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Evaluation of the Intestinal Absorption of Maltol-Zn and Ethyl maltol-Zn Complexes in Rat

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Zinc deficiency in man is characterized by skin lesion, alopecia and has been linked to growth retardation and mental disturbance. For supplementation purposes zinc is currently presented as its sulfate salt, from which uptake by the body is poor, necesseting the administration of high doses. This leads to a range of unpleasant side effects. In order to increase the bioavailability of zinc, several zinc complexes such as malthol-Zn, ethyl maltol-Zn have been designed and synthesized. The distribution coefficients (K_{part}) of the complexes between 1-octanol and water (pH 7.4) were also determined. The intestinal absorption (I.A) of complexes and zinc sulfate was determined using Everted Gut Sac (E.G.S) method at different concentration of (0-1000µg/lit) zinc and different times (0-120 min).The results showed that**,** at the concentration of 800 µg/lit zinc and after 120 minutes, the absorption was reached to maximum for both complexes and zinc sulfate.

Zinc uptake from zinc sulfate, maltol-Zn and ethyl maltol-Zn complexes were 210, 220 and 235 µg/lit respectively. The higher zinc uptake from ethyl maltol-Zn complex may be attributed to its higher lipophilicity (K_{part} = 0.18) than maltol-Zn complex ((K_{part} = 0.5).

The results showed that, in spite of higher zinc uptake of ethyl maltol-Zn complex in comparison with zinc sulfate, statistically there is no significant difference between them. This investigation indicated that ethyl maltol-Zn complex could be considered as a suitable zinc complex for the treatment of zinc deficiency in replacement of zinc sulfate.

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Introduction

Zinc is an essential trace element in man; its major biochemical role being as a constituent in more than 3oo metalloenzyme systems with catalytic, regulatory and structural functions [1, 2]. It is a structural ion of biological membranes and closely related to protein synthesis, mitosis, cell division and DNA synthesis [3-6]. Zinc also interacts with important hormones involved in bone growth such as somatomedin-c, osteocalcin, testosterone, thyroid hormones, and insulin [7]. Table 1 shows the Recommended Dietary Allowance (RDA) of zinc proposed by the National Research Council in 1989 [8, 9].

Table 1. Recommended dietary allowances proposed by the National Research Council of United States

Subjects	Recommended dietary zinc/day
Infants	5mg
Children 1-10 years old	10mg
Males >10 years old	15 mg
Females >10 years old	12 mg
Pregnant	15 mg
Lactating women 1st semester	19 mg
Lactating women 2nd semester	16 mg

Zinc deficiency in humans is common in many developed and developing countries [10]. It is noteworthy that although newborn, children, pregnant women and old people are considered the main risk groups; zinc deficiency may affect the whole population [11]. The nutritional causes such as, decreased zinc intake or the consumption of poor zinc content foods are the most important and common factors in zinc deficiency [4, 12-15]. In addition, several syndromes related to metabolic or genetic malfunctions such as malabsorption syndromes, acordermatitis, enteropathica, Crohn's disease, alcoholism, liver cirrhosis, chronic renal disease, sickle cell anemia and gastrointestinal disorders also provoke zinc deficiency [11, 15-18].

The clinical manifestations of zinc deficiency include growth retardation, hypogonadism in males, neurosensory disorders, skin lesions, intercurrent infections, altered immune response, increased absorption risk, complications during delivery, prematurity pregnancy, neural tube defects of fetus, loss of appetite, delayed wound healing, abnormal dark adaptation, alopecia, oligospermia, anorexia, delayed sexual maturation, weight loss, mental lethargy, taste abnormalities, emotional disorders and hyperammonaemia [16, 19- 29].

There are three possible strategies to overcome zinc deficiency: supplementation, fortification, and dietary modification and diversification. Supplementation is appropriate for populations where zinc status must be improved over a relatively short period and the requirements cannot be met from habitual dietary sources. The current recommended doses in such cases are listed in Table 1. Food fortification with zinc is recommended when its deficiency is endemic or when it is targeted in specific region or for certain high-risk groups within a country. The third strategy involves changes in food-selection patterns or traditional household methods for preparing and processing foods with the aim of enhancing the availability, access, and utilization of foods with a high content and bioavailability of zinc.

The forms of zinc used as nutritional supplementations or in food fortifications are zinc sulfate, zinc chloride, zinc gluconate, zinc oxide, and zinc stearate [1, 27, 30, 31].

These five zinc salts have been approved as safe by the US Food and Drug Administration (FDA) [1]. The total quantity of zinc salts used has notably increased since 1970, and the compounds used most are zinc sulfate and zinc oxide [1, 27]. At present, zinc gluconate has been got more attention for food fortification or supplementation procedures as a proper zinc compound [11]. The main disadvantages of these zinc salts are their unacceptable taste and resulting nausea and dyspepsia. Uptake of zinc sulfate by the body is poor necessity the administration of high doses. This leads to a range of unpleasant side effects, including abdominal pains, nausea, and vomiting and severe headaches. Zinc oxide is poorly absorbed, and it precipitates in the nutritional matrix when zinc oxide is used to fortify liquid foods [1, 11, 30- 33].

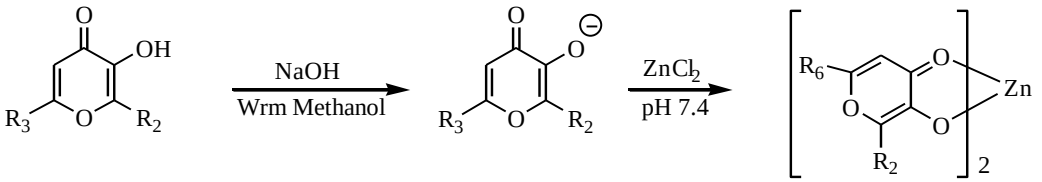
Based on previous study which suggested that chelating agents such as EDTA increased the

bioavailability of zinc [34], together with the fact that certain zinc chelating agents such as 3-hydroxypyranones are nontoxic [35]. In order to increase the bioavailability of zinc, three zinc complexes using with hydroxypyranones (such as maltol, ethyl maltol and kojic acid) were synthesized [36] as dietary supplements. In this project, we investigated the intestinal absorption of only two complexes (namely maltol-Zn and ethyl maltol-Zn which possess suitable lipophilicity) in comparison with zinc sulfate by using Everted Gut Sac (E.G.S) method. Therefore, the kojic acid-Zn(II) complex was not included in further intestinal absorption experiments due to its very low lipophilicity.

Material and methods

Complexes of maltol-Zn, ethyl maltol- Zn and kojic acid -Zn were synthesized as previously described [36]. Other chemicals used in this project were obtained from Aldrich (Gillingham, UK). Melting points are uncorrected. IR spectra were recorded on a perkin-Elmer 1420. ^{1}HNMR spectra were determined with Bruker 400 MHz spectrometer (Germany). Chemical shifts (δ) are reported in ppm downfield from the internal standard tetramethylsilane(TMS). Elemental analyses were performed by atomic absorption 2380 and CHN analyzer 2400 Perkin-Elmer

The procedure for the synthesis of the desired compounds is described in Fig 1.



1a: $R_2 = CH_3$, $R_6 = H$ (Maltol)

1b: $R_2 = C_2H_5$, $R_6 = H$ (Ehtyl maltol)

1c: $R_2 = H$, $R_6 = CH_2OH$ (Kojic acid)

(2)

3a: Maltol-Zn Complex)

3b: (Ehtyl maltol-Zn Complex)

3c: (Kojic acid-Zn Complex))

Fig. 1. Synthesis of Zn-complexes

Chemistry

*Synthesis of maltol- zinc (II) complex (**3a**).* To a solution of maltol (**1a**) (6.3 g, 0.05 mol) in warm methanol (50 ml) was added sodium hydroxide (2.0 g, 0.05 mol) dissolved in water (5.0 ml) and the mixture was stirred for 10 min. With continued heating/stirring a solution of zinc chloride (3.4 g, 0.025 mol) in 10.0 ml water was added drop wise over a period of 20 min. The pH of solution was adjusted to 7.4 with NaOH. Storage of this solution at 4 ºC for 24 h caused a precipitate to form, filtration followed by freeze-drying giving zinc maltol as a white powder. The resulting compound was purified by recrystallization from $CHCl_3$ / hexane. The complex was. heated in a vacuum oven (0.1mmHg, 120 ºC) for 12 h to give white maltol-Zn(II) complex 5.0 g (63%), 279-280 ºC (dec.), ^{1}H NMR (DMSO-d_6): δ 2.4 (s, 3H, 2-CH_3), 6.4 (d, j = 5.6 Hz, 1H, 5-H), 7.9 (d, j = 5.7 Hz, 1H, 6-H). IR (KBr): 1625 (C=O), 1565 (C=C) cm^{-1}.

Anal. Calcd for $C_{12}H_{10}O_6Zn$: C, 45.68; H, 3.17; Zn, 20.72%. Found: C, 45.50; H, 3.10; Zn, 20.57%.

*Synthesis of ethyl maltol-Zn (II) complex (**3**).* The procedure used was as described for synthesis of maltol-Zn (II) complexexcept ethyl maltol (7.0 g, o.o5 mol) (**1b**) was used in place of maltol (**1a**) in the reaction mixture. The resulting complex was purified in $CHCl_3$ / hexane and then heated in a vacuum oven (0.1mmHg, 120 ºC) for 18 h to give

white ethyl maltol-Zn(II) complex, 9.9 g (55%).%), 273-274 °C (dec. ^{1}H NMR (DMSO-d_6): δ 1.2 (t, 3H, 2-CH_2-**CH_3**), 2.7 (q, 2H, 2-**CH_2**-CH_3), 6.4 (d, j = 5.7 Hz, 1H, 5-H), 8.1 (d, 1H, j = 5.7 Hz, 6-H). IR (KBr): 162
(C=O) cm^{-1}.
Anal. Calcd for $C_{14}H_{14}O_6Zn$: C, 48.95; H, 4.08; Zn, 19.03%. Found: C, 49.05; H, 4.13; Zn, 19.12%.
*Synthesis of kojic acid -Zn(II) complex (**3c**).* The procedure used was as described for synthesis of zinc (II)-maltol complex except kojic acid (7.1 g, o.o5 mol) (**1c**) was used in place of maltol (**1a**) in the reaction mixture. The resulting complex was purified in $CHCl_3$ / hexane and then heated in a vacuum oven (0.1mmHg, 120 °C) for 24 h to give white kojic acid -Zn(II) complex, 7.8 g (45%).%), 284-285 °C (dec.), ^{1}H NMR (DMSO-d_6): δ 4.2 (d, 2H, 2-**CH_2**-OH), 5.5 (t, 1H, 2-CH_2-**OH**), 6.5 (d, j = 5.7 Hz, 1H, 3-H), 8.3 (d, 1H, j = 5.7 Hz, 6-H). IR (KBr): 1621 (C=O), 1565 (C=C) cm^{-1}.
Anal. Calcd for $C_{12}H_{10}O_8Zn$: C, 41.47; H, 2.88; Zn, 18.82%. Found: C, 41.39; H, 2.84; Zn, 19.01%.

Determination of partition coefficients

Partition coefficients (K_{part}) of the zinc (II) complexes were determined using the automated method as previously described [37]. The system was comprised of a computerised program which controlled anautoburette and an UV/Vis spectrophotometer, as well as performing all calculations of partition coefficients. All K_{part} values were performed usinganalitical grade reagents in a sealed titration vessel (250 ml) at a laboratory constant temperature (25 ± 0.5°C). The two phases used, were MOPS [3-(N-morpholino)propane sulphonic acid] buffer (50 mM, pH 7.4, prepared by the use of distilled water and n-octanol, each of which was pre-equilibrated with the other phase before use due to the limited solubility of water in n-octanol (2.3 M) [37]. The buffer (100 µL) was circulated through a spectrophotometric flow-cell, which was returned to the mixing chamber with the aid of a peristaltic pump at a flow rate of 1 ml/min. The aqueous phase was separated from two-phase system (n-octanol/MOPS) by means of a hydrophilic cellulose filter. A known volume (normally 20-100 ml) of MOPS buffer (saturated with *n*-octanol) was taken in the glass-mixing chamber. A base-line absorption value of the solution was used as a reference absorbance. A 10^{-4} M solution of the complex was prepared in the aqueous phase (typically 40 ml) to give an absorbance between 1.5-2.0 at the preselected wavelength (~ 310 nm). Upon commencement of the computer program, absorbance measurements were automatically recorded at preselected time intervals. When the absorbance readings were stabilized, a suitable volume of *n*-octanol was added to the aqueous phase from the automatic burette. Absorbance readings were subsequently recorded until the system reached to the equilibrium again, at which point a further aliquot of *n*-octanol was added. This cycle is repeated for at least 5 additions of 1-octanol. At each stage of 1-octanol addition the corresponding partition coefficient is calculated using the following formula:

$$K_{part} = \frac{A_0 - A_1}{A_1} \times \frac{V_w}{V_0}$$

Where
A_0 = Initial absorbance
A_1 = Absorbance at equilibrium after addition of 1-octanol
V_w = Volume of MOPS buffer
V_0 = Total volume of 1-octanol which has been added to glass vessel
Finally, a mean partition coefficient value and standard deviation is calculated. The K_{part}values for zinc (II) complexes of 1-2 are shown in Table 2.

Table 2. K_{part} values of zinc complexes (**3a-c)** between 1-octanol and MOPS buffer at pH 7.4. (Number of determinations = 5)

Zn(II)-Complex	K_{part} of Zn-Complex
3a	0.05 ± 0.004
3b	0.18 ± 0.01
3c	< 0.001

Intestinal absorption (Everted Gut Sac)

In this study, the intestinal absorption (I.A) of two complexes was determined using Everted Gut Sac

(E.G.S) method [38]. For this purpose, male wistar rats purchased from Tehran Pasteur. Institute and were kept in faculty animal house at standard conditions and fed on until their weights reached between 250-300 grams.

Animals were killed by cervical dislocation. Small intestine was removed, cleaned from debries, washed, blotted dried and weighted. The intestine cut into small pieces (between 7-8 cm). The segments were everted. The everted gut sac were filled with 200 tris buffer (pH 7.4) and suspended in tris buffer medium and zinc compounds. The incubation mixture was capped and gassed with $0.2/CO_2$ = 95/5 on water bath shaker at 37 ºC. At the time of intervals, the reaction mixture was removed and the concentration of zinc products inside the sacs was determined. Zinc concentration was measured using atomic absorption Perkin Elmer-model: 2380-USA at wavelength (λ_{max}) 213 nm, the corresponding concentrations were then determined by elaborating the Beer-Lambert standard curves.

Results and Discussion

In order to investigate the intestinal absorption of both ligands, by E.G.S method, the effect of incubation time on this process was studied first. To fallow this, E.G.S was prepared and incubated in three series of canonical flasks in tris medium containing 500 µg/lit zinc complexes and/or zinc sulfate. At intervals time E.G.S was removed from medium and the concentration of compounds (based on zinc element) inside the sacs were determined. The results showed that maximum zinc uptake (for both complexes and zinc sulfate) occurred after 120 min of incubation time (Table 3). The level of zinc uptake was then decreased, suggesting that the mucosal cells gradually loose their ability to take up zinc.

Table 3 :Effect of incubation time on zinc uptake at concentration of 500µg/lit from complexes of maltol-Zn and ethyl maltol-Zn and zinc sulphate by E.G.S. (Number of determinations = 3)

Time (Min)	Concentration of zinc (µg/lit) from maltol-Zn absorption	Concentration of zinc (µg/lit) from znicsulfateabsorption	Concentration of zinc (µg/lit) from ethyl maltol-Zn absorption
0	0	0	0
15	30 ± 3	37 ± 4	32 ± 3
30	56 ± 5	61 ± 5	62 ± 5
45	83 ± 6	90 ± 7	95 ± 5
60	105 ± 5	111 ± 8	117± 7
90	143 ± 7	150 ± 6	152 ± 8
120	166 ± 8	173 ± 9	183 ± 7
150	162 ± 7	170 ± 6	179 ± 7

In order to determine whether zinc uptake by E.G.S was dependent on the concentration of these compounds, to three series of canonical flasks varying concentrations of complexes (0-1000 µg/lit) were added. The sample solutions were incubated for 120 min at the same conditions mentioned in the method. At the end of incubation time, E.G.S from each flask was removed and zinc concentration inside the sacs was determined. The data presented in Fig 2-4 showing that there was a gradual increase in zinc uptake by E.G.S up to 800 µg/lit and then the level remaining unchanged. The maximum and minimum zinc uptake was found in ethyl maltol-Zn complex (235 µg/lit) and maltol-Zn complex (210 µg/lit) respectively (Fig 5).

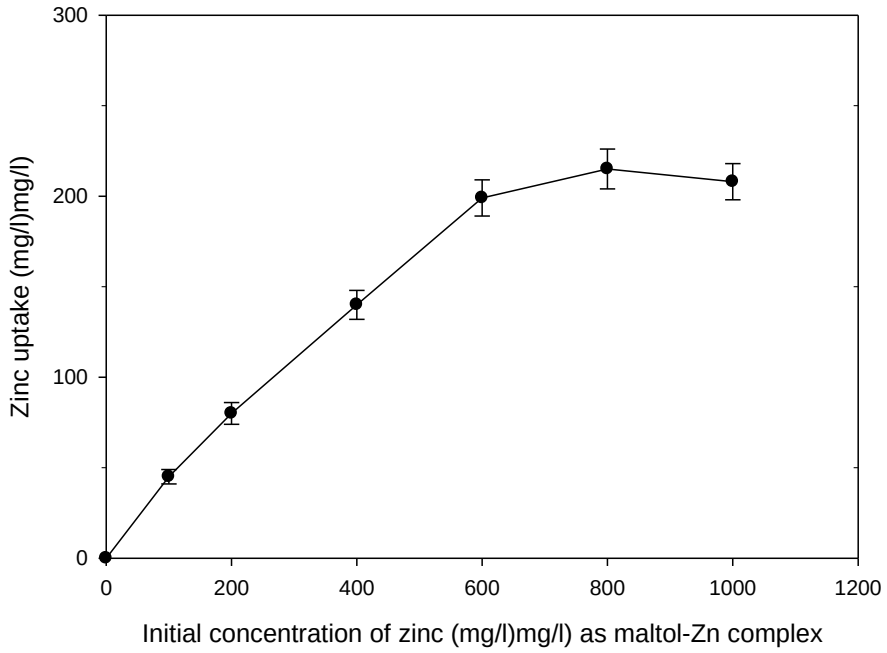


Fig. 2. Optimum concentration of zinc absorption from malthol-Zn complex after 120 min by E.G.S method.

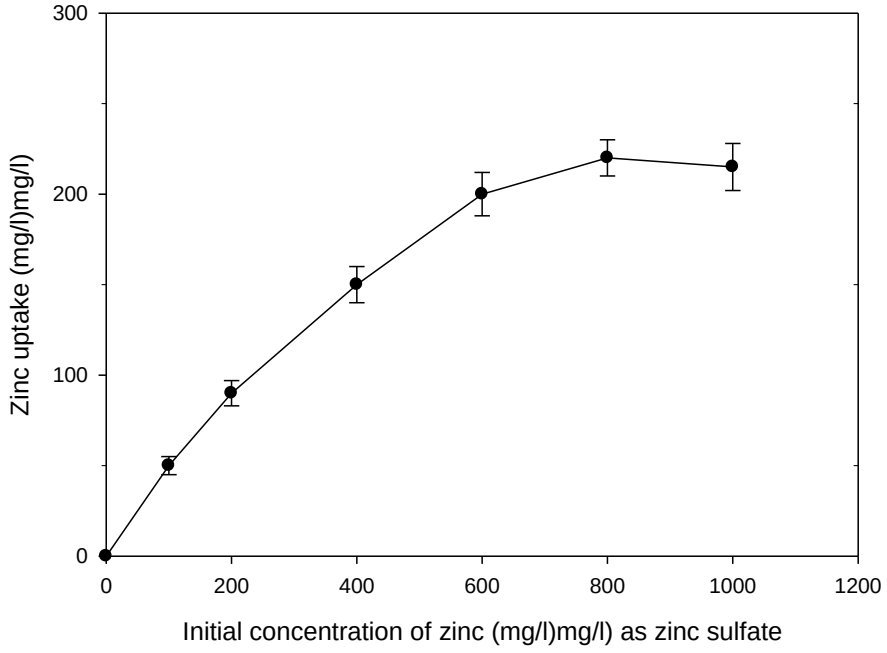


Fig 3. Optimum concentration of zinc absorption from zinc sulfate after 120 min by E.G.S method.

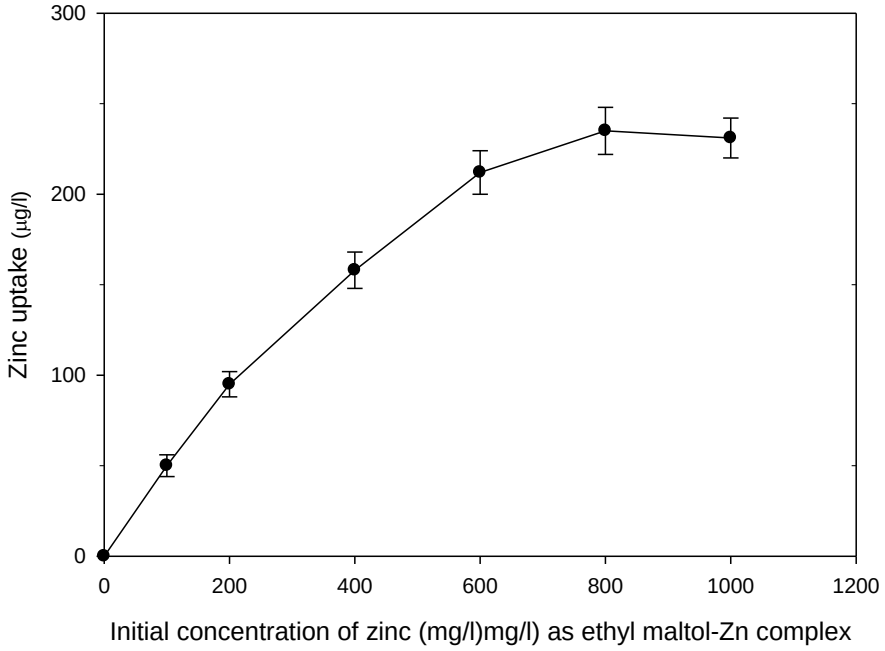


Fig. 4. Optimum concentration of zinc absorption from ethyl malthol-Zn complex after 120 min by E.G.S method.

Hence the key property for an orally active iron chelator is its ability to be efficiently absorbed from the gastrointestinal tract and to cross biological membranes thereby gaining access to the desired target sites as the liver. There are three major factors which influence the ability of a compound to freely permeate a lipid membrane, namely, lipophilicity, ionization state and molecular size.

In order to achieve efficient oral absorption, the complex should possess appreciable lipid solubility which may faciliate the molecule to penetrate the gastrointestinal tract (octanol/water partition coefficient greater than 0.1) [39]. Membrane permeability can also be affected by the ionic state of the compound. Uncharged molecules penetrate cell membranes more rapidly than charged molecules. It should be noted that both zinc complexes which investigated in this study are uncharged molecules. Molecular size is another factor which influence the rate if drug absorption. Generally, molecules with molecular masses >400 Da only poorly penetrate biological membranes by simple diffusion [39]. The synthesized complexes possess the molecular-mass less than 400 Da. Thus by virtue of their lower molecular masses, zinc complexes are likely to have a sufficient bioavailability.

The partition coefficient of maltol-zinc and ethyl maltol-zinc complexes are 0.05 and 0.18 respectively (Table 2). This data shows that the lipophilicity of ethyl maltol-zinc is much higher than the lipophilicitymaltol-zinc. As mentioned above, this factor plays and important role in drug absorption. For this reason, we expected that ethyl maltol-zinc complex (235 µg/lit) indicates higher intestinal absorption (zinc uptake of zinc-maltol complex is 210 µg/lit). The results presented in Fig 5, showed that in spite of higher zinc uptake of ethyl maltol-zinc complex (235 µg/lit) in comparison with maltol-Zn complex (210 µg/lit) and zinc sulfate, statistically there is no significant difference between them.

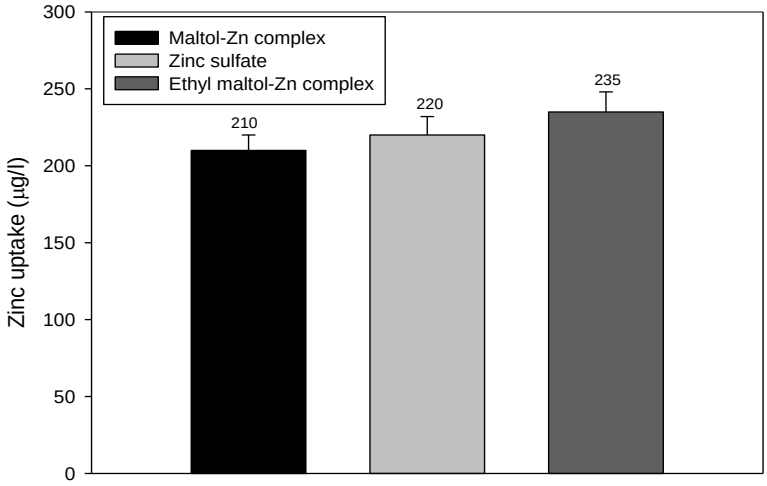


Fig. 5. Zinc absorption from Zn-complexes in comparison of zinc sulfate in optimal conditions by E.G.S. method

Conclusion

This study showed that zinc-ethyl complex could be considered as a suitable zinc complex for the treatment of zinc deficiency in replacement of zinc sulfate.

Conflict of interest

Authors certify that there isno actual or potential conflict ofinterest in relation to this article.

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