VOLUME 9 ISSUE 2 2023

ISSN 2454 – 3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 9, No. 2, 384-395 (2023)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Genotoxic Effect and Oxidative Damage in the Chicken Embryo Caused by Bisphenol A

Arwa N. Al-Sharifi[1]*, Rabeea Hazim Mohammed[2] and Rayya G. Alsultan[1]

[1]Department of Biology, College of Education for Girls, University of Mosul, Iraq
[2] Department of Biology, College of Education for Pure-science, University of Mosul, Iraq

**Corresponding Author*

Received: 2nd June, 2023; **Accepted:** 16th July, 2023; **Published online:** 21st August, 2023

https://doi.org/10.33745/ijzi.2023.v09i02.042

Abstract: The present study aimed to evaluate the Bisphenol A (BPA) effect on chicken embryos. 240 fertilized eggs of Ross 308 were divided into eight groups (n=30 for each), including two control groups. Embryos were injected on day 2 with 100 µl of increasing concentrations of BPA then dissected on days 21-22. The livers were removed from the chicken to evaluate DNA damage (using the PCR technique) and oxidative stress biomarkers. The results showed that BPA induces DNA damage and extensively increases oxidative stress. These confirmed that BPA can affect embryos at early stages by alteration in the genetic materials and changing liver enzymes.

Keywords: Bisphenol A, Ross 308, Genotoxicity, Oxidative stress, DNA damage, Liver, Biomarkers

Citation: Arwa N. Al-Sharifi, Rabeea Hazim Mohammed and Rayya G. Alsultan: Genotoxic effect and oxidative damage in the chicken embryo caused by Bisphenol A. Intern. J. Zool. Invest. 9(2): 384-395, 2023.

https://doi.org/10.33745/ijzi.2023.v09i02.042

Introduction

Bisphenol A (BPA), used to make certain plastics and resins, is colorless to white crystalline material with a mild phenolic odour. This industrial chemical has a widespread use in our life and variety of common products, including medical devices, water pipes, food production, safety equipment, incubators, motorcycle helmets and eyeglasses. BPA risk was readily detected in sweat, urine and blood associated with broad human exposure to BPA (Lee *et al*., 2018). BPA has an ability to cross barriers of the blood-brain and placenta then accumulate in the body organs including liver, cord blood, seminal fluid saliva and breast milk (Cao *et al*., 2012). This is a particular concerning given that BPA interferes with several biological receptors, such as androgen receptor, estrogen receptor and thyroid hormone receptor. Mimic of BPA with various hormones may disrupt the hypothalamic and pituitary gland function (Gorini *et al*., 2020) and cause various negative impacts on human body. It was detected that exposure to BPA leads to precocious puberty, lung

cancer, prostate, male reproductive cancer and infertility etc. (Tse *et al*., 2017). Recent data of Sang *et al*. (2021) revealed that BPA un-regulates cell proliferation and induces metastasis and ovarian cancer by interaction with estrogen receptor-α pathways. Indeed, several studies have been made to understand other adverse influences of BPA. In zebrafish embryos and *Xenopus laevis*, BPA causes severe disorders in craniofacial, growth, eye defects and increases mortality rates (Arancio *et al.,* 2019, Pinto *et al*., 2019). In mice, it was detected that BPA change the histological structure in the liver and lung (Al-Mustafa and Al-Sultan, 2022). It was also detected that BPA delays the neurulation and disrupts formation of the neural tube at hours-incubation (Atay *et al*., 2020). Up to now, the impact of BPA exposure on birds still needs more information. Since the liver is the target organs of BPA, potential hepatotoxic influences are of high relevance. Consequently, the current work was designed to investigate *in ovo* the genotoxic and enzymatic effects of BPA in chicken embryogenesis.

Materials and Methods

Chemical:

BPA was purchased from the Solarbio Life Science, Beijing and dissolved in corn oil according to Atay *et al.* (2020).

Animals:

The study was conducted on a group of 21-day-old ROSS 308 chicken embryos. Fresh fertilized chicken (Ross 308) eggs were obtained from a Bashiqa hatcheries in the Bashiqa region of Mosul district in Nineveh Governorate, northern Iraq. The weight of eggs ranged between 60-68 g. The eggs were incubated in a rotary incubator at a temperature of 37.7 °C and a relative humidity of 80%. The eggs were treated with 100 µl of 2,4-Dinitrophenol using an insulin needle at day two of incubation. On the 21st day of incubation, the liver was extracted from the embryos and stored in a foil-covered container at -20°C until the use.

Dose and treatment of BPA for genotoxicity study:

240 Fertilized eggs weighing between 55 to 60 g were divided into eight groups: unopened control group (CL1 n=30), corn oil (CL2 vehicle control) (n = 30) and treated groups injected with 100 µg/egg of increasing concentrations of BPA, including CS1-0.003, CS2-0.005, CS3-0.01, CS4-0.02, CS5-0.03 and CS6-0.1 (n=30 experimental eggs for each). Eggs were cleaned by 70% ethanol before injection for removing any external matter. 100 µl of vehicle (control) and BPA material was injected into the yolk sac by sterile syringes (26Gx1/200, Beybi), eggs were then covered with selotape and incubated under growth condition (37°C with 75 and 80% humidity). Ethics was according to Mosul university.

Extraction of DNA:

DNA was extracted from control and treated samples of each concentration following protocol of Genomic DNA Extraction Kit provided by Promega, a Canadian company, Electrophoresis was performed of the extracted DNA samples from the liver on a 1g agarose gel at a voltage difference of 100 volts. To determine the purity and concentration of the extracted DNA, a Nanodrop device was used (Fig. 1).

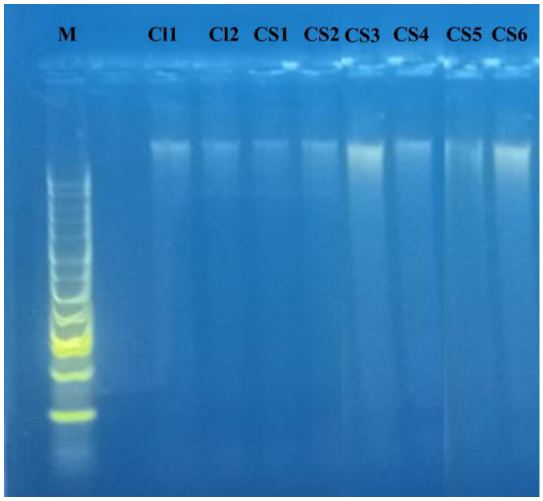

Fig.1: Extraction of DNA by gel electrophoresis.

The Amplification of DNA samples was performed using the PCR-RAPD technique. Eight random primers, designed by the National Center for Biotechnology Information (NCBI) GenBank were used as listed in Table 1. The PCR reactions

Table 1: Randomly designed primers with their nucleotide sequences and annealing temperature

Source	TM	Primer relay 5(Sequence) to 3	Primers	arrangement
Istiak el at.,2018	39	TGGACCGGTG	OPC-08	1
	39	AGGCGGGAAC	OPL-07	2
	39	ACCACCCACC	OPL-18	3
Eissa el at.,2014	32	GTGATCGCAG	OPA-10	4
	36	AGGTGACCGT	OPA-18	5
	32	TGATCCCTGG	OPB-02	6
	36	CTGCTGGGAC	OPB-10	7
	36	AGATGCAGCC	OPE-06	8

begin with an initial denaturation step at 98°C for 2 min to denature the double-stranded DNA template, followed by 35 cycles, each consisting of three steps performed at temperatures 98, 50 and 72°C for different times 20 sec, 45 sec and 2 minutes, respectively. The PCR products are then loaded onto pre-made agarose gels, then the electrophoresis was performed. The agarose gel was transferred to a UV trans illuminator, where the DNA bands are visualized through UV imaging.

Enzymatic Efficiency and Specificity:

Starter efficiency % = (number of duplicated bands)/(total duplicated bands) × 100 and Starter specificity % = (number of different bands) /(total different bands) × 100. Recorded the volume and type of each band for each starter using Excel software, and entered the number 1 if a band is present and 0 if it is absent.

Evaluation the activity of antioxidant enzymes:

The activity of antioxidant enzymes was measured according to manufacturing protocol. 0.5 mg of frozen liver sample was added to the eppendorf tube containing 1 ml of B-PER lysis. Samples were then placed to the incubator under 37°C for 20 min. The mixture was vortexed until it became a homogenized serum-containing solution. This process was repeated several times. The Eppendorf containing the serum was then transferred to a centrifuge to isolate the serum from the liver homogenate. Liver enzyme (AST, ALT, ALP, GGT) assays were performed using a kit provided by Fuji Films.

Statistical analysis:

The data of the present work was analyzed according to Duncan (1955) followed by SPSS. $p < 0.01$ was considered significant and differences were accepted at this value.

Results and Discussion

Bisphenol A Effects on Genetic material:

PCR is among the best techniques for detecting the changes in the structure of genetic material. In this study, eight random primers were designed by GenBank and used. Seven of these primers produced number of amplified bands, for RAPD-PCR results and based on electrophoresis, including 38 polymorphic bands, 18 unique bands, and one monomorphic band (Table 2). The total number of bands which were produced by the seven primers in all treatments was 57, while by all primers was 116. The OPC-08 primer produced the lowest number of polymorphic bands (2 out of 4), while the OPA-10 primer produced the highest number of polymorphic bands (7 out of 10). The OPE-06 primer produced the highest percentage of repetitive bands, reaching 75%.

The prefixes generated a total of 49 bands including both polymorphic and unique bands, for the 6 groups. Among these, 29 were polymorphic bands, 20 were unique bands, and 1 was a monomorphic band, all of which were generated by the prefix OBP-10, as shown in Table 3.

As in Table 3, the discriminatory power of OPC-08 primer was 6.90%, while its relative efficiency was 8.16% based on the percentage of

Table 2: Total number of amplified bands and number of monomorphic, unique, and polymorphic bands for each primer based on RAPD-PCR results

Primer / Ranges	OPC-8	OPL-07	OPL-18	OPA-10	OPA-18	OBP-02	OBP-10	OPE-06	Total
CL1	1	0	2	3	2	2	3	1	41
CL2	1	0	3	2	1	2	2	1	21
CS1	1	0	3	2	2	2	2	1	31
CS2	1	0	2	2	3	2	2	1	31
CS3	1	0	2	1	4	2	2	1	31
CS4	1	0	3	6	3	2	2	1	81
CS5	1	0	3	5	3	2	2	1	71
CS6	1	0	2	5	2	2	3	1	61
The total number of bands/Primer	8	0	20	26	20	16	18	8	116
The number of individual bands of the groups/ Primer	8	0	0	1	1	0	0	8	18
Number of single multiple bands/ Primer	0	0	8	7	7	8	8	0	38
Number Monomorphic	0	0	0	0	0	0	1	0	1

Table 3: Total number of duplicated, polymorphic bands and unique bands generated by each prefix as well as their discriminative power and relative efficiency

Primer	Total Number of Duplicate Bands	Polymorphic bands	Unique	Discriminating%	Efficiency %
OPC-08	4	2	2	.906	8.16
OPL-07	0	0	0	0	0
OPL-18	8	4	4	13.79	16.33
OPA-10	10	7	3	24.14	20.41
OPA-18	01	6	4	20.69	20.41
OBP-02	7	4	3	13.79	14.29
OBP-10	6	3	3	10.34	12.24
OPE-06	4	3	1	10.34	8.16
Total	49	29	02	99.99	99.99

polymorphic bands that the primer showed out of the total number of polymorphic bands revealed by RAPD-PCR. The primers generated the lowest number of duplicate bands compared to other primers were 2 polymorphic bands and 2 unique bands. Both CL2 control and the treated groups (CS3, CS4, and CS5) showed a band with a molecular weight of 70 bp. While CS1 and CS2 treatments showed 50 bp and the polymorphic bands showed 70-50 bp. The 90 bp band was unique and distinguished to CS6 treatment, while the unopened control group showed 80 bp bands (Table 4; Fig. 2).

The 90 bp band was unique and distinguished the CS6 treatment, while the 80 bp band distinguished the negative control group (Table 5).

As in Table 6, OPL-18 primer showed a group of 8 duplicated bands, 4 polymorphic bands an 4 for unique bands. All samples CS2, CS3, CS4, CS5, and CS6 shared the two polymorphic bands with molecular weights of 230 bp and 180 bp, whereas the two polymorphic bands of 150-200 bp were shared with CS1 and control groups. In the unique

Table 4 : OPC-08 primer bands resulting from electrophoresis

OPC-08									
Band type	band size	CL1	CL2	CS1	CS2	CS3	CS4	CS5	CS6
Polymorphic bands	70	0	1	0	0	1	1	1	0
	50	0	0	1	1	0	0	0	0
Unique bands	90	0	0	0	0	0	0	0	1
	80	1	0	0	0	0	0	0	0

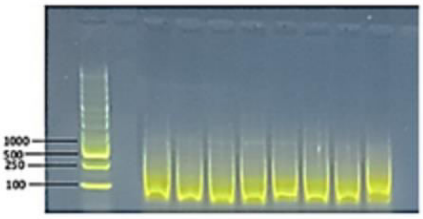

Fig. 2: Production of primer OPC-08 from thermal degradation.

Table 5: OPL-07 primer bands resulting from electrophoresis

OPL-07									
Band type	band size	CL1	CL2	CS1	CS2	CS3	CS4	CS5	CS6
Polymorphic	0	0	0	0	0	0	0	0	0
Unique	0	0	0	0	0	0	0	0	0

Table 6: OPL-18 primer bands resulting from electrophoresis

OPL-18									
Band type	band size	CL1	CL2	CS1	CS2	CS3	CS4	CS5	CS6
Polymorphic bands	230	0	0	0	1	1	1	1	1
	200	1	1	1	0	0	0	0	0
	180	0	0	0	1	1	1	1	1
	150	1	1	1	0	0	0	0	0
Unique bands	300	0	0	0	0	0	1	0	0
	250	0	0	0	0	0	0	1	0
	130	0	1	0	0	0	0	0	0
	110	0	0	1	0	0	0	0	0

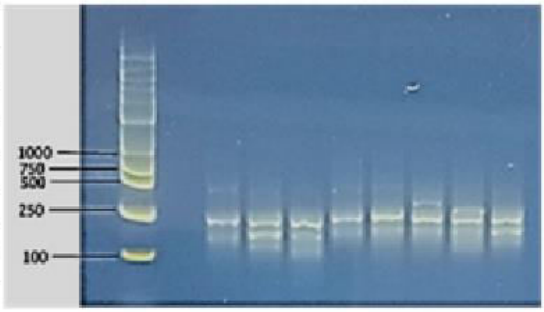

Fig. 3: Production of OPL-18 from thermal degradation

bands, CL2 control group was characterized by 130 bp, CS1 sample was characterized by 110 bp, while the CS4 and CS5 were 300 and 250, respectively (Table 6; Fig. 3). OPL-18 possessed a discrimination power of 13.79% and a relative efficiency of 16.33% (Table 3).

OPA-10 primer showed a discriminatory power of 24.14% and a relative efficiency of 20.41% (Table 3). This primer produced a number of duplicated bands, the variable bands were 7, while the unique were 3. CS6, CS4, and CS5 shared two consecutive bands with a molecular weight of 250-200 bp**.** CS4 and CS5 were distinguished by two consecutive bands with a molecular weight of 150-100 bp**.** CS2 showed 100 bp, while 75 bp was for both control groups. All of treatments (CS1-CS6) produced 50 bp band, which was unique for CL1 and CL2 groups. All of these bands were variable, while the unique one was distinguished by CS1 which showed 120 bp band, CS4 500 bp and 300 bp band was with CS6 (Table 7; Fig. 4).

This OPA-18 exhibited both relative efficiency (20.41%) and discriminatory power (20.69%) (Table 3).The results of this primer showed multiple variable (6) and unique (4) bands. The variable bands included the CS3 and CS5 treatments with a molecular weight 2000bp, while the CS2, CS3, and CS4 treatments shared two

Table 7 : OPA-10 primer bands resulting from electrophoresis

OPA-10

Band type	band size	CL1	CL2	CS1	CS2	CS3	CS4	CS5	CS6
Poly Morphic Bands	250	0	0	0	0	0	1	1	1
	200	1	0	0	0	0	1	1	1
	150	0	0	0	0	0	1	1	0
	100	0	0	0	1	0	1	1	1
	75	1	1	0	0	0	0	0	0
	50	0	0	1	1	1	1	1	1
	40	1	1	0	0	0	0	0	0
Unique bands	500	0	0	0	0	0	1	0	0
	300	0	0	0	0	0	0	0	1
	120	0	0	1	0	0	0	0	0

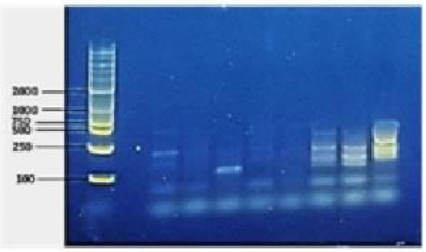

Fig. 4 : Production of primer OPA-10 from thermal degradation.

Table 8 : OPA-18 primer bands resulting from electrophoresis

OPA-18

Band type	band size	CL1	CL2	CS1	CS2	CS3	CS4	CS5	CS6
POLY Morphic bands	2000	0	0	0	0	1	0	1	0
	1500	0	0	0	1	1	1	0	0
	1000	0	0	1	0	1	0	0	0
	800	0	0	0	1	0	1	0	0
	400	0	0	0	1	1	1	0	1
	300	1	1	0	0	0	0	0	1
Unique Bands	3500	0	0	0	0	0	0	1	0
	2800	0	0	0	0	0	0	1	0
	500	1	0	0	0	0	0	0	0
	150	0	0	1	0	0	0	0	0

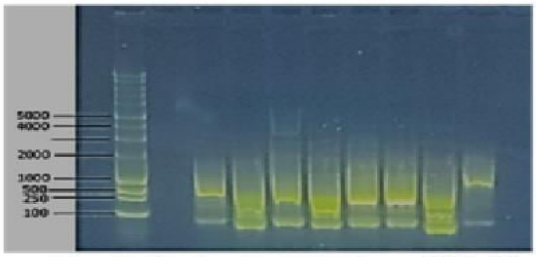

Fig. 5 : Production of primer OPA-18 from thermal degradation

Table 9 : OPB-02 primer bands resulting from electrophoresis

OBP-02

Band type	band size	CL1	CL2	CS1	CS2	CS3	CS4	CS5	CS6
Poly Morphic bands	200	0	0	1	0	1	1	0	0
	100	0	1	0	1	0	0	1	0
	90	1	0	0	0	0	0	0	1
	70	0	1	1	1	1	1	0	0
Unique bands	600	0	0	0	0	0	0	0	1
	300	1	0	0	0	0	0	0	0
	50	0	0	0	0	0	0	1	0

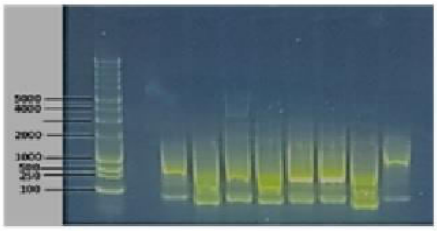

Fig. 6 : Production of primer OPB-02 from thermal degradation

bands with molecular weights 1500 and 400bp. Also, CS6 treatment showed a band at 400bp, whereas CS1 and CS3 treatments were characterized by 1000bp band. On the other hand, CS2 and CS4 produced a band at 800bp. Both control groups shared a band with the CS6 treatment at 300bp. All of these bands were of the variable type. The unique bands were identified in CS5 treatment with two consecutive unique bands at 3500 and 2800bp. CL1 control group produced a 500bp band, while CS1 treatment showed a unique band at 150bp (Table 8; Fig. 5).

In terms of OPB-02, this primer produced 4 distinct bands. CS1, CS3, and CS4 shared a band with a molecular weight 200bp, while CS2 and CS5 shared a band with CL2 control group in 100bp. CS6 was distinguished with CL1 control group with a band of molecular weight 90bp. Moreover, CL1 shared a band with the CS1, CS2, and CS3 in a weight of 70bp. The unique band was distinguished by CS6 with a molecular weight of 600bp, while CL1 control group was distinguished with a band of 300bp. CS5 showed a band of molecular weight 50 bp (Table 9; Fig. 6). This

Table 10 : OPB-10 bands of the primer from the electrophoresis.

OPB-10

Band type	band size	CL1	CL2	CS1	CS2	CS3	CS4	CS5	CS6
Polymorphic bands	90	0	0	0	1	0	0	1	0
	80	0	1	1	0	1	0	0	1
	50	1	0	0	0	0	0	0	1
Unique Bands	150	1	0	0	0	0	0	0	0
	70	0	0	0	0	0	1	0	0
Monomorphic Band	40	1	1	1	1	1	1	1	1

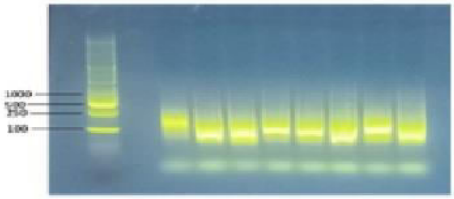

Fig. 7: Production of primer OPB-10 from thermal degradation

Table 11 : OPE-06 primer bands resulting from electrophoresis

OPE-06

band type	band size	CL1	CL2	CS1	CS2	CS3	CS4	CS5	CS6
Polymorphic	130	0	0	0	0	0	1	1	1
	70	1	0	0	0	1	0	0	0
	50	0	1	1	0	0	0	0	0
Unique	100	0	0	0	1	0	0	0	0

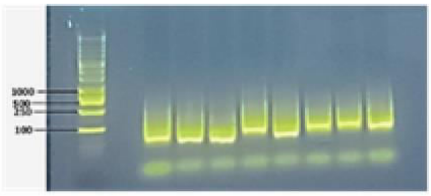

Fig. 8: Production of OPE-06 primer from thermal degradation

primer showed a discriminatory power of 13.79 % and relative efficiency of 14.29% (Table 3).

The OPB-10 primer produced diverse of bands for each treatment. CS2 and CS5 were associated with 90bp of the molecular weight, while CL2 control group was associated with CS1, CS3 and CS6 in 80bp band. CS6 was also associated with CL1 control group in 50bp band. The unique bands distinguished CL1 control group in 150bp band, while CS4 showed 70bp band. However, OPB-10 primer had a general band with a molecular weight of 40bp (Table 10; Fig. 7). This primer produced a discriminatory power of 10.34% and relative efficiency of 12.24% (Table 3).

OPE-06 primer exhibited a discrim-inatory power of 10.34% and a relative efficiency of 8.16% (Table 3). This primer produced a distinctive banding pattern characterized in CS4, CS5, and CS6 treatments in a molecular weight 130bp. The CS3 treatment showed similarity with CL1 control group in 70bp. In addition, CS1 treatment showed 50bp which is also similar with CL2 control group. Interestingly, the unique banding pattern was only observed with CS2 treatment in 100bp band (Table 11; Fig. 8).

The results of the electrophoresis of liver DNA samples from both the control group and the treated groups with the same primers and conditions showed several duplicated bands. The difference in the molecular weight of the bands may be attributed to the non-specific binding sites between the primers and the DNA sample or may be due to the oxidative stress induced by the material, which can cause negative changes in the cells and damage the DNA. Additionally, the changes in the molecular composition of the treated DNA may lead to the deviation and instability of the chromosomes, resulting from exposure to toxic substances that affect the genes. Moreover, weak and faint bands were observed when loading the PCR product. The OPL-07 primer did not show any bands, which may be due to a lack of compatibility between the nucleotide sequences of the primer and the sample. This finding is consistent with a study on the identification of local pigeon breeds and the variation in their nucleotide sequences using RAPD-PCR. Blood samples were taken from different male and female breeds and subjected to RAPD-PCR, which resulted in diverse and unique bands. These findings differ from the study itself,

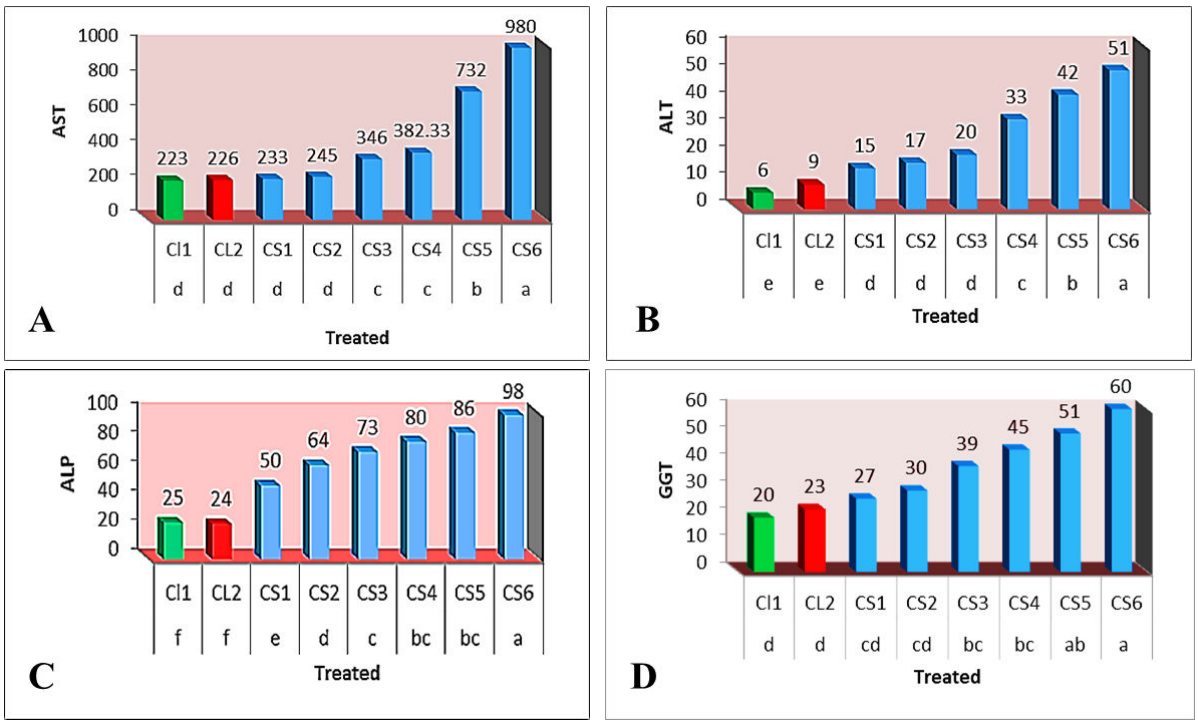

Fig. 9: Level of liver enzymes.

which showed a common band for the OBP-10 primer.

Effect of Bisphenol A on bio-chemical parameters:

AST Enzyme:

The results of the present study showed significant differences at a probability level of P≤0.01 compared with control groups and between treatment (CS3, CS4, CS5 and CS6) with values of (u/l) 980, 732, 382, 346, 226, and 223, respectively. There was no significant difference in CS1 (233) and CS2 (245) groups in comparison with CL1 and CL2 (223 and 226 respectively). However, compared with control groups, significant differences were observed between CS1 and CS2 with the remaining groups CS3, CS4, CS5, and CS6 with values of (u/l) 346, 282.33, 732 and 980, respectively (Fig. 9A).

ALT Enzyme:

In terms of ALT enzyme, there was significant differences at a probability level of P≤0.01 for ALT in comparison with CL1 and CL2 control groups and between each other of the treated groups CS1, CS2, CS3, CS4, CS5, and CS6. The highest value (51u/l) was observed in CS6 while the lowest value (6 u/l) was noticed in CL1 control group (Fig. 9B). Significant differences were observed when comparing groups CS1, CS2, and CS3 with each other.

ALP Enzyme:

The current study also observed significant differences at a probability level of P≤0.01 when comparing between control groups (CL1, CL2) and the treated groups (CS1, CS2, CS3, CS4, CS5, CS6). The highest value of ALT was observed in the group CS6 (98u/l) while the lowest value was shown in control groups (24 and 25u/l). When comparing the treatment groups with each other, no significant differences were observed between groups CS3, CS4, and CS5. However, significant differences were observed between groups CS1,

CS2, and CS6, with values of 50u/l, 64u/l, and 98u/l, respectively (Fig. 9C).

GGT enzyme:

The Findings of the current work showed no significant differences between CS1 and CS2 compared to CL1 and CL2 control groups. However, a significant difference was observed at a level of P≤0.01 in CS3 (39), CS4 (45), CS5 (51) and CS6 (60). In comparison with each of CS1, CS2, CS3, and CS4, no significant differences were observed (Fig. 9D).

Most of wildlife species can be exposed to concentrations of biologically active compounds endocrine-disrupting chemicals, such as BPA. These compounds may disrupt some of biological processes including gonads growth, development of embryos and sexual differentiation by binding to specific hormone receptors and preventing their response (Flint *et al*., 2012). Many studies have documented that people are exposed to several toxic chemicals, including pesticides, heavy metals, nicotine, epoxy, perfluorinated, phthalates and BPA. The latter, has an ability to cause health hazard and change developmental plasticity by disrupting the endocrine system (Heindel *et al*., 2015). In the present study, different concentrations of BPA were administered to evaluate the potential activity of this chemical material. *In ovo* study documented that BPA has adverse effects on biological systems and injecting the egg with low dose of BPA leads to formation of ovo-testis (Kandil and Emrah, 2018). Several studies proposed chicken embryos as an experimental model to evaluate the risk and toxicity of environmental chemical compounds, drugs and infections related diseases (Sommerfeld *et al*., 2022). In the present study, chicken embryos were exposed to BPA at early embryonic developemnt. Thus, the exposure of BPA at this period may severely influence organogenesis and disrupt the normal development in some organs. It has been found that exposure to BPA after fertilization delays the neurulation in chick embryos (Atay *et al*., 2020). The effect of bisphenol A at early stages of embryos is poorly understood, and exposure at stages through early embryonic development are sensitive period (Halevy, 2020). Here, the current work aimed to evaluate *in ovo* and the genotoxic effects of BPA and biochemical changes in chicken embryos during the development employing PCR assay and following oxidative stress protocol. The molecular techniques, including PCR, are among the best techniques for detecting any changes in the structure of genetic material. In this study, eight random primers designed by the GenBank were used, and seven of them produced a number of amplified bands for RAPD-PCR results, based on electrophoresis images. Eissa (2014) reported that six out of 21 random primers produced repeatable amplified bands. Through the PCR RAPD technique, any changes in the DNA molecule can be detected. This technique works by amplifying or increasing the size of the oxygen-depleted DNA molecule and is one of the breakthroughs that has revolutionized molecular biology and opened up areas of research in diagnosis. The results of the electrophoresis of liver DNA samples from both the control group and the treated groups with the same primers and conditions showed several duplicated bands. The difference in the molecular weight of the bands may be attributed to the non-specific binding sites between the primers and the DNA sample or may be due to the oxidative stress induced by the material, which can cause negative changes in the cells and damage the DNA. Faheem *et al*. (2019) showed that BPA stimulates mRNA transcription and causes damage to vital organs by altering the levels of enzymes. Langthasa *et al*. (2022) showed an increase in DNA damage of peripheral blood in chick embryos exposed to BPA. Using a comet assay, Malladi *et al*. (2007) also demonstrated that BPA is potentially enough to induce DNA damage in blood. It was found that BPA can cause genetic damage in estrogen receptors (ERs) and its genotoxicity is dependent on hormone receptors expression. It was revealed that BPA induces chromosomal aberrations and micronucleus frequencies in bone marrow and also causes DNA damage in rat lymphocytes (Fic *et al*., 2013).

The statistical analysis of the current work demonstrated that BPA significantly increased the level of liver enzymes (AST, ALT and ALP) with no significant change with GGT compared to the control groups. This is probably because that BPA can change architecture of the liver, indicative of function defects. Our results are consistent with studies performed on rats by Ateş and Hatipoğlu, (2022) and chicken embryos by Esuola *et al*. (2022). They found that diphenol A (100 mg/kg of body weight) caused hepatic toxicity and elevation in AST, ALT, ALP and GGT. It has been reported that BPA changes hepatocytes structure, leading to infiltration of white blood cells, cytoplasmic vacillation, swelling of the rough endoplasmic reticulum, mitochondrial degeneration, nuclear enlargement, fat droplet deposition and elevation of liver enzymes (Ahmed Zaki *et al*., 2021). A high level of BPA was associated with renal insufficiency and kidney diseases (Kataria *et al*., 2017). It can also stimulate oxidative stress and accumulation of lipid in the body. BPA could also decrease liver enzymes activities and accumulation of hepatic lipid (Lee *et al*., 2018). Therefore, BPA correlated to the inflammation of liver and its diseases, such as fatty liver and non-alcoholic diseases. Hassan *et al*. (2012) documented that BPA causes hepatotoxicity by reducing the levels of antioxidant enzymes gene expression in liver tissue, while Esuola *et al*. (2022) showed a significant increase in the liver enzyme activity in the chick embryos exposed to BPA. The increase in liver enzymes may be attributed to damage of liver cells or other tissues, such as the heart and muscles. Mahajan *et al*. (2018) confirmed that elevation in liver enzymes activity is due to oxidative stress, which reacts with cellular molecules including proteins, lipids and nucleic acids, leading to their oxidation and releasing these enzymes into the blood stream. Based on this information, our results confirmed that BPA has a potential role in oxidative stress by increasing the level of liver enzyme activities. Based on this study, by binding with ERs, BPA mimics hormones -like endocrine disrupting structure, thus alters the endocrine system function and causes health defects with abnormal expression (Atay *et al*., 2020). Damage of DNA in lymphocyte, kidney, and liver cells causes an increase in oxidative stress (Akram *et al*., 2021). Therefore, our results revealed that BPA has adverse effects on chick embryonic development by damaging genetic materials and elevating oxidative stress of liver. Since chicken embryos were a model of the current study, it possible that findings of our study suggested that BPA may cause various disorders in animals and humans, such as infection, DNA damaging and physiological effects.

Conclusion

According to our results, the exposure to BPA even at low concentration has a potential impact by altering level of liver enzymes and damaging of genetic material in chicken embryos. Exposure to BPA should be used with attention because it may cause several of health risks in domestic animals or humans.

Acknowledgements

We would like to express our deepest gratitude to the authorities at University of Mosul and College of Education for Girls as well as College of Education for Pure Science /Department of Biology for providing facilities for this work.

References

Abd El-Gwaad HMS, Abd El-Wahab HM, Mohamed EAK, Sharaf EHAA and Osman AAHM. (2020) Modulatory effect of dry orange (*Citrus sinensis*) peel powder on bisphenol A-induced hepatic and splenic toxicity in rats. J Basic Appl Zool. 81(1): 1-9.

Ahmed Zaki MS, Haidara MA, Abdallaa AM, Mohammed H, Sideeg AM and Eid RA. (2021) Role of dietary selenium in alleviating bisphenol A toxicity of liver albino rats: Histological, ultrastructural, and biomarker assessments. J Food Biochem. 45(5): e13725.

Akram, R, Iqbal R, Hussain R, Jabeen F and Ali M. (2021) Evaluation of oxidative stress, antioxidant enzymes and genotoxic potential of bisphenol A in fresh water bighead carp (*Aristichthys nobils*) fish at low concentrations. Environ Poll. 268: 115896.

Al-Mustafa SS and Al-Sultan RG. (2022) Histological changes of liver and lung induced by bisphenol A in

pregnant mice. Int J Drug Delivery Technol. 12(2): 678-681.

Arancio AL, Cole KD, Dominguez AR, Cohenour ER, Kadie J, Maloney WC, Cilliers C and Schuh SM. (2019) Bisphenol A, Bisphenol AF, di-n-butyl phthalate, and 17β-estradiol have shared and unique dose-dependent effects on early embryo cleavage divisions and development in *Xenopus laevis*. Reprod Toxicol. 84: 65-74.

Atay E, Ertekin A, Bozkurt E and Aslan E. (2020) Impact of Bisphenol A on neural tube development in 48-hr chicken embryos. Birth Defects Res. 112(17): 1386-1396.

Ateş MB and Hatipoğlu D. (2022) Effect of *Nigella sativa* oil on Bisphenol A-induced hepatotoxicity in wistar albino rats: histopathological and biochemical investigation. Int J Agricult Environ Food Sci. 6(3): 402-409.

Cao XL, Zhang J, Goodyer CG, Hayward S, Cooke GM. (2012) Bisphenol A in human placental and fetal liver tissues collected from Greater Montreal area (Quebec) during (1998–2008). Chemosphere 89: 505-511.

Duncan, D.B. (1955). Multiple range and multiple F test. Biometrics 11(1): 1-42.

Eissa EA, Farahat GS, Mahmoud BYF and El-Full EA. (2014) Productive performance and molecular genetic characterization of brown and white Japanese quail genotypes using RAPD and ISSRs-PCR markers. Egypt J Genet Cytol. 43(1): 73-98

Esuola LO, Esan O, Maikifi AS, Ajibade TO, Adetona MO, Oyagbemi AA, Omobowale TO, Oladele OA, Oguntibeju OO and Yakubu MA. (2022) Naringin mitigates Bisphenol A-induced hepatotoxicity in cockerel chicks. https://doi.org/10.21203/rs.3.rs-2129508/v1.

Faheem M, Khaliq S and Lone KP. (2019) Effect of bisphenol-A on serum biochemistry and liver function in the freshwater fish, *Catla catla*. Pak Vet J. 39(1): 1-5.

Fic A, Sollner Dolenc M, Filipič M and Peterlin Mašić L. (2013) Mutagenicity and DNA damage of bisphenol A and its structural analogues in HepG2 cells. Arhiv za Higijenu Rada i Toksikologiju 64(2): 189-199.

Flint S, Markle T, Thompson S and Wallace E. (2012) Bisphenol A exposure, effects, and policy: A wildlife perspective. J Environ Managem. 104: 19-34.

Gorini F, Bustaffa E, Coi A, Iervasi G and Bianchi F. (2020) Bisphenols as environmental triggers of thyroid dysfunction: clues and evidence. Int J Environ Res Public Hlth. 17(8): 26-54

Halevy O. (2020) Timing is everything-- the high sensitivity of avian satellite cells to thermal conditions during embryonic and posthatch periods. Frontiers Physiol. 11: 235.

Hassan ZK, Elobeid MA, Virk P, Omer SA, ElAmin M, Daghestani MH and AlOlayan EM. (2012) Bisphenol A induces hepatotoxicity through oxidative stress in rat model. Oxid Med Cell Longev. 2012: 194829.

Heindel JJ, Balbus J, Birnbaum L, Brune-Drisse MN, Grandjean P, Gray K, Landrigan PJ, Sly PD, Suk W, Cory Slechta D, Thompson C and Hanson M. (2015) Developmental origins of health and disease: integrating environmental influences. Endocrinology 156(10): 3416-3421.

Istiak MS, Mollah MBR and Ali MS. (2018) Analysis of genetic diversity in Bangladeshi quail populations using rapd markers. IOSR J Agricult Vet Sci. 11(1): 42-47.

Kandil B and Emrah SUR. (2018) The light microscopic investigation of the effects of in-ovo administered bisphenol A (BPA) on the development of testes. Ankara Üniversitesi Vet Fakültesi Dergisi 65(3): 273-281.

Kataria A, Levine D, Wertenteil S, Vento S, Xue J, Rajendiran K, Kannan K, Thurman JM, Morrison D, Brody R, Urbina E, Attina T, Trasande L and Trachtman H. (2017) Exposure to bisphenols and phthalates and association with oxidant stress, insulin resistance, and endothelial dysfunction in children. Pediatr Res. 81(6): 857-864.

Langthasa P, Barhoi D, Devi SH, Giri A and Giri S. (2022) Genotoxic and cytotoxic potential of Bisphenol A in the early developing stages of chick embryo (*Gallus gallus domesticus*) evidenced by HET-MN and Comet assay. Res J Biotechnol. 17: 1.

Lee S, Lee HA, Park B, Han H, Park BH, Oh SY, Hong YS, Ha EH and Park H. (2018) A prospective cohort study of the association between bisphenol A exposure and the serum levels of liver enzymes in children. Environ Res. 161: 195-201.

Mahajan L, Verma PK, Raina R, Pankaj NK, Sood S and Singh M. (2018) Alteration in thiols homeostasis, protein and lipid peroxidation in renal tissue following subacute oral exposure of imidacloprid and arsenic in Wistar rats. Toxicol Rep. 5: 1114-1119.

Malladi SM, Bhilwade HN, Khan MZ and Chaubey RC. (2007) Gamma ray induced genetic changes in different organs of chick embryo using peripheral blood micronucleus test and comet assay. Mutation Res Genetic Toxicol Environ Mutagen. 630(1-2): 20-27.

Pinto C, Hao R, Grimaldi M, Thrikawala S, Boulahtouf A, Aït-Aïssa S, Brion F, Gustafsson JÅ, Balaguer P and Bondesson M. (2019) Differential activity of BPA, BPAF and BPC on zebrafish estrogen receptors in

vitro and in vivo. Toxicol Appl Pharmacol. 380: 114709.

Sang C, Song Y, Jin TW, Zhang S, Fu L, Zhao Y, Zou X, Wang Z, Gao H and Liu S. (2021) Bisphenol A induces ovarian cancer cell proliferation and metastasis through estrogen receptor-α pathways. Environ Sci Pollut Res Int. 28(27): 36060-36068.

Sommerfeld S, Mundim AV, Silva RR, Queiroz JS, Rios MP, Notário FO, Medeiros Ronchi AA, Beletti ME, Franco RR, Espindola FS, Goulart LR and Fonseca BB. (2022) Physiological changes in chicken embryos inoculated with drugs and viruses highlight the need for more standardization of this animal model. Animals (Basel) 12(9): 1156.

Tse LA, Lee PMY, Ho WM, Lam AT, Lee MK, Ng SSM, He Y, Leung KS, Hartle JC, Hu H, Kan H, Wang F and Ng CF. (2017) Bisphenol A and other environmental risk factors for prostate cancer in Hong Kong. Environ Int. 107: 1-7.