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Enhancement effects of ethanolic extract of *Fagonia cretica* on Bisphenol A (BPA)-induced genotoxicity and biochemical changes in rats

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Abstract

Background: *Fagonia cretica* L. was considered to be a medical plant that was used for the treatment of different diseases, so the current study was designed to clarify whether *Fagonia cretica* extract (FCE) can avoid Bisphenol A (BPA)-induced genotoxicity and biochemical alterations in rats. Sixty-three male rats were used in this experiment. These animals were distributed into nine groups (seven rats each): negative control, control of corn oil, positive control that were administrated BPA in corn oil (10 mg/kg. b.w.) for 3 weeks, three protection groups received the same dose of BPA in corn oil at the same period together with FCE (3.3, 4.2 and 5.0 g/kg) daily for 3 weeks, and three therapeutic groups received FCE alone at the same doses for 10 days after cessation of BPA treatment. Genetic and biochemical studies were conducted. Genetic studies involved DNA comet assay, micronucleus test, chromosome examination, and mitotic index analysis. Biochemical studies involved liver function (AST, ALT, ALph, and Bilirubin), kidney function (urea and creatinine contents), protein profile, MDA, and endogenous antioxidative system (SOD, CAT, and ACHE) as well as nucleic acid (DNA and RNA) contents in the liver, kidney, and brain tissues.

Results: The results demonstrated that the treatment with BPA induced a significant elevation in genetic abnormalities and deleterious effects in biochemical parameters in relation to untreated control. FCE treatment was found to be significantly diminished the massive damage in the genetic constituents and dangerous alterations in biochemical parameters with respect to BPA treatment alone. These enhancements were increased by increasing the dose level of FCE. Moreover, better results were clarified by utilizing FCE as a protective agent than its utilization as a therapeutic agent especially by using the high dose (5.0 g/kg), in which mostly genetic and biochemical alterations were observed to be restored towards natural levels.

Conclusions: These findings clarified a new insight into the protective effect of FCE in minimizing BPA-induced genotoxicity and biochemical changes in rats detecting the capability of such medicinal plant for alleviating the adverse effect of BPA.

Keywords: Bisphenol A, *Fagonia cretica*, Genetics, Biochemistry, Rats

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Introduction

Bisphenol A (BPA) was found to be a chemical component that widely used and spread in the polycarbonate plastic manufacture and epoxy resins for several years (Hunt et al., 2003; Allard and Colaiacovo, 2010; Strong et al., 2016). This component has a major application in the plastic food products, beverage containers baby bottles, coating of food cans, medical devices, and dental sealants (Eid et al., 2015; Aghajanjpour- Mir et al. , 2016). So, people of different ages were observed to be inevitably exposed to BPA in daily life. Moreover, BPA was detected in different environmental samples involving dust, sewage, water, indoor and outdoor air (Vandenberg et al., 2007). Several studies reported that BPA was observed in the human amniotic fluid (Ikezuki et al., 2002), breast milk (Sun et al., 2004), human placenta (Schonfelder et al., 2002), cord blood (Wan et al., 2010), and fetal liver (Cao et al., 2012). However, the exposure to BPA was revealed to be accompanied by inducing serious environmental pollution and causing adverse effects on human and animal health (Eid et al., 2015). The hazardous or the adverse effects of BPA were detected to be associated with different diseases such as diabetes, neurobehavioral, abnormal karyotypes (Vandenberg et al., 2007; Haighton et al., 2012; Mirmira and Evans-Molina, 2014; Aghajanjpour-Mir et al., 2016), inflammatory cytokine dysregulation (Ben- Jonathan et al., 2009), and mitochondrial-mediated apoptosis in the hepatic tissue (Xia et al., 2014). Thus, scientific attempts must be considered to face the adverse effect of BPA. Natural products especially the extractions of medical plants are essential to save materials that are preferred for using against the hazardous effects of different toxicants.

The plant *Fagonia cretica L.* was considered to be a member of the family Zygophyllaceae (Hussain et al., 2007). The distribution of such plant species in Egypt is mostly found in the Mediterranean Coastal region (for example, Ras El Hikma, Burg El Arab). A concise description is given as a glabrous plant with prostrate quadrangular branches, small spiny undershrub (Shalaby, 1958; Sharkawy, 1961). In the scientific and folkloric literature, *Fagonia cretica L.* is reputed to be a medical plant that is used for the treatment of different diseases such as tumor, cancer, fever, thirst, vomiting, asthma, dysentery, liver trouble, stomach troubles, and skin diseases (Hussain et al., 2007). Moreover, *Fagonia cretica* has other effects including analgesic, anti-inflammatory, and antipyretic activities (Puri and Bhandari, 2014). *Fagonia cretica* has been reported to contain many antioxidant constitutions such as flavonoids, alkaloids, saponins, terpenoids, sterols, coumarins, proteins, and amino acids (Saleh et al., 2011; Puri and Bhandari, 2014). So, the present work was designed to study the protective and therapeutic effects against BPA-induced genotoxicity

and biochemical changes in rats. Genetic studies involved DNA comet assay, micronucleus test, chromosome examination, and mitotic index analysis. Biochemical parameters included superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), acetylcholinesterase (ACHE), liver function, and kidney function. Also, nucleic acid (DNA and RNA) contents in the liver, kidney, and brain tissues were investigated.

Materials and methods

1. Chemicals

Bisphenol A (BPA) was purchased from Sigma (St. Louis, Mo, USA). BPA, a water-insoluble powder was dissolved in corn oil, for daily uptake by gastric gavage to rat animals. A total of 5 g of BPA were dissolved in 500 ml corn oil (as 1% v/w). BPA doses: dose of 10 mg/kg.b.w. were selected based on the National Toxicology Program, 1985, 2008.

Plant material

Fagonia cretica L. had been collected in March 2018 from Burg El Arab desert region and identified in the Department of Horticultural Crop Technology, National Research Centre, Egypt. Fresh aerial parts of this plant were rinsed with distilled water and kept under shade till drying.

Preparation of extract of *Fagonia cretica* (FCE)

According to the method of Hussain et al. (2007), extraction was carried out by a simple maceration process. Aerial parts (490 g) were ground and merged in 3.5 L ethanol. Homogenate was kept for 4 weeks at room temperature ($25 \pm 2^\circ\text{C}$) in the extraction bottle. After 4 weeks, the mixture was filtered twice, first using ordinary filter paper and then Whatman-41 filter paper. Ethanol was completely evaporated at room temperature. In total, 21 g dried ethanolic extract of aerial parts was obtained.

Experimental animals

Male albino rats of Sprague–Dawley strain weighing about 120 g were obtained from the Animal House, National Research Centre, Egypt. Animals were housed at an ambient temperature of $25 \pm 3.2^\circ\text{C}$ on light/dark cycle of 12/12 h. All rats were kept in clean polypropylene cages and administered food and water ad libitum.

All experimental procedures involving animals were conducted in accordance with the ethical guidelines of the Medical Ethical Committee of the National Research Centre in Egypt (IAEC, 2010).

Experimental design

A total of 63 rats were used and divided into 9 equal groups (G):

G1 (control group) received only a basal diet for 3 weeks.

G2 (oil group) received a basal diet and gavage (orally) corn oil (10 mg/kg.b.w.) daily for 3 weeks.

G3 (BPA group) received (orally) BPA in corn oil at a dose of 10 mg/kg.b.w. daily for 3 weeks.

G 4–6 received (orally) BPA in corn oil in the same dose and way previously mentioned. Starting on the first day of BPA administration, rats in 4–6 groups were treated (orally) daily for 3 weeks with FCE (3.3 g/kg., 4.2 g/kg., and 5.0 g/kg., respectively). These groups (4–6) were used to evaluate the protective role of FCE against the toxicity of BPA.

G 7–9 received (orally) BPA in corn oil in the same dose and way previously mentioned and for the same period, and then the rat groups were treated (orally) with FCE (3.3 g/kg., 4.2 g/kg., and 5.0 g/kg., respectively) for 10 days. These groups (7–9) were used to evaluate the therapeutic effect of FCE against BPA toxicity.

DNA comet assay

Comet assay was performed referring to the protocol mentioned previously by Blasiak et al. (2004). The rat liver cells of each treatment were mixed with low-melting-point agarose (ratio of 1:10 v/v), then pipetted to pre-coated slides with normal-melting-point agarose. The slides were kept flat at 4 °C for 30 min in a dark environment. The third layer of low-melting-point agarose was then pipetted on slides, left to solidify at for 30 min at 4 °C. The slides were transferred to a pre-chilled lysis solution, kept for 60 min at 4 °C. After that, slides were immersed in freshly prepared alkaline unwinding solution at room temperature in the dark for 60 min. Slides were subjected to electrophoresis run at 25 V, 300 mA at 4 °C for 30 min. The slides were rinsed in neutralizing solution followed by immersion in 70% ethanol and then air-dried. Ethidium bromide was used for slides stain then and visualized by using Zeiss epifluorescence microscope (510–560 nm, barrier filter 590 nm) with a magnification of 400×. For each animal, about 100 cells were examined to determine the percentage of cells with DNA damage that appear like comets. The non-overlapping cells were randomly selected and were visually assigned a score on an arbitrary scale of 0–3 (i.e., class 0 = no detectable DNA damage and no tail; class 1 = tail with a length less than the diameter of the nucleus; class 2 = tail with length between 1× and 2× the nuclear diameter; and class 3 = tail longer than 2× the diameter of the nucleus) based on perceived comet tail length migration and relative proportion of DNA in the nucleus (Collins et al., 1997).

Cytogenetic analyses

Micronucleus test

Bone marrow slides were prepared according to the method described by Krishna and Hayashi (2000). The bone marrow was washed with 1 ml of fetal calf serum and then smeared on clean slides. The slides were left to air dry and then fixed in methanol for 5 min, followed by staining in May-Grunwald-Giemsa for 5 min then washed in distilled water and mounted. For each animal, 2000 polychromatic erythrocytes (PCEs) were examined for the presence of micronuclei.

Chromosome preparation

For chromosome analysis, both treated and control animals were sacrificed by cervical dislocation at the end of the experiment. One hour and a half or 2 h before sacrifice, rats were injected i.p. with 0.5 g colchicine/kg. b.w. The femurs were removed and the bone marrow cells were aspirated using saline solution. Metaphase spreads were prepared using the method of Preston et al. (1987). Fifty metaphase spreads per animal were analyzed for scoring the different types of chromosome aberrations.

Mitotic index

Mitotic index was determined by scoring at least 1000 cells from each animal, and then MI was calculated by a ratio of mitotic cells to total cell number in 1000 cells of each rat, then the coefficient of division is obtained according to the following formula: mitotic index (%) = the number of the dividing cells × 100/the total number of cells (Shubber and Juma, 1999).

Biochemical study

At the end of the first stage (protection treatment) and the end of the second stage (therapeutic treatment), blood samples were collected and centrifuged at 3000g to obtain the serum for the determination aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphate (ALPh) (according to Steven, 1996; Scandiatvien, Soretty 1974; Walter and Gerade, 1970, respectively) as indicators of liver functions. The levels of urea (Fawcett and Soctt, 1960) and creatinin (Bartles and Bohmer, 1972) were also determined as kidney function. Serum protein profile, as well as endogenous lipid peroxidation (MDA) and antioxidant system including superoxide dismutase (SOD), catalase (CAT) and acetylcholinesterase (ACHE) were determined according to Ohkama et al. (1979), Nishikimi et al. (1972), Aebi (1984) and Henry (1974), respectively.

Moreover, nucleic acid (DNA and RNA) contents were determined in the liver, kidney and brain tissues according to Pearse (1985).

Statistical analysis

Statistical analysis was carried out with SPSS software. Data were analyzed using a one-way analysis of variance (ANOVA) followed by Duncan's post hoc test for comparison between different treatments. The values were expressed as mean \pm S.E. and differences were considered significant when $P < 0.05$.

Results

Comet assay of DNA

The results of the present work (Fig. 1 and Table 1) clarified significant increases ($P < 0.01$) of DNA damage in rats that exposed to BPA as compared to those found in the normal control group. In the protective treatments with FCE, the present findings showed a decrease in the values of DNA damage induced by exposure to BPA. The DNA damage decreased by increasing the level of FCE dose, where the statistical analysis exhibited a slight decrease in the DNA damage using low dose (3.3 g/kg.b.w.), while the DNA damage significantly lowered by using medium (4.2 g/kg.b.w.) or high (5.0 g/kg.b.w.) doses. The highest dose (5.0 g/kg.b.w.) caused the lowest values of DNA damage. On the other hand, the results of therapeutic treatments with three doses (3.3, 4.2, and 5.0 g/kg) of FCE displayed insignificant decreases of DNA damage produced by exposure to BPA, in spite of these decreases of DNA damage were shown to be increased by increasing the dose level. Moreover, the BPA-intoxicated rats that were treated with a high dose had the lowest value of DNA damage.

Micronucleus test

The results of the present experiment (Fig. 2 and Table 2) showed significant elevation in values of micronucleated

polychromatic erythrocytes (MNPCE) in the rats that exposed to BPA relation to that of unexposed normal control. In the male rat groups that were exposed to BPA and received FCE doses as protective or therapeutic agents, the induced values of MNPCE significantly reduced as compared to the male rat group that was treated with BPA alone. This reduction of induction of MNPCE was increased by increasing the dose level of FCE. The present findings displayed that the treatment with a high dose (5.0 g/kg.b.w.) produced the lowest value of MNPCE. Moreover, the utilizing of FCE as a protective agent especially by using the highest dose ameliorated more the genetic material recovery (by decreasing the induced values of MNPCE) and resulted in the best results than the utilization of FCE as a therapeutic agent.

Chromosome examination

The reported data of the present results (Table 3) revealed that the treatment with BPA had induced a highly significant elevation in the frequencies of chromosome aberrations comparing with the control group. The male rat groups that were treated with BPA and gavages the FCE doses as protective or therapeutic agents had a significant reduction of the frequencies of chromosome aberrations in relation to the rat group that was treated with BPA alone. The reduction in the frequencies of chromosome aberrations was found to be increased by increasing the dose level of FCE. The present data exhibited that the utilizing of the highest dose (5.0 g/kg.b.w.) of FCE as a protective or therapeutic agent gave the lowest values of the frequencies of chromosome aberrations. Furthermore, the utilization of FCE as a protective agent especially by using the highest dose led to more amelioration in the genetic material recovery by decreasing the

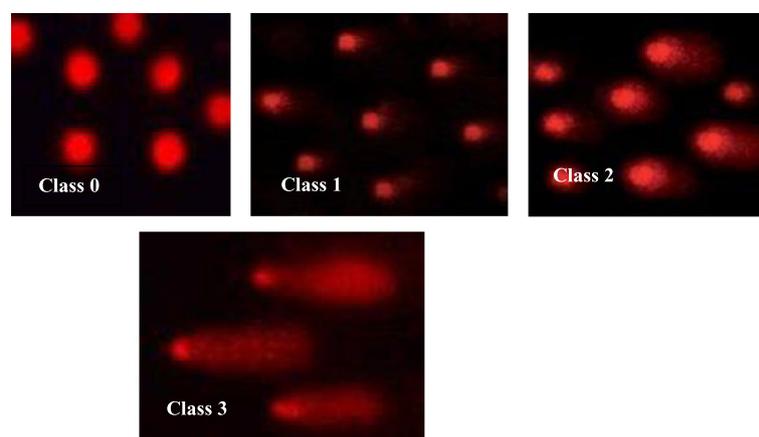


Fig. 1 Shows photographs of the comet assay classes of extended DNA damage in the rat liver. The examination of the varying extent of DNA damage in normal control, BPA-intoxicated rats, protection groups of FCE, and therapeutic groups of FCE was recorded in Table 1. Class 0 = no tail; class 1 = tail length < diameter of the nucleus; class 2 = tail length between 1 \times and 2 \times the diameter of the nucleus; and class 3 = tail length > 2 \times the diameter of the nucleus

Table 1 Comet assay analysis shows the enhancement role of *Fagonia cretica* (FCE) on Bisphenol A-induced DNA damage in rats

Treatment	No of animals	No. of analyzes cells*	Comets	Comet classes**				DNA damaged cells % (mean ± SEM)
				0	1	2	3	
Normal control	5	500	31	469	19	12	0	6.2 ± 1.30 ^c
Control oil treatment	5	500	34	466	21	13	0	6.8 ± 1.30 ^c
Bisphenol A (BPA)	5	500	139	361	42	53	44	27.8 ± 2.59 ^a
Protective low dose of FCE	5	500	124	376	36	42	46	24.8 ± 0.84 ^a
Protective medium dose of FCE	5	500	86	414	31	26	29	17.2 ± 1.92 ^b
Protective high dose of FCE	5	500	77	423	28	27	22	15.4 ± 2.61 ^b
Therapeutic low dose of FCE	5	500	126	374	39	45	42	25.2 ± 1.92 ^a
Therapeutic medium dose of FCE	5	500	113	387	34	44	35	22.6 ± 1.52 ^{ab}
Therapeutic high dose of FCE	5	500	106	394	31	38	37	21.2 ± 1.64 ^{ab}

*Number of cells examined per a group, **Class 0 = no tail; Class1 = tail length < diameter of the nucleus; Class 2 = tail length between 1× and 2× the diameter of the nucleus; and Class 3 = tail length > 2× the diameter of the nucleus

All data are expressed as mean ± SE. Means with different superscripts letters a, b, c are significantly different at $P \leq 0.05$

chromosome damages and resulted in better findings than the utilization of FCE as a therapeutic agent.

Mitotic index

In the present experiment, the mitotic index results (Table 4 and Fig. 3) showed a significant mitotic delay ($P < 0.01$) in the BPA-intoxicated rats relation to that of the normal control group. Whereas in the protective treatment, FCE doses gave elevation in the mitotic activity, this elevation was statistically insignificant at low dose treatment as compared to the BPA treatment alone. However, the mitotic activity was significantly increased in BPA-intoxicated rats that treated with medium ($P < 0.05$) or high ($P < 0.01$) doses of FCE with respect to those of BPA treatment alone. The treatment with the highest dose (5.0 g/kg.b.w.) of FCE recovered the mitotic

activity to the approximate baseline of normal control. In the therapeutic treatment, the present findings showed no improvement in the mitotic activity by using the low dose of FCE. However, in treatment with medium or high doses of FCE for BPA-intoxicated rats, the mitotic activities were enhanced, this enhancement was insignificant by utilizing the medium dose of FCE treatment and significant ($P < 0.05$) by using the high dose of FCE treatment in relation to that of BPA treatment alone.

Biochemical analysis

The results of biochemical study (Table 5) showed that the administration of BPA to rats significantly decreased the total protein as well as reduced the SOD, CAT, and ACHE activities as compared to the control group. In

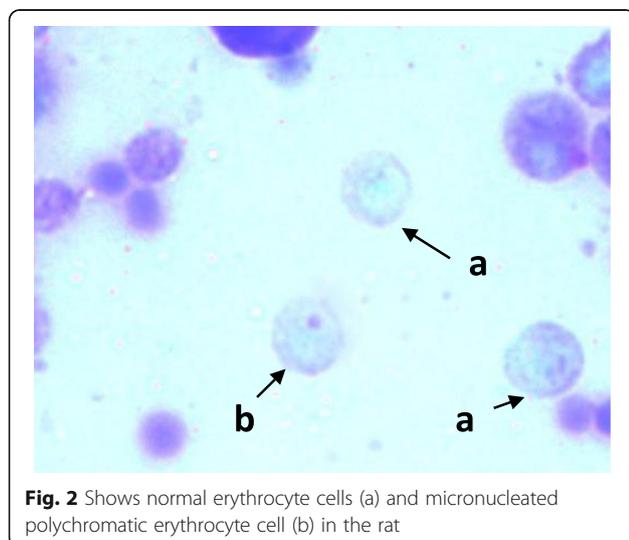


Fig. 2 Shows normal erythrocyte cells (a) and micronucleated polychromatic erythrocyte cell (b) in the rat

Table 2 Shows the enhancement role of *Fagonia cretica* extract (FCE) on Bisphenol A-induced micronucleated polychromatic erythrocytes (MNPCE) in rats

Treatment	MNPCE
Normal control	6.0 ± 0.55 ^g
Control oil treatment	5.40 ± 0.68 ^g
Bisphenol A (BPA)	36.20 ± 1.20 ^a
Protective low dose of FCE	27.60 ± 0.81 ^{bc}
Protective medium dose of FCE	22.80 ± 0.97 ^d
Protective high dose of FCE	13.60 ± 0.75 ^f
Therapeutic low dose of FCE	29.80 ± 0.58 ^b
Therapeutic medium dose of FCE	62.20 ± 0.74 ^c
Therapeutic high dose of FCE	20.0 ± 0.89 ^e

All data are expressed as mean ± SE. Means with different superscripts letters a, b, c, d, e, f, g are significantly different at $P \leq 0.05$

Table 3 Shows the enhancement role of *Fagonia cretica* extract (FCE) on Bisphenol A-induced chromosomal aberrations in rats

Treatment	Structural chromosomal aberrations						Aneuploidy	Total aberrations
	Gap	Break	Deletion & fragment	Centromeric attenuation	Endomitosis	Total structural		
Normal control	1.0 ± 0.32 ^d	0.40 ± 0.24 ^f	0.40 ± 0.25 ^d	0.60 ± 0.40 ^e	0.20 ± 0.20 ^d	2.60 ± 0.81 ^f	0.60 ± 0.25 ^d	3.20 ± 0.80 ^f
Control oil treatment	1.20 ± 0.37 ^d	0.80 ± 0.37 ^{ef}	0.40 ± 0.25 ^d	1.0 ± 0.45 ^e	0.60 ± 0.25 ^{cd}	4.0 ± 0.44 ^f	0.60 ± 0.25 ^d	4.60 ± 0.51 ^f
Bisphenol A (BPA)	5.40 ± 0.75 ^a	3.60 ± 0.51 ^a	4.80 ± 0.74 ^a	7.20 ± 0.37 ^a	3.0 ± 0.32 ^a	24.0 ± 1.22 ^a	5.40 ± 0.68 ^a	29.40 ± 0.68 ^a
Protective low dose of FCE	2.80 ± 0.37 ^c	2.20 ± 0.20 ^{bcd}	1.40 ± 0.40 ^{cd}	5.40 ± 0.51 ^b	2.20 ± 0.49 ^{ab}	14.0 ± 0.63 ^{cd}	3.40 ± 0.40 ^b	17.40 ± 0.51 ^c
Protective medium dose of FCE	2.80 ± 0.20 ^c	1.80 ± 0.37 ^{cde}	1.40 ± 0.25 ^{cd}	4.40 ± 0.40 ^{bc}	2.0 ± 0.32 ^{ab}	12.0 ± 0.63 ^d	2.40 ± 0.51 ^{bc}	14.40 ± 0.81 ^d
Protective high dose of FCE	2.0 ± 0.32 ^{cd}	1.20 ± 0.37 ^{def}	1.0 ± 0.32 ^{cd}	2.40 ± 0.51 ^d	1.60 ± 0.51 ^{bc}	8.60 ± 0.87 ^e	1.80 ± 0.37 ^{cd}	10.40 ± 1.0 ^e
Therapeutic low dose of FCE	5.0 ± 0.32 ^{ab}	3.0 ± 0.45 ^{ab}	4.20 ± 0.37 ^{ab}	3.40 ± 0.40 ^{cd}	2.40 ± 0.51 ^{ab}	18.0 ± 1.18 ^b	5.20 ± 0.66 ^a	23.20 ± 0.86 ^b
Therapeutic medium dose of FCE	4.0 ± 0.32 ^b	2.60 ± 0.26 ^{abc}	3.20 ± 0.20 ^b	3.0 ± 0.55 ^d	3.0 ± 0.32 ^a	15.60 ± 0.75 ^{bc}	3.60 ± 0.51 ^b	18.80 ± 0.86 ^c
Therapeutic high dose of FCE	2.20 ± 0.37 ^{cd}	2.20 ± 0.37 ^{bcd}	2.0 ± 0.32 ^c	2.60 ± 0.40 ^d	2.80 ± 0.20 ^a	11.80 ± 1.0 ^d	2.60 ± 0.51 ^{bc}	14.40 ± 0.41 ^d

All data are expressed as mean ± SE. Means with different superscripts letters a, b, c, d, e, f are significantly different at $P \leq 0.05$

contrast, the level of MDA was significantly elevated in BPA-intoxicated rats than those found in untreated rats.

In the male rat groups that were treated with BPA and gavage. The FCE doses as protective or therapeutic agents, the harmful effects of above biochemical parameters were significantly alleviated and these effects were dose-dependent. The values of total protein, SOD, CAT, and ACHE were significantly increased in the BPA group that was treated with *Fagonia* doses as protective or therapeutic agents than those found in the BPA group alone. In contrast, the values of MDA in the intoxicated rats that received *Fagonia critica* doses were significantly reduced with respect to those observed in the intoxicated control rats. These improvements were pronounced in the use of a high dose of *Fagonia* treatment (5.0 g/kg. b.w.). Furthermore, better results were

observed in BPA-intoxicated rats that were treated with *Fagonia* doses as protective agents than those revealed in BPA-intoxicated rats that received *Fagonia* doses as therapeutic agents.

Liver function analysis

The results of the present experiment (Table 6) clarified a significant rise in AST, ALT, ALph, and bilirubin in BPA-intoxicated rats in comparison with untreated control. The male rat groups that received BPA and treated with FCE doses as protective or therapeutic agents had a significant decrease of the values of AST, ALT, ALph, and bilirubin as compared to BPA-intoxicated rat group alone. The amelioration of the above liver function parameters was increased by increasing the dose levels of FCE. The present findings found that the use of the highest dose (5.0 g/kg. b.w.) of FCE as a protective or therapeutic agent gave the lowest values of AST, ALT, ALph, and bilirubin. Furthermore, the utilization of FCE as a protective agent especially by using the highest dose caused more improvements in previous parameters of liver functions and resulted in better findings than the utilization of FCE as a therapeutic agent.

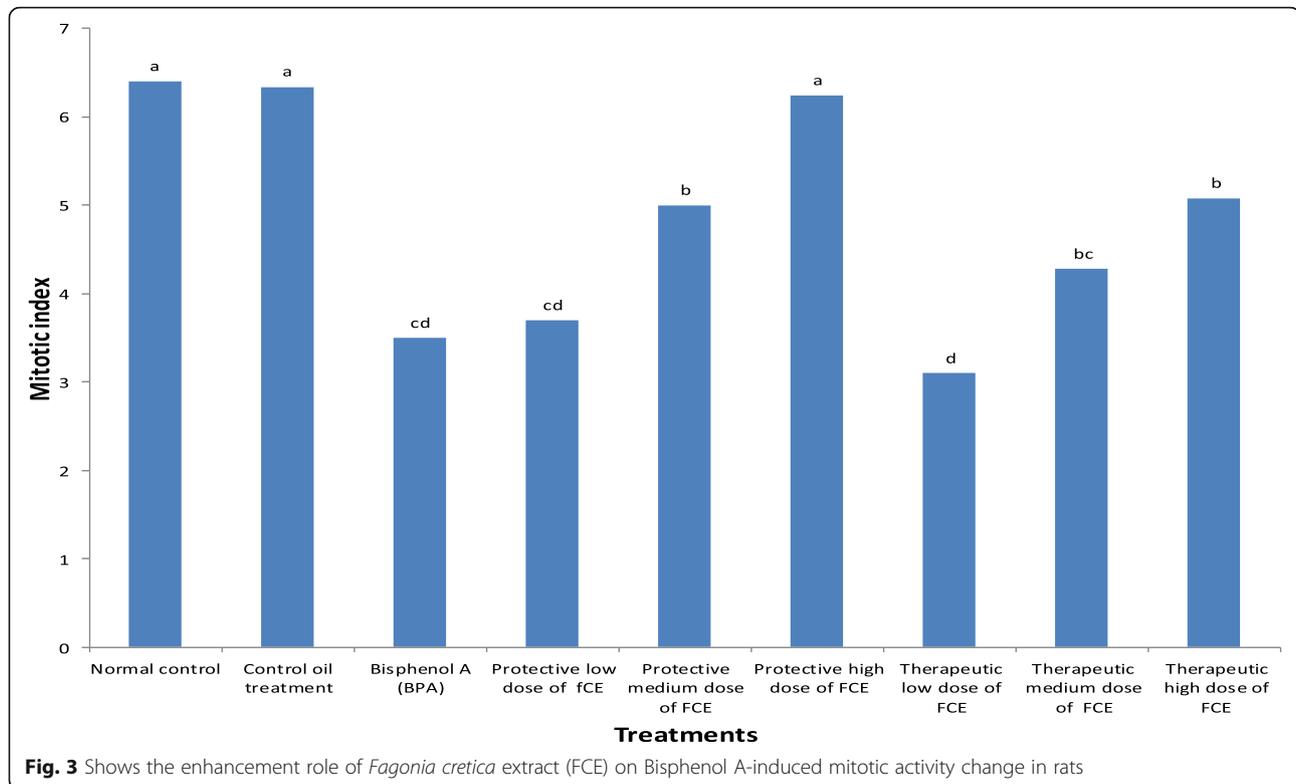
Kidney function analysis

The results of the present work (Table 6) observed a significant elevation in the values of urea and creatinine in BPA-intoxicated rats as compared to those found in the control. The rat groups that exposed to BPA and gavage FCE doses as protective or therapeutic agents had a significant reduction of the values of urea and creatinine as compared to the rat group that was treated with BPA alone.

Table 4 Shows the enhancement role of *Fagonia cretica* extract (FCE) on Bisphenol A-induced mitotic activity change in rats

Treatment	Mitotic index
Normal control	6.40 ± 0.62 ^a
Control oil treatment	6.34 ± 0.51 ^a
Bisphenol A (BPA)	3.50 ± 0.32 ^{cd}
Protective low dose of FCE	3.70 ± 0.07 ^{cd}
Protective medium dose of FCE	5.0 ± 0.20 ^b
Protective high dose of FCE	6.24 ± 0.36 ^a
Therapeutic low dose of FCE	3.1 ± 0.28 ^d
Therapeutic medium dose of FCE	4.28 ± 0.30 ^{bc}
Therapeutic high dose of FCE	5.08 ± 0.29 ^b

All data are expressed as mean ± SE. Means with different superscripts letters a, b, c, d are significantly different at $P \leq 0.05$



The amelioration in the above kidney function parameters in *Fagonia* groups increased by increasing the dose levels of FCE. Furthermore, the use of FCE as a protective agent especially by using the highest dose ameliorated more the above kidney function parameters and resulted in the best results than the use of FCE as a therapeutic agent.

The present data (Table 7) revealed significant decreases in the DNA and RNA contents in the BPA-intoxicated rats as compared to those found in the control. The BPA-intoxicated rats that were treated with

FCE doses as protective or therapeutic agents had improvements of the DNA and RNA contents as compared to BPA-intoxicated rat group alone. These improvements were increased by increasing the dose level of FCE. The present findings clarified that the treatment with the high dose (5.0 g/kg, b.w.) gave the highest values of DNA and RNA contents. Moreover, the treatment with FCE as a protective agent especially by using the highest dose improved more the genetic material contents and resulted in better results than the use of FCE as a therapeutic agent.

Table 5 Shows serum protein profile as well as endogenous lipid peroxidation and antioxidant system of serum in Bisphenol A-intoxicated rats treated with *Fagonia cretica* extract

Treatment	Protein g/dl	MDA N mol/ml	SOD % (U/ml)	Catalase (U/ml)	Acetylcholinesterase (U/ml)
Normal control	13.86 ± 0.44 ^a	8.34 ± 0.25 ^e	9.11 ± 0.27 ^a	813.25 ± 3.27 ^a	7439.63 ± 45.31 ^a
Control oil treatment	13.98 ± 0.45 ^a	8.00 ± 0.27 ^e	9.24 ± 0.29 ^a	828.78 ± 2.23 ^a	7493.46 ± 37.71 ^a
Bisphenol A (BPA)	5.82 ± 0.31 ^g	23.66 ± 0.60 ^a	2.85 ± 0.18 ^f	403.90 ± 6.23 ^g	3512.54 ± 161.87 ^g
Protective low dose of FCE	10.22 ± 0.24 ^{cd}	12.02 ± 0.39 ^c	7.63 ± 0.18 ^{bd}	684.45 ± 6.02 ^d	6208.54 ± 47.35 ^d
Protective medium dose of FCE	10.73 ± 0.22 ^c	10.88 ± 0.32 ^d	6.94 ± 0.10 ^c	713.031 ± 9.61 ^c	6535.61 ± 61.24 ^c
Protective high dose of FCE	11.62 ± 0.22 ^b	10.74 ± 0.25 ^d	6.13 ± 0.15 ^d	767.44 ± 11.11 ^b	6931.00 ± 84.30 ^b
Therapeutic low dose of FCE	8.24 ± 0.25 ^f	14.50 ± 0.21 ^b	5.74 ± 0.11 ^d	600.28 ± 3.60 ^f	5845.27 ± 60.21 ^f
Therapeutic medium dose of FCE	9.25 ± 0.27 ^e	13.82 ± 0.28 ^b	5.17 ± 0.15 ^e	615.794 ± 2.961 ^f	5985.03 ± 22.72 ^{ef}
Therapeutic high dose of FCE	9.63 ± 0.19 ^{de}	12.60 ± 0.57 ^c	4.66 ± 0.12 ^e	641.36 ± 4.55 ^e	6117.85 ± 2573 ^{de}

All data are expressed as mean ± SE. Means with different superscripts letters a, b, c are significantly different at $P \leq 0.0$

Table 6 Shows liver and kidney functions of serum Bisphenol A-intoxicated rats treated with *Fagonia cretica* extract

Treatment	AST (IU/l)	ALT (IU/l)	Alph (IU/l)	Bilirubin (g/dl)	Urea mg/dl	Creatinine mg/dl
Normal control	47.40 ± 0.92 ^f	51.20 ± 0.86 ^g	153.91 ± 0.79 ^h	0.28 ± 0.01 ^d	22.93 ± 0.37 ^g	0.623 ± 0.01 ^d
Control oil treatment	47.20 ± 0.86 ^f	50.80 ± 0.86 ^g	153.43 ± 0.87 ^h	0.27 ± 0.01 ^d	21.04 ± 0.40 ^g	0.625v0.01 ^d
Bisphenol A (BPA)	81.00 ± 1.22 ^a	101.40 ± 1.72 ^a	415.99 ± 5.02 ^a	1.33 ± 0.1 ^a	54.82 ± 0.48 ^a	1.27 ± 0.06 ^a
Protective low dose of FCE	52.00 ± 0.70 ^c	62.80 ± 0.86 ^c	213.06 ± 2.17 ^e	0.36v0.01 ^{bcd}	28.96 ± 0.38 ^d	0.719 ± 0.01 ^{bc}
Protective medium dose of FCE	58.20 ± 1.39 ^g	61.00 ± 0.70 ^{ef}	199.43 ± 1.79 ^f	0.34 ± 0.01 ^{cd}	27.52 ± 0.28 ^e	0.719 ± 0.17 ^{bc}
Protective high dose of FCE	55.00 ± 0.70 ^c	58.60 ± 0.51 ^f	187.37 ± 1.18 ^g	0.31 ± 0.01 ^{cd}	25.63 ± 0.50 ^f	0.68 ± 0.005 ^{cd}
Therapeutic low dose of FCE	69.00 ± 0.70 ^b	73.80 ± 0.58 ^b	243.87 ± 4.72 ^b	0.45 ± 0.14 ^b	34.11 ± 0.35 ^b	0.783 ± 0.01 ^b
Therapeutic medium dose of FCE	68.00 ± 0.94 ^b	70.60 ± 0.51 ^c	234.27 ± 2.40 ^c	0.41 ± 0.01 ^{bc}	31.90 ± 0.50 ^c	0.758 ± 0.01 ^{bc}
Therapeutic high dose of FCE	64.00 ± 0.70 ^c	67.20 ± 0.86 ^d	223.23 ± 1.93 ^d	0.39 ± 0.01 ^{bc}	30.88 ± 0.39 ^c	0.737 ± 0.01 ^{bc}

All data are expressed as mean ± SE. Means with different superscripts letters a, b, c are significantly different at $P \leq 0.05$

Discussion

Genetic study

The effect of BPA on genetic changes

DNA damage In the present work, the comet assay analysis showed that the treatment with BPA had induced an elevation in DNA strand breaks causing greater DNA migration out of the nucleus into the tail of the comet in the hepatic tissue. This massive DNA damage was observed to be significantly increased in rats exposed to BPA in relation to those of normal control. These findings were found to be in agreement with the report of Eid et al. (2015) who observed by using comet assay method a significant elevation of DNA damage in hepatic tissue of rats received BPA as compared to those found in the normal control. Also, similarly in the previous study, Parry et al. (2002) demonstrated DNA damage in human cell line (MCF) after BPA exposure with respect to untreated MCF cells. Mourad and Khadrawy (2012) detected massive DNA damage in cell line from hamster ovary (CHOKI) that was treated with BPA relative to those observed in normal control.

Cytogenetic alterations The obtained data in the present study revealed that the exposure to BPA treatment had induced significant increases in cytogenetic alterations that involved micronuclei, chromosome anomalies, and mitotic activity changes with respect to normal control as follows:

- Chromosome aberrations

Concerning the inducing of chromosome aberrations, the present findings were similar to that reported of Aghajanzpour-Mir (2016) who illustrated massive chromosome damage in MCF-7 cell line that exposed to BPA in comparison with that found in the normal control. These chromosome anomalies included structural and numerical aberrations. Structural aberrations involved chromosomal gaps and breaks, chromatid gaps and breaks, fragments, ring chromosomes, and chromosome rearrangements such as triradials. Chromosome aneuploidies were the most frequent numerical aberrations. Also, previous several studies by Pfeiffer et al. (1997), Tsutsui et al. (1998), Ochi (1999) and Tsutsui et al. (2000) demonstrated DNA adduct

Table 7 Shows the nucleic acid (DNA and RNA) contents in Bisphenol A-intoxicated rats treated with *Fagonia cretica* extract

Treatment	DNA content in tissues of			RNA content in tissues of		
	Liver	Kidney	Brain	Liver	Kidney	Brain
Normal control	0.66 ± 0.01 ^a	0.47 ± 0.02 ^a	0.51 ± 0.00 ^a	0.41 ± 0.0 ^a	0.31 ± 0.0 ^a	0.35 ± 0.0 ^a
Control oil treatment	0.67 ± 0.01 ^a	0.46 ± 0.01 ^a	0.51 ± 0.01 ^a	0.41 ± 0.01 ^a	0.32 ± 0.01 ^a	0.36 ± 0.0 ^a
Bisphenol A (BPA)	0.28 ± 0.00 ^f	0.21 ± 0.01 ^g	0.23 ± 0.01 ^g	0.22 ± 0.0 ^g	0.16 ± 0.01 ^f	0.15 ± 0.01 ^f
Protective low dose of FCE	0.50 ± 0.01 ^c	0.39 ± 0.01 ^{cd}	0.40 ± 0.00 ^c	0.32 ± 0.0 ^d	0.26 ± 0.01 ^c	0.25 ± 0.0 ^c
Protective medium dose of FCE	0.56 ± 0.00 ^b	0.40 ± 0.01 ^{bc}	0.46 ± 0.01 ^b	0.35 ± 0.0 ^c	0.27 ± 0.0 ^b	0.28 ± 0.0 ^b
Protective high dose of FCE	0.59 ± 0.01 ^b	0.42 ± 0.01 ^b	0.48 ± 0.01 ^b	0.38 ± 0.0 ^b	0.29 ± 0.01 ^b	0.29 ± 0.0 ^b
Therapeutic low dose of FCE	0.41 ± 0.00 ^e	0.33 ± 0.0 ^f	0.32 ± 0.0 ^f	0.27 ± 0.0 ^f	0.20 ± 0.0 ^e	0.20v0.01 ^e
Therapeutic medium dose of FCE	0.44 ± 0.00 ^d	0.35 ± 0.0 ^{ef}	0.35 ± 0.0 ^c	0.28 ± 0.0 ^{ef}	0.21 ± 0.0 ^e	0.22 ± 0.1 ^{cd}
Therapeutic high dose of FCE	0.47 ± 0.00 ^d	0.37 ± 0.01 ^{de}	0.38 ± 0.0 ^d	0.30 ± 0.0 ^e	0.23 ± 0.01 ^d	0.23 ± 0.0 ^{cd}

All data are expressed as mean ± SE. Means with different superscripts letters a, b, c are significantly different at $P \leq 0.05$

formation and chromosome anomalies in different somatic mammalian cells that were treated with BPA as compared to normal control. Moreover, Machtinger et al. (2013); Liu et al. (2013) and Fic et al. (2013) observed that BPA treatment could induce numerical chromosome anomalies such as aneuploidy through disruption of meiotic or mitotic process and also genomic structural aberrations such as DNA breakage. Allard and Colaiacovo (2010) clarified that BPA impairs the double-strand break repair machinery causing chromosome aberrations. Also, the same authors (Allard and Colaiacovo, 2010) reported that BPA exposure resulted in down-regulation of double-strand break repair (DSBR) genes in both somatic and germinal cells, thereby induce impairing maintenance of genomic integrity during meiosis and mitosis divisions. Furthermore, synaptic defects could be induced due to the BPA treatment causing chromosome anomalies (Susiarjo et al., 2007; Aghajanzpour-Mir, 2016).

- Micronuclei and mitotic changes

Concerning the inducing micronuclei and mitotic activity changes in the current work, the toxic effects of BPA that induced such abnormalities had not been previously reported in vivo of the mammalian cells in the literature. However, Aghajanzpour-Mir et al., (2016) reported invitro study that the direct genotoxic effects due to BPA treatment included the inducing of micronuclei (MNPEC) and mitotic activity changes in MCF-7 cell line.

In the present study, the inducing DNA damage and cytogenetic changes due to BPA treatment might be due to that BPA during its metabolites in liver produce reactive oxygen species (ROS) and nitric oxide (NO) (Chitra et al., 2003; Asahi et al., 2010; Moon et al., 2012; Eid et al., 2015). These constituents (ROS and NO) were found to be strong oxidants that are capable of attacking and modifying lipids, proteins, and DNA as well as depleting antioxidant defenses inducing genotoxic effects that include DNA damage and cytogenetic changes in mammalian cells (Moon et al., 2012; Eid et al., 2015).

Also, Bauer (2000), Kabuto et al. (2003), and Eid et al. (2015) demonstrated that BPA treatment had induced lowering in the antioxidant enzyme activities (SOD, CAT, GPx) and increase in levels of ROS and NO and consequently lead to genotoxic effect in different mammalian tissues. Also, in the previous study, Grattagliano et al. (1999) clarified that NO is considered to be highly diffusible free radicals. This component can react with superoxide (O₂) leading to the formation of peroxy-nitrite (ONOO) which is a highly reactive free radical resulting in more harmful effects on the mammalian tissues such as nitrosative stress and consequently induce genotoxic effects.

Effect of using FCE on genetic changes

In the current study, the results exhibited that the utilization of FCE as a protective or therapeutic agent could minimize and inhibit the genotoxic effects induced by BPA in rat tissues. The occurring of tail lengths of DNA comets, inducing of micronuclei, chromosome aberration frequencies, and mitotic activity changes were significantly diminished compared with BPA treatment alone. Best results were obtained by utilizing FCE as a protective agent than its use as a therapeutic agent. So, the present findings proved that this natural product (FCE) has potent effects on the maintenance of the genetic materials against BPA genotoxicity. In spite of the literature investigated, the extensive traditional utilize of *Fagonia cretica*, its antigenotoxicity profile in mammalian cells remains not obvious. However, several studies recorded that FCE was found to have significant anticancer and antitumor activity (Saeed, 1969; Hussain et al., 2007), anti-inflammatory, and wound healing property (Saleh et al., 2011). Moreover, Rawal et al. (2004) found that *Fagonia cretica L.* extract was effective in enhancing the GSH levels, improving gene expression of gamma-glutamylcysteine ligase and Cu-ZnSOD genes against oxidative stress in rats.

Furthermore, the use of extract of such medicinal plant was found to be significantly minimized the expression of iNOS gene which plays a main role in neuronal injury during hypoxia-ischemia (Rawal et al., 2004; Puri and Bhandari, 2014). Also, in rats, Rawal et al. (2004) observed potent free radical scavenging properties of FCE against reactive oxygen and nitrogen species that studied by electron paramagnetic resonance spectroscopy. Lam et al. (2012) revealed that FCE could cause cell cycle arrest and apoptosis via P⁵³-dependent and independent mechanisms, with activation of the DNA damage response. Also, their results clarified that FCE contains anticancer factors that act singly or in combination against the proliferation of breast cancer cells via DNA damage-induced FOXO3a and P⁵³ expression.

Biochemical study

Liver and kidney functions of serum Bisphenol A-intoxicated rats

In the present work, the biochemical data observed significant biochemical alterations in liver and kidney functions in rats exposed to BPA. The activities on the levels of ALT, AST, ALP, and bilirubin in the serum were shown to be very great elevation, as compared to those found in the control. These harmful effects in such biochemical parameter indicator of acute hepatic necrosis and hepatobiliary disease, as well as the elevation of AST and ALT levels, indicate initial hepatocellular damage (Michalowicz and Duda, 2007; Darwish et al., 2011).

Several studies reported in different mammalian cells that exposure to BPA led to an increase of oxidative stress causing inhibition of mitochondria and microsome functions in the liver, also hydroxylated metabolites are induced and consequently, excess of reactive oxygen species (ROS_s) are formed. These ROS_s attack biological compounds in the living cells such as lipid, protein, nucleic acids, and other compounds (Pigeolet et al., 1990; Eid et al., 2015; Poormoosavi et al.; 2018; Shirani et al., 2019) leading to adverse effect on the parameters of ALT, AST, ALP, and bilirubin.

The results of the present experiment on rats revealed that the treatment with BPA resulted in a significant increase in serum uric acid and creatinine. These findings suggest that BPA treatment induce deleterious changes in kidney cells. These adverse effects are indicators of the occurring of impairments of kidney functions (Ye et al., 2008, Ahmed et al., 2015). Similarly, Calafat et al. (2005) and Ye et al. (2008) observed in humans that the exposure to BPA led to an increase of urea level than the normal level in 90% of the population worldwide. Also, Ahmed et al. (2015) reported in adult male rats that the treatment with BPA had induced adverse effect on kidney tissues causing an increase levels of urea and creatinine as compared to the untreated group. Shirani et al. (2019) demonstrated in the recent study that the kidney weight in the male rats that received BPA significantly decreased as compared to those found in the control.

Lipid peroxidation(MDA) and antioxidant system of serum in Bisphenol A-intoxicated rats

From the present results, it was seen that activities of SOD, CAT, and ACHE were significantly reduced under the exposure to BPA with respect to normal control. In contrast, the level of MDA in BPA-intoxicated rats was significantly elevated in comparison with those found in untreated rats (control group). These findings are in agreement with that reported in different mammalian cells by Pigeolet et al. (1990); Eid et al. (2015) and Poormoosavi et al. (2018) who observed that the exposure to BPA led to reduction in the antioxidant enzyme activities and increase of MDA level. Also, in studies on rats by Bindhoumol et al. (2003); Hassan et al. (2012 b) and Kourouma et al. (2014); Kamel et al. (2018); Abdel-Rahman et al. (2018) and Ozaydin et al. (2018), the obtained results revealed that the treatment with BPA led to increase of percentages of MDA and decrease of values of SOD, CAT, and ACHE. These studies suggested that the major biochemical influence of BPA is the formation of oxidation systems resulting in hydroxylated metabolites. These hydroxylated metabolites with ROS radicals can cause deterioration in the different metabolic pathways in living cells in which the level of

MDA is increased, but the activities of SOD, CAT, and ACHE are decreased.

Nucleic acid (DNA and RNA) contents and serum protein profile

The present data on rats showed that exposure to BPA led to a significant reduction in the nucleic acid contents. Some studies by Sangai and Verm (2011) and Abdel-Rahman et al, (2018) suggested that during the metabolic processes for BPA in the liver, the intracellular ROS_s are formed. These reactive oxygen species involved superoxide anion (O⁻), hydroxyl radical (-OH) and hydrogen peroxide (H₂O₂). These constituents of radicals interact with cell components such as nucleic acids (DNA and RNA), protein, and lipids inducing a reduction of nucleic acids and protein contents in living cells. Also, Atkinson and Roy (1995) and Collins and Harrington (2002) reported that the reduction of DNA, RNA, and protein contents by exposure to BPA might be due to the formation of DNA adducts as a result of interaction between ROS_s and DNA structure. These adducts inhibit the transcription processes and can prevent the formation of mRNA leading to inhibition of protein synthesis. Also, our findings are in agreement with those observed by Sangai and Verma (2011) who observed that the treatment with BPA in mice induced a significant decrease in DNA, RNA, and protein contents in liver and kidney tissues relative to untreated mice (control group).

Moreover, Shirani et al., (2019) reported that the exposure to BPA caused an excess of ROS and MDA leading to DNA damage in different tissues in the mammalian organs including the brain organ.

Abdel-Rahman et al. (2018) revealed that the elevation of the percentage of MDA in rats due to the treatment with BPA induced increase in comet tail DNA%.

Effect of FCE utilization on biochemical alteration

In the present study, it was found that the BPA-intoxicated rats that received different doses of FCE as protective or therapeutic agents had enhancement in liver and kidney functions than intoxicated control rats. This study showed better results that were seen by utilizing FCE as a protective agent than its use as a therapeutic agent. FCE attenuates the oxidative stress and enhances the levels of activity of antioxidative enzymes such as SOD, CAT, and ACHE. Moreover, it improved the nucleic acids (DNA and RNA) and protein contents in the liver, kidney, and brain tissues. In contrast, FCE treatment induced inhibition of the extent of lipid peroxidation (MDA) and reduced the values of ALT, AST, ALP, and bilirubin as well as it minimized the percentages of urea and creatinine. The present ameliorations of FCE treatment on mentioned biochemical parameters

might be owing to its containing high percentages of natural antioxidants which have important properties as processes of antioxidation and antigenotoxicity (Alam, 2011; Samreen et al., 2014; Salah Eldin et al., 2015; Azam et al., 2018). These processes of antitoxicity caused suppression of the extent of lipid peroxidation (MDA), stimulated the hydroxyl radical scavenging activity, and prevented the formation of DNA adduct (Lam et al., 2012; Salah Eldin et al., 2015; Azam et al., 2018). Moreover, the treatment with FCE was used to cure each of kidney injury (Kamran et al., 2017) and hepatic ailments (Azam et al., 2018). Also, FCE exhibited antimicrobial activity (Sajid et al., 2011), antibreast cancer (Matthew, 2012), antitumor (Alam, 2011) and hemorrhagic inhibitor (Razi et al., 2011).

Conclusion

These findings clarified a new insight into the protective effect of FCE in minimizing BPA-induced genotoxicity and biochemical changes in rats detecting the capability of such medicinal plant for alleviating the adverse effect of BPA.

Abbreviations

ACHE: Acetylcholinesterase; ALPh: Alkaline phosphate; ALT: Alanine transaminase; AST: Aspartate transaminase; BPA: Bisphenol A; CAT: Catalase; FCE: *Fagonia cretica* extract; MDA: *Malondialdehyde*; MNPCE: Micronucleated polychromatic erythrocytes; PCEs: Polychromatic erythrocytes; SOD: Superoxide dismutase

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Ethics approval and consent to participate

Authors declare that the work is ethically approved and consent to participate.

Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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