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Role of N-acetyl cysteine and serratiopeptidase against hormonal imbalance and oxidative damage in methotrexate-exposed rat testes

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***Abstract*— N-acetyl cysteine (NAC) and serratiopeptidase are two compounds with distinct yet complementary mechanisms that play a crucial role in protecting testicular function and maintaining reproductive health. This combination holds significant promise in safeguarding testicular tissue from external insults, such as those induced by chemotherapeutic agents like methotrexate. A total of 35 adult male albino rats (*Rattus*), weighing an average of 200 ± 20 grams and aged between 8 to 10 weeks, were used in this study. The animals were divided into five groups, with each group consisting of 7 rats. Methotrexate was administered at a dose of 100 µg/kg per day via intraperitoneal (IP) injection for two weeks. N-acetyl cysteine (NAC) was given orally at a dose of 600 mg/kg/day for the same duration, while serratiopeptidase(SER) was administered orally at a dose of 5 mg/kg/day for two weeks as well.**

5 ml of serum was collected to conduct clinical tests for hormones and oxidative stress after 2 weeks . The results showed a significant decrease in testosterone levels in the methotrexate (MTX) group compared to the control group, reflecting the negative impact of MTX on Leydig cells responsible for hormone production. However, an improvement in testosterone levels was observed in the groups treated with NAC and NAC + SER, indicating the role of these treatments in mitigating damage. For LH and FSH hormones, LH levels decreased in the MTX group compared to the control, while they improved in the NAC and NAC + SER-treated groups. As for FSH, a slight increase was observed in the MTX group, whereas its levels decreased in the treated groups, suggesting improved testicular function. Regarding oxidative stress markers, GSH levels decreased and MDA levels increased in the MTX group compared to the control, while these levels improved in the NAC and NAC + SER-treated groups, demonstrating the role of these treatments in reducing oxidative stress and enhancing testicular health.

Keywords: N-acetyl cysteine; Hormone; Methotrexate; Rat

INTRODUCTION

Methotrexate (MTX), a cornerstone chemotherapeutic agent, is widely used in the treatment of various malignancies and autoimmune diseases. However, its therapeutic benefits are often overshadowed by severe side effects, particularly on male reproductive health(1). MTX has been shown to induce hormonal imbalances and oxidative damage in the testes, leading to reduced testosterone production, impaired spermatogenesis, and compromised fertility (2). The drug's toxicity is primarily attributed to its ability to generate excessive reactive oxygen species (ROS), which disrupts the delicate balance of antioxidant defenses and damages testicular cells (3). In this context, N-acetyl cysteine (NAC), a potent antioxidant, and serratiopeptidase, an anti-inflammatory enzyme, have garnered attention for their potential protective roles. NAC mitigates oxidative stress by replenishing intracellular glutathione levels and scavenging free radicals, thereby preserving testicular integrity (4,5). Serratiopeptidase, on the other hand, reduces inflammation by degrading inflammatory proteins and promoting tissue repair, which is crucial for maintaining testicular function (6). Recent studies have demonstrated that the combination of NAC and serratiopeptidase can synergistically alleviate MTX-induced testicular damage by addressing both oxidative stress and inflammation. This study aims to explore the efficacy of NAC and serratiopeptidase in restoring hormonal balance and reducing oxidative damage in the testes of MTX-exposed rats. By investigating their therapeutic potential, this research seeks to contribute to the development of strategies that protect male reproductive health during chemotherapy, offering hope for improved quality of life for patients undergoing such treatments (7,8).

MATERIALS AND METHODS

The study was conducted at the College of Veterinary Medicine, University of Kerbala, in the physiology department's animal house. A total of 35 adult male albino rats (*Rattus*), weighing 200 ± 20 g and aged 8–10 weeks, were

used. The animals were acclimatized for two weeks before the experiment. They were housed in plastic cages (15 × 35 × 50 cm), with 7 rats per cage, under controlled conditions: temperature (22–25°C), a 14:10-hour light-dark cycle, and free access to water and a standard pellet diet (supplied by the Institute for Public Accuracy, IPA). Body weight was recorded at the start and end of the experiment to monitor weight gain.

STATISTICAL ANALYSIS

The Anova test was conducted using the statistical tool Graph Pad Prism 8.0, and a significance threshold of P≤0.05 was used. The analytic data is presented as the mean± standard deviation.

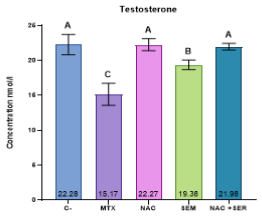

Figure 1. Comparison of serum Testosterone level in rat after exposure to MTX (Group 2), MTX + NAC (Group 3), MTX + SER (Group 4), and MTX + NAC + ESR(Group 5) with the control group (Group 1) The different letters refer to the significant change between groups ($p \leq 0.05$)

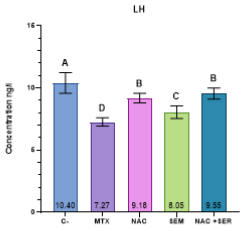

Figure 2. Comparison of serum Luteinizing hormone (LH) level in rat after exposure to **MTX** (Group 2), **MTX + NAC** (Group 3), **MTX + SER** (Group 4), and MTX + NAC + ESR(Group 5) with the control group (Group 1) The different letters refer to the significant change between groups ($p \leq 0.05$)

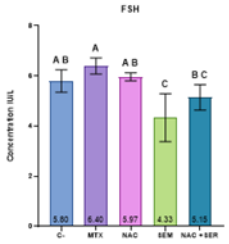

Figure 3. Comparison of serum Follicle stimulation hormone (FSH) level in rat after exposure to **MTX** (Group 2), **MTX + NAC** (Group 3), **MTX + SER** (Group 4), and MTX + NAC + ESR(Group 5) with the control group (Group 1). The different letters refer to the significant change between groups ($p \leq 0.05$)

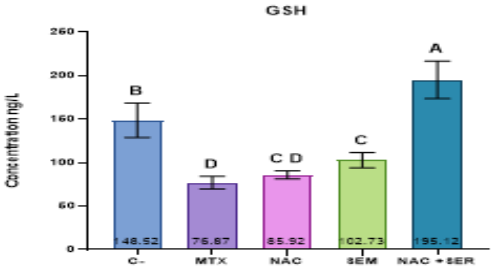

Figure 4. Comparison of serum Glutathione stimulation hormone (GSH) level in rat after exposure to **MTX** (Group 2), **MTX + NAC** (Group 3), **MTX + SER** (Group 4), and MTX + NAC + ESR(Group 5) with the control group (Group 1).
The different letters refer to the significant change between groups ($p \leq 0.05$)

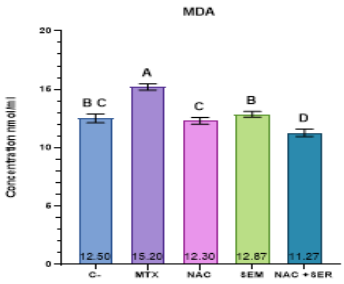

Figure 5. Comparison of serum malondialdehyde (MDA) level in rat after exposure to **MTX** (Group 2), **MTX + NAC** (Group 3), **MTX + SER** (Group 4), and MTX + NAC + ESR(Group 5) with the control group (Group 1). **The different letters refer to the significant change between groups ($p \leq 0.05$)**

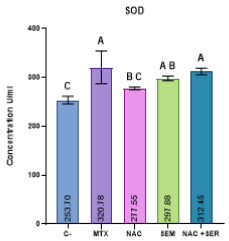

Figure 6. Comparison of serum Superoxide dismutase (SOD) level in rat after exposure to **MTX** (Group 2), **MTX + NAC** (Group 3), **MTX + SER** (Group 4), and MTX + NAC + ESR(Group 5) with the control group (Group 1). **The different letters refer to the significant change between groups ($p \leq 0.05$)**

RESULT & DISCUSSION

The results showed a significant decrease in testosterone levels in the MTX group (15.16 ± 1.57) compared to the control group C- (22.28 ± 1.46). This decline reflects the negative impact of Methotrexate on Leydig cells in the testes, which are responsible for testosterone production. These findings are consistent with the results of Babakhanzadeh,*et al*, (9), who demonstrated that MTX causes a reduction in testosterone levels due to increased oxidative stress and damage to hormone-producing cells. However, a noticeable improvement in testosterone levels was observed in the groups treated with NAC (22.26 ± 0.87) and NAC + SER (21.98 ± 0.47), indicating that these treatments helped mitigate the damage caused by MTX. These results align with the findings of Aldini *et al*. (10), who reported that NAC acts as an antioxidant and improves testicular function by reducing oxidative stress. Additionally, Serratiopeptidase contributed to reducing inflammation caused by MTX, leading to improved testicular function, which is consistent with the findings of Jadhav et al.2020(11). In the MTX group, a significant decrease in LH levels (7.26 ± 0.34) was observed compared to the control group (10.4 ± 0.82). This result is in agreement with the study by Shrestha *et al.* (12) who found that MTX negatively affects the pituitary gland, leading to reduced secretion of gonadotropins. However, LH levels improved in the groups treated with NAC (9.18 ± 0.38) and NAC + SER (9.55 ± 0.45), suggesting that these treatments helped restore hormonal balance. As for FSH, a slight increase was observed in the MTX group (6.40 ± 0.32) compared to the control group (5.80 ± 0.45), which can be explained by the body's attempt to compensate for the damage caused by MTX through increased stimulation of the testes. This explanation is supported by the findings of Dashti *et al.* (13). However, FSH levels decreased in the groups treated with NAC and NAC + SER, indicating improved testicular function and responsiveness to gonadotropins.

The results showed a significant decrease in GSH levels (76.86 ± 7.23) and an increase in MDA levels (15.20 ± 0.26) in the MTX group compared to the control group (148.51 ± 19.69 and 12.50 ± 0.37, respectively). These findings are consistent with the study by Ansari *et al.* (14),who demonstrated that MTX increases the production of free radicals and reduces levels of endogenous antioxidants such as GSH. However, GSH and MDA levels improved in the groups treated with NAC and NAC + SER, indicating that these treatments helped reduce oxidative stress. This improvement aligns with the results of Raghu *et al.* (15), who found that NAC acts as a source of GSH, thereby enhancing antioxidant defenses. Additionally, Serratiopeptidase contributed to reducing inflammation associated with oxidative stress, leading to improved testicular health, which is consistent with the findings of Premrajan *et al.* (16).

The results of this study highlight the detrimental effects of methotrexate (MTX) on testicular function, particularly in terms of hormonal imbalance and oxidative stress, as well as the protective roles of N-acetyl cysteine (NAC) and serratiopeptidase (SER) in mitigating these effects. The significant decrease in testosterone levels in the MTX group (15.16 ± 1.57) compared to the control group (22.28 ± 1.46) can be attributed to the toxic impact of MTX on Leydig cells, which are responsible for testosterone synthesis. MTX is known to induce oxidative stress, leading to cellular damage and impaired steroidogenesis (17). The improvement in testosterone levels in the NAC (22.26 ± 0.87) and NAC + SER (21.98 ± 0.47) groups underscores the antioxidant properties of NAC, which mitigate oxidative damage and restore Leydig cell function (18). Additionally, the anti-inflammatory effects of serratiopeptidase likely contributed to reducing testicular inflammation, further supporting hormonal recovery (19).

The observed decrease in luteinizing hormone (LH) levels in the MTX group (7.26 ± 0.34) compared to the control group (10.4 ± 0.82) suggests that MTX negatively affects the hypothalamic-pituitary-gonadal (HPG) axis, impairing the secretion of gonadotropins (20). The restoration of LH levels in the NAC (9.18 ± 0.38) and NAC + SER (9.55 ± 0.45) groups indicates that these treatments help normalize pituitary function, possibly by reducing oxidative stress and inflammation. The slight increase in follicle-stimulating hormone (FSH) levels in the MTX group (6.40 ± 0.32) compared to the control group (5.80 ± 0.45) may reflect a compensatory mechanism by the pituitary gland to stimulate the damaged testes (21). The subsequent decrease in FSH levels in the treated groups suggests improved testicular responsiveness to gonadotropins, likely due to the protective effects of NAC and SER on testicular tissue (22)

The significant reduction in glutathione (GSH) levels (76.86 ± 7.23) and increase in malondialdehyde. (MDA) levels (15.20 ± 0.26) in the MTX group compared to the control group (148.51 ± 19.69 and 12.50 ± 0.37, respectively) highlight the oxidative damage induced by MTX. MTX generates reactive oxygen species (ROS), depleting endogenous antioxidants like GSH and increasing lipid peroxidation, as indicated by elevated MDA levels (23). The improvement in GSH and

MDA levels in the NAC and NAC + SER groups demonstrates the efficacy of NAC in replenishing GSH stores and reducing oxidative stress (10,24). Furthermore, serratiopeptidase's anti-inflammatory properties likely contributed to reducing oxidative damage by mitigating inflammation-associated ROS production (25).

In conclusion, the findings of this study demonstrate that MTX-induced testicular damage is mediated through oxidative stress and hormonal disruption. The protective effects of NAC and serratiopeptidase are attributed to their antioxidant and anti-inflammatory properties, which restore hormonal balance and reduce oxidative damage. These results align with previous studies and provide a physiological basis for the use of NAC and serratiopeptidase as adjunct therapies to protect male reproductive health during MTX treatment. Further research is warranted to explore the long-term benefits and mechanisms of these interventions.

CONCLUSION

Through the current study conclude that as the amount of rice provided to broiler chickens increases leads to a decrease in the length, width, and crept depth of the villi. Therefore,

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