ORIGINAL ARTICLE

CONTENTS

Reproductive physiological amelioration of BPA - induced toxicity by resveratrol: Sperm quality preservation, DNA damage mitigation, and testicular protection

Saad Mashkoor Waleed[1], Balsam G. Hassan[2], Hussam H. Tizkam[2], Mohamed A. Zarka[3]

[1]DEPARTMENT OF PHARMACOLOGY, COLLEGE OF PHARMACY, UNIVERSITY OF AL KAFEEL, NAJAF, IRAQ

[2]DEPARTMENT OF PHARMACEUTICS, SCHOOL OF PHARMACY, ALSAFWA UNIVERSITY COLLEGE, IRAQ

[3]PHARMACOGNOSY DEPARTMENT, COLLEGE OF PHARMACY, ISLAMIC UNIVERSITY IN NAJAF, NAJAF, IRAQ

ABSTRACT

Aim: The object of the present study aimed to investigate the impact of Bisphenol A on the fragmentation, and histopathological changes, and evaluating the protective effects of resveratrol against Bisphenol A.

Materials and Methods: The study used forty adults male Wistar rats were that randomly divided into four groups: a control group, a Bisphenol A group administered at 50 mg/kg/day, a resveratrol group at 50 mg/kg/day, and a Bisphenol + resveratrol group at the same dosages for 60 consecutive days. The blood and tissue samples were collected for evaluating the sperm parameters, DNA integrity, Luteinizing hormone, Testosterone hormone, total antioxidant capacity, and histopathological section.

Results: The results showed that Bisphenol A exposure causes significantly disrupted in sperm motility, concentration, morphology, and DNA integrity, with severe histopathological change in testicular tissue and causes significantly increasing in luteinizing hormone and decreased testosterone levels. In contrast, the results illustrated the resveratrol play as protective effects by conserved the sperm quality, testicular histopathology and reducing DNA fragmentation.

Conclusions: The Bisphenol A-induced significantly increased in oxidative stress while resveratrol play significant protection against Bisphenol A which that induced testicular damage. The present finds proved that resveratrol is a potential therapeutic agent that can be used as an intervention against chemical substances in the environment and preserve the fertility

KEY WORDS: antioxidant, Bisphenol A, DNA fragmentation, infertility, reproduction, resveratrol, sperm quality, testicular tissue

Wiad Lek. 2025;78(11):2286-2296. doi: 10.36740/WLek/212542 DOI

INTRODUCTION

Interestingly, infertility is a world health challenge, which affects about 15% of couples in the world, since, that is several factors participate to infertility, including chemical substances of the environment that exposure to the reproductive system, and it is one of the most important factors affecting the sperm parameters [1-2]. Bisphenol A (BPA), a widely used industrial chemical found in plastics, food containers, and everyday items, has appeared as a significant environmental threat to male reproductive health. With its ability to mimic estrogen and disrupt androgen function, BPA interferes with the hypothalamic-pituitary-gonadal axis, altering hormonal balance and impairing critical processes such as spermatogenesis [3]. Studies in both humans and animal models have linked BPA exposure to decreased testosterone levels, reduced sperm quality, DNA damage, and oxidative stress, raising concerns about its long-term impact on fertility [4-5]. Resveratrol (RES) is a natural polyphenolic compound that found in grapes, berries, and peanuts [6]. Moreover, the RES have a potent antioxidant property, which scavenges oxidative stress and decreases the adverse effects that are due to environmental toxins such as BPA [7]. Generally, the study illustrated that RES protects the tissue by scavenging reactive oxygen species, which improves the activity of endogenous antioxidant included enzymes, and subsequently reduces the lipid peroxidation, which finally that prevents cellular damage in the tissue [3, 8]. Additionally, the last studies illustrated that RES improved the mitochondrial function by increasing the production of ATP and by stabilizing the membrane of mitochondrial, then subsequently lead to enhance the sperm motility and viability. Furthermore, that RES play a critical role in inhibiting of the pro-apoptotic pathways which that promoting the activation of survival

signaling pathways, thus the RES improved the germ cell integrity and enhancing the spermatogenesis [9]. Several studies showed that RES acts as an anti-inflammatory which protects the testicular tissue from exposure to environmental contaminants and protects reproductive health [10]. Sperm parameters, including concentration, motility, viability, and morphology, are critical indicators of male fertility and are highly susceptible to environmental toxins which BPA disrupts endocrine function, leading to hormonal imbalances that impair spermatogenesis. Studies have consistently shown that BPA exposure reduces sperm concentration [5] compromises motility by impairing mitochondrial function and ATP production [9,11] decreases viability through oxidative stress-induced apoptosis [8,10] and increases abnormal morphology [3]. Thus, the present study aimed to evaluation the protective effect of the RES against the adverse effect of BPA that exposure the reproductive system in rats, which assessments the levels of luteinizing and testosterone hormonal, evaluation of sperm parameters (motility, morphology, and concentration), sperm DNA fragmentation and total antioxidant capacity levels. Additionally, evaluate the Histopathological Examination of Testicular Tissue.

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

This present study included 40 adults' male Wistar rats that weighted (225–275 g) that housed under typical conditions with a 12/12-hour light-dark cycle and free access to food and water. After adaptation in two-week period, the rats were randomly divided into four equal groups (10 per group): a control group (C) receiving no treatment; a BPA group administered BPA at 50 mg/kg/day [3]; a resveratrol group (RES) administered RES at 50 mg/kg/day [12]; and combined group a BPA + resveratrol group (BPA + RES) co-administered BPA (50 mg/kg/day) and resveratrol (50 mg/kg/day). All treatment solutions were given orally via gavage for 60 consecutive days.

ETHICS

All the procedures in this study, including animal husbandry, handling, and scarifying were performed according to the guidelines instructed by the Animal Ethics Committee of The Islamic University, Najaf, Iraq (2024-12-1/M12a). Ethical considerations were paramount throughout all stages of the study, and efforts were made to minimize potential harm to the experimental animals.

TREATMENT PROTOCOL

To prepare the RES solution 3 g of carboxymethylcellulose (CMC) powder in 97 g of distilled water to create the stock solution of 3% CMC then stirred continuously at room temperature for 4 hours to ensure dissolved. separately, the amount of RES was dissolved in a 1%ethanol to enhance its solubility then RES-ethanol solution was then added to the 3% CMC solution and stirring continuously for 30–60 minutes in water bath at 37 °C. The final volume of the mixture was adjusted with distilled water to achieve the desired concentration, followed by an additional 15 minutes of stirring to ensure complete homogeneity and stored at room temperature and [13]. To prepare the BPA solution, the amount of BPA was weighed and dissolved in normal to dosing at 50 mg/kg body weight. The prepared BPA solution was stored in an amber container at room temperature until administration via oral gavage [3, 10].

ANIMALS AND SAMPLE COLLECTION

At the endpoint of experimental at the 60-day experimental period, the animals were euthanized through an intramuscular overdose of a combination of Xylazine (Micopite, USA, 40 mg/kg) and Ketamine (Alpha Than, Netherlands, 90 mg/kg). The Blood samples about 5 mL were collected via cardiac-puncture and then the scrotal area was carefully cleansed with sterile normal saline before harvesting bilateral testicular and epididymal tissues [14-16]. The epididymal tail was incubated in 2 mL of normal saline at 37°C then by using anatomical micro-scissors, the tissue was divided into segments, and spermatozoa were extracted for subsequent evaluation of sperm motility, concentration, morphology, and DNA Integrity following the study's standardized methodology [17].

SPERM EVALUATION

The sperm motility, morphology, and concentration were assessed as followed: for evaluate sperm progressive motility, a 10 µL aliquot of sperm suspension was placed on a dry, warmed slide and examined under a light microscope at 400× magnification [14], while the sperm morphology, 10–20 µL of sperm was mixed with an 20 µL eosin-nigrosine stain, smeared onto a warm slide, air-dried, and observed under a microscope, where sperm heads stained pink [16-18]. Sperm concentration was determined by weighing testicular tissue, mincing it, and suspending it in 1 mL of PBS. A diluted aliquot (1:1000) was prepared using a solution of normal saline 95%, formaldehyde 4%, and eosin stain 1% following the methodology of Smith and Mayer (1955)

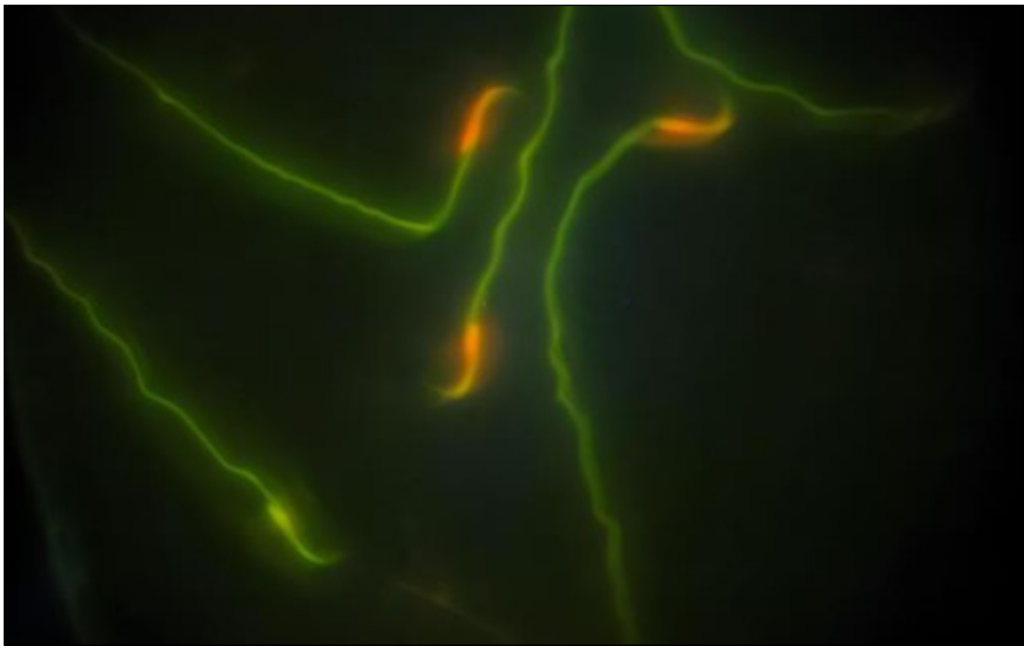

Fig. 1. Assessment of The Sperm Chromatin Integrity under Fluorescence that staining by Acridine Orange Staining. A Comparative Analysis of Condensed Chromatin (Green Fluorescence) and Denatured Chromatin (Red Fluorescence) 400X
Source: Own materials

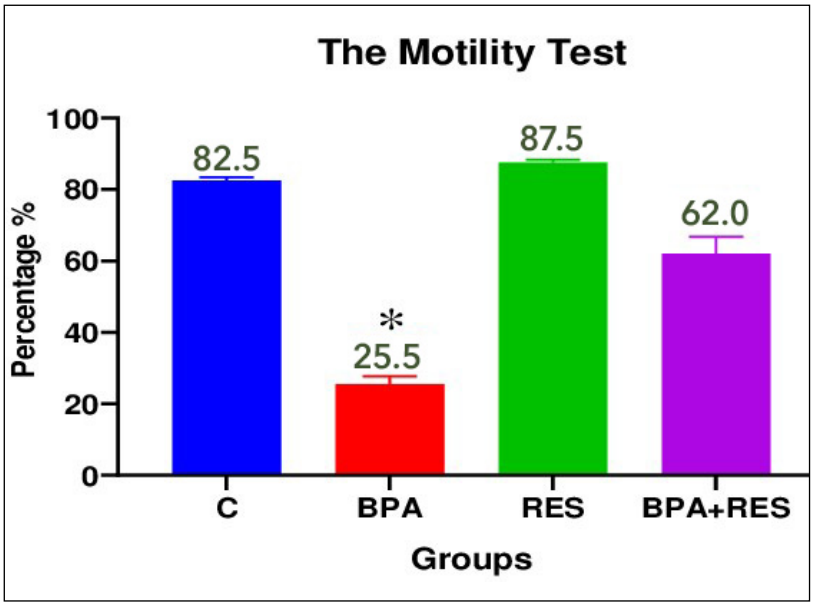

Fig. 2. Effects of Bisphenol A (BPA) and Resveratrol on Sperm Motility (%)
Control (C), BPA-treated (BPA), Resveratrol-treated (RES), and combined BPA + Resveratrol-treated (BPA + RES) groups. Error bars represent the standard error of the mean (SEM), *Indicates significant differences between groups at $p<0.05$
Source: Own materials

[19]. Sperm counting was performed using a Neubauer hemocytometer, adhering to the procedures outlined in previous studies [20].

SPERM DNA FRAGMENTATION TEST

For assessment the DNA fragmentation the sperm were fixation by drying a 10 µL of sample on a slide, and then immersing in a 3:1 methanol-acetic acid solution for 5 minutes, and fully dry [21]. After fixation the slide was stained by 0.01 of acridine orange for 2 minutes and washed with distilled water and left to dry. Finally, the slides examined under a fluorescent microscope showed sperm nuclei the green color mean with intact, and red that indicating fragmented, less condensed DNA, figure (1) [22].

EVALUATION OF CIRCULATING LUTEINIZING AND TESTOSTERONE HORMONES

For evaluation the LH and testosterone the blood was analyzed by using assay kits provided by (Sun Long Biotech Co., Ltd., China; SL1061Ra kit used for testosterone and the SL1093Ra kit for LH analysis.

REDOX SYSTEM EVALUATION

The redox system evaluation included the assessment of total antioxidant capacity (TAC) using specific assay kits (Cat No: BC1315) by using a spectrophotometer. The procedure involved sample preparation, serum dilution of the $FeSO_4$ standard solution, and preheating the spectrophotometer and reagents were added according to the protocol, and absorbance readings were used to construct a standard curve and calculate TAC values.

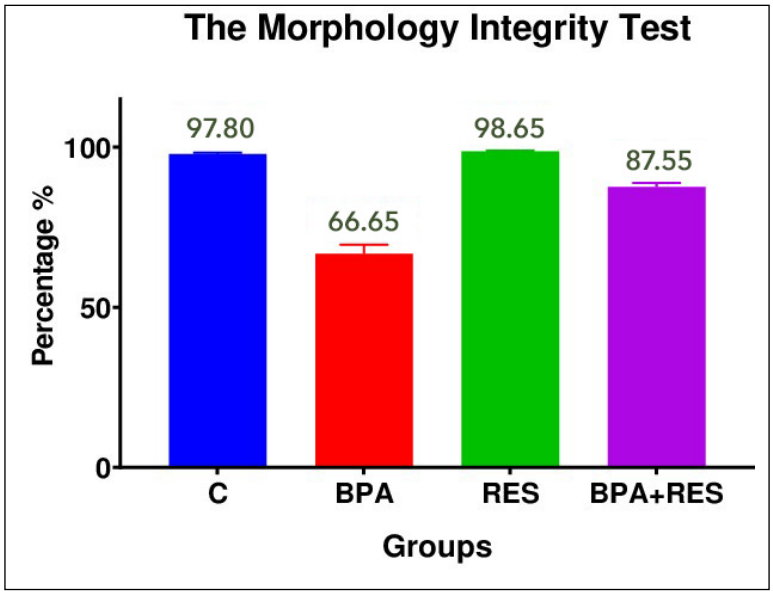

Fig. 3. Effects of Bisphenol A (BPA) and Resveratrol on Sperm Morphology (%)
Control (C), BPA-treated (BPA), Resveratrol-treated (RES), and combined BPA + Resveratrol-treated (BPA + RES) groups. Error bars represent the standard error of the mean (SEM), *Indicates significant differences between groups at $p<0.05$
Source: Own materials

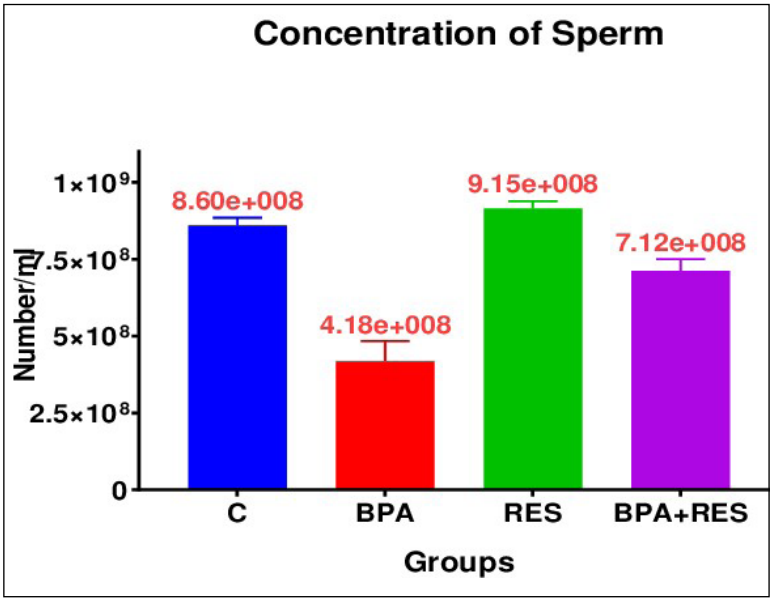

Fig. 4. Effects of Bisphenol A (BPA) and Resveratrol on Sperm concentration (%)
Control (C), BPA-treated (BPA), Resveratrol-treated (RES), and combined BPA + Resveratrol-treated (BPA + RES) groups. Error bars represent the standard error of the mean (SEM), *Indicates significant differences between groups at $p<0.05$
Source: Own materials

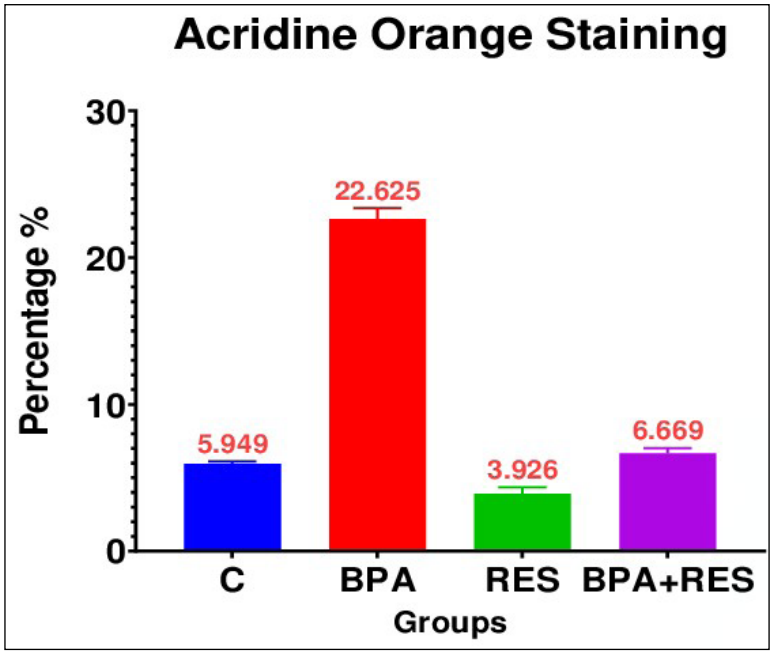

Fig. 5. Effects of Bisphenol A (BPA) and Resveratrol on Sperm DNA Fragmentation (%)
Control (C), BPA-treated (BPA), Resveratrol-treated (RES), and combined BPA + Resveratrol-treated (BPA + RES) groups. Error bars represent the standard error of the mean (SEM), *Indicates significant differences between groups at $p<0.05$
Source: Own materials

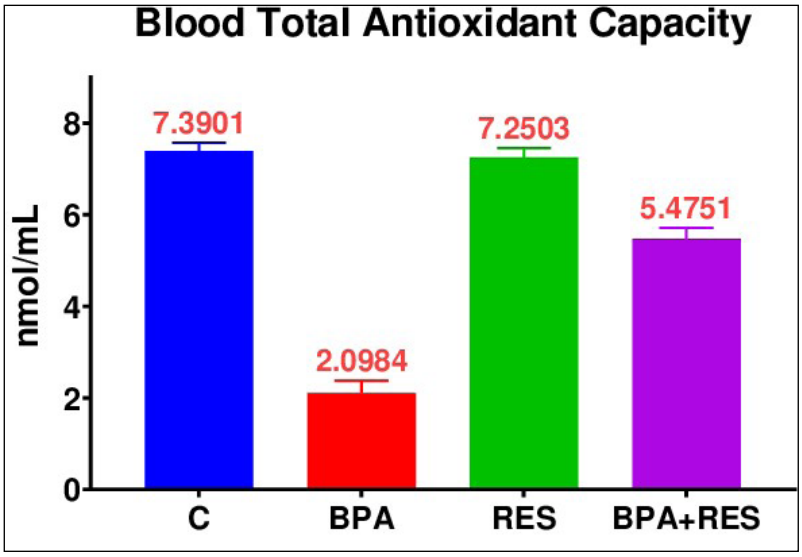

Fig. 6. Effects of Bisphenol A (BPA) and Resveratrol on Blood total Antioxidant capacity
Control (C), BPA-treated (BPA), Resveratrol-treated (RES), and combined BPA + Resveratrol-treated (BPA + RES) groups. Error bars represent the standard error of the mean (SEM), *Indicates significant differences between groups at $p<0.05$
Source: Own materials

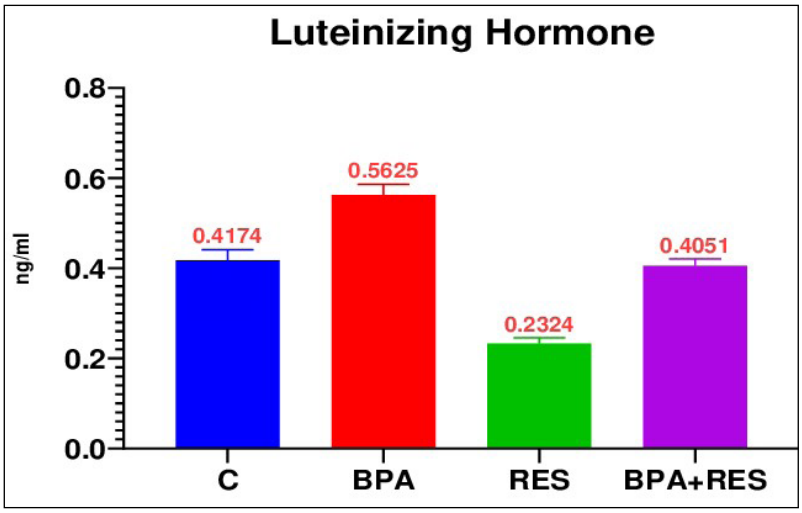

Fig. 7. Effects of Bisphenol A (BPA) and Resveratrol on LH
Control (C), BPA-treated (BPA), Resveratrol-treated (RES), and combined BPA + Resveratrol-treated (BPA + RES) groups. Error bars represent the standard error of the mean (SEM), *Indicates significant differences between groups at $p<0.05$
Source: Own materials

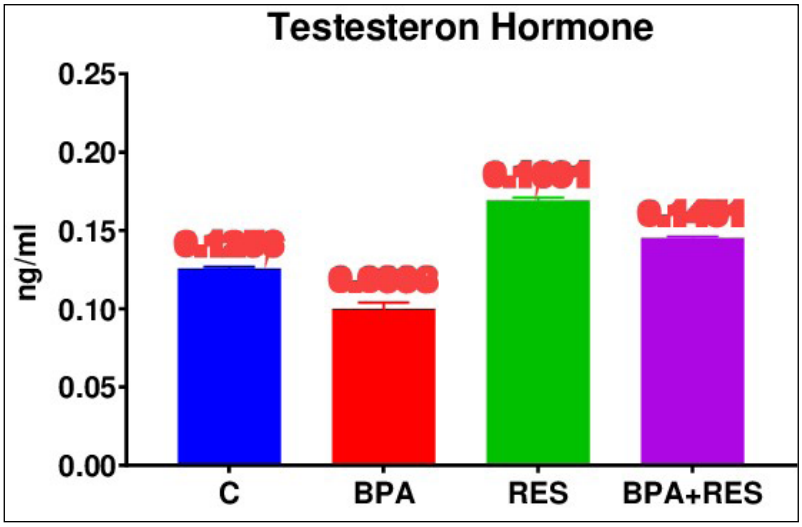

Fig. 8. Effects of Bisphenol A (BPA) and Resveratrol on Testosterone
Control (C), BPA-treated (BPA), Resveratrol-treated (RES), and combined BPA + Resveratrol-treated (BPA + RES) groups. Error bars represent the standard error of the mean (SEM), *Indicates significant differences between groups at $p<0.05$
Source: Own materials

HISTOPATHOLOGICAL EXAMINATION OF TESTICULAR TISSUE

The testicular tissue was excised, longitudinally opened, and preserved in 10% buffered formalin for fixation. Following standardized histological procedures [23] the samples were fixed at room temperature for 48 hours. The fixed tissues underwent graded alcohol dehydration, clearing in two stages of xylene, and embedding in liquid paraffin maintained at 56°C for two hours. Thin sections, 5 micrometers thick, were

prepared using a microtome. These sections were then dewaxed and stained with Harris Hematoxylin and Eosin (H&E) for detailed histological analysis. The stained slides were examined under a light microscope using magnifications of X4, X10, and X40, allowing for a comprehensive evaluation of the testicular tissue's histological structure.

RESULTS

SPERM EVALUATION

The Figure 2 illustrated the progressive sperm motility which showed significant variations among the experimental groups since C group exhibited the highest motility, with a mean value of (82.5 ± 0.83) indicating normal physiological function, conversely, the BPA group showed a significant reduction in motility (25.5 ± 2.167), reflecting the detrimental effects of BPA on sperm motility. Interestingly, the RES group demonstrated the highest motility among all groups (87.5 ± 0.8333), and nonsignificant different compared with the control group. The combined treatment group (BPA + RES) showed a partial restoration of motility (62 ± 4.726), indicating that resveratrol mitigated, but did not fully counteract, the negative effects of BPA.

The evaluation of sperm morphology in Figure 3 revealed significant differences among the treatment groups. The control group exhibited morphology percentage (97.8 ± 0.4163), closely matched and non-significantly different from the used resveratrol-only RES group with 98.65 ± 0.2242, indicating no adverse impact on sperm structure. In contrast, the BPA group showed a significant decrease in sperm morphology (66.65 ± 2.861), highlighting the detrimental effects of BPA exposure on sperm structure. Notably, the co-administration of BPA and resveratrol BPA+RES demonstrated a marked improvement in morphology (87.55 ± 1.248) compared to the BPA group, suggesting the protective role of resveratrol.

The evaluation of sperm concentration in figure (4) showed significant differences among the experimental groups since the C group showed a sperm concentration (8.60×10^8 sperm/mL), indicating normal spermatogenesis, in contrast, the BPA group showed significantly reduced in sperm concentration (4.18×10^8 sperm/mL), that mean BPA's toxic effects on spermatogenesis while on the other hand, the RES group exhibited the normal concentration (9.15×10^8 sperm/mL), a nonsignificant difference among the control group, suggesting that resveratrol not only counteracts oxidative damage but may enhance spermatogenic efficiency. Notably, the co-administration group (BPA+RES) demonstrated normal sperm concentration (7.12×10^8 sperm/mL) compared to the BPA group, highlighting the protective role of resveratrol.

SPERM DNA FRAGMENTATION

The assessment of sperm DNA fragmentation in figure (5) revealed the significant differences across the experimental groups which the C group exhibited low levels of DNA fragmentation (5.949 ± 0.1742), indicative of intact chromatin integrity, in contrast, the BPA group showed a dramatic increase in DNA fragmentation (22.63 ± 0.7494), reflecting BPA's adverse effects on sperm DNA stability. The RES demonstrated the lowest levels of DNA fragmentation (3.926 ± 0.4412), suggesting a strong protective effect of resveratrol on chromatin integrity. The co-administration BPA+RES group exhibited a marked reduction in DNA fragmentation (6.669 ± 0.3551) compared to the BPA group, indicating the partial restorative capacity of resveratrol.

TOTAL ANTIOXIDANT CAPACITY LEVELS

The result of blood total antioxidant capacity (TAC) test showed in figure (6) that illustrated the C group exhibited the highest TAC levels (7.3901 nmol/mL), indicating antioxidant capacity under normal physiological conditions while in contrast, the BPA group showed a significant decreased in TAC (2.0964 nmol/mL), that proved the oxidative stress induced by BPA, in other hands RES group showed TAC levels (7.2503 nmol/mL) while he co-administered BPA + RES group exhibited TAC levels in (5.4751 nmol/mL) compared to the BPA group, indicating the partial ameliorative effect of resveratrol against BPA-induced oxidative stress.

HORMONAL ANALYSIS

SERUM LEVELS OF LUTEINIZING

The result of analysis of serum LH levels in the figure (7) explained that is significant change between groups which C group (0.4174 ± 0.02326), indicative of normal level of LH which BPA group showed significant increase in LH levels (0.5625 ± 0.02311), which is indicated the disruptive effect of BPA on endocrine signaling pathway, in contrast, the RES group exhibited the normal LH levels (0.2324 ± 0.01295) that proved that resveratrol enhance androgen feedback. In other hands, BPA + RES group showed a reduction in LH levels (0.4051 ± 0.01547) significant different with the BPA group.

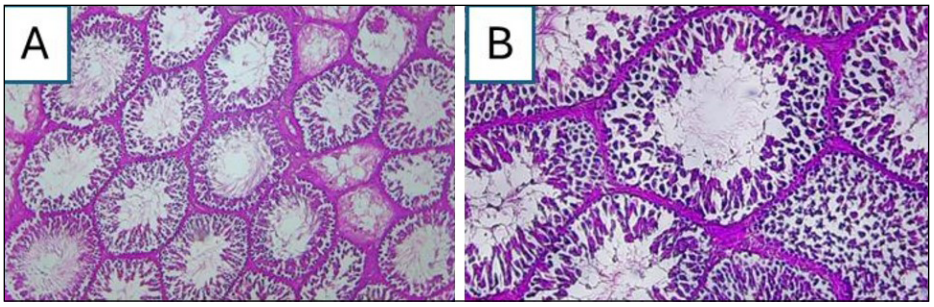

Fig. 9. Histological Section of Control Group Testicular Tissue (Normal histological architecture of testicular tissue), A. Rats testicular under 10X, B. Rats testicular under 40X
Source: Own materials

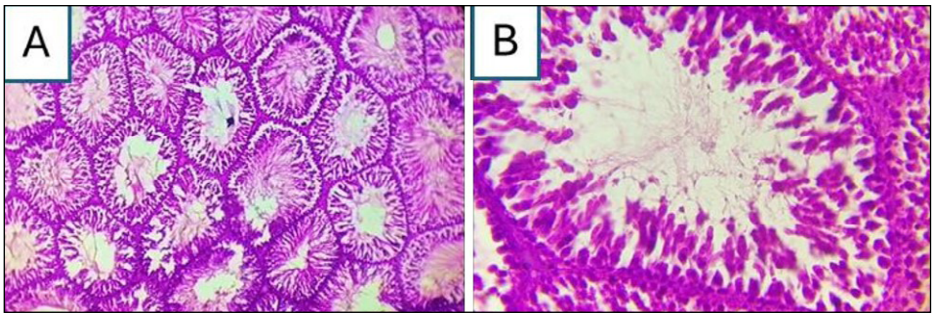

Fig. 10. Histological Section of RES Group Testicular Tissue (Normal histological architecture of testicular tissue), A. Rats testicular under 10X, B. Rats testicular under 40X
Source: Own materials

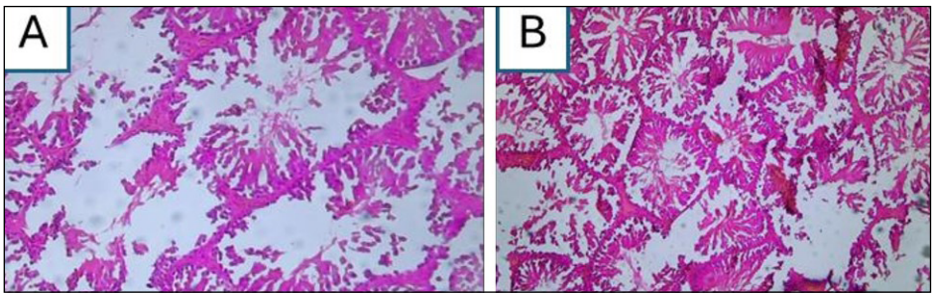

Fig. 11. Histological Section of BPA Group Testicular Tissue (Abnormal histological architecture of testicular tissue), A. Rats testicular under 10X, B. Rats testicular under 40X
Source: Own materials

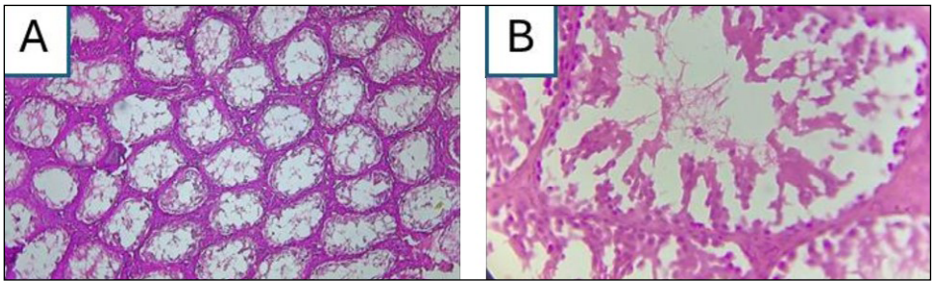

Fig. 12. Histological Section of BPA+RES group Testicular Tissue (Abnormal histological architecture of testicular tissue), A. Rats testicular under 10X, B. Rats testicular under 40X
Source: Own materials

SERUM LEVELS OF TESTOSTERONE

The analysis of serum testosterone levels across the experimental groups revealed significant alterations due to Bisphenol A (BPA) exposure and the potential restorative effects of resveratrol. The control group (C) exhibited baseline testosterone levels (0.1256 ± 0.1256), reflecting normal androgen function and hypothalamic-pituitary-gonadal (HPG) axis activity. BPA exposure resulted in a reduction in serum testosterone levels (0.09979 ± 0.09979), indicating the detrimental effects of BPA on Leydig cell function and androgen synthesis. Conversely, the resveratrol-only group (RES) showed an increase in testosterone levels (0.1691 ± 0.1691), suggesting that resveratrol enhances testosterone production, likely through its antioxidative and protective mechanisms. The co-administration group (BPA+RES) displayed an intermediate level of testosterone (0.1451 ± 0.1451), indicating that resveratrol partially mitigates BPA-induced disruptions (Fig. 8).

HISTOPATHOLOGICAL EVALUATION OF TESTICULAR TISSUE

The histopathological examination of testicular tissue of experimental groups sowed significant structural variations, reflecting the effects of BPA exposure and RES potential protective role. The C group, as shown in figure (9A-B), in contrast, the RES group figure (10A-B) displayed improved structural integrity, demonstrating the beneficial effects of resveratrol. Conversely, the BPA

group figure (11A-B) showed severe histopathological alterations, including disrupted seminiferous tubules and reduced germ cell populations. The BPA + RES group figure (12A-B) exhibited partial restoration of normal architecture, with noticeable improvement compared to the BPA group, though some abnormalities persisted.

DISCUSSION

The present discussion highlights the findings related to BPA's detrimental effects on male reproductive parameters and the protective role of resveratrol, supported by comparisons with previous research which observed reduction in sperm motility in the BPA group aligns with previous studies demonstrating that BPA exposure significantly impairs sperm quality which disrupts mitochondrial function and ATP production by inducing oxidative stress and damaging mitochondrial DNA [11]. Additionally, BPA alters calcium ion homeostasis, which is crucial for flagellar movement and motility regulation [10]. The RES group showed an increase in motility, that surpassed the C group, which is in agreement with previous findings from studies that illustrated the antioxidant properties of RES which enhance the mitochondrial function and lead to increase the protection of ATP; moreover, RES scavenging of ROS, and protecting sperm from oxidative damage [10]. In the BPA + RES group, the protective effect of RES enmeshment the motility percentage which that suggests decreased the adverse effect of BPA. Which RES play critical role in upregulated the endogenous antioxidant enzymes and reduced oxidative stress in the sperm cell [3], however, the incomplete restoration indicates that the chronic exposure to BPA cause irreversible damage to sperm cells or mitochondrial dysfunction beyond the protective role capabilities of resveratrol [7, 10, 24].

The BPA-induced oxidative stress further damages the plasma membrane and axonemal structures essential for motility while the resveratrol mitigates these effects by targeting oxidative stress and inflammation that due to its antioxidant action prevents lipid peroxidation of sperm membranes and restores mitochondrial function. The present result agreement with previous studies where BPA is shown to disrupt sperm morphology by inducing oxidative stress, leading to lipid peroxidation, DNA fragmentation, and impaired chromatin condensation [7, 25-26]. The protective role of RES in the BPA+RES group is attributed to RES potent antioxidant properties, which decreased oxidative damage by enhancing the activity of antioxidant enzymes like SOD and catalase, reducing MDA levels, and preserving cellular integrity [27-28]. Similar studies have documented resveratrol's ability to counteract BPA-induced damage, supporting its role in maintaining sperm structural integrity [7]. These findings align with previous studies showing that BPA induced oxidative stress, mediated by ROS and lipid peroxidation, damages testicular tissue and germ cells [7, 25], while Resveratrol's effectiveness is attributed to its potent antioxidant and anti-inflammatory properties, which mitigate ROS, enhance the activity of antioxidant enzymes the SOD and catalase, and preserve mitochondrial function. Previous research corroborates these results, emphasizing resveratrol's ability to restore sperm production by protecting Sertoli cells and Leydig cells from oxidative stress [2, 29].

The results of DNA Fragmentation in figure 5 are consistent with previous findings that highlight BPA's ability to induce DNA damage through the generation of ROS and oxidative stress, which disrupt chromatin structure and increases DNA fragmentation [3,8,30]. The mechanism of BPA adverse effect lead to increased lipid peroxidation, mitochondrial damage, and subsequently causes activation of apoptotic pathways [11, 30]. On other hands, RES have antioxidant properties that scavenging the ROS and improved the endogenous antioxidant enzymes finally prevent oxidative damage [31-32]. Previous studies proved that RES plays a critical role in improving sperm DNA integrity by stabilizing chromatin and reducing oxidative stress [22].

The findings of antioxidants in figure 6 approbate with previous research, where BPA exposure is associated with a decrease in TAC due to excessive production of ROS, which overwhelm the body's antioxidant defenses and lead to oxidative damage [10-11]. The RES plays a critical role in the enmeshment at the TAC level, which that due to the activity of endogenous antioxidant enzymes sand its ROS-scavenging properties [3, 33, 34]. The partial recovery observed in the BPA+RES group underscores the potential of resveratrol to mitigate oxidative stress, though complete restoration requires further exploration.

The LH findings of present study agreement with previous studies, which proved that BPA causes disrupting of the hypothalamic-pituitary-gonadal axis that by binding to estrogen and androgen receptor and lead to impairing normal feedback pathway and subsequently increased in the LH levels [10, 30]. As description in the present result showed significant increased TCA and inflammation that induced by BPA may further dysregulate hypothalamic function, contributing to defect LH levels [14-15, 35]. RES play a critical role in restoring hormonal balance by its antioxidant and anti-inflammatory properties, which protect the hypothalamus and pituitary from oxidative damage [3, 9]. The testosterone level in the result findings are consistent with previ-

ous studies showing that BPA disrupts testosterone synthesis by impairing Leydig cell function through oxidative stress, mitochondrial dysfunction, and direct interference with steroidogenic enzymes. BPA's estrogenic activity also disrupts the feedback mechanisms of the hypothalamic-pituitary-gonadal axis, leading to reduced luteinizing hormone (LH) stimulation and subsequent declines in testosterone. Resveratrol's ability to restore testosterone levels is attributed to its potent antioxidant and anti-inflammatory properties, which protect Leydig cells from oxidative damage and enhance steroidogenesis by normalizing enzymatic activities involved in androgen production. The results highlight the potential of resveratrol as a therapeutic agent to counteract BPA-induced reproductive toxicity. The histological section of the tests showed significant deferent which C group, as shown in figure (9), which exhibited normal histological structure. Furthermore, the histological section of seminiferous tubules that are enveloped by a thin basement membrane and intact interstitial blood vessels, in additionally the showed intact and mature spermatozoa within the lumens of tubules. While the epithelial layer maintained its structural integrity with Sertoli and Leydig cells and germ cells at various stages of spermatogenesis. That proved RES in 50 mg/kg for 60 days preservation of testicular tissue integrity, and protective the testicular histology. Furthermore, the antioxidative and anti-inflammatory properties of RES play a critical role in mitigating oxidative stress and preventing cellular damage [36–38]. These findings agreement to previous studies, which showed RES scavenge free radicals, reduce oxidative stress, and protect against testicular damage caused by environmental pollution [7, 39-40]. In contrast, the BPA group Figure (11) showed severe histopathological damage, which that is irregularly shaped and atrophied seminiferous tubules, and a reduction in germ cell count. While the tubular lumens of seminiferous showed immature germ cells and sperm debris, due to impaired spermatogenesis. The oxidative stress causes disrupt of Leydig and Sertoli cell that leads to DNA fragmentation and apoptosis in sperm cells [10-11]. The BPA + RES group in the figure (12) showed amelioration in the histopathological section which observed in the BPA group. While some of seminiferous tubule shrinkage, interstitial expansion, and basement membrane thickening were noted but in lower effect. That illustrated structural improvements by RES. Resveratrol's antioxidant mechanisms, including its ability to enhance endogenous antioxidant enzyme activity and neutralize reactive oxygen species, likely contributed to the observed recovery [12, 41-42].

CONCLUSIONS

This study demonstrates the profound adverse effects of Bisphenol A (BPA) on male reproductive health, evidenced by disrupted hormonal balance, reduced sperm quality, increased DNA fragmentation, and compromised testicular histopathology. BPA exposure significantly impaired testosterone levels, sperm motility, concentration, morphology, and DNA integrity, emphasizing its role as an endocrine-disrupting chemical that induces oxidative stress and cellular damage. The findings align with existing research indicating BPA's detrimental effects on the hypothalamic-pituitary-gonadal axis and testicular structure. Importantly, resveratrol (RES) exhibited remarkable protective effects against BPA-induced reproductive toxicity. Resveratrol treatment not only restored sperm quality and DNA integrity but also mitigated histopathological abnormalities in testicular tissue. Its antioxidant, anti-inflammatory, and anti-apoptotic properties were pivotal in reducing oxidative stress, enhancing mitochondrial function, and preserving germ cell integrity. The partial recovery observed in the BPA+RES group underscores resveratrol's potential as a therapeutic agent to counteract environmental toxicants like BPA. In conclusion, this study highlights the dual impact of BPA on male reproductive parameters and the promising protective role of resveratrol. Future research should further explore the mechanisms of resveratrol's action and its potential in preventing or reversing reproductive damage induced by other environmental toxins, aiming to preserve fertility and male reproductive health.

REFERENCES

1. Khayat MT, Zarka MA, El-Telbany DFA, El-Halawany AM, et al. Intensification of resveratrol cytotoxicity, pro-apoptosis, oxidant potentials in human colorectal carcinoma HCT-116 cells using zein nanoparticles. Sci Rep. 2022;12(1):15235. doi.org/10.1038/s41598-022-18557-2.
2. Bustani G, Al-Humadi N, and Alghetaa H. Aryl Hydrocarbon Receptor Modulation and Resveratrol Influence on Dnah1 Expression: Implications for Male Reproductive Health. Iraqi J Vet Med. 2024; 48: 15–24. doi.org/10.30539/p5wv8002.
3. Cariati F, D'Uonno N, Borrillo F, Iervolino S, Galdiero G, Tomaiuolo R. Bisphenol a: an emerging threat to male fertility. Reprod Biol Endocrinol. 2019;17:1–8. doi.org/10.1186/s12958-018-0447-6.
4. Palak E, Lebiedzińska W, Anisimowicz S, Sztachelska M, et al. The association between bisphenol A, steroid hormones, and selected microRNAs levels in seminal plasma of men with infertility. J Clin Med. 2021;10;24: 5945. doi.org/10.3390/jcm10245945.

5. Castellini C, Museli M, Parisi A, Totaro M, et al. Association between urinary bisphenol A concentrations and semen quality: a meta-analytic study. Biochem Pharmacol. 2022;197: 114896. doi.org/10.1016/j.bcp.2021.114896.
6. Bustani G, and Alghetaa H. Aryl hydrocarbon receptor signaling in male fertility: Protective role of resveratrol and disruptive effects of CH223191 in adult male rats. Vet World. 2025;18(5):1274. doi.org/10/14202/vetworld.2025.1274-1287.
7. Bordbar H, Yahyavi S-S, Noorafshan A, Aliabadi E, Naseh M. Resveratrol ameliorates bisphenol A-induced testicular toxicity in adult male rats: a stereological and functional study. Basic Clin Androl. 2023; 33: 1. doi.org/10.1186/s12610-022-00174-8.
8. Lin J, Deng L, Sun M, Wang Y, et al. An in vitro investigation of endocrine disrupting potentials of ten bisphenol analogues. Steroids. 2021;169:108826. doi.org/10.1016/j.steroids.2021.108826.
9. Rybczyńska-Tkaczyk K, Skóra B, and Szychowski KA. Toxicity of bisphenol A (BPA) and its derivatives in diverse biological models with the assessment of molecular mechanisms of toxicity. Environment Sci Pollut Res. 2023; 30(30): 75126–75140. doi.org/10.1007/s11356-023-27747-y.
10. Chaichian S, Khodabandehloo F, Haghighi L, Govahi A, et al. Toxicological impact of bisphenol A on females' reproductive system: review based on experimental and epidemiological studies. Reprod Sci. 2024;31(7):1781–1799. doi.org/10.1007/s43032-024-01521-y.
11. Barbonetti A, Castellini C, Di Giammarco N, Santilli G, Francavilla S, and Francavilla F. In vitro exposure of human spermatozoa to bisphenol A induces pro-oxidative/apoptotic mitochondrial dysfunction. Reprod Toxicol. 2016; 66: 61–67. doi.org/10.1016/j.reprotox.2016.09.014
12. Gu T, Wang N, Wu T, Ge Q, and Chen L. Antioxidative stress mechanisms behind resveratrol: a multidimensional analysis. J Food Qual. 2021;2021:1–12. doi.org/10.1155/2021/5571733.
13. Amanat S, Taymouri S, Varshosaz J, Minaiyan M, and Talebi A. Carboxymethyl cellulose-based wafer enriched with resveratrol-loaded nanoparticles for enhanced wound healing. Drug Deliv Transl Res. 2020;10:1241–1254. doi.org/10.1007/s13346-020-00711-w.
14. Al-Amery AKM, Obaid FN, Jabbar MK, and Bustani GS. Protective Effect of Ocimum Tenuiflorum against Negative Effect Filgrastim on Sperm Parameters of Male Rats. REDVET. 2022;22–27. doi.org/REDVET/article/view/171.
15. Bustani GS, Jabbar MK, AL-Baghdady HF, Al-Dhalimy AMB. Protective effects of curcumin on testicular and sperm parameters abnormalities induced by nicotine in male rats. In: AIP Conference Proceedings AIP Publishing. 2022. doi.org/10.1063/5.0066855.
16. Al-Mousaw M, Bustani GS, Barqaawee MJA, AL-Shamma YM. Evaluation of histology and sperm parameters of testes treated by lycopene against cyclophosphamide that induced testicular toxicity in Male rats. AIP Conference Proceedings AIP Publishing LLC. 2022;20040. doi.org/10.1063/5.0067059.
17. Ngaha Njila MI, Massoma Lembè D, Koloko BL, Yong Meng G, et al. Sperm parameters quality and reproductive effects of methanolic extract of Alchornea cordifolia leaves on senescent male rats. Andrologia. 2019;51:e13359. doi.org/10.1111/and.13359.
18. K Abdulameer A, S Alkhayyat A, Talib Abbas M, A Habeeb I, and Sabah Bustani G. The Effect of Palm Pollen and Pomegranate Peel Supplementation on Sperm Development in Male Rabbits. Arch Razi Inst. 2022. doi.org/10.22092/ari.2022.357616.2069.
19. Vrinda P and Sneha J. Comparing sperm concentration measurement in human semen using improved Neubauer hemocytometer and Makler counting chamber. Int J Acad Med Pharm 2023;5 (3);108-113. doi.org/10.47009/jamp.2023.5.3.24
20. Vinnikov D, Syurin S. Nickel and human sperm quality: a systematic review. BMC Public Health. 2024; 24(1):3545. doi.org/10.1186/s12889-024-21119-y
21. Al-Zaiyadi SM, Salih AM, Sabr IA, Al-Murshidi SY, et al. Impact of fertilization rate on ICSI outcome and pregnancy rate for unexplained subfertile couples. Ind J Public Health. 2019;10(11). doi.org/10.5958/0976-5506.2019.03887.
22. de la Iglesia A, Jodar M, Oliva R, Castillo J. Insights into the sperm chromatin and implications for male infertility from a protein perspective. WIREs Mech Dis. 2023 Mar;15(2):e1588. doi.org/10.1002/wsbm.1588.
23. Sead FF, Mohammed HJ, Hamzah AI. Potentiometric ion-selective electrode determination cobalt (II) based on crafted thia polymeric crown ether SPCE-Co2+. AIP Conference Proceedings. 2023;2839(1). doi.org/10.1063/5.0176748
24. Boudalia S, Bousbia A, Boumaaza B, Oudir M, and Lavier MCC. Relationship between endocrine disruptors and obesity with a focus on bisphenol A: A narrative review. Bioimpacts. 2021; 11: 289. doi.org/10.34172/bi.2021.33
25. Meng Y, Lin R, Wu F, Sun Q, Jia L. Decreased capacity for sperm production induced by perinatal bisphenol a exposure is associated with an increased inflammatory response in the offspring of C57BL/6 male mice. Int J Environ Res Public Health. 2018;15:2158. doi.org/10.3390/ijerph15102158.
26. Zhou Y, Xu W, Yuan Y, Luo T. What is the impact of bisphenol A on sperm function and related signaling pathways: a mini-review? Curr Pharm Des. 2020;26:4822–4828. doi.org/10.2174/1381612826666200821113126.
27. Assunção CM, Mendes VRA, Brandão FZ, Batista RITP, et al. Effects of resveratrol in bull semen extender on post-thaw sperm quality and capacity for fertilization and embryo development. Anim Reprod Sci. 2021; 226: 106697. doi.org/10.1016/j.anireprosci.2021.106697.
28. Shati AA. Resveratrol improves sperm parameter and testicular apoptosis in cisplatin-treated rats: Effects on ERK1/2, JNK, and Akt pathways. Syst Biol Reprod Med. 2019;65:236–249.
29. Alibraheemi NAA, Bustani GS, Al-Dhalimy AMB. Effect of Curcumin on LH and FSH Hormones of Polycystic Syndrome Induced by Letrozole in Female Rats. Lat. Am. J. Pharm. 2021;40(special issue):179–83 doi.org/10.1080/19396368.2018.1541114.

30. Tian Z, He Z, Zhang Q, Ding L, et al. The relationship between semen quality in male infertility clinic patients and bisphenol A: A Chinese cross-sectional study. Heliyon. 2024;10(16):e30257. doi.org/10.1016/j.heliyon.2024.e30257.
31. Khanvilkar S and Mittra I. Copper imparts a new therapeutic property to resveratrol by generating ROS to deactivate cell-free chromatin. Pharmaceuticals. 2025;18(1):132. doi.org/10.3390/ph18010132.
32. Liu W-C, Shyu J-F, Lin Y-F, Chiu H-W, Lim PS, Lu C-L, et al. Resveratrol rescue indoxyl sulfate-induced deterioration of osteoblastogenesis via the aryl hydrocarbon receptor/MAPK pathway. Int J Mol Sci. 2020; 21: 7483. doi.org/10.3390/ijms21207483.
33. Zhou J, Yang Z, Shen R, Zhong W, et al. Resveratrol improves mitochondrial biogenesis function and activates PGC-1α pathway in a preclinical model of early brain injury following subarachnoid hemorrhage. Front Mol Biosci. 2021;8:620683. doi.org/10.3389/fmolb.2021.620683.
34. Meng T, Xiao D, Muhammed A, Deng J, Chen L, He J. Anti-inflammatory action and mechanisms of resveratrol. Molecules. 2021;26:229. doi.org/10.3390/molecules26010229.
35. Al-Garawi NAD, Suhail AA, Kareem HA, Bustani GS. Study of Lipid Profile and Leptin hormone and Adiponectin hormone hypertensive patients in Najaf Governorate. Revista Electronica de Veterinaria. 2022; 45–51. Index.php/REDVET/article/view/174.
36. Qu Y, Han J, Han H, Ma X, et al. Advances in the study of Benzo[a]pyrene on male reproductive toxicity: exploring underlying mechanisms and mitigant. Authorea. 2024; doi.org/10.22541/au.172975349.99426264/v1 (Preprint).
37. Haunschild R, Marx W. On health effects of resveratrol in wine. Int J Environment Res Public Health. 2022;19(5):3110. doi.org/10.3390/ijerph19053110.
38. Bordbar H, Yahyavi S-S, Noorafshan A, Aliabadi E, Naseh M. Resveratrol ameliorates bisphenol A-induced testicular toxicity in adult male rats: A stereological and functional study. Basic Clin Androl. 2023 Jan 6;33(1):1. doi.org/10.1186/s12610-022-00174-8.
39. Assefa EG, Yan Q, Gezahegn SB, Salissou MTM, et al. Role of resveratrol on indoxyl sulfate-induced endothelial hyperpermeability via aryl hydrocarbon receptor (AHR)/Src-dependent pathway. Oxid Med Cell Longev. 2019 Nov 27;2019:5847040. doi.org/10.1155/2019/5847040.
40. Sener TE, Atasoy BM, Cevik O, Cilingir Kaya OT, Cetinel S, Degerli AD, et al. Effects of resveratrol against scattered radiation-induced testicular damage in rats. Turk J Biochem. 2020;46: 425–433. doi.org/10.1515/tjb-2020-0320.
41. Liu J, Zeng L, Zhuang S, Zhang C, Li Y, Zhu J, et al. Cadmium exposure during prenatal development causes progesterone disruptors in multiple generations via steroidogenic enzymes in rat ovarian granulosa cells. Ecotoxicol Environ Saf. 2020 Sep 15;201:110765. doi.org/10.1016/j.ecoenv.2020.110765.
42. Hamzah AI, Hussein NM, Salem KH, Saraswat SK, Kaur M, Kaur H, Zainul R. Adsorption behavior of hydroxyurea drug on pristine, Si, and Ge-doped carbon nitride nanosheet: A computational study. Inorganic Chemistry Communications. 2024;170:113186. doi.org/10.1016/j.inoche.2024.113186.

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTHOR

Mohamed A. Zarka
Pharmacognosy Department, College of Pharmacy,
Islamic University in Najaf, Najaf, Iraq
e-mail: dr.m.abdulaal84@gmail.com

ORCID AND CONTRIBUTIONSHIP

Saad Mashkoor Waleed: 0000-0002-4151-2219 B C
Balsam G. Hassan: 0009-0000-2081-9230 D E
Hussam H. Tizkam: 0000-0003-3896-6954 D E
Mohamed A. Zarka: 0000-0002-0526-6268 A F

A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

RECEIVED: 02.05.2025
ACCEPTED: 13.10.2025