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Protective effects of n-acetyl serotonin against experimental testicular ıschemia–reperfusion ınjury: An experimental study

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Testicular torsion (TT) is a urological emergency that occurs as a result of twisting of the spermatic cord, leading to reduced or completely obstructed blood flow and consequent testicular ischemia. Prolonged ischemia may result in germ cell loss and infertility, particularly due to oxidative stress induced during ischemia–reperfusion (I/R) injury. N-acetyl serotonin (NAS), a precursor of melatonin, is known for its potent antioxidant and anti-inflammatory properties. In this study, the potential of NAS, which has strong antioxidant properties, to prevent oxidative stress occurring during testicular ischemia and the associated development of infertility was investigated. In this study, 24 adult male Wistar Albino rats (200–250 g) were randomly divided into three groups (n = 8): control, ischemia–reperfusion (I/R), and ischemia–reperfusion treated with NAS (I/R+NAS). Testicular torsion was induced for 2 h followed by detorsion, and NAS (10 mg/kg, i.p.) was administered after surgery. Testicular tissues were evaluated histopathologically and biochemically 24 h after reperfusion. Histopathological examination revealed that edema, congestion, and hemorrhage were significantly reduced in the NAS-treated group compared with the I/R group (P<0.05). Improvements in mean testicular biopsy score (MTBS) were accompanied by better organization of the spermatogenic cell layers and more regular seminiferous tubule contours. Biochemically, total antioxidant capacity (TAC) was significantly increased, whereas total oxidant capacity (TOC) and protamine-2 (PRM2) levels were significantly decreased in the treatment group compared with the I/R group (P< 0.05). In addition, androgen-binding protein (ABP) levels were significantly higher in the NAS-treated group than in the I/R group (P<0.05). In conclusion, these findings suggest that NAS exerts protective effects against oxidative damage in testicular tissue exposed to ischemia–reperfusion injury and may serve as a potential therapeutic candidate for reducing I/R-associated testicular damage.

Keywords: Infertility, N-acetyl serotonin, Oxidative stress, Testis, Rat

Introduction

Testicular torsion is a common urological emergency in newborns and adolescents and is characterized by circulatory failure caused by twisting of the testicular pedicle around its own axis[1-3]. In cases diagnosed late, testicular dysfunction and even permanent infertility are inevitable[2,3]. The fundamental pathology is ischemia resulting from torsion[2]. Previous studies have demonstrated that loss of spermatogenesis following testicular torsion is primarily associated with germ cell–specific apoptosis[3-5].

Surgical testicular detorsion combined with targeting oxidative stress is considered an important strategy to reduce the detrimental effects of ischemia–reperfusion injury[6]. Therefore, the efficacy of antioxidant agents in the medical treatment of testicular ischemia–reperfusion injury has been intensively investigated in recent years[7,8]. The main nuclear proteins in sperm are known as protamines (PRMs). Protamines are divided into two types: Protamine 1 (PRM-1) and Protamine 2 (PRM-2), each produced by a single gene[9,10]. Changes in protamine expression in sperm are thought to be one of the main causes of male infertility[10,11]. It has been shown that alterations in androgen-binding protein (ABP) levels induce apoptosis in spermatocytes[12]. High concentrations of ABP, which is a glycoprotein, are found in the rat epididymis[13,14]. This protein is secreted by Sertoli cells in the rat[14]. ABP may contribute to the provision of androgenic hormones required for normal spermatogenesis[13].

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N-acetyl serotonin (NAS), a metabolite of L-tryptophan (Trp), is a precursor to melatonin synthesis[15,16]. This molecule stands out for its antioxidant and anti-apoptotic effects and has been shown in numerous studies to protect ischemically damaged tissues[16-21]. Several studies have found that NAS has a protective effect on many diseases through antioxidant stress, anti-apoptosis, autophagy dysfunction, anti-inflammatory, and other mechanisms, and that NAS is superior to melatonin as an antioxidant[22,23]. NAS is considered a potential therapeutic agent that significantly improves molecular changes and organ damage associated with many ischemic diseases[22-24].

In light of these considerations, the present study aimed to investigate the histopathological and biochemical effects of N-acetyl serotonin in an experimental testicular ischemia–reperfusion model.

Materials and Methods

This study was approved by the Hatay Mustafa Kemal University Local Animal Experiments Ethics Committee (HADYEK) on 28/03/2024 with decision number 2024/03-03. The study was conducted at the HMKU Experimental Research Application and Research Centre. A total of 24 healthy adult male Wistar albino rats, aged 3 months and weighing 200–250 g, bred in accredited experimental animal facilities, were used in this study. The animals had ad libitum access to standard chow and filtered water and were housed individually in clean plastic cages under controlled temperature and humidity conditions (20–22 °C) with a 12 h light/12 h dark photoperiod.

In this study, rats were randomly divided into three groups (n = 8 per group). Group 1 was defined as the control group; no medication or surgical procedure was performed during the experiment, and the left testes were collected at the end of the study, followed by euthanasia. Testicular torsion was induced in rats in Groups 2 and 3. The testes were then returned to their normal position and ischemia-reperfusion injury was assessed. Group 2 (I/R group): This was the ischemia-reperfusion (I/R) group. Rats in this group underwent testicular torsion for 2 hours and were detorsioned 2 hours later, but no treatment was administered. The rats were euthanized 24 hours later. Group 3: This was the treatment (I/R+NAS) group. Rats in this group underwent testicular torsion for 2 hours and were detorsioned after 2 hours. NAS (Ambeed-Cat. No.: A158904-1gr-Purity: 98% N-Acetylserotonin, ABD) (10 mg/kg IP) was administered intraperitoneally after surgery. The selected NAS dose and timing of administration were based on previous studies[21]. All surgical interventions were conducted under deep anesthesia, and the animals were closely monitored throughout the study. The rats were euthanized 24 hours after the procedure by intracardiac blood collection under deep anesthesia.

Surgical Procedure

Surgical procedures were performed under sterile conditions using a combination of xylazine/ketamine (10/50 mg/kg, i.p.) (Alfazyne 2% enj. 50 ml, EGE-VET, Türkiye, Alfamine 10% enj. 50 ml, EGE-VET, Türkiye). After ensuring the asepsis and antisepsis of the testicular tissue, the testes of rats in Groups 2 and 3 were removed through the left inguinal-scrotal incision. The left testis was then rotated 720 degrees clockwise and fixed to the scrotum with 5/0 polyglycolic acid sutures for 2 hours. Following the ischemic process, the testis was returned to the detorsion position (Fig. 1). After detorsion, the testis was left in its normal anatomical position for 24 hours to assess reperfusion injury[25]. Subsequently, a left orchiectomy was performed for histopathological and biochemical examination of the testicular tissue.

Histopathological Analyses

A post-mortem examination was performed on all animals after euthanasia. The testicles were removed and fixed in 10% buffered formalin. Subsequently, following routine methods, they were passed through alcohol and xylene series, embedded in paraffin, and sections 5 μm thick were taken. These were deparaffinized in xylenes and passed through 100, 96, 80, and 70% alcohol series before being stained with Hematoxylin and Eosin (H & E)[26]. After examination under a light microscope (Olympus CX31), microphotographs were taken

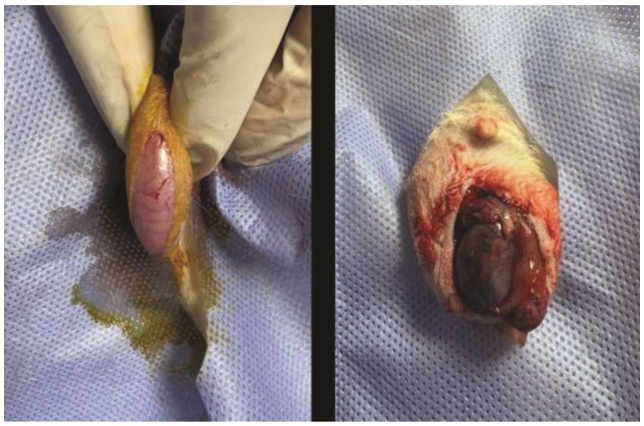

Fig. 1 — Testicular incision and torsion model.

Table 1 — Testicular Biopsy Scores (MTBS)

Score	Description
1	No cells
2	Sertoli cells without germ cells
3	Only spermatogonia
4	Only a few spermatocytes
5	Many spermatocytes
6	Only a few early spermatids
7	Many early spermatids without differentiation
8	Few late spermatids
9	Many late spermatids
10	Full spermatogenesis

Table 2 — Jonhsen testicular biopsy scoring

Score	Description
1	No germ cells or Sertoli cells present.
2	No germ cells present. Only Sertoli cells present.
3	Only spermatogonia present.
4	Very few spermatocytes present.
5	No spermatozoa or spermatids present. However, numerous spermatocytes present.
6	Only a few spermatids are present.
7	No spermatozoa are present. However, a large number of spermatids are present.

(Olympus DP12). Testicular parenchymal damage such as hemorrhage, edema, and congestion was scored as normal (0), mild (1), moderate (2), and severe (3)[27,28]. Seminiferous tubules structure, spermatogenesis, and sperm cell maturation were scored using Johnsen's scoring system (MTBS: Mean testicular biopsy score) and are presented in the following tables (Table 1 and Table 2)[28,29]. Spermatogenesis was scored on a scale of 0–10 based on epithelial maturity.

Biochemical analyses

Preparation of homogenate

The testis tissues were washed with PBS (0.01 M, pH 7.4) and then divided into 0.1 g tissue pieces. Subsequently, using a tissue homogeniser, they were homogenised in 1 ml of RIPA buffer solution (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM PMSF) using a tissue homogeniser. The homogenised mixture was incubated at +4°C with occasional vortexing for 30 minutes. It was then centrifuged at +4°C at 10,000xg for 10 minutes, and the supernatant was collected and stored for subsequent studies.

Total antioxidant capacity determination

The total antioxidant capacity (TAC) was determined colorimetrically using a commercial kit (Elabscience E-BC-K801-M, USA) from the samples taken. Briefly, this method is based on the reduction of the green ABTS+ radical to colorless ABTS in the presence of antioxidants. The TAC values of the samples can be calculated by measuring the absorbance at 660 nm and comparing it to the standard curve. Trolox was used as the reference molecule for the determination of total antioxidant levels.

Total oxidant capacity detection

The total oxidant capacity (TOC) of the samples were also determined colorimetrically using a commercial kit (Elabscience E-BC-K802-M, USA). The basic principle of the method is based on the oxidation of Fe+2 ions to Fe+3 by oxidant agents present in the samples and the measurement of the absorbance of the colored complex formed with xylenol orange at 590 nm. The absorbance values of the samples are directly proportional to the oxidant molecules they contain. Standard graphs were plotted to calculate the total oxidant levels from the absorbance values of the samples.

ELISA analyses

Protein levels of protamine-2 (PRM2) and androgen-binding protein (ABP) were measured in the tissue samples obtained using commercially available ELISA kits (Abbexa, Netherlands).

Statistical analysis

The variables to be obtained were examined using the Shapiro Wilk test for normality and the Levene test for homogeneity of variances before undergoing significance tests. The differences in histopathological parameters between groups were evaluated using the Kruskal Wallis test. The multiple Dunn test was used as a post hoc test for histopathological parameters found to be significant. The differences in biochemical parameters and proteins between groups were determined using one-way analysis of variance (ANOVA). The Duncan test was used as a post hoc test for biochemical parameters and proteins found to be significant. All statistical analyses were performed using the IBM SPSS 23.0 statistical package program and evaluated using the $P<0.05$ criterion.

Results

Histopathological results

The histopathological evaluation was performed using a blinded assessment method. The effect of NAS on changes in the testicular ischemia-

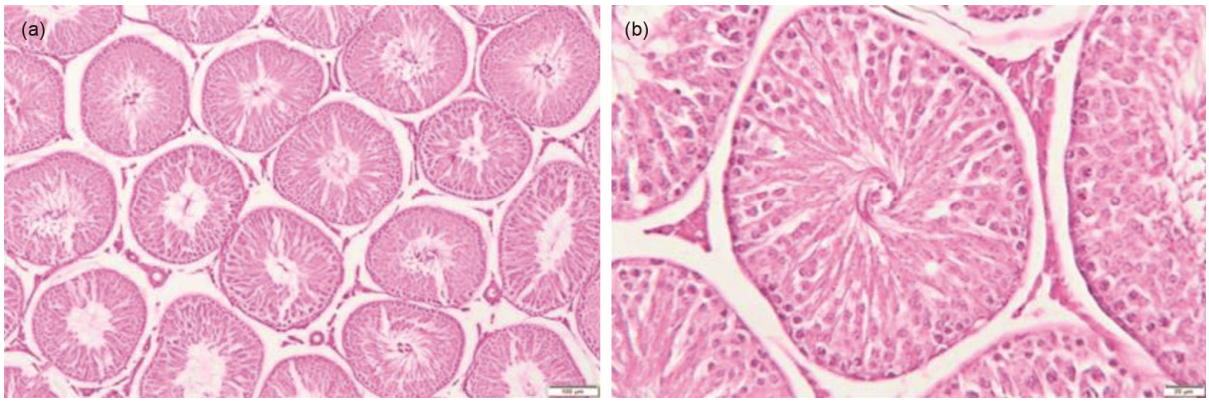

Fig. 2 — Histopathological findings observed in the control group in the testes, HE. (a) Seminiferous tubules with normal histological structure, Control group, (100x). (b) Seminiferous tubules with normal histological structure, Control group, (20x).

Table 3 — Congestion, hemorrhage, edema and MTBS scores (Median (Min-Max))

Group/ Parameter	Congestion	Haemorrhage	Edema	MTBS
Control	0 (0-0) ^{B}	0 (0-0) ^{B}	0 (0-1) ^{C}	9 (8-10) ^{A}
I/R	1 (0-2) ^{A}	2 (1-3) ^{A}	2 (1-3) ^{A}	5,5 (2-8) ^{B}
NAS Treatment	0 (0-1) ^{B}	0 (0-1) ^{B}	1 (0-2) ^{B}	7 (6-9) ^{B}
P value	0,013	<0,001	<0,001	<0,001

Statistically significant differences were found between groups in terms of congestion and hemorrhage ($p=0.013$ and $p<0.001$, respectively). In both histopathological examinations, the I/R group was statistically higher than the other groups. Statistically significant differences were determined between groups in terms of edema ($p<0.001$). The I/R group was found to have a statistically higher score than all other groups ($p<0.05$). The difference in MTBS between groups was found to be statistically significant ($p<0.001$). The MTBS score was higher in the treatment group compared with the I/R group; however, this increase did not reach statistical significance.

reperfusion model is presented in Table 3. In the control group, the testes had a normal histological structure (Fig. 2a & Fig. 2b). In the I/R group, mild congestion was present (Fig. 3a). Edema and hemorrhage ranging from mild to severe were noted between the seminiferous tubules. A statistically significant decrease in the MTBS score, indicating disruption in the arrangement of spermatogenic series cells, was observed (Fig. 3a & Fig. 3b). In the treatment group, a statistically significant decrease in edema, congestion, and hemorrhage was observed compared to the I/R group. Furthermore, improvement in the MTBS score was accompanied by a decrease in irregularities in the seminiferous tubule contours and improvements in the arrangement of spermatogenic series cells (Fig. 3c & Fig. 3d).

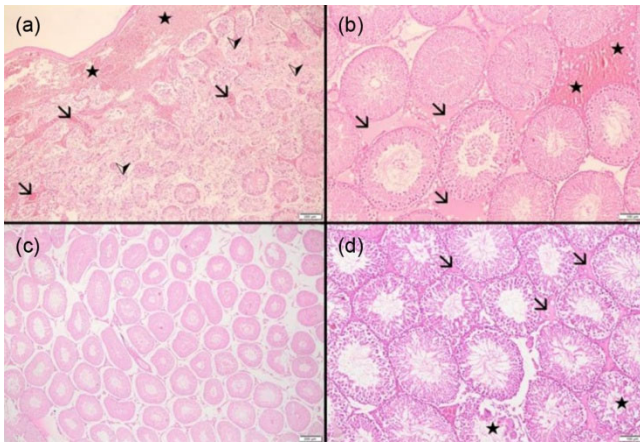

Fig. 3 — Histopathological findings observed in the I/R group and treatment groups in the testes, HE. (a) Mild congestion in the vessels (arrows), moderate hemorrhage in the intertubular region (stars) and alterations in the seminiferous tubules structures (arrowheads), I/R group, (200x). (b) Haemorrhage (stars) and edema (arrows) between seminiferous tubules, (100x). (c) Seminiferous tubules with almost normal histological structure, Treatment group, (200x). (d) Mild edema between seminiferous tubules, Treatment group, (100x).
* $P \leq 0.05$ indicates statistical significance.

Biochemical results

At the end of the experimental study, TOC and TAC levels in the samples were determined using commercially available kits (Elabscience E-BC-K802-M, USA; Elabscience E-BC-K801-M, USA, respectively). PRM-2 and ABP protein levels were investigated using ELISA kits (Abbexa, NL). Fig 4 shows the oxidative stress parameters, while Fig. 5 shows the protein analyses.

Discussion

In the present study, testicular ischemia–reperfusion injury resulted in marked histopathological damage and oxidative imbalance in

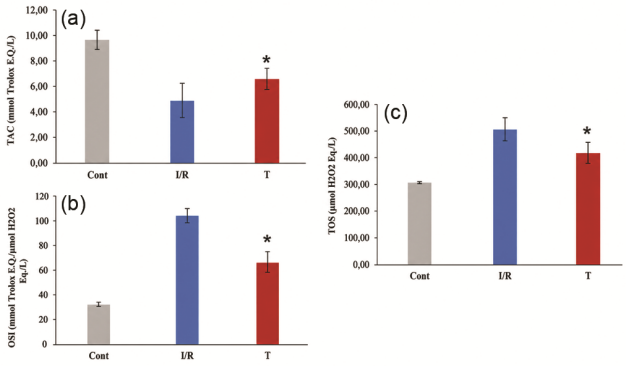

Fig. 4 — In the I/R group, TAC levels in testicular homogenates decreased by 49% compared to the control group, whereas a 33.5% increase was observed in the treatment group compared to the I/R group ($P \leq 0.05$), (a) TOC levels increased by 65% in the I/R group compared to the control group and decreased by 17% in the treatment group relative to the I/R group ($P \leq 0.05$) (b). OSI values increased by 235% in the I/R group compared to the control group, while a 38% decrease was observed in the treatment group compared to the I/R group (c). * $P \leq 0.05$ indicates statistical significance.

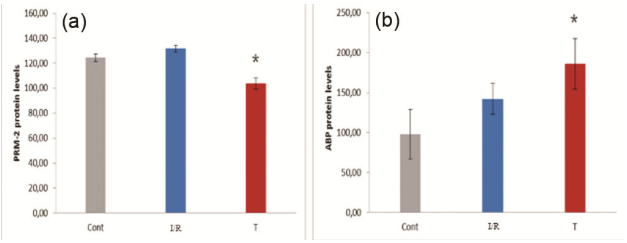

Fig. 5 — In the I/R group, PRM2 protein levels were 6% higher than those in the control group, whereas a 21% decrease was observed in the treatment group compared to the I/R group ($P \leq 0.05$) (a). ABP protein levels increased by 46% in the I/R group compared to the control group and showed a further 30% increase in the treatment group relative to the I/R group ($P \leq 0.05$) (b).

testicular tissue, consistent with previous reports describing the detrimental effects of torsion–detorsion on testicular structure and function[1-3]. These findings further support the concept that delayed restoration of blood flow following torsion is a critical determinant of testicular injury.

In the present study, we investigated the TOC, TAC, and OSI values of different groups in testicular tissue homogenates. According to the data obtained, we found that TOC levels were significantly increased in the ischemia/reperfusion (I/R) group compared to the control, while NAS treatment significantly decreased the TOC levels compared to the I/R group. Conversely, TAC values were significantly reduced in the ischemia–reperfusion group and markedly increased following NAS treatment, indicating an improvement in antioxidant capacity. Consistent with these findings, OSI values reflected a pronounced oxidative imbalance in the ischemia–reperfusion group and showed significant improvement following NAS treatment.

The term ischemia refers to an inadequate blood supply to tissues resulting from obstruction of arterial blood flow. Ischemia–reperfusion (I/R) injury is primarily associated with increased free radical generation and the resulting oxidative stress[30–32]. Preclinical studies have demonstrated that suppression of reactive oxygen and nitrogen species (RONS) production can attenuate I/R injury by limiting oxidative damage[33]. I/R injury develops in two sequential phases. During the ischemic phase, prolonged oxygen deprivation exceeding the cellular adaptive capacity leads to cellular dysfunction and irreversible injury or necrosis. Although reperfusion restores blood flow to ischemic tissue, it paradoxically exacerbates tissue damage through excessive reactive oxygen species (ROS) production triggered by inflammatory and immune responses[30,34]. ROS, including superoxide, hydroxyl radicals, singlet oxygen, and hydrogen peroxide, are highly reactive molecules that regulate physiological cellular signaling and energy metabolism but also play a central role in the pathophysiology of I/R injury[35–39]. Excessive ROS generation disrupts cellular homeostasis and promotes oxidative stress, leading to lipid peroxidation, protein oxidation, and cellular injury. Wei *et al.*[40] demonstrated excessive ROS production in an experimental testicular I/R model. Superoxide dismutase (SOD) represents the first line of antioxidant defense against superoxide radicals[41], whereas glutathione (GSH) is an endogenous antioxidant involved in free radical scavenging, immune regulation, and DNA protection[41,42]. Consistent with our findings, previous experimental I/R studies have reported significant reductions in GSH levels, SOD activity, and other antioxidant defense mechanisms[43,44].

Similar to the findings in our study, Şen *et al.* (2020)[45] demonstrated that in their experimentally created in vivo kidney I/R model, the TOC value was significantly increased in rats subjected to I/R compared to the control group, while the TAC value was statistically significantly decreased. Similarly, Güzel *et al.* (2022)[44] reported that in their experimental renal ischemia-reperfusion study, TAC values decreased in rats with I/R compared to the

control group, while TOC values increased significantly.

As a metabolite of tryptophan, NAS possesses notable antioxidant properties[46]. Plasma concentrations of NAS have been reported to be at nanomolar levels, which are higher than those of melatonin[47]. Recent studies have shown that NAS provides protection against oxidative damage in neurons, erythrocytes, retinal cells, lung epithelial cells, lymphocytes, and enterocytes[15,48]. Furthermore, NAS administration has been observed to prevent I/R-induced mucosal damage and protect tissues in the jejunum and ileum of rats[48]. 4-Hydroxy-2-nonenal (4-HNE), a metabolite of lipid peroxidation in response to intracellular damage, is associated with oxidative stress in various cells and tissues[49]. Liang *et al*. (2020)[16] demonstrated that NAS alleviates oxidative damage and intestinal dysfunction and contributes to mucosal barrier function by regulating Nrf2 signalling, which stimulates antioxidant production, against 4-HNE-induced oxidative stress. This study also emphasised that NAS increased glutathione, an important element of the antioxidant system, in a dose-dependent manner. Similarly, Yoo *et al.* (2017)[19] demonstrated in their study that NAS exhibits neuroprotective efficacy in oxidative stress-induced neuronal damage by regulating the Akt/NRF2 pathway and stimulating the antioxidant system. Similar to the findings in our study, Yu *et al*. (2013)[17] showed that NAS significantly reduced the oxidative marker malondialdehyde (MDA) compared to the I/R group in an acute hepatic ischemia-reperfusion model and, at the same time, significantly increased SOD antioxidant enzyme activity. In another study similar to our findings, Zhang *et al.* (2024)[50] demonstrated that NAS exhibited potent antioxidant activity through stimulation of the Akt/Nrf-2 signal in a retinal ischemia-reperfusion model.

Our study also investigated PRM2 protein levels. Our findings revealed that PRM2 protein levels in testicular tissue were significantly increased in groups subjected to ischemia-reperfusion compared to the control group. Literature reviews revealed that studies on testicular tissue PRM2 protein levels in testicular I/R models are very rare, and data regarding the effects of N-acetyl serotonin on PRM2 expression are scarce. Oxidative stress, resulting from an increase in ROS or insufficient antioxidant defence systems, is one of the important factors affecting reproductive performance in males[51]. While low ROS levels are normally necessary for spermatic function, excessively increased ROS in cases of oxidative stress causes adverse effects such as lipid peroxidation, protein oxidation, and DNA damage, leading to structural and functional impairments in sperm cells and significantly reducing fertility[51-53]. Protamines are proteins rich in arginine and cysteine residues. The positively charged arginine amino acid allows protamines to easily bind to negatively charged DNA. Cysteine residues, on the other hand, play an active role in stabilising this binding through the disulphide bonds they form. It is known that protamines have important protective effects in the preservation of the sperm genome in males. They particularly facilitate DNA hypercondensation and genome protection by replacing histones during spermatogenesis. Therefore, deficiencies or disruptions in the protamination process negatively affect fertility[55]. Most mammalian species studied possess only PRM1. Humans, mice, and horses are exceptions to this rule; sperm nuclei in these species contain PRM2 protamines, a second protamine family[56]. Therefore, the increased PRM2 protein levels observed in the I/R group may reflect alterations in sperm chromatin-associated proteins under conditions of oxidative stress. Furthermore, the reduction in PRM2 protein levels following NAS treatment, together with the improvements observed in oxidative stress parameters and histopathological findings, suggests that the antioxidant properties of NAS may contribute to the preservation of normal spermatogenic processes. Nevertheless, further studies are required to clarify the biological significance of PRM2 alterations and the underlying mechanisms involved in testicular ischemia–reperfusion injury.

In humans and many rodents, it has been reported that two important members of the protamine family, PRM1 and PRM2, are expressed in species-specific ratios[57,58]. Merges *et al.* (2022)[9] reported in their study that the PRM1 protein in mice is effective in the maturation and functioning of PRM2 and plays an important role in DNA hypercondensation. In a study investigating the long-term effects of oxidative stress on the testes, epididymis, and spermatozoa in rats, it was reported that the epididymis was more affected than the testes during treatment and that high oxidative stress markers were observed. Furthermore, it was reported that the peroxiredoxins (PRDX1 and PRDX6) and the catalase enzyme, which are important antioxidant enzymes, were upregulated as

an antioxidant response, but this was insufficient to prevent oxidative damage induced by tert-butyl hydroperoxide (t-BHP) in spermatozoa[59]. It is thought that the controlled production of ROS (mild oxidative stress) in low amounts has an important effect on many cellular processes in spermatozoa, including the acquisition of fertilisation capacity and signal transduction[60-62]. In a study conducted on PRM2-deficient mice, a decrease in the gene expression of SOD1 and PRDX5 enzymes was observed, along with a decrease in sperm antioxidant capacity and the formation of oxidative stress-mediated DNA damage in epididymal sperm[55]. Hamidian *et al.* (2020)[63] reported a significant increase in PRM1 and PRM2 gene expression following vitamin C administration in male partners of couples experiencing recurrent pregnancy loss. It is thought that the differences in PRM2 levels between groups in our study may vary depending on the duration of the study and the severity of oxidative stress. These findings support the notion that modulation of oxidative stress may influence PRM2 regulation, although the experimental conditions and species differ. In our study, when examining testicular ABP values, we found that they increased in the I/R group compared to the control group. Furthermore, our findings indicate that there was no ABP expression in the serum of the experimental groups. Literature reviews have observed a positive correlation between ABP levels and antioxidant system elements. Our findings support the literature. In other words, the increase in ABP in the I/R group may have occurred in response to increased ROS. Bourebaba *et al.* (2023)[64] showed that the levels of SOD2, Catalase (Cat), and Glutathione Peroxidase (GPx) were significantly upregulated in response to excessive ROS production under EMS conditions. Again, the absence of ABP in serum samples is thought to be related to changes in the tissue-organ axis produced depending on the life cycle of rats. ABP is almost undetectable in rat pups and reappears at low levels in the blood of male rats just before weaning, originating from Sertoli cells before the maturation of the blood-testis barrier[65]. The absence of ABP transcripts in the livers of adult rats[66] and the undetectable plasma ABP in their blood[65] present an interesting puzzle, as this suggests that ABP is not required for the transport or regulation of sex steroids in rodents after birth.

In our study, we also investigated androgen-binding protein (ABP) levels and found that they were increased in the I/R group compared with the control group. Previous studies have suggested a positive association between ABP and antioxidant capacity. ABP is a 90–100 kDa homodimeric glycoprotein produced by hepatocytes and adipocytes that binds circulating steroid hormones and regulates their bioavailability[67–69]. Bourebaba *et al.* (2023)[64] reported that ABP treatment reduced nitric oxide and ROS production while enhancing antioxidant activity and regulating the expression of endogenous antioxidant enzymes, including SOD1, SOD2, CAT, and GPx, under oxidative stress conditions. In addition, oxidative stress associated with insulin resistance has been linked to decreased ABP levels together with impaired antioxidant status and increased oxidative stress markers[70-72]. Based on these findings and the results of the present study, NAS may indirectly increase ABP levels through its antioxidant effects.

In previous studies on the model of testicular torsion-detorsion, histopathological changes such as degeneration, desquamation, irregularities in germ cells, interstitial edema, and hemorrhage have been reported in the testes[27,28]. Similarly, in our study, edema and hemorrhage were observed between the seminiferous tubules in testicular torsion-detorsion. In addition, irregularities in the contours of the seminiferous tubules and disruption in the arrangement of spermatogenic series cells were noted.

N-acetyl serotonin has been reported to exert protective effects against ischemia–reperfusion injury in various experimental models, primarily through modulation of oxidative stress–related mechanisms[22,23]. NAS is considered a potential therapeutic agent that significantly improves molecular changes and organ damage associated with many ischemic diseases[22-24]. In our study, histopathological examination revealed that NAS reduced ischemia-induced edema, congestion, and hemorrhage in the testes. Furthermore, this study found that NAS caused improvements in the irregularities of seminiferous tubule contours and the arrangement of spermatogenic series cells in this model.

Conclusions

In conclusion, according to the results obtained from biochemical tests in our study, total oxidant capacity and oxidative stress index increased significantly after application in the experimental testicular tissue ischemia-reperfusion model, while

antioxidant capacity decreased significantly compared to other groups. However, it was found that N-acetyl serotonin, administered as part of the treatment, effectively reversed the adverse effects on these parameters, which are important in oxidative stress. Based on these findings, N-acetyl serotonin may represent a potential protective agent against I/R-induced oxidative stress in testicular tissue. Furthermore, while it was determined that I/R increased the levels of important ABP proteins in DNA packaging in gamete cells compared to the control group, this value was found to be even higher in the treatment group. Based on these data, it was concluded that a reactive increase may occur as a protective reaction in I/R application and that the greater increase in the treatment group suggests that N-acetyl serotonin may be considered an effective agent in the reproductive system. In addition, it was determined that I/R increased PRM2 protein levels in DNA packaging in gamete cells compared to the control group, while this value decreased in the treatment group. When reviewing the literature, it should be considered that the expression levels of isoforms such as PRM1 within the PRM gene locus should also be investigated in order to fully discuss efficacy. Further experimental studies are therefore needed to better elucidate the biological effects of N-acetyl serotonin in testicular ischemia–reperfusion injury. The findings obtained in the study demonstrate the potential protective effects of the investigated treatment and suggest that more comprehensive evaluations may be conducted in future studies.

Funding statement

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Conflict Of Interests

The authors declare no conflicts of interest.

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