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Protective Role of Gallic Acid in Restoring miR-34c-5p Expression in Testicular Tissue of Cadmium-Induced Reproductive Damage in Adult Male Mice

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Abstract

Cadmium, a known environmental toxin, is associated with significant reproductive damage, including the dysregulation of key genetic pathways. This study investigated the protective role of gallic acid in mitigating cadmium-induced testicular damage by regulating miR-34c-5p gene expression. Eighteen healthy male Balb/c mice were divided into six groups: Group 1 (control) received normal saline, Groups 2 and 3 were treated with gallic acid (50 mg/kg and 100 mg/kg, respectively), Group 4 received cadmium (3 mg/kg), Group 5 was administered cadmium (3 mg/kg) with gallic acid (50 mg/kg), and Group 6 received cadmium (3 mg/kg) with gallic acid (100 mg/kg). Treatments were administered intraperitoneally over five weeks, with cadmium given weekly and gallic acid twice weekly. Results revealed a significant reduction in miR-34c-5p expression in cadmium-exposed mice compared to the control group ($p < 0.0001$). Gallic acid alone (50 mg/kg or 100 mg/kg) did not significantly alter miR-34c-5p expression compared to the control ($p > 0.05$). However, co-administration of gallic acid (50 mg/kg) with cadmium significantly increased miR-34c-5p expression ($p < 0.05$), although levels remained lower than in the control group ($p < 0.01$). Similarly, cadmium-treated mice receiving gallic acid (100 mg/kg) showed an increase in miR-34c-5p expression, but this was not statistically significant ($p > 0.05$). In conclusion, gallic acid demonstrates a dose-dependent protective effect against cadmium-induced suppression of miR-34c-5p expression in testicular tissue. These findings highlight the potential of gallic acid as a therapeutic agent in mitigating cadmium-associated reproductive toxicity.

Keywords: Gallic Acid, Cadmium, miR-34c-5p, Testicular Tissue

Introduction

Environmental exposure to heavy metals, particularly cadmium, poses a significant and growing threat to human and animal health (1). Cadmium, a toxic pollutant, is introduced into the environment through industrial processes, agricultural activities, and cigarette smoke, leading to widespread contamination (2). Once absorbed, cadmium accumulates in various tissues, including the reproductive organs, where it disrupts normal physiological processes. It exerts its toxicity through mechanisms such as oxidative stress, inflammation, and apoptosis, while also interfering with endocrine signaling and compromising the integrity of the blood-testis barrier (3-6). These disruptions collectively result in impaired spermatogenesis, testicular damage, and infertility. Despite the well-documented reproductive toxicity of cadmium, effective therapeutic strategies to mitigate its adverse effects remain elusive.

Understanding the molecular pathways disrupted by microRNAs (miRNAs), small non-coding RNA molecules cadmium is essential for developing targeted interventions to protect reproductive health (7).

that regulate gene expression, have emerged as key players in cellular homeostasis and stress responses, including in the context of reproduction (8, 9). Among these, miR-34c-5p is recognized as a pivotal regulator of spermatogenesis and testicular function (10). It plays a crucial role in germ cell development and the maintenance of testicular homeostasis. Dysregulation of miR-34c-5p expression has been implicated in testicular damage and impaired fertility, particularly under conditions of oxidative stress triggered by environmental toxicants such as cadmium (11).

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Natural antioxidants have garnered increasing attention in recent years for their potential to counteract damage caused by toxicants and various conditions affecting the reproductive system (12, 13).

These compounds, found abundantly in fruits, vegetables, and other plant-based sources, play a crucial role in neutralizing free radicals and reducing oxidative stress, which can significantly impair reproductive health (14, 15). Research has shown that antioxidants such as vitamins C and E, flavonoids, and polyphenols can enhance fertility, improve sperm quality, and protect against the adverse effects of environmental pollutants and harmful lifestyle factors (16, 17). By mitigating oxidative damage, natural antioxidants not only support the overall functioning of the reproductive system but also contribute to improved outcomes in both male and female fertility, highlighting their importance as a complementary approach to conventional reproductive health treatments (18).

Gallic acid, a phenolic compound abundant in fruits, vegetables, and medicinal plants, exhibits potent antioxidant, anti-inflammatory, and anti-apoptotic properties (19).

Mechanistically, gallic acid mitigates oxidative stress by scavenging free radicals, enhancing the activity of endogenous antioxidant systems, and modulating key molecular pathways involved in cellular defense and repair, including the Nrf2 and NF-κB signaling pathways (20, 21).

These properties position gallic acid as a promising candidate for protecting against cadmium-induced reproductive toxicity. However, its interactions with specific molecular markers of testicular damage, such as miR-34c-5p, remain poorly understood.

This study aimed to investigate the protective effects of gallic acid on cadmium-induced testicular toxicity in mice. Specifically, we examined the expression of miR-34c-5p in testicular tissue as a molecular marker of cadmium toxicity and the potential therapeutic effects of gallic acid. By exploring the interplay between cadmium exposure, miR-34c-5p dysregulation, and gallic acid treatment, this research addresses a critical gap in the understanding of natural antioxidant-based therapies.

These findings contribute to a growing body of evidence supporting the use of natural compounds in safeguarding reproductive health. They may inform the development of novel strategies to mitigate the public health burden of environmental toxicants on fertility.

Materials and Methods

Animals

For this study, 18 healthy male Balb/c mice, each weighing approximately 35 ± 2 g and aged 8 weeks, were obtained from the animal facility at Shiraz University of Medical Sciences (SUMS). The mice were housed in standard cages (three animals per cage) under controlled conditions, with a temperature of 22 ± 2°C, 55 ± 10% relative humidity, and a 12-hour light/dark cycle. They were provided unrestricted access to a standard rodent chow diet and water. The animals were acclimatized to the laboratory environment for one week before the experiment. The research protocols were reviewed and approved by the SUMS institutional ethics committee (IR.SUMS.REC.1399.730).

Experimental Design

Following a two-week acclimatization period, 18 Balb/c mice were randomly divided into six experimental groups (Groups 1 to 6, n = 3 per group). Group 1 served as the control group and received intraperitoneal (i.p.) injections of normal saline. Groups 2 and 3 were treated with gallic acid at concentrations of 50 mg/kg and 100 mg/kg, respectively. Group 4 received cadmium chloride ($CdCl_2$) at a concentration of 3 mg/kg. Group 5 was administered both cadmium (3 mg/kg) and gallic acid (50 mg/kg), while Group 6 received cadmium (3 mg/kg) and gallic acid (100 mg/kg).

The experimental period lasted five weeks. Cadmium chloride was administered intraperitoneally once per week, while gallic acid was administered twice weekly, dissolved in normal saline to the appropriate concentrations for each group. Figure 1. demonstrates the study design.

At the end of the treatment period, the mice were euthanized using a carbon dioxide (CO_2) chamber following standard ethical protocols. Testes were carefully dissected, snap-frozen in liquid nitrogen, and stored at -70°C in anitrogen tank for subsequent RNA extraction and molecular analyses.

Real Time PCR

MicroRNAs were extracted from testicular tissue using RNX-Plus (Cinnagen, Iran). Complementary DNA was synthesized with the RB-Micro RNA Synthesis kit (RNA Biotechnology Company, Iran), employing specific stem-loop primers and 500 ng of total RNA in a 25-µL reaction, following the manufacturer's instructions.

Real-time PCR was performed using the Step-One ABI Applied Biosystems (Life Technologies), with U6 snRNA as a control for assessing the expression levels of miR-346-5p. The PCR reactions were conducted under the following conditions: an initial denaturation at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds and annealing/extension at 63°C for 60 seconds. Each sample was analyzed in triplicate, and the average cyclic threshold (Ct) values were utilized for further analysis. The Livak method ($2^{-\Delta\Delta Ct}$) was applied to evaluate the expression levels of miR-346-5p in the samples.

Table 1. Details of primers used for Real Time PCR

Genes	Primer (5′→3′)
U6	Forward: TGCTTCGGCAGCACATATAC Reverse: AGGGGCCATGCTAATCTTCT
miR-346-5p	Forward: AGGCAGTGTAGTTAGC Reverse: GTGCAGGGTCCGAGGT

Statistical Analysis

The GraphPad Prism software (version 9.0, Inc. La Jolla, California, USA) was used for statistical analyses, and the results were mentioned as mean±SEM. $P<0.05$ was considered statistically significant.

Results

The expression of miR-34c-5p was significantly reduced in mice exposed to cadmium compared to the control group ($p < 0.0001$), indicating a strong suppressive effect of cadmium on this molecular marker (Figure 2).

In mice treated with gallic acid at doses of 50 mg/kg or 100 mg/kg without cadmium exposure, miR-34c-5p expression showed a slight decrease compared to the control group. However, this reduction was not statistically significant ($p > 0.05$), suggesting that gallic acid alone does not alter miR-34c-5p expression under normal conditions.

Co-administration of 50 mg/kg gallic acid with cadmium significantly increased miR-34c-5p expression compared to the cadmium-only group ($p < 0.05$). However, expression levels remained significantly lower than those observed in the control group ($p < 0.01$). At a higher dose (100 mg/kg), gallic acid also increased miR-34c-5p expression in cadmium-exposed mice, but this change was not statistically significant ($p > 0.05$). Nonetheless, miR-34c-5p expression in this group remained significantly suppressed compared to the control group ($p < 0.001$).

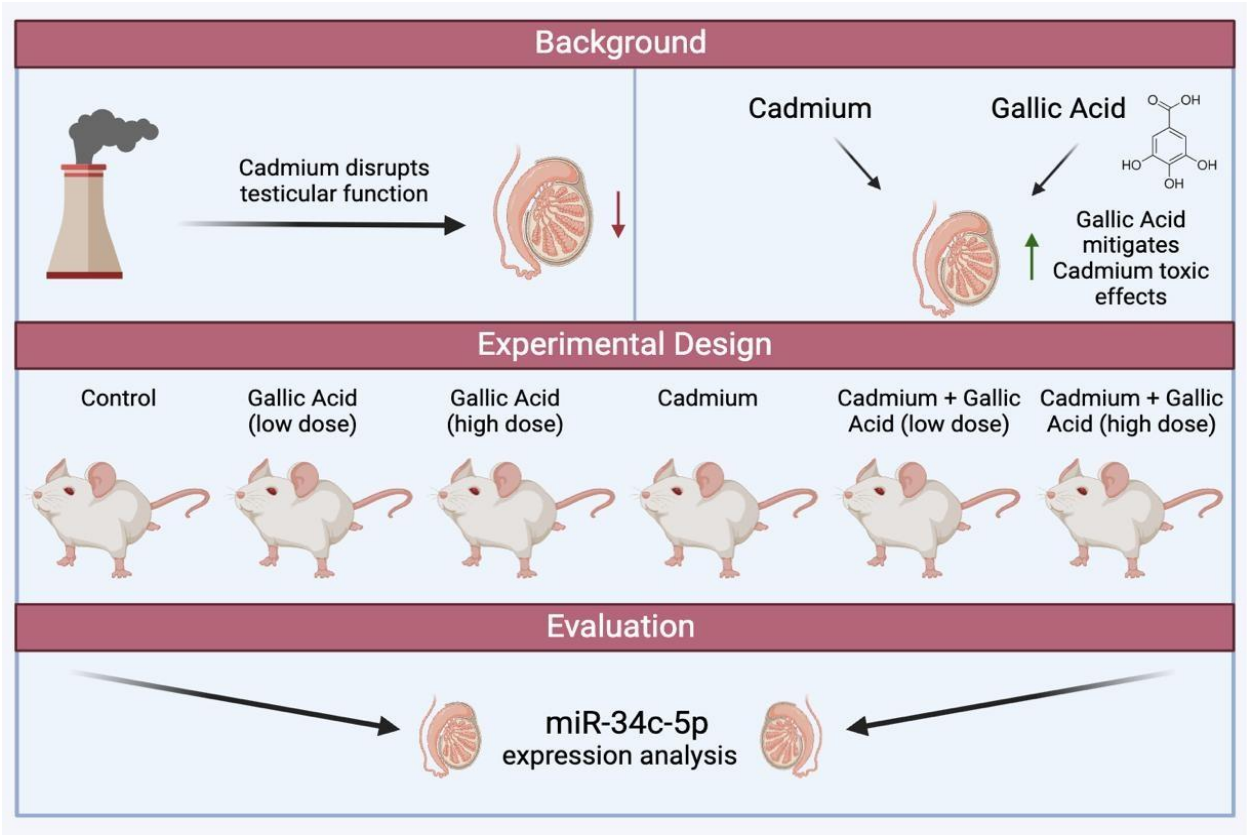

Figure 1. The Study background, design, and evaluation

Direct comparisons between cadmium-treated groups demonstrated a significant improvement in miR-34c-5p expression with both 50 mg/kg and 100 mg/kg gallic acid co-treatment compared to cadmium alone ($p < 0.0001$ for both doses). These results suggest that gallic acid exhibits a dose-dependent protective effect, with the lower dose (50 mg/kg) showing statistically significant restoration of miR-34c-5p expression.

Discussion

Cd is a well-documented environmental toxicant with profound adverse effects on male fertility (22). Its toxicological mechanisms involve the disruption of various biological processes, including the regulation of gene expression (23).

In this study, cadmium exposure significantly suppressed the expression of miR-34c-5p in testicular tissue, consistent with previous research demonstrating cadmium-induced dysregulation of miRNA profiles (24, 25). Dysregulated miRNAs contribute to cadmium's toxic effects by impairing testicular function and spermatogenesis, underscoring their importance in reproductive health.

The suppression of miR-34c-5p observed in this study is particularly notable, given its established role in regulating cellular stress responses, apoptosis, and oxidative stress (26, 27). As a critical regulator of spermatogenesis and testicular function, downregulation of miR-34c-5p provides a mechanistic insight into the reproductive toxicity associated with cadmium exposure (28, 29).

Natural compounds have gained significant attention as promising agents in mitigating the adverse effects of

toxicants on the reproductive system and alleviating infertility conditions (30-32). These bioactive compounds, derived from plant-based sources such as fruits, vegetables, herbs, and spices, exhibit potent free-radical-scavenging properties (31). By neutralizing oxidative stress, a primary mechanism through which toxicants impair cellular function, natural antioxidants protect reproductive tissues from damage (33, 34).

Emerging studies on natural compounds validate their efficacy, highlighting their association with significant improvements in reproductive tissue health, reduced oxidative damage, and enhanced fertility outcomes in both toxin-exposed models and infertility-related conditions (35-37).

Among these, gallic acid—a polyphenolic compound with potent antioxidant properties—has been shown to counteract oxidative stress and reduce cellular damage in various toxicity models (19, 38). In our study, gallic acid alone did not significantly alter miR-34c-5p expression in healthy mice. However, when administered at 50 mg/kg following cadmium exposure, it significantly restored miR-34c-5p expression. This suggests that gallic acid can mitigate cadmium-induced toxicity by counteracting oxidative stress and restoring normal cellular functions. Previous studies have also highlighted the protective effects of gallic acid against cadmium toxicity, including its ability to preserve testicular structure and function (21, 39, 40).

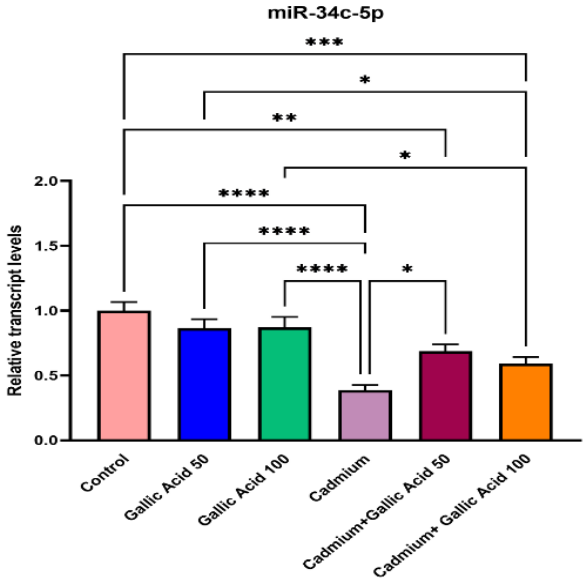

Figure 2. The gene expression of miR-34c-5p in all experimental groups. Values are expressed as means ± standard error (SE). Statistical significance is denoted as follows: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

For instance, Onuoha et al. demonstrated that gallic acid ameliorates cadmium-induced testicular damage by restoring testosterone levels, enhancing glutathione reductase activity, and preserving DNA integrity, both alone and in combination with other phenolic compounds (34). Similarly, Rotimi et al. reported that gallic acid mitigates cadmium-induced reproductive toxicity in female rats by improving antioxidant status and preserving ovarian function (21). These findings collectively highlight the broad-spectrum protective effects of gallic acid against cadmium toxicity across different reproductive contexts.

Interestingly, our results revealed a dose-dependent response to gallic acid treatment. While the 50 mg/kg dose significantly restored miR-34c-5p expression, the 100 mg/kg dose led to a non-significant increase. This aligns with previous findings by Akbarzadeh et al., who reported a dose-dependent effect of gallic acid in reducing freezing-induced damage to human sperm (35). The observed plateau effect may reflect a balance between the antioxidant capacity of gallic acid and the overwhelming toxicity of cadmium at higher exposures, warranting further investigation.

The significant suppression of miR-34c-5p in cadmium-treated mice compared to those co-treated with gallic acid underscores the compound's potential to restore miRNA homeostasis. This restoration of miRNA expression could be a critical mechanism by which gallic acid mitigates the toxic effects of environmental stressors such as cadmium.

Conclusion

Overall, our results demonstrate that while cadmium exposure markedly reduces miR-34c-5p expression, the administration of gallic acid has the potential to modulate this effect, particularly at lower doses. Future research should explore the underlying mechanisms behind miR-34c-5p regulation and the therapeutic potential of gallic acid in models of cadmium toxicity, with a focus on optimising dosage and treatment regimens to enhance reproductive health.

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