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Original Research

Effect of Zinc Supplementation on Biometry of Testis in Wistar Rats

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Abstract

The study was conducted on 72 weaned Wistar male rats for 8 weeks from 4 to 12 weeks of age and they were divided into 3 groups as control: rats fed diet without zinc supplementation, T1 and T2: rats fed diet containing zinc sulphate @ 50mg and 100mg/kg body weight/day, respectively. After induction of proper anaesthesia, the rats were sacrificed and testes were collected from each rat of all the 3 groups on 6, 8, 10 and 12 weeks of experiment and tissue pieces were collected. The length, width, thickness and weight of testes were recorded. In the present study it was observed that in zinc-supplemented rats, the biometrical parameters of epididymis were found higher than control group. Biometrical studies revealed that all the measured dimensions (length, width, thickness, weight and volume) showed increasing trend with progression of age. In zinc-supplemented rats, the biometrical parameters i.e., length, width, thickness, weight and volume of testes were found higher when compared to the control group. The study revealed increased T1 values as compared to T2 were found in the later phase of zinc supplementation (mainly 10 and 12 weeks of zinc supplementation).

Keywords: Biometry, Testis, Wistar Rats, Zinc Supplementation

Introduction

Relatively few studies have been conducted on male animal responses to supplementation of important minerals at their critical phase of growth and reproduction. Earlier studies on zinc substantiate that it is directly involved in anatomical development and normal function of male reproductive organs (Devi *et al*., 2011). Testes have a strict zinc (Zn) requirement and severe zinc deficiency compromises spermatogenesis, sperm viability, motility and fertility in human beings (Croxford *et al*., 2011). Zinc deficient mice included delayed sexual maturation, testicular disruption, impaired spermatogenesis and infertility (Dissanayake *et al*., 2004). Deficiency of zinc in diet might lead to delay in testicular development (Underwood and Somer, 1977). It was felt that a scientific study on the growth and functional responsiveness of male genital organs in Wistar rat to zinc supplementation at their active phase of growth would yield valuable scientific information regarding possible role of dietary zinc in regulating the initiation of germ cell multiplication in the seminiferous tubules and optimizing suitable bio-environment in the epididymis for maturation of germ cells. Therefore, the present study was undertaken to elucidate the effect of zinc supplementation in two different doses on testicular biometry in Wistar rats during growth period.

Materials and Methods

The study was conducted in 72 weaned Wistar male rats for a period of 8 weeks from 4 to 12 weeks of age. The experimental rats were divided in to three groups as control: rats fed diet without zinc supplementation, T1 (treatment 1): rats fed diet containing zinc sulphate @ 50 mg/kg body weight/day and T2 (treatment 2): rats fed diet containing zinc sulphate @ 100 mg/kg body weight/day. The rats were procured from Indian Institute of Integrative Medicine, CSIR Laboratory, Jammu. They were provided standard pelleted ration and clean drinking water *ad libitum* and maintained under standard managemental conditions. Prior to start of experiment, the rats were acclimatized in the laboratory conditions for a period of one week.

After induction of proper anaesthesia, the rats were sacrificed and the reproductive organs (testes and epididymidis) were collected from each rat (Fig. 1 and 2) of all the 3 groups on 6, 8, 10 and 12 weeks of experiment. The testis was separated from the epididymis (Fig. 3 and 4) and length, width, thickness by using verniercallipers, weight by using monopan electronic balance and volume by water displacement method (Barnwal and Sinha, 1981) were recorded.

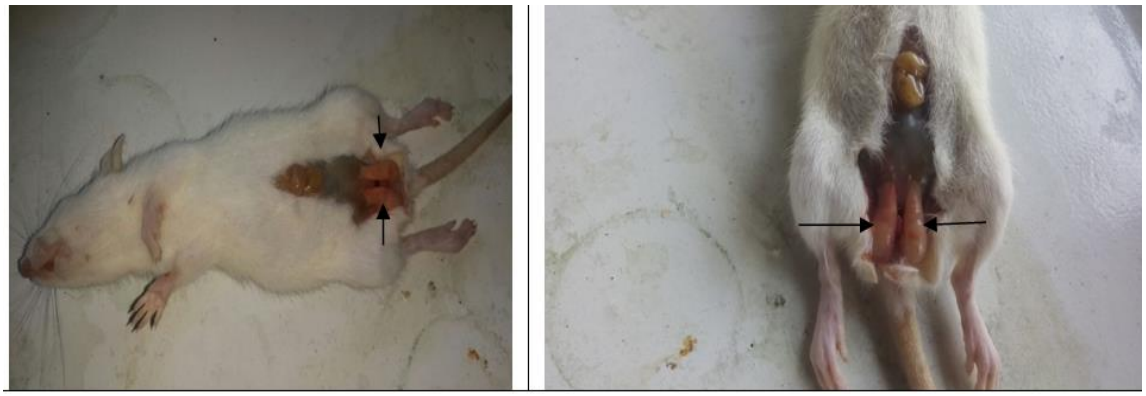

Figure 1: Photographs showing in situ testes and epididymidis (arrows) in rats.

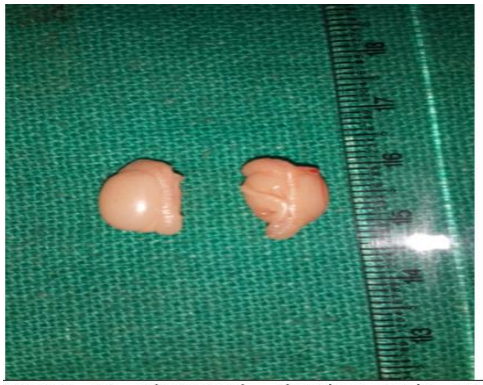

Figure 2: Photograph showing testis & epididymis (intact)

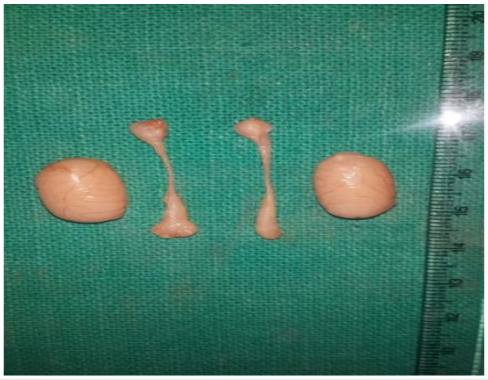

Figure 3: Photograph showing testis & epididymis (separated)

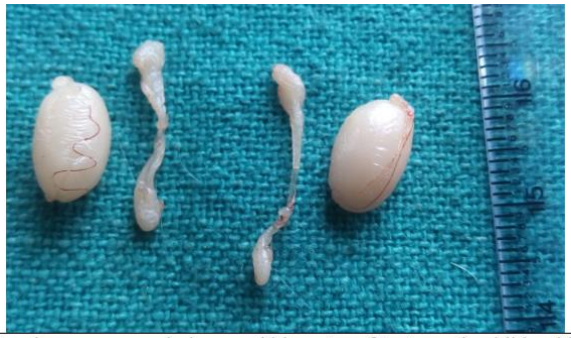

Fig. 4: Photographs showing gross morphology and biometry of testes and epididymidis in rats

For all the observed data in the present experiment, the standard statistical procedures recommended by Snedecor and Cochran (2004) have been followed. The data were presented by showing mean and standard error. The significant differences of values for different parameters studied were assessed by the test of one way and two-way analysis of variance depending upon the data. The significant values of different groups and weeks were calculated by Tukey's test. All the above calculations were carried out using SPSS software version 16.0.

Results

Length of Testis

In between control, T1 and T2 groups, significant difference ($P<0.01$) of overall testicular length was found in 6 weeks in T2 group as compared to control and T1 groups (Table 1). A definite trend of increase of testicular length was found within the group as measured at fortnightly interval from 6 to 12 weeks of age. The overall testicular length were found significantly higher ($P<0.01$) at 8, 10 and 12 weeks as compared to 6 weeks in control, T1 and T2 groups. When comparing right and left testes length, significantly higher ($P<0.05$) length were recorded in left testis in T1 group at 6 weeks of age, in control and T2 groups at 8 weeks of age and in all three groups at 10 and 12 weeks of age.

Width of Testis

When comparing the treatment groups (Table 1), significantly higher ($P<0.01$) overall value was recorded in T1 group as compared to other two groups at 10 weeks of age. At 12 weeks, significantly higher ($P<0.01$) values recorded in both treatment groups (T1 & T2) as compared to control, however no significant difference was found between the values of T1 and T2. At 6 weeks, significantly higher ($P<0.01$) value was recorded in T2 group as compared to control. The width of testes showed an increasing trend as age advanced from 6 to 12 weeks in all three groups. In T1, the values were varied significantly ($P<0.01$) with each other between different ages. In control and T2 groups, significantly higher ($P<0.01$) values were recorded from 8 to 12 weeks as compared to 6 weeks; there was no significant variation found between 8 and 10 weeks and higher significant value was observed at 12 weeks as compared to other age groups. When comparing right and left testes width, significantly higher ($P<0.05$) values were recorded in left testis in control group at 6 weeks of age, in control and T1 groups at 8 and 10 weeks of age and in T2 group at 12 weeks of age.

Table 1: Biometry of testes in male Wistar rats following zinc supplementation

Groups	Age in weeks											
	6			8			10			12		
	Right	Left	Overall	Right	Left	overall	Right	Left	Overall	Right	Left	Overall
Length of testes (cm, Mean ± S.E)												
Control	1.195 ±0.094	1.185±0 .097	1.190^{aA} ±0.095	1.728^{p} ±0.005	1.858^{q} ±0.014	1.793^{b}± 0.008	1.785 ^{p}±0.015	1.843^{q} ±0.012	1.814^{b}± 0.009	1.788^{p}± 0.019	1.865^{q} ±0.017	1.826^{b}± 0.012
Treatment (T1)	1.238^{p} ±0.021	1.328^{q}± 0.016	1.283^{aAB} ±0.017	1.767±0 .032	1.86±0.0 29	1.813^{b}± 0.030	1.873^{p} ±0.015	1.925^{q} ±0.007	1.899^{b}± 0.007	1.882 ^{p}±0.018	1.955^{q} ±0.012	1.918^{b}± 0.012
Treatment (T2)	1.342±0.0 35	1.408±0 .034	1.375^{aB} ±0.033	1.762 ^{p}±0.010	1.875^{q}± 0.008	1.818^{b}± 0.007	1.812 ^{p}±0.016	1.877^{q} ±0.013	1.844^{b}± 0.012	1.852 ^{p}±0.011	1.905^{q} ±0.019	1.878^{b}± 0.008
Width of testes (cm, Mean±S.E)												
Control	0.551^{p}±0. 010	0.620^{q}± 0.019	0.585^{aA} ±0.011	0.800^{p}± 0.013	0.900^{q}± 0.023	0.850^{b}± 0.016	0.831^{p}± 0.012	0.905^{q} ±0.007	0.868^{bA} ±0.005	0.938± 0.013	0.963± 0.011	0.950^{cA} ±0.008
Treatment (T1)	0.601±0.0 19	0.601±0 .019	0.601^{aAB} ±0.019	0.823^{p}± 0.004	0.923^{q}± 0.006	0.875^{b}± 0.005	0.946^{p}± 0.009	1.091^{q} ±0.019	1.019^{cC}± 0.010	1.003± 0.033	1.19±0. 071	1.096^{dB} ±0.031
Treatment (T2)	0.628±0.0 20	0.68±0. 039	0.654^{aB} ±0.026	0.848±0 .021	0.928±0. 030	0.888^{b}± 0.010	0.925± 0.011	0.96±0. 010	0.942^{bB} ±0.009	1.020^{p}± 0.031	1.060^{q} ±0.048	1.040^{cB} ±0.035
Thickness of testes (cm, Mean±S.E)												
Control	0.603±0.0 15	0.641±0 .018	0.622^{aA} ±0.013	0.941^{p}± 0.003	0.966^{q}± 0.006	0.954^{b}± 0.004	0.943± 0.015	0.966± 0.006	0.955^{bA} ±0.006	1.068^{p}± 0.019	1.143^{q} ±0.019	1.105^{cA} ±0.018
Treatment (T1)	0.693±0.0 23	0.721±0 .017	0.707^{aB} ±0.017	0.956±0 .009	0.975±0. 006	0.965^{b}± 0.007	1.051± 0.011	1.083± 0.021	1.067^{cB}± 0.008	1.318^{p}± 0.048	1.440^{q} ±0.020	1.379^{dC} ±0.028
Treatment (T2)	0.775±0.0 30	0.851±0 .034	0.813^{aC} ±0.030	0.945±0 .008	0.965±0. 012	0.955^{b}± 0.007	1.09±0. 019	1.126± 0.008	1.108^{cB}± 0.012	1.21±0. 050	1.286± 0.048	1.248^{dB} ±0.035
Weight of testes (gm, Mean±S.E)												
Control	0.683±0.0 30	0.721±0 .014	0.702^{aA} ±0.013	0.798±0 .025	0.803±0. 004	0.800^{bA} ±0.013	0.855^{p}± 0.017	1.016^{q} ±0.045	0.935^{cA} ±0.025	1.043^{p}± 0.037	1.166^{q} ±0.040	1.105^{dA} ±0.026
Treatment (T1)	±0.033	0.881±0 .032	0.856^{aB} ±0.009	1.05±0. 034	1.116±0. 030	1.083^{bB} ±0.024	0.966^{p}± 0.033	1.150^{q} ±0.042	1.058^{bB} ±0.037	1.216^{p}± 0.033	1.325^{q} ±0.030	1.270^{cB} ±0.019
Treatment (T2)	0.868±0.0 18	0.89±0. 022	0.879^{aB} ±0.020	1.016±0 .030	1.016±0. 030	1.016^{bB} ±0.030	0.983± 0.030	1±0.02 5	0.991^{bAB} ±0.015	1.241± 0.020	1.283± 0.016	1.262^{cB} ±0.015
Volume of testes (ml, Mean±S.E)												
Control	0.515±0.0 37	0.533±0 .013	0.524^{aA} ±0.016	0.61±0. 027	0.613±0. 008	0.611^{aA} ±0.012	0.658^{p}± 0.018	0.810^{q} ±0.043	0.734^{bA} ±0.022	0.858± 0.039	0.975± 0.046	0.916^{cA} ±0.021
Treatment (T1)	0.643±0.0 29	0.705±0 .023	0.674^{aB} ±0.012	0.861±0 .031	0.958±0. 055	0.910^{bB} ±0.039	0.778^{p}± 0.031	0.941^{q} ±0.041	0.860^{bB} ±0.035	1.016± 0.044	1.1±0.0 34	1.058^{cB} ±0.024
Treatment (T2)	0.67±0.03 2	0.7±0.0 26	0.685^{aB} ±0.028	0.828±0 .033	0.836±0. 035	0.832^{bB} ±0.032	0.808± 0.035	0.815± 0.021	0.811^{bA} ±0.018	1.041± 0.030	1.1±0.0 28	1.070^{cB} ±0.028

Thickness of Testis

Table 1 represent the average testicular thickness of right and left testes and overall values at different weeks of age in Wistar rats. In between control, T1 and T2 groups, the values varied significantly ($P<0.01$) with each other at 6 and 12 weeks. At 10 weeks, significantly higher ($P<0.01$) overall values recorded in treatment groups as compared to control, however, no significant difference found between the treatment groups. Highest significant ($P<0.01$) values were found in T1 group as compared to T2 at 12 weeks of age and the values were recorded as 1.379±0.028 cm in T1 and 1.248±0.035 cm in T2 groups. The thickness of testes showed a definite increasing trend as the age advanced from 6 to 12 weeks in all three groups. Significantly higher ($P<0.01$) overall values were observed in 8 to 12 weeks as compared to 6 weeks in control group. However, at 8 and 10 weeks, the values were not varied significantly. It was observed that left testicular thickness was higher than the right testicle at 6 and 10 weeks, but the values were not varied significantly. Left testes values were significantly higher ($P<0.05$) than the right testes in control group at 8 weeks and in control and T1 at 12 weeks.

Weight of Testis (gm)

Table 1 represent the average weight of right and left testes and overall weight at different ages in weeks in Wistar

rats. In between three treatment groups, significantly (P<0.01) higher overall testicular weight were found in treatment groups (T1 and T2) than control group at 6, 10 and 12 weeks. At 8 weeks, T1 value was significantly higher (P<0.01) than that of control. The values of T1 and T2 did not varied significantly with each other. In all three groups, there was a definite trend of increase of testicular weight within the group as measured at fortnightly interval from 6 to 12 weeks of age. In control group, values were varying significantly (P<0.01) with each other. Whereas, in T1 and T2 groups, overall testicular weight were significantly higher (P<0.01) in 8 to 12 weeks as compared to 6 weeks; no significant difference was observed between 8 and 10 weeks and highest significant (P<0.01) values were observed in 12 weeks as compared to other age groups. Between left and right testicular weight, significantly higher (P<0.05) values were found in left than right one at 10 and 12 weeks in control and T1 groups.

Volume of Testis (ml)

Table 1 represent the average volume of right and left testes and overall volume at different ages in weeks in Wistar rats. When compared between three treatment groups, significantly higher (P<0.01) overall values were found in treatment groups at 6, 8 and 12 weeks as compared to control. At 10 weeks, significantly higher (P<0.01) values found in T1 as compared to other two groups. There was a definite trend of increase of overall testicular volume within the group at various ages as measured at fortnightly interval from 6 to 12 weeks in all the groups. Significantly higher (P<0.01) values found at 12 weeks of age as compared to other age groups. Between left and right testicular volume, significantly higher (P<0.05) values were found in left than right one at 10 weeks in control and T1 groups. Almost all the values showed that left volume of testis was higher than the right one.

Discussion

Zinc played an important role in normal testicular development and maintenance of the germinal epithelium (Anderson *et al.,* 1993). Zinc was directly involved in anatomical development and normal function of male reproductive organs. Deficiency of zinc in the diet delayed testicular development (Underwood and Somers, 1977). In the present study also, it was observed that in zinc-supplemented rats, the biometrical parameters i.e., length, width, thickness, weight and volume of testes were found higher when compared to the control group. There was positive correlation between the testicular size and the incidence of production of better-quality sperm and severely zinc-deficiency caused delayed testicular development (Bedwal and Bahuguna, 1994), smaller testes (Kumari *et al*., 2010) and arrest of spermatogenesis and depletion of Leydig cells (Taneja and Nirmal, 1980). Zinc deficiency affected spermatogenesis and the development of primary and secondary sex organs in the male (Underwood, 1971). El Hendy *et al.* (2001) reported that due to deficiency of zinc, the weight of the testis decreased significantly. Omu *et al*. (2015) found zinc deficiency caused 42% reduction in testicular volume and weight compared to zinc supplemented group and control. In another study (Al-Ani *et al*., 2015), the testicular weight increased significantly (P<0.001) in adult rats receiving zinc with cadmium as compared to only cadmium supplementation. Increased testicular development might be due to stimulatory effect of zinc on the hypothalamo-pituitary axis to increase the production of testosterone through gonadotropic hormone which in turn helped in the development of male genital organs. Importance of zinc supplement was also revealed in the earlier studies, since deprivation of zinc caused reduction in secretion of GnRH from the hypothalamus in animals (Martin and White, 1992). The present study revealed increased values in T1 as compared to T2 were found in the later phase of zinc supplementation (mainly 10 and 12 weeks of zinc supplementation). Hence, this study indicated that zinc had definite role to play in the anatomical development of testis, especially zinc @ 50 mg/kg body weight/day had better effect on testicular growth as reflected in the present study shown in Table 1, showing increased testosterone concentrations in T1 as compared to T2 group.

Biometrical studies revealed that all the measured dimensions (length, width, thickness, weight and volume) showed increasing trend with progression of age. The finding of the present study was in good agreement with the earlier findings carried out by different workers in rats and mouse (Taneja & Nirmal, 1980; Anderson *et al*., 1993; Omu *et al*., 2015). These findings matched the present observations that increase size of testis synonymous to higher concentration of spermatozoa. Dun (1955) reported that testicular development had a relation with increased body weight and age and it could be used as a basis for estimating sexual maturity. Ferreira *et al.* (2003) also found that body weight and testicular weight were significantly correlated and they increased significantly (P<0.05) with age in pigs. There was positive correlation between testicular size and sperm production and more sperm production occurred in larger and heavier testes.

Conclusion

In the present study it was observed that the biometrical parameters of epididymis were found higher in zinc-supplemented rats than the control group. Also, it was revealed that the growth of the epididymis in terms of its various biometrical values was found to be even more in rats supplemented with zinc @50 mg /kg body weight/day as compared to the rats supplemented at 100 mg /kg body weight/day during the later phases. It is concluded that supplementation of zinc @50 mg /kg body weight/day promotes better development of the epididymis in rats.

Conflict of Interests

There is no conflict of interest.

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