Research Article

Histopathological and PCNA-Based Immunohistochemical Assessment of Pumpkin and Grape Seed Extracts in Protecting Male Rat Accessory Glands Under Chemotherapeutic Stress

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Abstract | Cyclophosphamide (CP) is a widely prescribed anticancer agent with significant gonadotoxic side effects that compromise male reproductive function. Natural antioxidants may offer protection against such toxicity. This study investigated the potential protective effects of pumpkin seed extract (PSE) and grape seed extract (GSE) against CP-induced histological and functional alterations in male rat accessory sexual glands. Thirty-six adult male albino rats were randomly divided into six groups (n=6): control (saline), CP (20 mg/kg), PSE (600 mg/kg), GSE (300 mg/kg), CP+PSE, and CP+GSE. The extracts were administered orally from the first day of the experiment for 14 days, while CP was injected intraperitoneally for 7 consecutive days starting from day 8. Histological examination, organ weight analysis, and Proliferation cell nuclear antigen (PCNA) immunohistochemistry were performed to assess treatment outcomes. CP administration induced severe histopathological alterations in accessory sex glands, characterized by vascular congestion, interstitial edema, epithelial vacuolation, and reduced luminal spermatozoa in the epididymis. The seminal vesicles and prostate exhibited marked atrophy, decreased secretory material, epithelial hyperplasia, and necrosis, accompanied by significant organ weight reduction and diminished PCNA immunoexpression. Pretreatment with either PSE or GSE significantly ameliorated these CP-induced alterations, restored organ weights, and enhanced PCNA immunoexpression. Both PSE and GSE demonstrate promising protective potential against CP-induced reproductive toxicity, suggesting their possible therapeutic application in preserving male reproductive function during chemotherapy.

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Introduction

Male fertility relies on a complex interplay of reproductive organs, with the epididymis and accessory glands (prostate and seminal vesicles) serving as crucial components in this intricate system. The epididymis plays a dual role by not only storing sperm but also providing essential protection through the secretion of antioxidant enzymes that neutralize reactive oxygen species O'Flaherty (2019). These specialized glands contribute significantly to seminal plasma composition, producing vital secretions that ensure sperm mobility, provide nourishment, facilitate capacitation, and promote survival (Verze *et al*., 2016; Noda and Ikawa, 2019; Wang *et al*., 2020).

In the context of cancer treatment, chemotherapy poses significant challenges to male reproductive health, often resulting in temporary or permanent fertility impairment. Cyclophosphamide (CP), a widely prescribed alkylating agent in the treatment of neoplastic and autoimmune diseases, exemplifies this therapeutic challenge (Ghafouri-Fard *et al*., 2021; Ayza *et al*., 2022). Despite its therapeutic efficacy, CP's clinical application is constrained by its pronounced reproductive dysfunction and cytotoxic effects Pavin *et al*. (2018).

The reproductive toxicity of CP manifests through multiple pathways, including disrupted gonadotropin secretion, hormonal dysregulation, severe oxidative stress in tissues, extensive cellular damage, ATP depletion, and genotoxic effects Ghobadi *et al*. (2017). Mechanistically, CP functions as a prodrug, undergoing hepatic metabolism to generate two active compounds: Phosphoramide mustard, responsible for therapeutic effects, and acrolein, which induces toxicity through apoptosis and necrosis in normal tissues Gu *et al*. (2017). To mitigate these adverse effects while potentially enabling higher therapeutic doses, antioxidant supplementation has emerged as a promising strategy Cetik-Yildiz *et al*. (2015).

Natural compounds with antioxidant properties have gained attention as potential protective agents. Grape (*Vitis vinifera* L.), one of the world's most cultivated fruit crops, offers exceptional nutritional and therapeutic benefits due to its rich antioxidant profile. The seed extract has a strong antioxidant activity since it contains 60–70% polyphenolics Moosavi *et al*. (2015). Grape seed extract (GSE) is characterized by high concentrations of vitamins C and E and bioflavonoids, providing robust protection against oxidative injury, inflammatory mediators, and carcinogenic effects Alkhedaide *et al*. (2016). Beyond its antioxidant properties, GSE demonstrates diverse pharmacological activities, including antibacterial, antiviral, anti-inflammatory, anti-allergic, and vasodilatory effects, while also showing promise in protecting reproductive function Martins *et al*. (2021).

Similarly, pumpkin (*Cucurbita pepo*) has emerged as a significant medicinal plant with considerable nutritional value Shaban and Sahu (2017). Pumpkin seed extract (PSE) presents a rich nutritional profile, containing essential trace elements like zinc, vital vitamins (carotenoids, tocopherol), proteins, phytosterols, and polyunsaturated fatty acids Stevenson *et al*. (2007). Notably, PSE's high oleic acid content, a monounsaturated fatty acid, helps protect reproductive organs against lipid peroxidation Shaban and Sahu (2017). The therapeutic applications of pumpkin extend to antineoplastic, antioxidant, antibacterial, anti-diabetic, and anti-obesity properties Wadi *et al*. (2021). Research has demonstrated that PSE's antioxidative properties can enhance fertility and prevent reproductive dysfunction (Aghaei *et al*., 2014; Mohamadi *et al*., 2014).

Given the established antioxidant and protective properties of both GSE and PSE, coupled with the significant reproductive toxicity associated with cyclophosphamide treatment, this study aims to investigate the potential protective effects of these natural extracts against CP-induced gonadotoxicity. This research seeks to provide insights into possible therapeutic strategies for preserving male reproductive function during chemotherapy.

Materials and Methods

Experimental animals

Adult male Albino rats (n=36, weight: 270 ± 20 g) were obtained from the Animal House Facility, Faculty of Veterinary Medicine, Zagazig University, Egypt. Animals were maintained under controlled laboratory conditions (temperature: 25±5°C; relative humidity: 55 ± 5%; 12-hour light/dark cycle) with standard laboratory diet and ad libitum access to water. Prior to experimentation, animals underwent a one-week acclimatization period. All experimental procedures were conducted in accordance with institutional

guidelines and approved by the Institutional Animal Care and Use Committee (IACUC) Faculty of Nursing, Modern University for Technology, and Information (MTI), Egypt with a formal approval number (FAN/140/2024).

Pharmaceuticals and extract preparation

Cyclophosphamide: Cyclophosphamide (Endoxan®, Baxter Oncology GmbH, Germany) was obtained in its commercial form and prepared according to manufacturer's specifications.

Grape seed extract (GSE) preparation: Grape seeds (*Vitis vinifera* L.) were manually isolated, shade-dried (25–30˚C, 7 days), and pulverized to fine powder using an electric grinder. The powder underwent maceration in 70% ethanol (1:4 w/v ratio) for 72 hours at room temperature (25 ± 2˚C) with intermittent agitation. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate was evaporated to dryness under reduced pressure using a rotary evaporator (temperature: 40˚C) to obtain GSE powder modified from Badavi *et al*. (2013).

Pumpkin seed extract (PSE) preparation: Cucurbita pepo seeds were manually collected, washed with distilled water, and air-dried. The dried seeds were finely ground, and the powder (100 g) was extracted with 80% ethanol (1:5 w/v) under continuous agitation. The mixture underwent vacuum filtration through Whatman No. 2 filter paper, followed by concentration using a rotary evaporator (40˚C, under reduced pressure). The resulting PSE was reconstituted in sterile distilled water prior to administration modified from Xanthopoulou *et al*. (2009).

Experimental design

Thirty-six rats were randomly allocated into six experimental groups (n=6/group):

- Group I (Control): Received physiological saline (0.5 mL/kg, i.p.) daily from day 8-14
- Group II (CP): Administered cyclophosphamide (20 mg/kg, i.p.)
- Group III (PSE): Received pumpkin seed extract (600 mg/kg, p.o.)
- Group IV (GSE): Administered grape seed extract (300 mg/kg, p.o.)
- Group V (CP+PSE): Received CP (20 mg/kg, i.p.) + PSE (600 mg/kg, p.o.)
- Group VI (CP+GSE): Administered CP (20 mg/kg, i.p.) + GSE (300 mg/kg, p.o.)

GSE and PSE were administered orally from the first day of the experiment for 14 consecutive days. CP administration was initiated on day 8 and continued for 7 consecutive days. Dosages were selected based on previous studies (Sabik and Abd El- Rahman, 2009; Aghaei *et al*., 2014; Hussien *et al*., 2013).

Histopathological and immunohistochemical analyses

Tissue processing and histopathology: The epididymis, seminal vesicle, and prostate were carefully excised and fixed in Bouin's solution (24 hours). Tissues were processed according to standard histological protocols and embedded in paraffin. Serial sections (5 µm thickness) were prepared and stained with: a) Hematoxylin and Eosin (H&E) for routine histopathological examination b) Masson's trichrome for evaluation of extracellular matrix deposition Bancroft and Layton (2019).

Immunohistochemical analysis: PCNA immunostaining was performed using mouse monoclonal anti-PCNA antibody (Clone PC 10, DAKO A/S, Denmark; 1:200 dilution in Tris-buffered saline). Deparaffinized sections underwent overnight incubation with primary antibody (4˚C), followed by visualization using an avidin-biotin-peroxidase detection system. 3,3'-Diaminobenzidine (DAB) was used as chromogen, and sections were counterstained with hematoxylin. All immunohistochemical procedures were performed simultaneously under standardized conditions D'Andrea *et al*. (2008).

Microscopic analysis: Histopathological and immunohistochemical evaluations were performed using a Nikon E800 Eclipse light microscope equipped with a digital imaging system.

Statistical analysis

Data analysis was performed using GraphPad Prism software (version 9, La Jolla, CA, USA). Results are expressed as mean ± standard error of the mean (SEM). Intergroup comparisons were conducted using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. Statistical significance was set at $P < 0.05$.

Results and Discussion

Effect of PSE and GSE on epididymal, prostatic and seminal vesicle weight

Compared with control group, CP group showed a significant ($p < 0.001$) reduction in epididymal and prostatic weights as well as seminal vesicle weight ($p < 0.0001$). Meanwhile, rats pretreated with GSE or PSE showed a significant improvement ($p < 0.05$) in epididymal and ($p < 0.01$) in prostate and seminal vesicle when compared to the CP group.

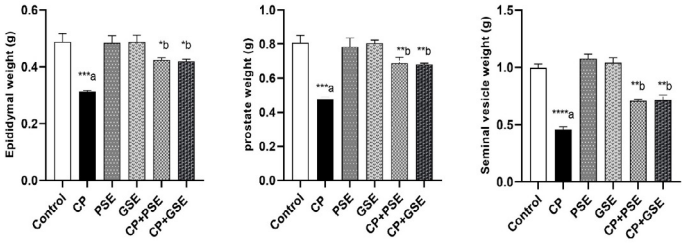

Figure 1: *Effect of CP, PSE and GSE on epididymal, prostatic and seminal vesicle weight ****p < 0.0001, ***p < 0.001, *p < 0.05. (a) versus the control group, while (b) versus the CP.*

Histopathological assessment of epididymal tissue

Histological examination of the epididymis showed circular and oblong epididymal tubules that are lined with stereociliated columnar epithelium, properly surrounded by fibromuscular connective tissue. Abundant healthy spermatozoa were visible in the lumen of the epididymal tubules in the control group (Figure 2A). Similar histological structure was observed in PSE and GSE groups. Administration of CP resulted in severe disruption in the epididymal tissue evidenced by congestion accompanied by edema and hemorrhage in between the epididymal tubules. Vacuolation, disorganization, and desquamation of the epididymal epithelial cells with necrotic sperm in the tubular lumen was also observed. Some epididymal tubules suffered from hyperplasia as well as severe necrosis of their lining epithelial cells with thickening in the connective tissue layers in between epididymal tubules that confirmed by Masson trichrome stain (Figure 2B-H). While rats given CP and pretreated with GSE restored the structural architecture of epididymal tubules with mild oedema in between these tubules, and some necrotic sperm inside the lumen (Figure 2I-J). The pretreated rats with PSE showed a greater improvement with mild vacuolation in the epithelial lining of epididymal tubules with mild oedema between the affected tubules (Figure 2K-L).

Histopathological assessment of prostatic tissue

Histopathological examination of the prostate gland in the control group showed closely packed acini of different sizes. The acini were lined with tall columnar cells with oval nuclei and occasional papillary infoldings.

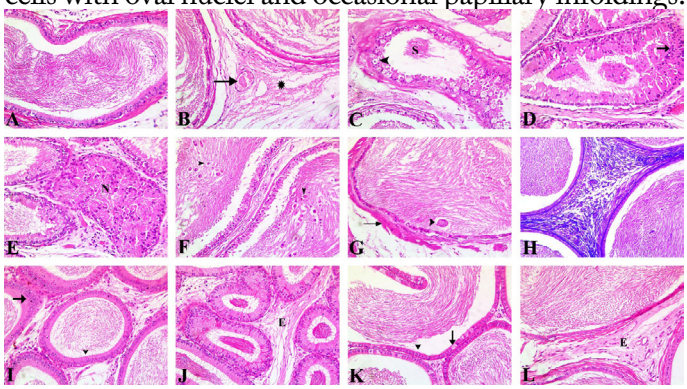

Figure 2: *H & E-stained sections of epididymis of different experimental groups, x200. (A) The control group showed the normal histological structure of epididymal tissue. (B-H) The CP-intoxicated group, (B) showed wide interstitial spaces with congested blood vessels (arrow), edema and hemorrhage in between the epididymal tubules (asterisk), (C) Vacuolated epididymal epithelium (arrowhead) with necrotic sperm (S) in their lumen, (D) Some epididymal tubules displaying either hyperplasia (arrow), or (E) necrosis (N) in their lining epithelium, (F) Desquamated epithelial cells (arrowhead), (G) giant cell formation (arrowhead) and fibroplasia (arrow), (H) that stained blue with Masson Trichrome stain. (I, J) The epididymal tissue in pretreated with GSE showed (I) restored histoarchitecture of epididymal tubules lined with stereociliated columnar epithelium (arrowhead) with mild degree of hyperplasia (arrow), (J) some necrotic sperm inside the lumen and mild interstitial edema (E). (K, L) PSE pretreated group showed (K) nearly normal epididymal lining epithelium (arrowhead) with only mild vacuolation in lining epithelial cells (arrow), (L) mild oedema (E) in between the epididymal tubules.*

The lumina was filled with homogenous acidophilic secretion. Narrow inter-acinar spaces occupied by fibro-muscular stroma separated the acini (Figure 3A). Similar histological structure in GSE, and PSE groups. In the CP group, Significant histological changes in the prostate tissue's acini and stroma. The affected acini displayed destructed epithelial cells, which had small pyknotic nuclei and detached into the lumen (Figure 3B). Some prostatic acini revealed marked hyperplasia of their lining epithelium with papillary projection into the acinar lumen. These arborization folds have darkly stained nuclei with vacuolated cytoplasm (Figure 3C). Occasionally, some acini lost their wall integrity and appeared atrophied with a reduction in their size, remarkable thinning of the lining epithelium, and increased stromal prominence (Figure 3D). Concerning the fibromuscular stroma separating the acini was thicker than the control group and exhibited stromal hyperplasia with hyaline plaques (Figure 3E). Moreover, some stromal areas revealed broad inter-acinar spaces, noticeable hemorrhage, faint proteinaceous edematous fluid, and infiltration of inflammatory cells. Most acini

had scanted or absent prostatic secretions, some containing inflammatory cells in their lumina (Figure 3F). While rats given CP and pretreated with GSE restored the normal appearance of prostatic folding and secretion with some stromal widening (Figure 3G). Furthermore, a group given CP and pretreated with PSE revealed a great improvement in the prostate structure with abundant eosinophilic secretion in the lumen (Figure 3H). Masson's trichrome staining was used to assess the fibromuscular stroma that separated the acini, which was shown by collagen deposition. The prostatic interstitial tissue of the CP group showed a significant accumulation of collagen in comparison to the normal control group. In contrast to the CP group, the pretreatment group's either PSE or GSE prostatic tissue had substantially less collagen deposition (Figure 3I-L).

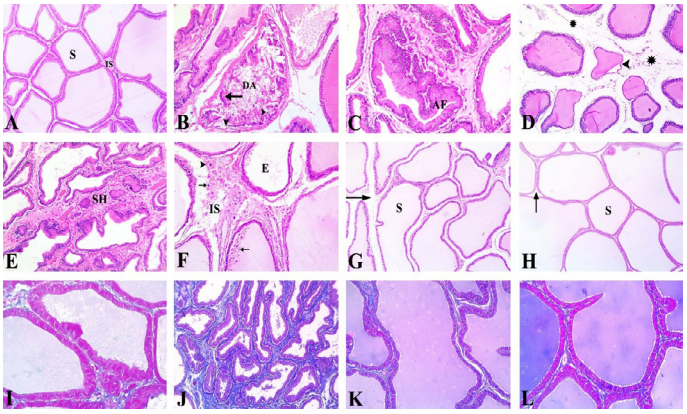

Figure 3: *Photomicrograph of H&E-stained (A-H) and Masson Trichrome stained (I-L) prostate section of different experimental groups, x200. (A) Control group showing different sizes of acini lined with tall columnar cells with oval nuclei and occasional papillary infoldings. Homogenous acidophilic secretion (S) fills the lumen of acini. Narrow inter-acinar spaces (IS) occupied by fibro-muscular stroma separated the acini. (B-F) CP group, (B) Destructed acini (DA) filled with detached epithelial cells (small arrow) with small pyknotic nuclei (arrowhead), (C) Marked epithelial hyperplasia with arborization folds (AF) into the acinar lumen, (D) Atrophied prostatic acini with marked thinning of the epithelial lining (arrowhead) and increased stromal prominence (asterisk), (E) Fibromuscular stromal hyperplasia (SH) with hyaline plaques, (F) Wide inter-acinar spaces (IS) with noticeable hemorrhage (arrowhead), empty prostatic acini (E) and inflammatory cells (arrow) infiltration. (CP+GSE) and (CP+PSE) groups (G, H) respectively show the normal appearance of prostatic folding and secretion (S) with some stromal widening (arrow). (I) Control group showing little collagen in interstitial tissue. (J) CP group shows excessive bluish stained collagen. (K, L) CP+GSE and CP+PSE groups showing decreased collagen in the interstitial tissue.*

Histopathological assessment of seminal vesicles

The histological structure of the seminal vehicles was found to be normal in the control group. The gland was divided into lobes of varying shapes, sizes, and numbers by well-developed fibromuscular capsules and trabeculae. The parenchyma had a honeycomb-like appearance with visible anastomosing mucosal folds. The mucosa is formed of pseudostratified columnar epithelium and lamina propria. The columnar epithelial cells had acidophilic cytoplasm, and basal basophilic nuclei. Additionally, deeply stained luminal acidophilic secretion was observed (Figure 4A). Similar histological structure was detected in GSE and PSE treated groups. The CP-treated group revealed a significant atrophy of the seminal vesicles. The glands exhibited a reduction in glandular components and lining epithelial cells with the formation of small papillary folds, along with prominent fibromuscular stroma (Figure 4B). Furthermore, some alveoli exhibited a reduction in mucosal fold height, and papillary folds appeared flattened with diminished epithelial cells size (Figure 4C). In other examined sections of seminal vesicle, the lining epithelium had necrosis, with detached epithelial cells in the lumina. These lumina showed little acidophilic secretion. Additionally, there was a prominent fibromuscular stroma and monocellular inflammatory cell infiltrations (Figure 4D). The variant areas of fibromuscular stromal prominence expressed positive color by Masson trichrome stain (Figure 4E). Also noted widespread congestion and edema between the degenerated lobules (Figure 4F). Obviously, these alterations were seen in that group

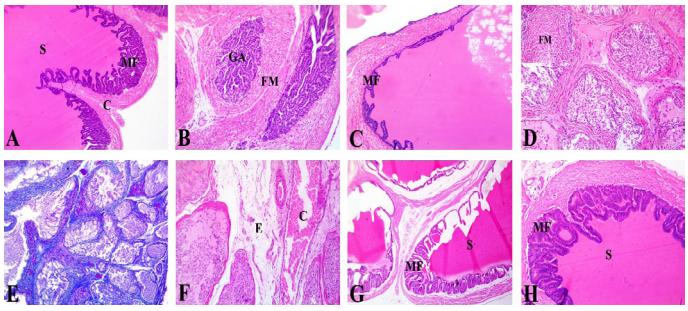

Figure 4: *Photomicrograph of H&E-stained seminal vesicle section of different experimental groups, x200. (A) Control group showing normal histological structure with acidophilic secretion (S) in the lumen supported by a well-developed fibromuscular capsule (C). The parenchyma showed anastomosing mucosal folds (MF). (B- F) CP group, (B) Glandular atrophy (GA), with a reduction in glandular components, and prominent fibromuscular stroma (FM), (C) Alveoli showing a reduction in mucosal fold height (MF), with flattened papillary folds, (D) Necrosis of the lining epithelium with detached epithelial cells and little acidophilic secretion, Inset showing prominent fibromuscular stroma (FM) and monocellular inflammatory cell infiltrations, (E) Fibromuscular stroma showing positive color for Masson trichrome stain, (F) widespread congestion (C) and edema (E) between the degenerated acini. (G, H) CP+GSE and CP+PSE group respectively, showing normal epithelium, mucosal folds (MF), and secretion (S) with restoration of most of the normal architecture*

of seminal vesicle.

were improved in the pretreated groups with GSE or PSE. These pretreated groups showed a restoration of the seminal vesicle's tissues to their normal histological features, including normal epithelium, mucosal folds, secretion, and the reversal of most of the necrotic changes (Figure 4G, H).

Immunohistochemical assessment of epididymis, prostate, and seminal vesicle

Immunohistochemical examination of epididymis, prostate and seminal vesicle revealed a significant reduction in the number of immunopositive cells observed in CP treated group in comparison with the control group. In contrast, the pretreated groups (CP+GSE, CP+PSE) showed a significant increase in the number of PCNA-positive cells compared to the CP group (Figure 5).

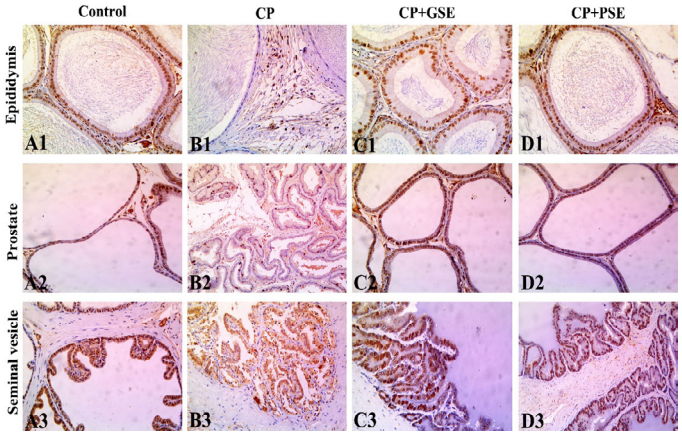

Figure 5: *Photomicrographs of PCNA expression in the epididymal, prostatic and seminal vesicle tissues, showing an intensive brown positive reaction in the nuclei of epithelial cells in the control group (A1, A2, A3), weak positive reaction in an intoxicated group with CP (B1, B2, B3), While rats given CP and pretreated with GSE (C1, C2, C3) or PSE (D1, D2, D3) had a greater increase in PCNA expression in the nuclei of epithelial cells. All magnifications were x200.*

Chemotherapy is an effective cancer treatment. However, it is related to cytotoxic effects on normal cells and tissues, especially in the reproductive system. Chemotherapy causes changes in fertility, organ structure, sexual hormones, and function, as well as quality of life Haghi-Aminjan *et al.* (2017). The susceptibility of the genital organ and accessory glands to chemotherapeutic agents is related to their high content of polyunsaturated membrane lipids, low oxygen tensions, and abundant ROS-generating systems Agarwal *et al.* (2014). Cyclophosphamide is an effective anticancer and immunosuppressive agent, but it is severely limited by reproductive toxicity as documented in a variety of species Ghobadi *et al.* (2017). This study highlights the possible protective roles of GSE and PSE in male rat accessory glands exposed to chemotherapy.

In the present study, CP induced various histopathological disruptions in the examined epididymis, prostate, and seminal vesicle glands. This result was consistent with Iqubal *et al.* (2020). These damages induced by CP were reflected in the wights of the examined organs that significantly decreased when compared to the control group. This finding correlated with Das *et al.* (2002) who noticed that CP caused a decrease in the male accessory gland weights due to inhibition in testicular androgenesis secretion. In the present investigation, the epididymal tissue of CP-treated rats exhibits a reduction in the luminal spermatozoa with hyperplasia, vacuolation, and necrosis of the epithelial lining cells that sloughed into the lumen. There was thickening in the fibromuscular connective tissue layer of almost all the epididymal tubules. Such findings coincide with those of previous reports Aghaei *et al.* (2014). The prostatic acini were destructed and atrophied with flattened epithelial lining. Some acini displayed hyperplastic lining epithelium with papillary projection into the acinar lumen. This result was consistent with Abdel-Hafez *et al.* (2017). The seminal vesicle of CP-treat rats was atrophied with necrosed detached lining epithelium along with little acidophilic secretion in the lumen. Additionally, there was a prominent fibromuscular stroma infiltrated with inflammatory cells. Similar results were recorded by Iqubal *et al.* (2020). The prominent fibromuscular stroma could be a contributing factor to the development of glandular atrophy, where the epithelial cells of the seminal vesicles are replaced by muscular tissue Do Nascimento *et al.* (2020). This deleterious effect of CP is mediated by oxidative stress, and DNA damage of the cells Fusco *et al.* (2021). Oxidative stress is considered a main modulator of inflammation, myofibroblast differentiation and extracellular matrix production (Richter and Kietzmann, 2016; Thuan *et al.*, 2018). That explained the observed interstitial inflammatory cell infiltration, congested capillaries, edema, abundant smooth muscles, and hyaline material deposition in the examined organs. However, the epithelial hyperplasia in this work represents either a form of cellular adaptation to stress, or unfortunately, it takes place at the expense of normal function (Kumar *et al.*, 2017).

In the current work revealed positive expression of PCNA in all groups in this study except in CP treated group. This could be related to the necrotic and atrophic changes observed in the epithelium Oltra *et al*. (2014). On the other hand, a significant increase in PCNA-positive cells was noticed in the pretreated groups with GSE or PSE indicating cellular proliferation. Earlier studies confirmed immunohistochemical findings using different markers for cellular proliferation, specifically Ki-67 in the cisplatin-treated group, Ki-67 was expressed in a low number of epithelial cells in the affected glands Abou-Elghait *et al*. (2022).

In the current study, pretreatment with GSE significantly reduced epididymal, prostatic, and seminal vesicle histological injury caused by CP and restored the weights of these organs. These findings were in parallel with Hasseeb *et al*. (2013) who detected the ameliorating effect of the GSE especially in high levels against the induced lesions of the Acrylamide in the male reproductive organs of the rats. This could be attributed to the high antioxidant activity of GSE due to the presence of phenolic compounds such as catechin, gallic acid, epicatechin, and proanthocyanidins which present a higher antioxidant capacity than vitamin C or E Gupta *et al*. (2020). Furthermore, it possess anti-inflammatory, and anti-apoptotic properties Martins *et al*. (2021).

Pretreatment with PSE markedly reversed the epididymal histological alterations induced by CP with abundant healthy luminal spermatozoa. These findings were in collaboration with Aghaei *et al*. (2014). As well as the pretreatment with PSE negated the adverse effect of CP on the prostate gland and seminal vesicle histological disruptions with restoration of the weight of these organs. The ameliorative effects of PSE could be contributed to presence of trace minerals such as selenium and zinc, which are powerful antioxidants Dowidar *et al*. (2020). Prostate health and function are dependent on high levels of zinc, which are required for the production and secretion of citrate, a major component of prostatic fluid Costello and Franklin (2016).

Furthermore, pumpkin seeds contain high antioxidant vitamins like tocopherol and carotenoid that playing a protective role against toxic substances and free radicals Lestari and Meiyanto (2018). Pumpkin seeds have an abundance of Phytosterols, they are used in benign hypertrophic prostate disease in which they preventing the transformation of testosterone to the more efficient androgen dihydrotestosterone (DHT) Arora *et al*. (2023).

Conclusions and Recommendations

Based on histopathological and immunohistochemical findings obtained from this study, it could be concluded that CP caused marked disruption in the accessory male sexual glands and epididymis that may be minimized by the pretreatment of GSE and PSE. The ameliorative effect of these seed extracts was related to their antioxidant efficacy.

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Novelty Statement

This study provides novel evidence of the protective effects of pumpkin seed extract (PSE) and grape seed extract (GSE) against cyclophosphamide-induced reproductive toxicity in male rats. The research highlights the histopathological and PCNA immunohistochemical findings, demonstrating that both extracts significantly restore accessory gland structure and function, suggesting their potential as therapeutic agents to mitigate chemotherapy-induced gonadotoxicity.

Author's Contribution

Ibrahim Elmaghraby conceptualized the study, designed the methodology, and performed the histopathological analysis. Zeinab Said contributed to the experimental design, data interpretation, and manuscript writing. Marwa Darweish conducted the laboratory work, including immunohistochemistry and data acquisition. Doaa Galal El-Sahra performed the statistical analysis, literature review, and manuscript editing. Ahmed Ibrahim El-Nemr supervised the research, reviewed the final manuscript, and handled

correspondence. All authors read and approved the final manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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