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# THE EFFECTS OF HIGH ZINC AND COPPER ON AST, ALT, LDH,CK, GLUCOSE, TOTAL PROTEIN AND ALBUMIN IN RATS.

(With 2 Table)

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## SUMMARY

Zinc and copper have a role in a lot of metabolic events and in many enzymes function. In our study it has been aimed to investigate the effects of both high doses and combine forms of relevant heavy metals on some physiological parameters. In this study 77 Wistar albino rats were used. Rats were separated into six groups as one control (n=13) and five experimental (n=12) groups. While tap water was given to the control group (C), 70mg/kg day zinc, 80 mg/kg day zinc, 10 mg/kg day copper, 20 mg/kg day copper and 70 mg/kg day zinc+10mg/kg day copper were given to the experimental groups called $E_1$, $E_2$, $E_3$, $E_4$ and $E_5$ for 4 weeks. As a result it was found that while giving high doses of zinc to the rats increased glucose and albumin levels, also lactic dehydrogenase (LDH) concentration decreased in zinc received group same as in the group which copper was added into the water, also giving both zinc and copper statistically decreased the plasma aspartate aminotransferase (AST) level. When all data were evaluated, it has been concluded that high doses of zinc and copper we applied were tolerated by the animals.

***Key words:*** *High copper, zinc AST, ALT, LDH,CK, glucose, total protein, albumin, rats*

## INTRODUCTION

Copper is obligatory for the structure and functions of the immune system, nervous system, skeleton system and also for the

biochemical and main physiologic functions. In addition it is required as a cofactor of proteins and enzymes which are necessary for the constancy of healthiness and normal cellular homeostasis (Panemangalore and Bebe, 1996) Beside zinc has a role in a lot of metabolic events like anabolism and catabolism of connective tissue, nucleic acids, protein, lipid, carbohydrate and enzymes, also it is involved in the functioning of more than 300 enzymes (Prasad, 1995). Briefly, different haematological defects may occur on the deficiency or excess of the zinc and copper (Sanstead, 1995) which are essential in many events during the growth of living organisms (Prasad, 1985).

It has been reported that giving 100, 500, 1000 and 2000 ppm zinc orally to rats for 22 days statistically increased serum AST, alanin aminotransferase (ALT), alkaline phosphatase (ALP) levels except for the group which was given 100 ppm zinc (Tekeli *et al.*, 2002). Also it has been reported that giving single high dose zinc intraperitoneally increased serum AST and ALT levels in mice (Syzmanska *et al.*, 1991). The rats which were experimentally induced cirrhosis by thiocetamide, were treated by 227 mg zinc sulphate and it was observed that increased AST, ALT, gama glutamylatransferase (GGT) and bilirurbin levels due to effects of cirrhosis decreased and reached to the normal levels after 14 weeks of treatment. Also it was announced that increased copper level decreased by receiving zinc (Dashti *et al.*, 1996). Goel *et al.* (1994) have reported that similarly in the rats which were exposed to the carbontetrachlorur toxicity, the activity of high serum AST and ALT decreased by rising zinc levels in the diet. At the end of the two study (Goel *et al.*, 1994 and Dashti *et al.*, 2001) it was emphasized that zinc provides repairing and renovating in liver. It has been determined that in the mallards received between 7and 27 mg/kg/day zinc with the diet for 30 days, serum AST level increased but glucose and protein levels decreased (Levengood *et al.*, 2000).

It has been determined that in the male rats which were given different doses (100, 500, 1000, 2000ppm) of zinc, glucose decreased, total protein amount increased, however albumin level increased in the groups which were given 100 and 2000ppm zinc and did not change in the other groups (Tekeli, 2002).

During the test 500mg zinc oxide were given 12 times to the male Ivesi lambs. And it has been determined that total protein amount did not change but serum copper level increased in the blood samples which were taken on 0, 14, 28, 42 and 84 days (Aksoy *et al.*, 2002).

In a study which investigated the effects of high copper levels it was found that giving 500mg/kg/bw copper by orally for 5 days a week during 8 weeks in rats, caused statistical increase in ALT level (Zhang *et al*., 2000). In an other research related to the subject 15,5 mg/kg copper was injected subcutaneously to mutant mice which were experimentally induced Monkeys' Kinky-hair disease. 29,5 mg/kg copper was given to the normal mice, 23,5 mg/kg copper was given to heterozygote mice. It has been found that in the mutant mice AST and LDH activity increased comparing with both two other groups but creatinine kinase (CK) level did not change (Shiraishi *et al*., 1991).

It has been determined that serum AST level related with the copper intoxication increased in sheep (West *et al*., 1987; Kerr and McGavin, 1991; Sansinanea *et al*., 1994 and Mairoka *et al*., 1997), cows (Struharikova *et al*., 1989; Perrin *et al*., 1990 and Auza *et al*., 1999), calf (Steffen *et al*., 1989) and lama (Junge and Thornburg, 1989), ALT increased in lambs (Mairoka *et al*., 1997), decreased in cow (Struharikova *et al*., 1989), LDH level increased in lama (Junge and Thornburg, 1980), CK concentration increased in cow (Perrin *et al*., 1990) and sheep (Gooneratne and Howell, 1980) and in an other research CK decrease was determined in calf (Struharikova *et al*., 1989).

It has been reported that as a result of giving dehydrated activated sludge (includes 1,820 zinc, 0,273 nickel 0,017 lead, 0,053 copper, 0,006 chromium and 0,005 mg/g cadmium) to rats, serum and liver AST levels increased, ALT levels statistically decreased in all experimental groups, serum LDH levels did not change in the group received %5 sludge but decreased in other groups (Bag *et al*., 1999).

Bires *et al*. (1995) are suggested that death cases were observed and also serum AST, ALT, and CK concentrations increased in the sheep which exposed to industrial wastes from the zinc and copper fabrics. Also it has been reported that giving heavy metals mixed with zinc and copper to the fish for 30 days significantly decreased total serum protein and serum glucose amount (Dhanapakiam and Ramasamy, 2001).

Although the effects of zinc or copper on liver enzymes were investigated frequently, there are limited studies about the effects of combine form of two elements on both liver enzymes and LDH, CK, glucose, total protein and albumin. 70-80 mg/kg/day zinc and 10-20mg/kg/day copper which are high doses for rats (Rhee & Dunlap, 1990 and Turner & Soliman, 2000) were used in the studies but the

effects of these doses on the haematological values which is important to define the animal health has not been investigated. Because of these reasons in this study it was aimed to investigate the effects of both different doses of zinc and copper and combine forms on some haematological parameters mentioned above.

## MATERIAL and METHODS

In this study 77 Wistar albino rats were used. rats were separated into 6 groups as one control (n=13) and five experimental (n=12) groups. While tap water was given to the control group (C), 70mg/kg/day zinc, 80mg/kg/day zinc, 10mg/kg/day copper, 20mg/kg/day copper, 70mg/kg/day zinc +10mg/kg/day copper were given to the experimental groups called $E_1$, $E_2$, $E_3$, $E_4$ and $E_5$ for 5 days a week during 4 weeks period.

At the end of 4 weeks all animals' serum AST, ALT, LDH, CK, glucose, total protein, albumin values were measured on a Ciba Corning Express Plus auto analyser by using Bio Clinica Commercial kits.

The averages about all data, standard error of the averages and the importance of checking of the differences between groups were measured on SPSS 9,01 statistical packet program by using Duncan test.

## RESULTS

It has been found that while there was no change observed on ALT, CK, total protein of all zinc applied experimental groups, AST decreased in zinc+copper received group, LDH decreased in both two groups zinc was given, comparatively with the other groups, glucose increased only in $E_2$ group, and albumin increased in the groups different doses of zinc was given comparatively with the group zinc+copper was given ($p<0,05$) (Table 1).

In Table 2 it was shown that LDH decreased in the group which was given 10mg/kg/day copper, ALT increased in the groups copper loaded ($E_3$, $E_4$) ($p<0,05$). While there was not any change in experimental groups' glucose amount comparatively with control group, it was determined that glucose amount decreased in $E_4$ group which was loaded with high concentration of copper and in zinc+copper received group ($p< 0,05$).

## DISCUSSION

Zinc and copper mostly accumulate in the liver (Metha and Eikum, 1989; Saito, 1996). Some researchers suggest that AST, GGT, glutamate dehidrogenaze and the other enzyme levels can not be used for the diagnosis of the functional damages in liver which are formed after accumulation of the heavy metals (Duncan & Prasse, 1986; Bohman *et al.*, 1987; Sutherland *et al.*, 1992; Botha *et al.*, 1993 and Sansinanea *et al.*, 1995). However there are some studies does not support this and suggest that zinc and copper loading which may lead to intoxication can increase liver enzymes levels (Metha & Eikum, 1989; and Saito, 1996 and Levengood *et al.*, 2000).

We can explain the reason of unaffected AST, ALT enzyme levels in rats after giving different doses of zinc and copper (Table 1-2) by using less zinc and copper doses than other researches (Shiraishi *et al.*, 1991; Syzmanska *et al.*, 1991; Zhang *et al.*, 2000 and Tekeli *et al.*, 2002) and these doses have not damaged the tissues and liver. Also the announcements indicated that loading +/-10 ppm copper to the different blood sheep races increased AST level but this increase linked with copper accumulation in liver support our results in susceptible races (vand der Berg *et al.*, 1983).

Defined AST levels in control and experimental groups except the group both zinc+copper were given, are close to the normal limits for the rats. Although at first the significant decrease of the AST level in $E_5$ group comparatively with the other experimental groups and control makes us think that giving zinc with copper may cause a damage in liver and soft tissues, unchanged enzymes like ALT, LDH, CK which are specific to the damages in these tissues do not support this idea. Our finding about AST in zinc+copper group is in contradiction with the report announced that AST level increased as a result of giving many heavy metals together to the rats (Bag *et al.*, 1999). But Bag et al. (1999) explained this increase by functional mitochondrial and respiratory disorders.

When the studies made in recent years was considered, it has not been encountered an article about the effects of giving zinc to rats on LDH level. Therefore we could not discuss our findings. But unchanged LDH in $E_5$ group is consistent with the findings of research that heavy metals were given together to rats. The reason of different decreases between both experimental groups copper was given and the literatures

(Junge & Thornburg, 1989 and Shiraishi *et al.*, 1991) could be explained by giving different doses of copper and zinc and applying them in a different way. Likewise, it has been reported that the effects of zinc and copper on haematological could vary depending on the level of this element in the diet, application way and animal species (Cordova *et al.*, 1993 and Singh *et al.*, 1994).

Gooneratneand and Howell (1980) have explained the CK increase related with the copper toxication by the temporary increase in muscle membrane permeability and occurred hemolyze. However in our study CK level did not change in all experimental groups. (Table 1-2). But affected CK levels by applying similar copper doses with rats to mice (Shiraishi *et al.*, 1991) support our results.

Although glucose level did decrease in mallards (Levengood *et al.*, 2000) and rats (Tekeli, 2002) by taking high doses of zinc (Bock *et al.*, 1995) Cordova (1994) reported a direct relation between glucose and zinc levels in blood and also an increase in the zinc and glucose rates in blood, relating to the increase of zinc rate in the feed in rats and Faye *et al.* (1995) also found similar results in female camels.

We can explain the increased glucose levels after receiving zinc (Table 1) by decreased insulin secretion due to zinc application. Likewise Yamaguchi and Takahashi (1984) reported that a single oral administration of zinc, sufficient to induce hypocalcaemia, caused a decrease in glucose tolerance in fasted rats and this decrease resulted from reduction of insulin secretion. Statistically insignificant decrease (Table 1-2) of glucose levels in the group, which zinc and copper mixture was given, is similar with the findings obtained from researches which were used fish as material (Dhanapakiam and Ramasamy, 2001).

It was observed that application of zinc and copper had different effects on total protein and albumin levels in the studies different animal species were used. For example the excess of zinc had no effects on lambs total protein level (Aksoy *et al.*, 2002) and it increased albumin level in rats (Tekeli, 2002) and camels (Faye *et al.*, 1995). There is a correlation between this results and ours. However our results are in contradiction with the reports announced that zinc decreased total protein in mallards (Levengood *et al.*, 2000) increased in rats (Tekeli, 2002), zinc and copper mixture decreased total protein in fishes (Duncan and Prasse, 1986), however increased in camels (Faye, 1995). However we can explain this contradiction between the studies by differences between animal species, heavy metal doses and application ways.

As a result it was found that while the zinc level applied to rats increased glucose and albumin levels, it decreased plasma LDH concentration as in Cu application, however zinc+copper mixture statistically decreased plasma AST level.

Consequently, we think that further studies focused on application of zinc+copper at different doses to different animals are required and findings of these studies would put a light on contradictory results of previous studies.

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Table 1: The effects of high zinc ($E_1$, $E_2$) and zinc+copper ($E_5$) on some parameters in rats.

| Parameters | $E_1$ (n=13) x | Sx | $E_2$ (n=13) x | Sx | $E_5$ (n=13) x | Sx | C (n=12) x | Sx |
|---|---|---|---|---|---|---|---|---|
| AST (µ/l) | 96.91$^{a}$ | 9.66 | 106.00$^{a}$ | 7.48 | 44.92$^{b}$ | 9.49 | 102.54$^{a}$ | 11.47 |
| ALT (µ/l) | 49.82$^{a}$ | 4.54 | 57.20$^{a}$ | 3.46 | 57.25$^{a}$ | 2.46 | 49.28$^{a}$ | 2.70 |
| LDH (µ/l) | 28.96$^{b}$ | 4.52 | 26.87$^{b}$ | 2.35 | 51.85$^{a}$ | 5.01 | 50.91$^{a}$ | 3.71 |
| CK (µ/l) | 46.92$^{a}$ | 9.73 | 33.61$^{a}$ | 5.54 | 50.60$^{a}$ | 8.34 | 55.94$^{a}$ | 7.32 |
| Glikoz (mg) | 98.38$^{ab}$ | 5.66 | 115.15$^{a}$ | 7.10 | 76.60$^{b}$ | 4.32 | 91.75$^{b}$ | 36.51 |
| Total Protein (g/dl) | 5.73$^{a}$ | 0.10 | 6.05$^{a}$ | 0.11 | 5.84$^{a}$ | 0.20 | 5.73$^{a}$ | 0.11 |
| Albumin (g/dl) | 2.52$^{ab}$ | 0.23 | 2.84$^{a}$ | 0.12 | 1.84$^{c}$ | 0.23 | 1.90$^{bc}$ | 0.28 |

$^{a,b,c}$: In each lines, the differences between the means having the different letters are significant ($P<0.05$).

Table 2: The effects of high copper ($E_3$, $E_4$) and zinc+copper ($E_5$) on some parameters in rats.

| Parameters | $E_3$ (n=13) x | Sx | $E_4$ (n=13) x | Sx | $E_5$ (n=13) x | Sx | C (n=12) x | Sx |
|---|---|---|---|---|---|---|---|---|
| AST (µ/l) | 119.15$^{a}$ | 11.13 | 111.58$^{a}$ | 6.41 | 44.92$^{b}$ | 9.49 | 102.54$^{a}$ | 11.47 |
| ALT (µ/l) | 51.92$^{a}$ | 2.54 | 62.16$^{a}$ | 3.75 | 57.25$^{ab}$ | 2.46 | 49.28$^{b}$ | 2.70 |
| LDH (µ/l) | 40.75$^{ab}$ | 5.96 | 36.61$^{b}$ | 3.78 | 51.85$^{a}$ | 5.04 | 50.91$^{a}$ | 3.71 |
| CK (µ/l) | 45.40$^{a}$ | 10.42 | 35.68$^{a}$ | 5.29 | 50.60$^{a}$ | 8.34 | 55.94$^{a}$ | 7.32 |
| Glikoz (mg) | 118.77$^{a}$ | 13.64 | 84.46$^{b}$ | 8.24 | 76.60$^{b}$ | 4.32 | 91.75$^{ab}$ | 36.51 |
| Total Protein (g/dl) | 5.91$^{a}$ | 0.10 | 5.89$^{a}$ | 0.18 | 5.84$^{a}$ | 0.20 | 5.73$^{a}$ | 0.11 |
| Albumin (g/dl) | 2.43$^{a}$ | 0.12 | 1.96$^{a}$ | 0.21 | 1.84$^{a}$ | 0.23 | 1.90$^{a}$ | 0.28 |

$^{a,b,c}$: In each lines, the differences between the means having the different letters are significant ($P<0.05$).