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Effects of swimming on the testicular histomorphology of alcoholized rats

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ABSTRACT. The objective of this study was to examine whether the practice of long-duration, low-intensity physical exercise helped reverse changes observed in the testes of rats subjected to alcohol dependence induced by semi-voluntary intake. Forty male rats were distributed into four groups: alcohol no exercise (ANE); alcohol with exercise (AWE); no alcohol no exercise (NANE); no alcohol with exercise (NAWE). The cachaça ("51" brand) was offered in the following dilutions: 10% (10 days), 15% (11 days), 20% (12 days), 25% (12 days), 30% from the 45th to 120th day, when the alcohol was replaced by drinking water. On the 120th day, the groups NAWE and AWE were assigned to swim in individual tanks with water temperature around 30ºC for 20 minutes, 5 days week^{-1} for 8 weeks. In the ANE group there was a depletion of the seminiferous epithelium, while in AWE rats the majority of seminiferous tubules were unaltered. Compared to the AWE group, there were reductions in tubular diameter and the height of the seminiferous epithelium in the ANE group. The results showed lesser testicular atrophy in animals that exercised than in sedentary ones. Physical exercise after the discontinuation of alcohol seems to accelerate the process of recovery of damaged tissue.

Keywords: exercise, testis, alcoholism, swimming.

Efeitos da natação na histomorfologia testicular de ratos alcoolizados

RESUMO. O objetivo deste estudo foi examinar se a prática do exercício físico de longa duração e baixa intensidade reverte as alterações observadas nos testículos de ratos submetidos à dependência alcoólica induzida pela ingestão semivoluntária. Foram distribuídos 40 ratos machos em quatro grupos: alcoolizado não-exercitado (ANE); alcoolizado com exercício (ACE); não-alcoolizado e não-exercitado (NANE); não-alcoolizado com exercício (NACE). A aguardente (marca '51') foi oferecida nas seguintes diluições: 10% (10 dias), 15% (11 dias), 20% (12 dias), 25% (12 dias), 30% do 45º ao 120º dia, quando o álcool foi substituído por água. No 120º dia, os animais dos grupos NACE e ACE foram transferidos para nadar em tanques individuais com água na temperatura de 30º por 20 min., cinco dias semana^{-1}, durante oito semanas. No grupo ANE houve depleção do epitélio seminífero, enquanto nos ratos do grupo ACE, a maioria dos túbulos seminíferos estava inalterado. Comparado ao grupo ACE, houve redução no diâmetro tubular e na altura do epitélio seminífero no grupo ANE. Os resultados mostraram menor atrofia testicular nos animais que foram exercitados do que nos sedentários. O exercício físico, após a interrupção da ingestão de álcool, parece acelerar o processo de recuperação de tecidos lesados.

Palavras-chave: exercício, testículo, alcoolismo, natação.

Introduction

Alcoholic beverages are classified as products capable of causing bodily damage through mechanisms of direct and/or indirect toxicity that act on several body systems. The effects of their use can include acute intoxication and severe chemical dependency. Human involvement with alcohol presents a history of cultural, social, religious, political and even biological interference. Social drinking, binge drinking and dependency may have multifactorial causes (BABOR et al., 2003) such as genetic vulnerability, gender, and individual biological and psychological traits, as well as sociocultural factors affecting patterns of alcohol consumption (LARANJEIRA et al., 2000).

According to Tadic et al. (2000), men who chronically abuse alcohol may display a spectrum of endocrine abnormalities including hypogonadism and feminization; they also present elevated serum estradiol and low serum testosterone due to action of the enzyme Aromatase. However was reported

that ethanol-induced hypogonadism is not dependent on activation of the HPG axis (EMANUELE et al., 2001).

The effects of alcohol on pituitary-gonadal axis hormones depend, however, on the gender and sexual maturation of the subjects (FRIAS et al., 2002). Kim et al. (2003) demonstrated that prolonged administration of ethanol resulted in a profound inhibition of the reproductive activity in the male rat at all levels of HPG axis.

Most alcoholics are often found to have fertility abnormalities such as low sperm count and/or impaired sperm motility (MANEESH et al., 2006), although the mechanisms of alcohol-induced testicular damage have yet to be fully explained. As examples of such mechanisms, Emanuele and Emanuele (2001) cite opioids and the mechanism of oxidation and cell damage. Testicular opioids are messenger molecules similar to morphine that, when produced in the testes suppress testosterone synthesis and may increase apoptosis at the gonadal level, resulting in the death of both Leydig and seminiferous cells, which are involved in sperm cell formation and maturation. Oxidation occurs as a result of alcohol metabolism, which generates byproducts called oxidants that contribute to cell death, such as reactive oxygen species (ROS). On a cellular level, damage due to the mechanism of lipid peroxidation may also contribute to gonadal dysfunction. According to Rengarajan et al. (2003) the steroidogenic activity of Leydig cells is suppressed after ethanol exposure, since the binding of LH to its receptors on Leydig cells surface is impaired.

Currently, many clinicians who treat cases of acute and chronic alcoholism favor the implementation of a physical exercise program to facilitate recovery from the effects of alcohol abuse. This practice was first proposed by Juhlin-Dannfelt et al. (1977) under the premise of causing a general improvement in body function and also an improvement in those functions directly affected by the chronic use of alcohol, such as hepatic metabolism and cognitive functions.

Buckworth and Dishmann (2002) report that the benefits of resistance exercise (weight training) – maintaining and/or gaining muscle mass – can bring about a restoration of lost mass in alcohol-dependent subjects.

Thus, the purpose of this study was to determine whether, after the discontinuation of alcohol ingestion, the practice of low-intensity long-duration physical exercise (swimming) facilitated recovery from changes observed in the testes of rats subjected to alcohol dependence induced by semi-voluntary intake.

Material and methods

Animals

This experiment was carried out on 40 male, 90-day-old Wistar rats (300 ± 30.0g body weight). All animals were randomly divided into four experimental groups: alcohol and no exercise (ANE), alcohol with exercise (AWE), no alcohol and no exercise (NANE) and no alcohol with exercise (NAWE). The inducement of alcohol dependence by semi-voluntary intake for the ANE and AWE groups proceeded according to the model proposed by Pereira and Conegero (2004), so that the only source of liquid available for the animals was an aqueous solution of cachaça in water ('51' brand, 39 proof - G1, Muller Industries, Pirassununga, São Paulo State, Brazil). The liquor was offered to the animals in the following dilutions: 10% for 10 days, 15% for 11 days, 20% for 12 days, 25% for 12 days, 30% from 45 days, continuing until 120 days, when the alcohol solution was replaced with pure drinking water. The groups NANE and NAWE were provided *ad libitum* access to drinking water throughout the experiment.

Animals were housed in cages in a temperature and humidity controlled environment (22-25ºC, 50-75%) under a standard 12h light-dark cycle.

Physical training

Beginning on the 120th day, the NAWE and AWE groups were submitted to the modified swimming protocol proposed by Lancha Jr. et al. (1995), featuring low-intensity long-duration physical exercise. Each animal was put in a separate tank with water temperature of around 30º and allowed to swim for 20 minutes, 5 days a week, for 8 weeks. During this period, animals in the groups NANE and ANE remained sedentary in their cages.

Histological processing

At the end of training, all animals were weighed and euthanized by inhalation of saturated anesthetic ether. The testes were collected, weighed and fixed in Bouin's solution. The usual histological routine was carried out, with stages of dehydration, diaphanization and embedding in paraplastic (Oxford-Labware, St. Louis, MO, USA). Histological sections 5μm thick were stained using the H-E and PAS techniques and examined under a light microscope (Leica model DMLS).

Morphometric analysis

The weight (g) and net volume (mL) of each testis were determined. The latter parameter was calculated by subtracting the weight of the albuginea

and testicular mediastinum from the gross weight of the gonad (FRANÇA; GODINHO, 2003).

Tubular diameter and height of seminiferous epithelium

Tubular diameter was obtained by the random measurement of 30 cross-sections of seminiferous tubules having the most circular contour possible. The same sections used to measure the tubular diameter were used to measure the height of the seminiferous epithelium, which was regarded as the distance from the basement membrane to the tubular lumen. For these measures, we used the software LEICA QWinV3 at 10X.

Gonadosomatic index (GSI)

Having determined the weight of the left and right testicles of each animal, it was also possible to calculate the gonadosomatic index (percentage of the body occupied by the gonad) using the following formula:

$$\text{Gonadosomatic index (\%)} = [(RTw + LTw(\text{g}) / Bw(\text{g}))] \; x \; 100$$

Being:
RTw = weight of right testis;
LTw = weight of left testis;
Bw = body weight

Volumetric density of testicular parenchyma (seminiferous tubules) and interstitial tissue

The volumetric densities of testicular tissue were determined by light microscopy using a 441-intersection grid placed in the eye-piece of the light microscope. Fifteen sections chosen randomly (6.615 points) were scored for each animal at 400X magnification.

The volumes of basic tissues (seminiferous tubules and interstitial tissue) of the testes were calculated in mL, from the percentage for each gonad, and were obtained with the density of tissue volume and total testicular volume.

Distribution of Leydig cells

We calculated the number of sections needed to find 1000 incidences of Leydig cells (cytoplasm or nucleus) in the interstitial tissue. For this calculation we used a 100-point reticle attached to a binocular optical microscope, with a final magnification of 1000X.

Statistical analysis

The experimental results obtained in each treatment group were compared statistically using the program INSTAT 3.0 (GraghPad Software) through analysis of variance (ANOVA) complemented, *a posteriori*, with the Tukey-Kramer test ($p < 0.05$).

Results

Histological analysis

Testes analysis under optical microscopy demonstrated that the structure of seminiferous tubules showed morphological integrity in the groups NANE (Figure 1 A) and NAWE (Figure 1 B), characterized by the presence of several cell layers in the germinal epithelium and spermatozoa in the lumen.

In the testes of some animals in the ANE group, seminiferous tubules showed atrophy and it was impossible to identify the spermatogenic cell line (Figure 1 C). However, the seminiferous tubules of most of the testes from that group showed a less pronounced depletion, with visible epithelial cell debris and vacuolization processes (Figure 1 D).

In non-sedentary alcohol-dependent rats (AWE) the majority of seminiferous tubules showed a clear recovery (Figure 1 E), but still presented a number of seminiferous tubules with loss of germinal elements such as late spermatids. The most abundant cells were in the epithelium, and the nuclei of Sertoli cells showed more condensed chromatin. Some tubules also had visible epithelial cell debris and vacuolization processes (Figure 1 F).

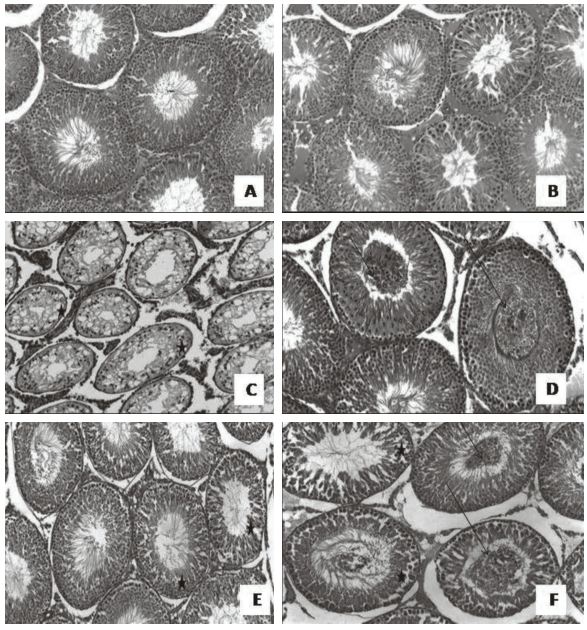

Figure 1. Histological section of rat testis stained with haematoxylin & eosin (10X objective) of groups: NANE (A), NAWE (B), ANE (C and D) and AWE (E and F). Note reduced interstitial tissue in C and D and atrophic seminiferous tubules in D. In E and F, seminiferous tubules with architecture restored after suspension of alcohol in combination with physical exercise, but with vacuolated epithelium (*) and exfoliation.

Distribution of Leydig cells

In the non-alcoholic control groups, Leydig cells were normally distributed throughout interstitial tissue cells, totaling 1000 cells in 130.5 fields. In the experimental group ANE, however, 166.5 additional sections needed to be reviewed, and in the AWE group 72.5 additional sections were necessary to accumulate 1000 Leydig cells.

Morphometric analysis

Data analysis of animal weight, testicular weight, gonadosomatic index (Table 1) and morphometric data of the seminiferous tubules (tubule diameter and height of the seminiferous epithelium) demonstrated a significant difference ($p < 0.05$) in the sedentary alcoholic group (ANE) for animal weight, tubular diameter and epithelium height compared to other groups.

Volumes: total testicular, parenchymal and interstitial tissue

Total testicular volume was reduced in the animals of the alcohol no exercise group (ANE). Data analysis of the volumes of the parenchyma and interstitial tissue showed that there was a significant reduction ($p < 0.05$) in average volume of parenchyma in the ANE and AWE groups. For the alcohol without exercise group (ANE), the percentage of testicular parenchyma of the gonad was lower than in the alcohol with exercise (AWE) group. After the discontinuation of ethanol in the swimming group, the testicular parenchyma was approximately 74.2% of the total organ volume, whereas in the group that did not exercise the volume was only 50.9% (Table 1).

Table 1. Variables in assessing the effect of alcohol on the reproductive system profile of Wistar rats.

Groups	NANE	ANE	NAWE	AWE
Weight (g)				
Body	347.5 ± 31.05[a]	283.0 ± 5.15[ab]	370.5 ± 12.11[c]	321.50±20.82[ac]
Testicle	1.40 ± 0.10[a]	1.33 ± 0.25[a]	1.55 ± 0.14[c]	1.34±0.09[a]
IG (%)	0.74 ± 0.04[a]	0.82 ± 0.12[a]	0.78 ± 0.03[a]	0.86 ± 0.04[a]
Volume (mL)				
Testicle	1.35 ± 0.10[a]	1.08 ± 0.23[b]	1.44 ± 0.09[a]	1.32± 0.09[a]
Parenchyma	1.01 ± 0.08[ac]	0.55 ± 0.12[ac]	1.16 ± 0.14[c]	0.98 ± 0.09[ab]
Interst. Tissue	0.34 ± 0.03[ac]	0.53 ± 0.11[ac]	0.28 ± 0.04[c]	0.34 ± 0.06[ab]
Diameter T.S. (μm)	292.8 ± 1.40[a]	177.2 ± 10.70[b]	280.2 ± 15.60[a]	296.0 ± 4.50[a]
Epithel Height (μm)	79.4 ± 0.68[a]	39.4 ± 0.76[b]	83.5 ± 3.41[a]	79.8 ± 2.03[a]

Values express the mean ± SEM; IG: Gonadosomatic index; Parench.: Parenchyma; Int: Interstitial; Diam.T.S.: Seminiferous Tubule Diameter; Ep.: Epithelium.* Equivalent letters in the same column do not differ statistically ($p < 0.05$).

Discussion

Several studies have been conducted regarding addiction and drug abuse in Brazil. Experimental studies about alcoholism in the literature have been primarily focused on humans (GIANCOLA, 2004).

It is known that ethanol affects reproductive function in adult rats and that the deleterious effects are manifested as testicular atrophy, cellular damage in the germinal epithelium, reduced weight of the prostate, seminal vesicles and epididymis, as well as decreased sperm motility (ANDERSON JR. et al., 1983). Koh and Kim (2006) showed that ethanol intake increases apoptotic cell death in testicular germ cells. Studies have demonstrated that prenatal ethanol exposure in rats interferes with the neurobehavioral sexual differentiation of the male, attenuating the postnatal testosterone surge required by the male brain for normal sexual differentiation (FAKOYA; CAXTON-MARTINS, 2004). Lee et al. (2010) suggested that chronic ethanol affects rat testis through a reduction of testicular GnRH and GnRH-RmRNA expression, particularly during puberty.

El-Sokkary (2001) observed the same deleterious effects on seminiferous tubules and Leydig cells as a result of the metabolism of ethanol by alcohol dehydrogenase.

The histological analysis showed intense lesions in some seminiferous tubules of alcoholized animals even after the discontinuation of alcohol intake, demonstrating that ethanol can cause irreversible changes in the spermatogenic process. However, eight weeks after discontinuation, the seminiferous tubular morphology of most testes of animals given alcohol showed few changes, including the occurrence of intraepithelial vacuoles. This was probably due to exfoliation and seminiferous tubule atrophy. By observing a large number of vacuoles in the seminiferous epithelium of animals exposed prenatally, Fakoya and Caxton-Martins (2004) found that recovery from the consumption of alcohol in affected testicular morphology varies according to dose, age, individual metabolism and even lifestyle. Therefore, it is clear that other procedures in addition to medical treatment, such as physical exercise, are necessary to improve recovery outcomes.

Theoretically, the use and/or abuse of alcohol can lead to chemical dependency. Medical treatment for addiction is complex, usually multidisciplinary, and administered on an individualized, case-by-case basis.

The reduction of deleterious effects on male reproductive function begins by suspending ethanol use. Sustained moderate-intensity exercise for the purpose of organ and tissue recovery shows significant positive effects when properly implemented (BOSCO et al., 2004). In this study,

we found that the consumption of alcohol affected testicular morphology by observing reductions in both tubular diameter and seminiferous epithelium height of sedentary alcoholized animals, resulting in, along with inhibited spermatogenesis, a 49.13% reduction in the diameter of the tubules and a 50.62% reduction in the thickness of the germinal epithelium compared to animals of the alcoholized group that exercised. The changes to tubular morphology after discontinuation of ethanol consumption, although still present, almost returned to normal. However, there were significant differences between the average volume of the parenchyma, tubular diameter and the height of the seminiferous epithelium in sedentary alcoholized rats compared to those that exercised. These variations show that physical exercise after the discontinuation of alcohol seems to accelerate the process of damaged tissue recovery. Likewise, results demonstrated that sedentary animals only lost weight while those that underwent physical exercise began to regain body mass after they stopped drinking alcohol.

Data in literature indicate that the chronic intake of ethanol significantly reduces plasma levels of testosterone (OLIVA et al., 2006). This reduction may explain the observed changes in tubular morphology and the display of possible functional damage in Leydig cells. In this experiment, in order to obtain a count of 1000 Leydig cells, it was necessary to run more sections in the interstitium for alcoholized rats than for controls. Animals subjected to physical exercise required a smaller number of sections to achieve a count of 1000 Leydig cells according to El-Sokkary (2001), whereas the quantitative results and nuclear volume of Leydig cells showed a highly significant decrease in the mean number of cells mm^{-2} (unit area) and in the mean volume of nuclei in alcoholized rats versus controls.

Several studies have shown that exercised rats have increased testosterone concentration (MARIN; JÚNIOR, 2007), although the concentration of testosterone after exercise may be an important biomarker of training stress.

According to Urhausen et al. 1998), the increased concentration of plasma testosterone is due to increased activity of the hypothalamic-pituitary-gonadal axis during exercise. Although the plasma concentration of testosterone was not examined in this study, data in the literature strengthens the hypothesis that moderate-intensity exercise increases levels of circulating testosterone which could be responsible for accelerating the process of testes recovery after interrupting alcohol intake.

Conclusion

After the discontinuation of ethanol use, the rats subjected to exercise showed less testicular atrophy than their sedentary counterparts, which indicates that the effects of physical exercise on testicular structure can help accelerate regeneration.

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