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Influence of constant lighting, melatonin and geldanamycin under mice testicle over 60 days

Influência de constante iluminação, melatonina e geldanamicina sob o testículo de rato por mais de 60 dias

Influencia de la iluminación constante, melatonina y geldanamicina bajo el testículo de ratones durante 60 días

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Abstract
Melatonin (N-acetyl-5-methoxytryptamine) is a hormone secreted by the pineal gland. In men, melatonin affects reproductive regulation in three main ways. First, it regulates the secretion of GnRH and LH. Second, it regulates testosterone synthesis and testicular maturation. Third, as a potent deputare of free radical that is both lipophilic and hydrophilic. The mammalian testis is an organ susceptible to environmental and therapeutic toxic agents that compromise spermatogenesis, the present study aimed to analyse the influence of constant illumination, melatonin and geldanamycin under the testis of rats treated for 60 days. Males were randomly divided into 4 groups, each consisting of 5 animals, which are: Group I - control rats (untreated); Group II - rats subjected to constant illumination for 60 days; Group III - rats subjected to constant illumination for 60 days and treated with melatonin for 60 days; Group IV - rats subjected to constant illumination for 60 days, treated with melatonin and geldanamycin for 60 days. Exposure to constant illumination affects the development of germinal epithelium, as well as decreased weight of the testicles, Leydig, Sertoli, and testosterone cells. Melatonin and geldanamycin treatment have a protective effect against constant illumination, reducing testicular damage when taken together.
Keywords: Testis; Melatonin; Constant illumination; Geldanamycin; Leydig.

Resumo
A melatonina (N-acetil-5-metoxitriptamina) é um hormônio secretado pela glândula pineal. Nos homens, a melatonina afeta a regulação reprodutiva de três maneiras principais. Primeiro, ele regula a secreção de GnRH e LH. Em segundo lugar, regula a síntese de testosterona e a maturação testicular. Terceiro, como um potente representante do radical livre que é tanto lipofílico quanto hidrofílico. O testículo de mamíferos é um órgão suscetível a agentes tóxicos ambientais e terapêuticos que comprometem a espermatogênese, o presente estudo teve como objetivo analisar a influência da iluminação constante, melatonina e geldanamicina sob o testículo de ratos tratados por 60 dias. Os machos foram divididos aleatoriamente em 4 grupos, cada um composto por 5 animais, sendo eles: Grupo I - ratos controle (não tratados); Grupo II - ratos submetidos à iluminação constante por 60 dias; Grupo III - ratos submetidos à iluminação constante por 60 dias e tratados com melatonina por 60 dias; Grupo IV - ratos submetidos à iluminação constante por 60 dias, tratados com melatonina e geldanamicina por 60 dias. A exposição à iluminação constante afeta o desenvolvimento do epitélio germinativo, bem como a diminuição do peso dos testículos, células de Leydig, Sertoli e testosterona. O tratamento com melatonina e geldanamicina tem um efeito protetor contra a iluminação constante, reduzindo os danos testiculares quando tomados em conjunto.
Palavras-chave: Testículo; Melatonina; Iluminação constante; Geldanamicina; Leydig.

Resumen
La melatonina (N-acetil-5-metoxitriptamina) es una hormona secretada por la glándula pineal. En los hombres, la melatonina afecta la regulación reproductiva de tres formas principales. Primero, regula la secreción de GnRH y LH. En segundo lugar, regula la síntesis de testosterona y la maduración testicular. En tercer lugar, como un potente deputare de radicales libres que es tanto lipofílico como hidrofílico. El testículo de mamífero es un órgano susceptible a agentes tóxicos ambientales y terapéuticos que comprometen la espermatogénesis, el presente estudio tuvo como objetivo analizar la influencia de la iluminación constante, melatonina y geldanamicina debajo de los testículos de ratas tratadas durante 60 días. Los machos se dividieron aleatoriamente en 4 grupos, cada uno de los cuales constaba de 5 animales, que son: Grupo I - ratas de control (sin tratar); Grupo II: ratas sometidas a iluminación constante durante 60 días; Grupo III: ratas sometidas a iluminación constante durante 60 días y tratadas con melatonina durante 60 días; Grupo IV: ratas sometidas a iluminación constante durante 60 días, tratadas con melatonina y geldanamicina durante 60 días. La exposición a una iluminación constante afecta el desarrollo del epitelio germinal, así como la disminución del peso de los testículos, las células de Leydig, Sertoli y de testosterona. El tratamiento con melatonina y geldanamicina tiene un efecto protector contra la iluminación constante, reduciendo el daño testicular cuando se toman juntas.
Palabras clave: Testículo; Melatonina; Iluminación constante; Geldanamicina; Leydig.

1. Introduction

Every form of life that has developed has had to adapt to variations in the light-dark cycle and that is why it is said that there was an evolutionary need for rhythm. According to their frequency, these rhythms can be classified into circadian, ultradian and infradian. Among the different biological rhythms, the most studied and most frequent in living organisms are circadian rhythms (circa = about; diem = day), which adjust to the cyclical variations of day and night (Moore 2013).

The variation in body temperature, the levels of cortisol and melatonin and mental performance are rhythmic phenomena that present circadian frequency, with the sleep-wake cycle being the most evident among circadian rhythms (LeGates et al. 2014).

In mammals, the circadian timer system is responsible for the genesis and maintenance of rhythms, with the suprachiasmatic nucleus (NSQ) as a central pacemaker capable of synchronizing our endogenous rhythms with those of the environment (Moore 2013).

Light is the main zeitgebers, a German term that means (which donates or marks time), capable of curling our rhythms because, on the retina, we have cones and rods, cells specialized in detecting light, as well as their spectrum characteristics and intensity and, through the hypothalamic retinal tract (HRT), this message reaches the NSQ (LeGates et al. 2014).

It is known that seeing sunlight at dawn can raise blood pressure and blood glucose in preparation for routine in daytime animals. Not least, the dark period is also essential for the proper physiology of the organism (Vásquez-Ruiz et al. 2014). The pineal gland, which receives innervation from the NSQ, produces and releases melatonin in the absence of the light stimulus (Reiter 1991).

Among the processes under circadian control are neurotransmission, metabolism, immunity and endocrine signaling, such as those of the pineal gland, pituitary and adrenal glands. The pineal is a gland located in the brain approximately 5 mm long, 1-4 mm thick and weighing around 100mg that receives afferents from the paraventricular nucleus (NPV) of the hypothalamus. This gland has two cell types: neuroglial cells and, predominantly, pinealocytes, whose main product is the hormone melatonin (Wu & Swaab 2004).

Melatonin (N-acetyl-5-methoxytryptamine) is a hormone secreted by the pineal gland, which production and concentration in the body is always linked at night - rhythm regulated by signals sent from the suprachiasmatic nucleus (SCN) to the paraventricular nucleus (PVN) and from this to pineal -,(Cajochen, Kräuchi & Wirz-Justice, 2003). The high production of this hormone is known to be maintained during the dark phase of the light / dark cycle as long as there is no light in the environment, because light blocks melatonin production at night (Brainard et al. 1983; Shalin et al. 2013).

In men, melatonin affects reproductive regulation in three main ways. First, it regulates the secretion of two major neurohormones, GnRH and LH. Second, it regulates testosterone synthesis and testicular maturation. Third, as a potent deputare of free radical that is both lipophilic and hydrophilic, it avoids testicular damage caused by environmental toxins or inflammation (Chunjin; Zhou, Xu, 2015). To be brief, literature data indicate that melatonin affects the secretion of gonadotropins, testosterone and improves sperm quality. This implies that it has important effects on the regulation of testicular development and male reproduction (Li, Chunjin; Zhou, Xu, 2015).

The structural organization of the testis is highly conserved among mammals and it contains two main compartments: the tubular, avascular, without innervations and constituted by the proper tunic (myoid cells, collagen fibers and the basement membrane), seminiferous epithelium and lumen; and the interstitial or intertubular cells in which the Leydig cells that produce steroids, blood and lymph vessels, nerves, fibroblasts, connective tissue fibers, macrophages, lymphocytes and, occasionally, mast cells are located (Pelletier et al. 2003).The mammalian testis is an organ susceptible to environmental and therapeutic toxic agents that compromise spermatogenesis and the analysis of seminiferous tubules (morphological and morphometric parameters), is a simple strategy to evaluate changes in this process (Pannocchia 2008). Public concern has been expressed that environmental factors, including drug abuse, may adversely affect testicular and sperm function (Nudell et al. 2002).

The Geldanamycin (GA) belonging to the ansamycin family from the secondary metabolism of actinobacteria and is an anticancer agent produced by Streptomyces hygroscopicus (Silva 2016). It is a substance that induces the degradation of mutant proteins in tumors that affect testicular infertility. This effect is the result of inhibition of HSP90 which normally acts to maintain correct folding of mutant proteins (Silva et al. 2018). Although the exact mechanism of action of geldanamycin

against the testis is not clearly understood, the present study aimed to analyse the influence of constant illumination, melatonin and geldanamycin under the testis of rats treated for 60 days.

2. Methodology

2.1 Experimental Animals

The present research considered exploratory with use of quantitative methodology according to Pereira et al. (2018). All experimental procedures were conducted in accordance with the principles for the Guide for the Care and Use of Laboratory Animals (8th ed., 2011) and had been approved by the ethics committee of the Federal Rural University of Pernambuco with protocol number nº. 23082.009629 / 2010. Twenty 90-day-old Wistar albino rats (Rattus norvegicus albinus) from the Department of Animal Morphology and Physiology, in Federal University of Pernambuco, were used. These animals were kept in cages with food and water ad libitum.

2.2 Experimental Design

Males were randomly divided into 4 groups, each consisting of 5 animals, which are:

Group I - control rats (untreated);

Group II - rats subjected to constant illumination for 60 days;

Group III - rats subjected to constant illumination for 60 days and treated with melatonin for 60 days;

Group IV - rats subjected to constant illumination for 60 days, treated with melatonin and geldanamycin for 60 days.

2.3 Constant Light Stimulation

Using a wooden box with an area of 0.5 m3, with slots to allow ventilation, containing two 40 W PHILLIPS fluorescent lamps, which provided adequate and sufficient light, around 400 lux. This box remained during the experiment in the laboratory animal house at a temperature of approximately 22 ° C, containing the animals from groups II to IV.

2.4 Melatonin Treatment

Melatonin treatment (Sigma, St. Louis, MO, USA) was performed according to the methodology proposed by (Prata Lima; Baracat; Simões, 2004). It was administered at a dose of 200 μg melatonin per 100g body weight of the animal by intraperitoneal injections in the evening (18: 00h) for 60 days. Melatonin was dissolved in a volume of ethanol (0.02 mL) and diluted in saline (0.9% NaCl). Control animals received 0.9% NaCl solution and 0.02 mL ethanol, respectively.

2.5 Geldanamycin Treatment

A 17-Dimethylaminopropylamino-17-demethoxy-geldanamycin (17-DMAP-GA / Invivogen - USA) was used at the 10 μM dose (Hinzpeter et al. 2006) intraperitoneally.

2.6 Testis Weight

The testicles were weighed on a precision analytical balance immediately after extraction.

2.7 Histological and Histochemical Analysis

The fragments were dehydrated in ethyl alcohol (increasing concentrations), diaphanized by xylol and embedded in paraffin. Then, the sections were submitted to staining with Hematoxylin-Eosin (HE), Mallory Trichrome for collagen fibers and Schiff Periodic Acid (PAS) for acid glycosaminoglycans analysis (GAGs). The material were analysed using an

OLYMPUS BX-49 and photographed in an OLYMPUS BX-50 photomicroscope. Histochemical analysis was classified as intense (++), moderate (±) and weak (+) reaction.

2.8 Morphometric Analysis of the Testicles

Tubular diameter and epithelium height were measured in 100X magnification using linear micrometric reticulum (10mm / 100 - Olympus) calibrated with a standard micrometer. The average tubular diameter for each rat was obtained from the measurement of fifteen tubules at various stages of the seminiferous epithelium cycle, randomly chosen, with round or rounded profiles. The height of the epithelium was obtained from the same tubules used to determine the tubular diameter. For this, two diametrically opposite measurements were taken, having as reference the proper tunic and the limit between the fire and the germinal epithelium, determining the average height of the seminiferous epithelium. Volumetric data of testicular parenchyma composition were obtained using couting of point by systematic allocation of micrometric graticule (Olympus) with 441 points of intersection over histological preparation of testicle at 400X magnification. Fifteen sites will be counted randomly totaling a total of 6615 points for each animal. The testis is divided into two compartments, tubular and intertubular or interstitial. The first will be evaluated the proper tunic, the seminiferous epithelium and the lumen; while in the second will be investigated Leydig cells, cells and fibers of connective tissue, blood and lymphatic vessels. As the density of the testis is around 1.03 to 1.04, the weight of the testis will be considered equal to its volume. The volume of each testicular component expressed in µL were established from the product between the volumetric density of the testicular constituents (%) and the net weight of the testis (mg). The value of the latter will be obtained by subtracting 6.5% for the albuginean gross testicular weight (Russell & França, 1995). The total length of the seminiferous tubules (TL) per testis expressed in meters will be estimated from the knowledge of the volume occupied by the seminiferous tubules in the testis and the average tubular diameter obtained for each animal. The following formula will be employed: TL = TVS / pR2, where TVS = Total volume of seminiferous tubules; pR2 = Area of cross-section of seminiferous tubules (R = tubular diameter / 2). The estimation of the different cell types that make up the seminiferous epithelium at stage 7 of the cycle, classified according to the acrosome method (RUSSELL et al., 1990) were made from counts of germ cell nuclei and Sertoli cell nucleoli. At these counts, 5 transverse sections of seminiferous tubules will be used for each animal. The nuclei of the following cell types will be counted: spermatocytes I, in the preleptotene / leptotene phase (SPT I Pl / L); spermatocytes I, in the pachytene phase (SPT I P); rounded spermatids (SPD Ar); nucleoli of Sertoli cells. Excluding Sertoli cell nuclei, the counts obtained will be corrected for nuclear diameter and histological section thickness, using the Abercrombie's formula (1946) modified by Amann and Almiquist (1962):

$$\text{Corrected number} = \text{Count result} \times \frac{\text{Section thickness}}{\text{Section thickness} + \sqrt{\left(\frac{DM2}{4}\right) - \left(\frac{DM2}{2}\right)}}$$

The average nuclear diameter (A.D.) represents the average of the diameter measurements of 10 nuclei of the studied cell type, for each animal. Nuclear diameters will be measured with the aid of linear micrometric reticulum (10mm / 100 - Olympus) adapted to one of the 10x eyepieces and attached to the 100x objective, providing a final magnification of 1000x. In the case of spermatogonia type A with ovoid nuclei, the value used were that obtained by the mean between the largest and smallest nuclear diameter. Sertoli cell numbers will be corrected for nucleolar diameter and histological section thickness, for this reason, Sertoli cells with visible nucleoli will be counted exclusively, which will provide application of the same formula mentioned previously. The total Sertoli cell population per testis will be obtained from the corrected number of Sertoli cell

nucleoli per seminiferous tubule cross section at stage VII and the total length of seminiferous tubules per testis according to the applied formula by Hochereau-de Reviers and Lincoln (1978): Number of Sertoli cells per testis = [Total length of Seminiferous Tubules (mm) X Corrected number of Sertoli cell nucleoli by cross-section] / Cut thickness (mm). Daily sperm production (DSP) per testis and per gram of testis will be obtained according to Rocha et al. (1999) and Silva Júnior et al. (2006): PED = Total No. of Sertoli cells per testis X the proportion of round spermatids in stage VII x stage VII round spermatids with relative frequency / stage duration (days).

2.9 Hormone Dosage

Hormone dosing of testosterone was performed by ELISA Test Kit.

2.10 Data Analysis

All weight data, morphometric data and hormone levels were analysed using nonparametric Kruskal-Wallis test, followed by post-hoc Dunn tests ($p < 0.05$) through the software Prism Graphic Pad Version 3.0.

3. Results

Histological analyzes performed on the testicles revealed that there were no changes in the control animals (GI), in that case, the manipulations to which the animals in the control group were submitted did not trigger any pathological process associate to the light microscopy. Abundant seminiferous tubules with normal round shape were observed. These showed normal spermatogenesis, including various stages of germ cell maturation. Many spermatogonia at the base of both type A and type B seminiferous tubules were observed. Spermatocytes I in the leptotene and pachytene phase were also observed in normal quantities. The initial spermatids were in large quantity and with distinguishing round euchromatic nucleus, the mature spermatids formed normal cell groups with elongated heterochromatic nucleus. The seminiferous tubules had many sperm in the tubular lumen. Intertubular tissue was normal with Leydig cells in a characteristic coronal pattern near the seminiferous tubules and abundant blood vessels.

Animals exposed to constant light (GII) showed pathological lesions in several seminiferous tubules such as scaling of the seminiferous epithelium, degenerate germ cells in the tubular lumen, and tail edema in elongated spermatids. However, many seminiferous tubules still retained their normal characteristics. Despite the tubular changes, the intertubular tissue was normal under light microscopy and Leydig cells retained their characteristics, including their rounded nucleus appearance and eosinophilic cytoplasm with vesicular pattern due to lipid droplets.

Animals exposed to constant light and treated with melatonin for 60 days (Group III) presented pathological lesions in some seminiferous tubules, such as desquamation of the seminiferous epithelium and degenerate germ cells in the tubular lumen. The intertubular tissue presented normal characteristics. This shows that with the replacement of exogenous melatonin the reproductive cells of the experimental groups presented positive changes in their conformation, since the pathological lesions found were smaller.

Animals exposed to constant light, treated with melatonin and geldanamycin for 60 days (Group IV) presented similar characteristics to those of the control group. The intertubular tissue presented normal characteristics.

The weight of the testicles showed a significant difference between the GII animals when compared with the other groups (Table 1).

**Table 1: ***Means ± standard deviation of testis weights of animals from each experimental group.

Weight/Group	GI	GII	GIII	GIV
Testis	0.126±0.0013a	0.106±0.0023b	0.117±0.0008a	0.126±0.0017a

* Means followed by distinct letters in the column differ significantly from each other by the Wilcoxon-Mann-Whitney test (p <0.05). Source: Authors.

Histochemical analysis of the testicles did not show significant alteration regarding Mallory Trichrome and PAS staining (Table 2).

Table 2: Testicular histochemistry in experimental groups. Intense (++), moderate (±) and weak (+) reaction.

	GI	GII	GIII	GIV
Collagen fibers	++	++	++	++
Acid GAGs	+	+	+	+

Source: Authors.

The morphometric analysis of the testis of the experimental animals revealed a decrease in the tubular diameter of the GIII and GIV groups when compared to the other groups (Table 3). Epithelium height showed a significant decrease in GIII group when compared to control group.

Table 3: *Means ± standard deviation of morphometric analysis of testis of animals of each experimental group.

Parameters/ Groups	GI	GII	GIII	GIV
Tubular Diameter	552,9±42,65a	462,56±51,50a	320,15±55,37b	370,62±25,79b
Epithelium Height	216,24±50,41a	142,23±25,13a	118.54±39,03b	129,21±25,46a
Testicular Volume	224210,98±31764a	130849,99± 29457a	60921± 16112b	63805,38± 6427,9b
Tunica propria	5,41±2,05a	3,75±1,11a	6,12± 2,36a	5,27±1.85a
Tubular Lumen	202,05±54,68a	214,61±50,37b	117,28±33,04a	120,38±35,84a
Leydig cells	397,98±2.65a	214,65±2,72b	399,86±2,32a	410,80±1,95a
Connective Tissue Cells	0,94±0,30a	0,90±0,36a	0,99±0,31a	0,97±0,30a
Vases	0,714±0,32a	0,789±0,25a	0,799±0,29a	0,798±0,27a

* Means followed by distinct letters in the column differ significantly from each other by the Wilcoxon-Mann-Whitney test (p <0.05). Source: Authors.

The testicular volume also presented significant difference where a decrease of this parameter was observed in the groups GIII and GIV. The tubular lumen did not present significant difference. Leydig cell morphometry showed a significant decrease in the GII group when compared to the other groups, which could suggest that regarding Leydig cells and their important role in reproduction, constant light negatively affected the testes.

The morphometric analysis of the seminiferous tubule area and height of the seminiferous epithelium did not present significant differences between the studied groups. However, the number of sertoli cells showed a significant decrease in GII when compared to other groups (Table 4).

Table 4: *Means ± standard deviation of morphometric analysis of testis of animals of all experimental groups.

Parameters/ Groups	**GI**	**GII**	**GIII**	**GIV**
Seminiferous Tubule Area	15380±1046[a]	13926±780[a]	14652±986[a]	15210±1006a
Seminiferous Epithelium Height	49,15±1,61[a]	45,37±2,20[a]	49,10±1,76[a]	48,86± 1,99a
Sertoli Cells	10,98±2.65[a]	5,96±2,72b	11,24±2,32[a]	11,02±1,95a

*Means followed by distinct letters in the column differ significantly from each other by the Wilcoxon-Mann-Whitney test ($p < 0.05$). Source: Authors.

Hormone dosage of testosterone demonstrated a significant decrease in testosterone in group II when compared to the other experimental groups (Table 5).

Table 5: *Means ± standard deviation of testosterone hormone dosage analysis of all experimental groups.

Hormone / Groups	GI	GII	GIII	GIV
Testosterone	13.65±3.08[a]	6.73±1.01b	12.96±3.52[a]	13,31±3.12a

*Means followed by distinct letters in the column differ significantly from each other by the Wilcoxon-Mann-Whitney test ($p < 0.05$). Source: Authors.

4. Discussion

As noted, (GII) had a low testicular weight, a result already observed in the study by Malekinejad H et al. 2011; Ji YL et al. 2012; Rao MV 2012, in which acute exposure to cadmium resulted in apoptosis of germ cells and cellular stress in the testicles of rats and these effects were attenuated by treating 5 mg / kg with melatonin intraperitoneally. Exposure to di (2-ethylhexyl) phthalate (DEHP) causes a reduction in body and testicle weights. DEHP also reduced the number of sperm, primary spermatocytes and Sertoli cells, as well as sperm vitality and progressive motility; these toxic effects have been associated with changes in serum hormone levels. Melatonin improved the changes induced by DEHP in hormonal levels, in the number of Sertoli cells, in spermatogonia and in sperm viability and motility (Bahrami, Nosrat et al., 2018).

Melatonin plays a role in testicular protection (Li, Chunjin; Zhou, Xu, 2015). High throughput is maintained during the dark phase of the light / dark cycle as long as there is no light in the room as night light blocks melatonin production

(Brainard et al., 1983; Shalin et al., 2013). In the exposure to constant illumination for 60 days, (GII) did not produce melatonin, when it is produced in the dark and acts at the germinal epithelium level. The study indicates the continuous light does not affect the endocrine function of the testis, but eliminates the suppressive effects of melatonin on stereogenic activity of the testis in rats (Maitra, Saumen; Ray, Arun, 2000).

Testicular torsion is a form of genital trauma that frequently occurs during the peripuberal period. It must be diagnosed accurately and quickly, in order to avoid damage resulting from abnormal hormonal production, subfertility and potentially even complete infertility (Woodruff DY et al. 2010). Studies in animal models have shown that the damage caused by testicular torsion is related to ischemia-reperfusion duration (I / R) (Uguralp et al. 2005). The pathological effects of this trauma are largely due to the formation of reactive oxygen species (ROS) during ischemia-reperfusion (Wei SM et al. 2013). ROS can cause DNA damage, impaired protein function and lipid peroxidation (Mylonas C, Kouretas D, 1999). Mammalian testes are rich in polyunsaturated fatty acids that are read by ERO (Koeberle et al. 2012).

Several studies showed that antioxidants are effective in reducing the damage caused by testicular torsion due to their ability to "clean up" excess ERO. Melatonin has an amphiphilic character passing rapidly through cell membranes and is also a powerful endogenous antioxidant (Yang Y, Sun Y, Yi W, et al. 2014) and can also be safely taken exogenously (Rocha et al. 2015).

In a rat model with an artificially induced varicocele, melatonin treatment reduced the severity of damage to epithelium and seminiferous tubules, increasing the activity of antioxidant enzymes and reducing nitric oxide (NO) levels, which can impair sperm function (Semercioz et al. 2003). Melatonin could also increase the responsiveness of Sertoli cells to FSH during testicular development, which may help to prevent damage to the testicles (Heindel et al. 1984).

One of the functions of melatonin is the regulation of testosterone synthesis and testicular maturation. It is only produced in the dark, since the GII was exposed to constant illumination for 60 days, we deduce that it produced no melatonin predisposing them to pathological changes and lower proliferative activity, as seen in the slides. This implies that it has important effects on the regulation of testicular development and male reproduction (Li, Chunjin; Zhou, Xu, 2015). This suggested mechanism is certainly the cause for significant weight reduction in this experimental group.

The testicular volume also presented significant difference where a decrease of the parameter is observed in the groups GIII and GIV. The tubular lumen did not present significant difference. Leydig cell morphometry showed a significant decrease in the GII group when compared to the other groups, which may suggest that through the Leydig cells and their important role in reproduction, constant light negatively affected the testes. These results corroborate the study by Rashed, Mohamed, & Ei-Alfy, (2010), it was reported in histological and ultrastructural analyses that rats treated with melatonin had small testicles and low spermatid numbers.

The area and height of the Seminiferous tubule of (GII) showed significant differences compared to (GI), a result that corroborates with the studies carried out by Lima et al. (2013), in which he was seen at 60 days, it was possible to observe significant changes in the seminiferous epithelium, showing to be thicker and with an increase in the lumen of the seminiferous tubules, concluding that the constant illumination interferes, proportionally to the exposure period, in the offspring gonads promoting structural changes.

Melatonin has direct actions on testicular somatic cells. Leydig cells synthesize and secrete testosterone and are regulated by Sertoli cells. These two cell types can work together to regulate the production of testicular androgens. Studies showed that Leydig cell androgen synthesis can be dramatically increased by Sertoli cells in the presence of melatonin, which can regulate the secretory function of Leydig and Sertoli cells. However, the molecular Mechanism of androgen Leyding cell production regulate by melatonin is not clear yet. (Frungieri et al. 2017; Yu 2018; Kun et al. 2018).

Melatonin acts as a local modulator of endocrine activity in Leydig cells. In Sertoli cells, melatonin influences cell growth, proliferation, energy metabolism, and oxidation status and, consequently, may regulate steroidogenesis and spermatogenesis. These data shows melatonin as an essential molecule in the regulation of testicular physiology (ie steroidogenesis, spermatogenesis). These studies suggest, melatonin as a key molecule in the regulation of steroidogenesis (Frungieri et al. 2017; Yu 2018; Kun et al. 2018). Since GII animals were exposed to constant illumination without treatment with exogenous melatonin, it resulted in a decrease in Leydig and Sertoli cells.

High production is maintained during the dark phase of the light / dark cycle, on the condition there is no light in the room as night light blocks melatonin production (Brainard et al. 1983; Shalin et al. 2013). In men, melatonin affects reproductive regulation by regulating testosterone synthesis and testicular maturation. As noted, GII was subjected to constant illumination and this affected testosterone production. Overall, literature data indicate that melatonin affects the secretion of gonadotropins and testosterone and improves sperm quality. This implies that it has important effects on the regulation of testicular development and male reproduction (Li, Chunjin; Zhou, Xu, 2015).

The histopathological examination previously reported the presence of several morphological changes in the testicles, characterized by degeneration of spermatogonia / spermatocytes, decrease in the number of early spermatogenic cells and vacuolization related to treatment with geldanamycin (Kim et al. 2014). Geldanamycin induced structural changes, such as desquamation of the seminiferous epithelium, vacuoles and gaps in the epithelium, nuclear pycnosis and atrophic changes in some tubules (Kilarkaje 2008; Khaki et al. 2009). As well as germ cell depletion, germ cell necrosis, especially in spermatogonia, Leydig cells, presenting an abnormal space between neighboring sertoli cells, mitochondria appeared without ridges and vacuolized. In addition, GA also affects sperm, affecting their number, motility and morphology (Kilarkaje 2008) but none of these effects were evident in the present study. These results do not corroborate with the present study, since there was morphological conservation of the testicle in (GIV), a group that was tested with melatonin and geldanamycin.

5. Conclusions

Exposure to constant illumination affects the development of germinal epithelium, as well as decreased weight of the testicles, Leydig, Sertoli, and testosterone cells. Melatonin and geldanamycin treatment have a protective effect against constant illumination, reducing testicular damage when taken together.

In view of the main findings of this research, it is necessary to investigate which melatonin receptors are involved as well as to evaluate the production of melatonin in other organs such as the testicle.

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