INVESTIGATION OF ANTIOXIDANT EFFECT OF ZINC BIOCHEMICALLY AND HISTOPATHOLOGICALLY IN RATS

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ABSTRACT
In this study, biochemically (Total anti oxidant capacity (TAC), Alanin aminotransferaz (ALT) ve Aspartat aminotransferaz (AST)) and histopathologically effects investigated in CCl₄ - treated rats and the effects of intraperitoneal zinc administration on these parameters. Cirrhosis was induced in 40 rats by intraperitoneal injections of CCl₄ twice a week over 6 weeks. Forty and ten additional animals were used as controls. Both groups were further subdivided to receive standard diet. TAC, ALT and AST were also worked out. Masson’s trichrome stain was applied to evaluate the fibrosis in liver. The results were evaluated by means of Student-t and Mann Whitney U tests in SPSS 6.0. TAC in plasma was significantly different into experimental goups ( P <0.05). Value of TAC was lower in B group than in C and D groups ( P <0.05), however differences between control and A,C,D groups was not important (P> 0.05). It is found out that the TAC in the groups which treated with CCl₄ + ZnSO₄ were higher than the groups those treated with only CCl₄. This finding support that zinc plays an important role in regeneration of the damaged cells and antioxidant defense mechanism. Moreover, the histopatologic findings of our study were revealed this hypothesis.

Introduction
Environmental effects such as smoke, gases, cause cellular damages in the Living organisms by forming free radicals metabolites (1, 2). These free radicals cause increase in permeability by affecting the structures of membranes. Cellular expansions, vacuolization and calcium accumulations occur in the affected cells due to the increase in permeability. At the further stages of cellular damaging, cell deaths occur (3). Therefore, antioxidant defense mechanisms against to free radical attacks get importance (4). A liver tissue consists of 1 to 2 mm diameters hexagonal lobules. Central vein, a branch of hepatic vein, is located in the center of these lobules. The portal area that involves hepatic vein, branches of hepatic artery and gall ducts are situated on the periphery of the hepatic lobules. The border between portal area and liver parenchymatous cells is known as limiting plate. Liver parenchymatous cells are ranged from central veins to periphery of the lobules, radially (5). Carbon tetrachloride is an important chemical in the world and especially used in dry cleaning (2). It forms extremely toxic free radicals with P-450 in the cells (CCl₃ + Cl). These free radicals cause the autooxidation of “Polyenic” fatty acids present in membrane phospholipids (3, 4). Therefore, rapid structural and functional defeats in endoplasmic reticulum and a decrease in protein synthesis rate of liver cells occur in 30 minutes and rough endoplasmic reticulum
TABLE 1

<table>
<thead>
<tr>
<th>Weeks</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>ZnSO₄</td>
<td>ZnSO₄</td>
<td>ZnSO₄</td>
<td>ZnSO₄</td>
<td>ZnSO₄</td>
<td>ZnSO₄</td>
</tr>
<tr>
<td>Group B</td>
<td>CCl₄</td>
<td>CCl₄</td>
<td>CCl₄</td>
<td>CCl₄</td>
<td>CCl₄</td>
<td>CCl₄</td>
</tr>
<tr>
<td>Group C</td>
<td>ZnSO₄</td>
<td>ZnSO₄</td>
<td>ZnSO₄ +CCl₄</td>
<td>ZnSO₄ +CCl₄</td>
<td>ZnSO₄ +CCl₄</td>
<td>ZnSO₄ +CCl₄</td>
</tr>
<tr>
<td>Group D</td>
<td>CCl₄</td>
<td>CCl₄</td>
<td>CCl₄+ZnSO₄</td>
<td>CCl₄+ZnSO₄</td>
<td>CCl₄+ZnSO₄</td>
<td>CCl₄+ZnSO₄</td>
</tr>
<tr>
<td>Control</td>
<td>Control</td>
<td>Control</td>
<td>Control</td>
<td>Control</td>
<td>Control</td>
<td>Control</td>
</tr>
</tbody>
</table>

release from ribosome and smooth endoplasmic reticulumes swell in two hour (2). Then, mitochondrial damages occur and permeability of the cellular membranes begin to increase. Calcium amount, taken into cell, increase rapidly due to the increase in permeability and cells begin to die consequently. An increase occur in collagen synthesis and at the next stage, cirrhosis develops in liver (1, 5, 6, 7). In the study, biochemical and histopathological investigations on the antioxidantal effects of zinc against to cellular damaging were carried out experimentally. According to these results, the opinion that zinc plays an important role in regeneration of the damaged cells and antioxidant defense mechanism was became more meaningful because the total antioxidant levels in the study groups those treated with CCl₄ + ZnSO₄ were higher than the groups those treated with only CCl₄ and also the histopathologic findings obtained from our research work is revealed this phenomena.

Materials and Methods

Each of the experimental groups consisted of six animals. The study was conducted in 30 male Wistar-Albino rats weighing 240 ± 30 g. One week before the start of the experiment, animals were placed in illuminated animal rooms for 12 hours cyclus. All the animals fed with a standard chow(Korkutelli feed-food industry/ Antalya- Turkey) and tap water.

0.5 mL of CCl₄ diluted in olive oil 1 : 1 (vol/vol) were given to animals by intraperitoneal injections. 3mL of ZnSO₄ (227 mg/Lt) were given to animals daily. 3mL cardiac blood samples were drawn from rats at the times shown in the Table 1 and collected into tubes with heparin. The plasma was seperated and samples were stored at -70 °C. Samples were taken from liver tissues stored in 10% formaldehyde. At the end of six weeks, sampling processes were completed in all groups. Total antioxidant capacity in plasma samples were measured with total antioxidant kits in Hitachi-902 otoanalyzator. Alanin aminotransferaz (ALT) ve Aspartat aminotransferaz (AST) in plasma were measured with kits of Dade Behring.

Livers of rats were removed and stored in 10% formaldehyde. Then, paraffin blocks were prepared with convenient samples taken from liver tissues. Microtome sections have thicknesses of 4 micron and prepared from these paraffin blocks were stained with hemotoxylin-eosin and also to examine the fibrosis developed in liver, Masson’s trichome stain was applied.

Results and Discussion

Total antioxidant levels and test results on the functions of liver

Prior to application, CCl₄ was given to experimental groups, and liver tissue was evaluated by histopathologically and, serum total antioxidant capacity (TAC), Alanin transferase (ALT)-Asparagine transferase enzyme levels were evaluated biochemically, and these valves were compared. TAC, ALT, AST values and histopathological result were found unsignificant statistically (p <0.05) within the group
Fig. 1. Total antioxidant levels of the groups, only ZnSO₄-treated (group A), only CCl₄-treated (group B), treated with ZnSO₄ for the first and second weeks and then treated with CCl₄ (group C), and treated with CCl₄ for the first and second weeks and then treated with ZnSO₄ (group D) are shown in the graphic. As the graphic reveals, in-group B, only CCl₄-treated, total antioxidant level was lower than the other groups. (P <0.05).

Fig. 2. ALT (alaninamino transferaz) and AST (aspartataamino transferaz) levels in groups. ALT levels in CCl₄-treated rats were higher than other groups. The increase on ALT levels in-group D indicates the improvement in the functions of liver. But AST levels in CCl₄-treated rats were not important different than other groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>TAC (nmol/lt)</th>
<th>ALT(U/lt)</th>
<th>AST(U/lt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.05±0.11</td>
<td>48</td>
<td>153</td>
</tr>
<tr>
<td>B</td>
<td>0.65±0.21</td>
<td>460</td>
<td>417</td>
</tr>
<tr>
<td>C</td>
<td>1.00±0.10</td>
<td>196</td>
<td>530</td>
</tr>
<tr>
<td>D</td>
<td>0.90±0.07</td>
<td>55</td>
<td>303</td>
</tr>
<tr>
<td>Control</td>
<td>0.93±0.06</td>
<td>46.5</td>
<td>122</td>
</tr>
</tbody>
</table>

TABLE 2 Total antioxidant levels and test results on the functions of liver
TABLE 3

Histopatological findings

<table>
<thead>
<tr>
<th>Groups</th>
<th>Hyperemia in hepatocytes</th>
<th>Degenerations in lobules</th>
<th>Vacuolization</th>
<th>Degenerations in lobules</th>
<th>Accumulation of gall water</th>
<th>Fibrosis</th>
<th>Cirrhosis</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>-</td>
<td>+++</td>
<td>+</td>
<td>3 Rats</td>
</tr>
<tr>
<td>C</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D</td>
<td>+</td>
<td>Light degeneration</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>1 Rat</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Fig. 3. Histological appearance of liver tissue of ZnSO$_4$-treated rats (Group A)

AST values are higher in the group B than in Group D.

TAC results were evaluated by statistically, the difference between group B and control was found significant (p<0.05). The difference between other groups was found unsignificant (p>0.05). The difference between group B and D was found significant according to the results of liver function tests ALT and AST (p<0.05).

Histopatological finding

In the study, histopatology effects of carbon tetrachloride and the reductive role of zinc sulfate on these effects were investigated. There were not any other findings except the serious hyperemia in central veins in ZnSO$_4$-treated rats (group A). Serious hyperemia in central veins, wide vacuolizations, degenerations in liver lobules and increases in fibrosis tissues were observed in CCl$_4$-treated rats in liver lobules. There were pseudolobules those have non-radial hepatocytes with no central veins, and surrounded with fibrosis bands in the livers of these rats. Fibrosis were determined by staining Masson’s trichrome stain in porto-portal, porto-central and central areas. When these findings were concluded it is clear that CCl$_4$ caused cirrhosis in these rats. Wide hyperemia, serious degenerations and vacuolization in hepatocytes were determined in rats (group C) those were treated with ZnSO$_4$ and CCl$_4$ in 3rd, 4th, 5th and 6th weeks after the ZnSO$_4$ treatment for the first two weeks. Irregular structures and small-scale fibrosis around the central veins and in portal areas were observed in lobules by staining with Masson’s trichrome stain. Wide hyperemia, serious degenerations and vacuolization in
Recent experimental studies showed that reactive oxygen species and lipid peroxidation have an important role in liver cirrhosis pathogenesis (10-12). Because of this event, antioxidants gained value both therapeutically and clinically in order to prevent cirrhosis (9). In chronic zinc deficiency, oxidation of lipids, proteins and DNA as a result of oxidative stress, and some changes occur at enzyme levels which has role in antioxidant defense system (13). Possible involvement of zinc in the oxidant defense system that prevention of cells from oxidative damage has been studied in different in vitro and in vivo models (14-23). Similarly, it was claimed that there were many mechanisms about the antioxidant activity of zinc in biological systems (24). One of these mechanisms is the exchange of zinc with transition metals. This exchange prevents redox cycle of metals, production of reactive oxygen species and oxidation of macromolecular in the environment. For the first time Girotti et al. defined that zinc prevents iron containing red blood cells against lipid peroxidation (25). In spite of the definition of role of zinc in the prevention of oxidation of macromolecules related with Fe and Cu, antioxidant properties of zinc can’t be explained completely (8). Capacity of zinc in order to prevent the membrane lipids from oxidation in the presence of different initiators by M. Paolo et al. (13). They defined the membrane and zinc...
interaction and its results in membrane rheology, probable prevention in the linkage of Fe to membrane and potential role of zinc in the prevention of membrane from oxidation as a part of the antioxidant chain. It was observed that zinc could not prevent the lipid peroxidation in the presence of different chemical and physical initiators. They claimed that zinc does not have a cleaning capacity of oxidant species. In the same study that stated that zinc prevented the lipid oxidation initiated by Fe$^{2+}$ and Cu$^{2+}$. It was reported that zinc has a more protective effect in the situation when Fe$^{3+}$ is used in excess amount Cu$^{2+}$ as initiators (26). In the same study it was reported that inhibitory effect of zinc is related with the high amount of negative charge of membrane in the lipid peroxidation membrane in the lipid peroxidation initiated by Fe$^{2+}$. Zinc shows an inhibitory effect by making the binding site of Fe$^{2+}$. Role of zinc in antioxidant defense mechanism was reported in many studies. In your study we investigated that zinc had effect whether by taking role in the mechanism which prevents the oxidation formation or by taking role in the fixing the damage formed. We used CCl$_4$ as oxidation initiators. Prior to application, CCl$_4$ was given to experimental groups, and liver tissue was evaluated by histopathologically and, serum total antioxidant capacity (TAC), Alanine transferase (ALT)-Asparagine transferase enzyme levels were evaluated biochemically, and these valves were compared. TAC, ALT, AST values and histopathological result were found unsignificant statistically ($p >0.05$) within the group C which zinc was applied before CCl$_4$ application and group D which zinc was applied after CCl$_4$ application. Yoshia et al explained by observation that high concentrations of zinc prevented the lipid peroxidation. They stated that they support the idea that zinc has a membrane protective role against the oxidation resulting from metals. In our histopathological and biochemical result it is seen that prior zinc application has no protective role. As a result, total antioxidant level (TAC) has observed significantly lover in the group supplied by CCl$_4$ for 6 weeks (Group B) them in the group supplied by CCl$_4$ + ZnSO$_4$ for 6 weeks (Group D), ($p<0.05$). This situation shows that CCl$_4$ weakens the antioxidant defense mechanism. Similarly, ALT and AST values are higher in the group B than in Group D. Histopathological results supports these results. When TAC results were evaluated by statistically, the difference between group B and control was found significant ($p<0.05$), the difference between other groups was found unsignificant ($p>0.05$). The difference between group B and D was found significant accor-
According to the results of liver function tests ALT and AST (p<0.05). According to the reports of M. Paola at all, Zn does not have role on oxidant cleaner in the presence of different initiators, but prevents the lipid oxidation initiated by Fe²⁺ and Cu²⁺. In the studies that we used CCl₄ as initiator, it was observed that Zn has no oxidant preventer role. According to our results, detection of total antioxidant level higher in the groups supplied with CCl₄ + ZnSO₄ than groups supplied with CCl₄, being the difference of ALT within the groups insignificant, show that Zn has an important role in the damaged cell fixing and antioxidant defense system. Total antioxidant levels in CCl₄-treated rats (group B) were meaningful lower than the levels in CCl₄ + ZnSO₄-treated rats for the last 4 weeks (group D). (p<0.05). This result indicates the negative effect of CCl₄ on antioxidant defense mechanism. Similarly, results of ALT and AST in-group B were higher than of group D. In addition, histological findings support these results. When total antioxidant levels were considered statistically, important differences between group B and group D were determined (P<0.05). Differences between other groups were minimal (P > 0.05). Differences between group B and group D were important according to the results of test on liver functions, ALT and AST (P< 0.05).

**Conclusions**

These results, higher total antioxidant levels in CCl₄ + ZnSO₄-treated rats compared to only CCl₄-treated rats and meaningful differences in the results of ALT and AST between these groups, reveal the important role of zinc in the regeneration of damaged cells and antioxidant defense mechanism.

**REFERENCES**