

Investigation of the Effects of Calcium Fructoborate on Testicular Structure in Rats within the Framework of Biochemical Parameters, Testosterone Hormone and DNA Damage in Cadmium Chloride Induced Toxicity

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ABSTRACT

Background: The present study aims to investigate the effects of calcium fructoborate on testicular DNA damage and testicular tissue biochemical markers and serum testosterone levels after cadmium chloride administration. **Materials and Methods:** 28 Wistar albino rats (200-220 g) in the study were divided into 4 groups with an equal number. These groups are; Control group (No chemicals applied), calcium fructoborate (100 mg) group, Cadmium chloride (200 mg/L) + calcium fructoborate (100 mg), Cadmium chloride (200 mg/L) group. The study lasted 28 days and both chemicals were applied daily with oral gavage. **Results:** While 8-hydroxy-2-deoxyguanosine (8-OHdG) expression was moderate in the cadmium chloride + calcium fructoborate group, the expression in the cadmium chloride group was severe. In the cadmium chloride group, testicular tissue glutathione (GSH) and 8-OHdG levels, superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities and serum testosterone levels were significantly decreased compared to other groups. It is determined that the reversal of the change in the parameters listed in the calcium fructoborate group. **Conclusion:** The reversal of the change in the parameters listed in the calcium fructoborate group indicates the positive strength of the present chemical. It is our suggestion to transfer calcium fructoborate into life practice by conducting further clinical studies and evaluating different parameters.

Key words: Cadmium chloride, Calcium fructoborate, Rat, 8-OHdG, Testosterone.

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INTRODUCTION

Pollution in the atmosphere is a serious problem for both human health and other ecosystems. In this pollution, people, transportation vehicles and heavy metals play a big role.¹ Cadmium, a heavy metal, has been reported to be abundant especially in industrial areas,² causing injury in the liver, kidneys and testicles.³⁻⁹ Especially, in the toxicity it causes in the testicles, it has been reported that spermatogenesis is impaired and there are decreases in gonadotropic hormones and testosterone

hormone.¹⁰⁻¹² It has also been reported to cause apoptosis in the male reproductive cell.⁶ There are many studies in which oxidant / antioxidant balance is troubled after cadmium exposure.^{3-6,8,9}

Boron is an essential nutrient in animals as much as necessary for the normal growth and development of plants.^{13,14} It is emphasized that some fruits (Banana, apple, grape, peanut, hazelnut) and vegetables (carrot, potato, bean, broccoli, cabbage) have rich boron content.¹⁵ In boron deficiency, it



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has been reported that the number of sperm decreases in the frogs, sperm morphology is impaired and causes atrophy in the testicles and ovaries.¹⁶ Boron increases the level of testosterone in men,¹⁷ is important in the immune system,^{18,19} prevents osteoporosis²⁰ and protects against some types of cancer.^{21,22} Boron contains many features such as anti-inflammatory, anticancer, disinfectant, antioxidant and antiapoptotic.^{23,24}

Efforts are being made to find safe and convenient antioxidants from some plant extracts.²⁵ Calcium fructoborate was obtained in 1998 as a US patent and was later developed.²⁶ The calcium fructoborate form of boron, consisting of approximately 3% boron, 5% calcium and 92% fructose, is found especially in grapes, plums, celery and broccoli.^{26,27} In the USA, calcium fructoborate is added to diets for bone health.²⁸ Calcium fructoborate has been reported to have antioxidant, anti-inflammatory and antitumor effects.²⁹⁻³¹

In this study, we tried to determine what effects calcium fructoborate will have on some of the biochemical and immunohistochemical problems that may occur after cadmium chloride application. In this context, from a biochemical point of view; testicular tissue glutathione (GSH) superoxide dismutase (SOD) and glutathione peroxidase (GPx) were evaluated. Immunohistochemically also; 8-OHdG expression levels, an important marker for oxidative DNA damage, were determined.

MATERIALS AND METHODS

Chemicals

Cadmium chloride (CdCl₂) (655198 Sigma-Aldrich) was used as the cadmium source and FruiteX-B® Brand Calcium Fructoborate as the source of calcium fructoborate. The chemicals listed below were used immunohistochemically in our study: 8-OHdG (Abcam, Catalog No ab62623); HRP (Thermofischer, Catalog number: TP-125-HL). In addition to these, the following chemicals were used biochemically: GSH (Catalog no: SG-20391), SOD (Catalog no: SG-10188), GPx (Catalog no: SG-20976), 8-OHdG (catalog no: YLA0061RA).

Animals and experimental design

The study was performed on 28 (200-220 g) wistar albino male rats. In rats supplied from the Van Yuzuncu Yil University Experimental Research Laboratory, ethical rules were observed in all applications. Prior to the study, the necessary permission was obtained from the Van Yuzuncu Yil University Ethics Committee (authorization number: 2020/41). Standard conditions existed in the care units of the rats. There was no

restriction on feed intake. Care was taken for cleaning and ventilation.

Control group: No medication was applied.

Calcium fructoborate group: Calcium fructoborate 100 mg was administered orally. In the study, both cadmium chloride and calcium fructoborate were applied daily for 28 days.

Cadmium chloride + calcium fructoborate group: Freshly prepared 200 mg / L cadmium chloride in drinking water was administered orally to 7 rats in this group. In addition, 100 mg of calcium fructoborate was administered orally.

Cadmium chloride group: Freshly prepared 200 mg / L cadmium chloride in drinking water was administered orally to 7 rats in this group.³²

At the end of the 28 day working period, rats 75 mg / kg i.p. They were anesthetized by applying ketamine. The testicles were taken for biochemical parameter evaluation and immunohistochemical evaluation. The testis taken for immunohistochemistry was left in a 10% neutral formalin solution.

Immunohistochemical analysis

Testicular tissues detected in neutral formalin (10%) solution were subjected to alcohol-xylol treatment. Then, paraffin was taken into blocks. Sections of 5 µm taken on polylysine slides were subjected to xylol and alcohol processes. It was washed with PBS and then endogenous peroxidase inactivation (10 m) was achieved in 3% H₂O₂. Treated with antigen retrieval solution (500 watts for 2x5 m). Then, washing was done with PBS. Then, 8-OHdG (Abcam, Catalog No ab 62623, 1/100 dilution rate) was incubated at room temperature (20 m) with the primary antibody. Secondary; Large Volume Detection System: Anti-Polyvalent, HRP (Thermofischer, Catalog number: TP-125-HL) was used as recommended by the manufacturer. DAB (3,3'-Diaminobenzidine) was used as a chromogen. After contrasting with Mayer's Hematoxylin, it was closed with entellan and examined under a light microscope. The examination was made as no (-), mild (+), moderate (++) and severe (+++) in the testicular tissues.

Biochemical analysis

It was studied by ELISA method using GSH (Catalog no: SG-20391), SOD (Catalog no: SG-10188), GPx (Catalog no: SG-20976) and 8-OHdG (catalog no: YLA0061RA) commercial kit.

Statistical analysis

The data obtained were analyzed with SPSS 20.00 (SPSS, Inc., Chicago, IL, USA) program. In terms of

immunohistochemistry, the difference between the groups was determined by Kruskal Wallis, one of the nonparametric tests and the group that formed the difference was by the Mann Whitney U test ($p < 0.05$). Analysis of the biochemical data was carried out by one-way ANOVA followed by post hoc Tukey multiple comparison tests. Differences were considered significant at $p < 0.05$

RESULTS

Immunohistochemical findings

In testicular tissues, a significant difference was detected between the groups in terms of 8-OHdG immunopositivity (Table 1, $p < 0.05$). Immunopositivity of 8-OHdG was not significantly determined in testicular tissues of the control group and calcium fructoborate group (Figure 1). While 8-OHdG expression level was moderate in testicular tissues of cadmium chloride + calcium fructoborate group rats, 8-OHdG expression was found to be severe in testicular tissues of cadmium chloride group rats (Figure 1).

Biochemical findings

Control group testicular tissue GSH and 8-OHdG levels and SOD and GPx activities were significantly higher than the cadmium group. The cadmium group testicular tissue GSH and 8-OHdG levels and SOD and GPx activities were significantly lower than the cadmium + calcium fructoborate group. Serum testosterone level was significantly higher in the calcium fructoborate group than in the other groups. Serum testosterone

level was significantly lower in the cadmium treated group than in the other groups. The serum testosterone level of the cadmium + calcium fructoborate group was significantly higher than the cadmium group. The findings obtained biochemically are presented in Table 2.

DISCUSSION

Cadmium is a industrial environmental pollutant, is taken by contaminated water, food and air and accumulates in various organs.^{33,34} Approximately 4500 mg / kg dose of boric acid creates toxicity in testicles in mice and rats. However, sugar compounds such as boron calcium fructoborate are practically non-toxic.²⁶ It has even been reported that calcium fructoborate has an antioxidant effect by reducing the production of intracellular reactive oxygen species.²⁹ Oxidative stress and inflammation have been reported to play a role in the pathogenesis of cadmium-induced testicular toxicity.³⁵ Therefore, it was used in our calcium fructoborate study, which has antioxidant properties.

As a result of experimental studies, it has been reported that cadmium causes changes in reproductive hormone levels, disrupts spermatogenesis, causes DNA damage, induces apoptosis.^{6,10,11} In many studies, it has been reported that oxidative stress is induced in almost all organs of the body in acute or chronic cadmium exposure. In these studies in the literature list, we have learned that cadmium causes decreased levels of the indicator related to testicular oxidation in the testicular tissue.^{4,5,7-9} In our current study, in cadmium chloride

Table 1: Effect of cadmium chloride and calcium fructoborate on 8-OHdG expression levels.

Parameters	Control	Calcium fructoborate	Cadmium chloride + calcium fructoborate	Cadmium chloride
8-OHdG	0.16±0.40 ^a	0.33±0.51 ^a	1.83±0.40 ^b	2.83±0.40 ^c
P	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$

Note: The subscript letters (a, b, c) in the same column indicate significant differences between groups ($p < 0.05$).

8-OHdG: 8-hydroxy-2-deoxyguanosine

Table 2: Effect of cadmium chloride and calcium fructoborate on testicular tissue biochemical parameters and serum testosterone levels.

Parameters	Control	Calcium fructoborate	Cadmium chloride + calcium fructoborate	Cadmium chloride
GSH (ng/L)	137.74±5.10 ^a	136.00±5.51 ^a	137.100±4.71 ^a	124.00±2.68 ^b
SOD (pg/ml)	249.47±7.50 ^a	223.87±4.70 ^b	206.10±8.53 ^c	196.35±6.91 ^d
GPx (IU/ml)	35.10±2.63 ^a	33.14±3.01 ^b	31.30±2.87 ^b	25.23±2.20 ^c
8-OHdG (ng/ml)	0.60±0.04 ^a	0.41±0.03 ^b	0.42±0.04 ^b	0.23±0.03 ^c
Testosterone (nmol/l)	2.30±0.31 ^a	14.54±1.50 ^c	7.48±0.51 ^b	1.99±0.49 ^a

Note: The subscript letters (a, b, c, d) in the same column indicate significant differences between groups ($p < 0.05$).

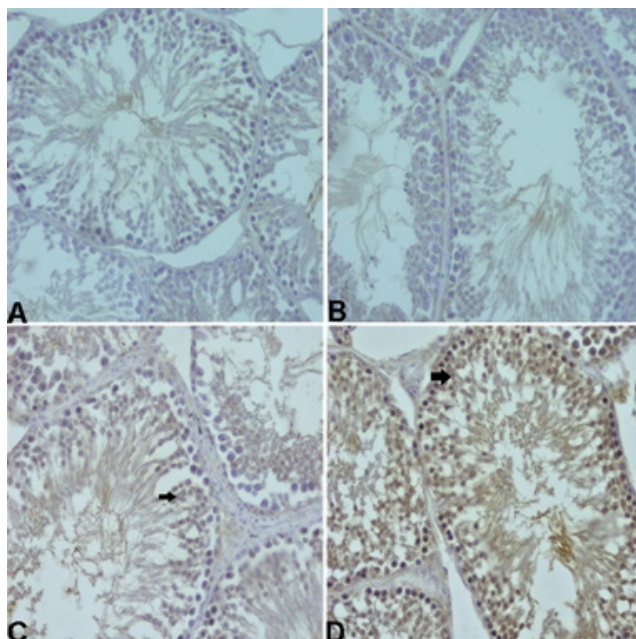


Figure 1: A (Control) and B (calcium fructoborate) group. Moderate 8-OHdG expression (arrow) in the C (cadmium chloride + calcium fructoborate) group testicle seminiferous tubules. Severe expression of 8-OHdG in the D (cadmium chloride) group testicle seminiferous tubules (arrow). Testicular-IHC.

group, SOD and GPx activity and GSH and testosterone levels were significantly lower than the control group. Our results support the previous studies. The interaction of cadmium with the metallic components of SOD may have been effective in the reduction of SOD activity. Because this can lead to inhibition of the enzyme.^{36,37} In the calcium fructoborate + cadmium chloride group, the calcium fructoborate significantly increased GSH and testosterone levels and SOD and GPx activities compared to the cadmium chloride group. In our current study, calcium fructoborate reduced the negative effects of cadmium chloride as much as possible by eliminating or minimizing free oxygen radicals and strengthening the antioxidant defense system in the testicle. We see more clearly the effect of calcium fructoborate, which we use to reduce or eliminate possible damage to the testicular tissue of cadmium compared to the group that only cadmium is administrated

In our study, we evaluated immunohistochemical 8-OHdG expression levels to detect DNA damage in testicular tissue, apart from biochemical parameters. We found that 8-OHdG expression levels were severe in the cadmium chloride group. Our findings with 8-OHdG expression levels, which are a good marker for DNA damage, support previous studies.^{6,12} It was determined that the severity of 8-OHdG expression levels decreased in the cadmium chloride + calcium fructoborate group.

This may be due to the antioxidant effect of calcium fructoborate.

Based on our findings, we can say that calcium fructoborate, a boron compound, reduces the severity of oxidative stress and DNA damage in cadmium chloride induced testicular tissue. In order to test different activities of calcium fructoborate, we can suggest new studies to be done by changing the dosage and application times of calcium fructoborate and using different animals.

CONCLUSION

Based on our findings, we can say that calcium fructoborate, a boron compound, reduces the severity of oxidative stress and DNA damage in cadmium chloride induced testicular tissue. In order to test different activities of calcium fructoborate, we can suggest new studies to be done by changing the dosage and application times of calcium fructoborate and using different animals.

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CONFLICT OF INTEREST

There is no conflict of interest for the current study.

ABBREVIATIONS

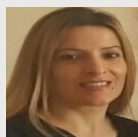
DAB: 3,3'-Diaminobenzidine; **GPx:** Glutathione peroxidase; **GSH:** Glutathione; **H₂O₂:** Hydrogen peroxide; **i.p:** Intra peritoneal; **PBS:** Phosphate buffer solution; **8-OHdG:** 8-hydroxy-2-deoxyguanosine; **SOD:** Superoxide dismutase; **µm:** Micro meter.

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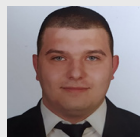
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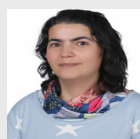
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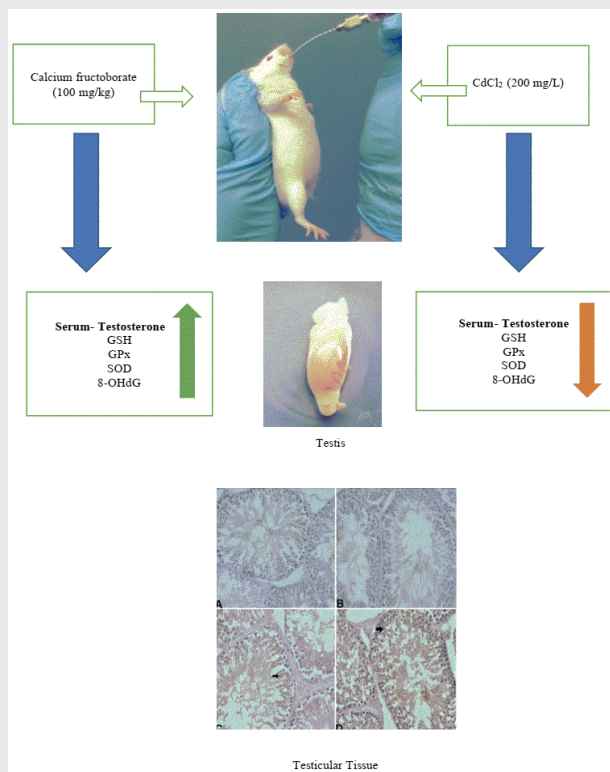


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PICTORIAL ABSTRACT



SUMMARY

In this study, it was determined that calcium fructoborate had positive effects on DNA damage and biochemical markers and serum testosterone levels in testicular tissue after application of cadmium chloride. The protective effect of calcium fructoborate was confirmed as a result of the finding listed below after daily oral gavage application in study that continued for 28 days.

1- Expression of 8-hydroxy-2-deoxyguanosine (8-OHdG) was moderate in cadmium chloride + calcium fructoborate group, while expression was severe in cadmium chloride group.

2- In the cadmium chloride group, testicular tissue glutathione (GSH) and 8-OHdG levels, superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities and serum testosterone levels were significantly lower than other groups.

The reversal of the change in the parameters listed in the calcium fructoborate group had indicated the positive strength of the present chemical.

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