Effects of clinoptilolite treatment on oxidative stress after partial hepatectomy in rats

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Summary  Background/Objective: Clinoptilolite is a natural zeolite crystal. Cytoprotective effects of clinoptilolite have been reported. However, so far there are no data about the effects of clinoptilolite treatment on oxidative stress after partial hepatectomy. In this experimental study, the effects of clinoptilolite treatment after partial hepatectomy on oxidative stress were evaluated.

Methods: There were four experimental groups (n = 8): Group S, the sham group; Group H, the hepatectomy group; Group HC, the clinoptilolite treatment after partial hepatectomy group; and Group CS, the clinoptilolite-treated sham group. A 70% partial hepatectomy was performed for Group H and HC. Clinoptilolite (5 mg/kg) was given to the rats orally (via gavage tube) twice a day for 10 days after hepatectomy. Malondialdehyde (MDA), Cu-Zn super oxide dismutase (SOD), and glutathione (GSH) levels were assessed to evaluate oxidative stress.

Results: Plasma and liver tissue MDA levels of Group HC were significantly lower than the H group (p = 0.018 and p = 0.000, respectively). Liver tissue Cu-Zn SOD activity and GSH levels of Group HC were significantly higher than Group H (p = 0.003, p = 0.007, respectively).

Conclusion: Clinoptilolite administration reduces oxidant activity and supports antioxidant response after partial hepatectomy.

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1. Introduction

Liver surgery has been improved with advanced techniques and sophisticated perioperative care. However, liver failure following partial hepatectomy is still a matter of debate. Functional activity of the remnant liver and the presence of pre-existing liver disease such as cirrhosis, chronic hepatitis, and fatty liver disease are important parameters for posthepatectomy liver failure. Interestingly, liver insufficiency may also occur in patients who had no additional liver pathology after hepatectomy. Oxidative stress is one of the causes of liver injury after hepatectomy. Reduction of oxidant activity could prevent posthepatectomy liver failure. Applications of antioxidants could support the functions of the remnant liver.

Clinoptilolite (Na₆ [(AlO₂)₆(SiO₂)₃0]·24 H₂O) is a natural zeolite crystal that can be synthesized in laboratory conditions. Zeolites are hydrated microporous crystals with well-defined structures containing AlO₄ and SiO₄ tetrahedra linked through the common oxygen atoms. Natural micronized clinoptilolites have a positive influence on the immunologic and the inflammatory processes through the action on superoxide anions and nitric oxide.

Clinoptilolite also have antibacterial and anti diarrheic effects. There are no data about the effects of clinoptilolite administration following partial hepatectomy on oxidative stress. Hereby, in this experimental study, the effects of clinoptilolite administration after partial hepatectomy on oxidative stress were evaluated.

2. Methods

2.1. Experimental design

The Ethical Committee of the Animal Care Review Board of Istanbul University for the Experimental Medicine Research Institute approved the study. Adult Sprague-Dawley male rats, weighting 200–250 g, were obtained from Istanbul University, Cerrahpaşa Medical Faculty, Experimental Animal Research Laboratory. The animals were housed in accordance with National Legislation and the Council Directive of the European Communities on the Protection of Animals Used for Experimental and Other Scientific Purposes (L358/1, November 24, 1986). The rats were kept in standard cages (15 × 25 × 40 cm) under controlled conditions, including temperature of 23 ± 2°C, light (12-hour light to 12-hour darkness), and humidity of 50–55%. The animals were fed with standard rat chow and tap water ad libitum during the experimental procedure.

There were four experimental groups: Group S, the sham group (n = 8); Group H, the hepatectomy group (n = 8); Group HC, the clinoptilolite treatment after partial hepatectomy group (n = 8); and Group CS, the clinoptilolite-treated sham group (n = 8). Clinoptilolite (Froximun, Froxpharma Ilac Medikal Ltd. Sti., Istanbul, Turkey) 5 mg/kg was given to the rats of the HC and the CS groups orally (via gavage tube) twice a day for 10 days after partial hepatectomy. The S and the H groups received the same volume of oral saline solution (via gavage tube) at the same time.

To assess the optimum dose of clinoptilolite, we performed a preliminary evaluation in normal rats with different doses of oral clinoptilolite form 1 mg/kg to 100 mg/kg. We had observed no toxic effects of clinoptilolite in any dose and the results were similar between 5 mg/kg to 100 mg/kg for the antioxidant parameters [super oxide dismutase (SOD), and glutathione (GSH)]. A total of 5 mg/kg was the minimum dose of clinoptilolite that showed antioxidant activity.

The animals were sacrificed by cervical dislocation 10 days after partial hepatectomy. The liver tissue samples and the blood samples were collected for the histologic and the biochemical analyses. The plasma and the liver tissue samples were stored at −70°C until the biochemical analysis. The liver tissue samples were fixed in 10% formaldehyde solution for the histologic analyses.

2.2. Surgical procedure

The procedures were performed under ketamine (40 mg/kg, intraperitoneal) and xylazine (5 mg/kg, intramuscular) anaesthesia. The abdominal wall was cleansed with povidone iodine solution after shaving and a median abdominal incision was performed. Standard 70% partial hepatectomy was performed in the H and the HC groups, whereas laparotomy without liver resection was done in the S and the SC groups. The left and median lobes of the liver were exposed according to the standard 70% hepatectomy technique in the rats. The peduncle of the left and median lobe was ligated with 4/0 silk initially, and the lobes were resected. Right and caudate lobes of the liver were left in place in all of the rats. The abdominal incision was closed with 2/0 silk continuous sutures. No preoperative or postoperative deaths occurred in the rats during the experiment.

2.3. Biochemical procedure

2.3.1. Assays of plasma aspartate aminotransferase and alanine aminotransferase levels

Plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were measured by enzymatic methods using commercial kits on an Olympus AU800 analyzer (Olympus, Hamburg, Germany).

2.3.2. Assay of malondialdehyde

Lipid peroxidation levels in the plasma and the liver tissue were measured with thiobarbituric acid (TBA) reaction. This method was used to obtain a spectrophotometric measurement of the color produced during the reaction to TBA with malondialdehyde (MDA) at 535 nm. The coefficients of intra- and interassay variations for the MDA assay were 3.2% (n = 8) and 5.1% (n = 8), respectively.

2.3.3. Assay of GSH

The liver tissue GSH concentrations were estimated in accordance with the method of Beutler and colleagues. One milliliter of erythrocyte preparation and tissue homogenate were deproteinized and then centrifuged. After the addition of dithiobis-nitrobenzoate and phosphate buffer (pH 8.0), into the clear supernatants of the samples, the color that developed was read at 412 nm. GSH
concentrations of the samples were calculated using $1.36 \times 10^4 \text{ M} \cdot \text{cm}^{-1}$ as the molar absorption coefficient. The intra- and interassay coefficients of variation for GSH were 3.4% ($n = 8$) and 3.5% ($n = 8$), respectively.

2.3.4. Assay of Cu–Zn SOD
The plasma and the liver tissue Cu-Zn SOD superoxide dismutase (SOD) activities were determined by the method of Sun and colleagues\(^1\) with the inhibition of nitroblue tetrazolium reduction with xanthine/xanthine oxidase used as a superoxide generator. One unit of SOD was defined as the amount of protein that inhibited the rate of nitro blue tetrazolium (NBT) reduction by 50%. The intra- and interassay coefficients of variation for GSH were 3.6% ($n = 8$) and 3.8% ($n = 8$), respectively.

2.4. Histologic procedure

The liver tissues were fixed in 10% buffered formalin embedded in paraffin. Each section in 4-ı`m thickness was stained with hematoxylin and eosin for the light microscopic assessment. An arbitrary scope was given to each microscopic field at a magnification of $20 \times$, $40 \times$, and $100 \times$. Cellular lipoidosis, lipid deposition, cellular swelling, focal necrosis, mitosis, increase of Kupffer’s cell account, inflammation in portal area, presence of granuloma, evaluation of the central vein and the surrounding liver parenchyma, and cells with double nuclei were the principle criteria for the histopathologic evaluation.

2.5. Statistical analysis

The data were expressed as means ± standard deviation and 95% confidence intervals. The data were compared between the groups using one-way analysis of variance and posthoc Tukey’s test. SPSS 12.0 (SPSS: Statistical Package for Social Sciences, Chicago, IL, USA) was used for assessing the significance of differences between the groups. A $p$ value of $< 0.05$ was considered significant.

3. Results

The results of the biochemical parameters were summarized in Table 1. Plasma AST and ALT levels of the H group were significantly higher than the S group ($p = 0.000$). Plasma MDA levels of the S group were significantly lower than the H group ($p = 0.000$) and the HC group ($p = 0.000$). Liver tissue MDA levels of the S group were significantly lower than the H group ($p = 0.000$) and the HC group ($p = 0.003$). Plasma Cu-Zn SOD activity of the S group was significantly lower than the CS group ($p = 0.015$). The liver tissue Cu-Zn SOD activity of the S group was significantly higher than the H group ($p = 0.000$) and the HC group ($p = 0.009$). The liver tissue GSH levels of the S group were significantly higher than the H group ($p = 0.000$).

Plasma AST and ALT levels of the HC group were significantly lower than the H group ($p = 0.000$). The plasma MDA levels of the H group were significantly higher than the HC group ($p = 0.018$). The liver tissue MDA levels of the H group were significantly higher than the HC group and the CS group ($p = 0.000$). The liver tissue Cu-Zn SOD activity of the H group was significantly lower than the HC group ($p = 0.003$) and the CS group ($p = 0.000$). The liver tissue GSH levels of the H group were significantly lower than the HC group ($p = 0.007$) and the SC group ($p = 0.000$).

There was no difference regarding the histologic parameters between the experimental groups (Fig. 1).

4. Discussion

Oxidative stress is one of the important factors diminishing hepatocyte functions. Additional risk factors such as fatty liver disease or septicemia could predispose to the liver failure after hepatectomy by aggravating oxidant and inflammatory responses.\(^{12-14}\) MDA is one of the end products of lipid peroxidation. Tissue concentrations of MDA were assayed as an index of the membrane oxidative damage.\(^{15}\) Low amounts of reactive oxygen species (ROS) are generated in the mitochondria during the physiologic processes.\(^{16}\) The conditions that diminish the mitochondrial respiration can increase ROS production.\(^{17}\) We observed increased lipid peroxidation after partial hepatectomy. It has been suggested that ROS occurs after partial hepatectomy during the early phase of the liver regeneration.\(^{18}\) Oxidative stress and consequent lipid peroxidation cause harmful effects, which have been associated with the pathogenesis of the liver injury.\(^{19,20}\) Increased plasma AST and ALT levels of the H group confirmed this suggestion. It is well known that the ability of a cell to maintain functional homeostasis depends on the rapid induction of protective

Figure 1  (A) Normal liver tissue histology of the S group; (B) clinoptilolite had caused no harmful effect on hepatocytes in the HC group. (Hematoxylin & eosin staining, 40× magnification.)
antioxidant enzymes, and intracellular GSH levels play a central role in defending cells against oxidative stress.\(^\text{21,22}\) GSH is a coenzyme for various enzymes. SOD catalyses the conversion of two O\(_2^-\) molecules into H\(_2\)O and O\(_2\). GSH and SOD protect cells against oxygen radicals and toxic compounds.\(^\text{23}\) Depression of the mitochondrial GSH has been suggested as a cause of production of ROS levels following partial hepatectomy.\(^\text{24}\) As a result of reduced activity of the scavengers and the antioxidant protective systems, ROS becomes dominant and harmful.\(^\text{25}\) In our study, the liver tissue GSH levels and Cu-Zn SOD activity were reduced after partial hepatectomy. The oxidant effects of partial hepatectomy via inducing lipid peroxidation have been reported previously.\(^\text{26}\)

Antioxidant supplementation would be beneficial after partial hepatectomy. Fish oil and vitamin E have showed antioxidant response after partial hepatectomy. These agents are also supports the liver regeneration.\(^\text{22}\) Additionally, it has been demonstrated that vitamin C and vitamin E had hepatoprotective effects by attenuating lipid peroxidation.\(^\text{29}\) However, these proverbial antioxidants have various adverse events when they used for longer time periods in high doses. Researchers are looking for harmless antioxidants. It has been reported that clinoptilolite is one of the most abundant forms of zeolite. No toxic effects of clinoptilolite have been documented.\(^\text{29–31}\) In our study, clinoptilolite application reduced lipid peroxidation and normalized the liver functions after partial hepatectomy. Both natural and synthesized zeolites are characterized by the ability to lose and gain water reversibly, to absorb molecules of appropriate diameter, and to exchange their constituent cations without major change of their structure; the zeolites are also protective against the mycotoxins.\(^\text{32,33}\) Clinoptilolite is also has antitumoral effect via its immunomodulator activity similarly to superantigens.\(^\text{34}\) But, there are few in vivo studies has been published about the effects of clinoptilolite. We showed in vivo antioxidant properties of clinoptilolite against hepatectomy induced oxidative stress. High GSH levels and increased Cu-Zn SOD activity was observed in the remnant liver tissue of clinoptilolite treated rats. GSH and Cu-Zn SOD are involved in the antioxidant system and are important for protection from the oxidative damage. MDA and GSH were used as markers in monitoring therapy of the liver pathologies.\(^\text{35,36}\) Positive effects of clinoptilolite treatment on the parameters were seen in this study. However, the exact antioxidant mechanisms of clinoptilolite are not well defined. It has been described that the zeolites and the zeolite systems tend to neutralize the solutions, acting either as proton acceptors or donors.\(^\text{37}\) As a zeolite, clinoptilolite can modulate the disturbances in a redox state and could intercept the production of the peroxides and the free radicals via its amphoteric character, such as the clinoptilolite-Fe oxidase system.\(^\text{38}\)

Oxidants cause hepatocyte dysfunction after liver resection.\(^\text{1}\) We aimed to investigate the antioxidant activity of clinoptilolite against hepatectomy induced oxidative stress. The present study revealed that clinoptilolite administration reduces oxidant injury and increases antioxidant capacity after partial hepatectomy. After showing nontoxic and beneficial effects of clinoptilolite on the remnant liver, evaluation of the effects of clinoptilolite on liver regeneration in various pathologic conditions such as cirrhotic or fatty liver could be the aim of further studies. The use of clinoptilolite in clinical practice for liver-related pathologies should be assessed after a definitive experimental evaluation because the current data are not enough to reach an ultimate implication.

### References


### Table 1  Biochemical parameters of the groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Plasma MDA (nmol/ml)</th>
<th>Plasma Cu-Zn SOD (nmol/ml)</th>
<th>Liver tissue MDA (nmol/mg protein)</th>
<th>Liver tissue Cu-Zn SOD (nmol/mg protein)</th>
<th>Liver tissue Cu-Zn SOD (U/mg protein)</th>
<th>Plasma ALT (U/L)</th>
<th>Plasma AST (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>2.30 ± 0.28</td>
<td>23.44 ± 2.52</td>
<td>0.98 ± 0.13</td>
<td>0.70 ± 0.14</td>
<td>30.20 ± 2.86</td>
<td>24.4 ± 4.22</td>
<td>34.4 ± 3.9</td>
</tr>
<tr>
<td>S (p vs CS)</td>
<td>p = 0.151</td>
<td>p = 0.015</td>
<td>p = 0.296</td>
<td>p = 0.622</td>
<td>p = 0.565</td>
<td>p = 0.776</td>
<td>p = 0.809</td>
</tr>
<tr>
<td>CS</td>
<td>2.37 ± 0.26</td>
<td>27.50 ± 3.24</td>
<td>0.78 ± 0.29</td>
<td>0.76 ± 0.12</td>
<td>31.90 ± 3.63</td>
<td>28.78 ± 5.6</td>
<td>38.2 ± 6.9</td>
</tr>
<tr>
<td>H</td>
<td>3.62 ± 0.16</td>
<td>21.40 ±2.80</td>
<td>2.56 ± 0.32</td>
<td>0.36 ± 0.15</td>
<td>21.60 ± 2.72</td>
<td>110.6 ± 15.76</td>
<td>120.8 ± 20.98</td>
</tr>
<tr>
<td>p (H vs HC)</td>
<td>p = 0.018</td>
<td>p = 0.895</td>
<td>p = 0.000</td>
<td>p = 0.003</td>
<td>p = 0.007</td>
<td>p = 0.000</td>
<td>p = 0.000</td>
</tr>
<tr>
<td>HC</td>
<td>3.26 ± 0.31</td>
<td>22.30 ± 2.83</td>
<td>1.41 ± 0.25</td>
<td>0.53 ± 0.35</td>
<td>26.80 ± 2.25</td>
<td>37.8 ± 10.8</td>
<td>41.8 ± 5.92</td>
</tr>
</tbody>
</table>

ALT = alanine transaminase; AST = aspartate aminotransferase; GSH = glutathione; MDA = malondialdehyde; NS = not significant; SOD = superoxide dismutase.
Clinoptilolite administration after hepatectomy