Original Article

Effect of Zinc Supplementation on Heat Induced Gross Changes in Testes of Adult Mice

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ABSTRACT

Objective: To compare the effects of Zinc supplementation on heat induced gross changes in testes of adult mice with placebo.

Study Design: Experimental study

Place and Duration of Study: This study was conducted at the Anatomy Department ANMC Islamabad and Anatomy Department Sialkot Medical College, Sialkot in collaboration with (N.I.H) Islamabad from January 2016 to May 2017.

Materials and Methods: Fifty-four adult male BALB/c mice were divided into two main groups. Control group of 18 mice and Experimental group of 36 mice. Control group was further divided into Normal control group of 9 mice and Experimental control group of 9 mice. Normal control group was given neither heat nor given any drug or placebo and were sacrificed on day 0 of the experiment. Experimental control group was given limited body heat and then sacrificed after 48 hours of heat exposure. Experimental group was further divided into two groups, Group C and Group D. Group C was further subdivided into C1, C2, C3.C1 was given limited body heat then placebo (Normal Saline) for 15 days, C2 for 30 days and C3 for 60 days. Group D was further subdivided into subgroups D1, D2, and D3. D1 was given limited body heat then $ZnSO_4$ for 15 days, D2 for 30 days and D3 for 60 days. 6 mice from group C were sacrificed on 15, 30 and 60 days each. 6 mice from group D were sacrificed on 15, 30 and 60 days each. Testes were dissected out for gross examination.

Results: Consistency was firm after heat exposure, weight was reduced but it regained after drug treatment.

Conclusion: Zinc supplementation completely reverses the gross changes produced by limited heat in testes of adult mice.

Key Words: Normal Saline, placebo, Testes, Zinc supplementation, $ZnSO_4$

Citation of article: Ahmad M, Hassan B, Abbass S, Butt NI, Sabir M, Ali. Effect of Zinc Supplementation on Heat Induced Gross Changes in Testes of Adult Mice. Med Forum 2020;31(11): 72-75.

INTRODUCTION

The testes are paired organs that are present in scrotum in the lower part of anterior abdominal wall. Each testis is divided into lobules by incomplete connective tissue septa that project from capsule[1]. Each lobule of testis consists of seminiferous tubules which are highly convoluted. These seminiferous tubules contain Spermatogenic cells and sertoli cells.

The Spermatogenic cells are derived from primordial germ cells which originate in yolk sac during early development of testis[2].Sperms are produced in these tubules. In the interstitial spaces between seminiferous tubules are present Leydig cells, these Leydig cells secrete testosterone under effect of anterior pituitary gland[3].

In humans testis develop in abdominal region and at 26th week of gestation descend into scrotum. During descent from abdomen to scrotum testes carry with them the blood vessels, nerves, lymphatics, ductus deferens and an extension of abdominal peritoneum called tunica vaginalis[2]. Within scrotum temperature is 2c to 8c low than core body temperature[4]. This lower body temperature is essential for spermatogenesis but low temperature is not required for steroidogenesis[5].

Thermoregulation in testis is very important as slight increase in temperature can cause disruption of spermatogenesis and ultimately problems with fertility. With the exception of elephants and whales most mammals have a scrotum and scrotal temperature is always lower than that of abdomen[6]. The tone of dartos muscle in scrotum and countercurrent heat exchange between testicular artery & pampiniform plexus play their role in maintaining testicular temperature at low[7].

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Received: April, 2020
Accepted: August, 2020
Printed: November, 2020

Occupational exposure to high temperatures in men impairs testicular function[8]. Depending on period of arrest it leads to partial or complete Spermatogenic arrest in these workers. After heat stress increased metabolism in testes may not be met with sufficient increase in blood flow and testes become hypoxic. Hypoxia then results in cell cycle arrest and apoptosis[9].
Although testes require Zinc for spermatogenesis, sperm viability and motility, and its deficiency compromise fertility in men[10]. Intracellular zinc functions to improve sperm oxygen uptake, sperm capacitation and in vitro fertilizing ability of spermatozoa, yet insufficient work has been done to see the reversal effects of Zinc on heat induced changes on testes in our country. Therefore, current study has been done to observe the effects of zinc supplementation on heat induced gross changes in testes of adult mice.

MATERIALS AND METHODS

This experimental study was performed at Anatomy Department Al-Nafees Medical College and Hospital Islamabad, in collaboration with National Institute of Health (N.I.H) Islamabad from January 2016 to May 2017 after approval from IRBC (letter number F.2/IUIC-ANMC/EC-86/2015).54 adult male BALB/c mice, with age of 50-100 days were procured from NIH Islamabad. The mice were segregated by simple random selection and then divided into control and experimental groups.
The mice of group A (Normal Control Group) were neither given heat nor were they given any drug or placebo. The mice were sacrificed by cervical dislocation[11] at the start of experiment on day 0. Testes were dissected out, gross features were noted.
The mice of group B (Experimental Control Group) were anesthetized by giving ketamine 100mg/kg and xylazine 10mg/kg[12]**.** Then mice of this group were exposed to limited heat by submerging the hind legs, scrotal area and tail in a water bath which was maintained at temperature 43˚Cfor 15 minutes on the day 0 of the experiment. They were sacrificed by cervical dislocation [11] 48 hours after heat exposure. Testes were dissected out, gross features were noted.
The mice of group C were anesthetized by giving ketamine 100mg/kg and xylazine 10mg/kg[12]. Then mice of this group were exposed to limited heat by submerging the hind legs, scrotal area and tail in a water bath which was maintained at temperature 43˚C on the day 0 of the experiment. Then they were divided into subgroups C1, C2 and C3. Mice of subgroup C1 were given 0.2ml orally 0.9%NaCl for 15 days. Mice of subgroup C2 were given 0.2ml orally 0.9%NaCl for 30 days, Mice of subgroup C3 were given0.2ml orally 0.9%NaCl for 60 days.
The mice of group D were anesthetized by giving ketamine 100mg/kg and xylazine 10mg/kg[12]**.** Then mice of this group were exposed to limited heat for 15 minutes by submerging the hind legs, scrotal area and tail in a water bath which was maintained at temperature 43˚C on the day 0 of the experiment. Then they were divided into subgroups D1, D2 and D3. Mice of subgroup D1 were given $ZnSO_4$ orally for 15 days, Mice of subgroup D2 were given $ZnSO_4$ orally for 30 days. Mice of subgroup D3 were given $ZnSO_4$ orally for 60 days, testes were dissected out, gross features were noted., consistency was noted using gloved hands and weight was measured using an electronic weight machine. %ages of mice were calculated for consistency, while weight was measured in mg for testes, mean ±SD were calculated.
Data Analysis: Data were analyzed using SPSS version 20. Student's t-test was applied and results with p values≤0.05 were taken as statistically significant.

RESULTS

Consistency of testes: Consistency was firm in group B (100%), in subgroup C1 83.33% was soft and 16.67% was firm, subgroup C2 83.33% was soft and 16.67% was firm, subgroup C3 100% was soft. In subgroup D1 83.33% was soft and 16.67% was firm, in subgroups D2 and D3 consistency was soft 100% which shows that limited body heat changed the consistency of testes from soft to firm (Group B). After placebo (Normal Saline) given consistency returned to soft after 60 days (group C) and after $ZnSO_4$ treatment consistency of testes returned to soft form in 15 to 30 days.
Weight of testes: On comparison of weight of experimental control group B with subgroups of group C and D, statistically significant results were achieved. Limited body heat decreased the weight of testes (Group B). by giving placebo (Normal Saline) weight of testes returned towards normal in 60 days (Group C) and by giving $ZnSO_4$ the weight of testes returned to normal in 15 days.

Table No.1: Comparison of Mean Weight of Testes in mg ± SD

Group	Mean weight in mg ± SD	p-Value
A	0.2133±0.13	0.01*
B	0.210±0.01	
B	0.210±0.01	0.01*
C1	0.196±0.13	
B	0.210±0.01	0.03*
C2	0.196±0.13	
B	0.210±0.01	0.02*
C3	0.211±0.01	
B	0.210±0.01	0.04*
D1	0.211±0.01	
B	0.210±0.01	0.03*
D2	0.212±0.01	
B	0.210±0.01	0.02*
D3	0.2133±0.01	

Table No.2: Comparison of Consistency of Testes in Control and Experimental Group

Group	Consistency			
	Soft	Firm	Firm to Hard	Hard
A	18 / 18 = 100%	0 / 18	0 / 18	0 / 18
B	0 / 18	18 / 18 = 100%	0 / 18	0 / 18
C1	10 / 12 = 83.33%	2 / 12 = 16.67%	0 / 12	0 / 12
C2	10 / 12 = 83.33%	2 / 12 = 16.67%	0 / 12	0 / 12
C3	12 / 12 = 100%	0 / 12	0 / 12	0 / 12
D1	10 / 12 = 83.33%	2 / 12 = 16.67%	0 / 12	0 / 12
D2	12 / 12 = 100%	0 / 12	0 / 12	0 / 12
D3	12 / 12 = 100%	0 / 12	0 / 12	0 / 12

DISCUSSION

This study is of experimental type. This study highlights the significance of zinc supplementation for treatment of infertility especially in subjects working in extremes of temperature. Out of various techniques used in past for germ cell apoptosis, we used technique of submerging hind legs, tail and scrotal areas of mice in a water bath maintained at 43˚C for 15 minutes after anesthetizing animals[13]. Zinc has its well defined role in spermatogenesis, sperm viability and motility yet sufficient work was not done in our country to see the effects of zinc on heat induced changes in testes of mice. Therefore, current study has been done to see effects of zinc supplementation on heat induced changes in testes of mice. Our study shows that limited body heat to testes reduces weight. A similar study done by Kanter M. et al in 2013 showed decrease in weight of rat testes by 40% after local heat of 43c for 30 minutes[14]. Another study done by Mura M. et al in 2002 showed decrease in testicular weight by 50-60% after 14 days of scrotal heat[15]. Still another study done in 2008 by Paul C. et al showed reduction in weight of testes by 40% and 60% after 7 and 14 days post heat exposure to testes respectively[16]. The weight of testes is reduced after exposure to heat due to increase in loss of germ cells[17].

In a study done on rat testes by Yetertopcu et al. in 2009 Carnitine was used as antioxidant after apoptosis was done by irradiation. At 21, 44 and 70 days post irradiation the rat testes showed significant increase in weight as compared to controls[18].Carnitine has antioxidative activity and it protects cellular DNA and membranes against free radical injury[19].

Future recommendations for our study are that electron microscopic studies should be done on testes of mice after limited body heat. In this way the cellular changes can be evaluated for decrease in weight of testes and change of consistency of testes to firm after heat exposure. And after treatment with zinc how these cellular changes are reversed so that weight of testes increases back to normal weight of testes and consistency of testes changes back to soft after zinc treatment.

CONCLUSION

Our study showed that zinc supplementation completely reversed the heat induced gross changes in testes of adult mice and on comparing its effect with placebo, zinc supplementation performs its function more rapidly and early.

Author's Contribution:

Concept & Design of Study:	Mushtaq Ahmad
Drafting:	Bilal Hassan, Saif Abbass
Data Analysis:	Nauman Idris Butt, M Sabir, Liaqat Ali
Revisiting Critically:	Mushtaq Ahmad, Bilal Hassan
Final Approval of version:	Mushtaq Ahmad

Conflict of Interest: The study has no conflict of interest to declare by any author.

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