El Makawy et al. Bulletin of the National Research Centre (2018) 42:5
https://doi.org/10.1186/s42269-018-0007-4

Bulletin of the National Research Centre

RESEARCH Open Access

Flaxseed oil as a protective agent against bisphenol-A deleterious effects in male mice

Aida El Makawy[1*], Fawzy Eissa[2], Mahmoud EL-Bamby[2] and Osama Elhamalawy[2]

Abstract

Background: Flaxseed oil is one of the most vital oils that contain a high content of omega-3 polyunsaturated fatty acids (PUFAs) recognized as a high-quality nutrition for health benefits. In addition, a source of vitamin E and β-carotene has a positive health effect in a variety of pathologies. The goal of the present work was to investigate the protecting character of flaxseed oil against bisphenol-A deleterious effects in male mice. Animals were gavaged with BPA for 28 successive days. They were orally administered flaxseed oil either before, with, or after treatment of BPA. DNA damage was evaluated in the liver, testes, and bone marrow cells using comet and micronucleus assays. Liver and testes histopathological examination as well as sperm physical characters were also investigated.

Results: The results showed that BPA induced a significant raise in DNA damage that obviously appear in the increase of tail length, tail DNA percentage, and tail moment in the liver and testes. In addition, there was an increase in the frequency of micronuclei in bone marrow cells. Liver and testes histopathological alterations and sperm count and motility significant comedown were seen in male mice exposed to BPA as well. Conversely, flaxseed oil oral administration through the three regimens of treatment effectively attenuated the abovementioned effects. Moreover, administration of flaxseed oil before BPA treatment was the best protocol in the attenuation of BPA toxic effects.

Conclusions: Flaxseed oil successfully attenuated the BPA genotoxicity, sperm defects, and histological alterations in male mice that may be referred to its antioxidant property.

Keywords: Flaxseed oil, Bisphenol-A, DNA damage, Histopathological alterations, Sperm character

Background

Bisphenol-A (BPA) is an important manufacturing chemical that is intensively used in many consumer products, such as polycarbonate plastics, epoxy resins, medical instruments, baby toys, water pipe, dental sealants, food packaging liners, and coatings for tins (Huang et al. 2012; Makris et al. 2014). It has known that BPA annual production more than 4 million tons (Michalowicz 2014). Numerous studies established its occurrence in different ecological samples (Fu and Kawamura 2010; Rocha et al. 2013). Human BPA exposure sources including atmosphere, water, sewage, food, and dust (Michalowicz 2014). However, food is mainly the vital resource of common population contact to BPA (Vandenberg et al. 2007). BPA exposure was linked with sperm DNA damage increase and a decline in semen quality among men (Meeker et al. 2010). Moreover, it has been revealed that BPA was able to cause oxidative stress by forming reactive oxygen species (ROS) (Yi et al. 2011) that may cause genetic toxicity (Tiwari et al. 2012).

Consequently, there is a critical need to find a protective agent, which has possible roles in intra- and extracellular defense against oxygen radicals and lipid peroxides induced by oxidative stress. Among natural products, flaxseed oil (FXO) became a matter of choice for our investigation. It is obtained from *Linum usitatissimum* that belongs to the family Linaceae, and it is commonly known as Flax or Linseed. It is one of the most vital oils, which contain high content of omega-3 polyunsaturated fatty acids (PUFAs), specifically α-linolenic acid recognized as a high-quality nutrition for health benefits (Barcelo-Coblijn and Murphy 2009). Recently, studies report that a high intake of saturated fats was inversely associated with sperm physical characters. The higher intake of omega-3 polyunsaturated fats

* Correspondence: aelmakawy@yahoo.com
[1]Department of Cell Biology, National Research Center, 33 El-Bohouth St.-Dokki, P.O.12622, Giza, Egypt
Full list of author information is available at the end of the article

(PUFAs) was positively associated with sperm morphology (Safarinejad 2011; Attaman et al. 2012; Olsen and Ramlau-Hansen 2012). It is also a source of vitamin E and β-carotene (Hana and Saed 2013). Sargi et al. (2013) revealed that flaxseed is an excellent source of alpha-linolenic acid that is a precursor of long-chain PUFAs metabolically synthesized in the human body. It has a higher level of omega-3 and omega-6 and showed higher antioxidant capacity. Earlier studies have reported that FXO-supplemented diets have a positive health effect in a variety of pathologies (Lin et al. 2002; Newairy and Abdou 2009).

DNA damage in single cells can be determined by numerous standard methods like micronucleus (MN) test or the comet assay. The rodent erythrocyte MN assay in the bone marrow is considered as a primary assay to assess the genotoxic potential. The alkaline comet assay is being proposed by testing and regulatory agencies as a second in vivo genotoxicity bioassay to complement the MN assay, since it can detect DNA repair and a broad spectrum of DNA damage (Kang et al. 2013).

Hence, the in-progress work aimed to investigate the deleterious effects of BPA on DNA integrity in liver, testis and femoral bone marrow; sperm count and motility; and histopathological alterations in the liver and testis of male mice and the protecting effects of FXO on these parameters.

Methods

Chemicals

Bisphenol-A (BPA) (≥ 99%) was purchased from Sigma–Aldrich Company (St. Louis, MO, USA). Flaxseed commercial oil (FXO) was purchased from EL Captin Company (Al Obour City, Cairo, Egypt). All other chemicals were of analytical grade and purchased from standard commercial suppliers.

Dose preparation

BPA was dissolved in absolute ethyl alcohol (95%) and diluted with corn oil [1:20 alcohol to corn oil (vehicle)] to obtain a final concentration of BPA 50 mg/kg bw (Tyl et al. 2008).

Animals

Male Swiss albino mice (26 ± 5 g) were purchased from Theodor Bilharz Research Institute, Giza, Egypt, at 10–12 weeks of age. All animals were housed in polycarbonate cages with stainless steel covers in an air-conditioned room (12-h light/dark cycle with a temperature of 23 ± 0.5 °C and a relative humidity of 55 ± 5%). All animals received humane care in compliance with the guidelines of the Animal Care and Use Committee of the National Research Centre and the National Institutes of Health (NIH publication 86-23 revised 1985).

Experimental design

Seventy male Swiss albino mice were randomly divided into seven groups ($n = 10$), according to approximately equal mean body weight. Animals were administered orally with BPA (50 mg/kg bw) and/or FXO (1 mL/kg bw) according to Kaithwas and Majumdar (2012) either before, with, or after BPA administration for 28 successive days as follows: group 1 (control), group 2 (vehicle), group 3 (FXO), group 4 (BPA), group 5 (FXO before BPA), group 6 (FXO with BPA), and group 7 (FXO after BPA).

Oil analysis

Fatty acids analysis

The fatty acids profile was determined as fatty acid methyl esters by Thermo Scientific TRACE 1310 Gas Chromatograph attached with ISQ LT single quadrupole mass spectrometer (GC–MS). Preparation of fatty acid methyl esters (FAMEs) was performed according to the procedure of AOAC (2000). A sample of oils (50 mg) and 1% H2SO4 in absolute methanol was put in a screw-cap vial (2 ml). The vial was covered under a stream of nitrogen before heating in an oven at 90 °C. Finally, 1 μl of the solution thus obtained was injected into the GC–MS system after addition of the internal standard. FAME composition analysis was performed in a Thermo Scientific TRACE 1310 Gas Chromatograph attached with ISQ LT single quadrupole mass spectrometer with DB1, 15 m; 0.25 mm ID (J&W scientific) capillary column. Helium was used as a carrier gas with flow rate at 1.5 mL min^{-1}, and the injector temperature was maintained at 200 °C. Oven temperature was programmed with an initial temperature of 115 °C, holding for 1 min, and then increasing to 280 °C by 7.5 °C min^{-1}, holding for 3 min.

Total phenolic content

Total phenolic content (TPC) was determined using the Folin–Ciocalteau's reagent according to the method reported by Lin and Tang (2007) at 760 nm with a spectrophotometer (UV-1601; Shimadzu, Tokyo, Japan), and the quantification was done on the basis of the standard curve of gallic acid concentration ranging between 10 to 80 mg/mL ($r^2 = 0.99$).

Determination of total tocopherol (vitamin E)

High-performance liquid chromatography (HPLC) system (1100 series, Agilent Technologies) was used for the quantification of vitamin E based on a method described by Gimeno et al. (2000). The oil sample was diluted in hexane (1:10). Thereafter, 200 μl was transferred to a screw-capped tube, where 600 ml of methanol and 200 ml of the internal standard solution (300 μg/ml of α-tocopherol acetate in ethanol) were added. After being

vortex-mixed and centrifuged (3000*g*, 5 min), the samples were filtered through a 0.45-mm pore size filter and an aliquot of the overlay was directly injected into the chromatograph.

Measurement of antioxidant activity

The ability of FXO at 200 μL to scavenge 2.9 mL of 1,1′-diphenyl-1–2-picrylhydrazyl (DPPH) free radicals was estimated by the method of Singh et al. (2002).

DNA damage evaluation

Comet assay in cells of the liver and testes

The comet assay was performed in liver and testes cells according to Bandyopadhyaya et al. (2008). Briefly, 50 μL of cell suspension was mixed with 100 μL of 1% low melting point (LMP) agarose and added to fully frosted slides coated with 80 μL of 1% normal melting point (NMP) agarose. The cells were then incubated in a lysis solution (2.5 mol L^{-1} NaCl, 100 mmol L^{-1} EDTA, 10 mmol L^{-1} Tris-HCL, 1% Triton X-100, pH 10) at 4 °C for at least 2 h, at which the slides were placed into an alkaline solution (300 mmol L^{-1} NaOH, 1 mmol L^{-1} EDTA, pH 13) at 4 °C for 20 min so as to allow DNA unwinding, and electrophoresed at 25 V (300 mA) for 20 min. Finally, the slides were neutralized in a 400 mmol L^{-1} Tris buffer (pH 7.5) for 15 min and stained with ethidium bromide (5 μg mL^{-1}). Images of 50 randomly selected nuclei per experimental group were captured using a fluorescence microscope (Eclipse 800, Nikon, Tokyo, Japan) and analyzed with image analysis software (Comet Assay IV, Perceptive Instruments, Suffolk, UK). Scored parameters included tail length, DNA percentage in tail, and olive tail moment (OTM). Tail length is the maximum distance that the damaged DNA migrates from the center of the cell nucleus. DNA percentage in tail is the DNA content that migrates from the nucleus into the comet tail. OTM is the product of the tail length and percentage of DNA, which gives a more integrated measurement of overall DNA damage in the cell.

Micronucleus assay

The micronucleus test was carried out in mice femoral bone marrow cells according to Chauhan et al. (2000). Numbers of normochromatic and polychromatic erythrocytes and micronuclei were evaluated in the control and treated groups. For micronuclei evaluation, 2000 polychromatic erythrocytes were scored per animal. Both normochromatic erythrocytes (NCEs) and polychromatic erythrocytes (PCEs) were scored in 500 erythrocytes for determination of the PCEs to NCEs ratio according to the OECD No. 474 guideline of mammalian erythrocyte micronucleus test for chemical testing (OECD 1997).

Sperm collection and analysis

The epididymides from each mouse were removed, and sperm was collected as quickly as possible after dissection. Epididymides was excised and minced in 1 mL of phosphate-buffered saline (pH 7.2) to obtain sperm suspension that was filtered through a nylon mesh. The sperm count was assessed from the right cauda epididymides while sperm motility was analyzed from the left one. Sperm count was performed according to Narayana et al. (2002) using a Neubauer hemocytometric chamber. Sperm motility was assessed by counting the progressive motile sperms. Approximately 10 μL of sperm suspension was layered onto a warmed microscope slide. In each semen sample, at least 10 microscopic fields were examined with at least 100 sperm/field counted. The total number divided by the number of motile sperm cells in each field and the average of the fields was calculated. The percentage of motile sperm was determined as described by Kvist and Bjorndahl (2002).

Histopathological examination

The liver and testes from each sacrificed mouse were dissected out and trimmed of excess fat tissues. Tissues were fixed in 10% buffered formalin and processed for paraffin sectioning by dehydration in different concentrations of alcohol, cleared with xylol and embedded in paraffin blocks. Sections of about 5 μm thickness were stained with Harris hematoxylin and eosin (H&E) for histological study (Delafield 1984).

Statistical analyses

Statistical analyses were performed with SPSS 16 software. Experimental data were analyzed using one-way analysis of variance (ANOVA). Duncan's multiple range test was used to determine the significant differences between means. All values were expressed as mean ± SD, and the significance level was set at $p \leq 0.05$.

Results

Flaxseed oil analyses

As summarized in (Table 1), the main fatty acids in FXO are stearic (2.30 ± 0.26), oleic (20.60 ± 1.49), linoleic (26.90 ± 3.30), linolenic (43.26 ± 5.55), and palmitic (4.70 ± 0.65). Besides, some traces of myristic (0.47 ± 0.15), pentadecanoic (0.37 ± 0.15), heptadecanoic (0.53 ± 0.15), and arachidic (0.87 ± 0.81) are found. In addition, data of oil analysis illustrate that the FXO's phenolic and vitamin E content was (18 ± 1.0) and (20.63 ± 0.31), respectively. In addition, FXO at 200 μL gave an antioxidant activity of (69.17 ± 0.42).

Genetic investigation

BPA DNA damage

The toxic effects of BPA on a variety of comet assay parameters are tabulated in (Table 2). Compared to the

Table 1 Chemical composition and antioxidant activity of flaxseed oil

Fatty acids	Value (%)
Myristic	0.47 ± 0.15
Palmitic	4.70 ± 0.65
Heptadecanoic	0.53 ± 0.15
Pentadecanoic	0.37 ± 0.15
Stearic	2.30 ± 0.26
Oleic	20.60 ± 1.49
Linoleic	26.90 ± 3.30
Linolenic	43.26 ± 5.55
Arachidic	0.87 ± 0.20
Total Phenolic Content (mg gallic acid/100 g oil)	18.0 ± 1.00
Vitamin E Concentration (μg/mL)	20.63 ± 0.31
Percentage of antioxidant activity (200 μL)	69.17 ± 0.42

control and vehicle, the mean values of tail length, tail DNA percentage, and olive tail moment in liver and testes cells appeared at a significant increase ($P \leq 0.05$) following the treatment of male mice with BPA. Regarding the micronucleus test, the micronucleated polychromatic erythrocyte (MNPCE) frequencies were significantly increased ($P \leq 0.05$) in male mice treated with BPA (50 mg/kg) for 28 successive days. The PCEs to NCEs ratio was significantly decreased as compared to that in control mice (Table 3).

The protective role of FXO against BPA's DNA damage

As illustrated in Table 2, administration of FXO through the three regimens of treatment produced a significant reduction ($P \leq 0.05$) in the mean values of tail length, DNA tail percentage, and olive tail moment than the treatment with BPA in liver and testes cells of male mice. Moreover, treatment with the three regimens of FXO significantly alleviated the deleterious effects of BPA on the frequency of PCEs and NCEs, and MNPCEs and PCEs to NCEs ratio (Table 3). In addition to that, data clearly illustrated that the treatment of mice with FXO before BPA was the best regimen in restoring the observed DNA alterations in various tested cells followed by administration of FXO after BPA whereas the co-administration of both FXO and BPA was the least.

Sperm quality

BPA effect on sperm physical characters

BPA showed a significant decrease ($P \leq 0.05$) in the mean values of sperm count (281.33 ± 32.01) and motility percentage (55.00 ± 5.00) of treated mice compared to control (385.00 ± 32.18 and 90.00 ± 5.00, respectively) as in (Table 4).

The protective role of FXO against BPA sperm defects

The means of the caudal epididymal sperm count and motility percentage of BPA- and/or FXO-treated male mice are illustrated in (Table 4). Results showed that supplementation of FXO through the three regimens of treatment caused a significant increase ($P \leq 0.05$) in the mean values of sperm count and motility compared with the treatment with BPA in mice. Meanwhile, administration of FXO before BPA was the best regimen in the elevation of sperm count and motility.

Histopathological examination

BPA histopathological alterations

Liver section examination of BPA-treated mice exhibited marked congestion of the hepatic blood vessels as well as marked hepatocytes vacuolar degeneration with many necrotic cells that showed without any nuclear composition (Fig. 1b, c). Regarding the examined testicular tissue of BPA-treated mice, it revealed an obvious defective spermatogenesis that was characterized by severe necrosis and loss of spermatogonial layers with multiple spermatid giant cell formation in the majority of seminiferous tubules. Interstitial blood vessel congestion was observed as well. Some seminiferous tubules showed destructed materials in the lumen with multiple spermatid giant cell formation (Fig. 2b, c).

Table 2 Comet assay parameters in liver and testes cells of treated male mice with BPA and/or FXO for 28 consecutive days

Treatments	Tail length (μm)		Tail DNA (%)		Olive tail moment (μm)	
	Liver	Testes	Liver	Testes	Liver	Testes
Control	7.66 ± 0.80^{e}	6.73 ± 0.46^{e}	13.53 ± 0.51^{d}	14.26 ± 0.20^{d}	1.03 ± 0.08^{d}	0.98 ± 0.11^{e}
Vehicle	8.56 ± 0.41^{e}	7.26 ± 0.30^{de}	13.15 ± 0.22^{d}	15.00 ± 0.50^{d}	1.12 ± 0.04^{cd}	1.09 ± 0.08^{e}
FXO (1 mL/kg bw)	8.16 ± 1.04^{e}	7.06 ± 0.20^{de}	12.70 ± 0.95^{d}	14.30 ± 0.51^{d}	1.03 ± 0.14^{d}	1.01 ± 0.04^{e}
BPA (50 mg/kg bw)	17.30 ± 1.08^{a}	9.83 ± 0.15^{a}	19.93 ± 0.58^{a}	23.56 ± 0.45^{a}	3.63 ± 0.52^{a}	2.37 ± 0.05^{a}
FXO before BPA	12.43 ± 0.45^{d}	8.33 ± 0.15^{c}	15.13 ± 0.23^{c}	16.43 ± 0.35^{c}	1.87 ± 0.04^{c}	1.36 ± 0.02^{d}
FXO with BPA	15.23 ± 0.55^{b}	9.23 ± 0.25^{b}	16.56 ± 0.64^{b}	17.80 ± 0.26^{b}	2.51 ± 0.16^{b}	1.65 ± 0.05^{b}
FXO after BPA	13.90 ± 0.26^{c}	8.60 ± 0.26^{c}	15.33 ± 0.30^{c}	17.26 ± 0.49^{b}	2.09 ± 0.40^{c}	1.48 ± 0.01^{c}

Data are expressed as means ± SD. Mean values in the same column within each parameter bearing superscript do not differ significantly ($P \leq 0.05$)

Table 3 Frequencies of MN PCEs and PCEs/ NCEs ratio in bone marrow cells BPA and/or FXO treated male mice for 28 consecutive days

Treatments	MNPCEs	PCEs	NCEs	PCEs/NCEs ratio
Control	10.40 ± 2.96^{d}	1438.00 ± 32.95^{a}	562.00 ± 32.95^{c}	5.13 ± 0.43^{a}
Vehicle	10.00 ± 4.00^{d}	1434.40 ± 57.36 ^{a}	565.60 ± 57.36^{c}	5.13 ± 0.75^{a}
FXO (1 mL/kg bw)	9.20 ± 2.28^{d}	1451.60 ± 47.01^{a}	548.40 ± 47.01^{c}	5.33 ± 0.65^{a}
BPA (50 mg/kg bw)	66.40 ± 9.94^{a}	1149.60 ± 56.80^{c}	850.40 ± 56.80^{a}	2.71 ± 0.31^{c}
FXO before BPA	22.40 ± 4.56^{c}	1363.60 ± 57.64^{b}	636.40 ± 57.64^{b}	4.31 ± 0.57^{b}
FXO with BPA	34.40 ± 8.05^{b}	1302.80 ± 54.38^{b}	697.20 ± 54.38^{b}	3.75 ± 0.45^{b}
FXO after BPA	26.00 ± 7.07^{c}	1344.40 ± 62.79^{b}	655.60 ± 62.79^{b}	4.14 ± 0.59^{b}

Data are expressed as means ± SD. Mean values in the same column within each parameter bearing the same superscript do not differ significantly ($P \leq 0.05$)

The protective role of FXO against BPA histopathological alterations

Administration of FXO through the three regimens of treatment with BPA obviously attenuated the liver and testes histopathological alterations. The examination of liver of FXO-treated mice for 28 successive days before BPA treatment revealed mild degenerative changes of the hepatocytes with few necrotic cells (Fig. 1d). Liver sections of co-treated mice with FXO and BPA revealed a mild degree of restoration of a large number of hepatic cells with mild vacuolar degeneration and dispersed necrotic cells (Fig. 1e). In addition, liver histoarchitecture of FXO-treated mice for 28 successive days after BPA treatment showed moderate hepatocellular degeneration and necrosis especially in the centrilobular area (Fig. 1f). Concerning examination of the testes tissues, mice given FXO before BPA treatment revealed a moderate degree of preservation of a large number of the spermatogonial cells and mild interstitial edema (Fig. 2d). In regard to the testes, examination of FXO and BPA co-treated mice showed marked defective spermatogenesis and presence of extensive debris in the lumen of the seminiferous tubules (Fig. 2e). In addition, examination of the testes tissues of mice given orally FXO after BPA treatment displayed congested blood vessels, nuclear pyknosis, and destruction of many spermatogonial cells with slightly active spermatogenesis in scanty seminiferous tubules (Fig. 2f).

Table 4 Mean values of sperm count and motility in treated male mice with BPA and/or FXO for 28 consecutive days

Treatments	Sperm count (10^{6}/ml)	Sperm motility (%)
Control	385.00 ± 32.18^{ab}	90.00 ± 5.00^{ab}
Vehicle	382.00 ± 37.80^{ab}	93.00 ± 2.64^{a}
FXO (1 mL/kg bw)	385.00 ± 33.42^{ab}	88.33 ± 2.88^{ab}
BPA (50 mg/kg bw)	281.33 ± 32.01^{c}	55.00 ± 5.00^{d}
FXO before BPA	387.67 ± 7.63^{a}	88.00 ± 2.64^{ab}
FXO with BPA	340.00 ± 5.00^{b}	79.00 ± 1.00^{c}
FXO after BPA	365.00 ± 6.08^{ab}	84.00 ± 1.00^{bc}

Data are expressed as means ± SD. Mean values in the same column parameter bearing the same superscript do not differ significantly ($P \leq 0.05$)

Discussion

Flaxseed is abundant in many nutrients, such as polyunsaturated fatty acid, protein, and lignans, and it is eminent by α-linolenic acid in high content, which recently has been found as chiefly vital for human organism (Wang et al. 2007). The results revealed that the main fatty acids in FXO are stearic, oleic, linoleic, linolenic, and palmitic, in addition to its own high content of vitamin E and phenolic compounds. These results were confirmed by Kakilashvili et al. (2014) whose investigation showed that in flaxseed oil linoleic and linolenic acids were predominant and together constitute the major basis of research composition, while palmitic and stearic acids in less quantity. In addition, flaxseed oil is a source of many vitamins, and the main plentiful vitamins that constitute flaxseeds are tocopherols (α, β, and γ forms). Vitamin E as an antioxidant nutrient keeps cell constituents from free radical damaging effects that might lead to cancer development (Winter and Vitamin 2013).

DNA damage is an important initial event in carcinogensis. DNA lesions can change in nucleotide sequence, causing mutagenesis and other cellular mechanisms (Lord and Ashworth 2012). In the current study, liver and testes comet assay and bone marrow micronucleus assay evaluated DNA damage. The alkaline comet assay was used as a quantitative and illustrative technique to determine DNA strand breaks (Gedik et al. 1998). The results of the present study indicated that BPA significantly increased the DNA damage in the tested tissues. These results are in coincidence with the findings of Tiwari et al. (2012) who found a significant elevation in the incidence of micronuclei and chromosomal abnormality in bone marrow and lymphocyte DNA damage in BPA-exposed rats. In addition, Xin et al. (2015) found significant increases in the content of DNA damage, frequencies of micronucleus, and conventional chromosome aberrations in CHO cells exposed to BPA. Growing evidence indicated that the oxidative stress caused by BPA could be its mechanisms to cause genetic toxicity (Tiwari et al. 2012; Meeker et al. 2010). In addition, the induction of micronuclei could be owing to BPA

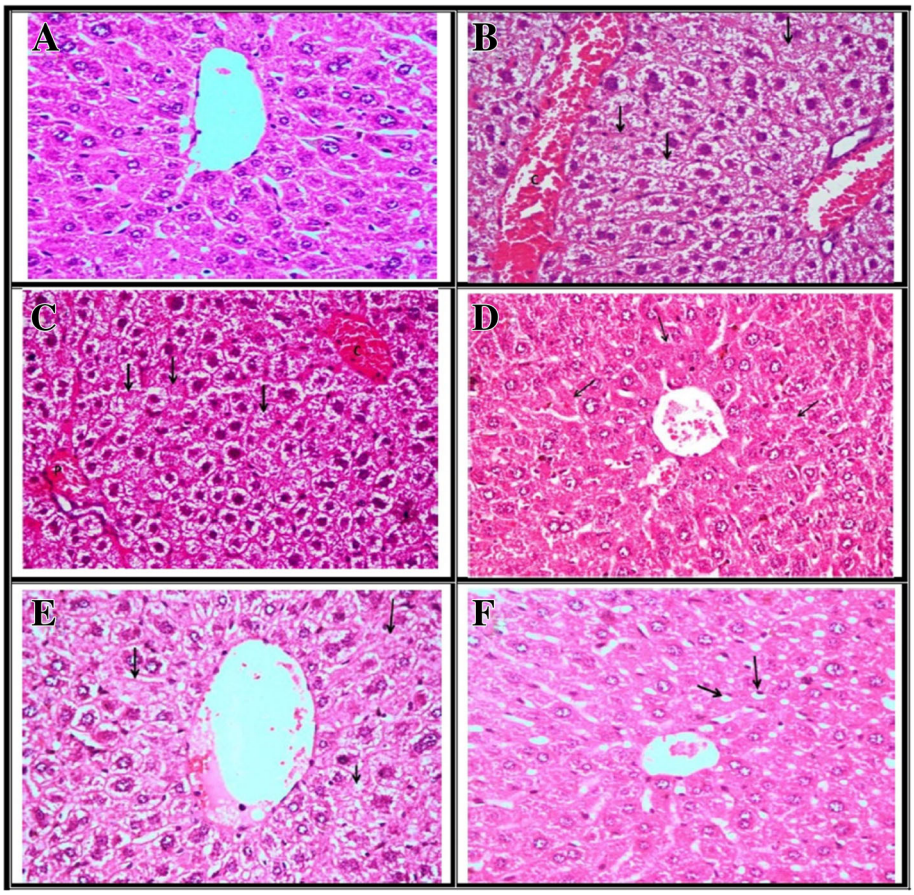

Fig. 1 **a** Liver of control mice showing normal hepatic parenchyma. **b** Liver of BPA-treated mice showing congestion (C) of the hepatic blood vessels and marked vacuolar degeneration of the hepatocytes with many necrotic cells (arrow). **c** Liver of BPA-treated mice showing congestion of the central vein (C) and portal blood vessels (P) with marked hepatocellular vacuolar degeneration and necrosis (arrow). **d** Liver of FXO-treated mice before BPA treatment showing mild degenerative changes of the hepatocytes with few necrotic cells (arrow). **e** Liver of FXO-treated mice concurrently with BPA showing a mild degree of restoration of a large number of hepatic cells with mild vacuolar degeneration (short arrow) and scattered necrotic cells (long arrow). **f** Liver of FXO-treated mice after BPA treatment showing moderate hepatocellular vacuolation (arrow) and necrosis especially in the centrilobular area. (H&E, × 400)

aneugenic effect (Quick et al. 2008). Moreover, treatment with the three regimens of FXO significantly alleviated the deleterious effects of BPA on DNA integrity. Several studies are in agreement with these results; Abd El-Rahim and Hafiz (2009) revealed that pretreatment with FXO (0.1 mL/kg bw) for 14 days prior to an IP injection of cyclophosphamide (25 mg/kg bw) significantly diminished the cyclophosphamide chromosomal aberrations and DNA fragmentation percentage in mice. In addition, the protecting effect of FXO on lead acetate-induced DNA fragmentation in the hepatic tissue of rats was reported by Abdel-Moneim et al. (2011). The protection afforded by FXO may be due to its intrinsic antioxidant and/or free radical scavenging properties associated with its constituent bioactive components as omega-3 and lignans (Burdge and Calder 2005; Newairy and Abdou 2009). FXO's lignans exhibited strong antioxidant and protective effects in quenching the free radical and inhibiting peroxyl-radical-mediated damage of plasmid DNA (Hu et al. 2007). Furthermore, the presence of polyphenols and vitamin E in FXO may have contributed to its therapeutic role against BPA's DNA damage. Polyphenols were revealed to ameliorate cell injury and protect DNA from lesion induced by reactive oxygen species (ROS) owing to their capacity to scavenge free radicals (Urquiaga and Leighton 2000). In addition, vitamin E has antioxidant and enzymatic activities that have the ability to prevent DNA damage (Songthaveesin et al. 2004).

In addition, BPA exhibited reduction in the count and motility of sperms. These results are in coincidence with the findings of Chitra et al. (2002) who reported that BPA at dose levels of 0.2–20 μg/kg bw showed a dose-dependent decline in rats' epididymal sperm motility and count. BPA is an estrogenic endocrine disruptor, which interfere with processes related to

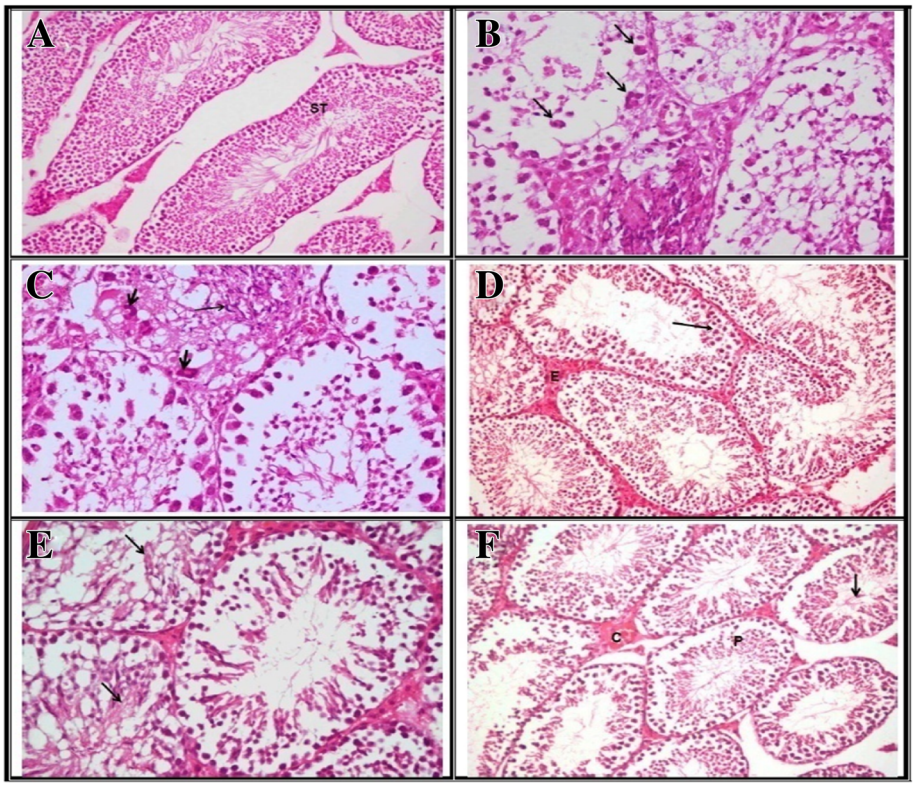

Fig. 2 **a** Testis of control mice showing normal spermatogenesis within the seminiferous tubules (ST). **b** Testis of BPA-treated mice showing severe necrosis and loss of the spermatogonial layers with multiple spermtide giant cells formation (arrow) in most of the seminiferous tubules. **c** Testis of BPA-treated mice showing congestion of the interstitial blood vessel, destructed and abnormal spermatid formation (thin arrow), spermatid giant cells (thick arrow), and loss of most of the spermatogonial cell layers. **d** Testis of FXO-treated mice before BPA treatment showing a moderate degree of preservation of a large number of the spermatogonial cells (arrow) and mild interstitial edema (E). **e** Testis of FXO-treated mice concurrently with BPA showing marked defective spermatogenesis and presence of extensive debris (arrow) in the lumen of the seminiferous tubules. **f** Testis of FXO-treated mice after BPA treatment showing congested blood vessels (C) nuclear pyknosis (P) and destruction of many spermatogonial cells with slightly active spermatogenesis in scanty seminiferous tubules (arrow). (H&E, × 400)

spermatogenesis, such as androgen production and Sertoli cell activity (Akingbemi et al. 2004; Salian et al. 2009). In addition, it has been reported that BPA generate ROS that cause oxidative damage and the oxidative stress that causes sperm damage (Chitra et al. 2003). FXO supplementation through the three regimens of treatment caused a significant increase ($P \leq 0.05$) in the mean values of sperm count and motility compared with the BPA treatment in mice. These findings are in accordance with those reported by Abd El-Rahim and Hafiz (2009) who found that pretreatment with FXO for 14 days prior to an IP dose of cyclophosphamide improved the sperm count percentage in treated mice. The increase in sperm count and motility may be attributed to the antioxidant nature of vitamin E present in FXO, which is believed to be the primary component of the spermatozoa antioxidant system and the major membrane protectants against ROS and lipid peroxidation attack (Hsu and Guo 2002). Furthermore, FXO has high content of linolenic acid. Comhaire and Mahmoud (2003) reported that linolenic acid deficiency is related with impaired sperm motility and causes sperm abnormalities.

BPA treated mice exhibited marked histopathological alteration s in the tissue of liver and testes. These results are in concurrence with Korkmaz et al. (2010) who noticed hepatic necrosis and congestion in the liver of male rats treated with BPA at the dose of 25 mg/kg/day three times a week for 50 days. In addition, Kalb et al. (2016) found that BPA (3000 μg/kg bw) via breast milk caused testicular erosion and complete aplasia in some seminiferous tubules. The observed histopathological alterations in liver and testes tissues may be related to DNA damage induced by BPA in these tissues. Moreover, Sangai and Verma (2012) found that BPA caused changes in the activities of ATPase in the liver and kidney of mice thereby causing a reduction in ATP that cause necrosis. Moreover, it has reported that BPA oxidative damage as a result of ROS generation (Kabuto et al. 2004). In addition, the great histopathological alterations in testes tissues caused by BPA may be due to its estrogen-mimicking

(Korkmaz et al. 2010). Meanwhile, administration of FXO through the three regimens of treatment with BPA obviously attenuated the liver and testes histopathological alterations. These observations are in accord with results obtained by Abdel-Moneim et al. (2011) who indicated that treatment of rats with FXO largely prevented lead-induced liver histopathological changes as indicated by a reduction in inflammatory cellular infiltration and hepatocytic damages. In addition, Karaca and Eraslan (2013) reported that FXO attenuated cadmium-induced testicular oxidative damage in male rats. The improvements in histopathological alterations afforded by FXO may be due to the polyunsaturated fatty acids (PUFAs) that may reduce cellular tendency to lipid peroxidation and alter membrane fluidity (Best et al. 2003). In addition, the presence of oleic acid, a monounsaturated fatty acid in FXO, reduces the susceptibility of the testes to lipid peroxidation (Bourre et al. 2004). Moreover, flaxseed is a high-quality supply of dietary fiber and phytoestrogenic lignans that have antioxidant properties (Zanwar et al. 2010).

Conclusions

Flaxseed has dietary and functional properties. The content of compounds such as polyunsaturated fatty acids, essential amino acids, vitamin E, lignans, and dietary fibers makes flaxseed a supply to satisfy essential needs in human diet and health maintenance. As mentioned above, FXO administration through the three regimens of treatment with BPA successfully attenuated the genotoxicity, sperm defects, and histological alterations induced by BPA in male mice that may be referred to its antioxidant property. In addition, the treatment of mice with FXO before BPA was the best regimen in restoring bisphenol-A deleterious effects. Flaxseed antioxidant may have prospective application in the food and health industry as a food stabilizer and nutraceutical.

Funding

The research was financed by authors.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

AE, FE, and OE developed the concepts of the study, design, and material preparation and collected the literature research. AE, FE, OE, and ME analyzed and interpreted the data and manuscript preparation. AE, FE, and OE wrote the manuscript. AE and FE revised the manuscript. All authors read and approved the manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

Author details

[1]Department of Cell Biology, National Research Center, 33 El-Bohouth St.-Dokki, P.O.12622, Giza, Egypt. [2]Department of Environment and Bio-Agriculture, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.

Received: 9 June 2018 Accepted: 16 August 2018
Published online: 03 September 2018

References

Abd El-Rahim AH, Hafiz NA (2009) Investigation on the protective effect of grape seed and linseed oils against cyclophosphamide induced genotoxicity in mice. Global Veterinaria 3(5):377–382

Abdel-Moneim AE, Dkhil MA, Al-Quraishy S (2011) The redox status in rats treated with flaxseed oil and lead-induced hepatotoxicity. Biol Trace Element Res 143:457–467

Akingbemi B, Sottas C, Koulova A, Klinefelter G, Hardy M (2004) Inhibition of testicular steroidogenesis by the xenoestrogen bisphenol A is associated with reduced pituitary luteinizing hormone secretion and decreased steroidogenic enzyme gene expression in rat Leydig cells. Endocrinol 145:592–603

AOAC (2000) Association of Official Analytical Chemists. Official methods of analysis. 17thed. Gaithersburg, M.D. In: U.S.A

Attaman JA, Toth TL, Furtado J, Campos H, Hauser R et al (2012) Dietary fat and semen quality among men attending a fertility clinic. Hum Reprod 27: 1466–1474

Bandyopadhyaya G, Sinha S, Chattopadhyay BD, Chakraborty A (2008) Protective role of curcumin against nicotine-induced genotoxicity on rat liver under restricted dietary protein. Euro J Pharmacol 588:151–157

Barcelo-Coblijn G, Murphy EJ (2009) Alpha-linolenic acid and its conversion to longer chain n-3 fatty acids: benefits for human health and a role in maintaining tissue n-3 fatty acid levels. Prog in Lipid Res 48:355–374

Best CA, Cluette-Brown JE, Teruya M, Teruya A, Laposata M (2003) Red blood cell fatty acid ethyl esters: a significant component of fatty acid ethyl esters in the blood. J Lipid Res 44:612–620

Bourre JM, Dumont O, Durand G (2004) Dose-effect of dietary oleic acid: oleic acid is conditionally essential for some organs. Reprod Nutr Dev 44(4):371–380

Burdge GC, Calder PC (2005) Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. Reprod Nutr Dev 45:581–589

Chauhan LK, Pant N, Gupta SK, Srivastava SP (2000) Induction of chromosome aberrations, micronucleus formation and sperm abnormalities in mouse following carbofuran exposure. Mut Res 465:123–129

Chitra KC, Latchoumycandane C, Mathur PP (2002) Effect of nonylphenol on the antioxidant system in epididymal sperm of rats. Arch Toxicol 76:545–551

Chitra KC, Latchoumycandane C, Mathur PP (2003) Induction of oxidative stress by bisphenol A in the epididymal sperm of rats. Toxicol 185:119–127

Comhaire FH, Mahmoud A (2003) The role of food supplements in the treatment of the infertile man. Reprod BioMed Online 7:385–391

Delafield F (1984) Haematoxylin and eosin for general staining. Staining of the animal tissues practical and theoretical. Oxford University Press, London

Fu P, Kawamura K (2010) Ubiquity of bisphenol A in the atmosphere. Environ Poll 158:3138–3143

Gedik CM, Wood SG, Collins AR (1998) Measuring oxidative damage to DNA: HPLC and the comet assay compared. Free Radic Res 29:609–615

Gimeno E, Castellote AI, Lamuela-Raventos RM, Torre MC, Lopez-Sabater MC (2000) Rapid determination of vitamin E in vegetable oils by reversed phase high-performance liquid chromatography. J Chromatography A 881:251–254

Hana RS, Saed N (2013) Alteration in oxidants, antioxidants and cytokines levels in blood of malathion exposed human and animal groups and the effect of flaxseed oil in alleviating malathion toxic effects. Euro J Biotechnol Biosci 1(1):8–19

Hsu PC, Guo YL (2002) Antioxidant nutrients and lead toxicity. Toxicol 180:33–44

Hu C, Yuan YV, Kitts DD (2007) Antioxidant activities of the flaxseed lignan secoisolariciresinol diglucoside, its aglycone secoisolariciresinol and the mammalian lignans enterodiol and enterolactone *in vitro*. Food Chem Toxicol 45(11):2219–2227

Huang Y, Wong C, Zheng J, Bouwman H, Barra R, Wahlstrom B, Neretin L, Hong M (2012) Bisphenol A (BPA) in China: a review of sources, environmental levels, and potential human impacts. Environ Int 42:91–99

Kabuto H, Amakawa M, Shishibori T (2004) Exposure to bisphenol A during embryonic/fetal life and infancy increases oxidative injury and causes underdevelopment of the brain and testis in mice. Life Sci 74:2931–2940
Kaithwas G, Majumdar DK (2012) In vitro antioxidant and in vivo antidiabetic, antihyperlipidemic activity of linseed oil against streptozotocin-induced toxicity in albino rats. Eur J Lipid Sci Tech 114:12–45
Kakilashvili BI, Zurabashvili DZ, Turabelidze DG, Shanidze LA, Parulava GK (2014) The fatty acid composition of ordinary flax seed oil (Linum usitatissimum L.) cultivated in Georgia and its byological activity. Georgian Med News 227:86–88
Kalb AC, Kalb AL, Cardoso FT, Fernandes CG, Corcini CD, Junior ASV, Martine PE (2016) Maternal transfer of bisphenol a during nursing causes sperm impairment in male offspring. Arch Environ Contam Toxicol 70:793–801
Kang HS, KwonYJ LKJ, Seo RY (2013) Recent advances in in vivo genotoxicity testing: prediction of carcinogenic potential using comet and micronucleus assay in animal models. J Cancer Prevent 18(4):277–288
Karaca S, Eraslan G (2013) The effects of flaxseed oil on cadmium-induced oxidative stress in rats. Biol Trace Elem Res 155:423–430
Korkmaz A, Ahbab MA, Kolankaya D, Barlas N (2010) Influence of vitamin C on bisphenol A, nonylphenol and octylphenol induced oxidative damages in liver of male rats. Food Chem Toxicol 48(10):2865–2871
Kvist U, Bjorndahl L (2002) Manual on basic semen analysis. In: ESHRE Monographs, vol 2. Oxford University Press, Oxford
Lin JY, Tang CY (2007) Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. Food Chem 101:140–147
Lin X, Gingrich JR, Bao W, Li J, Haroon ZA, Demark-Wahnefried W (2002) Effect of flaxseed supplementation on prostatic carcinoma in transgenic mice. Urol 60: 919–924
Lord CJ, Ashworth A (2012) The DNA damage response and cancer therapy. Nature 481:287–294
Makris KC, Andra SS, Jia A, Herrick L, Christophi CA, Snyder SA, Hauser R (2014) Association between water consumption from polycarbonate containers and bisphenol A intake during harsh environmental conditions in summer. Environ Sci Technol 47:3333–3343
Meeker J, Calafat A, Hauser R (2010) Urinary bisphenol A concentration in relation to serum thyroid and reproductive hormones in men from an infertility clinic. Environ Sci Technol 44:1458–1463
Michalowicz J (2014) Bisphenol A-sources, toxicity and biotransformation. Environ Toxicol Pharmacol 37:738–758
Narayana K, D'Souza UJA, Rao KPS (2002) Ribavirin induced sperm shape abnormalities in Wistar rat. Mut Res 513:193–196
Newairy AS, Abdou HM (2009) Protective role of flax lignans against lead acetate induced oxidative damage and hyperlipidemia in rats. Food Chem Toxicol 47(4):813–818
OECD (1997) Test No. 474: Mammalian Erythrocyte Micronucleus Test. OECD Publishing, Paris
Olsen J, Ramlau-Hansen HC (2012) Dietary fats may impact semen quantity and quality. Asian J Androl 14(4):511–512
Quick EL, Parry EM, Parry JM (2008) Do oestrogens induce chromosome specific aneuploidy *in vitro*, similar to the pattern of aneuploidy seen in breast cancer. Mut Res 651:46–55
Rocha S, Domingues V, Pinho C, Fernandes V, Delerue-Matos C, Gameiro P, Mansilha C (2013) Occurrence of bisphenol A,estrone, 17β-estradiol and 17α-ethinyl estradiol in Portugalese rivers. Bulletin Environ Contam Toxicol 90:73–78
Safarinejad MR (2011) Effect of omega-3 polyunsaturated fatty acid supplementation on semen profile and enzymatic anti-oxidant capacity of seminal plasma in infertile men with idiopathic oligoasthenoteratospermia: a double blind, placebo-controlled, randomized study. Andrologia 43:38–47
Salian S, Doshi T, Vanage G (2009) Neonatal exposure of male rats to bisphenol A impairs fertility and expression of sertoli cell junctional proteins in the testis. Toxicology 265:56–67
Sangai NP, Verma RJ (2012) Effect of quercetin on bisphenol A-caused alterations in succinate dehydrogenase and adenosine triphosphatase activities in liver and kidney of mice. Acta Poloniae Pharmaceutica-Drug Res 69(6):1189–1194
Sargi CS, Silva CB, Santos CMH, Montanher FP, Boeing SJ, Junior SOO, Souza EN, Visentainer VJ (2013) Antioxidant capacity and chemical composition in seeds rich in omega-3: chia, flax, and perilla. Food Sci Technol Campinas 33(3):541–548
Singh RP, Murthy KN, Jayaprakasha GK (2002) Studies on the antioxidant activity of pomegranate *(Punica granatum)* peel and seed extracts using *in vitro* models. J Agric Food Chem 50:81–86
Songthaveesin C, Saikhun J, Kitiyananta Y, Pavasuthipaisit K (2004) Radio protective effect of vitamin E on spermatogenesis in mice exposed to gamma-irradiation: a flow cytometric study. Asian J Androl 6:331–336
Tiwari D, Kamble J, Chilgunde S, Patil P, Maru G, Kawle D, Bhartiya U, Joseph L, Vanage G (2012) Clastogenic and mutagenic effects of bisphenol A: an endocrine disruptor. Mut Res 743:83–90
Tyl RW, Myers CB, Marr MC, Sloan CS, Castillo NP, Veselica MM, Seely JC, Dimond SS, Van Miller JP, Shiotsuka RN, Beyer D, Hentges SG, Waechter JM Jr (2008) Two-generation reproductive toxicity study of dietary bisphenol A in CD-1 (Swiss) mice. Toxicol Sci 104(2):362–384
Urquiaga I, Leighton F (2000) Plant polyphenol antioxidants and oxidative stress. Biol Res 33:55–64
Vandenberg LN, Hauser R, Marcus M, Olea N, Welshons WV (2007) Human exposure to bisphenol A (BPA). Reprod Toxicol 24:139–177
Wang B, Li D, Wang L, Huang Z, Zhang L, Chen XD, Mao Z (2007) Effect of moisture content on the physical properties of fibered flaxseed. Int J Food Eng 3(5):1–11
Winter R. Vitamin E: Your protection against exercise fatigue, weakened immunity, heart disease, cancer, aging, diabetic damage, environmental toxins. Crown Publishing Group, 2013
Xin L, Lin Y, Wang A, Zhu W, Liang Y, Su X, Hong C, Wan J, Wang Y, Tian H (2015) Cytogenetic evaluation for the genotoxicity of bisphenol-A in Chinese hamster ovary cells. Environ Toxicol Pharmacol 40:524–529
Yi B, Kasai H, Lee HS, Kang Y, Park JY, Yang M (2011) Inhibition by wheat sprout (*Triticum aestivum*) juice of bisphenol A-induced oxidative stress in young women. Mut Res/Genet Toxicol Environ Mutagen 724:64–68
Zanwar AA, Hegde MV, Bodhankar SL (2010) In vitro antioxidant activity of ethanolic extract of *Linum usitatissimum*. Pharmacol Online 1:683–696