

The aphrodisiac herb *Tribulus terrestris* does not influence the androgen production in young men

V.K. Neychev*, V.I. Mitev¹

Department of Chemistry and Biochemistry, Medical University, 2 Zdrave str., Sofia-1431, Bulgaria

Received 16 September 2004; received in revised form 9 May 2005; accepted 21 May 2005

Available online 1 July 2005

Abstract

Objective: The aim of the current study is to investigate the influence of *Tribulus terrestris* extract on androgen metabolism in young males. **Design and methods:** Twenty-one healthy young 20–36 years old men with body weight ranging from 60 to 125 kg were randomly separated into three groups—two experimental (each $n=7$) and a control (placebo) one ($n=7$). The experimental groups were named TT1 and TT2 and the subjects were assigned to consume 20 and 10 mg/kg body weight per day of *Tribulus terrestris* extract, respectively, separated into three daily intakes for 4 weeks. Testosterone, androstenedione and luteinizing hormone levels in the serum were measured 24 h before supplementation (clear probe), and at 24, 72, 240, 408 and 576 h from the beginning of the supplementation.

Results: There was no significant difference between *Tribulus terrestris* supplemented groups and controls in the serum testosterone (TT1 (mean \pm S.D.: 15.75 ± 1.75 nmol/l); TT2 (mean \pm S.D.: 16.32 ± 1.57 nmol/l); controls (mean \pm S.D.: 17.74 ± 1.09 nmol/l) ($p > 0.05$)), androstenedione (TT1 (mean \pm S.D.: 1.927 ± 0.126 ng/ml); TT2 (mean \pm S.D.: 2.026 ± 0.256 ng/ml); controls (mean \pm S.D.: 1.952 ± 0.236 ng/ml) ($p > 0.05$)) or luteinizing hormone (TT1 (mean \pm S.D.: 4.662 ± 0.274 U/l); TT2 (mean \pm S.D.: 4.103 ± 0.869 U/l); controls (mean \pm S.D.: 4.170 ± 0.406 U/l) ($p > 0.05$)) levels. All results were within the normal range. The findings in the current study anticipate that *Tribulus terrestris* steroid saponins possess neither direct nor indirect androgen-increasing properties. The study will be extended in the clarifying the probable mode of action of *Tribulus terrestris* steroid saponins.

© 2005 Elsevier Ireland Ltd. All rights reserved.

Keywords: *Tribulus terrestris*; Steroid saponins; Testosterone; Luteinizing hormone; Androstenedione

1. Introduction

Erectile dysfunction is considered as one of the most important public health problem, since it affects great percentage of men. Despite the increasing availability of effective conventional medical treatments, plant-derived and herbal remedies continue to provide a popular alternative for men seeking to improve their sexual life.

Tribulus terrestris (TT) herb has been commonly used in folk medicine to energize, vitalize and improve sexual function and physical performance in men. Although different effects of TT on animals (Gauthaman et al., 2002; Gauthaman et al., 2003; Arcasoy et al., 1998) and men (Brown et al., 2000; Brown et al., 2001; Kohut et al., 2003; Antonio et al., 2000) have been evaluated and many active compounds from TT extract have been established (Huang et al., 2003; De Combarieu et al., 2003; Cai et al., 2001; Conrad et al., 2004) the mode of its action and efficacy remains uncertain and controversial. It is widely believed that TT affects strongly the androgen metabolism increasing significantly testosterone or testosterone precursor levels. The aim of the current study is to investigate the influence of TT extract on androgen metabolism in young males.

* Corresponding author. Tel.: +359 889 49 51 25 (personal)/359 2 51 66 528 (office); fax: +359 2 95 20 345.

E-mail address: neychev@dir.bg (V.K. Neychev).

¹ Tel.: +359 888 44 33 53 (personal)/359 2 51 66 528 (office); fax: +359 2 95 20 345.

2. Materials and methods

2.1. *Tribulus terrestris* extract

Tribulus terrestris (origin Bulgaria) extract encapsulated in gelatinous capsules each containing 200 mg of the dry extract approved by Bulgarian Ministry of Public Health under no. 04-2003 with ext. ref. no. 4465-1809/021003 were purchased from “Vemo 99” Ltd., Sofia, Bulgaria. The steroid saponins concentration has been determined to be 60% of the dry matter.

2.2. Subjects and treatment

Twenty-one healthy 20–36 years old men were randomly separated into three groups – two experimental (each $n = 7$) and a control one ($n = 7$). They were placed in an academic research environment for 4 weeks. The body weight of the subjects ranged from 60 to 125 kg. The experimental groups were named TT1 and TT2 and the subjects were assigned to consume 20 and 10 mg/kg body weight per day of TT extract, respectively, separated into three daily intakes for 4 weeks.

Informed consent from all volunteers was obtained. All experiments were performed in compliance with the guidelines of the declaration of Helsinki and Tokyo for humans, and were approved by the Ethical Committee of Medical University in Sofia.

2.3. Investigated hormones and specimen preparation

The total testosterone (T), luteinizing hormone (LH) and androstenedione (A) levels in the serum were measured 24 h before supplementation (clear probe), and at 24, 72, 240, 408 and 576 h from the beginning of the supplementation. These probes were named P-1, P-2, P-3, P-4, P-5, and P-6, respectively. The blood samples were obtained in the morning, since A and T show high diurnal variability with their highest levels measured in the morning.

All recommendations for collecting, handling and storing blood samples furnished by the National Committee for Clinical Laboratory Standards were followed.

2.4. Principles of the assay

ACS: 180[®] (Automated Chemiluminescence System) for T assay was used. This is a competitive immunoassay using direct, chemiluminescent technology. T in the subject sample competes with acridinium ester-labeled T in the Lite reagent for a limited amount of polyclonal rabbit anti-testosterone antibody bound to monoclonal mouse anti-rabbit antibody which is coupled to paramagnetic particles in the solid phase. A testosterone-releasing agent to release bound T from the endogenous binding proteins in the samples was used. An inverse relationship exists between the amount of total T in the subject samples and the amount of relative light units detected by the system.

DELFLIA[®] LH Spec assay for quantitative determination of human LH in the serum is a solid phase, two-site fluoroimmunoassay based on the direct sandwich technique, in which two monoclonal antibodies (derived from mice) are directed against two separate antigenic determinants on the LH molecule. The fluorescence is proportional to the concentration of LH in the sample.

DRG androstenedione ELISA assay for quantitative determination of human A is a direct competitive solid phase enzyme immunoassay with a peroxidase conjugate as label and tetramethylbenzidine as chromogen.

2.5. Statistics

Statistical analyses of the data were performed using the treatment repeated measures analysis of variance (ANOVA) with software available on www.physics.csbsiu.edu/stats/anova.html. An analysis of covariance (ANCOVA) was also performed using basal hormone concentrations as the covariate.

3. Results

Testosterone, androstenedione and luteinizing hormone were measured in nmol/l, ng/ml and U/l, respectively. The referent values for the assay by the quoted-above methods in men are from 8.7 to 28.7 nmol/l for the T, from 1.0 to 8.4 U/l for the LH, and from 0.35 to 3.15 ng/ml for the A. The results of the ANCOVA revealed no effect of basal serum hormone concentrations on the hormonal response to TT extract supplementation. Therefore, actual, rather than adjusted means from the ANCOVAs are presented throughout the text.

The results from P-1 (obtained 24 h before supplementation) of both the control and the two experimental groups fell in referent values. The results from P-2, P-3, P-4, P-5, and P-6 were also within the normal range (Figs. 1–3).

Serum testosterone (Fig. 1), androstenedione (Fig. 2) and luteinizing hormone (Fig. 3) concentrations were not significantly altered by TT extract supplementation: testosterone TT1 (mean \pm S.D.: 15.75 \pm 1.75 nmol/l), TT2 (mean \pm S.D.: 16.32 \pm 1.57 nmol/l), controls (mean \pm S.D.:

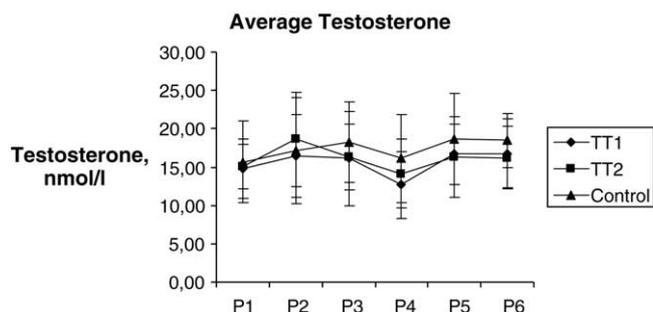


Fig. 1. Average testosterone levels.

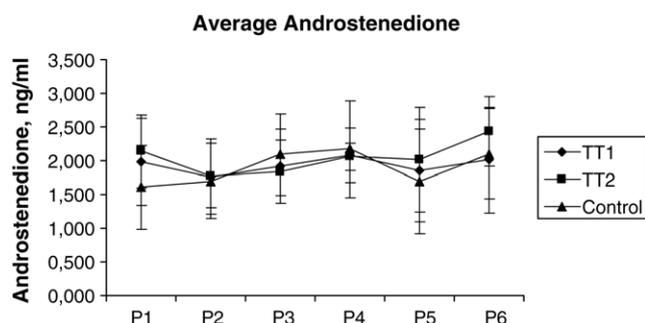


Fig. 2. Average androstenedione levels.

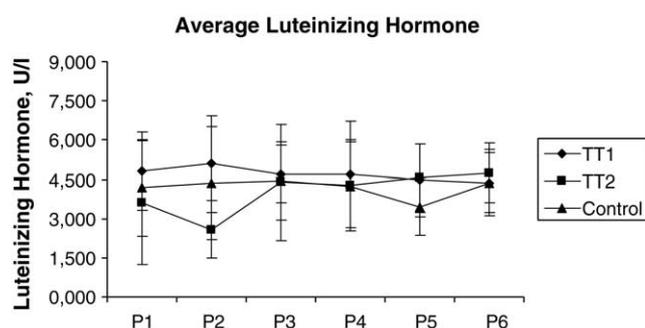


Fig. 3. Average luteinizing hormone levels.

17.74 ± 1.09 nmol/l) ($p > 0.05$); androstenedione (TT1 (mean ± S.D.: 1.927 ± 0.126 ng/ml), TT2 (mean ± S.D.: 2.026 ± 0.256 ng/ml), controls (mean ± S.D.: 1.952 ± 0.236 ng/ml) ($p > 0.05$)) and luteinizing hormone (TT1 (mean ± S.D.: 4.662 ± 0.274 U/l), TT2 (mean ± S.D.: 4.103 ± 0.869 U/l), controls (mean ± S.D.: 4.170 ± 0.406 U/l) ($p > 0.05$)).

4. Discussion

4.1. Arguments in favor of testosterone, androstenedione, and luteinizing hormone as indicators in this study

The measurement of androstenedione was preferred in this study instead of dehydroepiandrosterone (DHEA), and/or dehydroepiandrosterone sulphate (DHEA-S).

Indeed, the principal adrenal steroids circulating in male plasma are the androstenedione, DHEA, and DHEA-S. Nevertheless, androstenedione is synthesized from DHEA by the enzyme 3,17- β -hydroxysteroid dehydrogenase and, in addition, it is the immediate and major precursor to testosterone in the intrinsic synthetic pathways of androgens (Horton and Tate, 1966; Longcope et al., 1969; Weinstein et al., 1974). Furthermore, there is strong correlation between DHEA-S and androstenedione, but not between DHEA-S and testosterone in men (Phillips, 1996). Therefore, the measurement

of androstenedione would be indicative for DHEA and/or DHEA-S production even in the case of probable conversion of steroid saponins from *Tribulus terrestris* into DHEA mentioned in a study (Gauthaman et al., 2002). On the other hand the measurement of DHEA or DHEA-S could not be indicative for the androstenedione production in case of eventual conversion of *Tribulus terrestris* steroid saponins into androstenedione.

The measurement of total testosterone was used instead of free testosterone in the study.

Changes in the total testosterone concentration produce relatively minor changes in the size of the free fraction because, as it known, excess sex hormone-binding globulin (SHBG) binding sites modulate extreme variations in hormone concentrations (Blight et al., 1989). In contrast, changes in the SHBG concentration result in large shifts in the free and albumin bound testosterone fractions on the background of an unchanged total testosterone (Selby, 1990). Therefore, the eventual changes in testosterone production could not be precisely defined via measurement of free testosterone fraction solely. On the other hand the measurement of total testosterone will surely show the alterations in the testosterone production including its free (active) fraction.

Finally, LH secreted by basophil cells of the anterior pituitary controls testosterone production from Leydig cells through negative feedback of the hypothalamic-pituitary-adrenal loop. Therefore, if the *Tribulus terrestris* steroid saponins influence androgen biosynthetic pathways directly or have indirect mode of action increasing LH concentration, we could establish that by measuring T, A and LH concentrations.

4.2. Results versus past studies

The effectiveness of a nutritional supplement, designed to enhance serum testosterone concentrations and to prevent the formation of dihydrotestosterone and estrogens from the ingested androgens has been investigated in healthy 30–59 years old men (Brown et al., 2001). Subjects have been randomly assigned to consume DION (300 mg androstenedione, 150 mg dehydroepiandrosterone, 540 mg saw palmetto, 300 mg indole-3-carbinol, 625 mg chrysin, and 750 mg *Tribulus terrestris* per day) for 28 days. An increase in the serum concentrations of androstenedione (342%), free testosterone (38%), dihydrotestosterone (71%), and estradiol (103%) has been determined at unchanged total testosterone. In another study the effects of a dietary supplement containing dehydroepiandrosterone, androstenedione and herbal extracts (TT including) on immune function in middle-aged men had indicated that this supplement had minimal effect on men immune function, but significantly increased serum levels of androstenedione, free testosterone, and estradiol (Kohut et al., 2003).

In our opinion, the presence of androgens in the above-mentioned nutritional supplements questions the causal role

of TT extract for the increase in the serum androgen (testosterone) concentrations. Most probably, the elevation of the free testosterone levels at an unchanged total testosterone observed in those studies is a result of SHBG decreasing properties of the ingested DHEA and androstenedione, since ingestion of androstenedione decreases SHBG and results in shift of the free testosterone fraction (Beckham and Earnest, 2003; Leder et al., 2000; Selby, 1990). Furthermore, the serum concentrations of free and total testosterone have been unchanged in a similar study in which the above mentioned nutritional supplements including *Tribulus terrestris* extract have been used to investigate the effect of anabolic precursors on serum testosterone concentrations (Brown et al., 2000).

In another two studies, sexual behavior and intracavernous pressure (ICP) have been investigated in both normal and castrated rats to further understand the role of TT as an aphrodisiac (Gauthaman et al., 2002; Gauthaman et al., 2003). Decreases in body weight, prostate weight and ICP have been observed among the castrated groups of rats compared to the intact group. An overall reduction in the sexual behavior parameters in the castrated groups of rats as reflected by decrease in mount and intromission frequencies (MF and IF) and increase in mount, intromission, ejaculation latencies (ML, IL, EL) as well as post-ejaculatory interval (PEI) have also been determined. Compared to the castrated controls, treatment of the castrated rats (with either testosterone or TT extract) had showed increase in the prostate weight and ICP. A mild to moderate improvement in the sexual behavior parameters as seen by the increase in MF and IF and the decrease in ML, IL and PEI have also been determined. The authors have concluded that TT extract appears to possess aphrodisiac activity (observed also in their earlier study on primates) probably due to the androgen increasing property of TT. A conversion of TT steroid saponins, in particular protodioscine, into DHEA has been supposed.

On the basis of the results from our research and current knowledge of androgen metabolism and action we found this observation controversial. Although a study indicates the DHEA intrinsic androgenic activity that is potentially independent of metabolic conversion to other androgens (Lu et al., 2003), DHEA is known as a principal metabolic precursor of androstenedione and there is strong correlation between DHEA and androstenedione (Phillips, 1996). Therefore, if TT steroid saponins undergo conversion to DHEA increasing its concentration, then testosterone and/or androstenedione levels will be also elevated. In the current study, we did not find such elevation (Figs. 1 and 2). Furthermore, we found no changes of luteinizing hormone concentrations during supplementation (Fig. 3).

5. Conclusion

Altogether, the findings in the current study anticipate that chronic ingestion of either 10 or 20 mg/kg body weight of

Tribulus terrestris extract influence neither directly nor indirectly androgen production in young males.

This study will be extended to clarify the probable mode of action of TT steroid saponins.

References

- Antonio, J., Uelmen, J., Rodriguez, R., Earnest, C., 2000. The effects of *Tribulus terrestris* on body composition and exercise performance in resistance-trained males. *International Journal of Sport Nutrition and Exercise Metabolism* 10, 208–215.
- Arcasoy, H.B., Erenmemisoglu, A., Tekol, Y., Kurucu, S., Kartal, M., 1998. Effect of *Tribulus terrestris* L. saponin mixture on some smooth muscle preparations: a preliminary study. *Bollettino Chimico Farmaceutico* 137, 473–475.
- Beckham, S.G., Earnest, C.P., 2003. Four weeks of androstenedione supplementation diminishes the treatment response in middle-aged men. *British Journal of Sports Medicine* 37, 212–218.
- Blight, L.F., Judd, S.J., White, G.H., 1989. Relative diagnostic value of serum non-SHBG-bound testosterone, free androgen index and free testosterone in the assessment of mild to moderate hirsutism. *Annals of Clinical Biochemistry* 26, 311–316.
- Brown, G.A., Vukovich, M.D., Martini, E.R., Kohut, M.L., Franke, W.D., Jackson, D.A., King, D.S., 2001. Effects of androstenedione-herbal supplementation on serum sex hormone concentrations in 30–59 years old men. *International journal for vitamin and nutrition research* 71, 293–301.
- Brown, G.A., Vukovich, M.D., Reifenrath, T.A., Uhl, N.L., Parsons, K.A., Sharp, R.L., King, D.S., 2000. Effects of anabolic precursors on serum testosterone concentrations and adaptations to resistance training in young men. *International Journal of Sport Nutrition and Exercise Metabolism* 10, 340–359.
- Cai, L., Wu, Y., Zhang, J., Pei, F., Xu, Y., Xie, S., Xu, D., 2001. Steroidal saponins from *Tribulus terrestris*. *Planta Medica* 67, 196–198.
- Conrad, J., Dinchev, D., Klaiber, I., Mika, S., Kostova, I., Kraus, W., 2004. A novel furostanol saponin from *Tribulus terrestris* of Bulgarian origin. *Fitoterapia* 75, 117–122.
- De Combarieu, E., Fuzzati, N., Lovati, M., Mercalli, E., 2003. Furostanol saponins from *Tribulus terrestris*. *Fitoterapia* 74, 583–591.
- Gauthaman, K., Adaikan, P.G., Prasad, R.N., 2002. Aphrodisiac properties of *Tribulus Terrestris* extract (Protodioscin) in normal and castrated rats. *Life Sciences* 71, 1385–1396.
- Gauthaman, K., Ganesan, A.P., Prasad, R.N., 2003. Sexual effects of puncturevine (*Tribulus terrestris*) extract (protodioscin): an evaluation using a rat model. *The Journal of Alternative and Complementary Medicine* 9, 257–265.
- Horton, R., Tate, J.F., 1966. Androstenedione production and interconversion rates measured in peripheral blood and studies on the possible site of conversion to testosterone. *The Journal of Clinical Investigation* 45, 301–313.
- Huang, J.W., Tan, C.H., Jiang, S.H., Zhu, D.Y., 2003. Terrestriins A and B, two new steroid saponins from *Tribulus terrestris*. *Journal of Asian Natural Products Research* 5, 285–290.
- Kohut, M.L., Thompson, J.R., Campbell, J., Brown, G.A., Vukovich, M.D., Jackson, D.A., King, D.S., 2003. Ingestion of a dietary supplement containing dehydroepiandrosterone (DHEA) and androstenedione has minimal effect on immune function in middle-aged men. *Journal of the American College of Nutrition* 22, 363–371.
- Leder, B.Z., Longcope, C., Catlin, D.H., Ahrens, B., Schoenfeld, D.A., Finkelstein, J.S., 2000. Oral androstenedione administration and serum testosterone concentrations in young men. *The Journal of the American Medical Association* 283, 779–782.
- Longcope, C., Kato, T., Horton, R., 1969. Conversion of blood androgens to estrogens in normal adult men and women. *The Journal of Clinical Investigation* 48, 2191–2201.

- Lu, S.F., Mo, Q., Hu, S., Garippa, C., Simon, N.G., 2003. Dehydroepiandrosterone upregulates neural androgen receptor level and transcriptional activity. *Journal of Neurobiology* 57, 163–171.
- Phillips, G.B., 1996. Relationship between serum dehydroepiandrosterone sulfate, androstenedione, and sex hormones in men and women. *European Journal of Endocrinology* 134, 201–216.

- Selby, C., 1990. Sex hormone binding globulin: origin, function and clinical significance. *Annals of Clinical Biochemistry* 27, 532–541.
- Weinstein, R.L., Kelch, R.P., Jenner, M.R., Kaplan, S.L., Grumbach, M.M., 1974. Secretion of unconjugated androgens and estrogens by the normal and abnormal human testis before and after human chorionic gonadotropin. *The Journal of Clinical Investigation* 53, 1–6.