Original Article

The Prevention of Reproductive Problems Brought on by Bisphenol A *In Vivo* by *Alpinia officinarum* Extract

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Abstract

Background: Male fertility has been declining globally for a significant period, particularly in nations where a high presence of chemicals known as endocrine disruptors. **Objective:** The well-known endocrine disruptor bisphenol A (BPA), which is employed as a monomer in the manufacture of plastic materials, significantly affects the male reproductive system and male fertility. The purpose of this work was to determine whether an extract from the rhizomes of *Alpinia officinarum* (*A. officinarum*) could protect against the reproductive harm that BPA causes. **Materials and Methods:** Male test rats received the following doses after their division into three equal groups: 0.2 mL of olive oil was administered daily to each rat in the control group; BPA (50 mg/kg) was given to the BPA group; and *A. officinarum* extract (400 mg/kg) was administered to the protected group first, followed by BPA (50 mg/kg) after an hour. For 60 days, the various doses were given orally. After the animal was killed, blood samples were taken to gauge sex hormone levels, and testes and epididymis were removed for analysis of sperm parameters and histological examinations. **Results:** Testosterone (T), follicle-stimulating hormone (FSH), and luteinizing hormone serum levels decreased in BPA-exposed rats; and sperm parameters (concentration, motility, viability) also decreased. Additionally, contrasted with the control group, histological investigation revealed a significant reduction in the epithelial thickness and diameter of the seminiferous and caudal tubules in this group. In contrast, the protected group's data show a marked decline in all prior biochemical and histological problems. No significant differences were observed in T and FSH levels, sperm concentration, and sperm motility in the protected group as compared to the control group. **Conclusion:** These results suggested that *Alpinia officinarum* extract is effective at lowering the male reproductive toxicity due to its antioxidant properties and subsequently offering protection against the harm caused by oxidative stress brought on by BPA exposure.

Keywords: *Alpinia officinarum*, bisphenol A, endocrine-disrupting chemicals, oxidative damage

Introduction

For a very long time, there has been a global drop in male fertility, with the biggest declines occurring primarily in the USA, European nations, and Australia. This is particularly true in nations that have frequently reported a proliferation of substances that are deemed to disturb the endocrine system, whether in the environment or in human tissues.[1] Many aspects of endocrine homeostasis and growth control can be disrupted by endocrine-disrupting chemicals (EDCs), a wide class of man-made exogenous compounds.[2,3] Occupational and environmental exposure to EDCs in humans occurs continually through a variety of channels, including ingestion, inhalation, and skin contact.[4] By way of their effects on testicular development, potential epigenetic effects, effects on estrogen and androgen receptors, formation of reactive oxygen species (ROS), and direct effects on spermatogonia and other cells of the testicular tissue, EDCs are linked to altered reproductive system functions in both male and female animal models as well as in humans. According to recent scientific data, exposure to persistent and nonpersistent endocrine disruptors including bisphenol A (BPA) and perfluoroalkyl substances is linked to an increase in male infertility.[1,2]

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People regularly come into contact with the chemical BPA, which is widely utilized in industrial areas. Because of its status as an endocrine disruptor, BPA is predicted to affect how the chemical estrogen receptor and other nuclear hormone receptors work.[5] This substance is still typically present in consumer products, where it then escapes into the environment through food, water, and air. The presence of BPA has been found in human milk, urine, and blood.[6,7] It is well known that BPA causes the body to produce ROS, including hydroxyl radicals, hydrogen peroxide, and superoxide anions.[8] Moreover, BPA has the potential to be hazardous to reproduction and harm spermatogenesis by generating ROS and degrading natural antioxidants.[9]

People have used plants as a source of healing medicines for millennia.[10] The *Alpinia* is a genus in the family Zingiberaceae that has long been known for both its therapeutic and nontherapeutic applications.[11] *Alpinia officinarum*, likewise called Lesser galangal, is a significant Zingiberaceae plant that grows widely in Southeast Asia's tropical and subtropical climates. Traditional medicine employs its rhizomes as analgesics, anti-inflammatory, and antioxidant agents.[12] For *A. officinarum* in diabetic rats, the extract has been shown to reduce testicular histopathological damage and sperm parameters.[13] It has been shown to have a number of elements with antioxidant, anti-inflammatory, and antimicrobial properties.[14,15] The primary components of this plant, galangin, kaempferide, and quercetin, have been researched and found to have antioxidant activity. As a result, they can reduce the oxidative stress and reproductive damage brought on by BPA.[16,17]

Materials and Methods

Plant collecting and extract preparation

A plant taxonomist from Karbala University, College of Education for Pure Sciences identified *A. officinarum* rhizomes; they were bought at the community market. After cleaning them with water from suspended impurities and then air drying, they were soaked in 70% ethyl alcohol for 3 days at ambient temperature. Alpin powder extract and solvent were combined in a ratio of 1:5 to produce *A. officinarum* extract. After filtering using a Whitman filter, the extract was concentrated until dry using a rotatory evaporator set at 45°C. About 12.5% of the dry extract was produced.[18]

Animals

The experiment was conducted on 18 adult male albino rats, weighed 200–220 g and aged 12 weeks, prepared in the Animal Home Lab at Karbala University's College of Education for Pure Sciences. These animals were kept in typical laboratory settings with a 12-h light/dark cycle and water available at will. They were also fed a regular meal.

Design of experiment

The experimental animals were randomly divided into three equal groups ($n = 6$), and then they were treated with the following doses orally using an intragastric tube for a period of 60 days:

Group I: (control group) was given olive oil (200 µL/animal/day).

Group II: (BPA group) was given BPA (50 mg/kg/day) dissolved in 200 µL of olive oil.

Group III: **(protected group)** was given *A. officinarum* extract (400 mg/kg/day) dissolved in 200 µL of normal saline, followed by (an hour later) BPA (50 mg/kg/day) dissolved in 200 µL of olive oil.

The amount of BPA was chosen using the Alboghobeish *et al.*'s[19] approach, whereas the amount of *A. officinarum* extract and olive oil was chosen using Abass *et al.*'s[20] and Munir *et al.*'s approaches,[21] consecutively.

Choosing samples

The animals were killed and heart blood samples were taken for biochemical tests after 24 h had passed since the last dose. In order to evaluate sperm parameters and make histological preparations, reproductive organs (the testis and epididymis) were immediately removed.

Estimation of serum sex hormones

The hormones testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were measured using ELISA kits from BT Lab China.

Parameter evaluation for sperms

Each rat's epididymis cauda was removed from its testicles, and after being cut up into tiny parts, 3 mL of ordinary saline is added to it. Small drop of the aforementioned solution was placed on a Neubauer hemocytometer (HBG Henneberg-Sander GmbH, Gießen, Germany) for sperm counting, which was done manually with high-resolution microscope. The sperm count per milliliter was used to calculate the data.[22] An aliquot of 50 µL of semen suspension was obtained to test the motility of the sperm, which was then viewed under a light microscope at ×40 on a glass slide that had been prewarmed to 37°C. A technician assessed the motility of 100 sperm/field in total. The sperm sample was mixed with a drop of eosin and nigrosin to test for viability. A 10-µL sample was put on a warmed glass slide and examined at a magnification of ×100. Within 10 microscopic fields, 100 sperm/field were examined for eosin staining, and the proportions of living and dead sperm were calculated. For each sample, the average value was recorded and expressed as a proportion of living sperm.[23]

Histological studies

After being separated and treated with formalin solution (10%), the right testis and epididymis were put in paraffin. Hematoxylin and eosin staining was employed to generate tissue sections measuring 5–7 µm for histological investigation. After calibration, the diameter and epithelial height of the seminiferous and epididymis tubules were measured under a light microscope. Photos of the slides were taken at a magnification of about ×40.[13]

Statistical analysis

The results of the data analysis were presented as mean ± standard error (SE) using IBM Statistical Package for the Social Sciences software (version 26, Chicago, Illinois). The one-way analysis of variance and *post-hoc* least significant difference tests were used to analyze group differences. The threshold for statistical significance was fixed at $P < 0.05$.

Ethical approval

All procedures involving animals were carried out consistently with the regulations established by the Supervision Committee of the Biology Department, the Education College of Pure Sciences, Karbala University. The study protocol was reviewed and approved by a local ethics committee according to document number 1818/14 on June 7, 2022.

Results

Alpinia officinarum extract and bisphenol A's impact on sex hormones

Serum levels of testosterone, FSH, and LH were considerably lower in the BPA group in contrast to the control group ($P < 0.05$) and significantly higher in the protected group compared to the BPA group ($P < 0.05$). However, there was no significant difference ($P > 0.05$) in the levels of testosterone hormones and FSH in the protected group compared to the control group, while the level of LH was significantly low [Table 1].

Alpinia officinarum extract and bisphenol A's effects on sperm characteristics

When compared to the control group, the BPA group had a significantly lower sperm count, sperm motility, and sperm viability. In addition, all of these indicators increased in the protected group relative to the BPA group by a substantial amount ($P < 0.05$). In contrast to the control group, the protected group did not exhibit significant differences ($P > 0.05$) in sperm count and sperm motility, but the percent sperm viability was significantly low [Table 2].

Alpinia officinarum extract and bisphenol A's effects on testicular planimetry and histopathological alterations

Results demonstrating that the diameter and epithelial thickness of seminiferous and caudal tubules were significantly decreased ($P < 0.5$) in the BPA group compared to the control group. Also, compared to the BPA group, the protected group's epithelial thickness and tubular diameter were considerably higher ($P < 0.5$). However, they are still significantly lower ($P < 0.5$) in the protected group as compared to control group [Table 3, Figures 1 and 2].

Histopathologically, the testicular tissue in the control group displays a typical arrangement of seminiferous tubules with rounded or oval shapes and a typical pattern of spermatogenesis. Spermatozoa were present in the lumens of these tubules [Figure 1A]. In contrast, the BPA group's testis showed histological alterations including lowering of spermatozoa differentiation, atrophy, and separation of germinal layer in seminiferous tubules. Some tubule lumens do not seem to contain any sperm [Figure 1B]. The majority of the earlier changes in the

Table 1: Sex hormones levels and the effects of BPA and an extract from *A. officinarum*

Groups	Testosterone (ng/mL)	FSH (mIU/mL)	LH (mIU/mL)
Control	4.62 ± 0.19^{a}	3.46 ± 0.12^{a}	2.52 ± 0.11^{a}
BPA	2.30 ± 0.21^{b}	2.32 ± 0.08^{b}	1.56 ± 0.08^{c}
Protected	4.23 ± 0.10^{a}	3.28 ± 0.11^{a}	2.26 ± 0.09^{b}

Means ± SE were used to express values

When values in the same column with different letters are significant, the significance level is $P < 0.05$

Table 2: *A. officinarum* extract and BPA's effects on sperm parameters

Groups	Sperm count (106)	Sperm motility (%)	Viability (live %)
Control	88.61 ± 3.05^{a}	83.10 ± 2.42^{a}	87.13 ± 1.82^{a}
BPA	62.05 ± 0.51^{b}	68.23 ± 2.03^{b}	53.71 ± 1.81^{c}
Protected	84.65 ± 2.24^{a}	78.40 ± 1.53^{a}	74.52 ± 1.48^{b}

Means ± SE were used to express values

When values in the same column with different letters are significant, the significance level is $P < 0.05$

Table 3: *A. officinarum* extract and BPA's effects on epithelial height and diameter of caudal and seminiferous tubule

Groups	Seminiferous tubule diameter (μm)	Seminiferous tubule epithelial height (μm)	Caudal tubule diameter (μm)	Caudal tubule epithelial height (μm)
Control	238.33 ± 6.57[a]	83.83 ± 3.13[a]	296.33 ± 5.90[a]	25.83 ± 1.35[a]
BPA	181.66 ± 3.98[c]	51.16 ± 2.52[c]	264.16 ± 5.39[c]	17.66 ± 0.88[c]
Protected	219.17 ± 7.14[b]	74.33 ± 2.09[b]	279.83 ± 3.77[b]	21.83 ± 0.70[b]

Means ± SE were used to express values

When values in the same column with different letters are significant, the significance level is $P < 0.05$

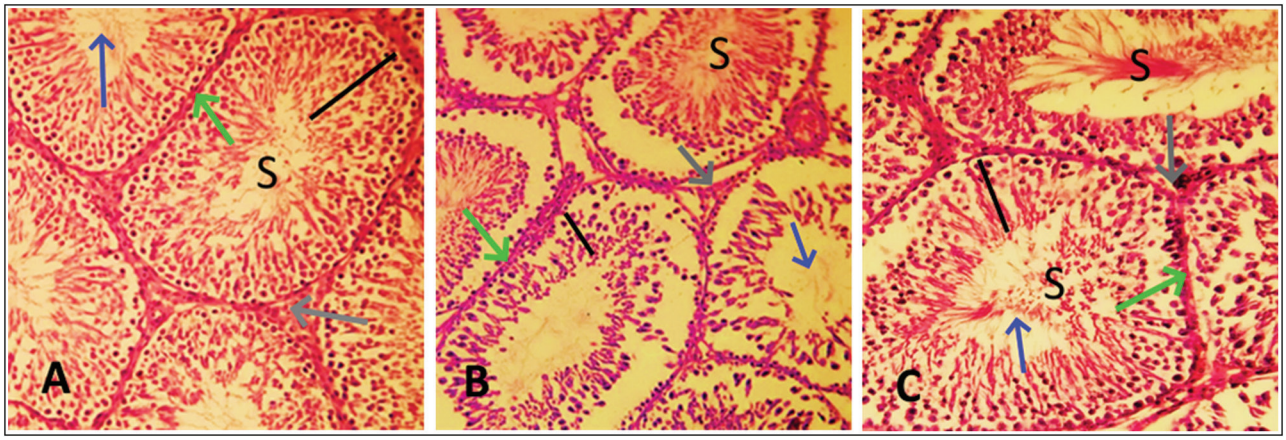

Figure 1: *A. officinarum* extract and BPA's effects on the testicular tissue of several experimental groups are shown in a photomicrograph (H&E, ×40). (A) Control group; (B) BPA group; and (C) protected group. Signs indicate basement membrane of seminiferous tubules (green arrow), lumens of tubules (blue arrow), interstitial tissue (gray arrow), germinal layer (black line), and spermatozoa (s)

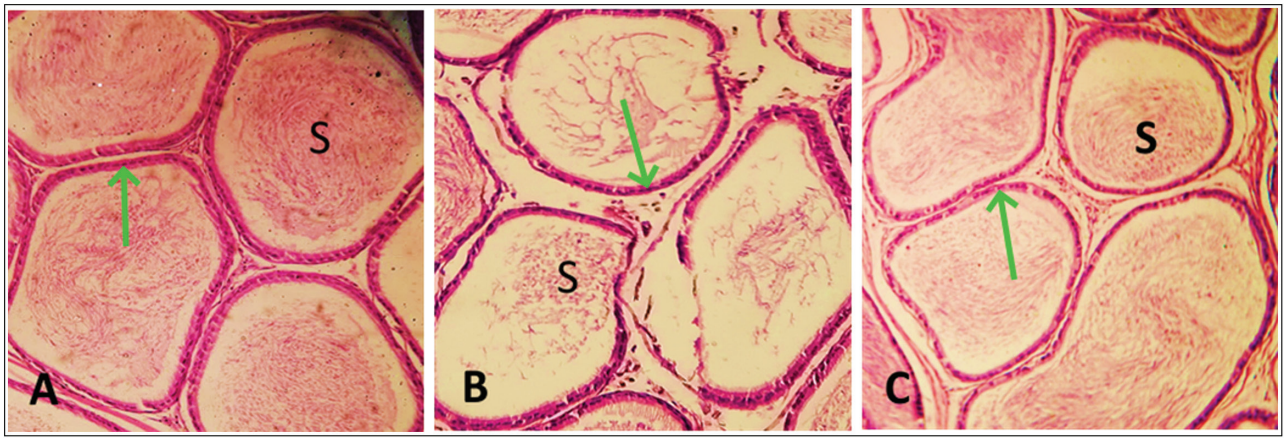

Figure 2: BPA and *A. officinarum* extract effects on cauda epididymis tissue of several experimental groups are depicted in a photomicrograph (H&E, ×40). (A) Control group, (B) BPA group, (C) protected group. Signs indicate epithelial lining of caudal tubule (green arrow) and spermatozoa (s)

BPA group, however, were dramatically reduced in the protected group [Figure 1C].

Microscopic examination also reveals the usual cauda epididymis anatomy in the control group [Figure 2A], including the tight arrangement of the tubules and the abundance of sperm in the lumens. The BPA group, on the other hand, saw morphological alterations that included loose organization, wall disintegration in some tubules, and a reduction in the number of sperm in their lumens [Figure 2B]. The protected group saw a marked reduction in these changes [Figure 2C].

Discussion

Drugs and environmental pollutants such as BPA often have a significant negative impact on the reproductive organs. Many studies have documented their harmful effects on reproduction.[22,24] The goal of the current work was to determine whether an extract of *A. officinarum*'s rhizomes could protect adult male rats from the reproductive toxicity and testicular impairment caused by BPA. This is based on the plant's ability to scavenge ROS through the presence of polyphenols, flavonoids, and the chemical diarylheptanoid.[23]

Our research has shown administering BPA results in decreased levels of testosterone, FSH, and LH. These findings were in agreement with a number of earlier research.[19,23] The administration of BPA dramatically decreases circulation levels of gonadotropins and/or testosterone as well as the expression of the gonadotropin-releasing hormone (GnRH) gene in cells of the preoptic region of hypothalamus.[25] According to Jin *et al*.,[26] BPA impairs Sertoli cell function and disrupts the hypothalamic–pituitary–gonadal axis. Akingbemi *et al*.[27] revealed that BPA affects the pituitary gland, altering testicular function and decreasing LH flow. Nanjappa *et al*.[28] suggested that interfering with the growth and proliferation of Leydig cells may be the cause of the decreased testosterone in the BPA-treated rats. Also, our findings revealed that the protected group's testosterone, FSH, and LH levels significantly increased in comparison to the BPA group [Figure 1]. This result may be attributable to *A. officinarum*'s direct or indirect impact on oxidative cell damage. Al-Qarawi[29] mentioned that *A. officinarum* extract directly affects the testicles, increasing testosterone production. An increase in LH levels explains this spike in testosterone levels.[30] Similar research demonstrated that when rats were given an alcoholic extract of *Alpinia galanga*, their serum testosterone levels considerably increased.[31]

The study's second part examines how exposure to BPA affects some sperm parameters. One of the most crucial signs of male infertility is sperm concentration.[32] Reduced sperm count is thought to be a factor in male infertility.[33] Moreover, sperm motility is necessary for sperm transportation through the female reproductive system.[34] The results of this study show that the BPA group's sperm count, sperm motility, and sperm viability were considerably less than those in the control group. This result is in line with several previous research. Alboghobeish *et al*.[19] recorded that male rats exposed to BPA had fewer sperm afterward, whereas Grami *et al*.[24] reported a significant drop in the rat test subjects' sperm parameters (number, motility, and vitality) following treatment with 100 mg/kg BPA. In contrast, our study found a significant increase in all sperm parameters compared to the BPA group in the protected group, which may be due to the ROS in the sperm was suppressed. Kolangi *et al*.[18] explained that *A. officinarum* increased sperm quantity and quality without any negative consequences. These effects have been linked to the antioxidant and radical-scavenging properties of plant substances like galangin.[35] Reid *et al*.[36] indicated that the flavonoids extracted from *A. officinarum* are crucial for preventing oxidative damage by promoting the development of antioxidant proteins. Our histology analysis revealed that rats exposed to BPA had significantly smaller seminiferous and caudal tubule diameters and epithelial heights in their testis and epididymis. These outcomes were in agreement with a number of previous investigations.[19,37-39] Low blood levels of testosterone may be the root of this effect. In contrast, it was discovered that *A. officinarum* extract in the protected group led to positive shifts of these alterations in the direction of normal. The inhibition of oxidative damage to the testis, and the rise in testosterone and LH concentrations may both contribute to the protective effects of *A. officinarum*.

Conclusion

In conclusion, rats' testis and epididymis undergo histological and functional alterations as a result of BPA exposure. BPA consumption can harm the reproductive system by oxidative stress, which lowers sex hormone levels, sperm parameters (count, motility, and viability), and seminiferous and epididymal tubule diameter and epithelial height. *A. officinarum* extract has been proven to be protective against BPA-induced reproductive toxicity and oxidative damage. According to our findings, *A. officinarum* makes a strong candidate for protection against the reproductive damage caused by BPA.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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