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Impact of *Tribulus terrestris* Alcoholic Extract on Domestic Adult Male Cats of the Local Breed's Serum Levels of Luteinizing Hormone, Follicle-Stimulating Hormone, and Testosterone

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Abstract The current study aims to evaluate the effects of an alcoholic extract of Puncturevine (*Tribulus terrestris*) on the testosterone, luteinizing, and follicle-stimulating hormone levels in adult male domesticated local breed cats.

Twenty male cats of the domesticated local breed, aged among14±1.2 months and weight from 2.4±0.13 kg, participated in the current study. Five males were randomly assigned in each of the four groups of the study animals.

Three groups received oral doses of alcoholic extract of puncturevine (*Tribulus terrestris*) once daily for a period of 30 days (T1, T2, and T3) at concentrations of 100, 200, and 300 mg/kg body weight respectively. As a control, the first group (C) was retained. Blood samples were collected in serum separator tubes for blood parameters analysis including; luteinizing hormone, follicle-stimulating hormone, and testosterone. The results of T2 group showed a significant increasing in levels of serum testosterone and luteinizing hormone (LH) compared with other groups, while T1 and control groups did not differ significantly, according to the study. On the other hand, the T3 group's results showed significant decrease compared with other groups. Follicle-stimulating hormone (FSH) levels remained unchanged in the current study in all animals of experiment.

Conclusions: The extract clearly affects in the levels of serum luteinizing hormone and testosterone. The dose 200 mg/kg body weight is the recommended dosage while excessive concentration of extract causes negative effects on serum hormone levels. There was no discernible impact of the alcoholic extract on the follicle-stimulating hormone (FSH) levels.

Keywords: *Tribulus terrestris,* male cats, FSH, LH, testosterone

Introduction Some hormone deficiencies impact male reproductive function in all breeding in domesticated animals (1). A deficiency in testosterone impacts sexual desire absolutely and sperm generation (spermatogenesis) (2). The levels of serum luteinizing hormone deficiency impacts reproductive performance by effect on testosterone with feedback mechanism (3). As a result, several investigations have been carried out to treat the lack of sexual hormones to increase the reproductive efficiency (4). The breeding season in domestic local cats clearly affects the testicles' histological and behavioral changes, as well as the hormone levels; luteinizing hormone and testosterone levels rise, and Leydig and Sertoli cells become more active (5). The most studies look at the effects of using hormonal medical treatments to increase testosterone and luteinizing hormone levels (6). In addition to that using of nutritional supplements to enhance reproductive efficiency (7), and the use of medical herbs and their extracts to improve reproductive function in order to reduce side effects (8). For instance, *Tribulus terrestris* is a medical herb that can raise intracavernous pressure, which may be linked to its proerectile aphrodisiac effects. Nitric oxide may be released from the nerve endings supplying the corpus cavernous as a result of an increase in androgen levels. The weight gain and better sexual behavior seen in rats may be due to the androgen-boosting effects of *Tribulus terrestris* (9). Gonadal processes (such as ovarian folliculogenesis and spermatogenesis), sexual behavior, pituitary and gonadal hormones and their receptors, fecundity, the quality and quantity of gametes, and the central nervous system can all be stimulated by *Tribulus terrestris* extract. (10).

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The present study was to evaluate the effects of an alcoholic extract of Puncturevine (*Tribulus terrestris*) on the augmentation of testosterone, luteinizing hormone, and follicle-stimulating hormone levels in adult male domesticated local breed cats.

Material and methods

Ethical approval

The project was approved (2385 in 2/6/2025) by the Committee for Research Ethics at the College of Veterinary Medicine, University of Al-Qadissiyah, Iraq. The National Research Council's guidelines for the use and care of laboratory animals were followed in the conduct of the current study. This experiment was approved by the University of Al-Qadisiyah's College of Veterinary Medicine's Ethical Council.

Ethical Acceptance: All applicable national, international, and/or institutional criteria were followed when caring for and using the animals. Every method employed in animal research adhered to the ethical standards established by the institution or practice carrying out the investigation. The ethical status of the animals used in this investigation was thoroughly explained in the materials and method section, along with the sources we cited.

Animals of the study Twenty adult toms of local breeds, weighing between 2.4±0.13 kg and aged about 14±1.2 months included in this study. Each group consist of 5 males, which kept in stainless steel cages by individually fed commercial cat food (Reflex-Turkey) and given unlimited access to water. The animals were kept in a controlled setting with artificial lighting.

Design of study Twenty male adult domestic cats (local breed) were used and split into four groups, each with five male cats; animals were dosed orally once daily for thirty day as following:

- The untreated control group (C), animals received distal water only.
- The first treatment group (T1), animals administered a dose of 100 mg/kg body weight of alcoholic extract of Puncturevine
- The second treatment group (T2), animals administered a dosage of 200 mg/kg body weight of alcoholic extract of Puncturevine.
- The third treatment group (T3), animals received 300 mg/kg body weight of alcoholic extract of Puncturevine.

The study employed the Tosoh AIA-360 equipment (Tosoh Company, Japan) to use radioimmunoassay to measure hormones, evaluating the extract's effect on testosterone, luteinizing hormone, and follicle-stimulating hormone levels.

Preparation of alcoholic extract The continuous extraction device (Soxhlet) (Korean company WISD) was filled with 40 g of crushed plant leaves in order to create an alcohol-based extract of *Tribulus terrestris*. After adding 500 milliliters of 70% ethanol alcohol to the device's beaker, the device was allowed to run at 70 degrees Celsius for six to eight hours. A rotary evaporator set to 60 degrees Celsius was then used to dry the filtrate after the solution had been filtered through Whatman filter paper No. 1. Using an incubator set to 37°C for 48–72 hours, the remaining liquid was dried to obtain the final extract. The extract was then stored in a refrigerator at 4°C until it was used (11).

Statistical analysis

The statistical analysis of the study's findings was conducted using the Statistical Package of Social Science (SPSS) program at a probability level of (0.01) the Independent F test is used to identify significant differences in the rates (12).

Results and discussion

Testosterone Hormone The T2 group had the highest serum testosterone hormone levels (2.877 ng/ml), while the T3 group had the lowest (1.215 ng/ml). According to these findings, there is no discernible difference between the control and T1 groups. While T2 groups was compared to other groups as reveal in Table-1 and Fig.1 which recorded a significant increase in effect.

Our results concur with those of (13), who has found that *Tribulus terrestris* enhancing the number of Leydig cells, and agree with (13, 14, 15), whose observed that serum testosterone levels were elevated when rats were treated with *Tribulus terrestris*. The study's findings supported those of (16, 17, 18, and 19), which concluded that *Tribulus* extract plasma has a stimulatory effect on testosterone level. The results similar with the findings of (16), who discovered that *Tribulus terrestris* stimulated serum testosterone and rat reproductive behavior.

The findings supported the findings of (20), who recorded that the extract from *Tribulus terrestris* plant raises the level of testosterone hormone in male wistar rats. The male rabbits' reproductive organs showed negative histological changes at higher extract dosages, as shown in (21), which is in line with the idea that excessive doses can have detrimental effects.

Luteinize Hormone The serum luteinize hormone assessment revealed (3.554, 3.435, 4.984, and 2.256 ng/ml), respectively, in Table-1 and Fig.2. Our results indicate that the T2 group increased significantly, but there was no discernible difference between the T1 and control groups. Additionally, our findings clarified why the luteinizing hormone (LH) level in T3 was decline. These findings concur with those of

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(13, 14, and 15); who has pointed to that, rats treated with *Tribulus terrestris* had higher serum levels of LH. The current study's findings concurred with those of (22), who recorded that using *Tribulus terrestris* raises the levels of LH in Sprague Dawley rats. The study's findings agreement the conclusion drawn by (23) that administering the extract raises the amount of luteinizing hormone in male rats.

Follicular Stimulated Hormone The current study's findings demonstrated that the follicular stimulating hormone concentrations in the animal groups under investigation were, respectively (1.616, 1.618, 1.628, and 1.609 ng/ml). There was no discernible difference between the treated groups and the control group in the current study on follicular stimulating hormone, according to Table-1 and Figure-3. The study's findings were in line with those of (13, 24, 15), which demonstrated that giving *Tribulus terrestris* to rats raised their levels of LH (but not FSH). The results corroborated (16), which showed that *Tribulus* extract increased rat plasma testosterone but not LH or FSH

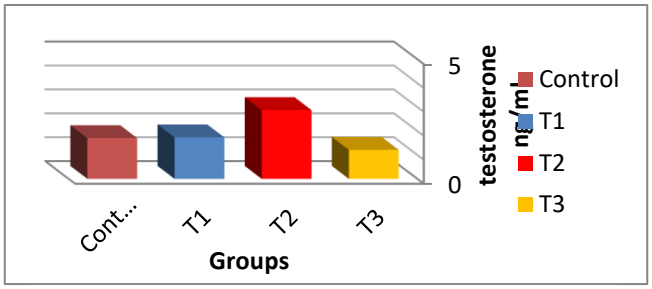

Fig.1: Levels of testosterone in animal groups

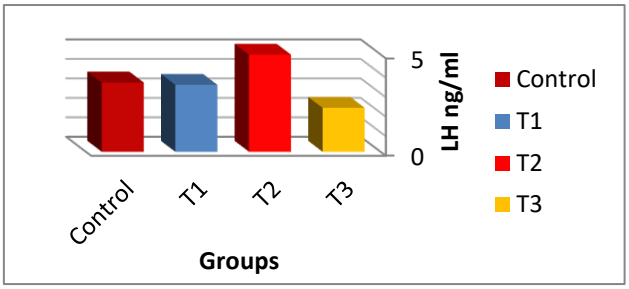

Fig.2: Levels of LH in animal groups

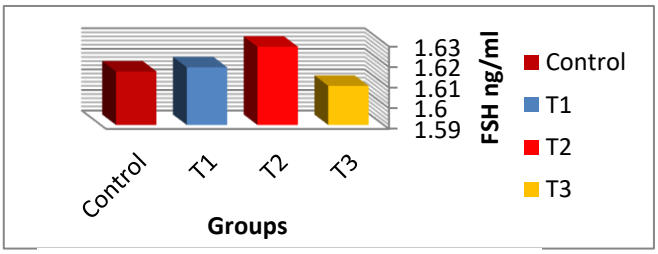

Fig.3:Levels of FSH in animal groups

Table (1): *Tribulus terrestris* alcoholic extract's impact on adult domestic male cats' hormones (mean±SE)

Parameter / Groups	Control	T1	T2	T3
Testosterone (ng/ml)	1.706±0.123 [a]	1.735±0.015 [a]	2.877±0.876 [b]	1.215±0.980 [c]
LH (ng/ml)	3.554±0.054 [a]	3.435±0.154 [a]	4.984±0.063 [b]	2.256±0.097 [c]
FSH (ng/ml)	1.616±0.055 [a]	1.618±0.089 [a]	1.628±0.088 [a]	1.609±0.062 [a]
Different letters mean significance variances P≤0.01				

Conflicts of Interest: No other peer-reviewed journal has published this material in a substantially similar or identical form, and it is not currently being considered for publication anywhere.

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