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ORIGINAL ARTICLE

Effect of zinc on some selected blood parameters and reproductive performance of male mice

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Abstract

Background: The effect of zinc supplementation on birth weight, litter size, total erythrocyte count (TEC), hemoglobin estimation, total leukocyte count (TLC), blood glucose level, abnormal sperm count and histopathology of testis in mice was evaluated.

Methods: 45 albino mice were randomly divided into 3 equal groups viz. control group, A; 10 mg zinc/kg feed treated group, B; 20 mg zinc/kg feed treated group, C. Each group was comprised of 10 male mice and 5 female mice. Then they are allowed to breed for about 25 days. After breeding males were withdrawn from each group and treated with zinc for 30 days. Then male mice were transferred back to female cage and were allowed to breed for 25 days.

Results: Mice of group B and C showed a significant increase ($p<0.01$) in total erythrocyte count (TEC), total leukocyte count (TLC), hemoglobin content, litter size and decreased abnormal sperm count. No significant increase in body weight, even a decrease in glucose content was recorded in group B and birth weight was found increased in group C. In the histopathological study, no significant change found with different doses of zinc supplementation except reactive cell infiltration and slight tissue degeneration in the mice fed with 20 mg zinc supplement/kg feed was recorded.

Conclusion: Supplementing zinc @ 10-20 mg/kg feed was found to enhance hematological parameters, increase litter size and decrease abnormal sperm count in mice.

Keywords: TEC, TLC, Hb, Sperm count

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Introduction

Zinc is an essential nutrient that is required in humans and animals for many physiological functions (Mohamed *et al*., 2011). Zinc plays an effective role in reproduction in both male and female rat. Dose dependent administration of zinc improves the sexual performance (Dissanayake *et al*., 2009). It is particularly important in cellular function because it is an integral component of numerous proteins, including metalloenzymes, structure proteins and transcriptional factors (Zhou *et al*., 2008). Zinc also considered as a cofactor and is a constituent of many enzymes which are involved in macronutrient metabolism and cell replication like lactate dehydrogenase, alcohol dehydrogenase, glutamic dehydrogenase, alkaline phosphatase, carbonic anhydrase, carboxypeptidase, superoxide dismutase, retinene reductase, DNA and RNA polymerase (Arinola, 2008). Metals are naturally occurring in an inorganic elements which are present in very small amounts in the living tissues but are important for the vital processes of life (Kazi *et al*., 2008). Some metals (e.g. magnesium) are known as macro-metals and are found in high amount in the body tissues, therefore they are also called macro-nutrients (Simsek *et al*., 2007). Zn concentration is high in the seminal fluid and has a multifaceted role in the sperm's functional properties. It has been suggested that Zn acts as an important anti-inflammatory factor and that it is involved in the sperm's oxidative metabolism. Zn has many important functions in the spermatozoa physiology, including effects on lipid flexibility and sperm membrane stabilization (Chia *et al*., 2000). It also has a regulated role in capacitation and the acrosome reaction of sperm and is essential for conception and embryonic implantation (Eggert *et al*., 2002). High concentrations of zinc found in the sex glands including testis. So it may play a crucial role in the reproductive system. Zinc may be involved in functions that are important for sperm physiology. Therefore, the present work was taken to evaluate the effect of zinc supplementation on male mice in reproduction.

Materials and Methods

The experiment was carried out in the Department of Physiology, Faculty of Veterinary Science, Bangladesh Agricultural University (BAU), Mymensingh to study the effect of zinc supplementation on birth weight, litter size, total erythrocyte count (TEC), hemoglobin estimation (Hb), total leukocyte count (TLC), blood glucose level, abnormal sperm count and histopathology of testis in Swiss Albino mice(*Mus musculus*) during the period from July 05, to October 07, 2012.

A total of 45 albino mice aged 60 days and weighing 20-25g were used and purchased from ICDDR, B Mohakhali, Dhaka. After 7 days adaptation 45 the mice were randomly divided into: 3 equal groups viz. A: control group; B: 10 mg zinc /kg feed treated group; C: 20 mg zinc /kg feed treated group. Each group consists of 10 male mice and 5 female mice. Then they are allowed to breed for about 25 days with normal ration. After breeding males were withdrawn from each group. Among them 5 were sacrificed and blood and testis samples were collected from each group. Rest males are treated with zinc at the dose stated for group B, and C for 30 days. Pups weight from each group just after birth was recorded. The male mice were transferred back to female cage and were allowed to breed for 25 days. Then male mice were sacrificed for collecting blood and testis samples from each group. After second breeding pups were collected to detect the weight and litter size. Zinc in the form of Zinc Sulfate (Baby Zinc, ICDDR, B) were mixed with feed and supplied twice a day (morning and evening).

The mice were sacrificed for collecting blood samples in double oxalate containing vials for hematological examination. TEC, Hb and TLC were performed as per methods indicated by Lamberg and Rothstein (1977). Serum samples were prepared for measurement of blood glucose level. Testis was collected for abnormal sperm count and histopathology was determined as per technique described by Sutherland and Shastry (1983).

In Sperm morphology, caudal epididymidis of male mice was removed from each side and minced in a phosphate-buffered saline solution (PBS) and passed through an 80-mesh stainless-steel filter. Then smears were prepared for suspension and allowed to dry in air. Slides were stained in 1% eosin-Y solution for 45 minute. A total of 100 spermatozoa were observed for each animal; the spermatozoa were scored 'blind' as normal or abnormal but the specific type of abnormality was not categorized within each group. Only intact spermatozoa were included in the analyses. All slides were scored by the same individual.

Data obtained were analyzed statistically for mean, standard deviation (SD) and Student's 't' test according to the standard procedures as described by Snedecor and Cochran (1980).

Results

Litter size and pups weight of different groups of mice at pre- and post- treatment in Table 1. The mice supplemented with both 10 mg zinc /kg feed and 20 mg zinc /kg feed showed significant change in the number of offspring in between pre-treated and post-treated values. But the amplitude of decrease in the number of offspring was found significantly lower in mice supplemented with 20 mg zinc /kg feed, possibly it is due to positive impact of zinc on sperm.

Table 1. Effect of zinc on litter size and pups weight (g) in mice

Parameters	Groups	Pre-treatment	Post-treatment	Level of significance
Litter size	A	6.80±0.84	7.60±1.14	NS
	B	6.40±1.14	10.20±0.84	**
	C	6.60±0.89	9.60±0.55	**
Pups weight	A	1.60±0.08	1.57±0.20	NS
	B	1.42±0.06	1.45±0.09	NS
	C	1.51±0.03	1.56±0.015	NS

Data are shown as mean±SD of n=5 samples per group. NS=Non significant.
*=Significant at 5 percent level (P<0.05), **=Significant at 1 percent level (P<0.01).

Post-treatment value of pups body weight differs non-significantly from their respective pre-treated value. There was no significant difference among all group of mice A (1.57±0.20 g), B (1.45±0.09 g) and C (1.56±0.015 g). It seemed that there was no significant effect of zinc supplement on birth weight at this level.

The results of hematological and biochemical values of the mice are shown in table 2. TEC, hemoglobin value and TLC were significantly (*P* <0.01) increased compared with control. So zinc is essential elements for hemopoiesis. But the number of TEC and hemoglobin were found lower in mice supplemented with 20mg zinc/kg feed group. Possibly it is due to negative impact of excess zinc on TEC and hemoglobin. Mice of group both B and C showed a significant (P<0.01) increase in total leukocyte count after 30 days of pre-treatment. But in the control group there was non-significant relationship between pretreatment (8.36±0.11) and post-treatment (8.37±0.09) groups. The blood glucose level in mice with supplement receiving zinc was approximately as same as before. Administration of zinc@20mg/kg feed changed the blood glucose level significantly (P<0.05) decreased after treatment. The significantly decreased level of glucose in mice might be a due to high performance of pancreas in utilizing of glucose and higher physical activity at adult age. Zinc supplementation may stimulate for over activity of pancreas.

Table 2. Effect of zinc on total erythrocyte count (TEC), hemoglobin value, total leukocyte count (TLC), blood glucose content and abnormal sperm count in mice

Parameters	Groups	Pre-treatment	Post-treatment	Level of significance
Total erythrocyte count (TEC) millions /mm^3	A	5.54±0.19	5.33±0.31	NS
	B	5.56±0.09	7.34±0.43	**
	C	5.59±0.08	7.17±0.32	**
Hemoglobin value (g /dL)	A	6.58±0.20	6.53±0.10	NS
	B	6.57±0.22	8.65±0.49	**
	C	6.59±0.19	8.54±0.52	**
Total leukocyte count (TLC) millions /mm^3	A	8.36±0.11	8.37±0.09	NS
	B	8.35±0.26	9.01±0.46	**
	C	8.38±0.21	9.40±0.54	**
Blood glucose content (mg /dL)	A	4.67±0.25	4.67±0.32	NS
	B	4.65±0.28	4.58±0.23	*
	C	4.67±0.24	4.59±0.29	**
Abnormal sperm count (million /mL)	A	9.60±2.60	9.80±2.39	NS
	B	9.60±2.30	5.20±1.64	**
	C	9.80±2.59	5.80±1.30	**

Data are shown as mean±SD of n=5 samples per group. NS=Non significant.
*=Significant at 5 percent level ($p<0.05$), **=Significant at 1 percent level ($p<0.01$).

The mice supplemented with both 10 mg zinc /kg feed and 20 mg zinc /kg feed showed significant ($p<0.01$) change in the number of blind sperm in between pretreated and post treated values. But the amplitude of decrease in number of blind sperm was found in group B which was significantly lower ($p<0.01$) from mice supplemented with 10 mg zinc /kg feed. The result showed that, the application of 10 mg zinc /kg feed have significant effect in decreasing the number of blind sperm in mice. From this result it is evident that 30 days supplement of 10 mg zinc /kg feed zinc is beneficial to sperm morphology.

Histopathological Study

In the present study, testis samples were examined by histopathologically for the detection of any pathological lesion. No specific lesions were found in the testis of group B and group C zinc treated groups as compared with the control group.

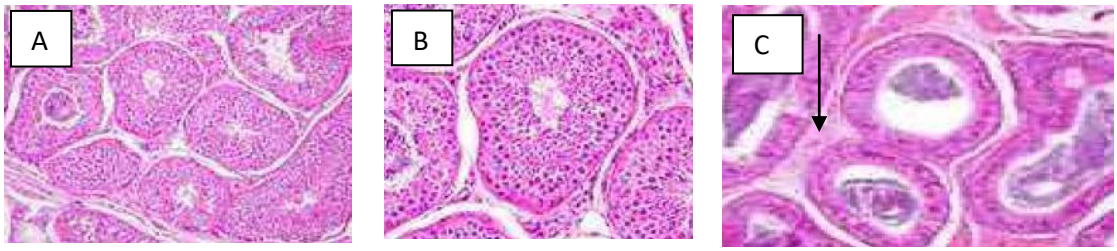

Figure 1. Histopathological section of testis of mice (H&E x 100), **(**A) Control group; **(**B) 10mg zinc /kg feed treated group (arrow indicates the inflammatory cells); (C) 20 mg zinc /kg feed treated group (arrow indicates the inflammatory cells and slight necrosis).

However; these inflammatory changes are not statistically significant in the treated group as compared with the control. The non-specific lesions were recorded in the testis of 10 mg zinc / kg feed and 20 mg zinc /kg feed treated groups. The lesion includes bulky fluid content within testis and slightly swollen cells. Some group of inflammatory cells were scattered in parenchyma of testis in both the zinc treated groups. Slight

degenerative tissue changes were observed in 20 mg zinc /kg feed treated groups.

Discussion

Trace elements are important for proper functioning of a number of enzymes and proteins which are involved in many physiological, biochemical and metabolic processes that contribute to growth and production. Overall, trace elements improve immune competence and productive by Yatoo *et al*. (2013).

The mice supplemented with both 10 mg zinc /kg feed and 20 mg zinc /kg feed showed significant change in the number of offspring in between pre-treated and post-treated values. But the amplitude of decrease in number of offspring was found significantly lower in mice supplemented with 20 mg zinc /kg feed. Possibly it is due to positive impact of zinc on sperm morphology. This finding is almost similar to reports made by Ismail *et al.* (2016) and Fillah *et al*. (2018). (Dissanayake *et al*. (2009) showed that zinc plays an effective role in reproduction in both male and female rat.

Table 2 showed that in all the treated groups TEC, hemoglobin value and TLC were significantly ($p<0.01$) increased compared with control. So zinc is essential elements for hemopoiesis. TEC and hemoglobin value were found lower in mice supplemented with 20mg zinc/kg feed group. Possibly there is negative impact of excess zinc on TEC and hemoglobin value. This finding is similar to that of Boscolo *et al*. (2005) and Tuncer *et al*. (2011) who stated that dietary excess of zinc induces signs of copper deficiency, including anemia, which can be prevented by adding copper to the diet. Mice of group both B and C showed a significant ($p<0.01$) increase in total leukocyte count after 30 days of pre-treatment. But in the control group there was non-significant relationship between pretreatment (8.36±0.11 millions /mm^3) and post-treatment (8.37±0.09 millions /mm^3) groups. It could also be compared with the study of Boscolo *et al*. (2005) who showed in a study that the inhibitory effects of cadmium on peripheral blood mononuclear cell proliferation and cytokine release can be reversed by zinc and selenium salts. The blood glucose level in mice with supplement receiving zinc was approximately as same as before. Administration of zinc@20 mg /kg feed changed the blood glucose level significantly ($p<0.05$) decreased after treatment. The significantly decreased level of glucose in mice might be a due to high performance of pancreas in utilizing of glucose and higher physical activity at adult age. Zinc supplementation may stimulate for over activity of pancreas. Fillah *et al*. (2018) and Ming *et al*. (1998) stated that zinc supplementation alleviated the hyperglycemia of obese (ob/ob) mice, which may be related to its effect on the enhancement of insulin activity.

The mice supplemented with both 10 mg zinc /kg feed and 20 mg zinc /kg feed showed significant ($p<0.01$) change in the number of blind sperm in between pretreated and post treated values. But the amplitude of decrease in number of blind sperm was found in group B which was significantly lower ($p<0.01$) from mice supplemented with 10 mg zinc/kg feed. The result showed that, the application of 10 mg zinc /kg feed has significant effect in decreasing the number of blind sperm in mice. From this result it is evident that 30 days supplement application of 10 mg zinc /kg feed zinc is beneficial to sperm. This type of work done by Jones *et al.* (1996). He stated that, high levels of zinc are required to maintain hormone synthesis, spermatogenesis, and sperm chromatin structure.

However, the inflammatory changes are not statistically significant in the treated group as compared with the control. The lesions in the testis of 10 mg zinc / kg feed and 20 mg zinc /kg feed treated groups were recorded non-specific. Bulky fluid content, slightly swollen cells, inflammatory cells were found in parenchyma of testis in both the zinc treated groups. Slight degenerative tissue changes were observed in 20 mg zinc /kg feed treated groups. Similar finindings were observed by Tuncer *et al*. (2011) and Fillah *et al*. (2018).

Conclusion

The present study is a preliminary work on effect of zinc on male reproduction in laboratory animal

in Bangladesh. Zinc treated mice showed a significant increase ($p<0.01$) in total erythrocyte count (TEC), total leukocyte count (TLC), hemoglobin content, litter size and decreased abnormal sperm count.

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