day, for 8 weeks and demonstrated that BPA-induced liver damage and fibrosis are related to inflammation, oxidative stress and apoptosis.

Conclusion

The concluded result of the present study from male albino rats that were administered BPA (25, 50 and 100 mg/kg b.wt.) for 35 days revealed a significant ($P \leq 0.01$) decrease in all fertility hormones and semen parameters, with a significant ($P \leq 0.01$) increase in some liver function enzymes and hepatic injuries. These results strongly recommend that individuals and those exposed to BPA have their liver functions periodically monitored.

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Authors contributions

All authors are equally contributed to this study.

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Conflict of interest

All authors declare that they have no conflict of interest.

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