

## Effect of Aqueous Extract of *Tribulus terrestris* orally administration on some Biochemical Parameters in Castrated Male Rabbits

Abeer Uthman Moosa<sup>1</sup>; Rasha Hussein Kuba<sup>2</sup> and Yasser A.H. AL- Issa

<sup>1</sup>Department of Biology, College of Science, University of Baghdad <sup>2</sup>Department of Chemistry, College of Science, University of Baghdad, Iraq

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### Summary

The present study is aimed to investigate the influence of an aqueous extract of *Tribulus terrestris* on some biochemical parameters namely, alkaline phosphatase (ALP), Iron, phosphate (PO<sub>4</sub>), sodium (Na<sup>+</sup>), potassium (K<sup>+</sup>), aldosterone, and cortico steroid in unilateral and bilateral castrated male rabbits. Thirty mature male rabbits which are divided randomly in to six groups. group 1: is control, group2: orally administrated 150 mg / kg / day of an aqueous extract of *T. terrestris*, group3: is bilateral castrated rabbits received D. W., group4: is bilateral castrated rabbits received (150 mg / kg / day) of the extract, group 5: unilateral castrated rabbits received D. W., group 6 is unilateral castrated rabbits received 150 mg / kg / day of the extract, all doses were given orally every day for 30 days. At the end of the experiment, sera were collected and biochemical tests measured. The results showed a significant (P<0.05) increased in ALP level in group 2 and in PO<sub>4</sub> level in group 5 in comparison with group1, while PO<sub>4</sub> level tended to be decreased significantly (P<0.05) in group 6 when it compared with group 5. The results also showed a significant(P<0.05) increased in (K<sup>+</sup>) and (Na<sup>+</sup>) in group 2 and group 6 in comparison with group1 while there was a significant(P<0.05) decreased in (K<sup>+</sup>) and (Na<sup>+</sup>) in group 4 when it compared with group 2. In addition there was a significant (P<0.05) increased in aldosterone concentration in group 6 compared with group 1 and group 2. While there was a significant decreased in cortico steroid concentration in group 3 , 5 and 2 compared with group 1 but the cortico steroid showed a significant (P<0.05) increased in group 6 compared with group 1. There were no significant differences between groups concerning the iron concentration.

**Keywords:** Aqueous Extract, *Tribulus terrestris*, orally administration, Biochemical Parameters, Castrated Male Rabbits.

### تأثير المستخلص المائي لنبات القطب *Tribulus terrestris* L في بعض المعايير الكيموحياتية في ذكور الأرانب المخصية

عبيد عثمان موسى<sup>1</sup> و رشا حسين كبة<sup>2</sup> وياسر عبد الحسين جعفر

<sup>1</sup>قسم علوم الحياة - كلية العلوم - جامعة بغداد <sup>2</sup>قسم علوم الكيمياء - كلية العلوم - جامعة بغداد - العراق

### الخلاصة

تهدف الدراسة الحالية التحري عن تأثير المستخلص المائي لثمار نبات القطب *Tribulus terrestris* في بعض المعايير الكيموحياتية وهي الفوسفاتيز القاعدي والحديد والفوسفات والصوديوم والبوتاسيوم والألدوستيرون والكورتيكو ستيرويد في ثلاثين أرنباً من الذكور البالغة. قسمت حيوانات التجربة على ستة مجاميع. المجموعة الأولى هي مجموعة السيطرة، و المجموعة الثانية جرعت ب 150 ملغم/كغم/يوم من المستخلص المائي للقطب، والمجموعة الثالثة تتكون من أرانب مخصية كلياً أعطيت ماء مقطر، و المجموعة الرابعة تتكون من أرانب مخصية كلياً جرعت ب 150 ملغم/كغم/يوم من مستخلص القطب، والمجموعة الخامسة تتكون من أرانب مخصية جزئياً أعطيت ماء مقطر، وأخيراً المجموعة السادسة تتكون من أرانب مخصية جزئياً جرعت ب 150 ملغم/كغم/يوم من المستخلص. استمرت المعاملة ثلاثين يوماً، وبعد انتهاء مدتها تم جمع عينات الدم لغرض قياس المعايير الكيموحياتية. أظهرت النتائج ارتفاع معنوي (P<0.05) لمستوى ALP في المجموعة الثانية وفي مستوى PO<sub>4</sub> في المجموعة الخامسة عند مقارنتها مع مجموعة السيطرة. لوحظ حدوث إنخفاض معنوي (P<0.05) في مستوى PO<sub>4</sub> في المجموعة السادسة عند مقارنتها مع المجموعة الخامسة. أظهرت النتائج حصول إرتفاع معنوي (P<0.05) في مستوى تركيز كل من K<sup>+</sup> , Na<sup>+</sup> في المجموعة الثانية والسادسة عند

مقارنتها مع مجموعة السيطرة ، في حين حصل انخفاض معنوي ( $P<0.05$ ) في مستوى  $K^+$  ,  $Na^+$  في المجموعة الرابعة عند مقارنتها مع المجموعة الثانية. فضلاً عن حصول انخفاض معنوي ( $P<0.05$ ) في مستوى تركيز الألدوستيرون في المجموعة السادسة بالمقارنة مع مجموعة السيطرة والمجموعة الثانية ، وحصول انخفاض معنوي لمستوى الكورتيزول في المجموعة الثانية والثالثة والخامسة بالمقارنة مع مجموعة السيطرة ، بينما حدث ارتفاع معنوي لمستوى الكورتيزول في المجموعة السادسة بالمقارنة مع مجموعة السيطرة. في حين لم يظهر الحديد أي فروق معنوية بين المجاميع المذكورة آنفاً. بينت نتائج الدراسة الحالية التأثيرات الموجبة لمستخلص نبات القطب في تركيز الفوسفاتيز القاعدي و الصوديوم واليوتاسيوم والفوسفات و الألدوستيرون و الكورتيزول الضرورية في كثير من وظائف الجسم.

**الكلمات المفتاحية:-** المستخلص المائي , نبات القطب , المعايير الكيموحياتية , ذكور الأرانب المخصية.

## Introduction

The requirements of the present market for ecological production laid the use of plant extracts in the feed industry as an alternative nutritive antibiotics and synthetic hormones (1). Recently one of the most popular phytoproducts is an extract of annual herb *Tribulus terrestris* L. (Zygophyllaceae). The occurrence of glycosides, steroids, saponins, flavonoides, alkaloids, tannins, vitamins, unsaturated fatty acid, cinammic acid, and amides has been reported in *Tribulus terrestris* (2). This plant has been used in the folk medicine by Arabs (3) and it has played an important role as aphrodisiac plant in east countries and Bulgaria, and for treatment of erectile dysfunction, diabetes, tumors, cardiovascular-, respiratory-, and other diseases (4). There are many clinical investigations about the effect *T. terrestris* on humans and labor animals were carried out, but there are no data concerning the effect of *T. terrestris* extract on Iron.

The objectives of this study are to examine the effect of aqueous extract of *T. terrestris* on some biochemical parameters of blood serum in normal and unilateral, bilateral castrated rabbits.

## Materials and Methods

*T. terrestris* fruit was collected from the garden of Baghdad University in Al-Jadiria, then cleaned, dried in room temperature and ground in to fine powder by electric grinder and stored in 4 °C until using them.

Preparation of an aqueous extract for *T. terrestris* fruits was carried out as follows, 100 gm of dried fruits was transferred in to a conical flask and 500 ml of distilled water was in a rate of 1:5 w:v shaken by electric shaker for 2 hours and left in room temperature for 24 hours, the mixture was filtered by four layers of gauze and put in plan tube and centrifuged mixture in centrifuge at 2000 rpm for 10 minute. The supernatant was filtered by Whatmann no.4 filter paper. Filtrate mixture was concentrated by oven for 72 hours to obtain crude extract. This extract was stored in dark sterile screw bottle 4 °C until use (5).

Thirty mature male rabbits weighted 1350 – 1775 g were obtained from local markets, housed in Biology Department/ College of Science/ University of Baghdad.

The room temperature was between 20-25°C. animals were fed by special food of laboratory animal (pellets) and clover, and were gave water and food ad labium.

Castration was done as following steps: Twenty mature male rabbits were anesthetized by intramuscular injection xylazine 5 mg / kg and Ketamine 15 mg / kg / BW (6). Sterilizing the scrotum by alcohol and a median skin incision in about 1 cm was made at the tip of scrotum. The testes were removed surgically, then the scrotum was closed by sewing it by a sterilized silk and sprayed by Medoxy spray after sterilized it by non-existent alcohol to prevent the area from contamination. The rabbits were left out for 15 days to recover from the surgery. The previous steps also used for unilateral castration rabbits but one testis was isolated. Animals were divided into six groups (5 rabbits per group) Group 1: control animals received distilled water (D. W.) Group 2: normal animals orally administrated an aqueous extract of *T. terrestris* in a dose of 150 mg / kg / day. Group3: bilateral castrated rabbits received D. W. Group 4: bilateral castrated rabbits received 150 mg / kg / day of an aqueous extract of *T.*

*terrestris*. Group 5: unilateral castrated rabbits received D. W. Group 6: unilateral castrated rabbits received 150 mg / kg / day an aqueous extract of all doses were given orally every day for 30 days.

After 30 days, blood samples were taken by heart puncture. Sera were separated by centrifugation 3000 rpm for 15 min. then they kept in -20°C until using them, to measure ALP, Na<sup>+</sup>, K<sup>+</sup>, Fe, PO<sub>4</sub>, cortico steroid and aldosteron by using biochemical tests.

Statistical analysis was done by using statistical package of social sciences (SPSS) version 17 (Inc., Chicago, IL, USA) computer software. Differences between groups were analyzed using an analysis of variance (ANOVA), (P < 0.05) was regarded as statistically significant.

### Results and Discussion

The results of the analysis showed a significant (P<0.05) increased in Alkaline phosphatase (ALP) concentration in the group that received aqueous extract of *T. terrestris* in comparison with control. This results agreed with Rajendar, *etal.*, (7) who concluded that *T. terrestris* offered a protective effect against Cd-induced testicular damage in rats. The beneficial effect of *T.terrestris* on testes can be attributed to antioxidant and metal chelating effect of *T. terrestris*. In many cases, either ALP was increased slightly or it did not change at all (Table1). In addition to that, phosphorous (PO<sub>4</sub>) showed a significant (P<0.05) increased in unilateral castrated rabbits group compared with control and this results agreed with Nerurkar and Sahasrabundhe, who observed that (PO<sub>4</sub>) is increased in castrated mice (8). While in unilateral castrated rabbits received aqueous extract of *T. terrestris* , the concentration of (PO<sub>4</sub>) was significantly (P<0.05) decreased , and that may be due to the protective effect *T. terrestris* by metal chelator activity (7).

The results also showed a significant (P<0.05) increased in Potassium (K<sup>+</sup>) and Sodium (Na<sup>+</sup>) in normal rabbits received extract group and unilateral castrated rabbits administrated extract compared with control, because of *T. terrestris* extract increased in the membrane potential (9). While there were a significant (P<0.05) decreased in K<sup>+</sup> and Na<sup>+</sup> in by lateral castrated rabbits administrated extract group compared with normal rabbits received extract group because castration (deficiency of testosterone) caused a lowering in the membrane potential (10), resulted from flow of positive sodium ions into the cell (11).

The unilateral castrated rabbits group showed no significant (P<0.05) increased in aldosteron concentration comparing with control, this result may be due to the effect of castration which leads to decrease testosterone levels as a result of absence of testis (12), and some articles refers to antagonistic relationship between testosterone and aldosteron levels (13).

While in The unilateral castrated rabbits received extract there is a significant (P<0.05) increased when compared with control, because of the effect of extract which stimulate adrenal gland to secret aldosteron and this could probably due to direct conversion of *T. terrestris* extract in to Dehydroepiandrosterone (DHEA) (14).

In addition to that, there was a significant(P<0.05) decrease in cortico steroid concentration in unilateral and bilateral castrated rabbits and normal rabbits that orally received extract compared with control, this result disagree with Fisher, *et al.*, (15) who reported that castration induced increases in cortico steroid. In many researches that achieved on human suggested a significant negative relationship between cortico steroid and testosterone (16). While Lubbers *et al.*, (17) who found similar cortico steroid concentrations measured in castrated and control calves. While, the unilateral castrated rabbits administrated *T. terrestris* extract, showed a significant (P<0.05) increased in comparison with control group, this may be due to the significant role of *T. terrestris* in induction of endogenous steroid production (18).

The results also showed no significant differences between all groups in Iron concentration, this results disagreed with Hussein *et al*, (19) who concluded that castration caused a marked decreased in Iron and other metals.

**Table 1: Effect of aqueous extract of *Tribulus terrestris* on ALP, Iron, PO<sub>4</sub>, Na<sup>+</sup>, K<sup>+</sup>, aldosterone, and cortico steroid concentration in different groups of rabbits**

	ALP U/L	Iron Umg/ dl	PO <sub>4</sub> Mg/dl	K <sup>+</sup> Mmol/l	Na <sup>+</sup> Mmol/l	Aldosterone Mg/dl	Cortico steroid Ng/dl
Group 1	34.50	205.13	3.47	3.89	159.80	41.00	40.25
Group 2	104.25*	269.50	4.90	6.00*	187.80*	41.00	21.33*
Group 3	78.20	264.35	4.78	4.84*	147.60*	42.00	18.85*
Group 4	84.73	312.3	4.92	4.57*	147.63*	43.00	41.50*
Group 5	74.00	265.38	5.80*	4.70*	150.57*	44.50	22.35*
Group 6	54.40	265.38	3.90*	5.34*	177.95*	47.50*	70.45*

Values represent means and (\*) represents a significant differences (P<0.05)

Group 1: control animals received distilled water (D. W.) ,Group 2: normal saline received an aqueous extract of *T. terrestris* in a dose of 150 mg / kg / day, Group3: bilateral castrated rabbits received D. W., Group 4: bilateral castrated rabbits received 150 mg / kg / day of an aqueous extract of *T. terrestris*, Group 5: unilateral castrated rabbits received D. W., Group 6: unilateral castrated rabbits received 150 mg / kg / day an aqueous extract of all doses were given orally every day for 30 days.

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