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# Protective Effects of Rutin and Hesperidin on Cisplatin-Induced Testicular Dysfunction in Rats

Ruby Shahzad<sup>1</sup>, Ali Abbas<sup>2</sup>, Kajal<sup>3</sup>, Anchal<sup>4</sup>, Asma Hafeez<sup>5</sup>, Areesha Shaikh<sup>6</sup>

|                                                                                        | Authors' Contribution                                                                        |
|----------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|
| 1 Assistant Professor, Department of Biochemistry, Isra University, Hyderabad Pakistan | Study conception, design, data analysis, and manuscript drafting and final manuscript review |
| 2 Associate Professor, Department of Pharmacology, Isra University, Hyderabad Pakistan | Data collection, literature review, manuscript preparation, and final manuscript review      |
| 3 Medical Student, Isra University, Hyderabad Pakistan                                 | Data collection, manuscript, preparation, and final manuscript review                        |
| 4 Medical Student, Isra University, Hyderabad Pakistan                                 | Data collection, manuscript, preparation, and final manuscript review                        |
| 5 Medical Student, Isra University, Hyderabad Pakistan                                 | Data collection, manuscript, preparation, and final manuscript review                        |
| 6 Medical Student, Isra University, Hyderabad Pakistan                                 | Data collection, manuscript, preparation, and final manuscript review                        |

Dr. Ali Abbas

Correspondence: Associate Professor, Department of Pharmacology, Isra University, Hyderabad Pakistan  
Email: alee.abbas@hotmail.com

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## ABSTRACT

**Background:** Cisplatin is a common chemotherapeutic agent that is known to cause testicular toxicity by oxidative stress, inflammation, and apoptosis, resulting in impaired reproductive function in male patients. Natural flavonoids such as rutin and hesperidin have been shown to have potential protective effects due to their antioxidant properties. **Objective:** To compare the protective effect of rutin and hesperidin against cisplatin-induced testicular dysfunction in rats. **Study Design:** Quasi-experimental animal study. **Setting:** Isra University, Hyderabad Pakistan. **Duration:** June to November 2025. **Methods:** A total of 50 adult male Wistar rats were randomly divided into 5 groups (n = 10). Group A was the control, Group B received cisplatin (7 mg/kg, one intraperitoneal dose), and Groups C, D, and E received cisplatin followed by hesperidin (50 mg/kg/day), rutin (75 mg/kg/day), and their combination, respectively, for one month. Serum reproductive hormone levels, oxidative stress markers, sperm parameters, and histopathological changes were assessed. One-way ANOVA and Tukey's post hoc test were used to conduct the statistical analysis. **Results:** Cisplatin significantly decreased the body and testicular weights, reproductive hormones, and sperm parameters, and increased oxidative stress and histopathological damage ( $p < 0.001$ ). The combination of rutin and hesperidin significantly enhanced these parameters ( $p < 0.05$ ), with the combination group showing near-normal restoration and the most significant protective effects. **Conclusion:** Rutin and hesperidin are effective in reducing cisplatin-induced testicular dysfunction, possibly via antioxidant and cytoprotective actions, with combination therapy proving most effective.

**Keywords:** Cisplatin, Hesperidin, Rutin, Oxidative stress, Spermatogenesis, Testicular diseases.

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## INTRODUCTION

About one in six couples worldwide are affected by infertility, which continues to be a major global health concern and imposes a considerable psychological and social cost.<sup>1</sup> Cisplatin is one of the most common chemotherapeutic agents and has markedly enhanced survival rates in several malignancies such as testicular, ovarian, and lung cancer.<sup>2</sup> Although it has clinical efficacy, its use is often restricted by dose-related toxicities of several organ systems.<sup>3</sup> Testicular toxicity has emerged as one of these, with growing concern over its impact on male fertility and reproductive health. Testicular dysfunction induced by cisplatin is marked by impaired spermatogenesis, disruption of steroidogenesis, hormonal derangements, oxidative stress, and seminiferous tubule damage, etc.<sup>4</sup> Such negative effects not only deteriorate

reproductive ability, but also adversely affect the quality of life of cancer survivors, especially among the young population.

The mechanism underlying pathophysiological changes in cisplatin-induced gonadotoxicity is multifactorial, with oxidative stress as a core component. Cisplatin stimulates the overproduction of reactive oxygen species (ROS), which causes lipid peroxidation, protein denaturation, and DNA damage of testicular tissue.<sup>5</sup> This oxidative injury interferes with the integrity of the germ cells and the Sertoli cells, which ultimately affects spermatogenesis. Moreover, cisplatin has been shown to cause an inflammatory response and activate apoptotic pathways, including up-regulation of pro-apoptotic proteins such as Bax and caspase-3 and down-regulation of anti-apoptotic proteins such as Bcl-2. All these mechanisms lead to progressive testicular damage and functional loss.<sup>6</sup>

The use of natural antioxidants to reduce chemotherapy-induced toxicities has attracted increasing interest in recent years. Flavonoids are a heterogeneous family of polyphenolic compounds commonly found in plants that have shown strong antioxidant, anti-inflammatory, and anti-apoptotic effects. Two of them, rutin and hesperidin, have become promising candidates, as their pharmacological profiles are well documented. Rutin, a glycoside of quercetin, is reported to act as a free radical scavenger, improve the endogenous antioxidant defense, and prevent oxidative damage of cell membranes.<sup>7</sup> Likewise, hesperidin, a citrus flavonoid, is also a powerful antioxidant and is reported to regulate inflammatory mediators and apoptotic signaling pathways.<sup>8</sup>

Past experimental research has alluded to the fact that rutin and hesperidin can have protective properties against many types of organ toxicity such as renal toxicity, hepatic toxicity, and reproductive toxicity. Nevertheless, there is limited data on their contribution to cisplatin-induced testicular dysfunction, and there has been little comparison or combination of their effects. The complementary mechanism of action between rutin and hesperidin, which may act synergistically, could be an interesting therapeutic modality for reducing gonadotoxicity.

Thus, the current research aimed to evaluate the protective effects of rutin and hesperidin, administered individually and in combination, against cisplatin-induced testicular dysfunction in rats by assessing reproductive hormone levels, testicular oxidative stress markers, sperm parameters, and histopathological changes.

## METHODS

This is a quasi-experimental animal study conducted from June to November 2025, after approval from the ERC/ERB vide letter NO. IU/CP.REC(FBMS)/2025/-075 Dated. 16<sup>th</sup> June 2025. Fifty healthy rats with a weight of 200-250 g were used. A standard power analysis approach was employed to determine the sample size based on prior research.<sup>9,10</sup> Animals were kept under controlled conditions (22 ± 2°C, 12-hour light-dark cycle, 50-60% humidity) with free access to standard pellet food and water.

Animals were randomly assigned to five groups (n = 10 each). Group A (control group) was fed a basal diet and distilled water over a period of one month and was intraperitoneally injected with normal saline. Group B (cisplatin group): cisplatin (20 mg/kg body weight) was administered intraperitoneally (one injection) to cause testicular dysfunction. Group C (cisplatin + hesperidin group) received cisplatin as described, followed by oral hesperidin (50 mg/kg/day) the next day for 1 month. Group D (cisplatin + rutin group) received cisplatin, followed by rutin (75mg/kg/day) orally for 1 month. Group E (cisplatin + combination group) was administered cisplatin, then administered hesperidin and rutin at the same doses over a period of one month. Hesperidin, rutin, and cisplatin doses were chosen based on previously published studies reporting their pharmacological and toxicological effects.<sup>11-13</sup>

At the conclusion of the experiment, heart punctures were used to obtain blood samples from the sedated experimental animals. The serum was centrifuged and preserved for hormone analysis.

The animals were then sacrificed, and testes were carefully excised, cleaned of surrounding tissue, and weighed. A single testis from each animal was homogenized in phosphate-buffered saline to analyze biochemical parameters, and the other was fixed in 10 per cent neutral-buffered formalin to examine histopathology.

Following the manufacturer's instructions, commercially available enzyme-linked immunosorbent assay kits were used to measure the amounts of testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) in the serum. Testicular oxidative stress was measured using standard spectrophotometric techniques to estimate malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH).

To analyze the sperm, the cauda epididymis was removed, chopped in physiological saline, and left to incubate to allow the dispersion of the sperm. Standard microscopic techniques were used to assess sperm count, motility, and morphology in accordance with established protocols. Paraffin-embedded sections of the testicles were stained with hematoxylin and eosin and examined histopathologically. The Johnsen scoring method was used to assess spermatogenesis, and a semi-quantitative scoring system from a prior study was used to grade the severity of tissue alterations (such as seminiferous tubular degeneration, germ cell loss, and interstitial edema) as none (0), mild (I), moderate (II), and severe (III).<sup>14</sup>

One-way analysis of variance was used to conduct the statistical analysis with a post hoc test (Tukey) to carry out multiple comparisons.

## RESULTS

The administration of cisplatin (Group B) led to a significant decrease in final body weight, testicular weight, and gonadosomatic index relative to the control group (Group A) ( $p < 0.001$ ).

Hesperidin (Group C) and rutin (Group D) treatments had a significant positive effect on all parameters compared with the cisplatin group ( $p < 0.05$ ), yet the values were lower than the control. The combination therapy (Group E) had the highest protective effect, with values comparable to the control group and significantly better than those of individual treatments.

**Table 1: Body and testicular weights**

| Parameter               | Group A     | Group B                  | Group C                  | Group D                  | Group E                   | F-value | p-value |
|-------------------------|-------------|--------------------------|--------------------------|--------------------------|---------------------------|---------|---------|
| Final Body Weight (g)   | 245 ± 8     | 198 ± 10 <sup>a</sup>    | 228 ± 9 <sup>b</sup>     | 225 ± 11 <sup>b</sup>    | 238 ± 7 <sup>bc</sup>     | 52.4    | <0.001  |
| Testicular Weight (g)   | 1.62 ± 0.08 | 1.08 ± 0.07 <sup>a</sup> | 1.42 ± 0.09 <sup>b</sup> | 1.38 ± 0.08 <sup>b</sup> | 1.53 ± 0.06 <sup>bc</sup> | 88.6    | <0.001  |
| Gonadosomatic Index (%) | 0.66 ± 0.03 | 0.54 ± 0.04 <sup>a</sup> | 0.62 ± 0.03 <sup>b</sup> | 0.61 ± 0.04 <sup>b</sup> | 0.65 ± 0.02 <sup>bc</sup> | 24.7    | <0.001  |

Data are presented as mean ± SD (n = 10). One-way ANOVA followed by Tukey's post hoc test was used. Different superscript letters indicate statistically significant differences between groups ( $p < 0.05$ )

The treatment with cisplatin (Group B) led to a drastic reduction in serum testosterone, LH, and FSH levels compared with the control group (Group A) ( $p < 0.001$ ).

Hesperidin (Group C) and rutin (Group D) treatments significantly restored hormone levels compared with the cisplatin group ( $p < 0.05$ ), but did not completely reach control levels. The combination group (Group E) showed the most significant improvement, with hormone levels close to normal and much higher than those in the treatment groups separately.

**Table 2: Serum Reproductive Hormones**

| Parameter            | Group A     | Group B                  | Group C                  | Group D                  | Group E                   | F-value | p-value |
|----------------------|-------------|--------------------------|--------------------------|--------------------------|---------------------------|---------|---------|
| Testosterone (ng/mL) | 4.82 ± 0.45 | 1.62 ± 0.38 <sup>a</sup> | 3.75 ± 0.41 <sup>b</sup> | 3.58 ± 0.47 <sup>b</sup> | 4.35 ± 0.39 <sup>bc</sup> | 136.2   | <0.001  |
| LH (mIU/mL)          | 2.85 ± 0.30 | 1.15 ± 0.28 <sup>a</sup> | 2.34 ± 0.26 <sup>b</sup> | 2.21 ± 0.31 <sup>b</sup> | 2.72 ± 0.24 <sup>bc</sup> | 94.7    | <0.001  |
| FSH (mIU/mL)         | 3.12 ± 0.35 | 1.38 ± 0.32 <sup>a</sup> | 2.65 ± 0.29 <sup>b</sup> | 2.58 ± 0.34 <sup>b</sup> | 2.95 ± 0.27 <sup>bc</sup> | 89.3    | <0.001  |

Data are presented as mean ± SD (n = 10). One-way ANOVA followed by Tukey's post hoc test was used. Different superscript letters indicate statistically significant differences between groups ( $p < 0.05$ ).

The administration of cisplatin (Group B) led to a marked increase in MDA levels and significant decreases in antioxidant enzymes (SOD, CAT, and GSH) compared with the control group (Group A) ( $p < 0.001$ ), indicating increased oxidative stress.

Hesperidin (Group C) and rutin (Group D) treatments significantly reduced MDA levels and antioxidant enzyme activities compared with the cisplatin group ( $p < 0.05$ ). Still, the values remained partially lower than in the control. The combination group (Group E) showed the greatest improvement, with oxidative stress markers approaching normalization and results much better than those from single treatments.

**Table 3: Testicular oxidative stress markers**

| Parameter             | Group A     | Group B                  | Group C                  | Group D                  | Group E                   | F-value | p-value |
|-----------------------|-------------|--------------------------|--------------------------|--------------------------|---------------------------|---------|---------|
| MDA (nmol/mg protein) | 2.18 ± 0.22 | 6.82 ± 0.55 <sup>a</sup> | 3.25 ± 0.31 <sup>b</sup> | 3.48 ± 0.37 <sup>b</sup> | 2.64 ± 0.28 <sup>bc</sup> | 312.4   | <0.001  |
| SOD (U/mg protein)    | 9.85 ± 0.71 | 4.12 ± 0.63 <sup>a</sup> | 7.84 ± 0.66 <sup>b</sup> | 7.56 ± 0.72 <sup>b</sup> | 9.21 ± 0.58 <sup>bc</sup> | 228.6   | <0.001  |
| CAT (U/mg protein)    | 62.4 ± 4.1  | 31.6 ± 3.8 <sup>a</sup>  | 51.2 ± 3.5 <sup>b</sup>  | 48.9 ± 4.0 <sup>b</sup>  | 58.6 ± 3.2 <sup>bc</sup>  | 197.3   | <0.001  |
| GSH (μmol/g tissue)   | 7.62 ± 0.52 | 3.28 ± 0.44 <sup>a</sup> | 6.15 ± 0.47 <sup>b</sup> | 5.88 ± 0.53 <sup>b</sup> | 7.05 ± 0.39 <sup>bc</sup> | 241.8   | <0.001  |

**Table 5: Histopathological scoring**

| Parameter                       | Group A (Control) | Group B (Cisplatin)   | Group C (Cis + Hesperidin) | Group D (Cis + Rutin)    | Group E (Combination) |
|---------------------------------|-------------------|-----------------------|----------------------------|--------------------------|-----------------------|
| Tubular Degeneration            | 0-I (None-Mild)   | III (Severe)          | II (Moderate)              | II (Moderate)            | I (Mild)              |
| Germ Cell Loss                  | 0-I (None-Mild)   | III (Severe)          | II (Moderate)              | II (Moderate)            | I (Mild)              |
| Interstitial Edema              | 0 (None)          | III (Severe)          | II (Moderate)              | II (Moderate)            | I (Mild)              |
| Johnsen Score (Spermatogenesis) | Normal (9-10)     | Severely Reduced (≤5) | Moderately Reduced (7-8)   | Moderately Reduced (7-8) | Near Normal (8-9)     |

Data are presented as mean ± SD (n = 10). One-way ANOVA followed by Tukey's post hoc test was used. Different superscript letters indicate statistically significant differences between groups ( $p < 0.05$ ).

There was a major reduction in sperm count, sperm motility, and normal morphology between Group B (Cisplatin administration) and the Control group (Group A) ( $p < 0.001$ ), indicating a severe impairment of spermatogenesis.

All sperm parameters (hesperidin Group C and rutin Group D) were significantly improved ( $p < 0.05$ ) when treated with hesperidin or rutin, but not as high as the control levels. The combination group (Group E) showed the greatest improvement, and the sperm parameters were approaching normal values and were much higher than those observed when the individual treatments were applied.

**Table 4: Sperm parameters**

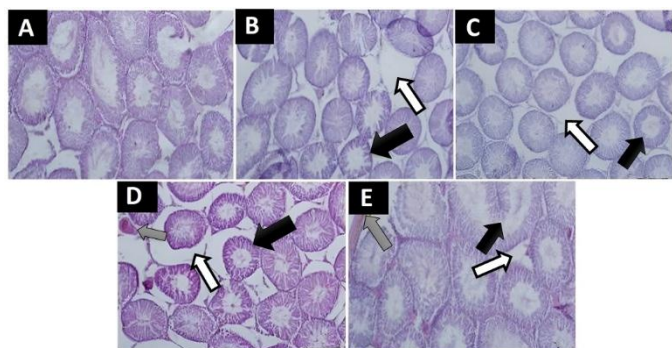
| Parameter                | Group A    | Group B                 | Group C                 | Group D                 | Group E                  | F-value | p-value |
|--------------------------|------------|-------------------------|-------------------------|-------------------------|--------------------------|---------|---------|
| Sperm Count (million/mL) | 68.4 ± 6.2 | 22.5 ± 4.1 <sup>a</sup> | 51.6 ± 5.3 <sup>b</sup> | 48.9 ± 5.8 <sup>b</sup> | 61.8 ± 4.7 <sup>bc</sup> | 183.7   | <0.001  |
| Motility (%)             | 82.3 ± 5.4 | 29.6 ± 4.8 <sup>a</sup> | 65.8 ± 5.2 <sup>b</sup> | 63.4 ± 5.6 <sup>b</sup> | 75.2 ± 4.3 <sup>bc</sup> | 216.9   | <0.001  |
| Normal Morphology (%)    | 88.6 ± 4.7 | 41.2 ± 5.9 <sup>a</sup> | 72.4 ± 5.1 <sup>b</sup> | 69.8 ± 6.2 <sup>b</sup> | 81.7 ± 4.8 <sup>bc</sup> | 174.2   | <0.001  |

Data are presented as mean ± SD (n = 10). One-way ANOVA followed by Tukey's post hoc test was used. Different superscript letters indicate statistically significant differences between groups ( $p < 0.05$ ).

The testicular architecture of the control group (Group A) was not significantly distorted. There were no or slight alterations (Grade 0 -I) of all parameters, and the Johnsen score was high, reflecting normal spermatogenesis. Conversely, Group B, which was treated with cisplatin, exhibited severe histopathological changes (Grade III), including extensive tubular degeneration, widespread loss of germ cells, and intense interstitial edema, along with a much lower Johnsen score, indicating disruption of spermatogenesis.

There was significant improvement in treatment groups. The hesperidin (Group C) and rutin (Group D) groups showed moderate histopathological alterations (Grade II), partial recovery of the seminiferous tubule architecture, and less cell damage. The combination group (Group E) showed few changes (Grade I), with an almost normal histological picture and significant recovery of spermatogenesis, as evidenced by an increased Johnsen score.

**Figure 1: Histopathological changes in testicular tissue across experimental groups (H&E staining, ×400)**



(A) Control: normal seminiferous tubules. (B) Cisplatin: severe tubular degeneration and germ cell loss. (C) Cisplatin + Hesperidin: partial recovery. (D) Cisplatin + Rutin: moderate improvement. (E) Combination: near-normal architecture. Arrows indicate areas of damage and recovery

## DISCUSSION

The current work revealed that cisplatin treatment led to considerable testicular dysfunction, which was manifested by the decrease in reproductive hormones, abnormal sperm parameters, oxidative stress, inflammatory factors, and substantial histopathological changes. Treatment with hesperidin and rutin, separately and combined, had a strong mitigating effect on these harmful effects, with the combination being the most noticeable.

Gonadotoxicity induced by cisplatin in this study is consistent with the previous study by Heibashy *et al.*, which reported that cisplatin affects sperm count, motility, and testosterone levels, and causes structural damage to testicular tissue and high levels of oxidative stress.<sup>15</sup> Equally, it was demonstrated that cisplatin causes testicular damage by majorly causing the production of reactive oxygen species (ROS) that cause lipid peroxidation, germ cell apoptosis, and steroidogenesis impairment by Meghji *et al.*<sup>5</sup> As a platinum-based chemotherapy drug, cisplatin shares toxicodynamic characteristics with heavy metals (such as lead), especially in its capacity to produce reactive oxygen species, cause oxidative stress, and interfere with spermatogenesis.<sup>16</sup> These results closely match the current findings, which support the role of oxidative stress as a key pathway in cisplatin-induced reproductive toxicity.

The significant rise in malondialdehyde levels and the reduction in antioxidant enzymes (SOD, CAT, and GSH) in the cisplatin group further substantiate the role of oxidative imbalance in mediating testicular injury. Oxidative stress impairs cellular integrity by damaging lipids, proteins, and DNA, and eventually leads to impaired spermatogenic activity.<sup>5</sup> The same changes have been observed in oxidative stress indicators in experimental studies on testicular toxicity caused by a variety of agents, reflecting the susceptibility of testicular tissue to oxidative stress.<sup>17</sup>

In the current study, treatment with hesperidin and rutin greatly restored antioxidant enzyme activity and reduced lipid peroxidation. These results are consistent with prior literature indicating that both compounds are strong antioxidants that reduce oxidative stress by replenishing antioxidant enzymes

and decreasing malondialdehyde levels in models of drug-induced testicular toxicity. Ileriturk *et al.* reported that hesperidin effectively reduces oxidative stress by restoring antioxidant enzyme levels and lowering malondialdehyde levels in drug-induced testicular toxicity models.<sup>18</sup> Likewise, Wang *et al.* reported that rutin has dose-dependent protective effects that increase antioxidant defenses and minimize oxidative damage in testicular tissue.<sup>19</sup> The reported increase in the parameters of oxidative stress in the present study can thus be explained by the free radical scavenging property of these flavonoids.

Besides oxidative stress, inflammation is also an important factor in cisplatin-induced testicular damage. The pro-inflammatory cytokines TNF- $\alpha$  and IL-6 were elevated in the cisplatin group, indicating activation of inflammatory pathways. This is in line with earlier events in which inflammatory mediators have been massively overexpressed after toxic insult and more so, the anti-inflammatory effect of hesperidin in down-regulating NF- $\kappa$ B signaling and pro-inflammatory cytokines and further, the anti-inflammatory nature of hesperidin was observed in inhibiting major inflammatory processes.<sup>18,20</sup> The decreased level of TNF- $\alpha$  and IL-6 in treated groups in the current study supports the anti-inflammatory activity of rutin and hesperidin.

This enhancement in sperm parameters and the hormonal profile after treatment with rutin and hesperidin is presumably due to decreased oxidative stress and inflammation. The re-establishment of testosterone, LH, and FSH levels indicates the restoration of hypothalamic-pituitary-gonadal axis activity, which is necessary for normal spermatogenesis. These results are consistent with the existing literature showing improved sperm quality and hormonal balance following antioxidant therapy. Abdel-Aty *et al.* and Heibashy *et al.* both showed significant changes in reproductive parameters after flavonoid treatment.<sup>15,17</sup>

The degeneration of seminiferous tubules, the loss of germ cells, and the damage in the interstitial area, which were severe in the cisplatin group, were significantly reduced in the treated groups, especially in the combination group. The same has been observed in prior research studies on the protective effect of flavonoids, which have shown that normal testicular architecture is restored after treatment with hesperidin and rutin.<sup>15</sup>

Interestingly, the combination of hesperidin and rutin provided better protection than either agent alone. This increased effectiveness could be explained by their complementary mechanisms of action, such as synergistic antioxidant and anti-inflammatory activities. Combination therapy likely provides broader cellular protection by acting on multiple pathways that contribute to testicular damage.

## CONCLUSION

In conclusion, the findings of this study suggest that hesperidin and rutin effectively mitigate cisplatin-induced testicular dysfunction by modulating oxidative stress. The combination therapy demonstrated superior protective effects, highlighting

its potential as a therapeutic strategy to prevent chemotherapy-induced reproductive toxicity.

## LIMITATIONS

This study has several limitations. First, the experiment was conducted in an animal model; therefore, the findings cannot be directly extrapolated to humans. Second, only a single dose of cisplatin and fixed doses of rutin and hesperidin were evaluated, precluding assessment of dose-response relationships. Third, molecular markers involved in inflammation and apoptosis were not investigated. Finally, the relatively short duration of the study did not permit evaluation of long-term reproductive recovery or fertility outcomes.

## SUGGESTIONS / RECOMMENDATIONS

Further studies should investigate the molecular mechanisms underlying the protective effects of rutin and hesperidin, including the expression of oxidative stress, inflammatory, and apoptotic signaling pathways. Dose-response studies and long-term experiments evaluating fertility outcomes are also warranted. Clinical studies are ultimately required to determine the safety and efficacy of these flavonoids as adjunctive therapies for preserving male reproductive function in patients receiving cisplatin chemotherapy.

## CONFLICT OF INTEREST / DISCLOSURE

The authors declare that they have no conflict of interest regarding the publication of this study.

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## REFERENCES

1. Abbas A, Meghji KA, Hanif MS, Baloch MS, Channar SPP, Aslam A. Effects of Escitalopram and Citalopram on Semen Parameters in Wistar Albino Rats. *Ann Punjab Med Coll.* 2019 Dec;13(4):272-276.
2. Ranasinghe R, Mathai ML, Zulli A. Cisplatin for Cancer Therapy and Overcoming Chemoresistance. *Heliyon.* 2022 Sep;8(9):e10608.
3. Meghji KA, Siddiqui S, Javed S, Thallo AA, Kalhor R, Memon AA. Dose-Dependent Protective Effects of Sesamin Against Renal Inflammation and Oxidative Stress in Cisplatin-Treated Rats: An Experimental Study. *Gomal J Med Sci.* 2025;23.
4. Aly HA, Eid BG. Cisplatin Induced Testicular Damage Through Mitochondria Mediated Apoptosis, Inflammation and Oxidative Stress in Rats: Impact of Resveratrol. *Endocr J.* 2020 Sep;67(9):969-980.
5. Meghji KA, Memon TF, Aslam A, Noor N, Abbas A, Memon RA. The Novel Role of Resveratrol in Attenuating Cisplatin Induced Testicular Toxicity in Rats. *Prof Med J.* 2020 Sep;27(9):2023-2029.
6. Altayr AE, Albadrani GM, Farouk SM, Alamoudi MK, Sayed AA, Mohammedsahleh ZM, et al. The Antioxidant, Anti-Inflammatory, and Anti-Apoptotic Effects of Sesamin Against Cisplatin-Induced Renal and Testicular Toxicity in Rats. *Ren Fail.* 2024 Jul;46(2):2378212.
7. Zielińska D, Starowicz M, Wronkowska M, Zieliński H. Multifaceted Biological Activity of Rutin, Quercetin, and Quercetin's Glucosides. *Molecules (Basel).* 2025 Jun;30(12):2555.
8. Rahmani AH, Babiker AY, Anwar S. Hesperidin, a Bioflavonoid in Cancer Therapy: A Review for a Mechanism of Action Through the Modulation of Cell Signaling Pathways. *Molecules (Basel).* 2023 Jun;28(13):5152.
9. Charan J, Kantharia ND. How to Calculate Sample Size in Animal Studies? *J Pharmacol Pharmacother.* 2013 Oct;4(4):303-306.
10. Hanif MS, Baloch MS, Meghji KA, Abbas A, Kashif S, Qureshi R. Histopathological Changes in the Gastric Mucosa Induced by Carbaryl Toxicity: An Experimental Rat Model. *Khyber Med Univ J.* 2020 Jun;12(2):137-142.
11. Meghji KA, Talpur RA, Uqaili AA, Nizammani YM, Kazi N, Nizammani GS. Resveratrol Attenuates Oxidative Stress in Chemotherapy Induced Acute Kidney Injury: An Experimental Rat Model. *Khyber Med Univ J.* 2019 Jun;11(2):85-89.
12. Kasem SE, Abdelnaby AA, Mohammed PA, Hemdan SB, Abd El-Fattah RM, Elsayed RM. Protective Effect of Hesperidin on Kidneys and Testes of Adult Male Rats Exposed to Bisphenol A. *Egypt J Hosp Med.* 2022 Jul;88:3005-3013.
13. Jahan S, Munawar A, Razak S, Anam S, Ain QU, Ullah H, et al. Ameliorative Effects of Rutin Against Cisplatin-Induced Reproductive Toxicity in Male Rats. *BMC Urol.* 2018 Nov;18(1):107.
14. Meghji KA, Memon TF, Ahmed I, Memon SG, Noor N, Abbas A. Nephroprotective Effects of L-Arginine Against Chemotherapy Induced Acute Kidney Injury in Wistar Rats. *J Islamabad Med Dent Coll.* 2020 Dec;9(4):249-255.
15. Heibashy DMI, Morsy WA. Effectiveness of Hesperidin and Rutin on Cisplatin-Induced Testicular Dysfunction in Rats. *J Basic Appl Zool.* 2025 Jul;86(1):56.
16. Memon TF, Meghji KA, Kashif S, Nadeem F, Memon AA, Thallo AA. Protective Effects of Melissa Officinalis Extract Against Lead-Induced Toxicity in Hepatic, Renal and Testicular Tissues of Male Rats. *Gomal J Med Sci.* 2025 Oct-Dec;23(4):412-417.
17. Abdel-Aty DM, Ibrahim MA, Mohamed SR, El-Khadragy MF, Abdel Moneim AE, Fathalla AS, et al. Rutin and Moringa Oleifera Leaf Extract Prevent Monosodium Glutamate-Induced Testicular Toxicity in Adult Male Albino Rats. *Front Vet Sci.* 2025 May;12:1566471.
18. Ileritürk M, Kandemir O, Akaras N, Simsek H, Genc A, Kandemir FM. Hesperidin Has a Protective Effect on Paclitaxel-Induced Testicular Toxicity Through Regulating Oxidative Stress, Apoptosis, Inflammation and Endoplasmic Reticulum Stress. *Reprod Toxicol.* 2023 Jun;118:108369.
19. Wang Y, Hua Z, Hui JQ, Qiu D. Rutin as a Potential Therapeutic Agent for Arsenic-Induced Testicular Damage: Antioxidant, Anti-Inflammatory, and Anti-Apoptotic Effects. *Invest Clin.* 2025 Dec;66(4):408-425.
20. Yan L, Wang J, Dai D, Zhang Y, Li Y, Xiao W. Testicular Protective Effects of Hesperidin Against Chemical and Biological Toxicants. *Toxicol Res (Camb).* 2024 Jun;13(3):tfae078.