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Could Licorice prevent Bisphenol A-Induced Biochemical, Histopathological and Genetic Effects in the Adult Male Albino Rats?

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Abstract Bisphenol A (BPA) is an ecological estrogenic endocrine disruptor used commonly in polycarbonate plastics. This study aimed to investigate the biochemical, histopathological and genetic effects of BPA at different doses and to evaluate the protective role of licorice against such effects. Thirty Wistar male albino rats were divided into five groups administered BPA daily at 2.4 µg/kg and 500 mg/kg orally with or without licorice (150 mg/kg) for 4 weeks. The results revealed that the high toxic dose decreased GSH, SOD and catalase levels and increased MDA level significantly. Serum TNF-α, testosterone and testicular cholesterol levels were significantly decreased while serum alkaline phosphatase was significantly increased. Histopathological changes were observed in testes, lungs and stomach. Alteration in the expression of NF-κB1 gene in lung occurred. These results suggested that BPA induced oxidative stress; resulted in its complication in the examined rats and treatment with licorice alleviated the toxicity induced by BPA.

Keywords | Bisphenol A, licorice, oxidative stress, genetic

Introduction

Bisphenol A (BPA) is an ecological estrogenic endocrine disruptors with similar chemical structure to estradiol and diethylstilbestrol. It is one of the most common worldwide chemicals (Ritter, 2011) that have been commonly used in polycarbonate plastics as drinking bottles, toys and dental sealant and in epoxy resins lining containers for food and beverage (Fenichel et al., 2013).

Exposure of polycarbonate products to high temperature, acidic or basic compounds and repeated washing hastens hydrolysis of the ester bond linking BPA molecules and degradation of the polymers leading to leakage of BPA from food and beverage containers (Lim et al., 2009). Nowadays, BPA are everywhere in the environment. Human at different ages become in contact with BPA continuously through packaged food, drinking water, dermal exposure, and inhalation of household dusts (Lakind and Naiman, 2011).

BPA and their metabolites have been detected in about 92.6 % of the human population bodies (Kitraki et al., 2015). European Food Safety Authority set 0.05 mg/kg body weight as the standard tolerable daily dose of BPA. Recently, the safe daily human exposure limit to BPA has been lowered to 4 µg/kg/day (EFSA, 2015).

As a result of increasing evidence for the hazards of exposure to BPA, more and more studies have been conducted to prove the side effects of BPA on different body organs either in vivo or in vitro (Ahbab et al., 2017; Fang et al., 2015).

The adverse effects of BPA are mostly due to its estrogenic activity (Kurosawa et al., 2002). Their actions are mediated by endocrine signaling pathways that resulting in large changes in the cell functions even at very low concentrations (Welshons et al., 2003). These changes have deleterious effects on health including prostate and breast cancer (Prins et al., 2008; Pupo et al., 2012). It has been reported that BPA induces oxidative stress in different body organs (Kabuto et al., 2004; Mourad and Khadrawy, 2012). Furthermore, other effects of BPA have been recorded such as mitochondrial mediated apoptosis in the hepatic tissue (Xia et al., 2014) and inflammatory cytokine dysregulation (Ben-Jonathan et al., 2009).

Herbal medicine has been used for treatment of several diseases even after the evolution of modern medicines. Licorice, from the dried roots of Glycyrrhiza glabra L.(Fabaceae), is one of the common herbal medicines that are grown and used broadly in different regions of the world (Karkanisa et al., 2016). Several countries have used extracted licorice as a sweetening additive in candies, tobacco, and beverages (Hesham et al., 2012).

Licorice has many components, including chalcones, flavonoids, isoflavonoids, glycyrrhizic acid and glycyrrhizin which is considered the major biologically active one (Huo et al., 2011). Previous studies have reported that licorice root has many medical activities such as antimicrobial, anti-atherosclerotic, anti-ulcer, anticancer, anti-viral, anti-inflammatory, antioxidant effects (Gafner et al., 2011; Liao et al., 2012; Fu et al., 2013) and free-radical scavenging activities (Di Mambro and Fonseca, 2005). It has been used as a medical raw material to reduce weight gain because of its diuretic effect (Wang et al., 2013), to increase white blood cell count, and as an antidote, a relaxant (Jung et al., 2016) and an expectorant (Takhshid et al., 2012).

Rahnama et al. (2013) proved that licorice is effective in the detoxification and protection of the liver. Moreover, licorice root contain liquiritin apioside which is a potent antitussive compound (Kamei et al., 2003).

The aim of the present study to investigate the biochemical, histopathological and genetic effects of BPA at different doses and to evaluate the protective role of licorice against such effects.

Materials and Methods

Animals:

Thirty Wistar male albino rats weighing 200-220 gm, were obtained from the National Research Center, Cairo, Egypt. Rats were housed and left in their cages for one week to allow appropriate acclimatization to the animal house conditions (45± 5% humidity, 25±2 ˚C temperature and 12 h lighting cycle) and had free access to tap water and standard rodent chow (El-Nile Company, Egypt).

Chemicals

Bisphenol A (BPA) was purchased from Sigma Aldrich (Saint Louis, MO, USA) in the form of crystalline white powder (CAS number: 80-05-7, purity of 99%). Licorice (Glycyrrhizic acid ammonium salt ($C_{42}H_{62}O_{16}.NH_3$) from glycyrrhiza root) was purchased from Sigma Aldrich (CAS Number: 53956-04-0, purity of ≥ 95.0%).

Animal treatment schedule:

The rats were randomly divided into 5 equal groups:

Group I (control group): was received the standard diet with corn oil (0.5 ml/day).

Group II (Licorice group): was received the standard diet with licorice at a dose of 150 mg/kg b.w. (body weight) dissolved in water, according to Takhshid et al., (2012).

Group III (BPA-low dose group) & Group IV (BPA-high dose group) were received the standard diet with BPA at doses of 2.4 µg/kg/day (Tiwari et al., 2012) and 500 mg/kg/day (Kattaia and Abdel Baset, 2014) respectively dissolved in corn oil.

Group V (treated group): was received the standard diet with BPA at a dose of 500 mg/kg b.w./day and licorice at the same previous dose.

The treatments were given orally by gastric tube for 4 weeks. Corn oil was given to the control group as it is the vehicle of BPA.

The selected low dose of BPA in our study was based on Howdeshell et al., (1999) who decided that the environmental level of BPA at 2.4 µg/kg b.w. Previous researches have reported that 2.4 µg/kg BPA has reproductive toxicity and same biological effects of its environmental amount which gives attention to the hazard of human exposure to BPA (Vomsaal and Hughes, 2005; Salian et al., 2009a). The high dose of BPA was about 10% of the LD50 value of BPA (LD50 oral for rats was 5000 mg/kg body weight) according to Chapin et al. (2008).

Animals, at the end of the experiment, were anesthetized with ether and sacrificed. Blood samples were suitably collected from each rat and centrifuged (centrifuge Jantezki, T30, Germany), at 5000 rpm for 10 minutes for serum collection. Then sera were separated and kept at -80°c until estimation of MDA (malondialdhyde), SOD (superoxide dismutase), CAT (catalase), GSH (reduced glutathione), TNF-α, ALP (alkaline phosphatase) and testosterone hormone. The whole lungs, stomach and testes were dissected out.

Preparation of Tissue Homogenates

Specimens from testes, lung and stomach were homogenized separately in phosphate buffer solution (prepared by dissolving 8.01g NaCl, 0.20g KCl, 1.78g Na_2HPO_4, $2H_2O$ and 0.27g KH2PO4 in 1 liter of distilled water and pH was adjusted at 7.4) using homogenizer (Tri-R Stir-R homogenizer, Tri-R Instruments, Inc., Rockville Centre, NY). The ratio of tissue weight to homogenization buffer was 1:5. Centrifugation of the homogenates was done at 5000 rpm for 10 min at 4°C. The resulted supernatant was kept at -80˚c until assessment of MDA level in lung, stomach and testes and cholesterol level in testes.

Biochemical Analysis:

At the end of the experiment, the concentrations of Alkaline phosphatase and cholesterol (BioMed, Egypt), catalase and reduced glutathione (Biodiagnostic, Egypt), TNF-α [ELISA kit (IDlabs™ inc. Biotechnology, Canada)] and testosterone [ELISA kits (Enzo Biochem Inc., USA)] were estimated by using diagnostic kits. The activities were detected according to the manufacturers' guidelines using commercially available kits.

The index of lipid peroxidation [malondialdehyde (MDA)] was detected by using 1,1,3,3-tetramethoxypropane as standard (Buege and Aust, 1978). Superoxide dismutase (SOD) activity was determined according to the previously described

method (Marklund and Marklund, 1974) based on the fact that the autoxidation of pyrogallol is inhibited by SOD. Gastric mucosal malondialdehyde (MDA) level was measured by the method of Mihara and Uchiyama, (1978).

Analysis of the gastric juice:

Gastric juice from each animal was collected and centrifuged at 1000 rpm for 10 min to get rid of any solid debris and the volume of the supernatant was determined. Next, the supernatant was assayed for total acid concentration and pepsin concentration.

Determination of total acidity of the gastric juice:

The total acidity was determined according to the method of Hara et al., (1991).

Determination of the proteolytic activity:

This was determined by a modified spectrophotometric method (Sanyal et al., 1971). The pepsin activity is the main factor concerned with the proteolytic activity of gastric secretion. The measured pepsin activity represent the amount of liberated tyrosine in micromole per 1 ml of gastric juice per minute using 1:100 diluted gastric juice and 2% bovine serum albumin in 0.01 NHCL as substrate.

Determination of the mucin concentration: This was determined by Winzler, (1955).

Histological Examination:

Samples from testes, lung and stomach were fixed in 10% buffered formalin, set in paraffin wax, sectioned and stained with hematoxylin and eosin. Slides were examined under Olympus (U.TV0.5XC-3) light microscopy and photographed by Olympus digital camera. Adobe Photoshop was used to process the images.

Gene Expression Analysis

Reverse transcription (RT) followed by polymerase chain reaction (PCR) is considered the ideal method for the detection and quantification of mRNA. Quantitative real time PCR (RT-qPCR) is progressively more used due to its high sensitivity, accuracy, excellent reproducibility, and broad dynamic quantification range (Cikos and Koppel, 2009).

Extraction of total RNA and cDNA synthesis

Lung tissues of male rats were used for isolation of the total RNA by TRIzol® Reagent (Invitrogen, Germany) Kit according to the manufacturer's guidlines of the above Kit. Aliquots of RNA were ready after isolation to be reverse transcribed into complementary DNA (cDNA). The reaction volume was done in 20 µl and prepared according to the protocol of the RevertAid First Strand cDNA Synthesis Kit (MBI Fermentas, USA). The reverse transcription (RT) reaction was done for 10 min at 25°C. Then, the tubes of the reaction were kept in thermo-cycler machine for 60 min at 42°C. Finally, the reaction was terminated for 5 min at 99°C. The PCR products containing the cDNA were preserved at -20°C till DNA amplification.

Quantitative real time-PCR:

Evaluation of the copy of the cDNA of male rats was done by a StepOne Real-Time PCR System (Applied Biosystem, USA) to identify the expression values of the tested genes. The PCR reaction was described previously by El-Baz et al., (2016).

Briefly, 25 µL of reaction mixtures was prepared containing 12.5 µl of SYBR® green (TaKaRa, Biotech. Co. Ltd.), 0.5 µl of 0.2 µM forward and reverse primers, 6.5 µL DNA-RNA free water, and 2.5 µl of the synthesized cDNA. Propagation of the cDNA was done by reaction program in three steps. Firstly: incubation of the PCR tubes at 95°C for 3 min. Then: the reaction program had 50 cycles. Each cycle had 3 sub-steps: (a) 15 sec at 95°C; (b) 30 sec at 60°C; and (c) 30 sec at 72°C. Finally: the reaction program contained 71 cycles, with temperature ranged from 60 °C to 95 °C that increased in steps of 0.5°C/10 s. A melting curve analysis of the reaction followed these cycles was done for each qRT-PCR termination at 95.0°C to detect the quality of the primers. The qRT-PCR reaction without any contamination was confirmed by using PCR tubes containing non template control. The $2^{-\Delta\Delta CT}$ method was used to determine the relative quantification of the target genes to the reference (β-Actin).

Statistical analysis

The collected data from all groups were analyzed using SPSS 16 (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA). The data was expressed as means ± standard deviation. One-way ANOVA was applied to compare the means of the quantitative parameters among the examined groups. Post hoc Tukey's test was applied to identify which groups' means differed.

Results

Biochemical analysis:

Serum GSH, SOD and catalase were significantly decreased while level of TNF-α showed a significant increase following BPA (500 mg/kg/day) for 4 weeks as compared to the control group. Meanwhile, treatment of rats with licorice in combination with BPA (500 mg/kg/day) caused significant increase in serum levels of GSH, SOD and catalase and a significant decrease in level of TNF-α as compared to high dose BPA group **(Table I).**

The level of MDA in serum, lung, testis and stomach in BPA- high dose group increased significantly in comparison to the control group. On the other hand, administration of licorice in combination with BPA (500 mg/kg/day) showed a significant decrease in level of MDA in serum, testis and stomach as compared to BPA (500 mg/kg/day) group **(Table II).**

Serum testosterone and testicular cholesterol were significantly decreased while serum alkaline phosphatase was significantly increased in BPA (500 mg/kg/day) group as compared to the control group. Licorice in group V caused a significant increase in both serum testosterone and testicular cholesterol and a

significant decrease in serum alkaline phosphatase as compared to BPA (500 mg/kg/day) group **(Table III).**

Bisphenol A in a dose of 500 mg/kg/day caused a significant increase in the levels of total acidity and pepsin, however, the level of mucin was significantly reduced as compared to the control group. While, significant decrease in the levels of total acidity and pepsin with significant increase in mucin was recorded in rats treated with licorice in combination with BPA (500 mg/kg/day) as compared to BPA (500 mg/kg/day) group **(Table IV).**

Histological examination

In the present study, histological examination of testis sections of group I (control group; **Fig. 1A1 & A2)**, group II (Licorice group; **Fig. 1B**) and group III (BPA-low dose; **Fig. 1C**) showed the seminiferous tubules (T) lined with regularly arranged rows of spermatogenic cells. The interstitial cells of leydig (ic) located in between the seminiferous tubules. (1. mature sperms, 2. Spermatid, 3. primary spermatocytes, 4. Spermatogonium, 5. Sertoli cell). Figure 1A2 showed higher magnification of spermatogenic cells. Group IV (BPA-high dose) showed marked degenerated seminiferous tubules (T) with depletion of the spermatogenic cells which are irregularly arranged (*). Many tubules are filled with cellular debris (arrows) **(Fig. 1D).** Group V (treated group) showed few seminiferous tubules with degenerated spermatogenic cells (arrow), others resemble normal (T) **(Fig. 1E).**

Examination of lung sections of group I (control group; **Fig. 2A**), group II (Licorice group; **Fig. 2B**) and group III (BPA-low dose; **Fig. 2C**) showed normal lung architecture. The alveoli (a) appeared inflated with thin interalveolar septa (arrow). The bronchioles (b) appeared normal with intact epithelial lining. Group IV (BPA-high dose group; **Fig. 2D**) had marked morphological changes in the lung tissue. Extravasated red blood cells appeared in the alveolar lumen and in the interstitium (black arrow). The alveolar septa increased in thickness (green arrow). Degenerated bronchiolar epithelium was observed in the bronchiolar lumen (b). On the other hands, improvement of the morphological changes was noticed in group V (treated group; **Fig. 2E**). Patent alveolar lumen, few areas of septal thickening and inflammatory cells infiltration were noticed (arrow). Scattered bronchioles showed degenerated bronchiolar cells.

Examination of stomach sections of group I (control group; **Fig. 3A**), group II (Licorice group; **Fig. 3B**) and group III (BPA-low dose; **Fig. 3C**) showed regularly arranged fundic glands (g) in the lamina propria, normal intact gastric pits (arrow), muscularis mucosa (mm) and musculosa (m). Group IV (BPA-high dose; **Fig. 3D**) showed severe erosions in the fundic glands and ulcerated mucosa (star) associated with distorted normal architecture with discontinuity of muscularis mucosa (mm), and musculosa (m). Apparent decrease in the fundic glands` length (line) was noticed. Also, detached cells (arrows) and inflammatory cell infiltration (circle) in lamina propria were noticeable. Group V (treated group; **Fig. 3E**) showed visible intact fundic glands, lamina propria, muscularis mucosa (mm) and musculosa but with wide fundic pits (arrows). The reactive fundic glands hyperplasia (h), mucosal infiltration (circle) and congestive blood vessels (bv) were obvious.

Gene Expression Analysis

The expression values of the inflammation related gene (NF-κB1) in lung tissues of male rats treated with BPA and/or licorice were quantified by real-time RT-PCR. The results revealed that exposure of male rats to BPA exhibited several effects on the expression of NF-κB1 gene depending on the dose of exposure used in this study. The expression level of NF-κB1 was increased in male rats exposed to low dose of BPA (group III) compared with those in control rats, however, the differences were not significant. While, the expression level of NF-κB1 was increased significantly in male rats exposed to high dose of BPA (group IV) as compared with those in control group. Moreover, administration of BPA-high dose with licorice (group V) showed significant decrease in the expression level of NF-κB1 as compared with group IV (BPA-high dose). **(Table V)**

Table I: One-way ANOVA statistical analysis of the effect of BPA at different doses, licorice and their combination on serum GSH, catalase, SOD and TNF-α in the examined rats (n=6).

Mean ± SD Group	Serum GSH (mg/dl)	Serum catalase (U/dl)	Serum SOD (U/l)	Serum TNF-α (pg/ml)
Group I	57.83 ± 8.44	47.33 ± 8.89	75.42± 10.66	13.08 ± 3.26
Group II	51.17 ± 9.24	42.17 ± 7.16	69.36 ±8.31	16.33±2.33
Group III	53.67 ± 8.02	44.16 ± 8.93	66.94± 7.04	17.00± 5.60
Group IV	25.83 ± 5.70	15.67 ± 5.78	27.95± 6.40	39.83±3.25
Group V	46.83 ± 6.88	31.67± 7.15	58.55± 6.93	20.08 ± 5.86
P value	0.87^{***}	0.88^{***}	0.79^{***}	0.30^{***}

****P<0.0001: Very highly significant, SD : Standard deviation*

Table II: One-way ANOVA statistical analysis of the effect of BPA at different doses, licorice and their combination on serum, lung, testis and gastric MDA in the examined rats (n=6).

Mean ± SD Group	Serum MDA (nmol/ dL)	Lung MDA (nmol/gm tissue)	Testis MDA (nmol/gm tissue)	Gastric MDA (nmol/gm tissue)
Group I	182.71± 9.72	38.39±6.46	53.90±4.80	24.55±8.25
Group II	196.02 ± 15.11	38.95±4.92	51.46±6.32	26.11±7.24
Group III	200.31±13.36	39.66±4.69	54.29±8.09	25.51±9.84
Group IV	384.13 ± 11.83	80.41±7.97	81.16±3.57	72.72±9.11
Group V	203.92± 12.25	47.15±7.77	60.22±4.24	35.49 ± 9.20
P value	0.92***	0.70***	0.41***	0.97***

****P<0.0001: Very highly significant , SD: Standard deviation*

Table III: One-way ANOVA statistical analysis of the effect of BPA at different doses, licorice and their combination on serum testosterone, alkaline phosphatase and testicular cholesterol in the examined rats (n=6).

Mean ± SD Group	Serum testosterone (pg/ml)	Serum alkaline phosphates (U/L)	Testicular cholesterol (mg/gm tissue)
Group I	4.93± 1.24	125.95± 12.42	34.27±7.98
Group II	5.25±1.16	129.36 ± 18.79	35.62±7.82
Group III	4.79±1.39	132.43 ± 17.90	33.11± 9.35
Group IV	1.84±0.96	186.87± 10.46	15.49± 3.73
Group V	4.24 ± 1.65	156.36±8,.66	26.02± 6.41
P value	0.82***	0.41***	0.44***

****P<0.0001: Very highly significant, SD: Standard deviation*

Table IV: One-way ANOVA statistical analysis of the effect of BPA at different doses, licorice and their combination on total acidity, pepsin and mucin in the examined rats (n=6).

Mean ± SD Group	Total acidity (mEq/3h)	Pepsin (µg/ml tyrosine)	Mucin (mg % hexose)
Group I	47.50± 9.33	116.50±11.09	104.83± 7.83
Group II	48.66± 9.07	119.83±11.80	102.60±7.63
Group III	47.76± 7.97	118.50±11.05	106.33± 8.29
Group IV	96.00± 8.25	183.83±6.05	65.50 ± 17.98
Group V	57.33± 11.54	135.16±6.55	99.33±11.18
P value	0.93***	0.49***	0.25***

****P<0.0001: Very highly significant, SD : Standard deviation*

Table V: One-way ANOVA statistical analysis of the effect of BPA at different doses, licorice and their combination on the expression of NF-κB1 gene in the lung tissue of the examined rats (n=6).

Group	Mean ± SD
Group I	0.63 ± 0.01^{c}
Group II	0.59 ± 0.05^{c}
Group III	0.93 ± 0.02^{bc}
Group IV	1.93 ± 0.02^{a}
Group V	1.13 ± 0.047^{b}
P value	0.13***

****P<0.0001: Very highly significant, SD : Standard deviation*

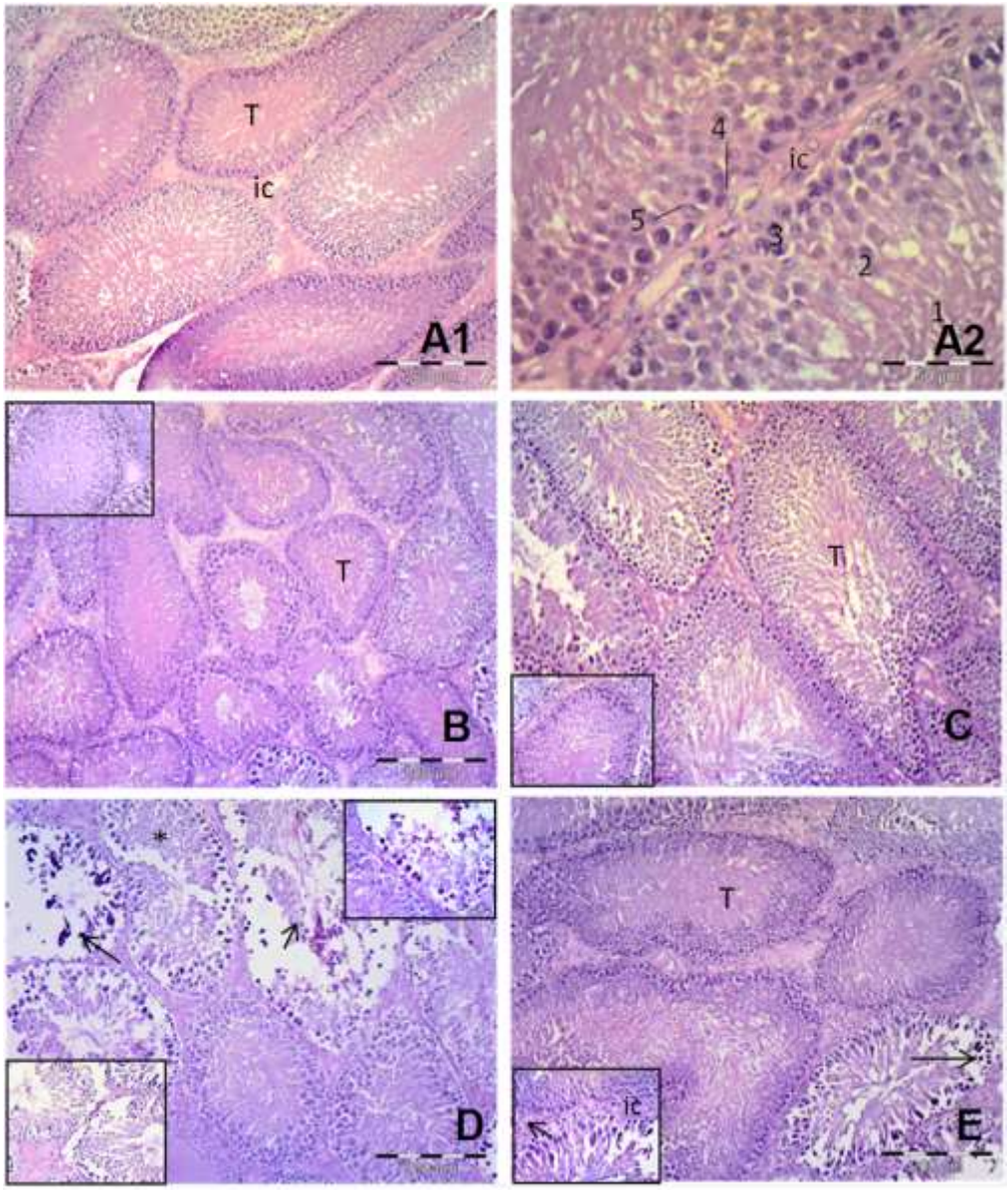

Figure (1): Photomicrograph of a section of testis A) Control group, B) Licorice group and C) BPA-low dose group showing the seminiferous tubules (T) lined with regularly arranged rows of spermatogenic cells. The interstitial cells of leydig (ic) located in between the seminiferous tubules. Inset key (1. mature sperms, 2. Spermatid, 3. primary spermatocytes, 4. Spermatogonium, 5. Sertoli cell). A2) showing higher magnification of spermatogenic cells. D)BPA-high dose showing marked degenerated seminiferous tubules (T) with depletion of the spermatogenic cells which are irregularly arranged (*). Many tubules are filled with cellular debris (arrows). E) Treated group showing few seminiferous tubules with degenerated spermatogenic cells (arrow), others resemble normal (T). H&Ex100 & Insets x400

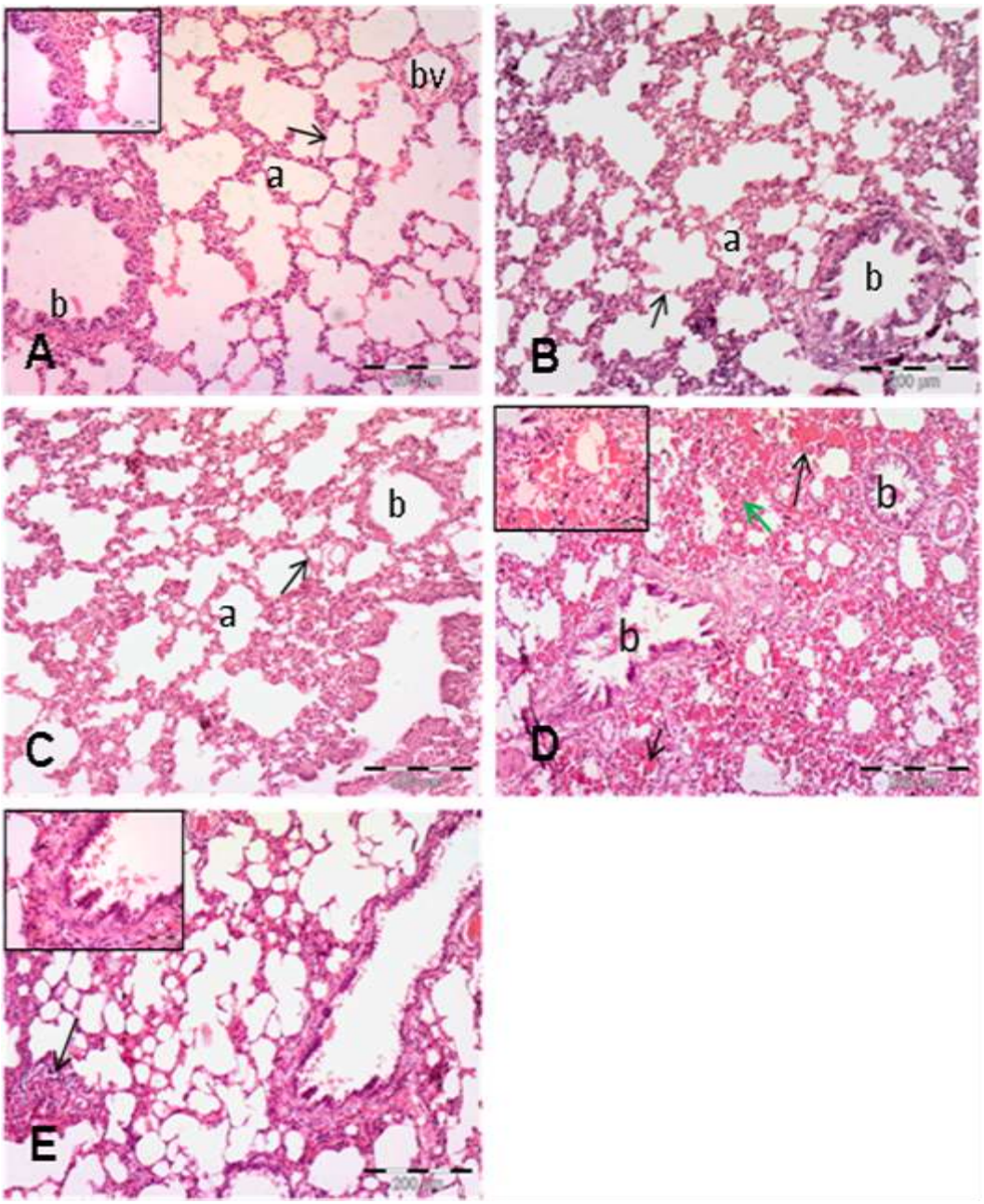

Figure (2): Photomicrograph of a section of lung A) control group, B) Licorice group and C) BPA-low dose showing normal lung architecture. The alveoli (a) appeared inflated with thin interalveolar septa (arrow). The bronchioles (b) appeared normal. D) BPA-high dose group showing marked morphological changes in the lung tissue. Extravasated red blood cells appeared in the alveolar lumen and in the interstitium (black arrow). The alveolar septa increased in thickness (green arrow). Degenerated bronchiolar epithelium was observed in the bronchiolar lumen (b). E) Treated group showing improvement of the morphological changes. Patent alveolar lumen, few areas of septal thickening and inflammatory cells infiltration were noticed (arrow). Scattered bronchioles showed degenerated bronchiolar cells. H&Ex100 & Insets x400

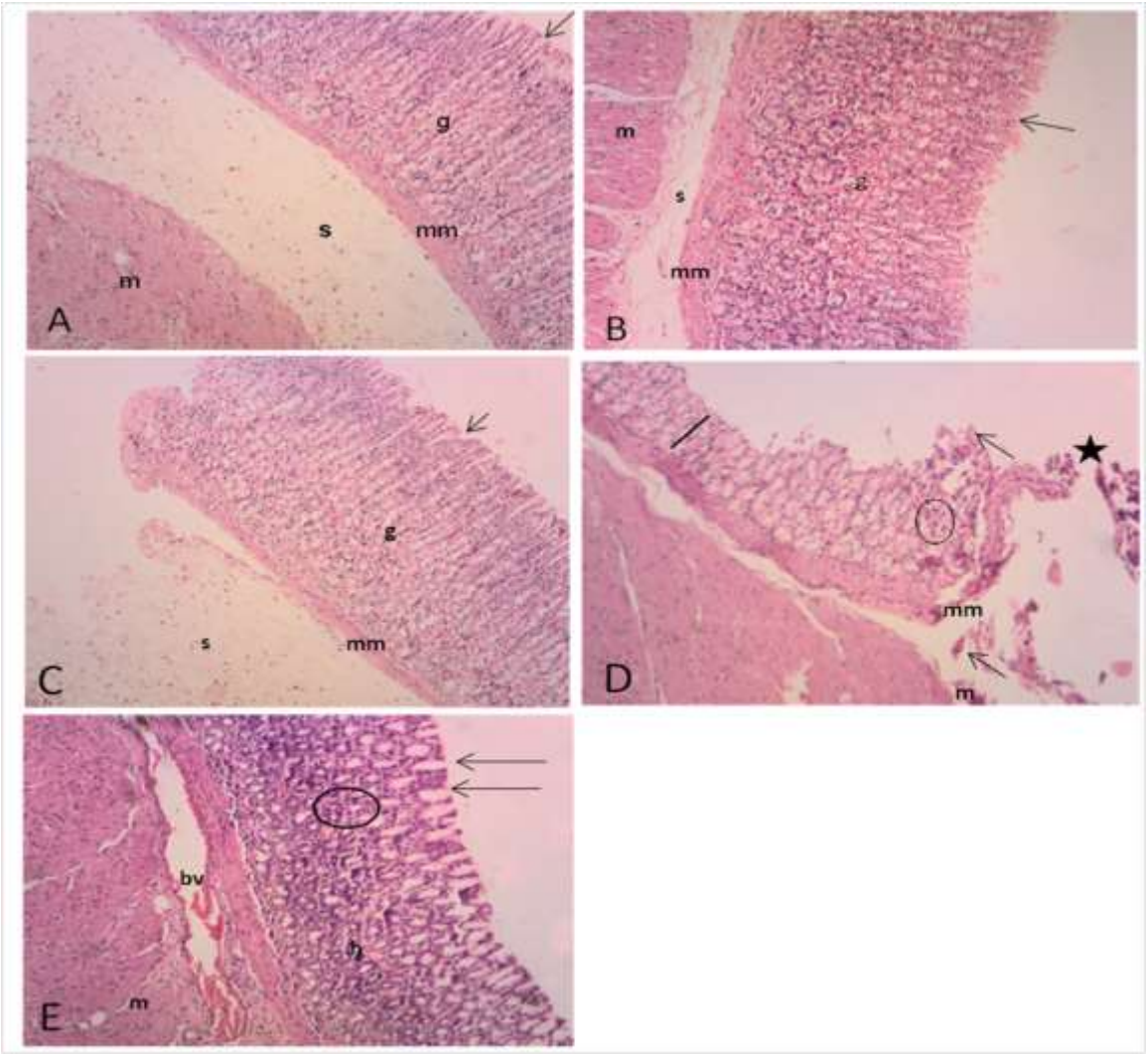

Figure (3): Photomicrograph of a section of stomach A) control group, B) Licorice group and C) BPA-low dose group showing regularly arranged fundic glands (g) in the lamina propria, normal intact gastric pits (arrow), muscularis mucosa (mm) and musculosa (m). D) BPA-high dose group showing severe erosions in the fundic glands and ulcerated mucosa (star) associated with distorted normal architecture with discontinuity of muscularis mucosa (mm), and musculosa (m). Apparent decrease in the fundic glands` length (line) was noticed. Also, detached cells (arrows) and inflammatory cell infiltration (circle) in lamina propria were noticeable. E) treated group showing visible intact fundic glands, lamina propria, muscularis mucosa (mm) and musculosa but with wide fundic pits (arrows). The reactive fundic glands hyperplasia (h), mucosal infiltration (circle) and congestive blood vessels (bv) were obvious. H&Ex100.

Discussion

The widespread exposure of human to BPA-containing products attracts attention of the scientists to investigate their detrimental effects on the human health (Mourad and Khadrawy, 2012). Our study showed decrease in the activities of superoxide dismutase, catalase and reduced glutathione in serum while there was an increase in MDA level; lipid peroxidation marker in serum, lung, testes and stomach after 4 weeks treatment with BPA in high dose.

Accordingly, these results demonstrated that exposure to high dose of BPA induced oxidative stress and this is in agreement with several previous studies inspite of different doses and different duration of exposure such as Tiwaria et al. (2012) who reported that BPA treatment (50 mg/kg b.w.) caused significant increase in the levels of lipid peroxidation and decrease in the concentration of GSH activity. Tamilselvan et al. (2013) revealed the same results in testes of rats after treatment with 200 mg/kg BPA for 30 days. Moghaddam et al. (2015) showed an increase in MDA level and a deacrease in GSH levels in serum and pancreas and a decrease in SOD and CAT levels in pancreas.

On the other hand, the present results are in contrast in a part to Kabuto et al. (2003) who showed that SOD activity increased and catalase activity decreased

significantly in the liver after intraperitoneal injection of 25 mg/kg and 50 mg/kg BPA for 5 days but SOD activity showed no significant change in brain, lung, kidney, liver, fat body, testes, and blood. This difference may be due to different doses and different duration of treatment.

It has been suggested that oxidative stress induced cytotoxicity through direct membrane damage and generally increases the membrane permeability. This leads to rising in the intracellular calcium which disturbs mitochondrial function and ultimately stimulates reactive oxygen species (ROS) production (Demuro et al., 2005; Gleichmann and Mattson, 2011).

The ROS are formed in profuse in pro-oxidant states and play an essential role against pathological conditions; however overproduction of ROS may harm the body tissues. The antioxidant enzymes act against ROS toxicity (Birben et al., 2012). Superoxide anion radical has been dismutased by Superoxide dismutase (SOD) to hydrogen peroxide, which is further degraded by catalase through reduced glutathione (Hayyan et al., 2016). The decrease in antioxidant enzymes activities explained the malfunction of antioxidant system to overcome the free radicals formed after exposure to BPA (Lobo et al., 2010). Sajiki (2001) suggested that bisphenol A is metabolized to a reactive metabolite 4,5-bisphenol O-quinone and bisphenol radical by oxygen radical reactions.

Alkaline phosphatases (ALP) are a group of enzymes that found in several body tissues such as liver, kidney, intestine, bone and white blood cells. The main function of alkaline phosphatase is transporting across the cell membranes (Celik et al., 2009). Hence, destruction of these tissues results in leakage of ALP into the blood. Our study showed a significant increase in ALP serum level which may be attributed to oxidative stress induced by BPA treatment that cause tissue damage and release of the enzymes from tissues in serum. This result is in agreement with Eshak and Osman (2014). Sangai and Verma (2012) found that BPA may serve as a plasma membrane labilizer. The raise in ALP activities can comprise a danger to the cell life which is reliant on phosphate esters for its vital processes.

The current study showed a significant increase in the serum level of TNF-α after exposure of rats to high dose of BPA for 4 weeks. This result revealed that exposure to high dose of BPA induced inflammatory reaction which is in accordance with Zhu et al. (2015). High TNF-α level may affect the apoptotic pathways in various organs and increase the susceptibility to autoimmunity (Zhang and An, 2007).

In the present study, significant decrease in testicular cholesterol level was demonstrated after exposure of examined rats to 500 mg/kg BPA. This result had been explained by Negri-Cesi (2015) who reported that BPA can disturb the hypothalamic neuroendocrine functions which is partly involved in regulation of fat metabolism (Dieguez et al., 2011).

Decrease in testicular cholesterol level accompanied by reduction in serum testosterone level. Similar to these results, Eshak and Osman (2014) revealed that BPA induced endocrine and reproductive toxicity. Cholesterol is a precursor of all sex hormones. Testosterone synthesis process is a series of events through which conversion of cholesterol into testosterone occurs when luteinizing hormone (LH) triggers the testicular leydig cells (Miller and Auchus, 2011). The reduction in plasma testosterone level may be due to reduction in cholesterol level and/or degeneration of Leydig cells in testes (Nanjappa et al., 2012) that is revealed by histopathological examination of exposed rats in our study. Moreover, it has been reported that BPA acts as estrogen agonist and androgen antagonist activity (Acconcia et al., 2015).

The testosterone is the major androgen hormone which is important for maintenance of spermatogenesis and inhibition of germ cell apoptosis (Dohle et al., 2003). Akingbemi et al. (2004), Nakamura et al. (2010) and Gurmeet et al. (2014) are in agreement with our study, they showed reduction in serum testosterone level after treatment with BPA. While Jahan el al. (2016) are party in contrast, they revealed that BPA (50 mg/kg) induced decrease in serum testosterone and increase in serum cholesterol levels. This difference may be due to different doses of exposure to BPA.

These results were supported by marked histological changes of testes after administration of 500 mg/kg BPA. These changes have been clarified by Gurmeet et al. (2014) who supposed that the decreased testosterone level may be the cause of alteration of spermatogenesis and disturbance of the seminiferous epithelium. Additionally, the disturbances in hypothalamic-pituitary-gonadal axis and decrease in Sertoli cell phagocytic function that is induced by BPA administration could affect the histological structure of the testes (Vandenberg et al., 2009). The cytotoxic agents can affect the spermatogenic cells due to their high mitotic activity (Endo et al., 2003). Our results are in accordance with Li et al. (2009), Jahan et al. (2016) and Tian et al. (2017). They showed morphological damages in testis after administration of BPA and provide the evidence for their toxicity on the male reproductive system.

The current microscopic examination of the lung showed severe morphological disruption in the lung after chronic administration of 500 mg/kg BPA. This result is in agreement with Amaravathi et al. (2012) and Kattaia and Abdel Baset (2014). Previous researchers suggested that BPA induced inflammation by production of different cytokines which are secreted by macrophages (Wetherill et al., 2007). These cytokines resulted in endothelial cell adhesion molecules that provoked the

adhesion and transport of neutrophils from the vascular space into parenchyma. This lead to production of proteases and oxidants that damaged the vascular endothelial cells and clarified the extravasated RBCs of the lung tissue and degenerated bronchial epithelium found in the present study (Wetherill et al., 2007). The thickened alveolar septa may be due to marked deposition of interstitial collagen fiber and severe cellular infiltration with eosinophils, neutrophils, lymphocytes and macrophages (Zidan, 2011).

The current study also revealed that the high dose of BPA resulted in gastric ulcer formation, inflammatory cell infiltration and distortion of the normal architecture of the gastric tissue after 4 weeks. It has been reported that a disturbance between aggravating and protective factors leads to induction of gastric ulcer formation. Gastric acid, pepsin and oxidant agents are considered as aggravating factors, while mucous, and antioxidant agents are protective factors (Suleyman, 2012). To our knowledge, there are no studies have been reported about the effect of BPA on the stomach.

Moreover, our study reported that high dose of BPA increased the gastric juice total acidity and pepsin activity significantly. This effect may be attributed to several processes; generation of ROS, enhancement of lipid peroxidation, infiltration of leukocytes and initiation of apoptosis (Bech et al., 2000). Also, a significant reduction in mucin was recorded in group IV. These results were evidently supported our findings in the histological examination of the stomach. Mucin is essential for gastric mucosa against ulcerogens and promotes the repair of the damaged gastric epithelium by decrease in the stomach wall friction during peristalsis and serving as an important barrier to back diffusion of hydrogen ions (Sevak et al., 2002). Furthermore, mucus is able to act as an antioxidant as it can minimize mucosal hurt enhanced by oxygen free radicals (Repetto and Llesuy, 2002).

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) is a protein complex that is present in approximately all cell types. NF-κB is involved in DNA transcription and cell survival (Gilmore, 2006). It is concerned with cellular responses to different stimuli for example; cytokines, viral or bacterial antigens, oxidative stress and free radicals (Perkins, 2007). Additionally, NF-κB has an important role in controlling the immune response to infection (κ light chains are essential structures of antibodies). The mammalian NF-κB family has five proteins; NF-κB1, NF-κB2, RelA, RelB and c-Rel (Nabel and Verma, 1993). False regulation of NF-κB may be due to viral infection, inflammation, septic shock, cancer, and autoimmune diseases (Meffert et al., 2003). Hence, we aimed to study NF-κB transcription and activation.

NF-κB is remained in the cytoplasm in unstimulated cells. Once the cells are activated, NF-κB leads to nuclear translocation, which is responsible for regulation of certain cytokines genes, like TNF-alpha (Oeckinghaus et al., 2011). Our study revealed a significant increase in the expression level of NF-κB after exposure to high dose of BPA as compared to the control group. Li et al. (2012) determined that BPA can modify different transcriptional factors, such as Sp1 (specificity protein 1) that affect the combining site of NF-κB and hence change the transcriptional capacity of NF-κB. Moreover, Jung et al. (2016) reported that oxidant generation enhanced activation of NF-κB which resulted in raising the TNF-α level that lead to tissue injury and this is in concomitant with the results in the present study. Previous studies supposed that of NF-κB activation could stimulate invasion of cancer cells (Palumbo et al., 2007; Wu et al., 2009).

The current study revealed that low dose of BPA (2.4 µg/kg) had no significant effect on the exposed rats and this is in agreement with Tiwari et al. (2012) and Kattaia and Abdel Baset (2014). The International Food Safety Authorities Network (INFOSAN) reported that 5 mg/kg/day showed no-observed adverse- effect level (NOAEL) for BPA. NOAEL is the maximum dose that did not stimulate any adverse effect in the examined animals (INFOSAN, 2009). But, this result disagrees with Salian et al. (2009a, 2009b) and Akingbemi et al. (2004) who reported that the environmental dose of BPA induced adverse effects on testicular functions of experimental animals.

The current study revealed that treatment with licorice improved the oxidative stress induced by high dose of BPA, raised the levels of cholesterol and testosterone to normal and decreased the level of TNF-α significantly. Also, it improved the histopathological changes induced by high dose of BPA in testis, lung and stomach in group V in comparison to group IV. These results indicated that antioxidant and anti-inflammatory effect of licorice. This is in agreement with Yousaf et al. (2016) and Rashwan and Anfenan (2012) who contributed the antioxidant properties of licorice extract to metabolism of glycyrrhizic acid to glycyrrhetinic acid that has an antioxidant activity (Marí et al., 2009). It also may be due to their content of glabridin (Asl and Hosseinzadeh, 2008). Glabridin inhibits oxidation of LDL by binding to it, minimizes NADPH oxidase activation and increases cellular glutathione that results in reduction of cellular oxidative stress (Rosenblat et al., 2002).

It has been reported that the anti-inflammatory effect of licorice may be as a result of its effect on the adrenal gland which is responsible for production of cortisol (Konovalova et al., 2000). Previous studies suggested that licorice extract has different bioactive compounds which inhibit NO production and inflammatory cytokine (Chung et al., 2014; Yu et al., 2015). Our results are in accordance with Takhshid et al.

(2012) and Jung et al. (2016) who clarified that licorice-derived compounds enhance prostaglandins concentration in the digestive system and this increases secretion of mucus from the stomach which has healing effects.

Furthermore, licorice treatment led to a significant reduction in the expression level of NF-κB1 as compared with group IV (BPA-high dose). Previous research demonstrated the efficiency of Licorice extract in vitro to prevent the signaling pathways leading to NF-κB activation (Schröfelbauer et al., 2009).

According to the authors` knowledge, there is no previous studies have investigated the protective effect of licorice for inhibition and/or reduction of toxicity induced by BPA.

Conclusion

In conclusion, 4 weeks exposure to high level of bisphenol a (BPA) induced oxidative stress, inflammatory reaction, histopathological changes in different body organs, and genetic effect. The licorice can reduce and/or prevent the toxic effects induced by BPA due to its antioxidant, anti-inflammatory, anti-atherosclerotic, anti-ulcer effects and free-radical scavenging activities. Hence, we can use the licorice as a prophylaxis against BPA toxicity, taking into consideration the dose and duration of treatment.

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Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

Animals and their care were approved according to the animal care Committee of Faculty of Medicine - Minia University, Egypt. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

This article does not contain any studies with human participants performed by any of the authors.

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الملخص العربى

هل يستطيع العرق سوس منع التأثيرات البيوكيميائية و الهستولوجية و الجينية الناتجة عن مادة البسفينول أ

ولاء يحيى عبد الظاهر١ و داليا محمد على٢ و وجدى خليل٣

ان البسفينول أ هو معطل للغدد الصماء الايكولوجية الاستروجينية الذى يستخدم عادة فى البلاستكات البولى كاربونية. هدفت هذه الدراسة إلى اختبار التأثيرات البيوكيميائية و الهستولوجية و الجينية الناتجة عن جرعات مختلفة لمادة البسفينول أ و لتقييم التأثير الوقائي المحتمل للعرق سوس ضد هذه التأثيرات. تم تقسيم ٣٠ من الفئران البيضاء الى خمس مجموعات ، أعطيت مادة البسفينول يوميا بجرعات 2.4 ميكروجم/كجم & ٥٠٠ مجم/كجم عن طريق الفم مع أو بدون العرق سوس (١٥٠ مجم/كجم) لمدة ٤ أسابيع. وأظهرت النتائج أن الجرعة السمية العالية انقصت مستوى الجلوتاثيون والسبوبر أكسيد دسميوتيز و الكاتالايز و زادت مستوى المالونديالدهايد بشكل ملحوظ. انخفضت مستويات عامل نخر الورم- الفا و هرمون التستوستيرون و الكولسترول بالخصية بشكل ملحوظ في حين أن الفوسفاتيز القلوية في الدم زادت بشكل ملحوظ. وقد لوحظت التغيرات الهستولوجية في الخصيتين والرئتين والمعدة. حدث تغيير في التعبير عن الجين NF-κB1 في الرئة. وأشارت هذه النتائج أن الإجهاد التأكسدي الناجم عن البسفينول أ أدى إلى هذه المضاعفات في الفئران المفحوصة وأن العلاج بالعرق السوس لطف من السمية الناجمة عن البسفينول أ.

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