Long term excessive zinc supplementation in diet induced alteration in serum lipids, hormones and minerals profile in wistar rats and has carry over effect in their F1 generation rats

Tanushree Das¹, Sudeep Ghatani², Kshetrimayum Birla Singh³*

¹,² Department of Zoology, Sikkim University, Gangtok-737102, Sikkim, India.
³ Department of Life Sciences (Zoology), Manipur University, Canchipur-795003, Manipur, India.

*Corresponding author: Kshetrimayum Birla Singh (Email: birla.kshetri@gmail.com)

Abstract

The use of the zinc in various areas such as animal husbandry, agricultural techniques and multivitamin supplements has increased in recent decades and will increase over time. In this study, the impact of Zn overload for long period in the body, and their carry-over effect on the next generation is determined. Three groups of rats were fed on basal diets having 20 mg Zn in one kg diet (Group-1), control group, 50 mg Zn in one kg diet (Group-2) and 80 mg Zn in one kg diet (Group-3) for 180 days. The F1-generation rats obtained from each group of parent rats were fed only on rat pellet diet. The investigation revealed that body weight rise with increased concentration of Zn in parent and F1-generation rats. The blood lipid profile in serum showed an increase in triglycerides, cholesterol, very-low-density lipoprotein (VLDL) cholesterol whereas lowered level of high-density Lipoprotein (HDL) cholesterol levels was recorded in both generation of rats. A significant increase in serum Insulin, HbA1c, and C-reactive protein level was recorded in group-2 and 3 rats while no significant changes in the F1-generation of rats. In F1-rats, Zn showed increase in its level while decreased level of Mn and Cu in the kidney and liver of group-F1-2 and F1-3 was observed in comparison to their control counterparts. The results of the study suggest long term treatment of diet rich in Zn induced alteration in serum lipids, hormones and profile of minerals and has carry over effect to their F1-generations.

Keywords: Blood hormones, Blood lipid profile, F1-generation rats, HDL, Metallomics, Nutrition, Serum lipids, VLDL.

DOI: 10.53894/ijirss.v6i1.1175
Funding: This research is supported by the Science and Engineering Research Board, Department of Science and Technology, Government of India. (Grant number: EEQ/2016/000120 Dated 20the Feb, 2007).
History: Received: 10 October 2022/Revised: 29 November 2022/Accepted: 16 December 2022/Published: 5 January 2023
Copyright: © 2023 by the authors. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).
Authors’ Contributions: Tanushree Das (TD), Sudeep Ghatani (SG) and Kshetrimayum Birla Singh (KBS) designed the study, TD and SG analyzed and interpreted data and wrote the manuscript, and KBS finalized the manuscript and supervised the study. All authors read and agreed to publish the manuscript in the present form.
Competing Interests: The authors declare that they have no competing interests.
Transparency: The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained.
Ethical Statement: This study followed all ethical practices during writing.
Publisher: Innovative Research Publishing
1. Introduction

Zinc (Zn) has been utilized indiscriminately in various practices since last few decades such as in the various practices of agriculture and husbandry as well as in diet of baby and vitamin supplements with the belief that zinc is nontoxic and activates body growth and weight in consumers. The mechanisms of Zn absorption and excretion are regulated to maintain the body's homeostatic level. It has been discovered that the body does not store a significant amount of Zn and that the limited amount that does remain is redistributed to address the problem of zinc-deficient symptoms [1]. Because Zn cannot be retained in the body, excessive Zn intake leads to increased excretion and decreased absorption [2]. Over the last couple of years, in the United States, there is an increase in the consumption of Zn, either from zinc enriched foods [3] or from meat food stuffs and vegetables and loaded with more Zn as reported in some regions of India [4, 5] and this will continue to rise over certain period of time. Furthermore, there is an increase in Zn concentration in both animal and plant products due to treatment with elevated concentrations of ZnSO₄ in various animal feeds and fertilizers besides growth hormones and its analogs in order to get higher yields of plants and animal products for easy and quick commercial is being used in large-scale livestock. As a result of this, the people of India and many other regions of the world have been eating products laced with excess Zn. Even though Zn is an essential micronutrient, health specialists advise against consuming too much of it because it might lead to deficiencies of other trace elements and induce mineral imbalances in the body [6-9].

Previous research in this area indicates that excessive Zn in the diet, when consumed for extended periods alters blood lipid metabolisms and tissue mineral status [5, 6]. It has also been demonstrated that when both low-Zn diet and high-zinc diet were fed on chicks for short and long term respectively had evident deleterious impacts in a mouse model and excessive dietary Zn levels can impair pancreatic exocrine function in chicks [10]. Although the impact of increases Zn load for long period on various biochemical parameters in the human and animal models have been studied before, its impact on hormones, mineral status and their carry over effect on the next generation is yet to be determined. This prompted us to investigate effects of increased Zn load for a long period on the various blood serum parameters and status of minerals in the tissue of the body both in parent and their carry over effect in the F1 generation rats. Therefore, the investigations was undergone to see the influence of excess Zn supplementation at a quantity within pharmacologically prescribe doses if supplemented for a longer time triggers any alteration in the various blood lipids and mineral levels in the rats and their F-1 generation rats which does not have any genetical predisposition. The present communication contains the findings of the investigation.

2. Methodology

Composition of Basal Diet – The male and female rats in this investigations were fed a basal diet enriched with fat and refined sucrose as per Orgebin-Crist, et al. [11] instead of a rat pellet feed consisting of conventional ingredients to maintain the consistency composition of diet specially content of sucrose, micronutrients and fat in the course of the experiment. It also helps in avoiding the conditions where fibres and phytates in the diet may binds to Zn and may lower the bioavailability of Zn in the digestive tract [12, 13]. Otherwise, the time taken to manifest the effects of high Zn in the body may longer in the experimental conditions. The diet given above were divided into 3 parts and modified as follows: control diet containing 20 mg Zn in one kg diet (per se) for Group-1(control), Zn enriched diet-I containing 50 mg Zn in one kg diet for Group-2, and Zn enriched diet-II containing 80 mg Zn in one kg diet for Group-3 by increasing amount of ZnSO₄·7H₂O in the diet accordingly.

Diet Preparation- Minerals and vitamins which are soluble in water were finely crushed for each diet group and vitamins which are soluble in fat were dissolved in corn oil. Agar was dissolved in 25ml double distilled and warm water (60°C) and will be used as binder. In a separate container containing agar solution, each diet components were thoroughly mixed after cooling to 40°C. The resulting dough was placed in Petri dishes and refrigerated to solidify. The diet which has been solidified was then cut into small pieces 2 × 2 × 2 cm and putted in a container at a temperature > -4°C till used.

Design of Experiment: 24 Wistar albino rats weighing between 80-100g were obtained from Chakraborty Enterprise, a CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Ministry of Animal and Husbandry, Government of India registered breeder in Kolkata, India. The rats were kept in cages which is plastic and having steel top grills at 26°- 28°C with room temperature of 10:14 hr with L: D cycles of 70-80 percent Relative Humidity (RH), following the regulations of the Institutional Animals Ethics Committee (IAEC), Sikkim University, Gangtok, India and the globally accepted norms for laboratory animal use and care.

After acquiring the rats, they were fed on pellet feed for one week to acclimatize to the new laboratory conditions. Thereafter, the rats were separated into 3 groups viz. group 1, 2 and 3 with mean initial body weights that were nearly identical in each group. For 180 days, the animals were fed their individual diets and given proper access to double distilled deionized water. The body weights and food intakes were observed at the start of the Zn treatment and then every week after that.

Production of F1-generation rats: In order to get F1- generation rats, 1 male and 2 female wistar rats (3 sets for each group) from each group-1, 2 and 3 parent rats were kept in separate cages and fed on their individual diets for 75 days. During this feeding period, urine was examined using Benedict’s test. The urine of the group 2 & 3 rats reacted positively with Benedict’s test on 56th day onward to till day 75 indicating glucosuria in these group of rats. 1 male and 2 females/cage of rats in each group were allowed to mate and tested for pregnancy by observing the female rats for the presence of sperms in their vaginal smears. After confirming the presence of sperms, the female rats were separated from the males and counted as day 1(one) of the pregnancy. The number of pups delivered and other abnormalities in the pups if any were recorded after gestation period of 21 days. The F1-generations rats delivered were label as Group-F1-1(Control), Group-F1-2 and Group-F1-3 for those obtained from parent Group-1(Control), Group-2 and Group-3 respectively. For the F1
generation experimental rats, the rats of each group were fed on a pellet rat diet instead of the semi-synthetic diet of the parents. The intention of supplementation of conventional pellet rat feed was to assess the carry-over effect of excessive Zn treatment in the F1 generation in a real-life situation. During the experiment, the animals were given nothing but their diet and double-distilled water. Both the parent and F1 generation rats were fed their individual diet for 180 days and 60 days respectively.

Assessment of Blood Lipid Profile- Blood was obtained from the lateral saphenous vein of the rats after the diet treatment was completed. By centrifuging blood samples for 15 minutes at 2500 rpm, the blood serum was prepared. The freshly prepared serum was examined for cholesterol [14, 15] triglycerides [16, 17] HDL-cholesterol [18] and HbA1c and CRP (all by using analysis kits- ERBA diagnostic Mannheim GmbH, Mannheim, Germany) and total lipids [19]. Friedewald's equation was used to compute low-density lipoprotein (LDL) and VLDL cholesterol [20].

Assessment of Tissue metal status- The levels of Zn, Cu, and Mn was determined using an Atomic Absorption Spectrophotometer (AAS) and hollow cathode lamps (213.9 nm, 324.8 nm, and 279.5 nm for Zn, Cu, and Mn respectively).

Kidney and liver samples were digested separately in nitric and perchloric acids (in a ratio of 3:1 v/v) on a hot plate until white ash was produced. Before analysis, the digested ash was dissolved in 10 mM HNO₃ [213.9 nm, 324.8 nm, and 279.5 nm for Zn, Cu, and Mn respectively]. The data of observations are presented as means ± SEM of 6 observations each.

3. Results

Body Weight - The data of this investigation demonstrated significant increase in body weights (monthly) (P < 0.05) in groups 2 and 3 of parent rats after a 150-day dietary treatment (5 months). Following that, significant decrease in body weights was observed in Group 2 and 3 after 30 days Zn supplementation Table 1. Similarly, F1 generation rats of all the three groups showed an increase in body weight, though it was not significant Table 2. The gain in body weight in each group was higher concerning their initial body weight.

Table 1. Month-wise body weight and food intake of rats of Group-1 ([Fed on a basal diet (Control)], Group-2 (fed on Zn-supplemented diet-I), and Group-3 (Fed on Zn supplemented diet-II) during 180 days of dietary treatment. [Values are mean ± SEM of 6 observations each].

<table>
<thead>
<tr>
<th>Time duration</th>
<th>Group-1</th>
<th>Group-2</th>
<th>Group-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>93.16 ± 1.88</td>
<td>-</td>
<td>94.67 ± 1.35 N</td>
</tr>
<tr>
<td>Month 1 (30 days)</td>
<td>156 ± 2.32</td>
<td>298.8 ± 2.13</td>
<td>192.50 ± 1.82 b</td>
</tr>
<tr>
<td>Month 2 (60 days)</td>
<td>168.50 ± 1.70</td>
<td>308.0 ± 4.53</td>
<td>200.50 ± 1.96 b</td>
</tr>
<tr>
<td>Month 3 (90 days)</td>
<td>191.34 ± 1.17</td>
<td>322.6 ± 6.21</td>
<td>213.5 ± 2.52 b</td>
</tr>
<tr>
<td>Month 4 (120 days)</td>
<td>198.83 ± 1.01</td>
<td>326.5 ± 5.47</td>
<td>227.83 ± 2.16 b</td>
</tr>
<tr>
<td>Month 5 (150 days)</td>
<td>211.67 ± 1.30</td>
<td>328.1 ± 5.84</td>
<td>257.83 ± 2.68 b</td>
</tr>
<tr>
<td>Month 6 (180 days)</td>
<td>256.83 ± 1.57</td>
<td>343.6 ± 3.51</td>
<td>251.83 ± 2.71 b</td>
</tr>
</tbody>
</table>

Note: Units: Gram, P values: < 0.05; < 0.001 [Values of the group 2 and group 3 were compared with group-1] *Non-significant (Values of the group 2 and group 3 were compared with group-1).

Table 2. Month-wise body weight of rats of F1 generation rats of Group F1-1(Control), group F1-2 and group F1-3 fed on pallet rat diet during 60 days. [Values are mean ± SD of 6 observations each].

<table>
<thead>
<tr>
<th>Time duration (In weeks)</th>
<th>Group F1-1 (Control)</th>
<th>Group F1-2</th>
<th>Group F1-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight</td>
<td>6.34 ± 0.33</td>
<td>7.16 ± 0.16 N</td>
<td>6.67 ± 0.71 N</td>
</tr>
<tr>
<td>Month 1 (30 days)</td>
<td>47.67 ± 1.28</td>
<td>49.20 ± 1.39 N</td>
<td>50.16 ± 1.74 N</td>
</tr>
<tr>
<td>Month 2 (60 days)</td>
<td>93 ± 1.43</td>
<td>95.16 ± 1.95 N</td>
<td>98.5 ± 3.96 N</td>
</tr>
</tbody>
</table>

Note: Units: Gram; P values: < Non-significant (Values of the group F1-2 and group F1-3 were compared with group F1-1).

3.1 Changes in Food Intake in Parent rats- The food intake data revealed an increased Zn concentration in basal diet and increased bodyweight of the rats were directly linked to increased food intake in the parent rats Table 1.

Table 3. Blood profile of parent rats of group-1 [Fed on a basal diet (Control)], group-2 (Fed on Zn-supplemented diet-I), and group-3 (fed on Zn supplemented diet-II) during 180 days of dietary treatment. [Values are mean ± SD of 6 observations each].

<table>
<thead>
<tr>
<th>Blood parameters</th>
<th>Group-1 (Control)</th>
<th>Group-2</th>
<th>Group-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum triglyceride</td>
<td>48.19 ± 2.32</td>
<td>64.21 ± 1.39 b</td>
<td>92.75 ± 1.24 b</td>
</tr>
<tr>
<td>Serum cholesterol</td>
<td>92.75 ± 1.90</td>
<td>115.97 ± 1.89 b</td>
<td>121.81 ± 2.12 b</td>
</tr>
<tr>
<td>Serum HDL</td>
<td>67.15 ± 2.92</td>
<td>64.51 ± 2.97 a</td>
<td>60.5 ± 2.98 a</td>
</tr>
<tr>
<td>Serum LDL</td>
<td>15.95 ± 1.15</td>
<td>38.61 ± 2.98 b</td>
<td>42.76 ± 2.01 b</td>
</tr>
<tr>
<td>Serum VLDL</td>
<td>11.66 ± 9.63</td>
<td>13.85 ± 2.84 a</td>
<td>17.49 ± 2.55 a</td>
</tr>
<tr>
<td>Serum insulin</td>
<td>15.01 ± 1.30</td>
<td>24.2 ± 1.16 b</td>
<td>30.5 ± 2.15 b</td>
</tr>
<tr>
<td>HbA1c</td>
<td>4.95 ± 1.25</td>
<td>6.34 ± 1.73 a</td>
<td>7.58 ± 1.45 a</td>
</tr>
<tr>
<td>CRP</td>
<td>5.20 ± 1.20</td>
<td>8.08 ± 1.02 a</td>
<td>10.5 ± 1.25 a</td>
</tr>
</tbody>
</table>

Note: Units: * mg/dl, * uL/ml, %, * mg/L; P values: < 0.05; < 0.001 [Values of the group 2 and group 3 were compared with group-1].

187
Changes in Blood lipid and hormone Profile in Parent rats and F1 generation- After six months of the Zn treatment, the lipid parameters of the blood displayed significant rise in triglycerides, cholesterol, LDL and VLDL- cholesterol whereas significant decrease in the level of HDL-cholesterol in the group-2 and group-3 parent rats as compared to control group-1 (P< 0.05) revealing changes in lipid metabolism in blood in Zn treated groups Table 3. We obtained similar results in the case of the F1 generation rats where significant rise was observed in triglycerides, cholesterol, VLDL-cholesterol and decreased in HDL-cholesterol levels in group F1-2 and F1-3 compared to their control counterpart Group-1-F1 Table 4. The hormone profile reveals a significant increase in serum insulin, HbA1c, and CRP level in group-2 and 3 rats of parent rats while an insignificant increase in HbA1c, and CRP level in group-F1-2 and group-F1-3 rats in comparison to their respective control groups. However, in case of serum insulin, similar significant rise was observed in both the groups of F1 generation in comparison to the control group.

Table 4. Blood profile in F1 generation rats of group F1-1 (Control), Group F1-2 and group F1-3 fed on pallet rat diet during 60 days. [Values are mean ± SD of 6 observations each].

<table>
<thead>
<tr>
<th>Blood parameters</th>
<th>Group F1-1(Control)</th>
<th>Group F1-2</th>
<th>Group F1-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum triglyceride*</td>
<td>45.7 ± 1.06</td>
<td>63.58 ± 2.46 *</td>
<td>85.69 ± 1.5 *</td>
</tr>
<tr>
<td>Serum cholesterol*</td>
<td>88.3 ± 2.47</td>
<td>105.3 ± 2.95 *</td>
<td>108.2 ± 1.96 *</td>
</tr>
<tr>
<td>Serum HDL*</td>
<td>58.2 ± 2.51</td>
<td>57.41 ± 2.1 N</td>
<td>51.7 ± 5.02 N</td>
</tr>
<tr>
<td>Serum LDL*</td>
<td>20.9 ± 7.27</td>
<td>35.1 ± 1.88 b</td>
<td>39.30 ± 3.86 b</td>
</tr>
<tr>
<td>Serum VLDL*</td>
<td>9.15 ± 0.81</td>
<td>12.71 ± 1.89 a</td>
<td>17.13 ± 1.92 b</td>
</tr>
<tr>
<td>Serum insulin *</td>
<td>20.05 ± 0.04</td>
<td>30.12 ± 1.29 b</td>
<td>35.11 ± 1.09 b</td>
</tr>
<tr>
<td>HbA1c *</td>
<td>2.85 ± 0.42</td>
<td>3.27 ± 0.93 N</td>
<td>4.00 ± 1.31 N</td>
</tr>
<tr>
<td>CRP *</td>
<td>4.01 ± 0.84</td>
<td>4.5 ± 0.95 N</td>
<td>4.62 ± 1.13 N</td>
</tr>
</tbody>
</table>

Note: Units: *, mg/dl; a, ul/ml; %, / - mg/L; P values: < 0.05; b<0.001; N< Non-significant value (Values of the group F1-2 and group F1-3 were compared with group F1-1).

Minerals status in Liver and Kidney (μg/g tissue)- The Zn content of the liver and kidney increased significantly in Groups F1-2 and F1-3 compared to Group F1-1 (Control). However, there was a significant drop in Mn and Cu concentration in both kidney and liver in Group F1-2 and Group F1-3 while compared to Group F1-1 (Control).

Figure 1. Mean Zinc (Zn), Copper (Cu), and Manganese (Mn) in liver of F1 generation rats of group F1-1(Control), group F1-2 and group F1-3 fed on pallet rat diet during 60 days of dietary treatment. [Values are mean ± SD of 6 observations each]. Unit: μg/g tissue; p-values: *< 0.05; (Values of group-F1-2 and group-F1-3 were compared with group-F1-1).
hly potent in inducing observe state of metabolic ds, triglycerides, cholesterol; LDL by that these F1 pellet rat in generations rats. The rise in serum triglycerides, cholesterol and VLDL indicating the development of Cu and Mn compared to the control investigation carried out to see their effect on the metal status in the next generations. The evaluation of metal status in the current taking excessive dosages of supplementary zinc for long periods of time of first and second wistar rat generations has negative effects on the metabolism of leptin and glucose, resulting in the resistance of insulin and of the hypertension, cardiovascular diseases, dyslipidimia and depression can be observed of rats. These observations are consistent with the report that among the offspring of dia infliction with metabolic syndrome of abnormalities and lowered level of HDL group level of HDL human studies which reported compared to the control group total lipids, triglycerides c, VLDL c and lowered HDL c and lowered the level of HDL cholesterol. As observed in parent rats, the profile of blood lipid of F1 generation rats in groups F1-1 and F1-3 rats also showed an increase in the level of total lipids, triglycerides, cholesterol; LDL-c, VLDL-c and lowered HDL-c level after 180 days of treatment of Zn as compared to the control group Table 1. These findings are in corroborating with already reported data from animal and human studies which reported that supplementation of Zn increased the level of both LDL-c and VLDL-c and lowered the level of HDL cholesterol. As observed in parent rats, the profile of blood lipid of F1 generation rats in group F1-1 (control) and group F1-3 rats also showed an increase in the level of total lipids, triglycerides, cholesterol, LDL and VLDL-cholesterol and lowered level of HDL-cholesterol while compared with group F1-1 (control). Our finding indicates a carryover effect of abnormalities associated with the parents to their offspring suggesting thereby that these F1-generation rats were inflicted with metabolic syndrome-X like their parents even though these first-generation rats are fed on standard pellet diet of rats. These observations are consistent with the report that among the offspring of diabetic patients, an increased chances of the hypertension, cardiovascular diseases, dyslipidimia and depression can be observed [22]. In a study, it has been reported that a hypercaloric diet rich in fat, carbohydrate, and other nutrients while fed in rats influences the metabolism, has negative effects on the metabolism of leptin and glucose, resulting in the resistance of insulin and also affects fertility of first and second wistar rat generations [23, 24]. Several studies proved that copper deficiency is commonly related with taking excessive dosages of supplementary zinc for long periods of time [25-27]. But no such investigations have been carried out to see their effect on the metal status in the next generations. The evaluation of metal status in the current investigation indicated a rise in Zn while decrease in the content of Cu and Mn in Groups F1-2 and Group F1-3 rats compared to the control Group F1-1 (control) rats in both liver and kidney like their parents (data not shown here) indicating the development of Cu and Mn deficiencies or an increase in the Cu/Mn to Zn ratio in the various tissues in F1 generations rats. The rise in serum triglycerides, cholesterol and VLDL-cholesterol after 60 days of feeding on standard pellet rat in Groups F1-2 and Group F1-3 rats corresponds very well with the reduction in Cu content in these tissues. Cu

Figure 1 and Figure 2 illustrates the significant increase in Zn level and significant decrease in Cu and Mn level in liver and kidney of F1 generation rats of Group F1-2 and Group F1-3, respectively in comparison to rats of Group F1-1(Control)

4. Discussion

The data of the present investigation revealed that increasing Zn concentration to 50mg and 80mg Zn/kg diet in group-2 and group-3 parent rats respectively increased body weight in the rats. Higher Zn concentration in the diet and increased bodyweight of the rats were directly linked to increased food intake in the parent rats Table 1. The urine of rats in Group-2 and Group-3 also showed positive reaction with Benedict’s test indicating the presence of sugar in them. This implies that Zn is highly potent in inducing observe state of metabolic syndrome-X in rats that are not genetically predisposed to it as reveals in the present study. It has been found that ICR (a strain of albino mice) mice fed on high Zn, high fat diet gained significantly more body weight similar to obese mice than their control counterparts fed on a low Zn, high fat indicating a link between absorption of Zn and fat deposition in obese mice and ICR mice fed on a diet with high-fat [21] as reported in the present investigation. The mating of animals in groups 2 and group-3 parent rats produced offspring with no visible outward abnormality in the neonates and they finished their weaning phase as healthy pups. However, the body weight in the F1-generation rats reveals a non-significant rise in body weight in Groups F1-2 and Group F1-3 as compared to Group F1-1 Table 2 though they are supplemented with conventional pellet rat diet just like in real situation. Since obesity as well as metabolic syndrome-X is considered a genetic illness, it’s unclear if the offspring born from such diabetic mother will develop abnormalities just like their parents.

The profile of blood lipid of Zn supplemented rats in group-2 and group-3 revealed significant increase in the level of total lipids, triglycerides, cholesterol; LDL-c, VLDL-c and lowered HDL-c level after 180 days of treatment of Zn as compared to the control group Table 3. These findings are in corroborating with already reported data from animal and human studies which reported that supplementation of Zn increased the level of both LDL-c and VLDL-c and lowered the level of HDL cholesterol. As observed in parent rats, the profile of blood lipid of F1 generation rats in group F1-1 and group F1-3 rats also showed an increase in the level of total lipids, triglycerides, cholesterol, LDL and VLDL-cholesterol and lowered level of HDL-cholesterol while compared with group F1-1 (control). Our finding indicates a carryover effect of abnormalities associated with the parents to their offspring suggesting thereby that these F1-generation rats were inflicted with metabolic syndrome-X like their parents even though these first-generation rats are fed on standard pellet diet of rats. These observations are consistent with the report that among the offspring of diabetic patients, an increased chances of the hypertension, cardiovascular diseases, dyslipidimia and depression can be observed [22]. In a study, it has been reported that a hypercaloric diet rich in fat, carbohydrate, and other nutrients while fed in rats influences the metabolism, has negative effects on the metabolism of leptin and glucose, resulting in the resistance of insulin and also affects fertility of first and second wistar rat generations [23, 24]. Several studies proved that copper deficiency is commonly related with taking excessive dosages of supplementary zinc for long periods of time [25-27]. But no such investigations have been carried out to see their effect on the metal status in the next generations. The evaluation of metal status in the current investigation indicated a rise in Zn while decrease in the content of Cu and Mn in Groups F1-2 and Group F1-3 rats compared to the control Group F1-1 (control) rats in both liver and kidney like their parents (data not shown here) indicating the development of Cu and Mn deficiencies or an increase in the Cu/Mn to Zn ratio in the various tissues in F1 generations rats. The rise in serum triglycerides, cholesterol and VLDL-cholesterol after 60 days of feeding on standard pellet rat in Groups F1-2 and Group F1-3 rats corresponds very well with the reduction in Cu content in these tissues. Cu
deficiency has been linked to hypercholesterolemia in both animals and humans [28, 29]. In addition to this, Mn deficiency has been demonstrated to raise triglycerides, cholesterol, and VLDL-cholesterol while decreasing HDL-cholesterol [30]. Another possible explanation for the change in lipid parameters in rats with Mn-deficient is that in the endoplasmic reticulum of the cell the level of lipid peroxidation increases which is considered as site for lipoprotein production. This shows that an increase in the amount of triglycerides, cholesterol and VLDL-cholesterol found in Groups FI-2 and Group FI-3 rats in the present study was caused by combined Cu and Mn deficiencies in tissues caused by presence and absorption of excess Zn from the diet. The current data reveal that Zn, even in therapeutic quantities in the diet substitutes Cu and Mn over time, resulting in leaching and more excretion in urine even though an adequate level of these metals are present in the diet as recorded in the liver and kidney of Groups FI-2 and Group FI-3 rats. It has been reported that the descendants of type-2 diabetes mellitus and cardiovascular patients possess a higher tissue Zn which is 2 to 4 times higher and low Cu concentration than their counterparts of non diabetic parents and they are destined to develop type-2 diabetes mellitus and heart disease. This observations suggested that Cu and Zn imbalance in the body continues for a period of time which after exceeding threshold level manifest as disease [31].

Our present study reveals a significant rise in the insulin and haemoglobin Alc (HbA1c) both in Zn supplemented group of rats in comparison to group-1 (control). We also observed similar trend in the F1 generation rats like their parent rats however the increase in their level is non-significant Table 3 & Table 4. The rise in insulin hormone level in both parent and F1-generation rats in our study might be due to deficiency of Cu in their body as low level of Cu can disrupts the metabolism of glucose by its influence of normal consumption of glucose resulting in intolerance of glucose which leads to impaired glucose clearance, hyperinsulinemia, and insulin resistance [32-34]. Mn is also known to be an essential role in homeostasis of glucose and sensitivity of insulin, and its deficiency impacts transport of glucose and their metabolism in adipose cells. As deficiency in Mn is also recorded in the rats from Groups FI-2 and Group FI-3 there might be fewer insulin receptors per cell and is most likely due to a reduction in the number of glucose transporters in Mn deficiency in rat's adipose tissue [35]. Overall, the findings point to a link between increased Zn intake, poor glycemic management, and insulin resistance caused by Cu and Mn deficiency in tissues. Cu and Mn supplementation in the diet appears to be a viable option for avoiding and treating hyperglycemia and hyperinsulinemia caused by high Zn bioavailability [36]. As the glucose level increased in all the group of rats both in parent and F1-generation rats as compared to the control counterpart (data not shown here), it may be the reason for the increase in HbA1c level in the present study and corroborate with study reported by Rohlfing, et al. [37]. The C-reactive protein was found to be raised in the both the groups of parent rats which are fed on Zn enriched diet in respect to Group-I (control) while there were no changes observed in the Group-FI-2 and Group-FI-3 than Group-FI-1 (control) in the current investigation Table 3 & 4.

Since rats in the Group-2 and 3 were inflicted with metabolic syndrome-X, as expected the CRP level of CRP was found to be raised significantly in both group of parent rats. It is reported from other studies that plasma CRP level was significantly elevated with the increase in the metabolic syndrome-X, diabetes mellitus and dyslipidaemia conditions. The main mechanism for rise of this inflammatory protein (CRP) as seen in the present investigation might be due to increased abdominal fat associated with conditions of obese in metabolic syndrome-X. It has been reported that a significant amount of various inflammatory cytokines like and IL-6 and tumor necrosis factor alpha (TNF-α) are formed from the adipose tissues which in turn influence the production of CRP from the liver [38, 39]. The non-significant changes observed in the CRP level in the Group FI-2 and Group FI-3 in our study could imply that the amount of Zn obtained by the F1- rats through placental and milk during weaning period from their Zn-supplemented parents was not greater enough to elicit a change in C-reactive protein status in these young rats.

5. Conclusion
From the present investigation, it can be inferred that long-term exposure to excessive Zn in the diet has several negative implications in the rats and their F1 generations rats. The rising prevalence of cardiovascular diseases, even among younger generations, during the last few decades may be linked to excessive Zn intake from Zn-rich meals, which may affect the body's trace mineral and lipoprotein metabolism to the point where diseases can be manifested. As a result, when Zn intakes are continued for longer period of time, it may rise various potential negative impacts even if such intakes within pharmacological dosage. Based on the observations, we can say that the effect of excessive Zn in the diet is programmed early in the offspring during pregnancy and even after the early phase of gestation, and this may be the explanation for the observed carry-over effect of parents to offspring in the current study.

References


S. Taneja, M. Mahajan, and P. Arya, "Excess bioavailability of zinc may cause obesity in humans," *Experience*, vol. 52, no. 1, pp. 31-33, 1996. https://doi.org/10.1007/bf01922412

A. Sukumar and R. Subramanian, "Elements in hair and nails of urban residents of New Delhi, CHD, hypertensive, and diabetic cases," *Biological Trace Element Research*, vol. 34, no. 4, pp. 89-97, 1992. https://doi.org/10.1007/bf02783901


