Research Article

Ameliorative Role of Ethanolic Extract of *Fagonia cretica* on BPA-Induced Genetic Alterations and Histological Changes in Liver and Kidney Tissues of Rats

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Abstract | In the last decade, bisphenol A (BPA) has received heightened attention because of its ubiquitous presence in our living environment. However, it was revealed that this component has potent genotoxicity, causing various disorders to human and animal health. Hence, the present work is designed to investigate the capacity of *Fagonia cretica* extract (FCE) to inhibit bisphenol A-induced genotoxicity and histopathology in rats. In the genetic study, the assaying of expressions of IkBα and COX-1 genes in liver tissues, as well as the expressions of mdr1a and COX-1 genes in kidney tissues are evaluated. In addition, the histological architectures of these organs are examined. Bisphenol A (BPA) treatment causes over-expressions of the above mentioned genes, and induces massive damage to the histological architectures of liver and kidney tissues. In contrast, FCE treatment with different doses (3.3 g/Kg., 4.2 g/kg. and 5.0 g/kg) can inhibit the upregulation of such gene expressions and enhance the histopathological changes of liver and kidney organs. These ameliorations increase by increasing the dose of FCE, through its utilization either as a protective or therapeutic agent. Using FCE as a therapeutic agent, particularly in the treatment with the highest dose (5.0 g/Kg), produces the best results, where some genotoxic and histopathological parameters are restored to the normal level or natural status. The present investigation confirms the important role of *Fagonia cretica* in overcoming the harmful BPA-induced effects on animal cells, since the extraction of this medicinal herb can modulate the over-expressions of IkBα, mdr1a and COX-1 genes to favorable or normal levels in rats. Moreover, it may be able to markedly ameliorate or remedy the histopathological cases.

Keywords | *Fagonia cretica*, Bisphenol A, Gene expression, Histopathology, Rats

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INTRODUCTION

Pretense of bisphenol A (BPA) in our living environment receives a great attention. This component is used in polycarbonate plastics and epoxy resins production. The resins of BPA revealed to be essential in coating the inside of food and beverage cans, and in dental composites and sealants (Vanderberg et al., 2007; Ye et al., 2009; Cabaton et al., 2011). However, it was observed that the BPA component is unstable in changed PH, in acidic and basic

solutions, and with the extended exposure to sunlight or UV light. Under these conditions, the polymorphic form of BPA may convert to the dangerous monomeric forms released into foods, beverages, or our living environment (Talsness et al., 2009). Thus, most individuals in the general population are widely exposed to BPA, due to its long-term release from food product containers. In addition, every year, hundreds of tons of this component are released into the atmosphere, causing various disorders to human health, due to its toxic effect (Liu et al., 2013).

The toxic effect of BPA is revealed through inducing oxidative stress via generation of certain reactive radicals, such as reactive oxygen species (ROS), quinines, and nitric oxide (NO) (Bindhumol et al., 2003; Moon et al., 2012; Kourouma et al., 2015). These radicals are considered to be cytotoxic agents that cause impairment of prooxidant/antioxidant balance (McMillian et al., 2004; Videla, 2009; Eid et al., 2015; Kazemi et al., 2016; Aghajanpour-Mir et al., 2016).

Moreover, some investigations reported the toxic effect of BPA on human and animal cells, by decreasing the gene expression of antioxidant genes leading to an increased oxidative activity in various body organs (Bindhumol et al., 2003; McMillian et al., 2004; Videla, 2009; Moon et al., 2012; Kourouma et al., 2015). In this light, Hassan et al. (2012) found significant reduction of the expression levels of antioxidant genes, glutathione transferase (GST), and glutathione reductase (GR) in the liver tissues of rats treated with various doses of BPA (0.1, 1.0, 10 and 50 mg/kg). Such reduction of gene expression increased by increasing the dose levels of BPA, causing a decrease in the reduced glutathione (GSH) and superoxide dismutase (SOD), and an increase in TBARS and nitric oxide (NO) levels.

On the other hand, the exposure to BPA demonstrated to be a main cause of the increase in the expressions of oxidative stress-related genes, such as Ho-1 and GADD45B genes, where the over-expression of these genes caused elevating of the oxidative activity, and stopping cell cycle survival, apoptosis, and repairing of DNA in different mammalian cells (McMillian et al., 2004; Liu et al., 2008; Kim et al., 2014; Kazemi et al., 2016).

According to the previously mentioned reports, scientific attempts must be made to face the adverse effects of BPA on human and animal health. Since ancient time, the medicinal herbs or plants have provided natural remedies for human ailments. Moreover, these plants are usually preferred in overcoming the hazardous effects of various toxicants.

Fagonia plant, a genus of family Zygophyllaceae is considered a good medicinal herb. This genus contains about 40 species. *Fagonia cretica* (FC) revealed to be an important medicinal species of the fagonia genus. In Egypt, FC was described and identified by El-Hadidy (1966). It was pointed that this species is restricted to the region of the Mediterranean coast, especially in the western littoral zone (Batanouny and Batanouny, 1970). The characterization of this plant is described as perennial, dark-green, and glabrous with prostrate quadrangular branches. Its leaves are trifoliate on short petioles. Its fruit is about 10 mm wide, 7 mm long, with thick and reflexes peduncles, and deciduous calyx. Its spines are shorter than petioles (Batanouny and Batanouny, 1970). The medicinal properties of this herb may attribute to the number of major phytoconstituents or antioxidant types it possesses, which exhibited strong free radical scavenging capacity against reactive oxygen and nitrogen species. The phytoconstituents include saponins, alkaloids, steroids, carbohydrates, flavonoid, and triterpenoidal glycosides, proteins and amino acids, coumarins, sulphates, anthraquinones, cyanogenic glycosides, and trace elements (Saleh et al., 2011; Kasture et al., 2014; Puri and Bhandari, 2014).

The pharmacological activity and medicinal applications of FC include anticancer, antimicrobial, antiviral, analgesic, anti-inflammatory, and antipyretic, activities as well as applications in; anti-skin diseases and anti-urinary tract infections (Kasture et al., 2014). FC is also used for the treatment of neurological, dendrochronological and hematological disorders (Saeed and Wahid, 2003; Jahala et al., 2014; Kasture et al., 2014), and as remedy to kidneys, liver (Puri and Bhandari, 2014) and thalassemia (Seyal et al., 2013) diseases.

Furthermore, it was found that FC had the treatment and the ability to modulate the expression of some gene that related to avoiding or inducing some diseases. In this respect, Lam et al. (2012) clarified that, in breast cancer, FC treatment could decrease the expression of P53 gene, and increase the expression of FOXO3a genes, where these suitable modulations of gene expression was associated with the activation of DNA damage response, causing cell cycle arrest and apoptosis in two phenotypically distinct breast cancer lines, MCF-7 and MDA-MB-231 cells. In rat hippocampal slices subjected to oxygen-glucose deprivation (OGD), Rawal et al. (2004) mentioned that the FC treatment was effective in elevating the expression of antioxidant genes (gamma-glutamylcysteineligase and Cu-Zn SOD) and minimizing the expression of oxidation gene (iNOS). In addition, it enhanced the reduced glutathione (GSH) level, which had a crucial role in the regulation of expressions of several anti-inflammatory genes. These appropriate modulations of such gene

expressions led to the reduction of oxidant levels, via direct scavenging of the reactive oxygen and nitrogen species, amelioration of the peroxide scavenging enzyme, and proving the neuroprotective properties of FC. Despite the various literatures clarifying the extensive traditional use of FC for the treatment of different diseases and ailments, FC utilization against the dangerous effects of toxicants remains scant.

Therefore, the present investigation aims to evaluate the ameliorative role of ethanolic extract of *Fagonia cretica* on BPA-induced genetic alterations and histopathological changes in liver and kidney tissues of rats. The genetic study involves the assay of gene expression of IkBα and COX-1 genes in liver tissues and Mdrla and COX-1 genes in kidney tissues. The histological examinations of such tissues are also conducted.

MATERIALS AND METHODS

Chemicals

Component of Bisphenol A as a powder state (BPA; 4, 4'-propane-2, 2-diyldiphenol, CAS no. 80-05-7) with purity > 99% was purchased from Sigma (St. Louis, MO, USA). 5g of BPA were dissolved in 500 ml corn oil (as 1% v/w) for daily uptake by gastric gavages to rats.

The BPA doses

According to National Toxicology Program (1985, 2008), a dose of 10 mg/kg. B.w. is chosen.

Preparation of ethanolic extract of fagonia plant

FC plant collected from desert region of Burg El Arab City, Egypt. It was identified in the Department of Horticultural Crop Technology, National Research Centre. The ethanolic preparation of FC extract was carried out according to Hussain et al. (2007), by a simple maceration process as follows: The collected plant, the fresh of aerial parts were rinsed with distilled water and kept under the shade until drying. The dried aerial parts were ground and merged in 3-5 L ethanol, then, in sterilized and clean bottles, the mixture were kept for 4 weeks at room temperature (25±2°C). The mixture was filtered twice, using ordinary filter paper in the first time and through Whatman-41 filter paper in the second time. The ethanol was completely evaporated at room temperature. The quant of 21 g dried ethanolic extract of aerial parts is obtained.

Experimental animals

Sixty-three male Sprague-Dawley rats that have body weight about 120 g were used. The healthy rats were chosen and obtained from Animal House, National Research Centre, Egypt. It housed in an ambient temperature of 25±3.2°C on alight/dark cycle of 12/12h, and kept in clean polypropylene. In addition, they had access to food and water ad libitum. In this work, the animals were anesthetized and slaughtered according to the ethical guidelines of the Medical Ethical Committee of the National Research Centre in Egypt (IAEC, 2010).

Experimental design

The 63 rats were divided randomly into 9 equal groups (7 animals each). These groups included negative control (G1), oil group (G2), BPA group (G3), protective groups (G4 to G6), and remedy groups (G7 to G9). G1 fed on a basal diet for 3 weeks, G2 administrated a basal diet and corn oil (10 mg/kg. B.w.) orally and daily for 3 weeks, G3 received orally with BPA in corn oil at a dose of 10 mg/kg. B.w. daily for 3 weeks, G4 to G6 received BPA, that soluble in corn oil at the same dose orally. At the first day of BPA administration, the animals received FCE (3.3 g/kg., 4.2 g/kg, and 5.0 g/kg, respectively) orally and daily for 3 weeks. These groups were used for evaluating the protective role of FCE against BPA toxicity. In G7 to G9, the rats received BPA soluble in corn oil orally in the same dose, and for the same period followed by FCE (3.3 g/kg., 4.2 g/Kg., and 5.0 g/kg., respectively) for 10 days. The groups (G7 to G9) were used for evaluating the remedy role of FCE against BPA toxicity.

Genetic study

Semi-quantitative RT-PCR

Collection of liver and kidney samples: After animal scarifications, liver and kidney samples were collected from the rats and immediately stored at -80°C until RNA extraction.

RNA extraction

The total RNA is extracted from 50 mg of liver and kidney tissues using TRIzol® Reagent (Invitrogen, Germany) according to the manufacturer instructions. The concentration of RNA is measured spectrophotometrically at A_{260}. Also, the spectrophotometric 260/280 nm ratio was assessed the purity of total extracted RNA and determined between 1.8 and 2.1. To protect RNA from damaging, the aliquots were either used immediately for reverse transcription (RT) or stored at - 80°C.

Complementary DNA (cDNA) synthesis

According to the instructions of the Revert Aid™ First Strand cDNA Synthesis Kit, the complete poly(A)$^+$ RNA isolated from liver and kidney tissues was reverse transcribed into cDNA using 5 µg of the total RNA in a total volume of 20 µl of the reaction mixture (RM). The constituents of the reaction mixture were 50 mM $MgCl_2$, 5x reverse transcription (RT) buffer (50 mM KCL; 10mM Tris-HCL; pH 8.3), 10mM of each dNTP's, 50 µM

Table 1: Primer sequences that used for qRT-PCR amplification of IkBα and COX-1 genes in liver tissues.

Gene	Primer sequences (5'-----'3)	
IkBα	F- GACGAGGATTACGAGCAGAT	R- CCTGGTAGGTTACTCTGTTG
COX-1	F- TTGCACAACACTTCACCCACCAG	R- AAACACCTCCTGGCCCACAGCCAT
β-actin	F- TCGTGCGTGACATTAAAGAG	R- ATTGCCGATAGTGATGACCT

F: Forward R: Reverse

Table 2: Primer sequences that used for qRT-PCR amplification of Mdr1a and COX-1 genes in kidney tissues.

Gene	Primer sequences (5'-----'3)	
Mdrla	F- GGGCCACATGATCAAGACGG	R- AGCGTCATTGGCAAGCCTGG
COX-1	F-TTGCACAACACTTCACCCACCAG	R-AAACACCTCCTGGCCCACAGCCAT
β-actin	F- TCGTGCGTGACATTAAAGAG	R- ATTGCCGATAGTGATGACCT

F: Forward R: Reverse

oligo-dT primers, 20 Uribonuclease inhibitor (50 kDa recombinant enzyme to inhibit RNase activity) and 50 UM-Mu LV reverse transcriptase. The reaction mixture of each sample was centrifuged for 30 sec at 1000g, and transferred to the thermocycler.

Reverse transcription reaction was carried out at 25°C for 10 min, followed by 60 min at 42°C and terminated with a denaturation step at 99°C for 5 min. Consequently, the reaction tubes (or PCR products) containing RT preparation (or cDNA) were kept at -20°C until being used for DNA amplification through semi-quantitative RT-PCR (Farag et al., 2008; Klopfleisch and Gruber, 2009; Eshak et al., 2013, 2015; Aboelhassan et al., 2018).

Primers

The sequences of specific primers (Tables 1 and 2) used for assessing the expression of IkBα, Mdrla, COX-1 genes, and the housekeeping gene β-actin in liver and kidney tissues were designed based on the genomic sequences available in GenBank by the primer program (http://frodo.wi.mit.edu/cgi-bin/primer3/Primer3www.cgi) and tested using the BLAST program (http/www.ncbi.nlm.nih.gov/BLAST/).

Quantitative polymerase chain reaction (PCR)

The first-strand of cDNA was used as a template for RT-PCR with a pair of specific primers. The reaction mixture (RM) for RT-PCR was performed in a volume of 25 µL containing 10 mM dNTP's, 50 mM $MgCl_2$, 10x PCR buffer, 1 µL Taq polymerase, 0.5 µL 0.2 µM sense primer, 0.5 µL 0.2 µM antisense primer and autoclaved water. The reaction program was performed in three steps: PCR tubes in the first step were incubated at 95°C for 3 min. Second step was performed by 40 cycles, each cycle included 3 sub-steps: (a) at 95°C for 15 sec.; (b) at 55.0°C for 30 sec. and (c) at 72.0°C for 30 sec. Third step was conducted by 71 cycles, starting at 60.0°C and the increased about 0.5°C every 10 sec. up to 95.0°C. After that the PCR products (10 µL) were detected on a 2.0% agarose gel with PCR products derived from β-actin. Of the different rat samples, the RT-PCR (sq.RT-PCR) values of each gene were normalized and determined on the bases of β-actin gene.

Histological investigation

Liver and kidney samples of all examined animals had been dissected immediately after scarification followed by fixing in 10%, neutral-buffered formalin saline for 72 hours at least. The specimens were washed in tap water for half an hour. The samples were dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin. Serial sections of 6 um thick were cut and stained with haematoxylin and eosin for histological examinations using light microscope.

Statistical analysis

The present data of gene expressions for IkBα, mdr1a, and COX-1 genes were analyzed using the General Liner Models (GLM) procedure of Statistical Analysis System (SAS, 1982) which followed by Scheffe-test to evaluate the significance between groups. All values were expressed as mean±SEM. The significance statements had been based on probability of ($P<0.05$).

RESULTS AND DISCUSSION

Gene expression results

Analysis of gene expression of IkBα and COX-1 genes in liver tissues: The results of gene expression of IkBα and COX-1 genes in liver tissues were recorded in Figures 1 and 2, respectively. The expressions of such genes were verified in male rat liver tissues for BPA treatment. Moreover, the effect of different doses of *Fagonia cretica* extraction (FCE) as a protective or therapeutic agent on the expression levels of IkBα and COX-1 genes was investigated. The gene expressions of both genes were successfully detected in all liver tissues of all the treated groups, and normalized with the expression of the house-keeping β-actin gene.

Results showed that the rats treated with BPA had over-expression with high significance (P<0.00001), for each of IkBα and COX-1 genes, compared to normal control. On the other hand, the present findings demonstrated that the treatment with FCE doses as a protective or therapeutic agent resulted in significant inhibition of the upregulation of gene expressions induced by BPA treatment. These ameliorations of the gene expression levels were observed to increase by increasing the dose of FCE. Furthermore, the FCE doses that were utilized as a therapeutic agent produced the best results. These results were further clarified using the highest dose (5.0 g/kg), in which the gene expressions of both genes relatively recovered to their normal expressions comparable with the control group.

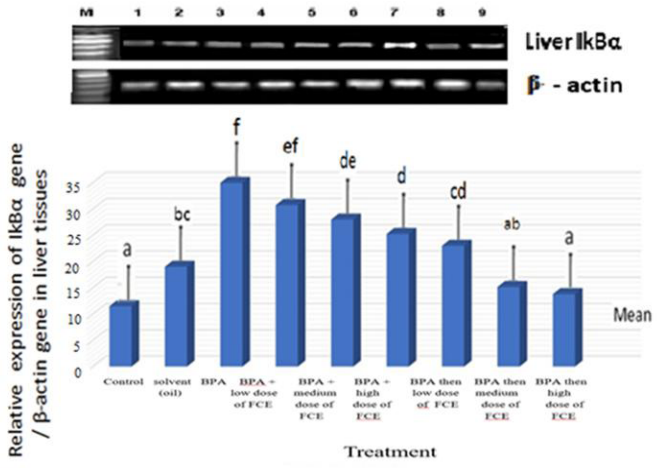

Figure 1: Gene expression levels of IkBα gene in liver tissues of male rats that had been treated with FCE against BPA. These expression levels had been assayed by semi-quantitative RT-PCR. Recovery rate of mRNA was estimated as the ratio between the intensity of IkBα gene and the β-actin gene. The means with different letters are significantly different (P<0.05). M=DNA ladder. Lane 1=normal 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.

Analysis of gene expression of mdr1a and COX-1 genes in kidney tissues

As reported in the previous liver results, semi-quantitative RT-PCR was also used to assay the expressions of mdr1a and COX-1 genes in kidney tissues of male rats. These gene expressions examined for BPA and FCE (as a protective or therapeutic agent) treatments. In all kidney tissues of all the treated groups, the expressions of mdr1a and COX-1 genes (Figures 3 and 4, respectively) were successfully clarified and normalized with the expression of the house-keeping β-actin gene. These results revealed that BPA treatment caused over-expression with high significance for mdr1a (P<0.000001) and COX-1 (P<0.00001) genes, with reference to the normal control. Contrastingly, the FCE treatment with different doses (as a protective or therapeutic agent) significantly minimized the upregulation of gene expressions of both of the mentioned genes, which produced by BPA treatment alone. However, the only exception was the minimization of the upregulation of COX-1 gene expression using low dose of FCE (as a protective agent), which was not significant in compared to BPA treatment alone. The improvements in expression levels of mdr1a and COX-1 genes were increased by increasing the doses of FCE as a protective or remedy agent. These improvements were pronounced when utilizing the FCE treatment as a remedy agent giving the favorable results. Additionally, the highest dose (5.0 g/kg) of FCE resulted in the best results, since this dose caused further minimization in the over-expression induced by BPA treatment alone, than observed using low or medium doses.

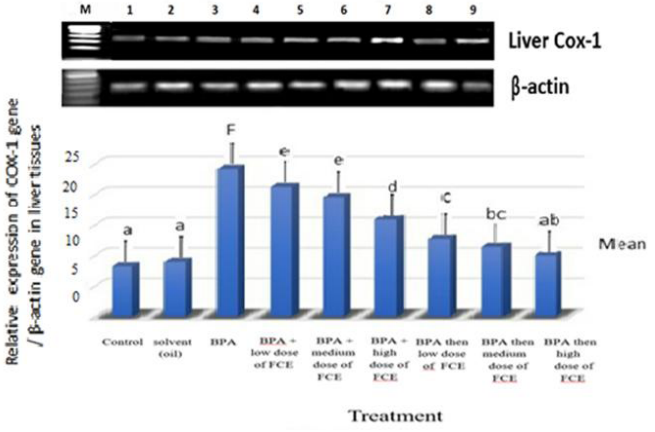

Figure 2: Gene expression levels of COX-1 gene in liver tissues of male rats that had been treated with FCE against BPA. These expression levels had been assayed by semi-quantitative RT-PCR. Recovery rate of mRNA was estimated as the ratio between the intensity of COX-1 gene and the β-actin gene. The means with different letters are significantly different (P<0.05). M=DNA ladder. Lane 1=normal 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.

Histological results

The present results were obtained from examining the liver samples of all groups, revealing that BPA caused a damaging effect on liver tissue in the form of cellular necrosis, acidophilia of cytoplasm, fibrosis and dilatation of blood sinusoids with cellular infiltration (Figure 5C and D).

Using *Fagonia cretica* as a protective or a therapeutic agent ameliorated these effects in a dose-dependent manner as

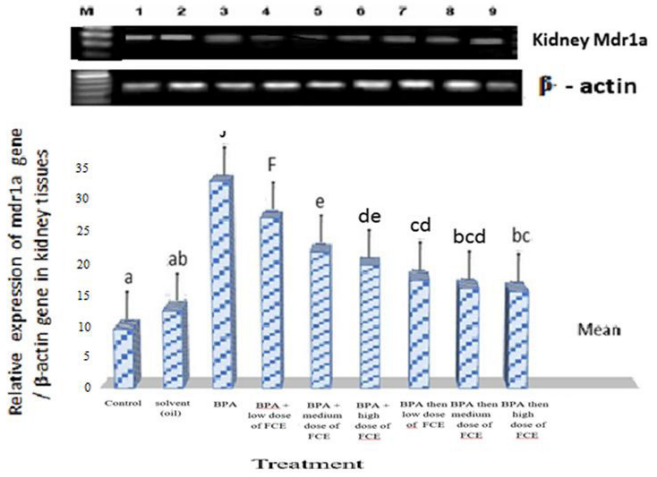

Figure 3: Gene expression levels of Mdr1a gene in kidney tissues of male rats that had been treated with FCE against BPA. These expression levels had been assayed by semi-quantitative RT-PCR. Recovery rate of mRNA was estimated as the ratio between the intensity of Mdr1a gene and the β-actin gene. The means with different letters are significantly different (P<0.05). M=DNA ladder. Lane 1=normal 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.

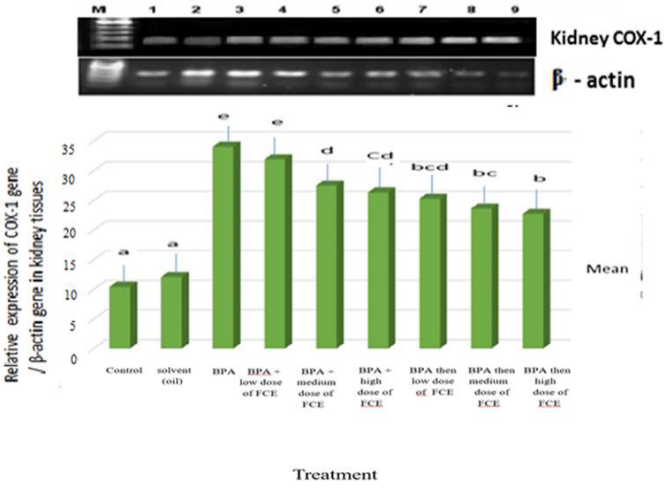

Figure 4: Gene expression levels of COX-1 gene in kidney tissues of male rats that had been treated with FCE against BPA assayed by semi-quantitative RT-PCR. Recovery rate of mRNA was estimated as the ratio between the intensity of COX-1 gene and the β-actin gene. The means with different letters are significantly different (P<0.05). M=DNA ladder. Lane 1=normal control. Lane2=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.

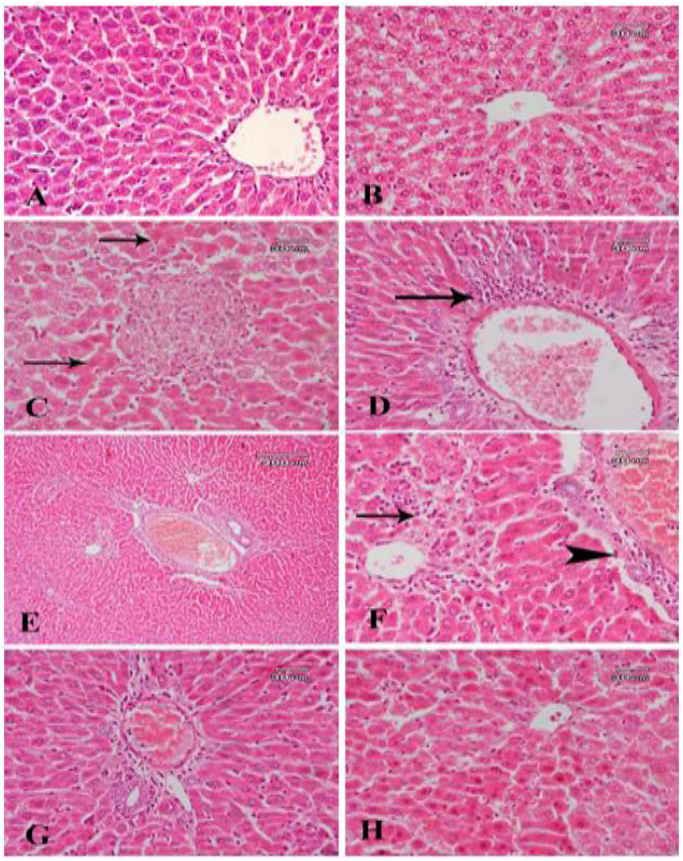

Figure 5: A photomicrograph of sections from liver tissue: (A) of control -ve group shows the normal structure of the hepatic lobule. (B) of a rat treated with corn oil shows a quite normal structure of liver tissue. (C) from a rat treated with bisphenol shows an area of necrotic cells with acidophilic hepatic cells (arrow) in the area around. (D) Another section for the same group shows dilatation of central vein with fibrosis and cellular infiltration around (arrow). (E) From a rat treated with bisphenol and *Fagonia cretica* (low dose) shows dilatation of central vein with fibrosis around is still noticed. (F) A higher magnification for a part of the previous section shows a slight reduction of fibrous tissue around vein (arrowhead) and dilated blood sinusoids with cellular infiltration (arrow). (G) A section from a rat treated with bisphenol and *Fagonia cretica* (medium dose) shows marked reduction of fibrous tissue and cellular infiltration around main blood vessels. (H) A section from a rat treated with bisphenol and *Fagonia cretica* (high dose) shows no fibrous tissue or cellular infiltration, but some acidophilic hepatic cells are still observed.

seen in (Figures 5E, F, G, H and 6A, B, C, D), respectively. Examining renal tissue samples of all the animal groups revealed that BPA had a similar damaging effect on renal tissue as that of the liver. Bisphenol A caused a marked distortion of the renal tissue, due to the increased fibrous tissue component. It also caused dilatation of renal tubules with atrophy of the lining epithelium (Figure 7C and D). *Fagonia cretica* ameliorated these effects in a dose-dependent manner, either as a protective or therapeutic agent (Figures

7E, F, G, H and 8A, B, C, D). In both cases of liver and renal tissues, the ameliorating effect of *Fagonia cretica* as a therapeutic agent was better than that as a protective agent.

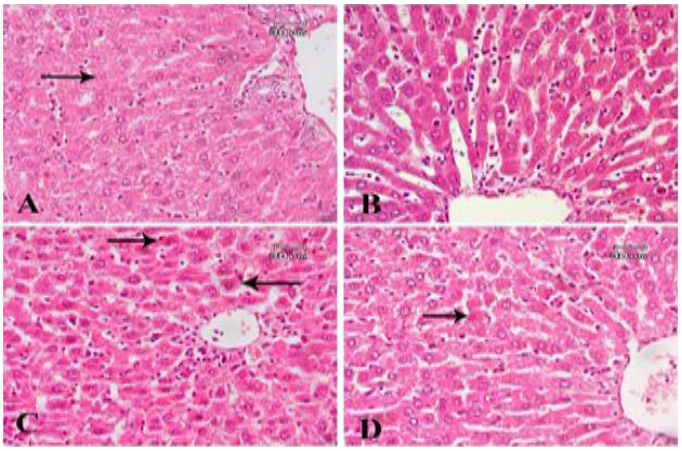

Figure 6: A photomicrograph of sections from liver tissue: (A) section of rat treated with bisphenol and then *Fagonia cretica* (low dose) shows mild fibrosis around main blood vessels. Some hepatocytes show karryolysis (arrow). (B) Another section for the same group shows mild dilatation and congestion of blood sinusoids. (C) A section of rat treated with bisphenol and then *Fagonia cretica* (medium dose) shows slight cellular infiltration. Some hepatocytes show acidophilic color (arrow). (D) A section of rat treated with bisphenol and then *Fagonia cretica* (high dose) shows marked amelioration of liver tissue, only a few acidophilic hepatocytes are observed (arrow).

Genetic study

This work was performed on three genes of which their expressions were associated with regulating the function activity in liver and Kidney cells. Such important genes included IkBα, mdr1a and COX-1 genes. Gene expression of IkBα and COX-1 genes was studied in liver cells, while gene expression of Mdr1a and COX-1 genes was demonstrated in Kidney cells. IkBα (inhibitor of nuclear factor Kappa B) gene expression in liver was found to be a major activator for the nuclear factor-KB (NF-KB), which is responsible for the activation of the expressions at least 150 genes, some of which are anti-apoptotic (Häcker and Karin, 2006; Kameyama et al., 2008). Furthermore, IkBα gene expression was observed to play a major role in the regulation of multiple drug resistance (mdr) gene activity (Bierhaus et al., 200l; Kameyama et al., 2008), and involved in propagating the cellular response to inflammation (Häcker and Karin, 2006).

Cyclooxygenase (COX-1) gene expression in hepatic tissues showed to be a key enzyme that catalyzed the oxygenation of arachidonic acid (AA) to produce prostaglandins (PGs) and thromboxane A2 (TXA2). It was noted that PGs and TXA2 mediated a wide range of physiological and pathophysiological responses (Graupera et al., 2003; Blobaum and Marnett, 2007; Perrone et al., 2010; Aid and Bosetti, 2011; Liedtke et al., 2012). Moreover, COX-1 gene expression in Kidney tissues was responsible for physiological Kidneys function, playing important role in the regulation of renal homeostasis. In normal conditions and in distinct regions of the kidneys, it was demonstrated that COX-1 generated prostaglandins which were a main factor in vasodilatation, and minimization of the vascular resistance, ensuring adequate blood flow (Moro et al., 2017). Concerning the genes of multiple drug resistance (mdr) in genomes of human and monkey, there is a single group of the gene (denoted mdr1) in rodents genomes contain two groups (denoted mdr1a and mdr1b) (Brady et al., 2002). These genes are known to be Xenobiotic transports and to encode the P-glycoprotein (Pg-gp) (Brady et al., 2002; Kameyama et al., 2008).

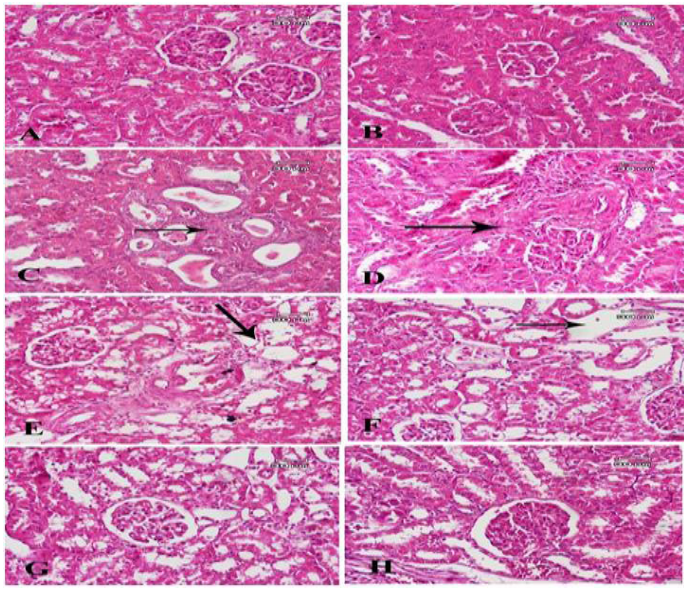

Figure 7: A photomicrograph of sections from renal tissue: (A) From a control -ve group shows the normal structure of the renal glomeruli and the different types of tubules. (B) from a rat treated with corn oil shows no abnormal structure in renal tissue. (C) from a rat treated with bisphenol shows many dilated tubules with necrotic materials within their lumens and increased connective tissue component around them (arrow). (D) Another section for the same group shows massive distortion of renal tissue due to marked increase of connective tissue components (arrow). (F) From a rat treated with bisphenol and *Fagonia cretica* (low dose) shows slight decrease of fibrosis, but dilated tubules are still noticed (arrow). (F) Another section of the same group shows marked reduction of fibrous, but many tubules show marked dilatation of their lumens with atrophy of lining epithelium (arrow). (G) A section from a rat treated with bisphenol and *Fagonia cretica* (medium dose) shows many tubules are still dilated with atrophied epithelial lining and no fibrosis around. (H) A section from a rat treated with bisphenol and *Fagonia cretica* (high dose) shows marked reduction of dilated tubules with no fibrosis.

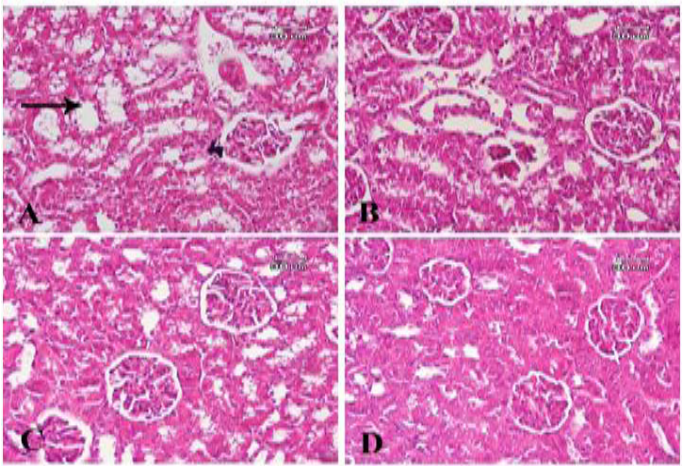

Figure 8: A photomicrograph of sections from renal tissue: (A) A section of rat treated with bisphenol and then *Fagonia cretica* (low dose) shows mild dilatation of renal tubules (arrow). (B) Another section for the same group shows the same results. (C) A section of rat treated with bisphenol and then *Fagonia cretica* (medium dose) shows slight dilatation of some tubules with no fibrosis. (D) A section of rat treated with bisphenol and then *Fagonia cretica* (high dose) shows renal tissue close to normal.

In kidneys, P-gp was located in brush border membranes of kidney-proximal tubules (Fajo et al., 1987; Thiebaut et al., 1987), and responsible for resistance to some cancer chemotherapeutic drugs, naturally occurring toxins and efflux of Xenobiotic of cells (Brady et al., 2002; Semeniuk et al., 2020). Nevertheless, the expression changes of the above mentioned genes, due to the exposure to abnormal conditions, could induce deleterious effects, leading to different diseases of the liver and kidney tissues (Kameyama et al., 2008; Lin et al., 2017; Moro et al., 2017; Semeniuk et al., 2020).

Thus, the expressions of such genes in the present work are considered good markers for diagnosis of pathogenesis cases as a result of exposure to BPA toxicant, as well as good targets for evaluating the protection or remedy of FCE medicinal herb against such toxicant.

The genotoxic effects of BPA on mammalian cells were documented (Cavalieri and Rogan, 2010; Sakuma et al., 2010; Fic et al., 2013; Kourouma et al., 2015), since this component through its biotransformation in liver caused oxidative stress by forming a lot of free radicals, such reactive oxygen species (ROS), quinines, and nitric oxide (NO) (Bindhumol et al., 2003; McMillian et al., 2004; Videla, 2009; Schmidt et al., 2013). These radicals could react with cell constituents involving DNA, proteins and lipids (Cavalieri and Rogan, 2010; Moon et al., 2012; Kourouma et al., 2015), inducing impairments, particularly genetic alterations, including the changes of gene expressions (Xu et al., 2010; Chung et al., 2011; Castro et al., 2013; Wisniewski et al., 2015). The findings of the present study noted that the expressions of IkBα and COX-1 genes (in liver tissues) as well as mdr1a and COX-1 genes (in kidney tissues), in rats treated with BPA were significantly higher than those found in the normal control. To the best of the researchers' knowledge, the present work is the first report to show the effect of BPA on the expressions of the above mentioned genes.

Furthermore, these results are supported by several investigations, which reported that exposure to abnormal conditions, including toxicants treatments, could induce over-expressions of IkBα (Häcker and Karin, 2006; Kameyama et al., 2008), and COX-1 (Lin et al., 2017) genes in hepatic tissues, causing the upregulation of expressions of mdr1a (Brady et al., 2002; Fouassier et al., 2007; Kameyama et al., 2008) and COX-1 (Moro et al., 2017) genes in kidney tissues, and leading to different liver and kidney diseases (Kameyama et al., 2008; Lin et al., 2017; Moro et al., 2017; Semeniuk et al., 2020). Moreover, in previous studies, Burt and Thorgeirsson (1988) showed that the treated rats with 2, 3, 7, 8-tetrachlorodibenzo-P-dioxin, TCDD (microsomal enzyme inducers) had an increase of mRNA levels of mdr genes, in liver tissues, compared to normal control. Dexamethasone (Dex) treatment in rat, mice and human hepatoma cell lines also led to the elevation of mdr1a mRNA levels with referenced to untreated cells (Zhao et al., 1993; Schuetz et al., 1995; Seree et al., 1998).

Furthermore, Brady et al. (2002) observed the over-expression of mdr1a mRNA in gastrointestinal tract of rats treated with different microsomal enzyme inducers. On the other hand, many studies demonstrated that exposure to BPA led to the upregulation of expression of different genes related to various functions, including brain functions-related genes (P450 arom5α-Reductase and Tph2 genes) (Castro et al., 2013), and oxidation genes (FK bps) (Kitraki et al., 2015). BPA treatment was capable of stimulating the mitogen-activated protein Kinase (MAPK), signaling the pathway, and inducing over-expression of cancer and proliferation-related genes (Lan et al., 2015). Wisniewski et al. (2015) also revealed that in the pituitary- testicular axis of adult Wistar rats, BPA treatment caused the over-expression of genes of gonadotropin releasing hormone receptor (Gnrhr), luteinizing hormone beta follicle stimulating hormone beta (Fshb), estrogen receptor beta (Esr2), and androgen receptor (Ar), resulting in a state of hypogonadotropichypogonadism. Furthermore, Abd-El-Moneim et al. (2020) demonstrated that in BPA-intoxicated adult rats, there was a significant decrease in the nucleic acid (DNA and RNA) contents in brain, liver, and kidney tissues, in contrast with those of the normal control. These results may indicate the alteration of the expressions of various genes in such tissues.

The present results clarified that FCE treatment could minimize the BPA-induced alterations of gene expressions of some genes in liver and kidney tissues. The treatment with FCE, especially utilizing the high therapeutic dose (5.0 g/kg), was capable of significantly downregulating the over-expressions induced by BPA treatment for IkBα and COX-1 genes in liver tissues, and for mdrla and COX-1 genes in kidney tissues. These findings (to the best of the reserchers' knowledge) were the first to demonstrate the ameliorative role of FCE treatment against the deleterious effects of BPA treatment on the expressions of IkBα, mdr1a and COX-1 genes in mammalian cells. Nevertheless, the present results are supported by previous studies that showed the ameliorative role of FCE treatment on some gene expressions against the adverse effects caused by the exposure to abnormal and pathological conditions. For example, the treatment with FCE could be used in cancer cases, due to its effect on the expression of cancer and proliferation-related genes. In this respect, Lam et al. (2012) found that FCE could ameliorate the expression of FOXO3a gene and diminish the expression of P53 gene in two phenotypically distinct breast cancer lines (MCF-7 and MDA-MB-231 cells), causing the activation of DNA damage response and inducing cell cycle arrest and apoptosis.

Moreover, previous work reported that, the FCE treatment could enhance the expression of genes related to antioxidant properties, and minimize the expression of genes correlated with oxidation processes causing immunity enhancement against the exposure to abnormal conditions. In this light, Rawal et al. (2004) proved that in rat hippocampal slices subjected to oxygen-glucose deprivation (discriminated with high oxidation stress), FCE treatment could increase the expression of antioxidant genes (gamma-glutamylcysteine ligase and Cu-ZnSOD), diminish the expression of oxidation gene (i NOS) and elevate the reduced glutathion (GSH) level. GSH has an important role in regulating the expressions of several redox-sensitive antioxidant and anti-inflammatory genes, for which their ameliorations led to the reduction of oxidant levels (via direct scavenging of the reactive oxygen and nitrogen species), and the enhancement of the peroxide scavenging enzyme.

In another study, Abd-El-Moneim et al. (2020) revealed the significant improvements of nucleic acid (DNA and RNA) contents in brain, liver and kidney tissues of BPA-intoxicated rats treated with FCE, as compared to those found in BPA treatment alone. These findings suggest the reason for ameliorations in the expressions of various genes in such tissues. Moreover, FCE was found to be rich in antioxidant constituents (Saleh et al., 2011; Kasture et al., 2014; Puri and Bhandari, 2014), that might be the main factors ameliorating the expressions of IkBα, mdr1a and COX-1 genes against the adverse effect of BPA in the present study.

Effect of treatment with BPA on histological examination

The present work clarified that the exposure to BPA induced deleterious effects on the histological architectures of liver and kidney tissues. The toxic effect of BPA was demonstrated in several studies on human and animal health, through the generation of reactive oxygen species (ROS) such as superoxide, hydroxyl and proxy radicals, these which were able to stimulate oxidative stress, causing significant reduction of the expressions of antioxidant genes, and the upregulation of oxidation genes. These events were found to induce impairment of prooxidant/antioxidant balance, and leading to histopathological conditions in different body organs, including the liver and kidneys (Bindhumol et al., 2003; McMillian et al., 2004; Videla, 2009; Hassan et al., 2012; Kourouma et al., 2015; Kazemi et al., 2016). The findings are in agreement with those reported by Rönn et al. (2013), who clarified that exposure to BPA caused hepatic damage in rats, disrupted the integrity of cellular membranes in liver tissues, and led to leakage of cytoplasmic liver enzymes. Furthermore, the present results coincide with those revealed by Thoene et al. (2017), who observed vacuolar degeneration in the liver tissues of Juvenile Porcine Models, after exposure to BPA component. Moreover, the particular histopathologies in the present study were similar to those commonly revealed after chemical exposure, in literatures on the effects of long-term ethanol or narcotics usage (Cederbaum et al., 2009; Manzo-Avalos and Saavedra-Molina, 2010; Diab et al., 2020; Fahmy etal., 2020). On the other hand, the over-expressions of the genes under study might be the major cause for inducing the histopathological conditions. It was found that the upregulation of the expressions of IkBα (Häcker and Karin, 2006; Kameyama et al., 2008), and mdrla (Brady et al., 2002; Fouassier et al., 2007; Kameyama et al., 2008) were accompanied by increases in P-glycoproteins, correlated with oxidative stress, by producing the reactive oxygen species (ROS), which was the main factor in inducing injury in the liver, kidneys, and other organs (Bindhumol et al. 2003; Semeniuk et al., 2020).

It was also found that the upregulation or the over activation of COX-1 gene expression was a major contributor in including the over-expression of vasoconstrictor thromboxane A2 (TXA 2), which was an essential regulator of hepatic endothelial dysfunction causing hepatic disorders (Lin et al., 2017). Moreover, in kidney tissues, it was observed that in abnormal situations, the function of COX-1 gene might be altered causing imbalanced changes in prostaglandin generation, and leading to kidney diseases (Moro et al., 2017).

Effect of FCE treatment on BPA-induced histopathology

In the present work, the histological examination showed that the treatment with FCE led to improvements in histopathological cases produced by BPA in liver and kidney tissues. It was observed that these ameliorations increased by increasing the dose of FCE. The highest dose (5.0 g/kg) that was utilized as a therapeutic agent gave the best results, in which the histopathological parameters were restored to normal level, or reverted nearly to natural status. FCE had an ameliorative role because its possessed a lot of phytochemical constituents or antioxidants, including saponins, polyphenolic compound, alkaloid, flavonoids, terpenoids, sulphates, cyanogenic glycosides, sterol, proteins and amino acids, coumarins, and trace elements (Khalik et al., 2001; Saeed and Wahid, 2003; Kasture et al., 2014). These chemical constituents had pharmacological activity and medicinal properties, such as anticancer, anti-inflammatory, antimicrobial, analgesic, and antipyretic effects and wound healing properties (Saleh et al., 2011; Puri and Bhandari, 2014; Kasture et al., 2014). These phytochemicals were found to enhance the activities of antioxidant enzymes that acted against the oxidative stress induced by various toxicants, including BPA, by increasing the rates of free radical scavengers (Rawal et al., 2004; Puri and Bhandari, 2014; Kasture et al., 2014). Moreover, Hussain et al. (2007) showed significant antitumor activity on potato disc, using FCE against the tumor- inducing Agrobacterium strains (At 6, At 10 and at 77); and the maximum tumor inhibition (77.04%) was revealed against at 10. Moreover, Lam et al. (2012) proved that FCE could act against breast cancer cell proliferations via activation of DNA damage and cell cycle, arrest as well as inducing apoptosis in such cancer type.

CONCLUSIONS AND RECOMMENDATIONS

The present investigation confirmed the important role of *Fagonia cretica* in overcoming the harmful BPA-induced effects on animal cells. The extraction of this medicinal herb could modulate the over-expressions of IkBα, mdr1a and COX-1 genes to favorable or normal levels in rats. Moreover, it could markedly ameliorate or remedy the histopathological cases.

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Ethical guidelines

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

NOVELTY STATEMENT

The novelty of our work entitled "Ameliorative Role of Ethanolic Extract of Fagonia cretica on BPA-Induced Genetic Alterations and Histological Changes in Liver and Kidney Tissues of Rats" can be summarized as: This investigation proved the important role of FC in overcoming the harmful effect of BPA on animal cells, where FCE could modulate the over-expressions of IkBα, mdr1a and COX-1 genes and histopathological cases to favorable or normal levels in rats.

AUTHOR'S CONTRIBUTION

IF: Established the idea of the article, the experiment design, supervising the breeding of rats, collecting the samples from the rat liver and kidney tissues, statistical design and writing the paper.
IG: Shared in collecting the samples from the rat liver and kidney tissues of rats, RNA isolation from the studied tissues samples, RNA transcription into cDNA, PCR amplification for the studied genes, beside gel electrophoresis analysis and capturing the photos, measuring the concentration of the resulted amplified bands, performed the statistical analysis and writing the paper.
DA: Collecting data concerning with the point of study, shared in RNA isolation from the rat liver and kidney tissues samples, RNA transcription into cDNA, PCR amplification for the studied genes, beside gel electrophoresis analysis and capturing the photos.
NI: Shared in collecting the samples from the required liver and kidney tissues of rats, RNA isolation from the studied tissues samples, RNA transcription into cDNA, PCR amplification for the studied genes, beside gel electrophoresis analysis and capturing the photos.
NSH: performed the histopathology analysis for the cells of rat liver and kidney tissues.
ME: Shared in the design of the experiment and reviewed the paper
AA: Offered the plant extract of *Fagonia cretica*.
All authors read and approved the final manuscript.

Conflicts of interests

The authors have declared no conflict of interest.

REFERENCES

Abd-El-Moneim OM, Abd El-Rahim AH, Mohamed AA, Farag IM, Abdalla AM (2020). Enhancement effects of ethanolic extract of *Fagonia cretica* on Bisphenol A (BPA)-induced genotoxicity and biochemical changes in rats. Bull. Natl. Res. Centre, 44(67): 1-13. https://doi.org/10.1186/s42269-020-00295-y

Aboelhassan DM, Hafiz NA, Darwish HR, Shabana ME, Eshak MG, Hassanane MM, Farag IM, Abdalla AM (2018). Enhancing effects of Moringa oleifera leaf extract on carcinogenic aflatoxin Bl-induced genetic alterations, haematoxicity and histological changes in liver and Kidney of rats. Biosci. Res., 15(2): 814-833.

Aghajanpour-Mir SM, Zabihi E, Akhavan-Niaki H, Keyhani E, Bagherizadeh I, Biglari S, Behjati F (2016). The genotoxic and cytotoxic effects of Bisphenol - A (BPA) In MCF-7 cell line and Amniocytes. Int. J. Mol. Cell Med. Winter, 5(1): 19-29.

Aid S, Bosetti F (2011). Targeting cyclooxygenases-l and -2 in neuroinflammation: Therapeutic implications. Biochimie, 93: 46-51. https://doi.org/10.1016/j.biochi.2010.09.009

Batanouny K, Batanouny M (1970). Autecology of common Egyptian *Fagonia* species. Phyton (Austria), 14(1-2): 79-92.

Bierhaus A, Schiekofer S, Schwaninger M, Andrassy M, Humpert PM, Chen J, Hong M, Luther T, Henle T, Kloting I, Morcos M, Hofmann M, Tritschler H, Weigle B, Kasper M, Smith M, Perry G, Schmidt AM, Stern DM, Häring HU, Schleicher E, Nawroth PP (2001). Diabetes-associated sustained activation of the transcription factor nuclear factor-kappaB. Diabetes, 50: 2792-2808. https://doi.org/10.2337/diabetes.50.12.2792

Bindhumol V, Chitra KC, Mathur PP (2003). Bisphenol A induces reactive oxygen species generation in the liver of male rats. Toxicology, 188(2-3): 117-124. https://doi.org/10.1016/S0300-483X(03)00056-8

Blobaum AL, Marnett LJ (2007). Structural and functional basis of cyclooxygenase inhibition. J. Med. Chem., 50: 1425-1441. https://doi.org/10.1021/jm0613166

Brady JM, Cherrington NJ, Hartley DP, Buist SC, Ning LI, Klaassen CD (2002). Tissu diatribution and chemical induction of multiple drug resistance genes in rats. Drug Metab. Dispos., 30: 638-844. https://doi.org/10.1124/dmd.30.7.838

Burt RK, Thorgeirsson SS (1988). Coinduction of MDR-1 multidrug-resistance and cytochrome P-450 genes in rat liver by xenobiotics. J. Natl. Cancer Inst., 80: 1383-1386. https://doi.org/10.1093/jnci/80.17.1383

Cabaton NJ, Wadia PR, Rubin BS, Zalko D, Schaeberle CM, Askenase MH, Gadbois JL, Tharp AP, Whitt GS, Sonnenschein C, Soto AM. (2011). Perinatal exposure to environmentally relevant levels of bisphenol A decreases fertility and fecundity in CD-1 mice. Environ. Health Perspect., 119: 547-552. https://doi.org/10.1289/ehp.1002559

Campbell LG (1982). Reference filing system utilizing the Statistical Analysis System (SAS). J. Agric. Educ., 11(1): 51-53. after Saleh 2011

Castro B, Sánchez P, Torres JM, Preda O, Del Moral RG, Ortega E (2013). Bisphenol A exposure during adulthood alters expression of aromatase and 5α-reductaseisozymes in rat prostate. PLoS One, 8(2): e55905. https://doi.org/10.1371/journal.pone.0055905

Cavalieri EL, Rogan EG (2010). Is Bisphenol A a weak carcinogen like the natural estrogens and diethylstilbestrol? IUBMB Life, 62: 746-751. https://doi.org/10.1002/iub.376

Cederbaum AI, Lu Y, Wu D (2009). Role of oxidative stress in alcohol induced liver injury. Arch Toxicol., 83: 519-548. https://doi.org/10.1007/s00204-009-0432-0

Chung E, Genco MC, Megrelis L, Ruderman JV (2011). Effects of bisphenol A and triclocarban on brain-specific expression of aromatase in early zebrafish embryos. Proc. Natl. Acad. Sci. USA, 108(43): 17732-17737. https://doi.org/10.1073/pnas.1115187108

Diab KA, Ibrahim NE, Fahmy MA, Hassan EM, Omara EA (2020). Inhibitory activity of flaxseed oil against $CdCl_2$ induced liver and kidney damage: Histopathology, genotoxicity and gene expression study. Toxicol. Rep., 7: 1127-1137. https://doi.org/10.1016/j.toxrep.2020.08.023

Eid JI, Eissa SM, El-Ghor AA (2015). Bisphenol A induces oxidative stress and DNA damage in hepatic tissue of female rat offspring. J. Basic Appl. Zool., 71: 10-19. https://doi.org/10.1016/j.jobaz.2015.01.006

El-Hadidy MN (1966). The genus *Fagonia* L. in Egypt. Candollea, 2: 13-54.

Eshak MG, Farag IM, Fadel M, Stino FKR (2013). Effect of distillery vinasse on the productive performance, DNA damage, expression of IGFBPs gene and histopathological changes in Japanese Quail fed diets contaminated with phenol. Glob. Vet., 11(5): 657-673.

Eshak MG, Farag IM, Nada SA, Khalil WKB (2015). Effect of Amaryl and variety of dietary supplementations on gene expression alteration in pancreas, liver and ovarian tissues of hyperglycemic rats. Int. J. Pharm. Sci. Rev. Res., 33(2): 93-102.

Fahmy MA, Hassan EE, Ibrahim NE, Hassan EM, Hassan ZM, Omara EA (2020). Protective role of Ficus carica extract against Hepato-Testicular side effects and genotoxicity induced by Cisplatin. Pharmacogn. J., 12(3): 645-656. https://doi.org/10.5530/pj.2020.12.96

Fajo AT, Ueda K, Slamon DJ, Poplack DG, Gottesman MM, Pastan I (1987). Expression of a multidrug-resistance gene in human tumors and tissues. Proc. Natl. Acad. Sci. U. S. A., 84: 265-269. https://doi.org/10.1073/pnas.84.1.265

Farag IM, Nada SA, Gamil KA, Shoeib AR, El-Nahass E (2008). Molecular genetic study on coagulation factor IX (FIX) in human and some Egyptian murine species. Egypt. Med. J. Natl. Res. Centre,s 7(2): 3-9.

Fic A, Zegura B, Dolenc MS, Filipic M, Masic P (2013). Mutagenicity and DNA damage of bisphenol A and its structural analogues in HepG2 cells. Arh. High Rada Toksikol., 64: 189-200. https://doi.org/10.2478/10004-1254-64-2013-2319

Fouassier L, Beaussier M, Schiffer E, Rey C, Barbu V, Mergey M, Wendum D, Callard P, Scoazec JY, Lasnier E, Stieger B, Lienhart A, Housset C (2007). Hypoxia-induced changes in the expression of rat hepatobiliary transporter genes. Am. J. Physiol. Gastroint. Liver Physiol., 293: G25-G35. https://doi.org/10.1152/ajpgi.00175.2006

Graupera M, García-Pagán JC, Parés M, Abraldes JG, Rosello J, Bosch J, Rodés J. (2003). Cyclooxygenase-1 inhibition corrects endothelial dysfunction in cirrhotic rat livers. J. Hepatol., 39: 515-521. https://doi.org/10.1016/S0168-8278(03)00347-7

Häcker H, Karin M (2006). Regulation and function of IKK and IKK-related kinases. Sci. STKE. 2006(357): https: //

pubmed. ncbi.nih.gov/17047224. https://doi.org/10.1126/stke.3572006re13

Hassan ZK, Elobeid MA, Virk P, Omer SA, Maha ElAmin M, Daghestani MH, AlOlayan EM. (2012). Bisphenol A induces hepatotoxicity through oxidative stress in rat model. Oxidat. Med. Cell. Longevity, 2012; Article ID 194829; 6 pages. https://doi.org/10.1155/2012/194829

Hussain A, Zai M, Mirza B (2007). Cytotoxic and antitumor potential of *Fagonia cretica* L. Turk. J. Biol., 31: 19-24.

IAEC (Institutional Animal Ethics Committee) (2010). Commit for the purpose of control, supervision of experiments on animals (CPCSEA) CPCSEA is guidelines for laboratory animal facility.

Jahala OAM, Izzeldin OM, Abdalla RE (2014). Effect of Fagoniacreticalinnethanolic extract on different hematological parameters in albino rats in Sudan. Pharma Innov. J., 3(9): 89-93.

Kameyama N, Arisawa S, Ueyama J, Kagota S, Shinozuka K, Hattori A, Tatsumi Y, Hayashi H, Takagi K, Wakusawa S (2008). Increase in P-glycoprotein accompanied by activation of protein kinase C α and NF-k Bp65 in the livers of rats with streptozotocin-induced diabetes. Biochim. Biophys. Acta, 1782: 355-360. https://doi.org/10.1016/j.bbadis.2008.02.005

Kasture VS, Gosavi SA, Kolpe JB, Deshapande SG (2014). Phytochemical and Biological Evaluation of Fagonia species: A review. World J. Pharm. Pharm. Sci., 3(5): 1206-1217.

Kazemi S, Mousavi SN, Aghapour F, Rezaee B, Sadeghi F, Moghadamnia AA (2016). Induction effect of Bisphenol A on gene expression involving hepatic oxidative stress in rat. Oxidat. Med. Cell. Longevity, Article ID 6298515, 5 pages. https://doi.org/10.1155/2016/6298515

Khalik A, Miyase SM, Melek T, Ashaal HA (2001). Saponins from *Fagonia critica*. Dipharmaazie, 56: 247-250.

Kim JH, Qu A, Reddy JK, Gao B, Gonzalez FJ (2014). Hepatic oxidative stress activates the Gadd45b gene by way of degradation of the transcriptional repressor STAT3. Hepatology, 59(2): 695-670. https://doi.org/10.1002/hep.26683

Kitraki E, Nalvarte I, Alavian-Ghavanini A, Rüegg J (2015). Developmental exposure to bisphenol A alters expression and DNA methylation of Fkbp5, an important regulator of the stress response. Mol. Cell. Endocrinol., 417: 191-199. https://doi.org/10.1016/j.mce.2015.09.028

Klopfleisch R, Gruber AD (2009). Differential expression of cell cycle regulators p21, p27 and p53 in metastasizing canine mammary adenocarcinomas versus normal mammary glands. Res. Vet. Sci., 87: 91-96. https://doi.org/10.1016/j.rvsc.2008.12.010

Kourouma A, Quan C, Duan, P, Hassan ZK, Elobeid MA, Virk P, Qi S , Yu T, Wang Y, Yang K. (2015). Bisphenol A induces apoptosis in liver cells through induction of ROS. Adv. Toxicol., Article ID 901983; 10 pages. https://doi.org/10.1155/2015/901983

Lam M, Carmichael AR, Griffiths HR (2012). An aqueous extract of Fagoniacretica induces DNA damage, cell cycle arrest and apoptosis in breast cancer cells via FOXO3a and P^{53} expression. PLoS One, 7(6): 1-11. https://doi.org/10.1371/journal.pone.0040152

Lan HC, Lin IW, Yang ZJ, Lin JH. (2015). Low-dose bisphenol A activates Cypllal gene expression and corticosterone secretion in adrenal gland via the JNK signaling pathway. Toxicol. Sci., 148(1): 26-34. https://doi.org/10.1093/toxsci/kfv162

Liedtke AJ, Crews BC, Daniel CM, Blobaum AL, Kingsley PJ, Ghebreselasie K, Marnett LJ (2012). Cyclooxygenase-1 selective inhibitors based on the (E)-2-Des-methyl-sulindac sulfide scaffold. J. Med. Chem., 55: 2287-2300. https://doi.org/10.1021/jm201528b

Lin L, Cai M, Deng S, Huang W, Huang J, Huang X, Huang M, Wang Y, Shuai X, Zhu K (2017). Amelioration of cirrhotic portal hypertension by targeted cyclooxygenase-1 siRNA delivery to liver sinusoidal endothelium with polyethylenimine grafted by hyaluronic acid. Nanomed. NBM, 13: 2329-2339. https://doi.org/10.1016/j.nano.2017.06.019

Liu C, Duan W, Li R, Xu S, Zhang L, Chen C, He M, Lu Y, Wu H, Pi H, Luo X, Zhang Y, Zhong M, Yu Z, Zhou Z. (2013). Exposure to bisphenol A disrupts meiotic progression during spermatogenesis in adult rats through estrogen-like activity. Cell Death Dis., 4: article e676. https://doi.org/10.1038/cddis.2013.203

Liu J, Yu L, Tokar EJ, Bortner C, Sifre MI, Sun Y, Waalkes MP (2008). Arsenic-induced aberrant gene expression in fetal mouse primary liver-cell cultures. Annls N. Y. Acad. Sci., 1140(1): 368-375. https://doi.org/10.1196/annals.1454.028

Manzo-Avalos S, Saavedra-Molina A (2010). Cellular and mitochondrial effects of alcohol consumption. Int. J. Environ. Res. Publ. Health, 7: 4281-4304. https://doi.org/10.3390/ijerph7124281

McMillian M, Nie AY, Parker JB, Koo YD, Ann HY, Lee KJ, Kim SH, Yoon YC, Cho B, Park KS, Jang HC, Park YJ . (2004). A gene expression signature for oxidant stress/reactive metabolites in rat liver. Biochem. Pharmacol., 68(11): 2249-2261. https://doi.org/10.1016/j.bcp.2004.08.003

Moon MK, Kim MJ, Jung IK, Koo YD, Ann HY, Lee KJ, Kim SH, Yoon YC, Cho B, Park KS, Jang HC, Park YJ . (2012). Bisphenol A impairs mitochondrial function in the liver at doses below the no observed adverse effect level. J. Korean Med. Sci., 27(6): 644-652. https://doi.org/10.3346/jkms.2012.27.6.644

Moro MG, Sanchez PKV, Lupepsa AC, Baller EM, Franco GCN (2017). Cyclooxygenase biology in renal function. Literature review. Rev. Colomb. Nefrol., 4(1): 27-37. https://doi.org/10.22265/acnef.4.1.263

National Toxicology Program (1985). Carcinogenesis Bioassay of Bisphenol A (CAS No. 80- 05-7) in F344 Rats and B6 C3 F_1 Mice (Feed Study). Tech. Rep. Ser., 215: 1-116.

National Toxicology Program. (2008). NTP- CERHR monograph on the potential human reproductive and developmental effects of Bisphenol A. NTP Cerhr Mon., 22: 1-64.

Perrone MG, Scilimati A, Simone L, Vitale P (2010). Selective COX-1 inhibition: A therapeutic target to be reconsidered. Curr. Med. Chem., 17: 3769-3805. https://doi.org/10.2174/092986710793205408

Puri D, Bhandari A (2014). Fagonia: A potential medicinal desert plant. J. NPA., XXVII(I): 28-33. https://doi.org/10.3126/jnpa.v27i1.12147

Rawal AK, Muddeshwar MG, Biswas SK (2004). Rubiacordifolia, Fagoniacretica Linn and Tinosporacordifolia exert neuroprotection by modulating the antioxidant system in rat hippocampal slices subjected to oxygen glucose deprivation. BMC Complement. Altern. Med., 4: 11. https://doi.org/10.1186/1472-6882-4-11

Rönn M, Kullberg J, Karlsson H, Berglund J, Malmberg F, Örberg J, Lind L, Ahlström H, Lind MP (2013). Bisphenol A exposure increases liver fat in juvenile fructose-fed Fischer 344 rats. Toxicology, 303: 125-132. https://doi.org/10.1016/j.tox.2012.09.013

Saeed MA, Wahid SA (2003). Effects of *Fagonia cretica* L constituent on various hematological parameter in rabbits. J. Ethopharmacol., 85: 195-200. https://doi.org/10.1016/S0378-8741(02)00365-3

Sakuma S, Nakanishi M, Morinaga K, Fujitake M, Wada S, Fujimoto Y (2010). Bisphenol A 3; 4-quinone induces the conversion of xanthine dehydrogenase into oxidase *in vitro*. Food Chem. Toxicol., 48: 2217-2222. https://doi.org/10.1016/j.fct.2010.05.051

Saleh IA, Hasan SY, Aftab A (2011). Anti-inflammantory and wound healing activity of Fagonia Schweinfurthi alcoholic extract herbal gel on albino rats. Afr. J. Pharmacol., 5(17): 1996-2001. https://doi.org/10.5897/AJPP11.190

Schmidt J, Kotnik P, Trontelj J, Knez Z, Mašič LP (2013). Bioactivation of bisphenol A and its analogs (BPF; BPAF; BPZ and DMBPA) in human liver microsomes. Toxicol. *In Vitro*, 27: 1267-1276. https://doi.org/10.1016/j.tiv.2013.02.016

Schuetz EG, Schuetz JD, Thompson MT, Fisher RA, Madariage JR, Strom SC (1995). Phenotypic variability in induction of P-glycoprotein mRNA by aromatic hydrocarbons in primary human hepatocytes. Mol. Carcinog., 12: 61-65. https://doi.org/10.1002/mc.2940120202

Semeniuk M, Cere LI, Ciriaci N, Bucci-Munoz M, Villanueva SSM, Mottino AD, Catania VA, Rigalli JP, Ruiz ML (2020). Regulation of hepatic P-gp expression and activity by genistein in rats. Arch. Toxicol., https://doi.org/10.1007/s00204-020-02708-3

Seree E, Villard PH, Hever A, Guigal N, Puyoou F, Charvet B, Point-Scomma H, Lechevalier E, Lacarelle B, Barra Y (1998). Modulation of MDR1 and CYP3A expression by dexamethasone: evidence for an inverse regulation in adrenals. Biochem. Biophys. Res. Commun., 252: 392-395. https://doi.org/10.1006/bbrc.1998.9662

Seyal RA, Tareen SM, Awan HM (2013). Can we really treat Thalassemia Major? Proc. Pak. Acad. Sci., 50(4): 315-325.

Talsness CE, Andrade AJM, Kuriyama SN, Taylor JA, Saal FSV (2009). Components of plastic: Experimental studies in animals and relevance for human health. Philos. Trans. R. Soc. B Biol. Sci., 364(1526): 2079-2096. https://doi.org/10.1098/rstb.2008.0281

Thiebaut F, Tsuruo T, Hamada H, Gottesman MM, Pastan I, Willingham MC (1987). Cellular localization of the muludrug-resistance gene product P-glycoprotein in normal human tissues. Proc. Natl. Acad. Sci. U.S.A., 84: 7735-7738. https://doi.org/10.1073/pnas.84.21.7735

Thoene M, Rytel L, Dzika E, Włodarczyk A, Kruminis-Kaszkiel E, Konrad P, Wojtkiewicz J (2017). Bisphenol A causes liver damage and selectively alters the neurochemical coding of intrahepatic parasympathetic nerves in juvenile porcine models under physiological conditions. Int. J. Mol. Sci., 18: 2726. https://doi.org/10.3390/ijms18122726

Vanderberg LN, Hauser R, Marcus M, Olea N, Welshons WV (2007). Human exposure to bisphenol A (BPA). Reprod. Toxicol., 24: 139-177. https://doi.org/10.1016/j.reprotox.2007.07.010

Videla LA (2009). Oxidative stress signaling underlying liver disease and hepatoprotective mechanisms. World J. Hepatol., 1(1): 72-78. https://doi.org/10.4254/wjh.v1.i1.72

Wisniewski P, Romano RM, Kizys MML, Oliveira KC, Kasamatsu T, Giannocco G, Chiamolera MI, Dias-da-Silva, MR, Romano IMA (2015). Adult exposure to bisphenol A (BPA) Wistarrats reduces sperm quality with disruption of the hypothalamic pituitary testicular axis. Toxicology, 329: 1-9. https://doi.org/10.1016/j.tox.2015.01.002

Xu XH, Wang YM, Zhang J, Luo QQ, Ye YP, Ruan Q (2010). Perinatal exposure to bisphenol-A changes N-D-aspartate receptor expression in the hippocampus of male rat offspring. Environ. Toxicol. Chem., 29(1): 176-181. https://doi.org/10.1002/etc.18

Ye X, Pierik FH, Angerer J, Meltzer HM, Jaddoe VW, Tiemeier H, Hoppin JA, Longnecker MP. (2009). Levels of metabolites of organophosphate pesticides; phthalates; and bisphenol A in pooled urine specimens from pregnant women participating in the Norwegian Mother and Child Cohort Study (MoBa). Int. J. Hyg. Environ. Health, 212: 481-491. https://doi.org/10.1016/j.ijheh.2009.03.004

Zhao JY, Ikeguchi M, Eckersberg T, Kuo MT (1993). Modulation of multidrug resistance gene expression by dexamethasone in cultured hepatoma cells. Endocrinology, 133: 521-528. https://doi.org/10.1210/endo.133.2.8102093