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Basic Study

Fertaric acid amends the toxicity, DNA breakdown, and histopathology of liver, kidney, and testis induced by bisphenol A exposure

Fertaric acid in bisphenol exposure

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Abstract

BACKGROUND

Bisphenol A (BPA) presents in many plastic products and food packaging. On the other hand, ¹feraric acid (FA) is a hydroxycinnamic acid.

AIM

This study aims 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 5 equal groups (6 rats/group); Control and paraffin oil groups: oral administration with 1 mL distilled water and 1 mL paraffin oil, respectively. FA-, BPA-, and FA+ BPA-treated groups: oral administration 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) dissolved in 1 mL distilled water then after 1 h these rats orally intake with BPA (4 mg/kg, bw) dissolved in 1 mL paraffin oil, respectively. All these treatments were orally adminstrated once a day for 6 wk.

RESULTS

The results showed that the BPA induced significant decrease in serum alkaline phosphatase, acid phosphatase, sodium, potassium and chloride, testosterone, dehydroepiandrosterone sulfate, glucose-6-phosphate dehydrogenase, 3 β -hydroxysteroid dehydrogenase, and testis protein levels but highly ¹significant increase in serum aspartate aminotransferase, alanine aminotransferase, γ -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 prohibited the degradation of liver, kidney, and testis DNA content. Oral administration with FA to BPA-treated rats restored all the above parameters to normal levels.

CONCLUSION

FA ameliorates liver, kidney, and testis-related toxicity, DNA breakdown, and histopathology in BPA exposure

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

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Core Tip: BPA induced significant decrease in serum alkaline phosphatase, acid phosphatase, sodium, potassium and chloride, testosterone, dehydroepiandrosterone sulfate, glucose-6-phosphate dehydrogenase, 3 β -hydroxysteroid dehydrogenase, and testis protein levels but highly significant increase in serum aspartate aminotransferase, alanine aminotransferase, γ -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 liver, kidney, and testis. Oral intake of FA to BPA-treated rats restored all the above parameters to normal levels. Therefore, FA ameliorates liver, kidney, and testis-related toxicity, DNA breakdown, and histopathology in BPA exposure.

INTRODUCTION

Industrial pollutants such as bisphenol A (BPA), octylphenols, and nonylphenols are known as endocrine-disrupting compounds^[1]. BPA presents 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] increases to more than 2.2 million tons in 2009^[5] and becomes 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 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 BPA doses (1µg/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. So, 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 level. So, low doses of BPA (0.2 µg/mL) exposure declined mice sperm quality by damaging germ cell proliferation, leading to declined male fertility^[11]. The dose used in this study (4 mg/kg/day) 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 (RfD), by dividing the rodent “lowest effect” level of 50 mg/kg/day 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/day in rats= oral administration of 4 µg/kg/day 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 where BPA is already present in water bottles and food packaging therefore human expose to BPA toxicity day and night. The use of herbal plants in the medicine was known for a long time ago and today it has made a comeback in all over the world. This is because of its minor side effects and has well therapeutically performed. ¹ 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 coutaric acids from grape pomace. The purity of FA, ¹ caftaric acid, and coutaric acid were 90.4%, 97.0%, and 97.2%, respectively. Moreover, Koriem and Arbid^[16] proved that FA ameliorated liver function, antioxidants, and inflammatory cytokines in 4-tert-octylphenol toxicity. In addition, Wetchakul *et al*^[17] stated that Thai traditional preparation (Jatu-Phala-Tiga, JPT where FA is a major constituent in JPT) exhibited strong antioxidant activities so 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 determinate FA in 173 wines made from 4 red and 6 white grape varieties. Moreover, Abdallah *et al*^[19] isolated FA with 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 design of this study is to investigate the protective effect of FA to ameliorate the toxicity, DNA breakdown, and histopathology in liver, kidney, and testis tissues in male rats exposed to BPA oral administration.

MATERIALS AND METHODS

Materials

The kits used for the detection of liver function were obtained from Stanbio Laboratory Company, USA. The kidney function and serum electrolytes (sodium, potassium, and chloride) were measured by analytical kits from Bio-diagnostics kits, United Kingdom. Testosterone (Ts), luteinizing hormone (LH), follicle stimulating hormone (FSH), and dehydroepiandrosterone sulfate (DHEA-SO₄) kits were purchased from BioSource Co., Nivelles, Belgium. The sex hormone binding globulin (SHBG), γ -glutamyl transpeptidase (γ -GT), glucose-6-phosphate dehydrogenase (G6PD), and 3 β -hydroxysteroid dehydrogenase (3 β HSD) kits were obtained from IBL Co., Hamburg, Germany. BPA (purity= 99%) was obtained from Sigma-Aldrich, USA 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, 12010 \pm g). 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 sustained on a rat 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 = 27-30°C, and experimental room humidity = 40-70%.

Experimental design

Thirty male albino rats were divided into 6 equal groups (6 rats/ group) as follows; (1) **Control group:** daily oral intake of 1 mL distilled water once a day for 6 wk period. (2) **Paraffin oil group:** daily oral intake of 1 mL paraffin oil once a day for 6 wk. The choose of paraffin oil due to this oil without any antioxidant activity in contrast to corn oil, olive oil, and safflower oil which contains vitamin E with antioxidant effect. (3) **FA-**

treated group: daily oral intake of FA (45 mg/kg bw of FA)^[16] dissolved in 1 mL distilled water once a day for 6 wk. (4) **BPA-treated group:** daily oral intake of BPA (4 mg /kg, bw)^[21] dissolved in 1 mL paraffin oil once a day for 6 wk. The 4 mg/kg of BPA (=10% of the LD₅₀ of BPA where the median lethal dose (LD₅₀) of BPA = 40 mg/kg^[22] and 10% of the LD₅₀ is a safe dose^[23,24]). (5) **FA+ BPA-treated group:** daily oral intake of FA (45 mg/kg, bw,) dissolved in 1 mL distilled water. Then, after 1 h the same rats administrated orally with BPA (4 mg /kg, bw) dissolved in 1 mL paraffin oil. Both FA and BPA were administrated orally once a day for 6 wk

The animals were observed daily for any clinical symptoms or animal death. During the experimental periods, the food ingesting, 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 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, these blood samples were centrifuged at 4000 rpm for 15 minutes to obtain the blood serum. These serums were stored at -80°C for detection of liver and kidney functions and male sex hormones.

Liver, kidney, and testicular tissues 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. Then, 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 homogenated in a homogenizer apparatus for 30 minutes and the resulting liver, kidney, and testis homogenates were stored at -80 °C for the detection of liver, kidney, and testis DNA, as well as, testicular hormones.

These procedures were summarized as follows; 0.5 g of liver, kidney, and testis tissues were dissolved in 2.5 mL of Tris buffer solution. Then, these tissues were homogenated in the homogenizer. Finally, liver, kidney, and testis homogenates were centrifuged for 30 min at 7000 rpm to obtain the supernatant which used for liver, kidney, and testis DNA, as well as, testicular hormones detection.

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 by Kind and King^[27]. Serum γ -glutamyl transferase (γ -GT) activity was done according to 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 Walter and Gerard^[30] method. Serum urea was calculated according to the method of Patton and Crouch^[31]. Serum creatinine was performed by the kinetic method as described by Houot^[32]. Serum uric acid was done 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 of sodium, potassium and chloride levels 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 determined on the method of Drupt^[39]. Serum Ts was determined according to Maruyama *et al*^[40]. Serum LH was calculated depending on the method of Knobil^[41]. Serum FSH was estimated according to Odell *et al*^[42]. Serum DHEA-SO₄ was obtained according to De-Peretti and Forest^[43] method. Serum SHBG was evaluated according to Selby^[44]. Testicular G6PD was determined according to Chan *et al*^[45] method. Testicular 3 β HSD was calculated depending on 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×). 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) has been reached. The results were presented as a frequency histogram on the monitor through 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] to; (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 normal control. Liver, left kidney and left testis were used in DNA determination.

Histopathological investigation

The liver, right kidney, and right testis tissues were fixed at 10% formalin solution and then processed for routine technique by embedding in paraffin. These blocks were sectioned (5 µm thick) and then stained with hematoxylin and eosin stains for histopathological examination under light microscopy.

Statistical analysis

The results obtained were expressed as mean ± standard deviations (SD). Data distribution was tested by the Kolmogorov-Smirnov test. The statistical analysis was calculated through one-way analysis of variance (ANOVA), using SPSS program followed by a *post-hoc* test using Tukey's analysis. The *P* value ≤ 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 BPA exposure rats

The effect of FA on body weight, food and water intake, urine volume, and urinary protein and albumin excretion in BPA-treated group was arranged in table (1). BPA

induced significant decrease in body weight, food intake, and water consumption while causing significant increase in urinary volume, protein, albumin, and albumin/globulin ratio compared to control. On the other side, FA oral intake with BPA administration increased body weight, food intake, and water consumption while decreased urinary volume, protein, albumin, and albumin/globulin ratio in BPA-treated group to approach the control levels. Furthermore, paraffin oil and FA oral administration showed insignificant changes in body weight, food intake, and water consumption, urinary volume, protein, albumin, and albumin/globulin ratio compared to control. There was not any edema or hair loss or death or any other clinical symptoms were observed in animals throughout the experimental period of the study.

Protective effect of FA on liver, kidney, and testis toxicity in BPA exposure

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

The protective effect of FA on kidney toxicity and serum electrolytes in BPA-treated rats displays in table (3). It is clear from the data in this table that the oral intake with distilled water, paraffin oil, and FA to 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 intake with 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 after BPA oral intake compared with control rats. Furthermore, the oral intake with FA to BPA-treated rats caused a decrease in serum 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 BPA-treated group.

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

Protective effect of FA on liver, kidney, and testis DNA content in BPA exposure

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

The data occurs in table (6) displays the DNA content of kidney in male rats. It is clear from the data in this table that control rats demonstrated [72.90% (2C) diploid cells, 14.95% (3C) triploid cells (medium proliferation index), 0.0% (4C) tetraploid cells and 0.0% (>5C) aneuploid cells (diploid-medium proliferation index)]. BPA-treated group, the kidney tissue indicated [19.81% (2C) diploid cells, 31.13% (3C) triploid cells (high

proliferation index), 28.30% (4C) tetraploid cells, and 20.76% (>5C) aneu-ploid cells (tetraploid-high proliferation index)]. In rats received FA before BPA exposure, kidney tissue exhibited [57.80% (2C) diploid cells, 29.36% (3C) triploid cells (medium proliferation index), 5.51% (4C) tetraploid cells, and 7.34 % (>5C) aneuploid cells (diploid-medium proliferation index)].

The data presents in table (7) presents the DNA content of testis tissue of male rats. It is clear from the data in this table that control rats displayed [66.37% (2C) diploid cells, 12.39% (3C) triploid cells (medium proliferation index), 0.89% (4C) tetraploid cells and 0.0% (>5C) aneuploid cells (diploid-medium proliferation index)]. BPA-treated group, the testis tissue detected [23.85% (2C) diploid cells, 11.01% (3C) triploid cells (high proliferation index), 27.52% (4C) tetraploid cells, and 37.62% (>5C) aneu-ploid cells (tetraploid-high proliferation index)]. In rats administrated with FA then received BPA, testis tissue revealed [36.94% (2C) diploid cells, 22.52% (3C) triploid cells (medium proliferation index), 28.83% (4C) tetraploid cells, and 11.71 % (>5C) aneuploid cells (diploid-medium proliferation index)].

Protective effect of FA on liver, kidney, and testis histopathology in BPA exposure

Figure (1) exhibits the histology of liver tissue in control, paraffin oil, and FA, BPA, and FA + BPA-treated groups. It is clear from this figure that in control, paraffin oil, and FA-treated groups the hepatocytes are large in size, rounded, and bounded by a distinct nuclear envelope. The structure of the liver in control, paraffin oil, and FA-treated groups showed normal hepatocytes, vascular sinusoids, and centro-lobular vein (Figures 1A, 1B, and 1C). The oral intake with BPA caused hoop of edema in the periportal area which compressed the surrounding hepatocytes. Intra-cytoplasm vaculation was also found after BPA oral intake (Figure 1D). The oral intake with FA to BPA-treated rats showed preserved hepatic lobular architecture and normal structure of the hepatocytes and dilated hepatic sinusoids where the hepatocytes are within normal limits and preserved its plate pattern (Figure 1E).

Figure (2) displays the histology of kidney tissue in control, paraffin oil, and FA, BPA, and FA + BPA-treated groups. It is clear from this figure that in control, paraffin oil, and

FA-treated groups the glomeruli showed normal size with normal tubules (Figures 2A, 2B, and 2C). Figure (2D) reveals BPA-treated-group showed widespread coagulated necrosis with dilatation, vacuolar degeneration, epithelial desquamation and intraluminal cast formation. Figure (2E) shows FA+BPA-treated group revealed marked improvement in the histological picture which is comparable to that of the control group.

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

DISCUSSION

BPA is an environmental pollutant belongs to the endocrine disrupting chemicals. BPA presents 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 is to evaluate the protective effect of FA on the toxicity, DNA breakdown, and histopathology of liver, kidney, and testis induced by BPA oral administration.

BPA induced significant decrease in body weight, food intake, and water consumption while causing significant increase in urinary volume, protein, albumin, and albumin/globulin ratio compared to control. On the other side, FA oral administration with BPA oral administration increased body weight, food intake, and water consumption while decreased urinary volume, protein, albumin, and albumin/globulin ratio in BPA-treated group to approach the control levels. These results are in

agreement with that of Kazemi *et al*^[52] who found that oral intake with 5, 25 and 125 µg/kg of BPA for 35 days decreased body weight of rats and this weight loss was more evident at doses of 25 and 125 µg/kg. On the other hand, FA oral administration to BPA-treated rats pushed all the above mentioned parameters to approach the normal levels and these effects are similar to the effect of FA (45 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 enzymes 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 with BPA changes the hepatocytes and liver metabolism and liver toxicity occurred. Moreover, all liver enzymes such as serum ALP, ACP γ-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 γ-GT), inflammatory cell infiltration and hepatocyte necrosis. The authors of this paper^[57] used 500 mg/kg BPA which was higher than the dose of BPA in this research (4mg/kg) but Kazemi *et al*^[52] used oral dose of 5, 25 and 125 µg/kg of BPA (induced liver toxicity in adult rats) which was lower than our dose. Moreover, Sun *et al*^[57] found an increase in ALP as a result of liver toxicity after BPA oral administration but Kazemi *et al*^[52] reported a decrease in ALP level after lower doses (5, 25 and 125 µg/kg) oral administration of BPA-related liver toxicity and these observations are in parallel to our result. Also, Akçay *et al*^[58] who found that BPA is a reason of liver steatosis, which lead to the formation of metabolic syndrome. Further, Elswefy *et al*^[59] who found that BPA induced hepatic damage and fibrosis. On the contrary, the oral intake of FA to BPA-treated groups returned all the above mentioned liver function to approach the control levels and this effect related to the ability of FA to protect the liver against BPA harmful effects. Such results are in agreement of that Koriem and Arbid^[16] who proved that FA at a dose of 45 mg/kg ameliorated liver

function, antioxidants and inflammatory cytokines in endocrine-disrupting chemical 4-tert-octylphenol toxicity. The authors proved that FA ameliorated serum AST, ALT, γ -GT, LDH, ALP, ACP, and bilirubin. Also, Sochorova *et al*^[20] who found that FA has antioxidant activity, therefore it quenches BPA-related oxidative stress and increases the antioxidant effect of the cells to fight against BPA-harmful effects.

³ 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 ⁷ 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 are an indication of kidney toxicity caused by BPA exposure. Such observations are in accordance with Jiang *et al*^[60] who found that BPA induced kidney toxicity in rats after 5 wk of BPA treatment. Also, Esplugas *et al*.^[61] who found that BPA (25 μ g/kg bw) caused renal and liver damage evidenced by oxidative stress in mice. Furthermore, Ola-Davies and Olukole^[62] who found ⁶ that orally administered with BPA at 10 mg/kg for 14 days 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 ¹¹ in BPA-treated rats.

BPA-exposed rats revealed ¹¹ renal dysfunction and histopathological abnormalities, oxidative stress, apoptosis, mitochondrial functional impairment, mitochondrial dynamic, and mitophagy disproportion. ⁵ 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 the 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 FA+BPA-treated group indicate the ability of FA to treat kidney organ against harmful effect of BPA. Such observations are in accordance of that Koriem 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] who found that FA has antioxidant activity therefore it quenches BPA-related oxidative stress and increases the antioxidant effect of the cells to fight against BPA-related oxidative stress.

The testis is the male reproductive organ. The function of the testis is to produce both sperm and androgens (Testosterone). Testosterone (Ts) is controlled by LH but sperm production is controlled both by FSH and Ts. The testis is well known to be very sensitive to injury, especially from endocrine disturbing chemicals such as BPA. These due to endocrine disturbing chemicals such as BPA can affect the size and function of the testis. The oral intake with BPA in this study caused a decrease in serum Ts, DHEA'S, G6PD, 3 β HSD, and protein levels but an increase in serum LH, FSH, SHBG, and cholesterol levels. The decrease of Ts is attributed to (1) BPA inhibits human chorionic gonadotropin (hcG)-stimulated Ts biosynthesis by both cultured rat precursor and immature leydig cells^[64], or (2) through BPA to convert cholesterol to androstenedione through inhibiting 17- α -hydroxylase and 3 β -hydroxysteroid dehydrogenase-isomerase steps^[65]. The decrease of 3 β -hydroxysteroid dehydrogenase activity in this study is accompanied with an increase LH and FSH levels in BPA-treated rats. The increase in LH and FSH levels in BPA exposure is related to (1) LH induced leydig cell secretion of Ts (which incorporated in the regulation of

spermatogenesis by targeting androgen receptors in the germinal epithelium) and (2) FSH targets receptors inside sertoli cell to control spermatogenesis by stimulating many sertoli cell factors. The decrease of testicular cholesterol and protein in this study is linked to testicular dysfunction. On the contrary, FA oral intake to BPA-treated rats increased the number of leydig cells and their ameliorates Ts levels and consequently restores testicular function^[66]. Such observations are in agreement with that of Koriem 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] who found that FA has antioxidant activity, therefore it quenches BPA-related oxidative stress and increases the antioxidant effect of the cells to protect against BPA-harmful effects.

In this study, DNA content levels in liver, kidney, and testis were determined in BPA-treated rats and FA+BPA-treated rats. The results of liver, kidney, and testis DNA content exhibited that BPA caused a very high increase in DNA breakdown of these organs. ¹⁷ Such observations are in agreement with that of Moreover, Akram *et al*^[67] who found that BPA increased DNA damage potential of BPA in liver, kidney, and brain tissues. ¹⁰ The very low concentrations BPA causes toxic effects *via* turbulences in physiological and biochemical parameters in multiple tissues of fish. Also, Panpatil *et al*^[68] who found that BPA-treated groups exhibited significantly higher mean levels of DNA damage in the liver and kidney as compared to the untreated control group. ⁸ Furthermore, Pan *et al*^[69] who found that BPA declined sperm chromatin integrity while increased DNA damage in mice spermatogenic cell. On the contrary, the oral intake with FA to BPA-treated rats showed that DNA content of liver, kidney, and testis tissues restored to the normal diploid level. These observations were recorded due to FA antioxidant activity. These results are in accordance with Koriem and Arbid^[16] and Sochorova *et al*^[20] who found that FA has antioxidant activity which increases the antioxidant activity in liver, kidney, and testis of BPA-treated rats. ² 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

endocrine-disrupting chemical 4-tert-octylphenol. Where FA prevented the degradation of liver DNA, and consequently DNA reformation occurred. Also, Sochorova *et al*^[20] who found that FA has antioxidant activity therefore it quenches BPA-related oxidative stress and increases the antioxidant effect of the cells to protect against BPA-harmful effects.

The mechanism sustaining FA protective effect on BPA-induced liver, kidney, and testis-related toxicity, DNA breakdown, and histopathology is 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 histopathology due to FA has antioxidant activity to quenches BPA-related oxidative stress^[16,20].

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

CONCLUSION

In conclusion, this paper proved that FA can be used as a protective agent in ameliorating the toxicity, DNA breakdown, and histopathology of liver, kidney, and testis organs induced by BPA exposure, which suggest the use of this acid in preventing BPA toxicity which presents in plastic industry such as water bottles and food packages.

ARTICLE HIGHLIGHTS

Research background

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

Research motivation

a challenging responsibility to find a safe and effective way to overcome the bishenol A (BPA) toxicity where BPA is already present in water bottles and food packaging therefore human expose to BPA toxicity day and night. The use of herbal plants in the medicine was known for a long time ago and today it has made a comeback in all over the world. This is because of its minor side effects and has well therapeutically performed.

Research objectives

This study aims to investigate the effect of fertaric acid on bisphenol A-related liver, kidney, and testis toxicity, DNA breakdown, and histopathology in male rats.

Research methods

¹ Thirty male albino rats were divided into 5 equal groups (6 rats/group); Control and paraffin oil groups: oral administration with 1 mL distilled water and 1 mL paraffin oil, respectively. FA-, BPA-, and FA+ BPA-treated groups: oral administration 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) dissolved in 1 mL distilled water then after 1 h these rats orally intake with BPA (4 mg/kg, bw) dissolved in 1 mL paraffin oil, respectively. All these treatments were orally adminstrated once a day for 6 wk.

Research results

The results showed that the BPA induced significant decrease in serum alkaline phosphatase, acid phosphatase, sodium, potassium and chloride, testosterone, dehydroepiandrosterone sulfate, glucose-6-phosphate dehydrogenase, 3β-

hydroxysteroid dehydrogenase, and testis protein levels but highly ¹ significant increase in serum aspartate aminotransferase, alanine aminotransferase, γ -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 prohibited the degradation of liver, kidney, and testis DNA content. Oral administration with FA to BPA-treated rats restored all the above parameters to normal levels.

Research conclusions

This is the first study of fertaric acid in bisphenol A toxicity and the new theories that this study proposes that fertaric acid can amends the bisphenol A toxicity.

The new methods that this study proposed is fertaric acid amends the toxicity, DNA content, and histopathology of liver, kidney, and testis.

Research perspectives

The direction of the future research is to applied in clinical study and it will be interesting to prove that fertaric acid can amends the bisphenol A toxicity.

ACKNOWLEDGEMENTS

This research has no acknowledgment.

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SIMILARITY INDEX

PRIMARY SOURCES

- 1 www.tandfonline.com 300 words — 5%
Internet
- 2 Khaled M.M. Koriem, Adley Y. Selim, Ramzy A. Mazen. "N-acetylcysteine-amide improves tissue oxidative stress, DNA damage, and proteins disappearance in methamphetamine toxicity more efficiently than N-acetyl-L-cysteine", Toxicologie Analytique et Clinique, 2021 267 words — 4%
Crossref
- 3 Khaled M. M. Koriem, Islam B. Gad. "Sinapic acid restores blood parameters, serum antioxidants, and liver and kidney functions in obesity", Journal of Diabetes & Metabolic Disorders, 2022 200 words — 3%
Crossref
- 4 www.ncbi.nlm.nih.gov 82 words — 1%
Internet
- 5 www.findhealthnews.com 41 words — 1%
Internet
- 6 backend.orbit.dtu.dk 38 words — 1%
Internet
- 7 Khaled M.M. Koriem, Mahmoud S. Arbid, Kawther R. Emam. "Therapeutic effect of pectin on octylphenol induced kidney dysfunction, oxidative stress and 26 words — < 1%

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| <div style="background-color: #0056b3; color: white; display: inline-block; width: 30px; height: 30px; text-align: center; line-height: 30px; margin-bottom: 5px;">12</div> | Huiqing Hu, Lihong Wang, Qingqing Wang, Liya Jiao, Weiqi Hua, Qing Zhou, Xiaohua Huang. "Photosynthesis, chlorophyll fluorescence characteristics, and chlorophyll content of soybean seedlings under combined stress of bisphenol A and cadmium", Environmental Toxicology and Chemistry, 2014
<small>Crossref</small> | 15 words — < 1% |
|---|--|-----------------|
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|---|---|-----------------|
| <div style="background-color: #ff0000; color: white; display: inline-block; width: 30px; height: 30px; text-align: center; line-height: 30px; margin-bottom: 5px;">13</div> | Sohrab Kazemi, Seydeh Narges Mousavi Kani, Leyla Rezazadeh, Mahdi Pouramir, Maryam Ghasemi-Kasman, Ali Akbar Moghadamnia. "Low dose administration of Bisphenol A induces liver toxicity in adult rats", Biochemical and Biophysical Research Communications, 2017
<small>Crossref</small> | 15 words — < 1% |
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|---|--|-----------------|
| <div style="background-color: #ff00ff; color: white; display: inline-block; width: 30px; height: 30px; text-align: center; line-height: 30px; margin-bottom: 5px;">14</div> | Thangarasu Rajakumar, Pachiappan Pugalendhi, Subbaiyan Thilagavathi, Dhanabalan Ananthakrishnan, Krishnaswamy Gunasekaran. "Allyl isothiocyanate, a potent chemopreventive agent targets | 15 words — < 1% |
|---|--|-----------------|

-
- 15 Khaled M. M. Koriem, Mahmoud S. Arbid. "Evaluating of β -carotene role in ameliorating of favism-induced disturbances in blood and testis", Journal of Complementary and Integrative Medicine, 2018 14 words — < 1%

Crossref

-
- 16 "Implementation of the evidence - based risk assessment for the re - evaluation of Bisphenol A: preparatory work on Mode of Action studies in mammalian, human and/or in vitro models.", EFSA Supporting Publications, 2021 13 words — < 1%

Crossref

-
- 17 Khaled M. M. Koriem, Mahmoud S. Arbid, Nawal E. Gomaa. "Supplementation of α -Tocopherol Attenuates Minerals Disturbance, Oxidative Stress and Apoptosis Occurring in Favism", Indian Journal of Clinical Biochemistry, 2016 13 words — < 1%

Crossref

-
- 18 A. MURTAZA. "Hypocalcemia and Hyperphosphatemia in Severely Dehydrated Children with and without Convulsions", Acta Paediatrica, 3/1988 12 words — < 1%

Crossref

-
- 19 XueXia Liu, ZhiXin Wang, Fujun Liu. "Chronic exposure of BPA impairs male germ cell proliferation and induces lower sperm quality in male mice", Chemosphere, 2021 12 words — < 1%

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