

Effect of plant extract of *Tribulus terrestris* and probiotics on the reproductive performance, total cholesterol and testosterone hormone levels of rams

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Abstract

The objective of this experiment was to compare between the effects of tribulus terrestris extract and probiotics on scrotal circumference, testicular volume, reaction time, semen characteristics, serum total cholesterol and testosterone hormone levels of rams. The study was carried out from February to July 2013 on eight sexually mature Rahmani rams. The eight rams were used as control group for one month before treatment (weekly assessment for all the parameters). Rams were divided into two groups, each group included 4 animals. The first group received extract from tribulus terrestris plant (1.5 gm/ head /day, orally in 100 ml water suspension) once daily for two months. The second group received probiotics (10 gm. / head/ day, orally in the form of 100 ml water suspension) for two months. In the second, third and fourth months from administration of tribulus terrestris extract and probiotics; scrotal circumference, testicular volume, reaction time, semen characteristics, serum total cholesterol and testosterone hormone levels were assessed weekly in all the studied rams. Results showed that scrotal circumference, sexual desire, hydrogen ion concentration, sperm morphology (total and secondary abnormalities percent), and total sperm number per ejaculate increased significantly ($P<0.01$) in tribulus terrestris group as compared to probiotics and control groups. Ejaculate volume, mass motility, individual motility, alive sperm percentage, sperm morphology, sperm cell concentration and acrosome integrity percentage increased significantly ($P<0.01$) in tribulus terrestris and probiotics groups as compared to control one. Serum total cholesterol level decreased significantly ($P<0.01$) in tribulus terrestris group as compared to the control group. Serum testosterone level exhibited a significant increase ($P<0.05$) in tribulus terrestris group as compared to control and probiotics groups. It was concluded that administration of tribulus terrestris extract resulted in an improvement in the sexual performance, semen quality, testosterone level and decreased total cholesterol levels of rams.

Keywords: Ram semen, *Tribulus terrestris*, probiotics, cholesterol, testosterone

Introduction

Tribulus terrestris is a natural herb used for many purposes as aphrodisiac, analgesic, astringent, stomachic, antihypertensive, diuretic, and urinary anti-infective (Aldein, 1986 and Majeed and Mahmood, 1988). It is a member of the Zygophyllaceae family, and an annual herb found in many tropical areas of the world. It contains steroidal saponins and protodioscin that act as a natural testosterone and sexual enhancer. *Tribulus terrestris* increases testosterone through increasing interstitial cell stimulating hormone (LH). There is a good

confidence that *Tribulus terrestris* is useful as a sexual enhancement herb (Gauthaman, et al. 2003). Probiotics are alive microbial feed additives (containing viable, defined micro-organisms in sufficient number) which beneficially affect the health of animal host by improving the intestinal microbial balance (Schrezenmeir and de Vrese, 2001). Therefore, probiotics increased feed conversion efficiency, live weight gain and resistance to diseases in growing animals (Sissons, 1989). The most common microorganisms included in probiotic products are *Lactobacillus*,

Streptococcus, *Bacillus* and strains of yeast belonging to the *Saccharomyces* species, which are normal inhabitants in the digestive tract of healthy animals (Piva and Rossi, 1999). They act through encouraging the proliferation of these micro-organisms in the intestinal tract, Therefore they improve the animal performance (Von Wright, 2005 and Anadon, et al., 2006). The aim of the present study was directed to compare between the effects of plant extract from tribulus terrestris and probiotics on the reproductive performance, total cholesterol and testosterone hormone levels of ram

Material & Methods

This experiment was carried out in the period from February to July 2013. in the Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt.

Eight sexually mature Rahmani rams aged 1.6-1.9 years and a mature Rahmani ewe aged 1.3 years was also used. Their average body weight ranged from 55-60 kg at the beginning of the study. Animals were apparently healthy and had apparently normal external genitalia.

The ration consisted of Egyptian clover, wheat straw, yellow corn, wheat bran, broken horse beans, common salt, vitamin and mineral premix (NRC, 1985). Each ram received 0.4 Kg of concentrates per day. Water was offered ad libitum.

A- Scrotal circumference, testicular volume, reaction time, semen characteristics, serum total cholesterol and testosterone hormone levels were assessed weekly in all the studied rams for one month before treatment (control).

B- Tribulus terrestris group: Tribulus terrestris extract was prepared as follow: Tribulus terrestris fruits were obtained from Egypt (natural habitats) and it was undergo an extraction procedure which included; Air dried plant samples rinsed with water and dried. After evaporation of the water, the residues were powdered (250 gm) and

extracted with 500ml, 70% ethanol in a soxhlet apparatus and the extracts were evaporated to dryness by a rotary evaporator at 65° c according to Hussain et al. (2009). Rams were divided into two groups, each group included 4 animals. The first group received tribulus terrestris (1.5 gm/ head /day, orally in 100 ml water suspension) in the form of alcoholic extract once daily for two months (Kistonova, 2005).

C- Probiotics (AGRETEK-MISR CO., 8738, Egypt) used in this study composed of *Saccharomyces cerevisiae*, *Lactobacillus acidophilus*, *Lactobacillus Plantarum*, *Streptococcus Faecalis* (1×10^5 , 10^6 , 10^4 and 10^5 CFU/ gm., respectively).

The second group received probiotics (10 gm./ head/ day, orally in the form of 100 ml water suspension) for two months according to Arcos-Garcia et al. (2000). In the second, third and fourth months for tribulus terrestris and probiotic groups, scrotal circumference, testicular volume, reaction time, semen characteristics, serum total cholesterol and testosterone hormone levels were assessed weekly in all the studied rams.

Results

Table (1): Effect of tribulus terrestris and probiotics on scrotal circumference and testicular volume of ram

Groups	Scrotal circumference (Cm)	Testicular volume (Cm ³)
Control (n=32)	25.78 ±0.56 ^b	118.35±3.47 ^a
Tribulus terrestris (n=48)	28.80 ±0.28 ^a	128.72±3.33 ^a
Probiotics (n=48)	28.18±0.45 ^a	119.00±3.63 ^a

Different superscripts between columns represent a highly significant difference at ($P < 0.01$). n= number of measurements

As illustrated in Table (1), the mean scrotal circumference of the studied rams increased significantly ($P < 0.01$) in tribulus terrestris and probiotics treated groups as compared to control groups. While the studied rams revealed a non-significant increase in testicular volume in tribulus terrestris and

probiotics treated groups as compared to the control one.

Table (2): Effect of tribulus terrestris and probiotics on reaction time and semen characteristics of rams (Mean ± SE).

Parameters	Control (n=32)	Tribulus terrestris (n=48)	Probiotics (n=48)
Reaction time (seconds)	18.98±1.72 ^{ab}	15.27±0.65 ^b	22.41±1.35 ^a
Volume (ml)	0.79±0.05 ^b	1.05±0.95 ^a	0.96±0.86 ^a
pH	6.50±0.01 ^a	6.43±0.01 ^c	6.46±0.01 ^b
Mass motility (grades)	4.58±0.06 ^b	4.85±0.05 ^a	4.75±0.06 ^a
Individual motility (%)	76.22±1.20 ^b	82.19±1.17 ^a	80.41±1.31 ^a
Alive sperm (%)	70.41±0.80 ^b	74.26±0.45 ^a	73.07±0.57 ^a
Total abnormalities (%)	9.73±0.33 ^a	5.45±0.31 ^c	6.32±0.33 ^b
Primary sperm abnormalities (%)	1.74±0.16 ^a	1.12±0.26 ^b	0.86±0.11 ^b
Secondary sperm abnormalities (%)	7.99±0.34 ^a	4.33±0.21 ^c	5.46±0.33 ^b
SP.C. C. (10 ⁶ / mm ³)	2.63±0.09 ^b	3.72±0.13 ^a	3.60±0.09 ^a
Acrosome integrity (%)	91.40±0.51 ^b	93.39±0.39 ^a	92.20±0.45 ^{ab}
Total sperm number / ejaculate (10 ⁹ / ml)	194.46 ±2.02 ^c	402.51 ± 2.44 ^a	343.76 ± 1.69 ^b

Different superscripts between rows represent a highly significant difference at (P<0.01). n= number of ejaculate

The obtained results in Table (2) showed a highly significant (P<0.01) improvement in sexual desire, hydrogen ion concentration, sperm morphology (total and secondary abnormalities percent), and total sperm number per ejaculate in tribulus terrestris group as compared to probiotics and control group. Data in Table (2) also revealed a **Table (3): Effect of tribulus terrestris and probiotics on serum total cholesterol and testosterone levels of rams**

Group	Cholesterol (mg/dl)	Testosterone (ng/ml)
Control (n=32)	63.98±4.03 ^a	3.65±0.61 ^B
Tribulus terrestris (n=48)	49.38±3.35 ^b	4.55±0.57 ^A
Probiotics (n=48)	59.03±4.47 ^{ab}	3.73±0.48 ^B

Different small superscripts between columns represent a highly significant difference at (P<0.01). Different capital superscripts between columns represent a significant difference at (P<0.05). n= number of serum samples

In Table (3), tribulus terrestris group demonstrated a highly significant (P<0.01) decrease in serum total cholesterol level as compared to the control group. Serum testosterone level exhibited a significant increase (P<0.05) in tribulus terrestris group as compared to control and probiotics groups.

Discussion

highly significant (P<0.01) improvement in ejaculate volume, mass motility, individual motility, alive sperm percentage, sperm morphology (primary sperm abnormalities percent, sperm cell concentration and acrosome integrity percentage in tribulus terrestris and probiotics groups as compared to control one.

These previous results regarding scrotal circumference were in harmony with those of **Fernandez et al. (2004) and Kerban (2008)** who demonstrated that scrotal circumference in rams was significantly greater in probiotics fed group than control one. Our data regarding the improvement of scrotal circumference in probiotics treated rams may be attributed to the fact that administration of probiotics in the diets of sheep improved the feed intake and feed conversion efficiency (**Fernandez et al., 2004; Kheradmand et al., 2006 and Kerban, 2008**). Our results regarding the increase in testicular volume in tribulus terrestris treated rams may be attributed to the suggestion that the number of sertoli cells increased within the seminiferous tubules of tribulus terrestris treated animals as compared to the controls ones (**Viktorov**

et al., 1994). Testicular volume increased slightly in probiotics treated rams than the control ones as probiotics improved the feed intake and feed conversion efficiency (Lachowski, 2001; Rao et al., 2001 and Sara et al., 2002). The improved semen characteristics and serum testosterone level in tribulus terrestris treated rams in the current study could be related to the fact that tribulus terrestris plant increased the secretion of interstitial cell stimulating hormone (LH) from the pituitary gland due to presence of saponins. LH hormone is also a special stimulant for production of testosterone and hence can improve sexual functions in the form of increased sperm production, quality of semen, improved erectile function and increased libido (Xu et al., 2001 and Hussain et al., 2009). The highly significant decrease in serum total cholesterol was in line with those of Li et al. (2001) and Chu et al. (2003) who mentioned that in mice, supplementation of diet by tribulus terrestris extract resulted in a significant decrease in total serum cholesterol level. Also Petkova et al. (2002) and Grigorova et al. (2008) recorded that in bulls and cocks, respectively administration of tribulus terrestris revealed a significant decrease ($p < 0.05$) in serum cholesterol level as compared to the control groups. On the other hand, Kimoto et al. (2002); Begle et al. (2006) and Lay et al. (2010) cited that probiotics supplementation in human resulted in lowering the serum total cholesterol level. The reduced serum cholesterol level in probiotics treated rams may be attributed to its mechanism that includes enzymatic deconjugation of bile acids by bile-salt hydrolase of probiotics. Bile acids are water-soluble end product of cholesterol in the liver. Cholesterol is used to synthesize new bile acids in a homeostatic response, resulting in lowering of serum cholesterol as demonstrated by Begley et al. (2006).

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