THE ENVIRONMENTAL INJURIES ARISING FROM ZINC DEFICIENCY IN THE EGYPTIAN ENVIRONMENT AND METHODS OF THEIR CIRCUMVENTION

By
Sherif El-Sayed Aly Badr
B.Sc. Ain Shams University, 1986

A Thesis Submitted in Partial Fulfillment
Of
The Requirement the Master Degree
In
Environmental Sciences

Department of Biology and Natural Sciences
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Under The Supervision of:

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LIST OF ABBREVIATIONS

BPH	Benign Prostatic Hyperplasia.
[Ca]	Calcium concentration
$CaNa_2EDTA$	Calcium di sodium Ethylene Di amine Tetra Acetate
D-	Dextrorotatory
d^{-1}	Per day
DHT	dihydrotestosterone
DL-	Racemic mixture
dl	Deciliter
EDTA	Ethylene Di amine Tetra Acetic acid
H & E	Hematoxylin and Eosin
IEC	The International Electronical Commission.
ISO	The International Organization for Standerization.
L-	Levorotatory
mm	Millimeter
mmol	Millimole
Na_2EDTA	Di sodium Ethylene Di amine Tetra Acetic acid
NaFeEDTA	Sodium ferric ethylene di amine tetra acetate
ND	Non Detective
nm/min.	Nanometer per minuet
P	Significance level
pH	- log $[H^+]$
Ph / Zn	Phytic acid to zinc ratio
[Phytate]	Phytate concentration
redox	Reduction- Oxidation Activities.
U/l	Unite per liter
[Zn]	Zinc concentration
Zn-EDTA	Zinc Ethylene Di amine Tetra Acetic acid
$ZnNa_2EDTA$	Zinc di sodium ethylene di amine tetra acetat

ABSTRACT

The environmental injuries arising from zinc deficiency in the Egyptian environment and methods of their circumvention; Sherif El-sayed Aly Badr; M.D. thesis, Institute of Environmental Studies and Research; Biology and Natural Sciences.

Zinc deficiency in human is widespread throughout the world. After iron, zinc is the second most abundant trace metals in the human body where, an average 70-kg adult human contains 2.3g of zinc. The food surveys which were carried out by the agricultural sector in Egypt, which is the first responsible for providing food for citizens, exemplified in some institutes of agricultural research center, confirmed that, there is a great zinc deficient in pre school children (2-6 years old) and also in women (pregnant- lactating- who don't work). It was found also that, the low zinc intake is varied from urban to rural as compared to the international recommended dietary allowance. 5-weeks-old Swiss Webster out bred male mice were caged individually in two different experimental designs, the first design was in stainless steel cages of *adlibitum* feeding. The analysis of the previously prepared 5- different diets were done after complete homogenization of their ingredients. Mice group (A) were fed zinc deficiency diet *adlibitum* of "9.6 mg Zn / kg diet, mice group (B) were fed zinc controlled *adlibitum* diet "31.3 mg Zn / kg diet", mice group (C) were fed zinc supplemented *adlibitum* diet "60mg Zn /kg diet", zinc source of last groups is $ZnSO_4.7H_2O$. On the other hand, mice groups (D) and (E) were fed zinc supplemented *adlibitum* diet included phytic acid in concentration of 1497.0 and 1505.4 mg / kg diet

respectively, as well as, their zinc sources are $ZnSO_4.7H_2O$ in concentration of 58 mg / kg diet in diet of mice group (D) and $ZnNa_2EDTA$ in concentration of "58.4 mg / kg diet" in diet of mice group (E). Experiments were initially designed to examine the simultaneous interaction between both zinc and other nutrient metals as calcium, iron and copper and the effect of dietary fiber of phytic acid on zinc absorption and study the impact of zinc absorption enhancement by using a chelating source of zinc as EDTA on the utilization of these nutrients. The duration of this experiment was 4- weeks divided into 2- intervals; 2- weeks for each interval. Serum calcium and iron were not affected by neither zinc deficiency nor zinc supplementation. Due to the aggressive competition between zinc and copper absorptions on the same cites of small intestine, copper absorption was positively highly affected by zinc deficient diet and recorded highly increasing in HDL- C "good cholesterol" and was negatively highly affected by zinc supplementation as hydrated zinc sulfate "$ZnSO_4 .7H_2O$" recorded high value of LDL- C "bad cholesterol". Zinc supplementation as "$ZnNa_2EDTA$" recorded highly increasing in absorption levels of zinc and copper in blood. the concentration of zinc levels 60mg and 58.4mg/kg diet of the diets of mice groups (C) and (E) respectively, were high in a degree that affected the functions of kidney {urea and creatinine}. The majority of liver cells were completely lost their cytoplasmic structure in mice of zinc deficient diet, either due to low intake of zinc or presence of dietary fiber of phytate. Phytate is known to form a complex with nutritional metals" Ca, Fe, Zn and Cu". Thus, the unavailable phytate and metallic nutrients complexed with it can not be utilized and were excreted.

The second design was in the metabolic cages where mice divided into 5- subjected mice groups. Three different zinc supplementation

sources of high bio- availability [they are ionic as zinc sulfate "$ZnSO_4.7H_2O$" in amount of 60mg Zn / kg diet, chelated as zinc di-sodium ethylene di-amine tetra acetate " $ZnNa_2EDTA$" 58.0 mg Zn / kg diet and finally, natural source as "pumpkin seeds : $ZnSO_4.7H_2O$" in amount of 54.57mg Zn / kg diet by ratio of 10.76:43.81mg Zn respectively] were added to fed 5-weeks-old Swiss Webster out bred male mice which caged individually in the metabolic cages and divided into 3- subjected mice groups, mice group (C) fed zinc supplemented diet as $ZnSO_4.7H_2O$, mice group (D) fed zinc supplemented diet as $ZnNa_2EDTA$ and mice group (E) fed zinc supplemented diet as (pumpkin seeds + $ZnSO_4.7H_2O$). The other 2- subjected mice groups were: mice group (A) fed zinc deficient diet (09.6mgZn/kg diet) and mice group (B) fed zinc controlled diet (31.3 mg Zn / kg diet) where, zinc source of the last two groups is $ZnSO_4.7H_2O$. All mice groups maintained at 22-24°C and 45-55% relative humidity (RH), diets and de-ionized water were provided fresh daily. The duration of these experiments were 8- weeks divided into 3- intervals; zero time period, depletion period (the 1^{St}- 4weeks) and repletion period (the 2^{nd}- 4weeks). By the end of each period, the bio- assay of zinc deficiency and it's injuries effect on the different metabolites were carried out by withdrawing blood samples and harvesting from mice of each group and isolated to determine calcium, iron, zinc and copper in the serum as well as, estimating of alkaline phospatase activity, HDL-, LDL-Cholesterol levels and kidney functions. Also body weights and food consumption of subjected mice groups were accurately weighted weekly to follow up the mice growth at the end of each period. The previous three zinc sources ($ZnSO_4.7H_2O$, $ZnNa_2EDTA$ and pumpkin seeds) are good supplementation zinc sources, which also not influence calcium and iron absorptions. In addition, copper

absorption enhanced with $ZnNa_2EDTA$, while inhibited with $ZnSO_4.7H_2O$. Generally; pumpkin seeds also were a good source of zinc supplementation without any definite effects on the other minerals absorption in blood, as well as, it rich in fat, protein and other minerals. Therefore; biscuit was fortified with zinc will be a strategy method and/or circumvention method to increase the zinc intake, that are known to be deficient in the Egyptian food and thus fortification with zinc contributes to reducing the incidence of zinc deficiencies. The three sources are added individually to wheat flour "to make fortified biscuit with zinc" in amount of 25mg Zn/kg wheat flour followed by addition of other ingredients of biscuit paste. pumpkin seeds were collected from different suburbs of five governorates; Cairo, Giza and Kaluobia "Great Cairo", Alexandaria and Marsa matrouh and the collected seeds were well homogenized, removed the hulls and dried, are rich in both fat, which they contain 43.3 % fat, and zinc which contain 9.32 mg Zn/100g. Each type of three fortified biscuits was analyzed for zinc content of biscuit before and after the baking process to study the effect of baking heat "170-180 ^oC" on zinc content of biscuit which resulted in, zinc content of the three types of fortified biscuits not affected by this range of the baking heat. Palatability of the fortified biscuit with $ZnNa_2EDTA$ was a highest grade followed by fortified biscuit with Pumpkin seeds and that with $ZnSO_4.7H_2O$. Hair analysis for minerals, gives a good indication of actual metal levels while blood levels may still be in the normal range.

We recommended in this thesis firstly by increasing the nutritional education for the people to be easy change of their attitude. We recommended also the people of different ages to eat at least pumpkin seeds as natural form of zinc as well as; fortifying the floor of the balady bread with zinc in EDTA form as strategy for the governorates which

people in their villages suffered from signs and symptoms of zinc deficiency.

Key Words:
.Zinc .Copper .Calcium .Iron .Bio- assay .Metabolites .Male mice .Metabolic cage . *adlibitum* .Depletion .Repletion .Zinc deficiency .Supplementation .HDL-, LDL- cholesterol levels .Phytic acid analysis .Pumpkin seeds .EDTA .Blood serum .Wheat bran .Biscuit fortification .Baking heat .Palatability .Urine analysis .Feces analysis .Hair analysis .Histopathological studies .Alkaline phosphatase .Kidney function . Fortification

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