Original Article

Protective Effect of Propolis and Vitamin E on Testicular Dysfunction in Male Rats Induced by Cadmium Chloride

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ABSTRACT

Background: Testicular dysfunction in male rats can manifest in various ways and may be caused by genetic, environmental, hormonal, and nutritional factors.

Objectives: This study aims to evaluate the effects of Propolis and vitamin E against induced testicular dysfunction by cadmium chloride ($CdCl_2$) in male rats.

Methods: Forty adult male rats were randomly divided into four groups containing 10 animals each as follows: Group 1 (negative control group): rats received distilled water; group 2 (positive control group): Rats received $CdCl_2$ at 1 mg/kg every 72 h via intraperitoneal (IP) injection; group 3 ($CdCl_2$+propolis): rats were treated with Propolis at 250 mg/kg body weight (BW) orally daily by a gavage needle, followed by $CdCl_2$ at 1 mg/kg IP injection every 72 h and group 4 ($CdCl_2$ + Vit E): rats were treated with Vit E at 100 mg/kg body weight (BW) orally daily by a gavage needle followed by $CdCl_2$ 1 mg/kg by IP injection every 72 h. After four weeks, the animals were sacrificed, and tissues were collected to measure experimental parameters, including histopathological study, testosterone level, tumor necrosis factor α (TNF-α), and caspase 3.

Results: The results showed a significant ($P \leq 0.5$) increase in caspase and TNF-α in G2 compared to G1, while testosterone showed a significant ($P \leq 0.5$) decrease in G2 compared to G1. Treatment with propolis and vitamin E enhanced testosterone, TNF-α, and caspase three concentrations compared with G2 (positive group). Histopathological analysis of Propolis and Vitamin E restored normal tissue structure compared to the positive control group, showing clear damage in the interstitial Leydig cells and severe damage in the interstitial connective tissue. In contrast, the Sertoli cells and primary spermatocytes showed clear necrotic changes.

Conclusion: The study concluded that propolis and vitamin E have neuroprotective effects against testicular dysfunction.

Keywords: Cadmium chloride ($CdCl_2$), Propolis, Testicular dysfunction, Vitamin E

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Introduction

Testicular dysfunction in male rats can manifest in various ways and may be caused by genetic, environmental, hormonal, and nutritional factors. Testicular dysfunction often refers to issues that affect the proper functioning of the testes, leading to impaired fertility or disruptions in reproductive health. Some potential causes and aspects of testicular dysfunction were observed in male rats (hormonal imbalances, genetic factors, nutritional deficiencies, and toxic exposures) (Agarwal et al., 2020; Usende et al., 2022; Sartorius & Handelsman, 2023).

Cadmium is divided into organic and inorganic compounds; it also belongs to the toxic substance group, along with lead, mercury, arsenic, and selenium. These substances are usually studied due to their use in a wide spectrum of sectors, especially agriculture, gas, and steelmaking. Vital applications include the discharge of cadmium waste into the environment and the consumption of food containing this element. With an increase in the amount of cadmium accumulated in the human body, the number of cases of poisoning due to this element also increases. Cadmium enters the body via the digestive or respiratory system (Bhattacharyya et al., 2023).

Cadmium usually accumulates in tissues, including the testes. Due to the long half-life of cadmium and its low excretion rate, it can cause a toxic effect that manifests in various ways, such as apoptosis, oxidative stress, and autographic cell death. Cadmium can alter Sertoli cells, which are crucial in the spermatogenesis process, and provide a barrier to the testis by damaging the cytoskeleton, leading to deformities in the structure of Sertoli cells, allowing the immune system to enter the testis and cause various problems, such as germ cell apoptosis, as well as decreased testosterone production by affecting Leydig cells by downregulating the genes that are responsible for testosterone production (Ali et al., 2022).

Propolis is a natural brownish-green resinous material that is produced by honeybees. Bees use Propolis to create a protective layer, fill cracks, and polish the cells of honeycomb walls (Zapata et al., 2022). Pro is a valuable natural compound capable of treating and preventing numerous illnesses (Handelsman, 2019). Many pharmacological actions of Propolis have been reported, such as hepatoprotective, anti-inflammatory, and antioxidant activities (Stojanović et al., 2020). Exhibiting immunomodulatory and anti-inflammatory properties is crucial to prevent testicular dysfunction since conditions, such as orchitis or testicular torsion are often accompanied by inflammation, and chronic inflammation can cause testicular dysfunction, due to its ingredients, phenols, flavonoids, and cinnamic acid derivatives (Magnavacca et al., 2022). Propolis is used to manufacture cosmetics and drugs to treat rhinitis, wounds, burns, and acne. It appears in various forms, including capsules, creams, mouthwash, toothpaste, and throat lozenges (Coppock, 2021). This study investigated the combined effects of Propolis and Vitamin E on cadmium chloride $CdCl_2$-induced testicular dysfunction in male rats.

Several studies have shown that propolis can enhance wound healing through numerous mechanisms, including stimulating granulation tissue, fibroblasts, and angiogenesis. It also depresses inflammation (Salrian et al., 2022). Propolis is also known to have antibacterial effects, especially against *Aenibacillus larvae* and *Melissococcus plutonius*. The synergistic effect of Propolis and different extracts may enhance its therapeutic potential (Toutiaee et al., 2023). Other studies have shown that Propolis may play a role in immunization since combining it with silver nanoparticles can increase interleukin-4 and immunoglobulin concentrations in the body (Sekhi & Al-Samarraae, 2023).

Vitamin E was chosen to combine with propolis due to its well-documented antioxidant properties, which are the main mechanisms of testicular dysfunction induced by $CdCl_2$ (Shi et al., 2024). While vitamin E provides antioxidant protection, and Propolis provides broader mechanisms to protect against testicular dysfunction, including anti-inflammatory and immunomodulatory pathways.

The present study was conducted to assess the potential therapeutic impact of Propolis and vitamin E in rats exposed to cadmium toxicity.

Materials and Methods

Experimental animals

The animal model of this study was Albino Wistar rats, with a weight range of 250±25 g, The animals were kept in plastic cages, where there were five animals in each cage, with free access to drinking water and food; the conditions were under control, the humidity levels were 45±15%, temperature 25±3 and light cycle (14/10, light/dark), each group also was consisted of two cages, since each group contains 10 animals and each cage contains only five animals.

Drugs used in this study

Xylazine hydrochloride (100 mg/mL) and ketamine hydrochloride (100 mg/mL) were purchased from the Dechra Pharmaceuticals Company (UK). Propolis was purchased from YS Eco Bee Farms Company, USA. Cadmium Chloride was purchased from Sigma-Aldrich (USA).

Group 1 (G1 or negative control): rats received distilled water.

Group 2 (G2 or positive control): Rats were administered 1 mg/kg of $CdCl_2$ every 72 h for four weeks via the intraperitoneal (IP) route (Areba, 2020).

Group 3 (G3 or $CdCl_2$ + propolis): Rats were treated with Propolis at 250 mg/kg body weight (BW) orally daily by a gavage needle (Singla et al., 2014), followed by $CdCl_2$ at 1 mg/kg by IP injection every 72 h for four weeks

Group 4 (G4 or $CdCl_2$+ Vit E): rats were administered a daily oral dose of 100 mg/kg BW of vitamin E using a gavage needle (Abouelghar et al., 2020), followed by $CdCl_2$ at 1 mg/kg via IP injection every 72 h for four weeks

Parameters for investigation

Tissue samples were collected at the end of the study; the experimental rats were ethically sacrificed using ketamine at 10 mg/kg BW and xylazine at 40 mg/kg BW. Testes were collected from the rats and stored at -80 ˚C for seventy-two hours. One test was used for histopathological analysis, and the other was homogenized using cold polyphenylene sulfide (PPS) (PH 7.5) mixed with protease inhibitor to obtain a homogenizing solution cocktail. The homogenizing solution was centrifuged at 3000 rpm for 15 minutes in a cold centrifuge. The supernatant was collected in a sterile Eppendorf tube, discarded, and stored at -20 ˚C for 24 h. Elisa used the supernatant to evaluate this caspase- 3, tumor necrosis factor α (TNF-alpha), and testosterone levels.

Results

Statistical analysis

All results were analyzed using a one-way analysis of variance (ANOVA) test for statistical analysis, and post-hoc comparisons were performed using Tukey's test to detect differences between groups.

The results of Caspase and TNF-α presented in Figures 1 and 2 demonstrated a significant (P≤0.05) increase in G2 compared to the control group, which indicates the toxic effect of $CdCl_2$ on testicular dysfunction. In contrast, testosterone in Figure 3 demonstrated a significant (P≤0.05) decrease in G2 compared to the control group. In contrast, caspase and TNF-α, presented in Figures 1 and 2, demonstrated a significant (P≤0.05) decrease in G3 and G4 compared to G2. Finally, testosterone in Figure 3 demonstrated a significant (P≤0.05) decrease in G3 and G4 compared to G2, which rules the therapeutic effect of Propolis.

Histopathological analysis

Figure 4 shows the histological sections of the testes in rats in the negative control group (Figure 4). The section shows the normal texture of testes, including seminiferous tubules (black arrow), interstitial cells of Leydig (red arrow), interstitial connective tissue (green arrow), spermatids (blue arrow), Sertoli cells (orange arrow), and primary spermatocytes (yellow arrow). The tissue is stained with hematoxylin and eosin (H&E), and the sections are captured using a digital camera and a light microscope at a 10X magnifier scale.

Figure 5 shows histopathological sections of the testes of rats treated with $Cdcdl_2$ at 1 mg/kg BW. The section shows clear damage in the interstitial Leydig cells (black

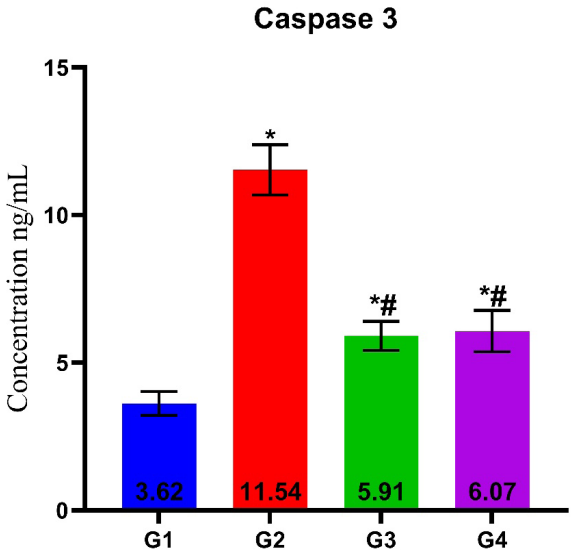

Figure 1. Concentrations of Caspase 3 in all groups

*P<0.05 compared to control, #P<0.05 compared to $CdCl_2$, $P<0.05 compared to Propolis.

Note: Results are expressed as Mean±SD.

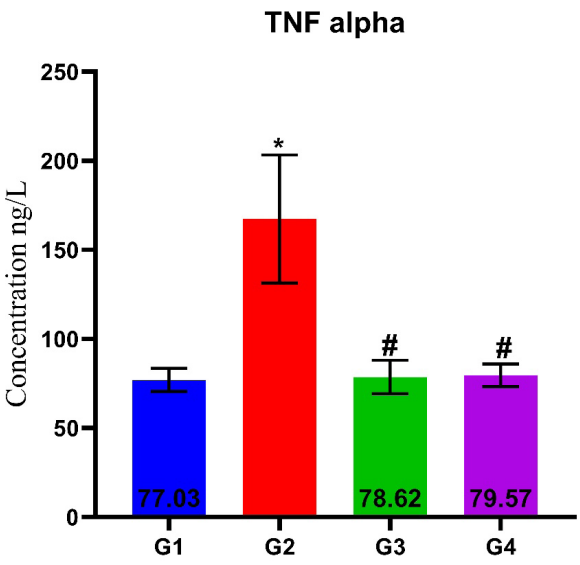

Figure 2. Concentrations of tumor necrotic factor (TNF) in all groups

*P<0.05 compared to control, #P<0.05 compared to $CdCl_2$, $P<0.05 compared to propolis.

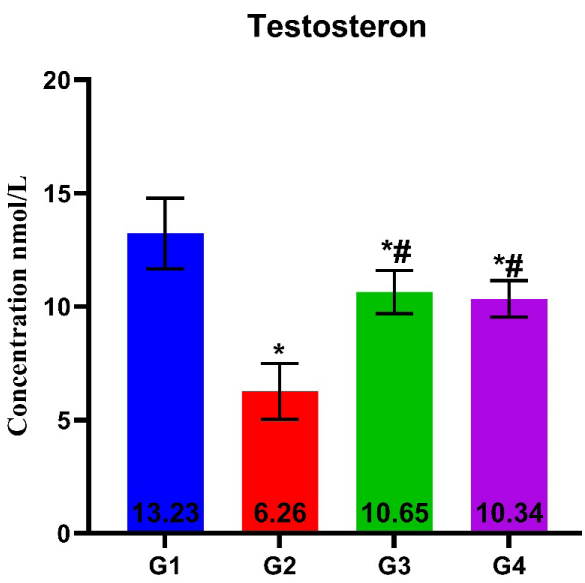

Figure 3. Concentrations of testosterone in all groups

*P<0.05 compared to control, #P<0.05 compared to $CdCl_2$.

Note: Results are expressed as Mean±SD.

arrows) and severe damage in the interstitial connective tissue (red arrows). Sertoli cells and primary spermatocytes show clear necrotic changes (green arrows). The tissue is stained with H&E, and the sections are captured using a digital camera and a light microscope at a 10X magnifier scale.

Figure 6 shows the histological sections of the testes in rats in the propolis-treated group. The section shows normal texture without any significant occupied lesions

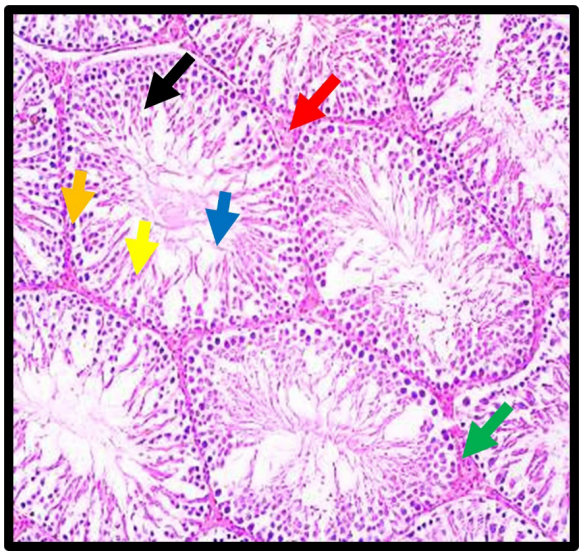

Figure 4. The histological section of testes in rat in control negative group

(SOL) of the testes, including seminiferous tubules (black arrow), interstitial cells of Leydig (red arrow), interstitial connective tissue (green arrow), spermatids (blue arrow), Sertoli cells (orange arrow) and primary spermatocytes (yellow arrow). The tissue is stained with H&E, and the section is captured using a digital camera and a light microscope at a 10X magnifier scale.

Figure 7 shows the histological sections of the testes in rats in the vitamin E-treated group. The section shows normal texture without any SOL of testes, including seminiferous tubules (black arrow), interstitial cells of Leydig (red arrow), interstitial connective tissue (green arrow), spermatids (blue arrow), Sertoli cells (orange

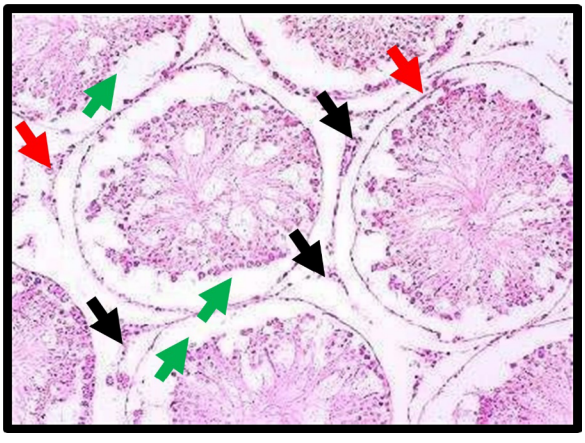

Figure 5. The histopathological section in testes of rats treating with $Cdcdl_2$ 1 mg/kg BW

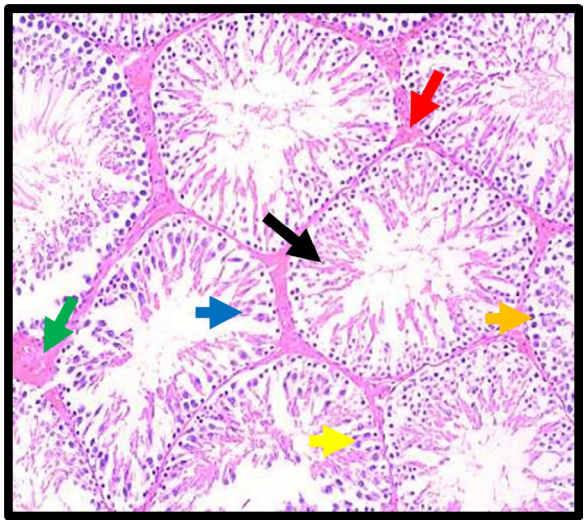

Figure 6. The histological section of testes in rat in propolis treated group

arrow) and primary spermatocytes (yellow arrow). The tissue is stained with H&E, and the sections are captured using a digital camera and a light microscope at a 10X magnifier scale.

Discussion

This study was conducted to evaluate the effectiveness of propolis and vitamin E in treating testicular dysfunction induced by $CdCl_2$ by evaluating concentrations of TNF-α, caspase 3, and testosterone. G2, which also received $CdCl_2$, showed a highly significant elevation in caspase and TNF-α levels compared to G1. Cadmium can exhibit toxic properties and the ability to disrupt cellular homeostasis to induce programmed cell death, as evidenced by an increase in caspase activity. (Đukić-Ćosić et al., 2020; Souza-Arroyo et al., 2022). Further evidence shows that the induction of a cellular immune response within the organism to cadmium exposure includes an increase in TNF-α, an inflammatory cytokine (Yang et al., 2021).

A significantly lower testosterone level observed in G2 than in G1 can thus provide an alert regarding the cadmium effect on reproductive performance. Testosterone is thus vital in many physiological processes of spermatogenesis and may reflect basic information on male fertility status. Its levels decline, probably due to the harmful cadmium influence on Leydig cells responsible for testosterone production. (Grande et al., 2022). This is supported by the findings of other authors, who showed that heavy metals can disrupt the endocrine system (Zhou et al., 2022).

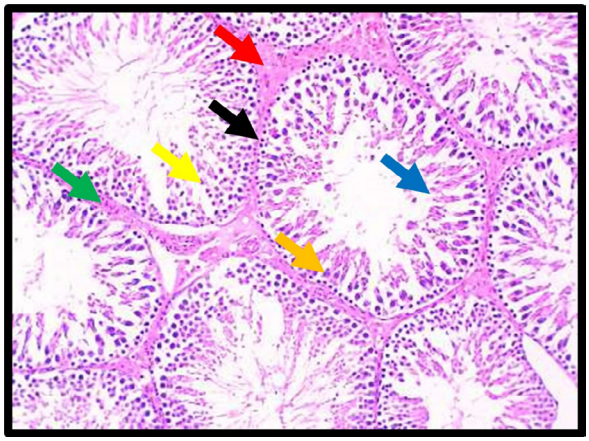

Figure 7. The histological section of testes in rat in vitamin E treated group

Moreover, regarding caspase and TNF--α levels, treatments with propolis and vitamin E, along with $CdCl_2$, have significantly lowered the levels in G3 and G4, respectively, compared to G2. This suggests that such treatments have protective effects against cadmium-induced toxicity. Propolis's antioxidative properties could reduce oxidative stress and inflammation, leading to a lesser degree of apoptosis as expressed in reduced caspase levels. Correspondingly, vitamin E, a well-accepted antioxidant, can stabilize the cell membrane and inhibit oxidative damage, thereby reducing inflammation and apoptosis. (Mohamed et al., 2021; Mohamed et al., 2022)

However, testosterone levels were significantly reduced in groups 3 and 4 compared to group 2. This may be attributed to the fact that although propolis and vitamin E can play a role in reducing inflammation and apoptotic responses to a certain degree, they did not bring testosterone to the normal value. This would mean that such agents afford partial protection but are inadequate in surmounting the severe endocrine disturbances caused by cadmium exposure. This intimate relationship among the pathophysiological mechanisms of oxidative stress, inflammation, and hormonal regulation also points to this fact.

The results underlined the noxious effect of cadmium on both inflammatory markers and testosterone levels, underlining the health hazards possibly linked to cadmium exposure. The possible therapeutic benefit of Propolis and vitamin E underlines further possibilities of intervention that would require further investigation into the underlying mechanism and other protective measures.

Histopathologically, interstitial Leydig cells and connective tissue deterioration is accompanied by necrotic changes in Sertoli cells and primary spermatocytes. Pathological changes in the testes may be caused by several factors, including toxins and testicular torsion (Adamczewska et al., 2020; Abarikwu et al., 2021). These results also showed serious disturbances in the normal pattern and function of the testes, which are harmful to sperm production and fertility. Propolis is a resinous product collected by honeybees that have been reported to possess many biological activities, including antioxidant and anti-inflammatory properties (Seven et al., 2020). Considering these aspects, propolis appears to be one of the agents that can protect testicular tissues from damage at both the morphological and functional levels (Martins et al., 2021; Mega et al., 2022).

The absence of notable pathological alterations in this segment indicated that propolis therapy may have averted or remedied the type of harm detected in the positive control group sample. Vitamin E is one of the established antioxidants studied for efficiency in protecting the testicles. (El-Kotb et al., 2020; Abdel-Wahab et al., 2021). This coherence between the established ability of vitamin E to reduce oxidative stress and preserve testicular structure across different studies closely aligns with structurally intact presentations of testicular components. (Nowicka-Bauer & Nixon, 2020) (Mondal & Bandyopadhyay, 2024). This result supports using vitamin E as a treatment to maintain testicle health.

Conclusion

This study demonstrated that propolis and vitamin E protected against $CdCl_2$ by improving the optimal results according to physiological parameters and pathohistological sections.

Ethical Considerations

Compliance with ethical guidelines

All procedures used in this study were reviewed and approved by the Scientific Committee of the Faculty of Veterinary Medicine, University of Kufa, Kufa, Iraq, in compliance with the ethical principles of animal welfare (Code: UK.VET.2023.27152).

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Authors' contributions

All authors equally contributed to preparing this article

Conflict of interest

The authors declared no conflict of interest.

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