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TADALAFIL'S ROLE IN TESTICULAR REGENERATION: ANTI-INFLAMMATORY AND ANTIOXIDANT EFFECTS POST HEAT STRESS

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ABSTRACT

Background and objectives: An effective phosphodiesterase inhibitor 5 (PDE-5) is tadalafil, which is frequently prescribed to treat erectile dysfunction. Its therapeutic potential, however, goes beyond this particular indication. This study looked at how tadalafil affected male Wistar rats' testicular parenchyma's ability to heal heat stress in the testicles. After undergoing testicular heat stress, 54 Wistar rats were randomised to receive either tadalafil therapy (TAD) or no treatment at all (control). Two different doses of TAD were given intraperitoneally: 0.9 mg/kg and 1.8 mg/kg. On days 7, 15, and 30 following heat shock, biometric parameters, testicular histopathology assessment, serum testosterone levels, oxidative stress, and interleukin levels were assessed.[1] PDE5 inhibitors are indicated as therapy for benign prostatic hyperplasia with lower urinary tract symptoms, pulmonary arterial hypertension and erectile dysfunction, and have also been shown to have an anti-inflammatory effect against tissue inflammation.[2] At the conclusion of each experimental period, the animals were put to sleep, and samples were taken up until day seven following the injury, TAD therapy preserved testicular weight and slowed the testicular degeneration process. On the other hand, serum testosterone levels were still lowered in the groups that received treatment on days 7 and 15 following heat stress. At varying doses, TAD also reduced TNF-α and NO levels, but had no effect on IL-6. Even though TNF-α and NO levels were systematically reduced, the use of TAD following heat shock showed anti-inflammatory and antioxidant capabilities; nonetheless, it did not stop the exacerbation of testicular lesions in subsequent periods.[1]

Because of this, the resumption of the spermatogenic process may be compromised because this specific PDE-5 inhibitor, at the dosages utilised, did not positively affect testosterone levels throughout the postthermal stress phase.[1]

KEYWORDS: Tadalafil, testicular, wistar rats.

INTRODUCTION

The testes, located outside the abdominal cavity, are crucial organs for a species' survival. The vascular cone around the testicular artery controls blood flow, countercurrent heat exchange, and radiation-induced heat loss. Despite these physiological defenses, male gonad degeneration can occur due to heat stress.[1]

Heat stress (HS) occurs when an animal is subjected to temperatures that exceed its physiological range and compensatory ability; it usually involves the entire body, although it may be confined to a particular organ or anatomic area.[7]

In mammals, testicular heat suppresses spermatogenesis, with testicular cells being particularly susceptible. High temperatures can cause apoptosis, mediated by the tumor gene suppressor protein p53. In response to heat stress, germ cells upregulate the synthesis of heat shock protein (HSP). PDE-5 inhibitors can lower intracellular cytochrome C and alter the Bcl-2/Bax9 ratio, affecting the number of apoptotic cells reduced in tissue injury. Injuries to testicular tissue that result in reperfusion encourage an upregulation of iNOS, eNOS, and NO, causing testicular germ cell death. However, the injection of tadalafil diminishes this rise. Direct heating of the testicles has provided new information about spermatogenesis damage and potential remedies. This study examined how PDE-5 inhibitors affected spermatogenesis's ability to recover from heat shock-induced testicular degeneration.[1]

FORMULATION AND COMPOSITION[1]

Experimental Draw

This study involved 90-day-old male Wistar rats from the Department of Animal Morphology and Physiology of the Federal Rural University of Pernambuco. The rats were housed in a controlled environment with standard conditions, including temperature, humidity, and light/dark cycles. The experimental protocol was approved by the Ethics Committee for the Use of Animals of the Federal University of Pernambuco and conducted at the Academic Center of Vitória. The rats were randomly assigned to one of three experimental groups:

thermal shock group, treated with a daily intraperitoneal dose of tadalafil (TAD), and thermal shock group. The rats were anesthetized with ketamine and xylazine, and then immersed in water at 43ºC. After recovery, the rats were given daily intraperitoneal applications of distilled water, 0.9 or 1.8 mg/kg-1 of TAD, and their body weight was measured daily.

Testicular Perfusion

After the trial came to an end, the rats received heparin (125 UI/100 g; Akzo Organon Teknika) and thiopental (50 mg/Kg; Roche) for anaesthesia. They were then put through intracardiac perfusion using a 0.9% NaCl solution that contained sodium chloride (500 UI/L) and heparin, nitroprusside (Sigma, 100 mg/L). Subsequently, glutaraldehyde 4% (Vetec) in sodium phosphate buffer (pH 7.2 and 0.01M) was perfused into the rats. After fixation, the testes were removed and weighed precisely to within 0.001g on a BEL Engineering scale (MARK 500 / BRA).

Testicular Histopathology

The study involved rats subjected to heparinization, anesthesia, and intracardiac perfusion. The testes were extracted and weighed, and testicular fragments were sliced and re-fixed. After soaking in phosphate buffer, dehydrated, and embedded in a plastic resin, histological sections of 4 µm thickness were obtained. Histological and morphometric analyses were performed, and the testicular components were evaluated histopathologically using an optical microscope. The study aimed to understand the effects of heparinization and anesthesia on testicular function.

Peripheral blood cytokine dosage

Cytokine dosage in blood supernatants collected from splenic cells was determined using the Cytometric Bead Array System (CBA) technique. The CBA was used for the quantitative measurement of the cytokines TNF-α, IL-2, IL-4, IL-6, IL-10, IL-17, IFN-γ by using BD™ Cytometric Bead Array (CBA) Mouse Th1/Th2/Th17 CBA Kit (Beckton Dickson) as recommended by the manufacturer with modifications in the final reaction volume to 60 uL (25 uL of beads mix, 25 uL of sample and 25 uL of detection reagent). The beads (2700 events) were acquired using the FACSCalibur flow cytometer (Beckton Dickson), located in the Technological Platforms Center (NPT)/IAM/Fiocruz, through the CellQuestPro software (Beckton Dickson) and analyzed in the FCAP Array 3.1 software (Beckton Dickson).

Statistical Analysis

The Shapiro-Wilk test was used to determine whether the data were normal, and then the Student Newman-Keus test and an analysis of variance (ANOVA) were used. The software STATISTICA for WINDOWS 3.11 was utilised. Two variables were compared using Pearson's correlation analysis. $P < 0.05$ indicated the differences were significant. The standard deviation and mean of each result were reported. The means and standard deviation of the data were reported. Additionally, Pearson's correlation analysis was carried out on variable pairs. A high intensity correlation was found between the variables with low intensity ($r < 0.30$), medium intensity ($r < 0.60$), and high intensity ($r > 0.61$) based on the significance of the linear correlation results. For every test, a probability level of 5% was employed.

Serum testosterone analysis

The study involved rats who underwent heparinization, anesthesia, and intracardiac perfusion to fixate their tissues. The testes were excised and weighed using a precision balance. Blood samples were collected through puncture at the convergence of the cranial and caudal vena cava, centrifuged, and stored in Eppendorf plastic containers. Hormonal levels were evaluated using the enzyme-linked immunosorbent assay (ELISA) method with absorbance reading at 405 nm. The standard curve was prepared by serially diluting testosterone to a concentration of 2.3 pg/50 µL in 250 µL of ELISA assay solution. The hormone conjugated with the HRP enzyme was also diluted. The substrate ELISA solution was prepared by combining 40 µL of 0.5 M H2O2, 125 µL of 40 mM ABTS, and 12.5 mL of substrate ELISA solution. For each well containing the study or control material, 100 µL was added, and the plates were covered and incubated at room temperature under agitation. The plasma testosterone levels were expressed in nMol/L, ng/mL, and through the relationship between concentration and volume (CV%) of the hormone.

RESULTS[1]

Assessment of body and testicular weights

According to Table 1, we can observe that there was no significant variation between the different experimental groups in terms of body weight.

Table 1. Body weight (g) of control Wistar rats and rats subjected to testicular heat stress, treated and not treated with different doses of TAD, and evaluated at 7, 15 and 30 days after heat stress (Mean ±Standard deviation).

Days	Control HS (n=6)	HS + Tadalafil (0.9 mg.Kg^{-1}) (n=6)	HS+ Tadalafil (1.8 mg.Kg^{-1}) (n=6)	*p*
7	236.00 ± 32.49	225.83 ± 23.04	243.67 ± 44.56	0.675
15	250.83 ± 13.12	231.83 ± 18.90	242.50 ± 21.13	0.221
30	280.66 ± 27.20	270.66 ± 41.54	272.83 ± 45.08	0.896

Testicular weight

When compared to animals receiving 0.9 mg.Kg-1 during the same period, the control group's testicular weight on day 7 post-heat shock was reduced by 17.5% and the treated group's by 19.5%, respectively, 1.8 milligrammes per kilogramme. The testicular weights of the various experimental groups did not differ during the other times. On the other hand, between days 7 and 15, there was a 40% decrease in testicular weight in the control group that received 0.9 mg/kg treatment. The decrease between days 7 and 15 was 51.7% in rats treated with the maximum dose of TAD and subjected to heat shock. Between days 15 and 30 of the trial, there was no difference in testicular weight between the periods that were examined.

Table 2. Testicular weight (g) of control Wistar rats and rats submitted to testicular heat stress, treated or not with different doses of TAD, and evaluated at 7, 15 and 30 days after heat stress (Mean ± standard deviation).

Days	Control HS (n=6)	HS + Tadalafil (0.9 mg.Kg^{-1}) (n=6)	HS+ Tadalafil (1.8 mg.Kg^{-1}) (n=6)	*p*
7	0.97 ± 0.10	0.95 ± 0.09	1.18 ± 0.09	0.015
15	0.58 ± 0.04	0.58 ± 0.47	0.57 ± 0.09	0.952
30	0.58 ± 0.10	0.58 ± 0.01	0.57 ± 0.09	0.952

HS: heat stress.

Histopathological analysis

According to histopathological findings observed in the testicular parenchyma on the 7th day after the thermal stress, the animals in the control group had lesions in the seminiferous epithelium compatible with testicular degeneration. Among the main alterations of the germinal epithelium, it was possible to notice vacuolation of Sertoli cells, syncytial giant cells of rounded spermatids. Some tubules had epithelium with little vacuolization and retained most of the germ cells, while others were made up only of Sertoli cells and germ cells from the basal compartment (Figure 1A and 1B).

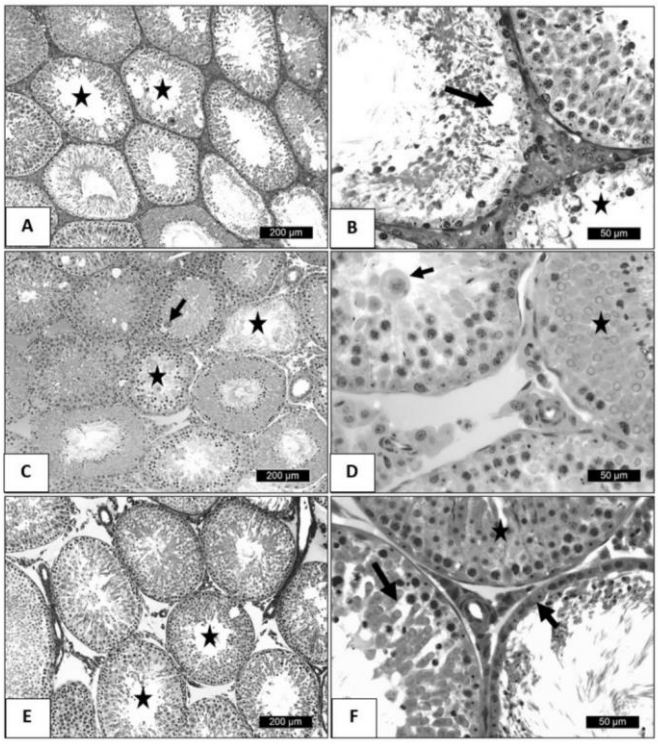
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Fig 1A.

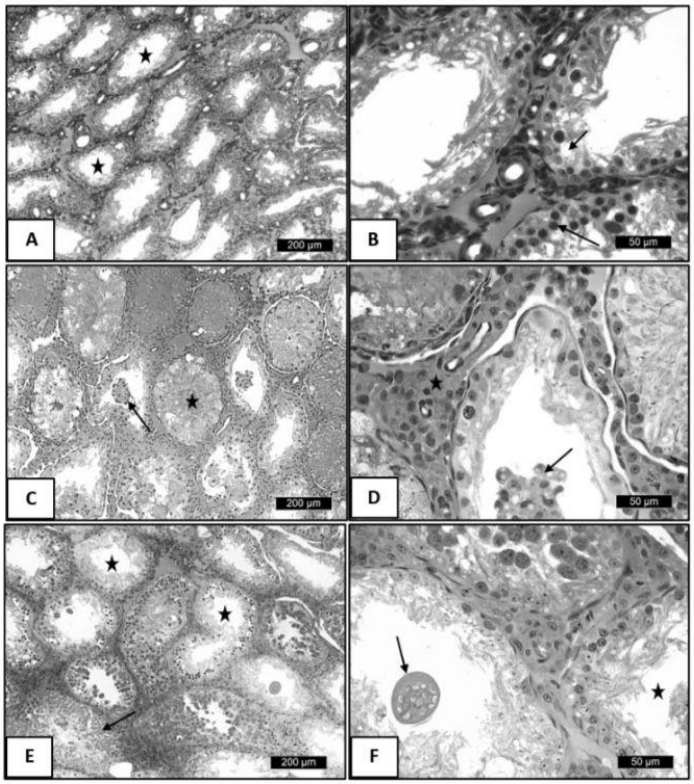
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Fig 2 B.

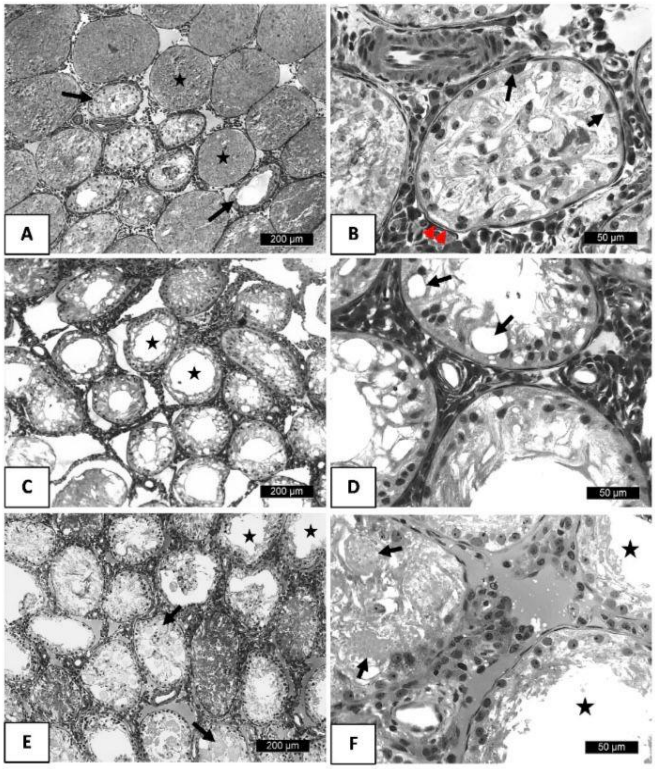


Fig 3.

Serum testosterone dosage

Plasma testosterone levels did not differ on day 7 post-heat shock between the control and TAD-treated groups. However, in animals that received 0.9 mg.Kg-1 was found 34% reduction in this parameter in relation to the control. On the other hand, with the dosage of 1.8 mg.Kg-1 the reduction was 27.5% compared to the control. Despite the absence of difference or statistical trend between groups, testosterone levels were lower in this post-heat shock phase, with the administration of DAT (Figure 4; Table 3).

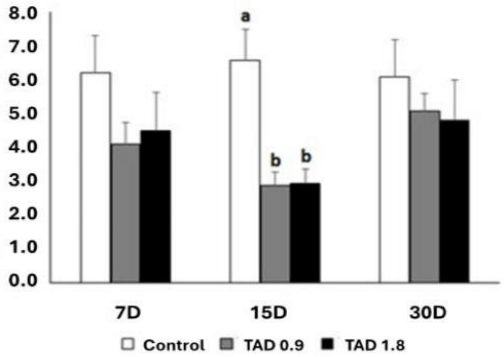
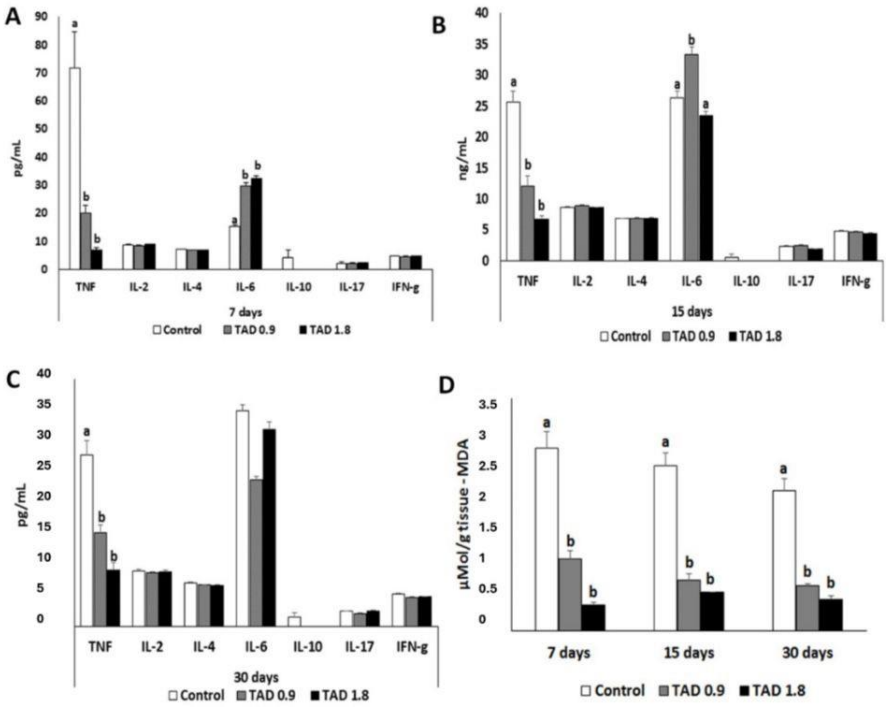


Table 3. Mean values and standard deviation of serum testosterone concentration in Wistar rats submitted to testicular thermal stress and treated or not with different doses of TAD

Groups	Time			General mean
	T-7 min	T-15 min	T-30 min	
	Testosterone (ng/mL)			
Controle HS	6.19 ± 1.11	6.55±0.91	6.08 ± 1.08	6.27 A
HS+Tadalafil (0.9 mg/kg^{-1})	4.08 ± 0.65	2.85±0.41	5.05 ± 0.62	3.99 B
HS+Tadalafil (1.8 mg/kg^{-1})	4.49 ± 1.11	2.91 ± 0.45	4.78 ± 1.20	4.63 B
Média Geral	4.92a	4.10a	5.31a	

On day 15 post-heat shock, plasma testosterone levels had an average reduction of 56% in the TAD-treated groups compared to the control. On the 3rd moment of evaluation of the plasmatic level of testosterone (day 30), there was no difference between the experimental groups. However, we can see an average recovery in serum testosterone levels of around 42% compared to that observed on day 15 in the groups treated with TAD (Table 3).

Hepatic/splenic oxidative stress and assessment of splenic cytokines



The study analyzed nitric oxide values and cytokine levels in Wistar rats exposed to testicular thermal stress. Results showed that rats treated with varying doses of TAD showed reduced TNFα levels, increased IL-6 levels, and no change in IL-6 levels compared to the control group. Lipid peroxidation levels also decreased in rats treated with TAD, indicating a reduction in oxidative stress.

Table 4. Mean values, standard deviation and significance level (Pr>F) of interleukins and nitric oxide in Wistar rats submitted to testicular heat stress and treated with different doses of TAD (0.9 e 1.8 mg.Kg^{-1}).

Variables	Groups			Pr>F
	Control HS	HS+Tadalafil (0.9 mg.Kg^{-1})	HS+Tadalafil (1.8 mg.Kg^{-1})	
TNF-α (pg/mL)	42.04 ± 5.61 a	15.81 ± 1.88 b	7.63 ± 0.86 c	<.0001
IL-2 (pg/mL)	8.78 ± 0.15	8.71 ± 0.28	8.80 ± 0.15	0.7228
IL-4 (pg/mL)	6.97 ± 0.11	6.81 ± 0.16	6.78 ± 0.09	0.0521
IL-6 (pg/mL)	22.88 ± 0.87 b	30.00 ± 0.96 a	30.96 ± 0.95 a	<.0001
IL-10 (pg/mL)	1.91 ± 0.98 a	0.00 ± 0.00b	0.00 ± 0.00 b	0.0085
IL-17 (pg/mL)	2.22 ± 0.28	2.22 ± 0.11	2.26 ± 0.07	0.8944
INF-γ (Unidade)	4.91 ± 0.11 a	4.66 ± 0.09 b	4.62 ± 0.13 b	0.0062
Nitric oxide (mMol/mL)	2.45 ± 0.21 a	0.84 ± 0.08 b	0.48 ± 0.04 c	<.0001

Different letters shows that there was a significant difference in the comparison, p<0.05.

Table 5. Significance levels of the analysis of variance for the variation factors (Groups and Times) and their interaction (Groups x Times) of the set of variables of Wistar rats submitted to testicular thermal stress and treated or not with different TAD doses.

Variables	Unit	Pr>F	ANOVA Variation Factors		
			Groups	Time	G x T
TNF-α	(pg/mL)	<.0001	<.0001	<.0001	<.0001
IL2	(pg/mL)	0.3343	0.7228	0.4641	0.1458
IL4	(pg/mL)	0.1176	0.0521	0.3988	0.2456
IL6	(pg/mL)	<.0001	<.0001	<.0001	<.0001
IL10	(pg/mL)	0.0424	0.0085	0.4678	0.5487
IL17	(pg/mL)	0.0008	0.8944	0.2545	<.0001
INF-γ	(pg/mL)	0.0031	0.0062	0.0190	0.1091
Nitric oxide	(mMol/mL)	<.0001	<.0001	0.0469	0.0359

The study found that TNF-α and Nitric Oxide levels were higher in the Control HS group, followed by lower values in the HS+TAD and HS+TAD groups. IL-10 and INF-α values were also higher in the HS control group. Interleukins IL-2, IL-4, and IL-17 were not influenced by the treatments. However, significant variations were observed in INF-α and interleukins IL-2 and IL-4 in the absence of interaction.

Table 6. Mean values, standard deviation and variation factors Time and Interaction Groups x Times of serum concentration of testosterone, nitric oxide and interleukins in Wistar rats submitted to testicular thermal stress and treated or not with different doses of TAD.

Groups	Time			Variation Factors	
	T-7 day	T-15 day	T-30 day	Time	G x T
		TNF-α (pg/mL)			
Control HS	71.83±12.81aA	25.68±1.75bA	28.61±2.32bA		
HS+Tadalafil (0.9 mg/kg^{-1})	20.21±2.67aB	12.10±1.63bB	15.12±1.34bB	<.0001	<.0001
HS+Tadalafil (1.8 mg/kg^{-1})	7.00±0.89aB	6.76±0.53aC	9.13±1.16aC		
Mean general	33.01	14.85	17.62		
		IL-2 (pg/mL)			
Control HS	8.72±0.15	8.68±0.09	8.95±0.20		
HS+Tadalafil (0.9 mg/kg^{-1})	8.51±0.16	8.89±0.09	8.72±0.06	0.4641	0.1458
HS+Tadalafil (1.8 mg/kg^{-1})	8.94±0.12	8.58±0.10	8.8±0.247		
Mean general	8.72	8.71	8.85		
		IL-4 (pg/mL)			
Control HS	7.06±0.11	6.82±0.06	7.03±0.16		
HS+Tadalafil (0.9 mg/kg^{-1})	6.83±0.06	6.90±0.10	6.70±0.09	0.3988	0.2456
HS+Tadalafil (1.8 mg/kg^{-1})	6.83±0.11	6.84±0.07	6.66±0.10		
Mean general	6.91	6.85	6.80		
		IL-6 (pg/mL)			
Control HS	15.30±0.47cC	22.75±1.10bC	30.58±1.03aA		
HS+Tadalafil (0.9 mg/kg^{-1})	29.85±1.07bB	33.28±1.31aB	26.88±0.50bB	<.0001	<.0001
HS+Tadalafil (1.8 mg/kg^{-1})	32.55±0.87aA	28.53±0.66bA	31.81±1.33aA		
Mean general	25.90	28.19	29.76		
		IL-10 (pg/mL)			
Control HS	4.18±1.66	0.55±0.55	1.00±0.72		
HS+Tadalafil (0.9 mg/kg^{-1})	0.00±0.00	0.00±0.00	0.00±0.00	0.4678	0.5487
HS+Tadalafil (1.8 mg/kg^{-1})	0.00±0.00	0.00±0.00	0.00±0.00		
Mean general	1.39	0.18	0.34		
		IL-17 (pg/mL)			
Control HS	1.20±0.70bA	2.31±0.10aA	2.36±0.05aA		
HS+Tadalafil (0.9 mg/kg^{-1})	2.18±0.16aA	2.40±0.12aA	2.08±0.06aB	0.2545	<.0001
HS+Tadalafil (1.8 mg/kg^{-1})	2.37±0.08aA	1.91±0.02bB	2.48±0.12aA		
Mean general	2.18	2.21	2.31		
		INF-γ (pg/mL)			
Control HS	4.70±0.14	4.81±0.09	5.22±0.11		
HS+Tadalafil (0.9 mg/kg^{-1})	4.61±0.11	6.81±0.08	4.69±0.07	0.0190	0.1091
HS+Tadalafil (1.8 mg/kg^{-1})	4.69±0.19	4.41±0.10	4.75±0.09		
Mean general	4.67b	4.63b	4.89a		
		Nitric oxide (mMol/mL)			
Control HS	2.75±0.25aA	2.48±0.20aA	2.11±0.18aA		
HS+Tadalafil (0.9 mg/kg^{-1})	1.08±0.12aB	0.76±0.10bB	0.68±0.02bB	0.0469	0.0359
HS+Tadalafil (1.8 mg/kg^{-1})	0.38±0.05aC	0.58±0.00aB	0.47±0.06aB		
Mean general	1.40	1.27	1.09		

HS: heat stress; TAD: tadalafil. Uppercase letters in the column and lowercase letters in the row differ at the 5% level of probability.

According to table 6, The experiment examined the effects of testicular heat stress on interleukins IL-2, IL-4, and IL-10. Interleukins TNF-α, IL-6, IL-10, and Nitric Oxide showed interaction effects between groups and times. The highest means of TNF-α were observed at

time T-7 of testicular heat stress for animals in the Control HS and HS+TAD groups. IL-6 gradually increased for animals in the HS control group over time, while the highest average was recorded at time T-15 for animals in the HS+TAD group. IL-17 had a lower concentration in animals in the HS control group at time T-7 and higher at other times. Nitric oxide had its highest concentration in animals from the HS+DAT group at time T-7, while a decrease was observed at other times. The highest averages were observed in the HS control group and decreased in other treatments.[1]

DISCUSSION

Spermatogenesis is a complex process that involves various stages of the spermatogenic cycle, including germinal, stem cell proliferation, meiotic division, and spermiogenesis. Elevation of testicular temperature can damage spermatogenesis, leading to structural changes that result in a reduction in testicular weight. In this study, the highest dose of Tadalafil (TAD) for 7 days after heat shock had a positive effect on testicular weight. However, a 40% reduction in testicular weight was observed in the control group and treated with 0.9 mg.Kg-1 between the 7th and 15th days. For animals subjected to heat shock that received the highest dose of TAD, the reduction was 51.7%. Degeneration of the seminiferous epithelium can occur quickly, but visible regeneration can occur 60 days after exposure to heat. In severe lesions where spermatogonia A is affected, azoospermia can occur. Testicular alterations detected in the present study are consistent with those observed by Kanter, who found that rats exposed to thermal shock had intratubular vacuoles, giant syncytial cells, pyknotic germ cells, and cells with apoptotic fragments.

The use of Tadalafil (TAD) for 7 days after heat shock provided the preservation of seminiferous tubules throughout the testicular parenchyma. Heat stress promotes an increase in reactive oxygen species (ROS) in different organs, including the testicles. The action of this specific PDE-5 inhibitor seems to have influenced the modulation of mechanisms that trigger degeneration by heat stress testis in this period, which may be directly related to the reduction of oxidative stress and anti-inflammatory properties.

The study found that the highest dose of Tadalafil (TAD) for 7 days after heat shock had a positive effect on testicular weight, while no significant difference was observed between groups. The study also found that heat stress can cause visible regeneration of the seminiferous epithelium, but severe lesions can lead to azoospermia. The testicular alterations observed in the study are consistent with those observed by Kanter, who found that rats

exposed to thermal shock had intratubular vacuoles, giant syncytial cells, pyknotic germ cells, and cells with apoptotic fragments. The use of DAT for 7 days after heat shock preserved seminiferous tubules throughout the testicular parenchyma, and its antioxidant capacity did not maintain testosterone levels. Heat stress can also influence serum concentrations of testicular testosterone and enzymes required for testosterone biosynthesis in Leydig cells, cytochrome P450 family 17 (CYP17), and steroidogenic acute regulatory protein. In the experimental model of cyclophosphamide-induced testicular degeneration, DAT prevented testicular dysfunction and reduction in testosterone levels in a dose-dependent manner.[1]

CONCLUSION

By acting as an antioxidant and anti-inflammatory, tadalafil postponed the onset of testicular degeneration. Even though TNF-α and NO levels were systematically reduced, it was unable to stop the testicular lesions from getting worse in later periods. It would be challenging to restart the spermatogenic process if this selective PDE-5 inhibitor did not positively impact the maintenance of testosterone levels during the post-thermal stress phase at the dosages used.[1]

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