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Effect of ethanolic extract of *Fagonia cretica* on BPA-induced genotoxicity and histopathology in brain and testis tissues of rats

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Abstract

The present investigation was aimed to evaluate the protective and therapeutic effect of *Fagonia critica* extract (FCE) against Bisphenol A (BPA) induced genotoxicity and histopathology in rats. Genetic study included expression of P450 aromatase and Tph2 genes in the brain tissues in addition to expression of P450 aromatase and COX-1 genes in testis tissues. Histological examination was documented in each of the brain and testis tissues. Our results demonstrated that the exposure to BPA had induced over-expressions of above genes and produced massive damage in histological architectures of brain and testis cells. Treatment with different doses (3.3 g/kg; 4.2 g/kg and 5.0 g/kg) of FCE in BPA-intoxicated rats markedly reduced the genotoxicity and histopathology parameters. These improvements increased by increasing the dose of FCE as a protective or therapeutic agent. Best results were found through utilization FCE as a therapeutic agent, especially by using the highest dose (5.0 g/kg), in which some genotoxic and histopathology parameters had been relatively restored towards the normal level or natural status. This study proved that the treatment with FCE has the capability for alleviating and facing BPA genotoxicity and histopathology. Since the treatment with extraction of this medicinal plant succeeded in overcoming BPA-induced over-expression of P450 aromatase, Tph2 and COX-1 genes, modulating them to approximately normal values and mitigating BPA histopathology status.

Keywords: Bisphenol A; *Fagonia cretica*; Gene expression; histopathology; rats.

Introduction

Bisphenol A (BPA; 4,4 propane-2,2 diyldiphenol) was found to be a synthetic xenoestrogenic chemical that is widespread in our living environment. Where this compound was revealed to be usually used in production of food contact materials such as food containers, protective coatings for canned food and beverages, baby bottles and metal lids on glass jars and bottles as well as for dental composites and sealants [1,2]. In spite of this importance, BPA was observed to be unstable in different environmental conditions such as extension of exposure to sunlight, higher temperature and changed pH of the contact medium. In these states, the polymorphic form of BPA may translate into monomer form by hydrolysis and consequently leach into food and beverages causing adverse health effects related to its endocrine-disrupting activity [3]. These endocrine disruptors might lead to development and/or reproductive disorders and carcinogenic effects through epigenetic events or through genotoxic effects [1]. The adverse effects of BPA had been confirmed and published in several reports of different countries including Canada, US States, Japan and European Union, where these reports clarified the toxic influence of such component on health of infants, children and mature humans. The toxic effect of BPA had been revealed through increase the oxidative stress via the forming of reactive quinone and ROS during biotransformation. These radicals can react with bio-constituents in the cells including DNA, protein and lipids causing various disorders in the body organs [4] including many areas of brain and testis tissues [5]. Also, O-quinone BPA could increase ROS generation and oxidize the guanine moiety of deoxyguanosine in the DNA inducing genotoxicity in the mammalian cells [6]. Moreover, the induction of oxidative stress due

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to exposure to BPA could elevate plasma levels of 8-hydroxyguanosine, increase lipid peroxidation and decrease glutathione activity [7]. The other pathway of BPA metabolism was clarified by Schmidt et al. [8] who reported that the hydroxylation could be induced in one of BPA symmetric phenyl rings forming catechol and o-oH forms. These forms can be oxidized to o-quinone BPA that react with DNA causing DNA adduct. Furthermore, many studies reported the modulation effect of BPA on the expression of genes responsible for preventing the oxidative activity where this effect was confirmed to reduce the expression of antioxidant genes causing generation of different species of free radicals inducing impairment of biological functions in cytological constituents [9]. The coincidence of this direction was demonstrated by Hassan et al. [10] who revealed that the BPA treatment in rats with various doses (0.1, 1.0, 10 and 50 mg/kg) significantly decreased the expression levels of antioxidant genes including glutathione transferase (GST) and glutathinone reductase (GR) in liver tissues. The decreased expressions of such genes were found to be increased by increasing the dose levels of BPA causing decrease of reduced glutathione (GSH) and superoxide dismutase (SOD) and increase of TBARS and nitric oxide (NO) levels. The exposure to BPA led to elevate the expressions of some genes such as Ho-1 and GADD 45 B, their over-expressions were responsible for increasing oxidative activity and affect stopping cell cycle survival, apoptosis and DNA repair in different mammalian cells [2,11]. Therefore, in the light of the above mentioned reports on the BPA toxicity, it must perform suitable investigations to face the deleterious effects of such component on human and animal health. Traditionally, the use of medicinal plants or herbs is preferred to face the hazardous effects of various toxicants. Where the utilization of these plants are benefit to living organisms without induction of the side ailments [12]. *Fagonia critica* (FC) plant is a species of Fagonia genus of family Zygophyllaceae that has been identified and documented to be an important medicinal herb. In Egypt, this species was found to be cultivated in the region of Mediterranean Coast especially in western littoral Zone [13]. The extracts of FC were exhibited to have a number of essential antioxidants that had been demonstrated to be a potent free radical against reactive oxygen and nitrogen species. These constituents contain carbohydrates, polyphenol flavonoid and compounds, triterpenoidal glycosides, saponins, alkaloids, steroids, proteins and amino acids, cyanogenic glycosides, coumarins, sulphates, anthraquinones and trace elements [12,14]. Moreover, this medicinal herb has been extensively used in the remedy of various abnormal types of hepatic, haematological and neurological conditions as well as for inflammatory and reproductive diseases [12,15]. On the other hand, some investigations demonstrated that FC treatment had capability to modulation the expression of a number gene that had been detected to prevent or induce some diseases. For example, Lam et al. [16] identified in the two phenotypically distinct breast cancer lines, MCF-7 and MDA-MB-231 cells, that FC treatment could reduce the expression of P53 gene and enhance the expression of FOXO3a gene. These appropriate modulations of such gene expressions had been found to be correlated with activation of DNA damage response causing cell cycle arrest and apoptosis. Also, Rawal et al. [15] found that in rat hippocampal slices subjected to oxygen-glucose deprivation (OGD), FC treatment could elevate the expression of antioxidant genes (gamma-glutamylcystein ligase and Cu-Zn SOD), diminish the expression of oxidation gene (iNOS) and increase the reduced glutathione (GSH) level that was observed to be associated with regulation the expression of several anti-inflammatory genes. These favorable modulations of such gene expressions had induced significant decrease of oxidative stress via direct scavenging the reactive oxygen and nitrogen species and clarified that FC has neuroprotective properties. Although, the treatment with FC extracts has been reportedly used as a remedial agent in a wide variety of human and animal diseases and ailments, however specific investigations with utilizations the FC against the deleterious effects of toxicants still few. So, the aim of the present work was to evaluate the modulatory role of ethanolic extract of *Fagonia cretica* on BPA-induced genotoxicity and histopathology in brain and testis tissues of rats. The genetic study included the assay of expression of P450 aromatase and Tph2 genes in brain tissues and expression of P450 aromatase and COX-1 genes in testis tissues. Also, the histological examinations in such tissues had been investigated.

Experimental

1. Chemicals:

Bisphenol A with purity greater than 99% was obtained from Sigma Chemical Company (St. Louis, MO, USA), a total of 5g of BPA (water-insoluble powder) was dissolved in 500 ml of corn oil (as 1 % v/w) for daily uptake by gastric gavages to rat animals.

1.1- The doses of BPA:

A dose of 10 mg/Kg b.w. had been chosen based on the National Toxicology Program, 1985, 2008.

1.2- Fagonia plant material:

Fagonia cretica L. had been obtained from Burg El Arab desert region and identified in the Horticultural Crop Technology Department, National Research Centre. The freshness of aerial parts of the collected

plant had been rinsed with distilled water and kept under shade till drying.

1.3- Ethanolic extract preparation of FC:

Ethanolic extract of FC was performed by a simple maceration process according to Hussain et al. [17]. The plant aerial parts were ground and merged in 3.5 L ethanol. The homogenate was kept at room temperature (25 ± 2°C) in sterilized and clean bottle for 4 weeks. The mixture was filtered twice, the first one was done by using ordinary filter paper, then the second time was made by using Whatman-41 filter paper. Ethanol was completely evaporated at room temperature. Totally, 21g dried ethanolic extract of aerial parts had been obtained.

2. Animals:

The experimental animals that used in this work were male Sprague-Dawley rats with body weight of about 120 gm. These animals were provided from Animal House, National Research Centre, Egypt. The rats were housed in an ambient temperature of 25±3.2 °C on a light/dark cycle of 12/12h. All animals were kept in clean polypropylene and they had access to food and water ad libitum. The all procedures were strictly in accordance with the ethical guidelines of the Medical Ethical Committee of the National Research Centre in Egypt (IAEC, 2010) [18].

2.1- Experimental design:

In the present study, 63 rats were used and divided into 9 equal groups (7 animals each) as follows:

- Control group (G1): The animals in this group fed only on a basal diet for 3 weeks.
- Oil group (G2): This group had been administrated on a basal diet and gavages (orally) corn oil (10 mg/kg. B.w.) daily for 3 weeks.
- BPA group (G3): In this group, the rats received orally soluble BPA at a dose of 10 mg/kg. B.w. daily for 3 weeks.
- Protection groups (G4-6): The rats received BPA in the same dose and way previously mentioned. At the first day of BPA administration, the rats had been received *Fagonia cretica* extract (FCE) as (3.3g/Kg, 4.2g/Kg, and 5.0 g/kg, respectively) orally and daily for 3 weeks and used for evaluating the protective role of FCE against BPA toxicity.
- Remedy groups (G7-9): The animals in these groups received BPA in the same dose and way previously mentioned and for the same period. Then the rat groups were administrated FCE as (3.3 g/ kg, 4.2 g/kg, and 5.0 g/Kg, respectively) for 10 days. These groups had been used for evaluating the remedy role of FCE against BPA toxicity.

3. Assay of gene expression:

3.1- Collection of brain and testis samples:

Brain and testis samples were collected from all rats and immediately stored at -80^{o}C till RNA extraction from tissues.

3.2- RNA Extraction:

Total RNA had been extracted from brain and testis samples using the standard TRI zol extraction method (Invitrogen, Germany) according to the manufacturer's instructions. The concentration of total RNA was measured at 260 nm using a spectrophotometer. The purity of total extracted RNA was assessed spectrophotometrically by the 260/280 nm ratio and was between 1.8 and 2.1. Moreover, integrity of total extracted RNA was verified with ethidium bromide-stain analysis of 28S and 18S bands using formaldehyde-containing agarose gel electrophoresis. RNA aliquots were either used immediately for reverse transcription (RT) or stored at -80°C.

3.3- Complementary DNA (cDNA) Synthesis:

Total RNA was reverse transcribed using oligo (dT) primer (Revert AidTM First strand cDNA Synthesis Kit) according to the manufacturers' instructions. Total volume of 20 µl reaction mixture was prepared as using 5µg of the total RNA and oligo-dT primer. The reaction tubes containing RT preparations had been flash-cooled in an ice chamber until being used for DNA amplification through semi-quantitative RT-PCR [19,20]. Specific primers were used for evaluating the expression of P450 aromatase, Tph2 and COX-1 genes in the brain and testis tissues as well as β-actin gene and designed according to the genomic sequence available in GenBank (http://frodo.wi.mit. Edu/cgi bin/primer 3/ primer 3 www.cgi) and tested using the BLAST program (http/www.ncbi.nlm.nih.gov/BLAST/). β-actin was used to normalize mRNA levels of the target genes (Tables 1 and 2).

3.4- Reverse transcription Polymerase Chain Reaction (RT-PCR):

The first strand cDNA was used as a template for semi-quantitative RT-PCR in a 25 µl reaction volume that consisted of 10 mM dNTP's, 50 mM MgCl2, 10x PCR buffer, l U µL Taq polymerase, 0.5 µL of 0.2 µM sense primer, 0.5 µL of 0.2 µM antisense primer and autoclaved water. A program of the reaction was allocated as follows: Incubation at 95°C for 3 min followed by 40 cycles, each cycle forms of denaturation at 95°C for 15 sec, annealing at 55°C for 30 sec and extension at 72°C for 30 sec. The final extension was performed at 72^{o}C. PCR products were tested on 1 % agarose gel. The values of RT-PCR of each gene were normalized and determined on the bases β-actin gene.

Table 1: Primers used for PCR amplification for some genes in brain tissues:

Gene	Primer sequences (5'-----'3)	
P450 aromatase	F- TGAGAAGAACGTTCCCTACAG	R- TCCTCATCTAGATGCAAGGAC
Tph2	F- CTCCAAGCTTCGCATCACAG	R- AGCACTTCAGGAAGCGTACC
β-actin	F- TCGTGCGTGACATTAAAGAG	R- ATTGCCGATAGTGATGACCT

Table 2: Primers used for PCR amplification for some genes in testis tissues:

Gene	Primer sequences (5'-----'3)	
P450 aromatase	F- GCCTGTCGTGGACTTGGT	R- GGTAAATTCATTGGGCTTG
COX-1	F- TTGCACAACACTTCACCCACCAG	R-AAACACCTCCTGGCCCACAGCCAT
β-actin	F- TCGTGCGTGACATTAAAGAG	R- ATTGCCGATAGTGATGACCT

4. Histological assessment studies:

Samples of all animals were dissected immediately after death and fixed at 10% neutral-buffered formalin saline for 72 hours at least. All the specimens were washed in tap water for half an hour and then dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin. Serial sections of 6μm thick were cut and stained with haematoxylin and eosin for histopathological investigation.

5. Statistical analysis:

Gene expression data was analyzed using GelquantNet program to evaluate the thickness of PCR product bands and analyzed using the General Liner Models (GLM) procedure of Statistical Analysis System [21] followed by Scheffe-test to assess significant differences between groups. The values are expressed as mean ± SEM. All statements of significance were based on probability of ($P \leq 0.05$).

Results

1. Gene expression analysis of P450 aromatase and Tph2 genes in brain tissues:

The results of P450 aromatase and Tph2 gene expressions in brain tissues were summarized in Figures 1 and 2, respectively. The expressions of such genes were verified using semi-quantitative RT-PCR in male rat brain tissues for the treatment with BPA. The effect of different doses of *Fagenia cretica* extraction (FCE) as a protective or therapeutic agent on the expression of P450 aromatase and Tph2 genes was examined. These gene expressions were detected successfully in all brain tissues within all treated groups and normalized with the expression of the housekeeping β-actin gene. The rat group that treated with BPA showed over-expressions with highly significance in P450 aromatase ($P<0.00001$) and Tph2 ($P<0.01$) genes as compared to normal control. Whereas, the treatment with FCE doses as a protective or therapeutic agent was observed to be significantly inhibited the up-regulation of the gene expressions produced from BPA treatment. These ameliorations of the expression levels were found to be increased by increasing the dose of FCE.

Moreover, the utilization of FCE as a therapeutic agent established the best results especially by using the highest dose (5.0 g/kg), where the expression of Tph2 gene of such dose relatively recovered to its normal expression comparable to the control group. Furthermore, the use of the highest dose of FCE (as a therapeutic agent) caused more reduction of over-expressions of P450 aromatase gene produced by BPA as compared to those observed by using low (3.3 g/kg) or medium (4.2 g/kg) doses of FCE.

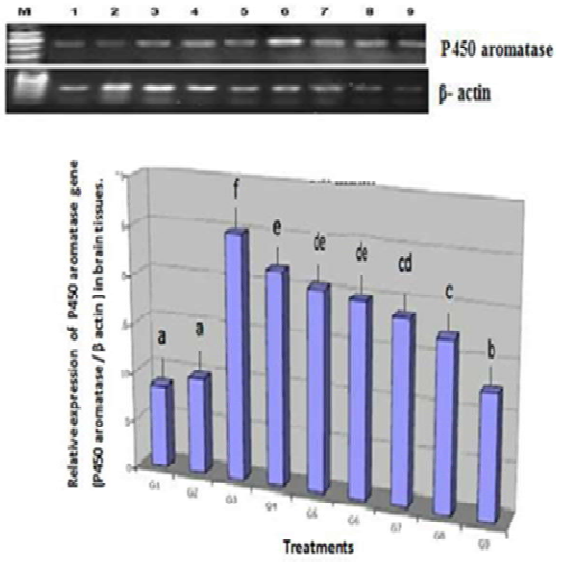

Figure 1: Gene expression of P450 aromatase gene in brain tissues of male rats treated with *Fagonia cretica* extract (FCE) against BPA determined by semi-quantitative RT-PCR. The recovery rate of mRNA was estimated as the ratio between the intensity of P450 aromatase gene and the β-actin gene. Means with different letters are significantly

different (P<0.05). M= DNA marker. Lane 1= control. Lane 2= solvent (oil). Lane 3= BPA. Lane 4= BPA+ low dose of FCE. Lane 5= BPA + medium dose of FCE. Lane 6= BPA + high dose of FCE. Lane 7= BPA then low dose of FCE. Lane 8= BPA then medium dose of FCE. Lane 9= BPA then high dose of FCE.

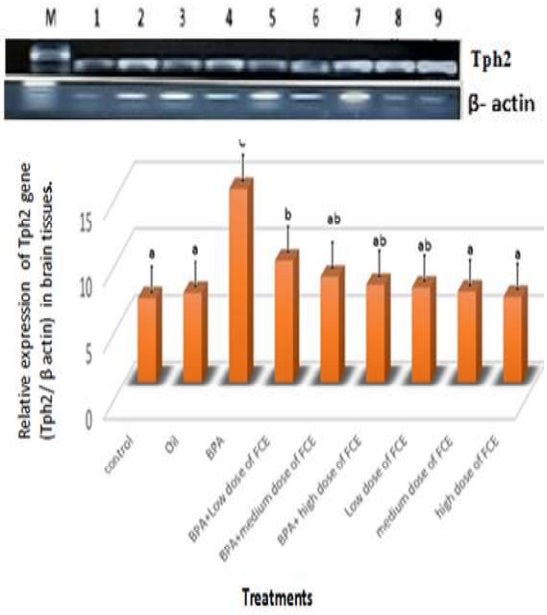

Figure 2: Gene expression of Tph2 gene in brain tissues of male rats treated with *Fagonia cretica* extract (FCE) against BPA determined by semi-quantitative RT-PCR. The recovery rate of mRNA was estimated as the ratio between the intensity of Tph2 gene and the β-actin gene. Means with different letters are significantly different (P<0.05). M= DNA marker. Lane 1= control. Lane 2= solvent (oil). Lane 3= BPA. Lane 4= BPA+ low dose of FCE. Lane 5= BPA + medium dose of FCE. Lane 6= BPA + high dose of FCE. Lane 7= BPA then low dose of FCE. Lane 8= BPA then medium dose of FCE. Lane 9= BPA then high dose of FCE.

2. *Gene expression analysis of P450 aromatase and COX-1 genes in testis tissues:*

The results of the P450 aromatase and COX-1 gene expressions in testis tissues were summarized in Figures 3 and 4, respectively. The obtained results demonstrated that the rat group that treated with BPA had higher significant (P<0.0001) of the expression level of P450 aromatase and COX-1 gene as compared to normal control. In contrast, the treatment with different doses of FCE as a protective or therapeutic agent significantly minimized the up-regulations of P450 aromatase and COX-1 gene expressions that were produced with BPA treatment alone. The only exception to this, the reduction of over-expressions of P450 aromatase gene by using low dose of FCE (as a protective agent) was not significant.

These improvements in expression levels of P450 aromatase and COX-1 genes were found to be increased by increasing the doses of FCE (as a protective or therapeutic agent). Moreover, the utilization of FCE as a therapeutic agent gave the best results, especially by using the highest dose (5.0 g/kg), where this dose caused more reduction of over-expressions (that were produced by treatment with BPA) of such genes than those found by using low or medium doses.

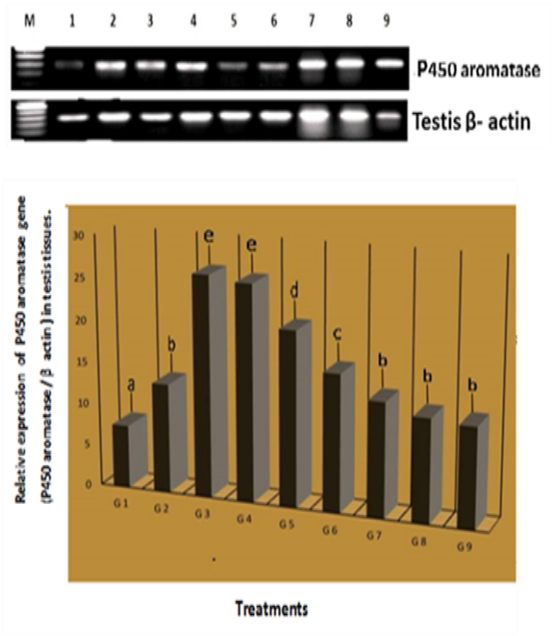

Figure 3: Gene expression of P450 aromatase gene in testis tissues of male rats treated with *Fagonia cretica* extract (FCE) against BPA determined by semi-quantitative RT-PCR. The recovery rate of mRNA was estimated as the ratio between the intensity of P450 aromatase gene and the β-actin gene. Means with different letters are significantly different (P<0.05). M= DNA marker. Lane 1= control. Lane 2= solvent (oil). Lane 3= BPA. Lane 4= BPA+ low dose of FCE. Lane 5= BPA + medium dose of FCE. Lane 6= BPA + high dose of FCE. Lane 7= BPA then low dose of FCE. Lane 8= BPA then medium dose of FCE. Lane 9= BPA then high dose of FCE.

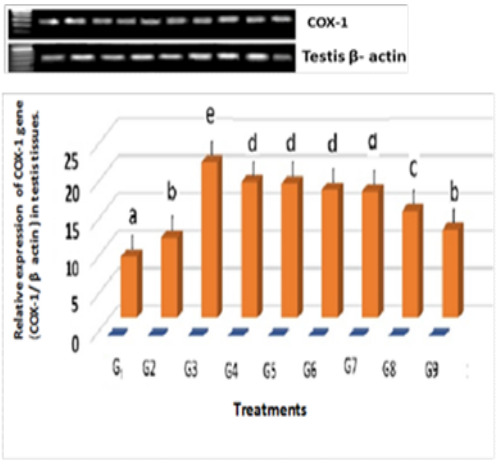

Figure 4: Gene expression of COX-1 gene in testis tissues of male rats treated with *Fagonia cretica* extract (FCE) against BPA determined by RT-PCR. The recovery rate of mRNA was estimated as the ratio between the intensity of COX-1 gene and β-actin gene. Means with different letters are significantly different ($P<0.05$). M= DNA marker. Lane 1= control. Lane 2= solvent (oil). Lane 3= BPA. Lane 4= BPA + low dose of FCE. Lane 5= BPA + medium dose of FCE. Lane 6= BPA + high dose of FCE. Lane 7= BPA then low dose of FCE. Lane 8= BPA then medium dose of FCE. Lane 9= BPA then high dose of FCE.

3. Histological results

Histological results were shown at figures from 5 to 7 (on brain tissues) and from 8 to 10 (on testis tissues). Brain tissues included cerebral cortex, hippocampus and cerebellum sections. Figure 5 shows the deleterious effect of BPA treatment (C) on cerebral cortex cells, while ameliorative role of FCE at different doses as protective agents (low, medium and high doses as described in D, E and F, respectively) or as therapeutic agents (low, medium and high doses as presented in G, H and I, respectively) on such area.

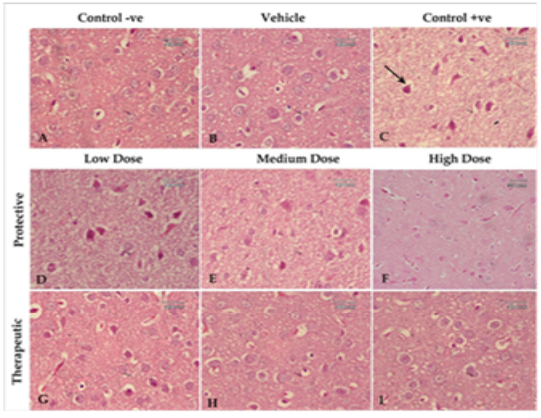

Figure 5: A photomicrograph of cerebral cortex sections from brain tissues of male rats treated with BPA + FCE (as a protective agent in D, E and F) or male rats treated with BPA and then FCE (as a therapeutic agent in G, H and I). In (A) Control -ve rat shows the normal cells with large vesicular nuclei in this area of brain. In (B) vehicle treated rat shows a close to normal structure. In (C) A bisphenol treated rat shows many neurons with deeply stained, smaller than normal nuclei (arrow). In (D) A rat treated with bisphenol + FCE (low dose) shows many cells with small deep nuclei are still observed. In (E) A rat treated with bisphenol + FCE (medium dose) shows a noticeable decrease in affected cells. In (F) A rat treated with bisphenol + FCE (high dose) shows only a few affected cells are seen, although many cells appear with smaller nuclei than normal. In (G) A rat treated with bisphenol and then FCE (low dose) shows some cells appear with small deeply stained nuclei, while rest of cells are normal. In (H) A rat treated with bisphenol and then FCE (medium dose) shows most of cells appear normal. In (I) A rat treated with BPA and then FCE (high dose) shows a quite normal cerebral cortex tissue.

Also, Figure 6 shows the deleterious effect of BPA treatment (C) on hippocampus cells and ameliorative role of FCE at different doses as protective agents (low, medium and high doses as described in D, E and F, respectively) or as therapeutic agents (low, medium and high doses as showed in G, H and I, respectively) on such area.

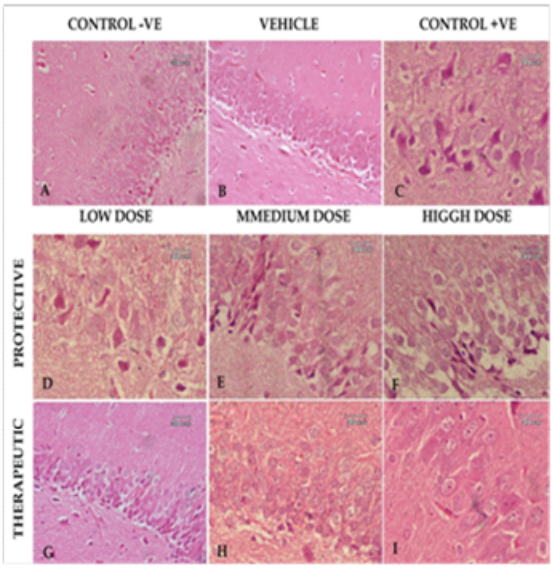

Figure 6: A photomicrograph of hippocampus sections from brain tissues of male rats treated with BPA + FCE (as a protective agent in D, E and F) or male rats treated with BPA and then FCE (as a

therapeutic agent in G, H and I). In (A) Control -ve rat shows normal cells arranged in many layers in this area of brain. In (B)A vehicle treated rat shows a hippocampal area that is close to normal. In (C) A bisphenol treated rat shows disorientation of cell layers with many neurons having deeply stained nuclei. In (D) A rat treated with bisphenol + FCE (low dose) shows some cells with small deep nuclei are still observed. Disarrangement of cells is still present. In (E) A rat treated with bisphenol + FCE (medium dose) shows some affected cells are still observed. Disorganization of cells is less marked. In (F) A rat treated with bisphenol + FCE (high dose) shows only a few cells with deep nuclei are observed in well-arranged cell layers. In (G) A rat treated with bisphenol and then FCE (low dose) shows some cells appear with small deeply stained nuclei in the area. No disarrangement is seen. In (H) A rat treated with bisphenol and then FCE (medium dose) shows most of cells appear normal. In (I) A rat treated with bisphenol and then FCE (high dose) shows quite normal well-organized cell layers.

Moreover, Figure 7 shows the deleterious effect of BPA treatment (C) on cerebellum cells and ameliorative role of FCE at different doses as protective agents (low, medium and high doses as described in D, E and F, respectively) or as therapeutic agents (low, medium and high doses as presented in G, H and I, respectively) on such area.

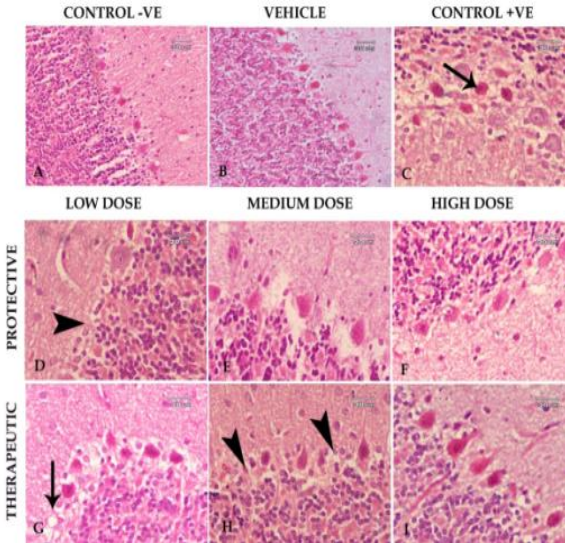

Figure 7: A photomicrograph of cerebellum sections from brain tissues of male rats treated with BPA + FCE (as a protective agent in D, E and F) or male rats treated with BPA and then FCE (as a therapeutic agent in G, H and I). In (A) Control -ve rat shows normal Purkinje cells arranged in a layer separating between molecular and granular layers. In (B) A vehicle treated rat shows a hippocampal area that is close to normal. In (C) A bisphenol treated rat shows many Purkinje cells with deeply acidophilic small nuclei (arrow). In (D) A rat treated with bisphenol + FCE (low dose) shows depletion of cells at some places (arrowhead). In (E) A rat treated with bisphenol + FCE (medium dose) shows increase in number of Purkinje cells. In (F) A rat treated with bisphenol + FCE (high dose) shows normal Purkinje cells. In (G) A rat treated with bisphenol and then FCE (low dose) shows small Purkinje cells with areas of depleted cells (arrow). In (H) A rat treated with bisphenol and then FCE (medium dose) shows increase in size of Purkinje cells. Areas of depleted cells are still observed (arrowhead). In (I) A rat treated with bisphenol and then FCE (high dose) shows normally shaped and size Purkinje cells with their dendrites.

In testicular tissues, histological examination had been performed on spermatogenic cells arranged in the seminiferous tubules. Figure 8 (C, D and E) shows the deleterious effect of BPA treatment on spermatogenic cells and seminiferous tubules. Whereas, Figure 9 shows the ameliorative role of FCE at different doses as protective agents (low, medium and high doses as described in A, B; C, D; and E, F; respectively). Also, Figure 10 shows the ameliorative role of FCE at different doses as therapeutic agents (low, medium and high doses as presented in A, B; C, D; and E, F; respectively).

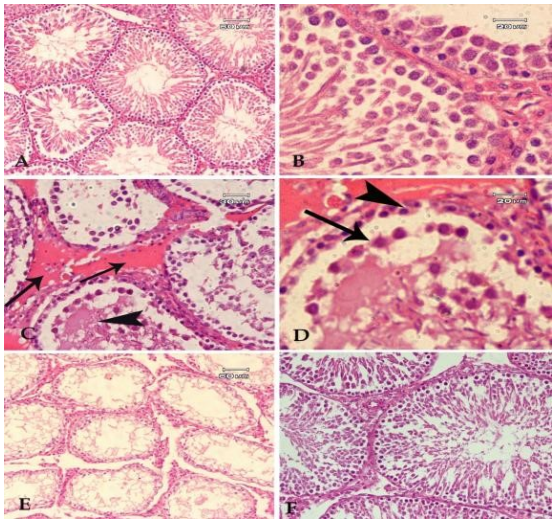

Figure 8: A photomicrograph of sections from testicular tissue of male control rats, rats treated with BPA, and rats received corn oil: In (A) In A section of control -ve group shows the normal structure of the testis and the seminiferous tubules. In (B) A higher magnification for the previous section shows

the different types of spermatogenic cells arranged in the seminiferous tubules. In (C) A section from a rat treated with bisphenol shows increased connective tissue component between the seminiferous tubules (arrow) and necrotic material in the lumen of tubules. In (D) A higher magnification for the previous section shows dark small nuclei of basal spermatogenic cells (arrowhead) and detached primary spermatocytes (arrow). In (E) another section of the same group shows severe damage to spermatogenic cells. The seminiferous tubules appear depleted from cells. In (F) A section from a rat treated with corn oil shows normal structure of testis.

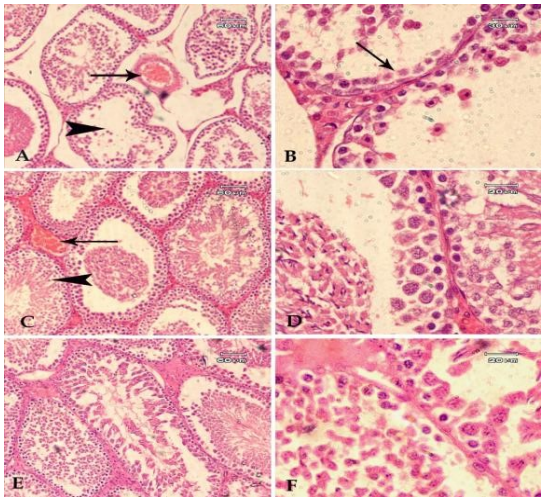

Figure 9: A photomicrograph of sections from testicular tissue of male rats treated with BPA combined with FCE (as a protictive agent). In (A) A section of rat treated with bisphenol + FCE (low dose) shows severe damage of seminiferous tubules, many of them are depleted (arrowhead). Dilated and congested blood vessels (arrow) are observed in increased connective tissue. In (B) A higher magnification for the previous section shows only basal spermatogenic cells (arrow) are attached to the basement membrane, the other cell layers are detached away. In (C) A section of rat treated with bisphenol + FCE (medium dose) shows some seminiferous tubules appear normal (arrowhead). Decrease of connective tissue is detected, but slightly dilated blood vessels (arrow) are still observed. In (D) A higher magnification for the previous section shows normal basal layer and primary spermatocytes, but the other layers are still not detected. In (E) A section of rat treated with bisphenol + FCE (high dose) shows most of the seminiferous tubules appear with normal cells although they are disorganized. In (F) A higher magnification for the previous section shows the same result.

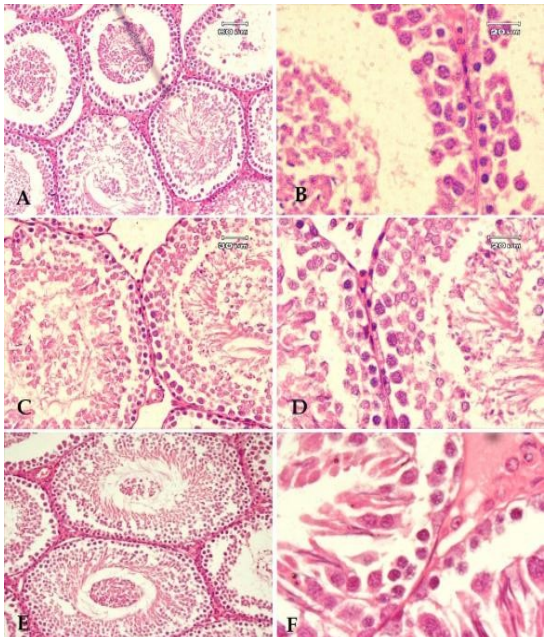

Figure 10: A photomicrograph of sections from testicular tissue of male rats treated with BPA and then FCE (as a therapeutic agent). In (A) A section of rat treated with bisphenol and then FCE (low dose) shows some tubules with detached cells (arrowhead) and other normal tubules. The connective tissue component is markedly decreased with no dilated blood vessels. In (B) A higher magnification for the previous section shows normal basal spermatogenic and primary spermatocytes, while the other cell layers are detached away. In (C) A section of rat treated with bisphenol and then FCE (medium dose) shows seminiferous tubules with many layers of spermatocytes, although they appear disorganized. In (D) A higher magnification for the previous section shows the same results. In (E) A section of rat treated with bisphenol and then FCE (high dose) shows completely normal seminiferous tubules. In (F) A higher magnification for the previous section shows the normal cell layers from basal to normal sperms attached to Sertoli cells.

Discussion

1. Genetic study:

The present study was conducted on three genes P450 aromatase, Tph2 and COX-1 that their expressions were found to have an important role for regulating the function activity in the brain and testis cells. The expression of P450 aromatase gene was studied in the brain and testis tissues, Tph2 gene expression was examined in the brain, whereas, COX-1 expression was evaluated in testis tissues. Castro et al. [22] clarified that the expression of P450 aromatase gene in brain tissues is responsible for

synthesizing the gamma-amino butyric acid (GABA) receptor modulators which are essential for prefrontal cortex (PFC) functions where it plays an important role in emotional learning and memory processes. In testis tissues, Castro et al. [23] revealed that this expression of P450 is a major factor in metabolization process for testosterone hormone and its conversion to estradiol. The later hormone had been established to be essential for controlling in reproduction and fertility processes.

Tryptophan hydroxylase-2 (Tph2) gene is one of two isoforms of tryptophan hydroxylases and specifically expressed in brain and responsible for synthesizing and regulating the serotonin (5-HT) neurotransmission, where the 5-HT system is essential for prefrontal cortex (PFC) function and it was closely related to stress response that is included in the pathophysiology of a wide spectrum of somatic and mental disorders [22,24].

Cyclooxygenase (COX-l) was found to be an ubiquitous in most body tissues including reproductive ones that express it [25,26]. COX-1 in testis cells was clarified to be a key enzyme in the conversion of polyunsaturated fatty acids (PSFA) and arachidonic acid to prostaglandin (PG) especially in the sperm acrosome reaction. The expression of COX-1 gene and PG were demonstrated to be important factors in the regulating of sperm metabolism and consequently the fertilization processes [26,27].

Therefore, the evaluation of the expression of previously genes in brain or testis tissues of the present study are considered to be the good markers for diagnosis of pathogenesis cases due to exposure to abnormal conditions and also they are the best targets for assaying the thereby or the protection of medicinal herbs in such disorder cases.

1.1- The effect of BPA treatment on gene expressions:

The results of the present investigation revealed that the expressions of P450 aromatase and Tph2 genes in brain tissues of adult rats treated with BPA were significantly higher ($P<0.0001$ or $P<0.01$) than those observed in the normal control. Our findings are in agreement with that reported by Castro et al. [22] who clarified that in adult rats treated with BPA caused up-regulation of the expression of both aromatase 450 and Tph2 genes in prefrontal cortex (PEC) of males and females leading to reduction of synthesis of GABA neurotrasmission and inducing physiopathology cases. The exposure to BPA increased the expression of P450 aromatase gene in other brain areas causing abnormalities of such organ tissues [28,29]. Further, Kitraki et al. [30] showed that the exposure to BPA could affect the expression of FK bp 5 gene and its methylation, clarifying its effective role on stress responses in the cells. Lan et al. [31] reported that BPA treatment could stimulate mitogen-activated protein kinase (MAPK) signaling pathway causing up-regulation of expression of different cancer genes.

Moreover, the expression of the Tph2 gene was found to be responsive in diversity environmental factors that include exogenous drugs or chemicals and variety diets. In this respect, Hageman et al. [32] revealed that high-fat diet (HFD) potently caused alterations in Tph2 mRNA expression of mouse adipose tissues. Also, Sullivan et al. [33] showed that maternal consumption of HFD during pregnancy induced over-expressions of Tph2 gene in the rostral raphe of nonhuman primate offspring.

Furthermore, in BPA-intoxicated adult rats, significant reduction of nucleic acid (DNA and RNA) contents in brain, liver and kidney tissues was clarified as compared to those of the normal control, and these results may indicate that there are alterations in the expressions of various genes in such tissues [34].

In the present study, the expressions of P450 aromatase and COX-1 genes in testis tissues of adult rats treated with BPA were significantly higher ($P<0.000l$) as compared to those found in the normal control. To our knowledge, the present results were observed to be the first report that demonstrated the effect of BPA on the expressions of P450 aromatase and COX-1 genes in testis cells. However, Bourguiba et al. [35] confirmed that the 450 aromatase transcripts were found in germ cells of adult rats and they were up-regulated by treatment with cyclic adenosine monophosphate (cAMP) or with dexamethasone (Dex). Wisniewski et al. [36] found in pituitary-testicular axis of adult rats that exposure to BPA induced over-expressions of genes of gonadotropin releasing hormone receptor (GnRHt), luteinizing hormone beta (LHb), follicle stimulating hormone beta (FSHb), estrogen receptor beta (ER2) and androgen receptor (AR) causing a state of hypogonadotropic hypogonadism.

Moreover, the BPA treatment could induce cytotoxicity or genetic alterations in germ cells, where the exposure to such toxicant led to disruption of each of meiotic process in human oocyte maturation in vitro and in meiotic progression during spermatogenesis of adult rats [37,38]. Concerning the alterations in the expression of COX-1 gene, some studies in abnormal conditions showed that the up-regulation of the COX-1 gene expression might be induced and the progressive PG imbalance had been revealed causing increase of oxidative stress with a consequent damage of mitochondria and nuclear sperm DNA and leading to fertility disorders, over-expressions of such gene in testicular cancer cells as compared to normal tissues were demonstrated [26,39]. Also, in diabetic and varicocele human sperm, Perrotta et al. [26] confirmed up-regulation

off COX-1 gene expression with respect to the healthy human sperms.

Biological mechanical processes of BPA for its deleterious effect on body cells had been explained by many investigations, where these works showed that the exposure to BPA compound lead to adverse biological effects through the alterations of endocrine signaling pathways causing pathogenesis cases of adult brain, sexual abnormalities, cognitive functions and infertility [40]. Also, several studies demonstrated that the BPA during its biotransformation could generate certain reactive radical species, especially reactive oxygen species (ROS) and quinones. These radicals can react with DNA, causing DNA damage and leading to genotoxic effects that include gene expression changes [1,8,22]. Moreover, during biotransformation of BPA, one of its symmetric phenyl rings is hydroxylated forming its catechol, o-OHBPA that can be oxidised to form o-quinones. O-quinones could elevate ROS formation and oxidise the guanine moiety of deoxy-guanosine in the DNA inducing DNA adduct and consequently lead to gene expression alterations [6,41].

1.2- Ameliorative role of FCE on gene expressions:

The present results demonstrated that the treatment with FCE could reduce the BPA - induced damage on expressions for some genes in brain and testis tissues. Where FCE treatment especially by using the high therapeutic dose (5.0 mg/kg) was able to significantly down-regulated the over-expressions that produced by BPA of P450 aromatase and TPh2 genes in the brain tissues and P450 aromatase and COX-1 genes in testis tissues. To our knowledge, this study was observed to be the first report that clarified the ameliorative role of FCE on gene expressions of P450 aromatase, Tph2 and COX-1 genes against adverse effect of BPA toxicant in mammalian cells. However, the present findings were supported by report of Rawal et al. [15] who revealed that the expression changes of some antioxidant and oxidation genes in rat hippocampal slices subjected to oxygen-glucose deprivation (OGD) (that discriminated with high oxidation stress), had been improved by FCE treatment. Their results proved that FCE treatment could increase the expression of gamma-glutamylcysteine ligase and Cu-Zn SOD antioxidant genes, reduce the expression of oxidation genes (iNOS), and enhance the reduced glutathione (GSH) level. GSH was found to have a crucial role in the regulation of expression of several redox-sensitive antioxidant and anti-inflammatory genes. These modulatory events of such gene expressions in OGD cases led to reduction of oxidant levels via direct scavenging reactive oxygen and nitrogen species and amelioration of the peroxide scavenging enzyme confirming the neuroprotective properties of FCE, and might be an effective therapeutic tool against ischemic brain damage [15]. Also, Lam et al. [16] found that the FCE treatment has an important role in affecting expression of cancer-related genes. This treatment could enhance the expression of FOXO3a gene and minimize the expression of p53 gene in the two phenotypically distinct breast cancer lines (MCF-7 and MDA- MB-231 cells), causing activation of DNA damage response and leading to arrest and apoptosis. Moreover, Abd-El-Moneim et al. [34] showed in adult rats that exposed to BPA and treated with FCE (as a protective or therapeutic agent) significant improvement in nucleic acid (DNA and RNA) contents in brain, liver and Kidney tissues as compared to those treated with BPA alone, and these results may indicate to amelioration the expressions of various genes in such tissues.

Concerning, the effect of FCE on reproductive conditions, to our knowledge there is no available information clarifies the effect of treatment with such medicinal herb on amelioration the expressions of genes responsible for reproductive processes including COX-1 gene against abnormal conditions. However, Abhirami et al. [42] revealed in male albino rats, that the FCE treatment led to significant androgenic activity represented in increase the weights of both seminal vesicles and ventral prostate as compared to control value.

Furthermore, in other studies, the expressions of P450 aromatase and Tph2 genes were observed to be enhanced through treatments with other protectors such drugs or extractions of different species of medicinal plants. In this direction, Roselli et al. [43] clarified in adult rats that the treatment with testosterone or dihydrotestosterone (DHT) after castration had enhanced the aromatase activity and mRNA levels in specific brain areas (preoptic area and medial basal hypothalamus) as compared to untreated animals. Also, in rat hindbrain, the treatment with Bacopa monniera leaf extract (a traditional Indian herbal medicine plant) had enhanced of Tph2 gene expression with respect to untreated cases [44] Additionally, the treatment with Wuzhuyutang (Evodiae prescription: a traditional Chinese herbal medicine plant) in migraine cases had affected Tph2 promoter activity in PC12 cells [45].

Thus, the importance of FCE in the present study was to improve the expressions of P450 aromatase, Tph2 and COX-1 genes against the deleterious effect of BPA in brain and testis tissues of rats. These improvements might be due to the high constituents of antioxidants in FCE, that are the potent factors for scavenging the free radical of reactive oxygen and nitrogen species and enhancing the various biological functions in animal body [12,14-16].

2- Histology study:

2.1- Effect of BPA treatment on histological architectures:

Our study demonstrated that the BPA treatment had produced massive damage in histological architectures of different cell types of brain and testis tissues:

2.1.1- The effect on brain tissues:

The effects of BPA on brain tissue showed a damaging effect of this compound on many areas of the brain which included cerebral cortex, hippocampus and cerebellum cells that concerned with various memory functions. These results go in agreement with results of Xu et al. [28] who reported that BPA can induce aggression, anxiety, cognitive deficits, and learning-memory impairment. BPA can also influence the display of juvenile social behaviors in mice [46].

Rochester [47] summarized the effects of BPA on many systems when stated that exposure to BPA is linked to cardiovascular disease, brain development abnormalities, obesity, hypertension, thyroid dysfunction, diabetes, breast cancer, infertility, etc., in human, terrestrial and aquatic animals.

2.1.2- Effect of BPA on testicular tissues:

This study showed that the BPA caused markedly damaging effect on the testicular tissue, where in the seminiferous tubules in the form of dark small nuclei of basal spermatogenic cells, detached primary spermatocytes and necrotic material in the lumen had been detected. Outside the tubules, we found increased connective tissue component between the seminiferous tubules. These results can be explained by the findings of Samova et al. [48] who declared that BPA treatment caused a significant decrease in serum testosterone level that was dose dependent in manner. He also reported that the oral administration of BPA causes a reduction in the activity of steroidogenic enzymes that leads to a reduction in the testosterone level. Many experimental studies upon laboratory animals showed that BPA can affect the development of gonads, accessory glands and spermatogenesis by the formation of reactive oxygen species [49,50]. Our results are also in agreement with those of Li et al. [51] who reported that BPA is a chemical with an estrogenic effect that can cause a significant dose-dependent reduction of steroid hormones in the serum of Swiss albino mice after 45 days of treatment.

Reduction in testosterone may cause sexual dysfunction via suppressing spermatogenesis, leading to low sperm count. Degeneration in Leydig cells can also affect the testosterone level [49,50], this effect can be caused through direct action on the interaction between Sertoli cells and germinal line cells [52]. Moreover, BPA is an endocrine disruptor. It interferes with hormone function via estrogenic, anti-androgenic, and anti-thyroid activity [5].

2.1.3- Ameliorative role of FCE on histopathological tissues:

The present histological results revealed that FCE could act against BPA-induced histopathology in the brain and testis tissues in rats by mechanism related to its anti-inflammatory and wound healing properties [12,53]. Also, some studies proved that FCE contains potential anti-cancer and anti-tumour agents that act either singly or in combination, these effects were shown against Agrobacterium strains (in potato disc) or brine shrimps and against breast cancer cell proliferation via DNA damage, cell cycle arrest and apoptosis in such cancer type [16,17].

Furthermore, in FCE treatment, the enhancement of histopathology parameters that showed in the present work might be due to the presence of higher rates of free radical scavengers (against reactive oxygen and nitrogen species) such as saponins, polyphenolic compounds, flavonoids, alkaloids, amino acids, triterpenoids and coumarins, where these constituents have been reported in other studies to reduce the toxic effects of various toxicants including BPA [12,16]. In the same direction, FCE was also found to increase the activities of antioxidant enzymes that offer strong evidence for the antioxidative efficiencies of such medicinal plant against oxidative stress induced by BPA [12,15,16].

Furthermore, FCE was extensively investigated by many studies regarding its medicinal utilization due to its contains a lot of antioxidant constituents, since this plant was antitumor, analgesic, astringent and febrifuge and it was also used for the treatment of thalassemia, fever, asthma, cancer in the indigenous system and neurological and Kidney diseases [12,15,17,53,54].

Conclusions

The present research proved that the treatment with FCE especially in case of its utilization as a therapeutic agent has the capability for alleviating and facing the BPA genotoxicity and histopathology. Since the treatment with extraction of this medicinal plant succeeded in overcoming the BPA-induced overexpression of P450 aromatase, Tph2 and COX-1 genes and modulating them to favourable levels or to approximately normal values. Also, FCE treatment demonstrated its ability for mitigating or curing the BPA histopathology status.

Conflicts of interest

There are no conflicts to declare.

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