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*World J Hepatol* 2022 March 27; 14(3): 482-646



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The *WJH* is now abstracted and indexed in PubMed, PubMed Central, Emerging Sources Citation Index (Web of Science), Scopus, Reference Citation Analysis, China National Knowledge Infrastructure, China Science and Technology Journal Database, and Superstar Journals Database. The 2021 edition of Journal Citation Reports® cites the 2020 Journal Citation Indicator (JCI) for *WJH* as 0.61. The *WJH*'s CiteScore for 2020 is 5.6 and Scopus CiteScore rank 2020: Hepatology is 24/62.

**RESPONSIBLE EDITORS FOR THIS ISSUE**

Production Editor: Rui-Rui Wu, Production Department Director: Xiang Li, Editorial Office Director: Xiang Li.

**NAME OF JOURNAL**

*World Journal of Hepatology*

**ISSN**

ISSN 1948-5182 (online)

**LAUNCH DATE**

October 31, 2009

**FREQUENCY**

Monthly

**EDITORS-IN-CHIEF**

Nikolaos Pylsopoulos, Ke-Qin Hu, Koo Jeong Kang

**EDITORIAL BOARD MEMBERS**

<https://www.wjgnet.com/1948-5182/editorialboard.htm>

**PUBLICATION DATE**

March 27, 2022

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<https://www.wjgnet.com/bpg/gerinfo/242>

**STEPS FOR SUBMITTING MANUSCRIPTS**

<https://www.wjgnet.com/bpg/GerInfo/239>

**ONLINE SUBMISSION**

<https://www.f6publishing.com>

## Basic Study

**Fertaric acid amends bisphenol A-induced toxicity, DNA breakdown, and histopathological changes in the liver, kidney, and testis**

Khaled Mohamed Mohamed Koriem

**Specialty type:** Gastroenterology and hepatology**Provenance and peer review:** Invited article; Externally peer reviewed.**Peer-review model:** Single blind**Peer-review report's scientific quality classification**

Grade A (Excellent): 0

Grade B (Very good): B

Grade C (Good): C

Grade D (Fair): 0

Grade E (Poor): 0

**P-Reviewer:** Pallottini V, Su XJ**Received:** September 27, 2021**Peer-review started:** September 29, 2021**First decision:** November 7, 2021**Revised:** November 10, 2021**Accepted:** February 23, 2022**Article in press:** February 23, 2022**Published online:** March 27, 2022**Khaled Mohamed Mohamed Koriem**, Department of Medical Physiology, National Research Centre, Giza 12622, Egypt**Corresponding author:** Khaled Mohamed Mohamed Koriem, PhD, Professor, Department of Medical Physiology, National Research Centre, 33 El-Buhouth Street, Dokki, Giza 12622, Egypt. [kkoriem@yahoo.com](mailto:kkoriem@yahoo.com)**Abstract****BACKGROUND**

Bisphenol A (BPA) is present in many plastic products and food packaging. On the other hand, fertaric acid (FA) is a hydroxycinnamic acid.

**AIM**

To investigate the effect of FA on BPA-related liver, kidney, and testis toxicity, DNA breakdown, and histopathology in male rats.

**METHODS**

Thirty male albino rats were divided into five equal groups (6 rats/group): Control, paraffin oil, FA-, BPA-, and FA + BPA-treated groups. The control and paraffin oil groups were administered orally with 1 mL distilled water and 1 mL paraffin oil, respectively. The FA-, BPA-, and FA+ BPA-treated groups were administered orally with FA (45 mg/kg, bw) dissolved in 1 mL distilled water, BPA (4 mg/kg, bw) dissolved in 1 mL paraffin oil, and FA (45 mg/kg, bw) followed by BPA (4 mg/kg, bw), respectively. All these treatments were given once a day for 6 wk.

**RESULTS**

BPA induced a significant decrease in serum alkaline phosphatase, acid phosphatase, sodium, potassium and chloride, testosterone, dehydroepiandrosterone sulfate, glucose-6-phosphate dehydrogenase, 3 $\beta$ -hydroxysteroid dehydrogenase, and testis protein levels but a highly significant increase in serum aspartate aminotransferase, alanine aminotransferase,  $\gamma$ -glutamyl transpeptidase, lactate dehydrogenase, bilirubin, urea, creatinine, uric acid, luteinizing hormone, follicle stimulating hormone, sex hormone binding globulin, blood urea nitrogen, and testis cholesterol levels. Also, FA inhibited the degradation of liver, kidney, and testis DNA content. Oral administration of FA to BPA-treated rats restored all the above parameters to normal levels.

**CONCLUSION**

FA ameliorates BPA-induced liver, kidney, and testis toxicity, DNA breakdown, and histopathological changes.

**Key Words:** Bisphenol A; Fertaric acid; Liver; Kidney; Testis; Toxicity; DNA

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**Core Tip:** BPA induced a significant decrease in serum alkaline phosphatase, acid phosphatase, sodium, potassium and chloride, testosterone, dehydroepiandrosterone sulfate, glucose-6-phosphate dehydrogenase, 3 $\beta$ -hydroxysteroid dehydrogenase, and testis protein levels but a highly significant increase in serum aspartate aminotransferase, alanine aminotransferase,  $\gamma$ -glutamyl transpeptidase, lactate dehydrogenase, bilirubin, urea, creatinine, uric acid, luteinizing hormone, follicle stimulating hormone, sex hormone binding globulin, blood urea nitrogen, and testis cholesterol levels. Also, FA inhibited DNA degradation in the liver, kidney, and testis. Oral administration of FA to BPA-treated rats restored all the above parameters to normal levels. Therefore, FA ameliorates BPA-induced liver, kidney, and testis toxicity, DNA breakdown, and histopathological changes.

**Citation:** Koriem KMM. Fertaric acid amends bisphenol A-induced toxicity, DNA breakdown, and histopathological changes in the liver, kidney, and testis. *World J Hepatol* 2022; 14(3): 535-550

**URL:** <https://www.wjgnet.com/1948-5182/full/v14/i3/535.htm>

**DOI:** <https://dx.doi.org/10.4254/wjh.v14.i3.535>

**INTRODUCTION**

Industrial pollutants such as bisphenol A (BPA), octylphenols, and nonylphenols are known as endocrine-disrupting compounds[1]. BPA is present in many consumer plastic products, food packaging, and in the dentistry for the manufacturing of resin materials[2]. The burning of dumped waste in an open air transfers BPA from plastic waste into the environment. The human and animal exposure to BPA is rapid and continuous[3]. The world production of BPA was 1 million tons in the 1980s[4], which increased to more than 2.2 million tons in 2009[5] and became 3.6 million tons of BPA-derived chemicals in 2015[6]. BPA is released into the surrounding environment by pre-consumer and post-consumer leakage. The pre-consumer leakage into the environment is directly from staining manufacturers, coat, and plastics. The post-consumer BPA is from wastewater treatment plants, agriculture irrigation pipes, ocean-borne plastic trash, and papers or materials recycling companies[7]. BPA affects reproduction, growth, and development of aquatic invertebrates, amphibians, reptiles, and fish at lower doses (1 $\mu$ g/L to 1 mg/L)[8]. BPA is a precursor to important plastics such as plastic bottles including baby bottles, water bottles, and food storage containers. BPA is a monomer that is part of polycarbonates and epoxy resins. However, it can improve the properties of other plastics, which is why it is found in many objects. BPA is similar in its structure to estrogen. Therefore, it interacts with estrogen receptors (in the cell membrane and in the cytoplasm/nucleus). It plays an important role in cardiovascular physiology and diseases such as hypertension[9]. BPA weakened liver function by increasing alkaline phosphatase, aspartate and alanine aminotransferases, triglyceride, cholesterol, globulin, and total bilirubin levels. BPA caused kidney damage by increasing blood urea nitrogen and serum creatinine levels. Histology study exhibited damages of the liver and kidney. The apoptosis of liver and kidney cells was increased by exposure to BPA[10]. BPA decreased sperm quality and serum testosterone (Ts) level. Exposure to a low dose of BPA (0.2  $\mu$ g/mL) impaired mouse sperm quality by damaging germ cell proliferation, leading to declined male fertility[11]. The dose used in this study (4 mg/kg/d) is not a high dose because the US Environmental Protection Agency (EPA) has calculated its human acceptable daily-intake level, known as the reference dose, by dividing the rodent "lowest effect" level of 50 mg/kg/d by 1000. This calculation is based on the assumption that humans are 10 times more sensitive than rodents to BPA exposure and a sensitive human is 10 times more sensitive than a typical human[12,13]. That is mean oral administration of 4 mg/kg/d in rats = oral administration of 4  $\mu$ g/kg/d in human. Furthermore, BPA has been in use commercially for over 50 years, and workers producing BPA and its products (such as epoxy resins) have been exposed to an average air levels of 10 mg over decades[13], which is equal to double and half the dose used in this research.

Therefore, it becomes a challenging responsibility to find a safe and effective way to overcome the BPA toxicity in regions where BPA is already present in water bottles and food packaging and people are therefore exposed to BPA toxicity day and night. The use of herbal plants in the medicine has been known for a long time and today it has made a comeback in all over the world. This is because of their

minor side effects and good therapeutic effects. A large number of secondary metabolites derived from natural sources are currently undergoing evaluation in clinical trials. Fertaric acid (FA) is a hydroxycinnamic acid found in grapefruit[14]. It is formed by the binding of ferulic acid with tartaric acid. FA publications are very rare. Maier *et al*[15] developed a method for the isolation of FA as well as caftaric and coumaric acids from grape pomace. The purities of FA, caftaric acid, and coumaric acid were 90.4%, 97.0%, and 97.2%, respectively. Moreover, Korier and Arbid[16] proved that FA ameliorated liver function, antioxidants, and inflammatory cytokines in the 4-tert-octylphenol-induced toxicity. In addition, Wetchakul *et al*[17] stated that Thai traditional preparation (Jatu-Phala-Tiga [JPT]; FA is a major constituent in JPT) exhibited strong antioxidant activities. Thus, FA is a promising agent for anti-aging and oxidative stress prevention. Furthermore, Lukić *et al*[18] used liquid chromatography with mass spectrometry method to determine FA in 173 wines made from 4 red and 6 white grape varieties. Moreover, Abdallah *et al*[19] isolated FA with a protective effect in ameliorating liver function and antioxidants in t-BHP-induced HepG2 hepatic carcinoma cells. Additionally, FA occurs in vine seeds (*Vitis vinifera* L.) and it has antioxidant activity. FA is among 14 antioxidant components in grape seeds [20].

The aim of this study was to investigate the protective effect of FA in ameliorating oral BPA-induced toxicity, DNA breakdown, and histopathological changes in liver, kidney, and testis tissues in male rats.

## MATERIALS AND METHODS

### Materials

The kits used for the detection of liver function were obtained from Stanbio Laboratory, United States. The kidney function and serum electrolytes (sodium, potassium, and chloride) were measured with analytical kits from Bio-Diagnostics, United Kingdom. Testosterone, luteinizing hormone (LH), follicle stimulating hormone (FSH), and dehydroepiandrosterone sulfate (DHEA-SO<sub>4</sub>) kits were purchased from BioSource Co., Nivelles, Belgium. The sex hormone binding globulin (SHBG),  $\gamma$ -glutamyl transpeptidase ( $\gamma$ -GT), glucose-6-phosphate dehydrogenase (G6PD), and 3 $\beta$ -hydroxysteroid dehydrogenase (3 $\beta$ HSD) kits were obtained from IBL Co., Hamburg, Germany. BPA (purity = 99%) was obtained from Sigma-Aldrich, United States, while FA (purity = 98.2%) was purchased from Riven International PVT, LTD, India.

### Animals

The animal house of the National Research Centre (NRC), Giza, Egypt provided the necessary animals for this study. This study included male albino adult rats of *Spargue Dawley* strains (10 wk old, 120  $\pm$  10g). These rats were preserved in plastic polycarbonate (without bisphenol A) cages [special cages were manufactured without PBA in Faculty of Agriculture, Cairo University, Giza, Egypt]. The rats were maintained with ordinary food and tap water. This research was started after the approval form was received from the ethical committee of NRC, Giza, Egypt and in accordance with the regulations for the suitable care and use of laboratory animals (NIH Publication No 85:23, revised 1985). The experimental conditions included 12 h light and 12 h dark cycle, laboratory temperature of 27-30 °C, and experimental room humidity of 40%-70%.

### Experimental design

Thirty male albino rats were divided into six equal groups (6 rats/ group) as follows: Control, paraffin oil, FA-, BPA-, and FA + BPA-treated groups. The control group was administered orally with 1 mL of distilled water once a day for 6 wk. The paraffin oil group was administered orally with 1 mL of paraffin oil once a day for 6 wk. Paraffin oil was chosen because this oil had no antioxidant activity in contrast to corn oil, olive oil, and safflower oil which contain vitamin E with an antioxidant effect. The FA-treated group was administered orally with FA (45 mg/kg body weight [bw])[16] dissolved in 1 mL of distilled water once a day for 6 wk. The BPA-treated group was administered orally with BPA (4 mg/kg, bw) [21] dissolved in 1 mL of paraffin oil once a day for 6 wk. The 4 mg/kg of BPA is equivalent to 10% of the LD<sub>50</sub> of BPA; the median lethal dose (LD<sub>50</sub>) of BPA is 40 mg/kg[22] and 10% of the LD<sub>50</sub> is a safe dose[23,24]. The FA+ BPA-treated group was initially orally administered with FA (45 mg/kg, bw) dissolved in 1 mL of distilled water. After 1 h, the rats were administered orally with BPA (4 mg/kg, bw) dissolved in 1 mL of paraffin oil. Both FA and BPA were administered orally once a day for 6 wk.

The animals were observed daily for any clinical symptoms or animal death. During the experimental period, the food ingestion, water drinking, and body weight were calculated and recorded daily until the end of this study.

### Determination of urine volume

The urine volume was determined according to the method of Kau *et al*[25], with minor modifications where urine of each rat was collected daily throughout the whole experiment and urine volume was calculated.

**Blood sampling and handling**

After 6 wk of the research, the blood samples were collected from the retro-orbital plexus of the animals. Then, the blood samples were transferred to capillary tubes. After the coagulation of the blood samples, the samples were centrifuged at 4000 rpm for 15 min to obtain the serum. These serum samples were stored at -80 °C for detection of liver and kidney function and male sex hormones.

**Liver, kidney, and testicular tissue preparation**

The next step following blood collection was the execution of the animals by cervical dislocation in this study. Liver, kidney, and testis tissues were collected from each group for histological and genetic analyses. Briefly, liver, kidney, and testis organs were taken and washed with saline solution. The filter papers were used to obtain dry liver, kidney, and testis organs. These organs were homogenized in a homogenizer apparatus for 30 min and the resulting liver, kidney, and testis homogenates were stored at -80 °C for the detection of liver, kidney, and testis DNA.

**Biochemical investigation**

Serum transaminases (AST and ALT) were determined according to Reitman and Frankel[26]. Serum alkaline phosphatase (ALP) and acid phosphatase (ACP) were determined as described by Kind and King[27]. Serum  $\gamma$ -glutamyl transferase ( $\gamma$ -GT) activity was measured according to the method of Szasz [28]. Serum lactate dehydrogenase (LDH) activity was estimated according to the method of Weisshaar *et al*[29]. Serum total bilirubin determination was performed according to the method of Walter and Gerard[30]. Serum urea was calculated according to the method of Patton and Crouch[31]. Serum creatinine was determined by the kinetic method as described by Houot[32]. Serum uric acid was measured according to the method of Kabasakalian *et al*[33]. Blood urea nitrogen was estimated according to the method of Zhu *et al*[34]. Serum electrolytes (sodium, potassium, and chloride) were analyzed colorimetrically according to the methods of Jooste and Strydom[35], Wang *et al*[36], and Hassan *et al*[37], respectively. Urinary and testicular proteins were determined according to the method of Gornall *et al*[38]. Urinary albumin was measured using the method of Drupt[39]. Serum Ts was determined according to the method of Maruyama *et al*[40]. Serum LH was calculated using the method of Knobil[41]. Serum FSH was estimated according to the method of Odell *et al*[42]. Serum DHEA-SO<sub>4</sub> was obtained according to the method of De-Peretti and Forest[43]. Serum SHBG was evaluated according to the method of Selby[44]. Testicular G6PD was determined according to the method of Chan *et al*[45]. Testicular  $\beta$ HSD was calculated using the method of Talalay[46]. Testicular cholesterol level was estimated according to the method of Kim and Goldner[47].

**Determination of DNA content in liver, kidney, and testis**

Feulgen-stained slides were prepared for the nuclear DNA analysis using the Leica Qwin 500 Image Analyzer (LEICA Imaging Systems Ltd, Cambridge, UK). The system was calibrated before each measurement session using the calibration slides provided with the system at high power magnification (400 $\times$ ). The optical density of the selected nuclei in each microscopic field was measured and automatically converted by the system into DNA content. The DNA fields were selected by the desired number of nuclei (100-150). The results are presented as a frequency histogram on the monitor by plotting the DNA content against the number of nuclei calculated. The DNA histograms were divided according to Danqu *et al*[48], Darzynkiewicz *et al*[49], Darzynkiewicz *et al*[50], and El-Gamal[51] into: (1) Diploid (DNA index ranging from 0.9-1.1), (2) tetraploid (DNA index ranging from 1.8-2.2), and (3) aneuploid (when at least 10% of the total events showed distinct abnormal peak outside the 2c or 4c) based on the amount of DNA related to the normal control. Liver, left kidney, and left testis tissues were used in DNA determination.

**Histopathological investigation**

The liver, right kidney, and right testis tissues were fixed in 10% formalin solution and then processed for routine technique by embedding in paraffin. The tissue blocks were sectioned (5  $\mu$ m thick) and then stained with hematoxylin and eosin for histopathological examination under a light microscope.

**Statistical analysis**

The results obtained are expressed as the mean  $\pm$  standard deviations (SD). Data distribution was tested by the Kolmogorov-Smirnov test. Statistical analyses were calculated through one-way analysis of variance (ANOVA) using SPSS program, followed by a *post-hoc* test using Tukey's analysis. A *P* value  $\leq$  0.05 was considered statistically significant.

## RESULTS

### ***Protective effect of FA on body weight, food and water intake, urine volume, and urinary protein, albumin, and albumin/protein ratio in rats exposed to BPA***

The effect of FA on body weight, food and water intake, urine volume, and urinary protein and albumin in the BPA-treated group is shown in [Table 1](#). BPA induced significant decrease in body weight, food intake, and water consumption while causing a significant increase in urinary volume, protein, albumin, and albumin/globulin ratio compared to the control group. On the other side, FA oral administration with BPA administration increased body weight, food intake, and water consumption, but decreased urinary volume, protein, albumin, and albumin/globulin ratio in the BPA-treated group to approach the control levels. Furthermore, paraffin oil and FA oral administration showed an insignificant impact on body weight, food intake and water consumption, urinary volume, protein, albumin, and albumin/globulin ratio compared to the control group. There was not any edema, hair loss, death, or other clinical symptoms observed in animals throughout the experimental period of the study.

### ***Protective effect of FA on liver, kidney, and testis toxicity following BPA exposure***

The protective effect of FA on liver toxicity in BPA-treated rats is shown in [Table 2](#). It is clear from the data in this table that the oral administration of distilled water, paraffin oil, and FA in normal rats did not induce any changes in serum AST, ALT, ALP, ACP,  $\gamma$ GT, LDH, and bilirubin levels. On the contrary, the oral administration of BPA caused a highly significant decrease in serum ALP and ACP but a highly significant increase in serum AST, ALT,  $\gamma$ GT, LDH, and bilirubin compared with control rats. Furthermore, the oral administration of FA in BPA-treated rats caused an increase in serum ALP and ACP levels and a decrease in serum AST, ALT,  $\gamma$ GT, LDH, and bilirubin levels compared to these liver parameters in the BPA-treated group.

The protective effect of FA on kidney toxicity and serum electrolytes in BPA-treated rats is shown in [Table 3](#). It is clear from the data in this table that the oral intake of distilled water, paraffin oil, and FA in normal rats did not induce any changes in serum urea, creatinine, uric acid, sodium, potassium, and chloride levels, as well as blood urea nitrogen. On the contrary, the oral administration of BPA caused a highly significant increase in serum urea, creatinine, uric acid, and blood urea nitrogen but a highly significant decrease in serum sodium, potassium, and chloride levels compared with control rats. Furthermore, the oral administration of FA in BPA-treated rats caused a decrease in serum urea, creatinine, uric acid, and blood urea nitrogen levels and an increase in serum sodium, potassium, and chloride levels compared to these kidney parameters in the BPA-treated group.

The protective effect of FA on male sex hormones in BPA-treated rats is shown in [Table 4](#). It is clear from the data in this table that the oral administration of distilled water, paraffin oil, and FA in normal rats did not induce any changes in serum Ts, LH, FSH, DHEA-S, and SHBG, as well as testicular G6PD, 3 $\beta$ HSD, cholesterol, and protein levels. On the contrary, the oral administration of BPA caused a highly significant decrease in serum Ts, DHEA-S, G6PD, 3 $\beta$ HSD, and protein levels but a highly significant increase in serum LH, FSH, SHBG, and cholesterol levels compared with control rats. Furthermore, the oral administration of FA in BPA-treated rats caused an increase in serum Ts, DHEA-S, G6PD, 3 $\beta$ HSD, and protein levels and a decrease in serum LH, FSH, SHBG, and cholesterol levels compared to these testicular parameters in the BPA-treated group.

### ***Protective effect of FA on liver, kidney, and testis DNA content after BPA exposure***

The data presented in [Table 5](#) exhibit the liver content in male rats. It is clear from the data in this table that control rats revealed 65.77% of diploid cells (2c), 11.71% of triploid cells (3c) (medium proliferation index), 0.90% of tetraploid cells (4c), and 0.0% of aneuploid cells (> 5c) (diploid-medium proliferation index). In BPA-treated rats, the liver tissue displayed 22.64% of diploid cells, 9.43% of triploid cells (low proliferation index), 31.13% of tetraploid cells, and 36.79% of aneuploidy cells (aneuploid-low proliferation index). In rats administered with FA before BPA exposure, the liver tissue presented 33.65% of diploid cells, 15.89% of triploid cells (high proliferation index), 40.19% of tetraploid cells, and 10.28% of aneuploid cells (diploid-high proliferation index).

The data presented in [Table 6](#) display the kidney content in male rats. It is clear from the data in this table that control rats demonstrated 72.90% of diploid cells, 14.95% of triploid cells (medium proliferation index), 0.0% of tetraploid cells, and 0.0% of aneuploid cells (diploid-medium proliferation index). In the BPA-treated group, the kidney tissue exhibited 19.81% of diploid cells, 31.13% of triploid cells (high proliferation index), 28.30% of tetraploid cells, and 20.76% of aneuploidy cells (tetraploid-high proliferation index). In rats treated with FA before BPA exposure, the kidney tissue exhibited 57.80% of diploid cells, 29.36% of triploid cells (medium proliferation index), 5.51% of tetraploid cells, and 7.34% of aneuploid cells (diploid-medium proliferation index).

The data presented in [Table 7](#) show the testis content in male rats. It is clear from the data in this table that control rats displayed 66.37% of diploid cells, 12.39% of triploid cells (medium proliferation index), 0.89% of tetraploid cells, and 0.0% of aneuploid cells (diploid-medium proliferation index). In the BPA-treated group, the testis tissue revealed 23.85% of diploid cells, 11.01% of triploid cells (high proliferation index), 27.52% of tetraploid cells, and 37.62% of aneuploidy cells (tetraploid-high proliferation index).

**Table 1 Protective effect of fertaric acid on body weight, food and water intake, urine volume, and urinary protein, albumin, and albumin/protein ratio in rats exposed to bisphenol A**

Parameter	Group				
	Control	Paraffin oil	FA	BPA	FA + BPA
Initial body weight (g)	132.5 ± 14.5	133.1 ± 15.6	131.8 ± 14.2	134.8 ± 13.9	135.0 ± 15.3
Final body weight (g)	185.0 ± 16.8	187.1 ± 17.2	186.3 ± 18.64	87.8 ± 12.5 <sup>d</sup>	183.75 ± 16.7 <sup>b</sup>
Initial food consumption (g/d)	11.5 ± 1.3	11.3 ± 1.0	11.4 ± 1.2	11.7 ± 1.1	11.6 ± 1.4
Final food consumption (g/d)	14.1 ± 1.1	14.3 ± 1.4	14.2 ± 1.3	5.7 ± 1.0 <sup>d</sup>	14.0 ± 1.2 <sup>b</sup>
Initial water intake (mL/d)	12.2 ± 1.5	12.4 ± 1.3	12.1 ± 1.2	12.3 ± 1.4	12.5 ± 1.0 <sup>b</sup>
Final water intake (mL/d)	15.4 ± 1.6	15.2 ± 1.3	15.3 ± 1.0	6.8 ± 0.9 <sup>d</sup>	15.1 ± 1.2 <sup>b</sup>
Urine volume (mL/100 g/8 h)	0.98 ± 0.07	0.96 ± 0.09	0.99 ± 0.08	1.26 ± 0.15 <sup>c</sup>	1.01 ± 0.08 <sup>a</sup>
Urinary protein excretion (g/dL)	4.06 ± 0.24	4.04 ± 0.21	4.07 ± 0.26	5.29 ± 0.19 <sup>c</sup>	4.08 ± 0.25 <sup>a</sup>
Urinary albumin excretion (g/dL)	2.30 ± 1.4	2.28 ± 1.1	2.32 ± 1.3	4.13 ± 1.5 <sup>d</sup>	2.31 ± 1.2 <sup>b</sup>
Urinary albumin/protein excretion ratio	0.57 ± 0.05	0.56 ± 0.03	0.57 ± 0.04	0.78 ± 0.02 <sup>c</sup>	0.57 ± 0.03 <sup>a</sup>

<sup>a</sup> $P \leq 0.05$  compared to bisphenol A (BPA).

<sup>b</sup> $P \leq 0.01$  compared to BPA.

<sup>c</sup> $P \leq 0.05$  compared to control.

<sup>d</sup> $P \leq 0.01$  compared to control. Number of animals = 6 rats/group. Initial body weight, food consumption, and water intake = body weight, food consumption, and water intake at the first day (day 0) of the experiment. Final body weight, food consumption, and water intake = body weight, food consumption, and water intake at the final day of the experiment. Values are expressed as the mean ± SD. FA: Fertaric acid; BPA: Bisphenol A.

**Table 2 Protective effect of fertaric acid on liver toxicity in rats exposed to bisphenol A**

Parameter	Group				
	Control	Paraffin oil	FA	BPA	FA + BPA
Serum AST (U/L)	121.8 ± 2.64	122.4 ± 3.26	124.0 ± 2.73	175.2 ± 0.72 <sup>d</sup>	130.4 ± 4.17 <sup>b</sup>
Serum ALT (U/L)	60.9 ± 2.37	61.9 ± 2.18	63.1 ± 2.59	86.7 ± 1.96 <sup>d</sup>	65.1 ± 2.9 <sup>b</sup>
Serum ALP (U/100 mL)	14.9 ± 1.27	14.7 ± 1.46	16.2 ± 1.56	5.65 ± 0.82 <sup>d</sup>	13.7 ± 1.22 <sup>b</sup>
Serum ACP (U/100 mL)	17.0 ± 2.79	16.2 ± 2.48	15.8 ± 2.75	6.86 ± 2.01 <sup>d</sup>	12.15 ± 2.34 <sup>b</sup>
Serum $\gamma$ GT (U/L)	8.42 ± 1.32	8.52 ± 1.25	9.90 ± 1.37	13.6 ± 2.30 <sup>d</sup>	10.2 ± 1.57 <sup>b</sup>
Serum LDH (U/L)	261.0 ± 43.6	257.9 ± 35.8	257.4 ± 36.8	755.9 ± 53.17 <sup>d</sup>	277.8 ± 52.8 <sup>b</sup>
Serum bilirubin (mg/dL)	0.53 ± 0.08	0.55 ± 0.09	0.58 ± 0.06	0.82 ± 0.05 <sup>d</sup>	0.54 ± 0.04 <sup>b</sup>

<sup>a</sup> $P \leq 0.05$  compared to bisphenol A (BPA).

<sup>b</sup> $P \leq 0.01$  compared to BPA.

<sup>c</sup> $P \leq 0.05$  compared to control.

<sup>d</sup> $P \leq 0.01$  compared to control. Number of animals = 6 rats/group. Values are expressed as the mean ± SD. FA: Fertaric acid; BPA: Bisphenol A; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; ACP: Acid phosphatase;  $\gamma$ -GT:  $\gamma$ -Glutamyl transferase; LDH: Lactate dehydrogenase.

eration index). In rats treated with FA and then BPA, the testis tissue revealed 36.94% of diploid cells, 22.52% of triploid cells (medium proliferation index), 28.83% of tetraploid cells, and 11.71% of aneuploid cells (diploid-medium proliferation index)].

### **Protective effect of FA on liver, kidney, and testis histopathology after BPA exposure**

Figure 1 exhibits the histology of liver tissue in the control, paraffin oil, and FA, BPA, and FA + BPA-treated groups. It is clear from this figure that in the control, paraffin oil, and FA-treated groups, the hepatocytes were large in size, rounded, and bounded by a distinct nuclear envelope. The structure of the liver in the control, paraffin oil, and FA-treated groups showed normal hepatocytes, vascular sinusoids, and centro-lobular vein (Figure 1A-C). The oral administration of BPA caused rim edema in

**Table 3 Protective effect of fertaric acid on kidney toxicity and serum electrolytes in rats exposed to bisphenol A**

Parameter	Group				
	Control	Paraffin oil	FA	BPA	FA + BPA
Serum urea (mg/dL)	26.5 ± 2.83	27.2 ± 2.54	25.8 ± 2.59	34.0 ± 2.59 <sup>c</sup>	28.2 ± 2.74 <sup>d</sup>
Serum creatinine (mg/dL)	0.75 ± 0.08	0.76 ± 0.06	0.74 ± 0.07	0.98 ± 0.09 <sup>c</sup>	0.76 ± 0.09 <sup>a</sup>
Serum uric acid (mg/dL)	8.21 ± 0.43	8.16 ± 0.52	8.28 ± 0.64	17.65 ± 0.82 <sup>d</sup>	9.16 ± 0.58 <sup>b</sup>
Blood urea nitrogen (mg/dL)	16.25 ± 1.54	16.47 ± 1.39	16.18 ± 1.52	34.42 ± 1.80 <sup>d</sup>	17.18 ± 1.71 <sup>b</sup>
Serum sodium (mmol/L)	154.60 ± 3.29	155.28 ± 3.61	153.81 ± 3.50	110.24 ± 4.16 <sup>d</sup>	144.9 ± 3.28 <sup>a</sup>
Serum potassium (mmol/L)	5.62 ± 0.19	5.71 ± 0.15	5.59 ± 0.24	2.71 ± 0.28 <sup>d</sup>	5.43 ± 0.18 <sup>a</sup>
Serum chloride (mmol/L)	102.83 ± 2.19	103.20 ± 2.34	102.36 ± 2.75	81.47 ± 1.85 <sup>d</sup>	101.26 ± 2.39 <sup>a</sup>

<sup>a</sup>*P* ≤ 0.05 compared to bisphenol A (BPA).<sup>b</sup>*P* ≤ 0.01 compared to BPA.<sup>c</sup>*P* ≤ 0.05 compared to control.<sup>d</sup>*P* ≤ 0.01 compared to control. Number of animals = 6 rats/group. Values are expressed as the mean ± SD. FA: Fertaric acid; BPA: Bisphenol A.**Table 4 Protective effect of fertaric acid on testicular toxicity in rats exposed to bisphenol A**

Parameter	Group				
	Control	Paraffin oil	FA	BPA	FA + BPA
Serum Ts (ng/mL)	5.98 ± 0.42	6.00 ± 0.51	5.96 ± 0.62	3.14 ± 0.49 <sup>c</sup>	5.89 ± 0.68 <sup>a</sup>
Serum LH (mIU/mL)	18.28 ± 1.85	18.31 ± 1.64	17.96 ± 1.93	33.30 ± 2.21 <sup>d</sup>	18.15 ± 5.14 <sup>b</sup>
Serum FSH (mIU/mL)	1.05 ± 0.13	1.03 ± 0.19	0.98 ± 0.15	2.32 ± 0.22 <sup>d</sup>	1.02 ± 0.18 <sup>b</sup>
Serum DHEA-SO <sub>4</sub> (μg/dL)	197.50 ± 23.29	198.25 ± 26.12	195.74 ± 21.84	154.25 ± 13.56 <sup>c</sup>	193.72 ± 19.71 <sup>a</sup>
Serum SHBG (nmol/L)	6.65 ± 0.49	6.67 ± 0.62	6.63 ± 0.51	9.06 ± 1.84 <sup>c</sup>	6.71 ± 4.95 <sup>a</sup>
Testis G6PD (U/g tissue)	11.92 ± 0.66	11.94 ± 0.86	11.89 ± 0.73	5.64 ± 0.43 <sup>d</sup>	10.86 ± 1.52 <sup>b</sup>
Testis 3βHSD (U/g tissue)	4.46 ± 0.86	4.48 ± 0.75	4.43 ± 0.62	2.15 ± 0.36 <sup>d</sup>	4.35 ± 0.88 <sup>b</sup>
Testis Chol (mg/g tissue)	130.67 ± 8.16	132.17 ± 6.90	128.86 ± 7.48	192.53 ± 8.44 <sup>d</sup>	129.21 ± 7.51 <sup>b</sup>
Testis protein (mg/g tissue)	290.6 ± 14.23	288.9 ± 16.51	292.19 ± 13.64	187.60 ± 15.18 <sup>d</sup>	289.45 ± 11.59 <sup>b</sup>

<sup>a</sup>*P* ≤ 0.05 compared to bisphenol A (BPA).<sup>b</sup>*P* ≤ 0.01 compared to BPA.<sup>c</sup>*P* ≤ 0.05 compared to control.<sup>d</sup>*P* ≤ 0.01 compared to control. Number of animals = 6 rats/group. Values are expressed as the mean ± SD. FA: Fertaric acid; BPA: Bisphenol A; Ts: Testosterone; LH: Luteinizing hormone; FSH: Follicle stimulating hormone; DHEA-SO<sub>4</sub>: Dehydroepiandrosterone sulfate; SHBG: Sex hormone binding globulin; G6PD: Glucose-6-phosphate dehydrogenase; 3βHSD: 3β-hydroxysteroid dehydrogenase; Chol: Cholesterol.

the periportal area, which compressed the surrounding hepatocytes. Intra-cytoplasm vacuolation was also found after BPA oral administration (Figure 1D). The oral administration of FA in BPA-treated rats resulted in preserved hepatic lobular architecture and normal structure of the hepatocytes and dilated hepatic sinusoids where the hepatocytes were within normal limits and preserved their plate pattern (Figure 1E).

Figure 2 displays the histology of kidney tissue in the control, paraffin oil, and FA, BPA, and FA + BPA-treated groups. It is clear from this figure that in the control, paraffin oil, and FA-treated groups, the glomeruli showed a normal size with normal tubules (Figure 2C). Figure 2D reveals that the BPA-treated group showed widespread coagulated necrosis with dilatation, vacuolar degeneration, epithelial desquamation, and intraluminal cast formation. Figure 2E shows that the FA + BPA-treated group revealed marked improvement in the histological picture which was comparable to that of the control group.

Figure 3 reveals the histology of testis tissue in the control, paraffin oil, and FA, BPA, and FA + BPA-treated groups. It is clear from this figure that in the control, paraffin oil, and FA-treated groups, the testis tissue revealed well-layered seminiferous tubules with germ cells (Figure 3C). In the BPA-treated group, the testis tissue showed disrupted basement membrane and tubular epithelium (Figure 3D). The

**Table 5 Liver DNA content in different groups**

Control				BPA				FA + BPA			
Range	Total cells	% cells	DNA index	Range	Total cells	% cells	DNA index	Range	Total cells	% cells	DNA index
All	111	100.0%	1.000	All	106	100.0%	2.374	All	107	100.0%	1.923
5cER	0	0.0%	-	5cER	39	36.79%	2.948	5cER	11	10.28%	2.815
< 1.5c	24	21.62%	0.679	< 1.5c	0	0.0%	-	< 1.5c	0	0.0%	-
1.5c-2.5c	73	65.77%	1.031	1.5c-2.5c	24	22.64%	1.045	1.5c-2.5c	36	33.65%	1.181
2.5c-3.5c	13	11.71%	1.359	2.5c-3.5c	10	9.43%	1.558	2.5c-3.5c	17	15.89%	1.595
3.5c-4.5c	1	0.90%	1.779	3.5c-4.5c	33	31.13%	2.033	3.5c-4.5c	43	40.19%	2.273

Number of animals = 6 rats/group. The results are presented as a frequency histogram on the monitor generated by plotting the DNA content against the number of nuclei calculated. 2c: Diploid cells containing two copies of DNA; 3c: Proliferation index (S-phase cells containing three strands of DNA); 4c: Tetraploid cells containing four copies of DNA; > 4c: Cells with more than 4c DNA content; < 1.5c: Cells containing less than 1.5c DNA content. FA: Fertaric acid; BPA: Bisphenol A.

**Table 6 Kidney DNA content in different groups**

Control				BPA				FA + BPA			
Range	Total cells	% cells	DNA index	Range	Total cells	% cells	DNA index	Range	Total cells	% cells	DNA index
All	107	100.0%	1.000	All	106	100.0%	1.628	All	109	100.0%	1.136
5cER	0	0.0%	-	5cER	22	20.76%	2.716	5cER	8	7.34%	-
< 1.5c	13	12.15%	0.650	< 1.5c	0	16.04%	0.623	< 1.5c	0	8.26%	0.663
1.5c-2.5c	78	72.90%	0.984	1.5c-2.5c	21	19.81%	0.984	1.5- 2.5c	63	57.80%	1.008
2.5c-3.5c	16	14.95%	1.364	2.5c-3.5c	33	31.13%	1.364	2.5c-3.5c	32	29.36%	1.411
3.5c-4.5c	0	0.0%	-	3.5c-4.5c	30	28.30%	1.988	3.5c-4.5c	6	5.51%	1.842

Number of animals = 6 rats/group. The results are presented as a frequency histogram on the monitor generated by plotting the DNA content against the number of nuclei calculated. 2c: Diploid (cells containing two copies of DNA); 3c: Proliferation index (S-phase cells containing three copies of DNA); 4c: Tetraploid cells containing four copies of DNA; > 4 c: Cells with more than 4c DNA content; < 1.5 c: Cells containing less than 1.5 c DNA content. FA: Fertaric acid; BPA: Bisphenol A.

FA + BPA-treated group (Figure 3E) exhibited normal seminiferous tubules with germ cells.

## DISCUSSION

BPA is an environmental pollutant that belongs to the endocrine disrupting chemicals. BPA is present in many consumer plastic products, such as water bottles and food packaging, and in the dentistry for the manufacturing of resin materials[2]. The burning of dumped waste in an open air transfers BPA from plastic waste into the environment and consequently the human and animal exposure to BPA is rapid and continuous[3]. On the other hand, FA is a hydroxycinnamic acid found in grapefruit.

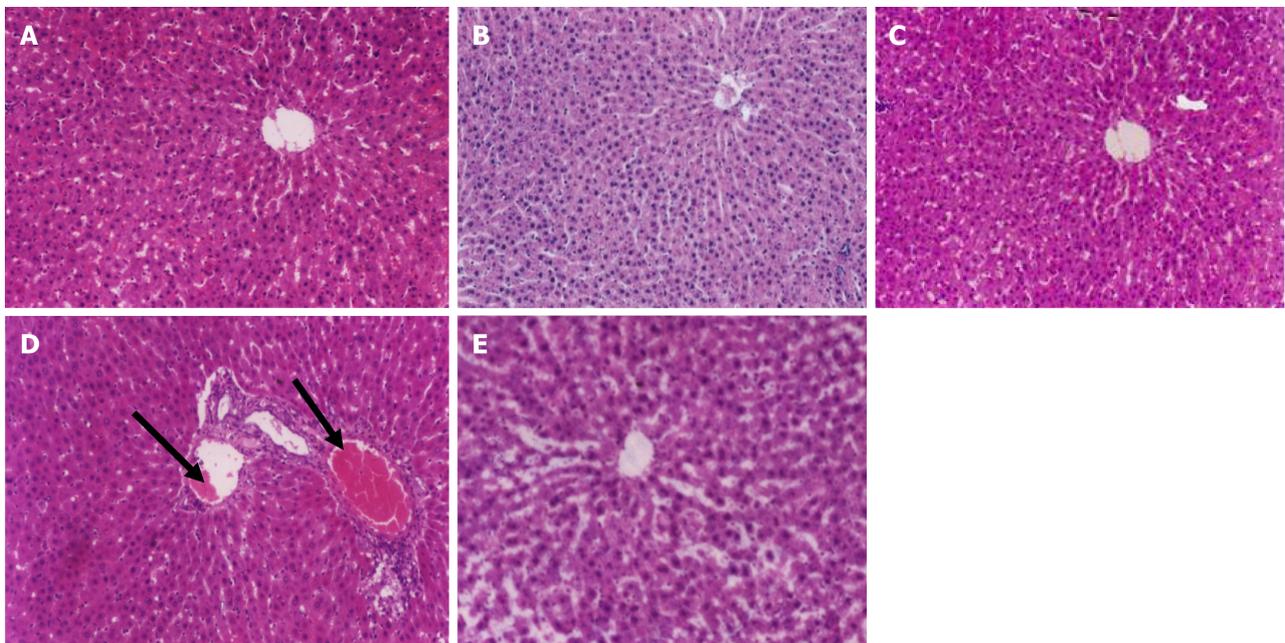
The aim of this study was to evaluate the protective effect of FA on the oral BPA-induced toxicity, DNA breakdown, and histopathological changes of the liver, kidney, and testis induced.

BPA induced a significant decrease in body weight, food intake, and water consumption while causing a significant increase in urinary volume, protein, albumin, and albumin/globulin ratio compared to the control group. On the other side, oral administration of FA increased the body weight, food intake, and water consumption while decreasing urinary volume, protein, albumin, and albumin/globulin ratio in BPA-treated rats to approach the control levels. These results are in agreement with that of Kazemi *et al*[52] who found that oral administration with 5, 25 and 125 µg/kg of BPA for 35 d decreased the body weight of rats and this weight loss was more evident at doses of 25 and 125 µg/kg. On the other hand, oral administration of FA in BPA-treated rats led all the above mentioned parameters to approach the normal levels and these effects are similar to the effect of FA (45

Table 7 Testis DNA content in different groups

Control				BPA				FA + BPA			
Range	Total cells	% cells	DNA index	Range	Total cells	% cells	DNA index	Range	Total cells	% cells	DNA index
All	113	100.0	1.000	All	109	100.0	2.614	All	111	100.0	1.951
5cER	0	0.0	-	5cER	41	37.62	2.953	5cER	13	11.71	2.726
< 1.5c	23	20.35	0.662	< 1.5c	0	0.0	-	< 1.5c	0	0.0	-
1.5c- 2.5c	75	66.37	1.071	1.5c- 2.5c	26	23.85	1.805	1.5c- 2.5c	41	36.94	1.250
2.5c- 3.5c	14	12.39	1.412	2.5c- 3.5c	12	11.01	1.741	2.5c- 3.5c	25	22.52	1.803
3.5c- 4.5c	1	0.89	1.503	3.5c- 4.5c	30	27.52	2.019	3.5c- 4.5c	32	28.83	1.948

Number of animals = 6 rats/group. The results are presented as a frequency histogram on the monitor generated by plotting the DNA content against the number of nuclei calculated. 2c: Diploid cells containing two copies of DNA; 3c: Proliferation index (S-phase cells contained three copies of DNA); 4c: Tetraploid cells containing four copies of DNA; > 4 c: Cells with more than 4c DNA content; < 1.5 c: Cells containing less than 1.5 c DNA content. FA: Fertaric acid; BPA: Bisphenol A.

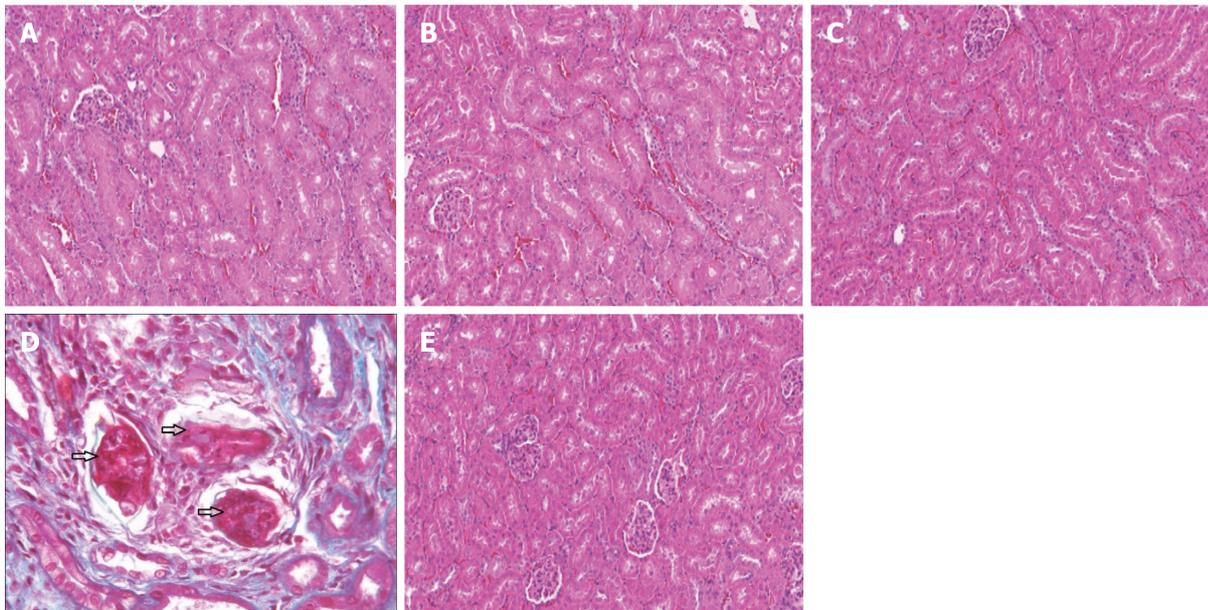


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**Figure 1 Pathological changes in the liver after treatment.** A: Control group; B: Paraffin oil-treated group; C: Fertaric acid (FA)-treated group; D: Bisphenol A (BPA)-treated group; E: FA + BPA-treated group. The control, paraffin oil, and FA-treated rats (A, B, and C; H&E staining, 200 ×) showed a normal hepatic architecture with preserved hepatic architecture. On the contrary, in BPA-treated rats (D), there was rim edema in the periportal area (black arrows) which compressed the surrounding hepatocytes. Intra-cytoplasmic vacuolation was noted. FA + BPA-treated rats (E) had a preserved hepatic lobular architecture.

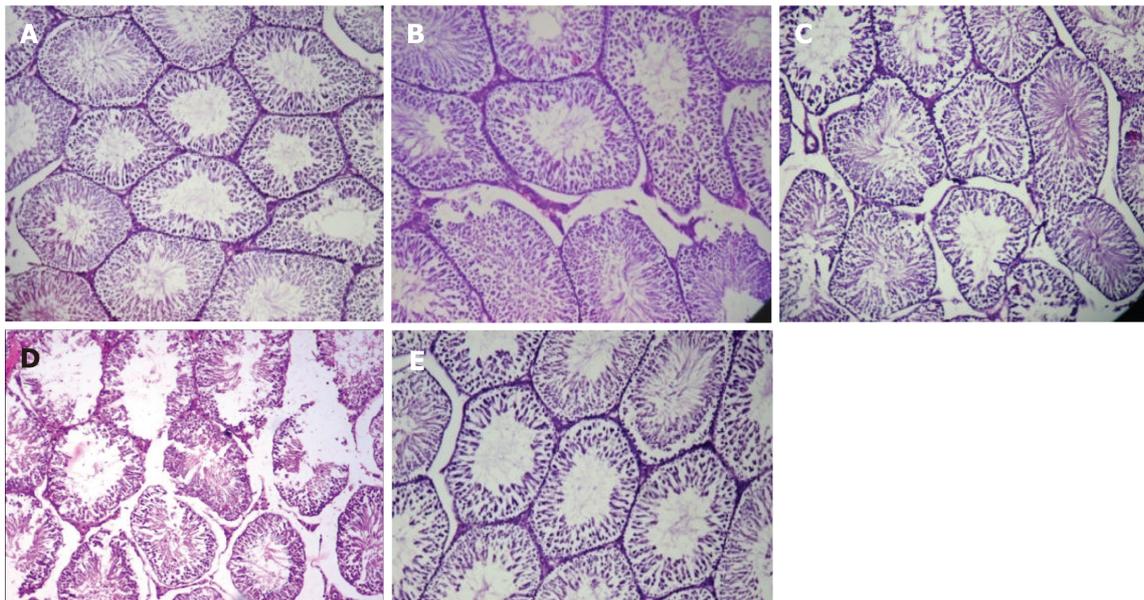
mg/kg) to increase food consumption, water intake, and body weight in endocrine disrupting chemicals exposed rats[16].

The liver is the main site of toxicity disposal or degradation in the human body. Therefore, any changes in the liver transaminases (AST and ALT) are indicators of liver dysfunction[53] and hepatic toxicity[54]. In this study, both AST and ALT activities showed a highly significant increase in BPA-treated rats. Thus, the oral intake of BPA changed the hepatocytes and liver metabolism and liver toxicity occurred. Moreover, all liver enzymes such as serum ALP, ACP  $\gamma$ -GT, LDH, and bilirubin were increased in this study, which indicated hepatic toxicity[55,56]. These observations are in agreement with that of Sun *et al*[57] who found that BPA induced an increase in liver enzymes (AST, ALT, and  $\gamma$ -GT), inflammatory cell infiltration, and hepatocyte necrosis. The authors of that paper[57] used 500 mg/kg BPA, which was higher than the dose of BPA in the present study (4 mg/kg), but Kazemi *et al* [52] used oral doses of 5, 25, and 125  $\mu$ g/kg of BPA (induced liver toxicity in adult rats), which were lower than our dose. Moreover, Sun *et al*[57] found an increase in ALP as a result of liver toxicity after



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**Figure 2 Pathological changes in the kidney after treatment.** A: Control group; B: Paraffin oil-treated group; C: Fertaric acid (FA)-treated group; D: Bisphenol A (BPA)-treated group; E: FA + BPA-treated rats. It is clear from these figures (H&E staining, 200 ×) that control, paraffin oil-, and FA-treated rats showed a normal size of glomeruli with normal tubules (A-C). BPA-treated rats (D) showed widespread coagulated necrosis with dilatation, vacuolar degeneration, epithelial desquamation, and intraluminal cast formation. FA + BPA-treated rats (E) revealed marked improvement in the histological picture which is comparable to that of the control group.



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**Figure 3 Pathological changes of the testis after treatment.** A: Control group; B: Paraffin oil-treated group; C: Fertaric acid (FA)-treated group; D: Bisphenol A (BPA)-treated group; E: FA+BPA-treated group. It is clear from these figures that control, paraffin oil-, and FA-treated rats revealed well-layered seminiferous tubules with germ cells (A-C). In BPA-treated rats, testis tissue showed disrupted basement membrane and tubular epithelium (D). FA + BPA-treated rats (E) exhibited normal seminiferous tubules with germ cells.

BPA oral administration but Kazemi *et al*[52] reported a decrease in ALP level after oral administration of lower doses (5, 25, and 125 µg/kg) of BPA, and these observations are in parallel to our result. Also, Akçay *et al*[58] found that BPA is a reason of liver steatosis, which leads to the formation of metabolic syndrome. Further, Elswefy *et al*[59] found that BPA induced hepatic damage and fibrosis. On the contrary, the oral administration of FA in the BPA-treated group returned all the above mentioned liver function to approach the control levels and this effect was related to the ability of FA to protect the liver against the harmful effects of BPA. Such results are in agreement of that of Korier and Arbid[16] who

proved that FA at a dose of 45 mg/kg ameliorated liver function, antioxidants, and inflammatory cytokines in the endocrine-disrupting chemical 4-tert-octylphenol-induced toxicity. The authors proved that FA ameliorated serum AST, ALT,  $\gamma$ -GT, LDH, ALP, ACP, and bilirubin. Also, Sochorova *et al*[20] found that FA had antioxidant activity, and it therefore quenched BPA-related oxidative stress and increased the antioxidant effect of the cells to fight against the harmful effects of BPA.

The kidney excretes many of waste products produced by metabolism into the urine. These include the nitrogenous wastes urea (from protein catabolism) and uric acid (from nucleic acid metabolism). The kidney participates in human homeostasis, regulating acid-base balance, electrolyte concentrations, extracellular fluid volume, and blood pressure. Therefore, any clinical and diagnostic changes are associated with the changes in kidney function (serum urea, creatinine, and uric acid) as mentioned by Martin and Friedman [55] and Plaa and Hewitt [56]. Thus, the increase in kidney function parameters (serum urea, creatinine, uric acid, and blood urea nitrogen) levels and the decrease in serum electrolytes (sodium, potassium, and chloride) levels in BPA-treated rats suggested an indication of kidney toxicity caused by BPA exposure. Such observation is in accordance with Jiang *et al*[60] who found that BPA induced kidney toxicity in rats after 5 wk of treatment. Also, Esplugas *et al*[61] found that BPA (25  $\mu$ g/kg bw) caused renal and liver damage evidenced by oxidative stress in mice. Furthermore, Ola-Davies and Olukole[62] found that oral administration of BPA at 10 mg/kg for 14 d in male rats increased renal reactive oxygen species and declined the antioxidant system. BPA induced significant increases in serum urea and creatinine in BPA-treated rats. Lesions of the kidney including inflammation, vascular congestion, and erosion of epithelial cells were also observed in BPA-treated rats.

BPA-exposed rats revealed renal dysfunction and histopathological abnormalities, oxidative stress, apoptosis, mitochondrial functional impairment, mitochondrial dynamic changes, and mitophagy disproportion. Sodium, chloride, and potassium are electrolytes that work together to regulate nutrients within the cells and regulate body fluids. Potassium is the main electrolyte in the fluid inside of cells, while sodium is the principal electrolyte in the fluid outside of cells. Chloride is an electrolyte that is important in keeping the suitable amount of fluids inside and outside the cells. The drastic decline of serum sodium, potassium, and chloride electrolytes after BPA exposure in this research was related to BPA exposure-stimulated accumulation of more sodium in the small intestine in male rats[63], which in turn decreased serum sodium and consequently both serum potassium and chloride decreased to keep sodium/potassium pump in normal state and to sustain body homeostasis of electrolytes. On the contrary, the decline in the levels of kidney function parameters and the increase in serum electrolytes in the FA + BPA-treated group indicated the ability of FA to protect the kidney against the harmful effect of BPA. Such observation is in accordance with that of Korierm and Arbid[16] who proved that FA at a dose of 45 mg/kg ameliorated serum and liver antioxidants such as serum and hepatic superoxide dismutase, glutathione peroxidase, and catalase. Also, Sochorova *et al*[20] found that FA had antioxidant activity and therefore quenched BPA-related oxidative stress and increased the antioxidant effect of the cells to fight against BPA-related oxidative stress.

The testis is a male reproductive organ. The function of the testis is to produce both sperm and androgens (Ts). Testosterone is controlled by LH but sperm production is controlled both by and Ts. The testis is well known to be very sensitive to injury, especially from endocrine disturbing chemicals such as BPA. These are because endocrine disturbing chemicals such as BPA can affect the size and function of the testis. The oral administration of BPA in this study caused a decrease in serum Ts, DHEA-S, G6PD,  $3\beta$ HSD, and protein levels but an increase in serum LH, , SHBG, and cholesterol levels. The decrease of Ts is attributed to: (1) The inhibitory effect of BPA on human chronic gonadotropin-stimulated Ts biosynthesis by both cultured rat precursor and immature Leydig cells[64]; or (2) the ability of BPA to convert cholesterol to androstenedione through inhibiting  $17\text{-}\alpha$ -hydroxylase and  $3\beta$ -hydroxysteroid dehydrogenase-isomerase steps[65]. The decrease of  $3\beta$ -hydroxysteroid dehydrogenase activity in this study was accompanied with an increase LH and levels in BPA-treated rats. The increase in LH and levels following BPA exposure is related to: (1) LH-induced Leydig cell secretion of Ts (which participated in the regulation of spermatogenesis by targeting androgen receptors in the germinal epithelium); and (2) targeting of receptors inside Sertoli cell to control spermatogenesis by stimulating many Sertoli cell factors. The decrease of testicular cholesterol and protein in this study was linked to testicular dysfunction. On the contrary, FA oral administration in BPA-treated rats increased the number of Leydig cells, ameliorated Ts levels, and consequently restored testicular function[66]. Such observations are in agreement with that of Korierm and Arbid[16] who proved that FA at a dose of 45 mg/kg ameliorated serum and liver antioxidants as well as inflammatory cytokines in endocrine disturbing chemical-exposed rats. Also, Sochorova *et al*[20] found that FA had antioxidant activity and therefore quenched BPA-related oxidative stress and increased the antioxidant effect of the cells to protect against the harmful effects of BPA.

In this study, content in the liver, kidney, and testis was determined in BPA-treated rats and FA + BPA-treated rats. BPA caused a very high increase in breakdown in these organs. Such observation is in agreement with that of Akram *et al*[67] who found that BPA increased damage in liver, kidney, and brain tissues. The very low concentrations of BPA caused toxic effects *via* affecting the physiological and biochemical parameters in multiple tissues of fish. Also, Panpatil *et al*[68] found that the BPA-treated groups exhibited significantly higher mean levels of damage in the liver and kidney as compared to the untreated control group. Furthermore, Pan *et al*[69] found that BPA declined sperm chromatin integrity

while increased damage in mouse spermatogenic cells. On the contrary, the oral administration of FA in BPA-treated rats resulted in the return of the content in liver, kidney, and testis tissues to the normal diploid level. These observations were recorded due to the antioxidant activity of FA. These results are in accordance with those of Koriem and Arbid[16] and Sochorova *et al*[20] who found that FA had antioxidant activity, which increased the antioxidant activity in the liver, kidney, and testis of BPA-treated rats. These results are in agreement with that of Koriem and Arbid[16] who proved that FA at a dose of 45 mg/kg counteracted the inhibitory action on the gene expression of liver proteins induced by the endocrine-disrupting chemical 4-tert-octylphenol, where FA prevented the degradation of liver, and consequently reformation occurred. Also, Sochorova *et al*[20] found that FA had antioxidant activity and therefore quenched BPA-related oxidative stress and increased the antioxidant effect of the cells to protect against the harmful effects of BPA.

The mechanism sustaining the protective effect of FA against BPA-induced liver, kidney, and testis-related toxicity, DNA breakdown, and histopathological changes depends on the antioxidant effect of FA. Therefore, FA increases serum and tissue superoxide dismutase, glutathione peroxidase, and catalase in BPA-treated rats. This will stop the BPA-related side effects such as liver, kidney, and testicular toxicity, DNA breakdown, and histopathological changes [16,20].

The implication of the results of this research to the human population is that daily oral administration of FA protects against the harmful effect of low-dose exposure to BPA. The significant impact of this research is that FA is available, very cheap, and without any side effects to protect against the toxicity related to daily exposure of babies, children, young, and elderly people to BPA. The FA dose used in this research is very useful to babies, children, and elderly people because these human groups are very susceptible to lower doses of BPA caused by daily exposure to cumulative amounts of BPA doses through foods, drinks, and inhalation.

## CONCLUSION

In conclusion, this study proved that FA can be used as a protective agent in ameliorating the BPA-induced toxicity, DNA breakdown, and histopathology of the liver, kidney, and testis, which suggests the use of this acid in preventing the toxicity of BPA that is present in plastic industry such as water bottles and food packages.

## ARTICLE HIGHLIGHTS

### Research background

Bisphenol A (BPA) is present in many plastic products and food packaging. On the other hand, fertaric acid (FA) is a hydroxycinnamic acid.

### Research motivation

It is a challenging responsibility to find a safe and effective way to overcome the toxicity of BPA toxicity in regions where BPA is already present in water bottles and food packaging and people are therefore exposed to BPA toxicity day and night. The use of herbal plants in the medicine has been known for a long time ago and today it has made a comeback in all over the world. This is because of their minor side effects and good therapeutic effects.

### Research objectives

To investigate the effect of FA on BPA-related liver, kidney, and testis toxicity, DNA breakdown, and histopathological changes in male rats.

### Research methods

Thirty male albino rats were divided into five equal groups (6 rats/group); Control, paraffin oil, FA-, BPA-, and FA + BPA-treated groups. The control and paraffin oil groups were administered orally with 1 mL distilled water and 1 mL paraffin oil, respectively. The FA-, BPA-, and FA+ BPA-treated groups were administered orally with FA (45 mg/kg, bw) dissolved in 1 mL distilled water, BPA (4 mg/kg, bw) dissolved in 1 mL paraffin oil, and FA (45 mg/kg, bw) followed by BPA (4 mg/kg, bw), respectively. All these treatments were given once a day for 6 wk.

### Research results

The results showed that BPA induced a significant decrease in serum alkaline phosphatase, acid phosphatase, sodium, potassium and chloride, testosterone, dehydroepiandrosterone sulfate, glucose-6-phosphate dehydrogenase, 3 $\beta$ -hydroxysteroid dehydrogenase, and testis protein levels but a highly significant increase in serum aspartate aminotransferase, alanine aminotransferase,  $\gamma$ -glutamyl

transpeptidase, lactate dehydrogenase, bilirubin, urea, creatinine, uric acid, luteinizing hormone, follicle stimulating hormone, sex hormone binding globulin, blood urea nitrogen, and testis cholesterol levels. Also, FA inhibited the degradation of liver, kidney, and testis DNA content. Oral administration of FA to BPA-treated rats restored all the above parameters to normal levels.

### Research conclusions

This study for the first time proposed that FA can amend the bisphenol A-induced toxicity, DNA content, and histopathological changes in the liver, kidney, and testis.

### Research perspectives

The direction of the future research is to apply FA in clinical study and it will be interesting to prove that FA can amend the BPA-induced toxicity clinically.

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## FOOTNOTES

**Author contributions:** Korier KMM designed the study, conceived of the manuscript, wrote and edited the first and final versions of the manuscript, conducted the literature search, and read and approved the final manuscript.

**Institutional review board statement:** The study was reviewed and approved by the Institutional Review Board of the National Research Centre (NRC), Giza, Egypt (Approval No. 21831).

**Institutional animal care and use committee statement:** All procedures involving animals were reviewed and approved by the Institutional Animal Care and Use Committee of the National Research Centre (NRC), Giza, Egypt (Approval No. 21831).

**Conflict-of-interest statement:** The authors declare that there are no conflict of interest to disclose.

**Data sharing statement:** No additional data are available.

**ARRIVE guidelines statement:** The authors have read the ARRIVE Guidelines, and the manuscript was prepared and revised according to the ARRIVE Guidelines.

**STROBE statement:** The authors have read the STROBE Statement-checklist of items, and the manuscript was prepared and revised according to the STROBE Statement-checklist of items.

**CONSORT 2010 statement:** The authors have read the CONSORT 2010 statement, and the manuscript was prepared and revised according to the CONSORT 2010 statement.

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**S-Editor:** Wang LL

**L-Editor:** Wang TQ

**P-Editor:** Wang LL

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