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Original Article

Investigating the Effects of *Alpinia officinarum* Alcoholic Extract on Rat Testis Tissue Exposed to Carboplatin

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Abstract

Introduction: In individuals receiving chemotherapy, reproductive impairment arises not only from the disease itself but also from the medications used to treat it. Carboplatin has been documented to affect reproductive function and sexual behavior in individuals who receive it. *Alpinia officinarum* contains flavonoids with various biological effects, including antioxidant properties and fertility-enhancing potential. The purpose of this study was to investigate the *in vitro* anti-infertility efficacy of *A. officinarum* alcoholic extract against carboplatin-induced testicular toxicity.

Methods: A total of 36 rats were randomly assigned to six groups: control, carboplatin alone (50 mg/kg), *A. officinarum* alone (50 and 100 mg/kg), and carboplatin combined with low -or high- dose *A. officinarum*. The treatment period lasted 28 days, after which the rats were weighed. Following dissection, their testicles were excised and weighed. The following parameters were assessed: tubular differentiation index (TDI), spermiogenesis index (SPI), repopulation index (RI), Sertoli cell index, meiosis index, seminiferous tubules integrity, and organization of the testicular epithelium.

Results: Animals in the carboplatin group exhibited a significant reduction in body weight and testis weight compared to the control group ($P<0.001$). Additionally, all markers of testicular integrity and activity differed significantly from those of the control group ($P<0.001$). Moreover, the groups receiving *A. officinarum* showed a remarkable improvement in these parameters compared with the carboplatin group ($P<0.001$).

Conclusion: *A. officinarum* can play a protective role against carboplatin-induced tissue damage in rats.

Keywords: Carboplatin, *Alpinia officinarum*, Testis tissue, Infertility, Gonadotoxicity

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Introduction

Infertility is a complex condition with multiple contributing factors that impacts approximately 15% of couples worldwide and is characterized by the failure to o achieve a clinical pregnancy after at least 12 months of regular, unprotected sexual intercourse (1, 2). Male-related factors account for approximately half of all infertility cases, contributing to reproductive impairment at a rate comparable to female-related factors (3, 4). Although male-related factors contribute substantially to infertility, recent global epidemiology indicates that infertility is more prevalent among women than among men (5). The clinical diagnosis of male infertility is typically established through the evaluation of conventional semen parameters (6). However, based on World Health Organization (WHO) criteria, a substantial proportion of infertile men still lack a definitive diagnosis and are therefore classified as having idiopathic or unexplained infertility (7). Low sperm count, impaired sperm quality, and testicular tissue damage account for over 90% of male infertility cases (8). Several studies have proposed various etiological factors, including exposure to chemotherapeutic agents, which frequently induce significant disruptions in the process of spermatogenesis (9).

As a primary target for toxic injury, the testis is highly susceptible to chemotherapeutic agents and environmental chemicals. Damage to germ cells from these exposures often leads to infertility, which is clinically diagnosed by assessing semen quality. It is estimated that over 100 chemical compounds, including various therapeutic drugs, can impair semen parameters (10). Platinum-derived drugs are now essential for treating many cancers, with most current toxicological data stemming from studies on cisplatin and carboplatin (11).

As a platinum-based drug, carboplatin differs structurally from cisplatin by having its chloride groups replaced with a 1,1-cyclobutane dicarboxylate ligand. It works by binding preferentially to guanine in DNA, creating cross-links that ultimately trigger cell death (12). Although highly effective against cancer, the clinical application of cisplatin and carboplatin is often limited by serious side effects, particularly hepatotoxicity, myelosuppression, alopecia, embryotoxic and teratogenic effects, and nephrotoxicity (11, 13, 14). While numerous studies have reported on the toxicity of cisplatin and carboplatin in various tissues, very few have examined their impact on fertility.

Traditional medicinal plants are widely used in different

parts of the world. With their low toxicity, these plants offer a promising natural alternative for fertility treatment and have increasingly captured researchers' attention (15).

Lesser galangal *(Alpinia officinarum Hance)* holds considerable importance as a member of the ginger family (Zingiberaceae) (16). The *A. officinarum* Hance plant has a reddish-brown tuberous rhizome with a strong aromatic odor and high medicinal and nutritional value. In Iran, the rhizome of *A. officinarum* Hance is often used in pickles and is ground and used as an aromatic spice in various stews (17). In traditional Iranian medicine, it has been suggested to improve libido, relieve back pain and sciatica, treat rheumatic diseases, support the immune system, and treat digestive disorders and stomachaches. Recent studies have shown its potential as an antioxidant, antimicrobial, anti-inflammatory, and anti-cancer agent (17). This plant is considered a protective agent against chemotherapy because of its antioxidant properties, and specifically due to its ability to donate hydrogen and precipitate metal ions (18). Galangal rhizomes contain abundant levels of the flavonol galangin (19) and have been used for centuries in traditional medicine to treat ulcers, diarrhea, and cancer (20), exhibiting analgesic effects, anti-inflammatory properties (21), antimicrobial (22), antiparasitic, anti-arthritic, and antitubercular activities (23), as well as antiplatelet effects (24). In traditional medicine, the rhizome of *A. officinarum* is used to boost male sexual health (25). Considering the importance of this topic, the present study investigated the role of *A. officinarum* Hance in mitigating the complications induced by carboplatin.

Material and Methods

Animal Model and Experimental Protocol

In this research, 36 male Wistar rats aged 10-12 weeks and weighing 150-220 grams were used. These animals were obtained from the Laboratory Animal Care and Maintenance Center of Yazd Infertility Center. All subjects were housed in controlled environments (22 ± 2°C; 12-h light/dark cycle) with free access to standard food and water. The animal cages were disinfected once a week, and their bedding was changed every two days. After the animals were acclimatized for one week, they were randomly allocated into six groups (n = 6 each): control (C), carboplatin (50 mg/kg) group (CA50), low-dose *A. officinarum* Hance (50 mg/kg) group (A50), high-dose *A. officinarum* Hance (100 mg/kg) group (A100), carboplatin (50 mg/kg) + low-dose *A. officinarum* Hance (50 mg/kg) group (CA50/A50), and carboplatin (50 mg/kg) + high-doses *A. officinarum* Hance (100 mg/kg) group (CA50/A100). Carboplatin administration was performed as a single dose on the first day. Carboplatin was dissolved in normal saline to achieve a concentration of 10 mg/mL. A single intraperitoneal injection of 50 mg/kg body weight was administered on the first day of the experiment, calculated based on each rat's body weight measured on that day. Subsequently, the alcoholic extract of *A. officinarum* Hance was administered to the receptive groups daily via oral gavage.

Sample Collection and Measurement of Body and Testicle Weights

The duration of the experiment was 28 days. After this period, the final body weight of the rats was measured using a high-precision scale. The animals were then anesthetized using chloroform. Next, the abdominal cavity was split by an easy pulling method, and the reproductive organs on both sides, including the testicles and the caput epididymidis, were carefully dissected. The left and right testicles of each rat were weighed with a precision of 0.0001 g. Finally, the testicles were placed in a 10% formalin solution to undergo processing for subsequent histological evaluation.

Preparation of Plant Material

In this study, 2.5 kg of rhizome of *A. officinarum* Hance was cut into small pieces in a mortar and then thoroughly powdered and softened using a grinder. The powdered material was transferred to a container, and 5.5 liters of 96% ethanol alcohol was added.

The extraction was carried out for 72 hours, and during this period, the solution was shaken thoroughly every three hours. At the end of the 72 hours, the entire solution was filtered using a funnel and filter paper, which lasted for 12 hours. The solution was then concentrated at 60 °C to remove excess solvent and obtain a semi-solid extract. The obtained extract was stored in a refrigerator. The required doses of 50 and 100 mg/kg body weight were prepared by dissolving the semi-solid extract in distilled water with a small amount of ethanol to improve solubility. The final concentration was adjusted so that each rat received 1 mL of solution per 100 g body weight via daily oral gavage (26).

Analysis of Spermatogenesis Markers in Testis Tissue

Spermatogenesis was quantified in testicular tissue using three established indices based on 100 seminiferous tubules: tubular differentiation index (TDI; tubules with at least three germ cell layers derived from type-A spermatogonia), repopulation index (RI; the ratio of active to inactive spermatogonia), and spermiogenesis index (SPI; the ratio of tubules containing spermatozoa to empty tubules) (27). Moreover, the Sertoli cell index was evaluated as the germ cell-to-Sertoli cell ratio (28). Finally, the meiosis index was expressed as the round spermatid-to-primary spermatocyte ratio (29).

Tissue Processing and Preparation

Testis samples were fixed in Bouin's solution, dehydrated through a graded ethanol series, and embedded in paraffin. Sections (5 µm) were cut, deparaffinized, stained with hematoxylin and eosin (H&E), and examined under a light microscope (30).

Statistical Analysis

Data were processed using SPSS 20 (IBM, SPSS Inc.).

Values are presented as mean ± standard error of the mean (SEM). Statistical significance among groups was determined by one-way ANOVA followed by Tukey's post hoc test, with $P < 0.05$ considered statistically significant.

Results

Examination of Body Weight

The CA50 group showed a significant decrease in final body weight compared with the control group ($P < 0.001$). Compared with the CA50 group, the CA50/A50 and CA50/A100 groups exhibited a significant increase in body weight ($P < 0.001$). The A50 and A100 groups also showed increased body weight compared with the CA50 group ($P < 0.001$); however, no significant difference was observed between the A50, A100, and control groups (Figure 1).

Measurement of Testicular Weight

The CA50 group exhibited a significant reduction in testicular weight compared with the control group ($P < 0.05$). Treatment with *A. officinarum* in the CA50/A50 and CA50/A100 groups significantly increased testicular weight compared with the CA50 group ($P < 0.01$ and $P < 0.001$, respectively). The A50 and A100 groups showed no significant difference in testicular weight compared with the control group, but both were significantly higher than the CA50 group ($P < 0.05$), as illustrated in Figure 2.

Measurement of Tubular Differentiation Index, Spermiogenesis Index, and Repopulation Index in Testicular Tissue

The CA50 group showed a significant decrease in TDI, SPI, and RI compared with the control group ($P < 0.001$ for all). Compared with the CA50 group, the CA50/A50 and CA50/A100 groups exhibited a significant increase in all three indices ($P < 0.001$). The A50 and A100 groups exhibited no significant difference in TDI or SPI compared with the control group, but both groups had significantly higher RI than the CA50 group ($P < 0.001$). Additionally, RI was significantly higher in the A50 and A100 compared with the control group ($P < 0.05$), as depicted in Table 1.

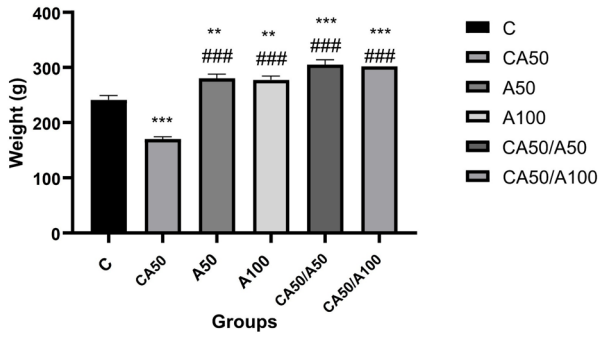


Figure 1. Final Rat Weight in Different Groups
Note. Groups: the control group (C), carboplatin-treated (50 mg/kg) group (CA50), low-dose *A. officinarum* Hance (50 mg/kg) group (A50), high-dose *A. officinarum* Hance (100 mg/kg) group (A100), carboplatin (50 mg/kg) + low-doses *A. officinarum* Hance (50 mg/kg) group (CA50/A50), carboplatin (50mg/kg) + high-dose (100 mg/kg) *A. officinarum* Hance group (CA50/A100) (n = 6). Data are presented as mean ± SEM. Group comparisons were made using one-way ANOVA followed by Tukey's post hoc test. *A. officinarum*: *Alpinia officinarum*; SEM: Standard error of the mean; $^{**}P < 0.01$; $^{***}P < 0.001$ vs. control group; ###$P < 0.001$ vs. CA50 group

Note. TDI: Tubular differentiation index; SPI: Spermiogenesis index; RI: Repopulation index; SEM: Standard error of the mean. Results are presented as mean ± SEM (n = 6). Group comparisons were made via one-way ANOVA followed by Tukey's post hoc test. Control group (C); carboplatin (50 mg/kg) group (CA50); low-dose *A. officinarum* (50 mg/kg) group (A50); high-dose *A. officinarum* Hance (100 mg/kg) group (A100); carboplatin (50mg/kg) + low-dose *A. officinarum* Hance (50 mg/kg) group (CA50/A50); carboplatin (50mg/kg) + high doses (100 mg/kg) *A. officinarum* Hance group (CA50/A100) (n = 6). Statistical comparisons between control and treatment groups: $^{*}P < 0.05$, †$P < 0.01$, ‡$P < 0.001$. Statistical differences between CA50 and other groups: §$P < 0.001$. *A. officinarum*: *Alpinia officinarum*; SEM: Standard error of the mean.

Measurement of Sertoli Cell Index and Meiosis Index

According to the obtained results, the CA50 group showed a significant decrease in Sertoli cell index compared with the control group ($P < 0.001$). Compared with the CA50 group, the CA50/A50 and CA50/A100 groups exhibited a significant increase in the Sertoli cell index ($P < 0.001$). The A50 and A100 groups showed no significant difference compared with the control group, but both were significantly higher than the CA50 group ($P < 0.05$), as illustrated in Figure 3a.

Regarding the meiosis index, the CA50 group revealed a significant decrease compared with the control group ($P < 0.05$). Compared with the CA50 group, the CA50/A50, CA50/A100, A50, and A100 groups all showed a

Table 1. Mean Indices of TDI, SPI, and RI in Testicular Tissue

Groups	Spermiogenesis Coefficients	Mean ± SEM
Control	TDI	91.833 ± 2.63
	SPI	91.50 ± 1.64
	RI	76.83 ± 2.63
CA50	TDI	29.00 ± 1.78‡
	SPI	32.16 ± 2.48‡
	RI	33.16 ± 2.48‡
A50	TDI	92.66 ± 3.61§
	SPI	92.66 ± 2.65§
	RI	90.50 ± 1.87*§
A100	TDI	93.66 ± 3.32§
	SPI	92.00 ± 1.67§
	RI	95.00 ± 1.09*§
CA50/A50	TDI	104.33 ± 4.54†§
	SPI	99.33 ± 0.81*§
	RI	103 ± 4.42†§
CA50/A100	TDI	106.16 ± 4.53†§
	SPI	117.50 ± 5.46†§
	RI	110.33 ± 5.53†§

significant increase ($P<0.05$). No significant difference was observed between any of the treatment groups and the control group, as seen in Figure 3b.

Histological Parameters of Testis

Examination of the testicular tissue in the control group (Figure 4A) showed normal histological architecture, with intact seminiferous tubules, no evidence of atrophy, and a normal population of seminiferous tubule cells.

In the CA50 group (Figure 4B1, B2), severe atrophy of the seminiferous tubules, along with disintegration and vacuolization of the germinal epithelium, hyperemia of blood vessels, collapse of the seminiferous tubules, and tissue edema (yellow arrow) were observed compared with the control group (Figure 4B1). Moreover, this group displayed homogenization and separation of the germinal epithelium from the basement membrane (yellow arrow) (Figure 4B2), reduced germinal epithelial layers, a decrease in the number of different types of germ cells and in spermatogenesis, a decrease in the number and irregularity of Sertoli cell nuclei, degeneration of Leydig cells, a decrease in the number of seminiferous tubule cells, shedding of germinal cells into the lumen, destruction of interstitial tissue, and a decrease in epithelial thickness.

In the A50 group (Figure 4C) and A100 group (Figure 4D), compared with the CA50 group, the testicular tissue showed no visible damage. These groups appeared similar to the control group, with no signs of the severe changes observed in the CA50 group.

In the CA50/A50 group (Figure 4E) and the CA50/A100 group (Figure 4F), compared with the CA50 group, cellular destruction, atrophy of seminiferous tubules, shedding of germinal cells into the lumen, and epithelial disorganization were markedly reduced, although these changes were still slightly more than those in the control group. Moreover, folds and irregularity of the basement membrane, disruption of the tunica albuginea, and destruction of the interstitial tissue in these groups were much less pronounced compared with the CA50 group. Finally, no sign of tissue edema was observed in the CA50/A100 group (Figure 4F).

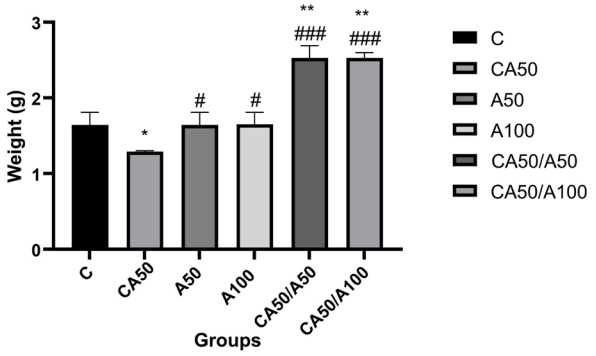
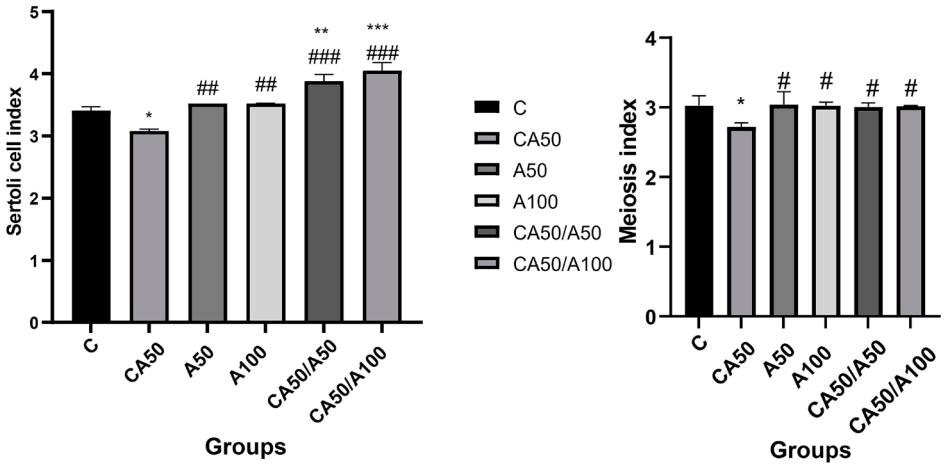


Figure 2. Measurement of the Testicular Final Rat Weight in Different Groups

Note. Groups: the control group (C), carboplatin (50 mg/kg) group (CA50), low-dose *A. officinarum* Hance (50 mg/kg) group (A50), high-dose *A. officinarum* Hance (100 mg/kg) group (A100), carboplatin (50 mg/kg) + low-dose *A. officinarum* Hance (50 mg/kg) group (CA50/A50), carboplatin (50 mg/kg) + high doses (100 mg/kg) *A. officinarum* Hance group (CA50/A100) (n = 6). Data are presented as mean ± SEM. Group comparisons were made using one-way ANOVA followed by Tukey's post hoc test. *A. officinarum*: *Alpinia officinarum*; SEM: Standard error of the mean; *$P<0.05$; **$P<0.01$ vs. control group; #$P<0.05$, ###$P<0.001$ vs. CA50 group

Discussion

Today, chemotherapy, as a cornerstone of cancer treatment, often induces changes in male reproductive function that may culminate in infertility. Chemotherapeutic agents do not act specifically, and in addition to damaging cancer cells, they also harm normal cells (31). Nearly all patients undergoing chemotherapy are at risk of temporary or permanent infertility. Carboplatin is one of the agents used in the treatment of many cancers (32). Based on previous research, carboplatin affects testicular morphology, and the processes of spermatogenesis are disturbed



a b

Figure 3. Measurement of Sertoli Cell Index (b) and Meiosis Index (b) in Testicular Tissue

Note. A. officinarum: *Alpinia officinarum*. Results are presented as mean ± SEM. Group comparisons were made using one-way ANOVA followed by Tukey's post hoc test. Control group, (C); carboplatin-treated group (CA50; 50 mg/kg; low-dose *A. officinarum* Hance group (A50; 50 mg/kg); high-dose *A. officinarum* Hance (A100; 100 mg/kg); carboplatin (50 mg/kg) + low-dose *A. officinarum* Hance (CA50/A50; 50 mg/kg;); carboplatin (50 mg/kg) + high-dose *A. officinarum* Hance group (CA50/A100; 100 mg/kg) (n = 6). *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs. control group; #$P<0.05$, ##$P<0.01$, ###$P<0.001$ vs. CA50 group

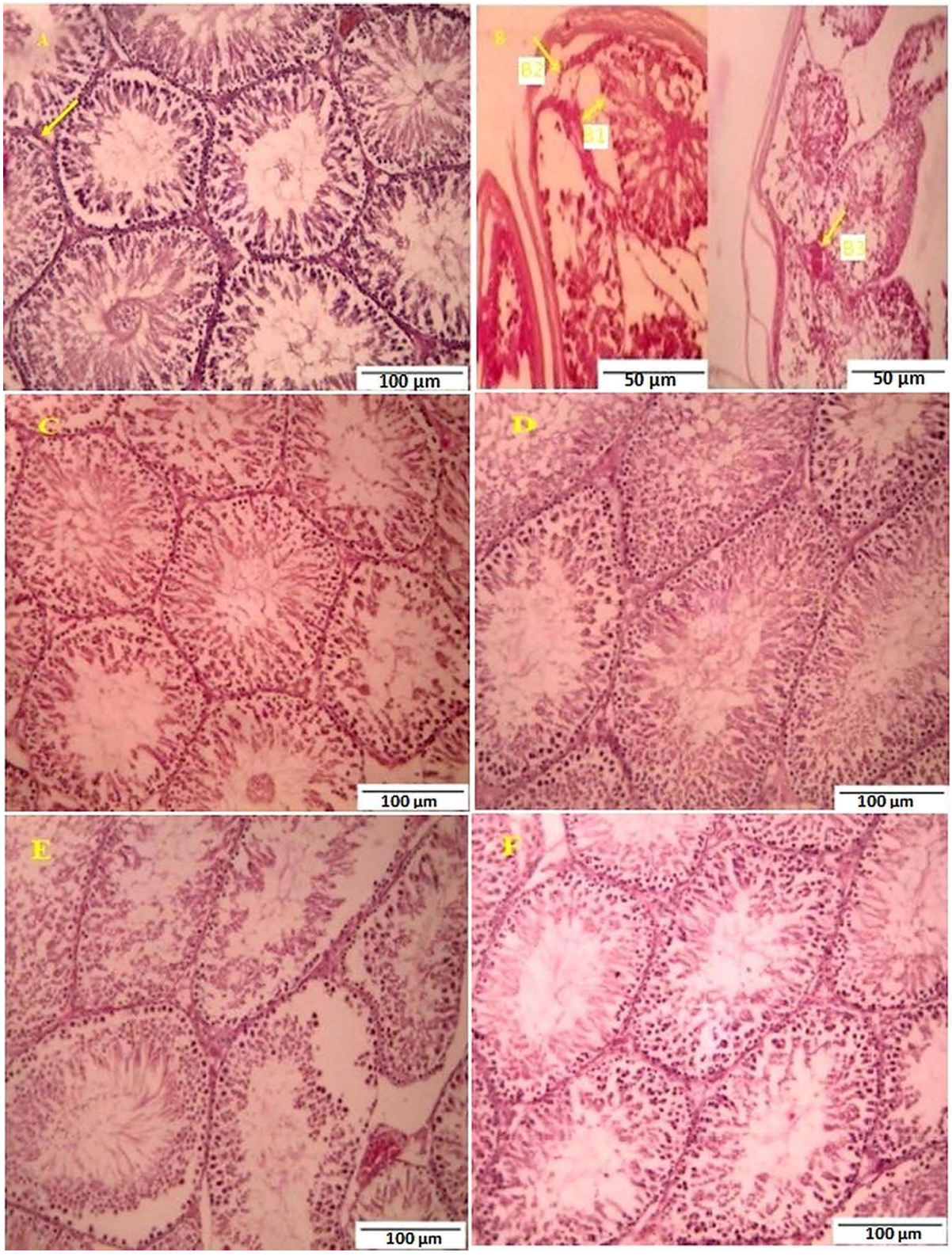


Figure 4. Histopathological Examination of Rat Testicular Tissue in All Experimental Groups (H&E staining × 50 magnification)
Note. Yellow arrows indicate areas of epithelial separation, vacuolization, and disorganization.
(A) Control group: Normal seminiferous tubules with intact germinal epithelium, organized interstitial tissue, and a regular basement membrane.
(B1) Carboplatin (CA50) group: Severe atrophy of seminiferous tubules, vacuolization and disintegration of the germinal epithelium, hyperemia of blood vessels, and tissue edema. **(B2)** Carboplatin (CA50) group: Homogenization and separation of germinal epithelium from the basement membrane, shedding of germinal cells into the lumen, and destruction of interstitial tissue.
(B3) Higher-magnification view of the carboplatin (CA50) group showing separation of the germinal epithelium from the basement membrane and vacuolization, as indicated by the yellow arrow.
(C) Low-dose *A. officinarum* (A50) group: Testicular tissue appears similar to that of the control group, with no visible damage.
(D) High-dose *A. officinarum* (A100) group: Normal testicular architecture comparable to that of the control.
(E) Carboplatin + low-dose *A. officinarum* (CA50/A50) group: Marked reduction in tubular atrophy and epithelial disorganization compared with the CA50 group, although mild changes remain.
(F) Carboplatin + high-dose *A. officinarum* (CA50/A100) group: Nearly normal testicular structure with no evidence of tissue edema

by its cytotoxic action, which is probably the result of molecular interactions between platinum compounds and DNA (33). Despite major medical advances, the side effects of synthetic chemotherapeutic agents continue to be a major challenge. Thus, there is growing interest in herbal medicines as adjuvant therapies to mitigate chemotherapy-induced toxicities, given their favorable safety profile and potential protective effects.

A. officinarum Hance has been shown to improve male fertility in rats by increasing testicular weight, testosterone levels, and sperm quality, likely through direct effects on testicular tissue (34).

In the present study, the body weight of rats that received low and high doses of *A. officinarum* Hance extract showed a remarkable increase. As an important indicator of nutritional status, body weight can help predict reproductive potential in animals (35). In a study conducted by Dailiah et al (36) on the growth rate of male and female rats following the use of extracts from several plants, it was found that after 45 days, rats treated with *A. officinarum* extract gained significantly more weight than the controls.

Segatelli et al (37) in 1998 stated that testicular weight is directly related to the number of germ cells and other cell types, such as Leydig and Sertoli cells, as well as tubular diameter, germinal epithelial thickness, seminiferous tubule cross-sectional area, tubular profile count per testicular area, and tubule numerical density (37). Since all parameters stated above were significantly reduced in the group receiving carboplatin, the decrease in testicle weight can be attributed to reductions in these parameters due to increased free radical production and oxidative stress in the testicular tissue.

As a highly active process involving massive cell division and differentiation, spermatogenesis produces significant amounts of free radicals. Therefore, testicular health and the progression of spermatocytogenesis, spermatogenesis, and spermiogenesis depend on a balance between free radical generation and antioxidant capacity. Consequently, the testes especially susceptible to oxidative stress (38), which can impair antioxidant defenses and lead to extensive tissue damage (39).

In 1992, Petra Kopf-Maier et al (40) at the Anatomy Department of the University of Berlin investigated the effects of carboplatin on rat testicular morphology compared with cisplatin administered at toxic dose levels. Their findings revealed that both compounds caused severe structural changes in Sertoli cells and disrupted the blood-testis barrier, thereby impairing both spermatogenesis and spermiogenesis. Furthermore, rats treated with a high dose of carboplatin exhibited significant weight loss (41). These results confirmed that carboplatin is as toxic to the testes as cisplatin and exerts negative effects on male fertility. Additionally, it was noted that testicular toxicity does not decrease with carboplatin use; rather, the structural damage to Sertoli cells and spermatogenic cells appears to be more severe and less reversible than that caused by carboplatin.

Pogach et al (42) similarly found that the administration of cisplatin, a carboplatin congener, resulted in biochemical and structural evidence of Sertoli cell failure, including leakage of the blood-testis barrier within one week of the initial dose. Furthermore, Vawda and Davis (43) reported that cisplatin damages testicular germ cells. In addition, they observed that the decrease in testicular DNA synthesis reflects the mechanism of action of cisplatin, noting that the inhibition of DNA synthesis occurs much faster than the inhibition of RNA synthesis. Consistent with these findings, the present study demonstrated that carboplatin administration significantly disturbed the TDI, SPI, RI, Sertoli cell index, and meiosis index.

In the current study, histological findings indicated that carboplatin induces substantial damage to testicular tissue, which is in line with previous studies (33, 44). In addition, Kopf-Maier (40) showed that within a few days after carboplatin injection, the tight junctions between neighboring cells were disrupted, intercellular spaces around Sertoli cells expanded, Sertoli cell cytoplasm disintegrated, and their nuclei became smaller. Chemotherapy drugs such as cisplatin and carboplatin can cause temporary, long-term, or permanent damage to the male gonads (45). Evidence also suggests that chemotherapy drugs induce damage to Leydig cells. The amount of testicular damage appears to be dependent on both the specific type of drug and the dose administered (46).

The protective effects of *A. officinarum* against carboplatin-induced testicular damage can be attributed to several underlying mechanisms. First, carboplatin is known to generate reactive oxygen species (ROS) and induce oxidative stress in testicular tissue, leading to lipid peroxidation, DNA damage, and germ cell apoptosis (47). The flavonoids present in *A. officinarum*, particularly galangin, possess potent free radical-scavenging properties that can neutralize ROS and restore the balance of endogenous antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase (48). Second, carboplatin triggers an inflammatory response in the testis, characterized by elevated levels of pro-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and IL-6 (49). *A. officinarum* has demonstrated anti-inflammatory activity through the inhibition of the COX-2 enzyme and the suppression of these inflammatory mediators (19). Third, carboplatin induces apoptosis in germ cells via activation of caspase cascades and disruption of mitochondrial membrane potential. The anti-apoptotic properties of *A. officinarum* may involve the upregulation of Bcl-2 (an anti-apoptotic protein) and the downregulation of Bax and caspase-3 (pro-apoptotic factors) (50). Fourth, as noted previously, the ability of *A. officinarum* to chelate metal ions may allow it to bind free platinum from carboplatin, thereby reducing its bioavailability to testicular tissue and limiting the formation of DNA cross-links in healthy germ cells (18). Collectively, these synergistic mechanisms—antioxidant, anti-inflammatory, anti-apoptotic, and metal-chelating—

contribute to the protective role of *A. officinarum* against carboplatin-induced reproductive toxicity.

In this study, intraperitoneal injection of carboplatin at 50 mg/kg decreased various parameters, including tubular diameter, germinal epithelial height, TDI, SPI, RI, Sertoli cell index, and meiosis index. In addition, *A. officinarum* Hance extract increased these parameters in the testes of rats receiving carboplatin. To the best of our knowledge, this is the first report to evaluate the effects of carboplatin and *A. officinarum* Hance on these parameters.

Conclusion

According to the present study and the obtained results, it can be concluded that carboplatin can cause damage to rat testicular tissue and exerts negative effects on male reproductive function. Furthermore, these findings reveal that *A. officinarum* Hance could be beneficial in reducing carboplatin-induced toxicity to a considerable extent. Therefore, the simultaneous use of *A. officinarum* Hance with carboplatin in chemotherapy is recommended.

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Authors' Contribution

Author contributions are clarified as follows:
Conceptualization: Maryam Thaghipoor, Elham Salehi, Majid Morovati-Sharifabad
Data Curation: Ali Abdollahi
Formal Analysis: Ali Abdollahi, Mohammad Saeid Heydarnejad
Investigation: Maryam Thaghipoor, Elham Salehi, Majid Morovati-Sharifabad
Methodology: Maryam Thaghipoor, Elham Salehi, Majid Morovati-Sharifabad
Project Administration: Maryam Thaghipoor, Elham Salehi, Majid Morovati-Sharifabad
Software: Ali Abdollahi
Supervision: Maryam Thaghipoor, Elham Salehi, Majid Morovati-Sharifabad
Validation: Mohammad Saeid Heydarnejad
Visualization: Ali Abdollahi
Writing – Original Draft: Maryam Thaghipoor, Elham Salehi
Writing – Review & Editing: Mohammad Saeid Heydarnejad

Competing Interests

The authors declare that they have no conflict of interests.

Ethical Approval

Animal husbandry and experimental procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals (No. 85-23, revised 1996) and were approved by the Ardakan University Animal Care Committee. All experimental procedures in this study were conducted in accordance with the Institutional Animal Care guidelines of Ardakan University and were approved by the Research Ethics Committee of Ardakan University, Ardakan, Iran (Ethical code: IR.YAZD.REC.1401.051).

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