

Enhancing Motility of Rainbow Trout (*Oncorhynchus mykiss*) Sperm by *Tribulus Terrestris* Extract Supplementation

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Abstract

Tribulus terrestris (TT) is a famous traditional plant of family Zygophyllaceae and widely distributes around the world. TT has potential to elevate the testosterone, testosterone precursor and luteinizing hormone levels. In this study, trials were conducted to assessment the impact of *Tribulus terrestris* addition on sperm motility of rainbow trout (*Oncorhynchus mykiss*) for the first time. In the trial, we used to different concentrations [0 µg L⁻¹ (Control), 200 µg L⁻¹, 400 µg L⁻¹, 600 µg L⁻¹, 800 µg L⁻¹ and 1000 µg L⁻¹] of *T. terrestris* extract. Sperm motility characteristics and longevity were determined. In addition, protodioscin content of *T. terrestris* extract was assessed. The present study revealed that the presence of *T. terrestris* caused to increase in sperm motility. The increases in duration (49.00±3.61 s) and motility rate (91.67±2.89%) at 400 µg L⁻¹ were statistically significant (p<0.05). Overall, addition of *T. terrestris* to activation solution can increase the sperm motility of rainbow trout.

Keywords: Herbal medicine, *Oncorhynchus mykiss*, spermatozoa, *Tribulus terrestris*.

Tribulus Terrestris Ekstraktının İlavesiyle Gökkuşacağı Alabalığının (*Oncorhynchus mykiss*) Sperm Motilitesinin Arttırılması

Öz

Tribulus terrestris (TT), Zygophyllaceae familyasına ait geleneksel bir bitkidir ve dünyada yaygın olarak dağılım gösterir. TT, testosteron, testosteron pekürsörü ve luteinize edici hormon seviyelerini yükseltme potansiyeline sahiptir. Bu çalışmada, *Tribulus terrestris* ilavesinin gökkuşacağı alabalığının (*Oncorhynchus mykiss*) sperm motilitesi üzerindeki etkisini değerlendirmek için ilk kez denemeler yapılmıştır. Denemede farklı konsantrasyonlarda [0 µg L⁻¹ (Kontrol), 200 µg L⁻¹, 400 µg L⁻¹, 600 µg L⁻¹, 800 µg L⁻¹ ve 1000 µg L⁻¹] *T. terrestris* ekstraktı kullanılmıştır. Sperm motilite özellikleri ve süresi belirlenmiştir. Ayrıca *T. terrestris* ekstraktının protodioscin içeriği değerlendirilmiştir. Bu çalışma, *T. terrestris* varlığının sperm motilitesinde artışa neden olduğunu ortaya koymuştur. 400 µg L⁻¹'de süre (49,00±3,61 s) ve motilite oranındaki (%91,67±2,89) artışlar istatistiksel olarak anlamlıydı (p<0.05). Sonuç olarak, aktivasyon solüsyonuna *T. terrestris* eklenmesi gökkuşacağı alabalığının sperm motilitesini artırabilir.

Anahtar Kelimeler: Bitkisel ürün, *Oncorhynchus mykiss*, spermatozoa, *Tribulus terrestris*

INTRODUCTION

Medicinal plants (MP) are important and promising candidates for new treatment options in basic and clinical researches instead of synthetic analogues in traditional medicine. Hence, herbal

medicine studies have been increased in the world due to their tolerability, minimum side effects, easiness to access, being economical and safety of treatments in recent years. *Tribulus terrestris* (TT) is

a famous traditional plant of family Zygophyllaceae and widely distributes around the world (Ma et al., 2017). It is naturally found in the Mediterranean region, desert and subtropical climate regions (Fatima and Sultana, 2017). The plant contains phenolic compounds, steroidal saponins, flavonol glycosides, flavonoids, amides, unsaturated fats, alkaloids, vitamins, amino acids and tannins (Ercan and El, 2016; Asadmobini et al., 2017; Basaiyye et al., 2017). TT has received great attention due to usage as widespread and indiscriminate in treatment of various diseases such as urinary infections, inflammations, sexual dysfunction and desire problems, leucorrhoea, erectile function, oedema, kidney stones, hypertension and coronary heart disease and ascites owing to possessing aphrodisiac, free-radical-scavenging, anti-inflammatory, anti-cancer, antispasmodic, anti-bacterial, antiurolithic and analgesic properties (Akram et al., 2011; Mohd et al., 2012; Hammada et al., 2013; Kamenov et al., 2017; Ma et al., 2017). Moreover, it has potential to elevate the testosterone, testosterone precursor and luteinizing hormone levels in humans (Asadmobini et al., 2017) and animals (Gauthaman and Ganesan, 2008; Omitoyin et al., 2013; Neychev and Mitev, 2016; Yeganeh et al., 2017). For these reasons, many studies have been focused on masculinization, growth and reproductive performance, survival and histopathology, feed utilization, hematological, immunological, and biochemical variables in different fish species (Cek and Turan, 2007; Cek et al., 2007; Kavitha and Subramanian, 2011; Omitoyin et al., 2013; Gültepe et al., 2014, Yilmaz et al., 2014; Hassona et al., 2020).

Sperm quality is important for aquatic life and most importantly, it affects fertilization and hatching success in aquaculture practices (Kutluyer et al. 2015, 2016; Kocabas et al. 2017a,b,c). Reducing in sperm quality can cause extinction or loss of populations. The literature reported that TT has been increased the number and motility of spermatozoa in human (Salgado et al., 2016; Khaleghi et al., 2017) and animals (rat, mice, mouse) (Gauthaman et al., 2002; Singh et al., 2012; Adaay and Mattar, 2012; Oliveira et al., 2015; Kumari and Singh, 2015; Kumar and Singh, 2015). Thus far, there is limited information about influences of TT supplementation on sperm motility. In this context, the purpose of the paper is to determine the impact of TT extract on sperm motility parameters and duration of rainbow trout.

MATERIAL AND METHODS

Obtainment of experimental animals

Six sexually mature males (three-year-old, 38.4±5.7 cm, 873.62±4.27 g, mean±SD) were obtained from a commercial fish farm (Göktepe Trout Production Facility, Tunceli, Turkey) (January, 2018). The water temperature was at 6.7±0.1°C, pH 8.28±0.1, and dissolved oxygen 8.2±0.1 mg L⁻¹ with natural photoperiod. Sperm were collected through gentle pressure on the abdomen after anesthetized using 2-phenoxyethanol (0.6 mL L⁻¹). Sperm samples were not used contaminated with water, urine, mucus, blood or fecal contamination. Sperm was collected in plastic tubes (50 mL) and placed on ice crush.

Experiments and evaluation of motility parameters

Different levels of 0 mM (Control), 0 µg L⁻¹ (Control), 200 µg L⁻¹, 400 µg L⁻¹, 600 µg L⁻¹, 800 µg L⁻¹ and 1000 µg L⁻¹ TT extract were added to activation solution (NaCl, 52 mM). Sperm colors, sperm volume, percent of motile spermatozoa, survival period of forward motility and spermatocrit were determined in this study. Motility analyses were performed with Sperm Class Analyzer system (Microptic S.L., Barcelona, Spain). Actively moved sperm was recorded as motile sperm percent. A chronometer was used for determination of duration of forward motility. Sperm samples with motility >80% and normal volume, pH was pooled for the trials. Burkner cell hemocytometer was used for assessment of spermatozoa density. Spermatocrit was determined according to the method described by Rurangwa et al. (2004).

Plant material and preparation of herbal extract

During summer season, TT was gathered from natural environment (Osmaniye, Turkey). Fruits and aerial parts of TT were cleaned and rinsed with water and, air-dried in shade. The dried material was pulverized (25 g). Ethanol (70%, 50 mL) was used for extraction (Ahmed et al., 2009) in a Soxhlet apparatus. The evaporation of the extracts was performed with a rotary evaporator for dryness under pressure at 45°C and stored in a freezer (-20°C) until experiments.

Instrumentation

Protodioscin content was assessed according to the method described by Shishovska et al. (2015)

using HPLC system (Shimadzu Prominence) equipped with: binary pump (LC-20AT), degasser (DGU-20A5), autosampler (SIL-20AHT), column oven (CTO-10ASVP) and a diode array detector (SPD-M20A). The separation was performed on HPLC column Fortis Universal HS C-18 (250x4.6 mm i. d.; particle size 5 μm). Optimization of chromatographic conditions are provided from mobile phase consisting of acetonitrile and water in a gradient mode (firstly, linear gradient with acetonitrile was used for 20 min from 10% to 60% (v/v), followed by an isocratic mode with 60% (v/v) acetonitrile for 5 min), pumped at a flow rate of 0.9 mL min⁻¹. Column temperature was set to 40°C. UV detection was realized at 200 nm.

Statistical analysis

Data on longevity and motility rate of sperm cells were presented as means \pm standard deviation (S.D.) with $p < 0.05$ significance level. The data were analyzed using One-way ANOVA with Duncan test. The statistical package SPSS for Windows (Ver:14.0) was used for computing all statistics.

RESULTS

Values for measured sperm parameters (mean \pm SD) are presented in Table 1. Motility percentage and duration of fresh sperm cells were 80.00 \pm 5.75% and 36.33 \pm 0.58 s, respectively. The data illustrated in Figure 1 and 2 showed the effect of different doses (200, 400, 600, 800 and 1000 $\mu\text{g L}^{-1}$) of aqueous extracts of TT. TT had sperm motility-enhancing effect. The trial indicated that the maximum increment in percent of motile spermatozoa (91.67 \pm 2.89%) and survival period of forward motility duration (49.00 \pm 3.61 s) was evoked at concentration of 400 $\mu\text{g L}^{-1}$ ($p < 0.05$). The motility rate and duration remarkably decreased after concentration 400 $\mu\text{g L}^{-1}$. High concentration (1000 $\mu\text{g L}^{-1}$) of the aqueous extracts of the TT caused a high significant reduction on motility rate and longevity ($p < 0.05$). The protodioscin content was 1762 mg kg⁻¹ in a raw plant material. The data indicated linear relationship between protodioscin quantity and peak area (the correlation coefficient value: $R^2 = 0.9997$) (Figure 3).

Table 1. Sperm motility parameters (mean \pm SD) of rainbow trout (*O. mykiss*) (n=6).

Parameters	Mean	Range
Colour	White	
Volume (mL)	11.25 \pm 6.45	1-20
pH	7.76 \pm 0.20	7.54-8.03
Spermatocrit (%)	49.75 \pm 11.09	40.00-71.43
Sperm density ($\times 10^9$)	9.27 \pm 0.56	9.17-9.37

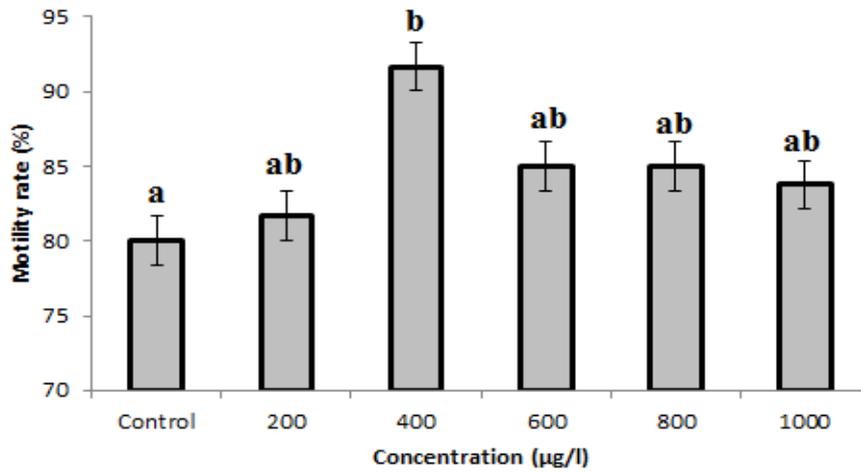


Figure 1. Mean (\pm S.D.) percentages of motility in *O. mykiss* sperm with different *T. terrestris* doses. Different letters show differences between treatments ($p < 0.05$). The bars and vertical lines represent mean \pm SD, $n = 6$.

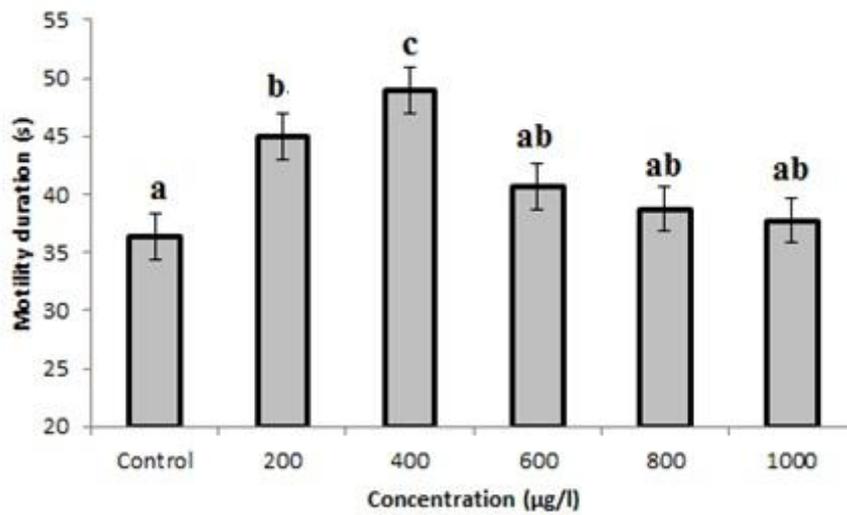


Figure 2. Mean (\pm S.D.) duration of motility in *O. mykiss* sperm with different *T. terrestris* doses. Different letters show differences between treatments ($p < 0.05$). The bars and vertical lines represent mean \pm SD, $n = 6$.

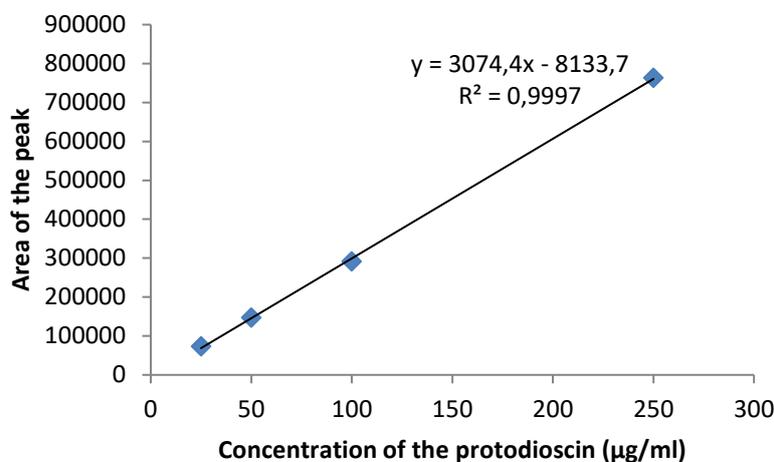


Figure 3. Calibration graph of protodioscin.

DISCUSSION

Our results about sperm parameters are different from reported data in previous studies. The differences may be related with ecological factors and broodstock (age and weight of the male), spawning (behavior of broodstock and stage of spawning), sampling method and term (Piironen and Hyvarinen 1983; Suquet et al. 1994; Suquet et al. 1998; Tekin et al. 2003; Kocabaş and Kutluyer 2017b).

The positive roles of TT on various diseases and disorders have been emphasized in previous studies owing to its aphrodisiac and free-radical-scavenging properties (Singh et al., 2012; Hammada et al., 2013; Keshtmand et al., 2014; Neychev and Mitev, 2016; Fernández-Lázaro et al., 2022). Even though there are no references on the use of TT as an agent for improving fish spermatozoa capacity during activation, the literature reported that TT has been increased the number and motility of spermatozoa in human and animals (rat, mice, mouse) (Gauthaman et al., 2002; Liu et al., 2004; Singh et al., 2012; Adaay and Mattar, 2012; Oliveira et al., 2015; Kumari and Singh, 2015; Kumar and Singh, 2015; Khaleghi et al., 2016; Salgado et al., 2016). The present results are going in line with previous studies in human and animals. Our data clearly showed that the sperm motility was increased by TT supplementation. Especially, presence of protodioscin as a steroidal saponin may be responsible for these positive effects of TT and, in this study, the content of protodioscin was

determined as 1762 mg kg⁻¹ in a raw plant material. Ganzera et. al. (2001) reported protodioscin content as 0.17-6.49%. In this study, protodioscin content of raw TT material were determined as 0.176% using HPLC. Our results confirm the chemical composition of the TT extracts and it was similar to the results of Ganzera et. al. (2001). In addition, TT might be provided protection against oxidative damage due to contain DPPH (2,2-di-(4-tert-octylphenol)-1-picrylhydrazyl), polyphenols, H₂O₂, and produce lower level of free oxygen radicals. Antioxidant activity is highly linked to the extract components (total polyphenols) of TT (Manach et al., 2005; Giovanelli and Buratti, 2009; Khaleghi et al., 2016; Qari and El-Assouli, 2017). Moreover, the reason could be its zinc and calcium content. The enzyme phosphodiesterase inhibition by CA⁺⁺ might be increased sperm motility due to prevent cyclic adenosine monophosphate (cAMP) degradation (Nassar et al., 1998; Keshtmand et al., 2014; Asadmobini et al., 2017). The improvement of sperm motility could be provided through protein synthesis and nuclear chromatin stabilization by zinc (Wang et al., 1990; Asadmobini et al., 2017).

CONCLUSION

Based on the results, *in vitro* addition of TT improved sperm motility in *O. mykiss*. Data from this study suggest that the addition of TT to activation media can be used as an alternative motility-inducing agent in *O. mykiss* and this study would be useful to evaluate the TT effect on short-

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term sperm storage and cryopreservation of other fish species owing to its minimum side effects, easiness to access, cost effective and safety of treatments. Future efforts are necessary about the mechanism and impacts of TT on fertility ability and embryonic and larval development.

CONFLICT OF INTEREST

The Authors report no conflict of interest relevant to this article

RESEARCH AND PUBLICATION ETHICS STATEMENT

The authors declare that this study complies with research and publication ethics.

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