



Research Article

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The effect of adding different levels of aqueous extract of *Tribulus terrestris* in the extender on sperm motility Afshari rams at 5°C

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ABSTRACT

As alternative medicine, medical and aromatic plants are getting popular in reproduction science and feed manufacturing sector since their appropriate usages have no any side effects on animal health. There have been huge studies in animal reproduction for investigating the effects of plant extracts and powders on the performance of animals as well as on sperm parameters in the World. Recently one of the most popular phytoproducts is an extract of the annual herb *Tribulus terrestris* L. (Zygophyllaceae). The occurrence of glucosides, steroids, saponins, flavonoids alkaloids, tannins, vitamins, unsaturated fatty acids, cinammic acid amides has been reported in *Tribulus terrestris*. Sperm survived for a short time in undiluted semen, and semen slow cooling to a temperature of 5°C will cause the death of many spermatozoa. Thus, extender is suitable which sperm parameters preserved during cooling and freezing. In this study, four Afshari rams with a mean of 5 ± 50 kg weight and 3 to 4 years, sperm was collected by electro ejaculation. After extraction of the medicinal plant at a concentration of 1%, 3% and 5%, each concentration was added to the sperm extender as separately and one group were considered as a control. Sperm motility were evaluated at zero time (immediately post ejaculation) and 24 hours post ejaculation by CASA software. The results of this study shows that progressive motility was significantly increased ($p < 0.05$) in treatment groups of 3% *Tribulus terrestris* extract compare to control and other treatment groups at time zero. In treatment group of 5% *Tribulus terrestris* extract compare to other treatment groups at 24 hours time, was significant higher motility ($P < 0.05$) than to other treatment groups. Therefore, the addition of *Tribulus terrestris* extract in extender was advisable to improve sperm parameters in afshari rams.

Keywords: Ram Sperm, *Tribulus terrestris*, Extender, Motility.

INTRODUCTION

Tribulus terrestris L (TTL) is a herbal plant native to Iran and China that has a long history as a powerful aphrodisiac and as a traditional medicine for treating male infertility [1]. Recently, a chemical compound isolated from TTL called protodioscin has been identified, purified and standardized as a phytochemical agent. In a multi-center, placebo-controlled, randomized, double-blind clinical trial, protodioscin proved to be an effective form of treatment for male infertility. It is known that sufficient dehydroepiandrosterone (DHEA) in the epididymis is necessary for the maturation process of spermatozoa [2]. Furthermore, it has been speculated that in some idiopathic oligoasthenoteratozoospermia Ram infertility is due to the low concentration of DHEA in the epididymis. *Tribulus terrestris* powder may be natural feed additive of broiler chicks by increasing testosterone level muscle mass and body strength, most likely due to LH production [3]. This plant contains alkaloids, resin, peroxidase, diastase, flavonoids, carbon-hydrate, protein, fructose, sucrose, sterodial saponins (protodioscin (0.17-6.49%) and protogracilin), glycosides and phytosteroids [4]. Sperm survived for a short time in undiluted semen, and semen slow cooling to a temperature of 5°C will cause the death of many spermatozoa. Thus, extender is suitable which sperm parameters preserved during cooling and freezing [5]. The aim of this study was to investigate the effect of *Tribulus terrestris* extract in extender on sperm motility of Afshari Ram.

EXPERIMENTAL SECTION

Extender preparation: Extender (10 ml) was prepared in autoclaved double distilled water (prepared in Biotechnology Laboratory of Islamic Azad University, Khorasgan branch) containing 0.244 g TRI vS, 0.136 g citric acid, 0.082 g D-fructose and 20 % v/v egg yolk [6].

Extract preparation: To accomplish this, we use the method of extraction by using Percolation (prepared in GOLDARU, Pharmaceutical Co) [7]. Then put the *Tribulus terrestris* extract for 48 hours at 30 to 40 °C and completely isolated environment free from microbes (oven). After the extracts were dried and then weighting using a Sodium chloride serum injection 9% can hold volume.

Experimental design: In this study, 4 Afshari rams with a mean of 50 ± 5 kg weight and 3 to 4 years, sperm was collected by electrical ejaculation. After extraction of the medicinal plant at a concentration of 1%, 3% and 5%, each concentration was added to the sperm extender as separately and one group were considered as a control. Sperm motility(TM%: Total motility, PM%: progressive motility) was evaluated at zero time (immediately post ejaculation) and 24 hours post ejaculation by CASA software. the collected data in each of the groups were analyzed by one-way ANOVA using SPSS software with $P < 0.05$ as the level of significance.

RESULTS AND DISCUSSION

The results of this study shows that progressive motility was significantly increased ($p < 0.05$) in treatment groups of 3% *Tribulus terrestris* extract compare to control and other treatment groups at time zero(table1), at zero time, with increasing concentration TT extract, motility was increased($p < 0.05$), but motility at a level of 5% is low. in treatment group of 5% *Tribulus terrestris* extract compare to other treatment groups at 24 hours time, was significant higher motility ($P < 0.05$) than to other treatment groups. The level of 5% TT extract was best impression in parameters of sperm motility, progressive in 24 hours after ejaculation.

Table 1: Effects of *Tribulus terrestris* on sperm motility in afshari ram measured by CASA at zero time (immediately after ejaculation)

Level of extract in extender	<i>Tribulus terrestris</i>			
	1%	3%	5%	control
Motility Parameters				
PM%	31 ± 6^a	39 ± 4.2^a	21.3 ± 1.02^b	28 ± 2^c
TM%	61 ± 0.9^b	65 ± 3.5^a	50 ± 2^c	53.8 ± 4.07^c

Mean percentages \pm SD of motility of fresh ram spermatozoa in Tribulus terrestris on extender
Different letters in the same Rows indicate a statistical difference ($P < 0.05$).

Table 2: Effects of *Tribulus terrestris* on sperm motility in afshari ram measured by CASA at 24 hours post ejaculation

Level of extract in extender	<i>Tribulus terrestris</i>			
	1%	3%	5%	control
Motility Parameters				
PM%	15 ± 0.5	26 ± 1.01^b	21.4 ± 1.9^c	24.3 ± 0.4^a
TM%	29 ± 4	38.85 ± 1.3^c	48 ± 1.6^a	41 ± 1.7^b

Mean percentages \pm SD of motility of fresh ram spermatozoa in Tribulus terrestris on extender
Different letters in the same Rows indicate a statistical difference ($P < 0.05$).

Tribulus also helps improve sperm motility (movement), thus another study on sheep showed that using 1.5 grams of *Tribulus terrestris* extract for 40 days increased spermatogenesis in sheep during breeding season. Moreover, the number of spermatozooids, sperms viability duration and motility increased and in the newborn sheep from those treated with *Tribulus terrestris* extract a significant improvement in libido and sexual behaviour was observed [8]. The positive effect of *Tribulus terrestris* on the reproductive system might be explained with the fact, that protodioscin in the *Tribulus* increases the level of luteinizing hormone, which in its turn stimulates testosterone secretion [9], but Furostanol is one of the saponins in *Tribulus terrestris* with stimulant effect on spermatogenesis and extender. This material significantly improves the quality and quantity of sperm motility [10]. European candidate plants for improving semen quality include *Tribulus terrestris*, better known for its aphrodisiac properties. This plant was shown to promote spermatids resistant to homogenisation by 22% in a study of rats [11]. Therefore, the addition of *Tribulus terrestris* extract in extender was advisable to improve sperm parameters in afshari rams.

CONCLUSION

The addition of extract of *Tribulus terrestris* to extender of Afshari ram spermatozoa had a positive effect on the quantitative and qualitative characteristics of their sperm. *Tribulus terrestris* increased spermatozooids motility, and this improves the productivity of the manufacturing industry in the future will be like sheep.

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