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Protective effect of the bee bread on cadmium-induced testicular toxicity in rats

Volkan Koşal[1]*, Turan Yaman[2], Çağlar Okulmuş[3]

[1] Department of Artificial Insemination, Faculty of Veterinary Medicine, Van Yüzüncü Yıl University, Van, Türkiye; [2] Department of Pathology, Faculty of Veterinary Medicine, Van Yüzüncü Yıl University, Van, Türkiye; [3] Department of Biochemistry, Faculty of Veterinary Medicine, Dokuz Eylül University, Izmir, Türkiye.

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Abstract

Cadmium (Cd) is a highly toxic environmental pollutant known to cause severe damage to the male reproductive system. This study aimed to investigate the protective effects of bee bread (BB), a natural product with anti-oxidant, anti-apoptotic, and anti-inflammatory properties, against Cd-induced testicular toxicity in male Wistar rats. A total number of 32 rats were divided into four groups, including control, BB (0.50 g kg^{-1}), Cd (5.00 mg kg^{-1}), and Cd + BB (5.00 mg kg^{-1} and 0.50 g kg^{-1}, respectively) groups. Administrations *via* oral gavage were performed for 4 weeks. Semen analysis revealed significant reductions in sperm motility and density along with increases in abnormal and dead sperm ratios in the Cd and Cd + BB groups compared to controls. Histopathological examination showed severe degeneration and desquamation of germ cells, tubular atrophy, and a decrease in spermatozoa in the Cd-treated groups. Polymerase chain reaction analysis indicated up-regulation of apoptotic markers (caspase-3, -8, and -9) and oxidative stress enzymes (catalase and superoxide dismutase) in the Cd group, signifying disrupted testicular function. The BB administration partially mitigated Cd-induced damage as evidenced by less severe histopathological changes and moderated gene expression alterations. However, the protective effects of BB were not sufficient to completely counteract the toxic impact of Cd. The present study concluded that while BB had potential in reducing Cd-induced testicular toxicity, its protective efficacy was limited, warranting further research to explore its therapeutic potential in combination with other protective agents.

Introduction

Pollen is collected by bees from plants, made sticky with saliva, and carried in a pollen basket to the hive. The pollen stored in the honeycomb cells undergoes bio-chemical changes due to the action of honey bee secretions, lactic acid bacteria, yeasts, moisture, and temperature. The resulting product is known as bee bread (BB; Perga).[1]

The composition of BB includes 24.00 - 35.00% carbohydrates, 20.00% proteins, 3.00% lipids, and 3.00% minerals and vitamins. In addition, it contains enzymes, such as phosphatase, glucose oxidase, sucrase, and amylase, and amino acids, like proline, glutamic acid, aspartic acid, histidine, arginine, valin, and alanine, as well as carotenoids, phenolic acids, and polyphenols.[2] Fourteen saturated and eleven unsaturated fatty acids have been identified in BB with α-linolenic acid being the most abundant at 25.00%, followed by arachidonic acid at 23.20%.[3] Trace elements, such as zinc, iron, manganese, selenium, and copper, function as cofactors for many anti-oxidant enzymes; therefore, considered important components in metabolism.[4]

Cadmium (Cd) is a toxic metal released into the environment through various human activities, including mineral mining, agriculture, and industry.[5] The Cd and its compounds were classified as carcinogenic to humans (group I) by the International Agency for Research on Cancer in 1993.[6] This metal shows increased levels in the blood, seminal plasma, and follicular fluid of elderly individuals and smokers.[7] The Cd affects cell proliferation, differentiation, and apoptosis and other cellular activities. It is also known to influence gene transcription and translation. Also, Cd interferes with DNA repair mechanisms in response to oxidative damage contributing to its genotoxic effects.[8] The Cd exerts toxic effects directly on the organism or by disrupting homeostatic mechanisms, such as the immune system.[9] Prolonged exposure to Cd can lead to structural and functional abnormalities in several organs, particularly male and female reproductive systems, kidneys, and endocrine system.[10]

***Correspondence:**

Volkan Koşal. PhD
Department of Artificial Insemination, Faculty of Veterinary Medicine, Van Yüzüncü Yıl University, Van, Türkiye
E-mail: volkankosal@yyu.edu.tr

The testes are highly sensitive to Cd following parenteral administration. Exposure to Cd results in hemorrhagic inflammation, atrophy, edema, necrosis, and dysfunction of the seminiferous tubules, causing permanent damage to these structures. Cadmium is a non-essential toxic element exerting toxic effects on many enzymes requiring iron as a cofactor, one of which is cytochrome P450. Leydig cells contain cytochrome P450 at levels 10 times higher than Sertoli cells, making them more susceptible to elevated Cd levels. Since cytochrome P450 is required for the functions of 17-hydroxylase and 7,20-lyase, its damage may affect testicular steroidogenesis.[11,12]

Due to its rich composition, BB has been studied for its anti-oxidant, anti-apoptotic, anti-inflammatory, anti-microbial, anti-cancer, and immunomodulatory properties in various diseases.[13-16] Given these properties, this study aimed to investigate the protective effects of BB against the adverse effects of Cd toxicity on the male reproductive system.

Materials and Methods

Experimental animals. In this study, male Wistar rats weighing 200 - 250 g and 3 months old were used. The animals were obtained from the Experimental Medicine Application and Research Center of Van Yüzüncü Yıl University, Van, Türkiye. The rats were housed in rooms with a 12 hr light/dark cycle, a temperature of 22.00 ± 2.00 ˚C, and 60.00% humidity with free access to tap water and fed on standard pellet rat chow. The study was undertaken under agreement No. 2024/05-07 of Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee, Van, Türkiye, dated 30/05/2024.

Bee bread. Freshly obtained BB was dried at 35.00 ˚ C for 4 hr, then ground into a fine powder using a blender, and stored at – 20.00 ˚C until use.[2] During the 4-week experimental period, BB was administered *via* daily oral gavage at a dose of 0.50 g kg^{-1} body weight.[17,18]

Cadmium. Cadmium as $CdCl_2H_2O$ (Carlo Erba, Val de Reuil, France) was administered *via* daily orogastric gavage at a dose of 5.00 mg kg^{-1} body weight during the experimental period.[18-20] A total number of 32 rats were divided into four groups, with eight rats in each group, including control group fed with standard pellet rat chow, BB group administered BB *via* daily orogastric gavage for 28 days at a dose of 0.50 g kg^{-1} body weight in 1.00 mL distilled water, Cd group received Cd *via* daily orogastric gavage for 28 days at a dose of 5.00 mg kg^{-1} body weight in 1.00 mL distilled water, and Cd + BB group received Cd *via* daily orogastric gavage for 28 days at a dose of 5.00 mg kg^{-1} body weight in 1.00 mL distilled water, as well as BB *via* daily orogastric gavage for 28 days at a dose of 0.50 g kg^{-1} body weight in 1.00 mL distilled water. The BB was given to rats 1 hr prior to the administration of Cd.

Anesthesia and euthanesia. Xylazine hydrochloride (Bioveta, Ivanovice na Hané, Czech Republic) at a dose of 3.00 mg kg^{-1} and ketamine hydrochloride (Richter Pharma, Ankara, Türkiye) at a dose of 90.00 mg kg^{-1} were administered intraperitoneally for anaesthesia. Rats were sacrificed by exsanguination method under anaesthesia.

Spermatological examination. After sacrification of the rats, semen was collected from the cauda epididymis by epididymis puncture immediately. Motility, density, abnormal sperm ratio, and live/dead sperm ratio analyses were performed immediately after sacrifice.[21]

Polymerase chain reaction (PCR). Collection of tissue samples for RNA isolation and preparation for analysis, RNA extraction and analysis, cDNA extraction, and real-time-qPCR analysis were performed according to Livak and Schimitten.[22] The target genes used are listed in Table 1.

Cadmium level in testis. Homogenized testis samples of about 0.20 g were taken into Teflon vessels. After this processes, 5.00 mL concentrated HNO_3 was added and resolubilized by microwave digestion. To obtain clear solutions, the final volume was completed to 10.00 mL

Table 1. Primary sequence of target genes.

Genes	Primary sequences	Amplicon size (bp)
Beta-actin	F: CTCCTCAAGGATGGCACC R: GCTCATTGTAGAAAGTGTGGT	91 bp
CAT	F: GGACGCTCAGCTTTTCATTC R: TTGTCCAGAAGAGCCTGGAT	119 bp
SOD1	F: GCTTCTGTCGTCTCCTTGCT R: CATGCTCGCCTTCAGTTAATCC	113 bp
AR	F: GTGAAATGGGACCTTGGATG R: TACTGAATGACCGCCATCTG	134 bp
CASPASE-3	F: TACCCTGAAATGGGCTTGTGT R: GTTAACACGAGTGAGGATGTG	115 bp
CASPASE-8	F: TAAGACCTTTAAGGAGCTTCATTTTGA R: AGGATACTAGAACCTCATGGATTTGAC	118 bp
CASPASE-9	F: GAGGGAAGCCCAAGCTGTTC R: GCCACCTCAAAGCCATGGT	103 bp

CAT: Catalase; SOD1: Superoxide dismutase; AR: Androgen receptor.

ultrapure water. The analyses were prepared in triplicate. The blank solutions were carried out in the same way. Cadmium concentrations were determined in a Perkin-Elmer AAnalyst 800 graphite furnace atomic absorption spectrometer (Waltham, USA).

Histopathological examination. Histological study of the testis was performed using Hematoxylin and Eosin stain to evaluate the degree of tubular degeneration and atrophy, as well as degeneration and desquamation of germ cells. Testes tissues were fixed in 10.00% neutral buffered formalin. Then, the fixed tissues were dehydrated in alcohol and embedded in paraffin, and 5.00-µm sections were cut. Four serial sections were prepared from each sample. Histopathological findings were evaluated subjectively as negative (–), mild (+), moderate (++), and intense (+++) by examining five different areas at least 100 µm apart on each section.[23]

Statistical analysis. The SPSS Software (version 20.0; IBM Corp., Armonk, USA) package program was used for statistical analysis. All data were expressed as mean ± standard deviation. After PCR analysis, gene expression levels were determined using $2^{-\Delta\Delta ct}$ log values based on the threshold cycle values. Statistical analyses of the groups were carried out statistically using the one way ANOVA, followed by *post hoc* multiple comparisons (Tukey's test) for comparative analysis between the groups. The $p < 0.05$ was regarded as statistically significant.

Results

Testicular Cd levels. The Cd concentration in the control group was 13.10 ± 0.33 ppb. In Cd group, testicular Cd levels increased to 287.10 ± 111.14 ppb, and this increase was statistically significant compared to the control group ($p < 0.001$). In BB group, the testicular Cd level was 13.30 ± 0.76 ppb, with no statistically significant difference compared to the control group ($p > 0.05$). In Cd + BB group, testicular Cd levels were measured as 249.50 ± 29.32 ppb, which was significantly higher than those in the control and BB groups ($p < 0.001$).

Sperm motilty and density, and abnormal and dead/live sperm rates. The results of semen analysis showed that motility and density were statistically decreased and abnormal and dead/live sperm ratios were statistically increased in Cd and Cd + BB groups (Table 2; $p < 0.001$).

PCR findings. As a result of testicular PCR analysis, androgen receptor (AR) level was found to be increased in Cd and Cd + BB groups compared to the control group and decreased in BB group (Fig. 1; $p < 0.001$). Caspase-3, -8, and -9, catalase, and superoxide dismutase levels were found to be increased in Cd group compared to the control group and decreased in BB group compared to the control group (Fig. 1; $p < 0.001$).

Histopathology. Normal histological structure of the testis was observed in the control group rats (Fig. 2A). In the Cd group, degeneration of seminiferous tubules, widening of interstitial spaces due to tubular atrophy, degeneration and desquamation of germ cells, and decrease in the number of spermatozoa were detected (Fig. 2B). While normal histology of the testis was observed in the BB group rats (Fig. 2C), it was determined that the changes resulting from Cd exposure were partially reduced in the Cd + BB group (Fig. 2D). The frequency and severity of histopathological lesions in testicular tissue in all of the groups are presented in Table 3.

Table 2. Results of semen analysis.

Groups	Motility (%)	Density (×10⁹)	Abnormal sperm (%)	Deal/live (%)
Control	81.42 ± 3.40[a]	1.96 ± 0.13[a]	17.25 ± 1.32[a]	17.48 ± 1.34[a]
BB	88.57 ± 3.77[a]	2.41 ± 0.07[a]	11.42 ± 1.39[a]	11.14 ± 1.21[a]
Cd	26.25 ± 25.15[b]	0.60 ± 0.45[b]	62.5 ± 19.63[b]	65.12 ± 21.14[b]
Cd + BB	24.44 ± 7.26[b]	0.56 ± 0.10[b]	59.88 ± 3.55[b]	58.55 ± 5.57[b]

BB: Bee bread, and Cd: Cadmium.
[ab] The differences between groups with different letters in the same column are significant ($p < 0.001$).

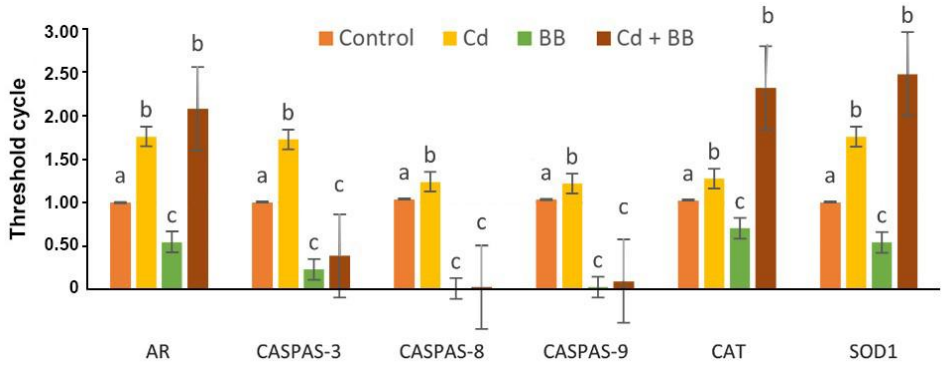

Fig. 1. Results of polymerase chain reaction results. AR: Androgen receptor, CAT: Catalase, SOD1: Superoxide dismutase, BB: Bee bread, and Cd: Cadmium. [abc] The differences between groups with different letters in the same column are significant ($p < 0.001$).

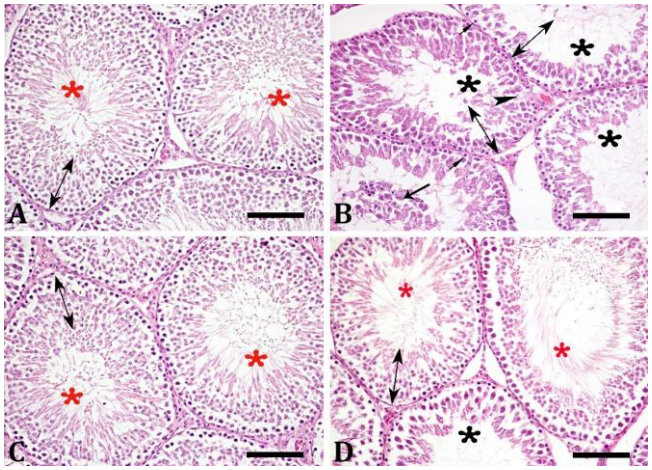

Fig. 2. Effects of bee bread (BB) on cadmium (Cd)-induced changes in testicular sections (Hematoxylin and Eosin staining, bars = 100 µm). **A)** Control group: Normal histological appearance of the testis with normal spermatogenesis (red stars). Germ cells are present and appeared normal (double-headed arrow); **B)** Cd group: Arrested spermatogenesis (black stars), narrowed germ cell line (double-headed arrows), degenerated germ cells (small arrows), and shed germ cells in the lumen of seminiferous tubules (long arrow) are seen. In some areas, germ cells are completely absent (arrowhead); **C)** BB group: Normal histological appearance of the testis is observed with normal spermatogenesis (red stars) and regular germ cells layer (double-headed arrow); **D)** Cd + BB group: Relatively reduced damage caused by Cd is observed. The spermatogenic cells (double-headed arrow) continued spermatogenesis in a partially normal manner (red stars). However, spermatogenesis arrest is present in some tubules (black star).

Table 3. Incidence and severity of the lesions in the testes of the control, bee bread (BB), cadmium (Cd), and Cd + BB groups.

Changes/lesions in testis	Control	BB	Cd	Cd + BB
Tubular atrophy				
Mild	0	0	3	4
Moderate	0	0	4	2
Intense	0	0	1	1
Degeneration of germ cells				
Mild	0	0	5	5
Moderate	0	0	2	2
Intense	0	0	0	1
Desquamated immature germ cells				
Mild	0	0	4	4
Moderate	0	0	1	2
Intense	0	0	3	0
Decrease in the number of spermatozoa				
Mild	0	0	3	5
Moderate	0	0	3	1
Intense	0	0	2	1

Discussion

The toxic effects of Cd on the testes, particularly in various animal species, have long been recognized.[24] It has been reported that the testes are the most sensitive organ to the acute toxic effects of Cd in rodents.[25] To prevent or mitigate Cd toxicity, various agents, such as selenium, vitamin E, vitamin C, lycopene, taurine, melatonin, acetylcysteine, progesterone, β-carotene, chlorpromazine, and glutathione, have been used.[15,24-27] In this study, the protective effects of BB against Cd-induced testicular toxicity were investigated.

In mammalian testes, the blood-testis barrier (BTB) is formed by specialized junctions between adjacent Sertoli cells along the basal membrane of the seminiferous tubules. The BTB is one of the primary targets of Cd. Cadmium has been shown to disrupt the BTB in rodent models.[28] In this study, it was found that Cd accumulated in the testes by atomic absorption analysis and testicular histopathological degenerations caused Cd to accumulate in the testes by disrupting the BTB.

Accumulated Cd in the testes caused histopathological changes, including tubular atrophy, degeneration, and desquamation. Previous studies documented that Cd induced numerous histopathological abnormalities in the testes, such as necrosis, germ cell loss, edema, hemorrhage, and degeneration.[5,14,29] In this study and similar studies using Cd, 5.00 mg kg^{-1} of Cd was applied. Testicular histopathological changes showed similarities. It was observed that BB did not treat the testicular histopathological changes detected due to Cd statistically. The administration of BB along with Cd did not show any positive effects on the histopathological changes.

Testosterone levels play a critical role in maintaining healthy spermatogenesis. Studies have reported that the number of Leydig cells, which are responsible for testosterone production, decreases and they undergo degeneration due to Cd exposure.[30-33] Androgen receptor levels are known to correlate with testosterone levels. In the present study, a decrease in AR levels was observed in BB group, where BB was administered alone, while Cd and Cd + BB groups exhibited a Cd-induced increase in AR levels.

In this study, Cd negatively affected sperm parameters, such as motility, density, abnormality, and live/dead sperm ratios consistent with findings from other studies.[13,16,17,32] Although BB alone showed positive effects on sperm parameters, no statistically significant difference was observed. In the Cd and Cd + BB groups, Cd altered the levels of apoptotic enzymes and caused histopathological changes.

The Cd replaces elements, such as zinc, calcium, copper, and iron in metalloenzymes, increasing the amount of these metals in unbound forms. The Cd also binds to the thiol and sulfhydryl groups of free radical scavengers, like glutathione, disrupting the activities of anti-oxidant enzymes, such as catalase, superoxide dismutase, and glutathione peroxidase.[8,34,35] Additionally, Cd enters the mitochondria within cells, affecting cellular respiration at the oxidative phosphorylation level and increasing the formation of lipid peroxidation products.[36]

The link between oxidative stress and apoptosis is well established.[37,38] This study comprehensively examined the toxic effects of Cd on the testes and the protective potential of BB against this toxicity. The findings revealed that Cd exposure led to significant impairments in spermatological parameters and severe histopathological changes in the seminiferous tubules, including degeneration and germ cell loss. Furthermore, PCR analyses confirmed that Cd disrupted anti-oxidant enzyme activities, and triggered apoptosis. Although BB partially mitigated these adverse effects, it did not provide complete recovery. The protective effect of BB against Cd-induced toxicity was found to be limited.

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Conflict of interest

The authors have no conflict of interest.

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