ORIGINAL ARTICLE

The Ameliorative Effect of Ginger on Cadmium Induced Histological Changes in Testes of Albino Rats

Naseer ud Din Sheikh[1], Rehana Rana[2], Shabana Ali[3], Hira Waqas Cheema[4], Huma Beenish[5], Arsalan Manzoor Mughal[6]

ABSTRACT

Objective: To study the ameliorative effect of ginger against histological changes induced by cadmium in testes of albino rats.

Study Design: Randomized control trial.

Place and Duration of Study: The research was conducted at animal house, National Institute of Health (NIH), Islamabad. The research was carried out for one year of duration from 1st November 2017 to 28 November 2018.

Material and Methods: Forty-five male adult albino rats were included via simple random sampling technique. Using balloting method, each subject was randomly allocated into one of the three groups. Distilled water was orally given to rats of control group I. The rats of group II were provided with cadmium chloride while group III received cadmium as well as ginger in selected doses orally for 28 days. Dissection was done after 28 days and testes were observed for histological changes.

Results: The use of ginger ameliorates the gross and microscopic changes induced by cadmium in testes of Albino rats of group III as compared to group II rats, which received only cadmium**.** Among microscopic qualitative parameters, infiltration, congestion and necrosis were markedly reduced by use of ginger in group III rats.

Conclusion: The use of ginger improves the histological changes induced by cadmium in testes of albino rats.

Key Words: *Cadmium Chloride, Ginger, Rats, Testis.*

Introduction

Testis is an important sexual organ which enables spermatogenesis and secretion of male sex hormones. This function is highly necessary for continuity of life. Among mammals, when considering a rat, the structure of rat testis is comparable to humans and has been a source of comparative study. Its testis measures 1x2x1.5cm.[1] Environmental toxins affect the function of testis; cadmium is one of them. It acts as catalyst in the production of reactive oxygen species; decreasing protein bound sulfhydryl groups and glutathione, thus increasing lipid peroxidation and oxidative stress.[2] However, the tissue damage caused by cadmium may be ameliorated by the use of certain herbal agents acting as antioxidants like ascorbic acid, zinc, garlic, turmeric, cinnamon and ginger.[3] Ginger has beneficial effects on cardiovascular system, reproductive system and gastrointestinal system.[4] The concentration of volatile oils present in ginger is 1% to 3%. The main constituents are sesquiterpenes, beta-bisabolene and zingiberene. Zingiberol and zingiberenol are other; all play an active role as anti-oxidants, anti-microbials and anti-inflammatory when introduced in mammals. Hence, ginger has been used in multiple clinical trials to check its efficacy as a protective agent against harmful substances.[5]

Cadmium is one of the important components used in rechargeable nickel- cadmium batteries, aircraft industry and television picture tubes. Hence, there's a certain degree of probable exposure to the personnel working in one of these industries.[6] Besides acute and chronic kidney damage, cadmium causes carcinoma of lung, kidney and prostate. Cadmium has acute and chronic effects on various organs of body including lungs, kidney, prostate and

[1,6]*Department of Anatomy*
Rawalpindi Medical University, Rawalpindi
[2,3,4,5] *Department of Anatomy*
Islamic International Medical College
Riphah International University, Islamabad

Correspondence:
Dr. Naseer ud Din Sheikh
Senior Lecturer
Department of Anatomy
Rawalpindi Medical University, Rawalpindi
E-mail: naseerrmc@gmail.com

Funding Source: Nil; Conflict of Interest: NIL
Received: September05, 2019; Revised: October 12, 2020
Accepted: October 13, 2020

bones.[7] Ginger has been proved to be an effective antioxidant for cadmium induced damage on organs such as liver[8] and kidenys.[9] However, there is minimal evidence in literature, specifically the local research base, which proves the advantages of ginger against cadmium in the reproductive system. Hence, the aim of this study was to assess the protective role of ginger against cadmium induced toxicity in testicular cells on a gross and microscopic level.

Material and Methods

The study was carried under supervision of animal house at National Institute of Health (NIH), Islamabad. The study was conducted from 1st November 2017 to 28th November 2018. A total of 45 male rats were procured by employing simple random sampling technique with balloting method[10] and were kept in a controlled standard living environment at the animal house of NIH. The study design was Randomized Controlled Trial. Prior to the initiation of the study, the research was approved by Ethical Review Committee (ERC) of Islamic International Medical College (IIMC).

Eight weeks old healthy male albino rats of Sprague Dawley strain weighing 140-220 gm were selected for the study, while those with any congenital or pathological abnormalities were excluded.

The rats were randomly allocated into 3 groups of 15 rats each and were kept in cages, in a controlled standard living environment in a well-ventilated room with cycles of 12 hour day and night, and temperature maintained between 20 to 26 °C. Group I (control) rats received distilled water as drinking water for 28 days. Group II rats received orally a solution of cadmium (03mg/kg/day) as cadmium chloride for 28 days. Group III rats consumed orally a solution of cadmium (03mg/kg/day) as cadmium chloride along with ginger 500mg/kg/day for 28 days. All rats were euthanized and dissected after 28 days. Testes were explored and taken out of scrotum. The tissues were fixed, embedded in paraffin blocks and stained with eosin and haematoxyline. The slides were examined in detail under 10X and 40X power of light microscope. Gross features including color, appearance, texture and weight were noted. Microscopic examination was performed to observe parameters including the presence of congestion of blood vessels in stroma, infiltration of inflammatory cells in stroma and change in epithelial height in seminiferous tubules in micrometers.

Data was entered and analyzed using Statistical Package for Social Sciences (SPSS) version 22. Mean and standard error were calculated for the quantitative variables. Categorical variables were represented by frequency and percentage. Chi square test was applied for comparison of qualitative variables. Data was tested for normality with Kolmogorov-Smirnov and Shapiro-Wilk tests. One way analysis of variance (ANOVA) was applied for the mean comparison of quantitative variables between control group I and experimental groups II and III.Post hoc Tukey's test was applied for multiple comparisons of these three groups. The results were analyzed and considered significant with P value less than 0.05.

Results

The color of testes ranged from pink, yellow and light pink in group I, II and III respectively. Group I showed normal appearance while group II and group III showed shrunken and swollen appearances respectively. The texture of the testes was soft in case of group I while group II and group III were having hard textures. (Table I) Regarding weight of the testes, in group II (Mean=1.96, SEM=0.074) it was reduced compared to group I (Mean=2.43, SEM=0.105) and again increased in group III (Mean=2.30, SEM=0.087) which received ginger ($p<0.001$). Table II shows the group wise multiple comparisons.

All blood vessels were normal and congestion (Figure 1a) was absent in all rats of group I while testes of rats of group II were congested. In group III, 47% of rats showed congestion of blood vessels while 53% showed normal blood vessels. There was significant

Table I: Color, Appearance and Texture of the Three Groups

	Group I	Group II	Group III	P-value
Colour	Pink (100%)	Yellow (100%)	Light Pink (100%)	<0.001
Appearance	Normal (100%)	Shruken (73.3%) Normal (26.7%)	Swollen (60%) Normal (40%)	<0.001
Texture	Soft (100%)	Hard (73.3%) Soft (26.7%)	Hard (46.7%) Soft (53.3%)	<0.001

Table II: Multiple Comparison of Weight (G) of Testis among Control and Experimental Groups of Albino Rats

Groups	Mean Difference	*p* value
I vs II	3.26	0.002
I vs III	1.24	0.349
II vs III	2.03	0.068

difference ($p<0.001$) between presence of congestion of blood vessels in all groups (Table III). Inflammatory cells were absent in all testes of rats of group I while present among all rats of group II. In group III, 53% of testes showed infiltration of inflammatory cells while 47% rat's testes showed no infiltration. Results were significant in all groups. ($p<0.001$) (Figure 1).All the testes of group I showed mean epithelial height (58.500µm) of seminiferous tubules. In group II the mean epithelial height of seminiferous tubules was 44.833 µm. In group III the mean epithelial height of seminiferous tubule was 54.000µm. significant difference was found in mean epithelial height in all groups.($p<0.0001$) (Table IV) while multiple groups comparison showed significant difference among all groups.

Table III: Group Wise Distribution of Congestion of Blood Vessels and Focal Necrosis among Control and Experimental Groups of Albino Rats

	Presence of Congestion of Blood Vessels n=15			Prensence of Inflamatory Cells in the Stroma n=15		
Groups	Present (%)	Absent (%)	*P*value	Present (%)	Absent (%)	*p* value
Group I	0 (0 %)	15 (100 %)	<0.001*	0 (0 %)	15 (100 %)	<0.001*
Group II	15 (100 %)	0 (0 %)		15 (100 %)	0 (0 %)	
Group III	7 (46.7%)	8 (53.3%)		7 (46.7%)	8 (53.3%)	

Table IV: Group Wise Distribution of Height of Seminiferous Epithelium among Control and Experimental Groups of Albino Rat's *p* ≤0.001

	Height of Seminiferous Epithelium in µm n=15				
Groups	Mean	SEM	Multiple comparisons		
I	58.50	1.39	I vs II	13.66	<0.001
II	44.83	1.30	I vs III	4.50	0.053
III	54.00	1.26	II vs III	9.16	<0.001
p value	<0.001				

Discussion

Exposure to cadmium is inevitable[11] and the subsequent deleterious effects on the testes are highly likely. Being an important organ for spermatogenesis, the testes also play an important role in production of male sexual hormones and male growth and development.[12] However, heavy metals such as arsenic, cadmium and nickel bring molecular changes in the structure of testicular cells which lead to decline in the functional ability of the organ.[13] However, as proven with the current study these effects can be reduced, if not prevented, by maintaining levels of herbs such as ginger. Hence, testicular viability, potency and function can be preserved. Other materials used in various studies have been proven to be potent in delaying testicular toxicity such as quercetin, grape seed extracts, zinc and magnesium.[14,16] Ginger, on the other hand, has been proved to have protective role by antagonizing reactive oxygen species.[17]

Congestion of vessels was seen as an effect of cadmium in our study, it was 100% in group II, but with the use of ginger it reduced down to 46.7%. In the study conducted by de Souza, cadmium was given to three different groups at different doses of 0.1 and 1.2mg per kg for 8 weeks, they showed congestion in blood vessels.[18]Manca D also conducted a study in which cadmium chloride was given in doses of 1mg and 10mg for 45 and 90 days respectively and testis showed congestion in blood vessels.[19]In a study by El-Sharkawya, when ginger extract was given, then congestion in blood vessels was remarkably decreased in testes of rats.[20]

The works of Schlaepfer[21] and Mouro[22]showed that seminiferous epithelium damage by cadmium results from hypoxia due to alteration in intratesticular blood flow. This effect leads to accumulation of inflammatory cells within the testis.[23]These findings were further validated with the current study in which group II had the highest degree of inflammatory cells, but since group III had reduced inflammatory cells, it proves the protective role of ginger in reducing the cadmium induced inflammatory response.

The study can be further extended by investigating the dose dependent effects of Cadmium. Moreover, the effects of route of administration and duration of use can be studied as well. As immunological effects

of Cadmium have been suggested at cellular level, so cyto-immunological analysis may be included.[23] Furthermore, the changes at the level of genetic expression may be considered. This would give a deeper view on the mechanisms behind the protective effects of ginger and may be extended towards other chronic autoimmune and inflammatory diseases.

Conclusion

Cadmium exposure causes gross and histological changes in the testes of albino rats. The use of ginger significantly reduces the development of the changes; effectively providing complete protection to at least half of the exposed population. Further larger scale and long-term trials are recommended to have an insight on exact mechanisms.

REFERENCES

1. Hegazy AA, Ahmad MM, Aziz JA, AbdAlmotaleb NA. Postnatal Changes in the Structure of Rat Testis. Cell Res. 2019; 3(2):1-9.
2. Marettová E, Maretta M, Legáth J. Toxic effects of cadmium on testis of birds and mammals: a review. Animal reproduction science. 2015; 155:1-10.
3. Khafaga AF, Abd El-Hack ME, Taha AE, Elnesr SS, Alagawany M. The potential modulatory role of herbal additives against Cd toxicity in human, animal, and poultry: A review. Environmental Science and Pollution Research. 2019; 26(5):4588-604.
4. Banihani SA. Ginger and testosterone. Biomolecules. 2018; 8(4):119.
5. Jeena K, Liju VB, Kuttan R. Antioxidant, anti-inflammatory and antinociceptive activities of essential oil from ginger. Indian J PhysiolPharmacol. 2013; 57(1):51-62.
6. Turner A. Cadmium pigments in consumer products and their health risks. Science of the Total Environment. 2019; 657:1409-18.
7. Đukić-Ćosić D, Baralić K, Javorac D, Djordjevic AB, Bulat Z. An overview of molecular mechanisms in cadmium toxicity. Current Opinion in Toxicology. 2020; 19:56-62.
8. Baiomy AA, Mansour AA. Genetic and histopathological responses to cadmium toxicity in rabbit's kidney and liver: protection by ginger (Zingiberofficinale). Biological trace element research. 2016; 170(2):320-9.
9. Gabr SA, Alghadir AH, Ghoniem GA. Biological activities of ginger against cadmium-induced renal toxicity. Saudi Journal of Biological Sciences. 2019; 26(2):382-9.
10. Lammers WJ, Babbie E. Sampling Techniques. Fundamentals of Behavioral Research Textbook. 2005:1-23.
11. Watanabe Y, Nogawa K, Nishijo M, Sakurai M, Ishizaki M, Morikawa Y, et al. Relationship between cancer mortality and environmental cadmium exposure in the general Japanese population in cadmium non-polluted areas. International Journal of Hygiene and Environmental Health. 2020 Jan 1; 223(1):65-70.
12. Chen Q, Deng T, Han D. Testicular immune regulation and spermatogenesis. Semin Cell Dev Biol. 2016; 59:157-65.
13. Rinaldi M, Micali A, Marini H, Adamo EB, Puzzolo D, Pisani A, et al. Cadmium, organ toxicity and therapeutic approaches: a review on brain, kidney and testis damage. Current medicinal chemistry. 2017; 24(35):3879-93.
14. Nna VU, Ujah GA, Mohamed M, Etim KB, Igba BO, Augustine ER, et al. Cadmium chloride–induced testicular toxicity in male wistar rats; prophylactic effect of quercetin, and assessment of testicular recovery following cadmium chloride withdrawal. Biomedicine & Pharmacotherapy. 2017; 94:109-23.
15. Alkhedaide A, Alshehri ZS, Sabry A, Abdel-Ghaffar T, Soliman MM, Attia H. Protective effect of grape seed extract against cadmium-induced testicular dysfunction. Molecular Medicine Reports. 2016; 13(4):3101-9.
16. Li R, Luo X, Li L, Peng Q, Yang Y, Zhao L, et al. The protective effects of melatonin against oxidative stress and inflammation induced by acute cadmium exposure in mice testis. Biological trace element research. 2016; 170(1):152-64.
17. Morvaridzadeh M, Fazelian S, Agah S, Khazdouz M, Rahimlou M, Agh F, et al. Effect of ginger (Zingiberofficinale) on inflammatory markers: A systematic review and meta-analysis of randomized controlled trials. Cytokine. 2020; 135:155-224.
18. de Souza Predes F, Diamante MA, Dolder H. Testis response to low doses of cadmium in Wistar rats. International journal of experimental pathology. 2010; 91(2):125-31.
19. Manca D, Ricard AC, Trottier B, Chevalier G. Studies on lipid peroxidation in rat tissues following administration of low and moderate doses of cadmium chloride. Toxicology. 1991; 67(3):303-23.
20. El-Sharkawya EE, El-Nisr BN. Lactational cadmium exposure induced alterations in the hematological indices and oxidative status in brain, liver and testes of rat pups. Scientific Journal of Veterinary Advances. 2012; 1(3):70-81.
21. SchlaepferWW.Sequential study of endothelial changes in acute cadmium intoxication.Laboratory investigation; a journal of technical methods and pathology. 1971; 25(6):556-64.
22. Mouro VG, Siman VA, da Silva J, Dias FC, Damasceno EM, do Carmo Cupertino M, et al. Cadmium-induced testicular toxicity in mice: subacute and subchronic route-dependent effects. Biological Trace Element Research. 2020; 193(2):466-82.
23. Deng B, Pakhomov OV, Bozhok GA. Long-term effects of acute cadmium exposure on testis immune privilege. RegulMechBiosyst. 2020; 11(2):180-5.