



Effect of Alcoholic Extract of Tribulus Terrestris on Fertility Parameters in Male Rabbit

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ABSTRACT

A total of fifteen male rabbits of mixed breeds, aged between 4-5 months and weighed 1.5–1.7Kg were used in this experiment to determine the effect of oral administration of alcoholic extract of Tribulus terrestris (Tt) on male fertility parameters. The rabbits were randomly allocated into 3 groups, the 1st and 2nd experimental groups were treatment orally with alcoholic extract of Tt in a dose of 75mg/kg/day or 150mg/kg/day respectively, the 3rd groups considered as a control groups and administrated distal water daily. Feed and water were served ad libitum throughout the period of 40 experimental days. At the end of the experiment, the male rabbit's weight and blood samples were taken by heart puncture. Sera were separated, kept at -20°C until using to determine the testosterone value of bucks, then slaughtering male rabbits and the pairs of testes were carefully dissected and transported into normal saline. The weight of testis and epididymis were measured, and the caudal spermatozoa were evaluated.

Result showed a significant ($P < 0.05$) effect of alcoholic extract at a dose of 75mg/kg/day and 150mg/kg/day on body weight, testicular weight, level of testosterone and sperm characteristics when compared with control group. This result showed that administration alcoholic extract (75mg/kg/day, 150mg/kg/day) increased fertility parameters in male rabbits, the dose of 150mg/kg/day gave best effect on fertility parameter in male rabbits.

KEYWORDS

Tribulus terrestris, male rabbit, fertility parameter

Introduction

Infertility is one of the major health problems in couple's lives, approximately 30% of couples infertilities are due to male factors (1). Several conditions can interfere with spermatogenesis and reduce sperm quality and production. Many factors such as drug treatment, chemotherapy, toxins, air pollution, and inadequate vitamin intake may have damaging effects on spermatogenesis and the normal production of sperm (2). Some studies have assessed the effect of *Tribulus terrestris* administration on male reproductive system and showed that the plant could positively influence on spermatogenesis (3).

A large number of plants have been verified throughout the world for the possible fertility regulatory properties (4). Some medicinal plants are widely used as aphrodisiac to relieve sexual dysfunction, or as fertility enhancing agents. They provide a boost of nutritional value thereby improving sexual performance and libido (5). *Tribulus terrestris* (Tt) is one of the traditional herbs with immense medicinal properties. It has long been used for different ailments of musculoskeletal, urogenital, gastrointestinal, central nervous and integumentary systems as it possesses diuretic, anti-inflammatory, and aphrodisiac properties (6). In traditional Chinese and Indian medicine, the plants fruit is used in the treatment of infertility, impotence, erectile dysfunction and low libido (7). Tt is a completely natural non-hormonal herbal, product extracted from the herb has been shown to enhance sexual behavior in an animal model (8). Studies demonstrating that Tt contains steroids, saponins, flavonoids, alkaloids, unsaturated fatty acids, vitamins, tannins, resins, nitrate potassium, aspartic acid and glutamic acid (9).

The objective of this investigation was to determine the effect of alcoholic extract of Tt fruits in different dose on fertility parameters in male rabbit.

Material and methods:

Plant fruit was harvested from the Garden of Baghdad University in AL-Jadiriya. The plant identified by Dr. Ali AL-Musawi, Department of Biology/College of Science /University of Baghdad under the name *Tribulus terrestris*, from Zygophyllaceae family. The fruits of plant were cleaned and dried in room temperature, dry fruits ground into fine powder by Electric

Grinder and stored in 4°C until using.

Fifty grams of Tt fruits powder were extracted with 500 ml of 70% ethanol alcohol under continuous string for 8hrs at room temperature, the suspension was filtered by whatman filter paper no 1, filtrate was concentrated by vacuum rotary evaporator (10). The crud extract was stored in sterile screw dark bottle at 4 C.

Fifteen mature male rabbits of mixed breed weighted 1.5-1.7 kg were obtained from local market. Rabbits were kept in cages of animal's house in Department of Surgery and Obstetrics, College of Veterinary Medicine, Baghdad University .The room temperature was between 20-25 °C. Animals were fed by Pellets, and gave water and food ad libitum. The animals preformed three weeks for adaptation, bucks were randomly divided into 3 groups (five rabbit per group) as follow:

Group 1 is the control group received daily 1 ml/ kg/day distal water orally.

Group 2 received an alcoholic extract of Tt in a dose of 75 mg/Kg daily.

Group 3 received an alcoholic extract of Tt in a dose of 150 mg/Kg daily.

All doses were administrated orally every day for 40 days. One day after receiving the last dose of the extract, male rabbit's weight, and blood samples were taken by heart puncture. Sera were separated by centrifugation at 3000 rpm for 15 min then they were stored at -20°C, pair of testes were isolated after slaughter, weighted and placed in normal saline transported to laboratory. Spermatozoa were collected by washing epididymis by 0.5 ml of warm normal saline solution 37- 40 °C to prevent cold shocks, after isolation of epididymis from testis, the sperms suspension was pulled by the RBCs pipette till 0.5 mark, and then diluted till 101 mark, so the dilution rate is 1:200. Diluting solution composed of 1 ml of 2% eosin, 1 ml of 3% sodium chloride and 50 ml of distilled water (11), it is used to determine of sperm concentration.

The percentages of individual motility, sperms alive and mor-

phologically abnormal sperms were measured according to the method of (12). Motility expressed as percentage by counting motile and immotile spermatozoa per unit area and quantitative alive sperms expressed as percentage by counting live and dead spermatozoa per unit area (13).

Statistical analysis

Statistical analysis was performed using SAS (Statistical Analysis System-version 9.1). Data were subjected to analysis using One Way Analysis of Variance (ANOVA) and least significant difference (LSD) was used to assess difference among means. $p < 0.05$ was considered statistically significant (14).

Results and Discussion:

The results of statistical analysis showed that the administration of alcoholic extract of *Tt* (75mg/kg/day and 150 mg/kg/day), for 40 days produced significant difference ($P < 0.05$) in body weight (1.746 ± 0.211 , 1.856 ± 0.187) kg respectively when compared with control group (1.680 ± 0.195) kg (Table 1). These results agree with (15) observed oral administration alcoholic extract herb causes a significant effect to body weight and sexual efficiency in the rat after treatment with different doses of *Tt*. *Tt* increases body ability to produce muscular mass and physical strength, furthermore, it causes an increase in production of red blood cells and improvement in circulation and oxygen transportation (16), also the results in table (1) illustrate that there is a significant increase ($P < 0.05$) in testes weight of buck when administrated with (75mg/kg/day and 150 mg/kg/day of *Tt* extract) to 292.4 ± 0.01 and 312 ± 0.02 gm respectively compared to control group (239 ± 0.01) gm. This result agree with (17) when administration *Tt*, the oral administration of *Tt* extract resulted an increase body and testicular weight which were statistically significant when compared with the control. This result can be explained by the androgenic effect of *Tt*, producing a stimulation in appetite. Androgens have a major role in the growth and differentiation of many tissues in addition to the organs of reproductions (18).

Also table (1) illustrated The results of statistical analysis which indicated a significant increase ($P < 0.05$) in serum testosterone level of bucks administrated with (75mg/kg/day or 150 mg/kg/day) of alcoholic extract (5.08 ± 0.24 , 7.05 ± 0.07) ng/ml respectively when compared with control group (3.98 ± 0.01) ng/ml. this result agree with (19) studies which shown that alcoholic extract of some parts of a plant in the family of *Tt* with the dose of 50 mg/kg could significantly increase serum level of free testosterone in the body. Furthermore, this extract has an Aphrodite activity which probably increases androgens. This result might explain the *Tt* plant increases secretion of luteotrophic hormone from pituitary gland due to containing saponins. Luteotrophic hormone is also a special stimulant for production of testosterone and hence can improve sexual function in forms of increased sperm production, improved erectile function and increased libido (20). In rat other studies concluded that *Tt* may improves the sexual activity, increase testosterone by intensification of leydig cells and my caused early puberty (21).

Table (1): Effect alcoholic extract *Tt* in body weight, weight of pair testes and serum testosterone level of mature male rabbits

parameters groups	Body weight. (kg)	Weight of testis (gm)	Serum testosterone (ng/ml)
Control	1.680 ± 0.195 c	239.6 ± 0.01 c	3.98 ± 0.01 c
Administered (75 mg/kg/day)	1.746 ± 0.211 b	292.4 ± 0.01 b	5.08 ± 0.24 b
Administered (150 mg/kg/day)	1.856 ± 0.187 a	312.4 ± 0.02 a	7.05 ± 0.07 a

Values are means \pm S.E

Different letters means Significant difference ($p < 0.05$) between groups.

Table (2) illustrate the results of administration of (75mg/kg/day, 150mg/kg/day), which represent a significant increase ($P < 0.05$) in sperms concentration, motility, live sperm percentage and significant decrease ($P < 0.05$) in the morphologically abnormal sperms when compared with control groups. This result agree with (22) when used aqueous extract of *Tt* (150 mg / kg / day) in rabbits, he concluded that the administration this *Tt* extract improve sperms characters by increasing the concentration and motility, and decreasing the morphologically abnormal and dead sperms percentage. This result illustrate the oral administration of *Tt* extract in laboratory animals resulted in the stimulation of spermatogenesis and the proliferation of spermatogonia, which involve cell division of spermatocyte and spermatid, these increase in cellular division were not accompanied by increase the diameter of the seminiferous tubules (23). In conclusion, the administration of *Tt* alcoholic extract to males rabbits increase some male parameter, such as body and testis weight, also increase the level of testosterone and improve semen characters (sperms concentration, sperms motility, decrease abnormal sperms and increase live sperms percentage).

Table (2) effect of alcoholic extract of *Tt* in semen characterize (sperms concentration, motility, lives sperms, morphologically abnormal) in epididymis sperms of male rabbits

parameters Groups	Sperms concentration (million /ml)	Motility Sperms %	Abnormal sperms%	Live Sperms %
Control	80.06 ± 1.12 c	60 ± 2.7 c	14.48 ± 1.37 a	61.20 ± 0.37 c
Administered (75mg/kg bw in day)	141.92 ± 1.04 b	67 ± 3.0 b	11.16 ± 0.49 b	69.40 ± 0.50 b
Administered (150mg/kg bw in day)	181.78 ± 0.75 a	82 ± 2.0 a	6.58 ± 0.62 c	85.40 ± 0.74 a

Values are means \pm S.E

Different letters means Significant difference ($p < 0.05$) between groups.

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