Cytogenetic and Biochemical Changes Induced by Bisphenol A on Testis of Albino Rats and Possible Protection of Black Seed Oil

Original Article

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ABSTRACT

Background: Bisphenol A (BPA), an estrogenic compound, is one of the world's highest production volume chemicals used in polycarbonate plastics in many consumer products and epoxy resins lining food containers. The BPA is known to have toxic effects on various systems in man and animals.

Objective: To evaluate the cytogenetic and biochemical changes induced by BPA on testis of albino rats and the possible protective effect of black seed oil.

Materials and Methods: This study was carried out on 80 rats, divided into four equal groups (20 rats each). Group I: negative control. Group II: received black seed oil (2ml/ kg BW), Group III: administered BPA (25mg/ kg BW), Group IV: administered BPA + black seed oil of same doses. Blood, testis and epididymis were collected for biochemical and cytogenetic evaluation.

Results: BPA caused a significant decrease in sperm count, total content of DNA, RNA and protein in the testis, with a significant increase of DNA damage of spermatocyte, head and tail abnormalities compared to controls. Co-administration of BPA+ black seed oil resulted in a significant improvement of sperm count, total content of DNA, RNA and protein with significant decrease in DNA damage of spermatocyte and head and tail abnormalities compared to BPA group. Also, BPA group showed a significant decrease in serum testosterone and catalase levels, with a significant increase of super-oxide dismutase (SOD) compared to controls. However, BPA + black seed oil group showed significant improvement of serum testosterone, catalase and SOD levels compared to BPA group.

Conclusion: Exposure of rats to BPA resulted in reproductive toxicity through inducing significant DNA damage and impaired synthesis of DNA, RNA and protein in testicular tissue. Black seed oil attenuate oxidative damage in rat testis via upregulating the activities of enzymatic antioxidants catalase and SOD.

Key Words: Comet assay, bisphenol A, black seed oil, rat, testis.

Received: 17 September 2019, **Accepted:** 05 October 2019

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ISSN: 2090-7265, Journal of Recent Advances in Medicine Vol 1 issue 1 January 2020

INTRODUCTION

Bisphenol A (BPA) is one of the world's highest production volume chemicals used in polycarbonate plastics in many consumer products, baby bottles, and epoxy resins lining drink and food containers, medical devices and dental sealants[1]. BPA is identified as a potential endocrine disruptor based on its estrogenic properties and toxic effects on germinal cells, which lead to disturbance in hormone production[2]. The possible genotoxicity of BPA has been tested in a variety of in vitro and in vivo studies, but the results are controversial[3].

Heat, repeated washing of polycarbonate products and contact with either acidic or basic compounds accelerate hydrolysis of the ester bond linking BPA molecules in polycarbonate plastics and resins resulting in an increase in the rate of leaching of BPA. BPA is absorbed from gastrointestinal tract into the blood and redistributed to other tissues[4]. The oxidative stress was proposed as another adverse cellular effect of BPA. BPA increased the generation of reactive oxygen species (ROS) and induced cellular apoptosis. It is known that several antioxidants can protect against BPA-induced toxicity[5].

Nigella sativa (black seed) is a widely used medicinal plant all over the world and has been used in the treatment of different diseases. Acute and chronic toxicity studies have confirmed the safety of Nigella sativa oil and its most abundant active component, thymoquinone, particularly when given orally[6,7].

 DOI: 10.21608/jram.2019.13342.1012

The aim of the present study was to evaluate the cytogenetic and biochemical changes induced by bisphenol A on the testis of adult male albino rats and the possible protective effect of black seed oil.

MATERIALS AND METHODS

Materials

1. Animals

The present study was carried out on 80 adult male albino rats, of three months age (180200- gram). The animals were obtained from Helwan animal breeding farm, Cairo, Egypt. The choice of the rats in this study was due to many metabolic similarities between rat and human[8]. The animals were housed in clean stainless-steel cages under the same environmental conditions. They were kept for acclimatization two week prior to the start of the experiment. The handling of animals followed the rules for the experimental research ethics approved by Research Ethics Committee at faculty of Medicine for Girls Al-Azhar University according to Kostomitsopoulos and Durasevic[9].

2. Chemicals

a. Bisphenol A (BPA) was supplied by Sigma Company USA as 97% purity in the form of white powder and suspended in distilled water, 2 ml of this suspension for each rat.

b. Black seed oil was purchased from El-captain Company (CAPPHARMA), the 6 October City, Egypt.

c. The reagents for the measurement of the different parameters were obtained from Bio diagnostic and Sigma Company.

Methods

Experimental Design

According to Bian et al. and AboulEzz *et al.*,[10,11] the animals have been divided into 4 groups 20 rates each. All groups have received the BPA by oral gavage daily for six weeks, five times/week.

Group I: Rats served as negative controls and were received distilled water.

Group II: Rats were received black seed oil (2ml/kg bw).

Group III: Rats were administered BPA at dose of 25 mg/kg bw.

Group IV: Rats were administered BPA + black seed oil (25mg /kg +2 ml /kg bw).

The animals were fasted overnight and lightly anaesthetized with diethyl ether. Blood samples were obtained from the retro orbital sinus puncture into heparinized capillary tubes from each rat before killing. Collected blood was left to clot at 37 º C incubator till clot retraction had started and serum separated from the clot. The clear supernatant serum was centrifuged at 4000 rpm for 15 minutes, then collected using a pastier pipette and stored at -20ºC for biochemical evaluation. All groups have been sacrificed using diethyl ether. Both testis and epididymis have been dissected and prepared to be subjected to the cytogenetic study".

The following parameters were done at Cell Biology Department of National Research Center, Dokki (Cairo).

Cytogenetic evaluation

a. Sperm count and their morphology

Evaluation of sperm count, and their morphology was done according to the technique described by Wyrobek *et al.*[12]. For each animal about 1000 sperms were examined for presence of morphological abnormalities (amorphous and banana heads) and (coiled and divided tails).

b. Comet assay

Endogenous DNA damage measured as the mean comet tail DNA of spermatocytes for the studied groups (20 rats each). The number of cells scored for each animal was 100 cells[13]. The comet assay was performed according to the procedure of Singh *et al.*[14] with modification of Klaude *et al.*[15] as described by Blasiak *et al.*[16].

c. Total Content of DNA, RNA and Protein

Extraction of Nucleic Acid

The nucleic acids were extracted from testes for determination of DNA and RNA concentration[17].

Determination of DNA Concentrations

Concentration of DNA was determined in the nucleic acid extracted by diphenyl amine method described by Dische and Schwartz[18]. Result of tissue DNA concentration (mg/gm tissue) was shown by stander DNA.

Determination of RNA Concentrations

Concentration of RNA was determined in the nucleic acid extracted by Orcinal procedure described by Dische[19]. Results of tissue RNA concentration (mg/ gm tissue) was shown in stander RNA.

Determination of Protein Concentrations

Colorimetric method described by Gornall *et al.*[20] was used for quantitative estimation of protein.

Biochemical studies

Serum testosterone level was done according to Rajkowski *et al*[21] and measured by Enzyme linked immunosorbent assay.

Quantitative estimation of serum superoxide dismutase (SOD)

The method described by Nishikimi *et al.*[22]was used for quantitative estimation of SOD (E C.1.15.1.1.).

Reagents

- Reagent (1): A bottle containing phosphate buffer pH 8.5
- Reagent (2): A bottle containing NBT reconstituted in 10 ml distilled water.
- Reagent (3): A bottle containing NADH reconstituted in 10 ml distilled water.
- Reagent (4): A bottle containing phenadinemetho sulphate (PMS) reconstituted in 10 ml distilled water.

Quantitative estimation of serum catalase (CAT)

The method described by[23] was used for quantitative estimation of CAT (E C.1.11.16-.).

Reagents

- Reagent (1): Chromogenic buffer: A bottle containing phosphate buffer (100mM /l, Ph.7.0).
- Reagent (2): A bottle containing H_2O_2 (substrate and standard) (0.5mM/l).
- Reagent (3): A bottle containing CAT inhibition.
- Reagent (4): A bottle containing peroxidase enzyme (>2000U/ D, 4-AAP (2mM/L).

Statistical analysis

Data were analyzed using Statistical Program for Social Science (SPSS) version 18.0. Quantitative data were expressed as mean± standard deviation (SD). Qualitative data were expressed as comparative statistical analysis (change of % between the studied groups)[24]. The results of the present study were represented in tables and photos. Independent-samples t-test of significance was used when comparing between two means, the level of significance at *p-value* ≤ 0.05.

RESULTS

Cytogenetic evaluation

a. Sperm count

Rats administered black seed oil alone showed just significant increase in sperm count 4.48%. while, rats administered BPA (25mg/kg) alone or BPA +black seed oil caused a significant decrease *P*<0.001 in sperm count 22.36% and 12.96% respectively compared to control. In addition, group III and group IV demonstrated a significant decrease in the sperm count 25.84% and 16.86% respectively as compared to group II. Furthermore, group IV showed a significant increase of sperm count 10.80% compared to group III as shown in (Table 1).

Table 1: Percentage of change between the studied groups of the effect of bisphenol A (BPA) on sperm count and serum testosterone level (ng/ ul) of adult male albino rats and the possible protection by black seed oil

Groups	Sperm count			Serum Testosterone level (ng/ ul)		
	% change	t-test	*P-value*	% change	t-test	*P-value*
Group (I) vs. Group (II)	4.48	2.828	0.047*	5.26	2.147	0.050*
Group (I)vs. Group (III)	22.36	9.500	0.001*	58.87	20.390	<0.001*
Group (I) vs. Group (IV)	12.96	7.778	<0.001*	29.71	9.642	<0.001*
Group (II) vs. Group (III)	25.84	11.500	<0.001*	61.04	25.127	<0.001*
Group (II) vs. Group (IV)	16.86	10.607	<0.001*	33.41	12.683	<0.001*
Group (III) vs. Group (IV)	10.80	4.000	0.025*	41.49	9.535	<0.001*

*significant *p-value*

Sperm head and tail morphology

BPA group showed a significant increase in abnormalities of sperm head morphology (amorphous and banana heads) 82.19% vs 77.99 and sperm tail morphology (coiled and divided tails) 80.00 vs 80.78 respectively compared to group I. While, group IV showed a significant decrease in abnormalities of (amorphous and banana heads) 67.30 vs 69.70% and (coiled and divided tails) 60.69% vs 56.54% respectively compared to BPA group as shown in (Table 2,3) and (Figure 1a-f).

Table 2: Percentage of change between the studied groups of the effect of bisphenol A (BPA) on sperm head morphology (amorphous & banana heads) of adult male albino rats and the possible protection by black seed oil

Groups	Amorphous head			Banana head		
	% change	t-test	*P-value*	% change	t-test	*P-value*
Group (I) vs. Group (II)	27.56	1.387	0.267	20.67	1.106	0.341
Group (I)vs. Group (III)	82.19	16.793	<0.001*	77.99	14.083	<0.001*
Group (I) vs. Group (IV)	45.52	3.467	0.002*	27.36	1.843	0.092
Group (II) vs. Group (III)	87.09	19.538	<0.001*	82.54	16.590	<0.001*
Group (II) vs. Group (IV)	60.53	19.538	<0.001*	42.37	16.590	0.011*
Group (III) vs. Group (IV)	67.30	13.336	<0.001*	69.70	13.259	<0.001*

*significant *p-value*

Table 3: Percentage of change between the studied groups of the effect of bisphenol A (BPA) on sperm tail morphology (divided& coiled tails) of adult male albino rats and the possible protection by black seed oil

Groups	Divided tail			Coiled tail		
	% change	t-test	*P-value*	% change	t-test	*P-value*
Group (I) vs. Group (II)	31.51	1.797	0.249	32.00	1.991	0.119
Group (I)vs. Group (III)	80.78	12.516	<0.001*	80.00	18.520	<0.001*
Group (I) vs. Group (IV)	55.76	6.549	<0.001*	86.40	5.064	<0.001*
Group (II) vs. Group (III)	86.83	13.847	<0.001*	82.54	20.708	<0.001*
Group (II) vs. Group (IV)	69.70	13.847	<0.001*	65.41	20.708	<0.001*
Group (III) vs. Group (IV)	56.54	8.761	<0.001*	60.69	13.094	<0.001*

*significant *p-value*

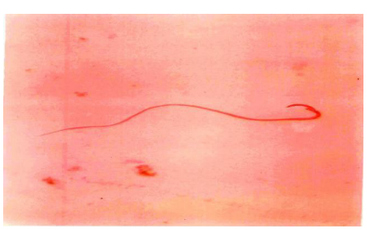

Fig. 1 a: Shows the normal sperm morphology in the control rat (Eosin stain, x 400).

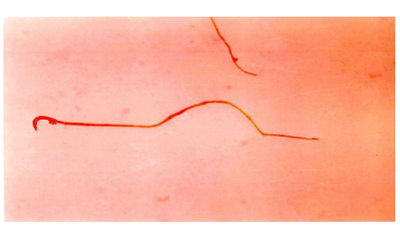

Fig. 1 b: Shows the normal sperm morphology in the control rat (Eosin stain, x 400).

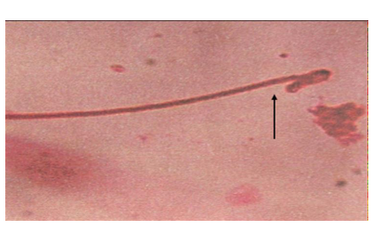

Fig. 1 c: Shows Amorphous head abnormality in rats exposed to bisphenol A (BPA) (Eosin stain, x 400).

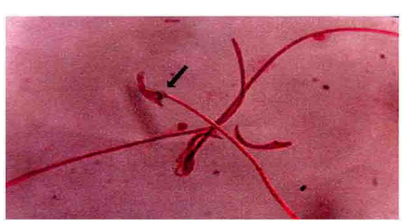

Fig. 1 d: Shows Banana shape head abnormality in rat exposed to bisphenol A (BPA) (Eosin stain, x 400).

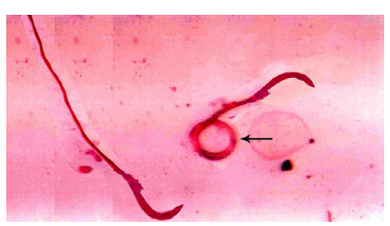

Fig. 1 e: Shows Coiled tail abnormalities in rats exposed to bisphenol A (BPA) (Eosin stain, x 400).

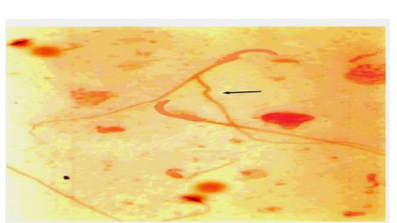

Fig. 1.f: Shows Divided tail abnormalities in rats exposed to bisphenol A (BPA) (Eosin stain, x 400).

b. Comet assay

The comet assay revealed that group I and group II showed undamaged spermatocyte, most of DNA located in comet head without tails are shown in (Figure 1g, h) which means no DNA damage is observed.

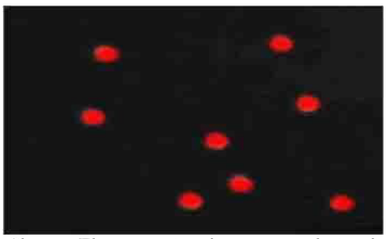

Fig. 1 g: Shows Fluorescent microscope photomicrograph of spermatocyte of group I show no migration out of the nucleus into the tail of the comet.

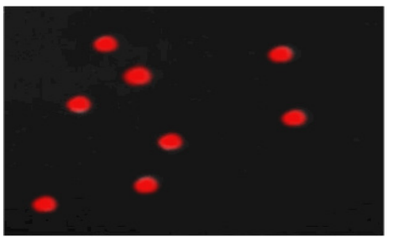

Fig. 1 h: Shows Fluorescent microscope photomicrograph of spermatocyte of group II showing no migration out of the nucleus into the tail of the comet as compared to group I.

BPA caused an increase in DNA strand breaks leading to greater DNA migration out of the nucleus into the tail of the comet in the spermatocyte compared to group I as shown in (Figure 1i). In group IV, there were lesser DNA migration out of the nucleus into the tail of comet as compared to group III as shown in (Figure 1j).

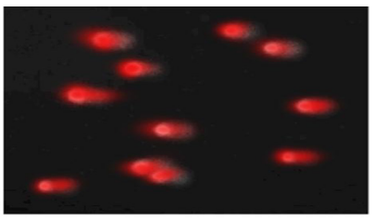

Fig. 1 i: Shows Fluorescent microscope photomicrograph of spermatocyte of group III showing greater DNA migration out of the nucleus into the tail of the comet as compared to group I.

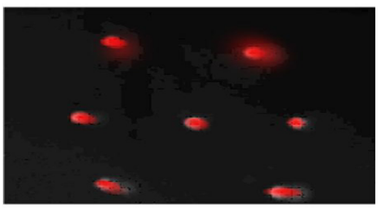

Fig. 1 j: Shows Fluorescent microscope photomicrograph of spermatocyte of group IV (BPA+ Black seed oil) showing lesser DNA migration out of the nucleus into the tail of the comet as compared to group III.

c. Total content of DNA, RNA and protein

Total content of DNA, RNA and total protein exhibited a significant decrease 42.22%, 41.38% and 37.10% respectively in BPA group. Whereas, no significant difference was observed in total content of DNA, RNA and total protein of group II and group IV compared to control. Furthermore, there were a significant decrease on total content of DNA, RNA and total protein in group III 49.51%, 37.04 and 41.23 and group IV 19.42%, 5.56% and 14.77% respectively compared to group II. Group IV showed a significant increase of total content of DNA, RNA and total protein 37.35%, 33.33% and 31.05% respectively as compared to the BPA group as shown in (Table 4).

Table 4: Percentage of change between the studied groups of the effect of bisphenol A (BPA) on total content of DNA, RNA (mg/ g tissue) and Total protein (g/dl) in testis of adult male albino rats and the possible protection by black seed oil

Groups	Total DNA content (mg/ g tissue) in testis			Total RNA content (mg/ g tissue) in testis			Total protein content (mg/ g tissue) in testis		
	% change	t-test	*P-value*	% change	t-test	*P-value*	% change	t-test	*P-value*
Group (I) vs. Group (II)	11.65	1.45	0.160	6.90	0.720	0.479	7.03	1.819	0.119
Group (I)vs. Group (III)	42.22	4.95	0.001*	41.38	12.53	0.001*	37.10	10.78	0.001*
Group (I) vs. Group (IV)	7.78	0.93	0.362	12.07	3.736	0.004*	8.78	2.46	0.001*
Group (II) vs. Group (III)	49.51	16.4	0.001*	37.04	4.250	0.001*	41.23	15.82	0.001*
Group (II) vs. Group (IV)	19.42	5.23	0.001*	5.56	0.772	0.448	14.77	5.35	0.001*
Group (III) vs. Group (IV)	37.35	10.6	0.001*	33.33	10.01	0.001*	31.05	11.84	0.001*

*significant *p-value*

Biochemical studies

Serum testosterone level

Group II showed just significant increase in testosterone level (5.26%). While, rats administered BPA alone or BPA +black seed oil caused a significant decrease in testosterone level 58.87% and 29.71% respectively compared to control. In addition, group III and group IV demonstrated a significant decrease in testosterone level 61.04% and 33.41% respectively compared to group II. Furthermore, group IV demonstrated a significant increase in testosterone level 41.49% compared to group III as shown in (Table 1).

Serum SOD and Catalase

Group III and group IV demonstrated a significant increase in serum SOD level 69.25% and 41.60% respectively compared with control. However, group IV showed a significant decrease in SOD 47.34% as compared to the BPA group. On the other hand, there was a significant decrease of serum Catalase level in group III & group IV 49.58% and 21.65% respectively as compared to control. Group IV showed a significant increase of Catalase 35.65% as compared to BPA group as shown in (Table 5).

Table 5: Percentage of change between the studied groups of the effect of bisphenol A (BPA) on serum level of superoxide dismutase (SOD) & Catalase (U/ml) of adult male albino rats and the possible protection by black seed oil

Groups	Serum SOD (U/ml)			Serum Catalase (U/ml)		
	% change	t-test	*P-value*	% change	t-test	*P-value*
Group (I) vs. Group (II)	12.44	3.217	0.004*	4.50	1.872	0.075
Group (I)vs. Group (III)	69.25	29.066	<0.001*	49.58	25.371	<0.001*
Group (I) vs. Group (IV)	41.60	13.364	<0.001*	21.65	11.298	<0.001*
Group (II) vs. Group (III)	73.07	30.996	<0.001*	51.85	23.223	<0.001*
Group (II) vs. Group (IV)	48.87	16.054	<0.001*	25.17	11.430	<0.001*
Group (III) vs. Group (IV)	47.34	18.109	<0.001*	35.65	16.676	<0.001*

*significant *p-value*

DISCUSSION

Bisphenol A is a common endocrine disruptor that can alter the synthesis, metabolism, transport, and elimination processes of endogenous hormones and, thus, of mimicking/antagonizing hormonal activities in the body[25]. Nigella sativa (Black seeds) and its derivatives have shown various pharmacological activities including therapeutic efficacy against different human diseases and antioxidant anti-inflammatory effects against environmental toxins[26].

Concerning the Sperm count the results of the present study indicated that black seed oil administration caused just significant increase in sperm count in compared to control. These could be explained by[27] as they stated that administration of Nigella sativa caused improvement in semen parameters in albino rat and increased sperm count among infertile men. The increased sperm concentration was due in part to the increase in testosterone and follicular stimulating hormone (FSH) levels in testicular tissue, since these two hormones were responsible for spermatocytogenesis.

In the current study, the oral administration of BPA to rats displayed a significant decrease in sperm count compared to control. This observation was generally in agreement with Karnam *et al.*[2] who revealed that there was significant reduction in the epididymal sperm count after administration of BPA as compared to control.

The mechanism by which BPA could affect sperm count was explained by Klaunig *et al*[28]who concluded that bisphenol A has been shown to accumulate in the fatty tissues and is metabolized by Cytochrome P450. Cytochrome P-450 has been shown to induce ROS that permanently impairs sperm function thereby resulting in decline of sperm counts in men and laboratory animals.

Another explanation was reported by Kadry *et al.*[29] who revealed that BPA-induced impairment of Sertoli cells had been reported by inhibiting endoplasmic reticulum Ca2+ homeostasis and the ectoplasmic specialization between Sertoli cells and spermatids. BPA may exert both anti-androgenic and estrogenic effects to impair sperm production[30]. Moreover, Thimmappa[31] stated that a dramatic decrease in caudal epididymal sperm numbers was previously considered to be the result of a lower sperm output by the testis and to increase sperm resorption (phagocytosis) in the rat testis and epididymis as a result of increase the number of mal-formed sperms.

This study revealed that administration of black seed oil and BPA showed a significant increase of sperm count in comparison to administration of BPA alone. This result is in accordance with Tawfeek *et al.*[32] as they showed a significant increase in the percentage of live/dead sperm of male albino rats in Nigella sativa oil joined to H_2O_2 compared with

H_2O_2 group. They concluded that Nigella sativa oil has radical scavenging properties, which may explain the increase in the percentage of live/dead sperms. The anti-oxidative action induced by oil is a result of a direct action or indirect effects, e.g. including antioxidative enzymes cascade.

Regarding the results of abnormalities in sperm head and tail morphology in the present study, there was a significant increase in abnormalities (amorphous and banana heads) and (coiled and divided tails) in rats administered BPA compared to control rats.

These results were in agreement with Karnam *et al.*[2] as they referred that head and tail abnormality percentages were significantly increased in rats of BPA group as compared to control. They suggested that BPA might have potential mutagenic effects on germ cells that led to abnormal sperm production.

Combination of BPA + black seed oil in the current study caused an improvement in the percentage of abnormalities (amorphous and banana heads) and (coiled and divided tail) as compared to rats administered BPA alone.

These results were in accordance with Tawfeek *et al.*[32] who demonstrated that the beneficial effect of Nigella sativa oil decrease in the percentage of morphologically abnormal sperms reflects its antioxidants effect that counteracts the H_2O_2 effect on sperms.

Also, these results were coincided with Hadi *et al.*[33] who reported that administration of the plants extracts mixture (Trigonella faenum-graecum seeds, Nigella sativa seeds, and Fraxinus ssp. seeds) to diabetic rats at three periods of study 45, 60, 75 days, respectively decreased sperms abnormalities compared with diabetic group.

Concerning DNA damage of spermatocyte evaluated by comet assay, result of this work revealed a significant decrease in DNA damage of spermatocyte in (groupII) as compared to control rats.

This result was in contrast to Al-Shdefat *et al.*[34] who demonstrated that thymoquinone (TQ) treatment, (5.0, 10, or 20 μ) increased DNA damage index in a concentration dependent manner compared with control. The geno- protective effect and genotoxic effect of TQ may be related to TQ quinine structure. TQ undergoes one or two electron reductions by cellular reductases. One-electron reduction results in the formation of semiquinones, which are converted to ROS when they react with molecular oxygen. Two-electron reductions produce the antioxidant hydroquinone.

The present study revealed a significant increase in DNA damage in BPA group compared to control. Result indicated that BPA is a testicular toxic substance and induced a significant DNA damage. This result was coincided with Wu *et al.*[35] who stated that BPA administration induced a significant increase in DNA migration within male germ cells. Also, the result of Ulutas *et al.*[36] reported a significant increase in tail length and tail moment of DNA in rat cells after administration of BPA and concluded that BPA may be genotoxic.

This result could be explained by[37,38] as they indicated that the mechanism of BPA genotoxicity may work through the induction of oxidative stress and the depletion of antioxidant enzymes. Scientific evidence supports the hypothesis that natural estrogens, synthetic estrogen diethylstilbestrol, as well as BPA generate reactive oxygen species (ROS) during biotransformation and that certain reactive species, predominantly quinones, can react with DNA and cause DNA damage. In addition, Sangai *et al.*[39] stated that BPA produces spermatotoxic effect through induced alteration in testicular DNA and sperm chromatin structure.

The present study revealed a significant decrease in DNA damage of spermatocyte in rats administrated BPA+ black seed oil compared to BPA group. These results could be explained by Kamarzaman *et al.*[40] who concluded that thymoquinone had significant high antioxidant activity in testicular tissue so protected DNA damage.

The present results of the total content of DNA, RNA and Protein in the spermatocyte of adult albino rats, showed a significant decrease in total content of DNA, RNA and Protein of rats administered (BPA) as compared to control.

These results were coincided with those of El-Beshbishy *et al.*[41] who reported that rats administered BPA orally, resulted in decreasing total testicular protein content. This might be due to the testicular fluid which contains several stimulatory and inhibitory factors that selectively altered the protein secretion, thus the changes in testicular protein level suggested that there was a reduction in the synthetic activity of testes.

Abdel-Wahab[42] added that bisphenol A is converted to bisphenol O-quinone that covalently binds to deoxyguanosine to form DNA adducts. The quinone intermediates of bisphenol A might be the ultimate DNA binding metabolites. This binding might prevent RNA polymerase from transcribing the DNA and can inhibit the formation of mRNA. A failure in mRNA formation can result in an inhibition of protein synthesis.

In the present study there was a significant increase of total content of DNA, RNA and Protein in the spermatocyte of rats administrated black seed oil+

BPA as compared to BPA group. This result could be explained by Juma and Abdulrahman[43] who revealed that Nigella sativa (Ns) increases thyroxin hormone that in turn increases growth hormone secretion affecting on total proteins synthesis in males, due to increased testosterone as an anabolic agent toward promoting the protein synthesis.

Regarding the serum testosterone level, the present study showed significant increase in testosterone level in rats administrated black seed oil compared to control. This observed result might be found explanation by Marbat *et al.*[27] as they found significant increase in serum testosterone in infertile men after 3 months administration of N. sativa. The increased testosterone level after N. sativa may be attributed to enhancing FSH and LH release, which leads to improvement in all semen parameters.

Oral exposure of rats to BPA induced a significant reduction in serum testosterone level as compared to control. Cha *et al.*[44] reported that the testosterone level in the painters decreased significantly, as the BPA exposure level increased. This result could be explained by Nakamura *et al.*[45] as they demonstrated that BPA and 17β-estradiol (E2) dose dependently decreased the expressions of steroidogenic acute regulatory protein (StAR), and steroidogenic enzymes such a P450 side-chain cleavage enzyme s (P450scc) and 17β- hydroxysteroid dehydrogenase (17β-HSD-mRNA).

An improvement of the serum testosterone level was observed in the present study in group IV compared to BPA rats. This result explained by Al-Seeni *et al.*[46] who concluded that the protective effect of nigella sativa oil on the testis might be due to its direct cytoprotective effect and/ or indirect antioxidant (potent superoxide anion scavenger) and androgen like activities.

Concerning the results of serum antioxidant enzyme SOD in the present study, BPA caused a significant increase in mean value of SOD of group III as compared to control. This result was in accordance with Kourouma *et al.*[47] as they stated a significant increase in SOD in BPA rats compared to control. The increased activity of SOD may be due to higher enzyme activity but doesn't mean better anti-oxidative protection of spermatozoa. This increase may be due to its induction by increased production of superoxide (O- 2), which has been implicated in cell dysfunction. In addition, increased superoxide activity has been shown to play an important part in the pathogenesis of different genetic and acquired forms of hypertension in experimental animals.

A significant decreased serum level of SOD after oral administration of BPA+ black seed oil as compared to BPA rats. Danladi *et al.*[48] who revealed that N. sativa treatment positively protects the alterations in biochemical variables SOD in the Carbon tetrachloride CCl4 + N. sativa-treated rats.

The present study demonstrated a significant decrease in catalase enzyme of BPA group as compared to control. Such results are matching with previous data, reported by Aboul Ezz *et al.*[11] who concluded that oral administration of BPA decreased catalase activity.

In the present study there was a significant improvement of serum level of catalase enzyme in rats administered BPA + black seed oil as compared to BPA rats. These data are in harmony with Al-Malki and Sayed[49] who stated that rats administered Cisplatin (CP) markedly reduced the catalase activity compared to the control while, administration of Thymoquinone (TQ) increased the activities. Mosbah *et al.*[50] reported that black seed oil exhibits cerebral, renal, liver and cardiac protective effect against many xenobiotics through its antioxidant action and ability to boost antioxidant enzymes activities in animals.

CONCLUSION

It could be concluded that

- Oral administration of BPA to adult male rats elicited reproductive toxicity and inducing a significant decrease in sperm count, serum testosterone level and antioxidant defense system.
- BPA showed an increase in head and tail abnormalities, impaired synthesis of DNA, RNA and protein in testicular tissue and significant DNA damage.
- The genetic DNA damage may be an initiation to multistep carcinogenesis later in life.
- Black seed oil induces favorable effects on reproductive system in BPA rats, and the beneficial effects may be attributable to its anti–oxidative and androgenic effects.

RECOMMENDATIONS

1. Using plastic products containing BPA should be avoided.
2. It is favorable to use the natural antioxidants (Black seed oil) to avoid the possible toxicity induced by BPA chemical compounds.
3. Further investigations are required to determine the affection of BPA on other systems of the human body.
4. Another study to demonstrate the effect of BPA administration on the pregnant female and offspring rats exposed during gestation and lactation.

FINANCIAL SUPPORT

There is no funding for this study.

CONFLICTS OF INTEREST

There are no conflicts of interest.

REFERENCES

1. Ritter S. Debating BPA‟s toxicity. Chem Eng News 2011; 89 (23):5–13.
2. Karnam SS, Ghosh RC, Mondal S and Mondal M. Evaluation of subacute bisphenol–A toxicity on male reproductive system. Veterinary world, 2015, 8.6: 738.
3. Jalal N, Surendranath AR, Pathak JL, Yu S, and Chung CY. Bisphenol A (BPA) the mighty and the mutagenic. Toxicol Rep. 2018; 5: 76–84.
4. Almeida S, Raposo A, Almeida-Gonz´alez M, and Carrascos C. Food Exposure and Impact on Human Health. Comprehensive Reviews in Food Science and Food Safety, 2018, 17.6: 15031517- Institute of Food Technologists®
5. Gassman NR. Induction of oxidative stress by bisphenol A and its pleiotropic effects. Environmental and molecular mutagenesis, 2017, 58.2: 6071-.
6. Randhawa MA, Alghamdi MS and Maulik SK. The effect of thymoquinone, an active component of Nigella sativa, on isoproterenol induced myocardial injury. Pak J Pharm Sci, 2013, 26.6: 12159-. https://www.ncbi.nlm.nih.gov › pubmed
7. Tavakkoli A, Ahmadi A, Razavi BM, and Hosseinzadeh H. Black seed (Nigella sativa) and its constituent thymoquinone as an antidote or a protective agent against natural or chemical toxicities. Iranian journal of pharmaceutical research: IJPR, 2017, 16.Suppl: 2.
8. Gad SC and Chengelis CP. The rats: Toxicology, pathology and metabolism. Cited in Animal models in toxicology 1992. New York. Basal. Hong Kong. USA. Chapter 1:21164-.
9. Kostomitsopoulos NG and Durasevic SF. The ethical justification for the use of animals in biomedical research. Archives of Biological Sciences, 2010, 62.3: 781787-.
10. Bayrak O, Kaya A, Bayrak R, Turgut FH, Bavbek N, Kanbay M, and Akcay A. Nigella sativa oil for prevention of chronic cyclosporine nephrotoxicity: an experimental model. American journal of nephrology, 2008, 28.3: 517522-.
11. Aboul Ezz HS, Khadrawy YA and Mourad IM. The effect of bisphenol A on some oxidative stress parameters and acetylcholinesterase activity in the heart of male albino rats. Cytotechnology. 2013; 67(1):14555-.
12. Wyrobek AJ, Watchmaker C- and. Gordon L. Sperm morphology testing in mice. Handbook of mutagenicity test procedures, 1984, 2nd Ed. Elsevier, Amsterdam, 739750-
13. Blasiak J, Arabaski M, Kzupa R, Wozniak M, Kasznicki M and Drzewoski J. DNA damage and repair in type 2 diabetes mellitus. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 2004, 554.1:2-304-297.
14. Singh NP, McCoy T, Tice RR and Schneider EL. A simple technique for of low levels of DNA damage in individual cells, Exp. Cell Res. 1988; 175(1):18491-.
15. Klaude M, Eriksson S, Nygren J and Ahnström G. The comet assay: mechanisms and technical considerations. Mutation Research/DNA Repair, 1996, 363.2: 8996-.
16. Blasiak J, Gloc E, Wo´zniak K, Drzewoski J, Zadrozny M, Skorski T, et al. Free radical scavengers can differentially modulate the genotoxicity of amsacrine in normal and cancer cells. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 2003, 535.1: 25- 34.
17. Pears AGE. Histochemistry. Theoretical and applied. Vol. 2: Analytical technology A. G. E. Pearse. Fourth edition. Churchill Livingstone, Edinburgh 1985, fourth Edition. Edinburgh, London Mellowric and New York. p4421055-.
18. Dische and Schwartz. Estimation of nucleic acids. In: Glick D (ed). Methods of Biochemical analysis 1954. Vol. I. New York: Inter science Publishers Inc. p: 299. Quoted from: Fahim FA, Esmat AY, Mady EA, Ibrahim EK. (2003): Antitumor activities of iodoacetate and dimethylsulphoxide against solid Ehrlich carcinoma growth in mice. Biol Res. 2003; 36(2):253- 62.
19. Dische. Determination of pentoses. In: Colourick S P and Kaplan NO (eds) Methods in Enzymology 1957, Vol. III. New York: Academic Press Inc p: 88. Quoted from: Fahim FA, Esmat AY, Mady EA, Ibrahim EK. (2003): Antitumor activities of iodoacetate and dimethylsulphoxide against solid Ehrlich carcinoma growth in mice. Biol Res. 2003;36(2):25362-.
20. Gornall AG, Bardawil CJ and David MM. (): Determination of serum proteins by means of the Biuret reagent. J. Biol. Chem. 1949;

177: 751756-. Quoted from: Pyun CW, Han KH, Hong GE and Lee CH. (2013): Effect of curcumin on the increase in hepatic or brain phosphatidylcholine hydroperoxide levels in mice after consumption of excessive alcohol. Biomed Res Int. 2013; 2013:242671.

21. Rajkowski KM, Cittanova N, Urios P and Jayle MF. An enzyme-linked immunoassay of testosterone. Steroids, 1977, 30.1: 129137-.
22. Nishikimi M, Roa NA and Yogi K. Measurement of superoxide dismutase Biochem. Biophys. Res. Common. 1972; 46:849854-. Quoted from: Madkour FF & Abdel-Daim, MM. (2013). Hepatoprotective and Antioxidant Activity of Dunaliella salina in Paracetamol-induced Acute Toxicity in Rats. Indian journal of pharmaceutical sciences, 75(6), 642–648. PMID: 24591738.
23. Aebi H. Catalase in vitro. In: Methods in enzymology. Academic Press, 1984. p. 121126-. Quoted from: Mbarki S, Dhibi S, Bouzenna, H, et al. (2016). Effects of MgCl2 supplementation on blood parameters and kidney injury of rats exposed to CCl4. Open Life Sciences, 11(1), pp. 250258-.
24. Abatan SM and Olayemi M. The Role of Statistical Software in Data Analysis. International Journal of Applied Research and Studies (iJARS) ISSN, 2014, 22789480-.
25. Rahman MS, Kang KH, Arifuzzaman S, Pang WK, Ryu DY, Song WH, et al. Effect of antioxidants on BPA-induced stress on sperm function in a mouse model. Scientific reports, 2019, 9.1: 10584
26. Karimi Z, Mirza Alizadeh A, Ezzati Nazhad Dolatabadi J, and Dehghan P. Nigella sativaand its Derivatives as Food Toxicity Protectant Agents. Advanced pharmaceutical bulletin, 2019, 9.1: 22
27. Marbat MM, Mohmed Abid Ali and Hadi AM. The use of Nigella sativa as a single agent in treatment of male infertility. Tikret Journal of Pharmaceutical Sciences, 2013, 9.1: 1929-.
28. Klaunig JE, Wang Z, Pu X and Zhou S. Oxidative stress and oxidative damage in chemical carcinogenesis. Toxicology and applied pharmacology, 2011, 254.2: 8699-
29. Kadry MS, Tarek KA and Mousa AA. Oxidative and Hormonal Disruptions Underlie Bisphenol A-Induced Testicular Toxicity in Male Rabbits. Int J Bio Food Vet Agri Engin, 2014, 8.11: 11741180-.
30. Hatef A, Zare A, Alavi SM, Habibi HR and Linhart O. Modulations in androgen and estrogen mediating genes and testicular response in male goldfish exposed to bisphenol A. Environmental toxicology and chemistry, 2012, 31.9: 20692077-
31. Kumar TR and Muralidhara. Induction of Oxidative Stress by Organic Hydroperoxides in Testis and Epididymal Sperm of Rats In Vivo. Journal of Andrology, 2007, 28: 41533-41545
32. Tawfeek FK, Ahmed SM and Kakel SJ. Effect of Nigella sativa oil treatment on the sex organs and sperm charactors in rats exposed to hydrogen peroxide. Mesopotamia Journal of Agriculture, 2006, 34.1: 28-
33. Hadi MA, Zaidan HK, Natah TM and Al-Saadi AH. Protective effect of plants extract mixture on sperm abnormalities, testicular and epididymal tissues in diabetic male rats. J Nat Sci Res, 2013, 3.9: 2837-
34. Al-Shdefat RI, Abd-ElAziz MA and Al-Saikhan FI. Genoprotective and genotoxic effects of thymoquinone on doxorubicin-induced damage in isolated human leukocytes. Tropical Journal of Pharmaceutical Research, 2014, 13.12: 20152020-
35. Wu HJ, Liu C, Duan WX, Xu SC, He MD, Chen HC, et al. Melatonin ameliorates bisphenol A-induced DNA damage in the germ cells of adult male rats. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 2013, 752.167-57 :2-
36. Ulutas OK, Yildiz N, Durmaz E, Ahbab MA, Barlas N and Cok I. An in vivo assessment of the genotoxic potential of bisphenol A and 4-tert-octylphenol in rats. Archives of toxicology, 2011, 85.8: 9951001-
37. Meeker JD, Calafat AM and Hauser R. Urinary bisphenol A concentrations in relation to serum thyroid and reproductive hormone levels in men from an infertility clinic. Environmental science & technology, 2009, 44.4: 14581463-
38. Cavalieri EL and Rogan EG. Is bisphenol A a weak carcinogen like the natural estrogens and diethylstilbestrol?. IUBMB life, 2010, 62.10: 746751-
39. Sangai NP, Verma RJ and Trivedi MH. Testing the efficacy of quercetin in mitigating bisphenol A toxicity in liver and kidney of mice. Toxicology and industrial health, 2014, 30.7: 581597-

40. Kamarzaman S, Sha'ban M and Abdul Rahman S. Effects on mouse spermatogenesis and DNA fragmentation following exposure to cyclophosphamide and thymoquinone. Eur Int J Sci Technol, 2013, 2: 119136-

41. El-Beshbishy HA, Aly HAA and El-Shafey M. Lipoic acid mitigates bisphenol A-induced testicular mitochondrial toxicity in rats. Toxicology and industrial health, 2013, 29.10: 875887-

42. Abdel-Wahab WM. Thymoquinone attenuates toxicity and oxidative stress induced by bisphenol A in liver of male rats. Pak J Biol Sci, 2014, 17.11: 115260-

43. Juma FT and Abdulrahman HMA. The effects of Nigella sativa oil administration on some physiological and histological values of reproductive aspects of rats. The Iraqi Journal of Veterinary Medicine, 2011, 35.2: 5260-.

44. Cha BS, Koh SB, Park JH, Eom A, Lee1 KM and Choi HS. Influence of occupational exposure to bisphenol A on the sex hormones of male epoxy resin painters. Molecular & Cellular Toxicology, 2008, 4.3: 230234-

45. Nakamura D, Yanagiba Y, Duan Z, Ito Y, Okamura A, Asaeda N, et al. Bisphenol A may cause testosterone reduction by adversely affecting both testis and pituitary systems similar to estradiol. Toxicology letters, 2010, 194.125-16 :2-

46. Al-Seeni MN, El Rabey HA, Al-Hamed AM, and Zamazami MA. Nigella sativa oil protects against tartrazine toxicity in male rats. Toxicology reports, 2018, 5: 146155-

47. Kourouma A, Peng D, Chao Q, Yaima M, Lopez T, Liu Changjiang L, et al. Bisphenol A induced reactive oxygen species (ROS) in the liver and affect epididymal semen quality in adults Sprague-Dawley rats. Journal of Toxicology and Environmental Health Sciences, 2014, 6.4: 103112-

48. Danladi J, Ahmed SA, Akpulu SP, Owolagba GK, Iduh MU and Mairiga AA. Protective effect of cool extraction of Black Seed (Nigella Sativa) oil against CCl4-Induced oxidative damages in wistar rats testis. IOSR-J Pharm Bio Sci, 2013, 5: 6874-

49. Al-Malki AL and Sayed AAR. Thymoquinone attenuates cisplatin-induced hepatotoxicity via nuclear factor kappa-β. BMC complementary and alternative medicine, 2014, 14.1: 282

50. Mosbah R, Djerrou Z, and Mantovani A. Protective effect of Nigella sativa oil against acetamiprid induced reproductive toxicity in male rats. Drug and chemical toxicology, 2018, 41.2: 206212-.

الملخص العربى

التغييرات الوراثية الخلوية والكيميائية الحيوية التي يسببها ثنائي فينول أ على خصية الفئران البيضاء والوقاية المحتملة لزيت الحبة السوداء

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الخلفية: ثنائي فينول أ،هو مركب كيميائي ذو تأثير استروجيني (له القدرة علي محاكاة هرمون الاستروجين)، هو واحد من أعلى المواد الكيميائية من حيث حجم الإنتاج في العالم والذي يستخدم في صناعة البلاستيك المسمى بولي كربونات، والراتينجات وطلاءِ الطبقة الداخلية التي تغلف العلبِ المستخدمةِ لحفظِ الطعامِ والمشروبات. ثنائي فينول أ معروف أن له تأثيرات سامة على الأنظمة المختلفة في الإنسان والحيوان. وكان الهدف من هذه الدراسة هو تقييم التغيرات الوراثية الخلوية والكيميائية الحيوية التي يسببها بيسفينول أ على خصية الفئران البيضاء والتأثير الوقائي المحتمل لزيت الحبة السوداء.

المواد والطرق: أجريت هذه الدراسة على (٨٠ فأر)، مقسمة إلى أربع مجموعات (٢٠ فأر) لكل منهم. المجموعة الأولى: الضابطة سلبية، المجموعة الثانية: تم إعطاؤها زيت الحبة السوداء (٢ مل / كجم من وزن الجسم)، المجموعة الثالثة: تم إعطاؤها ثنائي فينول أ (٢٥ مغ / كجم من وزن الجسم)، المجموعة الرابعة تم إعطاؤها ثنائي فينول أ + زيت الحبة السوداء مماثل للجرعات السابقة. تم جمع الدم والخصية والبربخ للتقييم الكيميائي والحيوي.

النتائج: تسبب ثنائي فينول أ في انخفاض كبير في عدد الحيوانات المنوية، والمحتوى الكلي للحمض النووي، الحمض النووي الريبي والبروتين في الخصية، كما تسبب في زيادة كبيرة في تلف الحمض النووي وتشوهات الرأس والذيل في الحيوانات المنوية، مقارنة مع المجموعة الضابطة. بينما لوحظ تحسن كبير في عدد الحيوانات المنوية، والمحتوى الكلي للحمض النووي، والحمض النووي الريبي والبروتين مع انخفاض كبير في تلف الحمض النووي من الحيوانات المنوية وتشوهات الرأس والذيل في المجموعة التي أعطيت ثنائي فينول أ + زيت الحبة السوداء مقارنة مع المجموعة التي أعطيت ثنائي فينول أ فقط. أيضًا، أظهرت مجموعة ثنائي فينول أ انخفاضًا كبيرًا في مستويات هرمون التستوستيرون والكاتالاز في الدم، في حين أن هناك زيادة كبيرة في سوبر أكسيد ديسموتاز (SOD)مقارنةً بالمجموعة الضابطة. ومع ذلك، أظهرت مجموعة زيت الحبة السوداء+ ثنائي فينول أ تحسنا ملحوظا في مستويات هرمون التستوستيرون والكاتلاز و سوبر أكسيد ديسموتاز(SOD) في الدم، مقارنة مع مجموعة ثنائي فينول أ.

االاستنتاجات: أدى تعرض الفئران إلى ثنائي فينول أ إلى حدوث سمية تناسلية من خلال إحداث تلف كبير في الحمض النووي وضعف تخليق الحمض النووي، الحمض النووي الريبي والبروتين في أنسجة الخصية. يخفف زيت الحبة السوداء الضرر التأكسدي في خصية الفئران عن طريق تنظيم أنشطة مضادات الأكسدة الإنزيمية.