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Araştırma Makalesi – Research Paper

CAN *GANODERMA LUCIDUM* BE AN ALTERNATIVE NUTRITIONAL SUPPLEMENT FOR ENHANCING SPERM MOTILITY RATE?

GANODERMA LUCIDUM SPERM MOTİLİTE ORANINI ARTIRMAK İÇİN ALTERNATİF BİR BESLENME DESTEĞİ OLABİLİR Mİ?

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Özet

Ganoderma lucidum (GL) yaygın olarak kullanılan tıbbi bir mantardır. Bu mantarın terapötik etkileri daha önceki birçok çalışmada kullanılmıştır. Bu çalışmanın amacı, düşük, orta ve yüksek doz uygulanan GL ekstresinin sıçanlarda testis dokusu, spermatogenic seri hücreleri ve sperm motilitesi üzerine etkisini araştırmaktır. Bu amaçla 40 adet Wistar albino cinsi erkek sıçan rastgele dört gruba ayrılmıştır. Grup 1'deki sıçanlara 2 ml serum fizyolojik, Grup 2, 3 ve 4'deki sıçanlara ise sırasıyla 500 mg/kg, 2500 mg/kg ve 5000 mg/kg *Ganoderma lucidum* ekstresi toplam dokuz gün ve günde bir kez gavaj yoluyla verilmiştir. Sperm motilitesi ve histopatolojik değişiklikleri değerlendirmek için epididimal sperm toplama işlemi yapılmıştır ve testis dokusundan örnek alınmıştır. Kan örnekleri ise biyokimyasal değerlendirme için alınmıştır. Grup 1 (Kontrol) grubu ile Grup 2, 3 ve 4 karşılaştırıldığında Johnsen skoru ve sperm motilitesi bu gruplarda artmış olarak bulunmuştur ve bu artış istatistiksel olarak anlamlıdır. Sonuç olarak, uygulanan düşük, orta ve yüksek doz GL ekstresinin spermatogenezi, epididimal total sperm sayısını ve progresif motil sperm sayısını artırdığı ortaya konmuştur. Ancak, yüksek dozlar testis dokusunda minimal hasara neden olduğu saptanmıştır, ayrıca sperm parametrelerindeki yükselme anlamlı bulunmamıştır. Bu nedenle, 2500 mg/kg'ın üzerindeki oral kullanım dozlarından kaçınılması gerektiği sonucuna varılmıştır.
Anahtar kelimeler: *Ganoderma lucidum*, erkek infertilitesi, sperm motilitesi, testis histopatolojisi.

Abstract

Ganoderma lucidum (GL) is a widely used medicinal mushroom. The therapeutic effect of this fungus on many diseases has been proven by studies. The aim of this study is to assess the effects of low, moderate and high dose GL extract administration on the testis tissue, spermatogenic series cells and sperm motility in rats. 40 Wistar albino rats were randomly divided into 4 groups. Rats in group 1, 2, 3 and 4 were administered 2 ml physiologic serum, 500 mg/kg, 2500 mg/kg, 5000 mg/kg Ganoderma lucidum extract 1 time per day via gavage for 9 days, respectively. For evaluation of sperm motility and histopathological changes, epididymal sperm collection and testis harvesting were done. Blood samples were collected for biochemical analysis. When the Group 1 (control group) is compared with Groups 2, 3 and 4, the Johnsen score and sperm motility in these groups increased and this increase was statistically significant. In conclusion, low, moderate, and high doses of GL extract administered to rats were revealed to increase spermatogenesis, epididymal total sperm count and progressive motile sperm counts. However, it is detected that high doses cause minimal damage to the testis and as the increase in sperm parameters wasn't significant, it's concluded that doses for oral use above 2500 mg/kg should be avoided.

Keywords: *Ganoderma lucidum*, Male fertility, Sperm motility, Testis histopathology.

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1. INTRODUCTION

Ganoderma lucidum (GL) is a medical mushroom known for many years in China, Japan, Taiwan and other Far East countries (Shiao, 2003, pp.172-180). It has been used to protect from diseases and to lengthen life since ancient times (Yuen & Gohel, 2005, pp.11-17). It is known as the "fungus that heals everything" for diseases (Engelbrecht & Volk, 2005).

Ganoderma lucidum has many effects including antitumor, antiangiogenic, antihypertensive, anti-HIV, antihistaminic, hepatoprotective, hypocholesterolemic, antidiabetic, antioxidant, anti-aging and immunomodulator properties (Cör et al., 2018, pp.649; Lin & Zhang, 2004, pp.1387-1395; Sun et al., 2004, pp.6646-6652; Wang et al, 2017, pp.691; Shevelev et al, 2018, pp.1-6; Ma et al.,2015, pp.109-113; Tang et al, 2006, pp.205-211; Sliva, 2003, pp.358-364).

Three important active components in GL are polysaccharides, peptidoglycans and triterpenes and these compounds are responsible for the various effect of the mushroom on health (Buswell et al, 2011, pp.232-248). Triterpenes in GL extracts show cytotoxic activity and form the antitumor effect of the fungus. Additionally, polysaccharides included in aqueous extracts show immunoenhancing activity and contribute to the antitumor effect (Lin & Zhang, 2004, pp.1387-1395).

Reactive oxygen species (ROS) such as superoxide anions and hydroxyl radicals are associated with many pathophysiological situations led by carcinogenesis. Studies have revealed that polysaccharides, polysaccharide-peptide complex and phenolic components are responsible for the antioxidant activity of GL. Especially, the *Ganoderma lucidum* peptide (GLP) contained in GL is mainly responsible molecule for the antioxidant and free radical scavenging effect of the mushroom (Sun et al., 2004, pp.6646-6652). GL polysaccharides were shown to have protective effect against brain trauma injury induced in rats in the literature (Özevren et al, 2017, pp.76-84).

The amino-polysaccharide fraction (G009) molecule obtained from GL has been shown to inactivate reactive oxygen species in dose-dependent fashion in studies. Additionally, GL in cell cultures has been shown to have a preventive effect on oxidative DNA damage by reducing the DNA chain breaks (Lee et al, 2001, pp.245-249).

Knowing the effects of this commonly-used GL on organs and systems in the body is very important to ensure correct and rational consumption of the mushroom. Knowing the effects of GL on the genital system may ensure more accurate knowledge of the situation present during assessment of fertility after treatment of cancer patients who use this mushroom. In the literature, there is one study encountered assessing the effects of GL on the female genital system. In rats given 600 mg/kg dose of GL extract for 15 days, biochemical and histologic

investigations revealed no toxic effect on the female genital system (Dönmez & Yılmaz, 2014, pp.141-145).

In the literature, the number of studies revealing the effects of GL on the male genital system is limited. A study researching the effect of GL spores on ROS injury mechanisms in the testis tissue of rats with induced Type 2 diabetes mellitus (non-insulin dependent diabetes mellitus=NIDDM) showed that GL spores reduced the malondialdehyde (MDA) levels and increased the activity of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD). In this way, GL spores were shown to reveal a preventive mechanism for the apoptosis of testis tissue caused by NIDDM (Zhong, Wang, Zhang, 2006, pp.000). Another study assessing the effects of GL spores on cytochrome C (Cyt-C) and mitochondrial calcium values in the testis of rats with induced NIDDM stated the mitochondrial Cyt-C and calcium levels significantly fell in the group with NIDDM given GL and this may have protective effect against testicular injury (Wang et al, 2006, pp.1072-1075). GL spores lowered the free radical levels in testis tissue in a rat model showing protective effect (Wang et al, 2008, pp.792-795).

In the literature, studies about the effects of GL on testis tissue all used a NIDDM model induced in rats. Additionally, there is no study involving detailed histopathologic assessment or about sperm parameters which are important for fertility assessment, especially.

The aim of this study is to assess the effects of low, moderate and high dose GL extract administration on the testis tissue, spermatogenic series cells and sperm motility in rats.

1. MATERIAL AND METHODS

This experimental protocol was approved by Çanakkale Onsekiz Mart University Institutional Animal Use and Care Committee (Approval No: 2017/04-23) and performed in accordance with the Guide for the Care and Use of Laboratory Animals and the Helsinki Declaration of World Medical Association recommendations on animal studies. A total of forty Wistar albino adult male rats were obtained from Çanakkale Onsekiz Mart University Experimental Research Application and Research Center with a mean age of four months and mean weight of 250-300 g. The rats were housed in standard cages in an animal room maintained at a standard humidity (50%-55%) and temperature 22±2°C with 12 hour light/dark cycles. All animals were fed standard food and water. Twelve hours before the study procedure feeding was stopped and the rats were only allowed to drink water. The entire experiment was conducted under half-sterile conditions.

2.1. Preparation of *Ganoderma lucidum* extract

In this study extracts obtained from GL mushroom were used. Before obtaining the extracts, dried GL pieces were arranged in 10-gram packets with filter paper, and extracted with a Soxhlet device (Wisd, Wise Therm). For 10 g of fungus, 300 ml solvent was used. At the end

of extraction lasting 12 hours, the filter paper and wrapped fungus pieces were removed from the device and left to cool in an oven. In this way, fungus samples cleaned of solvents were prepared for extraction with a second solvent. During extraction, ethyl alcohol (C2H6O, BP: 78,37 °C) and distilled water (H2O, BP: 100 °C) were respectively used. Later, solvents of extracts had evaporated with the aid of an evaporator (Spektral, Heidolph, Laborota 4001) at nearly 56 ºC. Extracts placed in the oven (50 ºC) were completely cleaned of solvents. The obtained raw extracts were stored in a fridge (0-4 ºC). Before testing, extracts prepared at different concentrations by dissolving in physiologic serum were sterilized with membrane filter (0.2 µm).

2.2. Experimental procedure

Wistar albino rats were randomly divided into 4 groups as follows:

Group I (control, n=10): 2 ml physiologic serum administered 1 time per day via gavage for 9 days.

Group II (500 mg/kg group, n=10): 500 mg/kg GL extract prepared in 2 ml physiologic serum administered 1 time per day via gavage for 9 days.

Group III (2500 mg/kg group, n=10): 2500 mg/kg GL extract prepared in 2 ml physiologic serum administered 1 time per day via gavage for 9 days.

Group IV (5000 mg/kg group, n=10): 5000 mg/kg GL extract prepared in 2 ml physiologic serum administered 1 time per day via gavage for 9 days.

2.3. Anesthesia

Rats were anesthetized with intraperitoneal (i.p.) ketamine hydrochloride (50 mg/kg, Ketalar®, Pfizer, Turkey) and xylazine (15 mg/kg, Rompun®, Bayer, Canada), and if required anesthesia was maintained with additional injections of ketamine hydrochloride.

2.4. Epididymal sperm collection

Epididymal sperm collection model was designed in a way parallel to previous trials (Naghdi et al, 2006). Rats administered anesthesia had the right cauda epididymis reached through a scrotal midline incision. After excising the cauda epididymis, it was placed in 1.5 ml phosphate-buffered saline (PBS pH=7) previously warmed to 37 °C and dissected. For distribution of spermatozoa, it was lightly shaken for 10 min and incubated at 37 °C for 20 min. After this time, the suspension was mixed with the aid of a micropipette and 10 µL semen was dropped onto a Makler counting chamber lamina. The Makler counting chamber was placed in a Zeiss Axio Scope A1 brand light microscope and sperm were counted in 10 random squares from among the 100 squares. This number was multiplied by 1 million to obtain the sperm

count per milliliter. Sperm motility was assessed according to the World Health Organization (Fifth edition) criteria.

2.5. Histopathological Evaluation

In order to investigate histopathologic changes, each animal's right tests were dissected, consecutively numbered and fixated in Bouin solution for 24 h, dehydrated, cleared in asetone and embedded in paraffin. Evaluation of the pathology specimens was done by a histology specialist who was blind to the four study groups. The paraffin blocks were cut in 5 µm thickness using Rotary Microtome (Leica RM2125 RTS) and the sections were stained with hematoxylin and eosin (H&E). Evaluation of the specimens was done by a histology specialist who was blind to the four study groups. The histopathologic sections were evaluated under a light microscope (Zeiss AxioScope A1) for the interstitisial hyalinisation, intercellular edema and cytoplasmic swelling.

2.6. Spermatogenesis assessment (Johnsen Scoring)

Johnsen scoring was performed to assess the seminiferous tubule structure and spermatogenesis in testis sections stained with H&E with a light microscope. With this aim, 2 sections were chosen from each testis taken from the rats with a total of 100 seminiferous tubules assessed for each group and scoring from 1 to 10 according to Johnsen criteria (Johnsen, 1970).

2.7. Biochemical analysis

Blood samples were collected into tubes with no anticoagulants. The blood samples were centrifuged at 4000 rpm for ten minutes for analyses. The resultant serum samples were collected and put into tubes for the biochemical analysis.

Rat serum Testosterone concentrations were measured with enzyme-linked immunosorbent assay (ELISA) kit (ER1462, Fine Test, Wuhan, China) according to the manufacturer's intructions. Results were expressed as nanograms in per liter (ng/L) of serum. The intra-assay and inter-assay coefficients of variations were <8% and <10% for Testosterone (ng/L), respectively.

Rat serum Dihydrotestosterone (DHT) levels were measured using ELISA kit (EU2551, Fine Test, Wuhan, China) according to the manufacturer's instructions. Results were expressed as picograms in per mililiter (pg/mL) of serum. The intra-assay and inter-assay coefficients of variations were <8% and <10% for Dihydrotestosterone (pg/mL), respectively.

2.8. Statistical analysis

Statistical analysis of data was completed using IBM SPSS Statistics Data Editor Version 21. Multiple comparisons between the groups were analyzed with the Kruskal-Wallis test. Two-way comparisons used the Mann-Whitney U test. A value of $p<0.05$ was accepted as statistically significant.

2. RESULTS

3.1. Histologic findings in testis and spermatogenesis assessment (Johnsen scoring)

When the testis sections from the Group 1 (control group) were investigated, the seminiferous tubule sections were observed with transverse, lengthened and oblique cross sections under the tunica albuginea wrapping the testis from the outside. The finer and loose intertubular connective tissue, or interstitium was present between the seminiferous tubules. The walls of the seminiferous tubules contained spermatogonia, type 1 and type 2 spermatocytes and spermatids regularly arranged in order within the germinal epithelium above the basal membrane. Early spermatids with rounded nuclei and late spermatids with entry into apical cytoplasm of Sertoli cells were observed.

In the interstitium between the seminiferous tubules, Leydig cells with eosinophil cytoplasm were present (Figure 1A).

When testis sections belonging to Group 2 are investigated, the seminiferous tubule structure had regular arrangement similar to the control group; however, there were small amounts of hyalinization observed in the interstitium between the tubules (Figure 1B).

In Group 3, in spite of germinal epithelium thickness and regular arrangement, occasional intercellular edema and cytoplasmic swelling were identified. Intense hyalinization in the interstitium was noted (Figure 1C).

In sections belonging to Group 4, the germinal epithelium in seminiferous tubules was regular as in other groups and all cells from the spermatogenic series were observed. However, in the cells between seminiferous tubules, interstitial edema, cytoplasmic swelling and hyalinization, even occasional vacuolization, in the interstitium was noteworthy (Figure 1D).

When all groups are assessed in terms of Johnsen scoring, there were statistically significant differences identified between the groups (Kruskall-Wallis test, $p=0.00$).

The results of two-way comparisons are as follows:

When the Group 1 is compared with Groups 2, 3 and 4, the Johnsen score in these groups increased and this increase was statistically significant (p values are 0.01, 0.00, and 0.00, respectively). When Group 2 and Group 3 are compared, the Johnsen score in Group 3 was increased and this increase was statistically significant ($p=0.017$). When Group 2 is compared

with Group 4, the Johnsen score in Group 4 was increased but this increase was not statistically significant (p=0.088). When Group 3 is compared with Group 4, though there was a numerical difference between the Johnsen score in both groups, this was not statistically significant (p=0.495).

3.2. Assessment of sperm count and motility

When all groups are assessed in terms of sperm motility, there were statistically significant differences identified between the groups (Kruskal-Wallis test, p=0.00).

When compared with Group 1, the progressive motile sperm, non-progressive motile sperm, non-motile sperm and total sperm counts had increased in Groups 2, 3 and 4 and this increase was significant. p values for progressive motile sperm counts of Groups 2, 3 and 4 are 0.014, 0.00 and 0.00, respectively. p values for non-progressive motile sperm counts of Groups 2, 3 and 4 are 0.00, 0.00 and 0.00, respectively. p values for non-motile sperm counts of Groups 2, 3 and 4 are 0.008, 0.00 and 0.00, respectively. p values for total sperm counts of Groups 2, 3 and 4 are 0.02, 0.00 and 0.00, respectively.

When Group 2 is compared with Group 3, the progressive motile sperm (p=0.011) and total sperm count (p=0.004) was increased in Group 3 and the difference between the groups was significant (Mann-Whitney U test). In Group 3, the non-progressive motile sperm and non-motile sperm counts were increased compared to Group 2 but the difference was not significant. When Group 2 is compared with Group 4, the increase in non-motile sperm count in Group 4 was not significant, but the difference between the groups in terms of progressive motile sperm, total sperm count and non-progressive motile sperm parameters was significant.

When Group 3 is compared with Group 4, in spite of an increase in sperm counts and motility parameters in Group 4, this difference was not statistically significant. p values for progressive motile, non-progressive motile and total sperm counts are 0.002, 0.019 and 0.002, respectively.

Sperm motility assessments for all groups can be seen in Figure 2.

3.3. Biochemical results

When assessed in terms of testosterone and DHT, there was a statistically significant difference identified between the groups (Kruskall-Wallis test, p=0.00).

When Group 1 is compared with Group 2, 3 and 4, the testosterone and DHT levels were reduced in Groups 2, 3 and 4; however, this reduction was not statistically significant in Group 2, and it was significant in Group 3 and 4 (Mann-Whitney U test). p values of Group 3 for testosterone and DHT are 0.001 and 0.001, respectively. p values of Group 4 for testosterone and DHT are 0.001 and 0.001, respectively.

When Group 2 is compared with Groups 3 and 4, testosterone and DHT levels had reduced and this reduction was significant. p values of Group 3 for testosterone and DHT are 0.03 and 0.013, respectively. p values of Group 4 for testosterone and DHT are 0.005 and 0.005, respectively.

When Group 3 is compared with Group 4, there was a reduction in testosterone level and this difference was statistically significant (p=0.046). Statistical analysis of all variables can be seen in Table 1.

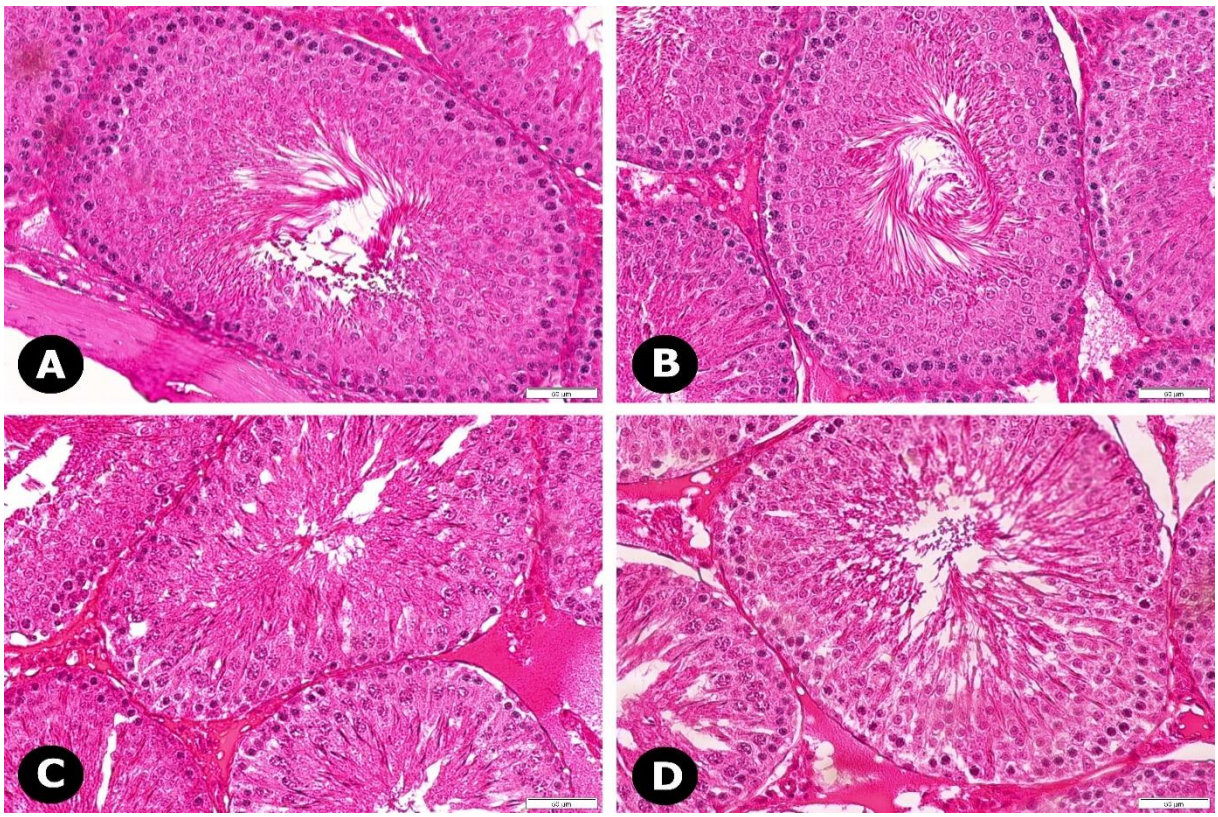

Figure-1: Histopathological evaluation of all groups seen in this figure. Histopathological changes for group 1,2,3 and 4 are seen in A, B, C and D, respectively. Hematoxylin and Eosin staining, X400 magnification.

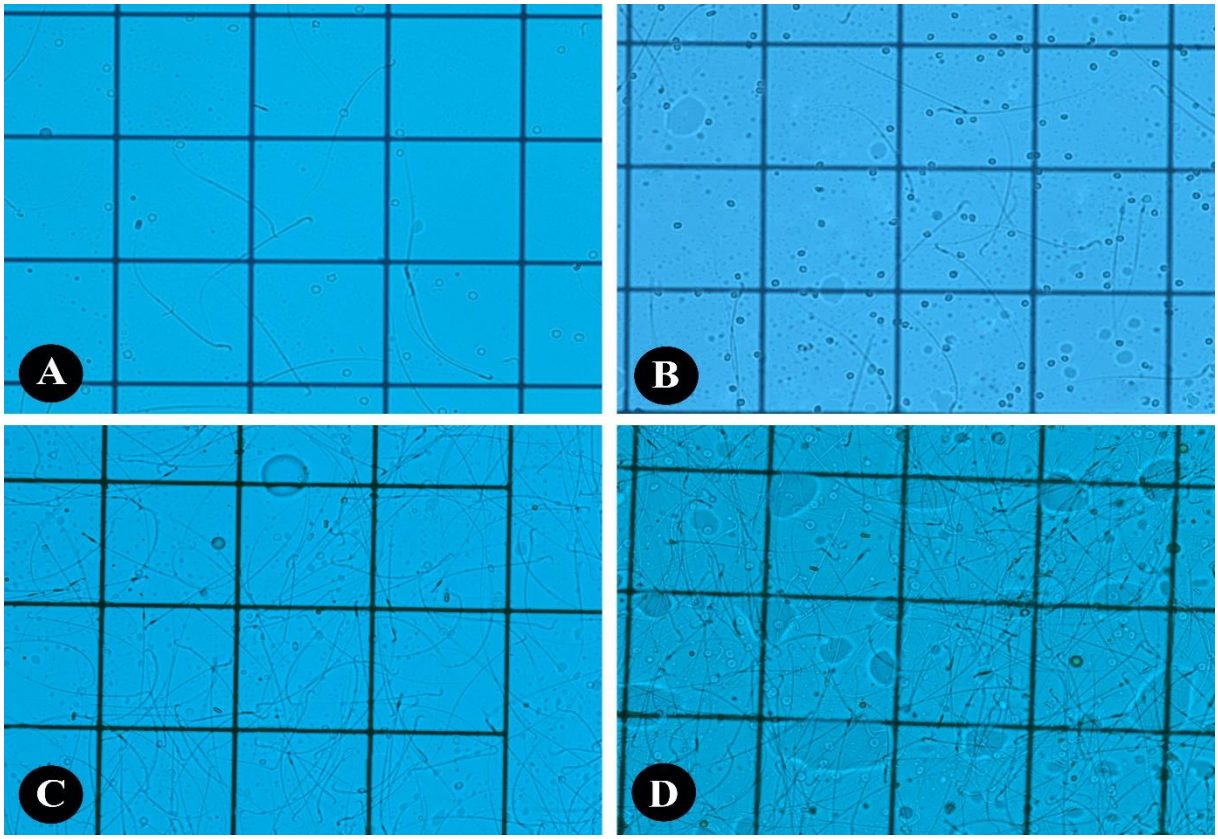

Figure-2: Sperm count and motility assessment of all groups. Group 1,2,3 and 4 are seen in A, B, C and D, respectively. From A to D, the amount of sperm seen in the Makler Counting Chamber increases (X200 magnification).

Table-1: Statistical analysis of all variables can be seen in table 1(SD: Standart deviation, Med: Median)

Groups	Johnsen Score	Progressive motile sperm (Mean±SD)	Non-progressive motile sperm (Mean±SD)	Non-motile sperm (Mean±SD)	Total sperm count (Mean±SD)	DHT (Mean±SD)	Testosteron (Mean±SD)
G1	9.18±0.20813 9.24 (Med)	5.40±5.038 4.00 (Med)	1.50±1.27 1.50 (Med)	10.30±6.06 8.50 (Med)	17.20±9.33 15.00 (Med)	1159.52±1129.21 1179.16 (Med)	15.39±1.88 15.93 (Med)
G2	9.44±0.23	9.13±5.167	10.13±11.679	34.50±27.69	53.75±29.678	1049.83±201.79	12.05±4.85

	9.48 (Med)	11.00 (Med)	4.50 (Med)	25.50 (Med)	41.50 (Med)	1103.37 (Med)	12.94 (Med)
G3	9.72±0.19 9.65 (Med)	49.43±32.341 45.00 (Med)	19.43±12.08 20.00 (Med)	50.29±18.98 41.00 (Med)	119.14±62.81 106.00 (Med)	797.73±177.23 780.6500 (Med)	5.67±2.23 4.79 (Med)
G4	9.66±.11 9.69 (Med)	73.33±46.16 83.00 (Med)	38.83±25.51 49.50 (Med)	50.67±24.06 41.00 (Med)	162.83±58.36 169.50 (Med)	487.14±296.75 453.09 (Med)	3.24±1.47 3.17 (Med)

3. DISCUSSION

Ganoderma lucidum is a medical mushroom that proven beneficial effects for many diseases (Cör et al., 2018, pp.649; Lin & Zhang, 2004, pp.1387-1395; Sun et al., 2004, pp.6646-6652; Wang et al, 2017, pp.691; Shevelev et al, 2018, pp.1-6; Ma et al., 2015, pp.109-113; Tang et al, 2006, pp.205-211; Sliva, 2003, pp.358-364). In recent years, it is important to know the in vivo effects due to common use globally. In our study, the effects of aqueous and alcoholic extracts of GL with oral administration of 500, 2500 and 5000 mg/kg doses on the testis morphology and sperm parameters were researched. GL administration over 9 days with 2500 mg/kg dose, increase the total sperm count and the progressive motile sperm count, which is especially important for fertility; however, the morphologic disruption of the testis began to be observed with further increases of the dose. Additionally, the increase in sperm counts and motility parameters with 5000 mg/kg dose were not significant and serum testosterone and DHT levels significantly fell compared to Group 1. The Johnsen score results used to assess spermatogenesis show a significant increase in Group 4 compared to Group 1, though this increase was not significant compared to Group 2. This leads to the conclusion that GL extract with dose above 2500 mg/kg is not effective on sperm production in the testis.

In the literature, the studies researching the effects of GL on the genital system are limited. A previous study investigated the effects of GL on the female genital system and administered 600 mg/kg dose of GL extract to female Sprague-Dawley rats for 15 days (Dönmez, Yılmaz, 2014, pp.141-145). At this duration and dose, GL had no histopathologic effects on the female genital system and GL was revealed not to be toxic for the female genital

system. In our study, male Wistar Albino rats were administered 500, 2500 and 5000 mg/kg doses of GL extract for 9 days. In our study, the group administered 500 mg/kg dose was similar to the study researching the effects on the female genital system in the literature, with this GL extract dose having no negative effect on testis morphology with increased sperm numbers and motility values in addition to increased spermatogenesis. From this aspect, different to GL's effects on females, it can be said to have positive effects on the male genital system. However, to reveal the effects of GL on the female genital system, this previous study administered GL for 15 days (Dönmez & Yılmaz, 2014, pp.141-145). In our study, 9 days administration was used and there is a need for advanced studies to reveal the effects of long-term use. However, in our study, 3 different doses of GL extract (low, moderate and high) were administered and the testicular and spermatogenic effects of increasing doses were revealed. Due to assessing the results of using different doses, we believe our study contributes to the literature.

In the literature there are three studies encountered about the effects of GL use as nutritional supplement on the male genital system (Zhong et al., 2006, pp.000; Wang, 2006, pp.1072-1075; Wang, 2008, pp.792-795). In these studies, rats with induced NIDDM were administered 250 mg/kg dose of GL spores and ROS damage, antioxidant enzymes and mitochondrial calcium levels were examined in testis. At the end of these studies, GL reduced the oxidant damage in testis tissue and showed antioxidant effect in NIDDM rats. However, these studies did not perform detailed histopathologic assessment and did not study hormone values. In our study, an attempt was made to reveal whether the use of GL as nutritional support product had any effect on rats with no testis injury and no additional disease that would affect sperm count and motility. These three studies in the literature did not assess spermatogenesis, sperm count and motility parameters which are very important for fertility. Our study performed detailed histologic assessment to reveal the potential for GL to cause injury to testis tissue. Additionally, spermatogenesis, sperm count and motility parameters were assessed and a certain dose of GL was proven to increase sperm parameters.

Couples who are sexually active and use no protection for a year but do not become pregnant are accepted by the World Health Organization as being infertile. For half of infertile couples, the cause is due to the male (Moskvin, Apolikhin, 2018, pp.1-15). There are a variety of causes of male infertility. Environment and nutritional causes, especially, may affect sperm count and motility causing infertility (Sukhn et al, 2018, pp.1-11; Sengupta et al, 2018, pp.247-255). As a result, material used for nutrition is very important for sperm parameters. GL is a mushroom with many beneficial effects proven and as a result has widespread use (Cör et al., 2018, pp.649; Lin & Zhang, 2004, pp.1387-1395; Sun et al., 2004, pp.6646-6652; Wang et al, 2017, pp.691; Shevelev et al, 2018, pp.1-6; Ma et al., 2015, pp.109-113; Tang et al, 2006, pp.205-211; Sliva, 2003, pp.358-364). In our study, GL extract significantly increased total sperm counts and motility values. From this aspect, use of GL may be beneficial for oligozoospermia, azoospermia and asthenozoospermia situations. It may be very beneficial to

reduce the need for assisted reproductive techniques to increase progressive motile sperm counts and achieve pregnancy through natural routes. In conclusion, GL administration may support improvement of sperm parameters in addition to a treatment plan to ameliorate disrupted sperm parameters causing male infertility. However, it is necessary to correctly determine the dose to be used. In our study, 5000 mg/kg dose caused minimal testicular damage in spite of increased sperm parameters. Additionally, 2500 mg/kg dose of GL extract, increases total sperm count and especially progressive motile sperm counts, but the increase in non-progressive motile and non-motile sperm counts was not statistically significant. As a result, whether used as a nutritional supplement or for treatment of male infertility, it is concluded that high doses of GL should be avoided. Using an initial dose of 500 mg/kg extract, it appears to be more appropriate that treatment continue by assessing sperm parameters and controlled increase of the dose, preferably without exceeding 2500 mg/kg dose.

In the literature, studies assessing the antiandrogenic effect of GL have revealed that GL administration reduces 5α-reductase enzyme activity lowering DHT levels and shows antiandrogenic effect in this way (Liu et al, 2009, pp.231-243; Fujita et al, 2005, pp.107-112). After testosterone is released from the Leydig cells found in the testis, 5α-reductase enzyme activity transforms it to DHT. DHT is a strong endogenous androgen and binds to androgen receptors more strongly than testosterone (Rhoades & Bell, 2017, pp.686-692). The Sertoli cells are found in the testis and are cells supporting the spermatogenic series cells. At the same time, sertoli cells release androgen binding protein (ABP) increasing local testosterone levels in seminiferous tubules and play an important role in maintaining spermatogenesis (Barrett et al, 2015, pp.420-421). Studies showing GL's 5α-reductase inhibition reported this mushroom can be used in treatment of diseases such as benign prostate hyperplasia and prostate carcinoma (Liu et al, 2009, pp.231-243; Fujita et al, 2005, pp.107-112). In our study, similarly, testosterone and DHT reduced and this reduction was observed to be significant especially at moderate and high doses administered to Groups 3 and 4. However, the simultaneous increase in sperm count and motility values lead to the consideration that the reduction in testosterone and DHT are not at levels to affect spermatogenesis. However, due to ABP released from Sertoli cells, the hormone levels in seminiferous tubules may not have fallen below the levels necessary for spermatogenesis. Additionally, in our study serum testosterone and DHT levels were analyzed. Advanced studies may reach more accurate information about spermatogenesis by examining hormone levels and ABP amounts in testis tissue. Again, in our study GL extract was administered to rats for 9 days. There is a need for more advanced studies with longer periods of administration to examine how hormone levels and sperm parameters change.

4. CONCLUSION

In conclusion, low, moderate and high doses of GL extract administered to rats was revealed to increase spermatogenesis, epididymal total sperm count and progressive motile

sperm counts. However, high doses caused minimal damage to the testis and as the increase in sperm parameters was not significant, it is concluded that doses for oral use above 2500 mg/kg should be avoided. However, due to the reduction in serum testosterone and DHT levels, care should be taken during long-term use and it is considered that controlled use is necessary. There is a need for advanced studies to determine the effects of long-term use of GL on testicular and other genital organs.

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