

The Effect of Oral Levothyroxine Sodium on Serum Zn, Fe, Ca and Mg Levels During Acute Copper Sulfate Toxication in Rabbits

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Abstract: This study was carried out to determine the effect of oral levothyroxine sodium (T_4) application on some serum mineral (Zn, Fe, Ca and Mg) concentrations during $CuSO_4$ toxication (1%). Forty male New Zealand rabbits (6 months-old, weighing 2.16 ± 0.08 kg) were allocated to 4 groups. All groups received 1% $CuSO_4$ ($*5H_2O$) (Copper (II) sulfate pentahydrate M1 02787) in drinking water. Except for control group (group A), T_4 was orally applied to 3 experimental groups at doses of 1.67 (group B), 3.33 (group C) and $6.67 \mu g kg^{-1}$ per bw per day (group D) for 2 days. Serum Cu measurements were performed by Atomic Absorption Spectrometer equipped with Flame system. Serum Zn levels in groups C and D were higher than in group A, while serum Ca levels in groups C and D were found to be lower than in group A ($p < 0.05$), on the last day. There were significant differences for all mineral levels with respect to time ($p < 0.001$). There was also a significant interaction between the dose of T_4 applied (groups A-D) and time ($p < 0.01$), except for Fe levels ($p > 0.05$). However, there were significant differences only in Zn and Ca levels in multiple comparisons ($p < 0.05$). A significant correlation was also found between T_4 and Zn ($r: -0.537$, $p < 0.001$) as well as T_4 and Ca ($r: -0.593$, $p < 0.001$) levels. In addition, administration of T_4 along with 1% $CuSO_4$ affected serum Zn, Ca and Mg levels significantly ($p < 0.05$). At the result, T_4 administration at different doses with 1% $CuSO_4$ caused an increase in serum Zn but a decrease in serum Ca levels. However, the administration of this combination showed no significant effect on serum Fe and Mg levels.

Key words: Thyroxine, $CuSO_4$ toxication, serum, Cu, Zn, Fe, Ca, Mg

INTRODUCTION

Stressful conditions like diseases could induce fluctuations in serum Cu levels (Karademir, 2007b, c; Orr *et al.*, 1990; Rahelie *et al.*, 2006). Soil and pastures with low Cu level can cause Cu deficiencies in grazing animals (Dargatz *et al.*, 1999; Gambling *et al.*, 2008; Kurt *et al.*, 2001). Similarly, different time periods of the year may affect serum Cu levels (Cimtay and Olcucu, 2000; Erdogan *et al.*, 2003). Cu is a component of the ceruloplasmin and Cu/Zn-SOD in the body and insufficient serum Cu level may cause inadequate activation of these structures (Bulbul *et al.*, 2008). Therefore, oral Cu supplementation may be required (Aziz *et al.*, 2008; Oztabak and Ozpinar, 2005; Sahin *et al.*, 2001; Uzlu *et al.*, 2007). However, over-dose of Cu may be applied involuntarily by animal owners or technicians. Additionally, Cu toxicity was reported to occur in some territories (Maclachlan and Jhonston, 1982). Thyroidal Hormones (TH), particularly thyroxine (T_4), could have a

great effect on the energy metabolism (Alcalde *et al.*, 1999; Braunlich, 1984; Canavan *et al.*, 1994; Dickson, 1996; Nakhoul *et al.*, 2000). Active transport mechanism, which is an energy dependent process is important for intestinal absorption of Cu and some other nutrients (Lonnerdal, 2008; McDowell, 1992). Thyroxine is used in hypothyroidism or in cases of thyroidal gland extirpation (Uzlu *et al.*, 2007; Van Hoek and Daminet, 2009; Dickson, 1996; Page *et al.*, 2009).

Zn, Fe, Ca and Mg are important minerals for animal organism (McDowell, 1992; Baxter, 1986). Minerals are involved in many metabolic functions (Karademir, 2006; Kurt *et al.*, 2001; O'Brien *et al.*, 2000) as well as in the formation of many enzymes (Aziz *et al.*, 2008). Interactions among minerals were reported to occur in the diet and/or within the body (Karademir, 2006; Kurt *et al.*, 2001; Witkowska *et al.*, 1991). It was also reported that administration of T_4 could increase serum Cu levels in a dose dependent manner (Karademir, 2009). Consumption of high Cu levels during T_4 therapy may occur in some

cases. However, there is no detailed information about serum mineral status during T₄ application along with high level CuSO₄. Therefore, the purpose of this study was to elucidate the effect of T₄ application and acute CuSO₄ toxication on serum Zn, Fe, Ca and Mg levels.

MATERIALS AND METHODS

Animals and procedures: Fourty male clinically healthy New Zealand Rabbits (6 months old, 2.16±0.08 kg) were used in this study. The animals were divided into 4 equal experimental groups. 1% Copper (II) sulfate pentahydrate (CuSO₄*5H₂O, Merck 102787) in drinking water, which is toxic dose (Chapman *et al.*, 1962), were given *ad-libitum* to all groups including control. Group A was kept as control and received tap water only. Levothyroxine sodium (T₄) (Levotiron tablet®) at doses of 1.67, 3.33 and 6.67 µg kg per bw per day in tap water was orally applied to group B-D, respectively. T₄ applied at ten o'clock daily at a single dose for 2 days (first and second days after the blood collections). The applications of T₄ and Cu were given simultaneously.

The animals were fed with a commercial animal food. The food and water were given *ad libitum* before and during the experiment. Cu, Zn, Fe, Ca and Mg contents of food and water was determined by flame system Atomic Absorption Spectrometer (FAAS) (Thermo Elemental S4, Thermo Electron Corporation, Cambridge, UK) (Karademir, 2007a, b). Levels of Cu, Zn, Fe, Ca and Mg in feed were found to be 12.56, 98.46, 475.83, 33715,4 and 2533.61 mg kg⁻¹ in Dry Matter (DM) and 0.013, 0.074, 0.058, 103.66 and 31.79 mg L⁻¹ in tap water, respectively.

The commercial food was purchased from Bayramoglu Yem ve Un San. Tic. A. S. ISO 9001:2000, ISO 22000:2005 and the composition is given in Table 1.

Blood collections and laboratory analysis: Two milliliters of the blood were collected via cardiac puncture under the light ether anesthesia on first, second and last days. Serum was separated by centrifugation at 3500 rpm for 15 min. Serum mineral status was also determined by FAAS (Karademir, 2007a, b). Standard solutions for Cu, Zn, Ca and Mg were supplied from Fluka Chemie GmbH, Switzerland (Fluka 61147, 96457, 21049 and 63046, respectively) and for Fe from Merck KgaA, Germany (119781).

Accuracy control of FAAS was performed using previously known standard solutions for all mineral measurements. These standard solutions were aspirated for 6 times per 10 samples during analyses and mineral levels were measured. Coefficients of Variations (CV) for

Table 1: Ingredients of diet given to rabbits

Diet composition	Units
Dry matter (%)	88
Crude protein (%)	17
Crude cellulose (%)	12
Crude ash (%)	10
Acid insoluble ash (%)	1
Calcium (%)	1.5
Phosphorus (%)	0.75
NaCl (%)	0.6
Vitamin A (IU kg ⁻¹)	5000
Vitamin D3 (IU kg ⁻¹)	600
Vitamin E (mg kg ⁻¹)	25
Metabolic energy (kcal kg ⁻¹)	2600

Raw-materials for this composition: Barly, corn, corn chaff, corn gluten, wheat, rye, craff, cottonseed meal, sunflower meal, dicalcium phosphate, vitamin, mineral

this parameter was calculated from this obtained findings. CV were found to be Cu: 4.16, Zn: 3.78, Fe: 2.36, Ca: 2.18 and Mg 1.73% (Karademir, 2007a, b). All lab-ware used were made of PTFE material.

Statistical analysis: Statistical analyses were performed using SPSS statistical software version 10.0.1 (SPSS, 1999). Data were presented as means±SEM.

One-Way ANOVA was used for comparison of days in each T₄ groups (A-D) (Ergun and Saktas, 2009). Differences of mineral levels throughout the experimental period as well as interaction between time and groups were analyzed by repeated measurement ANOVA (RM ANOVA). One-Way ANOVA were also used to compare the serum mineral levels between groups in days (for first, second, third, fourth and last days). Duncan test was employed for multiple comparisons. Pearson correlation test was used to determine the relationship between the dose of T₄ and individual average values of serum mineral levels on second and last days. Pearson correlation test was also employed for the analyses of relationship between serum Zn, Fe, Ca and Mg levels. Linear regression analysis was used to observe the effect of T₄ on serum Zn, Fe, Ca and Mg levels.

RESULTS AND DISCUSSION

Oral levothyroxine sodium at various doses with toxic dose copper sulfate was applied to the rabbits. The applications were continued for 2 days. One-Way ANOVA test results for differences among days (groups for days of first-last):

For Zn: There was no difference among days in groups A and B (p>0.05). However, differences were found between first day and other days in groups C and D (p<0.05 and p<0.01) (Fig. 1).

For Fe: There was no difference among the experimental days in all groups (A-B) (p>0.05) (Fig. 2).

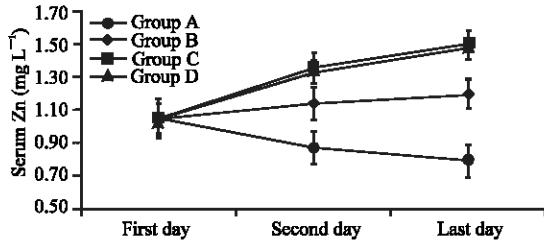


Fig. 1: Serum Zn levels of groups in relation to time

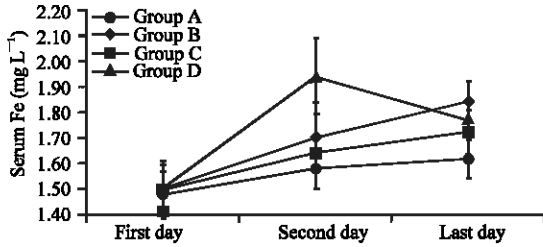


Fig. 2: Serum Fe levels of groups in relation to time

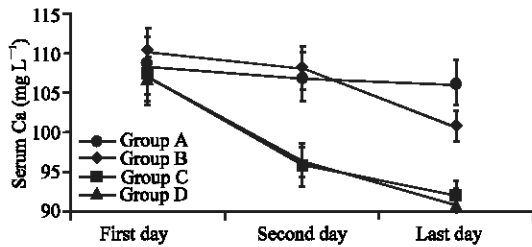


Fig. 3: Serum Ca levels of groups in relation to time

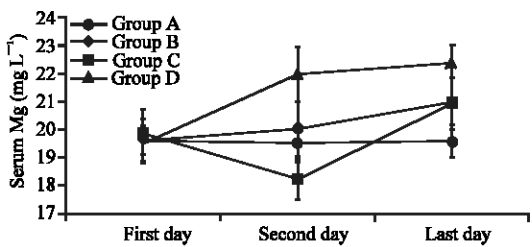


Fig. 4: Serum Mg levels of groups in relation to time

For Ca: There was no difference among days of group A ($p>0.05$). There were differences between last day and other days ($p<0.05$) in group B. There were also significant differences between first day and other days in groups C ($p<0.01$) and D ($p<0.001$) (Fig. 3).

For Mg: There was no difference among days in groups A and B ($p>0.05$). There was a significant difference between last and second day in group C ($p<0.05$), but no difference was found between first and other days in group C ($p>0.05$). There was a significant difference between first day and other days in group D ($p<0.05$) (Fig. 4).

Repeated measurements ANOVA (RM ANOVA) test results for differences among groups (A-D) within time

For Zn: There were significant differences within time ($p<0.001$). There were also significant interactions between time and mineral levels of groups ($p<0.001$). Post Hoc test results showed that there were significant differences between group A and other groups ($p<0.05$) (Fig. 1).

For Fe: There were significant differences within time ($p<0.001$), but no interaction was found between time and mineral levels of groups ($p>0.05$). There was also no difference among groups according to Post Hoc test ($p>0.05$) (Fig. 2).

For Ca: There were significant differences within time ($p<0.001$) and interactions were observed between time and mineral levels of groups ($p<0.01$). According to Post Hoc test, there were differences between groups A-B and C-D ($p<0.05$) (Fig. 3).

For Mg: There were significant differences within time ($p<0.001$) and interactions were observed between time and mineral levels of groups ($p<0.001$). However, there was no difference among groups according to Post Hoc test ($p>0.05$) (Fig. 4).

One-Way ANOVA test results for differences among groups within particular days (first-last days):

No significant differences were found in mineral levels of groups on the first days. However, significant differences were observed in mineral levels of groups on other days for minerals (Zn, Fe, Ca and Mg). These findings were given as follows:

For Zn: There was a significant difference between group A and other groups on second day ($p<0.01$). There were significant differences between group A and other groups as well as B and other groups on the last day ($p<0.001$) (Fig. 1).

For Fe: No differences were found in Fe levels for all days (Fig. 2).

For Ca: On the second day, Ca level of group A was similar to group B. In addition, group C was similar to group D. There was no difference between each other ($p>0.05$). However, there was a significant difference between group A and groups C, D as well as among group B and groups C, D ($p<0.01$). In contrast to second day, a difference was found between group A and B on the last day ($p<0.001$) (Fig. 3).

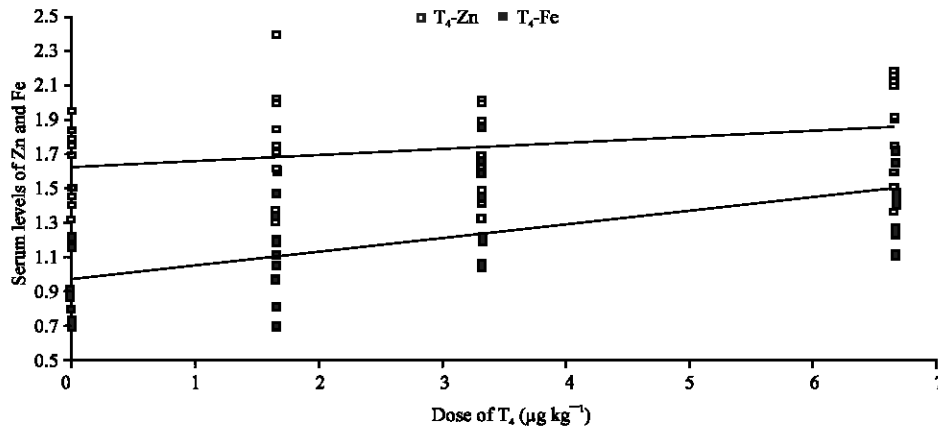


Fig. 5: Illustration of correlation test results between T₄-Zn and T₄-Fe according to average values of second and last days' data

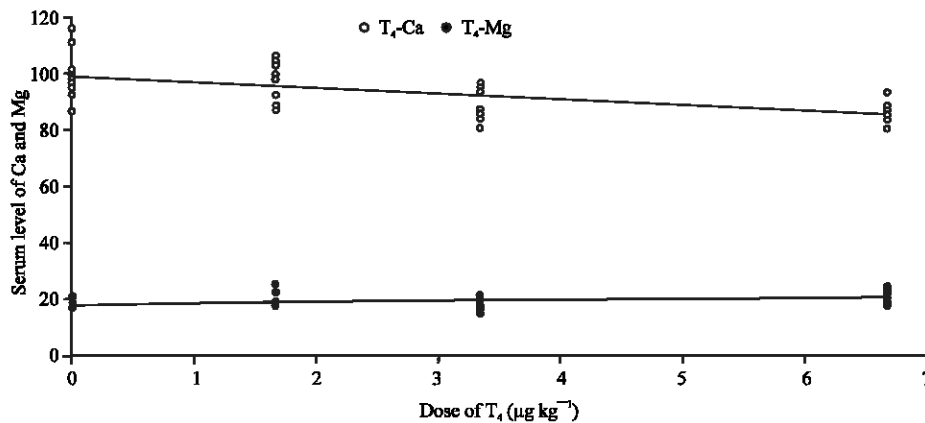


Fig. 6: Illustration of correlation test results between T₄-Ca and T₄-Mg according to average values of second and last days' data

Table 2: The effects of T₄ on serum Zn, Fe, Ca and Mg levels during 1% CuSO₄ toxication

Regression equation	r ² (%)	Significance
Zn = 0.968 + 0.0809 Applied T ₄	32.8	p<0.001
Fe = 1,63 + 0.0325 Applied T ₄	8.5	p>0.05
Ca = 106 - 1.12 Applied T ₄	35.2	p<0.001
Mg = 19.4 + 0.360 Applied T ₄	13.4	p<0.05

Unit of minerals: mg L⁻¹, unite of T₄: µg kg⁻¹ per BW per day

For Mg: On the second day, Mg level in group D was significantly different from those of other groups except group B (p<0.05). However, on the last day, there was a significant difference between group A and D (p<0.05) but no difference was observed between group A and B as well as group A and C (p>0.05) (Fig. 4).

The correlation between the dose of T₄ and average mineral levels in the second and last days was also evaluated by Pearson correlation test. It was found that there were significant correlation between the dose of T₄-Zn (r: 0.537, p<0.001) and T₄-Ca (r: -0.593,

p<0.001) but not between the dose of T₄-Fe (r: 0.291, p>0.05) and T₄-Mg (r: 0.336, p>0.05) levels (Fig. 5 and 6).

Regression analyses were employed to test the effect of T₄ on serum Zn, Fe, Ca and Mg levels during CuSO₄ toxication. T₄ affected the serum Zn, Ca and Mg levels, significantly (p<0.05) but not serum Fe level (p>0.05). The results of regression analyses are given in Table 2.

Average values of second and last days of Zn, Fe, Ca and Mg levels correlations test results were shown in Table 3. Only a negative correlation was detected between Zn and Ca levels.

Chemical and biological interactions among the minerals were reported to occur in many conditions (Karademir, 2006; Kurt *et al.*, 2001). These interactions were reported to be affected by intestinal absorption site, transport site in the intestinal mucosa, other membrane barriers, functional metal binding proteins, chemical forms, electrical structure of minerals (O'Dell, 1989).

Table 3: Correlation values between Zn, Fe, Ca and Mg levels of average values of second and last days (r)

Chemical components	Zn	Fe	Ca
Fe	0.114	-	-
Ca	-0.644*	-0.118	-
Mg	0.167	0.012	-0.113

*p<0.01

In the previous study, we showed that serum Cu levels were affected by oral T₄ applications along with 0.1% CuSO₄. During for 3 days, serum Cu levels were increased with the increased doses of T₄ (Karademir, 2009). The purpose of the present study was to investigate the effect of various doses of T₄ applications along with acute Cu toxication (1% CuSO₄) on some serum mineral levels (Zn, Fe, Ca and Mg). Findings of this study showed that some fluctuations occurred on serum Zn, Fe, Ca and Mg levels in a dose-dependent manner in response to T₄ treatment with toxic dose of CuSO₄ (1%). Possible mechanisms for these fluctuations may be associated with increased Cu levels in the body (Kurt *et al.*, 2001; Witkowska *et al.*, 1991) or the effect of T₄ on intestinal absorption of these minerals via altering the active transport system (Lonnerdal, 2008; McDowell, 1992) as well as altered mineral excretion via urinary system (Braunlich, 1984; Van Hoek and Daminet, 2009).

Group A (only 1% CuSO₄ applied group without T₄) showed slight decreases in levels of serum Zn and Ca (p>0.05), slight increase in Fe level (p>0.05) and a stable course in the level of Mg (p>0.05). However, T₄ treatments affected the serum Zn, Ca and Mg levels during 1-4 days (p<0.05) (Fig. 1-4).

Xin *et al.* (1993) measured serum Cu levels during, before and after 8 weeks of lactation with or without Cu supplementation. In the study, Zn levels were reported to be decreased with oral Cu supplementations.

The decrease was more prominent when Cu level was increased. Multiple type of interaction among Cu, Zn and Fe were reported for intestinal absorption or metabolism. There is a positive interaction between Cu and Fe, but a negative interaction could occur between Cu and Zn for absorption (O'Dell, 1989). Findings of the study were seen in group A (Cu supplementation without T₄) with the slight decrease of Zn and slight increase of Fe levels. Nevertheless, 3.33 µg kg⁻¹ and higher dose of T₄ applications significantly increased the levels of serum Zn (p<0.05). Serum Fe showed a slight increase in all groups (p>0.05). In addition, while serum Ca levels were decreased with all T₄ doses (p<0.05), serum Mg levels were increased with 3.33 µg kg⁻¹ and higher dose of T₄ applications (p<0.05). The possible cause of this mineral

fluctuations could be related to intestinal absorption (Lonnerdal, 2008; Nakhoul *et al.*, 2000), urinary excretion (Braunlich, 1984; Canavan *et al.*, 1994) or interactions of minerals (Gambling *et al.*, 2008; Dargatz *et al.*, 1999; Karademir, 2007b, c; 2009; Maclachlan and Jhonston, 1982).

Positive correlation was observed between T₄ and serum Zn levels (Fig. 5). On the other hand, opposite result could be expected due to the negative effect of increased Cu level on serum Zn level (Witkowska *et al.*, 1991; Xin *et al.*, 1993). This condition may be due to the effect of T₄ on absorption, distribution or excretion of body Zn level (Alcade *et al.*, 1999; Braunlich, 1984; Lonnerdal, 2008).

CuSO₄ toxication caused a non-significant slight decrease in serum Ca level of group A (p>0.05). The decrease in serum Ca level was found to be statistically significant with the T₄ application (p<0.05) (Fig. 3). Negative interaction between Cu and Ca is known (McDowell, 1992). The results of this investigation showed that the effect of negative interaction increased with the T₄ applications.

The cause of this condition may be expected from metabolic effect of T₄ on the organism (Dickson, 1996). Negative interaction between Zn and Ca levels are also known (Baxter, 1986) and the increased Zn levels may also contribute to the decreased Ca level seen in this study. The correlation results between T₄-Zn, T₄-Ca and Zn-Ca supported our opinion on the fluctuation of Zn and Ca under the effect of T₄ and CuSO₄ toxication (Fig. 5, 6 and Table 3). The effect of T₄ with CuSO₄ toxication on serum Zn and Ca levels were found highly significant (p<0.001) (Table 2).

Serum Mg is a macro mineral like Ca, whereas serum Fe is a micro mineral like Zn and Cu. Mg has a close relation to Ca in the living organism. Serum Fe has also a close relation with Zn and Cu (Karademir, 2009; O'Dell, 1989). T₄ applications with CuSO₄ toxication affected serum Mg and Fe less compared to serum Ca and Zn levels (Table 2, 3 and Fig. 4-6). The animal organisms have compensatory mechanisms for the fluctuations of some metabolic cases. The urinary system is one of them (Dickson, 1996). In addition, T₄ applications may affect this compensatory system (Alcalde *et al.*, 1999; Braunlich, 1984; Van Hoek and Daminet, 2009).

Differences for all minerals (Zn, Fe, Ca and Mg) among groups (A-D) were observed in relation to time during T₄ and CuSO₄ applications (p<0.001). Most prominent differences were observed in serum Zn and Ca levels. The serum levels of Zn were increased, while serum

Ca levels decreased in time with T₄ applications during CuSO₄ toxication. In group A, Zn level was lower, while Zn levels were increased with T₄ treatment in other groups. The differences between group A and groups B-C were significant (p<0.05) (Fig. 1).

Decreased serum Zn during Cu applications has been known (Witkowska *et al.*, 1991; Xin *et al.*, 1993). We observed in the present study that T₄ application increased the serum Zn levels in a dose dependent manner. Similarly, a negative interaction between Cu and Ca levels are known (Karademir, 2006; Kurt *et al.*, 2001; McDowell, 1992).

Nevertheless, T₄ applications effectively decreased the level of serum Ca compared to control group. Interactions between groups and time were significant within time (p<0.01) and according to Post Hoc analyses there were significant differences between A-B groups and C-D groups (p<0.05).

These results showed that high level T₄ is more effective than low dose on decreasing serum Ca level. T₄ with CuSO₄ increased serum Fe level within time (p<0.001), but no interaction was found between time and groups (p>0.05). Similarly, there was no difference between groups according to multiple comparisons (p>0.05).

These results suggest that there is no effect of T₄ along with CuSO₄ toxication on serum Fe levels. The elevation in serum Fe level may be due to positive interaction with Cu (O'Dell, 1989).

There was a significant difference in serum Mg levels within time period of the treatment (p<0.001). There was an interaction between time and groups (p<0.001). However, in multiple comparisons no significant differences between groups were found (p>0.05). The cause of this situation may be due to the wider standard deviations in groups.

The interaction between Serum Ca and Mg was reported in a previous study (O'Dell, 1989). However, levels of Mg showed no significant fluctuations (p>0.05). T₄ application may be a causative event for unaffected Mg level.

The results of One-way ANOVA for the analyses of differences between groups within particular days (first-last days) also supported the results obtained from RM ANOVA.

As the results of this study, oral T₄ administration increased serum Zn levels, but decreased serum Ca levels during CuSO₄ toxication. However, serum Fe and Mg levels were not significantly affected by these combinations.

T₄ therapy is widely used in the treatment of hypothyroidal functional disorders. Furthermore, there are many locations in the world which is Cu deficient or contains toxic level of Cu. Cases of Cu deficiencies may need Cu therapy. Consequently, simultaneous intake of T₄ and high amount of Cu could occur either voluntarily or involuntarily.

CONCLUSION

The elevation in serum Zn levels or Ca deficiency could occur during T₄ therapy along with CuSO₄ toxication. This combination can be utilized for the treatment of short time Zn deficiency or Ca toxication.

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