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Protective action of *Eruca sativa* leaves aqueous extracts against Bisphenol A-caused *in vivo* testicular damages

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Running title: Effect of ESAE on BPA-induced *in vivo* testicular damages.

Abstract

Eruca sativa action on the male reproductive system and fertility has not been precisely defined. In this study, the aim was to investigate the ameliorative activity of *Eruca sativa* aqueous extracts (ESAE) on reproductive toxicity associated with oxidative stress induced by Bisphenol A (BPA). Wistar rats were used and divided into 6 groups of animals each; control (0.4 ml of corn oil/rat), ESAE at the higher dose (200 mg/kg), BPA [100 mg/kg, body weight (b.w.), perorally (p.o.)] alone or in combination with varied doses of ESAE (50, 100 and 200 mg/kg, b.w, p.o.). The diverse doses were administrated orally for 30 consecutive days. The results showed that BPA-treatment produced a diminution of density, motility and viability of sperm with disruption of spermatozoa morphology and fertilizing potential as well as testosterone, luteinizing hormone and follicle stimulating hormone levels. These results were accompanied by testis and epididymis histological damages which were shown by an induction of testicular dysfunction as seen with a lower number of Leydig-cells and spermatocytes as well as a reproductive stress which was modeled. The oxidative stress was measured by malondialdehyde (MDA) production, thiol group (-SH) decline and antioxidant enzyme activities disturbance, in particular superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) in reproductive tissues. ESAE co-administration at the two lower doses improved all histological and biochemical parameter injuries. These finding suggested the ESAE ability to prevent the testicular damages in rats which might be linked to functional- bioactive substances such phenolic compounds with higher antioxidant capacity.

Key words: *Eruca sativa*; bisphenol A; reproductive system male toxicity; Wistar rats.

Abbreviations: BPA, Bisphenol A; BSA, bovine serum albumin; CAT, catalase; DTNB, 5,5′-dithiobis (2-nitrobenzoic acid); EDTA, ethylenediaminetetraacetic acid; ESAE, *Eruca sativa* aqueous extract; FSH, follicle-stimulating hormone; GSH, glutathione; GPx, glutathione peroxidase; LH, luteinizing hormone; MDA, malondialdehyde; ROS, reactive oxygen species; SOD, superoxide dismutase; TBA, thiobarbituric acid.

1. Introduction

Environment and diet influence spermatogenesis with known consequences on male fertility. In this context, a range of natural and synthetic components are named endocrine disturbing chemicals. Infertility is due to their ability to mimic, antagonize and disrupt the synthesis and metabolism of endogenous hormones and receptors which leads to adverse health outcomes in mammals. [1]

One of the most extensively studied endocrine disrupting chemicals is bisphenol A (BPA).[2] It is widely used in the manufacturing of polycarbonates plastics and epoxy resin. [3] It can be detected in baby bottles, food packages, diversity of household products (plastic windows, compact disks and consumer electronics), medical equipment (dental sealant) and thermal paper. BPA has detected in more than 90% of tested samples of body fluids and tissues such as amniotic fluid, blood and serum. [4] Various epidemiological research works have showed that BPA affects the male reproductive system and other diseases such obesity, hyperglycemia, cardiovascular diseases, thyroid disorder and cancer. [5] Other studies showed that oxidative stress, [6] lipid peroxidation [7,8] the modified motility [9], the changed morphology [10] and intracellular ATP content perturbation [6] were decreased after animal treatment with BPA.

The use of plant extracts in disease prevention and treatment is of great interest, particularly for reproductive alterations, where the therapeutic options are limited. Especially, Brassicaceae vegetables consumption has proved various health benefits, thank to the existence of diverse phytochemicals. [11] In this respect, *Eruca sativa* (Brassicaceae family) is biannual herb, originated in the Mediterranean region. [12] Rocket leaves are usually used as salads herb. [13] Some studies have shown that the major chemical compounds are flavonoids and glucosinolates. [14] It showed many pharmaceutical properties such as antioxidant, antiplatelet, antithrombotic, anticancer, antidiabetic and antiulcer activities. [15-19] *Eruca sativa* can activate the spermatogenesis mechanism. [20] It also encourages teste growth and improves the spermatozoalife and function. [21]

The research focused on the evaluation of the *Eruca sativa* aqueous extracts possessive effect against BPA-caused diverse male reproductive system toxicities, and the possible mechanisms of actions.

2. Materials and methods

2.1. Chemicals

Bisphenol A (2,2-Di (4-hydroxyphenyl) propane) 97% purity, trichloroacetic acid (TCA), acetylcholine iodide, S-butyrylcholine, butylhydroxytoluene (BHT), KOH, ethanol, bovine serum albumin (BSA), acetylthiocholine iodide, 5.5'-dithiobis-(2- nitrobenzoic acid) (DTNB), Triton X-100, eosin stain, RPMI (Roswell Park Memorial Institute) and tocopherol-stripped corn oilwere purchased from SigmaAldrich Co., St. Louis, US. All other chemicals were obtained from Biomaghreb (Ariana, Tunisia) and were ISO 9001 certified.

2.2. Eruca sativa leaves collection and ESAE preparation

The necessary permits for the area studies of *Eruca sativa* leaves were obtained from the Ministry of Agriculture in Tunisia. The leaves were picked from the central-west region of Tunisia (Kasserine) during December. Leaves were identified in the Institute of Biotechnology of Beja (University of Jendouba) by professional botanists. After that, fresh leaves were dehydrated at 40°C using a dry incubator for 72h and then ground into fine powder with an electric blender (Moulinex Ovatio 2, Serris, France). Powder was subsequently dissolved in double distilled-water and incubated at 40°C for 24h.Then, combined homogenates were filtered through a colander (0.5 mm mesh size), lyophilized using freeze vacuum drying (DFR-5N-B, ULVAC products) and stored at 80°C until used. Yield of the extract was calculated in grams and converted into percentage, and the percent yield of the *Eruca sativa* leaves extract was recorded approximately 10%.

2.3. Acute toxicity study

Orally-administration of ESAE at various doses ranging from 0.5 to 3.2 g/kg was used to study acute-toxicity in mice (n=6). Animals obtained from the Central Society of Pharmaceutical Industries of Tunisia (SIPHAT, Ben-Arours, Tunisia) were observed every 30min for 4h and then, occasionally for an additional period of 8h. Mortality of mice was obtained 24h after the treatment. The mice were also analyzed for other toxicity-signs such as motor co-ordination, righting reflex and respiratory changes. [22]

2.4. Male-Wistar-rats and treatment

Forty two animals (10 weeks old and weighing 200-250 g) were obtained from the Society of Pharmaceutical Industries of Tunisia (SIPHAT, Ben-Arours, Tunisia) and used consistent

with the NIH guidelines for the care and use of laboratory animals, and was approved by the Institutional Animal Care and Use Committee of National Institute of Health. [23] The animals were kept in separate metallic cages under stable conditions such as standard temperature (24 ± 2°C), humidity (55 ± 5%) and lighting (12h:12h Light:Dark. Animals were maintained with standard pellet diet (Society of Badr-Utique, Bizerte, Tunisia) containing 67% carbohydrates, 10% fat, and 23% protein as the energy sources (overall calories: 3.6 kcal/g) and water *ad-libitum*.

After adaptation-duration for 2 weeks, the animals were distributed into 6 groups (n = 7 in each group) and treated orally for 4 weeks, as followed: Group I (vehicle group): treated with 0.4 ml/kg/day of tocopherol-stripped corn oil in an equivalent volume to that given to the different experimental batches; Group II: rats treated with BPA at a dose of 100mg/kg dissolved in tocopherol-stripped corn oil; Group III: rats treated only with Eruca sativa aqueous extracts (ESAE) at a dose of 200 mg/kg; Group IV: rats treated with ESAE (50 mg/kg) combined with BPA (100 mg/kg); Group V: rats treated with ESAE (100 mg/kg) associated with BPA (100mg/kg); Group VI: rats treated with ESAE (200 mg/kg) and BPA (100 mg/kg).

After 4 weeks of treatment, the rats were sacrificed by decapitation, and the blood was collected in tubes. The serum was used for estimation of luteinizing hormone (LH), follicle-stimulating hormone (FSH) and testosterone levels. Hormone levels were measured using commercially available ELISA kits (rat testosterone ELISA kit Category No. E90243, rat luteinizing hormone ELISA KIT Category No. CK-E90904, rat follicle-stimulating hormone ELISA kit Category No. CK-30597) according to the manufacturer's instructions (Bio Pharm Co.). 50 min after adding a stopper solution, absorption of standards and samples at 450 nm wavelengths were read by ELISA (BioTeK-Synergy H1, US). In addition, the reproductive organs (testis and the epididymis) were immediately removed by dissection and homogenized in potassium phosphate-buffered saline (pH 7.4). The collected sperm was taken in 5-cc tubes. For biochemical parameter estimations, homogenates were centrifuged at 9000 x g for 10 min at 4°C using centrifuge Universal 320 R (Andreas Hettich GmbH, Co., Germany) and the supernatants were stored for 1 week at – 80°C until analysis.

2.5. Rats body and reproductive organs weights

Animal-body weights were assessed by the difference between initial and final body weights. However, reproductive organs absolute weights were determined after elimination of fatty

tissues and blood vessels. For toxicological criteria, reproductive organ damages were identified using clinical signs.

2.6. Sperm characteristics examination

2.6.1. Sperm-collection

After decapitation and laparotomy, reproductive organs (testis and epididymis) were delicately deleted. Sperm-count was determined from right cauda epididymis, but, sperm motility/morphology was investigated from the left one. Epididymis was cutted-up and minced in 1 ml of RPMI (1000 UI penicillin/ml, 1000 µg of streptomycin/ml) to obtain sperm suspension.[24]

2.6.2. Sperm-enumeration

Cauda epididymal sperm count was realized using the method of Vega et al. [25] It was expressed as number of sperms per epididymis. To minimize the error, the count of each sample was done in triplicate.

2.6.3. Sperm-motility

Ten microliters of sperm-suspensions were rapidly layered onto a warmed microscope slide. Their movements were evaluated using a computer Aided Sperm Analysis System counting all progressive motile (have the ability to move around or 'swim'), non-progressive motile (moving very slowly) and immotile spermatozoa (not moving at all). The motile-sperm-cells number in each field was divided by the total number, and the average of the fields was assayed. Sperm-motility means were expressed as a percent of motile sperm from the total sperm counted. [26]

2.6.4. Sperm-viability

Eosin stain method was used to determine sperm-viability. The coloration was realized using one drop of freshly collected semen (10 µl) combined with two drops (20 µl) of eosin. The live sperms (with capacity of mobility) were unstained (intact-cell-membrane) and dead sperms showed purple to red stained head with injured membranes. The dye exclusion was analyze in 100 spermatozoa. Sperm-viability was defined as the percentage of dead sperm cells. [27]

2.6.5. *Sperm-morphology*

The spreading of sperm solution was done on a slide, then, it was dried and made permanent. The smeared slide was colored using eosin (1%) according to Seed's method. [28] Sperm lesions were examined using an optical microscope. One hundred of spermatozoa obtained from different fields in each slide were analyzed and classified for measures of observed irregularities (head, tail, and tail-head). Anomalous sperm-cells were numbered and the percentage was calculated. [29]

2.6.6. *Sperm-production evaluation*

Sperm-production was estimated according to the procedure of Narayana et al. [30] with some change. Epididymal and testicular tissues (temperature varied between 25-27°C) were homogenized with saline solution combined with 0.05% (v/v) Triton X100. Obtained homogenates were associated with 1.5 ml of 0.9% NaCl; spermatozoa and spermatid were counted three times. Count means were used to achieve total number of spermatids per testis as well as sperm per epididymis; it was then divided by the testis or epididymis weight to calculate the number per g of reproductive organs.

2.7. *Oxidative indicators*

2.7.1. *Epididymis and testis proteins determination*

Protein concentration in the epididymis and testis supernatants was done by Hartree technique [31] with some modifications of the Lowry method using bovine serum albumin (BSA) as standard.

2.7.2. *Epididymis and testis lipid peroxidation (MDA)*

Testis and epididyms supernatants lipid peroxidation was measured by the double-heating method. Indeed, after proteins and trichloroacetic acid precipitations, the MDA that exists in supernatants reacts with TBA. The measurement with spectrophotometric-method of the produced-color was measured at 532 nm, and MDA levels were calculated using the absorbance coefficient of MDA-TBA complex: 1.56×10^5 $M^{-1}cm^{-1}$. [32]

2.7.3. *Epididymis and testis SH-groups measurement*

Testis and epididymis homogenates were mixed with 0.25 M Trizma base and 20 mM EDTA (pH 8.2). Then, the combined-solutions were mixed and their absorbances were read at 412 nm and the first value A1was noted. Then, DTNB (10 mM) was added, incubated for 15 min,

and a new value A2 of absorbance was determined. We calculated the concentration of thiol groups per tube using this expression: (A2-A1-B) × 1.57 mM. [33]

2.8. *Epididymis and testis antioxidant activities assays*

SOD activity was measured using the Misra and Fridovich technique. [34] Shortly, epididymis and testis homogenates were mixed with 2 ml of mixture solution containing 20 µl of epinephrine (5 mg/ml), 10 µl of bovine catalase (0.4 U/µl) and 62.5 mM of sodium carbonate/bicarbonate buffer pH 10.2. Absorbance modifications were analyzed at 480 nm. CAT-activity was determined following the method described by Aebi (1984). [35] The reaction mixture contained 33 mM H_2O_2 in 50 mM phosphate buffer pH 7.0 and CAT activity was calculated using the extinction coefficient of 40mM-1cm1 for H_2O_2. GPx activity was measured according to the procedure illustrated by Flohé and Günzler (1984). [36] For this reason, 1 ml of reaction mixture containing 0.2 ml of epididymis and testis supernatants, 0.4ml of H_2O_2 (5 mM), 0.2 ml of phosphate buffer 0.1 M pH 7.4 and 0.2 ml of GSH (4 mM) was incubated at 37°C and the reaction was interrupted by addition of 0.5 ml TCA (5%, w/v). After centrifugation at 1500 x g for 5 min, aliquot (0.2 ml) from supernatant was mixed with 0.5 ml DTNB (10 mM) and0.5 ml of phosphate buffer 0.1 M pH 7.4 and absorbance was measured at 412 nm. The GPx activity was expressed as nmol of GSH consumed/min/mg protein.

2.9. *Epididymis and testis histological examinations*

For histological examination, the testis and epididymis portions were fixed using 10% neutral buffered formalin solution, embedded in paraffin. Then, 5µm of thick segments were incised, deparaffinized, hydrated, and colored with hematoxylin-eosin. The final preparations were checked and micro-photographed using an Olympus BH2 microscope fitted with photographic attachment (Olympus C35 AD4), a camera (Olympus C40 AB-4) and an automatic light exposure unit (Olympus PM CS5P).

2.10. *Statistical methods*

The data was analyzed as mean ± S.E.M and presence of remark dissimilarities among means of the groups was done using one way ANOVA Software for significance. Values were designed significant when $P < 0.05$.

3. Results

3.1. Acute oral toxicity of ESAE

In acute-oral-toxicity study, mice given graded doses appeared normal and neither abnormal behavior nor mortality was observed during the experimentation period. Thus, the LD50 value was greater than 3.2 g/kg for the extracts.

3.2. Body and reproductive organs weights

BPA treated-group (100 mg/kg, b.w./day) showed a remarkably diminution ($p < 0.05$) of body weight associated to a reduction of food and water intake when compared to control and ESAE-treated-groups. These alterations were accompanied with a decrease of reproductive organs absolute weights such as seminal vesicle. ESAE-low-dose (50 mg/kg) administration was significantly ($p < 0.05$) protected against BPA-provoked decrease body weight and the reproductive related organs (Table 1and 2).

3.3. BPA and ESAE effects on epididymis and testis hormonal levels

Results for serum LH/FSH levels as well as testosterone of the distinctive groups are illustrated in Table 3. The data showed that BPA-treatment significantly ($p < 0.05$) reduced the serum-LH and testosterone levels. ESAE-low-dose (50 mg/kg) consummation significantly ($p < 0.05$) protected against BPA-induced serum-LH and testosterone levels depletion. Whereas, FSH level had no significant effect in all-group.

3.4. BPA and ESAE effects on reproductive performance quality

BPA/ESAE actions on reproductive performance quality are reported in Table 4. BPA-intoxication of during 30 days produced a significant reduction ($p < 0.05$) of epididymal spermatozoa count and testicular spermatid enumeration as well as the sperm motility. While, the number of dead sperms was increased when the data were compared to the control-group. Similarly, a strangely decline was showed in the percentage of the total sperm-viability. Abnormal cell proportions of spermatozoa, categorized by head or tail were significantly augmented in BPA-treated-groups. ESAE-low-dose (50 mg/kg) co-administration improved significantly all reproductive capacity and enforcement as well as semen irregularities caused by BPA-treatment.

3.5. BPA and ESAE actions on epididymis and testis lipid peroxidation, proteins and thiol group's content

BPA/ESAE effects on oxidative stress in both reproductive organs are reported in Figure 1A and B. Indeed, BPA-administration significantly ($p < 0.05$) increased testis and epididymis MDA concentrations as well as decreased the SH-group contents which explain the proteins oxidation process. ESAE-treatment protected against lipoperoxydation enhancement and SH-groups depletion created by BPA-intoxication.

3.6. BPA and ESAE effects on epididymis and testis antioxidant enzymes activities

The detection of the influence of BPA and aqueous extracts of Eruca sativa on antioxidant enzymes activities (Figure 2A and B) was performed. In this respect, bisphenol A treatment significantly ($p < 0.05$) produced an increase in testis/epididymis antioxidant enzymes activities such as SOD, CAT and GPx. ESAE-treatment significantly ($p < 0.05$) restored these enzyme-activities depletion as compared to control-group.

3.7. BPA and ESAE effects on epididymis and testis histological evaluation

The biochemical parameters of various rat-groups were confirmed by the histological studies. Indeed, morphological perturbations of seminiferous-tubules were distinguished in whole experimental-treated-rats (Figure 3). In this regard, remark intensification in the percentage of empty seminiferous-tubules was examined after treatment with ESAE. Histological assessment of testicular-tissues portions of the testis stained with hematoxylineosin in oil-control-group (Fig. 3 A and B) showed a regular and normal number of adjacent seminiferous tubules disjointed by conjunctive tissue and interstitial cells. These seminiferous-tubules were lined by stratified germinal-epithelium, which consists of dual separate cells-populations as spermatogenic/Sertoli cells. Spermatogenic-cells showed the diverse steps of spermatogenesis, with the spermatogonia resting on the basal-lamina and containing little and dark nuclei. Spermatocytes observed as immense cells with gross/oval nuclei. The spermatids were identified at their various phases of spermatogenesis and progressively, they became elongating spermatids that form spermatozoa with their structure proprieties. The same observations were showed in seminiferous-tubules of treated rats with 100 mg/kg of *Eruca sativa* in association with100 mg/kg of Bisphenol. However, in treated-group with 100mg/kg of Bisphenol (Fig. 3 I, J), hematoxylin-eosin stained testis segments demonstrated many histological variations when compared to control-group. The disorders of the germinal-

epithelium, with loss of the spermatogenic cells specially spermatocytes and spermatids were also noticed in most tubules. A degeneration and/or necrosis were observed in the spermatogenic-cells. Several abnormal/assemble-cells were detected in the lumen of seminiferous-tubule portions of treated-rats with 100 mg/kg of Bisphenol. These observations were found in treated-rats with 200 mg/kg of ESAE in combination with 100 mg/kg of Bisphenol (Fig. 3 G, H), which shows that this dose has no effect on the spermatogenesis restoration.

4. Discussion

The recent research was considered to investigate the pharmacological-influence of *Eruca sativa* against BPA-caused reproductive toxicities and testicular stress damages in adult male rats. Firstly, the phytochemical-analysis informed that ESAE is rich in bioactive compounds including; total polyphenols and tannins as well as flavonoids. Added to that, the chemical identification of the ESAE by LC-HRESIMS showed essentially the presence of flavonoids such as kampherol, acacetin, apegenin, cirsilineol as well as phenolic acid as gallic acid and protocatchuic acid. [37] In this context, several other studies have proved the existence of a number of chemical-constituents containing flavonoids, glucosinolates (GLs), carotenoids and vitamin C which having a high antioxidant activity. This antioxidant-ability may be ascribed in the form of free radical-scavenging-activity and protection in contrast to stress situation. [38,39] In according to preliminary results, using the DPPH-radical-scavenging measurement, it was established that ESAE possesses an immense scavenging-capacity. The existence of these bioactive nutrients as phenolic acid, flavonoids and tannins in leaves-ESAE is likely to be responsible for the free radical-scavenging capacities detected. [40] Nevertheless, ESAE adequately scavenged-ROS such as superoxide anion, hydroxyl radical, hydrogen peroxide (H_2O_2). Free-radical-scavenging ability of *Eruca sativa* extracts may partially be associated with glucoerucin. [38] About in vivo part, BPA-oral-administration at a dose of 100 mg/kg body weight /day was selected as poisonous dose. Previous researches announced that doses of BPA alter the reproductive-system is higher than the NOAEL (50 mg/kg body weight). [41] The data showed that exposure of male rats to BPA (100 mg/kg of PC)-induce reproductive toxicities and testicular-damages. [42,43] The results allow to find that BPA-consumption caused a diminution of body weight and absolute organs weights which is a significant indicator of reproductive-toxicity in male-animals. The results of the recent study are the same as previously study realized by Gurmeet et al. [44] that showed that the rats-body weights, after, exposure to 1, 5, 100 mg/kg diminished when compared to the control-group. Concerning this, other studies showed that BPA-high-dose (400 mg/kg) strongly depleted the rats-body weights. [45] Similar results as in the current study were founded in that the absolute weights of testis certainly reduced, even after low doses of BPA. [46] Also, Akingbemi et al.[47] showed that the paired rats-testis weight diminished due to perinatal and chronic postnatal exposure to BPA. Testis-weights were particularly related to the mass of the differentiated spermatogenic cells; the lessening in its weight may be due to decreased number of germ cells, reduction of

spermatogenesis, and steroidogenesis. This decrease of weight may also be due to a reduction in the bioavailability of testosterone, LH and FSH, whose levels in the bloodstream indicate the reproductive endocrine status of the male. [48] However, it was showed that the rat's body-weights and the paired testis weights in the ESAE-group (50 mg/kg) were significantly restored compared to the control-group. These data are identical with those accomplished by Farman (2013) [49] who showed that the male-albino-mice treatment with *Eruca sativa* extracts led to a significant increase in the weight of testis.

The histological observations showed that BPA treatment induce severe damages in the testis; including the reduction of the quantity of spermatogenic-cells, abnormality in the seminiferous-tubules, eruption of the germinal-membrane surrounding the tubule. Vacuolization in tubules and empty-lumen was detected in most of the tubules. Interlobular important areas were observed between these tubules. These finding are also in accordance with other studies that shown the same histological alterations and deteriorated testes action at high/low doses of BPA were explained in the rats-testis. [44,50] However, ESAE-treatment produces a significant augmentation in the diameter in the seminiferous tubules, spermatids and leydig-cells. The decreased interstitial zone was observed. This augmentation may be due to the capacity of ESAE to excite the testis-growth and intensify the maturation, proliferation, and differentiation of sperms as compared to the control-group. [21] The results shown also that the testosterone and Luteinizing hormone levels in serum were notably deceased in the BPA-treated-group compared to the control-group. The data are in accordance with some studies showed that the testosterone level was considerably decreased after BPA-exposure. On the other hand, Tohei et al. (2001) [51] have shown that BPA provokes fertility-injury but without effect on the luteinizing hormone. In contrast, further studies have shown that it depletes these hormones. [52] In addition, Nakamura et al. (2010) [53] noted that a dose dependent-reduction in serum testosterone and LH concentrations were recorded at various doses of BPA (20,100 and 200 mg/kg/day). ESAE-low-dose (50 mg/kg) reversed the depletion of serum levels decrease. A highest elevation in testosterone and LH hormones may be related to the effect of polyphenolic flavonoid compounds in leaves extracts. [54-56]

The current study showed that BPA administration induced reproductive function damage in both testis and epididymis which associated to oxidative-stress damage as showed by lipid and proteins oxidations causing MAD-production and sulfhydryl content depletion. These disruptions were related to antioxidant-enzyme-activities deficiency. Previous study showed that the BPA-treated-rats were under higher effect of oxidative-stress related to a significant

decrease in the sperm-quality parameters. [57,58] Spermatozoa are designed to be more sensitive to oxidative-stress due to the abundance of polyunsaturated fatty acids in this environment. [59] In this context, BPA can induce antioxidant-system perturbation and causing an increase in ROS-generation. [46,60] In this respect, it was found that ESAE-treatment induce an increase in sperm concentration, this may be attributed to the antioxidant effects of *Eruca sativa* which is responsible for its protective effect against hydrogen peroxide toxicity, so spermatozoa are protected by various antioxidant agents belongs to the phytochemical constituent of extracts. [61,62] Other researchers concluded the capability of *Eruca sativa* improve healthy sperm characteristics and fertility. [20,63]

More importantly, in a recent study, the examination of sperm parameters following the BAP-intoxication on human spermatozoa showed that the *E. sativa* extracts can exhibit a strong protective effect in BPA-induced a decrease on sperm motility/viability. Added to that, the use of a panel of fluorescent probes with specific sensitivity to the different cell membrane compartments, the data confirmed the direct influence of BPA on sperm mitochondria, with negligible involvement of plasma membrane potential. In contrast, at low concentration, the ESAE is able to generally compensate the alteration of the whole sperm redox system induced by BPA. [37]

5. Conclusion

The protective effect of ESAE against the reproductive damage and oxidative stress induced by BPA was confirmed. More investigations are needed to confirm the possible mechanisms of action. It is also recommended to fractionate the crude extracts and did experiments to identify the possible compounds responsible for the protective effect of *Eruca sativa* leaves extracts.

Conflict of interest

The authors declare that they have no competing interests

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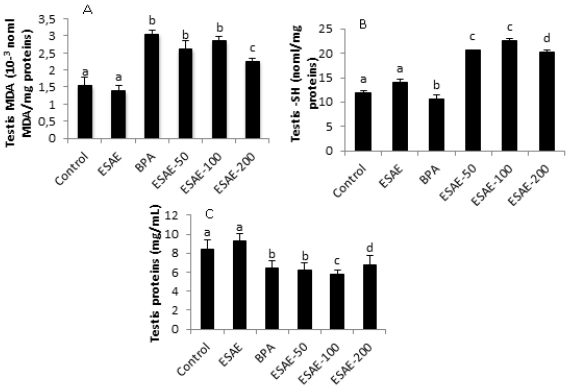
Legend of figures

Fig. 1 Effect of ESAE on BPA-induces changes in reproductive system (testis and epididymis) MDA, SH group and protein levels. Different letters: the difference is significant ($p < 0.05$). Similar letters: the difference is not significant ($p > 0.05$). Values are means ± SEM ($n = 7$).

Fig. 2 Effect of ESAE on BPA-induces depletion in reproductive system enzymatic antioxidant activities. Different letters: the difference is significant ($p < 0.05$). Similar letters: the difference is not significant ($p > 0.05$). Values are means ± SEM ($n = 7$).

Fig. 3 Histological examination of reproductive system (testis and epididymis): resection of control (A x 10, B x 40) testis control rat (oil) depicting normal stages of spermatogenesis. (C, D, E, F, G and H): Testis of treated-rat with ESAE (50, 100 and 200 mg/kg, respectively) and 100 mg/kg of Bisphenol. ESAE consumption (D, E) protects testis organ against different destructions. However, I and J represent the treated rats only with 100 mg/kg/BW of Bisphenol. Values are means ± SEM ($n = 7$).

Fig. 4 Histological investigation of epididymis: resection of control (x 40) epididymis control rat (oil) without disruption, disorder of histological structures in BPA-induces damage (B) and BPA-induced perturbation in rats (C) showed by many epididymis destructions. ESAE consumption (D, E) protects epididymis against different structure injuries. Values are means ± SEM ($n = 7$).



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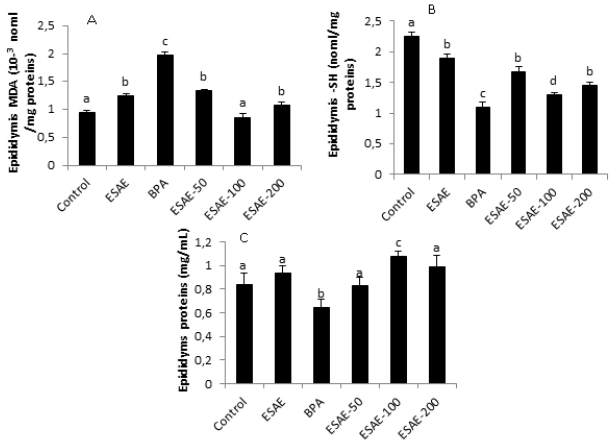

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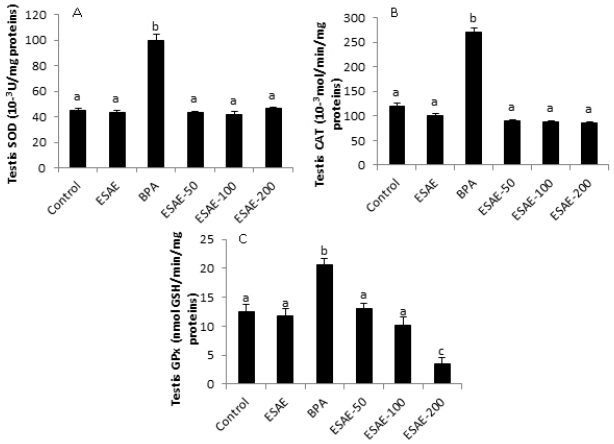

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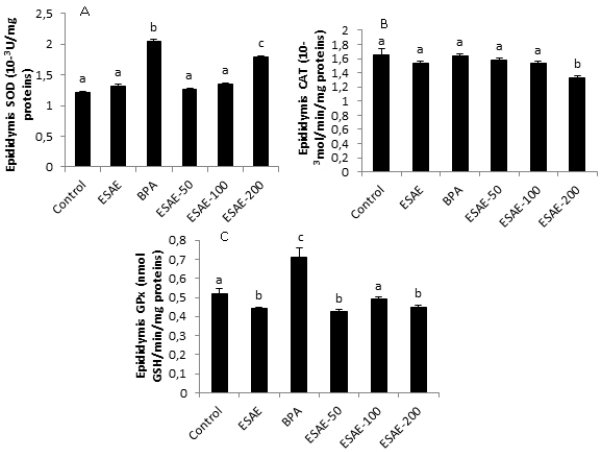

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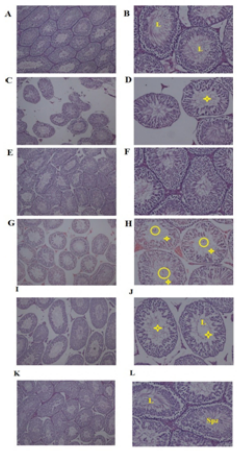

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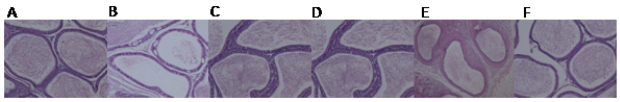

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Table 1

Effect of rocket leaves aqueous extract on the body weights and water/food intake after the sub-chronic treatment with bisphenol A at 100 mg/kg for 30 days.

	Weight gain ($x10^1$ g)	Food intake ($x10^2$ g/100g)	Water intake ($x10^2$ ml/day)
Control	2.10 ± 0.11^a	1.30 ± 0.11^a	2.13 ± 0.12^a
ESAE-200	1.33 ± 0.01^b	1.40 ± 0.13^a	2.01 ± 0.14^b
BPA	1.01 ± 0.03^c	1.10 ± 0.12^b	2.04 ± 0.12^b
BPA-ESAE-50	1.03 ± 0.02^c	1.24 ± 0.11^a	2.03 ± 0.11^a
BPA-ESAE-100	1.02 ± 0.04^d	1.22 ± 0.04^a	2.10 ± 0.14^b
BPA-ESAE-200	0.30 ± 0.01^e	1.14 ± 0.13^b	1.51 ± 0.11^c

The results are expressed as mean ± SEM (n = 7). Different letters: the difference is significant ($p < 0.05$). Similar letters: the difference is not significant ($p > 0.05$).

Table 2

Effect of rocket leaves aqueous extract on the reproductive organs weights after the sub-chronic treatment with bisphenol A at 100 mg/kg PC for 30 days.

	Absolute weights of organs			
	Epididymis (g)	Testis (g)	Prostate (g)	Seminal vesicle (g)
Control	1.01 ± 0.03[a]	1.20 ± 0.04[a]	0.30 ± 0.01[a]	1.02 ± 0.02[a]
ESAE-200	1.01 ± 0.04[a]	1.20 ± 0.1[a]	0.30 ± 0.03[a]	0.40 ± 0.01[b]
BPA	0.50 ± 0.01[a]	1.14 ± 0.03[a]	0.24 ± 0.02[a]	0.33 ± 0.02[b]
BPA-ESAE-50	0.50 ± 0.01[a]	1.22 ± 0.04[a]	0.30 ± 0.04[a]	0.40 ± 0.01[b]
BPA-ESAE-100	0.54 ± 0.02[a]	1.22 ± 0.1[a]	0.30 ± 0.03[a]	0.43 ± 0.01[b]
BPA-ESAE-200	0.50 ± 0.01[a]	1.20 ± 0.02[a]	0.30 ± 0.02[a]	0.34 ± 0.01[b]

The results are expressed as mean ± SEM (n = 7). Different letters: the difference is significant ($p < 0.05$). Similar letters: the difference is not significant ($p > 0.05$).

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Table 3

Effect of *Eruca Sativa* leaves extract on reproductive hormonal levels in rat.

	FSH (ng/ml of plasma)	LH (ng/ml of plasma)	Testosterone (ng/ml of plasma)
Control	0.10 ± 0.01^{a}	0.40 ± 0.01^{a}	9.12 ± 1.04^{a}
BPA	0.10 ± 0.01^{a}	0.20 ± 0.02^{b}	1.01 ± 0.10^{b}
ESAE-200	0.10 ± 0.02^{a}	0.40 ± 0.01^{a}	4.13 ± 1.03^{c}
ESAE-50	0.10 ± 0.03^{a}	0.30 ± 0.03^{a}	5.00 ± 0.23^{c}
ESAE-100	0.10 ± 0.01^{a}	0.21 ± 0.01^{b}	3.01 ± 0.21^{d}
ESAE-200	0.12 ± 0.02^{a}	0.20 ± 0.02^{b}	0.30 ± 0.11^{b}

Results are expressed as mean ± SEM; n = 7 in each group. Data was analyzed by Statview ANOVA. Different letters: the difference is significant ($p < 0.05$). Similar letters: the difference is not significant ($p > 0.05$).

Table 4

Effect of *Eruca Sativa* leaves extract on reproductive performance quality in rat.

	Control	BPA	ESAE-200	ESAE-50	ESAE-100	ESAE-200
Sperm Concentration ($x10^7$/ml)	6.42 ± 0.11^{a}	3.33 ± 0.11^{b}	6.02 ± 0.24^{a}	6.01 ± 0.32^{a}	6.02 ± 0.14^{a}	5.30 ± 0.31^{c}
Motility ($x10^1$ %)	8.51 ± 0.22^{a}	5.02 ± 0.23^{b}	9.03 ± 0.20^{a}	8.50 ± 0.25^{a}	7.45 ± 0.20^{c}	7.00 ± 0.20^{c}
Viability ($x10^1$ %)	8.33 ± 0.14^{a}	4.52 ± 0.10^{b}	9.04 ± 0.22^{a}	9.01 ± 0.21^{a}	8.01 ± 0.24^{a}	7.32 ± 0.25^{a}
Morphology (%)	4.40 ± 1.00^{a}	21.34 ± 1.42^{b}	5.23 ± 0.50^{a}	4.25 ± 0.70^{a}	13.24 ± 2.32^{c}	17.33 ± 3.00^{d}

Results are expressed as mean ± SEM; n = 7 in each group. Data was analyzed by Statview ANOVA. Different letters: the difference is significant ($p < 0.05$). Similar letters: the difference is not significant ($p > 0.05$).

Table 1

Effect of rocket leaves aqueous extract on the body weights and water/food intake after the sub-chronic treatment with bisphenol A at 100 mg/kg for 30 days.

	Weight gain ($x10^1$ g)	Food intake ($x10^2$ g/100g)	Water intake ($x10^2$ ml/day)
Control	2.10 ± 0.11^a	1.30 ± 0.11^a	2.13 ± 0.12^a
ESAE-200	1.33 ± 0.01^b	1.40 ± 0.13^a	2.01 ± 0.14^b
BPA	1.01 ± 0.03^c	1.10 ± 0.12^b	2.04 ± 0.12^b
BPA-ESAE-50	1.03 ± 0.02^c	1.24 ± 0.11^a	2.03 ± 0.11^a
BPA-ESAE-100	1.02 ± 0.04^d	1.22 ± 0.04^a	2.10 ± 0.14^b
BPA-ESAE-200	0.30 ± 0.01^e	1.14 ± 0.13^b	1.51 ± 0.11^c

The results are expressed as mean ± SEM (n = 7). Different letters: the difference is significant ($p < 0.05$). Similar letters: the difference is not significant ($p > 0.05$)

Table 1

Effect of rocket leaves aqueous extract on the body weights and water/food intake after the sub-chronic treatment with bisphenol A at 100 mg/kg for 30 days.

	Weight gain ($x10^1$ g)	Food intake ($x10^2$ g/100g)	Water intake ($x10^2$ ml/day)
Control	2.10 ± 0.11^a	1.30 ± 0.11^a	2.13 ± 0.12^a
ESAE-200	1.33 ± 0.01^b	1.40 ± 0.13^a	2.01 ± 0.14^b
BPA	1.01 ± 0.03^c	1.10 ± 0.12^b	2.04 ± 0.12^b
BPA-ESAE-50	1.03 ± 0.02^c	1.24 ± 0.11^a	2.03 ± 0.11^a
BPA-ESAE-100	1.02 ± 0.04^d	1.22 ± 0.04^a	2.10 ± 0.14^b
BPA-ESAE-200	0.30 ± 0.01^e	1.14 ± 0.13^b	1.51 ± 0.11^c

The results are expressed as mean ± SEM (n = 7). Different letters: the difference is significant ($p < 0.05$). Similar letters: the difference is not significant ($p > 0.05$).

Table 2

Effect of rocket leaves aqueous extract on the reproductive organs weights after the sub-chronic treatment with bisphenol A at 100 mg/kg PC for 30 days.

	Absolute weights of organs			
	Epididymis (g)	Testis (g)	Prostate (g)	Seminal vesicle (g)
Control	1.01 ± 0.03[a]	1.20 ± 0.04[a]	0.30 ± 0.01[a]	1.02 ± 0.02[a]
ESAE-200	1.01 ± 0.04[a]	1.20 ± 0.1[a]	0.30 ± 0.03[a]	0.40 ± 0.01[b]
BPA	0.50 ± 0.01[a]	1.14 ± 0.03[a]	0.24 ± 0.02[a]	0.33 ± 0.02[b]
BPA-ESAE-50	0.50 ± 0.01[a]	1.22 ± 0.04[a]	0.30 ± 0.04[a]	0.40 ± 0.01[b]
BPA-ESAE-100	0.54 ± 0.02[a]	1.22 ± 0.1[a]	0.30 ± 0.03[a]	0.43 ± 0.01[b]
BPA-ESAE-200	0.50 ± 0.01[a]	1.20 ± 0.02[a]	0.30 ± 0.02[a]	0.34 ± 0.01[b]

The results are expressed as mean ± SEM (n = 7). Different letters: the difference is significant ($p < 0.05$). Similar letters: the difference is not significant ($p > 0.05$).

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Table 3

Effect of *Eruca Sativa* leaves extract on reproductive hormonal levels in rat.

	FSH (ng/ml of plasma)	LH (ng/ml of plasma)	Testosterone (ng/ml of plasma)
Control	0.10 ± 0.01^{a}	0.40 ± 0.01^{a}	9.12 ± 1.04^{a}
BPA	0.10 ± 0.01^{a}	0.20 ± 0.02^{b}	1.01 ± 0.10^{b}
ESAE-200	0.10 ± 0.02^{a}	0.40 ± 0.01^{a}	4.13 ± 1.03^{c}
ESAE-50	0.10 ± 0.03^{a}	0.30 ± 0.03^{a}	5.00 ± 0.23^{c}
ESAE-100	0.10 ± 0.01^{a}	0.21 ± 0.01^{b}	3.01 ± 0.21^{d}
ESAE-200	0.12 ± 0.02^{a}	0.20 ± 0.02^{b}	0.30 ± 0.11^{b}

Results are expressed as mean ± SEM; n = 7 in each group. Data was analyzed by Statview ANOVA. Different letters: the difference is significant ($p < 0.05$). Similar letters: the difference is not significant ($p > 0.05$).

Table 4

Effect of *Eruca Sativa* leaves extract on reproductive performance quality in rat.

	Control	BPA	ESAE-200	ESAE-50	ESAE-100	ESAE-200
Sperm Concentration ($x10^7$/ml)	6.42 ± 0.11^{a}	3.33 ± 0.11^{b}	6.02 ± 0.24^{a}	6.01 ± 0.32^{a}	6.02 ± 0.14^{a}	5.30 ± 0.31^{c}
Motility ($x10^1$ %)	8.51 ± 0.22^{a}	5.02 ± 0.23^{b}	9.03 ± 0.20^{a}	8.50 ± 0.25^{a}	7.45 ± 0.20^{c}	7.00 ± 0.20^{c}
Viability ($x10^1$ %)	8.33 ± 0.14^{a}	4.52 ± 0.10^{b}	9.04 ± 0.22^{a}	9.01 ± 0.21^{a}	8.01 ± 0.24^{a}	7.32 ± 0.25^{a}
Morphology (%)	4.40 ± 1.00^{a}	21.34 ± 1.42^{b}	5.23 ± 0.50^{a}	4.25 ± 0.70^{a}	13.24 ± 2.32^{c}	17.33 ± 3.00^{d}

Results are expressed as mean ± SEM; n = 7 in each group. Data was analyzed by Statview ANOVA. Different letters: the difference is significant ($p < 0.05$). Similar letters: the difference is not significant ($p > 0.05$).

Table 3

Effect of *Eruca Sativa* leaves extract on reproductive hormonal levels in rat.

	FSH (ng/ml of plasma)	LH (ng/ml of plasma)	Testosterone (ng/ml of plasma)
Control	0.10 ± 0.01^{a}	0.40 ± 0.01^{a}	9.12 ± 1.04^{a}
BPA	0.10 ± 0.01^{a}	0.20 ± 0.02^{b}	1.01 ± 0.10^{b}
ESAE-200	0.10 ± 0.02^{a}	0.40 ± 0.01^{a}	4.13 ± 1.03^{c}
ESAE-50	0.10 ± 0.03^{a}	0.30 ± 0.03^{a}	5.00 ± 0.23^{c}
ESAE-100	0.10 ± 0.01^{a}	0.21 ± 0.01^{b}	3.01 ± 0.21^{d}
ESAE-200	0.12 ± 0.02^{a}	0.20 ± 0.02^{b}	0.30 ± 0.11^{b}

Results are expressed as mean ± SEM; n = 7 in each group. Data was analyzed by Statview ANOVA. Different letters: the difference is significant ($p < 0.05$). Similar letters: the difference is not significant ($p > 0.05$).

Table 4

Effect of *Eruca Sativa* leaves extract on reproductive performance quality in rat.

	Control	BPA	ESAE-200	ESAE-50	ESAE-100	ES
Sperm Concentration ($x10^7$/ml)	6.42 ± 0.11^{a}	3.33 ± 0.11^{b}	6.02 ± 0.24^{a}	6.01 ± 0.32^{a}	6.02 ± 0.14^{a}	5.30
Motility ($x10^1$ %)	8.51 ± 0.22^{a}	5.02 ± 0.23^{b}	9.03 ± 0.20^{a}	8.50 ± 0.25^{a}	7.45 ± 0.20^{c}	7.00
Viability ($x10^1$ %)	8.33 ± 0.14^{a}	4.52 ± 0.10^{b}	9.04 ± 0.22^{a}	9.01 ± 0.21^{a}	8.01 ± 0.24^{a}	7.32
Morphology (%)	4.40 ± 1.00^{a}	21.34 ± 1.42^{b}	5.23 ± 0.50^{a}	4.25 ± 0.70^{a}	13.24 ± 2.32^{c}	17.3

Results are expressed as mean ± SEM; n = 7 in each group. Data was analyzed by Statview ANOVA. Different letters: the difference is significant ($p < 0.05$). Similar letters: the difference is not significant ($p > 0.05$).

Figure Legends

Fig. 1 Effect of ESAE on BPA-induces changes in reproductive system (testis and epididymis) MDA, SH group and protein levels. Different letters: the difference is significant ($p < 0.05$). Similar letters: the difference is not significant ($p > 0.05$). Values are means ± SEM ($n = 7$).

Fig. 2 Effect of ESAE on BPA-induces depletion in reproductive system enzymatic antioxidant activities. Different letters: the difference is significant ($p < 0.05$). Similar letters: the difference is not significant ($p > 0.05$). Values are means ± SEM ($n = 7$).

Fig. 3 Histological examination of reproductive system (testis and epididymis): resection of control (A x 10, B x 40) testis control rat (oil) depicting normal stages of spermatogenesis. (C, D, E, F, G and H): Testis of treated-rat with ESAE (50, 100 and 200 mg/kg, respectively) and 100 mg/kg of Bisphenol. ESAE consumption (D, E) protects testis organ against different destructions. However, I and J represent the treated rats only with 100 mg/kg/BW of Bisphenol. Values are means ± SEM ($n = 7$).

Fig. 4 Histological investigation of epididymis: resection of control (x 40) epididymis control rat (oil) without disruption, disorder of histological structures in BPA-induces damage (B) and BPA-induced perturbation in rats (C) showed by many epididymis destructions. ESAE consumption (D, E) protects epididymis against different structure injuries. Values are means ± SEM ($n = 7$).