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Investigating the physiological Impact of Fenugreek Extraction on Gene Expression of Bcl-2 and p53 in Cadmium-Induced Testicular Toxicity in Male Rats

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Abstract

This study examined the protective and therapeutic effects of the fenugreek (trigonella Pheenum-grace) supplementation against cadmium-inspired testicular injury in male Vistarmus. Led adult male mice were divided into four groups: control, cadmium vigor (CD), pre-propellactic methi and under the cadmium exposure (pre-FG+CD) and cadmium exposure (CD+post-FG) after medical methi. Kadmium chloride (5 mg/kg body weight) was administered for two weeks, while the Methi supplement (5% weight/weight) was given during the 8-week experimental period in the diet or during the post-exposure phase (weeks 4-8). Cadmium exposure reduced the body and testicular weight and increased oxidative stress by increasing the level of freight slide (MDA) and reduced antioxidant enzyme activities (SOD, Cat, GPX). Jean-expression analysis revealed the significant decline of anti-apoptotic BCL-2 genes and an upgrade of the Pro-APPTIC P53 gene in the cadmium-powerful group. Prophylactic method supplements showed better protective effects than medical intervention, which significantly increases cadmium -induced changes in oxidative stress parameters and gene expression profiles. These findings suggest that Methi-Supplements, especially when administered, intensely administered, can provide protection against cadmium-inspired textile damage by reducing oxidative stress and changing the apoptic routes with potential implications for male reproductive health.

Keywords: Fenugreek, Bcl-2, p53, Cadmium, SOD, CAT, GPx and MDA.

I. INTRODUCTION

Exposure to heavy metals in the environment and commercial surroundings is an important public health issue globally. Kadmium (CD), a comprehensive environmental pollutant, has been identified as a very effective reproductive toxic that accumulates in biological systems due to the extended biological half -life (Siu et al., 2009). Industrial application, cigarette smoke, contaminated food and water are the main causes of exposure to human cadmium (Genchi et al., 2020). The testes exhibit significant sensitivity to cadmium poisoning, with exposure associated with disrupted spermatogenesis, diminished testosterone synthesis, and poor male fertility (Adamkovicova et al., 2016). The mechanism responsible for cadmium -inspired testicle damage includes complex pathophysiological procedures including oxidative stress, inflammation and deregulation of the apoptic routes (Nna et al.,). Apoptosis, or programmed cell death, is necessary to maintain testicular homeostasis in the correct spermatogenesis. Excessive apoptosis inspired by pollutants can interfere with this delicate balance (Aitken & Baker, 2013). The two main regulators of the apoptotic route are BCL -2, an anti -apoptotic protein that preserves mitochondrial membrane integrity and P53, a tumor restriction protein that facilitates apoptosis in response to cell damage. Cadmium exposure appears to modify the expression of these genes, resulting in an increase in death and the testicle damage to the Kim cell (Bu at al., 2011). Traditional medical flora has increased interest in the form of possible sources of protective compounds against reproductive toxins. Fennary (Trigonela Phinum-Grakom) is a large-scale annual herb cultivated with a rich historical application in traditional medicine in different cultures. Seeds include many bioactive components such as alkaloids, flavonoids, saponins and polyphenols that have antioxidants, anti -inflammatory and hepatoprotactive activities (Yadav & Baquer, 2014). Recent research indicates that the musical may have a protective effect against many types of tissue toxicity, including liver, kidney and tested injuries (Sakr et al., 2012; Hamden et al., 2010). Although several studies have investigated the overall preventive effects of fenugreek against heavy metal poisoning, the molecular passage that can clarify its

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protective role against cadmium -inspired testicular damage is not entirely understanding. The effect of fenugreek on the apoptotic regulator on cadmium -inspired testicle damage has not been studied extensively. The purpose of this study is to assess the effect of buckshorn clover extracts on the expression of the important apoptotic regulator, especially BCL -2 and P53, under the testicular tissue of male muses during cadmium exposure. In addition, we have purposefully examined the efficiency of the buckhorn clover extracts and medical administration and correlation of change in gene expression with antioxidant parameters. Understanding these molecular routes can lead to significant insight into potential natural agents for reproductive damage caused by heavy metals.

II. Materials and Methods

Experimental Animals

Forty ripe male vistarmus (8-10 weeks old, weighed 200-250 grams) was obtained from institutional animal facilities. Under standard laboratory settings (temperature 22 ° 2 ° C, 12-hour light/dark cycle, 50-60% moisture) the animals were adjusted to polypropylene cage (five mice per cage). Mouse had a habit for a week before the test started. All methods received approval from the Institutional Animal Ethics Committee (approval number IAC/2024/05) and were carried out in accordance with international standards for the use of animals.

Plant Material and Extract Preparation

Bukhorn clover seeds were obtained from a local market, confirmed by a botanist, and a coupon sample was deposited in the institutional herbari (coupon number TFG-2024-01). The seeds were clean, dried and found in a fine powder. 500 grams of powder was extracted using a soxhlet unit for 72 hours with 70% ethanol. The extract was filtered using rotating evaporation at 45 ° C under low pressure, then lyophilized to provide a dry extract (14.2% weight/weight). The extract was evaluated according to the total phenol material, flavonoids and saponin protocols (Ahmed et al., 2018).

Experimental Design

After acclimatization, rats were randomly divided into four groups (n=10 per group):

- **Group 1 (Control)**: Received standard diet and normal saline orally for 8 weeks
- **Group 2 (Cd)**: Received standard diet for 8 weeks with cadmium chloride ($CdCl_2$) administration (5 mg/kg body weight dissolved in saline) via oral gavage daily during weeks 2-3
- **Group 3 (Pre-Fg+Cd)**: Received fenugreek-supplemented diet (5% w/w) throughout the 8-week period with $CdCl_2$ administration during weeks 2-3
- **Group 4 (Cd+Post-Fg)**: Received standard diet during weeks 1-3 with $CdCl_2$ administration during weeks 2-3, followed by fenugreek-supplemented diet (5% w/w) during weeks 4-8

Sample Collection

At the end of the experimental period, mouse was weighed and stunned by intraperitoneal administration of ketamine (80 mg/kg) and xylazine (10 mg/kg). Blood tests were acquired by heart puncture, to coon at ambient temperatures, then centrifuge at 3000 rpm for 15 minutes to separate the serum. The animals were sacrificed through the cervical dispute, and both testicles were removed, weighed and prepared for further testing. The left testes were immediately frozen in liquid nitrogen and the gene expression was preserved at -80 ° C for profiling.

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Oxidative Stress Parameters

The measure malondialdehyde (MDA) levels as a marker of lipid peroxidation using the thiobarbituric acid reactive substances (TBARS) method. Antioxidant enzyme activities including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were determined using respective commercial assay kits (Cayman Chemical, USA).

RNA Extraction and Quantitative Real-Time PCR

Tricolor reagent (USA) was used to distinguish the total RNA from the testicle tissue according to the instructions provided by the manufacturer. The concentration and purity of RNA were assessed using a nanodrop spectrophotometer (Thermo Scientific, USA). RNA was considered suitable for subsequent analysis as the ratio of A260/A280 ranged from 1.8 to 2.0. Applied Biosystems, the United States, created an inverted transcripally high capacity, used to create an initial strict DNA, complementary DNA supplemented from 2 micrograms of total RNA. A quantitative real-time PCR (QRT-PCR) analysis was performed using a SYBR Green PCR Master Mix (USA) A SYBR Green PCR Master Mix (USA) using a SYBR Green PCR system (USA). The following primer sequences were used:

- Bcl-2: Forward 5'-GGTGGTGGAGGAACTCTTCA-3', Reverse 5'-GTGACGACATCTTCTCCCAC-3'
- p53: Forward 5'-CCCAGGTCCAGATGAAGCTC-3', Reverse 5'-CTCCGTCATGTGCTGTGACT-3'
- Reference gene : Forward 5'-CACCATGTACCCAGGCATT-3', Reverse 5'-ACTTGCGGTGCACGATGGA-3'

For the polymerase chain reaction (PCR), the following conditions were used: a 10-minute denaturation step at 95°C, 15 seconds of annealing at 58°C, and 30 seconds of extension at 72°C. Relative gene expression was calculated using the 2^(-ΔΔCt) method, with β-actin as the reference gene.

Statistical Analysis

Standard deviation (SD) was used to express the data. The SPSS statistical package (version 25.0, IBM Corp., USA) was used for the statistical analysis. A one-way analysis of variance (ANOVA) was used to analyse the difference between the groups. For several comparisons, Tukey's aftershock tests were used. To assess the association between gene expression, the piercen correlation coefficient was employed. It was deemed statistically significant if the p-value was less than 0.05.

III. Results

Body and Testicular Weights

Table 1 presents the body and testicle load of FG therapy and without control and cadmium-wisdom. Cadmium exposure (CD group) led a sufficient reduction in the latest body weight ($p < 0.01$), full testicular weight ($p < 0.001$), and relative test weight ($p < 0.01$) compared to the control group. Pre-Heavisure with FG (pre-FG+CD group) partly reduced the cadmium-inspired reduction in the latest body weight ($p < 0.05$) and full testicle weight ($p < 0.01$). The CD+post-FG group demonstrated remarkable recovery after treatment with FG, which arose from a significant increase in the latest body weight ($p < 0.05$) and full testicular weight ($p < 0.01$) compared to the CD group.

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Table 1. Body and testicular weights of control and experimental rats

Parameter	Control	Cd	Pre-Fg+Cd	Cd+Post-Fg
Initial body weight (g)	222.4 ± 12.6	225.1 ± 14.2	220.8 ± 13.4	223.7 ± 11.9
Final body weight (g)	310.6 ± 18.3	265.4 ± 21.7**	293.2 ± 17.5*	284.1 ± 19.2*
Absolute testicular weight (g)	3.12 ± 0.24	2.14 ± 0.31***	2.85 ± 0.27**	2.46 ± 0.29**
Relative testicular weight (g/100g bw)	1.00 ± 0.07	0.81 ± 0.09**	0.97 ± 0.08*	0.87 ± 0.10*

Values are expressed as mean ± SD (n=10). *p<0.05, **p<0.01, ***p<0.001 compared to control group.

Oxidative Stress Parameters

According to Table 2, there is a clear difference between the CD exposure group and the control group in the context of protein concentration (7.86 ± 1.12 nmol/mg protein against 2.34 ± 0.37 nmol/mg protein, p <0.001). The CD group showed much less activity of antioxidantenzymes compared to the control group (p <0.001), including superxide dismanton (Sods), Katalas (Cat) and glutation peroxide (GPX). The pre-FG+CD group, which was present with FG, significantly reduced CD-generated oxidative damage. In the pre-FG+CD group, the MDA level was the CD group (3.65 ± 0.58 nmol/mg protein against 7.86 ± 1.12 nmol/mg protein, p <0.05 against control; p <0.01 against CDs) much lower than. In addition, the pre-FG+CD group maintained the general level of SOD, CAT and GPX activities, indicating that FG Baketlefall has a protective effect against CD (p <0.01). Malondialdehyde (5.12 OL 0.87 nmol/mg protein, p <0.01 against control; p <0.05 against CD) and the CD group's relative antioxidant enzymatic activities fell in both CD+post-FG group after both FG. While the FG penalty had some protective effects, they were not as important.

Table 2. Testicular oxidative stress parameters in control and experimental rats

Parameter	Control	Cd	Pre-Fg+Cd	Cd+Post-Fg
MDA (nmol/mg protein)	2.34 ± 0.37	7.86 ± 1.12***	3.65 ± 0.58*##	5.12 ± 0.87**#
SOD (U/mg protein)	16.85 ± 2.13	7.23 ± 1.45***	13.76 ± 1.82*##	10.54 ± 1.64**#
CAT (U/mg protein)	42.37 ± 5.68	18.92 ± 3.74***	35.29 ± 4.85*##	27.63 ± 4.12**#
GPx (U/mg protein)	24.58 ± 3.12	10.36 ± 2.24***	20.14 ± 2.87*##	15.72 ± 2.46**#

Values are expressed as mean ± SD (n=10). *p<0.05, **p<0.01, ***p<0.001 compared to control group; #p<0.05, ##p<0.01 compared to Cd group.

Gene Expression Analysis

Cadmium exposure and fenugreek therapy affected the expression of BCL-2 and P53 genes in testicular tissue. Compared to the control group resulted in anti-apoptotic genes BCL-2 (0.28 ± 0.06-fold, p <0.001) and pro-apoptotic gene P53 (4.65 ± 0.72 fold, group resulted in anti-apoptotic genes. Propilactic treatment of fenugreek clearly reduced these changes, resulting in BCL-2 expression to 0.89 ± 0.12 times control (p <0.01 against CD group) and P53 exposure to 1.73 ± 0.31 fold (p <0.01 against CD group). Therapeutic treatment of fenugreek performed moderate growth in gene expression profiles, with BCL-2 levels 0.52 ± 0.09 fold control (compared to P <0.05 CD group) and P53 levels 2.86 ± 0.47-fold control (compared to P <0.05 CD group).

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Table 3. Relative gene expression of Bcl-2 and p53 in testicular tissue of control and experimental rats

Gene	Control	Cd	Pre-Fg+Cd	Cd+Post-Fg
Bcl-2 (fold change)	1.00 ± 0.11	0.28 ± 0.06***	0.89 ± 0.12^##^	0.52 ± 0.09**^#^
p53 (fold change)	1.00 ± 0.14	4.65 ± 0.72***	1.73 ± 0.31**^##^	2.86 ± 0.47***^#^

Values are expressed as mean ± SD (n=10). Gene expression levels are presented as fold change relative to control group. *p<0.05, **p<0.01, ***p<0.001 compared to control group; ^#^p<0.05, ^##^p<0.01 compared to Cd group.

IV. Discussion

This study examined the possible preventive and therapeutic effects of fenugreek extract against cadmium-induced testicular damage in male rats, concentrating on the modification of critical apoptotic regulatory genes. Our data indicate that cadmium exposure markedly disrupts testicular function, as shown by diminished testicular weight and altered gene expression. Significantly, fenugreek supplementation, especially when given prophylactically, greatly reduced these negative effects. The noted decrease in body and testicular weights after cadmium exposure corresponds with earlier studies (Alkhedaide et al., 2016; Jahan et al., 2014) and likely indicates the overall systemic toxicity of cadmium, together with its particular harmful effects on testicular tissue. Fenugreek's capacity to mitigate weight loss indicates a comprehensive preventive impact against cadmium toxicity, likely due to its various bioactive components such as flavonoids, alkaloids, and saponins (Yadav & Baquer, 2014). Oxidative stress is a recognised mechanism of cadmium-induced testicular damage (Nna et al., 2017). Our work demonstrated that cadmium exposure resulted in heightened lipid peroxidation (increased MDA levels) and diminished activity of antioxidant enzymes (SOD, CAT, and GPx), signifying a disturbance in the oxidant-antioxidant equilibrium. Fenugreek supplementation, particularly when given preventively, markedly improved these alterations, indicating that the antioxidant characteristics of fenugreek play a crucial role in its protective actions against cadmium-induced testicular injury.

The most innovative and important discovery of our research pertains to the impact of cadmium and fenugreek on the expression of critical apoptotic regulatory genes in testicular tissue. Apoptosis is essential for normal spermatogenesis but may be aberrantly induced by environmental toxicants (Aitken & Baker, 2013). Our findings indicate that cadmium exposure markedly decreases the expression of the anti-apoptotic gene Bcl-2 while increasing the expression of the pro-apoptotic gene p53, hence establishing a pro-apoptotic milieu in testicular tissue. These results correspond with those of Bu et al. (2011) and Agarwal et al. (2012), who documented analogous changes in apoptotic gene expression after to cadmium exposure. Significantly, fenugreek supplementation, especially when given prophylactically, markedly normalised these gene expression patterns, indicating a biological mechanism for its protective properties. Fenugreek's capacity to affect Bcl-2 and p53 expression may be ascribed to several bioactive components, such as flavonoids and polyphenols, which have demonstrated the ability to regulate apoptotic pathways in diverse tissues (Yadav & Baquer, 2014; Sakr et al., 2012).

Prophylactic administration of fenugreek consistently offered superior protection against cadmium-induced testicular damage compared to therapeutic therapy. This observation indicates that the preventive effects of fenugreek may be more pronounced when implemented before hazardous exposure, likely by augmenting cellular defence mechanisms and mitigating the early damage from cadmium exposure.

Conclusion

Cadmium exposure induces substantial testicular damage in male Wistar rats, resulting in reduced testicular weight, heightened oxidative stress, and modified expression of apoptosis-related genes. Fenugreek supplementation markedly mitigates this damage, with prophylactic treatment demonstrating superior protective effects compared to therapeutic intervention. Fenugreek's protective mechanism encompasses the

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reduction of oxidative stress, enhancement of antioxidant enzyme activities, and modulation of apoptotic pathway genes. Future study should concentrate on finding the bioactive constituents and molecular mechanisms, as well as evaluating their translational potential for men exposed to cadmium.

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