

# *Foeniculum vulgare* Extract Enhances Estrogen Levels, Total Antioxidant Capacity, and Protect Ovarian Histology in Rats Exposed to Bisphenol A

ORIGINAL ARTICLE  
Endocrinol Res Pract. 2023;27(4):227-232

## ABSTRACT

**Objective:** Bisphenol A (BPA) is an endocrine disruptor that interferes with estrogen (E<sub>2</sub>) levels, while *Foeniculum vulgare* L. (FV), a plant with phytoestrogen compounds and medicinal properties, has been suggested as a potential remedy against endocrine disruptors. This study aimed to evaluate the effect of FV extract on changes in estrogen levels, total antioxidant capacity (TAC), nitric oxide (NO), and ovarian structure in rats induced by BPA.

**Methods:** The study used 30 female rats that were divided into different treatment groups, including a control group, a BPA group, and 3 BPA-FV-treated groups with varying doses of FV (250, 500, and 1000 mg/kg). Blood samples were taken from the rats to measure estrogen, TAC, and NO levels, while their ovaries were analyzed.

**Results:** The results showed that BPA exposure caused a reduction in E<sub>2</sub>, TAC, and the number of ovarian follicles while increasing NO levels. However, treatment with FV extract resulted in increased E<sub>2</sub> and TAC levels and the number of ovarian follicles while reducing NO levels.

**Conclusion:** FV extract could be used as a natural supplement to mitigate the harmful effects of BPA.

**Keywords:** Bisphenol A, estrogen, stress oxidative, ovary, rat

## Introduction

Due to their wide use in everyday life, plastic materials have become ubiquitous and are associated with harmful compounds that have detrimental effects on the endocrine system, altering its function.<sup>1</sup> This system is also affected by endocrine-disrupting compounds, a group of synthetic agents that interfere with its normal functioning, including environmental estrogens (E<sub>2</sub>) and man-made chemicals. Bisphenol A (BPA) is a widely used compound in various industries that falls under this group of chemical compounds.<sup>2,3</sup>

BPA, also known as 2,2-bis (4-hydroxyphenyl) propane, is a type of polycarbonate and epoxy resin utilized to coat the inner surfaces of metal cans, food packaging, and various plastic products such as toys, water bottles, beverage containers, spectacle lenses, sports and medical equipment, pipes, and electronic products.<sup>4,5</sup> BPA has a structure that includes 2o phenolic unsaturated rings and has some similarities to E<sub>2</sub>, allowing it to mimic the actions of E<sub>2</sub> in the body. Polycarbonates are formed by bonding BPA with carbonate, and due to the high flexibility of these bonds, BPA may be released from polymerized epoxy resins under acidic or basic hydrolyzed environments and high-temperature conditions. As a lipophilic compound, most xenoestrogens, including BPA, can penetrate the human body through the skin and mucous membranes.<sup>6,7</sup>

BPA exposure has been linked to increased free radicals and impaired ovarian function, with studies showing that it disrupts meiosis progression, leading to oocyte degeneration and abnormalities in adult mice.<sup>8</sup> The ovary is particularly susceptible to oxidative stress, which can increase androgen production, destroy ovarian follicles, and damage ovarian tissue in patients with polycystic ovaries.<sup>9</sup> BPA can reduce E<sub>2</sub> levels, the number of primary and antral follicles, and the corpus luteum while increasing apoptosis in granulosa cells, which can prevent ovulation. Combining BPA with compounds that have estrogenic properties may help mitigate its effects, and the use of plants is increasingly popular due to their minimal side effects, compatibility with the body, and availability of diverse plant products.<sup>10</sup>

Fatemeh Khazaei<sup>1</sup> 

Leila Naseri<sup>2,3</sup> 

Mohsen Akbaribazm<sup>4</sup> 

Mozafar Khazaei<sup>5</sup> 

<sup>1</sup>Taleghani Hospital, Kermanshah University of Medical Sciences, Kermanshah, Iran

<sup>2</sup>Student Research Committee, Kermanshah University of Medical Sciences, Kermanshah, Iran

<sup>3</sup>Clinical Research Development Unit, Ayatollah Taleghani Hospital, Ilam University of Medical Sciences and Health Services, Ilam, Iran

<sup>4</sup>Department of Basic Medical Sciences, Khoy University of Medical Sciences, Khoy, Iran

<sup>5</sup>Fertility and Infertility Research Center, Health Technology Institute, Kermanshah University of Medical Sciences, Kermanshah, Iran

Corresponding author:  
Mozafar Khazaei  
✉ mkhazaei1345@yahoo.com

Received: June 7, 2023  
Revision Requested: July 4, 2023  
Last Revision Received: July 31, 2023  
Accepted: August 7, 2023  
Publication Date: October 5, 2023

Cite this article as: Khazaei F, Naseri L, Akbaribazm M, Khazaei M. *Foeniculum vulgare* extract enhances estrogen levels, total antioxidant capacity, and protect ovarian histology in rats exposed to bisphenol A. *Endocrinol Res Pract.* 2023;27(4):227-232.



Copyright © Author(s) – Available online at <http://endocrinolrespract.org>  
This journal is licensed under a Creative Commons (CC BY-NC-SA) 4.0 International License.

DOI: 10.5152/erp.2023.23269

*Foeniculum vulgare* L. (FV) is a flowering plant from the Apiaceae genus that has been studied for its various properties. Its fruit and essence have been found to reduce or block digestive system spasms, increase gastrointestinal secretion, and improve food digestion.<sup>11</sup> FV has also demonstrated a protective effect against CCl<sub>4</sub>-induced liver damage, as evidenced by a decrease in aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and bilirubin. Furthermore, FV has estrogenic effects, such as increasing milk secretion, libido, inducing preterm menstruation, accelerating labor, and exhibiting anti-hypertensive effects through diuretic and natriuretic processes.<sup>12</sup> Antol, a phytoestrogen found in FV, is responsible for its estrogenic properties, which have been shown to affect the female genital system and mammary gland.<sup>13</sup> The primary compounds in FV are trans-anethole and fenchone, with FV being the best source for trans-anethole extraction.<sup>14</sup> FV has been shown to exhibit therapeutic effects in cases of primary dysmenorrhea, leading to a decrease in total protein concentration, as well as acid and ALP activities in the testis and vasa deferens. Additionally, FV has been observed to increase the weight of the uterine tube and ovary. Additionally, FV has been shown to induce folliculogenesis in female mouse ovaries and increase the number of growing follicles.<sup>15,16</sup>

Phytosterols are natural compounds found in plants that are similar to cholesterol. They have the ability to interfere with cholesterol absorption and may play a role in reducing the synthesis of androgen hormones, particularly testosterone. Phytoestrogens, on the other hand, have both estrogenic and anti-androgenic effects, which can bind to E<sub>2</sub> receptors in the body and mimic the E<sub>2</sub> effects, potentially promoting follicle development and maturation.<sup>17</sup> A study by Choi and Hwang<sup>15</sup> demonstrated that FV contains genistein, formononetin, anethole, daidzein, palmitic acid, and  $\beta$ -sitosterol, which have anti-androgenic/phytoestrogen properties. Additionally, phytosterols such as isoflavonoids present in the FV have been reported to have antioxidant properties, which can protect the ovary from oxidative stress-induced damage. The long-term use of plant extracts containing phytoestrogens can lead to a reduction in testosterone levels by having a negative feedback effect on Luteinizing hormone (LH).<sup>18</sup>

In this study, an animal model was utilized to demonstrate the detrimental impact of BPA on female reproductive health as well as the potential protective effects of FV. Given the rising usage of BPA and its association with polycystic ovary syndrome (PCOS) in women, an animal model was employed to simulate human conditions. Given the beneficial effects of FV on the treatment of various diseases and its unique properties, including the presence of E<sub>2</sub>-like compounds,

the aim of this study was to investigate the effect of FV extract on BPA-induced changes in serum levels of E<sub>2</sub>, NO, and antioxidant capacity, as well as ovarian histology in rats.

## Materials and Methods

### Extract Preparation

We prepared the FV hydroalcoholic extract by grinding 100 g of FV seeds and adding them to 1000 mL of 70% ethanol. After stirring the mixture, we kept it in the dark for 48 hours. Next, we filtered the contents of the container using Whatman Grade 42 filter paper and evaporated the alcohol. Finally, we stored the resulting extract at 4°C.

### Study Design, Animal Grouping, and Treatment Protocol

All rats were housed under standard conditions with free access to food and water ad libitum and maintained at a temperature of 24 ± 3°C and 45 ± 5% humidity with a 12-hour light–dark cycle for 1 week. Rats in the estrous process of their reproductive cycle were selected and divided into 5 groups (n = 6):

- Group 1 (Control): received normal saline (500  $\mu$ L) by gavage for 56 days.
- Group 2 (BPA): received 25 mg/kg of BPA dissolved in olive oil (500  $\mu$ L) by gavage for 56 days.
- Groups 3-5 (250, 500, and 1000 FV treatment groups): received 25 mg/kg of BPA along with 1 of 3 doses (250, 500, or 1000 mg/kg body weight) of FV extract dissolved in normal saline (500  $\mu$ L) administered by gavage 3 times per week for 56 days.

Previous studies and a pilot study were used to select the best therapeutic and non-toxic FV and PCOS induction doses with BPA. Physical indicators, including permanent vaginal cornification in the vaginal epithelium and the presence of vaginal plaques, as well as an assessment of vaginal smears, were used to confirm PCOS.<sup>19,20</sup>

### Ethics Committee Approval

We obtained approval for this study from the Ethics Committee of Kermanshah University of Medical Sciences, Kermanshah, Iran (number: IR.REC.KUMS.1399.168). Since this study was conducted on an animal model of PCOS, it did not involve human subjects, so informed consent is not required.

### Serum Analysis

On the 57th day of the study, we sacrificed the rats using pre-anesthesia (100 mg/kg xylazine 2%) and anesthesia (15 mg/kg ketamine 10%) protocols and collected blood samples from the heart. We separated serum samples by centrifugation of blood samples at 10000 g for 15 minutes. We measured the levels of serum E<sub>2</sub> (Catalog Number: KA2330) hormone using a commercial ZennBio enzyme-linked immunosorbent assay (ELISA) kit (ZennBio, catalog number: 511103, Berlin, Lonssee, Germany) based on the colorimetric assay, following the manufacturer's instructions. We added 100  $\mu$ L of each standard, control, and sample to 96-well plates, followed by 200  $\mu$ L of enzyme conjugate to the sample and standard wells. After incubating the wells at room temperature for 120 minutes, we washed the wells 3 times with 400  $\mu$ L washing solution. Finally, we added 200  $\mu$ L of substrate solution to each well, and after 30 minutes, we added 100  $\mu$ L of stop solution to the resulting mixture. We measured the absorbance of the final mixture at a wavelength of 450 nm using an ELISA reader (Model Number: Spectronic 20; Milton Roy Company, Barcelona, Spain).<sup>21</sup>

## MAIN POINTS

- *Foeniculum vulgare* L. (FV) has the potential to promote folliculogenesis and prevent cystic follicle formation in bisphenol A (BPA)-treated groups, which is otherwise disrupted by BPA.
- FV has a protective effect on the normal tissue structure of the ovary and can prevent damage caused by BPA toxicity, which is known to impair ovarian function and cause structural changes.
- FV has the potential to mitigate the adverse effects of BPA toxicity on the endocrine system by regulating serum estrogen levels and reducing oxidative stress in the ovaries.

### Serum Ferric Reducing Antioxidant Power

Total antioxidant capacity (TAC) was assessed by the ferric reducing ability of plasma [ferric reducing antioxidant power (FRAP)] method based on the reduction of  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  ions in the presence of 2,4,6-tripyridyl-1,3,5-triazines by the sample antioxidants. Briefly, 200  $\mu\text{L}$  serum was added to 1500  $\mu\text{L}$  of FRAP reagents, including  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  solution (20 mmol/L) and acetate buffer (300 mM) and the absorbance was read at 593 nm using a spectrophotometer (Pharmacia, Novaspec II, Biochrom, London, England). TAC was calculated as  $\mu\text{mol}$  using a standard regression equation.<sup>21</sup>

### Nitric Oxide Measurement

Griess colorimetric assay was used for nitric oxide (NO) measurement. The serum sample (400  $\mu\text{L}$ ) was deproteinized by zinc sulfate (6 mg) and centrifuged at 12 000 rpm for 12 minutes. Vanadium chloride (100  $\mu\text{L}$ ) was mixed with 100  $\mu\text{L}$  of each deproteinized sample, and 50  $\mu\text{L}$  naphthyl ethylenediamine dihydrochloride and 50  $\mu\text{L}$  sulfonamides were added. Wells were incubated for 30 minutes. Sodium nitrate (0, 6.25, 12.5, 25, 50, 100, and 200  $\mu\text{L}$ ) as standard concentrations was selected. The sample absorbance was measured at 540 and 630 nm by an ELISA reader (Stat fax 100, Chicago, Illinois, USA). Each sample measurement was repeated 3 times.<sup>22</sup>

### Histologic Evaluation

Adipose tissue was removed from the ovaries, and their weight was recorded before they were fixed in 10% formalin. The ovaries were then sliced into 6  $\mu\text{m}$  sections and stained with hematoxylin and eosin stain (H&E) for histological analysis. The 5 largest sections from each ovary were examined to assess follicle development. Histopathological examination was conducted to evaluate the effects of FV on the average number of primary, preantral, and antral follicles. The samples were examined under an optical microscope to assess histological changes, and images were captured for further analysis using image processing software (Motic 2000, Shimadzu Corp., Kyoto, Japan).<sup>16</sup>

### Statistical Analysis

The normality of the data was assessed using the Kolmogorov-Smirnov test, and  $P$  values greater than .05 were considered statistically significant for normal and homogeneous data. A 1-way analysis of variance was used to compare the quantitative results between the study groups, followed by the Newman-Keuls post hoc test. Statistical significance was defined as a  $P$  value less than .05. The results are presented as means  $\pm$  standard deviation (SD). Data analysis was performed using Statistical Package for the Social Sciences statistics software version 16.0 (SPSS Inc.; Chicago, IL, USA), and graphs were created using GraphPad Prism software (Ver. 9; GraphPad Inc, San Diego, CA, USA).

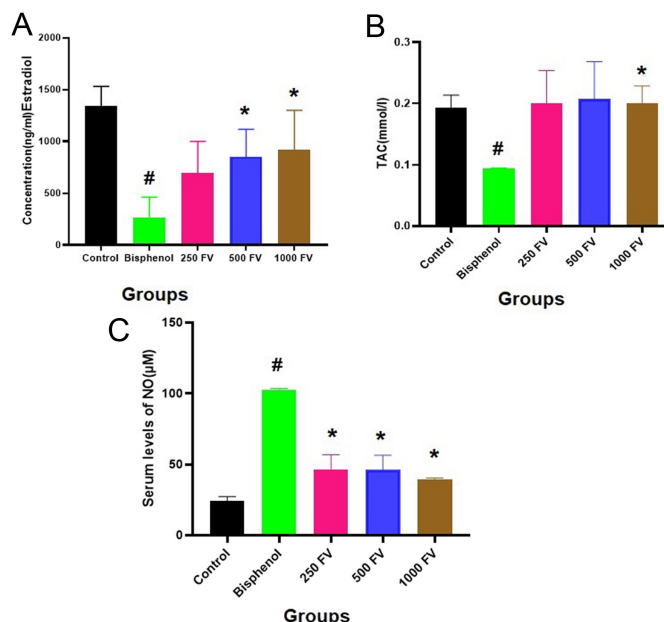
## Results

### Serum Estrogen Changes

The results indicated that exposure to BPA significantly reduced  $\text{E}_2$  levels compared to the control group ( $P=.002$ ). Furthermore, the groups treated with 500 ( $P=.02$ ) and 1000 ( $P=.006$ ) mg/kg FV showed significantly higher  $\text{E}_2$  levels compared to the BPA group (Figure 1A).

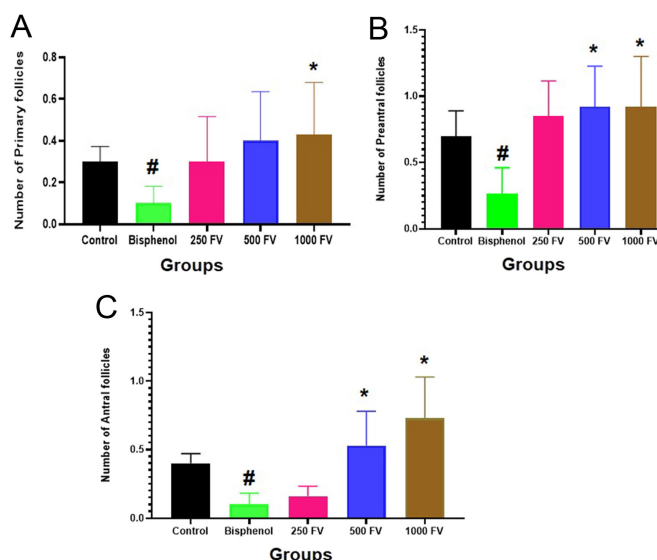
### Serum Total Antioxidant Capacity and Nitric Oxide Changes

The results showed that the TAC significantly decreased in the BPA group compared to the control group ( $P=.008$ ). However, treatment with different doses of FV increased TAC, with a statistically



**Figure 1.** Effect of BPA and FV on serum estrogen (A), total antioxidant capacity (TAC) (B), and nitric oxide (NO) (C) levels in control, bisphenol, and 250, 500, and 1000 mg/kg FV extract-treated groups. Significant decrease in estrogen (A), TAC (B), and NO (C) compared to the control group. #( $P < .05$ ) BPA group vs. normal control group; \*( $P < .05$ ) all treated groups vs. BPA group ( $n = 6$  rat/group; values are means  $\pm$  SD).

significant difference observed at the 1000 mg/kg dose ( $P=.01$ ) (Figure 1B). Additionally, NO levels significantly increased in the BPA group compared to the control group ( $P=.006$ ). However, in the FV treatment groups [250 ( $P=.02$ ), 500 ( $P=.01$ ), and 1000 ( $P=.008$ ) mg/kg], the levels of NO significantly decreased compared to the BPA group (Figure 1C).



**Figure 2.** Effect of BPA and FV on the number of ovarian follicles. Primary follicles (A), preantral follicles (B), and antral follicles (C). #( $P < .05$ ) BPA group vs. normal control group; \*( $P < .05$ ) all treated groups vs. BPA group ( $n = 6$  rat/group; values are means  $\pm$  SD).



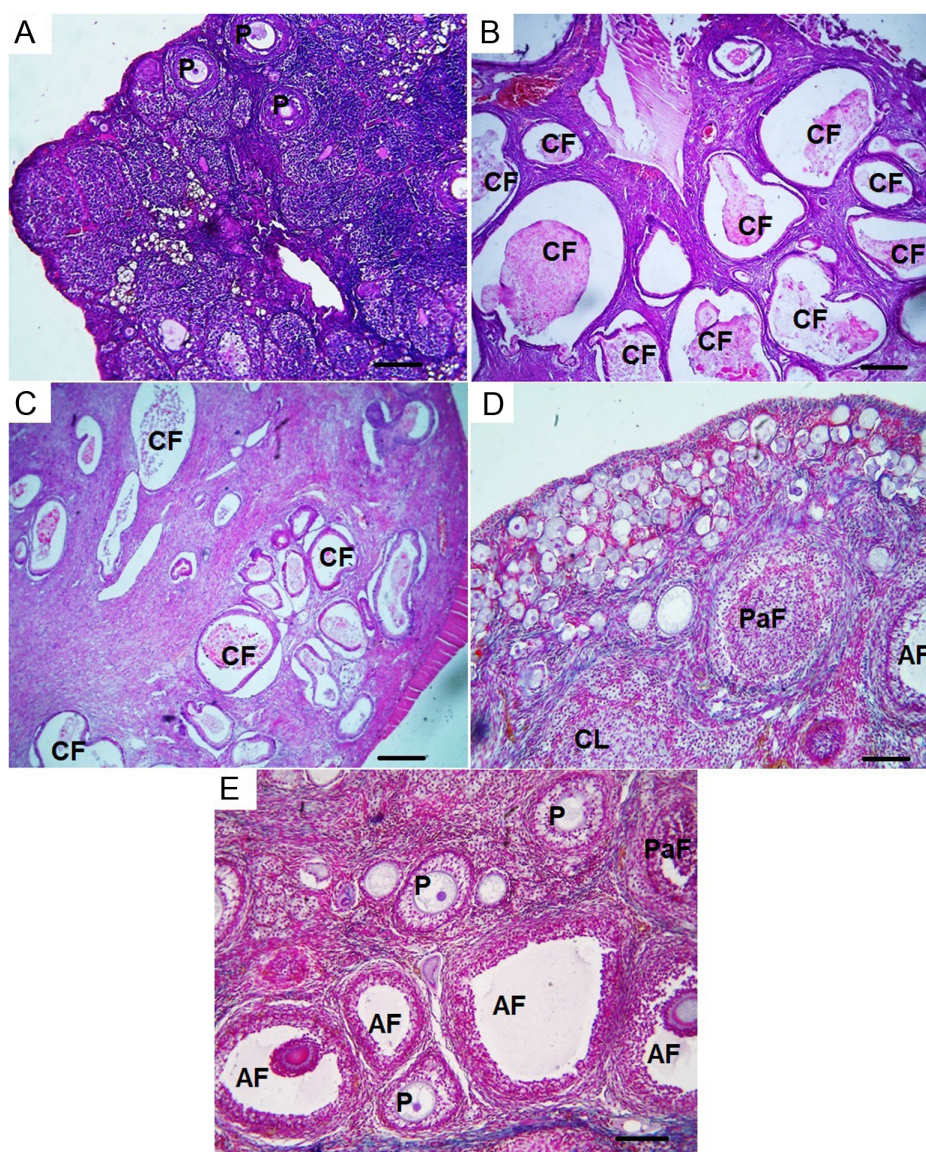
### Ovarian Tissue Changes

The results of this study demonstrate that exposure to BPA significantly decreased the number of primary ( $P=.009$ ), preantral ( $P=.02$ ), and antral ( $P=.006$ ) follicles compared to the control group. However, treatment with FV at 1000 mg/kg doses significantly increased the number of primary follicles compared to the BPA group ( $P=.02$ ). Preantral follicles in the 500 ( $P=.02$ ) and 1000 ( $P=.008$ ) mg/kg FV treatment groups also exhibited a significant increase compared to the BPA group. Moreover, the number of antral follicles significantly increased in the 500 ( $P=.02$ ) and 1000 ( $P=.01$ ) mg/kg FV treatment groups (Figures 2 and 3).

### Discussion

BPA exposure caused a reduction in serum levels of  $E_2$  and TAC as well as a decrease in the number of ovarian follicles while increasing serum NO levels. However, an increase in  $E_2$  and primary and

antral follicles was observed in the FV treatment groups, along with a decrease in NO levels compared to the BPA group. These effects were particularly noticeable at the 1000 mg/kg FV dose. BPA is a xenoestrogen compound that mimics  $E_2$  action and has been shown to have adverse health effects.<sup>6</sup> Several studies have reported its impact on the ovary, although the mechanisms are not fully understood.<sup>20,23</sup> The reduction in antral and primary follicles observed in this study is consistent with the findings of Zhou et al.<sup>23</sup> Patel et al.<sup>24</sup> demonstrated that rats exposed to BPA exhibited lower levels of estradiol, and the number of primary, pre-antral, and antral follicles was also reduced. Similarly, Peretz et al.<sup>25</sup> found that rats exposed to BPA exhibited suppressed differentiation of all types of follicles within 30 days. Additionally, atresia was observed after 70 days of exposure to BPA. In the present study, BPA disturbed folliculogenesis, resulting in the presence of incomplete and abnormal follicles, as well as atresia.



**Figure 3.** Histopathological changes in ovarian tissue in control (A); bisphenol (B); and 250 (C), 500 (D), and 1000 (E) mg/kg FV extract-treated groups. Primary (PF), pre-antral (PaF), antral (AF), cystic (CF) follicles, and corpus luteum (CL). (hematoxylin and eosin stain [H&E] staining  $\times 100$ , scale bar = 200  $\mu$ m).

To the best of our knowledge, this is the first study to examine the effects of FV on BPA-induced ovarian damage and how FV reduces the destructive impact of BPA on folliculogenesis by increasing antral and preantral follicles. Follicle growth is regulated by follicle-stimulating hormone (FSH), LH, and prolactin, as well as paracrine and autocrine factors.<sup>26</sup> Studies have demonstrated the presence of isoflavonoid compounds, such as genistein, formononetin, anethole, and daidzein in FV. These phytoestrogen compounds have been shown to regulate  $E_2$  synthesis by affecting the hypothalamus–pituitary–gonadal axis, as well as the internal single cells of developing follicles.<sup>12,13</sup>

Sadrefozalayi and Farokhi<sup>27</sup> conducted a study on rats exposed to estradiol valerate and found that 150 mg/kg of FV could regulate  $E_2$  levels and subsequently improve folliculogenesis. The study demonstrated that FV could enhance the function of internal single cells and effectively promote the differentiation of cystic follicles.<sup>16</sup> An increase in the number of follicles with FV extracts may be attributed to the presence of estrogenic compounds.<sup>28</sup> Consistent with previous studies, the present study also showed increased  $E_2$  levels in the FV treatment groups, indicating its estrogenic properties. The increased estradiol levels likely contributed to the increase in follicle numbers observed with FV treatment.

BPA has been shown to bind to classical  $E_2$  receptors alpha and beta, as well as non-classical  $E_2$  receptors such as G protein-coupled  $E_2$  receptor and  $E_2$ -related receptor gamma, altering the mechanism of these receptors and  $E_2$  levels in granulosa cells.<sup>29</sup> Laboratory studies have shown that BPA can disrupt the signaling pathway essential for fertility by affecting  $E_2$  in various ways.<sup>30</sup> BPA increases testosterone levels by upregulating enzymes like 17 $\alpha$ -hydroxylase and also binds to sex hormone-binding globulin, reducing aromatase (CYP19A1) expression, and increasing free androgens.<sup>31</sup> Additionally, BPA has been found to reduce aromatase expression (CYP19A1) and  $E_2$  production in human granulosa cells.<sup>20</sup> FV, which contains phytoestrogens, has been found to have negative feedback on LH, reducing testosterone levels and likely resulting in lower LH production and reduced dominance over FSH, restarting the natural cycle of sex hormones and possibly ovulation in patients.<sup>29</sup> In a study, FV doses of 100–200 mg/kg increased  $E_2$ , progesterone, and prolactin levels.<sup>26</sup> Consistent with previous studies, the present study also found that  $E_2$  levels increased in FV treatment groups. However, further research is needed to understand the mechanisms by which FV may counteract the effects of BPA.

The FV treatment groups in this study demonstrated an increase in TAC and a decrease in NO levels. Previous studies have shown that exposure to BPA can increase free radicals and decrease TAC levels.<sup>32</sup> BPA exposure can cause lipid peroxidation, decrease antioxidant ability, damage DNA, and increase abnormal chromosome numbers. By inducing free radical production, BPA can also inactivate the thiol group in actin protein, which is an oxidative stress target, ultimately causing cell deformation.<sup>10</sup> BPA-induced ovarian toxicity is associated with decreased TAC and increased malondialdehyde. Therefore, the results of this study suggest that FV may have a protective effect against BPA-induced oxidative stress in the ovaries by increasing TAC and decreasing NO levels.<sup>9</sup> Oxidative stress, due to the overproduction of reactive oxygen species (ROS) like NO and a decrease in antioxidants, can impact fertility.<sup>6,10</sup> Free radicals play essential roles in oocyte maturation, steroidogenesis, folliculogenesis, and ovulation.<sup>31</sup> The present study demonstrated that FV treatment increased

TAC and reduced NO levels, highlighting FV's antioxidant properties. However, the mechanisms by which FV counteracts the effects of BPA require further research. Overproduction of free radicals such as ROS and NO, combined with a decrease in the body's natural scavenging system for free radicals, can lead to oxidative stress.<sup>6</sup> This can contribute to various pathological conditions and diseases, including infertility, cancer, diabetes, cardiovascular disease, aging, and neurological disorders. Therefore, it is crucial to maintain a balance between ROS production and antioxidant defenses to prevent oxidative stress and its associated adverse health consequences.<sup>10,33</sup>

ROS concentrations play a crucial role in reproductive functions such as oocyte maturation, ovarian steroidogenesis, folliculogenesis, and ovulation.<sup>34</sup> Free radicals like NO are present in cumulus cells, follicular fluid, and oocytes, and their presence can have a negative impact on folliculogenesis by disrupting cell signaling, energy production, and DNA replication.<sup>10</sup> Excessive NO levels can be toxic to oocytes and granulosa cells, impairing oocyte quality and maturation. Therefore, it is important to maintain a balance between ROS production and antioxidant defenses to ensure optimal reproductive function. Oxidative stress, caused by an excess of ROS and insufficient antioxidants, can have a negative impact on fertility.<sup>31</sup>

FV seeds are rich in antioxidants and exhibit multiple antioxidant activities, including scavenging free radicals, superoxide anion radical scavenging, and hydrogen peroxide inhibition.<sup>35</sup> FV is rich in flavonoids like kaempferol and quercetin, which have the ability to eliminate free radicals, and reactive oxygen species. Numerous studies have confirmed the antioxidant properties of various components of FV, including its seeds, leaves, and fruits.<sup>12,14</sup> FV is recognized for its potent antioxidant properties, and its essential oil, which contains linoleic, palmitic, and oleic acids, has been shown to have strong antioxidant properties.<sup>13</sup> FV possesses numerous antioxidant properties that increase TAC, and in this study, its use resulted in an increase in TAC and a reduction in free radicals, including NO, emphasizing its antioxidant properties. These findings suggest that FV may protect against BPA-induced oxidative stress in the ovaries, although more research is required to understand its underlying mechanisms.

## Conclusion

FV extracts containing phytoestrogens and phenolic antioxidant compounds have shown promising effects in counteracting and reducing the adverse effects of BPA, improving  $E_2$  levels, and protecting the ovarian structure and function while decreasing oxidative stress, thus presenting a potential for further research and development as a natural remedy against BPA-induced ovarian toxicity.

**Ethics Committee Approval:** This study was approved by Ethics Committee of Kermanshah University of Medical Sciences, Kermanshah, Iran (Approval No: IR.REC.KUMS.1399.168, Date: 2020.05.12).

**Informed Consent:** There are no human subjects in this article and informed consent is not applicable.

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Concept – F.K., M.K.; Design – M.A., L.N.; Supervision – M.K., F.K.; Resources – M.K., L.N.; Materials – L.N., M.K.; Data Collection and/or Processing – M.A., F.K.; Analysis and/or Interpretation – L.N., M.A.; Literature Search – M.K., F.K.; Writing – L.N., M.A.; Critical Review – M.K., M.A.

**Declaration of Interests:** The authors have no conflict of interest to declare.



**Funding:** This study was supported by grants from Kermanshah University of Medical Sciences, Kermanshah, Iran (grant number: 990183).

## References

1. Flint S, Markle T, Thompson S, Wallace E. Bisphenol A exposure, effects, and policy: a wildlife perspective. *J Environ Manage*. 2012;104:19-34. [\[CrossRef\]](#)
2. Gharraei A, Ghorbani R, Khazaei M, et al. Altered pituitary hormone secretion in male rats exposed to bisphenol A. *Indian J Occup Environ Med*. 2006;10(1):24-27. [\[CrossRef\]](#)
3. Wang S-W, Kim J-M, Hwang K-G. Effect of gestational exposure to bisphenol A on neuronal stem cell differentiation in the neonatal rat hippocampus. *J Korean Child Neurol Soc*. 2011;19(3):218-230.
4. Cabado AG, Aldea S, Porro C, et al. Migration of BADGE (bisphenol A diglycidyl-ether) and BFDGE (bisphenol F diglycidyl-ether) in canned seafood. *Food Chem Toxicol*. 2008;46(5):1674-1680. [\[CrossRef\]](#)
5. Cao XL, Dufresne G, Clement G, Bélisle S, Robichaud A, Beraldin F. Levels of bisphenol A diglycidyl ether (BADGE) and bisphenol F diglycidyl ether (BFDGE) in canned liquid infant formula products in Canada and dietary intake estimates. *J AOAC Int*. 2009;92(6):1780-1789. [\[CrossRef\]](#)
6. Maffini MV, Rubin BS, Sonnenschein C, Soto AM. Endocrine disruptors and reproductive health: the case of bisphenol-A. *Mol Cell Endocrinol*. 2006;254-255:179-186. [\[CrossRef\]](#)
7. Heldring N, Pike A, Andersson S, et al. Estrogen receptors: how do they signal and what are their targets. *Physiol Rev*. 2007;87(3):905-931. [\[CrossRef\]](#)
8. Ptak A, Hoffmann M, Rak A. The ovary as a target organ for bisphenol A toxicity. *Bisphenol Expo Health Risks*. 2017;57.
9. Behmanesh MA, Poormoosavi M, Jannati S, Najafzadeh Varzi H, Sangtarash E. Aloe vera ameliorates ovarian toxicity induced by bisphenol A in Wistar rats. *J Adv Med Biomed Res*. 2018;26(119):17-23. [\[CrossRef\]](#)
10. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: impact on human health. *Pharmacogn Rev*. 2010;4(8):118-126. [\[CrossRef\]](#)
11. Mahboubi M. Foeniculum vulgare as valuable plant in management of women's health. *J Menopausal Med*. 2019;25(1):1-14. [\[CrossRef\]](#)
12. Al-Amoudi WM. Protective effects of fennel oil extract against sodium valproate-induced hepatorenal damage in albino rats. *Saudi J Biol Sci*. 2017;24(4):915-924. [\[CrossRef\]](#)
13. Ostad SN, Soodi M, Shariffzadeh M, Khorshidi N, Marzban H. The effect of fennel essential oil on uterine contraction as a model for dysmenorrhea, pharmacology and toxicology study. *J Ethnopharmacol*. 2001;76(3):299-304. [\[CrossRef\]](#)
14. Namavar Jahromi B, Tartifzadeh A, Khabnadideh S. Comparison of fennel and mefenamic acid for the treatment of primary dysmenorrhea. *Int J Gynaecol Obstet*. 2003;80(2):153-157. [\[CrossRef\]](#)
15. Choi EM, Hwang JK. Antiinflammatory, analgesic and antioxidant activities of the fruit of Foeniculum vulgare. *Fitoterapia*. 2004;75(6):557-565. [\[CrossRef\]](#)
16. Khazaei M, Montaseri A, Khazaei MR, Khanahmadi M. Study of Foeniculum vulgare effect on folliculogenesis in female mice. *Int J Fertil Steril*. 2011;5(3):122-127.
17. Hejmej A, Kotula-Balak M, Bilińska B. Antiandrogenic and estrogenic compounds: effect on development and function of male reproductive system. *Steroids-Clin Aspect*. 2011.
18. Nasri S, Oryan S, Haeri RSA, Amin GR, Taghizadeh M. The Effects of Vitex Agnus Castus Extract and Interaction with Bromocriptine on Luteinizing Hormone and Testosterone in Male Mice; 2005.
19. Wisniewski P, Romano RM, Kizys MM, et al. Adult exposure to bisphenol A (BPA) in Wistar rats reduces sperm quality with disruption of the hypothalamic-pituitary-testicular axis. *Toxicology*. 2015;329:1-9. [\[CrossRef\]](#)
20. Karampoor P, Azarnia M, Mirabolghasemi G, Alizadeh F. The effect of hydroalcoholic extract of fennel (Foeniculum vulgare) seed on serum levels of sexual hormones in female Wistar rats with polycystic ovarian syndrome (PCOS). *J Arak Univ Med Sci*. 2014;17(5):70-78.
21. Khazaei F, Ghanbari E, Khazaei M. Protective effect of royal jelly against cyclophosphamide-induced thrombocytopenia and spleen and bone marrow damages in rats. *Cell J*. 2020;22(3):302-309. [\[CrossRef\]](#)
22. Khazaei F, Farzaei MH, Khazayel S, Khazaei M. Trifolium pratense extract induces apoptosis and decreases nitric oxide secretion in human umbilical vein endothelial cell. *WCRJ*. 2019. [\[CrossRef\]](#)
23. Zhou W, Liu J, Liao L, Han S, Liu J. Effect of bisphenol A on steroid hormone production in rat ovarian theca-interstitial and granulosa cells. *Mol Cell Endocrinol*. 2008;283(1-2):12-18. [\[CrossRef\]](#)
24. Patel S, Brehm E, Gao L, Rattan S, Ziv-Gal A, Flaws JA. Bisphenol A exposure, ovarian follicle numbers, and female sex steroid hormone levels: results from a CLARITY-BPA study. *Endocrinology*. 2017;158(6):1727-1738. [\[CrossRef\]](#)
25. Peretz J, Craig ZR, Flaws JA. Bisphenol A inhibits follicle growth and induces atresia in cultured mouse antral follicles independently of the genomic estrogenic pathway. *Biol Reprod*. 2012;87(3):63. [\[CrossRef\]](#)
26. Sadeghpour N, Khaki AA, Najafpour A, Dolatkah H, Montaseri A. Study of Foeniculum vulgare (Fennel) Seed Extract Effects on Serum Level of Estrogen, Progesterone and Prolactin in Mouse; 2015.
27. Sadrefozalayi S, Farokhi F. Effect of the aqueous extract of Foeniculum vulgare (fennel) on the kidney in experimental PCOS female rats. *Avicenna J Phytomed*. 2014;4(2):110-117
28. Shabaniyan S, Bahmani M, Asadi-Samani M. The medicinal plants effective on female hormones: a review of the native medicinal plants of Iran effective on estrogen, progesterone, and prolactin. *J Chem Pharm Sci*. 2016;9(3):1270-1276.
29. Kwintkiewicz J, Nishi Y, Yanase T, Giudice LC. Peroxisome proliferator-activated receptor-γ mediates bisphenol A inhibition of FSH-stimulated IGF-1, aromatase, and estradiol in human granulosa cells. *Environ Health Perspect*. 2010;118(3):400-406. [\[CrossRef\]](#)
30. Gilibili RR, Wayne Vogl A, Thomas KH, Chang TKH, Bandiera SM. Localization of cytochrome P450 and related enzymes in adult rat testis and downregulation by estradiol and bisphenol A. *Toxicol Sci*. 2014;140(1):26-27.
31. Liu H, Jin H, Kim G, Bae J. A low dose of bisphenol A stimulates estradiol production by regulating β-catenin-FOXO2-CYP19A1 pathway in human ovarian granulosa cells. *Biochem Biophys Res Commun*. 2021;583(17):192-198. [\[CrossRef\]](#)
32. Ishido M, Suzuki J. Quantitative analyses of inhibitory effects of bisphenol A on neural stem-cell migration using a neurosphere assay in vitro. *J Health Sci*. 2010;56(2):175-181. [\[CrossRef\]](#)
33. Agarwal A, Gupta S, Sharma RK. Role of oxidative stress in female reproduction. *Reprod Biol Endocrinol*. 2005;3(1):28. [\[CrossRef\]](#)
34. Oktay M, Gülçin İ, Küfrevioğlu Ö. Determination of in vitro antioxidant activity of fennel (Foeniculum vulgare) seed extracts. *LWT Food Sci Technol*. 2003;36(2):263-271. [\[CrossRef\]](#)
35. De Marino S, Gala F, Borbone N, et al. Phenolic glycosides from Foeniculum vulgare fruit and evaluation of antioxidative activity. *Phytochemistry*. 2007;68(13):1805-1812. [\[CrossRef\]](#)