Efficacy of Modified Eggs and Chick Muscles on Oxidative Stress of Type-2 Diabetes Mellitus Induced Male Wistar Rats

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Abstract: A modified poultry egg (Indian Patent Application No. 2264/Del-2005) and chick muscle enriched with optimum minerals, vitamin E and omega-3 fatty acid were developed and its efficacy was studied on oxidative stress of Type-2 diabetes mellitus induced male wistar rats. In this study, two groups of rats were fed on semi-synthetic diet containing 20 mg Zn/kg (control, group-I) and 80 mg Zn/kg (group-II) diet respectively for a period of 6 months. The study revealed that the gain in body weight increased in rats in Zn concentration dependent manner. The urine examined on weekly basis showed glucosuria in group-II on week 8 and thereafter. The blood lipid profile displayed a significant rise in serum glucose, total lipids, cholesterol, triglycerides, LDL-cholesterol, VLDL-cholesterol whereas HDL-cholesterol showed a reduction in their levels in group-II rats than their control counter parts. They displayed higher lipid peroxidation products and activities of superoxide dismutase, catalase, glutathione-s-transferase, glutathione reductase, glutathione (reduced) and glucose-6-phosphate dehydrogenase were significantly lowered and revealed a higher Zn concentration and lower Cu, Mg and Mn both in liver and kidney. On day 90, the male rats in group-II after the establishment of type-2 diabetes mellitus, were divided in to two groups- group-IIA and group-IIB. Feeding on these eggs and chick muscles mixed diet in these groups of rats, all the abnormalities were restored and a considerable reduction in lipid peroxidation products and a significant increased in the activities of enzymes per se with reversal of Zn, Cu, Mg and Mn levels closer to the control group were recorded. The present data suggest that these modified egg and chick muscle are effective in ameliorating the oxidative stress in type-2 diabetes mellitus induced male rats.

Keywords: Zinc, modified eggs, chick muscles, oxidative stress, type-II diabetes mellitus.

1. INTRODUCTION

Diabetes mellitus is a global health problem predisposing to markedly increased in cardiovascular mortality and serious morbidity and mortality related to the development of nephropathy, neuropathy and retinopathy [1]. Recently there is increasing evidences in the literature that diabetes is accompanied by imbalances between proxidants and antioxidants factors and this oxidative stress could play a major role in the development of the diabetic complications [2]. The accumulation of lipid peroxide [3] increase in the titre of antibodies against the oxidized LDL have been demonstrated [4] while the activities of the antioxidant enzymes such as superoxide dismutase(SOD), glutathione peroxidise( GPx) may be weakened[5]. It has been seen that oxidative stress has been increasingly implicated in the pathogenesis of diabetes micro and macrovascular diseases [6].

There are reports that excessive Zn in diet induces obesity, diabetes, dyslipidemia and hypertension in experimental animals [7-9]. Higher concentration of Zn and lower concentration of Cu, Mg and Mn in the tissues have been reported to link the ionic imbalance of nutritionally important elements to the etiology of diabetes mellitus [10]. This toxic ionic imbalance has been reported to induce oxidative stress in the case of diabetes mellitus.

A number of dietary supplements having antioxidant activity such as vitamin-E [11], omega-3 fatty acids [12] have been reported to reduce the abnormalities associated with type-2 diabetes mellitus. Cu [13], Mg [14], and Mn [15] have also been shown to be useful in decreasing the severity of the oxidative stress related to this diseases. Therefore non pharmacological intervention is an ideal and attractive way to deal with diabetes mellitus related oxidative stress.

Keeping all these in mind, the characters of macro and micronutrients and their functional aspects, a modified egg (Indian Patent Application No. 2264/Del-2005) and chick were developed in which the essential micro and macronutrients have been optimized for individual inflicted with type-2 diabetes mellitus. Since the conjugated form of minerals and vitamins in natural food products are considered more effective than their synthetic forms, the eggs and chick muscles are the most convenient food items which can be modified by manipulation in their feed. The efficacy of these modified eggs and chick muscles on the oxidative stress of type-2 diabetes mellitus induced male wistar rats is reported in this paper.

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2. MATERIALS AND METHODS

The modified eggs and chick muscles used in the experiment were produced by the modification of the constituents of the bird feed at Jaya Health Care Poultry Farm, Panchkula (Haryana), India. Eggs and chicks obtained after feeding on this modified feed were labelled as modified eggs and chick and analyzed for their cholesterol, triglycerides, total lipids, vitamin-E, omega-3 fatty acids and essential minerals (Zn, Cu and Mg). Lipids were extracted from homogenates of eggs and chick muscles by the method of Folch et al. [16], using chloroform and methanol (2:1) as solvent. Total cholesterol [17], triglycerides [18], lipids [19] were estimated from the lipid extract. Proteins [20], carbohydrates [21] were also estimated. Vitamin-E was estimated according to the method of Taylor et al. [22], as detailed by Barker [23]. Omega-3 fatty acids (Linolenic acid) was estimated according to the method of Bindal and Wadhwa [24], using gas liquid chromatography operated at sensitivity of 1000 with column temperature of 150°C for 15 minutes and 225°C for 12 minutes. Zn, Cu and Mg in the eggs and chick muscles were estimated on atomic absorption spectrophotometer (Electronics Corporation of India Limited, Hyderabad-AAS-4139) using hollow cathode lamps (213.9nm, 324.8nm and 285.2nm for Zn, Cu and Mg respectively) Standards for Zn, Cu, and Mg were prepared by dilution in triple distilled water deionized water.

### 2.1. Experimental Designed

24 male wistar rats weighing 60-70g were procured from Central Animal House of the Panjab University Chandigarh. They were maintained in plastic cages with stainless steel top grill at room temperature (25-28°C) with 14:10 hours L: D cycles and 70-80% RH as per guidelines of Institutional ethics Committee. They were fed on standard pellet feed for one week to acclimatize. Thereafter the rats were divided in to 2 groups: group-I and II.

### 2.2. Preparation of Diet for Rats

For the present investigations, a semi-synthetic diet rich in fat and refined sucrose was preferred over standard rat pellet diet consisting of natural ingredients to keep consistency of the composition of diet particularly fat, sucrose and micronutrients throughout the experiment and to rule out the possibility of Zn-interactions with fibres and phytates which are known to reduce bioavailability of Zn by binding it in digestive tract and may take longer time duration feeding to manifest the impact of excessive Zn in diet [25]. Accordingly isocaloric semi-synthetic diet for the rats was prepared as prescribed by Orgebin-crist et al. [26] (Table 1).

### 2.3. Induction of Type-2 Diabetes Mellitus in Group-II Male Rats

Type-2 diabetes mellitus was induced in group-II male rats by increasing Zn concentration to 80mg

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**Table 1: Composition of Basal Diet**

<table>
<thead>
<tr>
<th>Diet Components</th>
<th>g/100g</th>
<th>*Vitamin Mixture (mg/ kg)</th>
<th>**Mineral Mixture (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>30</td>
<td>Ascorbic acid – 500</td>
<td>CaH₂PO₄ - 25.3</td>
</tr>
<tr>
<td>Agar</td>
<td>2.0</td>
<td>Biotin – 4</td>
<td>CoCl₂ – 0.04</td>
</tr>
<tr>
<td>Corn oil</td>
<td>5</td>
<td>Calcium- D- pentothenate – 320</td>
<td>CuCl₂ – 0.10</td>
</tr>
<tr>
<td>Cellulose</td>
<td>8</td>
<td>Choline chloride – 2500</td>
<td>FeSO₄.7H₂O - 0.60</td>
</tr>
<tr>
<td>Sucrose</td>
<td>51.0</td>
<td>Folic acid – 10</td>
<td>Mn SO₄.5H₂O - 0.31</td>
</tr>
<tr>
<td>*Vitamin mixture</td>
<td>0.50</td>
<td>Inositol – 1000</td>
<td>Mg SO₄.H₂O - 4.05</td>
</tr>
<tr>
<td>**Mineral mixture</td>
<td>3.50</td>
<td>Retinol - 0.31</td>
<td>NaF - 0.088</td>
</tr>
<tr>
<td>Total diet</td>
<td>100</td>
<td>Pyridoxine HCl – 80</td>
<td>KI - 0.004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Riboflavin -120</td>
<td>Na₂CO₃-1.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ergocalciferol - 0.0031</td>
<td>KCL – 3.430</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thiamin HCl – 200</td>
<td>Zn SO₄.7H₂O - 0.088</td>
</tr>
<tr>
<td></td>
<td></td>
<td>α-Tocopherol acetate (E) – 60</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cyanocobalamin - 0.40</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nicotinic acid – 300</td>
<td></td>
</tr>
</tbody>
</table>
Zn/kg in semi-synthetic diets rich in fat and refined sugar following the method of Taneja et al. [9]. This method was preferred over streptozotocin/alloxan treatment since zinc is a natural component of diet [27], promotes absorption of nutrients [28], induced proliferation of adipocytes [8] and is a component of insulin [29]. Accordingly, isocaloric semi-synthetic basal diet (Table 1) was prepared and divided into two parts: (i) Control diet (Diet-IC) consisting of basal diet (per se) containing with 20mg Zn/Kg semi-synthetic diet for Group-I (ii) Diabetes inducing diet (Diet-II-ZnDB) consisting of basal diet in which Zn was increased to 80 mg Zn/Kg semi-synthetic diet for Group-II so as to induce diabetes mellitus in this group.

In order to study efficacy of modified eggs and chick muscles, first chick muscles were cut in to small pieces and the quantity of modified eggs and chick muscles to be mixed were measured in a digital electronic balance (Denver, St-234) to maintain the accuracy of the weights and following diets were prepared: (1) Test diet-A: Four liquid modified eggs weighing 50g each mixed in 1kg of Diet-II-ZnDB for Group-IIA (2) Test diet-B: 200g of chick muscles mixed in 1 kg of Diet-II-ZnDB for Group-IIB. The modified egg typically has the following constituents per 50g liquid egg: protein, 6.5 ± 0.12 g; carbohydrates, 0.63 ± 0.003g; total lipids, 5.84 ± 0.043 g; triglycerides, 1.37 ± 0.008g; cholesterol, 110.05 ± 7.15, linolenic acid, 73.84 ± 3.75mg, vitamin-E, 10.35 ± 0.325 mg, zinc, 0.65 ± 0.002 mg; copper, 3.023 ± 0.045 mg; and magnesium, 2.02 ± 0.015 mg while the composition of chick muscle has total lipids: 1120 ± 0.924 mg/100g; Triglycerides: 587.7 ± 1.445 mg/100g; Cholesterol: 472.6 ± 0.621 mg/100g; Phospholipids: 361.8 ± 1.361 mg/100g; Vitamin-E: 1.20 ± 0.53 mg/100g; Linolenic acid: 62 ± 2.56 mg/100g; Zinc- 3.2 ± 0.016 mg/100g; Copper- 5.7 ± 0.680 mg/100g; Magnesium: 4.0 ± 0.788 mg/100g; Carbohydrates- 0.79 ± 0.036 mg/100g, Proteins: 15.83 ± 0.09 g/100g.

For each diet minerals and water soluble vitamins were ground in sucrose and fat soluble vitamins were dissolved in corn oil. Agar, which serve as a binder was dissolved in 25 ml of triple distilled deionized water, the contents of each diet were thoroughly mixed in separate containers. The dough so formed was mixed with warm water (60°C). On cooling to 40°C, the contents of each diet were thoroughly mixed in separate containers. The dough so formed was put in petridishes and solidified in refrigerator. The solidified diet was cut in to small pieces of 2 x 2 x 2 cm size and stored in the container at the temperature -4°C.

2.4. Feeding of Rats

The type-2 diabetes mellitus in Group-II male rats were found to establish on day 90 and was confirmed by hyperglycaemia state like presence of glucose in urine as tested with Benedict’s test and rise in blood pressure. All the rats (6 male rats) in Group-I (control) were fed on control diet (Diet-IC), Group-II (18 male rats) on diabetes inducing diet (Diet-II-ZnDB) ad libitum and triple distilled water was made freely available to them for a period of 90 days. On day 90, 12 male rats from Group-II were separated and subdivided into two groups each, which were Group-IIA (6 male rats) and Group-IIB (6 male rats). Group-I and II were fed on respective Diet-IC and Diet-II-ZnDB while those of Group-IIA and Group-IIB on Test diet-A and Test diet-B respectively. The experiment was run for all the groups for another 90 days completing 180 days from the start of the experiment. The body weight at the start of dietary treatment and thereafter every week was recorded. Ugbasile blood pressure recording instruments was used for recording the blood pressure and heart rates after anaesthetizing the animals by injecting thiopentone sodium (25mg/kg body weight) intraperitoneally. The urine samples were tested by Benedict’s test for the onset of diabetes every week. After the end of dietary treatment of 180 days, the male rats of each group were sacrificed using diethyl ether as anaesthesia.

The blood samples were collected by puncturing the heart. Blood serum was prepared by centrifuging blood at 2500 rpm for 15 minutes. The freshly prepared serum was analyzed for glucose [30], cholesterol [31], triglycerides [32], HDL-cholesterol [33] and total lipids [19]. The LDL and VLDL cholesterol were calculated by using Friedwald’s equation [34].

The liver and kidney of four groups of rats were removed for the study of enzyme activities. For this purpose, their homogenates were prepared in 50 mM Tris- HCL buffer (pH 7.4) and then centrifuged at 1000 g for 10 minute at 4°C to remove nuclei and debris. Supernatant was again centrifuged at 1000 g for 30 minute to obtain post mitochondrial supernatant (PMS). The levels of lipid peroxidation (LPO) products were evaluated by the method of Beuge and Aust [35] and glutathione (reduced) (GSH) by the method of Ellman [36] in their PMS fraction. Activities of superoxide dismutase (SOD) [37] catalase (CAT) [38], glutathione-s-transferase (Glu-s-T) [39] glutathione reductase (GRD) [40] and glucose- 6-phosphate dehydrogenase (G-6-PD) [41] were estimated in PMS of liver and...
kidney. Protein was evaluated as per of Lowry et al. [20].

Zn, Cu Mg and Mn were estimated on atomic absorption spectrophotometer (Electronic Corporation of India Limited, Hyderabad-AAS 4139) using hollow cathode lamps (213nm, 324nm, 285.2nm and 279.5 nm for Zn, Cu, Mg and Mn respectively). Samples of tissues were digested separately in 3:1 v/v nitric acid and perchloric acid on a sand bath until white ash was formed. The ash was dissolved in 6ml of 10mM HNO₃ and filtered through ash free filter paper before analysis. Standards of Zn, Cu, Mg and Mn (sigma) were prepared by dilution in distilled deionized water (TDW). The data were subjected to statistical analysis applying student’s t-test.

3. RESULTS

The result of the study revealed that during the first 150 days of dietary treatment in group-I & II, body weight increased with the increased in Zn concentration in diet. It was then observed that the body weight of the Group-II rats fall than that of the control counterpart during the next 30 days and at day 180 of the experiment. The blood pressure in the rats of group-II was significantly higher than those of the control group. In contrast, the body weight of the group-IIA and IIB rats showed no significant gained and the blood pressure were comparable with respect to control group at the time of the termination of the experiment (Table 2).

The urine of the animals in group-II continued to react positively throughout the experiment. The blood serum revealed significant increase in glucose, cholesterol, triglycerides, LDL and VLDL- cholesterol and lowered HDL-cholesterol than their control counterpart (Table 3) indicating that the male rats in group-II were diabetic and hypertensive and continued their displayed throughout the experiment. In contrast,

Table 2: Mean Month Wise Body Weight (g) and Blood Pressure (mm of Hg) of Male Rats in Group-I[( Fed on Diet-IC(Control)], Group-II (Fed on Diet-IIZnDB), Group-IIA( Fed on test diet-A) and Group-IIB ( Fed on test diet-B) During 180 Days of Dietary Treatment

<table>
<thead>
<tr>
<th>Time duration (in days)</th>
<th>Group-I(Control)</th>
<th>Group-II</th>
<th>Group-IIA</th>
<th>Group-IIB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Body weight</td>
<td>Blood pressure</td>
<td>Body weight</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>0</td>
<td>64.12 ± 1.20</td>
<td>-</td>
<td>65.10 ± 0.69</td>
<td>-</td>
</tr>
<tr>
<td>30</td>
<td>130.24 ± 1.50</td>
<td>92.86 ± 1.01</td>
<td>180.00 ± 1.12</td>
<td>140.30 ± 1.15</td>
</tr>
<tr>
<td>60</td>
<td>185.21 ± 0.56</td>
<td>96.50 ± 0.710</td>
<td>240.22 ± 1.90</td>
<td>158.51 ± 0.64</td>
</tr>
<tr>
<td>90</td>
<td>225.50 ± 1.70</td>
<td>98.62 ± 1.12</td>
<td>262.23 ± 1.02</td>
<td>170.12 ± 2.10</td>
</tr>
<tr>
<td>120</td>
<td>245.82 ± 1.25</td>
<td>96.10 ± 2.10</td>
<td>270.10 ± 0.82</td>
<td>187.20 ± 1.30</td>
</tr>
<tr>
<td>150</td>
<td>255.10 ± 1.70</td>
<td>104.24 ± 2.31</td>
<td>275.15 ± 1.34</td>
<td>205.12 ± 1.59</td>
</tr>
<tr>
<td>180</td>
<td>260.20 ± 2.20</td>
<td>105.20 ± 2.12</td>
<td>250.10 ± 2.02</td>
<td>220.15 ± 2.12</td>
</tr>
</tbody>
</table>

Values are mean ± SE of 6 observations each; a Animals shifted to test diet-A and B; P values: * < 0.001(Values of Group-II, Group-IIA and Group-IIB were compared with Group-I).

Table 3: Blood Profile of Male Rats of Group-I[( Fed on Diet-IC(Control)], Group-II (Fed on Diet-IIZnDB), Group-IIA( Fed on test diet-A) and Group-IIB ( Fed on test diet-B) after 180 Days of Dietary Treatment

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group-I(Control)</th>
<th>Group-II</th>
<th>Group-IIA</th>
<th>Group-IIB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose*</td>
<td>75.04 ± 1.24</td>
<td>180.12 ± 0.55</td>
<td>78.12 ± 1.12</td>
<td>80.10 ± 0.86</td>
</tr>
<tr>
<td>Total Lipids*</td>
<td>185.40 ± 1.24</td>
<td>272.18 ± 0.85</td>
<td>195.34 ± 0.46</td>
<td>200.22 ± 0.98</td>
</tr>
<tr>
<td>Cholesterol*</td>
<td>55.12 ± 1.52</td>
<td>96.0 ± 0.78</td>
<td>60.24 ± 0.54</td>
<td>62.24 ± 0.52</td>
</tr>
<tr>
<td>Triglycerides*</td>
<td>62.64 ± 1.12</td>
<td>110.50 ± 1.15</td>
<td>65.14 ± 0.26</td>
<td>70.02 ± 0.65</td>
</tr>
<tr>
<td>HDL-cholesterol*</td>
<td>16.35 ± 0.62</td>
<td>10.34 ± 0.25</td>
<td>14.12 ± 0.65</td>
<td>14.0 ± 0.24</td>
</tr>
<tr>
<td>VLDL-Cholesterol*</td>
<td>12.52 ± 0.96</td>
<td>22.10 ± 0.87</td>
<td>13.00 ± 0.96</td>
<td>14.00 ± 0.90</td>
</tr>
<tr>
<td>LDL-Cholesterol*</td>
<td>33.77 ± 0.60</td>
<td>63.56 ± 0.54</td>
<td>32.98 ± 0.24</td>
<td>34.00 ± 0.45</td>
</tr>
</tbody>
</table>

Units: *mg/dl; Values are mean ± SE of 6 observations each; a Estimations made after 90 days of dietary egg and chick muscle treatment; P values: * < 0.001, b < 0.01, c < 0.05, d Non significant values (Values of Group-II, Group-IIA and Group-IIB were compared with Group-I).
the reactivity of urine samples to Benedict's solution started declining in the rats of group-IIA and IIB and it reacted negatively at the end of the experiment. Their blood serum glucose, triglycerides, LDL-c, VLDL-c and HDL-c were comparable to those of the control group-I (Table 3).

The lipid peroxidation products were found to be significantly higher in liver and kidney of group-II comparable to those control group showing weak antioxidant defense system in this group of rats. Their higher levels in group-II male rats coincided with the enzymes of antioxidant defense system i.e. SOD, CAT, Glu-s-T, GRD, GSH and Glu-6-PD whose activities were evaluated significantly less in this group. On the contrary, all the activities of the enzymes mention per se in the rats of group-IIA and IIB fed on test diet A and B were comparable to those of their control counterparts suggesting an improved of antioxidant defense system as revealed by the reduction of lipid peroxidation products in them (Tables 4, 5).

The trace metal status in the liver and kidney of group-II male rats shows higher Zn concentration and lowered Cu, Mg and Mn that the control group-I. Their concentrations were restored in group-IIA and IIB closer to the control rats (Table 6).

4. DISCUSSION

The results revealed that supplementation of greater amount of Zn equal to 80mg/kg and not exceeding 100mg/kg diet to the male rats of Group-II resulted significance gain in body weight, significantly higher blood pressure and their urine reacted positively with Benedict's test suggesting the onset of obesity,
observations. Moreover, it lends support to the similar to presented by patients with coronary heart disease. Pathological cardiovascular abnormalities that are shown to induce a variety of physiological and biochemical effects. In animals, certain dietary elements such as copper restricted diets have been used to study the effects of copper deficiency on cardiovascular function. For example, rats fed on copper-deficient diets have been shown to have increased blood pressure and cholesterol levels, as well as decreased antioxidant capacity.

Hypertension and glucosuria in them. The blood profile also revealed higher total lipids, triglycerides, cholesterol, LDL-c, VLDL-c and glucose levels than those of the control group indicating that the rats in group-II fed on Diet-II-ZnDB were typically diabetic mellitus and dyslipidemia. In group-IIA and IIB male rats fed on test diet-A and B have been discussed. Further, these changes in the blood of male rats of group-I, IIA and IIB were typically diabetic mellitus, dyslipidemia and hypertension in rats feeding on increased amount of Zn have been discussed.

The reduction in the blood pressure in the group – IIA and group - IIB male rats fed on test diet A and B may be due to the availability of adequate levels of vitamins and minerals in the diet. In animals, certain elements such as copper, zinc, and chromium are involved in normal carbohydrate and lipid metabolism. Copper deficiency is associated with increased production of oxygen free radicals and oxidative stress which in turn is associated with the development of cardiovascular disease. Therefore, the consumption of foods rich in copper may help to lower blood pressure and cholesterol levels. Further, the lipid peroxidation products in the liver and kidney of male rats of group-I, IIA and IIB were compared with those of the control group indicating that these rats were under constant oxidative stress. There is an imbalance between free radical production and antioxidant capacity leads to oxidative stress which in turn is associated with the development of cardiovascular disease.

The blood profile of the group – IIA and IIB male rats revealed a reduction in the serum cholesterol, total lipids, triglycerides, LDL and HDL-c, glucose and triglycerides. Advanced glycation or glycosylations end products (AGEs) are the products of glycation and glycations. Advanced glycation or glycosylation end products (AGEs) are the products of glycation and glycations which are increased at an accelerated rate in diabetes mellitus. Increased lipid peroxidation in diabetes mellitus is due to an altered intracellular ratio between free radicals and antioxidant system. And the imbalance between free radical production and antioxidant capacity leads to oxidative stress which in turn is associated with the development of cardiovascular disease.
molecular antioxidants such as GSH and antioxidant scavenging enzymes such as cellular Cu, Zn-SOD, CAT, GPx and Glu-s-T [49-51]. The production of the lipid peroxidation products depends on the activities of the antioxidant enzymes such as SOD, CAT, Glu-s–T, GRD, GSH and Glu–6–PD which registered a significant reduction in liver and kidney of group-II male rats. These observations are in conformity with that of Kinalsks et al. [52], Muhammad et al. [53], Ugochukwu et al. [54] and Sailaja et al. [55] who had reported that patients with type–II diabetes mellitus have significant defect of antioxidant protection, which may cause vulnerability to such oxidative damage and development of the diabetic complications.

The decrease in the lipid peroxidation products in group -IIA and group - IIB male rats even lower than the control level is attributed to the essential minerals present in the modified eggs and chick muscles which led to the restoration of the minerals status in their tissues close to the control rats. The assessment of the mineral status estimated in the group-II male rats revealed an ionic balance wherein an elevated concentration of the Zn in both liver and kidney and that of the Cu, Mg and Mn were lowered while their concentrations were restored, even more than the control group-I in some cases in group-IIA and group-IIB male rats both in the liver and kidney. As a consequence of this, the activities of all the enzymes investigated were increased, even more, in group-IIA and group-IIB rats than control. Metallic ions being an integral component of these enzymes per se, the deficiency of some of the metals such as Cu, Zn, Mg and Mn have been reported to result in the reduction in enzymes activities of antioxidant defense system and increase in peroxidation products in diabetic rats [56, 57]. It was also reported by several studies that underlie the alteration of antioxidant micronutrient status in subjects with type- I and type- II diabetes mellitus [58-61] and may influence antioxidant enzymes activity through disturbances in micronutrient status [59]. SOD requires manganese, copper and zinc, GPx needs selenium and catalase contains haem as cofactor [62, 63]. In view of these, it has been suggested that the supplementation of trace elements such as Se, Cu, Zn and Mn, the essential component of the enzymes structures, may be useful in preventing the development of the diabetic complications [64]. The antioxidant system is a coordinated system in which the deficiencies in any one of the component will impact the efficiency of others. A deficiency in these micronutrients leads to the oxidative stress, which leaves body tissue open to the damaging effects of oxidative intermediates seen in many diseases.

5. CONCLUSION

Thus, the restoration trends noticed in antioxidant enzyme activity level on feeding modified eggs and chick muscle mixed test diet A and B in the rats of group -IIA and group –IIB male rats appear to be due to sparing action of optimized concentration of dietary minerals present in the modified eggs and chick muscles bestowed on their metalloenzymes complex as these minerals are the essential components of these antioxidants enzymes. The activities of the enzymes investigated were increased even more in these group of rats than that of the control group-I indicating thereby complete restoration of antioxidant defense system to the level of control group which in turn led to reduction of lipid peroxidation. This is evident from the data wherein inclusion of modified eggs and chick muscle in the group-IIA and IIB respectively resulted in the restoration of mineral concentration to the baseline. This resulted in the reduction of oxidative stress and other complications associated with type-2 diabetes mellitus. Since our indigenous antioxidant defense are not completely effective, it seems reasonable to propose that dietary micronutrients are particularly important in diminishing the cumulative effects of oxidative damage and the modified eggs and chick muscles serve this function. Therefore, a combination of micro and macronutrients in appropriate amounts in modified eggs and chick muscle is more potent in providing lasting effect than their presence singly and thus reduce the severity of the oxidative stress in Type-2 diabetes mellitus as is evident from the data per se.

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