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Use of alpha lipoic acid in preventing drug-induced cell damage in rat testicular tissue

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Abstract

This study aimed to evaluate the protective effects of alpha-lipoic acid (ALA), a potent antioxidant, on testicular oxidative damage induced by acitretin (ACT) and methotrexate (MTX) in rats, focusing specifically on the activities of cytosolic superoxide dismutase (SOD) and mitochondrial Mn-superoxide dismutase (MnSOD). *Wistar albino* rats were divided into four groups: control, ALA-only, ACT+MTX, and ACT+MTX+ALA. ACT (20 mg/kg/day), MTX (20 mg/kg/week), and ALA (50 mg/kg/day) were administered intraperitoneally. Rats were sacrificed on days 3, 5, and 7 post-treatment. SOD and MnSOD activities in testicular tissue were analyzed spectrophotometrically. Non-parametric Kruskal-Wallis tests were applied due to the non-normal distribution of data. The ACT+MTX group showed significant increases in both SOD and MnSOD activities, indicating strong oxidative stress induction. While ALA alone led to a non-significant, time-dependent increase in enzyme activities, its co-administration with ACT+MTX moderately reduced the oxidative stress response but failed to achieve statistical significance. The suppressive effect of ALA was more evident on mitochondrial MnSOD activity than cytosolic SOD, suggesting limited but tissue-specific antioxidant activity. The combination of ACT and MTX induces considerable oxidative stress in rat testicular tissue. ALA administration demonstrated a trend toward protective effects, particularly at the mitochondrial level, though not statistically significant. The findings suggest that ALA may offer partial cytoprotection, and further studies with adjusted dosing, extended durations, and broader antioxidant profiling are necessary to clarify its therapeutic potential in drug-induced reproductive toxicity.

Keywords: MTX; ACT; SOD; MnSOD; Alpha Lipoic Acid; Testicular Tissue

1. Introduction

Methotrexate (MTX) and acitretin (ACT) are systemic agents that have been used for treatment for many years longer than biological agents [1]. Methotrexate (MTX) is a folic acid antagonist antimetabolite that is widely used as an immunosuppressant in autoimmune diseases and in chemotherapy. Understanding the pharmacology of methotrexate supports its safe application in medicine in order to determine its optimum dose and to reduce its risks and side effects [2]. Acitretin belongs to a group of drugs known as retinoids, which include natural and synthetic compounds with activity similar to vitamin A. In addition to its effects such as regulation of the immune system, control of cellular growth, differentiation and proliferation, embryonic development, it also has immunological anti-inflammatory effects, induction of apoptosis and inhibition of tumor progression. [3]. Alpha-lipoic acid (ALA) is a bioactive molecule with specific antioxidant properties and important effects in health thanks to its oxidized form (ALA) and reduced form of dihydrolipoic acid (DHLA) [4]. Reactive oxygen species (ROS) are formed in cells as metabolic products and/or as a result of reactions in the mitochondrial respiratory chain. Free oxygen radicals produced under many physiological conditions are neutralized by the antioxidant defense system. [5]. Superoxide dismutase functions as an antioxidant enzyme that scavenges oxygen radicals with oxidation/reduction cycles at a very high reaction rate through transition metal ions in the active site. While superoxide dismutase (SOD), which contains copper-zinc in its active site, is located

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in the cytoplasm, superoxide dismutase (MnSOD), which contains manganese in its active site, is located in the mitochondrial matrix. [6].

In this study, the effects of ACT and MTX active substances and ALA on SOD and MnSOD activities in rat testicular tissue were investigated.

2. Material and Methods

The studies were approved by the Ethics Committee of Ondokuz Mayıs University (2018/ 13). *Wistar albino* type male rats produced and bred at Ondokuz Mayıs University, Experimental Animal Research Center (DEHAM) were used in the studies. Rats with an average weight between 200-250 gr were included in the study group. The organizations of experimental groups:

- Group 1 (Control): Control group with no treatment
- Group 2 (ALA group): Group given 50 mg/kg/day ALA
- Group 3 (ACT + MTX group): Group given 20mg/kg/day ACT and 20mg/kg/week MTX
- Group 4 (ACT + MTX + ALA): Group given 20mg/kg/day ACT, 20mg/kg/week MTX and 50mg/kg/day ALA

ACT, MTX and ALA were resolved in 0.9 % NaCl, ACT (20 mg /kg /day) (An et al. 2017), MTX (20 mg /kg /week) (Jingang et al. 2017), ALA (50 mg / kg / day) (Maritim et al.2003) and their combinations were given to the rats as intraperitonal injection (i.p) at the body weight level of each.

Rats from each of the experimental groups were sacrificed by cervical dislocation on the 3rd day, 5th day and 7th day after injection under general anesthesia. After this, perfusion was performed with 0.9% NaCl and the testicles were removed. Then, homogenization, sonication and centrifugation processes were carried out according to the procedures. The supernatants obtained after centrifugation at 15,000 rpm for 20 minutes at +4 °C were used for analysis.

SOD activity was determined by the spectrophotometric method of Mc Cord and Fridovich (1969) [7]. MnSOD activity was determined by modifying the Mc Cord and Fridovich (1969) [7] method by adding 10 mM NaCN.

One unit of SOD and MnSOD is determined as the amount of enzyme required for 50% inhibition of the reduction of cytochrome c by superoxide anion generated by the Xanthine/Xanthine Oxidase system. It is monitored spectrophotometrically at 550 nm.

2.1. Statistical Analysis

SPSS program was used for statistical analysis. Since the normality assumption was not provided in the analyses, the Kruskal Wallis test, which is the non-parametric equivalent of one-way analysis of variance, was performed.

3. Results

3.1. Effects on SOD Activity

After ALA alone was applied, a time-dependent increase in cytosolic SOD activity was observed; this increase reached 11% on day 3, 25% on day 5 and 27% on day 7. However, these changes were not found to be statistically significant ($p>0.05$) (Figure 1). The ACT+MTX combination provided a significant activation of approximately 3-fold on day 3 ($p<0.05$), but this effect decreased over time. This indicates that the ACT+MTX combination initially induced a strong oxidative response.

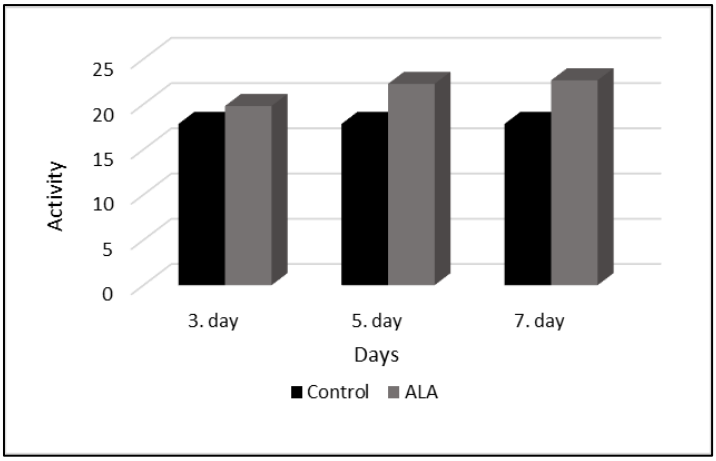

Figure 1 Time-dependent change in cytosolic SOD activity in ALA-administered rats

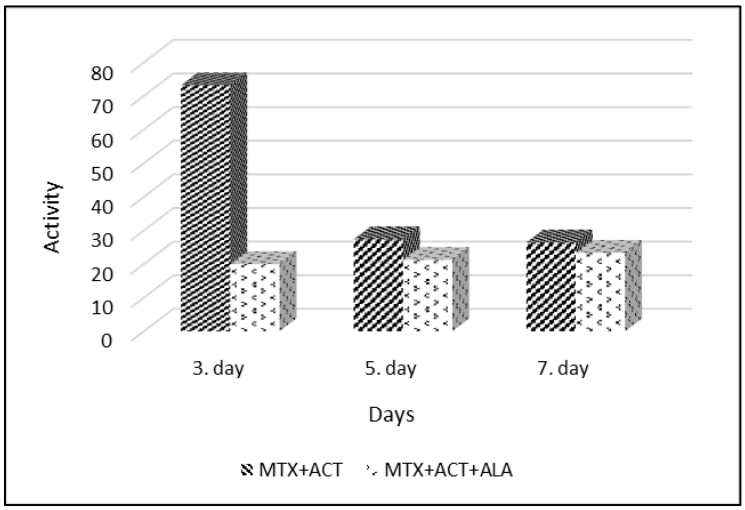

Figure 2 Time-dependent change in cytosolic SOD activity in rats given ACT+MTX and ACT+MTX+ALA

The combination of ACT+MTX+ALA caused a gradual increase in activity levels, with 11.70% activation on day 3, 18.60% on day 5, and 31% on day 7. However, these changes did not reach statistical significance. When ALA was added to the ACT+MTX group, 73% inhibition was observed on day 3, and this inhibition continued to decrease on the following days (Figure 2). These results show that ALA has the potential to suppress the ACT+MTX-induced oxidative response, but its efficacy remains limited and does not make a significant difference.

3.2. Effects on Mn-SOD Activity

A significant increase in Mn-SOD activity was observed as a result of the application of ALA alone. Approximately 88% activation was detected on the 3rd day, 83.20% on the 5th day and 75% on the 7th day (Figure 3). Although these increases were high, they were not found to be statistically significant ($p>0.05$). The ACT+MTX group reached the highest Mn-SOD activity, providing 91% activation on the 3rd day, 100% on the 5th day and 90% on the 7th day. In the ACT+MTX+ALA combination, it was determined that the activation levels were lower compared to the ACT+MTX group, remaining at 87%, 83% and 86%, respectively. When ALA was added to the ACT+MTX group, inhibition occurred at 28.70% on the 3rd day, 13.22% on the 5th day and 32% on the 7th day (Figure 4). However, these differences did not reach the level of statistical significance.

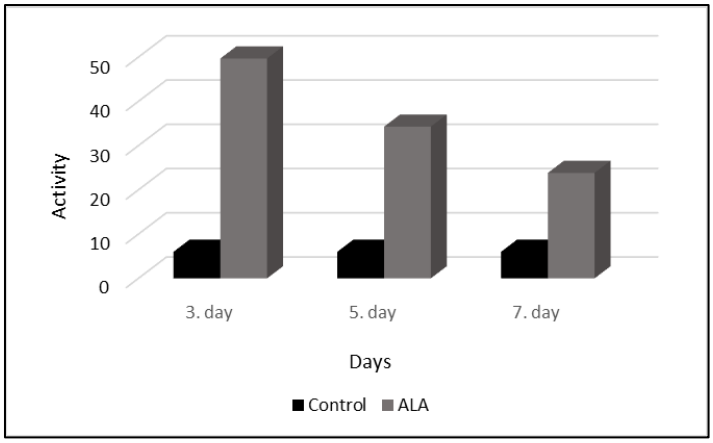

Figure 3 Time-dependent change in MnSOD activity in ALA-administered rats

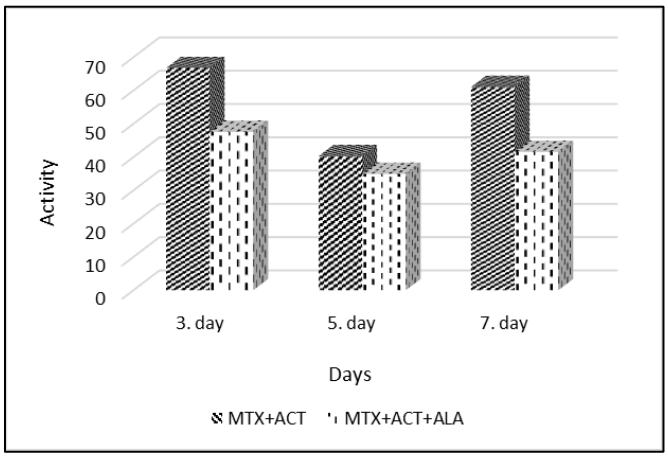

Figure 4 Time-dependent change in MnSOD activity in rats given ACT+MTX and ACT+MTX+ALA

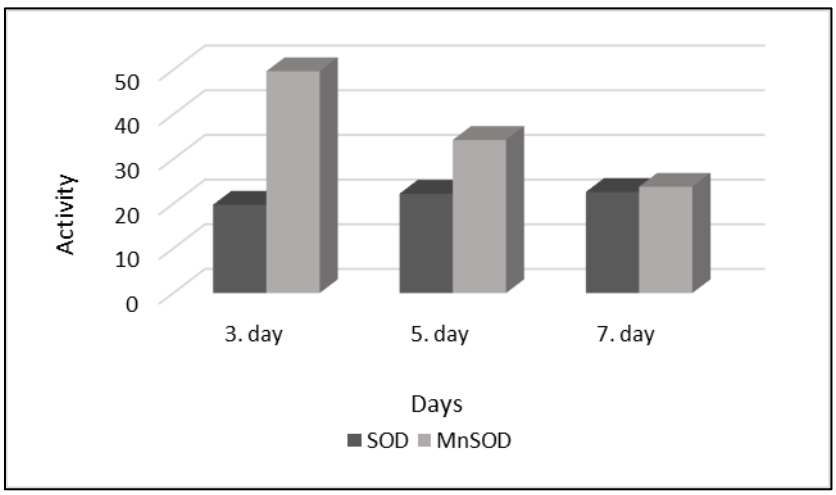

Figure 5 Time-dependent changes in SOD and MnSOD activities in rats given ALA

In the ALA group, MnSOD activity showed a maximum value on day 3 and decreased on days 5 and 7. In contrast, an increase in SOD activity was observed over time (Figure 5).

4. Discussion

According to the study results, it was observed that the combined application of ACT and MTX significantly increased both cytosolic and mitochondrial SOD activity in testicular tissue. This situation suggests that the ACT+MTX combination created high levels of oxidative stress in testicular tissue. It was observed that the application of ALA alone or in combination suppressed the response of the ACT+MTX combination to a limited extent, but did not create a statistically significant effect. Despite the known antioxidant effect of ALA, its insufficient effectiveness in this model can be explained by the dose used, application time, tissue response or drug interactions.

It is widely known in the literature that MTX causes oxidative stress in tissue regions such as the kidney, liver and testes. observed an increase in MDA and a decrease in enzyme activities in kidney tissue, indicating that MTX increases ROS production [8]. Similarly, Pınar et al. (2018) found a significant decrease in SOD, CAT and GPx values in rat testes treated with MTX, while this decrease was reversed with ALA [9]. Again, Aziz et al. (2020) encountered similar findings in lung tissue, showing that ALA brought SOD closer to its previous level [10].

Our findings are generally consistent with the literature data: MTX activates oxidative stress mechanisms, while ALA modulates these mechanisms, albeit to a limited extent.

In the literature, the antioxidant effect of ALA is also explained through the Nrf2/HO 1 axis [11]. For example, in studies conducted on cardio and hepatotoxic MTX models, ALA increased GSH, SOD, CAT activities and decreased MDA [12, 13]. On the other hand, in some clinical studies, additional ALA applications did not provide a significant increase in serum SOD [13]. In this study, ALA alone could not cause a strong SOD activation, but partially inhibited the high SOD increase caused by ACT+MTX. This indicates differences in the effect of ALA depending on the dose, duration and tissue specificity.

Mn-SOD directly responds to intracellular oxidative stress by detoxifying superoxide more rapidly. In this study, a very high Mn-SOD activity was observed with ACT+MTX. The decrease in activity was limited with the addition of ALA. This shows that the effect of ALA at the mitochondrial level is limited but present. On the other hand, it was concluded that the first response to ACT+MTX-induced radical damage is given at the mitochondrial level, and cytosolic SOD responds more slowly.

5. Conclusion

The toxicity profile of MTX use requires a tissue-protective approach in applications. At this point, the role of antioxidants such as ALA in combination applications becomes important. Further studies with larger samples, different doses and duration protocols are needed to evaluate the potential protective effects of ALA against ACT+MTX-induced damage. In addition, the inclusion of antioxidant enzymes other than SOD will provide a more comprehensive evaluation.

Compliance with ethical standards

Disclosure of conflict of interest

There is no conflict of interest between the authors.

Statement of ethical approval

The study was approved by the Ondokuz Mayıs University (Samsun, Turkey) Ethics Committee with the decision number 2018/13.

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