Journal of Animal & Plant Sciences, 36(3): 2026, Page: 798-807
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2026.3.0065

BIOACTIVE COMPOUNDS IN *COCOS NUCIFERA* L. WATER: A PROTECTIVE SHIELD FOR EPIDIDYMIS AGAINST BISPHENOL A-INDUCED OXIDATIVE STRESS IN SPRAGUE DAWLEY RATS

N. F. S. Shafie[1], N. Kabir[1], N. A. A. Aziz[2,3] and N. H. Hashim[1,2]

[1]Institute of Biological Sciences, Faculty of Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia.
[2]Glami Lemi Biotechnology Research Centre, Universiti Malaya, 71650 Jelebu, Negeri Sembilan, Malaysia
[3]Centre for Foundation Studies in Science, Universiti Malaya, 50603 Kuala Lumpur, Malaysia.
Corresponding author's email: nhhpasum@um.edu.my

ABSTRACT

Bisphenol A (BPA) is a prevalent endocrine disruptor which poses a serious risk to male reproductive health by inducing oxidative stress. Evidence links BPA exposure to impaired spermatogenesis, altered testicular morphology, and disrupted steroidogenesis. In contrast, its effects on the epididymis remain less clearly defined, highlighting the need for further investigation. While various strategies have been explored to counteract BPA- induced toxicity, natural interventions targeting the male reproductive system are still underexplored. Notably, *Cocos nucifera* L. (coconut) water, rich in polyphenols and bioactive compounds, is known for its antioxidative properties. This study investigates the oxidative stress and histopathological changes underlying BPA-induced epididymal damage and evaluates the protective potential of coconut water in counteracting these effects. Thirty Sprague-Dawley rats were allocated into five groups: control (C) received 0.5 mL of distilled water per day, vehicle (V) received 0.5 mL of corn oil per day, BPA (B) received 50 mg/kg per day, coconut water (CW) received 10 mL/kg per day, and co-administration of BPA and coconut water (CW+B) received both coconut water and BPA. The epididymides were harvested on day 31 for oxidative stress analysis and histological examination. BPA administration reduced glutathione levels, increased malondialdehyde levels, and induced histological alterations in the epididymis ($p < 0.001$). However, these parameters were significantly enhanced by the coconut water administration in CW+B group ($p < 0.001$). Conclusively, this study suggests that coconut water boosts antioxidant defences in rat epididymal tissue, offering protection against BPA-mediated harm.

Keywords: coconut water, bisphenol A, epididymis, oxidative stress, antioxidant

Published first online February 14, 2026 Published final May 05, 2026

INTRODUCTION

Environmental, genetic, and epigenetic factors, which are essential for spermatogenesis and sperm function, serve an important role in maintaining male reproductive health. Understanding oxidative stress in male reproduction is crucial, given its impact on male fertility and reproductive health. Recent statistics reveal that male infertility impacts roughly 7–10% of couples globally, with oxidative stress identified as a major contributing factor (Mannucci *et al.,* 2022). Sperm morphology, motility, count, and DNA integrity can be compromised by the dysregulation between reactive oxygen species (ROS) generation and antioxidant defences (Walke *et al.,* 2023).

Recently, there has been growing awareness of the extensive release of endocrine disrupting chemicals (EDCs) into the environment and their propensity to produce oxidative stress and endocrine system disruption, which can affect the male reproductive system (Mohajer *et al.,* 2025). Bisphenol A (BPA), an EDC primarily used in epoxy resins and polycarbonate plastics, is of concerns due to its ubiquitous presence and negative impact on male reproductive health (Cariati *et al.,* 2019). The overproduction of ROS and inadequate antioxidant defences results in its toxicity, which cause oxidative stress and organ damage (Santiago *et al.,* 2021).

Prior research has mainly focussed on the adverse impact of BPA on testicular damage or spermatozoa (Li *et al.,* 2023; Zhao *et al.,* 2025). Although BPA's testicular toxicity is well- established, its impact on the epididymis, a crucial organ for sperm maturation (Zhou *et al.,* 2018), is still inadequately investigated. Epididymal epithelial cells help maintain an acidic and hyperosmotic luminal environment, both essential for the proper development of sperm motility (Cooper, 2007; Shum *et al.,* 2011; Park *et al.,* 2017). While evidence found that BPA induces harmful oxidative damage and histological changes in testicular tissues (Tekin *et al.,* 2024), it is essential to ascertain its effects on the

epididymis. The epididymis comprises three primary regions: the head (caput), body (corpus), and tail (cauda). The epididymis is lined with pseudostratified columnar epithelial cells, which are composed of principal and basal cells. The principal cells are tall and possess long stereocilia. These stereocilia enhance the principal cells' function of absorbing luminal fluids by increasing their surface area (Johnson *et al.,* 2015). Understanding oxidative stress in the epididymis is essential, as it might disturb its microenvironment and potentially hinder sperm maturation and functionality (El Ghazzawy *et al.,* 2011).

The global demand for natural supplements with antioxidant properties is increasing, driven by the need to reduce the adverse effects of environmental pollutants on human and animal fertility (Ahmed *et al.,* 2022). Antioxidant supplementation involves the administration of exogenous antioxidants, individually or in combinations, to bolster the antioxidant defence mechanisms in the body and alleviate the impacts of oxidative stress (Walke *et al.,* 2023). Research indicates that dietary natural polyphenols derived from fruits, vegetables, and edible plants regulate ROS homeostasis, potentially improving male reproductive health (Di Giacomo *et al.,* 2020).

Quercetin, a polyphenolic flavonoid recognised for its potent antioxidant properties, mitigates testicular oxidative damage and germ cell apoptosis by reducing lipid peroxidation and enhancing the activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in rats (Yelumalai *et al.,* 2019). Resveratrol is another natural polyphenolic compound that markedly uncouples mitochondrial oxidative phosphorylation (Ferramosca *et al.,* 2021). Moreover, vitamin C is recognised as a major antioxidant vitamin that modulates free radicals in male reproduction by diminishing oxidative stress and improving antioxidant levels (Shabanian *et al*., 2017). Although promising, these interventions are constrained by the possibility of significant adverse effects at high doses and inconsistent efficacy (Bertoldo *et al.,* 2024; Doseděl *et al.,* 2021; Zou *et al.,* 2021). In contrast, *Cocos nucifera* L. (coconut) water offers a natural, highly nutritious alternative, featuring antioxidant benefits derived from its unique blend of vitamins, minerals, amino acids, and phytohormones (Tuyekar *et al.,* 2021). Its diverse combination of bioactive compounds removes the need for isolating certain active constituents, unlike other antioxidants.

To our knowledge, this is the first study which explores the role of polyphenols and bioactive compounds in coconut water in shielding against BPA-induced male reproductive damage, emphasizing oxidative stress and epididymal histology. Bridging this gap is critical for discovering cost-effective and accessible strategies to reduce BPA-induced oxidative stress and its adverse effects. Furthermore, the growing interest in alternative remedies for reproductive toxicity emphasises the potential of this research to deliver safer, non-synthetic treatments with fewer side effects than traditional medications. This development strategy assures that the study is not only innovative but also extremely relevant to practical reproductive health applications.

MATERIALS AND METHODS

Collection and preparation of coconut water: Fresh six-month-old coconuts from MATAG variety were harvested from a local farm. The coconut was de-husked, and the water was collected before being stored in a deep freezer at -20˚C. A treatment dose of 10 mL/kg body weight was chosen based on previous studies which demonstrated significant effects without causing adverse clinical symptoms (Olayinka *et al.,* 2022). Research suggests that a 30-day coconut water treatment can enhance reproductive health by raising epididymal weight and decreasing lipid peroxidation in tissues (Ommurugan *et al.,* 2021).

Collection and preparation of bisphenol A: Bisphenol A (≥99%, Sigma-Aldrich, CAS No. 80-05-7) was weighed and suspended in corn oil, a vehicle commonly used in BPA dissolution in toxicity studies (Mahmoud *et al.,* 2024). A BPA dose of 50 mg/kg body weight was selected based on previous research establishing its ability to induce reproductive damage in male rats (Li *et al.,* 2023). Treatment with BPA for 30 days has been demonstrated to inflict reproductive damage in male rats (Alboghobeish *et al.,* 2019).

Animal use and care: In this study, thirty male Sprague Dawley rats, weighing an average of 150 to 200 g and aged 5-7 weeks used. The rats were acquired from the animal husbandry unit in university and kept in the animal house under normal laboratory conditions, which included a regular photoperiod of 12 hours of light and 12 hours of darkness, at 25 ± 2°C. A week before treatment, they were acclimated to the laboratory environment. *Ad libitum* access to a commercial pellet diet and tap water was provided. The rats were randomly divided into five groups (n = 6) and treated as follows:

1) Control (C) group was given distilled water (0.5 mL/day).
2) Vehicle (V) group was given corn oil (0.5 mL/day).
3) BPA (B) group was given BPA suspended in the vehicle at 50 mg/kg body weight.
4) *Cocos nucifera* L. water (CW) group was given 10 mL/kg body weight of coconut water.
5) Co-administration of *Cocos nucifera* L. water and BPA group (CW+B) was given 10 mL/kg body weight of coconut prior to 50 mg/kg body weight of BPA.

Treatments were given once daily by oral gavage

for 30 consecutive days to replicate the primary human exposure route. Body weight was recorded every three days. Twenty-four hours after the final treatment, the rats were weighed again before being euthanized with ketamine (150 mg/kg) and xylazine (50 mg/kg). The left epididymides were immediately collected and fixed for histological examination, whereas the right epididymides were rinsed with ice-cold phosphate-buffered saline (PBS) and subsequently stored at −80°C for biochemical analysis.

Measurement of oxidative stress markers: Malondialdehyde (MDA) levels (ng/mL) were measured using an MDA ELISA Kit (Cat. No. E-EL-0060; Elabscience Biotechnology Co., Ltd., Wuhan, China), whereas glutathione (GSH) levels (ng/mL) were determined using a Rat GSH ELISA Kit (Cat. No. EK-ELK8577; ELK Biotechnology Co., Ltd., USA). All assays were performed following the manufacturer's instructions and run in duplicate.

The epididymides were prepared as homogenates prior to analysis using a slightly modified version of the procedure outlined by Oguntibeju *et al.* (2020). 100 mg of epididymal tissue per rat was weighed and homogenized in 1 mL of 10 mM PBS. Following that, the tissue and buffer were homogenized for 15 sec. To remove insoluble debris, the homogenates were centrifuged at 14,000 rpm for 15 minutes at 4°C and the supernatants were obtained for analysis.

Histological examination of epididymis: The collected epididymides were fixed in 10% neutral buffered formalin. The epididymal tissues were then dehydrated in alcohol-degraded solutions, infiltrated, and embedded into the paraffin blocks. Sections 5 µm thick were incised, deparaffinized, rehydrated, and stained with haematoxylin and eosin (H&E). Thirty-five tubules/group were visualized with magnification of ×100 and ×200 under a Nikon Eclipse 80i microscope (Nikon, Tokyo, Japan) that incorporates imaging software (Nikon's NIS-Elements), and a camera (DS-5M digital camera).

Biochemical analysis of coconut water: The coconut water sample was sent to external laboratory for analysis of total polyphenol, vitamin C, and minerals comprising of calcium (Ca), ferum (Fe), sodium (Na), potassium (K), copper (Cu), zinc (Zn), and magnesium (Mg). The concentrations of total proteins and amino acid profile were also determined.

The minerals were assayed by microwave digestion, followed by inductively coupled plasma mass spectrometry (ICP-MS). The amino acid profile was determined by acid hydrolysis using the AccQ•Tag Waters Method, followed by high-performance liquid chromatography with fluorescence detection (HPLC-FLD). Total polyphenol was analysed using Folin–Ciocalteu method while vitamin C was assayed by in-house method No: STP/Chem/A10-Titration method based on AOAC 20th Edition, 967.21.

Statistical analysis: The quantitative data are expressed as mean ± standard error (SE). Data were statistically analysed using IBM Statistical Package for the Social Sciences (SPSS), version 27. All percentage data were subjected to arcsine transformation prior to statistical analysis. One-way ANOVA was performed to analyse group differences, using Duncan's multiple range test for post hoc comparisons. The significance level was set at $\alpha = 0.05$.

Ethical Approval: All the experimental designs and procedures were approved by the animal ethics committee (Ethical No: S/12032023/02012023-01/R).

RESULTS

Measurement of oxidative stress markers: Table 1 shows a significant increase in MDA levels in the epididymides of the B group compared with C, V, CW, and CW+B groups after 30 days of treatment ($p < 0.001$). Conversely, the co- administration treatment of coconut water and BPA resulted in a significant decline of the MDA levels in the epididymides compared to the BPA-treated rats ($p < 0.001$). These levels, however, were not statistically different from those in the CW group.

GSH levels in epididymides were observed significantly reduced in the BPA-treated rats compared with the other four groups ($p < 0.001$). In contrast, GSH levels in the epididymides of the CW+B group were significantly higher than those in the B group, but no statistical difference was observed compared to the CW group.

Table 1: The concentration of malondialdehyde and glutathione in the epididymis of treated rats with coconut water and BPA.

Parameter / Treatment	Malondialdehyde (ng/mL)	Glutathione (µg/mL)
Control (C)	44.74±3.91^{a}	10.32±1.64^{b}
Vehicle (V)	47.15±2.99^{a}	8.97±0.63^{b}
BPA (B)	64.76±3.80^{b}	5.14±0.29^{a}
Cocos nucifera L. water (CW)	53.41±0.93^{a}	10.68±1.43^{b}
Cocos nucifera L. water + BPA (CW+B)	49.11±3.36^{a}	8.48±0.51^{b}

The data were analysed with one-way ANOVA and expressed as mean±SE (n=6). Mean with different superscripts within a same column shows significant difference ($\alpha = 0.05$).

Histological examination of epididymis: Figure 1 shows the histological features of epididymides in the groups C, V, and CW displayed normal features, with the majority of sections appearing as circular shapes of densely packed, folded tubules in the epididymides. These tubules were separated by normal inter-tubular spaces and lined with pseudostratified endothelial cells bearing long stereocilia extending into the lumen filled with spermatozoa. Principal cells were mainly columnar in shape, while the basal cells with rounded nuclei lay near the basement membrane. The sperm density in the lumen was observed to be densely packed, with no signs of cell debris in groups C, V, and CW (Figures 1 (A), (B), and (C), respectively). Sperm motility analysis for these animals was published previously (Shafie et al., 2025). Thus, this study focuses on histological endpoints, including luminal sperm density.

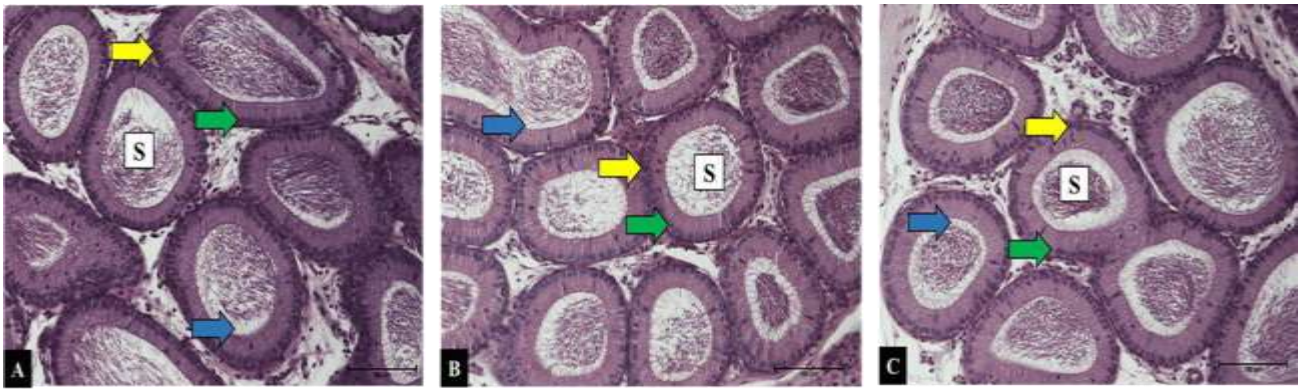

Figure 1. Photomicrograph of haematoxylin and eosin-stained of rat caput epididymis from A: control (C) group, B: vehicle control (V) group, C: *Cocos nucifera* L. water (CW) groups. The C, V, and CW groups exhibited normal tubular characteristics, defined by a columnar epithelial lining consisting of basal cells (yellow arrow) and principal cells (green arrow), accompanied by long and prominent stereocilia (blue arrow). The sperm density (S) was densely concentrated in the lumen, with no signs of cellular debris. The inter-tubular spaces in these groups appeared normal (Magnification of ×200).

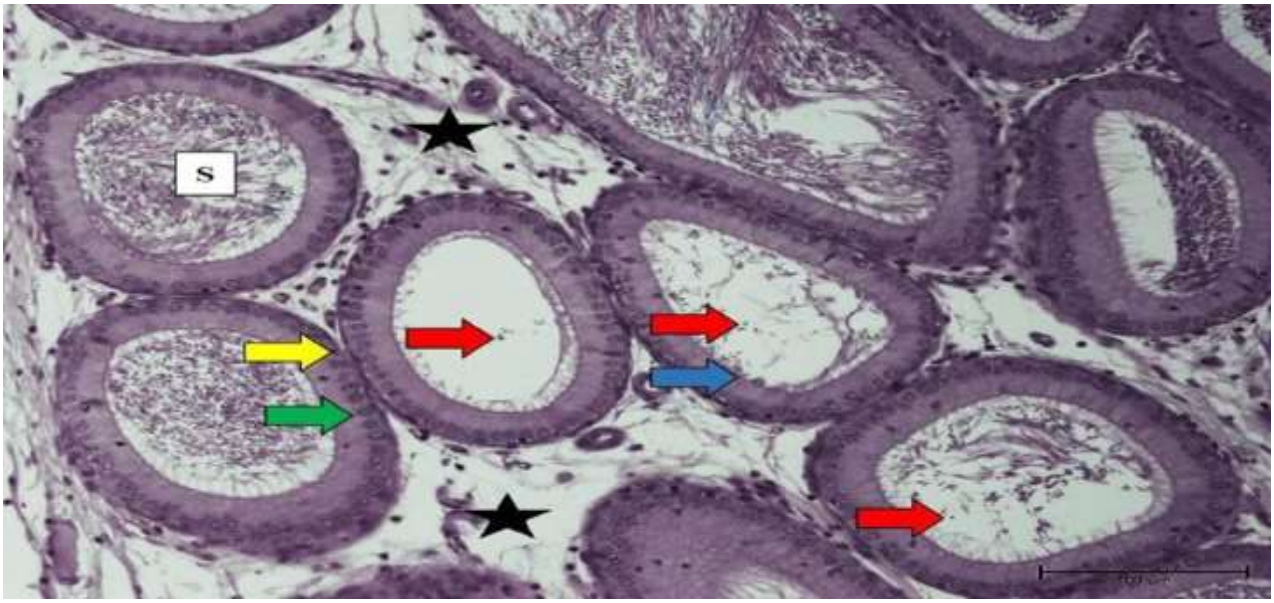

Figure 2. Photomicrographs of haematoxylin and eosin-stained of rat caput epididymis from BPA (B) group. The histoarchitecture of caput epididymis in the B group demonstrated mild alterations in the tubular characteristics, defined by a columnar epithelial lining consisting of basal cells (yellow arrow) and principal cells (green arrow), accompanied by shorter and less prominent stereocilia (blue arrow). Some lumens (S) displayed densely packed sperm, while others appeared sparse or completely empty, containing only cellular debris (red arrow). The inter-tubular spaces (black star) were significantly wider than those seen in the C, V, CW, and CW+B groups (Magnification of ×200).

As illustrated in Figure 2, the B group had mild changes in the histoarchitecture of caput epididymides along with enlarged inter-tubular spaces. Numerous tubules displayed lumina that were either completely empty or with scanty spermatozoa. Several lumens were filled with cellular debris. The stereocilia of the caput epididymal ducts in the B group were shorter and less prominent compared to those in the C, V, CW and CW+B groups.

In contrast, the co-administration of coconut water and BPA mitigated BPA-induced histoarchitectural alterations in the caput epididymides (Figure 3). This is proven by the near-normal histological appearance of the CW+B group, which is similar to that of the C, V, and CW groups. However, the inter-tubular spaces were slightly wider compared to the C, V, and CW groups, but narrower than those in the B group. The densely packed circular segments of epididymal tubules were lined with pseudostratified epithelial cells and characterized by long, prominent stereocilia. Moreover, the sperm density within the lumen in the CW+B group was significantly higher, with certain tubules showed minimal cellular debris. These data highlighted the effectiveness of coconut water in alleviating the detrimental impacts of BPA on the caput epididymis in rats.

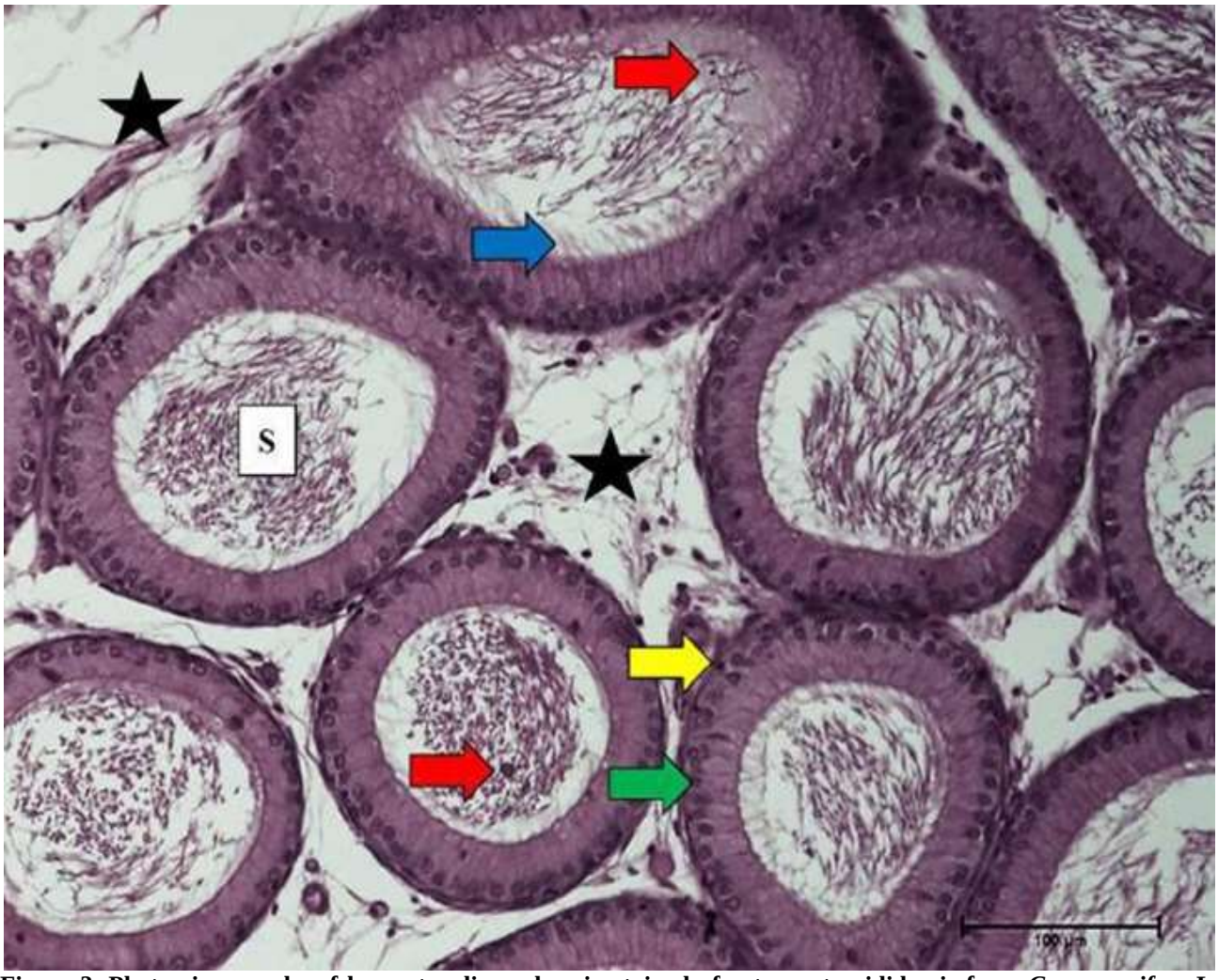

Figure 3. Photomicrographs of haematoxylin and eosin-stained of rat caput epididymis from *Cocos nucifera* L. water and BPA (CW+B) group. The histoarchitecture of caput epididymis in the CW+B group demonstrated near-normal tubular structures, defined by a columnar epithelial lining, which included basal cells (yellow arrow) and principal cells (green arrow). The principal cells were lined with long and prominent stereocilia (blue arrow). The sperm density (S) in the lumen was densely packed, however certain tubules exhibiting minimal cellular debris (red arrow). The inter-tubular spaces (black star) were slightly wider than those in the C, V, and CW groups but narrower than those in the B group (Magnification of ×200).

Biochemical analysis of coconut water: The component analysis was performed on frozen-thawed coconut water, and the biochemical results were presented in Table 2.

Table 2: Results of biochemical analysis of coconut water.

No	Test description	Unit	Results
1	Amino Acid		
	Hydroxyproline	g/100 mL	<0.001
	Aspartic Acid	g/100 mL	0.005
	Serine	g/100 mL	0.02
	Glutamic Acid	g/100 mL	0.006
	Glycine	g/100 mL	0.003
	Histidine	g/100 mL	<0.001
	Arginine	g/100 mL	0.004
	Threonine	g/100 mL	0.003
	Alanine	g/100 mL	0.002
	Proline	g/100 mL	0.003
	Tyrosine	g/100 mL	0.005
	Valine	g/100 mL	0.002
	Methionine	g/100 mL	0.007
	Lysine	g/100 mL	0.02
	Isoleusine	g/100 mL	<0.001
	Leusine	g/100 mL	<0.001
	Phenylalanine	g/100 mL	0.008
2	Minerals		
	Calcium	mg/L	151.30
	Ferum	mg/L	1.16
	Potassium	mg/L	2567.00
	Magnesium	mg/L	121.20
	Sodium	mg/L	63.38
	Zinc	mg/L	4.99
	Copper	mg/L	6.32
3	Vitamin C	mg/100 mL	0.98

DISCUSSION

Oxidative stress is a well-established major contributor to the detrimental effects of BPA on male reproductive health (Mubarak *et al.,* 2022). While the effects of BPA on testicular tissue are well-established, its impact on the epididymis remains unclear (Turner, 2008; Mazroa, 2011). The epididymis serves not only as a location for the passive transit and storage of spermatozoa but also creates a suitable microenvironment for sperm maturation (Zhou *et al.,* 2018). Consequently, any disruption in the anatomical and functional integrity of the epididymis may result in male infertility.

Malondialdehyde is a lipid peroxidation and oxidative stress marker, with its elevation typically correlating with decreases in antioxidant levels such as GSH (Saka *et al.,* 2024). In this study, BPA exposure resulted in oxidative stress in the epididymis, evidenced by significantly decreased GSH levels and elevated MDA levels in the B group relative to the C, V, CW, and CW+B groups ($p < 0.001$) (Table 1). This is consistent with Chitra *et al.* (2003), who found that oral BPA exposure (20 μg/kg for 45 days) elevated MDA levels and diminished antioxidant defences in the epididymis of rats on day 45 postnatally. Wu *et al.* (2020) also demonstrated that increased levels of 4-hydroxynonenal (4-HNE) in the caput and cauda epididymis indicate the onset of lipid peroxidation. Numerous studies have demonstrated that BPA can trigger the formation of reactive species, including hydroxyl, phenoxyl, superoxide, and peroxide radicals, by enzymatic or non-enzymatic pathways (Babu *et al.,* 2013). These free radicals trigger lipid peroxidation, leading to an increase in MDA levels and further establishing the relationship between oxidative stress caused by BPA in the epididymis and our findings.

Oxidative stress has also been reported as a potential causative factor for toxicity in reproductive organs. This study investigated the histoarchitecture of the caput epididymis, a region that is capable of creating a microenvironment that is responsible for early maturation of sperm, gain of motility, and fertilisation (Zhou *et al.,* 2018). This region contains a rich capillary network and experiences the greatest blood flow compared to other segments of the epididymis, rendering it more vulnerable to oxidative damage (Wu *et al.,* 2020). The vulnerability was evidenced by the significant BPA-induced alterations observed in the caput epididymis (Figure 2). The findings align with El Ghazzawy *et al*. (2011), who illustrated that BPA-induced oxidative stress triggered various degenerative stages that compromise the cell membrane integrity of the caput epididymis. Reactive oxygen species generated during oxidative phosphorylation in the cell membrane may have the capacity to disrupt intercellular junctional complexes integrity (El Ghazzawy *et al.,* 2011). Moreover, oxidative stress within the area might disrupt its metabolic environment, disrupting critical processes crucial for epididymal sperm maturation and functionality overall (Wu *et al.,* 2020).

Furthermore, the effect of BPA on aquaporin channels may augment the widened inter-tubular spaces in the caput epididymis observed in this study. Oestrogen controls the expression of aquaporin channel in the epididymis, necessary for fertility (Da Silva *et al.,* 2006; Menad *et al.,* 2021). The anti-estrogenic effect of BPA might inhibit the expression of aquaporin channels (Kurosawa *et al.,* 2002). This interrupted caput epididymal absorption caused fluid buildup, oedema, and widened inter-tubular spaces (El Ghazzawy *et al.,* 2011). Though not directly assessed in this study, the well-documented anti-estrogenic effects of BPA may explain the significant inter-tubular spaces seen in the B group.

Many tubules in the B group had lumens that were either empty or contained only scanty spermatozoa,

reflecting a possible arrest in spermatogenesis. Some tubules in this group also exhibited lumen filled with cellular debris. The cellular debris intermixed with mature sperm within the lumen appeared to originate primarily from the testis, as the tubule epithelium remained intact (Figure 2). Sloughed germ cells, either from spermatogenic disruptions or androgen deficiency, are the primary contributors to epididymal luminal debris (De Grava Kempinas and Klinefelter, 2014).

Besides, our recent findings indicated that the caput region of the epididymis in the B group had shorter and less prominent stereocilia compared to the C, V, CW, and CW+B groups. Stereocilia are actin-based organelles with microvillus-like progenitors (Barr-Gillespie, 2015). Rouyère *et al.* (2022) demonstrated that elevated ROS levels impair actin polymerisation and compromise cytoskeletal structure via actin oxidation. Given that BPA significantly induces oxidative stress in the epididymis, this may lead to the shortening and loss of stereocilia, reducing their prominence in the lumen.

Since epididymis is highly susceptible to oxidative damage, this requires a stronger antioxidant defence along with plant-derived antioxidants such as *Cordyceps militaris* fungus, *Eruca sativa* extract, *Ipomoea batatas* L. Lam., and polyphenols like quercetin, to counteract BPA-induced oxidative damage on epididymis (Majid *et al.,* 2019; Grami *et al.,* 2020; Sahu and Jena, 2024; Wang *et al.,* 2016). This led to the aim of the present study to examine the adverse effect of BPA-induced oxidative stress on the rat caput epididymis and the role of bioactive compounds in coconut water in mitigating this damage. The findings presented in Table 1 demonstrated that coconut water treatment significantly decreased MDA levels and elevated antioxidant GSH levels in the epididymis of CW+B-treated rats ($p < 0.001$).

The ability of coconut water to reduce testicular lipid peroxidation and improved the antioxidant defence response in experimental animals has been extensively reported (Ommurugan *et al.,* 2021; Kunle-Alabi *et al.,* 2021). This was due to its phytochemical composition, which includes powerful antioxidants including phenolic constituents, flavonoids, essential vitamins, minerals, and amino acids (Tuyekar *et al.,* 2021). A comparable protective effect may apply to the epididymis, which is similarly vulnerable to oxidative stress. Coconut water, abundant in powerful antioxidants such as total polyphenols and bioactive compounds, may counteract epididymal oxidative stress and maintain its microenvironment, potentially enhancing sperm maturation and function.

The increased of GSH levels in the epididymis may result from the GSH precursor administration like methionine, which enhances GSH production for optimal antioxidant defence. Methionine, a thiol-containing amino acid, found in coconut water (Table 2), is a precursor to cysteine. Cysteine is required for GSH synthesis, which acts as a cofactor in GPx activity (Zulaikhah and Sampurna, 2016). This likely justified the observed increase in GSH levels in the rat epididymis upon coconut water administration in the CW and CW+B groups.

The evidence also showed that the caput epididymis tubules in the CW+B group had only slightly wider inter-tubular spaces and minimal cellular debris compared to the tubules in the B group (Figure 3). The primary cause of cellular debris in the epididymal lumen has been identified as androgen deprivation in the testis (De Grava Kempinas and Klinefelter, 2014). Coconut water in the present study contains Zn (Table 2), a mineral essential for 5α-reductase activity. By modulating this Zn-dependent enzyme, coconut water may enhance dihydrotestosterone activity and account for the improved caput epididymal histological features.

Additionally, L-arginine likely elevated nitric oxide (NO) levels by increasing the endogenous arginine levels within the plasma and tissues (Saka *et al.,* 2021). Such an effect may have activated the Keap1/Nrf2 signalling pathway, which is a critical cellular defence mechanism for antioxidant and cytoprotective gene expression in response to oxidative stress, thereby boosting antioxidant activity and levels (Liang *et al.,* 2018). In this study, L-arginine in coconut water (Table 2) was detected, which may enhance L-arginine availability and promote NO production. By enhancing NO production, it assists in maintaining redox balance and decreasing oxidative stress in the epididymis of the CW+B-treated.

Moreover, polyphenol antioxidants can decrease ROS formation by inhibiting the enzymes responsible for their production and augmenting the body's antioxidant defence system (Kruk *et al.,* 2022). These polyphenols can chelate the transition metal ions, including Fe^{2+}/Fe^{3+}, thus restricting their availability for pro-oxidant reaction and free radical production (Appaiah *et al.,* 2016). The biochemical analysis in this study confirmed that presence of significant concentration of total polyphenols in coconut water (Table 2), further conferring its antioxidant protective effect on the rat epididymis. The restoration of oxidative balance in the epididymis likely accounts for the observed enhancement in caput epididymal histopathology in the present findings, as corroborated by Wang *et al.* (2016). Owing to its distinct blend of bioactive constituents, coconut water may help reduce oxidative stress and reproductive damage in BPA-exposed male rats, which suggests its potential as a natural therapeutic option.

Acknowledgement: The authors gratefully acknowledge the university for its facilities and the research fellows for their assistance during the experiments and analyses.

Data availability statement: The authors confirm that

all data relevant to the findings of this study are contained within the article.

Disclosure statement: All authors declare no conflicts of interest to disclose.

Funding: This research did not receive any funding.

Authors' contributions: Conceived and designed the work: NHH; data collection and analysis: NFSS; wrote the manuscript: NFSS; contributed reagents/materials/analysis tools: NHH, NAAA, and final editing of the paper: NHH, NAAA, NK.

REFERENCES

Ahmed, M.M., M.M. Hussein, T. Saber and Y.M. Abd-Elhakim (2022). Palliative effect of resveratrol against nanosized iron oxide-induced oxidative stress and steroidogenesis-related genes dysregulation in testicular tissue of adult male rats. Int. J. Environ. Res. Public Health. 19(13): 8171. https://doi.org/10.3390/ijerph19138171

Alboghobeish, S., M. Mahdavinia, L. Zeidooni, A. Samimi, A.A. Oroojan, S. Alizadeh, M.A. Dehghani, A. Ahangarpour and L. Khorsandi (2019). Efficiency of naringin against reproductive toxicity and testicular damages induced by bisphenol A in rats. Iran J. Basic Med. Sci. 22(3): 315-523. https://doi.org/10.22038/ijbms.2019.29757.7184 .

Appaiah, P., L. Sunil, A.G.K. Krishna and S.K. Gurusiddaiah (2016). Phytochemicals and antioxidant activity of testa extracts of commercial wet and dry coconuts and cakes. Int. Res. J. Pharm. 7(9): 9-13. http://dx.doi.org/10.7897/2230-8407.079106

Babu, S., S. Uppu, M.O. Claville and R.M. Uppu (2013). Prooxidant actions of bisphenol A (BPA) phenoxyl radicals: implications to BPA-related oxidative stress and toxicity. Toxicol. Mech. Methods. 23(4): 273-280. https://doi.org/10.3109/15376516.2012.753969

Barr-Gillespie, P.G. (2015). Assembly of hair bundles, an amazing problem for cell biology. Mol. Biol. Cell. 26(15): 2727-2732. https://doi.org/10.1091/mbc.E14-04-0940

Bertoldo, A., D. Pizzol, D.K. Yon, M. Callegari, V. Gobbo, P. Cuccurese, L. Butler, S. Caminada, J. Stebbing, F. Richardson and J. Gawronska (2024). Resveratrol and female fertility: A systematic review. Int. J. Mol. Sci. 25(23): 12792. https://doi.org/10.3390/ijms252312792

Cariati, F., N. D'Uonno, F. Borrillo, S. Iervolino, G. Galdiero and R. Tomaiuolo (2019). Bisphenol A: An emerging threat to male fertility. Reprod. Biol. Endocrinol. 17(1): 6. https://doi.org/10.1186/s12958-018-0447-6

Chitra, K.C., C. Latchoumycandane and P.P. Mathur (2003). Induction of oxidative stress by bisphenol A in the epididymal sperm of rats. Toxicology. 185(1-2): 119-127. https://doi.org/10.1016/s0300-483x(02)00597-8

Cooper, T.G. (2007). Sperm maturation in the epididymis: a new look at an old problem. Asian J. Androl. 9(4): 533-539. https:// doi.org/ 10.1111/j.1745-7262.2007.00285.x

Da Silva, N., C. Silberstein, V. Beaulieu, C. Piétrement, A.N. Van Hoek, D. Brown and S. Breton (2006). Postnatal expression of aquaporins in epithelial cells of the rat epididymis. Biol. Reprod. 74(2): 427-438. https://doi.org/10.1095/biolreprod.105.044735

De Grava Kempinas, W. and G.R. Klinefelter (2014). Interpreting histopathology in the epididymis. Spermatogenesis. 4(2): e979114. https://doi.org/10.4161/21565562.2014.979114

Di Giacomo, M., V. Zara, P. Bergamo and A. Ferramosca (2020). Crosstalk between mitochondrial metabolism and oxidoreductive homeostasis: A new perspective for understanding the effects of bioactive dietary compounds. Nutr. Res. Rev. 33(1): 90-101. https://doi.org/10.1017/s0954422419000210

Doseděl, M., E. Jirkovský, K. Macáková, L.K. Krčmová, L. Javorská, J. Pourová, L. Mercolini, F. Remião, L. Nováková, P. Mladěnka and Oemonom (2021). Vitamin C—sources, physiological role, kinetics, deficiency, use, toxicity, and determination. Nutrients. 13(2): 615. https://doi.org/10.3390/nu13020615

El Ghazzawy, I.F., A.E. Meleis, E.F. Farghaly and A. Solaiman (2011). Histological study of the possible protective effect of pomegranate juice on bisphenol-A induced changes of the caput epididymal epithelium and sperms of adult albino rats. Alexandria J. Med. 47(2): 125-137. https://doi.org/10.1016/j.ajme.2011.06.006

Ferramosca, A., S. Lorenzetti, M. Di Giacomo, P. Lunetti, F. Murrieri, L. Capobianco, V. Dolce, L. Coppola and V. Zara (2021). Modulation of human sperm mitochondrial respiration efficiency by plant polyphenols. Antioxidants. 10(2): 217. https://doi.org/10.3390/antiox10020217

Grami, D., K. Rtibi, I. Hammami, S. Selmi, L. De Toni, C. Foresta, L. Marzouki and H. Sebai (2020). Protective action of *Eruca sativa* leaves aqueous extracts against bisphenol A-caused in vivo testicular damages. J. Med. Food. 23(6): 600-610. https://doi.org/10.1089/jmf.2019.0170

Johnson, L.A.R.R.Y., N.H. Ing, K.O. Curley, J. Graham, T.H. Welsh and C. Staub (2015). Anatomy and physiology of the male reproductive system and potential targets of toxicants. Reference Module in Biomed. Sci. Elsevier. https://dx.doi.org/10.1016/B978-0-12-801238-3.02134-6

Kruk, J., B.H. Aboul-Enein, E. Duchnik and M. Marchlewicz (2022). Antioxidative properties of phenolic compounds and their effect on oxidative stress induced by severe physical exercise. J. Physiol. Sci. 72(1): 19. https://doi.org/10.1186/s12576-022-00845-1

Kunle-Alabi, O.T., O.O. Akindele and Y. Raji (2021). Coconut water improves testicular oxidative status in doxorubicin-treated male Wistar rats. African J. Med. Sci. 50(1): 19-30.

Kurosawa, T., H. Hiroi, O. Tsutsumi, T. Ishikawa, Y. Osuga, T. Fujiwara, S. Inoue, M. Muramatsu, M. Momoeda and Y. Taketani (2002). The activity of bisphenol A depends on both the estrogen receptor subtype and the cell type. Endocrine J. 49(4): 465-471. https://doi.org/ 10.1507/endocrj.49.465

Li, L., M.Y. Wang, H.B. Jiang, C.R. Guo, X.D. Zhu, X.Q. Yao, W.W. Zeng, Y. Zhao and L.K. Chi (2023). Bisphenol A induces testicular oxidative stress in mice leading to ferroptosis. Asian J. Androl. 25(3): 375-381. https://doi.org/ 10.4103/aja202266

Liang, M., Z. Wang, H. Li, L. Cai, J. Pan, H. He, Q. Wu, Y. Tang, J. Ma and L. Yang (2018). L-Arginine induces antioxidant response to prevent oxidative stress via stimulation of glutathione synthesis and activation of Nrf2 pathway. Food Chem. Toxicol. 115: 315-328. https://doi.org/10.1016/j.fct.2018.03.029

Mahmoud, A.A.E.D., A.O. Seliem, S.A. Selim and N.E. Ibrahem (2024). The sensitivity of adult male albino rat prostate to low-dose bisphenol A: A histological and immunohistochemical study. Zagazig Univ. Med. J. 30(1.1): 250-258. https://dx.doi.org/10.21608/zumj.2021.66437.2163

Majid, M., F. Ijaz, M.W. Baig, B. Nasir, M.R. Khan and I.U. Haq (2019). Scientific validation of ethnomedicinal use of *Ipomoea batatas* L. Lam. as aphrodisiac and gonadoprotective agent against bisphenol A induced testicular toxicity in male Sprague Dawley rats. BioMed R. Int. 2019(1): 8939854. https://doi.org/10.1155/2019/8939854

Mannucci, A., F.R. Argento, E. Fini, M.E. Coccia, N. Taddei, M. Becatti and C. Fiorillo (2022). The impact of oxidative stress in male infertility. Front Mol. Biosci. 8: 799294. https://doi.org/10.3389/fmolb.2021.799294

Mazroa, S. A. (2011). Effect of bisphenol A on the cauda epididymis of adult male albino rats and the possible protective role of quercetin: A histological and immunohistochemical study. Egyptian J. Histol. 34(2): 377-390. https://doi.org/10.1097/01.EHX.0000397468.63291.0c

Menad, R., M. Fernini, L. Lakabi, S. Smaï, T. Gernigon-Spychalowicz, K. Farida, X. Bonnet, E. Moudilou and J.M. Exbrayat (2021). Androgen and estrogen receptors immunolocalization in the sand rat (*Psammomys Obesus*) cauda epididymis. Acta Histochemica. 123(2): 151683. https://doi.org/10.1016/j.acthis.2021.151683

Mohajer, N. and M. Culty (2025). Impact of human-relevant doses of endocrine disrupting chemical and drug mixtures on testis development and function. Reproduction. 169(1): e240155. https://doi.org/10.1530/REP-24-0155

Mubarak, M., T.S. Omolaoye, M.N. Al Smady, M.N. Zaki and S.S. du Plessis (2022). Bisphenol A and male infertility: Role of oxidative stress. In Oxidative Stress and Toxicity in Reproductive Biology and Medicine: A Comprehensive Update on Male Infertility Volume II (pp. 119-135). Cham: Springer International Publishing.

Oguntibeju, O.O., Y. Aboua and P. Kachepe (2020). Possible therapeutic effects of vindoline on testicular and epididymal function in diabetes-induced oxidative stress male Wistar rats. Heliyon. 6(4): e03817. https://doi.org/10.1016/j.heliyon.2020.e03817.

Olayinka, A.T., A.A. Bakare and A.A. Oremosu (2022). Green coconut water supplementation attenuates flutamide-induced testicular damage in male prepubertal Wistar rats. Nigerian. J. Exp. Clin. Biosci. 10(4): 124-130. https://doi.org/10.4103/njecp.njecp_23_22

Ommurugan, B., S. Gangachannaiah, M.D. Nayak, R. Maradi, N, Khan and L.C. Pallavi (2021). Amelioration of methotrexate-induced testicular toxicity in rats by two varieties of coconut water: Effect on sperm characters, oxidative stress and testosterone levels. J. Nat. Remedies. 21(2): 133-140. http://dx.doi.org/ 10.18311/jnr/2021/26014

Park, Y.J., M.A Battistone, B. Kim and S. Breton (2017). Relative contribution of clear cells and principal cells to luminal pH in the mouse epididymis. Biol. Reprod. 96(2): 366-375. https://doi.org/10.1095/biolreprod.116.144857

Rouyère, C., T. Serrano, S. Frémont and A. Echard (2022). Oxidation and reduction of actin: Origin, impact in vitro and functional consequences in vivo. Eur. J. Cell Biol. 101(3): 151249. https://doi.org/10.1016/j.ejcb.2022.151249

Sahu, C. and G. Jena (2024). Combination treatment of zinc and selenium intervention ameliorated BPA-exposed germ cell damage in SD rats: Elucidation of molecular mechanisms. Naunyn-Schmiedebergs Arch. Pharmacol. 397(9): 6685-6704. https://doi.org/10.1007/s00210-024-03044-4

Saka, W.A., A.E. Adeogun, V.I. Adisa, A. Olayioye, Y.D. Igbayilola and R.E. Akhigbe (2024). L-arginine attenuates dichlorvos-induced testicular toxicity in male Wistar rats by suppressing oxidative stress-dependent activation of caspase 3-mediated apoptosis. Biomed. Pharmacother. 178: 117136. https://doi.org/10.1016/ j.biopha. 2024.117136

Saka, W.A., R.E. Akhigbe, A.O. Abidoye, O.S. Dare and A.O. Adekunle (2021). Suppression of uric acid generation and blockade of glutathione dysregulation by L-arginine ameliorates dichlorvos-induced oxidative hepatorenal damage in rats. Biomed. Pharmacother. 138: 111443. https://doi.org/10.1016/ j.biopha. 2021.111443

Santiago, J., J.V. Silva, M.A. Santos and M. Fardilha (2021). Fighting bisphenol A-induced male infertility: The power of antioxidants. Antioxidants. 10(2): 289. https://doi.org/ 10.3390/antiox10020289

Shabanian, S., F. Farahbod, M. Rafieian, F. Ganji and A. Adib (2017). The effects of vitamin C on sperm quality parameters in laboratory rats following long-term exposure to cyclophosphamide. J. Adv. Pharm. Technol. Res. 8(2): 73-79. https://doi.org/10.4103/japtr.japtr_153_16

Shafie, N.F.S., N.K. Nurul Bashar, N.A. Abd Aziz, R.Dasiman and N.H. Hashim (2025). Protective role of *Cocos nucifera* L. water on BPA-mediated oxidative stress and reproductive damage in male rats. Int. J. Environ. Health Res. 35(11): 1-13. https://doi.org/ 10.1080/ 09603123.2025.2484782

Shum, W.W., Y.C. Ruan, N. Da Silva and S. Breton (2011). Establishment of cell-cell cross talk in the epididymis: control of luminal acidification. J. Androl. 32(6): 576-586. https:// doi.org/10.2164/ jandrol.111.012971

Tekin, S., E. Sengul, S. Yildirim, E.H. Aksu, I. Bolat, B. Çınar, A. Shadidizaji, F. Çelebi and M. Warda (2024). Molecular insights into the antioxidative and anti-inflammatory effects of P-coumaric acid against bisphenol A-induced testicular injury: In vivo and in silico studies. Reprod. Toxicol. 125: 108579. https://doi.org/10.1016/ j.reprotox.2024.108579

Turner, T.T. (2008). De Graaf's thread: The human epididymis. J. Androl. 29(3): 237-250. https://10.2164/jandrol.107.004119

Tuyekar, S.N., B.S. Tawade, K.S. Singh, V.S. Wagh, P.K. Vidhate, R.P. Yevale, S. Gaikwad and M. Kale (2021). An overview on coconut water: As a multipurpose nutrition. Int. J. Pharm. Sci. Rev. Res. 68(2): 63-70. http://dx.doi.org/ 10.47583/ijpsrr.2021.v68i02.010

Walke, G., S.S. Gaurkar, R. Prasad, T. Lohakare and M. Wanjari (2023). The impact of oxidative stress on male reproductive function: Exploring the role of antioxidant supplementation. Cureus. 15(7): e42583. https://doi.org/ 10.7759/cureus.42583

Wang, J., C. Chen, Z. Jiang, M. Wang, H. Jiang and X. Zhang (2016). Protective effect of *Cordyceps militaris* extract against bisphenol A induced reproductive damage. Syst. Biol. Reprod. Med. 62(4): 249-257. https://doi.org/ 10.1080/ 19396368.2016.1182234

Wu, P.Y., E. Scarlata and C. O'Flaherty (2020). Long-term adverse effects of oxidative stress on rat epididymis and spermatozoa. Antioxidants. 9(2): 170. https://doi.org/10.3390/antiox9020170

Yelumalai, S., N. Giribabu, K. Karim, S.Z. Omar and N.B. Salleh (2019). In vivo administration of quercetin ameliorates sperm oxidative stress, inflammation, preserves sperm morphology and functions in streptozotocin-nicotinamide induced adult male diabetic rats. Arch. Med. Sci. 15(1): 240-249. https://doi.org/ 10.5114/aoms.2018.81038

Zhao, Q., J. Pan, Y. Bao, X. Wang and W. Shi (2025). Prenatal exposure to bisphenol A causes reproductive damage in F1 male rabbits due to inflammation and oxidative stress. Ecotoxicol. Environ. Saf. 290: 117735. https://doi.org/10.1016/j.ecoenv.2025.117735

Zhou, W., G.N. De Iuliis, M.D. Dun and B. Nixon (2018). Characteristics of the epididymal luminal environment responsible for sperm maturation and storage. Front Endocrinol. 9: 59. https://doi.org/10.3389/fendo.2018.00059

Zou, H., H. Ye, R. Kamaraj, T. Zhang, J. Zhang and P. Pavek (2021). A review on pharmacological activities and synergistic effect of quercetin with small molecule agents. Phytomedicine. 92: 153736. https://doi.org/10.1016/j.phymed.2021.153736

Zulaikhah, S.T. and S. Sampurna (2016). Tender coconut water to prevent oxidative stress due to mercury exposure. IOSR J. Environ. Sci. Toxicol. Food Technol. 10(6): 35-38. https://doi.org/ 10.9790/2402-1006023538 .