



## Significance of red ginseng extract pretreatment on cyclophosphamide-induced testicular damage in gamma-irradiated rats

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### ABSTRACT

Cancer is a complicated disorder with negative impacts on humans' lives that intensifies the mortality rate. Chemotherapy and/or radiotherapy (RT) are used for cancer treatment to achieve the most efficient management of cancer. Cyclophosphamide (CP) is a recommended medication for plentiful tumors. It has valuable anti-neoplastic and immunosuppressive impacts. The combination of CP and gamma radiation (R) can boost the curative benefits of some malignant diseases. However, this combination has some drawbacks, such as testicular damage. Korean red ginseng (*Panax ginseng*; KRG) is rich in ginsenoside phytochemicals that exhibit antioxidants and anti-inflammatory activities. The purpose of the current investigation was to determine whether orally administering KRG water extract might significantly protect against CP and/or R toxicities. KRG exhibited significant amelioration of the testicular architecture as well as sperm count and sperm motility; and restored the serum levels of male reproductive hormones (testosterone (TH), Follicle Stimulating Hormone (FSH), and Luteinizing Hormone (LH)). Moreover, it improved the levels of lactate dehydrogenase (LDH), acid phosphatase (ACP), calcium (Ca<sup>2+</sup>), malondialdehyde (MDA), nitric oxide (NO), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and glutathione reduced (GSH). Also, it boosted the superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) activities, lowered the elevated levels of tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin-6 (IL-6), nuclear factor-kappa B (NF- $\kappa$ B), interferon gamma (IFN- $\gamma$ ), and programmed death-ligand 1 (PD-L1), down-regulated the protein expression ratios of p38 mitogen-activated protein kinase (p38-MAPK) and epidermal growth factor receptor (EGFR), and regulated the gene expression ratios of androgen receptor (AR), inducible nitric oxide synthase (iNOS), p65 nuclear factor-kappa B (p65 NF- $\kappa$ B), miR-18a, and let7e in the testis tissues of R, CP, and R/CP intoxicated groups. It could be concluded that KRG may improve testicular impairment by attenuating oxidative stress and inflammatory responses via the regulation of PD-L1 and IFN- $\gamma$  levels, miR-18a and let7e genes' expression, and p38-MAPK and EGFR proteins' expression.

**Keywords:** Korean Red Ginseng; cyclophosphamide; gamma-radiation; testicular damage.

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## **INTRODUCTION**

Cancer is a complicated disorder that has negative impacts on humans' lives and intensifies the global risk of mortality. Cancer prevalence and mortality rates are rising all over the world (**De Rezende et al., 2019**). The routes for cancer treatment include chemotherapy (CT), radiotherapy (RT), hormonal therapy, or immunotherapy, etc. Sometimes a combination of these therapeutic approaches is used to achieve the most efficient management of cancer (**Saini and Twelves, 2021**). To protect against the development of secondary neoplasia, a combination of CT and RT was applied as a synergistic therapy for most malignant diseases. However, both therapeutic models demonstrate limitations, as they induce systemic toxicity, and critically affect normal cells (**Ganesh and Manjeshwar, 2018**). The combined CT and RT during cancer curing can give rise to testicular toxicity in male patients (**Meistrich, 2013**). Cyclophosphamide (CP) can be combined with gamma-radiation (R) to improve the curative benefits of malignant diseases (**Ganesh and Manjeshwar, 2018**). CP is a CT drug advised for plentiful tumors and some chronic non-malignant diseases due to its anti-neoplastic and immuno-suppressive properties (**Avgeropoulos and Newton, 2018, Araghi et al., 2018**). CP is metabolized into the phosphoramidate mustard then into aziridinium active forms in the liver of the patients. Aziridinium triggers DNA alkylation and develops DNA-DNA and DNA-protein cross-links to initiate the apoptosis of the malignant cell (**Stork and Schreffler, 2014**). The hepatic cytochrome P450 catalyzes the formation of acrolein, another metabolite of CP. Acrolein is responsible for the toxicity of CP through the induction of oxidative stress and apoptosis (**Araghi et al., 2018**). CP triggers toxicities in different organs, including gonadal toxicity, manifested by histo-biochemical changes, damage to the testes and epididymis tissues, disturbance in gonadotropin secretion, as well as a decline in testosterone level, which leads to infertility in men and experimental male animals (**Khamis et al., 2023**).

Ionizing radiation (IR) is an imperative modality to fight malignant diseases. About 50% of patients may be exposed to IR throughout their clinical management of cancer. Even so, IR affects normal and tumor tissues, which restricts the therapeutic process. IR exposure engender harmful free radicals, reactive oxygen species (ROS), and reactive nitrogen species (RNS), thus triggering oxidative stress in all cells and tissues. This oxidative stress triggers impairment of the cell structures and leads to various diseases mediated by several signaling pathways (**Ismail et al., 2023**). Exposure to IR can cause cell damage directly or through bystander effects. In the bystander effect, the irradiated cells communicate and share the acquired signals with neighboring cells, thus transferring the damaging effects of R to un-irradiated cells and organs. Exposure to IRs develops oxidative stress, inflammatory response, and apoptosis of the testicular tissues, depending on the radiation dose. This testicular toxicity causes alterations in the sperm production and function, sexual impairment, and the break-down of the spermatogenesis processes at high doses of radiation. Suppression of spermatogenesis processes causes sperm cell apoptosis and oligozoospermia, which can lead to azoospermia (**Zhang et al., 2019**).

Some antioxidant natural compounds demonstrated protective activity by reversing CP toxic effects (**Khamis et al., 2023**) or exhibiting radio-protective activity (**Targhi et al., 2017**). One of the extremely active natural drugs is Korean Red Ginseng (KRG, *Panax ginseng*). KRG is obtained after the steaming and drying of the fresh ginseng root. Accordingly, the biologically active Rg3, Rg5, Rh2, Rh3, Rh4, Rs3, and F4 ginsenoside phytochemical constituents were produced (**Park, 2017a**). The phytosterols, sesquiterpenes, flavonoids, polyacetylenes, alkaloids, and phenolic phytoconstituents are associated synergistically with ginsenosides to perform the biological activity of KRG (**Park et al., 2018**). Ginsenosides are steroidal triterpene saponins

that exhibit a variety of pharmacological activities. Ginsenosides regulate the inflammatory response by obstructing NF- $\kappa$ B signaling and down-regulating the expression of pro-inflammatory cytokines. Furthermore, KRG can encourage the activity of antioxidant enzymes and fight lipid peroxides, thus holding back oxidative stress (Jung *et al.*, 2023). KRG has a wide range of biological activities, including antioxidants (Park *et al.*, 2021), anti-inflammatory (Paul *et al.*, 2012), anti-diabetic (Chen *et al.*, 2019), hepatorenal protection (Elblehi *et al.*, 2023, Park *et al.*, 2017b), neuroprotection (Seo *et al.*, 2016), radioprotection (Kim *et al.* 2017), antineoplastic (Yun *et al.*, 2001), and immunologic benefits (Heo *et al.*, 2016). In addition, KRG and its ginsenoside exhibited anti-viral activity against the human coronavirus strain OC43 (Jeong *et al.*, 2023). Moreover, KRG is beneficial for improving libido and protecting against and treating infertility in men (Kopalli *et al.*, 2019). KRG demonstrated protection against testicular impairment and fertility in rats (Lee *et al.*, 2019), recovered testicular ineffectiveness in aging rats (Kopalli *et al.*, 2017a), attenuated doxorubicin-induced testicular dysfunction (Cha *et al.*, 2018, Kopalli *et al.*, 2016), and improved sperm property in Guinea pigs (Hwang *et al.*, 2004). KRG is a useful agent for the treatment of male infertility; however, the exact mechanism by which KRG improves spermatogenesis is still under investigation (Park *et al.*, 2016). According to these prominent facts, we investigated the protective role of the oral administration of Korean red ginseng water extract (KRG) in cyclophosphamide and gamma-radiation-induced testicular damage in rats, considering the role of let-7 in controlling the inflammatory and oxidative stress responses.

## MATERIALS AND METHODS

### Chemicals

KRG powder was purchased from the Imtenan herbal drug store. Cyclophosphamide (Endoxan, Baxter Germany, Westfalen/Halle, GmbH Oncology B) was used in this study.

### Irradiation facilities

Whole-body gamma radiation was achieved employing  $^{137}$ Cesium gamma Cell-40. At the time of exposure, the radiation dose rate was 623 rad/s (radiation-absorbed dosage per second). The animals received 6.0 gray (Gy) of radiation, 2 Gy daily for 3days starting from day 17.

### Preparation of Korean Red Ginseng water extract

Distilled water (dist. H<sub>2</sub>O<sub>2</sub>) was used to extracted KGR powder (1:10 wt./volume) at 90 °C for 48 h. Then, the filtrate was dried, yielding a dark brown water extract (Hwang *et al.*, 2004).

### Characterization of the Korean Red Ginseng extract

Total phenolic content (TPC) (Singleton *et al.*, 1999), total flavonoid content (TFC) (Chang *et al.*, 2002), total saponin content (TSC) (Hiai *et al.*, 1976), and total antioxidant capacity (TAC) (Zhang *et al.*, 2018) of KRG extract were assessed according to the mentioned references.

The result of TPC was demonstrated as mg gallic acid equivalents per g of dry KRG extracts (mg GAE/g). The result of TFC was demonstrated as mg quercetin equivalent per g of dry KRG extracts (mg QE/g). The TSC result was demonstrated as mg soya saponin Ba equivalents per g of dry KRG extracts (mg SBa E/g). The TAC was demonstrated as mg of ascorbic acid equivalent per gram of dry KRG extract (mg AAE/g).

### Doses of treatments

Rats received orally 100 mg of KRG extract suspended in dist. H<sub>2</sub>O<sub>2</sub>/kg b.wt., daily for 21 days (Kopalli *et al.*, 2016). On day 20, after the last dose of gamma-radiation, rats received intraperitoneally (IP) 300 mg of CP diluted with saline/kg b.wt. (Zhai *et al.*, 2018).

### **Animals and experimental design**

The NCRRT animal house's breeding facility provided the male *Wistar* albino rats, weighing between 200 and 220 grams. The animals were housed in plastic cages in standard practical conditions. Regular pellet food and fresh water were freely available to them at all times. The animals spent a week acclimation period.

Rats were randomly allocated into eight groups of ten, G1: control (C), G2: G, KRG treated rats, G3: R, gamma-irradiated rats, G4: CP, CP treated rats, G5: R/CP, R and CP treated rats, G6: G/R, G7: G/CP, and G8: G/R/CP.

Twenty-four hours after the last dose of CP and KRG extract administration, all animals were anesthetized with 1.2 g urethane (Sigma-Aldrich, St Louis)/kg b.wt. (Moheban et al., 2016). The blood was collected from the abdominal aorta, then kept on ice to clot, and finally centrifuged. The separated sera were used in the assessment of reproductive hormones (TH, FSH, and LH). The cauda epididymis from each rat was isolated and dissected in a 0.9% NaCl solution at 37 °C to prepare the sperm suspension (Faqi et al., 1998). The left testes were excised, rinsed in ice-cold saline, dried carefully, and stored at -80 °C. The right testes were fixed in 10% formalin for the histopathological checkup.

### **Histopathological examination**

Testicular tissues were collected from all animal groups, then washed with ice-cold saline and fixed in a 10% formalin solution for 24 to 48 hours. They were dehydrated in a graded ethanol series, then cleared in xylene, inserted in paraffin blocks, and sectioned at 4-6 µm thick. The gained tissue sectors were de-paraffinized by xylol and stained using hematoxylin and eosin (H&E). After then, examined under an electric light microscope (Bancroft and Layton, 2019).

The microscopic scoring was ranked on scales of mild (+), moderate (++) and severe (+++), which were applied to histological parameters [desquamation in germinal cells, disorganization in germinal cells, interstitial edema, degeneration in germinal cells, and reduction in germinal cell counts] (Tuglu et al., 2015).

### **Measurement of the body weight**

Prior to scarification, the body weights (BWT) of the experimental animals in each group were determined. Additionally, following scarification, the testes weights (TWT) were measured with an electronic balance.

### **Sperm count, motility, and abnormality**

The sperm count, motility, and abnormalities were assessed in the sperm suspensions.

### **Hormonal investigation**

The levels of the reproductive hormones were assessed in serum. Rat Elisa Kits were used for assessment of Testosterone (TH, ng/ml), Follicle Stimulating Hormone (FSH, ng/ml), and Luteinizing Hormone (LH, mIU/ml).

### **Lactate dehydrogenase and acid phosphatase activities**

The activities of lactate dehydrogenase (LDH) and acid phosphatase (ACP) were assessed in the testis tissues using rat ELISA kits.

### **Determination of calcium (Ca<sup>2+</sup>) level in the testis tissues**

The testis tissues of different studied groups were washed with ionized water, then digested in a mixture of concentrated nitric acid (HNO<sub>3</sub>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) (5:1 v/v) until overall digestion of the organic materials was achieved using Milestone MLS-1200 Mega, High-Performance Microwave Digester Unit, Italy. Ca<sup>2+</sup> level was determined in the prepared tissue samples using an ICP (OES), Perkin Elmer, Optima 2000 DV.

### **Preparation of the crude testes' tissues homogenates**

The testes were homogenized in ice-cold 50 mM Tris-HCl/150 mM KCl/0.25 M sucrose buffer (pH 7.4) (Kaushik *et al.*, 2018) to prepare a 10% (w/v) crude tissue homogenate.

### **Determination of the oxidative stress parameters and antioxidant enzymes in testis' tissues:**

Malondialdehyde (MDA), glutathione reduced (GSH), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and nitric oxide (NO) levels, as well as superoxide dismutase (SOD), catalase (CAT), and glutathione-peroxidase (GPX) activities were evaluated according to the described procedures using Biodiagnostic kits.

### **Assessment of the inflammatory markers level**

The levels of tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin-6 (IL-6), interferon gamma (IFN- $\gamma$ ), and nuclear factor-kappa B (NF- $\kappa$ B) were determined in the testicular tissues using ELISA kits for rats.

### **Western blotting analysis**

The relative protein expression ratios of p-38 MAPK and epidermal growth factor receptor (EGFR) compared to  $\beta$ -actin were assessed in the testis tissues using the Western Blot technique. TRIzol reagent was used to extract tissue proteins. The total protein concentration was estimated by Lowry's method (Lowry *et al.*, 1951). Twenty micrograms of protein per lane were isolated with 10% SDS polyacrylamide gel electrophoresis, then, transported to polyvinylidene fluoride (PVDF) membranes. Membranes were then incubated at room temperature for 2 h with a blocking solution (5% nonfat dried-milk/10 mM Tris-HCl/pH 7.5/ 100 mM NaCl/ 0.1% Tween 20). Membranes were incubated overnight at 4°C with the designated primary antibodies: p38 MAPK rabbit polyclonal antibody (Cat #PA1-30391), and EGFR rabbit polyclonal antibody (Cat #PA1-1110), and  $\beta$ -actin (Cat. No: MA5-1140), Invitrogen, Thermo Fisher Scientific. Then incubated with a mouse anti-rabbit secondary monoclonal antibody coupled to horseradish peroxidase, at room temperature for 2 h. Chemiluminescence detection was achieved using the Amersham detection kit. After each incubation process, the membranes were washed several times (with 10 mM Tris-HCl/pH 7.5/100 mM NaCl/0.1% Tween 20) at room temperature. Then, the amount of the analyzed protein was computed by densitometric analysis using BioRad software for image and gel analysis, USA. The results were standardized to  $\beta$ -actin protein expression.

### **Real-time quantitative reverse transcription polymerase chain reaction analysis**

The androgen receptor (AR), inducible nitric oxide synthase (iNOS), and p65 nuclear factor-kappa B (p65 NF- $\kappa$ B) mRNA relative to  $\beta$ -actin were assessed in the testis tissues using the real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR).

Total RNA from the frozen testis samples was extracted by a Qiagen kit (USA), isolated, and inversely transcript into complementary DNA (cDNA), employing Moloney murine leukemia virus (M-MLV) reverse transcriptase (Promega, Madison, USA). Step One Plus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) and an SYBR® Green PCR Master Mix (Applied Biosystems) were conducted in a 10  $\mu$ l final volume, programming the heating cycles: 95°C (10 min), then 40 cycles of 95°C (15 s) and 65 °C (1 min). The used sequences of PCR primer pairs were as follows: AR: androgen receptor: Forward: 5'-AATGGGACCTTGGATGGAGAACTA-3', Reverse: 5'-TCATAACATTTCCGGAGACGACAC-3', iNOS: inducible nitric oxide synthase: Forward: 5'-TCTTTGCTTCTGTGCTAATGCG-3', Reverse: 5'-GTTGTTGCTGAACTTCCAATCGT-3', p65 NF- $\kappa$ B: nuclear factor – kappa B subunit 1: Forward: 5'-CCAAAGACCCACCTCACC-3', Reverse: 5'-CGCTTCTTACACACTGGA-3', and the housekeeping reference gen;  $\beta$ -actin:

Forward: 5'-TCT ACA ATG AGC TGC GTG TG-3' and Reverse: 5'-TAC ATG GCT GGG GTG TTG AA-3'. The data were evaluated with the ABI Prism sequence detection system software and computed using v1.7 Sequence Detection Software, from PE Biosystems (Foster City, CA). The relative expression values of the studying genes were evaluated using the comparative threshold cycle method. All values were normalized to  $\beta$ -actin, applying the expression  $2^{-\Delta\Delta C_t}$  (Pfaffl, 2001).

#### **Assessment of micro-RNAs in the testis tissues**

Let7e mRNA and miR-18a relative to U6 were assessed in the testis tissues using RT-qPCR technique.

Total RNA containing micro-RNAs (miRNAs) was extracted from liver tissues by using acidic phenol/chloroform extraction (peqGOLD RNAPure; PEQLAB Biotechnologie, Erlangen, Germany) according to the manufacturer's instructions. For analyses of miRNAs, 2  $\mu$ g of RNAs were treated with DNase (Ambion, Austin, TX, USA). Using a volume of 2  $\mu$ mol/L oligo d (T) 16 primer (Promega, Mannheim, Germany) in addition to 200 U M-MLV reverse transcriptase (Promega) at a certain temperature of 42°C, RNAs were reverse transcribed. The cDNAs were quantified in real-time using primers. The qPCR volume was 13  $\mu$ L, whereas 6.5  $\mu$ L 2 $\times$ mastermix (Applied Biosystems, Foster City, CA, USA), 2.5  $\mu$ L primer mix (1.25  $\mu$ mol/L). miRNAs were reverse transcribed with a special primer (RT-primer). Then, for analysis of miRNAs extracted from liver tissues, 50 ng total RNA containing miRNAs, was reverse transcribed with 1  $\mu$ mol/L. The following PCR primers were used, for let7e: Forward: 5'-TGAGGTAGGAGGTTGTATAG-3'; Reverse: 5'-GAACATGTCTGCGTATCTC-3'), miR-18a: Forward: 5'-GCTGAGCTAAGGTGCATCTAG-3'; Reverse: 5'-TCAACTGGTGTCTGGAGT-3', and U6 RNA: Forward: 5'-ATACAGAGAAGATTAGCATGGCC-3', Reverse: 5'-GTCCAGTTTTTTTTTTTTTCGAC-3'). The let7e expression ratio was analyzed with the ABI Prism 7500 detection system (Applied Biosystems) and was performed according to the  $2^{-\Delta\Delta C_T}$  method (Pfaffl, 2001).

#### **Assessment of the Programmed death-ligand 1**

A rat ELISA Kit from MyBioSource was used for the assessment of programmed death-ligand 1 (PD-L1).

#### **Statistical analysis**

Microsoft Excel and the Statistical Package for Social Science Software (SPSS, version 23.0) were used to analyze the data. The data were explained as the mean  $\pm$  standard error (SE). To examine the variation in the variable means between groups, ANOVA (one-way analysis of variance) with the LSD (least significant difference) post hoc multiple comparisons was utilized. A *p*-value of less than 0.01 was deemed statistically high significant.

## **RESULTS**

### **Histopathological examination**

The testicular parenchyma of the C and G groups showed active spermatogenesis and normal-size seminiferous tubules. The spermatogenic cells composed of many layers: the spermatogonia, primary and secondary spermatocytes, and spermatids that rested on the thin basal lamina. The interstitial cells were found mostly in clusters between the seminiferous tubules with characteristically large and ovoid nuclei (Figures 1: A and B).

On the other side, in the R group, atrophied seminiferous tubules with a widening of the interstitial spaces and a reduction in the number of interstitial cells were observed. Disorganization and pyknosis of spermatogenic cells were noticed.

Seminiferous tubules showed decreases in tubular diameter, losses of germinal line, total or partial reductions in spermatogenesis, and the presence of abnormal spermatids (Figure 1C). However, animal groups treated with CP revealed shrunken seminiferous tubules with wide interstitial spaces and disorganization of spermatogenic cells. The cells in the spermatogenic series showed significant decreases in their numbers, as compared to the C group. Sertoli cells with vacuolated cytoplasm were seen (Figure 1D). Furthermore, animals in the R/CP group showed atrophy of seminiferous tubules and reductions in the number of interstitial cells. Disorganization and necrosis of spermatogenic cells were noticed. Degenerated and necrotic cells in the spermatogenic series with partial reductions of spermatogenesis were also noticed (Figure 1E).

However, in the animals in the G/R group, the testicular parenchyma showed active spermatogenesis in normal-size seminiferous tubules in comparison with the untreated group. The interstitial spaces showed clusters of interstitial cells between the seminiferous tubules. Mild interstitial edema and hyperplasia of interstitial cells were also seen (Figure 1F).

In addition, in the G/CP group, the seminiferous tubules showed features approximately like those of the C group. The spermatogenic cells appeared regularly arranged within the seminiferous tubules. Mild degrees of germinal cells degeneration and widening of the interstitial spaces were seen (Figure 1G).

Finally, in the G/R/CP group, testicular tissue sections revealed varied-size seminiferous tubules, and hyperplasia of interstitial cells. The seminiferous tubules showed a reduction of spermatogenic cells with hyperactivity in Sertoli cells. Some seminiferous tubules contained well-developed spermatids, and others were empty (Figure 1H).

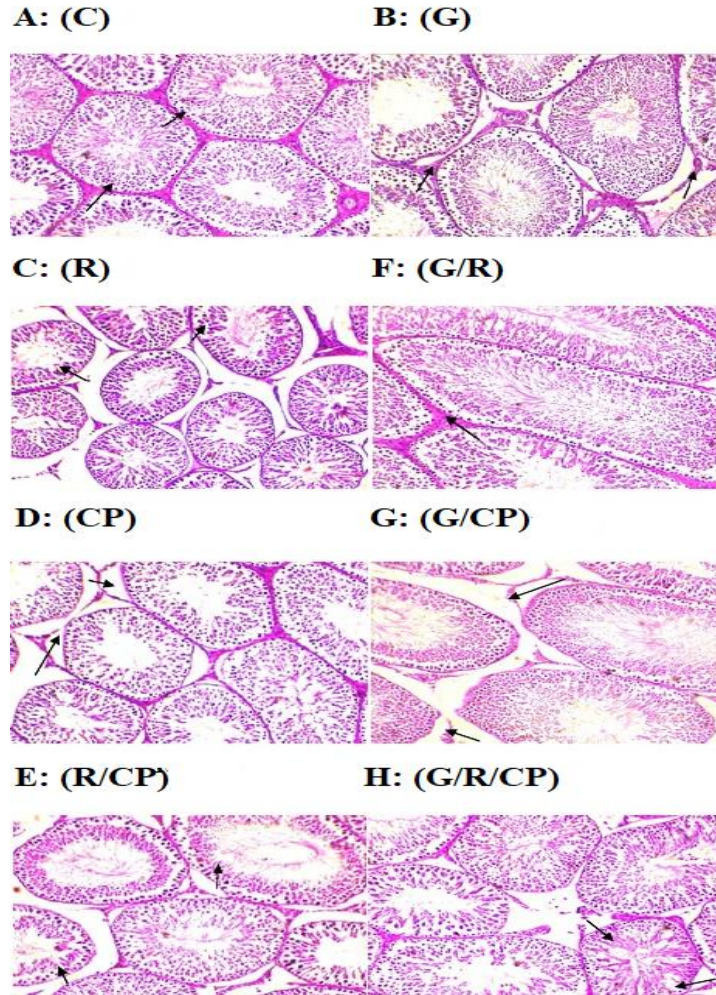
The degrees of testicular lesions for different groups are illustrated in Table (1).

**Table (1): The degrees of testicular lesions**

Parameters	Group	C	G	R	CP	R/CP	G/R	G/CP	G/R/CP
Desquamation in germinal cells		ND	ND	++	++	++	+	++	ND
Disorganization in germinal cells		ND	ND	+++	+++	+++	ND	++	ND
Interstitial oedema		ND	ND	+	++	+	ND	+	+
Degeneration in germinal cells		ND	ND	++	+++	+++	+	++	+
Reduction in germinal cell counts		ND	ND	++	++	+++	+	++	+

ND: Not detected, +: mild, ++: moderate, +++: severe.

C: control group, G: Korean Red ginseng (KRG) treated group, R: gamma-irradiated group, CP: cyclophosphamide treated rats, R/CP: rats exposed to gamma-radiation then treated with cyclophosphamide, G/R: KRG treated rats, then exposed to gamma-radiation, G/CP: rats treated with KRG, and cyclophosphamide, and G/R/CP: KRG treated rats, then exposed to gamma-radiation, and treated with cyclophosphamide.



**Figure 1 Photomicrograph of the testis histopathology (H&E X200)**

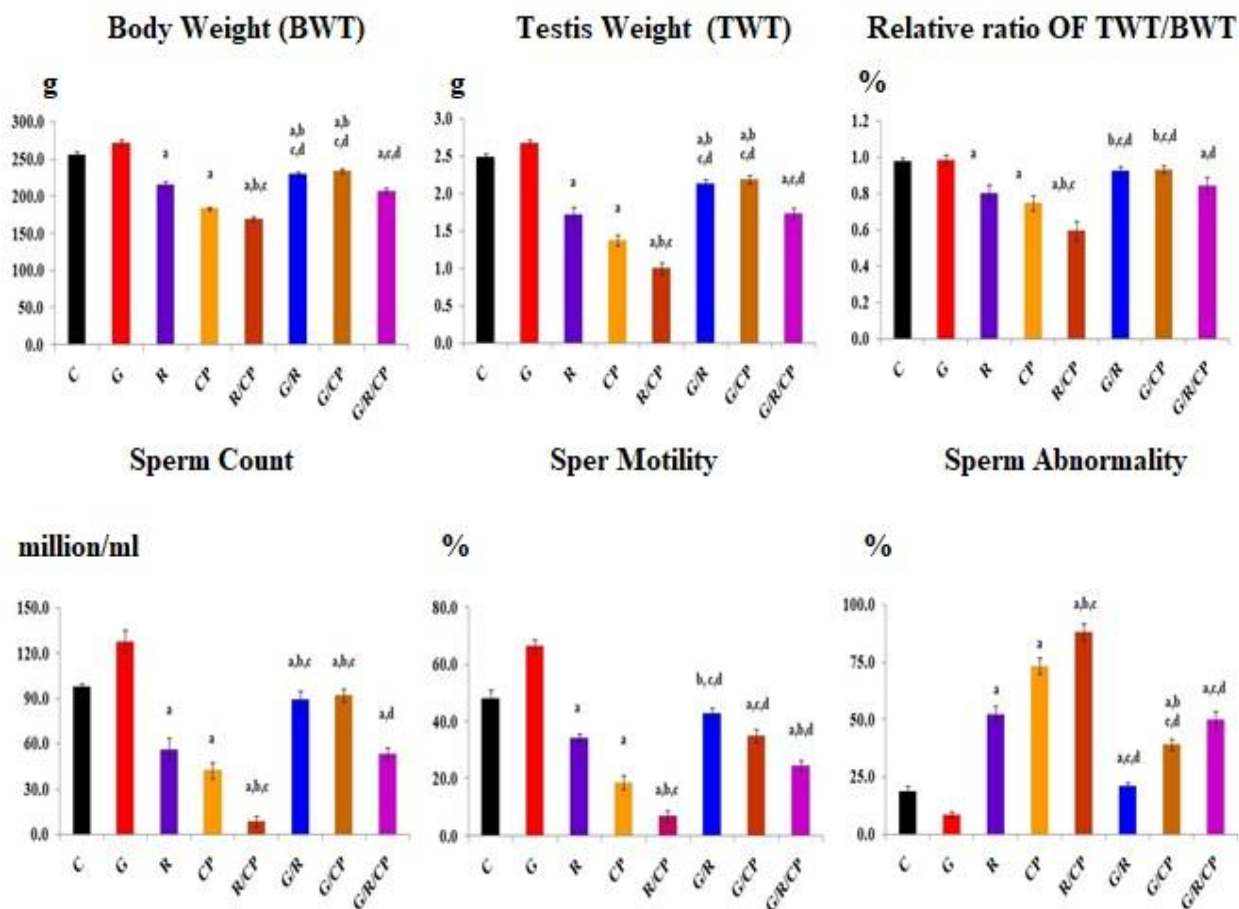
**Measurement of the body weight, and testis weight**

The body and testis weights, as well as their relative ratios (TWT/ BWT) of the R and CP groups showed high significant declines ( $p < 0.01$ ) that were augmented in the R/CP group, as evaluated to the controls. KRG extract improved the BWT, TWT, and TWT/ BWT ratio in the G/R, G/CP, and G/R/CP groups (Figure 2).

**Sperm count, motility, and abnormality**

The data demonstrated that the sperm count, sperm motility, and sperm abnormality showed high significant declines ( $p < 0.01$ ) in the R and CP-treated animals, which demonstrated more decline in the R/CP-treated group, as evaluated to the controls. KRG extract ameliorated the sperm count, sperm motility, and sperm abnormality in the G/R, G/CP, and G/R/CP groups (Figure 2).





**Figure 2. Body weight, Testes weight, Relative ratio of Testes weight to Body weight, and sperm count, motility, and abnormality.**

The results are expressed as Mean  $\pm$  SE (n = 10). a: Significance versus control (C), b: Significance versus radiation (R), c: Significance versus CP, d: Significance versus R/CP, at  $p < 0.01$ .

### Hormonal investigation

The data showed that the levels of TH and LH showed high significant ( $p < 0.01$ ) declines, but the levels of FSH revealed high significant ( $p < 0.01$ ) increases in the serum of the R, CP, and R/CP-intoxicated groups, as evaluated to the controls. KRG extract restored TH, FSH and LH levels in serum of G/R, G/CP, and G/R/CP groups (Figure 3).

### Lactate dehydrogenase and acid phosphatase activities in the testis tissues

The activity of LDH and ACP showed high significant ( $p < 0.01$ ) enhancements in the testis tissues of the R, CP, and R/CP-intoxicated groups, as evaluated to the controls. KRG extract ameliorated the activity of LDH and ACP in the testis tissues of G/R, G/CP, and G/R/CP groups (Figure 3).

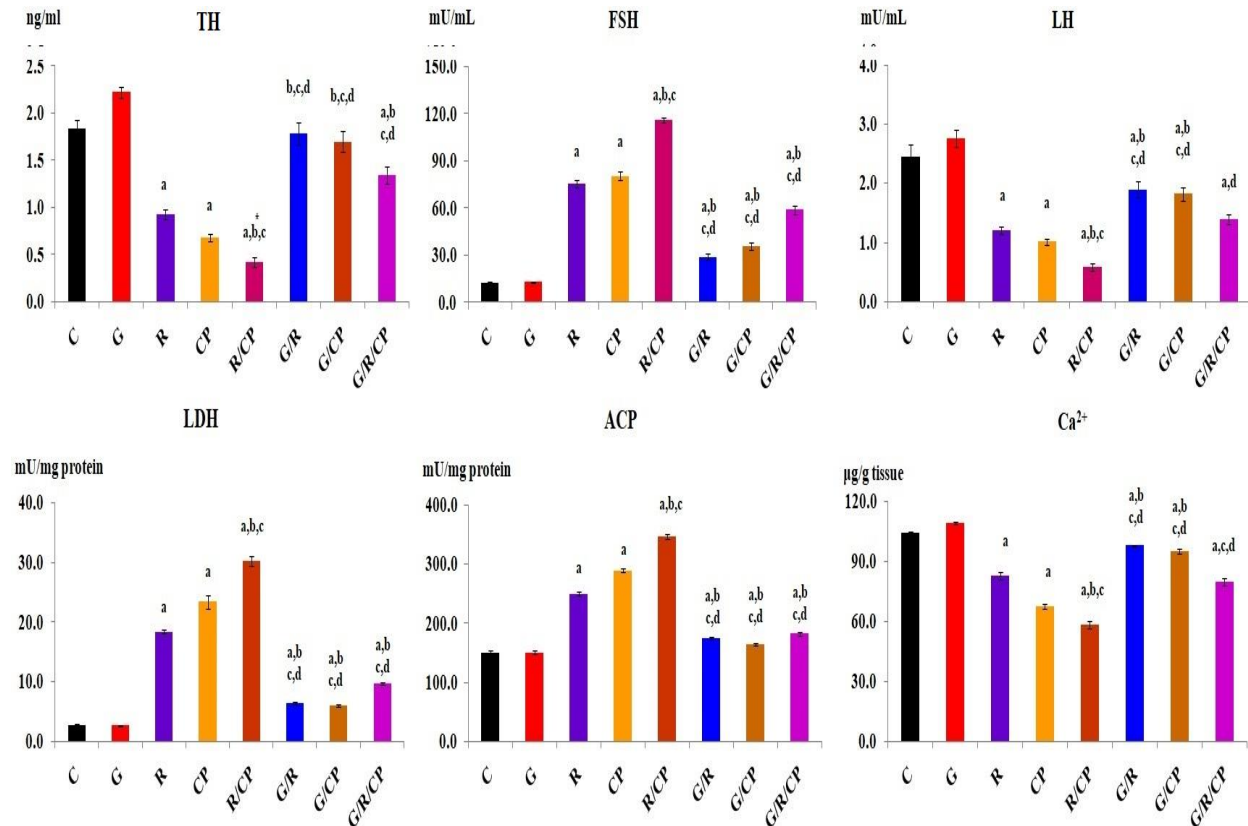


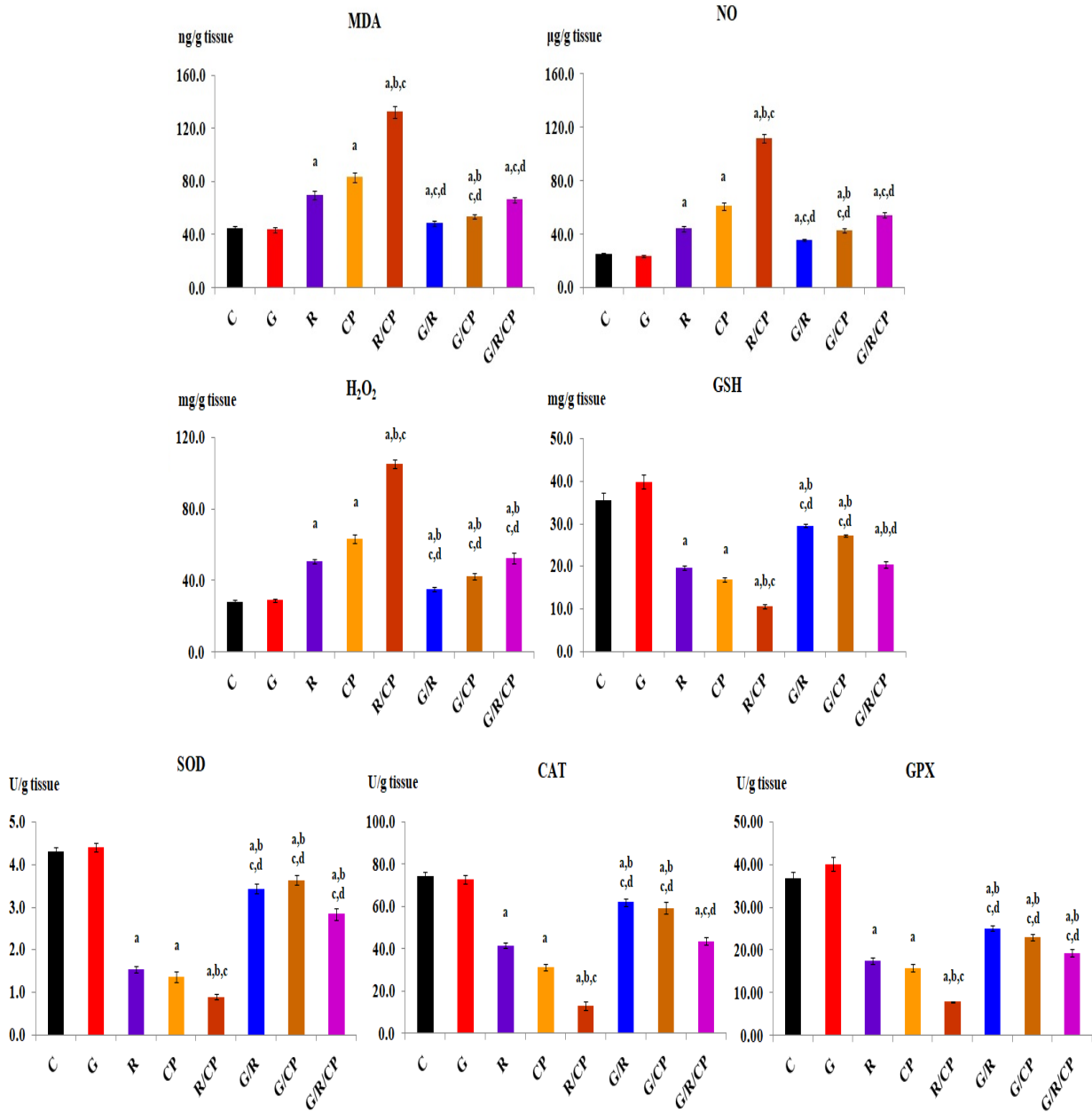
Figure 3. The levels of reproductive hormones, lactate dehydrogenase (LDH), acid Phosphatase (ACP), and calcium (Ca<sup>2+</sup>). Legend as figure 2.

#### Calcium level in the testis tissues

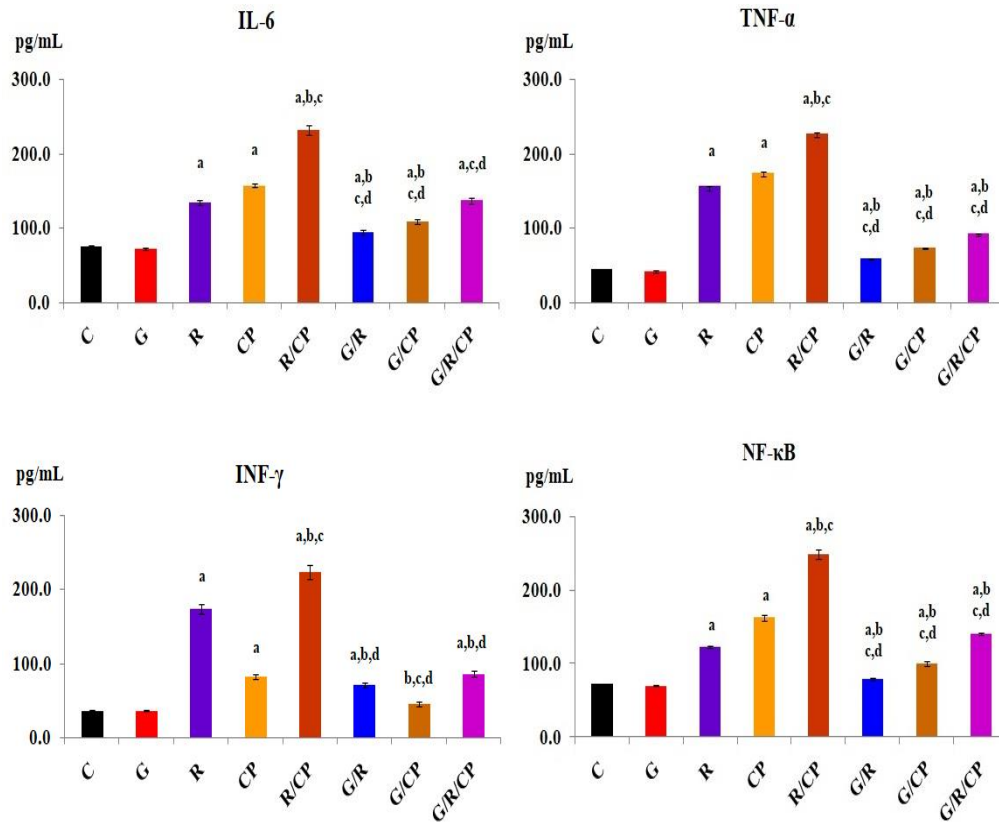
The level of Ca<sup>2+</sup> showed highly significant ( $p < 0.01$ ) decreases in the testis tissues of the R, CP, and R/CP intoxicated groups, as compared to the control animals. KRG extract ameliorated Ca<sup>2+</sup> levels in the testis tissues of G/R, G/CP, and G/R/CP groups (Figure 3).

#### The antioxidant status: the oxidative stress parameters and antioxidant enzymes

The levels of MDA, NO, and H<sub>2</sub>O<sub>2</sub> showed high significant amplification ( $p < 0.01$ ). While the GSH contents were highly significantly ( $p < 0.01$ ) decreased, and the SOD, CAT, and GPX activities showed high significant ( $p < 0.01$ ) suppressions in the testis tissues of the R, CP, and R/CP intoxicated groups, as evaluated to the controls. KRG extract reduced the MDA, NO, and H<sub>2</sub>O<sub>2</sub> levels, restored the GSH contents, and improved the activities of SOD, CAT and GPX enzymes in the testis tissues of G/R, G/CP, and G/R/CP groups (Figure 4).



**Figure 4.** The levels of oxidative stress parameters and activities of the antioxidant enzymes in the rats' testes. Legend as figure 2.



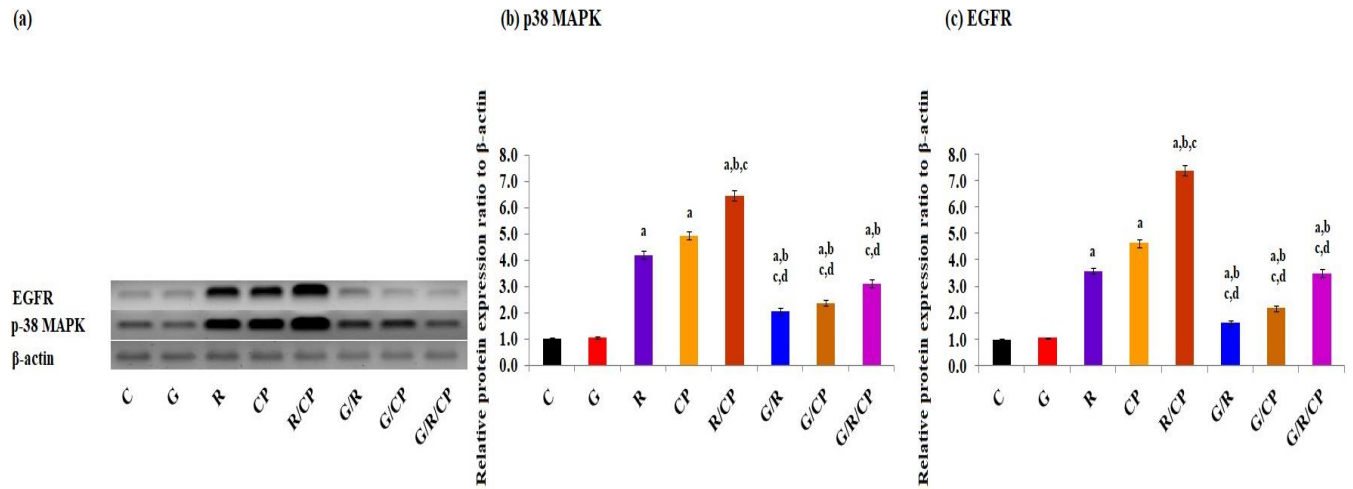
**Figure 5: The inflammatory Markers in the testis tissues.** Legend as figure 2.

**The anti-inflammatory Markers in the rats’ testes of different inspected animal groups**

The levels of TNF- $\alpha$ , IL-6, IFN- $\gamma$ , and NF- $\kappa$ B revealed high significant ( $p < 0.01$ ) increases in the testis tissues of the R and CP-intoxicated animals and demonstrated more significant ( $p < 0.01$ ) increases in their levels in the R/CP intoxicated animals, as compared to the controls. However, KRG extract showed significant regulations in the levels of TNF- $\alpha$ , IL-6, IFN- $\gamma$  and NF- $\kappa$ B in the testis tissues of the G/R, G/CP, and G/R/CP groups (Figure 5).

**Western blotting analysis**

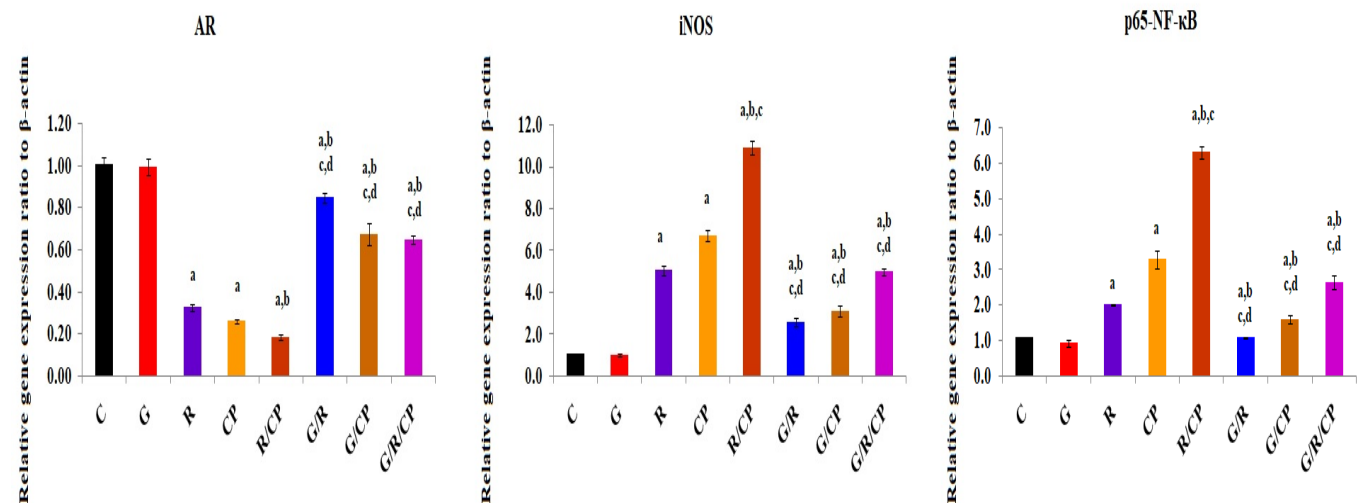
The relative protein expression ratios of p38 MAPK, and EGFR revealed high significant ( $p < 0.01$ ) up-regulations in the testis tissues of R and CP-intoxicated animals, and displayed more significant ( $p < 0.01$ ) up-regulations in their protein expression ratios in the R/CP intoxicated animals, as evaluated to the control ratios. KRG extract showed high significant ( $p < 0.01$ ) down-regulations in the p38 MAPK, and EGFR protein expression ratios in the testis tissues of G/R, G/CP, and G/R/CP groups (Figure 6).



**Figure 6: Western blot (a) and relative protein expression ratio of p-38 mitogen-activated protein kinases (p38 MAPK, b) and epidermal growth factor receptor (EGFR, c) to  $\beta$ -actin in the testis tissues. Legend as figure 2.**

### The relative gene expression ratios

The data demonstrated that the relative gene expression ratios of AR showed high significant ( $P < 0.01$ ) down-regulations in the testis tissues of the R and CP-intoxicated animals, and revealed more significant ( $p < 0.01$ ) down-regulations in the relative gene expression ratios in the R/CP-intoxicated animals, as evaluated to the control ratios. In addition, the relative gene expression ratios of iNOS and p65 NF- $\kappa$ B demonstrated high significant ( $p < 0.01$ ) up-regulations in the testis tissues of the R and CP-intoxicated animals, and exhibited more significant ( $p < 0.01$ ) up-regulations in the R/CP-intoxicated animals, as compared to the control ratios. However, KRG extract showed high significant ( $p < 0.01$ ) ameliorations in the relative gene expression ratios of AR, iNOS, and p65 NF- $\kappa$ B in the testis tissues of the G/R, G/CP, and G/R/CP groups (Figure 7).



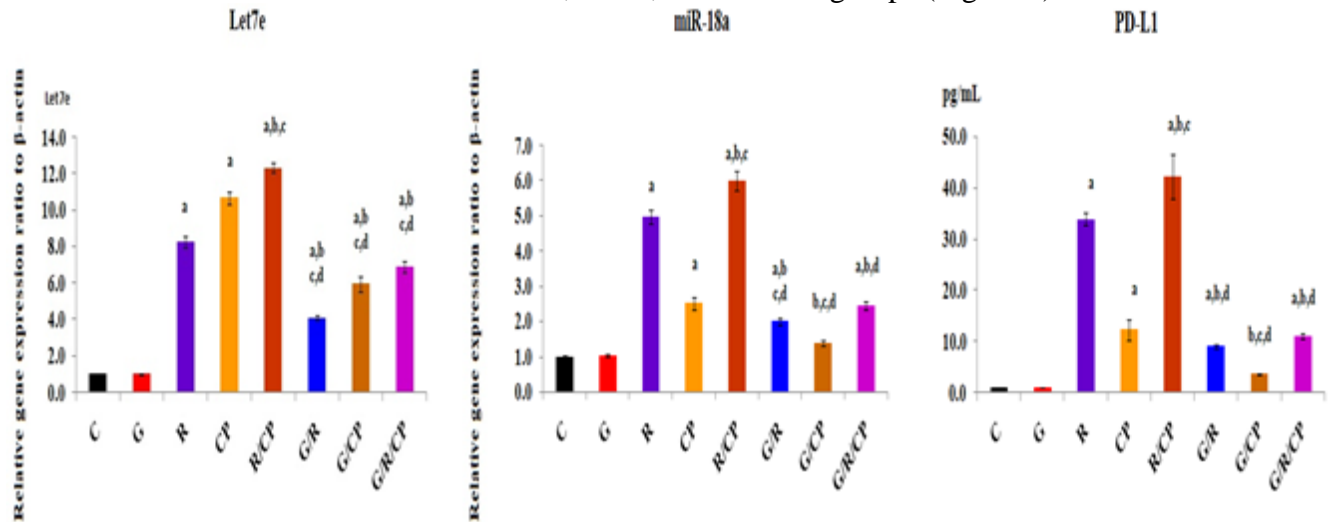
**Figure 7: Relative gene expression ratios of androgen receptor (AR), inducible nitric oxide synthase (iNOS), and p-65 nuclear factor-kappa B (p65-NF- $\kappa$ B) in the testis tissues. Legend as figure 2.**

**The relative gene expression ratio of micro-RNAs in the rats’ testes**

The data demonstrated that the relative gene expression ratios of let7e and miR-18a demonstrated high significant ( $p < 0.01$ ) up-regulations in the testis tissues of the R and CP-intoxicated animals, and exhibited more significant ( $p < 0.01$ ) up-regulations in the R/CP intoxicated animals, as evaluated to the control ratios. However, KRG extract showed high significant ( $p < 0.01$ ) ameliorations in the relative gene expression ratio of let7e and miR-18a in the testis tissues of the G/R, G/CP, and G/R/CP groups (Figure 8).

**The level of Programmed Death-Ligand 1 in the rats’ testes**

The levels of PD-L1 showed high significant ( $p < 0.01$ ) increases in the testis tissues of R, CP, and R/CP intoxicated groups, as evaluated to the control animals. KRG extract ameliorated the PD-L1 levels in the testis tissues of the G/R, G/CP, and G/R/CP groups (Figure 8).



**Figure 8: Relative gene expression ratio of let-7e, and miR-18a, and the programmed Death-Ligand 1 (PD-L1) level in the testis tissues. Legend as figure 2**

**DISCUSSION**

The chemotherapeutic drug CP and radiotherapy induce oxidative stress and inflammation in different organs and tissues. Oxidative stress and inflammation are correlated with each other, enhancing their effects and triggering different signaling pathways. CP and R toxicities triggers spermatogenesis dysfunction that is associated with some mRNA genes’ expressions and some protein expressions. However, the synergistic cancer therapy of combined chemotherapy and radiation is of great consequence during the treatment of most cancer types to avoid the development of secondary neoplasia. This treatment strategy has drawbacks, such as systemic toxicity and potentially harmful effects on normal cells (Ganesh and Manjeshwar, 2018, Zhao et al., 2024).

In the current study, the histopathological investigation showed alterations of the testis architecture in the radiation (R) and cyclophosphamide (CP) groups, which became worse due to the combined toxic effect in the R/CP group. These findings are consistent with previous studies, that cyclophosphamide and radiation induce testicular damage (Anan et al., 2018, Sharma et al., 2011). The results could be explained by the fact that CP testicular toxicity cause exfoliation and sloughing of germ cells, a decline in TH levels, and a diminution of the seminiferous tubule areas, resulting in widening of the interstitial spaces and substantial impacts on testicular

function (Anan *et al.*, 2018). Furthermore, the seminiferous tubules are very sensitive to free radicals (Said *et al.*, 2020), and exposure to gamma radiation triggers spermatid deterioration, leading to damage to the testicular tissues.

In the current study, animals treated with R and/or CP showed a decrease in BWT, TWT, sperm count and motility and changes in testosterone, FSH, and LH levels, showing the maximum changes in the R/CP group, which substantiate that, changes in BWT, TWT, reproductive hormones fluctuations, and the toxicity of CP and R, are inter-related. The results are in accordance with previous findings that CP poisoning (Abd El Tawab *et al.*, 2014, Afkhami-Ardakani *et al.*, 2018), as well as exposure to gamma radiation (Sharma *et al.*, 2011) cause declines in BWT, TWT, and their relative ratio TWT/BWT, along with aberrant sperm count and shape (Sharma *et al.*, 2011, Abd El Tawab *et al.*, 2014, Sivakumar *et al.*, 2006). In male animals, testicular weight serves as a useful indicator of reproductive toxicity. CP and/or radiation toxicities lead to gastrointestinal disturbances and reduce the amount of food and water consumed. Nonetheless, the germinal epithelial cells are unquestionably responsible for the decline in testicular weight and body-weight ratio (Sharma *et al.*, 2011, Abd El Tawab *et al.*, 2014). Low sperm assembly and decreased androgen levels result in a decrease in testis weight (Abd El Tawab *et al.*, 2014).

Serum reproductive hormones displayed notable changes in the R, CP, and R/CP intoxicated animal groups; higher amounts of FSH and decreased levels of TH and LH. The gonadotropin releasing hormone (GnRH) is produced by the hypothalamus' neurons. The pituitary gland, in response to GnRH, produces FSH and LH. FSH and LH are associated with regulating Leydig cell function and male TH assembly (Khamis *et al.*, 2023). The earlier research established the detrimental effects of CP and gamma radiation on the testicles, which included significant sperm count decreases, the induction of azoospermia and oligospermia, a high percentage of aberrant sperm forms, and changes in reproductive hormone levels (Anan *et al.*, 2018, Khamis *et al.*, 2023). CP interferes with cellular functions and impedes pituitary-testicular axis, resulting in inhibition of spermatogenesis (Khamis *et al.*, 2023). Significantly lower TH levels following gamma radiation exposure cause steroidogenesis inhibition and LH signaling disturbances in Leydig cells (Sivakumar *et al.*, 2006). In addition to producing hormonal disturbance, the induced oxidative stress and released ROS are responsible for sperm impairment and low count. The sperm's plasma membranes that made of polyunsaturated fatty acid are more responsive to ROS attack in hypoxic environments. This is because ROS can inactivate antioxidant enzymes and deplete GSH storage, which increases lipid peroxidation and impairs sperm function (Lu *et al.*, 2015). Additionally, oxidative stress leads to the degeneration of Leydig cells and affecting their steroidogenesis, which suppresses the secretion of TH (Zhou *et al.*, 2013).

In the current study, elevations of MDA and NO along with reduced levels of GSH in testicular tissues are indicative of oxidative stress. These alterations in MDA, NO, and GSH levels brought on by CP treatment are consistent with former studies (Abd El Tawab *et al.*, 2014, Anan *et al.*, 2018), or exposure to gamma radiation (Adeyi *et al.*, 2023). Down-regulation of gene expression and the generation of ROS and H<sub>2</sub>O<sub>2</sub> in male germ cells is a result of CP toxicity coincide with DNA fragmentation and the integration of these molecules into DNA regulatory mechanisms (Aguilar-Mahecha *et al.*, 2002). Furthermore, gamma radiation exposure increased the amount of ROS in the sperm, which resulted in sporadic alterations in the spermatozoa's structure and function. Gamma radiation exposure also contributes to testicular impairment by inducing MDA, NO, and H<sub>2</sub>O<sub>2</sub>, suppressing SOD, CAT, and GPX, activities, and depleting the GSH reservoir in the testicular tissues (Said *et al.*, 2020).

NO is also contributed to the testicular injury and spermatogenesis impairment. There is a correlation between infertility and increased RNS intensity in seminal plasma (**Abd El Tawab et al., 2014**).

The data obtained in the current study showed that the activities of LDH and ACP were increased in the testis tissues of the R, CP, and R/CP intoxicated groups. LDH and ACP are among the testicular marker enzymes that are thought to be functional indicators of spermatogenesis. The over-activity of LDH and ACP causes testicular atrophy, seminiferous epithelium, germ cells and Sertoli cells damage, leading to inhibition of steroidogenesis, and reduction in the testicular sperm assembly (**Abd El Tawab et al., 2014, Abarikwu et al., 2012**). The released ROS causes degeneration in the testicular tissues and catabolism of the germ cells, leading to the discharge of the ACP from the lysosomes (**Abd El Tawab et al., 2014**).

Alteration in AR is involved in male breast and prostate cancers (**Takuwa et al., 2018**). ROS triggers activation of AR signaling, which mediates the outgrowth and progression of the tumors into advanced stages (**Feng et al., 2020**). Anticancer drugs trigger spermatogenesis impairment via damage of DNA and RNA synthesis, which is mediated by ROS production (**Anan et al., 2018, Khamis et al., 2023**). The data showed that the ratios of androgen receptor (AR) gene expression were down-regulated in the testis tissues of R, CP, and R/CP groups.

The levels of TNF- $\alpha$ , IL-6, and NF- $\kappa$ B were increased in the testis tissues of R and CP-intoxicated animals showing the highest increase in R/CP-intoxicated animals. The induced oxidative stress by R and CP trigger the release of histamine, leading to induction of the pro-inflammatory cytokines' synthesis (**Ahmed et al., 2015**). This can explain the elevated levels of IL-6 and TNF- $\alpha$ . TNF- $\alpha$  associates with the production of inflammatory molecules, such as NO. TNF- $\alpha$  is involved in spermatogenesis impairment via reduction of-spermatogenesis, destruction of sperm membranes. Furthermore, TNF- $\alpha$  depresses semen quality, reduces sperm viability and motility, decreases the percentage of apoptotic spermatozoa, and sperm DNA integrity, and triggers mitochondrial dysfunction (**Zhang et al., 2021**). IL-6 mediates the activation of COX-2, iNOS, NF- $\kappa$ B, and MAPKs. Also, the stimulated p38-MAPK is associated with the expression of IL-6, TNF- $\alpha$ , NF- $\kappa$ B, and iNOS. The acute inflammatory response to CP toxicity is initiated by enhancing the levels of IL-6 and TNF- $\alpha$ . IL-6 and TNF- $\alpha$  are the principal pro-inflammatory cytokines that are affected by CP (**Anna et al., 2010**). Moreover, gamma-radiation triggers the inflammatory response and the release of pro-inflammatory cytokines due to up-regulation of their gene expressions. The triggered oxidative stress and increased ROS and the inflammatory response to CP and gamma-radiation are associated with male infertility. ROS is involved in NF- $\kappa$ B activation and encourages the assembly of pro-inflammatory cytokines. Radiation that triggers inflammation via NF- $\kappa$ B activation causes spermatogenesis impairment. The data in the current investigation demonstrated up-regulation of the NF- $\kappa$ B p65 subunit gene expression ratio in R, CP, and R/CP-intoxicated animals, which is consistent with **Said et al. (2020) and Iqbal et al. (2020)**. The up-regulation of the NF- $\kappa$ B p65 gene expression ratio is involved in up-regulation of NF- $\kappa$ B gene expression, leading to elevation of the levels of NF- $\kappa$ B and the pro-inflammatory cytokines in intoxicated groups and indicating the involvement of the inflammatory response in the testicular impairment as described previously (**Said et al, 2020, Iqbal et al., 2020**). In addition, iNOS gene expression was up-regulated in the R, CP, and R/CP intoxicated rats' group in the current investigation. iNOS is an inflammatory element involved in oxidative stress and the inflammatory response to toxicants. iNOS up-regulation encourages NO release, thus involving nitrosative stress and DNA damage to cells and organelles.



Moreover, iNOS triggers NF- $\kappa$ B activation and enhances the inflammatory response to R and CP toxicities (Ismail *et al.*, 2023, Khamis *et al.*, 2023).

The data demonstrated that p38 MAPK, and EGFR protein expression ratios, and let7e gene expression ratio were up-regulated in the testis tissues of R and CP-intoxicated animals and displayed greater up-regulations in R/CP-intoxicated animals. The results are in agreement with previous findings that p38 MAPK protein expression is induced in different tissues by CP administration (El-Kholy *et al.*, 2017, Abdelzaher *et al.*, 2023), and gamma-radiation (Fan *et al.*, 2017). The activation of p38 MAPK is a result of DNA damage and the intense production of ROS (Abdelzaher *et al.*, 2023). Also, the induced NF- $\kappa$ B and up-regulated iNOS expression are associated in p38-MAPK activation (El-Kholy *et al.*, 2017). Moreover, the level of Ca<sup>2+</sup> was decreased in the testis tissues, which could be related to the oxidative response of R, CP and R/CP intoxicated groups. Alteration of Ca<sup>2+</sup> levels is associated with the induction of p38 MAPK expression (Chang *et al.*, 2019). Lethal-7 (let-7) is a family of miRNAs, small non-coding RNAs that regulate eukaryotic cells' gene expressions. Let7e is involved in the inflammation process and triggers IL-6 overexpression, leading to an increase in IL-6 levels, as shown in the current investigation. Accordingly, disturbs the functional role of IL-6 in controlling Sertoli cell blood-testis barrier (BTB) dynamics and other testicular IL-6 functions, including stimulation of the ERK-MAPK pathways (Wei *et al.*, 2016, Zhang *et al.*, 2014).

On the other hand, EGFR overexpression is coupled with chemo- and radio-resistance. EGFR affects prostate growth and function, and tumor invasion and mechanisms involved in MAPK and AR pathway (Bonaccorsi *et al.*, 2004, Dittmann *et al.*, 2005). MAPKs signaling pathway activation is involved in oxidative stress, inflammation and apoptosis mechanisms and coupled with testicular tissue damage, including germ cells depletion, improper seminiferous tubule, alteration of the sex hormone secretion and reduction of the sperm's quantity and motility (Liu *et al.*, 2021).

In the current study, the data established that IFN- $\gamma$  and PD-L1 levels were increased. Moreover, the miR-18a gene expression were up-regulated in the testis tissues of the R, CP, and R/CP-intoxicated groups. miR-18a is extremely upregulated in activated T cells. miR-18a is a participant of the oncogenic miR-17-92a cluster (oncomiR-1). However, miR-18a performs as an oncogene and acts as a suppressor. Numerous genes that involved in differentiation, autophagy, cell cycle regulation, apoptosis, response to stress, and proliferation are regulated by miR-18a. Aberrations in miR-18a expression have been reported in a variety of illnesses and pathological conditions, including cancer. miR-18a is implicated in cancer progression and up-regulated in many cancers, including prostate cancer (Kolenda *et al.*, 2020). In addition, miR-18a upregulates prostate cancer cell lines (Hsu *et al.*, 2014). In activated T cells, miR-18a is the enormously intensely regulated member of the miR-17-92 cluster, triggering Th17 differentiation inhibition (Montoya *et al.*, 2017). However, boosting miR-18a stimulates PD-L1 expression, resulting in cancer proliferation and invasion (Danbaran *et al.*, 2020). The results of the current study corroborate previous findings that the increased levels of IFN- $\gamma$  and PD-L1 are direct responses to CP and or gamma irradiation treatments (Mangano *et al.*, 2010, Mittendorf *et al.*, 2020, Hussien, 2023) and that the increased levels of IFN- $\gamma$  provoke EGFR activation and enhance the up-regulation of miR-18a and PD-L1 (Mangano *et al.*, 2010, Danbaran *et al.*, 2020).

Consequently, fighting oxidative stress and inflammation responses to R exposure and CP administration is essential. In the current investigation, animals pre-treated with KRG exhibited protective effect against gamma-radiation exposure and CP administration, as well as, against

their combined toxicity in the R/CP group. The results showed that KRG water extract exhibited significant amelioration of the histological testicular architecture, animals and testis weights, sperm count, and sperm motility, restored the levels of TH, FSH and LH in the serum, improved the levels of LDH, ACP,  $\text{Ca}^{2+}$ , MDA, NO,  $\text{H}_2\text{O}_2$ , and GSH contents, boost the activity of SOD, CAT, and GPX, improved the elevated levels of PD-L1, TNF- $\alpha$ , IL-6, IFN- $\gamma$ , and NF- $\kappa$ B, down-regulated the protein expression ratios of p38 MAPK and EGFR, and regulated the gene expression ratios of AR, iNOS, p65 NF- $\kappa$ B, let7e and miR-18a in the testis tissues of R, CP, and R/CP-intoxicated groups.

Former studies showed that several crude extracts of natural substances decreased body weight loss in the animals due to radiation (**Chaudhary et al., 2008**). Administration of American ginseng recovered the serum TH and MDA levels and ameliorated the damage in the testicular seminiferous tubules triggered by CP (**Hosseini et al., 2018**). The results of extensive studies revealed that KRG regulated the animals' body weight, levels of NO and  $\text{Ca}^{2+}$ , protein expression ratios of TNF- $\alpha$ , IL-6, and NF- $\kappa$ B in aging rat mode, induced by D-galactose with high-fat diet-induced atherosclerosis (**Lee et al., 2014a**). KRG was reported also to restore the testis weight, sperm impairment, sex hormone receptors; including AR, and antioxidant-related enzymes; including GPX, in the testes of immobilization stress-induced rats (**Lee et al., 2019**), and to ameliorate the testicular damage induced by doxorubicin in rats by improving testicular histopathological architecture, the antioxidant system and hormonal imbalance (**Kopalli et al., 2016**). It was shown also to improve the levels of pro-inflammatory cytokines via regulation of the MAPK/NF- $\kappa$ B pathway (**Cha et al., 2018**), and sperm properties in Guinea pigs (**Hwang et al., 2004**). In an elegant study **Leung and Wong, (2013)** clarify that KRG improved the semen quality and restored sex hormone levels, whereas the ginsenosides phyto-constituents ameliorated the sperm count and motility. **Tsai et al. (2003)** showed that male rats' anterior pituitary glands secrete extra LH after being treated with ginsenoside Rb1. Furthermore, it was found that ginseng stem-leaf saponins, with antioxidant, anti-inflammation, and anti-aging effects, regulated the defects in the seminiferous tubules, the body weight, suppressed the oxidative stress and inflammatory response by depressing MDA levels, enhancing SOD and GPX activities, and regulating TNF- $\alpha$  and IL-6 levels, induced by D-galactose, as well as, regulating mouse sperm quality and quantity, the levels of the reproductive hormones, testosterone, FSH, and LH, suppressed MAPKs pathway activation in testes, and ameliorated reproductive impairment by blocking MAPKs signaling (**Zhang et al., 2021**). Saponins from stem and leaf of ginseng significantly suppressed the oxidative stress, drive down MDA level, by enhancing SOD, CAT, and GPX activities that altered by CP in chicken (**Yu et al., 2015**). Additionally, KRG administration was shown to attenuate busulfan-induced male reproductive dysfunction through regulation of sperm parameters, improvement of the testicular histological changes, and regulation of serum TH (**Jung et al., 2015**), and exhibit a considerable impact on testicular function via regulation of the histological alterations, antioxidant status; enhancing the antioxidant enzymes (SOD, CAT, GPX) activities, reversed MDA level, improved GSH content and modulated redox proteins in aged rats (**Kopalli et al., 2015**).

The ginsenoside compounds K and Rk1 are cytoplasmic inhibitors of NF- $\kappa$ B, they interact with I $\kappa$ B kinase, and inhibit the associated phosphorylation (**Chen et al., 2023**). The ginsenoside Rg1 significantly inhibit COX-2 and PGE2 production and reduce MDA levels. The ginsenosides Rb1 and Rb2 reduce the TNF $\alpha$  level. The ginsenoside Ro inhibit the TNF $\alpha$ -triggered NF- $\kappa$ B signaling pathway. The ginsenosides Rf, Rb2, Rg1, and Rd ameliorated the serum levels of IL-6, IL-1 $\beta$ , and TNF $\alpha$ . The ginsenoside Rb1 repress IL-1 $\beta$ . IL-1 $\beta$  elevation can trigger the expression

of COX-2/PGE2 and iNOS/NO involved in oxidative stress, inflammatory response, and DNA damage. Thus, KRG ginsenosides can suppress NF- $\kappa$ B and MAPK signaling pathways. Also, the ginsenoside Rh2 mixture (20(S)-Rh2, 20(R)-Rh2, Rk2 and Rh3) exhibited inhibitory activity on the expression of NF- $\kappa$ B signaling. Moreover, KRG hindered the activation of p38 MAPK signaling pathways in IL-1 $\beta$ -treated SW1353 cells. The ginsenoside Rb1 also enhanced the GSH contents, declined the MDA levels, inhibited COX-2 and iNOS expression, and NF- $\kappa$ B and p38 MAPK pathways. Furthermore, it up-regulated the nuclear factor (erythroid-derived 2)-like 2 (NRF2) pathways, which mediates the antioxidant and inflammatory responses (**Chen et al., 2023, Hossain et al., 2022, Kim et al., 2003, Lee et al., 2014b, Lee et al., 2018a, Lee et al., 2018b**). KRG-saponins fractions act synergistically with the ginsenoside saponins, potentiate pharmacological activity of the KRG, and improve male subfertility and spermatogenesis in aged rats (**Kopalli et al., 2017b**). In addition, the data showed that KRG regulated the levels of IFN- $\gamma$  and PD-L1, as well as, down-regulated the gene expression ratio of miR-18a. KRG extract demonstrated regulatory effects on IFN- $\gamma$ , miR-18a, and PD-L1 expressions. Moreover, the ginsenosides Rg3, Rh2, Rh4, and Rk1 are active constituents of KRG extract and can improve the immune checkpoints and regulate PD-L1 expression (**Lee et al., 2023**). However, ginsenoside Rd down-regulates miR-18a, and diminishes breast cancer metastasis (**Wang, et al., 2016**).

## CONCLUSION

Korean red ginseng water extract may improve testicular impairment induced by R, CP, or R/CP via attenuation of oxidative stress and inflammatory responses. Consequently, Korean red ginseng extract is recommended to protect against testicular dysfunction triggered by R, CP, or R/CP.

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## REFERENCES

- Abarikwu, S.O., Otuechere, C.A., Ekor, M., Monwuba, K., Osobu, D. (2012).** Rutin ameliorates cyclophosphamide-induced reproductive toxicity in male rats. *Toxicol Int*; 19(2):207-14.
- Abd El Tawab, A.M., Shahin, N.N., AbdelMohsen, M.M. (2014).** Protective effect of *Satureja montana* extract on cyclophosphamide-induced testicular injury in rats. *Chem.-Biol. Interact.* 224, 196–205.
- Abdelzaher, Y., Abdel-Gaber, S.A., Fawzy, M.A., Ali, A.H.S.A.B., Attya, M.E., Geddawy, A. (2023).** Atorvastatin protects against cyclophosphamide-induced thyroid injury in rats via modulation of JNK/ ERK/ p38 MAPK signaling pathway. *International Immunopharmacology* 124, 111061.
- Adeyi, O.E., Somade, O.T., James, A.S., Adeyi, A.O., Ogbonna-Eze, S.N., Salako, O.Q. et al (2023).** Ferulic acid mitigates 2-methoxyethanol-induced testicular oxidative stress via combined downregulation of FoxO1, PTEN, and modulation of Nrf2-Hmox1-NQO1 signaling pathway in rats, *Pharmacological Research - Modern Chinese Medicine*, 7, 100257.

- Afkhami-Ardakani, M., Hasanzadeh, S., Shahrooz, R., Delirezh, N., Malekinejad, H. (2018).** Antioxidant effects of *Spirulina platensis* (*Arthrospira platensis*) on cyclophosphamide-induced testicular injury in rats. *Veterinary Research Forum*; 9 (1) 35 – 41.
- Aguilar-Mahecha, A., Hales, B.F., Robaire, B. (2002).** Chronic cyclophosphamide treatment alters the expression of stress response genes in rat male germ cells. *Biol. Reprod.* 66 (4), 1024–1032.
- Ahmed, L.A., El-Maraghy, S.A., Rizk, S.M. (2015).** Role of the KATP channel in the protective effect of nicorandil on cyclophosphamide-induced lung and testicular toxicity in rats. *Sci. Rep.* 5, 14043.
- Anan, H.H., Zidan, R.A., Abd EL-Baset, S.A., Ali, M.M. (2018).** Ameliorative effect of zinc oxide nanoparticles on cyclophosphamide induced testicular injury in adult rat. *Tissue and Cell* 54, 80–93.
- Anna, C.B.D., Francisco, F., Larissa, F.M., (2010).** Protective effect of simvastatin in the cyclophosphamide-induced hemorrhagic cystitis in rats. *Acta Cir. Bras.* 25 43–46..
- Araghi, A., Golshahi, H., Baghban, F., Tabari, M.A. (2018).** Ameliorative action of farnesol on cyclophosphamide induced toxicity in mice. *J Herbmed Pharmacol.* 7(1): 37-43.
- Avgeropoulos, N.G., Newton, H.B. (2018).** Chapter 2 - Clinical Pharmacology of Brain Tumor Chemotherapy, Editor(s): Herbert B. Newton, *Handbook of Brain Tumor Chemotherapy, Molecular Therapeutics, and Immunotherapy* (Second Edition), Academic Press, Pages 21-44.
- Bancroft, J.D., Layton, C. (2019).** The hematoxylin and eosin (Ch 10), In Kim Suvarna S., Layton C., and Bancroft JD, Editors, *Theory and practice of histological techniques*, 8th ed. Churchill Livingstone, Edinburgh, London, Melbourne and New York, pp 126–138.
- Bonaccorsi, L., Muratori, M., Carloni, V., Marchiani, S., Formigli, L., Forti, G. et al. (2004).** The androgen receptor associates with the epidermal growth factor receptor in androgen-sensitive prostate cancer cells, *Steroids*, 69 (8–9), 549-552.
- Cha, K.M., Kopalli, S.R., Han, S.Y., Lee, S.H., Jeong, M.S., Cho, J.Y. et al. (2018).** Korean red ginseng attenuates doxorubicin-induced testicular dysfunction in rats by modulating inflammatory, oxidative, and autophagy responses, *J Functional Foods*, 40, 736-743,
- Chang, C.C., Yang, M.H., Wen, H.M., Chern, J.C. (2002).** Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food Drug Anal.* 10: 178-182.
- Chang, X.R., Yao, Y.L., Wang, D., Ma, H.T., Gou, P.H., Li, C.Y. et al. (2019).** Influence of hypothyroidism on testicular mitochondrial oxidative stress by activating the p38 mitogen-activated protein kinase and c-Jun NH2-terminal kinase signaling pathways in rats. *Human & Experimental Toxicology*; 38(1):95-105.
- Chaudhary, R., Jahan, S., Gupta, U., Goyal, P.K. (2008).** Radioprotective potential of *Trigonella foenum graecum* seeds extract, *Pharmacologyonline*, 2, 14–26.
- Chen, J., Huang, L., Liao, X. (2023).** Protective effects of ginseng and ginsenosides in the development of osteoarthritis (Review). *Exp Ther Med* 26: 465.
- Chen, W., Balan, P., Popovich, D.G. (2019).** Review of Ginseng Anti-Diabetic Studies. *Molecules.* 24(24):4501. [https://doi: 10.3390/molecules24244501](https://doi.org/10.3390/molecules24244501).
- Danbaran, G.R., Aslani, S., Sharafkandi, N., Hemmatzadeh, M., Hosseinzadeh, R., Azizi, G. et al (2020).** How microRNAs affect the PD-L1 and its synthetic pathway in cancer. *Int Immunopharmacol.*; 84:106594.

- De Rezendea, L.F.M., Leeb, D.H., Louzadac, M.L.D.C., Song, M., Giovannucci, E., Eluf-Neto, J. (2019).** Proportion of cancer cases and deaths attributable to lifestyle risk factors in Brazil. *Cancer Epidemiology* 59, 148–157.
- Dittmann K., Mayer, C., Fehrenbacher, B., Schaller, M., Raju U., Milas, L. et al. (2005).** Radiation-induced Epidermal Growth Factor Receptor Nuclear Import Is Linked to Activation of DNA-dependent Protein Kinase. *J Biol Chem.*, 280 (35), 31182-31189,
- Elblehi, S.S., Hafez, M.H., El-Far, A.H. (2023).** Panax ginseng ameliorates hepatorenal oxidative alterations induced by commercially used cypermethrin in male rats: experimental and molecular docking approaches. *Environ Sci Pollut Res Int.* 30(50):109702-109723.
- El-Kholy, A.A., Elkablawy, M.A., El-Agamy, D.S. (2017).** Lutein mitigates cyclophosphamide induced lung and liver injury via NF-kB/MAPK dependent mechanism. *Biomedicine & Pharmacotherapy* 92, 519–527.
- Fan, Z., Han, Y., Ye, Y., Liu, C., Cai, H. (2017).** L-carnitine preserves cardiac function by activating p38 MAPK/Nrf2 signalling in hearts exposed to irradiation, *European Journal of Pharmacology*, 804, 7-12.
- Faqi, A.S., Dalsenter, P.R., Mather, W., Heinrich-Hirsh, B., Chahoud, I. (1998).** Reproductive toxicity and tissue concentration of 3, 3',4,4'tetrachlorobiphenyl (PCB 77) in male adult rat. *Hum Exp Toxicol* 17:151–156.
- Feng, T., Zhao, R., Sun, F., Lu, Q., Wang, X., Hu J. et al. (2020).** TXNDC9 regulates oxidative stress-induced androgen receptor signaling to promote prostate cancer progression. *Oncogene* 39, 356–367.
- Ganesh, C.J., Manjeshwar, S.B. (2018).** The Combination Cyclophosphamide with Radiation Increases Tumour Cure in Ehrlich Ascites Carcinoma Bearing Mice as well as DNA Damage in the Normal Mice Bone Marrow Exposed to Different Doses of  $\Gamma$ -Radiation. *Curr Trends Biomedical Eng & Biosci.* 16(1): 555929.
- Heo, S.B., Lim, S.W., Jhun, J.Y., Cho, M.L., Chung, B.H., Yang, C.W. (2016).** Immunological benefits by ginseng through reciprocal regulation of Th17 and Treg cells during cyclosporine-induced immunosuppression. *J Ginseng Res*; 40:18-27.
- Hiai, S., Oura, H., Nakajima, T. (1976).** Color reaction of some saponinins and saponins with vanillin and sulfuric acid. *Planta Medica*, 29, 116–122.
- Hossain, M.A., Alam, M.J., Kim, B., Kang, C.W., Kim, J.H. (2022).** Ginsenoside-Rb1 prevents bone cartilage destruction through down-regulation of p-Akt, p-P38, and p-P65 signaling in rabbit. *Phytomedicine.*100 (154039).
- Hosseini, A., Zare, S., Borzouei, Z., Ghaderi, P.F. (2018).** Cyclophosphamide-induced testicular toxicity ameliorate by American ginseng treatment: an experimental study. *IJRM-International Journal of Reproductive Biomedicine*; 16 (11): 711-718.
- Hsu, T.I., Hsu, C.H., Lee, K.H., Lin, J.T., Chen, C.S., Chang, K.C. et al. (2014).** MicroRNA-18a is elevated in prostate cancer and promotes tumorigenesis through suppressing STK4 in vitro and in vivo. *Oncogenesis* 3, e99.
- Hussien, S.M. (2023).** The impact of cyclophosphamide versus high-level of ionizing radiation on the immune response. *Heliyon.* 9(7):e18025.
- Hwang, S.Y., Kim, W.J., Wee, J.J., Choi, J.S., Kim, S.K. (2004).** *Panax ginseng* improves survival and sperm quality in Guinea pigs exposed to 2,3,7,8-tetrachlorodibenzo- p - dioxin. *BJU International*; 94:663-668.

- Iqbal, A., Syed, M.A., Ali, J., Najmi, A.K., Haque, M.M., Haque, S.E. (2020).** Nerolidol protects the liver against cyclophosphamide-induced hepatic inflammation, apoptosis, and fibrosis via modulation of Nrf2, NF- $\kappa$ B p65, and caspase-3 signaling molecules in Swiss albino mice. *Biofactors*, 46(6):963–973.
- Ismail, A.F.M., Salem, A.A., Eassawy, M.M.T. (2023).** Rutin protects against gamma- irradiation and malathion- induced oxidative stress and inflammation through regulation of mir- 129- 3p, mir- 200C- 3p, and mir- 210 gene expressions in rats' kidney. *Env. Sci. Poll. Res.* 30, 72930–72948.
- Jeong, C.H., Kim, J., Kim, B.K., Dan, K.B., Min, H. (2023).** Antiviral effects of Korean Red Ginseng on human coronavirus OC43. *J Ginseng Res.* 47(2):329-336.
- Jung, E.M., Lee, S.H., Lee, G.S. (2023).** Protective effects of Korean Red Ginseng against toxicity of endocrine-disrupting chemicals, *Journal of Ginseng Research*, 47 (2), 193-198..
- Jung, S.W., Kim, H.J., Lee, B.H., Choi, S.H., Kim, H.S., Choi, Y.K. et al. (2015).** Effects of Korean Red Ginseng extract on busulfan-induced dysfunction of the male reproductive system. *J Ginseng Res*; 39(3):243-9.
- Kaushik, K., Kaushal, N., Kalla, N.R. (2018).** Conversion of apoptosis to necrosis and the corresponding alteration in the oxidative milieu of male germ cells of rat under acute heat stress: An experimental study. *Int J Reprod BioMed Vol.* 16. (9) 577-586.
- Khamis, T., Hegazy, A.A., El-Fatah, S.S.A., Abdelfattah, E.R., Abdelfattah, M.M.M., Fericean, L.M. et al. (2023).** Hesperidin Mitigates Cyclophosphamide-Induced Testicular Dysfunction via Altering the Hypothalamic Pituitary Gonadal Axis and Testicular Steroidogenesis, Inflammation, and Apoptosis in Male Rats. *Pharmaceuticals*, 16, 301.
- Kim, D.H., Moon, Y.S., Lee, T.H., Jung, J.S., Suh, H.W., Song, D.K. (2003).** The inhibitory effect of ginseng saponins on the stress-induced plasma interleukin-6 level in mice, *Neuroscience Letters*, 353, 1, 13-16.
- Kim, H.G., Jang, S.S., Lee, J.S., Kim, H.S., Son, C.G. (2017).** *Panax ginseng* Meyer prevents radiation-induced liver injury via modulation of oxidative stress and apoptosis. *J Ginseng Res.*; 41(2):159-168.
- Kolenda, T., Guglas, K., Kopczyńska, M., Sobocińska, J., Teresiak, A., Bliźniak, R. et al. (2020).** Good or not good: Role of miR-18a in cancer biology. *Rep Pract Oncol Radiother.* ;25(5):808-819.
- Kopalli, S.R., Cha, K.M., Hwang, S.Y., Jeong, M.S., Kim, S.K. (2019).** Korean Red Ginseng (*Panax ginseng* Meyer) with enriched Rg3 ameliorates chronic intermittent heat stress-induced testicular damage in rats via multifunctional approach. *J Ginseng Res.*;43(1):135-142.
- Kopalli, S.R., Cha, K.M., Ryu, J.H., Hwang, S.Y., Kim, S.K. (2017a).** Specific activity of Korean red ginseng saponin and non-saponin fractions in ageing-induced rat testicular dysfunction, *J Functional Foods*, 29, 226-237,
- Kopalli, S.R., Cha, K.M., Ryu, J.H., Lee, S.H., Jeong, M.S., Hwang, S.Y. et al. (2017b).** Korean red ginseng improves testicular ineffectiveness in aging rats by modulating spermatogenesis-related molecules, *Experimental Gerontology*, 90, 26-33.
- Kopalli, S.R., Hwang, S.Y., Won, Y.J., Kim, S.W., Cha, K.M., Han, C.K. et al. (2015).** Korean red ginseng extract rejuvenates testicular ineffectiveness and sperm maturation process in aged rats by regulating redox proteins and oxidative defense mechanisms. *Exp Gerontol*; 69:94-102.

- Kopalli, S.R., Won, Y.J., Hwang, S.Y., Cha, K.M., Kim, S.Y., Han, C.K. et al. (2016).** Korean red ginseng protects against doxorubicin-induced testicular damage: An experimental study in rats. *J. Functional Foods* 20, 96 – 107.
- Lee, E.J., Yang, J.H., Yang, H.J., Cho, C.K., Choi, J.G., Chung, H.S. (2023).** Antitumor Effect of Korean Red Ginseng through Blockade of PD-1/PD-L1 Interaction in a Humanized PD-L1 Knock-In MC38 Cancer Mouse Model. *Int. J. Mol. Sci.* 24, 1894.
- Lee J., Cho, J.Y., Kim, W.K. (2014a).** Anti-inflammation effect of Exercise and Korean red ginseng in aging model rats with diet-induced atherosclerosis. *Nutr Res Pract*; 8(3):284-291.
- Lee, J.H., Min ,D.S., Lee, C.W., Song, K.H., Kim, Y.S., Kim, H.P. (2018a).** Ginsenosides from Korean Red Ginseng ameliorate lung inflammatory responses: inhibition of the MAPKs/NF- $\kappa$ B/c-Fos pathways. *J Ginseng Res.* 42(4):476-484.
- Lee, M.J., Chang, B.J., Oh, S., Nah, S.Y., Cho, I.H. (2018b).** Korean Red Ginseng mitigates spinal demyelination in a model of acute multiple sclerosis by downregulating p38 mitogen-activated protein kinase and nuclear factor- $\kappa$ B signaling pathways. *J Ginseng Res.* 42(4):436-446.
- Lee, S.H., Choi, K.H., Cha, K.M., Hwang, S.Y., Park, U.K., Jeong, M.S. et al. (2019).** Protective effects of Korean Red Ginseng against sub-acute immobilization stress-induced testicular damage in experimental rats. *J Ginseng Res* 43, 125-134.
- Lee, Y.J., Han, J.Y., Lee, C.G., Heo, K., Park, S.I., Park, Y.S. et al. (2014b).** Korean Red Ginseng saponin fraction modulates radiation effects on lipopolysaccharide-stimulated nitric oxide production in RAW264.7 macrophage cells. *J Ginseng Res.* 38(3):208-14.
- Leung, K.W., Wong, A.S. (2013).** Ginseng and male reproductive function. *Spermatogenesis* 3(3):e26391.
- Liu, W., Leng, J., Hou, J.G., Jiang, S., Wang, Z., Liu, Z. et al. (2021).** Saponins derived from the stems and leaves of panax ginseng attenuate scrotal heat-induced spermatogenic damage via inhibiting the MAPK mediated oxidative stress and apoptosis in mice. *Phytother Res*; 35:311–23.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J (1951).** Protein measurement with the folin phenol reagent, *J. Biol. Chem.* 193 (1951) 265–275.
- Lu, W.P., Mei, X.T., Wang, Y., Zheng, Y.P., Xue, Y.F., Xu, D.H. (2015).** Zn (II)–curcumin protects against oxidative stress, deleterious changes in sperm parameters and histological alterations in a male mouse model of cyclophosphamide-induced reproductive damage. *Environ. Toxicol. Pharmacol.* 39 (2), 515–524.
- Mangano, K., Nicoletti, A., Patti, F., Donia, M., Malaguarnera, L., Signorelli, S., Magro, G., Muzio, V., Greco, B., Zaratin, P., Meroni, P., Zappia, M., Nicoletti, F. (2010).** Variable effects of cyclophosphamide in rodent models of experimental allergic encephalomyelitis. *Clin Exp Immunol.* 159(2):159-68.
- Meistrich, M.L. (2013).** Effects of Chemotherapy and Radiotherapy on Spermatogenesis in Humans. *Fertil Steril.* 100(5):1180-6.
- Mittendorf, E.A., Zhang, H., Barrios, C.H., Saji, S., Jung, K.H., Hegg, R. et al. (2020).** Neoadjuvant atezolizumab in combination with sequential nab-paclitaxel and anthracycline-based chemotherapy versus placebo and chemotherapy in patients with early-stage triple-negative breast cancer (IMpassion031): A randomised, double-blind, phase 3 trial. *Lancet.* 396(10257):1090-1100.

- Moheban, A.A., Chang, H.H., Havton, L.A. (2016).** The Suitability of Propofol Compared with Urethane for Anesthesia during Urodynamic Studies in Rats. *J Am Assoc Lab Anim Sci.*, 55(1): 89–94.
- Montoya, M.M., Maul, J., Singh, P.B., Pua, H.H., Dahlström, F., Wu N. et al. (2017).** A Distinct Inhibitory Function for miR-18a in Th17 Cell Differentiation. *J Immunol*; 199 (2): 559–569.
- Park, H. J., Choe, S., Park, N. C. (2016).** Effects of Korean red ginseng on semen parameters in male infertility patients: A randomized, placebo-controlled, double-blind clinical study. *Chin. J. Integr. Med.* 22, 490–495.
- Park, J., Song, H., Kim S.-K., Lee M. S., Rhee, D.-K., Lee, Y. J. (2017a).** Effects of ginseng on two main sex steroid hormone receptors: estrogen and androgen receptors. *J Ginseng Res* 2017b;41:215-221.
- Park, S.K., Hyun, SH, In G, Park CK, Kwak YS, Jang YJ, Kim B, Kim JH, Han CK (2021).** The antioxidant activities of Korean Red Ginseng (*Panax ginseng*) and ginsenosides: A systemic review through *in vivo* and clinical trials. *J Ginseng Res.* 2021;45(1):41-47.
- Park T. Y., Hong M., Sung H., Kim S., Suk, K. T. (2017b).** Effect of Korean Red Ginseng in chronic liver disease. *J Ginseng Res*;41:450e455
- Park, S.Y., Park, J.H., Kim, H.S., Lee, C.Y., Lee, H.J., Kang, K.S. et al. (2018).** Systems-level mechanisms of action of *Panax ginseng*: a network pharmacological approach, *J. Ginseng Res.*, 42 (1) 98-106.
- Paul, S., Shin, H.S., Kang, S.C. (2012).** Inhibition of inflammations and macrophage activation by ginsenoside-Re isolated from Korean ginseng (*Panax ginseng* C.A. Meyer). *Food Chem Toxicol.* 50:1354-61.
- Pfaffl, M.W. (2001).** A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res.* 29(9):2002-7.
- Said, R.S., Mohamed, H.A., Kassem, D.H. (2020).** Alpha-lipoic acid effectively attenuates ionizing radiation-mediated testicular dysfunction in rats: Crosstalk of NF- $\kappa$ B, TGF- $\beta$ , and PPAR- $\gamma$  pathways, *Toxicology*, 442, 152536, ISSN 0300-483X,
- Saini, K.S., Twelves, C. (2021).** Determining lines of therapy in patients with solid cancers: a proposed new systematic and comprehensive framework. *Br. J. Cancer* 125, 155–163.
- Seo, J.Y., Ju, S.H., Oh, J., Lee, S.K., Kim, J.S. (2016).** Neuroprotective and cognition enhancing effects of compound K isolated from red ginseng. *J Agric Food Chem.* 64(14):2855-64.
- Sharma, P., Parmar, J., Sharma, P., Verma, P., Goyal, P.K. (2011).** Radiation-Induced Testicular Injury and Its Amelioration by *Tinosporacordifolia* (An Indian Medicinal Plant) Extract. *Evidence-Based Complementary and Alternative Medicine*, 643847,
- Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M. (1999).** Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology*, Ch 14, 299, 152–178. Academic Press.
- Sivakumar, R., Sivaraman, P.B., Mohan-Babu, N., Jainul-Abideen, I.M., Kalliyappan, P., Balasubramanian K. (2006).** Radiation exposure impairs luteinizing hormone signal transduction and steroidogenesis in cultured human leydig cells. *Toxicol Sci*; 91(2):550-6.
- Stork, C.M., Schreffler, S.M. (2014).** Cyclophosphamide, Editor: Philip Wexler, *Encyclopedia of Toxicology (Third Edition)*, Academic Press, Pages 1111-1113.



- Takuwa, H., Tsuji, W., Shintaku, M., Yotsumoto, F. (2018).** Hormone signaling via androgen receptor affects breast cancer and prostate cancer in a male patient: A case report. *BMC Cancer* 18, 1282.
- Targhi, R.G., Homayoun, M., Mansouri, S., Soukhtanloo, M., Soleymanifard, S., Seghatoleslam, M. (2017).** Radio protective effect of black mulberry extract on radiation-induced damage in bone marrow cells and liver in the rat. *Radiation Physics and Chemistry* 130, 297–302.
- Tsai, S.C., Chiao, Y.C, Lu, C.C., Wang, P.S. (2003).** Stimulation of the secretion of luteinizing hormone by ginsenoside-Rb1 in male rats. *Chin J Physiol*; 46(1):1-7.
- Tuglu, D., Yuvanc, E., Yilmaz, E., Gencay, I.Y., Atasoy, P., Kisa, U. et al. (2015).** The antioxidant effect of dexmedetomidine on testicular ischemia-reperfusion injury. *Acta Cir Bras.* ;30(6):414-21.
- Wang, P., Du, X., Xiong, M., Cui, J., Yang, Q., Wang, W. et al. (2016).** Ginsenoside Rd attenuates breast cancer metastasis implicating derepressing microRNA-18a-regulated Smad2 expression. *Sci Rep* 6, 33709.
- Wei, Y. B., Liu, J. J., Villaescusa, J. C., Åberg, E., Brené, S., Wegener, G. et al. (2016).** Elevation of IL-6 is associated with disturbed let-7 biogenesis in a genetic model of depression. *Transl Psychiatry.* 6, e869.
- Yu, J., Chen, Y., Zhai, L., Zhang, L., Xu, Y., Wang, S. (2015).** Antioxidative effect of ginseng stem-leaf saponins on oxidative stress induced by cyclophosphamide in chickens. *Poult Sci*; 94:927–33.
- Yun, T.K., Choi, S.Y., Yun, H.Y. (2001).** Epidemiological study on cancer prevention by ginseng: are all kinds of cancers preventable by ginseng? *J Korean Med Sci.* 16(Suppl), S19-27.
- Zhai, J., Zhang, F., Gao S., Chen, L., Feng, G., Yin, J. et al (2018).** *Schisandra chinensis* extract decreases chloroacetaldehyde production in rats and attenuates cyclophosphamide toxicity in liver, kidney and brain. *Journal of Ethnopharmacology* 210, 223–231.
- Zhang, H., Yin, Y., Wang, G., Liu, Z., Liu L., Sun, F. (2014).** Interleukin-6 disrupts blood-testis barrier Through inhibiting protein degradation or activating phosphorylated ERK in Sertoli cells. *SCIENTIFIC REPORTS.* 4: 4260.
- Zhang, J., Yao, D., Zhang, J., Song, Y., Mo, F., Chu, X. et al (2019).** TGF- $\beta$  mediates thoracic radiation-induced abscopal effects of testis injury in rat, *Biochemical and Biophysical Research Communications*, Volume 514, Issue 3, Pages 678-683,
- Zhang, Q., Yang, C., Zhang, M., Lu, X., Cao, W., Xie, C. et al. (2021).** Protective effects of ginseng stem-leaf saponins on D-galactose-induced reproductive injury in male mice. *Aging (Albany NY).* 2021 Mar 10; 13:8916-8928.
- Zhang, X.C., Xu, B.J. (2018).** Phytochemical profiles and antioxidant capacities of white and red ginseng as affected by marinating media (vinegar, yellow wine, and Chinese liquor). *J Food Process Preserv.* 42:e13331.
- Zhao, Y., Wu, J., Li, X., Chen, Q., Hong, Z., Zheng, L. et al. (2024).** Protective effect of Huangqi-Guizhi-Wuwutang against cyclophosphamide-induced spermatogenesis dysfunction in mice by promoting steroid hormone biosynthesis, *J. Ethnopharmacol.*, 319 (2), 117260.
- Zhou, L., Beattie, M.C., Lin, C.Y., Liu, J., Traore, K., Papadopoulos, V. et al. (2013).** Oxidative stress and phthalate-induced down-regulation of steroidogenesis in MA-10 Leydig cells. *Reprod. Toxicol.* 42, 95–101.