

Effect of administration of green tea extract on regeneration of the testicular parenchyma of Wistar rats submitted to heat stress

Efeito da administração de extrato de chá verde na regeneração do parênquima testicular de ratos

Wistar submetidos ao estresse térmico

Efecto de la administración de extracto de té verde sobre la regeneración del parénquima testicular de ratos Wistar sometidas a estrés por calor

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Abstract

Objective: This study evaluated the effects of green tea extract (GTE) on the recovery of testicular parenchyma of adult Wistar rats submitted to heat stress (HS). **Methodology:** Animals (n=84) were distributed and treated, according to the experimental group (G1: unstressed and untreated; G2: stressed and untreated; G3: unstressed and treated; G4: stressed and treated). **Results:** The overall mean of testicular weight, epithelial height and tubular diameter were lower (P<0.05) in G2 and G4, and day 60 presented a higher value (P<0.05) to the two first parameters. The gonadosomatic index and the volumes of the seminiferous epithelium were lower (P<0.05) in G2 and G4. The overall means of proper tunica volume was lower (P<0.05) on day 30. The seminiferous tubule on days 15 and 30 presented lower values (P<0.05) in

G2 and G4, although with higher values ($P<0.05$) on day 60. The volume of lymphatic space was higher ($P<0.05$) in G2 and G4, being day 60 higher ($P<0.05$) than day 15. Greater preservation and recovery of seminiferous epithelium occurred in G4, compared to G2. Conclusion: Thus, the GTE administration is an effective way to improving tissue recovery after testicular damage, induced by short-term heat.

Keywords: Catechin; Gonads; Heat shock; Histology.

Resumo

Objetivo: Esse estudo avaliou o efeito do extrato de chá-verde (ECV) sobre a recuperação do parênquima testicular de ratos Wistar adultos, submetidos ao estresse pelo calor (EC). **Metodologia:** Os animais ($n=84$) foram distribuídos segundo os grupos experimentais (G1: não estressados e não tratados; G2: estressados e não tratados; G3: não estressados e tratados; G4: estressados e tratados). **Resultados:** As médias gerais do peso testicular, altura epitelial e diâmetro tubular foram menores ($P<0,05$) no G2 e G4, com o dia 60 maior ($P<0,05$) para os dois primeiros parâmetros. O índice gonadosomático e o volume do epitélio seminífero foram menores ($P<0,05$) no G2 e G4. A média geral do volume da túnica própria foi menor ($P<0,05$) no dia 30. Os túbulos seminíferos foram menores ($P<0,05$) para G2 e G4 nos dias 15 e 30, embora maiores ($P<0,05$) no dia 60. O volume do espaço linfático foi maior ($P<0,05$) no G2 e G4, com o dia 60 superior ($P<0,05$) ao 15. Maior preservação e recuperação do epitélio seminífero ocorreu no G4 em relação ao G2. **Conclusão:** Assim, a administração de ECV é uma forma eficaz de melhorar a recuperação do tecido após lesão testicular, induzida por calor de curta duração.

Palavras-chave: Catequinas; Gônadas; Choque térmico; Histologia.

Resumen

Objetivo: Este estudio evaluó el efecto del extracto de té verde (ECV) en la recuperación del parénquima testicular de ratas Wistar adultas sometidas a estrés por calor (EC). **Metodología:** Los animales ($n=84$) se distribuyeron según los grupos experimentales (G1: no estresados y no tratados; G2: estresados y no tratados; G3: no estresados y tratados; G4: estresados y tratados). **Resultados:** Las medias generales de peso testicular, altura epitelial y diámetro tubular fueron menores ($P<0,05$) en G2 y G4, siendo el día 60 mayor ($P<0,05$) para los dos primeros parámetros. El índice gonadosomático y el volumen del epitelio seminífero fueron menores ($P<0,05$) en G2 y G4. El volumen medio general de la túnica propia fue menor ($P<0,05$) el día 30. Los túbulos seminíferos fueron más pequeños ($P<0,05$) para G2 y G4 los días 15 y 30, aunque más grandes ($P<0,05$) el día 60. El volumen del espacio linfático fue mayor ($P<0,05$) en G2 y G4, siendo el día 60 mayor ($P<0,05$) que el 15. Se presentó mayor preservación y recuperación del epitelio seminífero en G4 en comparación con G2. **Conclusión:** Por lo tanto, la administración de ECV es una forma efectiva de mejorar la recuperación del tejido después de una lesión testicular inducida por calor a corto plazo.

Palabras clave: Catequinas; Gónadas; Choque térmico; Histología.

1. Introduction

Reproduction is essential for the perpetuation of species (Durairajanayagam et al., 2015; Carvalho et al., 2021). However, reproductive performance is influenced by multiple factors, with temperature being one of the most prominent elements (Boni, 2019; Schou et al., 2021), because its variations causes alterations in physiological parameters, how spermatogenesis functions, with consequent fertility impairment (Durairajanayagam et al., 2015). Heat stress is a problem experienced daily, especially in countries with a tropical climate, what has worsened with the climate change, marked by the global warming (Sinha et al., 2017; Schou et al., 2021).

Testicular degeneration can result from heat stress and determine spermatogenic cell death, defects in the DNA, and altered gene expression, with impairment in function and consequent failures in spermatogenesis (Lue et al., 1999). Structural and functional damage to male gonads and gametes, caused by exposure to excessive heat, are largely associated with the oxidative stress (Garcia-Oliveros et al., 2020) and reduction in antioxidant capacity (Fraczek et al., 2022), once that hyperthermia causes disruption of the oxidative homeostasis (Hamilton et al., 2016; Cilio et al., 2022; Wang et al., 2022).

As opposed to heat-induced damage and oxidizing agents, antioxidants may retard or inhibit oxidative stress, preventing the onset or continuation of the deleterious effects of this condition (Abshenas et al., 2011; Cilio et al., 2022). They can be acquired through diet such as green tea (*Camellia sinensis*), rich in polyphenolic compounds, especially catechins (Farhan, 2022; Nain et al., 2022), a class of flavonoids with antioxidant, anti-inflammatory, and anti-carcinogenic properties (Zanchi et al., 2015; Farhan, 2022),

as well as inhibitor of enzymes, as those involved in the apoptotic cascade (Bagherpour et al., 2019). Thus, the objective of this study was to evaluate the effects of green tea extract on the regeneration of the testicular parenchyma of Wistar rats submitted to heat stress.

2. Methodology

The study was carried out at the Physiology and Pathology laboratories, of the Federal Rural University of Pernambuco (UFRPE), after approbation by the Ethics Committee for Animal Experimentation (CEEUA/UFRPE 059/2015). A total of 84 adult Wistar rats (*Rattus norvegicus*, var. Albinus) from the bioterium of the Department of Animal Morphology and Physiology (UFRPE) were kept in an environment at $23 \pm 1^\circ\text{C}$, 50% humidity, and a 12-h light-dark cycle. Water and rodent feed were offered ad libitum.

At 120 days of age, the animals were divided through random probabilistic sampling into four experimental groups, each with 21 subjects (G1: not supplemented and not exposed to heat stress; G2: not supplemented and exposed to heat stress; G3: supplemented with green tea extract and not exposed to heat stress; G4: supplemented with green tea extract and exposed to heat stress). In turn, green tea extract (China-Hong Kong, via Pharma Nostra® handling establishment) was weighed and diluted daily in distilled water, so that each G3 and G4 animal received, every 24 hours, 100 mg/kg of extract in aqueous solution form, based on the amount of 50 mg/kg epigallocatechin gallate present in the extract used (Vieira et al., 2020).

On day 0 of the study, the animals in the G2 and G4 groups were anesthetized intraperitoneally with 80 mg/kg of 10% Ketamine Hydrochloride (Syntec) and 6 mg/kg of Xylazine Hydrochloride 2% (Syntec), placed in a support and their testicles immersed in a water bath at 43°C for 15 min. Subsequent to recovery from anesthesia, administration by oral gavage of aqueous solution of green tea extract or distilled water was initiated, according to the experimental groups. The treatment continued for 60 days, during which the animals were weighed daily to correct the administered dose of the extract, and without sample loss (Vieira et al., 2020).

Subgroups of animals ($n=07$ per group, totaling 28) were euthanized, through intraperitoneal administration of the anesthetic Thiopental Sodium 0.5 g, 50 mg/kg (Tiopentax® Cristália), on days 15, 30, and 60 after testicular heat stress and submitted to intracardiac perfusion with NaCl physiological solution (0.9%) for vascular lavage. Subsequently, the animals were perfused with 4% glutaraldehyde fixative solution (Vetec) in sodium phosphate buffer (pH 7.2 and 0.01 M). The testicles were removed and sectioned into fragments up to 2 mm thick, which were re-fixed in the same infusion solution, and immersed in a phosphate buffer for 2 h, dehydrated in increasing series of alcoholic solutions (70-100%), and incorporated in plastic resin composed of glycol methacrylate (Leica®). Histological sections 4 μm thick were used for slides preparation, stained with hematoxylin and floxin, and then analyzed morphologically and morphometrically.

The tubular diameter, height, and epithelial area were measured with an eyepiece micrometer (100X), calibrated with a standard micrometer. Twenty round or rounded tubular profiles were measured using the ImageJ program, with the mean determined for each animal. The morphometric data of the testicles composition were obtained using point counting, through random systematic allocation of an integrated graticule with 441 points of intersection, on the testicular parenchyma, with an increase of 400X. Approximately 6600 points were counted for each rat.

The volume of each testicle component was established from the product between the volumetric density (%) and the net volume of the testicle (net testicle weight). The specific gravity of the testicular tissue was considered as 1.0, for subsequent morphometric calculations. In order to calculate an exact measure of the net volume of the testicle, 6.5% of the capsule was extracted from its weight (Russell & França, 1995). The total length of the seminiferous tubules (meters) was obtained through the division of the tubule volume by πR^2 (França et al., 2000). In addition, from the weight of both testicles, it was possible to

calculate the gonadosomatic index, which refers to the percentage of body mass allocated in gonads (Caldeira et al., 2010).

The data were analyzed according to ProcMixed for mixed models (SAS Int. Inc., Cary, NY), with collection days as repeated measurements over time (15, 30, and 60 days). First, the data were analyzed in relation to the presence of normality of the residues (Shapiro-Wilk). Logarithmic or square root transformations ($x+1$) were necessary when the normality premise was not met. As for the data with response in percentage, they were submitted to angular transformation of the arcsine of the square root of the percentage value, according to Sampaio (1998). The statistical model includes a fixed treatment effect (T), days (D), and interactions (TxD). The means contrast was performed by the Student-Newman-Keuls test at the 5% probability level. The number of animals established in this design made it possible to determine the degrees of freedom, with treatments = $k - 1$; total = $kr - 1$; residue = $k(r-1)$, establishing, in this context, an adequate number of repetitions per treatment.

3. Results

The overall mean of testicular weight, seminiferous tubules diameter and height of the seminiferous epithelium (Table 1) were lower ($P<0.05$) in the G2 and G4 than in the G1 and G3. However, higher values were observed on day 60 ($P<0.05$) than on days 15 and 30 of the experiment to the first and last parameters. In addition, the gonadosomatic index (Table 1) on days 15, 30, and 60 was lower ($P<0.05$) in the G2 and G4, than in the G1 and G3, independent of the administration of green tea extract.

Table 1 - Means and standard deviations of body and testicular weight (g), gonadosomatic Index (%), seminiferous tubules diameter (μm), seminiferous epithelium height (μm) and total seminiferous tubule length (m) of Wistar rats submitted or not to testicular heat stress and treated or not with green tea extract at different times.

GROUPS / DAYS		D15 (n=7/group)	D30 (n=7/group)	D60 (n=7/group)	Overall Mean
Body weight	G1	353.43 \pm 36.80	389.57 \pm 33.03	365.00 \pm 32.38	369.33 \pm 35.87A
	G2	345.14 \pm 51.74	383.29 \pm 27.78	386.29 \pm 32.49	371.57 \pm 41.47A
	G3	357.43 \pm 36.27	356.57 \pm 32.84	382.86 \pm 32.24	365.62 \pm 34.44A
	G4	372.86 \pm 32.50	362.43 \pm 46.41	382.71 \pm 20.16	372.67 \pm 34.02A
	Overall Mean	357.21 \pm 39.09a	372.96 \pm 36.47a	379.21 \pm 29.34a	
Testicular weight	G1	1.49 \pm 0.19	1.65 \pm 0.13	1.58 \pm 0.25	1.57 \pm 0.20 ^a
	G2	1.03 \pm 0.43	1.19 \pm 0.33	1.45 \pm 0.19	1.22 \pm 0.36B
	G3	1.51 \pm 0.17	1.60 \pm 0.16	1.65 \pm 0.15	1.59 \pm 0.16 ^a
	G4	1.07 \pm 0.17	0.97 \pm 0.34	1.41 \pm 0.24	1.15 \pm 0.31B
	Overall Mean	1.28 \pm 0.34b	1.35 \pm 0.38b	1.52 \pm 0.22a	
Gonadosomatic Index	G1	0.42 \pm 0.06aA	0.43 \pm 0.04aA	0.43 \pm 0.06aA	0.43 \pm 0.05
	G2	0.29 \pm 0.09aB	0.31 \pm 0.07aB	0.37 \pm 0.03aB	0.32 \pm 0.07
	G3	0.43 \pm 0.05aA	0.45 \pm 0.03aA	0.43 \pm 0.02aA	0.44 \pm 0.04
	G4	0.29 \pm 0.05aB	0.27 \pm 0.08aB	0.37 \pm 0.06aB	0.31 \pm 0.08
	Overall Mean	0.36 \pm 0.09	0.36 \pm 0.10	0.40 \pm 0.05	
Seminiferous tubules diameter	G1	261.36 \pm 27.55	278.17 \pm 13.66	288.96 \pm 38.06	276.16 \pm 29.22A
	G2	222.68 \pm 54.67	225.17 \pm 61.44	233.40 \pm 34.67	227.08 \pm 49.11B
	G3	276.11 \pm 30.81	256.74 \pm 20.44	293.60 \pm 48.71	275.48 \pm 36.88A
	G4	225.22 \pm 25.46	236.07 \pm 70.57	262.22 \pm 36.79	241.17 \pm 48.45B
	Overall Mean	246.34 \pm 41.68a	249.04 \pm 50.06a	269.55 \pm 44.91a	
Seminiferous epithelium height	G1	92.59 \pm 11.41	101.42 \pm 7.08	107.47 \pm 14.94	100.49 \pm 12.66A
	G2	72.12 \pm 23.35	73.05 \pm 25.44	83.90 \pm 14.97	76.36 \pm 21.33B
	G3	96.01 \pm 11.75	94.07 \pm 4.90	106.11 \pm 14.96	98.73 \pm 12.04A
	G4	78.51 \pm 8.37	78.33 \pm 32.06	95.63 \pm 15.01	84.16 \pm 21.59B
	Overall Mean	84.81 \pm 17.22b	86.72 \pm 22.92b	98.28 \pm 17.28a	
Total seminiferous tubule length	G1	24.39 \pm 4.24	22.59 \pm 2.68	19.99 \pm 8.15	22.32 \pm 5.56A
	G2	23.67 \pm 11.51	24.59 \pm 6.15	26.98 \pm 5.22	25.08 \pm 7.83A
	G3	21.30 \pm 3.78	25.38 \pm 5.47	20.86 \pm 6.50	22.51 \pm 5.51A
	G4	22.83 \pm 5.81	17.08 \pm 5.74	22.38 \pm 5.57	20.76 \pm 6.04A
	Overall Mean	23.05 \pm 6.75a	22.41 \pm 5.91a	22.55 \pm 6.68a	

Different lowercase letters in the row demonstrate differences between days and different capital letters in the column demonstrate differences between groups. G1: not stressed and untreated; G2: stressed and untreated; G3: not stressed and treated; G4: stressed and treated. Fonte: Autores (2022).

With regard to volumetric characteristics of the seminiferous epithelium (Table 2), G1 and G3 presented higher volume ($P < 0.05$) than G2 on all days of the experiment, while the tunica propria (Table 2) demonstrated a lower overall mean volume ($P < 0.05$) on day 30 in relation to the other times. Moreover, the volume of the seminiferous tubules (Table 2), on days 15 and 30 was lower ($P < 0.05$) in G2 and G4 in relation to G1 and G3, where G2 and G4 presented higher values ($P < 0.05$) on day 60

than on the other days. In opposition, the lymphatic space (Table 2) presented higher volumes ($P < 0.05$) in the G2 and G4 groups compared to G1 on days 15, 30, and 60, and a higher volume ($P < 0.05$) was observed on day 60 in relation to day 15, in all groups. No statistical difference ($P > 0.05$) were observed for the other variables investigated.

Table 2 - Means and standard deviations of the volumetric characteristic of Leydig cells (ml), seminiferous epithelium (ml), lumen (ml), tunica propria (ml), seminiferous tubule (ml), connective cells (ml), vessels (ml), and lymphatic space (ml) of the testicular parenchyma of Wistar rats submitted or not to testicular heat stress and treated or not with with green tea extract at different times

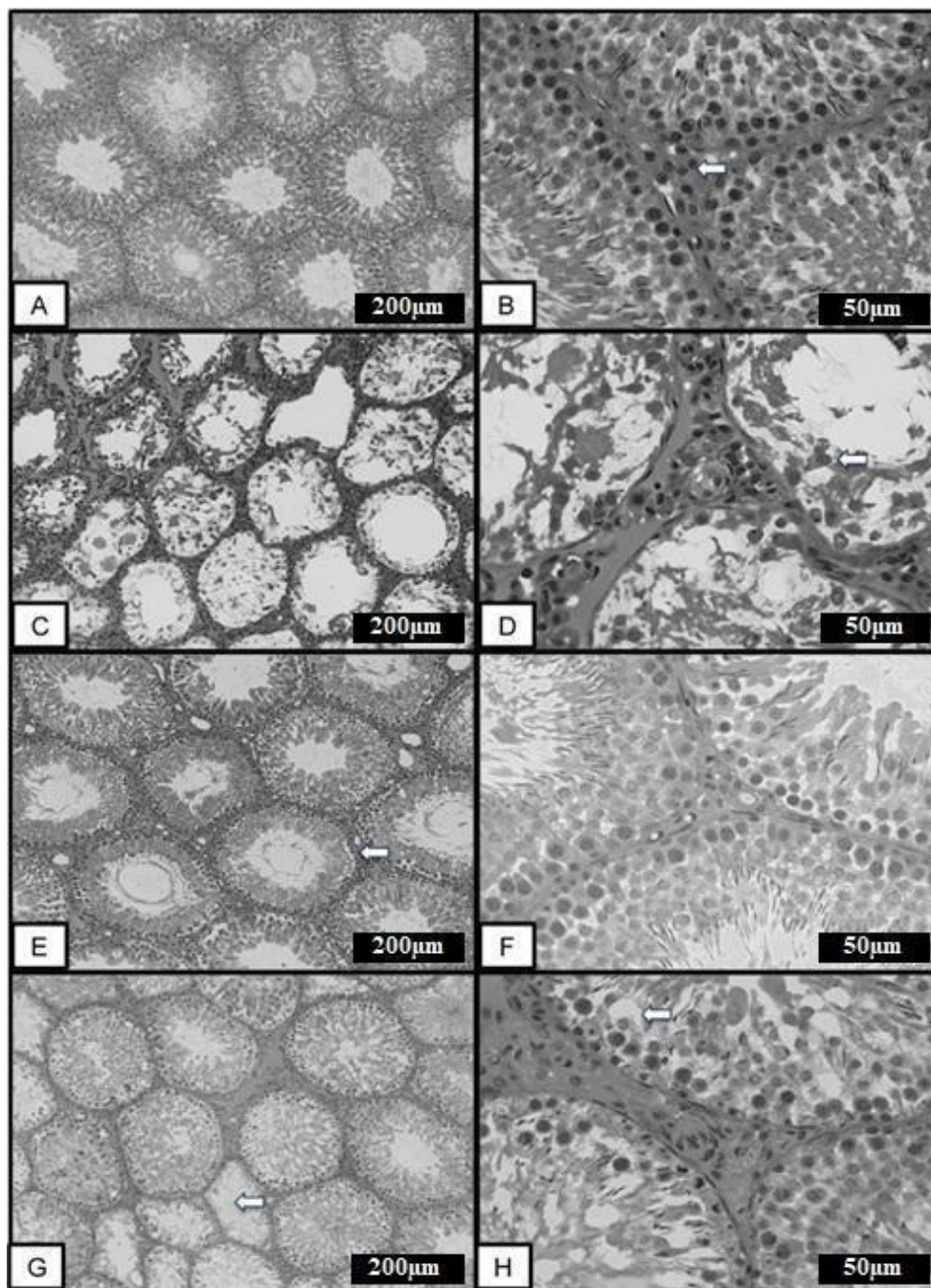
GROUPS / DAYS		D15 (n=7/group)	D30 (n=7/group)	D60 (n=7/group)	Overall Mean
Leydig cells	G1	0.011 ± 0.006	0.016 ± 0.006	0.020 ± 0.005	0.016 ± 0.01A
	G2	0.021 ± 0.016	0.020 ± 0.009	0.022 ± 0.006	0.021 ± 0.01A
	G3	0.018 ± 0.009	0.015 ± 0.006	0.018 ± 0.008	0.017 ± 0.01A
	G4	0.016 ± 0.006	0.020 ± 0.006	0.017 ± 0.004	0.017 ± 0.01A
	Overall Mean	0.016 ± 0.01a	0.018 ± 0.01a	0.019 ± 0.01a	
Seminiferous epithelium	G1	1.168 ± 0.165aA	1.284 ± 0.096aA	1.149 ± 0.195aA	1.200 ± 0.16
	G2	0.783 ± 0.383aB	0.848 ± 0.311aB	1.026 ± 0.216aB	0.886 ± 0.31
	G3	1.159 ± 0.125aA	1.207 ± 0.136aA	1.239 ± 0.123aA	1.202 ± 0.13
	G4	0.826 ± 0.265aAB	0.625 ± 0.285aAB	1.083 ± 0.198aAB	0.845 ± 0.31
	Overall Mean	0.984 ± 0.30	0.991 ± 0.35	1.124 ± 0.19	
Lumen	G1	0.082 ± 0.050	0.045 ± 0.017	0.039 ± 0.033	0.055 ± 0.04A
	G2	0.099 ± 0.159	0.058 ± 0.047	0.065 ± 0.038	0.074 ± 0.09A
	G3	0.055 ± 0.030	0.050 ± 0.032	0.044 ± 0.016	0.049 ± 0.03A
	G4	0.056 ± 0.016	0.070 ± 0.025	0.051 ± 0.017	0.059 ± 0.02A
	Overall Mean	0.073 ± 0.08a	0.056 ± 0.03a	0.050 ± 0.03a	
Tunica propria	G1	0.035 ± 0.008	0.036 ± 0.004	0.035 ± 0.015	0.036 ± 0.01A
	G2	0.031 ± 0.017	0.026 ± 0.008	0.039 ± 0.013	0.032 ± 0.01A
	G3	0.039 ± 0.009	0.029 ± 0.014	0.040 ± 0.014	0.036 ± 0.01A
	G4	0.029 ± 0.008	0.018 ± 0.004	0.036 ± 0.008	0.028 ± 0.01A
	Overall Mean	0.033 ± 0.01a	0.027 ± 0.01b	0.038 ± 0.01a	
Seminiferous tubule	G1	1.285 ± 0.150Aa	1.365 ± 0.097aA	1.233 ± 0.225aA	1.291 ± 0.17
	G2	0.913 ± 0.456bB	0.932 ± 0.305bB	1.130 ± 0.209aA	0.992 ± 0.34
	G3	1.253 ± 0.143aA	1.285 ± 0.123aA	1.323 ± 0.126aA	1.287 ± 0.13
	G4	0.911 ± 0.284bB	0.714 ± 0.272bB	1.170 ± 0.193aA	0.932 ± 0.31
	Overall Mean	1.090 ± 0.33	1.074 ± 0.34	1.212 ± 0.20	
Connective cells	G1	0.004 ± 0.002	0.002 ± 0.002	0.002 ± 0.001	0.002 ± 0.00A
	G2	0.002 ± 0.003	0.002 ± 0.001	0.003 ± 0.001	0.002 ± 0.00A
	G3	0.002 ± 0.001	0.001 ± 0.001	0.003 ± 0.001	0.002 ± 0.00A
	G4	0.002 ± 0.001	0.002 ± 0.001	0.002 ± 0.002	0.002 ± 0.00A
	Overall Mean	0.002 ± 0.00a	0.002 ± 0.00a	0.002 ± 0.00a	

Vessels	G1	0.010 ± 0.009	0.016 ± 0.009	0.020 ± 0.019	0.015 ± 0.01A
	G2	0.015 ± 0.014	0.007 ± 0.008	0.012 ± 0.008	0.012 ± 0.01A
	G3	0.021 ± 0.015	0.020 ± 0.012	0.016 ± 0.014	0.019 ± 0.01A
	G4	0.023 ± 0.019	0.011 ± 0.007	0.016 ± 0.013	0.017 ± 0.01A
	Overall Mean	0.017 ± 0.01a	0.014 ± 0.01a	0.016 ± 0.01a	
Lymphatic space	G1	0.082 ± 0.032bB	0.142 ± 0.043abB	0.216 ± 0.174aB	0.147 ± 0.11
	G2	0.151 ± 0.043bA	0.149 ± 0.038abA	0.184 ± 0.084aA	0.161 ± 0.06
	G3	0.120 ± 0.022bAB	0.171 ± 0.075abAB	0.182 ± 0.100aAB	0.158 ± 0.07
	G4	0.165 ± 0.097bA	0.161 ± 0.049abA	0.110 ± 0.100aA	0.145 ± 0.07
	Overall Mean	0.129 ± 0.06	0.156 ± 0.05	0.173 ± 0.11	

Different lowercase letters in the row demonstrate differences between days and different capital letters in the column demonstrate differences between groups. G1: not stressed and untreated; G2: stressed and untreated; G3: not stressed and treated; G4: stressed and treated. Fonte: Autores (2022).

In the histopathological analysis, 15 days after the testicular heat stress (Figure 1), the G2 group presented testicular degeneration, with loss of the germinal epithelium and desquamated cells. On the other hand, in G4 animals presented seminiferous tubules with reduced germ cell population and increased intertubular space.

Figure 1 - Transverse cut of testicles of adult Wistar rats submitted or not to testicular heat shock and treated or not with green tea extract at 15 days.

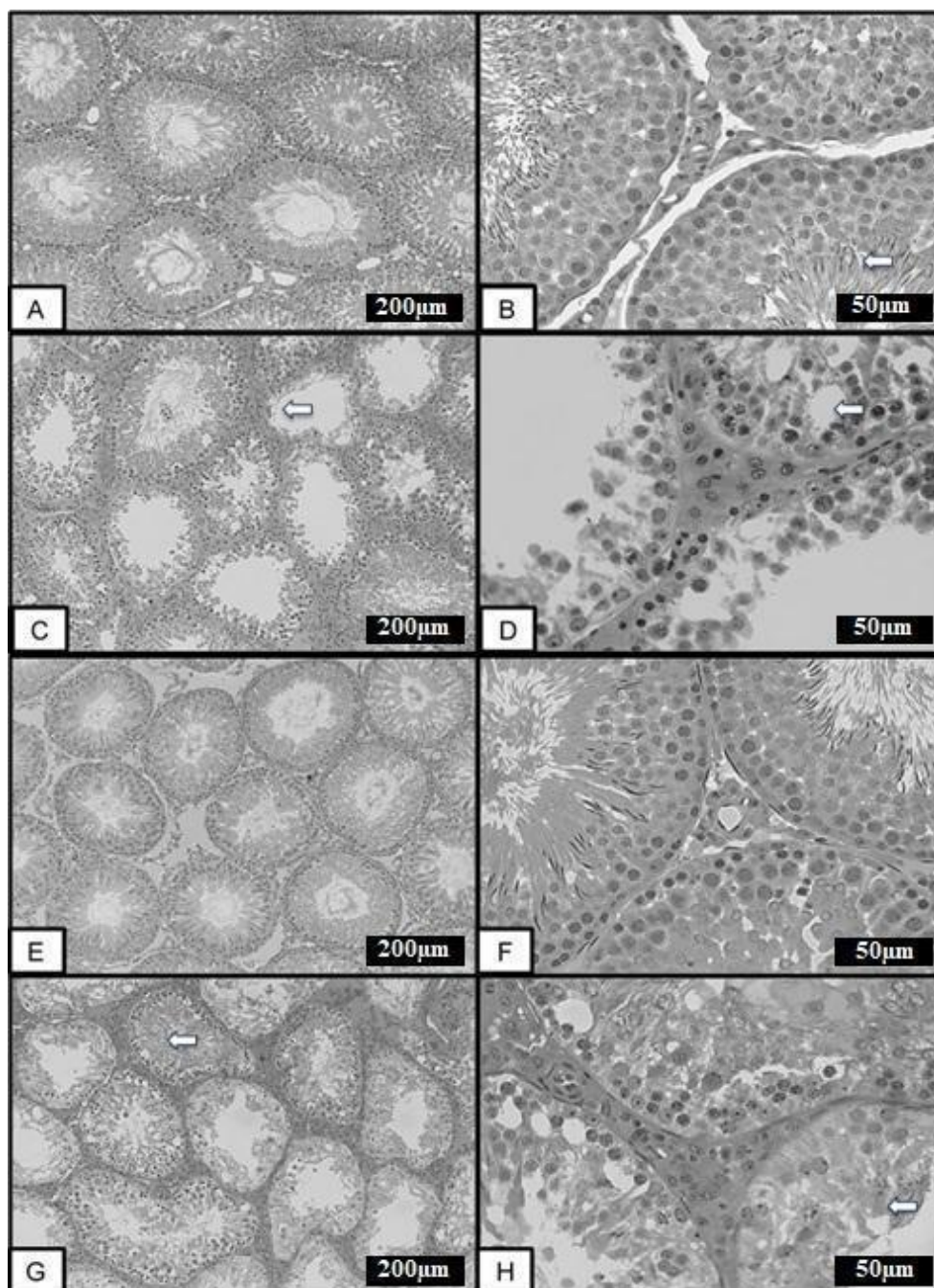


G1 (not stressed and untreated): A - Testicular parenchyma with most stages of the cycle of seminiferous epithelium without injury. B - Cycle of the seminiferous epithelium of three tubules in different stages and Leydig cells in the interstitium (arrow). G2 (stressed and untreated): C - Seminiferous tubules with loss of germinal epithelium. D - Desquamated cells from the germinal epithelium (arrow). G3 (not stressed and treated): E - Testicular parenchyma with most stages of the seminiferous epithelium cycle without any injury (arrow). F - Cycle of the seminiferous epithelium of three tubules in different stages. G4 (stressed and treated): G - Seminiferous tubules with reduced germ cell population and increased intertubular space, in addition to some tubules in advanced state of atrophy with desquamation of germ cells (arrow). H - Three seminiferous tubules with processes of degeneration, characterized by the process of loosening of the germ cells, formation of vacuoles (arrow) in the germinal epithelium, and desquamation. Fonte: Autores (2022).

On day 30 (Figure 2), G2 evidenced a reduction in the height of the seminiferous epithelium and the presence of cells desquamation inside the tubular lumen, in addition to loose and degenerate germinative cells and vacuolization of Sertoli cells. The G4 presented a reduction in the majority of the germ cell population, with long extensions and Sertoli cell vacuolization. Some G4 seminiferous tubules did not yet possess germ cells in the post-meiotic state, while others demonstrated recovery of

the spermatogenic process through the presence of spermatocytes in preleptotene and leptotene.

Figure 2. Transverse cut of testicles of adult Wistar rats submitted or not to testicular heat shock and treated or not with green tea extract at 30 days.

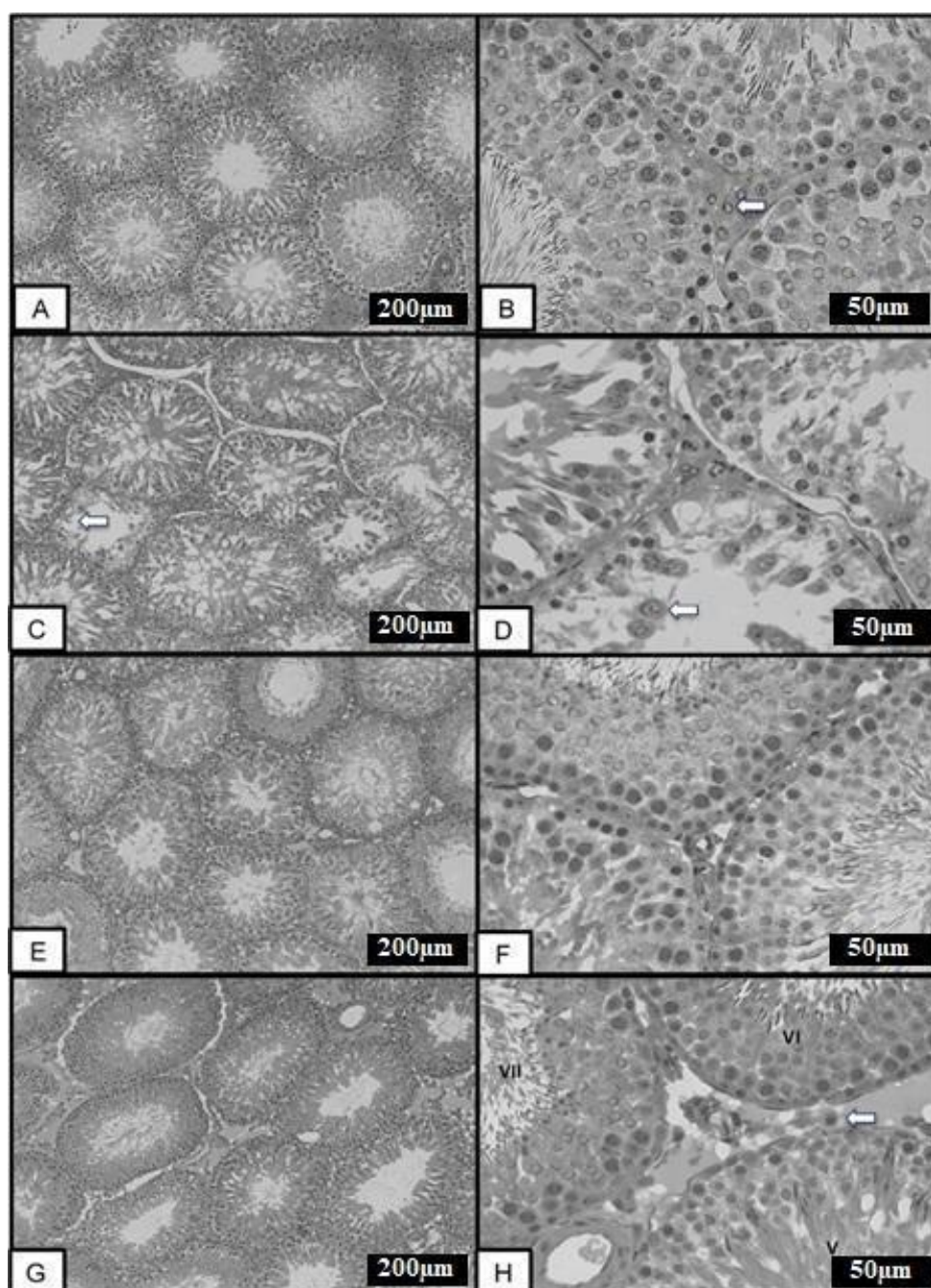


G1 (not stressed and untreated): A – Testicular parenchyma with most stages of the cycle of seminiferous epithelium without injury. B - Cycle of the seminiferous epithelium in stage VII (arrow). G2 (stressed and untreated): C – reduction in the height of the seminiferous epithelium (arrow) and presence of desquamated cells in the lumen. D – Loose germ cells and vacuolization of Sertoli cells (arrow), with degeneration. G3 (not stressed and treated): E - testicular parenchyma with the majority of stages of the cycle of seminiferous epithelium without injury. F – Cycle of the seminiferous epithelium of three tubules in different stages. G4 (stressed and treated): G – Seminiferous tubules with reduction in the majority of the germ cell population, with long extensions and vacuolization of the Sertoli cells, some seminiferous tubules still without germ cells in the post meiotic state and others with recovery of the spermatogenic process (arrow). H – Spermatocyte in pre-leptotene and leptotene, indicating recovery of spermatogenesis, some rounded spermatids, spermatogonia, and Sertoli cells, despite desquamation of the germinal epithelium (arrow). Fonte: Autores (2022).

Sixty days after the heat stress (Figure 3), G2 still maintained seminiferous tubules with a germ cell population in several stages and incomplete, in addition to characteristic findings of testicular degeneration, such as vacuolization of Sertoli cells,

desquamated germ cells, and atrophic seminiferous tubules without a germ cell population. Conversely, G4 demonstrated recovery of the spermatogenic process, marked by the filling of the seminiferous tubules with their respective cells, pertinent to each of the stages.

Figure 3. Transverse cut of testicles of adult Wistar rats submitted or not to testicular heat shock and treated or not with green tea extract at 60 days.



G1 (not stressed and untreated): A - Testicular parenchyma with most stages of the seminiferous epithelium cycle without injury. B - Seminiferous epithelium cycle of three tubules in different stages and Leydig cells in the interstitium (arrow). G2: (stressed and untreated) C - Seminiferous tubules with a population of germ cells in various incomplete stages and atrophic seminiferous tubules without germ cell population (arrow). D - Testicular degenerative process such as vacuolization of Sertoli cells, desquamated germ cells (arrow). G3 (not stressed and treated): E - Testicular parenchyma with the majority of stages of the cycle of seminiferous epithelium without injury. F - Cycle of the seminiferous epithelium of three tubules in different stages. G4 (stressed and treated): G - Full recovery of the spermatogenic process with filling of the seminiferous tubules with their respective cells pertinent to each of the stages. H - Stages V, VI, and VII, and presence of cells in the lymphatic space (arrow). Fonte: Autores (2022).

4. Discussion

No involvement from testicular heat stress and/or administration of green tea extract was observed on the body weight of male Wistar rats, a fact that corroborated with Hijazi et al. (2015). However, this diverges from literature reports on the lipolytic and weight-reducing effect of this compound, although it is a dose-dependent factor, influenced too by the experimental conditions employed (Chandra et al., 2011, Baláži et al., 2022). The body weight of the animals from the different experimental groups varied from 345.14 to 389.57 g, remaining within the standards for the species and sex (360 to 416 g), as described by Oliveira et al. (2015).

A reduction in testicle weights is an evident sign of their exposure to heat, whether acute or chronic (Yarmolenko et al., 2011; Yadav et al., 2017; Ngoula et al., 2020). According to Kanter et al. (2013) and Lue et al. (1999), a reduction in the testicular weight of rats can be observed one or two days after being submitted to 43 °C for 30 or 15 min, respectively, without recovery until day 35 (Kanter et al., 2013). This parameter resumes values similar to those of the control group only after 56 days (Lue et al., 1999). These observations are similar to that of the present study, where at 60 days after the heat shock the testicular weights were higher than on days 15 and 30, without positive or negative interference of the green tea extract.

The gonadosomatic index was widely compromised by the heat, regardless of the administration of green tea. How this parameter refers to the percentage of body mass allocated to the testicles of the animal (Capucho et al. 2022), its reduction is justified by the lower testicular weight of the heat stressed animals. Moreover, testicular hyperthermia decreased the diameter of the seminiferous tubules, like observed by Kanter et al. (2013) and Thanh et al. (2020), but without green tea protective effect, in opposition to Bagherpour et al. (2019) reports. Nevertheless, the mean tubule diameter indicates the development of the seminiferous epithelium (Kanter et al., 2013), representing an important parameter.

Regarding the volumetric characteristics of the testicular parenchyma, the lower volumes of the seminiferous epithelium and seminiferous tubules in the stressed groups, in relation to the groups without heat stress, confirm the harmful role of heat for the gonads, and the protective effect of the treatment. It is reinforced by the fact of EGCG, present in large amounts in green tea (Nain et al., 2022), can prevent inflammatory, apoptotic and oxidant effects in testicles (Al-Maghrebi et al., 2012; Bagherpour et al., 2019), with protection of the seminiferous tubules (Ding et al., 2015; Bagherpour et al., 2019).

The lymphatic space presented greater volume in the stressed groups than in the control group, due to the smaller diameter and height of seminiferous tubules as, consequently, the interstitial space becomes larger. Based on the foregoing, it can be seen that damages in the seminiferous tubules culminate in damages to spermatogenesis, increasing the possibility of infertility (Kanter et al., 2013; Thanh et al., 2020).

Although recovery of the seminiferous epithelium is possible, it depends on the presence of viable spermatogonia and Sertoli cells, from which improvement in the germinal epithelium is verified around 60 days after the removal of the cause of the stress (Gabaldi & Wolf, 2002). Thus, the temperature and time of exposure to testicular heat stress are determinant to the recovery of gonadal functions, since they are limiting factors to the survival of spermatogonia and Sertoli cells (Durairajanayagam et al., 2015).

Through the qualitative histological analysis on day 15, it was possible to identify that lesions of the testicular parenchyma, including Sertoli cells, were less intense in the group that received the green tea extract (G4) than in the group only exposed to elevated testicular temperature (G2), which was repeated on day 30. This was even more striking on day 60, when, while the G2 demonstrated typical findings of testicular degeneration, the G4 presented marked recovery in the spermatogenic process.

In addition to the above exposure, the presence of all stages of the seminiferous epithelium cycle, as verified on day 60 for the G4, is indicative of complete restoration of spermatogenesis (Jannes et al., 1998). Thus, the findings demonstrate the

protective effect of the catechins present in green tea, as previously reported, against the negative effects of testicular hyperthermia (Abshenas et al., 2011; Vieira et al., 2020), as well as the sensitivity of germ cells and Sertoli cells to the action of heat (Kanter et al., 2013; Thanh et al., 2020).

The results corroborate with previous reports that oral administration of green tea extract, after testicular thermal shock in mice (42°C/20 min), prevented the depletion of the seminiferous tubules, although the mechanisms by which this extract provides benefits to spermatogenesis are not fully elucidated (Abshenas et al., 2011). Thus, they have been largely associated to the antioxidant, anti-apoptotic (Bagherpour et al., 2019), and anti-inflammatory actions (Al-Maghrebi et al., 2012) of the EGCG. Therefore, research should be developed to prove the therapeutic activities and understand the mechanisms of the action of green tea, especially because this bioactive shows as an attractive alternative in the preservation of male fertility.

5. Conclusion

The administration of green tea extract is an effective way of improving tissue recovery after testicular damage induced by short-term heat stress in rat testicles, as it promotes full recovery of spermatogenesis in the second spermatogenic cycle.

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