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Beneficial effects of modified egg* on oxidative stress in F_1- generation of metabolic syndrome-X induced Wistar rat

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Congenital malformations of neonates are one of the adverse effects of diabetic pregnancy which can be prevented by supplementation of vitamin E and C. The survived neonates usually are at higher risk to diabetes, hypertension, dyslipidemia and cardiovascular diseases that may possibly be prevented through antioxidants administration. In view of this information, the efficacy of modified poultry egg enriched with optimum minerals, vitamin E and omega-3 fatty acids was studied on F_1-generation, which were made to survive by feeding them this modified egg to diabetic mothers of Wistar rats. The survived F_1-generation displayed hyperglycemia, dyslipidemia and hypertension like their parents, evaluated after three months of the experiment. Their mineral status revealed a higher Zn and lower Cu, Mg and Mn levels in liver and kidney. Their lipid peroxidation products were however higher and the enzyme activities of superoxide dismutase, catalase, glutathione-S-transferase, glutathione reductase, glutathione (reduced) and glucose -6 phosphate dehydrogenase were significantly lower. In the other group of F_1-generation, fed modified egg mixed diet, a significant reduction in the blood pressure, serum glucose, serum lipid profile, and the lipid peroxidation products, and a significant increase in the activities of enzymes *per se* with reversal of Zn, Cu, Mg and Mn levels closer to the control group were recorded. The data suggest that the modified egg can ameliorate the oxidative stress in F_1- generation of diabetic rats by improving the mineral status in their body.

Keywords: Cu, Diabetes mellitus, Omega-3 fatty acids, Mg, Mn, Modified egg, Oxidative stress, Vitamin- E, Zn

There are increasing evidences that excessive Zn in diet induces obesity, diabetes, dyslipidemia and hypertension in experimental animals[1-3]. Higher concentration of Zn and lower concentration of Cu, Mg and Mn in the tissues of humans of some populations have been reported to link the ionic imbalance of nutritionally important elements to the etiology of diabetes mellitus[4]. The diabetic mothers give birth to the congenital malformed neonates irrespective of the fact whether the diabetes is induced by streptozotocin (STZ) treatment or by administration of excessive Zn in diet or appeared due to genetic disposition[3, 5, 6].

The genetically predisposed diabetics humans and rats absorb and retain greater amount of Zn in their tissues resulting in Cu and Mg deficiencies due to their antagonistic interaction[4, 7-10]. Zn, Cu and Mg being the components of the enzymes of antioxidant defense system including superoxide dismutase (SOD), cytochrome c oxidase (CCO), catalase (CT) and glutathione peroxidase (GP_X)[11], their imbalances depress the activity of antioxidant enzymes and increase lipid peroxidation products inducing oxidative stress which have been implicated in the malformation in neonates of the diabetic mothers[5,6,12]. This lends support to the reports that vitamin E supplementation protects the pregnant rats and their offsprings of STZ induced diabetes and yields a reduction in embryo malformation by restoring fully diabetes induced lipid peroxidation in mother and thus improving gestational outcome[13-15].

The fate of the F_1 generation of diabetes induced rats that survive after vitamin E supplementation is not clear in spite of the fact that they can provide an important clue with respect to the mineral status and the degree of oxidative stress linking the juvenile diabetes mellitus. In view of this, the diabetes mellitus was induced by feeding excessive Zn in diet and the neonates of these diabetic mothers were made to survive by feeding them on a diet mixed with modified eggs (Indian Patent Application No. 2264/Del/2005) following the method of Taneja and Mandal[16]. The survived neonates were examined and they displayed diabetes, hypertension, dyslipidaemia associated with ionic imbalance like their parents. Addition of modified eggs in their diet ameliorated

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most of these abnormalities approaching close to the baseline. The results of the study are reported in this communication.

Materials and Methods

Induction of diabetes mellitus in parents rats—The diabetes mellitus in the parent rats was induced by increasing Zn concentration in semi-synthetic diet rich in fat and refined sugar[3]. This method was preferred over the streptozotocin/alloxan treatment since Zn, a natural component of the diet[17] promotes absorption of nutrients[18-20], induces proliferation of adipocytes[2] and is a component of insulin[21]. Accordingly, isocaloric semi-synthetic basal diet for inducing diabetes mellitus in parents was used following Taneja *et al*.[3] (Table 1). The basal diet was modified as:

(i) *Control diet-I* (Diet-I-C) consisted of basal diet (Table 1) containing 20mg Zn/kg semi-synthetic diet

(ii) *Diabetes inducing diet* (Diet-II-ZS-DB); Zn in the basal diet was increased to 80 mg/kg semi-synthetic diet so as to induce diabetes mellitus in the parent rats (Taneja *et al*[3].).

(iii) *Egg mixed diabetes inducing diet* (Diet-III- ZS-DB-EM): Four modified liquid egg (50 g/egg) were mixed in Diet-II-ZS-DB so as to prevent the malformation of neonates in FI-generation rats (Taneja and Mandal[16]).

The modified eggs used in the experiment were produced by the modification in constituents of the birds feed. Composition of modified egg vs. conventional eggs (50 g liquid egg) typically contained lower amounts of protein, 6.5 ± 0.12 vs 6.7 ± 0.14 (g); carbohydrate: 0.63 ± 0.003 vs. 0.69 ± 0.01(g); total lipids, 5.84 ± 0.43 vs. 7.8 ± 0.07 (g); cholesterol,110.05 ± 7.15 (7.5 mg/g yolk) vs. 234.10 ± 3.56 (mg) (12.0 mg/g yolk) triglycerides, 1.37 ± 0.008 vs. 5.5 ± 0.06 (g) and minerals including Zn, 0.65± 0.002 vs. 2.98 but higher amounts of Cu, 3.02 ± 0.045 vs. 1.36b ± 0.03 (mg/egg) and Mg, 2.02 ± 0.01 vs. 0.61 ± 0.01 (mg/egg) along with higher concentrations of Vitamin E, 10.35 ± 0.325 vs. 0.836 ± 0.03 (mg/egg) and linolenic acid, 73.84 ± 3.57 vs. 36 ± 0.01 (mg/egg).

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

Production of F_1-generation rats—For getting F_1-generation of the parent rats, 15 male and 30 female Wistar rats were obtained from Central Animal House, Panjab University, Chandigarh. They were maintained in plastic cages with stainless steel top grill at 25°-28°C with 10–14 hr L: D cycles at 70-80% RH as per guide lines of Institutional Animals Ethics Committee. They were fed on standard pellet rat feed for one week to acclimatize. Thereafter, the male and female rats were divided into 2 groups, group-I-C (5 male and 10 females) and group-II-ZS-DB (10 males and 20 females) in such a way that their mean initial body weights remained almost similar. The male and female rats in group-I and II were kept in separate

Table 1—Composition of basal diet

Diet components	(g/100g)
Casein	30
Agar	2.0
Corn oil	5
Cellulose	8
Sucrose	51.0
*Vitamin mixture	0.50
**Mineral mixture	3.50
Total diet	100

*Vitamin mixture (mg/ kg)
Ascorbic acid – 500
Biotin – 4
Calcium- D- pentothenate – 320
Choline chloride – 2500
Folic acid – 10
Inositol – 1000
Retinol - 0.31
Pyridoxine HCl – 80
Riboflavin -120
Ergacalciferol - 0.0031
Thiamin HCl – 200
α-Tocopherol acetate (E) – 60
Cyanocobalamin - 0.40
Nicotinic acid – 300

**Mineral mixture (g/kg)
CaH_2PO_4 - 25.3
$CoCl_3$ – 0.04
$CuCl_2$ – 0.10
$FeSO_4 7H_2O$ - 0.60
Mn $SO_4.5H_2O$ - 0.31
Mg $SO_4 .H_2O$ - 4.05
NaF - 0.088
Kl - 0.004
Na_2CO_3 -1.15
KCl – 3.430
Zn $SO_4 .7H_2O$ - 0.088

cages and fed diet-I-C and diet-II-ZS-DB for 75 days. During the course of the treatment, their urine samples were examined. On 56 days onward, the urine of the rats in group-II-ZS-DB gave positive Benedict's test and continued to occur till day 75 indicating the onset of the glucosuria in them.

On day 75, the group-II-ZS-DB rats were subdivided into two subgroups having 5 males and 10 females each. The rats in one of the subgroup-II were continued to feed on their parent diet- II-ZS-DB, the other subgroup-II was fed on egg mixed diet –III-ZS-DB-EM and labeled this group as group-III-ZS-DB-EM. They were fed on their respective diet for 30 days.

After 105 days of dietary treatment, the male and female parent rats in each group were allowed to mate (1 male and 2 females/cage) and the pregnancy was tested in the females by the presence of sperms in vaginal smears. The presence of sperms in the vaginal smears was considered as day-1 of the pregnancy and pregnant females were separated from the males. After gestation period of 21 days, the number of pups delivered and other abnormalities in the pups if any were recorded. The neonates of group-II-ZS-DB parents who did not received modified egg treatment died during weaning period. The details of this had been previously discussed[16]. In contrast the pups of the neonates of group-III-ZS-DB-EM parents that were fed egg mixed diet survived as healthy offspring. These survived offspring were used for the study of oxidative stress.

Feeding of F_1- generation—The survived F_1-generation of control group-I and group-III-ZS-DB-EM after weaning period were fed on pellet rat diet for 9 days. On day 30 after birth (after 9 days of weaning period), the male pups of group-I (group F_1-IC) were continued to feed a standard pellet rat feed and those of the parents of group-III-ZS-DB-EM were subdivided into two groups: i.e one such group was fed rat pellet feed like those of control group (Group-F_1-II-P-Db) while other subgroup; i.e. group F_1-III-P-EM was fed egg mixed rat pellet feed (4 liquid eggs, 50g each/kg diet). These three groups of F_1-generation were fed their respective diets for 120 days.

Data recording—During this period, the body weight and heart rates of the parents and their offspring were recorded. Ugobasile blood pressure recording instrument, installed at Pharmacology Division, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh, India, was used for this purpose after anaesthizing the animals by injecting thiopental sodium (25 mg/kg body weight, ip).

Bioassays of blood profile of F-1 generation rats—After the end of dietary treatment to groups F_1-IC, F_1-II-P-Db and F_1-III-P-EM of 120 days, the animals in each group were anaesthetized by ethylene ether. Their blood samples were collected by puncturing the heart and the blood serum was prepared by centrifuging blood at 2500 rpm for 15 min. Freshly prepared serum was analyzed for glucose[22], cholesterol[23, 24], triglycerides[25, 26]. HDL-cholesterol[27] (all by using commercially available kits-Reckon Diagnostics Pvt, Ltd, Baroda, India and ERBA Diagnostics Mannheim GmbH, Mannheim, Germany, supplied through Transasia Bio-Medicals LTD,Daman), total lipids[28] and phospholipids[29]. LDL-cholesterol and VLDL-cholesterol were calculated by Friedwalds equation[30].

Enzyme bioassay of F_1- generation rats—The liver and kidney of three groups of F-1 generation were removed for the study of enzyme activities. For this purpose, their homogenates were prepared in 50m*M* Tris- HCL buffer (*p*H 7.4) and then centrifuged at 1000 *g* for 10 min. at 4°C to remove nuclei and debris. Supernatant was again centrifuged at 1000 *g* for 30 min to obtain post mitochondrial supernatant (PMS). The levels of lipid peroxidation (LPO) products were evaluated by the method of Beuge and Aust[31] and glutathione (reduced) (GSH) by the method of Ellman[32] in their PMS fraction. Activiies of superoxide dismutase (SOD)[33] catalase (CAT)[34], glutathione-s-transferase (Glu-s- T)[35] glutathione reductase (GRD)[36] and glucose-6-phosphate dehydrogenase (G-6-PD)[37] were estimated in PMS of liver and kidney. Protein was evaluated as per of Lowry *et al*[38].

Estimation of minerals in F_1-generation rats—For the estimation of Zn, Cu, Mg and Mn, the samples of liver and kidney were digested separately in 3:1(v/v) nitric acid and perchloric acid on a sand bath until a white ash formed. The ash was dissolved in 6ml of 10m*M* HNO_3 and filtered through ash free filter paper before analysis. Zn, Cu, Mg and Mn were estimated on atomic absorption spectrophotometer (Electronic Corporation of India Limited, Hyderbad-AAS4139) using hollow cathode lamps (213.9, 324.8, 285.2 and 279.5 nm for Zn, Cu, Mg and Mn respectively). Standards of Zn, Cu, Mg and Mn from Sigma Chemical Co., USA were prepared by dilution in triple distilled deionised water (TDW).

Statistical analysis—All the results were analyzed by one way ANOVA.

Results

The results revealed that the supplementation of Zn in amount equal to 80 mg/kg in fat and refined sugar based semi- synthetic diet fed to the parents (Group-II-ZS-DB) resulted in significant higher gain in their body weight, displayed significantly higher blood pressure and heart rates (Tables 2, 3) and their urine reacted positively with Benedict's test suggesting the onset of obesity, hypertension and glucosuria in them. Their neonates had lower body weight than those of the control group-I-C at the time of birth. They displayed various malformations such as uncoordinated movement of body part, smaller eye size and the higher ratio of 'head' to 'rest of the body'. None of them could complete the weaning period and died at different time intervals. The rats of the group-III-ZS-DB-EM parents fed a modified egg mixed diet delivered healthy neonates, having more body weight than those of control rats. No apparent malformation was observed in them. This F_1-generation was used for the study of enzymes of oxidative stress.

The survived F_1-generation of pups of the two groups, i.e. group-F_1 and group-III-ZS-DB-EM were fed initially a pellet diet for 9 days. After 30 days of their birth, the F_1 pups in group-III-ZS-DB-EM were divided into 2 subgroups, i.e. group FI-II-P-Db and group F_1- III-P-EM. The F_1- rats of control group (F_1-IC) and F_1-II-P-DB were fed a rat pellet feed and that of the group- F_1-III-P-EM an egg mixed rat pellet feed.

No significant difference in the change of body weight among the three different dietary groups of F_1-generation was recorded during the three months of dietary treatment (Table 4). However the blood pressure and heart rate in rats of group- F_1-II-P-DB was 50 and 35% higher respectively than those of the control group FI-C (Table 5). In contrast, both BP and heart rate in the rats of group F_1-III-P-EM fed a egg mixed pellet diet were comparable with respect to control group at the time of the termination of the experiment (Table 5).

The urine of the animals in group F_1-II- P-Db continued to react positively to Benedict's test throughout the experiment. The blood serum revealed glucose higher by 57%, triglycerides by 62%, LDL-c by 167% and VLDL-c by 61% than their control counterparts (Table 6), indicaing that neonates which survived the weaning period were diabetic and hypertensive and continued their display throughout the experiment. In contrast, the reactivity of urine to Benedict's solution started declining after 30 days and onward in rats of group- F_1-III-P-EM and it reacted

Table 2—Mean initial and final body weights (g) of parent rats in Group-I (20mg Zn/kg diet) Group-II-ZS-DB (80mgZn/kg diet) and group-III-ZS-DB-EM (80mgZn+ 4 liquid egg/kg diet) after 105 days of dietary treatment

[Values are mean ± SE of 10 observations each]

	Group-I (Control)	Group-II-ZS-DB	Group-III-ZS-DB-EM
		Males	
Initial weights	63.5 ± 0.76	66.0 ± 1.00	64 ± 0.50
Final weights	273.0 ± 0.82	376.0 ± 0.06[a]	362.5 ± 1.50 [a]
		Females	
Initial weights	60.0 ± 1.05	64.0 ± 0.67	62.5 ± 1.53
Final weights	223.0 ± 0.82	261.5 ± 0.76[b]	250 ± 1.11[a]

P values: [b]< 0.001(Values of Group-II-ZS-DB and Group-III-ZS-DB-EM are compared with Group-I).Group-III-ZS-DB-EM was shifted to diet no-III-ZS-DB-EM after 75 days of dietary treatment of the diet given to Group-II-ZS-DB.

Table 3—Mean systolic blood pressure (mm. Hg) and heart rates (beats/min) in parent rats of Group-I (20mg Zn/kg diet) Group-II-ZS-DB (80mgZn/kg diet) and group-III-ZS-DB-EM (80mgZn+ 4 liquid egg/kg diet) after 105 days of dietary treatment

[Values are mean ± SE of 10 observations each]

	Group-I (Control)		Group-II-ZS-DB		Group-III-ZS-DB-EM	
Time duration in days	Blood Pressure	Heart Rate	Blood Pressure	Heart Rate	Blood Pressure	Heart Rate
			Males			
30	92.0 ± 0.82	184.8±4.57	143.0±0.89[a]	350.0±3.01[a]	142.5±0.83[a]	344.2±3.52[a]
105	101.4±0.92	232.1± 0.85	196.9±0.69[a]	400.0±3.08[a]	181.9±0.76[a]	390.6±3.60[a]
			Females			
30	86.5±0.76	181.2±3.54	140.0±1.05[a]	341.7±2.85[a]	138.0±0.82[a]	340.5±4.50[a]
105	94.1± 0.82	225.5±1.43	183.3±0.76[a]	390.0±4.20[a]	174.3±0.86[a]	380.8±3.10[a]

P values: [a]< 0.001(Values of Group-II-ZS-DB and Group-III-ZS-DB-EM are compared with Group-I).Group-III-ZS-DB-EM was shifted to diet no-III-ZS-DB-EM after 75 days of dietary treatment of same diet given to Group-II-ZS-DB.

negatively at the end of the experiment. Their blood pressure and heart rate declined and their blood serum glucose, triglycerides, LDL-c and VLDL-c levels were comparable to those of the control group (Table 6).

The lipid peroxidation products in F_1-generation were found to be higher in the liver and kidney of group F_1-II-P-DB and lesser in group F_1- III- P-EM comparable to those of control group F_1-IC showing weak antioxidant defense system in former than the latter two groups. Their higher levels in group-FI-II-P-DB 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, their activities in the rats of group F_1-III-P-EM were comparable to those of their control counterparts suggesting an improved status of antioxidant defense system as revealed by the reduction of lipid peroxidation products in them (Tables 7, 8).

The difference in the activities of the enzymes of antioxidant defense system and lipid peroxidation products coincided with the difference in mineral status in different groups. Zn concentration was higher and Cu, Mg and Mn concentrations were lower in the liver and kidney of the group-F_1-II-P-DB. Their concentration were restored in group-F_1-III-P-EM closer to control rats which indicated that the treatment of modified eggs ameliorated oxidative stress by improving the antioxidant defense system through restoring the mineral status in their tissues (Table 9).

Discussion

The data suggest that the increase of Zn concentration to 80 mg/kg in diet of parent rats resulted in an increase in the body weight, blood

Table 4—Mean body weight (g) of male rats of F_1-generation in Group F_1-IC (control) Group F_1-II-P-DB (fed on pellet rat feed) and Group F_1-P-EM (fed on eggs mixed pellet rat feed)

[Values are mean ± SE of 6 observations each]

Time duration (days)	Group F_1-IC (control)	Group F_1-II-P-Db	Group F_1-III-P-EM
30	98.16 ± 0.54	98.0 ± 0.57	97.0 ± 0.23
60	171.6 ± 3.47	170.0 ± 1.18 ^{N}	176.6 ± 2.38 ^{N}
90	223.3 ± 1.52	218.3 ± 0.96 ^{N}	232.5 ±1.95 ^{a}
120	251.2 ± 2.68	240.8 ± 0.76 ^{N}	260.0 ± 1.18 ^{a}

P values: ^{a}<0.05; ^{N}< Non significant value (values of Group F_1-II-P-DB and Group F_1-III-P-EM were compared with Group F_1-I-C)

Table 5—Mean blood pressure (mm. Hg) and heart rate (beats/min) of male rats of F_1-generation in Group F_1-IC (control), Group F_1-II-P-DB (fed on pellet rat feed) and Group F_1-III-P-EM (fed on eggs mixed pellet rat feed) recorded after 120 days

[Values are mean ± SE of 6 observations each]

	Blood pressure	Heart rate
Group F_1-IC (Control)	148.5 ± 4.00	280.3 ± 3.09
Group F_1-II-P-DB	220.7± 1.92 ^{a}	370.8 ± 2.99 ^{a}
Group F_1–III-P-EM	140.0 ±1.67 ^{N}	275.6 ± 2.72 ^{N}

P values: ^{a}<0.001 ^{N} < Non significant value(values of Group F_1-II-P-DB and Group F_1-III-P-EM were compared with Group F_1-I-C)

Table 6—Blood profile of male of F_1-generation in Group F_1–IC (control) Group-F_1-II-P-DB (fed on pellet rat feed) and Group-F_1-III-P-EM (fed on eggs mixed pellet rat feed).

[Values are mean ± SE of 6 observations each]

Parameters	Group F_1- IC (Control)	Group F_1 – II-P-DB	Group F_1-III-P-EM
Total lipids	210.12 ± 6.63	228.23 ±13.3 ^{a}	202.8 ± 4.18 ^{c}
Cholesterol	60.59 ± 4.11	108.1 ± 2.66 ^{a}	60.5 ± 2.77 ^{N}
Triglycerides	70.45 ± 3.98	114.46 ± 1.29 ^{a}	67.60 ± 2.50 ^{c}
Phospholipids	47.05 ±1.40	37.18 ± 0.92 ^{b}	45.57 ± 1.68 ^{N}
HDL-cholesterol	20.2 ± 0.99	15.9 ± 0.75 ^{c}	26.9 ± 1.18 ^{c}
LDL-cholesterol	26.2 ± 3.59	70.2 ± 2.13 ^{a}	20.1 ± 3.11 ^{c}
VLDL-cholesterol	14.1 ± 0.82	22.8 ± 0.38 ^{a}	13.4 ± 0.49 ^{N}
Glucose	66.0 ± 1.56	103.9 ± 4.8 ^{a}	64.6 ± 1.47 ^{N}

P values; ^{a}<0.001; ^{b}<0.01; ^{c}<0.05; ^{N}< Non-significant value (values of Group F_1-II-P-DB and Group F_1-III-P-EM were compared with Group F_1-I-C)

Table 7—Mean lipid peroxidation and enzyme activities in liver of male rats of F_1-generation in Group F_1-IC (control) Group F_1-II-P-DB (fed on pellet rat feed) and Group F_1-III-P-EM (fed on eggs mixed pellet rat feed).

[Values are mean ± SE of 6 observations each]

Parameters	Group F_1-IC (control)	Group F_1-II-P-DB	Group F_1-III-P-EM
Lipid peroxidation$^{\$}$	0.89 ± 0.04	1.15 ± 0.04 ^{a}	0.72 ± 0.02 ^{N}
Superoxide dismutase*	13.2 ± 0.13	10.9 ± 0.28 ^{a}	14.6 ± 0.18 ^{a}
Catalase**	47.03 ± 0.30	39.9 ± 0.94 ^{a}	50.95 ±1.14 ^{c}
Glutathione-s-transferase$^{\&}$	0.632 ± 0.01	0.522 ± 0.01 ^{c}	0.790 ± 0.02 ^{c}
Glutathione (Reduced)$^{\#}$	5.9 ± 0.14	4.5 ± 0.13 ^{a}	6.3 ± 0.17 ^{N}
Glutathione reductase $^{\#\#}$	5.29 ± 0.26	4.22 ± 0.17 ^{b}	5.38 ± 0.33 ^{N}
Glucose-6-phosphate dehydrogenase $^{\#\#\#}$	6.9 ± 0.29	5.15 ± 0.42 ^{a}	8.1 ± 0.36 ^{a}

Units: $^{\$}$: n mol MDA produced/ hr/ mg protein; *: unit/mg protein; **: µ mol H_2O_2 decomposed/ min /mg protein; $^{\&}$: µ mol of CDNB-GSH conjugate formed/min/mg protein; $^{\#}$: n mol GSH/mg protein; $^{\#\#}$: n mol NADPH oxidized/min/mg protein; $^{\#\#\#}$: n mol NADPH formed/min/mg protein. *P* values; ^{a}<0.001; ^{b}<0.01; ^{c}< 0.05; ^{N}< Non-significant value (values of Group F_1-II-P-DB and Group F_1-III-P-EM were compared with Group FI-I-C)

Table 8—Mean lipid peroxidation and enzyme activities in kidney of male rats of F_1-generation in Group F_1-I-C (control) Group F_1-II-P-DB (fed on pellet diet) and Group F_1-III-P-EM (fed on egg mixed pellet rat feed).

[Values are mean ± SE of 6 observations each]

Parameters	Group F_1-IC (control)	Group F_1-II-P-DB	Group F_1-III-P-EM
Lipid peroxidation $^{\$}$	0.69 ± 0.03	0.96 ± 0.02 ^{a}	0.53 ± 0.02 ^{N}
Superoxide dismutase*	12.1 ± 0.21	10.85 ± 0.25^{a}	13.5 ± 0.12 ^{a}
Catalase**	44.5 ±1.07	36.1 ± 0.68^{a}	48.01 ± 1.25 ^{b}
Glutathione-s-tansferase$^{\&}$	0.543 ± 0.01	0.427 ± 0.01^{c}	0.65 ± 0.01^{N}
Glutathione (Reduced) $^{\#}$	4.6 ± 0.06	3.65 ± 0.07^{a}	5.3 ± 0.07^{c}
Glutathione reductase $^{\#\#}$	3.98 ± 0.19	2.36 ± 0.16^{a}	3.82 ± 0.23^{N}
Glucose-6-phosphate dehydrogenase $^{\#\#\#}$	5.5 ± 0.23	3.7 ± 0.33^{a}	7.1 ± 0.51^{a}

Units: $^{\$}$: n mol MDA produced/ hr/ mg protein;*: unit/mg protein; **: µmol H_2O_2 decomposed/ min /mg protein; $^{\&}$: µ Mol of CDNB-GSH conjugate formed/min/mg protein; $^{\#}$: n mol GSH/mg protein; $^{\#\#}$: n mol NADPH oxidized/min/mg protein; $^{\#\#\#}$: n mol NADPH formed/min/mg protein.

P values: ^{a}<0.001; ^{b}<0.01; ^{c}< 0.05; ^{N}< Non significant value (values of Group F_1-II-P-DB and Group F_1-III-P-EM were compared with Group F_1-I-C).

Table 9—Mean Zinc(Zn), Copper(Cu), Magnesium(Mg) and Manganese(Mn) concentrations in the liver and kidney of the male rats of F_1-generation in Group F_1-I-C (control fed on pellet feed), Group F_1-II-P-DB (fed on pellet rat feed) and Group F_1-III-P-EM (fed on egg mixed pellet rat feed).

[Values are mean ± SE of 12 observations each]

Parameters (µg/g tissue)	Group F_1- I-C (control)	Group F_1-II-P-DB	Group F_1-III-P-EM
Liver Zn	9.1 ± 1.03	14.8 ± 0.95^{a}	9.6 ± 0.45^{N}
Liver Cu	19.7 ± 0.92	11.2 ± 0.50^{a}	20.6 ± 1.50^{c}
Liver Mg	90.0 ± 3.13	60.6 ± 1.60^{a}	94.9 ± 0.24^{N}
Liver Mn	57.2 ± 1.31	41.0 ± 1.86^{a}	69.2 ± 1.99^{a}
Kidney Zn	9.0 ± 0.76	12.5 ± 0.63^{c}	8.5 ± 0.97^{N}
Kidney Cu	42.0 ± 1.30	31.6 ±1.23^{a}	40.5 ± 1.31^{N}
Kidney Mg	72.2 ± 1.58	56.3 ± 2.41^{a}	82.0 ± 3.48^{a}
Kidney Mn	36.0 ± 0.82	28.2 ± 0.84^{a}	45.5 ± 0.32^{a}

P values: ^{a}<0.001; ^{b}< 0.01; ^{c}< 0.05; ^{N}< Non-significant value (values of Group F_1-II-P-DB and Group F_1-III-P-EM were compared with Group F_1-I-C).

pressure, heart rate and glucosuria. These disorders have been reported prerviously[3] and the contribution of Zn in induction of obesity, diabetes mellitus, dyslipidaemia and hypertension in wistar rats fed a similar diet containing either 40 mg or 80 mg Zn/kg diet as used in the present investigations have been discussed at length.

The mating of these diabetic parent rats in group-II-ZS-DB delivered offspring which showed certain degree of malformations and died during the course of the weaning period. Such complications in the rats fed high Zn content during pregnancy have been detailed earliar[16] and also in NIDDM pregnant women[39-41] which have been associated with weak antioxidant defense system, since maternal administration of antioxidants such as vitamin E and C can prevent diabetes induced dysmorphogenesis in mid and late pregnancy[41, 42]. The observed dosage dependency of the antioxidant treatments is related to successive reduction of lipid peroxidation in offspring supporting the view of disturbed metabolic handling in diabetic rats. Although vitamin E and C used in semi-synthetic diet during present study were adequate during the pregnancy of the control group-I mothers, their amount in group-II-ZS-DB were not sufficient enough to prevent the occurrence of adverse effect of excessive Zn during the embryogenesis or during the suckling period of the neonates. However, the addition of antioxidants rich modified eggs in the diet of the parent group-III-ZS-DB-EM a month prior to the mating resulted in the delivery of the normal neonates. These observations suggest that the requirement of antioxidants in Zn induced diabetic mothers is more than their non diabetic mothers. These modified eggs being rich in vitamin E, they helped in protecting the embryos against the onslaught of free radicals of oxygen species products by providing instantly the antioxidant substrate.

The survived F_1- generation of diabetic mothers in group F_1-II-P-DB when fed a standard rat pellet feed for the 120 days after the weaning period, gained weight little less than those of the control group F_1-IC. Their blood pressure and heart rates were higher and blood profile also revealed higher total lipid, triglycerides, cholesterol, LDL-c, VLDL- c and glucose levels than those of the control group FI-IC, suggesting thereby that the rats in group F_1-II-P-DB were typically diabetic with complications of hypertension and dyslipidemia like their parents[3]. In contrast, the control group-F_1-C pups were normal showing no signes of either hypertension or hyperglycemia or dyslipidemia in spite of the fact that they were fed on similar diet like those of the group-F_1-II-ZS-P-DB. These observations support the earlier report that there are increased chances of the hypertension, dyslipidemia and cardiovascular diseases, among the offspring of diabetic patients[43].

Persistence of hyperglycemia results in an increased production of free radicals of oxygen species through glucose auto-oxidation and non–enzymatic glycations[44]. Also, increased lipid peroxidation in the rats has been linked to altered intracellular ratio between free radicals and antioxidant defense system[45]. Since the group FI-II-P-DB rats were diabetic, as expected the lipid peroxidation products were evaluated higher in them than those of the control rats indicating that these rats were under constant oxidative stress.

The production of the lipid peroxidation products also 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 F_1-II-P-Db rats. These observations are in conformity with those of Maxwell *et al.*[46], Muhammad *et al.*[47], and Sailaja *et al.*[48], who had reported that patients with Type–II diabetes mellitus have significant defect of antioxidant protection, which may cause vulnerability to oxidative damage and development of the diabetic complications.

The assessment of mineral status in FI-generation revealed an ionic imbalance in group F_1II-P-DB wherein the Zn concentrations in liver and kidney were higher and that of Cu, Mg and Mn were lower compared to their control counter parts in spite of the fact that the control group F_1-IC and group-F_1-II-P-DB were fed a same rat pellet feed. This ionic imbalance may be attributed to the over expression of Zn metallotheionein gene during embryonic period and continued to exist during their growth phase. As a result of this, they absorbed and retained greater amount of Zn than their control counterpart leading to deficiencies of Cu, Mg, and Mn due to their interactions. This ionic imbalance resulted in hypertension, dyslipidemia and hyperglycemia in this group of F_1- generation rats and consistent with mineral status in NIDDM and hypertension as reported in some human populations[49-51] and some genetic diabetic animals[52, 53].

It has also been reported that the potentially susceptible young Indian women descendents of NIDDM[54] and myocardial infarct patients[55] have higher Zn and low Cu concentrations in their hair than those who have no family history of these diseases. This implies that ions metabolism in them is defectively programmed in early embryonic development in the offspring of such parents and develop the deficiencies of Cu, Mg and Mn as the growth advances. The deficiencies of these elements are linked to subsequent development of hyperglycemia, dyslipidemia and hypertension[56-60]. This may explain the possible reason of the transmission of diabetes in the offspring of the diabetic parents observed in the present study. These metallic ions being integral components of enzymes such as SOD, GPx and CAT, their imbalance/ deficiencies have been reported to reduce the activities of the enzymes of antioxidant defense system and increase in peroxidation products[9, 10, 61] as observed in the present study. Several reports underlie the alteration of antioxidants micronutrient status in subjects with type-I and type-II diabetes mellitus[61-64].

In view of this information, 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[65] as the damaging oxidative species (reactive oxygen, nitrogen and others) arise as by products of metabolism and physiological mediators and signaling molecules[66]. The levels of these oxidative intermediates may be held in check by antioxidant defense system. The components of this system are the macronutrients like vitamin-C and E or are dependent on dietary micronutrients such as Cu, Zn and Mn etc.

The decrease in the lipid peroxidation products in group F_1-III-P-EM rats even lower than that of the control level is attributed to the essential minerals present in the modified eggs which led to the restoration of the minerals status in their tissues. The activities of the enzymes investigated were increased, even more, in this group of rats than control group (F_1- IC) implying thereby a complete restoration of the antioxidant defense system to the levels of control group that in turn led to the reduction of lipid peroxidation products. This is evident from the data wherein the inclusion of modified eggs in the diet of group F_1-III-P-EM resulted in the recovery of the mineral concentrations in tissue to the baseline and ameliorated the deficiencies of Cu, Mn and Mg and reduced the level of Zn. This resulted in reduction of the oxidative stress and restored the blood profile status, blood pressure and heart rate close to the control.

In summary, the short treatment of these modified eggs to the diabetic mothers protects the embryos

from the malformations during pregnancy but do not ameliorate the diabetes, dyslipidemia and hypertension in the offspring like those of STZ induced diabetics[5]. After the weaning period, when their neonates (F_1-generation) are fed the modified egg mixed diet for a longer period, the restoration of minerals concentrations in tissues leads to the improvement of the enzymes of antioxidant defense system and associated defects of diabetes, dyslipidemia and hypertension are ameliorated in them. These eggs can serve as a dietary supplement to maintain the mineral status in the offspring of diabetic parents and can prevent the oxidative stress particular in areas where population is consuming food loaded with excessive Zn such as some states of India[4, 51].

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