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Original article

Comparative Study on the Ameliorative Influence of Melatonin and Pinostrobin on the Testicular Damage Induced by Paclitaxel in Adult Male Albino Rats: Histological and Immunohistochemical Study

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Abstract

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Background: paclitaxel (PAC) is a powerful chemotherapy; however, it lacks a focused action resulting in serious side effects. Melatonin (MLT) has potent antioxidant and cell-modulating abilities. Pinostrobin (PN) is an essential dietary flavonoid with numerous pharmacological potentials. This research aimed to assess the ameliorative role of MLT and PN on the testicular damage caused by paclitaxel in rats. **Subjects and Methods:** Fifty-four adult male albino rats were randomly separated into six equal groups; Control group, PN group, MLT group, PAC group, PAC+PN group, and PAC+ MLT group. After 30 days of treatment blood samples were collected for laboratory analysis and the testes were subjected to biochemical, immunohistochemical, and histological study. Results: Paclitaxel significantly decreased the serum testosterone level and lowered catalase (CAT) and superoxide dismutase (SOD) activities in the testicular tissues while increasing the level of malondialdehyde (MDA) in comparison to rats used as controls. On the other hand, blood testosterone and antioxidant enzyme levels were markedly increased in rats when MLT or PN was co-administered with PAC. Seminiferous tubule disarray and spermatogenic cell degradation were visible in the H&E-stained sections from the PAC group. Broad interstitial areas were also noted with few pyknotic Leydig cells. However, the groups receiving MLT or PN in line with PAC displayed restoration of the normal histological structure of the testicular tissue. The immunohistochemical results showed negative Bcl-2 immunoreaction and noticeable positive TNF-α expression in PAC-exposed rats. PAC+PN group presented moderate positive Bcl-2 immune reactivity and moderate reduction in TNF-α expression whereas, PAC+MLT group presented intense positive Bcl-2 and minimal TNF-α immuno-reactivity. **Conclusion**: Administration of MLT or PN to PAC exposed rats can protect the testicular tissues from the injurious effects of PAC by means of the anti-apoptotic and antioxidant qualities. However, administration of MLT was more efficient.

I. Background

Among the leading causes of death globally and a significant public health concern is cancer (Ferlay et al.,2018). Chemotherapy is a therapy alternative. It enhances cancer patients' quality of life and provides hope for cancer remission. Unfortunately, among of the most significant cancer drugs have nonspecific effects, killing both healthy and malignant cells, causing toxicity of other organs including gonadotoxicity (Borovskaya et al., 2009 and Zhang et al., 2018).

paclitaxel (Taxol) is a powerful chemotherapy medication, and it was derived from the Taxus brevifolia tree by Kathiravan et al., (2012). It sounded good in the treatment of several solid tumor types, including colon, stomach,

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breast, prostate, and bladder in addition to head and neck tumors (Wang et al., 2017).

However, like other chemotherapeutic medications, paclitaxel lacks a focused action and induces cell death, resulting in serious side effects (Malekinejad et al., 2017). According to earlier studies, paclitaxel causes testicular DNA damage, oxidative stress, and apoptosis in addition to aberrant alterations in sperm activity and motility (Abd-Elrazek et al., 2020). Therefore, to maximize the benefits of anti-cancer drugs while reducing their side effects, researchers are especially interested in mixing them with natural substances.

N-acetyl-5-methoxy tryptamine, or melatonin, is a naturally occurring tryptophan derivative. It was once believed to be exclusively generated by the pineal gland and to influence sleep and circadian rhythm like a hormone (Tan et al., 2003). Recently, it has been discovered that numerous cells have melatonin receptors and that many additional organs have melatonin-related enzymes (Aboelwafa et al.,2022).

Rossi et al., (2014) stated that melatonin has various indirect effects on the testicular somatic cells such as proliferation, cellular growth modulation, and the promotion of the secretory function of testicular cells. Because of its strong antioxidant properties, melatonin is a ubiquitous chemical affecting different biological processes, free-radical scavenging, and cell-modulating abilities (Luo et al.,2019).

An essential dietary flavonoid, pinostrobin is a significant component of *Pinus srobus* L. heartwood (Patel et al., 2016). It is present in several families of medicinal plants, including Fabaceae, Polygonaceae, Zingiberaceae, and Lauraceae (Al-Medhtiy et al.,2022). Pinostrobin is documented to possess anti-inflammatory, anti-fungal, gastroprotective, and antioxidant potentials (Hidajati et al., 2018; Kanchanapiboon et al., 2020). The recent investigation was planned to assess the valuable role of pinostrobin and melatonin in reducing testicular damage caused by paclitaxel in rats.

II. Methods

This research was carried out in the anatomy department of Benha University in Qaliobia governorate, Egypt, from December 2021 to December 2022.

All participants provided their written informed consent, and the research was authorized by the Benha University Faculty of Medicine's Human Research Ethics Council, Egypt (Ethical approval number RC 7-12-2023).

II.1 Study design:

Drugs and chemicals:

-Paclitaxel (PAC): Taxol vial of 100 mg (6 mg/mL) provided by Bristol-Myers Squibb, USA (product number NJ 08543).

- Melatonin (MLT): Was acquired from Sigma-Aldrich Biochemie GmbH, Germany. It was in the form of white to off-white powder with CAS number 73-31-4. It was dissolved in normal saline.
- Pinostrobin (PN): Also obtained from Germany (Sigma-Aldrich) in the form of powder (density 1.284 ± 0.06 g/cm3, boiling point 494.9 ± 45.0 ◦C). It was liquefied in 1% carboxymethylcellulose sodium (CMC-Na).
- carboxymethylcellulose sodium(CMC-Na) and normal saline were purchased from Sigma Chemical Co. (St. Louis, USA**)**

Experimental animals:

In this experimental work, fifty-four male adult albino rats, two months of age and 180– 200 g in weight, were employed. The laboratory animals unit of Benha University's Veterinary Medicine Faculty in Egypt donated the rats. The animals were given regular feed and water. They were kept in separate plastic cages with suitable environmental circumstances (light /dark cycle, temperature, and relative humidity).

Experimental design:

Fifty-four rats were housed for one week before being separated into six groups of nine rats each.

Group I (Control group): Nine rats were separated into three subgroups:

• **Group Ia**: Three of them received a normal diet with no treatment.

• **Group Ib**: Another three rats were injected with 1 mL of normal saline intraperitoneally (ip) daily for 30 successive days.

• **Group Ic:** The last three animals were given 1 mL of 1% CMC-Na daily for 30 days by oral gavage.

Group II (PN group): Nine rats received 10 mg/kg body weight (b.wt) of PN daily for 30 days via oral gavage **(Ijaz et al., 2023).**

Group III (MLT group): Nine Rats have injected IP with MLT (10 mg/kg b.wt) every day for 30 days at 8 am **(Kamsrijai et al.,2020).**

Group IV (PAC treated group): PAC was injected ip into nine rats once a week for 4 weeks (1st, 7th, 14th, and 21st day of the experiment) at a dosage of 5 mg/kg b.wt dissolved in 1 mL of normal saline **(Abd Elrazek et al., 2020).**

Group V (PAC+PN group): Nine rats were injected ip with PAC (5 mg/kg b.wt) as in group IV along with oral supplementation of PN (10 mg/kg b.wt) for 30 days via oral gavage.

Group VI (PAC+ MLT group): Nine rats were injected ip with both PAC (5 mg/kg b.wt) as in group IV and MLT (10 mg/kg b.wt) every day for 30 days**.**

II.2 Methodology

Blood and tissue collecting: After 30 days of therapy, all rats were dissected after being put to sleep by anesthesia

with 6 mg/kg of xylazine and 60 mg/kg of ketamine. Samples of blood were taken, placed in sterile containers which were heparinized, and then centrifuged for ten minutes at 3000 rpm. Testes were meticulously removed and then cleaned with physiological saline. While the left testes were fixed in formalin solution 10% for the histological and immunohistochemical investigation, the right testes were kept at -80°C for the biochemical assessment.

Biochemical markers

Testicular tissues were ground in liquid nitrogen for biochemical examination. Next, 1 ml of phosphate-buffered saline (PBS) was used to homogenize 50 mg of pulverized tissue. The homogenates were then centrifuged for five minutes at 5,000 × g to extract the supernatant, which was subsequently used for analysis. Using an ELISA kit (Elabscience, United States), the concentrations of malondialdehyde (MDA), catalase (CAT), and superoxide dismutase (SOD) were determined under the manufacturer's instructions (Chance and Maehly, 1995; Sun et al.,1998).

Hormonal assay

Enzyme-linked immunosorbent assay (ELISA) kits (Los Angeles, CA, USA) were used to measure the levels of plasma testosterone, luteinizing hormone (LH), and Follicle-Stimulating Hormone (FSH). The measurements of hormones were given in ng/mL (El-Azab and Elmahalaway 2019).

Histological study

Paraffin sections of 5μm thickness were spread out on glass slides, then stained with Haematoxylin and Eosin (H&E) to detect the overall histological structure as well as Masson Trichome to identify the presence of collagen fibers (Bancroft.& Layton, 2018).

At the Anatomy & Embryology department of the Faculty of Medicine at Benha University in Egypt, sections stained with H&E and Masson Trichome were studied under a light microscope (Olympus CX 41, Japan) and photographed using a digital device connected to the microscope.

Immunohistochemical study

1- Bcl-2 immunohistochemical staining: The primary antibody (mouse/IgG1, kappa) by Thermo Fisher Scientific, USA. Positive Bcl-2 immunoreaction appeared as brown discoloration in the cytoplasm (El-Azab and Elmahalaway, 2019).

2- TNF-α immunohistochemical staining: The primary antibody (rabbit polyclonal IgG) by Santa Cruz Biotechnology Inc., USA. Positive TNF-α immunoreaction appeared as brown discoloration in the cytoplasm (Ileriturk et al.,2021).

The avidin-biotin-peroxidase approach, previously published (Petrosyan et al., 2002), was utilized to conduct an immunohistochemical analysis. The chromogen was diamethoxybenzidine (Dakopatts, Glostrup, Denmark). Hematoxylin was used as a counterstain after the slides had been cleaned with distilled water. The standard immunostaining protocol was applied to the negative controls and the Phosphate buffered saline PBS was utilized in place of the primary antibody.

Histomorphometric Analysis

From the H&E-stained sections of all nine rats in each group, five randomly selected fields were used to measure the tunica albuginea thickness, seminiferous tubule diameter, germinal epithelium height, and the number of without sperms (Aboelwafa et al.,2022). To determine the mean area % of collagen fiber deposition, Bcl-2, and TNF- α positive immune reaction, morphometric analysis was also performed on five non-overlying fields from Masson Trichome-stained sections and immune stained sections from all the rats in each group. A Leica Qwin 500 image analysis computer system (Leica Microsystems Ltd., Cambridge, UK) was used for morphometric research.

II.3 Data analysis: The obtained values were presented as mean± standard deviation for every group. Statistical comparisons between different groups were assessed using one-way analysis of variance (ANOVA) and post hoc LSD test. Calculations were prepared with (the SPSS program; version 20.0 for Windows, SPSS Inc., Chicago,IL). The results were significant when the p-value ≤ 0.05.

III. Results Biochemical Results

Oxidative stress indicators

CAT and SOD activities were considerably diminished with the PAC treatment ($p \leq 0.05$), while the level of MDA significantly increased in comparison to the other groups ($p \leq 0.05$). In contrast to the PAC-intoxicated group, co-administration of PAC with PN or MLT led to a noticeable rise in CAT and SOD activities as well as an obvious decrease in MDA levels ($p \leq 0.05$). Moreover, the control group's mean values for these parameters were similar to those of the PN or MLT-alone supplemented groups (Table 1).

Table 1: Oxidative stress biomarkers in control and other studied groups.

Parameters	Group I control	Group II (PN)	Group III (ML	Group IV (PAC)	Group V (PAC + PN)	Group VI (PAC + MLT)	P value
Malondialdehyde (MDA) (nmol/g)	0.59±0.03	0.56±0.03 d & e	0.58±0.02 d & e	2.5±0.2 a,b,c,,e , f	1.7±0.2 a,b,c ,d & f	0.9± 0.1[d]	<0.001[a] 0.001[b] <0.001[c] <0.05[e] <0.02 [f]
Catalase (CAT) (U/mg protein)	8.48± 0.01	8.49±0.01 [d]	8.5±0.03 [d]	5.2±0.14 a,b,c ,e & f	7.6±0.02[d]	8.18±0.01[d]	<0.001[a] 0.001[b] <0.001[c] <0.04[e] <0.02 [f]
dismutase Superoxide (SOD) (U/mg protein)	7.4 ± 0.02	7.45±0.01[d]	7.46±0.02[d]	3.1±0.1 a,b,c ,e & f	6.9±0.07[d]	7.1±0.1[d]	<0.001[a] 0.001[b] <0.001[c] <0.03[e] <0.02 [f]

Data is presented as mean ±SD, *: significance ≤ 0.05; One way ANOVA method followed by Post-hoc Tukey's test
PN: Pinostrobin ; MLT: Melatonin ; PAC: paclitaxel.a: Significance vs Control, b: Significance vs Group II, c: Significance vs Group III, d: Significance vs group IV, e: Significance vs group V, f: Significance vs group VI.

Hormonal result

When compared to the other groups, the testosterone, LH and FSH plasma concentrations were considerably lower in the PAC treatment group (p≤ 0.05). Conversely, the PAC + PN group and the PAC + MLT group had significantly greater plasma concentrations of testosterone, LH, and FSH than the PAC group (P≤ 0.05). Furthermore, the hormonal levels in the groups administered only PN and MLT were close to those in the control group. (Table 2).

Table 2: Mean values ± SD of LH, FSH, and Plasma testosterone in all groups.

Parameters	Group I Control	Group II PN	Group III MLT	Group IV PAC	Group V (PAC + PN)	Group VI (PAC + MLT	P value
LH (ng/mL)	2.29± 0.03	2.31± 0.03 [d]	2.32± 0.02 d	1.15± 0.05 a, b,c, e & f	2.13± 0.4 [d]	2.21± 0.05 [d]	<0.001[a] 0.001[b] <0.001[c] <0.05[e] <0.02 [f]
FSH (ng/mL)	3.6± 0.1	3.5±0.1 [d & e]	3.6±0.07 d & e	1.4±0.05 [a, b,c, e & f]	2.9±0.2 a,b,c &d	3.1±0.2[d]	<0.001[a] 0.001[b] <0.001[c] <0.04[e] <0.02 [f]
Plasma osterone (ng/mL)	4.57± 0.03	4.59± 0.07 d & e	4.60± 0.08 d & e	2.2± 0.2 [a, b,c, e & f]	3.9± 0.05 a,b,c &d	4.1± 0.1[d]	<0.001[a] 0.001[b] <0.001[c] <0.03[e] <0.02 [f]

Data is presented as mean ±SD, *: significance ≤ 0.05; One way ANOVA method followed by Post-hoc Tukey's test; PN: Pinostrobin ; MLT: Melatonin ; PAC: paclitaxel. ; a: Significance vs Control, b: Significance vs Group II, c: Significance vs Group III, d: Significance vs Group IV, e: Significance vs Group V, f: Significance vs Group VI.

Histological results

Hematoxylin & Eosin (H & E) stain

The control group's testicular sections had a normal histological architecture, with a normal tunica albuginea enclosing multiple densely packed seminiferous tubules. Every tubule is lined with Sertoli cells and germinal epithelium, and its basement membrane is still intact. The germinal epithelium is composed of many layers of spermatogenic cells, including spermatogonia perched on the basement membrane, larger primary and secondary spermatocytes, spermatids, and sperms were seen actively releasing throughout the tubule lumen. The Sertoli cell had a huge vesicular nucleus. Seminiferous tubules with clusters of Leydig cells and normal blood arteries are divided by regular, thin interstitial spaces (Figures 1A& B). Similar to the control group, the PN and MLT groups displayed normal testicular anatomy. (Figures & B).

As regards the PAC group, The testicular sections showed clear histopathological changes. The tunica albuginea was thickened, and seminiferous tubules showed disorganization, a marked decrease in number, and empty lumens. Most tubules are lined with degenerated spermatogenic cells and spermatocytes with dark pyknotic nuclei. Other tubules displayed shedding of germ cells inside their lumen and distorted basement membrane. Wide interstitial spaces were detected filled with eosinophilic exudate fluid, few pyknotic leydig cells and congested blood vessels. (Figures 3A, 3B and 3C).

Sections from the PAC+ PN group showed improved histological structure of the testicular tissue with regular tunica albuginea. Most seminiferous tubules had stratified organization of spermatogenic cells and their lumen revealed mature sperms. Some tubules are still observed with nearly empty lumen. Narrow interstitial tissues having Leydig cells and congested blood vessel were also detected (Figures 4A and 4B).

Sections from the PAC+MLT group showed relatively normal testicular histoarchitecture with normal tunica albuginea. Seminiferous tubules are enclosed by an intact basement membrane and contain stratified germinal epithelium and Sertoli cells. Tubular lumen filled with mature sperms. Interstitial cells of Leydig were noticed normal within intact interstitial tissues (Figures 5A and 5B). **Masson trichome stain**

Masson trichome stained sections from the Control group, PN group, and MLT group exhibited regular distribution of a small amount of collagen fiber between the seminiferous tubules (Figures 6 A,B,C). On the other hand, sections from the PAC group revealed a thick layer of collagen fiber deposition in the interstitial tissues and around the congested blood vessel (Figure 6D). The Pac +PN group displayed a decreased amount of collagen fibers between seminiferous tubules in the intervening tissues (Figure 6E), while the PAC+MLT group displayed an amount of collagen fiber in the interstitial tissues(Figure 6F).

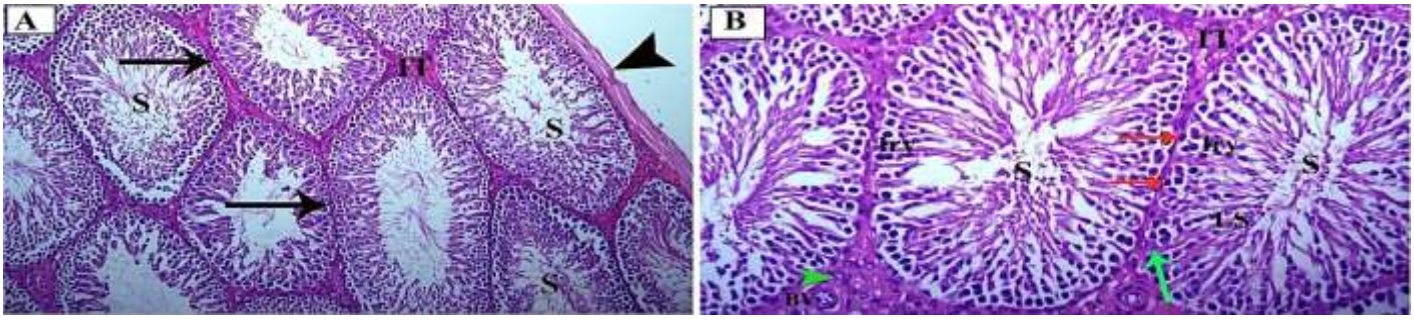

Figure (1): H&E-stained section photomicrographs from the control rats' testes: **(A)** showing normal tunica albuginea (black arrowhead), closely packed seminiferous tubules with intact basement membrane (black arrows). Narrow interstitial tissues (IT), and mature spermatozoa (S). (B) presenting spermatogonia (red arrows), primary spermatocytes (1ry), late spermatids (LS), and spermatozoa (S) are among the layers of spermatogenic cells lining normal seminiferous tubules. A Sertoli cell (green arrow). Tubules are divided by thin, regular interstitial tissue (IT), intact clusters of Leydig interstitial cells (green arrowhead), and normal blood vessels (BV) (A X 100; B X 200**).**

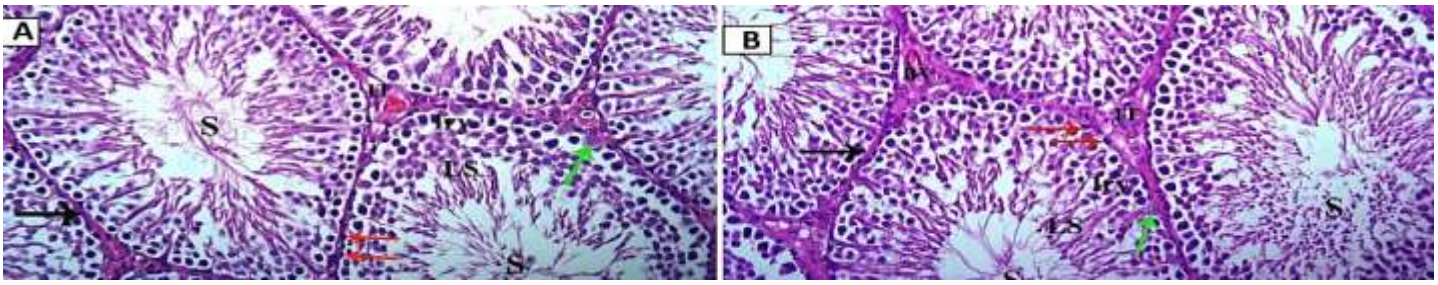

Figure (2): Photomicrographs of H &E-stained testicular sections from PN group (A) and MLT group (B) showing normal testicular architecture with densely crowded seminiferous tubules (black arrow). They are lined with stratified spermatogenic layers; spermatogonia (red arrow), primary spermatocytes (1ry), late spermatids (LS), Sertoli cell (green arrow), and spermatozoa (S) filling their lumen. Narrow intact interstitial tissue (IT) with normal blood vessels (BV) were observed (H&E X200).

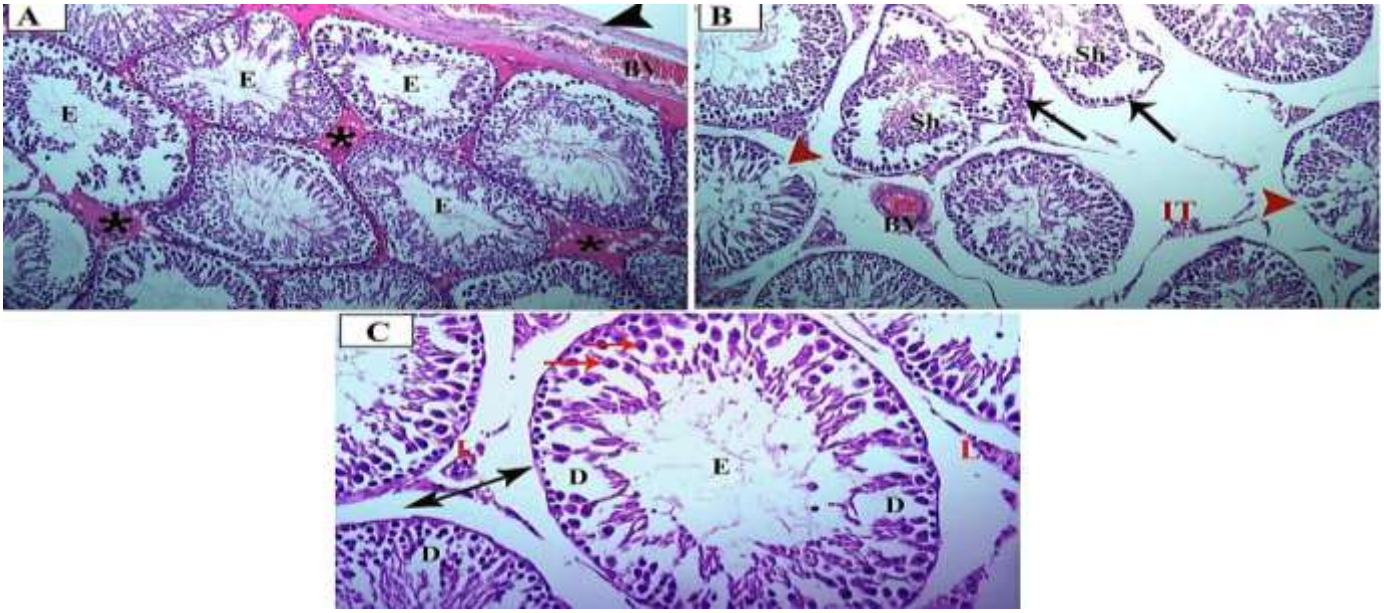

Figure (3): Photomicrographs of H&E-stained testicular sections from PAC group: (A) showing deteriorated testicular tissue with thickened tunica albuginea (arrowhead) and congested blood vessel (BV). The seminiferous tubules have degenerated germinal epithelium and nearly empty lumens (E). Wide interstitial spaces filled with eosinophilic exudate fluid (*). (B) presenting widely spaced disorganized seminiferous tubules (arrows), shedding of germinal cells (Sh), detached and distorted basement membrane (arrowhead). Note destructed interstitial tissue (IT) with congested blood vessels (BV). (C) displaying seminiferous tubules with empty lumen(E), degenerated spermatogenic cells (D), most spermatocytes have dark pyknotic nuclei (red arrows). Wide interstitial tissue spaces (double head arrow) with few pyknotic leydig cells (L) (A&B X 100; C X 200).

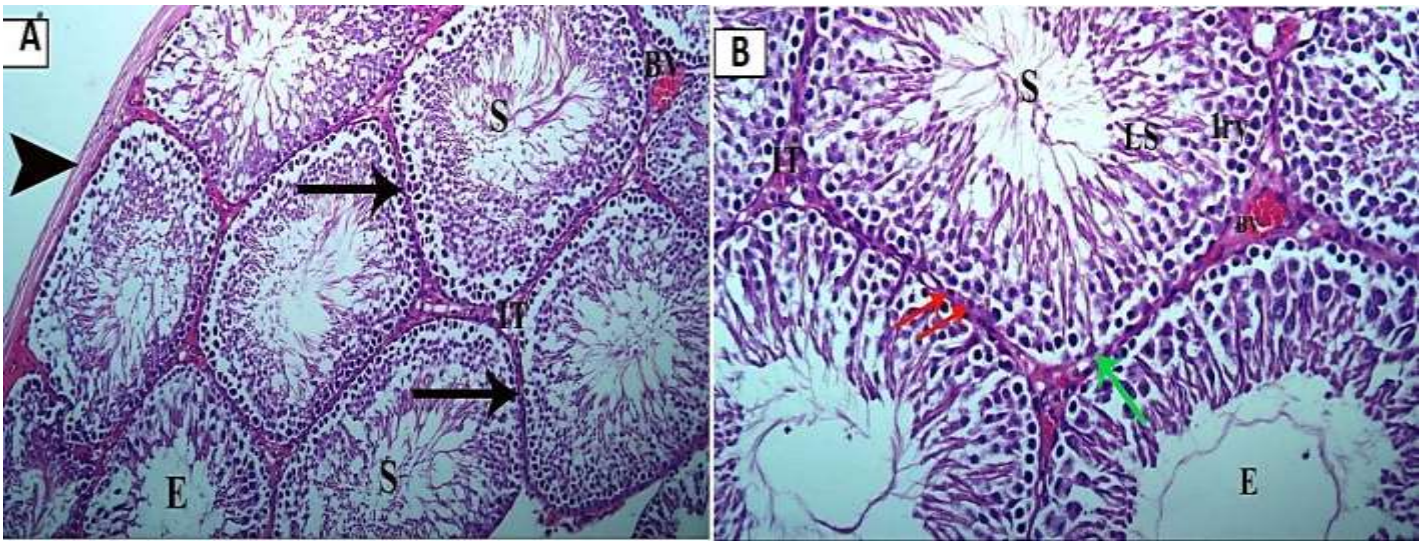

Figure (4): Photomicrographs of H&E-stained testicular sections from PAC+PN group: (A) presenting improved testicular architecture with regular tunica albuginea (arrowhead) enclosing closely packed seminiferous tubules (arrows) with mature sperms filling their lumens (S). Tubules are separated by narrow interstitial tissues (IT) with congested blood vessels (BV). Few tubules with an empty lumen (E). (B) presenting seminiferous tubules having stratified spermatogenic cells; spermatogonia (red arrow), primary spermatocytes (1ry), late spermatids (LS), and mature spermatozoa (S) fill the lumen, Sertoli cell (green arrow) also detected. Other tubules have empty lumen (E). Narrow interstitial tissue (IT) between the tubules with congested blood vessels (BV) (A X 100; B X 200).

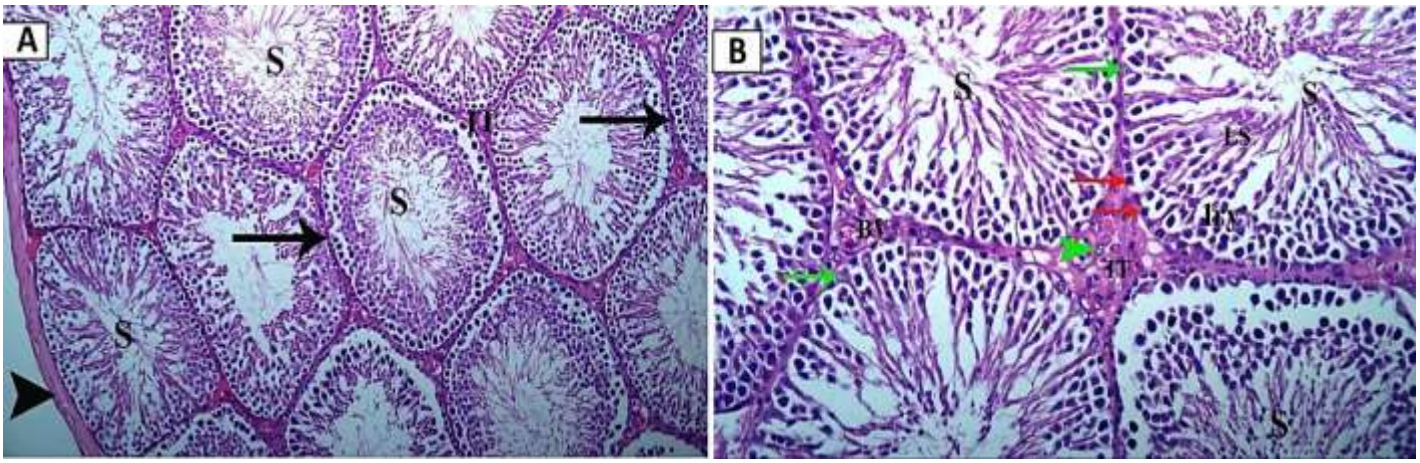

Figure (5): Photomicrographs of H&E-stained testicular sections from PAC+MLT group :(A) Showing testicular tissue nearly like the control group with normal tunica albuginea (arrowhead) surrounding closely packed, intact seminiferous tubules (arrows), their lumens filled with mature sperms(S). Narrow interstitial tissues (IT) with normal blood vessels are also seen between these tubules. (B) Showing seminiferous tubules containing regular Sertoli cells (green arrow), spermatogonia (red arrow) resting on basement membrane, primary spermatocytes (1ry), late spermatids (LS), and mature sperms(S) fill their lumen. Thin interstitial tissue (IT) with normal leydig cells (arrowhead) and normal blood vessels (BV) (A X 100; B X 200).

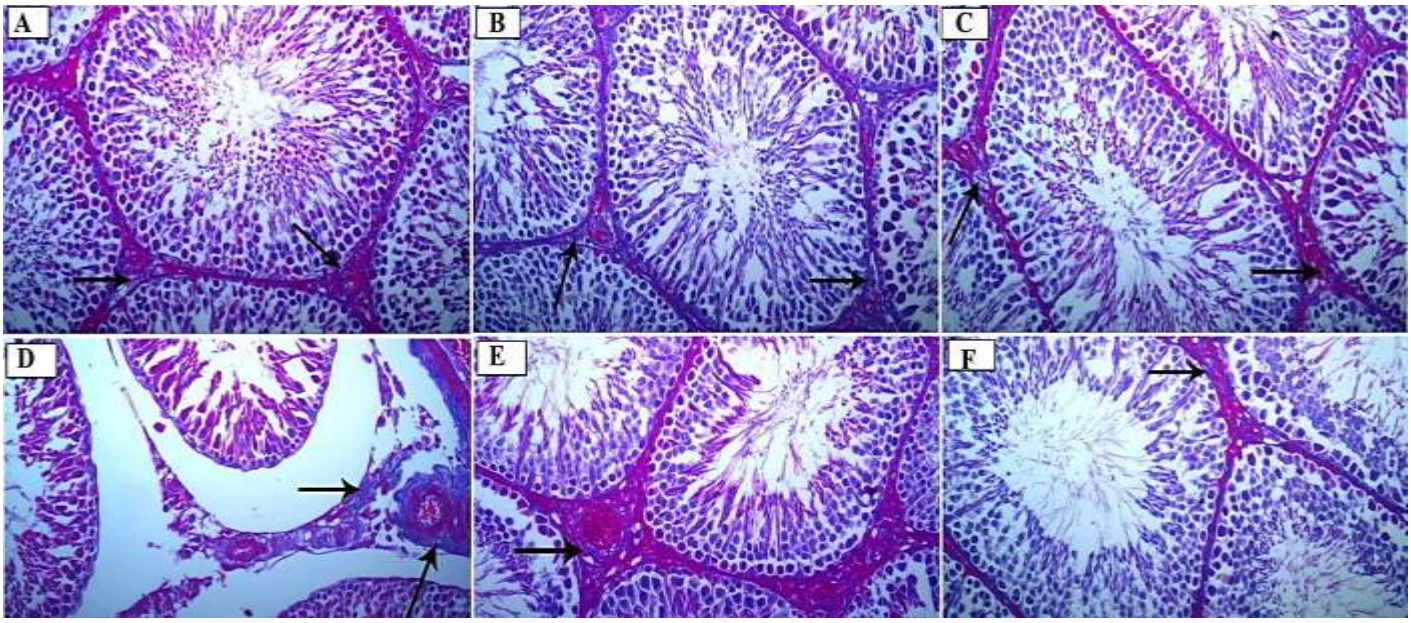

Figure (6): Photomicrographs of different sections from rat testes: (A) Control group; (B) PN group; and (C) MLT group demonstrating a normal distribution of a small quantity of collagen fiber in the interstitial tissue in between the seminiferous tubules. (D) The PAC group exhibits thick collagen fiber accumulation in the interstitial tissues and surrounding the dilated blood vessels. (E) The collagen fibers between the seminiferous tubules in the intervening spaces are moderately abundant in the PAC + PN group. (F) PAC + MLT group displaying few amounts of collagen fiber in the interstitial spaces (Masson trichome X200).

Immunohistochemical results

Bcl2

The control, PN and MLT groups showed intense positive Bcl-2 immunoreaction in the cytoplasm of all spermatogenic cells of seminiferous tubules as well as in Leydig cells (Figures 7A, B, & C). While negative Bcl-2 immunoreaction in spermatogenic cells and minimal positive immunoreaction in Leydig cells were observed in the PAC group (Figure 7D). PAC +PN group showed moderate positive Bcl-2 immunoexpression in basally located spermatogenic cells and Leydig cells (Figure 7E), but strong positive Bcl-2 immunoexpression was seen in the PAC +MLT group (Figure 7F).

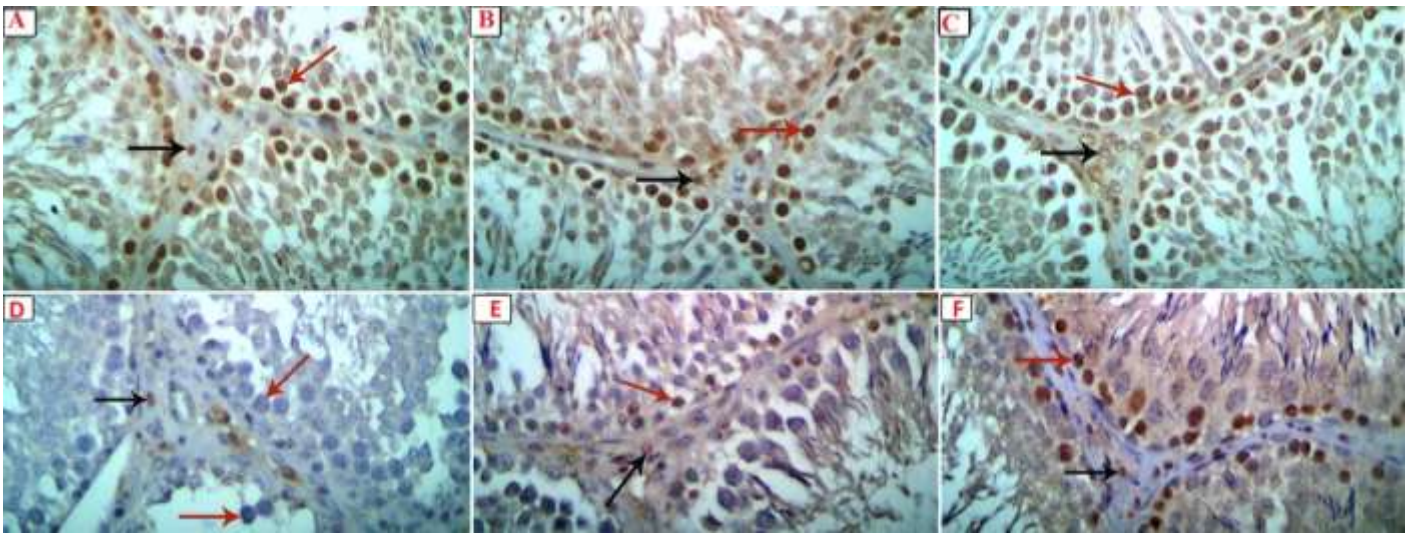

Figure (7): Photomicrographs of Bcl-2 immunostained testicular sections displaying: Intense positive Bcl-2 immunoreaction in the cytoplasm of all spermatogenic series (red arrow) as well as in Leydig cells (black arrow) in the control group (A), PN group (B) and MLT group (C). Negative Bcl-2 immunoreaction in all spermatogenic cells (red arrow) and minimal positive immunoreaction in Leydig cells (black arrow) were observed in the PAC group (D). Moderate positive Bcl-2 immunoexpression in basally located spermatogenic cells (red arrow) and Leydig cells (black arrow) were seen in the PAC +PN group (E). Intense positive Bcl-2 immunoexpression in basally located spermatogenic cells (red arrow) as well as in Leydig cells (black arrow) was seen in the PAC+MLT group (F) (Bcl-2 immunostaining X 400).

TNF-α:

Negative TNF-α cytoplasmic immunoreaction was observed in the interstitial tissues of the control group, PN group, and MLT group (Figures 8A,8B, and 8C). However, the PAC group showed marked TNF-α expression in the interstitial spaces between the seminiferous tubules (Figure 8D).PAC+PN group displayed moderate TNF-α expression (Figure 8E), while PAC+MLT group displayed minimal TNF-α expression in the interstitial areas (Figure 8F).

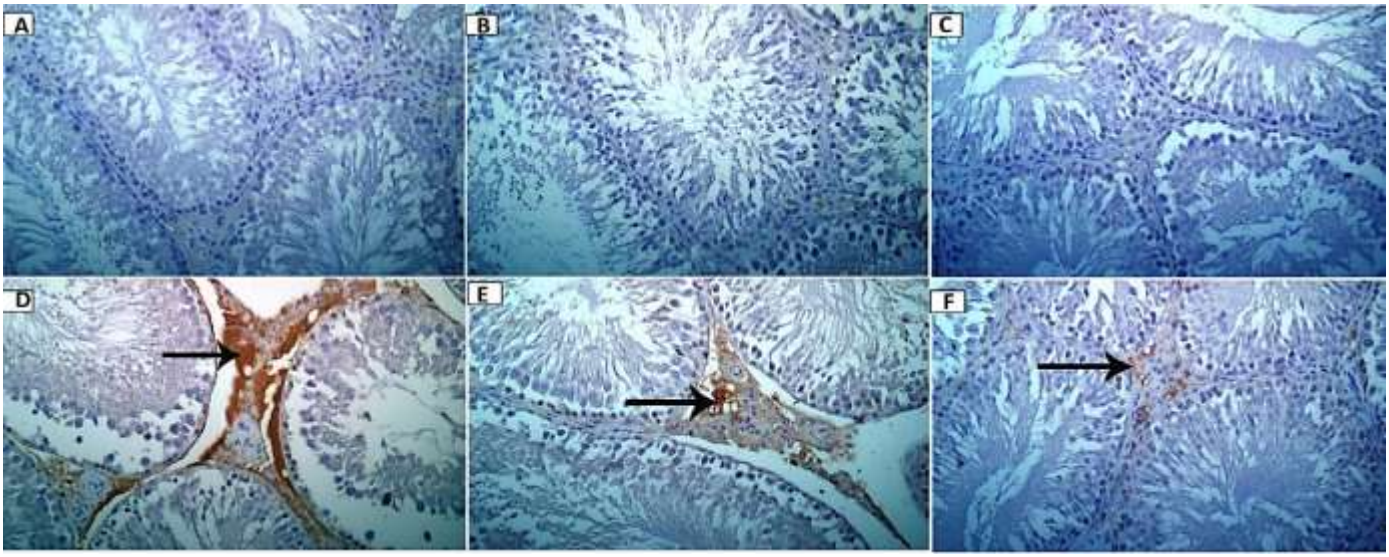

Figure (8): Photomicrographs of TNF-α immunoexpression in testicular tissues showing: Negative TNF-α cytoplasmic immunoreaction in the interstitial areas in control group (A), PN group (B) and MLT group (C). PAC group (D) presented marked TNF-α expression in the interstitial tissues (arrow). Moderate TNF-α expression (arrow) was observed in the PAC+PN group (E). While PAC+MLT group (F) presented minimal TNF-α immunoreaction (arrow)in the interstitial areas (TNF-α immunostaining X 200).

Histomorphometrically results

As presented in Table 3, rats given PAC treatment exhibited a notable rise ($p \leq 0.05$) in both the thickness of the tunica albuginea and the total number of empty seminiferous tubules, In contrast, it was discovered that there was a substantial ($p \leq 0.05$) decrease in the height of germinal epithelial cells and the widths of seminiferous tubules when in contrast to all other groups. However, these compromised testicular parameters were improved when PN or MLT supplementation was given to rats receiving PAC treatment. These groups showed a substantial decrease in tunica albuginea thickness in addition to the number of empty seminiferous tubules ($p \leq 0.05$); nonetheless, there was a significant increase in the diameters of seminiferous tubules and the height of germinal epithelial cells. ($p \leq 0.05$). Additionally, when contrasting the PAC+MLT-treated group to the control group, no discernible changes were seen in these parameters.

Table (4) showed that the PAC group's mean area% of collagen fiber accumulation was considerably larger than that of the control group ($p \leq 0.05$). As presented in the PAC+PN group and PAC+MLT group, the area percent of collagen deposition showed a major reduction ($p \leq 0.05$) relative to the PAC group.

As demonstrated in Table (5), the PAC group showed a notable increase in the mean area percentage of TNFα immunoreactivity in contrast to the control group ($p \leq 0.05$). This effect was significantly modified by co-administration of PN or MLT in the PAC+PN group and PAC +MLT group respectively ($p \leq 0.05$) comparable to the PAC group. On the other hand, the PAC group showed a major reduction in the mean area percent of BCL2 immunoreactivity relative to the control group ($p \leq 0.05$). Co-administration of PN or MLT in the PAC+PN group and PAC +MLT group significantly increased BCL2 mean area percent ($p \leq 0.05$). Additionally, PN only treated group and MLT only treated group showed no discernible changes in the levels of Bcl-2 immunoreactivity or TNFα compared to the control group.

Table 3: Histomorphometry analysis of testicular parameters in all groups.

Parameters	Group I control	Group II (PN)	Group III (MLT)	Group IV (PAC group)	Group V (PAC + PN)	Group VI (PAC + MLT)	P value
The thickness of tunica albuginea (mm)	30.6±0.3	29.2±0.2 d&e	29.7±0.2 d&e	56. 5±0.1 a, b,c, e & f	33.8±0.1 a,b,c &d	32.4±0.1 d	<0.001a 0.001b 0.001c <0.05e <0.02 f
Diameter of seminiferous tubules (mm)	356.6±0.8	356. 8±1 d&e	357.2±1.1 d&e	225.2±1.6 a, b,c, e & f	343.4±1 a,b,c &d	349.4±1.1 d	<0.001a 0.001b <0.001c <0.05e <0.02 f
Germinal epithelium height (mm)	85.17±0.2	85.87±0.4 d&e	86.01±0.2 d&e	55. 3±0.1 a, b,c, e & f	78.9±0.3 a,b,c &d	81.4±1d	<0.001a 0.001b <0.001c <0.04e <0.02 f
Number of seminiferous tubules without sperm	1.4 ±0.2	1.3 ±0.4 d&e	1.3 ±0.2 d&e	13.3 ±0.1 a, b,c, e & f	3.1 ±0.3 a,b,c &d	2.4 ±1.1 d	<0.001a 0.001b <0.001c <0.03e <0.02 f

Data is presented as mean ± SD, *: significance ≤ 0.05; One way ANOVA method followed by post-hoc Tukey's test, PN: Pinostrobin ; MLT: Melatonin ; PAC: paclitaxel, a: Significance vs Control, b: Significance vs Group II, c: Significance vs Group III, d: Significance vs group IV, e: Significance vs Group V, f: Significance vs Group VI

Table 4: area % ± SD of collagen fibre deposition in all different groups

	Group I (Control)	Group II (PN)	Group III (MLT)	Group IV (PAC)	Group V (PAC + PN)	Group VI (PAC + MLT)	P value
Collagen fiber deposition area%	5.3 ± 0.9	5.0 ± 0.4 d & e	4.3 ± 1 d & e	38.1 ± 2.6 a, b,c, e & f	10.4 ± 0.7 a,b,c ,d & f	8.5 ± 1.2 d	<0.001a 0.001b <0.001c <0.05e <0.02 f

Data expressed as mean±SD, *: significance ≤ 0.05; One way ANOVA method followed by post hoc Tukey's test; a: Significance vs Control, b: Significance vs Group II, c: Significance vs Group III, d: Significance vs Group IV, e: Significance vs Group V, f: Significance vs Group VI.

Table (5): All groups' mean area percent ± SD of TNFα and BCL2 immune expression.

	Group I Control	Group II (PN group)	Group III (MLT group)	Group IV (PAC group)	Group V (PAC+PN)	Group VI (PAC+MLT)	P value
TNFα area%	3.5 ± 0.6	2.8± 0.4 d & e	2.4 ± 1 d & e	52.6 ± 1.7 a, b,c, e & f	19.4 ± 0.7 a,b,c ,d & f	10.9 ± 1.3 d & e	<0.001[a] 0.001[b] <0.001[c] <0.04[e] <0.02 [f]
BCL2 area %	62.1 ± 2.9	62.07 ± 1.5 d & e	63.1 ± 4.1 d & e	11.8 ± 1.4 a, b,c, e & f	47.5 ± 1.6 a,b,c,d & f	55.2 ± 1.3 d & e	<0.001[a] 0.001[b] <0.001[c] <0.03[e] <0.02 [f]

Data expressed as mean±SD, *: significance ≤ 0.05; One way ANOVA method followed by Post-hoc Tukey's test, a: Significance vs Control, b: Significance vs Group II, c: Significance vs Group III, d: Significance vs group IV, e: Significance vs Group V, f: Significance vs Group VI

IV. Discussion:

Long-term anticancer medication therapy may result in problems with the reproductive organs. The common side effect of paclitaxel treatment is infertility.

This study assessed the detrimental consequences of PAC on the rat testis and compared the protective role of pinostrobin and melatonin against PAC-induced testicular impairment.

To evaluate oxidative stress, which is a key element in male infertility brought on by the harmful effects of paclitaxel, superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) levels were assessed in this study. According to (Schieber and Chandel,2014), oxidative stress is the outcome of an unbalance between the prooxidant and antioxidant systems, which produces excessive quantities of reactive oxygen species (ROS).

The testes express the antioxidant enzyme SOD, which regulates ROS levels and reduces possible harmfulness (Altintas et al., 2015; Wu et al., 2010). MDA is a persistent byproduct of lipid peroxidation brought on by ROS and is frequently employed as a ROS indicator. It indirectly reflects the harm done by oxygen-free radicals (Agarwal, Sengupta 2020). The antioxidant defense system of spermatozoa is weakened because of increased ROS generation (Scarlata and Flaherty, 2020). CAT is one of the important antioxidant enzymes that have a vital function in the catabolism of H2O2 (Tuzet et al., 2019).

The results of our investigations showed that PAC therapy dramatically raised MDA levels while extremely reducing CAT and SOD activities in testicular tissue. This may be due to the direct impact of paclitaxel on endothelial dysfunction and the production of free radicals (Serizawa et al., 2012; El Arem et al., 2017), and thus the oxidative stress damage caused by PAC reported by many studies (Kankılıç et al 2024; İleritürk et al., 2021)

The intake of PN in this study resulted in a substantial increase in SOD and CAT levels, which were subsequently decreased by paclitaxel and reduce the amount of MDA. Comparable outcomes were described in a work by (Ijaz et al., 2023) who clarified the role of PN against microplastic testicular toxicities in rats, which was explained by PN's antioxidant activity.

A similar effect was obtained when MLT and PAC were administered together, the SOD, and CAT activities increased and the amount of MDA decreased, demonstrating an antioxidant effect (Bonnefont & Collin, 2010; Najafi et al., 2018 and Aboelwafa et al ., 2022)

The hypothalamus pituitary-gonadal axis is a complicated network of signaling channels that primarily regulates testicular spermatogenesis. The pituitary gland's release of LH and FSH affects Sertoli cells and Leydig cells, which in turn control spermatogenesis and testicular development (Spaliviero et al., 2004).

According to our research, paclitaxel significantly decreased the mean testosterone, LH, and FSH plasma concentrations which are crucial for spermatogenesis. These outcomes are consistent with other earlier investigations (Afsar et al., 2017; Kianifard et al., 2019 and Balciolu et al., 2023). The results of our study manifested that the effects of PAC were reversed, by elevating the concentrations of testosterone, FSH and LH hormones , when PN was administered along with PAC, most likely via stabilizing the hypothalmic pituitary-gonadal axis and because of its anti-oxidant properties on the spermatogenesis process (liaz et al ., 2023).

Furthermore, these hormonal levels were nearly normalized by MLT treatment with PAC and this in agreement with the study conducted by(Ghasemi et al.,2010) on MLT against Busulfan toxicity and by (Hasanzadeh et al.,2017) on nicotine toxicity, which

linked melatonin's influence to its strong antioxidant actions on germ and somatic cells. This was further clarified by (Navid et al., 2017) who reported that melatonin influences Leydig cells, which in turn influences the testosterone secretion pathway and enhances the quality of sperm. Additionally, MLT controls secretory activity, growth, and proliferation of many testicular cells (Rossi et al., 2014) and makes Sertoli cells more sensitive to FSH during testicular development (Frungieri et al., 2005).

Specifically, we find that giving rats PAC for a month caused numerous histological alterations, such as the disarray of their seminiferous tubules, a significant reduction in their number, and even death of their germinal cells and desquamation of the cells within their lumen. Numerous studies have documented the detrimental effects of PAC on causing spermatogenic cell death (Okkay et al., 2021). Another study found that paclitaxel increased the quantity of aberrant spermatozoa while decreasing sperm motility and viability (Abd-Elrazek et al., 2020).

Few pyknotic Leydig cells were observed in this investigation in the wide interstitial gaps among the tubules. The basement membranes of certain tubules are deformed. The alterations align with the outcome of the study conducted by (Balcioglu et al., 2023), which demonstrated Leydig cell impairment in addition to mild anomalies and impaired epithelium in the paclitaxel-treated testicular tissue. It was determined that paclitaxel penetrating the testicular tissue through the interstitial space may be the cause of this injury. The close association between Leydig cells and their vasculature may be the cause of these problems since it puts them in danger of any exogenous toxin that causes a stop of spermatogenesis because of a lowering in testosterone secretion (Spaliviero et al., 2004). (Aboelwafa et al., 2023) reported wider intercellular gaps and apparent deformation of Sertoli cells in Taxol-treated testicular sections. In addition to spermatogenic cell death, the compromised Sertoli cells cause changes or reductions in the tubular secretory substances, which results in the sloughing or shedding of germ cells (Manivannan, et al., 2009).

Pinostrobin is an important dietary bioflavonoid found in fingerroot. It has been reported that PN showed several different bioactivities, including antibacterial and antioxidant (Atun et al., 2017; Marliyana et al., 2018), and anticancer effects by inhibiting cell development and encouraging cell death in several malignancies (Roman et al.,2017). It was found by this investigation that the histopathological damage caused by PAC was alleviated by the use of PN treatment and restored the architecture of testicular tissues. This matched with the study by (Ijaz et al.,2023) who reported that using of PN in contrast to the toxic effect of microplastics raised all germ cell counts and reverted entirely testicular damage. This may have been caused by PN's anti-inflammatory, anti-oxidative, anti-apoptotic, and androgenic properties (Hidajat et al., 2018; Patel et al., 2016). According to a prior study by (Gonz¬alez et al., 2022), PN can scavenge free radicals and reduce inflammation and relieve pain in mice's skin when cyclodextrins cause toxicity.

Our findings indicated that co-administration of PAC with MLT at a dose of 10 mg/kg b w to the rats for one month greatly diminished the histological injury of testicular tissue compared to PAC-treated rats. This is harmonized with the research of (Aboelwafa et al., 2022; Taslidere et al., 2023) who contend that the melatonin treatment mitigated the harmful consequences of chemotherapy on rodents' reproductive systems via its potent antioxidant influence.

The immunohistochemistry findings from this investigation demonstrated that the administration of PAC caused testicular apoptosis by decreasing the anti-apoptotic Bcl-2 protein and increasing the immunoexpressions of the apoptotic tumor suppressor protein TNF. Additionally, it has been reported that chemotherapeutics cause lipid, cholesterol, and protein peroxidation to cause male reproductive system oxidative stress. This leads to DNA destruction, apoptosis which causes faulty spermatogenesis and sterility (Ileriturk et al., 2023) PAC produced apoptotic damage in testicular tissues by inducing apoptotic factors and deactivating antiapoptotic proteins, according to a study by (İleritürk et al., 2021).

In The current study, it was found that when PN was administered in conjunction with PAC, the immunohistochemistry results revealed improvement that was linked to its anti-apoptotic action. Specifically, PN administration resulted in an upsurge in Bcl-2 expression and a decrease in TNF immunoexpressions (Ijaz et al., 2023).

Our immunohistochemistry results of the testicular tissues co-treated with MLT and PAC provided additional proof that MLT has anti-apoptotic properties against apoptosis induced by PAC. Significant increases in the immunoexpressions of active Bcl-2 protein and decreased TNF immunoexpressions supported these conclusions. This was in line with (Bozkurt et al., 2019) who illuminated in their research that melatonin significantly declines TNF α levels in melatonin-treated rats in comparison to diabetic rats.

In this investigation, we discovered that rats given PAC had significantly thicker tunica albuginea due to increased

fibrosis, which is a byproduct of PAC-induced cellular death. On the other hand, rat groups which received PN- concurrently with PAC and MLT co-administrated with PAC had control over PAC-induced cellular death, which in turn regained the thickness of the tunica albuginea to a nearly normal level. Therefore, the modification of tunica albuginea thickness may also have been influenced by the anti-fibrotic ability of MLT (Hu et al ., 2016).

The current study discovered that the injection of PAC reduced the diameter and epithelial height of seminiferous tubules, resulting in substantial structural damage to the testicles. However, by receiving PN- concurrently with PAC there was a significant increase in the diameters of seminiferous tubules and the height of germinal epithelial cells, in addition to that there was a substantial decrease in the number of empty seminiferous tubules. Furthermore, these previous histological changes including the diameter and height of seminiferous tubules were increased significantly and decreased the number of empty seminiferous tubules by Co-administration of PAC with MLT, and this matched with the study of (Ilbey et al., 2009) who proved the recovery influence of melatonin on the testicular damage induced by chemotherapy. MLT was discovered to transcend physiological barriers and prevent oxidative impact in both lipid and aqueous cellular settings, contrary to other antioxidants which are either hydrophilic or lipophilic. So it can shield spermatogonia and testicles from harmful chemicals, chemotherapeutic medications, and oxidative damage. (Cruz et al., (2014)

Nonetheless, it was registered that the rat group that received MLT concurrently with PAC had better histological characteristics, improved testicular parameters such as tunica albuginea thickness, seminiferous tubule diameters, and germinal epithelial heights, and the biochemical and hormonal levels to an extent were normalized in comparable to the rat group that received PN concurrently with PAC.

V. Conclusions: The outcomes of our research demonstrated that hazards of PAC were ameliorated as well as the hormonal levels were improved by the administration of PN or MLT in line with PAC. Therefore, it was suggested that using PN or MLT as a prophylactic medication while undergoing chemotherapy can reduce the risk of testicular damage. However, the improvement registered with MLT was higher than PN and this is probably attributed to the powerful antioxidant and anti-inflammatory effects of MLT.

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References

Abd-Elrazek, A. M.; El-dash, H. A.; & Said, N. I. (2020). The role of propolis against paclitaxel-induced oligospermia, sperm abnormality, oxidative stress and DNA damage in testes of male rats. *Andrologia*, *52*(1),.DOI: 10.1111/and.13394

Aboelwafa, H. R.; Ramadan, R. A.; El-Kott, A. F.; & Abdelhamid, F. M. (2022). The protective effect of melatonin supplementation against taxol-induced testicular cytotoxicity in adult rats. Brazilian Journal of Medical and Biological Research, 55 DOI: 10.1590/1414-431X2021e11614

Afsar, T.; Razak, S.; Khan, M. R.; & Almajwal, A. (2017). Acacia hydaspica ethyl acetate extract protects against cisplatin-induced DNA damage, oxidative stress, and testicular injuries in adult male rats. *BMC Cancer*, *17*(1), 883. https://doi.org/10.1186/s12885-017-3898-9

Agarwal, A.; & Sengupta, P. (2020). Oxidative stress and its association with male infertility. *Male infertility: contemporary clinical approaches, andrology, ART and antioxidants*, 57-68. https://doi.org/10.1007/978-3-030-32300-4_6

Al-Medhtiy, M. H.; Jabbar, A. A.; Aziz, P. Y.; & Abdulla, M. A. (2022). Hepatoprotective effect of Pinostrobin against Thioacetamide-induced liver cirrhosis in the rat. https://doi.org/10.21203/rs.3.rs-2289199/v1

Altintas, R.; Ciftci, O., Aydin, M.; Akpolat, N.; Oguz, F.; & Beytur, A.(2015). Quercetin prevents docetaxel-induced testicular damage in rats. *Andrologia*, *47*(3), 248–256. DOI: 10.1111/and.12253

Atun, S.; Handayani, S.; & Frindryani, L. F. (2017, August). Identification and antioxidant activity test of bioactive compound produced from ethanol extract of temukunci (Boesenbergia rotunda). In *AIP Conference Proceedings*.1868,(1). https://doi.org/10.1063/1.4995093 .

Balcioglu, E.; Göktepe, Ö.; TAN, F. C.; Bilgici, P.; Yakan, B.; & Özdamar, S. (2023). The role of curcumin against paclitaxel-induced oxidative stress and DNA damage in testes of adult male rats. *Turkish Journal of Medical Sciences*, *53*(1), 40-50. Doi**:** 10.55730/1300-0144.5556

Bonnefont-Rousselot, D.; & Collin, F. (2010). Melatonin: action as an antioxidant and potential applications in human disease and aging. *Toxicology*, *278*(1), 55-67. https://doi.org/10.1016/j.tox.2010.04.008

Bancroft, J.D.& Layton, C. (2018) The hematoxylin and eosin. In: Suvarna KS, Layton C, Bancroft JD (eds) Bancroft's theory and practice of histological techniques

e-book, 8th ed. Elsevier Health Science, pp 126–138. https:// books. google. com. eg/ books? id=CERPD wAAQB AJ

Borovskaya, T. G.; Goldberg, V. E.; Rumpel, O. A.; Pahomova, A. V.; Perova, A. V.; & Goldberg, E. D. (2009). The rat spermatogenesis after injection of paclitaxel (antitumor agent). Bulletin of Experimental Biology and Medicine, 147(6),715–718. DOI: 10.1007/s10517-009-0596-x

Bozkurt, A. S., Karabakan, M., Aydin, M., GÜMÜŞTAŞ, S., Onk, D., GÜRSUL, C., & EROL, H. S. (2019). Effects of melatonin treatment on the spermatogenesis and serum inflammatory cytokine levels in diabetic rats. DOI:10.5455/ANNALSMEDRES.2018.09.192

Chance, B. & Maehly, A.E. (1995) Assay of catalases and peroxidases, Meth. Enzym. 136 764–775. https://doi.org/10.1016/j.biopha.2023.114686

Cruz, M. H. C.; Leal, C. L. V.; da Cruz, J. F.; Tan, D.-X.; & Reiter, R. J. (2014). Role of melatonin on production and preservation of gametes and embryos: A brief review. Animal Reproduction Science, 145(3–4), 150–160. https://doi.org/10.1016/j.anireprosci.2014.01.011

El Arem, A.; Lahouar, L.; Saafi, E. B.; Thouri, A.; Ghrairi, F.; Houas, Z Neffati, F & Achour, L. (2017). Dichloroacetic acid-induced testicular toxicity in male rats and the protective effect of date fruit extract. *BMC Pharmacology and Toxicology*, *18*, 1-9. DOI:10.1186/s40360-017-0127-8

El-Azab, N. E. E.; & Elmahalaway, A. M. (2019). A histological and immunohistochemical study on testicular changes induced by Sliver nanoparticles in adult rats and the Possible protective role of Camel Milk. Egyptian Journal of Histology, 42(4), 1044-1058. DOI: 10.21608/ejh.2019.13507.1126

Ferlay, J.; Colombet, M.; Soerjomataram, I.; Mathers, C.; Parkin, D. M.; Piñeros, M., ... & Bray, F. (2019). Estimating the global cancer incidence and mortality in 2018: GLOBOCAN sources and methods. *International journal of cancer*, *144*(8), 1941-1953 https://doi.org/10.1002/ijc.31937

Frungieri, M. B.; Mayerhofer, A.; Zitta, K.; Pignataro, O. P.;Calandra, R. S.; & Gonzalez-Calvar, S. I. (2005). The direct effect of melatonin on Syrian hamster testes: Melatonin subtype 1a receptors, inhibition of androgen production, and interaction with the local corticotropin-releasing hormone system. Endocrinology, 146(3), 1541–1552. https://doi.org/10.1210/en.2004-0990

Ghasemi, F. M.; Faghani, M.; Khajehjahromi, S.; Bahadori, M.; Nasiri, E.; & Hemadi, M. (2010). Effect of melatonin on proliferative activity and apoptosis in spermatogenic cells in mouse under chemotherapy. *Journal of Reproduction and Contraception*, *21*(2), 79-94. Doi: 10.1016/S1001-7844(10)60016-8

González, A. S.; Tellini, V. H. S.; & Gutiérrez, D. M. B. (2022). Study of the dermal anti-inflammatory, antioxidant, and analgesic activity of pinostrobin. *Heliyon*, *8*(9). DOI:https://doi.org/10.1016/j.heliyon.2022.e10413

Hasanzadeh, S.; Khosh, A. H.; & Jalali, A. S. (2017). Ameliorative effects of Achillea millefolium inflorescences alcoholic extract on nicotine-induced reproductive toxicity in the male rat: Apoptotic and biochemical evidence. In *Veterinary Research Forum* (8 (2): 97 PMCID: PMC5524545

Hidajati, N.,; Tukiran, T.,; Setiabudi, D. A.; & Wardana, A. P. (2018). Antioxidant activity of palmitic acid and pinostrobin from methanol extract of Syzygium litoralle (Myrtaceae). In *International Conference on Science and Technology*. (183-187). Atlantis Press. DOI:10.2991/icst-18.2018.39

Hu, W.; Ma, Z.; Jiang, S.; Fan, C.; Deng, C.; Yan, X Di, S.; Lv, J.,;Reiter, R.J.& Yang, Y. (2016). Melatonin: the dawning of a treatment for fibrosis?. Journal of Pineal Research, 60(2), 121-131. https://doi.org/10.1111/jpi.12302

Ijaz, M. U.; Shahzadi, S.; Hamza, A.; Azmat, R.; Anwar, H.;Afsar, T.; ... & Razak, S. (2023). Alleviative effects of pinostrobin against cadmium-induced renal toxicity in rats by reducing oxidative stress, apoptosis, inflammation, and mitochondrial dysfunction. Frontiers in Nutrition, 10, 1175008. DOI: 10.3389/fnut.2023.1175008

Ilbey, Y. O.; Ozbek, E.; Simsek, A.; Otunctemur, A.; Cekmen, M.; & Somay, A. (2009). Potential chemoprotective effect of melatonin in cyclophosphamide-and cisplatin-induced testicular damage in rats. *Fertility and sterility*, *92*(3), 1124-1132. https://doi.org/10.1016/j.fertnstert.2008.07.1758

Ileriturk, M., Benzer, F., Aksu, E. H., Yildirim, S., Kandemir, F. M., Dogan, T., ... & Genc, A. (2021). Chrysin protects against testicular toxicity caused by lead acetate in rats with its antioxidant, anti-inflammatory, and antiapoptotic properties. Journal of Food Biochemistry, 45(2), https://doi.org/10.1111/jfbc.13593

Ileriturk, M.; Kandemir, O.; Akaras, N.; Simsek, H.; Genc, A.; & Kandemir, F. M. (2023). Hesperidin has a protective effect on paclitaxel-induced testicular toxicity through regulating oxidative stress, apoptosis, inflammation and endoplasmic reticulum stress. *Reproductive Toxicology*, *118*.DOI: 10.1016/j.reprotox.2023.108369

Kamsrijai, U.; Wongchitrat, P.; Nopparat, C.; Satayavivad, J.; & Govitrapong, P. (2020). Melatonin attenuates streptozotocin-induced Alzheimer-like features in hyperglycemic rats. *Neurochemistry International*, *132*, 104601. DOI: 10.1016/j.neuint.2019.104601

Kanchanapiboon, J.; Kongsa, U.; Pattamadilok, D.; Kamponchaidet, S.; Wachisunthon, D.; Poonsatha, S.; & Tuntoaw, S. (2020). Boesenbergia rotunda extract inhibits Candida albicans biofilm formation by pinostrobin and pinocembrin. *Journal of Ethnopharmacology*, *261*, 113193. DOI: 10.1016/j.jep.2020.113193

Kankılıç, N. A.: Küçükler, S.: Gür, C.: Akarsu, S. A.: Akaras, N.: Şimşek, H., ... & Kandemir, F. M. (2024). Naringin protects against paclitaxel-induced toxicity in rat testicular tissues by regulating genes in pro-inflammatory cytokines, oxidative stress, apoptosis, and JNK/MAPK signaling pathways. *Journal of Biochemical and Molecular Toxicology*, *38*(7). DOI: 10.1002/jbt.23751

Kathiravan, G.; Sureban, S. M.; Sree, H. N.; Bhuvaneshwari, V.; & Kramony, E. (2012). Isolation of anticancer drug TAXOL from Pestalotiopsis breviseta with apoptosis and B-Cell lymphoma protein docking studies. Journal of Basic and Clinical Pharmacy, 4(1), 14–19. DOI: 10.3389/fphar.2018.01253

Kianifard, D.; Ehsani, A.; Daneshgar, P. Z. (2019). Effect of monosodium glutamate on testicular tissue of paclitaxel-treated mice: An experimental study. *International Journal of Reproductive BioMedicine (IJRM)*, *17*(11), 819–830. DOI: 10.18502/ijrm.v17i10.5492

Küçükler, S.: Çomaklı, S.: Özdemir, S.: Çağlayan, C.: & Kandemir: F. M. (2021). Hesperidin protects against chlorpyrifos-induced chronic hepato-renal toxicity in rats associated with oxidative stress, inflammation, apoptosis, autophagy, and up-regulation of PARP-1/VEGF. *Environmental toxicology*, *36*(8), 1600-1617. **Doi:** 10.1002/tox.23156

Luo, C.; Yang, Q.; Liu, Y.; Zhou, S.; Jiang, J.; Reiter, R. J.; ... & Wang, X. (2019). The multiple protective roles and molecular mechanisms of melatonin and its precursor N-acetylserotonin in targeting brain injury and liver damage and in maintaining bone health. *Free Radical Biology and Medicine*, *130*, 215-233. DOI: 10.1016/j.freeradbiomed.2018.10.402

Malekinejad, H.; Fani, M.; Shafiee-Roodbari, S. K.; Delkhosh-Kasmaie, F.; & Rezaei-Golmisheh, A. (2017). Crosstalk between E2f1 and c-Myc mediates the hepato-protective effect of royal jelly on taxol-induced damages. *Human & Experimental Toxicology*, *36*(6), 626-637. DOI: 10.1177/0960327116660752

Manivannan, B.; Mittal, R.; Goyal, S.; Ansari, A. S.; & Lohiya, N. K. (2009). Sperm characteristics and ultrastructure of testes of rats after long-term treatment with the methanol subfraction of Carica papaya seeds. *Asian journal of andrology*, *11*(5), 583. doi: 10.1038/Aja.2009.25

Marliyana, S. D.; Mujahidin, D.; & Syah, Y. M. (2018). Pinostrobin derivatives from Prenylation Reaction and their antibacterial activity against clinical bacteria. In *IOP Conference Series: Materials Science and Engineering*. 349: 012057. DOI:10.1063/1.4995093

Najafi, D., Taheri, R. A., Najafi, A., Rouhollahi, A. A., & Alvarez-Rodriguez, M. (2018). Effect of Achillea millefolium-loaded Nanophytosome in the post-thawing sperm quality and oxidative status of roostersemen. *Cryobiology*, *82*, 37–42. DOI: 10.1016/j.cryobiol.2018.04.011

Navid, S.; Rastegar, T.; Baazm, M.; Alizadeh, R.; Talebi, A., Gholami, K., Khosravi-Farsani, S., Koruji, M., & Abbasi, M. (2017). In vitro effects of melatonin on the colonization of neonate mouse spermatogonial stem cells. Systems Biology in Reproductive Medicine, 63(6), 370–381. DOI: 10.1080/19396368.2017.1358774

Okkay, U.; Ferah Okkay, I.; Cicek, B.; Aydin, I. C.; Ertugrul, M. S.; Bayram, C& Hacimuftuoglu, A. (2021). Achillea millefolium alleviates testicular damage in paclitaxel-intoxicated rats via attenuation of testicular oxido-inflammatory stress and apoptotic responses. *Andrologia*, *53*(5**).** DOI: 10.1111/and.14028

Patel, N. K.; Jaiswal, G.; & Bhutani, K. K. (2016). A review on biological sources, chemistry and pharmacological activities of pinostrobin. *Natural product research*, *30*(18), 2017-2027. DOI: 10.1080/14786419.2015.1107556

Petrosyan, K.; Tamayo, R.; & Joseph, D. (2002). Sensitivity of a novel biotin-free detection reagent (Powervision+™) for immunohistochemistry. *Journal of Histotechnology*, *25*(4), 247-250. https://doi.org/10.1179/his.2002.25.4.247

Roman, W. A.; Gomes, D. B.; Zanchet, B.; Schönell, A. P.; Diel, K. A.; Banzato, T. P., ... & Santos, C. A. M. (2017). Antiproliferative effects of pinostrobin and 5, 6-dehydrokavain isolated from leaves of Alpinia zerumbet. *Revista Brasileira de Farmacognosia*, *27*, 592-598. https://doi.org/10.1016/j.bjp.2017.05.007

Rossi, S. P.; Windschuettl, S.; Matzkin, M. E.; Terradas, C.; Ponzio, R.; Puigdomenech, E.; ... & Frungieri, M. B. (2014). Melatonin in testes of infertile men: evidence for anti-proliferative and anti-oxidant effects on local

macrophage and mast cell populations. *Andrology*, *2*(3), 436-449. DOI: 10.1111/j.2047-2927.2014.00207.x

Rossi, S.; Windschuettl, S.; Matzkin, M.; Terradas, C.; Ponzio, R.; Puigdomenech, E.; Levalle, O.; Calandra, R.; Mayerhofer, A.; & Frungieri, M. (2014). Melatonin in testes of infertile men: Evidence for anti-proliferative and anti-oxidant effects on local macrophage and mast cell populations. Andrology, 2(3), 436–449. https://doi.org/10.1111/j.2047-2927.2014.00207.x

Scarlata, E.; & O'Flaherty, C. (2020). Antioxidant enzymes and male fertility: Lessons from knockout models. *Antioxidants & redox signaling*, *32*(8), 569-580. https://doi.org/10.1089/ars.2019.7985

Schieber, M.; & Chandel, N. S. (2014). ROS function in redox signaling and oxidative stress. *Current Biology*, *24*(10), R453-R462 doi: 10.1016/j.cub.2014.03.034

Serizawa, K.-I.; Yogo, K.; Aizawa, K.; Tashiro, Y.; Takahari, Y.; Sekine, K.; Suzuki, T.; Ishizuka, NIshida, H. (2012). Paclitaxel-induced endothelial dysfunction in living rats is prevented by nicorandil via the reduction of oxidative stress. Journal of Pharmacological Sciences, 119(4), 349–358. https://doi.org/10.1254/jphs.12067FP

Spaliviero, J. A.; Jimenez, M.; Allan, C. M.; & Handelsman, D. J. (2004).Luteinizing hormone receptor-mediated effects on initiation of spermatogenesis in gonadotropin-deficient (hpg) mice are replicated by testosterone. Biology of Reproduction, 70(1), 32–38. https://doi.org/10.1095/biolreprod.103.019398

Sun, J.; Chen, Y.; Li, M.,; & Ge, Z. (1998). Role of antioxidant enzymes on ionizing radiation resistance. *Free Radical Biology and Medicine*, *24*(4), 586-593. https://doi.org/10.1016/s0891-5849(97)00291-8

Tan, D. X., Manchester, L. C., Hardeland, R., Lopez-Burillo, S., Mayo, J. C., Sainz, R. M., & Reiter, R. J. (2003). Melatonin: a hormone, a tissue factor, an autocoid, a paracoid, and an antioxidant vitamin. *Journal of pineal research*, *34*(1), 75-78 DOI:10.1034/j.1600-079x.2003.02111.x

Taslidere, E. L. İ. F.; Esrefoglu, M.; Tok, O. E.; Taslidere, B.; & Bulut, H. (2023). Effects of melatonin on both testicular regeneration and recovery of spermatogenesis in busulfan-treated rats. *Brazilian Journal of Pharmaceutical Sciences*, *58*. Doi: 10.1590/s2175-97902022e20945

Tuzet, A.; Rahantaniaina, M. S.; & Noctor, G. (2019). Analyzing the function of catalase and the ascorbate–glutathione pathway in H2O2 processing: Insights from an experimentally constrained kinetic model. *Antioxidants & Redox Signaling*, *30*(9), 1238-1268. https://doi.org/10.1089/ars.2018.7601

Wang, Y.; Zhou, L.; Xiao, M.; Sun, Z. L.; & Zhang, C. Y. (2017). Nanomedicine-based paclitaxel induced apoptotic signaling pathways in A562 leukemia cancer cells. *Colloids and Surfaces B: Biointerfaces*, *149*, 16-22 https://doi.org/10.1016/j.colsurfb.2016.08.022

Zhang, Q. Y.; Wang, F. X.; Jia, K. K.; & Kong, L. D. (2018). Natural product interventions for chemotherapy and radiotherapy-induced side effects. Frontiers in Pharmacology, 9(6), 1253. DOI: 10.3389/fphar.2018.01253

Metwally, A., Kamal, K., Albanna, E. (2024). Comparative Study on the Ameliorative Influence of Melatonin and Pinostrobin on the Testicular Damage Induced by Paclitaxel in Adult Male Albino Rats: Histological and Immunohistochemical Study. *Zagazig Journal of Forensic Medicine and Toxicology*, 23 (1): 74- 89 doi: 10.21608/zjfm.2024.299711.1192