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The toxic effects of bisphenol a (BPA) on some biomarker blood of rats

Zaid Abdul-Hamza Abdul-Hassan
College of Science, University of Al-Qadisiyah, Iraq

Haider Mashkoor Hussein
College of Science, University of Al-Qadisiyah, Iraq

***Abstract*---**This study was conducted at the College of Science, University of Al-Qadisiyah, to study the relationship between the toxic environmental effects of BPA compound on some biochemical components of the blood of female and male rats. In this study, 60 male and female rats were used, They ranged in age from 8 to 12 weeks, and their weights ranged from 145-200 grams for females and 85-150 grams for males. Rats were divided into four groups according to the dose, with males and females separated. Two control groups: these two groups, three females and three males, dosed 5.0 ml/kg body weight of corn oil per day, group A. Nine females and nine males were fed BPA orally (every day after dissolving in corn oil at 50 mg./kg body weight). Group III (B): 9 females and 9 males were given BPA orally (every day with corn oil, 100 mg/kg body weight). In group C, nine females and nine males were orally injected with a dose of 200 mg/kg BPA dissolved in corn oil every day. The experiment lasted three months. After the end of the first month of animal adaptation, animals were dosed orally for 2 weeks daily. After the first test, the experiment continued for another eight weeks. At the end of that time, the mice were killed and blood and organ samples were taken for the last test. Biochemical parameters showed a significant increase ($P<0.05$) in (ALT, AST, ALP, and GGT) enzymes in female and male rats treated with 200, 100, and 50 mg/kg BPA for two weeks and eight weeks. Renal function tests showed a significant increase ($P<0.05$) in serum (urea, creatinine, and uric acid) levels and a decrease in protein in female and male rats treated with 200, 100, and 50, Antioxidant enzyme tests demonstrated an increase($P<0.05$) in serum (CAT, SOD, and GSH) levels and a decrease ($P>0.05$) in (actylcholinestra) enzyme activity in male and female rats given 200, 100, and 50 mg/kg BPA for two or eight weeks. Lipid profile testing showed an increase ($P<0.05$) in blood cholesterol, TG, and LDL levels and a decrease ($P>0.05$) in HDL in female and male rats treated with 200, 100, and 50 mg/kg BPA. Two to eight weeks after the experiment

started. This study indicated a substantial rise ($P<0.05$) in rat weights after BPA treatment in both female and male rats treated with 200, 100, and 50 mg/kg at the two-week collusion of the experiment.

***Keywords*---**toxic, bisphenol, biomarker, blood.

Introduction

BPA (2,2-di(p-hydroxyphenyl) propane, BPA) is one of the most widely used industrially manufactured chemicals in the world [1]. Its molecule is small (228 Da), its color is white, and at room temperature, it exists as a solid particle and has an odor of phenol[2]. BPA was first discovered in the year 1905, and since then, its commercial popularity has increased gradually with time. [3]. Besides food and liquid containers, polycarbonate that has BPA is used in things that aren't food like toys, pacifiers, and medical devices like eyeglass lenses, intravenous administration sets, syringes, and catheters. It's also used in things that aren't food like microwave ovenware and water pipes. [4]. Additionally, polycarbonate containing BPA is used to line food and beverage cans, to cover household drinking water storage tanks, and in resin-based powder paints, printing inks, flame retardants, and for medical uses, including dental composites and sealant materials.[3][5][4] are released into the atmosphere.[6]

The extensive use of BPA-containing products has resulted in high human exposure worldwide (Vandenberg et al., 2012). It appears that increased temperature leaches BPA into food and water products, as does the acidic pH of liquids[7]. Additionally, dermal contact with sales receipts and printer paper containing BPA compounds can lead to BPA exposure[7] [8]. The primary route of exposure to BPA is oral, mainly from eating or drinking products containing BPA. Other potential exposures include inhalation of dust particles and atmospheric exposure. Once the compound is introduced via the oral route, it is absorbed in the gastrointestinal tract and undergoes hepatic metabolism including oxidation and hydrolysis, which leads to the production of several metabolites including BPA monosulfate, BPA glucuronide, and BPA disulfate. The presence of BPA and its metabolites can be measured in bodily fluids, including urine and serum, by using the combined techniques of solid-phase extraction coupled with isotope dilution, high-performance liquid chromatography and mass spectrometry[6].According to research, BPA is said to be given from mother to child by intrauterine transmission during prenatal embryonic development and through breastfeeding during the early neonatal period.[9].

It has been claimed that BPA may operate as an antiestrogen, inhibiting estrogenic response by competing with estrogen receptor E2[10] Because of BPA's capacity to bind estrogen receptors, it has been linked to a number of diseases, including hypertension, atherosclerosis, liver dysfunction, diabetes, and obesity.[11][12][13]. Reactive oxygen species (ROS) are cytotoxic chemicals that induce oxidative damage to cells by damaging the membranes and DNA of the cells they enter. It has been proposed that inflammation, radiation, age, chemical compounds, and medications all contribute to an increase in ROS production and production. Antioxidants have the ability to mitigate the effects produced by ROS.

Excess ROS generation or low antioxidant levels in the body result in oxidative stress, which adds to the consequences of chronic diseases such as obesity, diabetes, and cardiovascular diseases [14][15].Many organs, including the liver, kidneys, and brain, have been shown to be effect by BPA, which has been linked to increased oxidative stress[14]

Despite substantial improvements in this field over the past few years, the toxicological, biochemical, and physiological impacts of BPA (BPA) remain a source of debate. Additional research is required to get a better understanding of these challenges. On the other hand, no research has been carried out in Iraq to establish the toxicity of BPA (BPA). As a result, the current study aims to investigate the toxic and detrimental effects of BPA (BPA) on the liver, kidneys, of both female and male rats in a laboratory setting. High-performance liquid chromatography (HPLC) technology will be used to measure the concentration of BPA (BPA) in the liver, kidneys, brain, and blood of both female and male rats.

Martials and methods

Animals

The current study involved 60 rats. 30 adult female rats, and 30 adult male rats of the type Albino rats, obtained from the animal house of the College of Science and College of Veterinary Medicine, University of Al-Qadisiyah, Iraq. Rats' ages ranged from 75 Days, weights ranged from 145–200 g for females and 85–150 g for males. The rats were housed in metal cages to avoid exposure to BPA, which rodents might be subjected to in the case of plastic cages. The cages were fitted with glass bottles to provide the animals with water. The animals were placed in the animal house for 30 days prior to the start of the experiment. This was with the aim of naturalization and adaptation to the atmosphere of the animal house and they were subjected to the light system. 12\12 hours of light and darkness and a temperature of (20-25 C) (20-25 C) The animals were raised in the animal house belonging to the College of Science at the University of Al-Qadisiyah.

Design of the experiment

In this experiment, rats were introduced to the animal house at the Faculty of Science at the University of Al-Qadisiyah. The rats were divided into eight groups after separating the males from the females, according to the figure.

- control groups: They were divided into two groups: 3 female rats and 3 male rats, which were classified as control groups, were given corn oil only at a concentration of 5.0 ml/kg of body weight every day.
- Groups (A): were split into two groups: 9 female rats and 9 male rats were given BPA orally (every day after dissolving it in corn oil at a dose of 50 mg/kg of body weight).
- groups (B): they were divided into two groups, 9 female rats and 9 male rats were given BPA orally (every day after dissolving it in corn oil at a dose of 100 mg/kg of body weight).
- Groups (C): they were divided into two groups, 9 female rats and 9 male rats, who were dosed with bisphenol-A orally (every day after dissolving it in

corn oil at a dose of 200 mg/kg of body weight).

The experiment began on (1/1/2022) and will end on (1/4/2022), The first month was devoted to the rat's adaptation to the new environment, the first rats were dosed orally on BPA daily for six weeks, after which blood samples were collected from the rats for the purpose of the latter test, as shown in Figures.

BPA compound

The trade name for the chemical is BPA (97.0 percent, CAS 80-05-7), and it was obtained from the Indian CDH Company. Pure corn oil was used in the dissolution of the BPA compound as well as in the dosing of the control group, A BPA solution was generated by dissolving BPA in maize oil on a weekly basis, and each group received a dose of this solution based on their body weight, with the control group receiving simply corn oil.

Sampling Blood collection

Rats were weighed and anesthetized by placing them in a cotton bag filled with anesthetic chloroform, then closed with a rubber band, and blood samples were taken by heart stab method. Blood samples were collected for primary tests. Samples were also collected eight weeks after BPA ingestion of phase II doses for final tests. Then half of the blood samples were placed in a sterile, dry tube, an anticoagulant container for blood tests, and the other half of the samples were placed in a tube that did not contain an anticoagulant, and then placed in a centrifuge (3000 cycles/min) for 15 minutes for the purpose of obtaining blood serum for the purpose of conducting blood and biochemical tests. The necessary tests were conducted directly, as the collection of blood samples was in two stages: two weeks for the first test, and the collection of other samples in six weeks after the initial test.

Determination antioxidants
Determination of Serum (GSH) [16]

TNB (5,5'-dithiobis(2-nitrobenzoic acid)), known as Ellman's Reagent, was developed for the detection of thiol compounds. DTNB and glutathione (GSH) react to generate 2-nitro-5-thiobenzoic acid and glutathione disulfide (GSSG). Since 2-nitro-5-thiobenzoic acid is a yellow colored product, the absorbance of the reduced chromogen is measured at 412 nm and is directly proportional to the GSH concentration [16]

Determination of serum SOD [17]

The method is based on the SOD ability to inhibit the Epinephrine oxidation to adrenochrome, The O2-. Substrate for SOD is generated indirectly in the oxidation of epinephrine at alkaline pH by the action of oxygen on epinephrine. As O2-. Builds in the solution, the formation of adrenochrome accelerates because O2-. Also reacts with epinephrine to form adrenochrome, SOD reacts with O2-. Formed during the epinephrine oxidation and therefor slows down the rate of formation of the adrenochrome as well as the amount that is formed. Because of

this slowing process, SOD can inhibit the oxidation of epinephrine. One unit of SOD activity was defined as the concentration of the enzyme in the serum that caused 50 % reduction in the auto-oxidation of epinephrine (Jewett and Rockling, 1993).

Determination of Serum Catalase (CAT) [18]

Catalase (CAT) which catalyze the decomposition of Hydrogen peroxide, therefore its activity was determined by the decrease in absorbance due to H2O2 consumption [19]

Determination of Serum Acetylcholinesterase Activity[20]
Product Description

Acetylcholinesterases (AChEs) are enzymes that hydrolyze the neurotransmitter acetylcholine (ACh) to acetate and choline. AChE is located at the synaptic cleft and functions to terminate synaptic transmission by catalyzing the breakdown of ACh allowing cholinergic neurons to return to a resting state after activation. Changes in AChE activity may result from exposure to certain insecticides, which act as cholinesterase inhibitors. Inhibitors of AChE are also used to treat certain conditions such as dementia. The Acetylcholinesterase Activity Assay kit provides a simple and direct procedure for measuring AChE levels in a variety of samples such as blood, serum, and plasma. This assay is an optimized version of the Ellman method in which thiocholine, produced by AChE, reacts with 5,5¢-dithiobis(2-nitrobenzoic acid) to form a colorimetric (412 nm) product, proportional to the AChE activity present.

Estimation of Liver Enzymes standards
Determination of Serum Alanine Aminotransferase (ALT)

ALT is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenyl-hydrazine [21]

Determination of Serum Alkaline Phosphatase (ALP)

Serum ALP was determined by colorimetric method [22][23] Free phenol liberated by hydrolysis of the substrate then reacts with 4-amino-antipyrine in the presence of alkaline potassium ferricyanide to form a red-colored complex with absorbance measured at 510nm is directly proportional to the ALP activity in the specimen. Sodium arsenate incorporated in the reagent abolishes further enzyme activity and prevents the dilution of the color inherent in the earlier methods.

Determination of Serum Aspartate Aminotransferase (AST)[21]

□-oxoglutarate + L-aspartae→ L-glutamate + oxaloacetate
AST is measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenyl-hydrazine

Determination of Serum Gamma-glutamyl transferase (GGT)

Gamma-glutamyl transferase (GGT) is clinically useful in detecting all forms of liver damage and diseases. Elevated levels are also associated with chronic alcoholism and drug abuse. GGT is a reagent set for the determination of GGT in human serum/plasma based on Substrate gamma-glutamyl-3-carboxy-4-nitroanilide (Glupa-C), recommended by the International Federation of Clinical Chemistry (IFCC). GGT is ready to use two liquid reagent system, using single-step reconstitution.[22]

HPLC Analysis
Animal sacrifice and organ collection

Prior to sacrifice, rats were weighed and anesthetized by placing them in a cotton-lid container tray filled with anesthetic chloroform, which was then closed with a rubber band, To access the organs used in the study, the abdominal cavity was opened by cutting the midline in the abdomen to make an opening. The kidneys, liver, and brain were taken from the animal cavity and stored at a high degree of freezing until the required tests could be performed on the organs using high-resolution liquid chromatography (HPLC).

Sample preparation

- tissue (170 mg) homogenized by tissue homogenizer
- serum samples (500ul) or the homogenized tissue were mixed with 3 ml of hexane and shacked for 3 min
- five ml of acetonitrile was added and shacked for 3 min
- the mixture was centrifuged at 2500 rpm FOR 15 MIN
- the acetonitrile layer (lower layer) was transferred and filtered by a syringe filter (0.2um)
- the filtrate was concentrated under vacuum to 500 ul and injected to HPLC system The High-Performance Liquid Chromatography (HPLC) system used in this study was an HPLC, the components of this system are according to the table below:

Table 1
The components of HPLC system

	Component	Model or version	Company and origion
1	Binary high-pressure gradient pump	P6.1L	Knuaer, Germany
2	Diode array detector	DAD 2.1L	Knuaer, Germany
3	Sample loop (20 µl) and injector	D1357	Knuaer, Germany
4	Analyses and system control software	Claritychrom, V 7.4.2.107	Dataapex, Czech Republic

The High-Performance Liquid Chromatography (HPLC) system used in this study was an HPLC Waters 2690 with an autosampler system and UV detector set to 210 nm. A 250 x 4.6 mm Sum Waters C18 Column was used to prepare the sample. Ten microliters of samples were put into the chromatographic system for analysis in isocratic clution at 1 ml/min at room temperature with a mobile phase of water/acetonitrile (40:60, v/v) for 17 minutes. When comparing the retention time and absorption spectra of each chemical to the standards, the detection of each compound was accomplished. The concentration was determined by using repeated concentrations of the external standard substances in order to construct a calibration curve between the concentration and the equivalent peak area of the external standard substances[24].

Result

Toxic Effect of BPA on antioxidant enzyme

The results of the current study showed that there was a decrease in significant differences at ($P < 0.05$) in CAT, SOD, and GSH, levels in all treatment groups compared to the control group, Between group A and group C and between group B and group C, and the results of the current study showed that there was a decrease in significant differences at ($P < 0.05$) in CAT, SOD, and GSH, levels in all treatment groups compared to the control group, where the results of our study showed that there is a significant increase between groups of females compared to With the male groups, the results of the current study showed that there was a decrease in significant differences at ($P < 0.05$) in CAT, SOD, and GSH, levels in all treatment groups compared to the control group, where the results of our study showed that there was a significant decrease at the second test in eight weeks of dosing compared to the first test In the two weeks of dosing. Table (3-1),

The results of the current study showed that there was an increase in the significant differences at ($P < 0.05$) in AchE levels in all treatment groups compared to the control group, Between group A and group C and between group B and group C, the results of the current study showed that there was an increase in significant differences at ($P < 0.05$) in AchE levels in all treatment groups compared to the control group, where the results of our study showed that there is a significant increase between groups of females compared to With the male groups, the results of the current study showed that there was an increase in the significant differences at ($P < 0.05$) in AchE levels in all treatment groups compared to the control group, where the results of our study showed that there was a significant increase at the second test in eight weeks of dosing compared to the first test In the two weeks of dosing. Table (3-1)

The results of the current study showed a completely negative correlation at (-1.0) in Antioxidants Enzymes During the exposure period BPA for male and female rats ranging from 2 to 8 weeks,the results of the current study showed that there is a completely negative correlation at (-1.0) in CAT, SOD, GSH and AchE, enzyme During the exposure period of BPA for males and female rats ranged from 2 to 8 weeks. Table (3-2)

Table 3-1
Toxic effect of different doses of BPA on antioxidant enzyme

MALE						
Period	Parameter Mg\dl	Control	A (200 mg\kg)	B (100 mg\kg)	C (50 mg\kg)	LSD
2 WEEKS	SOD	2.21±0.25 A	1.05±0.41 D	1.52±0.53 C	1.85±0.63 B	0.26
	GSH	2.51±0.22 A	1.03±0.24 D	1.62±0.36 C	2.01±0.45 B	0.33
	CAT	0.64±0.02 A	0.24±0.01 D	0.31±0.05 C	0.51±0.02 B	0.024
	AchE	1.65±0.52 D	3.68±0.66 A	3.01±0.45 B	2.53±0.66 C	0.51
FEMALE						
Period	Parameter Mg\dl	Control	A (200 mg\kg)	B (100 mg\kg)	C (50 mg\kg)	LSD
2 WEEKS	SOD	2.22±0.26 A	1.17±0.69 D	1.64±0.55 C	1.92±0.47 B	0.22
	GSH	2.55±0.63 A	1.16±0.24 D	1.74±0.33 C	2.13±0.47 B	0.32
	CAT	0.65±0.03 A	0.39±0.04 D	0.45±0.06 C	0.50±0.07 B	0.05
	AchE	1.64±0.26 D	3.52±0.33 A	3.18±0.48 B	2.85±0.59 C	0.31
MALE						
Period	Parameter Mg\dl	Control	A (200 mg\kg)	B (100 mg\kg)	C (50 mg\kg)	LSD
8 WEEKS	SOD	2.22±0.29 A	0.96±0.20 D	1.41±0.63 C	1.62±0.41 B	0.32
	GSH	2.51±0.58 A	0.98±0.41 D	1.40±0.63 C	1.86±0.57 B	0.42
	CAT	0.64±0.01 A	0.21±0.06 D	0.29±0.02 C	0.43±0.07 B	0.023
	AchE	1.65±0.45 D	4.66±0.44 A	3.85±0.56 B	2.98±0.68 C	0.56
FEMALE						
Period	Parameter Mg\dl	Control	A (200 mg\kg)	B (100 mg\kg)	C (50 mg\kg)	LSD
8 WEEKS	SOD	2.21±0.44 A	1.01±0.31 D	1.21±0.29 C	1.85±0.38 B	0.45
	GSH	2.52±0.65 A	1.02±0.48 D	1.42±0.44 C	1.98±0.25 B	0.34
	CAT	0.65±0.05 A	0.25±0.02 D	0.33±0.04 C	0.47±0.06 B	0.04
	AchE	1.63±0.42 D	5.12±0.96 A	4.21±0.85 B	3.56±0.66 C	0.58

Table 3-2
Correlation Coefficient between 2 weeks and 8 weeks of Dosing BPA on antioxidant enzyme

	Males			
Correlation Coefficient	SOD	GSH	CAT	AS.
	0.9829	0.9892	0.9835	0.9977
	Females			
	SOD	GSH	CAT	AS.
	0.9554	0.9867	0.9872	0.99375

Toxic Effect of BPA on liver enzyme

The results of the current study showed that there was an increase in the significant differences at (P < 0.05) in ALT, AST, ALP and GGT enzymes in all treatment groups compared to the control group, Between group A and group C and between group B and group C, the results of the current study showed that there was an increase in significant differences at (P < 0.05) in ALT, AST, ALP and GGT enzymes in all treatment groups compared to the control group, where the results of our study showed that there is a significant increase between groups of females compared to With the male groups, the results of the current study showed that there was an increase in the significant differences at (P < 0.05) in ALT, AST, ALP and GGT enzymes in all treatment groups compared to the control group, where the results of our study showed that there was a significant increase at the second test in eight weeks of dosing compared to the first test In the two weeks of dosing. Table (3-3). The results of the current study showed a completely negative correlation at (-1.0) in liver enzymes During the exposure period BPA for male and female rats ranging from 2 to 8 weeks, where the results of the current study showed that there is a completely negative correlation at (-1.0) in ALT, AST, ALP and GGT enzymes During the exposure period of BPA for males and female rats that ranged from 2 to 8 weeks. Table (3-4)

Table 3-3
Toxic effect of different doses of BPA on liver enzyme

MALE						
Period	Parameter Mg\dl	Control	A (200 mg\kg)	B (100 mg\kg)	C (50 mg\kg)	LSD
2 WEEKS	ALT	12.42±1.05 D	33.21±2.63 A	28.12±2.01 B	25.14±2.00 C	1.52
	AST	15.25±1.63 D	31.02±2.65 A	28.52±1.96 B	22.12±1.55 C	2.05
	ALP	12.25±1.12 D	22.14±2.09 A	19.56±1.88 B	17.42±1.67 C	1.58
	GGT	32.52±3.05 D	50.12±4.51 A	45.22±6.52 B	38.52±3.55 C	2.66
FEMALE						
Period	Parameter Mg\dl	Control	A (200 mg\kg)	B (100 mg\kg)	C (50 mg\kg)	LSD
2 WEEKS	ALT	12.04±1.95 D	35.28±3.22 A	31.05±3.01 B	28.51±2.98 C	2.88
	AST	14.56±1.65 D	30.52±3.11 A	27.45±2.96 B	22.54±2.45 C	1.45
	ALP	12.33±1.47 D	24.52±1.96 A	18.56±1.05 B	17.22±1.00 C	0.96
	GGT	31.41±3.26 D	49.88±4.11 A	41.02±4.03 B	38.65±1.96 C	3.25
MALE						
Period	Parameter Mg\dl	Control	A (200 mg\kg)	B (100 mg\kg)	C (50 mg\kg)	LSD
8 WEEKS	ALT	12.42±1.22 D	55.23±4.96 A	39.85±3.85 B	29.55±2.56 C	2.15
	AST	15.25±1.85 D	45.21±3.99 A	36.54±2.69 B	28.96±1.98 C	1.63
	ALP	12.25±1.21 D	33.21±2.85 A	22.41±2.14 B	18.55±1.96 C	2.58
	GGT	32.52±2.85 D	65.21±5.82 A	51.05±6.41 B	41.66±4.23 C	3.66
FEMALE						
Period	Parameter Mg\dl	Control	A (200 mg\kg)	B (100 mg\kg)	C (50 mg\kg)	LSD
8 WEEKS	ALT	12.04±1.41 D	58.52±5.69 A	48.63±4.33 B	35.21±2.54 C	3.48
	AST	14.56±1.41 D	47.25±5.01 A	38.63±2.56 B	29.52±1.96 C	1.56
	ALP	12.33±1.21	35.41±2.17	24.12±1.63	19.63±1.44	0.88

Table 3-4
Correlation Coefficient between 2 weeks and 8 weeks of Dosing BPA on antioxidant enzyme

Correlation Coefficient	Males			
	ALT	AST	ALP	GGT
	0.9709	0.9886	0.9464	0.9859
	Females			
	ALT	ALT	ALT	ALT
	0.97315	0.97315	0.97315	0.97315

Effect of BPA on some tissues of the body organs

The results of the current study showed an increase in the significant differences at ($P<0.05$) in the accumulation of BPA in some tissues of the body organs in all treatment groups compared to the other groups, where between group A and group B and between group B and group C, group A and group C, which showed The results of the current study that there is an increase in statistically significant differences at ($P < 0.05$) in the brain tissue of males and females rats in all treatment groups. The results of our study showed that there is a significant increase among female groups, compared to male groups, table (3-5)(3-6) The results of the current study showed an increase in the significant differences at ($P < 0.05$) in the accumulation of BPA in Liver tissue in all treatment groups compared to the other groups, were between group A and group B and between group B and group C, group A and group C, which showed The results of the current study that there is an increase in statistically significant differences at ($P < 0.05$) in the Liver tissue of males and females rats in all treatment groups. The results of our study showed that there is a significant increase among female groups, compared to male groups, table (3-5)(3-6)

Table 3-5
Effect of BPA through its accumulation in the tissues of the organs for male

Organs	Groups	BPA	ug/ml	ug/g tissue
Brain	A	27.20±2.53 A	0.7438±0.53 A	2.19±0.56 A
	B	19.67±1.96 B	0.5382±0.26 A	1.58±0.47 B
	C	8.55±2.12 C	0.2347±0.12 C	0.69±0.39 C
LSD	0.532			
Liver	A	26.34±3.02 A	0.7203±0.55 A	2.12±0.52 A
	B	5.83±1.02 B	0.1603±0.02 B	0.47±0.01 B
	C	5.52±0.89	0.152±0.01	0.45±0.02

		B	B	B
LSD	0.35			

Table 3-6
Effect of BPA through its accumulation in the tissues of the organs for male

Organs	Groups	BISPH.	ug/ml	ug/g tissue
Brain	A	22.42±2.13 A	0.6133±0.50 A	1.8±0.55 A
	B	7.71±1.85 B	0.2119±0.21 A	0.62±0.45 B
	C	7.68±2.05 B	0.211±0.10 A	0.62±0.31 B
LSD	0.78			
Liver	A	16.18±3.11 A	0.443±0.50 A	1.3±0.50 A
	B	12.52±1.14 B	0.343±0.01 A	1.01±0.02 A
	C	5.57±0.81 C	0.1535±0.03 B	0.45±0.05 B
LSD	0.57			

Discussion

The results of this study showed a significant decrease in the levels of SOD, GSH, in the serum of male and female rats who were given doses of 200, 100, and 50 mg/g BPA for two weeks at the first test. Serum SOD, GSH levels were measured at the second test in mice treated for six weeks at doses ranging from 200, 100, and 50 mg/kg, where a significant decrease in serum SOD, GSH levels was observed compared to SOD, GSH levels in the control group. These results are consistent with a study [25]which found that doses of 20 and 100 mg/kg body weight/day were effective. When male albino rats were exposed to BPA for 30 days, SOD and GSH levels decreased in their livers and testes. The results of this study showed that male and female rats that were administered 200, 100, and 50 mg/g of BPA for two weeks had a substantial decrease in blood CAT levels, and according to the same data, they discovered a significant decrease in CAT levels at the second test after six weeks of treatment with BPA. These results are consistent with a study conducted by [26]which found that 8-week-old male mice were exposed to BPA at dosages of 5, 50, and 500 g/kg body weight/day for 8 weeks. CAT levels in the liver were lowered by the high dose Enzymatic antioxidants like (SOD, CAT, and GSH) are capable of converting oxidized metabolic products into hydrogen peroxide and then water with the assistance of cofactors. [27][28].

In point of fact, living systems have varying degrees of antioxidant defenses, including those that prevent radical damage, scavenge radicals, or repair damage caused by radicals. These enzymes are responsible for the destruction of

potentially harmful compounds such as the superoxide radical, hydrogen peroxide (H2O2), and hydroperoxides (H2O or alcohol and O2). This class of proteins also includes metal ion-binding proteins like transferrin and caeruloplasmin, which inhibit free radicals from forming by binding to iron and copper, respectively, and preventing them from becoming unstable. [29][30] It has been hypothesized that lower antioxidant enzyme levels could be explained by the fact that these enzymes are used for the removal of ROS caused by BPA [15] Under healthy settings, the generation of ROS and its removal from cells occur in equilibrium with one another.

Damage to tissues and organs can be attributed to elevated levels of intracellular reactive oxygen species (ROS), also known as oxidative stress. This condition is caused when ROS impairs the normal function of biological molecules [15] By producing ROS, BPA is able to cause damage to the liver, kidneys, brain, and possibly other organs [31].This study showed a significant increase in the levels of ALT and AST enzymes in female and male rats treated with doses of 200, 100, and 50 mg/kg of BPA for two weeks. Female and male rats were treated with the same doses for eight weeks of exposure to BPA and found a significant increase in the levels of ALT and AST enzymes, and the results of this study were consistent with a study conducted (Morad and Khadraoui, 2012).. His study confirmed that there was a significant increase in enzyme activity (ALT and AST) in rats fed a dose of 10 mg/kg of BPA daily for six weeks compared to the control group. At a dose of 10 mg/kg, the activity of the two enzymes increased after 6 and 10 weeks of treatment.

This study concluded that oxidative stress, which was significant in the liver and testis after short and long-term exposure to high and low doses of BPA, is the mechanism underlying the toxicity produced by BPA. (Morad and Khadraoui, 2012). This study showed a significant increase in ALP enzyme levels in female and male rats treated with doses of 200, 100, and 50 mg/kg of BPA for two weeks. This study showed that female and male rats given the same doses for eight weeks of exposure to BPA observed a significant increase in ALP enzyme levels, and the results of this study were identical to those of the study [32]. which revealed that oral administration of 50 mg/kg bisphenol for four weeks to rats resulted in a significant increase in serum ALT and ALP levels, as well as elevation of serum AST, The elevated levels of ALP, ALT, and AST enzymes caused liver damage. which confirmed bisphenol is a toxic substance at a concentration of 5 mg, and it has no effect at the lowest concentration that can be found, The results of this research showed that female and male rats treated with doses of 200, 100, and 50 mg/kg of BPA for two weeks showed significantly higher levels of GGT enzyme. This study showed that female and male rats who were given the same doses for eight weeks of exposure to BPA had a significant increase in GGT enzyme levels. The results of this study were consistent with the study[33] that confirmed a significant increase in GGT enzyme levels. BPA levels lead to a marked increase in GGT enzyme levels. Serum levels of ALT, AST, ALP, and GGT were shown to be significantly increased after receiving a dose of 500 mg/kg over fourteen days, This study found that the liver function enzymes (AST, ALT, and GGT) in the blood were higher, the nuclei were contracting too much, and there were inflammatory cells in the liver tissue. All of these things show that long-term exposure to this environmental pollutant is bad for the body, Numerous studies

have shown that chronic exposure to BPA can damage the liver. This is mostly shown by abnormally high levels of AST and ALT in the serum as well as by histopathological changes in the liver that include too much death of hepatocytes and an influx of inflammatory cells (Meng *et al.,* 2019).BPA has been linked to a variety of hepatic conditions, including hepatic fibrosis, hepatic steatosis, liver cancers, and metabolic syndrome[34]. Additionally, mitochondrial dysfunction brought on by BPA exposure is a contributor to the toxin's hepatotoxicity in rats.[35] . It is interesting to note that it has been revealed that BPA was responsible for disrupting the defensive line against reactive oxygen species (ROS). In the meantime, oxidative stress has come to be seen as a likely way that BPA exposure could cause liver damage.[36]

Conclusions

From the results of the current study, it was concluded that Bisphenol A decreases the activity of the oxidative enzymes SOD, GSH, and CAT and increases the activity of the enzyme AchE, which makes free oxygen radicals and causes oxidative stress, Bisphenol A increases the activity of liver enzymes ALT, AST, ALP, and GGT, which causes damage and impairment in liver function, Females were more affected than males when exposed to bisphenol A because of the many disorders that were confirmed in this study in all biochemical indicators

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