Original Article

The Effect of *Tarantula cubensis* (Theranekron) on Some Reproductive Parameters in Male Rats

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How to Cite This Article Koşal, V., Keleş, O.F. (2023). The Effect of *Tarantula cubensis* (Theranekron) on Some Reproductive Parameters in Male Rats. *Iranian Journal of Veterinary Medicine, 17*(2), 107-112. http://dx.doi.org/10.32598/ijvm.17.2.1005343

doi: http://dx.doi.org/10.32598/ijvm.17.2.1005343

ABSTRACT

Background: *Tarantula cubensis* (theranekron), obtained from the poison of *Cuban Tarantula*, is a homeopathic drug frequently used in animal health.

Objectives: The effect of theranekron on reproductive parameters in male animals is unknown. In this study, the effect of theranekron on male reproductive parameters was investigated.

Methods: Three different groups of rats were created: control (n=8) (no application), theranekron I (n=8) (subcuteneous injection of 0.3 mg/kg on days 0, 5, and 10), and theranekron II (n=8) (subcuteneous injection of 0.3 mg/kg on days 0, 5, and 10). Control and theranekron I groups were sacrificed at the end of the 4 weeks, and the theranekron II group was sacrificed at the end of the 6 weeks. Sperm motility, density, abnormal rate, DNA damage, and testicular histopathology were examined.

Results: No statistical difference was observed in the parameters examined between the study groups (P>0.05). It was determined that the theranekron did not change sperm motility, density and spermatozoa ratio, also no histopathological changes, and sperm DNA damage.

Conclusion: There is no harm in using theranekron in male animals during the breeding and nonbreeding seasons.

Keywords: *Tarantula cubensis*, Theranekron, Sperm, Testis, Rat

Article info:
Received: 12 Dec 2022
Accepted: 10 Jan 2023
Publish: 01 Apr 2023

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1. Introduction

Tarantula cubensis extract, a homeopathic medicine, is obtained from the poison of the *Cuban tarantula* (Sencar et al., 2021). This drug is commercially available under the trade name theranekron® (Richter Pharma, Wels, Austria). Although the mechanism of action of *T. cubensis* venom is not clear, it is believed that it stimulates the immune system, activates vitality, and suppresses inflammation spontaneously in proliferative lesions (Iacopetti et al., 2020; Gültekin and Vural, 2007; Sencar et al., 2021). Theranekron helps in the treatment of inflammation and necrosis. It has been reported that it provides healing with demarcation, regeneration, resorption, and antiphlogistic action (Iacopetti et al., 2020; Gültekin & Vural, 2007; Sencar et al., 2021; Song et al., 2017; Karabacak et al., 2015; Adib-Hashemi et al., 2015; Güven et al., 2022).

Theranekronis used in the treatment of many genital diseases such as mastitis, breast edema, retention of secundinarium, papillomatosis, breast tumor, genital organ tumors, metritis (Kaçar et al., 2007; Oryan et al., 2012; Swamy et al., 2020; Sardari et al., 2007; Ghasemi et al., 2016; Lotfollahzadeh, 2020; Gürbulak et al., 2014; Stampa, 1986; Icen et al., 2011).

In the literature review, no study examined the effect of theranekron application on male reproductive parameters. This study aims to investigate the changes in the testis and spermatological parameters resulting from the theranekron application. It is the first study carried out in this context and has a pioneering character.

2. Materials and Methods

Study animals

Adult, pathogen-free, male Albino Wistar rats were obtained from Van Yüzüncü Yıl University. The rats aged 3-4 months on average and weighing 200-250 g were used in the study. Animals were fed ad libitum and kept under 12 hours of light and 12 hours of dark per day. The living areas had an average temperature of 26°C and 60% relative humidity.

Study groups

Twenty-four rats were randomly divided into 3 groups.

Control (n=8): No medication was administered. The rats were sacrificed after anesthesia (using ketamine+xylazine) at the end of the fourth week.

Theranekron I (n=8): theranekron (Richter-Pharma AG, Wels, Austria) was administered subcutaneously at a dose of 0.3 mg/kg on days 0, 5, and 10. The rats were sacrificed after anesthesia (using ketamine+xylazine) at the end of the fourth week (Dolapcioglu et al., 2013; Kozlu et al., 2021).

Theranekron II (n=8): theranekron (Richter-Pharma AG, Wels, Austria) was administered subcutaneously at a dose of 0.3 mg/kg on days 0, 5, and 10. The rats were sacrificed after anesthesia (using ketamine+xylazine) at the end of the sixth week (Dolapcioglu et al., 2013; Kozlu et al., 2021).

Anesthesia protocol

Xylazine hydrochloride (5 mg/kg) (Basilazine® 2%, Bavet, Turkey)+ketamine hydrochloride (80 mg/kg) (Ketasol®, Interhas, Turkey) were drawn into the same injector, administered intraperitoneally (Smith, 1993; Korkmaz & Sancak, 2022).

Sperm examination

Motility examination (progressive motility)

The sperm sample was obtained by epididymis puncture immediately after sacrifice and was placed on a glass slide on the heating table set to 38°C. The coverglass was closed at an angle of 45°, and motility (in %) was detected under microscope at 40x magnification. Uniform, linear, forward-moving spermatozoa were compared to immobile, swirling, and quivering spermatozoa (Hafez & Hafez, 2013).

Density analysis

After an epididymal puncture, 0.1 mL of sperm sample was added to eppendorf tubes with 0.5 mL Hayem solution (Norateks, Germany). Sperm count per mL was calculated on a thoma cell counting chamber (Hafez & Hafez 2013).

Abnormal sperm ratio

The sperm obtained by epididymis puncture was transferred to eppendorf tubes with 0.5 mL Hancock solution (Norateks, Germany). At least 400 sperm samples were examined at 40x magnification to determine the ratio. The proportion of spermatozoa with anomalies in the head, tail, and neck was determined (Hafez & Hafez, 2013).

Sperm DNA damage

DNA fragmentation index was assessed using the Halomax® (Spain) kit. Sperm DNA damage was calculated according to the protocol of the Halotech, Halomax HT-RN40 kit. A total of 600 sperms were counted for each group. Spermatozoa were evaluated according to Figure 1 (normal, fragmented, degraded). The damage ratio was calculated according to the Equation 1.

1) Sperm DNA fragmentation (SDF)=(fragmented+degraded)/(total sperm counted)

Histopathological examination

At the end of the experiment, necropsies of the rats were performed, and testis tissue samples were taken. After the tissue pieces were fixed in 10% buffered formaldehyde solution, routine tissue follow-up was performed and embedded in paraffin blocks, and 4 µm sections were taken with a microtome. They were stained with hematoxylin-eosin (H&E) and examined under a light microscope, and morphological findings were photographed and evaluated.

Statistical analysis

SPSS software, version 20 (Chicago, IL, USA) was used for statistical analysis. All data were expressed as Mean±SD. Statistical studies of the groups were conducted using the one-way ANOVA followed by post hoc multiple comparisons (Tukey test) for comparative analysis between the study groups. $P<0.05$ was regarded as statistically significant.

3. Results

Figure 2 shows the sperm DNA damages and DNA fragmentation ratio is mentioned in Table 1. In the histopathological examinations, normal histological structures of the testis were observed in all groups (A-B Control; C-D theranekron I; theranekron II E-F) (Figure 3). The ratio of motility, density, abnormal sperm ratios ratio are mentioned in Table 2.

4. Discussion

The effect of theranekron depends on spider venom in its structure, and this component is effective for a long time. Although theranekron is frequently used in treating many diseases in veterinary medicine, no research has investigated its effects on male reproductive parameters.

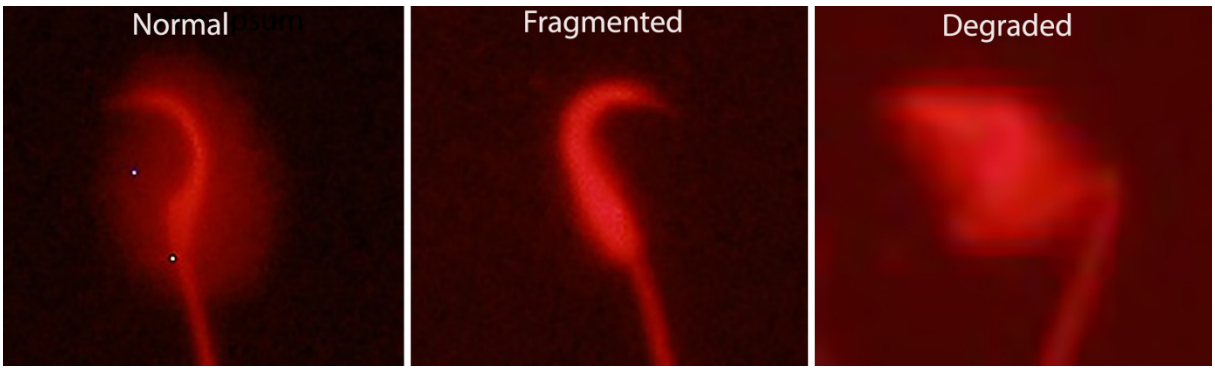

Figure 1. Dimensions of sperm DNA damage

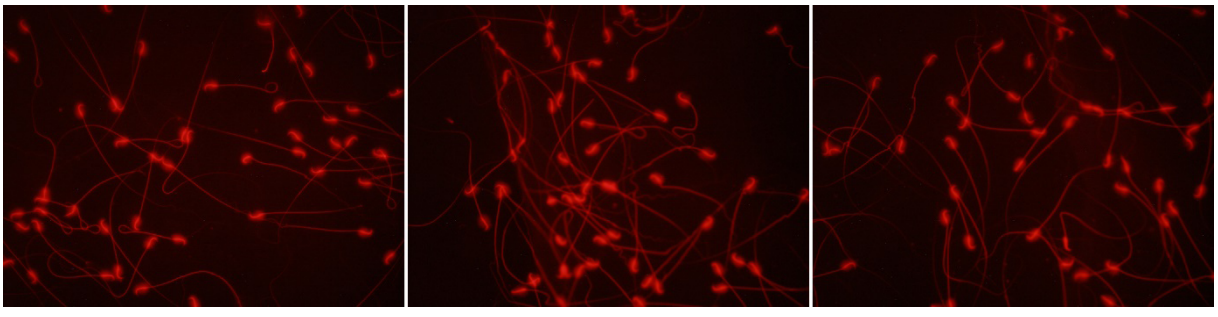

Figure 2. Sperm DNA fragmentation

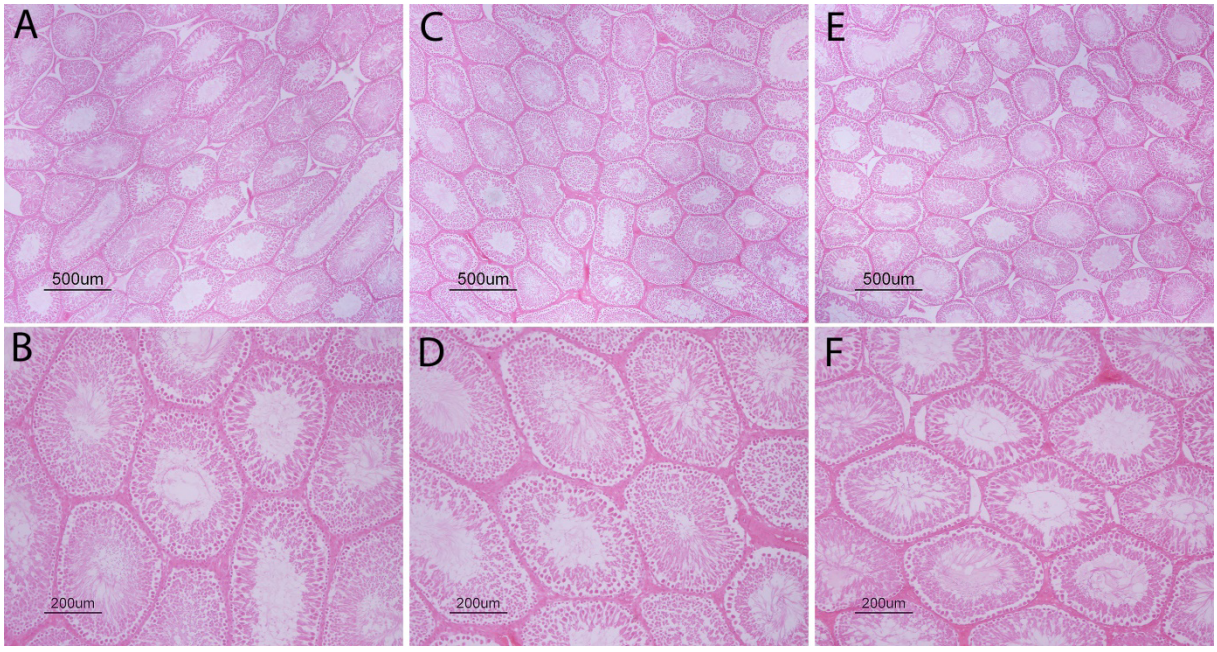

Figure 3. Testis histopatology

Table 1. Sperm DNA fragmentation (SDF) ratio

Group	Total	Fragmented	Degraded	SDF	%	P
Control	600	72	26	0.163333	16.3	>0.05
Theranekron I	600	65	28	0.155	15.5	>0.05
Theranekron II	600	75	25	0.166667	16.6	>0.05

There is no statistically significant difference between groups (P>0.05).

Table 2. Motility, density, abnormal sperm ratios

Variables	Mean±SD			P
	Control	Theranekron I	Theranekron II	
Motility (%)	86.25±5.17	85±5.34	86.87±4.35	>0.05
Density ($\times 10^9$)	2.08±0.08	2.09±0.07	2.07±0.08	>0.05
Abnormal sperm (%)	11.37±1.06	11.87±1.24	12.12±1.45	>0.05

There is no statistically significant difference between groups (P>0.05).

In this study, the impact of theranekron on reproductive parameters in male animals was investigated.

In the study, sperm motility, density, and abnormal sperm rates were examined. There was no statistical difference between the study groups (P>0.05). As a result of histopathological examination, no difference was observed between the groups in seminiferous tubules contours, Sertoli cells, Leydig cells, and spermatogonia. These results show that theranekron does not have a negative effect on spermatogenesis.

In examining sperm DNA damage, the ratios of breaks in the DNA chain, the proportion of DNA-damaged spermatozoa, and fragmented and degraded sperm do not differ statistically between groups (P>0.05).

5. Conclusion

Based on this study, theranekron did not have a negative effect on sperm motility, density, and abnormal spermatozoa rates; it did not cause a histopathological change, or affect the sperm DNA damage rate. For these reasons, theranekron does not have a negative effect on male reproductive parameters, and there is no harm in using it during breeding and non-breeding seasons.

Ethical Considerations

Compliance with ethical guidelines

The study was undertaken under agreement No. 2022/11-11 of Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee, dated 03/11/2022.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors' contributions

Conceptualization, supervision, investigation and writing-original draft: Volkan Koşal, and Ömer Faruk Keleş; Writing–review & editing:Volkan Koşal; Data collection: Ömer Faruk Keleş.

Conflict of interest

All authors declared no conflict of interest.

Acknowledgments

The authors would like to thank the Van Yüzüncü Yıl University.

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