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An Analytical and Clinical Study for Measure the Bioavailability of Zinc in Serum Using Flame Atomic Absorption Technique

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Abstract
An analytical and clinical study has been applied for measure the bioavailability of Zinc in serum of twenty adults healthy volunteers, using flame atomic absorption spectrophotometer (FAAS) at 213.9 nm. The calibration graph is linear in the ranges of 0.25-1.5 µg.mL^{-1} with correlation coefficient (R) 0.09996)µg.mL^{1-}and molar absorpitivites 22957.76(L.mol^{1-}cm^{-1}.The concentration of Zinc determined in serum of all volunteers before and after administered orally a tablet of 50 mg zinc sulphate, produced by Samara drugs company (SDI). All data were subjected to statistical analysis by calculating accuracy, precision in addition to other parameters. The results indicate that the average maximum concentration (C-max ± SD) of blood zinc was 0.8275 ± 0.0511mg.mL^{-1}after one hour of absorption (T-max ± SD), however one capsule has drug bioavailability through all day.
Keywords: Analytical, Clinical, Bioavailability, Zinc, Flam atomic abortion.

1. Introduction:

Zinc is an essential mineral for human cell growth, differentiation, and DNA synthesis (1). It is play a critical role in the development and maintenance of a healthy immune system (2). There are many associations with diarrhea and increased fecal zinc loss, negative zinc balance, and low tissue zinc concentrations (3). High zinc loss during diarrhea has been observed in infants)4,5) Zinc supplementation for the treatment of diarrhea may also be critical in improving overall immune function. Individual zinc deficiency is difficult to accurately assess. Population level estimates from national food balance sheets are able to determine the amount of bio-available zinc per person per day by country. These data estimate 21% of persons around the world are at risk of zinc deficiency)6). In 2002 the World Health Organization defined zinc deficiency as one of the major risks to child health (7).

Zinc is nutritionally an essential element and is required for the activity of a number of enzymes. About 20-30% of the ingested zinc is absorbed. Within mucosal cells zinc forms metal-protein complexes. Its known to be essential trace elements and a component of hundred of enzymes (8). Toxicity consequent upon excessive intake of this metal is rather uncommon (9).

In recent years, the importance of zinc for human health and development (10) and the possible widespread occurrence of suboptimal zinc intakes (11,12) have gained recognition. The fortification of staple foods with zinc may play an important role in achieving adequate zinc intakes in at-risk populations. two of the most commonly used zinc fortificants to date are zinc oxide and zinc sulfate(13).a few earlier studies compared the absorption of zinc between these chemical forms using the oral zinc tolerance test as a measure of bioavailability, but the results were conflicting. One of those studies reported a much lower plasma appearance from zinc oxide preparations compared with zinc sulfate, whereas the other reported no difference. A more recent study used stable isotope tracer techniques to measure zinc absorption in a group of Indonesian children; in that study, wheat flour dumplings were fortified with either zinc oxide or zinc sulphate(14) and zinc absorption did not differ between groups. Zinc is an essential trace element for humans and animals (15). The recommended daily allowance is 11 mg of zinc per day in diet of a human adult (16). Zinc deficiency produces profound physiological changes. Growth retardation, loss of taste acuity, skin ulcers, and sexual dysfunction has demonstrated in human studies as resulting from a deficiency of zinc. A deficiency is common in many diseases, including anemia, cancer and atherosclerosis. Patients with rosacea who attended the outpatient Clinic of Dermatology and Venereology in Baghdad Teaching Hospital were recruited into this. A disease severity score was calculated for each patient. The patients were randomly allocated to receive either zinc sulfate 100 mg or identical placebo capsules three times per day. Zinc sulfate and placebo capsules were given in a double-blind manner; following three months of starting the treatment. The patients crossed over, i.e. patients on placebo crossed over to zinc sulfate and those on zinc sulfate crossed over to placebo(17), and make study for acquired zinc deficiency disease of skin, two patients, who were on long term parenteral hyperalimentation, developed skin lesions similar to those seen in acrodermatitis enteropathica. Both patients were treated with oral zinc sulphate and their skin lesions cleared completely. These patients are presented as an acquired zinc deficiency syndrome (18).

In this work atomic absorption spectrophotometer, was used for measure the concentration of zinc in serum of human volunteers before and after treatment with 50 mg zinc sulphate tablet, and study the others parameters which affect the bioavailability of zinc in human body.

2. Materials and Methods:

2.1 Subjects:

Twenty volunteers (15 males and 5 females) their ages between (24-34) years with a mean ± SD of 28.5 ± 4.94 years, body weight between (58-84) kg with a mean ± SD of 66.90 ± 8.59 kg, and height between (160-174) cm with a mean ± SD of 168.5 ± 4.11 cm and Body Mass Index BMI (19) range between 0.7305±0.0759g.m^{-2} were collected from May to August 2013(Table 1). None of the subjects had a history of bone disease, peptic ulcer, enterectomy, regional enteritis, malabsorption, nephrolithiasis, liver cirrhosis, or renal disorder. Subjects had not taken zinc or vitamins, or any other drugs for the previous three months, which could affect zinc metabolism during the week preceding the start of the study. All study subjects were so instructed as to adhere to a daily tablet. All volunteers have zinc element deficiency in their blood. all volunteers took for one week a single dose of zinc sulphate tablet 50 mg produced by SDI which was administered orally to all volunteers who have deficiency in zinc levels, although the supplement of zinc tablet were taken for one week to reduce the deficiency of this element in their blood. Each subject appeared on the experimental day after an overnight fast.

2.2 Apparatus:

Zinc concentration was determined by using Phoenx-986, flame atomic absorption spectrophotometer (air/acetylene flame), K-PLC Series Centrifuge, Sartorius BL 210S balance, many plastic tubes, appendorf, stick, stirrer sterilized before used with demonized water and a Pentium 4 computer (DELL 1545) were used for data processing.

2.3 Blood Samples:

About three milliliters of venous blood were collected from each subject in the study after 12 hours fast. The blood samples were collected in plain tubes left at Rome temperate for 15 minute then centrifuge at 3000 rpm for 15 minute. Serum was separated and a liquated for subsequent measurement zinc level at different times 0.0, 0.5. 1.0. 2.0, 2.0, 3.0 and 6 hours intervals of oral administration after supplementation. The experiment was repeated one week later with the same volunteers and given the tablets from the same batch of zinc tablets. The serum was analyzed by flame atomic absorption spectrophotometer.

2.4 Analysis:

All samples were analyzed under stander curve which applied according to the instruction manual of spectrophotometer (Table2). The results were obtained from triplicate measurements. Standard solution for zinc covering the range of (0.25, 0.5, 1.0 and 1.5) µg.mL^{-1} was prepared by dilution of its stock solution (1000 µg.mL^{-1}) using de-ionized distilled water.

2.5 Data processing and Statistical analyses:

All obtained data were subjected to statistical analysis that was performed by using statistical software (STATISTICA 6.0), Stat-Soft Co. USA and Excel program office.

3. Results and Discussion:

3.1 Calibration graph:

Linear calibration graph for Zinc were obtained (Figure 1), which show that Beer's law was obey in the concentration range of (0.25-1.5) µ.mL^{-1}, The regression equations, correlation coefficients, molar absorptivities, and sandell sensitivities in addition to other parameters are given in (Table 3).

3.2 Accuracy and precision:

The accuracy and precision were confirmed by analyzing three replicate analyses of three different amounts of Zinc (within Beer's law) by calculating the relative error percentage and percentage relative standard deviation (RSD %).The results indicated good accuracies and precision at each of the studied concentration level (Table 4).

3.3 Supplementation and pharmacokinetic parameters:

Zinc is probably the most studied of the trace elements (16). Many clinical of zinc deficiency in humans have been reported including (nutritional dwarfism and hypogonadism). Also, appetite and taste are adversely affected by a Zinc deficiency. Zinc is also an important constituent for many enzymes. The recommended daily allowance is 15 mg/day for a male adult and 21 mg/day for lactating women (17). The results of the Zn supplementation study are shown in (Table 5). The results showed that the mean level of zinc in serum after treatment for one week at zero time was higher than the level before the treatment by about 7%. The volunteers had were recovered some of their deficiency form 0.6315 to 0.6775 µg.mL^{-1} throughout one week supplementation treatment. A simple method for the estimation of absorption rate constant (ka) after oral administration. The method is based on a previous work of Urso and Aarons known as the regression method of truncated areas for the estimation of absolute bioavailability for drug with a long elimination half-life. Table 6 shows the mean values of the pharmacokinetic parameters following oral administration of 50 mg zinc sulphate tablets SDI formula, were, absorption rate (Ka) mL.min^{-1}, Half

time of absorption (Ka 0.5t) $mL.min^{-1}$,elimination rate $mL.min^{-1}$ (Kelem), Half Time of Elimination(Kelem 0.5t) $mL.min^{-1}$, Maximum Time (T-max) $mg.mL^{-1}$, Maximum Concentration(C-max) and Auc: Area Under Curve, are calculated (20). All calculations were done according to Traezoidal Rule (21). The results indicated that (T-max), which corresponds to the time, required reaching a maximum concentration (C-max) equal one hour (Figure 2).

Table 1: Characteristics of the volunteers

Subject	Sex	Age/years	Height/cm	Weight/kg	BMI
1	M	23	170	68	23.529
2	M	24	165	65	23.875
3	M	26	173	63	21.050
4	M	29	166	70	25.403
5	M	20	174	80	26.424
6	M	35	172	78	26.366
7	M	32	171	74	25.307
8	M	27	167	75	26.892
9	M	33	169	73	25.559
10	M	29	173	84	28.066
11	M	30	168	72	25.510
12	M	40	174	69	22.790
13	M	24	160	58	22.656
14	M	23	164	57	21.193
15	F	24	166	60	21.774
16	F	26	168	55	19.487
17	F	29	170	59	20.415
18	F	30	161	60	23.148
19	F	32	165	58	21.304
20	F	34	165	60	24.655
Mean	-	28.5	168.05	66.90	23.770
±SD	-	4.937	4.110	8.59	2.410

Table 2: Operating Parameters for FAAS

Wave length(nm)	213.9
Slit(nm)	0.3-0.5
Lamp current(mA)	15
Fuel	C_2H_2
Flame high(mm)	6.0
Flame flow(mL/min)	1000

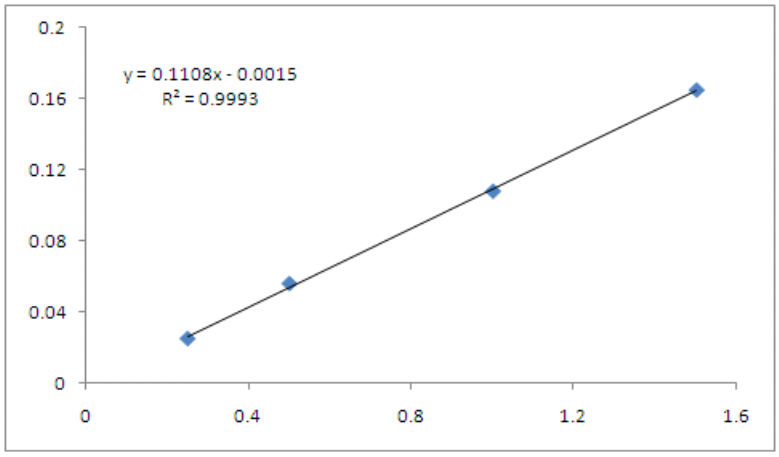

Figure (1): calibration curve for Zinc standard solution

Table 3: Spectral characteristics and statistical data of the regression equations for determination of Zinc in serum

Parameter	Zn
$_{max}$ (nm) λ	213.9nm
Linearity range (mg/L)	0.25-1.5
Molar absorpitivites ($l.mol^{-1}.cm^{-1}$)	76.22957
Regression equation	A = 0.1108 [Pb(µg.mL^{-1})] +0.0015
Calibration Sensitivity	0.1108
Sandell's Sensitivity (µg.cm^{-2})	0.0253
Correlation of Linearity (R^2)	0.9993
Correlation coefficient (R)	0.9996

Table 4: Evaluation the accuracy and precision of the method

Zinc Conc. (µg.mL^{-1})		Rel. Error %	R.S.D %
Taken	Found*		
0.5	0.496	-0.800	1.417
1.0	1.011	+1.100	1.347
1.5	1.507	+0.467	1.311

*Average of three determinations.

Table 5: Mean serum concentration of Zinc (µg.mL^{-1}) with time after oral administered of 50 mg Zinc sulphate tablets to 20 volunteers which have deficiency of zinc element in their bodies.

No.	Before Treatment (Time/Hours)	After Treatment (Time/Hours)					
		0.00	0.50	1.00	2.00	3.00	6.00
1	0.65	0.70	0.83	0.92	0.88	0.83	0.76
2	0.64	0.68	0.80	0.90	0.85	0.80	0.73
3	0.55	0.67	0.71	0.82	0.75	0.69	0.63
4	0.62	0.69	0.74	0.85	0.79	0.73	0.64
5	0.57	0.68	0.75	0.87	0.83	0.78	0.70
6	0.55	0.64	0.72	0.81	0.75	0.69	0.66
7	0.53	0.68	0.76	0.87	0.84	0.79	0.73
8	0.60	0.66	0.70	0.79	0.77	0.72	0.70
9	0.58	0.67	0.73	0.84	0.81	0.77	0.71
10	0.63	0.75	0.81	0.94	0.90	0.86	0.81
11	0.50	0.59	0.67	0.78	0.77	0.73	0.69
12	0.50	0.63	0.69	0.81	0.80	0.78	0.56
13	0.60	0.73	0.80	0.92	0.90	0.87	0.82
14	0.62	0.72	0.78	0.91	0.88	0.86	0.80
15	0.59	0.62	0.71	0.83	0.82	0.78	0.75
16	0.62	0.67	0.73	0.85	0.83	0.80	0.77
17	0.57	0.60	0.69	0.79	0.77	0.76	0.73
18	0.66	0.69	0.78	0.91	0.90	0.87	0.85
19	0.58	0.65	0.75	0.89	0.86	0.84	0.80
20	0.56	0.63	0.72	0.86	0.85	0.81	0.77
Mean	0.5860	0.6675	0.7435	0.858	0.8275	0.7880	0.7305
±SD	0.0436	0.0430	0.0393	0.0481	0.0511	0.0588	0.0759

Table 6: Pharmacokinetic parameters of Zinc sulphate(50mg tablet) after oral administered to 20 volunteers which have deficiency of zinc element in their bodies

No.	Ka ml/min	Ka 0.5t ml/min	Kelem ml/min	Kelem 0.5t ml/min	Tmax hour	Cmax mg/ml	AUC
1	0.341	2.034	0.039	17.718	1	0.88	4.985
2	0.325	2.136	0.041	17.065	1	0.85	4.776
3	0.116	5.974	0.045	15.579	1	0.75	4.289
4	0.140	4.952	0.057	12.285	1	0.79	4.677
5	0.196	3.536	0.046	15.180	1	0.83	4.638
6	0.236	2.941	0.025	27.536	1	0.75	4.248
7	0.246	2.816	0.046	15.000	1	0.84	4.362
8	0.263	2.634	0.046	15.164	1	0.77	4.821
9	0.176	3.938	0.037	18.780	1	0.81	4.317
10	0.154	4.501	0.028	24.972	1	0.90	5.133
11	0.254	2.725	0.028	24.937	1	0.77	4.333
12	0.246	2.817	0.042	16.422	1	0.80	4.658
13	0.198	3.500	0.037	18.579	1	0.90	4.472
14	0.216	3.208	0.040	17.412	1	0.88	4.832
15	0.271	2.555	0.021	33.426	1	0.82	4.638
16	0.172	4.039	0.019	36.603	1	0.83	4.755
17	0.153	4.523	0.044	15.894	1	0.77	4.633
18	0.188	3.686	0.027	25.858	1	0.90	4.962
19	0.278	2.491	0.039	17.953	1	0.86	4.225
20	0.267	2.594	0.025	27.709	1	0.85	4.787
Mean	0.2218	3.3800	0.0366	20.7036	-	0.8275	4.6271
±SD	0.0504	0.9762	0.0106	6.9858	-	0.0511	0.2630

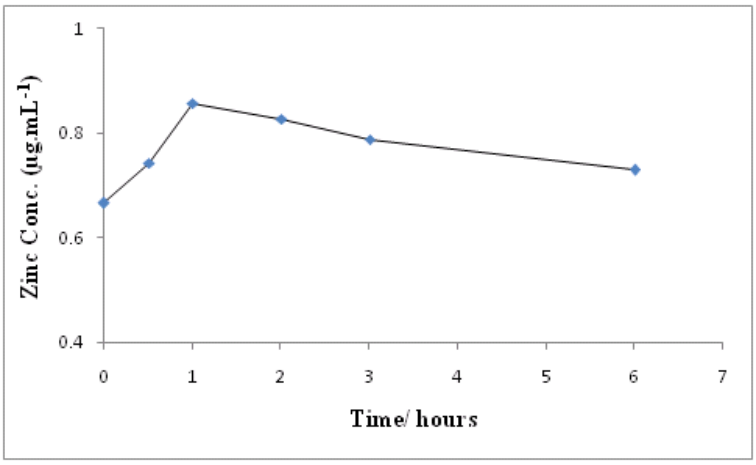

Fig 2: Mean serum concentration-time profile of Zinc (µg.mL^{-1}) after one week of continues supplementation a single dose of 50 mg Zinc sulphate tablet through 6 hours to 20 healthy volunteers

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