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Original Article

Protective Effect of Rosmarinic Acid Against Cisplatin-Induced Testicular Damage in Mice *via* Modulation of Inflammation, Oxidative Stress, and Apoptosis and Restoration of Nrf2/HO-1

Brendan P. Norman[1], Ammar A. Basabrain[2,3], Sahem M Alkharabsheh[1]

[1]Institute of Life Course & Medical Sciences, Faculty of Health and Life Sciences, University of Liverpool, University of Liverpool, Liverpool, UK.
[2]Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah, Saudi Arabia
[3]Hematology Research Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia

***Corresponding Author:** bnorman@liverpool.ac.uk

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Abstract: Cisplatin (CIS) is a promising chemotherapeutic drug widely utilized for the treatment of several malignancies though its role in inducing testicular injury is established. Rosmarinic acid (RA) is a polyphenolic whose health benefits are quite well known. This research explored the ability of RA to protect against CIS-induced testicular damage and the different mechanisms involved in this protective role. For this purpose, different groups of mice were given oral doses of RA (25 and 50 mg/kg/day) for two weeks and a single dose of CIS (7mg/Kg) intraperitoneally on the 8th day. CIS administration was found to cause a considerable reduction in sperm parameters like sperm count, viability and motility. It also mediated considerable histopathological changes in parallel to augmented MDA content and reduced GSH content and CAT and SOD activities in testis. Administration of RA in tissues with CIS-induced damage caused attenuation of sperm parameter alterations, indicating protection from testicular damage. RA administration was also found to alleviate NO and MDA levels and antioxidants in mice treated with CIS. An increment in NF-κB 65, TNF-α, IL-1β, Bax and caspase-3 was caused by CIS administration which also reduced Bcl-2 levels in testis. However, RA administration caused suppression of upregulation of NF-κB and concentrations of pro-inflammatory cytokines together with alleviation of apoptosis in testis. This resulted in the improvement of all sperm parameters. The protective efficacy of RA against CIS-induced testicular damage is quite clear from these results; however, further research in this connection is still required to strengthen the basis for the development of an RA-based therapeutic tool against CIS-induced testicular damage.

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1. INTRODUCTION

The drug cisplatin (CIS) is used extensively for the treatment of different tumors including bladder, ovarian, testicular, cervical, head and neck cancer (Manohar & Leung, 2018; Volarevic et al., 2019). CIS exerts its anticancer activity through binding to DNA, resulting in the formation of CIS-DNA adducts, thereby leading to inhibition of the cell cycle (García Sar et al., 2012). CIS also affects non-cancerous tissues, for example the testes, liver and kidneys in a dose-dependent fashion, which limits the drug's clinical utility (Al-Malki & Sayed, 2014; Aly & Eid, 2020; Howell & Shalet, 2005; Manohar & Leung, 2018). Organic cation transporters (OCT2) are involved in the uptake of CIS by testicular cells (Samodelov et al., 2020). The build-up of CIS in testicular cells such as Sertoli cells, Leydig cells and germ cells results in the generation of Reactive Oxygen Species (ROS). This activates the cellular antioxidant defense system leading to oxidative stress at the site (Altındağ & Meydan, 2021; Eren et al., 2020). Subsequent oxidative modification of DNA, proteins and lipids by ROS impairs testicular cell function. Testosterone generation by Lydig cells is also reduced due to this oxidative damage (El-Shafaei et al., 2018; Salem et al., 2012; J. Zhang et al., 2022). An association between reduced testosterone generation and decreased spermatogenesis as well as poor fertility has been established (Sharma et al., 2021).

Besides oxidative damage, the inflammatory response is also triggered by CIS which involves the increased generation of pro-inflammatory cytokines and nuclear factor kappa B (NF-κB) activation, thereby worsening the testicular injury (Asejeje et al., 2024; Negm et al., 2022; Tadagavadi & Reeves, 2010). This inflammatory and oxidative damage collectively results in apoptotic cell death particularly in germ cells which are vital for the generation of sperm (Azab et al., 2020; Ghafouri-Fard et al., 2021; Moradi et al., 2021). Testicular atrophy, reduction in the quality of sperm and eventually infertility are caused by the collective effect of cellular damage, decreased testosterone generation and germ cell apoptosis. This poses a serious threat to CIS-treated male cancer patients (Aly & Eid, 2020). In this connection, attenuation of oxidative stress together with the consequential apoptosis and inflammation can be viewed as a potential protective intervention for CIS-induced testicular damage.

It has been established that chemotherapy-induced tissue injury can be avoided by targeting the pathway of the nuclear factor erythroid 2-related factor-2 (Nrf2) (Aladaileh et al., 2019; Hamzeh et al., 2019; Khamis et al., 2023). Nrf2 is a cytoprotective transcription factor and master-regulator of genes involved in the antioxidant cellular response (Chen et al., 2018; Ding et al., 2021). Nrf2 modulation has proven to be an efficient therapeutic strategy to counter 5-FU-associated cardiotoxicity.

The beneficial effects of plant-based bioactive compounds on human health are well-established (Aladaileh et al., 2019; Khamis et al., 2023). Secondary plant metabolites like flavonoids and polyphenols are known for their antioxidant, anticancer, antibacterial and anti-inflammatory effects (Alaswad et al., 2021; Roy et al., 2022; Shamsudin et al., 2022; Z. Zhang et al., 2022). The polyphenolic chemical rosmarinic acid (RA; $C_{18}H_{16}O_8$) is found in oregano, lemon balm, clary sage, and

rosemary. Rosmarinic acid's cytoprotective, anti-inflammatory, anti-bacterial, anti-allergic, and antioxidant properties are well established (Al-khawaldeh et al., 2024; Noor et al., 2022). In animal models, RA has been shown to reduce inflammation, protecting the liver from the harmful effects of acetaminophen and damage caused by reperfusion/ischemia (Ramalho et al., 2014; Yu et al., 2021). Reports indicate that RA can also modulate p-Akt expression, resulting in enhanced energy metabolism in cardiomyocytes, reduction of ROS overproduction, and mitigation of reoxygenation/hypoxia-induced damage and cell death in cardiomyocytes (Li et al., 2014). RA has also been shown to significantly decrease lipopolysaccharide (LPS)-induced neuroinflammation and cobalt-induced hepatocyte damage *in vitro* (Jeon et al., 2014). In a separate study, RA has been demonstrated to activate Nrf2, consequently mitigating CCl4-induced liver injury in mice. By activating Nrf2, RA can also change how the liver reacts to oxidative stress and inflammation caused by CIS (Xiang et al., 2022).

Though numerous pharmacological aspects of RA have been established, its ability to offer protection for testicular damage induced by CIS requires detailed investigation. Therefore, the present study was designed to evaluate the protective effect of RA against CIS-induced testicular injury through modulation of oxidative stress, inflammation, and apoptosis. However, the protective effect of RA against cisplatin-induced testicular injury and its mechanistic modulation of the Nrf2/HO-1 axis have not been thoroughly investigated. Therefore, this study aims to elucidate whether RA can activate the Nrf2/HO-1 signaling pathway to mitigate CIS-induced testicular toxicity in mice.

2. MATERIALS AND METHODS

2.1. Experimental Design

30 Swiss albino male mice were included in the research as experimental subjects, and all were 6-8 weeks old. Water and food were accessible to all subjects throughout the day. They were kept in a setting with a 12-hour light/dark cycle where temperature and humidity were controlled at 23-25°C and 50-60% respectively. All subjects were kept under these conditions for at least seven days before their acclimatization for the experiment. The next step was a random grouping of all mice into 5 groups: each group with 6 mice (n=6).

Control Group: This experimental group comprised of mice which were orally administered with DMSO (0.5%) for 2 weeks. On 8th day, these mice were subjected to intraperitoneal administration of physiological saline.

Rosmarinic Acid Group: This group comprised of mice which were administered with RA for two weeks. On 8th day, these mice were subjected to intraperitoneal administration of physiological saline. Dose used for RA was 50mg/kg.

Cisplatin Group: This group comprised of mice which were orally administered with DMSO (0.5%) for 2 weeks. On 8th day, these mice were subjected to intraperitoneal administration of CIS (Sigma, USA) as suggested by (Nna et al., 2020). The cisplatin dose (7 mg/kg) was adopted from Nna et al. (2020).

Rosmarinic Acid (25)+Cisplatin Group: This group comprised of mice which were orally administered with RA for 2 weeks. On 8th day, these mice were subjected to intraperitoneal administration of Cis. Doses used for RA and CIS were 25mg/kg and 7mg/kg respectively.

Rosmarinic Acid (50)+Cisplatin Group: This group comprised of mice which were orally administered with RA for 2 weeks. On 8th day, these mice were subjected to intraperitoneal administration of CIS. Doses used for RA and CIS were 50 mg/kg and 7mg/kg respectively.

RA and CIS were obtained from Biosynth Carbosynth (Compton, UK) and Hikma Pharmaceuticals (Cairo, Egypt) respectively. CIS was dissolved in physiological saline and RA was dissolved in 0.5% DMSO for oral administration. RA was freshly prepared in 0.5% DMSO and administered orally via gavage once daily for 14 days. Cisplatin was administered intraperitoneally (i.p.) on day 8. The CIS dose used during this study (7 mg/kg) was the same as used by Nna et al., (2020). Similarly, RA doses (25 and 50 mg/kg) were the same as used by (Gautam et al., 2019) during their investigation of antioxidant and anti-inflammatory effects of RA.

The subjects were sacrificed and both testes were excised. 50mM cold phosphate-buffered saline (PBS) at pH 7 was then used for rinsing the testes. After rinsing, one testis was immediately submerged in 10% neutral-buffered formalin for immunohistochemical and histopathological analyses. The other testis was subjected to homogenization in cold PBS to achieve 10% w/v concentration. After centrifugation of the homogenates, the supernatant was separated and kept at -20 °C for biochemical tests. All experimental procedures involving animals were reviewed and approved by the Animal Ethics Committee (AEC/2024/015; March 15, 2024), in accordance with the guidelines of the National Institutes of Health (NIH Publication No. 85-23, revised 2011).

2.2. Determination of Sperm Cell Count, Viability, and Motility

Sperm analysis was performed using the procedure suggested by Han et al., (2019) after making some appropriate changes in the procedure (Han et al., 2019). For this purpose, sperm samples were collected from the cauda epididymis. After separating the right cauda epididymis, it was clipped in a centrifuge tube which containing pre-warmed PBS (5ml, pH: 7.4). After mixing the contents of the tube gently, tubes were kept for five minutes at 37°C thereby permitting sperms to swim out. The suspension became cloudy which indicated the presence of sperm. In addition, to avoid any cauda epididymis tissue particulates, a nylon mesh of 200 µm was used. A microscope and hemocytometer were used to count the sperm and percentage of motile sperms (Al-khawaldeh et al., 2024). Sperm viability was ascertained by eosin-nigrosin stain exclusion (Buranaamnuay, 2019).

2.3. Determination of Testicular Malondialdehyde (MDA) and Antioxidants

Lipid peroxidation in testis samples was ascertained by malondialdehyde (MDA) concentration following the procedure described by (Ohkawa et al., 1979). The method by Aebi (1987) was used to determined catalase (CAT) activity (Aebi, 1984). The method by Nishikimi et al. (1972) was utilized to determine superoxide dismutase (SOD) activity (Nishikimi et al., 1972). Reduced glutathione (GSH) in the testicular tissues were determined using the method proposed by (Griffith, 1980).

2.4. Determination of Pro-inflammatory Cytokines, Bcl-2, and Bax Contents in the Testis

Concentrations of pro-inflammatory cytokines and apoptotic markers in testis tissue were determined using commercial ELISA kits following the manufacturers' instructions. Kits from FineTest (Wuhan, Hubei, China) were used for IL-6 (Cat. No. EM012), IL-1β (Cat. No. EM0183), and TNF-α (Cat. No. EM0109), while kits from MyBioSource (San Diego, CA, USA) were used for Bax (Cat. No. MBS2509733) and Bcl-2 (Cat. No. MBS2512543).

2.5. Histology and Immunohistochemistry (IHC)

All histological and immunohistochemical analyses were undertaken by one researcher who was blinded to the experimental groupings. Testicular tissues were embedded in paraffin then divided into 5µm thick sections. Hematoxylin and eosin (H&E) staining was performed for analysis of potential histopathological changes using light microscopy (Leica microscope equipped with a Leica DFC camera). Immunostaining was performed to determine the caspase 3 and NF-κB p65 content. This analysis involved cutting of paraffin-embedded sections followed by deparaffinization and hydration. Then microwave antigen retrieval treatment was conducted. The sections were then incubated in 0.3% H_2O_2 solution to obstruct the activity of endogenous peroxidase.

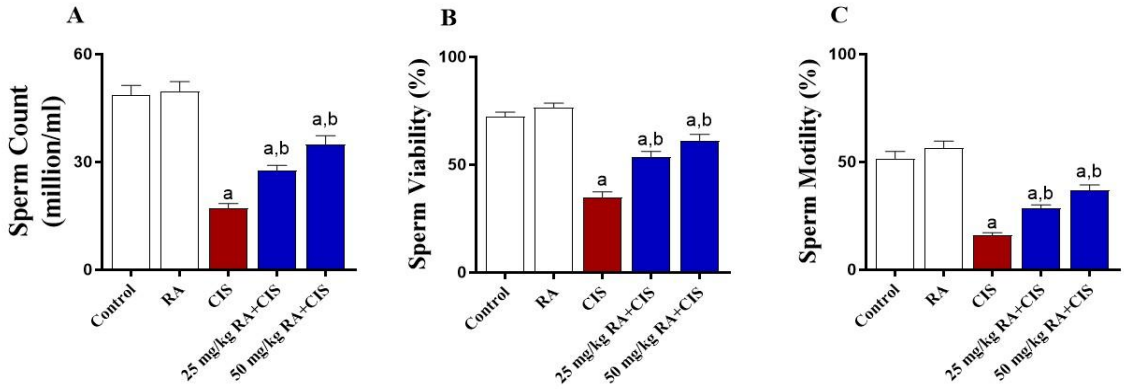

Figure 1. RA restores the CIS-induced decreased sperm parameters in mice. RA attenuated (A) sperm count, (B) sperm viability, and (C) sperm motility in CIS-injected mice. a P<0.05 vs. control; b P<0.05 vs. CIS, n=6. Data are presented as means ± SEM.

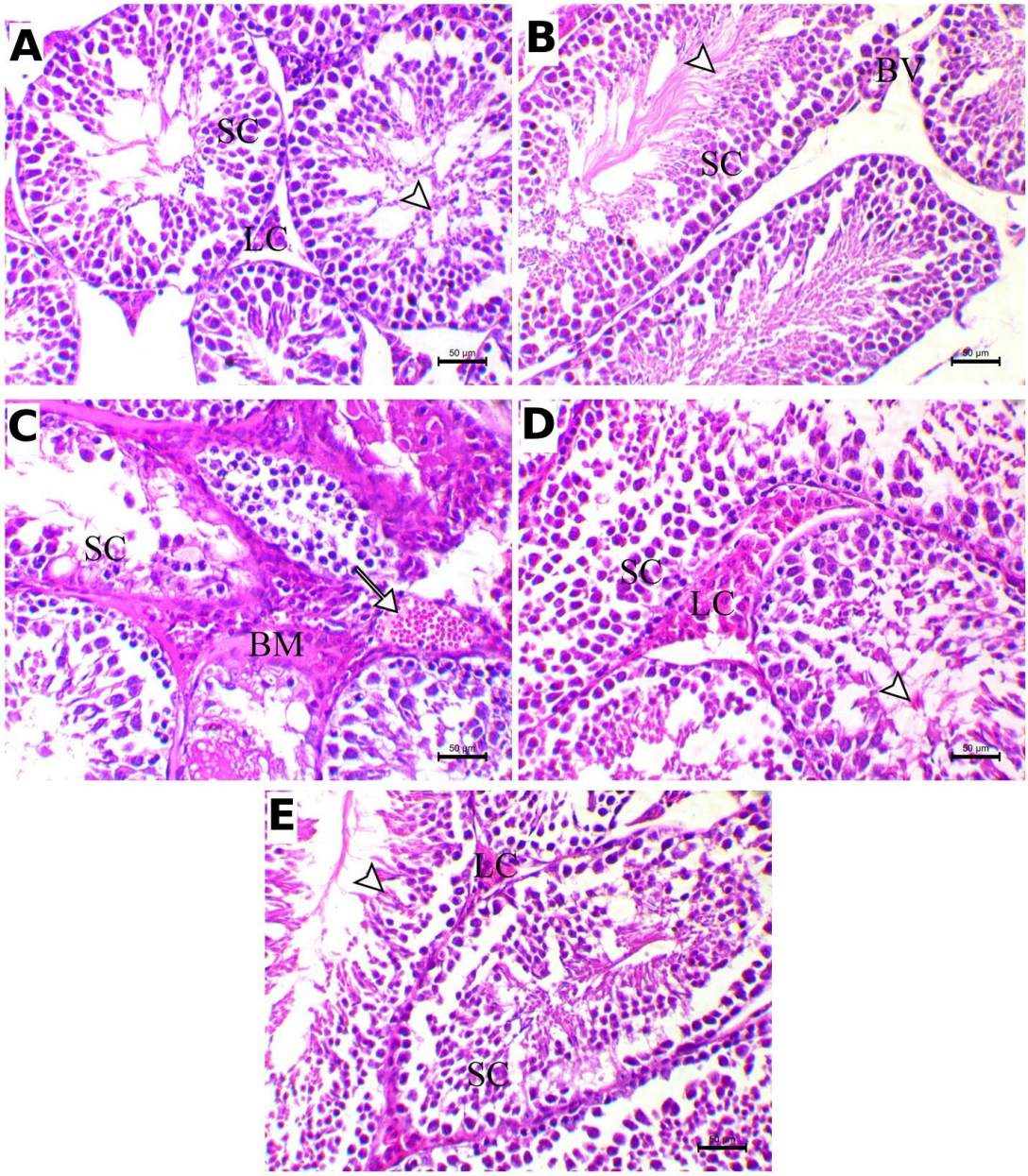

Figure 2: (A) Testis of control animal (G1) showing normal seminiferous tubules lined with normal spermatogenic cells (SC), normal Leydig's cells (LC), and free sperm within their lumen (arrowhead). (B) Testis of normal treated group (G2) showing normal seminiferous tubules lined with normal spermatogenic cells (SC), normal interstitial blood vessels (BV), and many sperm within their lumen (arrowhead). (C) Testis of diseased animal (G3) showing severe testicular degeneration with congestion of blood capillaries (arrow), necrosis of the germinal epithelium (SC), and thickening of the basement membrane (BM). (D) Testis of diseased and treated group (G4) showing fewer degenerative changes in spermatogenic cells (SC), mild hyperplasia of Leydig's cells, and free luminal spermatocytes (arrowhead). (E) Testis of diseased and treated group (G5) showing a marked decrease in degenerative changes in spermatogenic cells (SC) with a significant increase of normal spermatocytes (arrowhead). H&E stain, bar = 50 µm

Tissue sections were incubated for twenty minutes with serum to block nonspecific binding. Tissue sections were then incubated at 4ºC overnight with primary antibodies; 1:100 dilution of Nrf2 antibody (Invitrogen, Waltham, MA, USA), 1:100 dilution of caspase-3 antibody (Invitrogen, Waltham, MA, USA) and 1:100 dilution of NF-κB p65 antibody (Santa Cruz Biotechnology, Dallas, TX, USA). Tissue sections were then washed followed by incubation with secondary antibodies (EnVision+™ System Horseradish Peroxidase Labelled Polymer, Dako, Santa Clara, CA, USA). Tissues were then visualized using light microscopy. The proportions of caspase 3 and NF κB p65 positive cells per 1000 spermatogenic cells were determined using the ImageJ analysis software (NIH, Bethesda, MD, USA). Expression of target proteins in respective groups was quantified relative to the control group.

2.6. Statistical analysis

Statistical analyses were carried out using GraphPad Prism 8 (San Diego, USA). Mean ± SEM was computed. One-way Analysis of variance (ANOVA) was carried out to differentiate the groups. Tukey's post-hoc test was performed for the comparison of means using the significance level of $P<0.05$.

3. RESULTS

3.1. CIS-Induced Testicular Damage is Attenuated by RA in Mice

Different features of sperm were studied in RA-treated and untreated mice during this research to analyze the potential protective role of RA against the testicular damage induced by CIS. These features included sperm count, motility and viability as shown in Figure 1. Besides these, histological changes were also examined as shown in Figure 2. All sperm parameters were found to be significantly reduced by CIS treatment alone ($P<0.05$) (Figure 1 A-C). Administration of RA (25 or 50mg) significantly rescued the reduction in sperm parameters induced with CIS treatment (Figure 1 A-C). These parameters were not affected by the administration of RA alone in normal subjects ($P>0.05$). Histological examination supported these findings. Control and RA-only groups exhibited normal seminiferous tubules and intact spermatogenic epithelium, whereas CIS-treated mice showed marked degeneration, germinal epithelial necrosis, and basement membrane thickening. RA co-treatment ameliorated these alterations in a dose-dependent manner, with nearly normal testicular architecture in the high-dose group (Figure 2).

3.2. CIS-Induced Oxidative Stress in the Testis is Attenuated by RA in Mice

In this study, CIS-injected mice showed increased oxidative stress. This was indicated by higher levels of protein carbonyl (protein oxidation) and malondialdehyde (MDA; lipid peroxidation) (Figure 3A). CIS also led to a significant decrease in testicular glutathione (GSH) content (Figure 3B), superoxide dismutase (SOD) activity (Figure 3C), and catalase (CAT) activity (Figure 3D) with a P value less than 0.05. Treatment with RA significantly improved these oxidative stress markers in CIS-injected mice (Figure 3A–E) with a P value less than 0.05. In contrast, RA alone had no effect on oxidative stress markers in the testes of healthy mice.

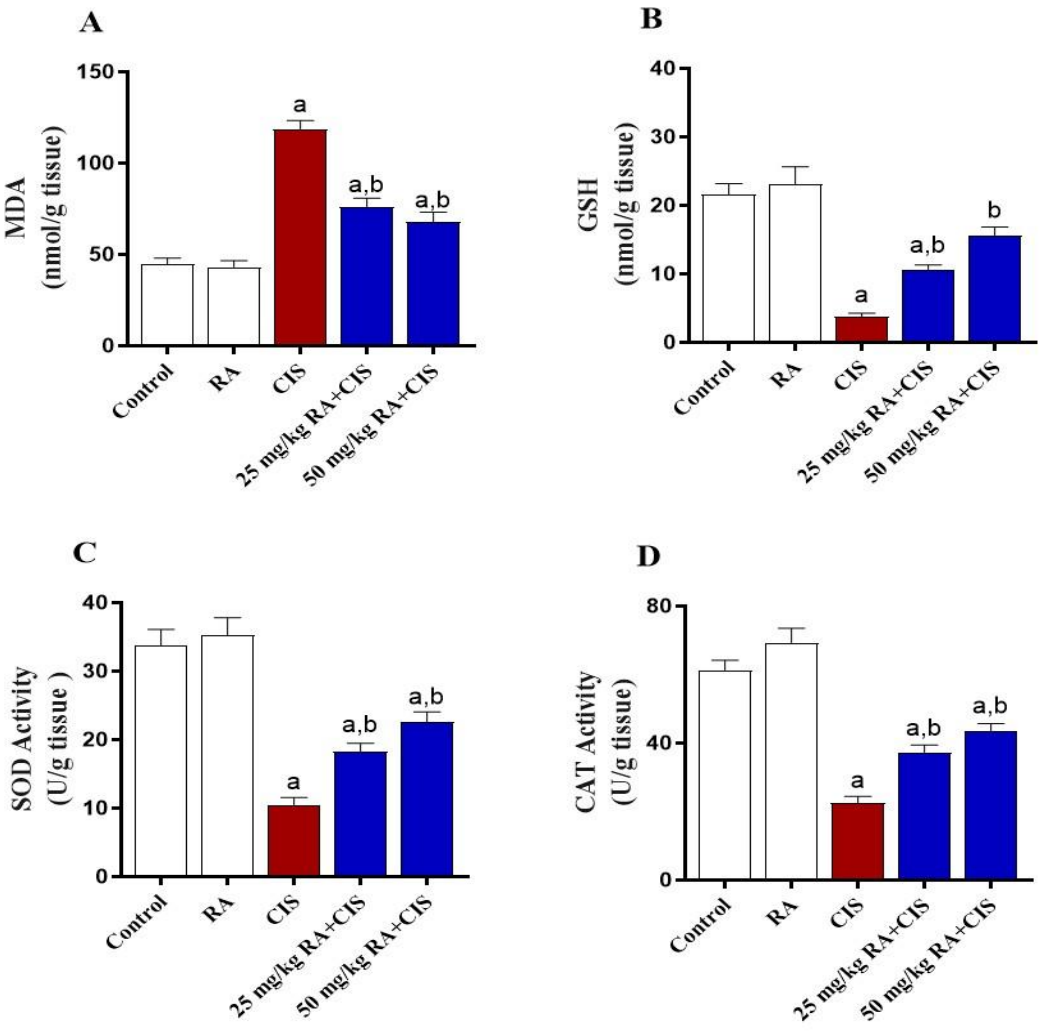

Figure 3. RA alleviates the CIS-induced oxidative stress in mouse testis. RA decreased (A) MDA contents in the testis. RA largely restored testicular (B) GSH contents and (C) SOD and (D) CAT activities. a $P<0.05$ vs. control; b $P<0.05$ vs. CIS, n=6. Data are presented as means ± SEM.

3.3. CIS-Induced Inflammation in the Testis is Mitigated by RA in Mice

Given the role of inflammation in CIS-induced testicular injury, we measured NF-κB p65 and its associated pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) in testicular tissue. CIS administration significantly increased the expression of NF-κB p65 (Figure 4) and the concentrations of TNF-α, IL-1β, and IL-6 (Figure 5A–C) compared to controls. Treatment with RA markedly ($P < 0.05$) reduced these inflammatory markers, while RA alone had no effect in healthy mice.

3.4. The CIS-Induced Apoptosis in the Testis is Suppressed by RA in Mice

Considering the crucial role played by inflammatory mechanisms and oxidative stress in instigating apoptosis in testicular tissues in response to CIS treatment, the presence of pro- and anti-apoptotic proteins in RA-treated and untreated subjects was analyzed. When testicular tissues of CIS-treated mice were subjected to IHC staining and ELISA, significant ($P<0.05$) reduction was recorded in the concentration of Bcl-2 protein; however, concentrations of caspase-3 and Bax were considerably increased as shown in Figure 6, pointing towards activation of apoptosis. Administration of RA in CIS-treated mice resulted in significant ($P<0.05$) attenuation of apoptosis in testicular tissues as shown in Figure 6. Nevertheless, administration of RA alone in the testis of healthy mice caused no effect on concentrations of caspase-3, Bax and Bcl-2 proteins

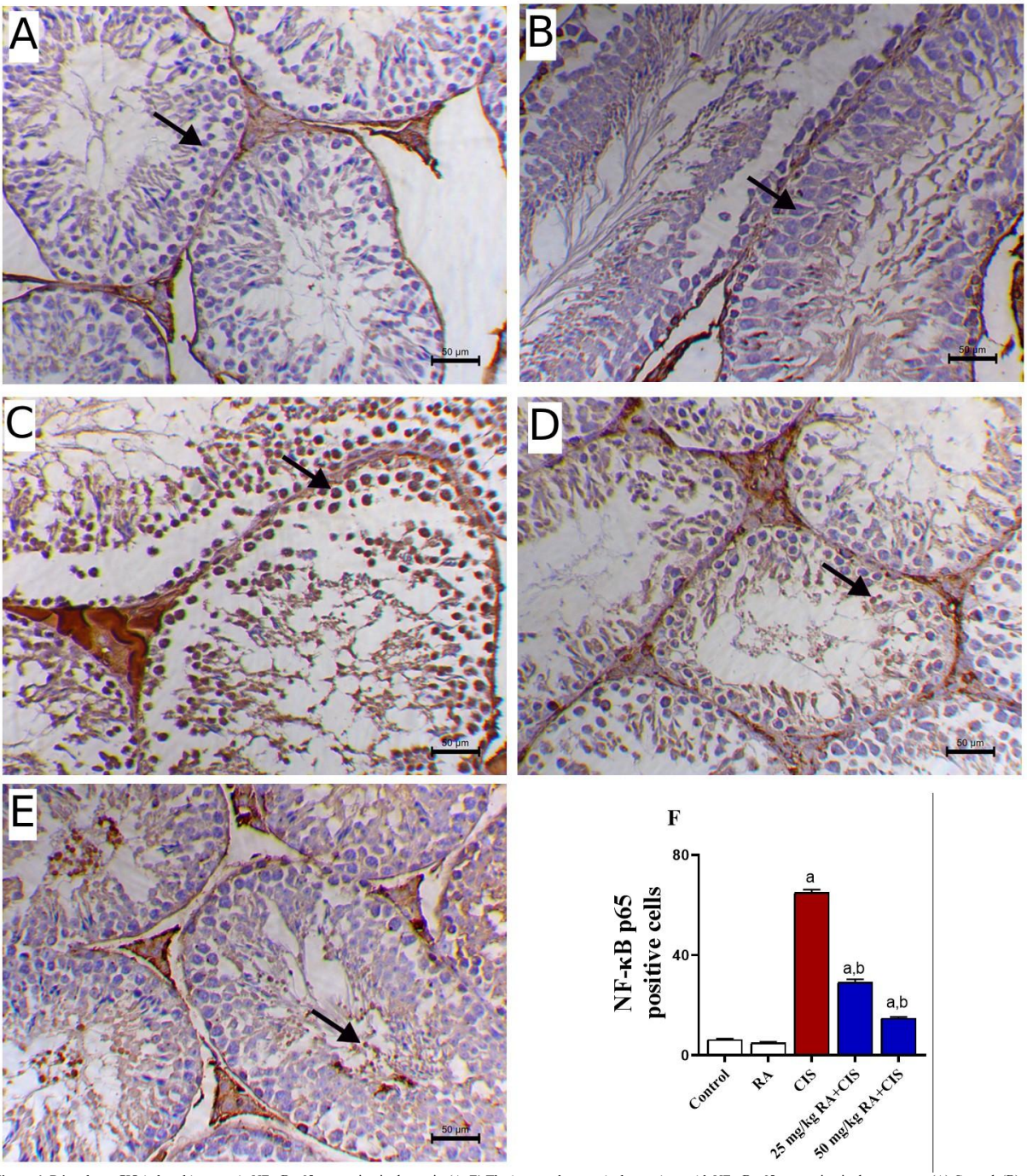

Figure 4. RA reduces CIS-induced increase in NF-κB p65 expression in the testis. (A–E) The images show testicular sections with NF-κB p65 expression in these groups: (A) Control, (B) RA alone, (C) CIS alone, (D) CIS + RA low dose, and (E) CIS + RA high dose. Arrows point to spermatogenic cells with positive NF-κB p65 staining. Scale bar = 50 µm. (F) We quantified NF-κB p65 expression using ImageJ software, comparing it to the control group. Data are shown as means ± SEM (n = 4). a $P < 0.05$ vs. control; b $P < 0.05$ vs. CIS.

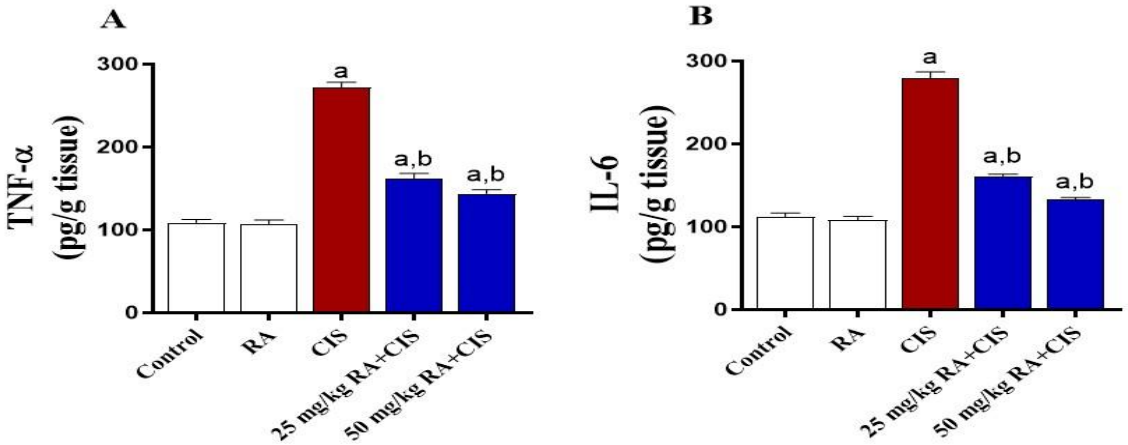

Figure 5. RA mitigates the CIS-induced pro-inflammatory production in the testis. RA attenuated the increase in testicular levels of (A) TNF-α, (B) IL-1β, and (C) IL-6 in the respective groups. a P<0.05 vs. control; b P<0.05 vs. CIS, n=6. Data are presented as means ± SEM.

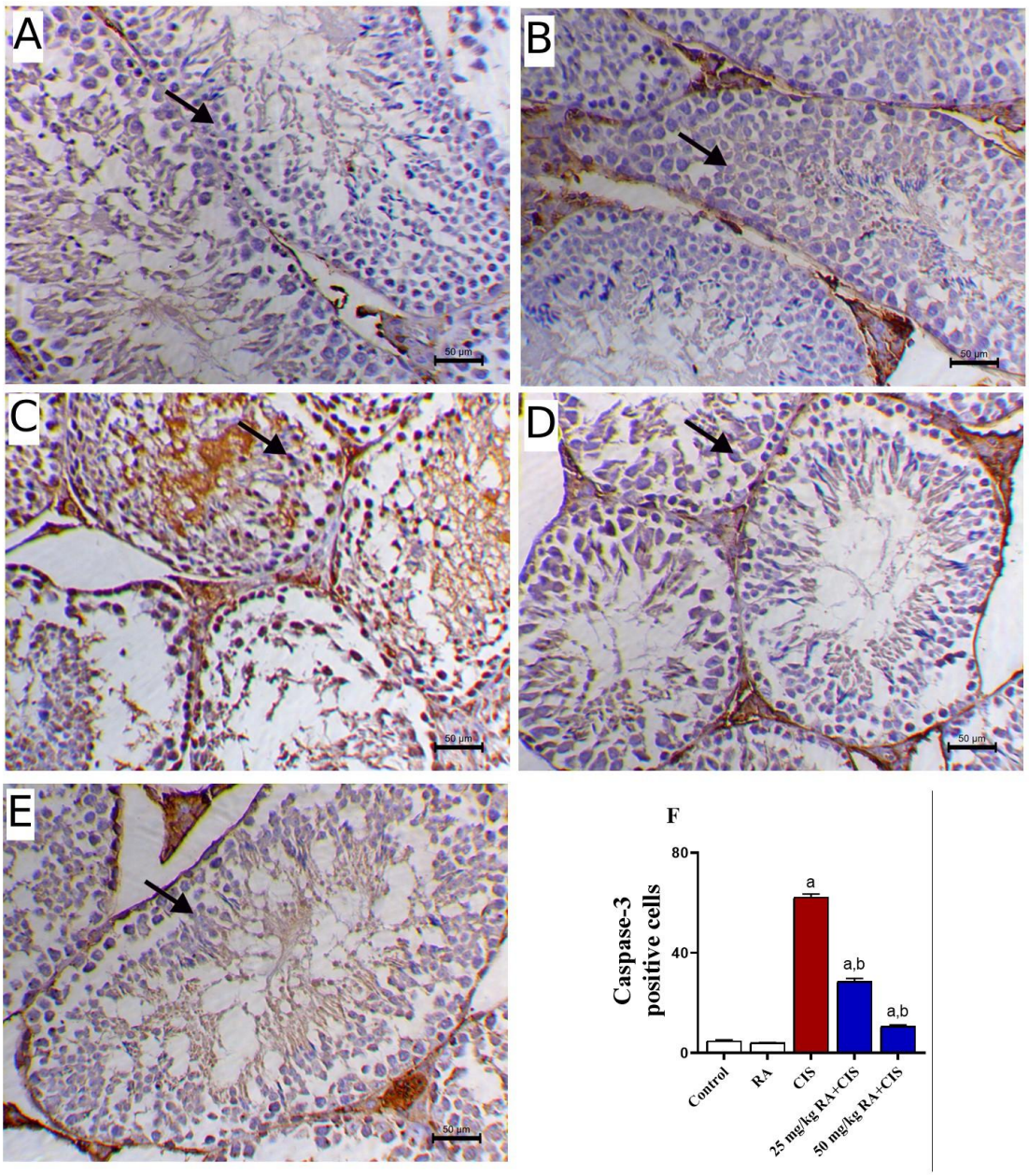

Figure 6. A restores Nrf2/HO-1 expression in the testes of CIS-injected mice. (A) Representative IHC staining of Nrf2 in testicular sections. (B) Quantification of Nrf2 expression across experimental groups. (C) Testicular HO-1 levels show a decrease with CIS administration and an increase following RA treatment. Data are presented as mean ± SEM (n = 6 per group). a P < 0.05 vs. control; b P < 0.05 vs. CIS.

3.5. RA pretreatment modulates testicular Nrf2/HO-1 signaling pathway

Expression of HO-1 and Nrf2 was found to be significantly ($P<0.05$) reduced in the testicular tissues of mice treated with CIS, as shown in Figure 6. These changes in concentration of HO-1 and expression of Nrf2 were reversed when CIS-treated mice were administered with RA (Figure 6). These findings confirm that activation of the Nrf2/HO-1 axis contributes to RA-mediated antioxidant and anti-apoptotic effects.

4. DISCUSSION

This study shows, for the first time, that RA significantly protects against cisplatin (CIS)-induced testicular damage. It does this by reducing inflammation, oxidative stress, and apoptosis, while restoring the Nrf2/HO-1 signaling pathway. Treatment with RA improved serum testosterone levels, sperm count, motility, and viability. It also reduced the histopathological damage caused by CIS in a dose-dependent way. These results suggest that RA could be a promising treatment for preventing reproductive toxicity related to chemotherapy.

Mechanistically, RA lowered the CIS-induced increase of NF-κB p65 and the related pro-inflammatory cytokines TNF-α, IL-1β, and IL-6. By reducing this inflammatory signaling, RA stopped the damage that leads to germ cell loss and disrupted spermatogenesis. RA also improved antioxidant defenses, as seen by restored glutathione levels, increased activities of SOD and CAT, and decreased lipid and protein oxidation. Additionally, RA treatment raised Nrf2 and its downstream target HO-1, indicating that activating this protective pathway is key to its effects on the testes. Moreover, RA positively adjusted the balance of apoptosis by lowering Bax and increasing Bcl-2 expression, thus preserving spermatogenic cells.

The finding that CIS alone harmed spermatogenesis, lowered testosterone, and caused degenerative histological changes matches previous studies (Abdel-Latif et al., 2022; Akhigbe et al., 2024; Aly & Eid, 2020). This consistency supports the validity of the current experimental design but does not present a new finding. The originality of this work lies in showing that RA can effectively counteract the known toxic effects of CIS through its combined actions on inflammatory, oxidative, and apoptotic pathways.

Overall, this study provides new evidence that RA offers multiple protective effects on the testis during chemotherapy-induced damage. By targeting NF-κB-related inflammation and boosting Nrf2/HO-1-driven antioxidant defense, RA helps restore testicular function and maintain spermatogenesis. These results underscore the potential of RA to protect male fertility during chemotherapy and highlight the need for further research, including dose-optimization studies and translational research in higher animal models and clinical settings.

The findings of this research were found to be in agreement with many previous reports (Abdel-Latif et al., 2022; Aly & Eid, 2020; Ismail et al., 2023). In particular, administration of CIS caused considerable decrement in the concentration of testosterone in sera and certain sperm parameters like its count, viability and motility. CIS administration also caused numerous histopathological changes like degenerative modifications in cells carrying out spermatogenesis. Loss of secondary and tertiary spermatogenic cells was also recorded which is linked with interstitial oedema. Likewise, a previous study has reported a reduction in serum concentration of testosterone as well as sperm count and sperm motility following CIS administration (Saral et al., 2016). Another research involving rabbit subjects reported a significant decrement in the concentration of epididymal sperms to the level of azoospermia together with reduced sperm viability and motility. Researchers also reported an association between these changes and reduced testosterone content in sera (Abdel-Latif et al., 2022). Spermatogenesis essentially requires testosterone as it triggers the synthesis of proteins in spermatogenic cells. Accordingly, a reduction in the concentration of testosterone as a consequence of oxidative stress in Leydig cells (Aly & Eid, 2020) impairs the process of spermatogenesis resulting in immature sperm generation (Rotimi et al., 2024). The CIS-induced testicular damage was effectively prevented with RA treatment by alleviating serum levels of testosterone and sperm count, viability and motility. The CIS-induced histological changes in the testis were also attenuated by RA in a dose-dependent fashion. These findings relating to the protective effect of RA are in agreement with the past in-vivo and in-vitro experiments (Li et al., 2014; Rahbardar et al., 2022; X. Zhang et al., 2018).

The role played by oxidative stress in causing CIS-induced testicular damage is confirmed (Mahran et al., 2025). Production of ROS serves as a crucial process in the pathology of CIS-induced testicular damage as it can lead to several outcomes like DNA oxidative damage, protein oxidation, lipid peroxidation and inactivation of antioxidants and other enzymes thereby ending up in cellular dysfunction and apoptosis (Abdel-Latif et al., 2022; Chiang et al., 2024). The potential of CIS for disturbing inner defense mechanisms is well-known; however, it can also damage mitochondrial DNA irreversibly thereby augmenting ROS generation as well as oxidative stress resulting in mitochondrial dysfunction and eventually causing cell death (Elmorsy et al., 2024; Katanić Stanković et al., 2023; Kleih et al., 2019). In concordance with previous reports (Abdel-Latif et al., 2022; Chiang et al., 2024), results recorded in this research indicated that the administration of CIS caused a considerable increment in MDA content which is linked with a reduction in GSH content and CAT and SOD activities. Physiological characteristics of cell membranes are modified due to lipid peroxidation resulting in the inactivation of membrane-bound proteins and loss of integrity of the membrane thereby leading to damage to the entire cell (Dasari & Tchounwou, 2014; Ognjanović et al., 2012). Likewise, the structure of protein gets changed due to oxidation resulting in aggregation or cleavage, impairment in function and hence catalytic activity is affected (Garrido Ruiz et al., 2022). Reduced semen quality, impairment in the function of sperm and DNA damage to sperm have proven to be some well-known outcomes of oxidative stress (Rezvanfar et al., 2013). Additionally, the degree of oxidative damage in the DNA of sperm can be augmented by oxidative stress which can also bring about germ cell apoptosis and inhibition of the production of testosterone in the Leydig cell (Asadi et al., 2021). Improvement in antioxidant defenses and alleviation of oxidative stress have therefore turned into crucial components of any therapeutic strategy meant to address complications associated with CIS. Administration of RA in CIS-treated mice caused a significant reduction in MDA and protein carbonyl levels besides restoring SOD and CAT activity as well as the concentration of GSH in the testis. Zych et al., (2019) have reported the role played by RA in protecting cardiac tissues from oxidative damage. The research involved female rats with type-2 diabetes (Zych et al., 2019). Likewise, Rahbardar et al., (2022) have carried out research that included both in-vivo and in-vitro experiments for exploring doxorubicin-linked cardiotoxicity. The researchers demonstrated the role of RA in mitigating MDA and GSH content (Rahbardar et al., 2022). Quan et al., (2021) also reported alleviation of ROS production and aconitase oxidative inactivation by RA in mice during myocardial reperfusion/ischmia injury (Quan et al., 2021). RA has also been reported to alleviate oxidative damage associated with acetaminophen hepatotoxicity in rodents (Yu et al., 2021). Besides these, RA can offer protection against liver damage associated with CIS and CCl4 (Y.-H. Lu et al., 2022), hepatic oxidative stress and DNA damage linked with chromium (Khalaf et al., 2020) and chlorpyrifos based renal oxidative stress (Abduh et al., 2023) through causing a decrement in lipid peroxidation. The ability of RA to scavenge free radicals enables it to demonstrate antioxidant properties (Frezza et al., 2019).

Numerous reports indicate activation of NF-κB by ROS production induced by CIS. Activation of NF-κB causes stimulation of generation of pro-inflammatory cytokines resulting in augmented oxidative stress and making the testicular

injury even worse (Akhigbe et al., 2024; Aly & Eid, 2020; Ismail et al., 2023; Katanić Stanković et al., 2023). Increment in concentrations of IL-6, IL-1 β, TNF-α and NF-κB p65 in the testis following treatment with CIS has been reported by numerous researchers (Akhigbe et al., 2024; Nna et al., 2020; Yalçın et al., 2024). According to Frungieri et al., (2002), inflammatory mechanisms can cause interference with normal spermatogenic activity, inhibition of testosterone generation by Leydig cells and disruption of gonadal steroidogenesis (Frungieri et al., 2002). Several researchers have put forward the proposition that CIS-induced inflammation and oxidative stress can evoke testicular apoptosis (Akhigbe et al., 2024; Aly & Eid, 2020; Ismail et al., 2023). Studies have also reported a considerable reduction in Bcl-2 concentration together with an increment in caspase-3 expression and concentration of Bax in tesis treated with CIS (Akhigbe et al., 2024; Aly & Eid, 2020; Nna et al., 2020). It has been suggested that apoptosis in testis under the effect of CIS is caused by the overproduction of ROS and impaired mitochondrial membrane potential which lead to the discharge of proapoptotic factors like cytochrome that ends up in the activation of caspase-3-dependent apoptotic cascade. Eventually male infertility occurs (Ghafouri-Fard et al., 2021; Hussein & Kamel, 2023; Nna et al., 2020). CIS-induced testicular damage can be attenuated by suppressing the inflammatory response (Hamdy et al., 1989; Hussein & Kamel, 2023). Administration of RA in testicular tissues of CIS-treated mice positively influenced the changes in levels of NF-κB p65, TNF-α, IL-1β, IL-6, caspase-3, Bax and Bcl-2. Researchers have reported a reduction in the inflammatory response in subjects with cardiac ischemia/reperfusion injury due to RA administration which addressed the changes in concentrations of heart proteins (p-NFκB and p-IκB-α) (Quan et al., 2021). Similarly, Zhang et al., 2019 demonstrated the prevention of apoptosis in cardiomyocytes through RA administration which inhibited the discharge of FasL in cardiac fibroblasts (J. Zhang et al., 2019). As stated by Kim et al. (2005), due to Adriamycin and overactivation of caspase protease, the RA treatment has the capability to address the decreased viability of H9c2 cell. A reduction in LPS- associated inflammation and cholestasis-associated oxidative stress were reported along with in inflammation in animal subjects via downregulating NF-κB (Kim et al., 2005). In addition, A role of RA in the modulation of NF-κB p65 and apoptosis mediators in kidneys was demonstrated by Abduh et al. (2023), which prevents inflammatory response and apoptosis linked with chlorpyrifos (Abduh et al., 2023).

The possible ways in the protection CIS-associated testicular damage by RA was discussed in this study. In this research, the expression of Nrf2 and concentration of HO-1 in the testis were explored. The protection of cells from oxidative stress and inflammatory response was successfully achieved by the role played by the transcription factor Nrf2 (Althunibat et al., 2022; Obeidat et al., 2022). It has been observed that after the CIS administration, the expression of Nrf2 and concentration of HO-1 has been decreased. The IκB-α proteasomal degradation and activation of NF-κB induced by oxidative stress are inhibited by Nrf2 thereby causing suppression of the inflammatory response mediated by NF-κB (Saha et al., 2020; Wardyn et al., 2015). Upregulation of Nrf2 is beneficial in alleviating CIS-linked testicular damage. RA administration in CIS-treated mice resulted in increased expression of Nrf2 and augmented concentration of HO-1 in the testis. This is in concordance with past studies which demonstrated mediation of Nrf2 by RA thereby offering protection from CCl4-associated liver damage (Y.-H. Lu et al., 2022), liver and kidney damage caused by cisplatin (Xiang et al., 2022) and kidney damage caused by chlorpyrifos (Abduh et al., 2023). According to Lu et al., (2017), suppression of ROS in hepatic stellate cells (HSCs) caused by RA was reduced by deletion of Nrf2 (C. Lu et al., 2017). Hence, activation of Nrf2 or HO-1 plays a pivotal role in the antioxidant and anti-inflammatory potential of RA in testicular damage caused by CIS administration.

Despite its strengths, this study has some limitations. It was conducted only in mice, and biochemical analyses were limited to testicular tissue without hormonal or fertility follow-up. Further studies in larger animal models and clinical settings are needed to confirm the translational relevance of these findings.

5. CONCLUSION

The well-established CIS-associated testicular damage can be avoided through utilization of RA. Here, we showed in CIS-treated mice, RA administration resulted in attenuation of the inflammation, oxidative stress and apoptosis implicated in CIS-mediated testicular damage. Changes in different sperm parameters like sperm count, sperm viability and mobility are also reversed due to RA administration. Same is the case with testosterone concentration in sera. Hence RA can be considered as a useful therapeutic tool to devise an innovative strategy for avoiding CIS-associated testicular injury and potentially other conditions involving these same pathological mechanisms.

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Data Availability

All data generated are represented in this manuscript.

Conflict of Interest

The authors declare no conflict of interest in this study

Author Contribution

BPN and AAB conceived and designed the study; AAB conducted the experimental work and collected the data; SMA performed data analysis and interpretation; BPN and SMA wrote the initial draft of the manuscript; BPN, AAB, and SMA critically reviewed and approved the final version of the manuscript. BPN supervised the entire research.

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