P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com

Research Article https://doi.org/10.47278/journal.ijvs/2025.121

N-acetylcysteine Attenuates the Deleterious Effects of Tramadol on Male Health and Semen Characteristics in Rabbits

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Article History: 25-227 Received: 09-Aug-25 Revised: 26-Sep-25 Accepted: 28-Sep-25 Online First: 20-Oct-25

ABSTRACT

Tramadol (TRM) is mainly used for pain relief but sometimes causes severe toxic effects in humans. N-acetylcysteine (NAC) is a beneficial treatment against the deteriorative effects of free radicals and reactive oxygen species. The present study investigates the possible impact of NAC administration on male health and semen characteristics in rabbits, as an animal model, treated with TRM. Thirty-two mature and healthy New Zealand white rabbit bucks were assigned to four equal groups (n=8) subcutaneously injected with 0.5mL of saline solution (control group), 20mg/kg tramadol (TRM group), 150mg/kg N-acetylcysteine (NAC group), or NAC and TRM (NAC+TRM group), respectively. The injection treatments were applied three times per week for 4 consecutive weeks. After that, blood samples were taken from the marginal ear vein to measure plasma testosterone (TS), total antioxidant capacity (TAC), malondialdehyde (MDA), alanine and aspartate aminotransferase enzymes (ALT and AST), creatinine (CR), and urea (UR). Furthermore, ejaculations were collected from the bucks to evaluate semen volume, concentration, sperm motility, and quality parameters. The results demonstrated that TRM treatment substantially ($P<0.05$) decreased the TS and TAC while increased the MDA, ALT, AST, CR, and UR, compared to the control. Moreover, TRM treatment dramatically ($P<0.05$) decreased the semen volume, concentration, sperm motility, and quality. In contrast, NAC administration markedly ($P<0.05$) improved all parameters in the NAC group and reduced the deleterious effects of TRM in the NAC+TRM rabbit groups. It is concluded that NAC treatment enhanced the male rabbit's health and alleviated tramadol's deterioration of male reproduction.

Keywords: N-acetylcysteine; Tramadol; Testosterone; Antioxidant biomarkers; Semen characteristics; Male rabbits

INTRODUCTION

Males contribute to 40-50% of infertility in animal species, and the precise prediction of male fertility is crucial for the economic success of the animal breeding industry (Kumaresan et al. 2020). It is suggested that reactive oxygen species (ROS) and oxidative stress (OS) play a pivotal role in male infertility by impairing sperm function through various molecular mechanisms, such as lipid peroxidation, DNA fragmentation, and protein oxidation (Wang et al. 2025). In contrast, antioxidant compounds suppress the excessive OS and ROS production and provide scavenger media for regular sperm functionality and fertilization during capacitation, hyperactivation, and fusion (Collodel et al. 2020).

Tramadol (TRM) is one of the practical and inexpensive analgesic drugs that is mainly used to ameliorate acute/chronic pain conditions via inhibiting neuronal activity in zoological animals (Ahmadian-Moghadam et al. 2021). However, bioactive metabolites of TRM may cause severe hepato-, nephro-, reno- and neurotoxic effects in rats and rabbits (Sheweita et al. 2018; Aghajanpour et al. 2020; Elsukary et al. 2022). It has been shown that tramadol induces OS that consequently harms sperm morphology, concentration, and motility in rat males (Koohsari et al. 2020). It also causes testicular mitochondrial dysfunction, apoptosis, inflammation, and histological degeneration (Feng et al. 2022). It was reported that tramadol decreased the levels of gonadotropin hormones and testosterone secretion in rats (El Sawy and Malak 2015; Mowaad et al. 2022) and rabbits (Sheweita et al. 2022).

N-acetylcysteine (NAC) is a sulfhydryl-containing amino acid extracted naturally from plant and animal products and it acts as a generic antioxidant for the

Cite This Article as: Al-Ahmad HM, Hassanien HA, Abbas AO and Mustafa ES, 2026. N-acetylcysteine attenuates the deleterious effects of tramadol on male health and semen characteristics in rabbits. International Journal of Veterinary Science 15(1): 179-187. https://doi.org/10.47278/journal.ijvs/2025.121

scavenging of ROS and OS in cell culture and/or animal experiments (Kulvinder Kochar et al. 2022; Geetika et al. 2025) . It was found that NAC therapy was effective in managing oxidative stress in dogs with chronic kidney disease, inducing a remarkable decrease in serum lipid peroxidation and an increase in glutathione peroxidase activity (Balakrishnan et al. 2023). NAC was also applied as a potential antioxidant, protecting the male rat testes from the adverse effects of fungicides (Mohammadi-Sardoo et al. 2018). In addition, NAC was suggested as a successful treatment against ROS and aflatoxin contamination in rabbits (Çam et al. 2008; Atef et al. 2016). Moreover, subcutaneous injection of NAC in New Zealand white rabbit males improved antioxidant status, promoted liver and kidney functions, and enhanced semen characteristics (Abdel-Rahman et al. 2022).

As far as we know, there are few studies concerning the protective role of NAC in alleviating the adverse effects of TRM in animal species. Only in albino rats, it was evidenced that NAC administration improves the TRM–induced nephrotoxicity (El-Aziz et al. 2023) and hepatotoxicity (Adikwu and Bokolo 2017). In view of the above information, the current study sought to assess the effects of NAC administration on plasma testosterone levels, antioxidant biomarkers, metabolites, and sperm kinetic and quality characteristics in TRM-treated rabbit males as an animal model.

MATERIALS AND METHODS

Animals and experimental design

Thirty-two mature and healthy New Zealand white rabbit bucks of 7 months old and 3.520±0.112kg at the initiation of the experiment were used. Bucks were maintained individually in a semi-closed rabbitry housing system during the trial. The bucks were kept under standard management conditions and fed commercial diets that meet rabbit nutritional requirements per kg diet of 185g crude protein, 132g crude fiber and 2500kcal digestible energy. The rabbits had free access to water and meals. The bucks were assigned to four equal treatment groups (n=8), which received either a subcutaneous injection of 0.5mL saline (Control group), a subcutaneous injection of 20mg/kg tramadol (Accord Healthcare, Whiddon Valley, UK) (TRM group), a subcutaneous injection of 150mg/kg N-acetylcysteine (Sigma, St Louis, MO, USA) (NAC group), or a subcutaneous injection of NAC and TRM (NAC+TRM group). The treatments of TRM and NAC were repeated three times per week for four consecutive weeks. The TRM and NAC doses implemented in the study were approved by previous studies (Atici et al. 2004; San-Miguel et al. 2006).

Blood sampling and analysis

Blood samples were drawn from each bucks' marginal ear vein at the conclusion of the therapy and put into heparinised tubes. After centrifuging the plasma at 1000×g for 15min at 4°C, the resultant plasma was kept at -20°C until it could be examined further.

Testosterone assay

ELISA kits (CUSABIO, Houston, TX, USA) were used to measure the levels of plasma testosterone (TS). The plate was incubated at 37°C for 60min after 50μL of standard or sample and 50μL of conjugate were added to each well in accordance with the manufacturer's instructions. Following incubation, unbound material was removed from the wells by washing them three times with 200μL of wash buffer. Each well was then filled with 50μL of HRP-avidin, and it was incubated for 30min at 37°C. Following three additional rounds of washing, 50μL of substrate solutions A and B were added to each well, and they were then incubated for 15min at 37°C. After adding 50μL of stop solution to each well, the optical density was measured within 10min at 450nm using a microplate reader. The assay's detection range was 0.6–10.0ng/mL, and its accuracy within and across assays was less than 15%.

Antioxidant biomarkers

Using colorimetric test kits, the levels of malondialdehyde (MDA) and total antioxidant capacity (TAC) in plasma were measured (Elabscience Biotechnology Inc., Houston, TX, USA). 100μL of plasma, 1mL of buffer reagent, 2mL of chromogenic reagent, and 0.5mL of ferric salt reagent were combined for the TAC test. After 30min of incubation at 37°C, 100μL of stop reagent was added to the mixture. Within 10min, the optical density was determined using a spectrophotometer calibrated to 520nm. The test has an intra-assay accuracy of 2.7% and an inter-assay precision of 8.2%, with a detection range of 0.6-145.2U/mL. 100μL of plasma, 100μL of clarity reagent, 3mL of acid reagent, and 1mL of chromogenic reagent were mixed together for the MDA test. After 40min of incubation at 100°C, the mixture was allowed to cool to room temperature before being centrifuged for 10min at 3000×g. After collecting the supernatant, a colorimetric spectrophotometer was used to detect absorbance at 532nm. With intra-assay accuracy of 4.9% and inter-assay precision of 8%, the detection range was 0.38−133.33nmol/mL.

Metabolites assay

The current research measured metabolites that indicate kidney function, such as urea nitrogen (UR) and creatinine (CR) concentrations, and liver function, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzymes. In accordance with the manufacturer's instructions, colorimetric test kits (Elabscience Biotechnology Inc., Houston, TX, USA) were used for all studies. In microplate wells, 5μL of sample or standard solution was combined with 20μL of substrate solution for the AST and ALT tests. The mixture was then incubated for 30min at 37°C. Following the addition of 20μL of chromogenic agent to each well, the wells were incubated at 37°C for 20min. After mixing and adding 200μL of alkali working solution, the plate was allowed to sit at room temperature for fifteenmin. A microplate reader was used to measure the optical density at 510nm. For AST and ALT, the detection ranges were 1.1–72.3IU/L and 0.8–72.3IU/L, respectively. For AST, the intra-assay accuracy was less than 7.9% and less than 5.5%, whereas for ALT, it was less than 5.7% and less than 9.4%. Twelve microlitres of the sample or standard, 180 microlitres of enzyme solution A and fivemin of incubation at 37°C were added to each well for the CR test. After that, 60μL of

enzyme solution B was added, and the mixture was incubated at 37°C for two moremin. At 515nm, absorbance was measured. In each well, 50µL of enzyme working solution was combined with 4µL of sample or standard for the UR test, which was then incubated for 10min at 37°C. Following the addition of 125µL of chromogenic and alkaline agents, the mixture was incubated for an additional 10min at 37°C. At 580nm, absorbance was measured. Both CR and UR have detection ranges of 20.5−400.0µmol/L and 0.3–35.0mmol/L, respectively. For CR and UR, the intra-assay and inter-assay precisions were less than 1.7 and 4.2%, respectively, and less than 3.3 and 4.5%.

Semen sampling and analysis

At the conclusion of the experiment, bucks were ejaculated utilising an artificial vagina. Within 30min of collection, the semen samples were processed in sterile glass tubes in a water bath at 37°C.

Semen volume and concentration

A graded tuberculin syringe was used to measure the sperm volume after the gel mass was removed, with an accuracy of 0.1mL. Five microlitres of semen were placed into a Neubauer hemocytometer and diluted with one millilitre of 1% formal saline in order to assess the concentration of sperm. Direct cell counting under a 400× magnification microscope was used to determine the sperm concentration.

Sperm kinetics

Using the procedures outlined by (Jimoh and Ewuola 2019), sperm kinetics were assessed. A tiny aliquot of recently ejaculated semen was put on a sterile glass slide and seen under a microscope with a 10× objective lens to measure mass motility. The degree of wave-like motion brought on by the collective movement of spermatozoa was used to grade mass motility subjectively; the range ranged from no apparent waves (+) to very turbulent motion (+++). Using a micropipette, a drop of semen was diluted with semen extender and put on a microscope slide that had been preheated in order to measure increasing motility. A coverslip was placed over the slide, and it was seen at 400x magnification. The proportion of spermatozoa displaying progressive forward migration was calculated on a subjective scale ranging from 0% to 100%. Every sample was examined in a minimum of five microscopic domains.

Sperm quality

The Eosin-Nigrosin staining technique was used to evaluate the viability of the sperm. 20µL of stain solution (0.6g Eosin-Y, 5g Nigrosin and 3g sodium citrate diluted in 100mL distilled water) was combined with 10µL of semen and applied to a heated slide. The slide was then left to air dry at room temperature. Viability was assessed using immersion oil and a phase-contrast microscope at 1000× magnification, looking at 200 sperm per slide. Complete or partial purple staining was regarded as non-viable, but spermatozoa that did not exclude the stain were categorised as viable. After staining with a buffered Giemsa solution for 90min, as explained by (Abdelatty et al. 2020), the sperm acrosomal integrity was assessed. The hypo-osmotic swelling test (HOST) was used to determine the sperm plasma membrane's functioning (Ducci et al. 2002). 100µL of semen and 900µL of a solution containing 60mOsmol/L fructose and 1% (w/v) Eosin Y were combined for the HOST test, which was then incubated for 30min at 37°C. After applying a drop of the mixture to a slide, it was examined at 400× magnification using a phase-contrast microscope. Out of 200 counted sperm per slide, the percentage of spermatozoa with inflated and coiled tails—a sign of intact plasma membranes—was noted.

Statistical analysis

IBM SPSS Statistics was used to determine the means and standard errors for every treatment group (IBM Corp., Armonk, NY, USA). One-way ANOVA was used to examine differences between treatment groups, and $P<0.05$ was used as the threshold for statistical significance.

RESULTS

Plasma testosterone

Fig. 1 shows the plasma TS levels as impacted by TRM and NAC treatments. When compared to the control, the TRM therapy substantially ($P<0.05$) reduced the plasma TS levels by 35.7%. TRM's harmful effects were considerably ($P<0.05$) mitigated by the NAC treatment, which also raised TS levels from 5.45ng/mL in the TRM group to 6.82ng/mL in the NAC+TRM group.

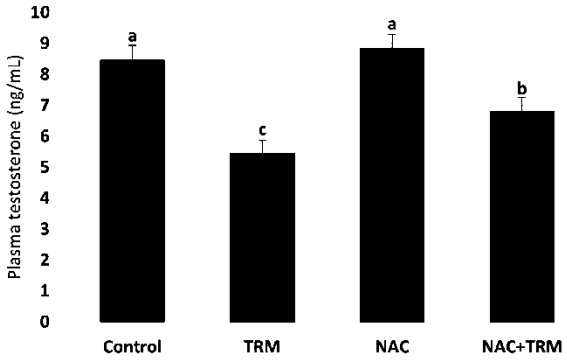

Fig. 1: Effect of tramadol (TRM) and N-acetyl cysteine (NAC) treatments on the plasma testosterone level in male rabbits. Treatment groups: New Zealand white rabbits were divided into four equal groups (n=8), subcutaneously injected with 0.5mL of saline solution (control group), 20mg/kg tramadol (TRM group), 150mg/kg N-acetylcysteine (NAC group), or NAC and TRM (NAC+TRM group), respectively. The injection treatments were applied three times per week for 4 consecutive weeks. Bars express means with standard error. Means with different superscripts significantly differ at P-value less than 0.05.

Antioxidant biomarkers

Fig. 2 displays the plasma TAC and MDA levels as impacted by TRM and NAC treatments. The findings showed that, in comparison to the control, TRM therapy substantially ($P<0.05$) raised the MDA by 31% and lowered the TAC by 19%. On the other hand, as compared to the control, NAC therapy substantially ($P<0.05$) raised the TAC by 46% and lowered the MDA by 23%. Additionally, the administration of NAC in the NAC+TRM group normalised the plasma TAC and MDA levels to those of the control and substantially ($P<0.05$) reduced the negative effects of TRM on the antioxidant biomarkers.

Plasma metabolites

Fig. 3 shows the plasma levels of AST, ALT, CR, and UR as affected by TRM and NAC therapy. The TRM therapy significantly (P<0.05) increased the levels of AST, ALT, CR, and UR. The NAC group's ALT and CR levels were much lower than those of the control group. Additionally, the NAC+TRM group's AST, ALT, CR, and UR levels were considerably (P<0.05) lower than those of the TRM group, but still greater than those of the control group.

Semen volume and concentration

Fig. 4 shows how TRM and NAC treatments affected the amount and concentration of semen. Semen volume dropped from 0.9mL to 0.3mL and concentration from 412.5 to 311.1 million sperm/mL as a result of the TRM therapy (P<0.05). In contrast, the concentration (420.7 million sperm/mL) remained the same, but the NAC therapy considerably (P<0.05) raised the semen volume to 1.1mL in comparison to the control. Additionally, NAC treatment substantially (P<0.05) reduced the NAC+TRM group's semen volume and concentration (0.8mL and 358.2 million sperm/mL, respectively) and increased them to values that were comparable to those in the control group.

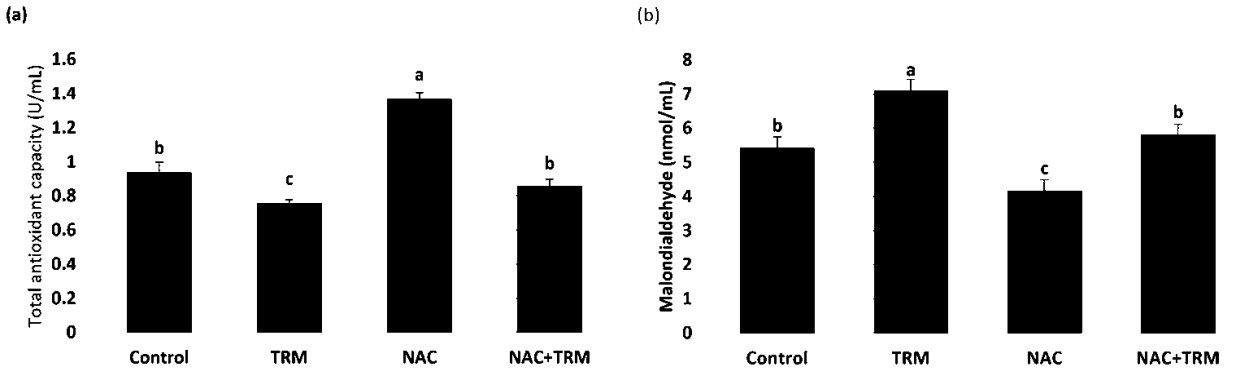

Fig. 2: Effect of tramadol (TRM) and N-acetyl cysteine (NAC) treatments on the plasma total antioxidant capacity (a) and malondialdehyde level (b) in male rabbits. Treatment groups: New Zealand white rabbits were divided into four equal groups (n=8), subcutaneously injected with 0.5mL of saline solution (control group), 20mg/kg tramadol (TRM group), 150mg/kg N-acetylcysteine (NAC group), or NAC and TRM (NAC+TRM group), respectively. The injection treatments were applied three times per week for 4 consecutive weeks. Bars express means with standard error. Means with different superscripts significantly differ at P-value less than 0.05.

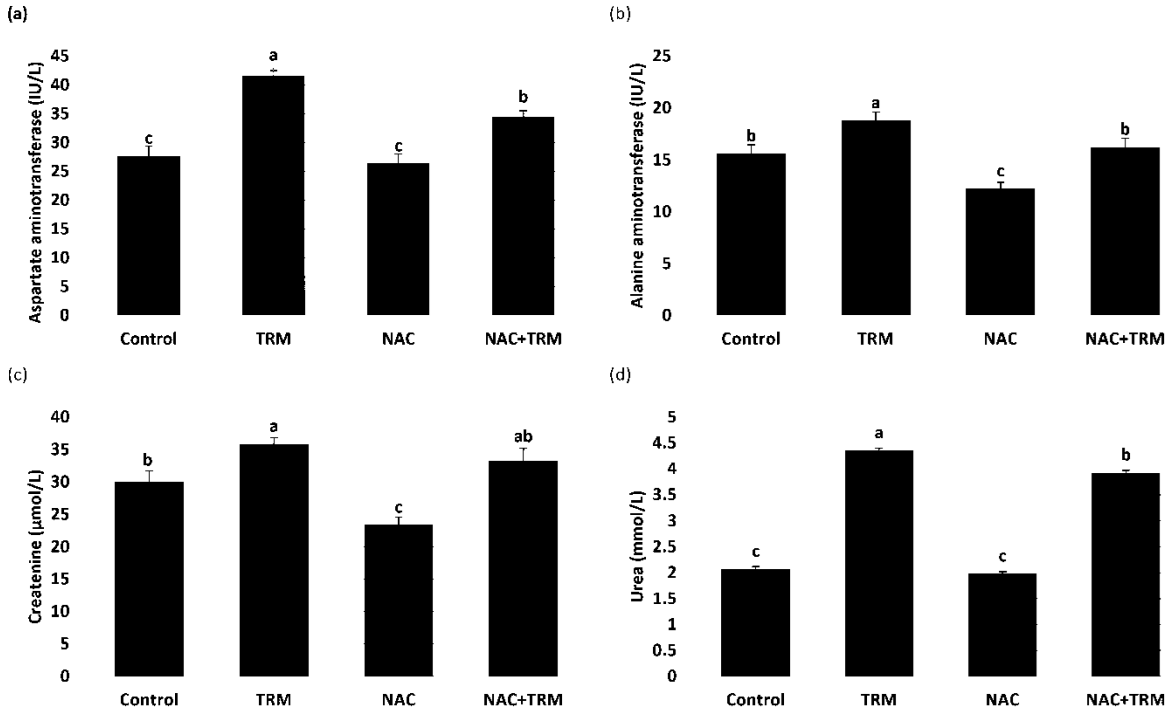

Fig. 3: Effect of tramadol (TRM) and N-acetyl cysteine (NAC) treatments on the plasma aspartate aminotransferase (a), alanine aminotransferase (b), creatinine (c), and urea (d) in male rabbits. Treatment groups: New Zealand white rabbits were divided into four equal groups (n=8), subcutaneously injected with 0.5mL of saline solution (control group), 20mg/kg tramadol (TRM group), 150mg/kg N-acetylcysteine (NAC group), or NAC and TRM (NAC+TRM group), respectively. The injection treatments were applied three times per week for 4 consecutive weeks. Bars express means with standard error. Means with different superscripts significantly differ at P-value less than 0.05.

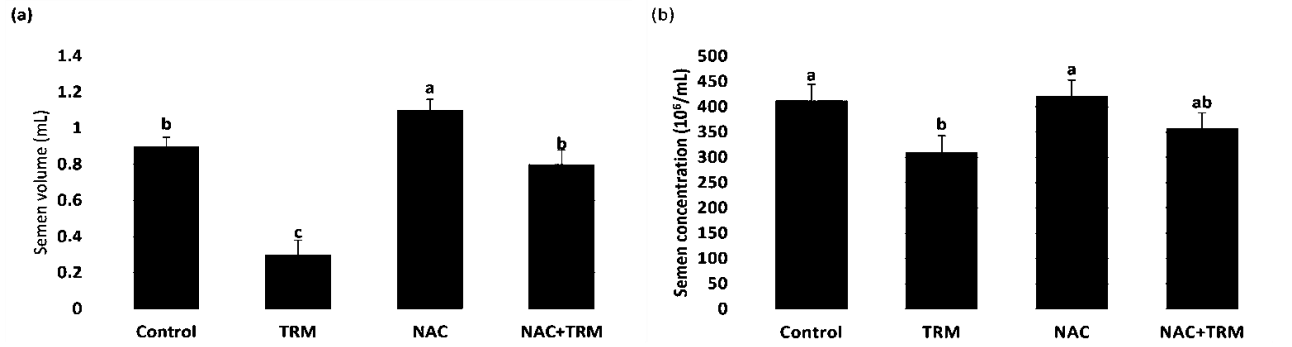

Fig. 4: Effect of tramadol (TRM) and N-acetyl cysteine (NAC) treatments on the semen volume (a) and concentration (b) in male rabbits. Treatment groups: New Zealand white rabbits were divided into four equal groups (n=8), subcutaneously injected with 0.5mL of saline solution (control group), 20mg/kg tramadol (TRM group), 150mg/kg N-acetylcysteine (NAC group), or NAC and TRM (NAC+TRM group), respectively. The injection treatments were applied three times per week for 4 consecutive weeks. Bars express means with standard error. Means with different superscripts significantly differ at P-value less than 0.05.

Sperm kinetics

Fig. 5 shows how TRM and NAC treatments affect the mass motility and progressive motility of sperm. In comparison to the control group, the TRM therapy substantially (P<0.05) decreased the mass and progressive motility by around 13 and 29% in the TRM group and 9 and 18% in the NAC+TRM group, respectively. On the other hand, NAC therapy considerably (P<0.05) improved the progressive motility by 6% while not affecting mass motility. Additionally, when comparing the NAC+TRM group to the TRM group, the NAC therapy substantially (P<0.05) increased the bulk motility by 5% and the progressive motility by 15%.

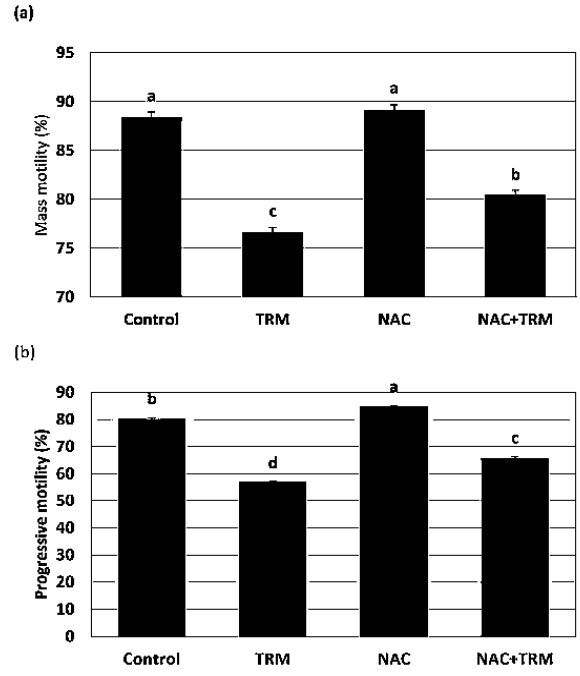

Fig. 5: Effect of tramadol (TRM) and N-acetyl cysteine (NAC) treatments on the mass motility. a) Mass motility and b) Progressive motility of spermatozoa in male rabbits. Treatment groups: New Zealand white rabbits were divided into four equal groups (n = 8) subcutaneously injected with 0.5 mL of saline solution (control group), 20 mg/kg tramadol (TRM group), 150mg/kg N-acetylcysteine (NAC group), or NAC and TRM (NAC+TRM group), respectively. The injection treatments were applied three times per week for 4 consecutive weeks. Bars express means with standard error. Means with different superscripts significantly differ at P<0.05.

Sperm quality

Fig. 6 shows the effects of TRM and NAC treatments on sperm viability, acrosome integrity and plasma membrane functioning. The TRM treatment considerably (P<0.05) decreased sperm viability by 17%, acrosome integrity by 11%, and plasma membrane functioning by 18% as compared to the control. In contrast, the NAC therapy dramatically increased sperm vitality by around 4%. Additionally, the NAC presence in the NAC+TRM group increased the viability and plasm membrane functioning by 8 and 10%, respectively, and substantially (P<0.05) mitigated the detrimental effects of TRM on the sperm quality.

DISCUSSION

Tramadol is considered one of the opiate family, such as codeine, morphine, and oxycodone (Niesters et al. 2013; Edinoff et al. 2021). These substances can disrupt male sex hormones and cause low fertility by interacting with hormone receptors and/or inhibiting the activity of several enzymes involved in steroidogenesis (Sheweita et al. 2017; Edinoff et al. 2021; Sheweita et al. 2022; Kafel et al. 2025). Our results indicated a substantial decrease in plasma testosterone after TRM treatment compared to the control. This reduction in plasma TS may be due to the damage induced by TRM on Leydig and Sertoli cells (Abd et al. 2020). Similar results of TS reduction were obtained when tramadol was administered to male rats (Abdellatief et al. 2015; Abd et al. 2020) and rabbits (Sheweita et al. 2022). The results also agree with previous studies (Tennese and Wevrick 2011; Fountas et al. 2018; Marudhai et al. 2020; Kafel et al. 2025), which reported a decrease in TS and gonadotropin hormones in males after administration of other opiates like morphine, cannabis, and methadone. The low TS levels in the TRM group may be responsible for the reduction of semen volume and concentration observed in the same group, as well as the low quality and motility of sperm. In addition, previous studies (Budin et al. 2017; Li et al. 2023; Oluwaseye et al. 2025) reported that high MDA production and low antioxidant enzyme activity in the testes may be the possible mechanisms involved in these deleterious effects of TRM on sperm production, motility, and quality traits, as also presented in this study.

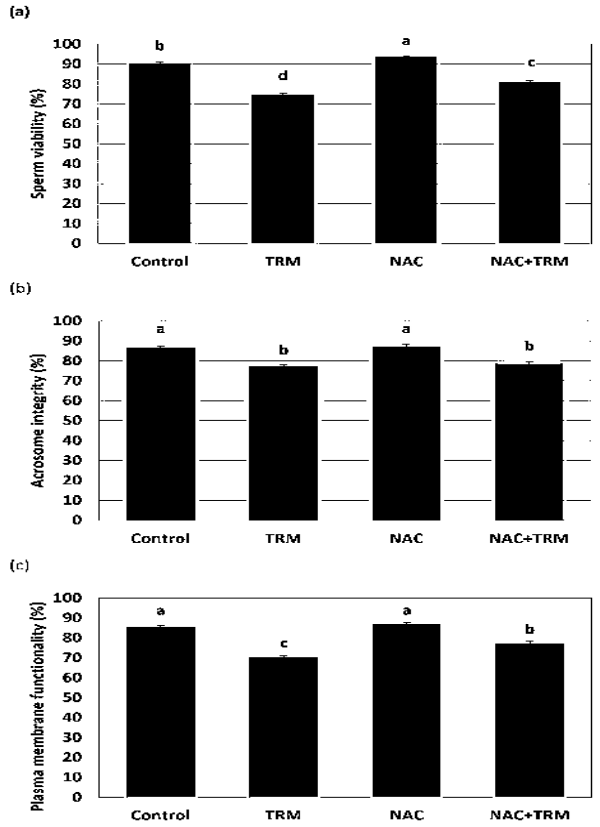

Fig. 6: Effect of tramadol (TRM) and N-acetyl cysteine (NAC) treatments on the sperm viability (a), acrosome integrity (b), and plasma membrane functionality (c) in male rabbits. Treatment groups: New Zealand white rabbits were divided into four equal groups (n = 8) subcutaneously injected with 0.5 mL of saline solution (control group), 20 mg/kg tramadol (TRM group), 150 mg/kg N-acetylcysteine (NAC group), or NAC and TRM (NAC+TRM group), respectively. The injection treatments were applied three times per week for 4 consecutive weeks. Bars express means with standard error. Means with different superscripts significantly differ at $P<0.05$.

Tramadol-induced lipid peroxidation can cause cellular dysfunction and structural damage (Abd et al. 2020). Antioxidants are molecules that can protect biological cells from oxidation and damage induced by exposure to ROS and free radicals. It was demonstrated that NAC has an antioxidant effect and can ameliorate oxidative stress and apoptosis induced by cytotoxic agents (Kulvinder Kochar et al. 2022; Balakrishnan et al. 2023; Ezzat et al. 2023). The current study indicated a 46% TAC increment and 23% MDA reduction in the NAC group compared to the control group. Our results agree with those obtained by a previous study (Abdel-Rahman et al. 2022), confirming the capability of NAC in lowering oxidative stress and supporting the antioxidant status of rabbits. Moreover, it was observed that NAC administration alleviated the deleterious effects of TRM on the antioxidant biomarkers and normalized the plasma TAC and MDA levels in the NAC+TRM group (Fig. 2).

Furthermore, NAC treatment improved the liver and kidney performance of rabbits in both NAC and NAC+TRM groups compared to their controls (Fig. 3). These results displayed a protective effect of NAC on the function of rabbit liver and kidneys, especially under stress conditions. Similarly, it was reported that NAC protected the renal function and lowered the CR and UR through normal excretion in the urine (Atef et al. 2016; Abdel-Rahman et al. 2022). In addition, it was found that NAC treatment declined the toxic effects of carbon tetrachloride on liver and kidney in rats, improving liver and kidney architecture and decreasing liver MDA, AST and ALT enzyme markers (Foaud et al. 2018).

Besides the substantial effect of NAC on protecting the liver and kidney against the TRM-induced dysfunction, there was a remarkable improvement in TS level, semen volume and concentration, and sperm kinetics and quality. It has been documented that NAC acts as a potent antioxidant directly through preventing pre-apoptotic gene expression and indirectly through elevating glutathione levels (Hashim et al. 2022). This, in turn, explains the ameliorative impact of NAC against hepatorenal toxicity (Elsayed et al. 2021) and testes damage (Ezzat et al. 2023) induced by toxic compounds in rats. In line with our results, it was shown that NAC can improve the testosterone level and sperm quantity, quality, motility and DNA integrity (Mao et al. 2010; Walczak-Jedrzejowska et al. 2013; Avdatek et al. 2018; Jannatifar et al. 2019; Abdel-Rahman et al. 2022; Feng et al. 2023; Wang et al. 2025). The positive effect of NAC could be attributed to its high capability of ROS scavenging and intracellular glutathione reduction (Kulvinder Kochar et al. 2022; Geetika et al. 2025). Likewise, the high TAC and low MDA observed in the current study's NAC groups may correlate positively with sperm viability, motility, plasma membrane, and acrosome integrity (De Flora et al. 2001; Abdel-Rahman et al. 2022; Wang et al. 2025). It was also reported that NAC improves sperm motility by enhancing membrane integrity and decreasing ROS generation in mitochondria (Gallo et al. 2018). In addition, free sulfhydryl groups in NAC, as a source of reducing equivalents, can quench radical species, convert to antioxidant glutathione, and therefore, protect sperm components from harmful peroxidation (Rushworth and Megson 2014; Pedre et al. 2021). Improving TS levels in rabbits treated with NAC may positively contribute to spermatogenesis, stability and quality of sperm production (Ruwanpura et al. 2010; Jimoh et al. 2021).

Conclusion

Our data suggest that NAC administration to male rabbits succeeded in modulating the deleterious effects of TRM on health and reproduction. This is indicated by the reduction of liver and kidney dysfunction metabolites in plasma and the potential improvement of the plasma TS and antioxidant biomarkers, as well as the sperm kinetics and quality traits.

DECLARATIONS

Funding: This work was supported by the Deanship of Scientific Research, Vice Presidency for Graduate Studies and Scientific Research, King Faisal University, Saudi Arabia (KFU251933).

Acknowledgement: The authors wish to express their gratitude to the Department of Animal and Fish production, College of Agricultural and Food Sciences, King Faisal University, Saudi Arabia.

Conflict of Interest: The authors declare that they have no competing interests.

Data Availability: All authors declare that the data supporting the findings of this study are available upon request.

Ethics Statement: All experimental procedures were conducted under the guidelines approved by the Ethical Research Committee for Animal Care and Use at King Faisal University (KFU-REC-2024-APRIL-ETHICS117).

Author's Contribution: Hossam M. Al-Ahmad: conceptualization, methodology, investigation, data curation, and writing original draft; Hesham A. Hassanien: conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing original draft, writing review, editing, supervision, project administration, and funding acquisition; Ahmed O. Abbas: conceptualization, methodology, validation, formal analysis, investigation, resources, data curation, writing original draft, writing review, editing, supervision, project administration, and funding acquisition; and Eman S. Mustafa: conceptualization, methodology, investigation, data curation, writing original draft, writing review, and editing.

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