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The effects of milled *Tribulus terrestris*, *Avena sativa*, and white ginseng powder on total cholesterol, free testosterone levels and testicular tissue in rats fed a high-cholesterol diet

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Summary: This study examines the effects of milled *Tribulus terrestris* (TT), *Avena sativa* (AS), white ginseng (WG) and triple-combination (TC) powders on sexual dysfunction parameters – such as serum total cholesterol, free testosterone levels and histopathological changes in testicular tissue – in rats fed a high-cholesterol diet. The study's animal material consisted of 42 male Wistar albino rats weighing 200–210 g divided into six groups. Group I was fed normal pellet feed, while the remaining groups were fed pellet feed containing 2% cholesterol. Group III, IV, V, and VI also received 0.6 g/kg/day of TT, 0.3 g/kg/day of AS, 0.2 g/kg/day of WG and 0.55 g/kg/day of TC (7.5% TT, 3.75% AS, 2.5% WG), respectively. After 90 days, the rats were sacrificed and blood and testicular tissue samples obtained. Serum total cholesterol and free testosterone levels were measured, and the Johnsen testicular biopsy score (JTBS) was calculated by a histopathological examination of testicular tissue samples. The high-cholesterol diet in Group II significantly caused increase in total cholesterol level and decrease in JTBS as compared to Group I. Although the groups' free testosterone levels were not statistically significant, WG and TC significantly prevented total cholesterol increase. TC significantly increased the JTBS compared to TT, AS and WG alone. Thus, it was concluded that TC might be particularly efficient for improving male sexual dysfunction.

Keywords: *Avena sativa*, ginseng, hypercholesterolemia, testosterone and sexual function, *Tribulus terrestris*.

Yüksek kolesterollü diyetle beslenen ratlarda öğütülmüş *Tribulus terrestris*, *Avena sativa* ve beyaz ginseng tozunun total kolesterol, serbest testosteron düzeyleri ve testis dokusu üzerine etkileri

Özet: Bu çalışma, öğütülmüş *Tribulus terrestris* (TT), *Avena sativa* (AS), beyaz ginseng (WG) ve üçlü kombinasyon (TC) tozlarının, yüksek kolesterollü diyetle beslenen ratlarda serum total kolesterol, serbest testosteron düzeyleri ve testis dokusunda histopatolojik değişiklikler gibi seksüel fonksiyon bozukluğu parametrelerine etkilerini araştırmak amacıyla yapılmıştır. Araştırmanın hayvan materyali 6 gruba ayrılan, 200-210 g ağırlığında, 42 adet erkek Wistar albino rattan oluştu. Grup I normal pelet yemle beslendi, buna karşın kalan gruplar %2 kolesterol içeren pelet yemle beslendi. Group III, IV, V, ve VI ayrıca sırasıyla 0.6 g/kg/gün TT, 0.3 g/kg/gün AS, 0.2 g/kg/gün WG ve 0.55 g/kg/gün TC (%7.5 TT, %3.75 AS, %2.5 WG) aldı. Doksan gün sonra ratlara ötenazi uygulandı ve kan ve testis doku örnekleri alındı. Serum total kolesterol ve serbest testosteron düzeyleri ölçüldü ve Johnsen testis biyopsi skoru (JTBS), testis doku örneklerinin histopatolojik muayenesi ile hesaplandı. Grup II'de yüksek kolesterollü diyet Grup I'e kıyasla önemli düzeyde total kolesterol düzeyinde artışa ve JTBS'de azalmaya neden oldu. Grupların serbest testosteron düzeyleri istatistiksel olarak önemli olmamasına rağmen, WG ve TC total kolesterol artışını önemli düzeyde önledi. TC tek başına TT, AS ve WG'ye kıyasla JTBS'yi önemli düzeyde artırdı. Dolayısıyla erkek cinsel fonksiyon bozukluğunu iyileştirmek için TC'nin özellikle etkili olabileceği kanısına varıldı.

Anahtar sözcükler: *Avena sativa*, ginseng, hiperkolesterolemi, testosteron ve seksüel fonksiyon, *Tribulus terrestris*.

Introduction

Male sexual dysfunction (MSD), such as erectile dysfunction (ED), is a common concern leading to sexual intercourse problems and decreasing quality of life (6). Chronic heart failure, high cholesterol, diabetes, smoking, alcoholism or drug dependence, stress, eating habits and increased age are among the factors that cause MSD. In epidemiologic studies, MSD appeared in high rates in developed countries is considered an important health concern (6).

In traditional folk medicine, *Tribulus terrestris* (TT), *Avena sativa* (AS) and white ginseng (WG) are used to improve sexual functions in humans. These substances also have been reported to have some aphrodisiac activities (12).

TT is a flowery plant belonging to Zygophyllaceae that grows in Mediterranean, subtropical and desert climates worldwide. In traditional medicine, it has been used as a treatment for urinary tract infections and as an aphrodisiac since ancient times (1). It is also revealed to decrease cholesterol and blood lipids (9). TT includes glycoside, alkaloid, flavonoids, and steroidal saponins (4). Protodioscin in TT extract increases testosterone levels by enhancing dehydroepiandrosterone (DHEA) in the body (5). Furthermore, ayurvedic formulations are used for the treatment of MSD (7).

AS is grown worldwide and is used as dietary supplements. It has various activities in the body such as antioxidant, anti-inflammatory, immunomodulatory, antidiabetic and anticholesterolaemic (22). Thus, AS has a beneficial role in human health (16).

Ginseng is used to promote human health for many years. It contains tetracyclic triterpenoid saponins. Ginseng has been used for the treatment of ED in traditional Chinese medicine for many years (13). Chen and Lee (3) have revealed that ginsenosides relax the corpus cavernosum via nitric oxide released from endothelial and neural cells, clarifying the aphrodisiac effects of *Panax ginseng*. In addition, black ginseng extracts have been found to decrease the total serum cholesterol and low-density lipoprotein levels in male rats fed a high-cholesterrol diet (19).

The extracts of TT, AS, WG and triple combinations (TC) have been sold in tablet form recently. This study utilized the milled TT, AS and WG powders, which are cheap and easily supplied, in the rates of extracts in a commercial product.

This study evaluates the effects of TT, AS, WG and TC on serum total cholesterol and free testosterone parameters and testicular tissue in male rats fed a high–cholesterol diet.

Materials and Methods

Experimental animals: This study was conducted at KOBAY Laboratories using 42 male Wistar albino rats, aged 2.5 months and weighing 200-210 g. The rats were kept under standard conditions (12 hours light and 12 hours dark at 25 °C), and acclimatized for 15 days. The study was approved by Kobay's Local Ethics Committee (26.06.2012, Protocol No: 41). In this study, six groups of rats were fed different diets for 90 days ad libitum. The control group (Group I) was fed a normal pellet feed. The diets of the remaining five groups were prepared manually and contained 2% cholesterol (Group II), 2% cholesterol + 15% TT (Group III), 2% cholesterol + 7.5% AS (Group IV), 2% cholesterol + 5% WG (Group V) and 2% cholesterol + 7.5% TT + 3.75% AS + 2.5% WG (Group VI) (19). Consumed daily diet amount of animals was measured.

At the end of the study, the rats were anaesthetised using ketamine (90 mg/kg) and xylazine (10 mg/kg), and blood samples were collected from the *aorta abdominalis*. Then, the rats were sacrificed, and testicular tissues were obtained. These tissues were fixed in a 10% neutral buffered formalin solution.

Blood samples were centrifuged at 3000 rpm for 10 minutes. The serum total cholesterol (COBAS-C501, Roche, Diagnostics GmbH D-68298 Mannheim, Germany) and free testosterone (Cusabio ELISA kit, Bayer, Tarrytown, NY, USA) levels were measured.

After a routine tissue follow-up, the testicular tissues were embedded in paraffin blocks, cut into 5 μm sections, stained with haematoxylin-eosin (H&E) and examined under a Nikon Eclipse Ni microscope. In the evaluation of the testicular tissues, a Johnsen testicular biopsy score (JTBS) was used (8). According to JTBS, the scoring was as follows: complete spermatogenesis and many spermatozoa – 10; mild impairment of spermatogenesis and many late spermatids – 9; fewer than five spermatozoa in each tubule and few late spermatids – 8; azoospermia, no late spermatids, many early spermatids –7; azoospermia, no late spermatids, few early spermatids – 6; azoospermia or no spermatids, many spermatocytes – 5; azoospermia or no spermatids, few spermatocytes – 4; spermatogonia only – 3; no germinal cells, sertoli cells only – 2; no seminiferous epithelium – 1.

Used substances:

Cholesterol: Two percent (2%) cholesterol was added to the pellet feed of five groups in this study (Sigma Aldrich C-75209).

Milled seeds and roots: The milled forms of 15% TT, 7.5% AS and 5% WG root were added to the pellet feed containing 2% cholesterol. The feed of the TC group was supplemented with 7.5% TT, 3.75% AS and 2.5% WG. The amounts of milled feeds and roots prepared by Ipekyolu Baharat, an Isparta company, included 400 mg TT, 200 mg AS and 150 mg WG similar the amounts of Kibarli Panax produced by Kibarli Bitkisel Takviye Urunler Company, Bayrampasa/Istanbul.

The SPSS 20.0 program was used for statistical analyses. It was determined that the parameters examined in this study were not distributed normally by Shapiro-Wilk test. The significance between groups was determined by a Kruskal-Wallis test. A Dunn-Bonferroni test of multiple comparisons was used to determine from which group the significance derived. $P<0.05$ was considered significant.

Results

Biochemical results: The serum total cholesterol and free testosterone levels of the six groups are given in Table 1. The serum total cholesterol levels were determined to significantly increase in Group II compared to Group I

(P<0.05). The serum total cholesterol levels were determined to significantly decrease in Groups V and VI compared to Group II (P<0.05). The serum free testosterone levels were determined not to significantly differ among the groups (P>0.05).

Histopathological results: The JTBS of the rats' testicular tissues are given in Table 1. The JTBS significantly decreased in Group II compared to Group I (P<0.05), significantly increased in Group VI compared to Group II (P<0.05), and had no significant difference between Group I and Group VI (P>0.05).

In the testicular tissue from the control group, the testis was surrounded with tunica albuginea and had normal seminiferous tubules and interstitial areas. There was no increase or decrease in seminiferous tubule dimensions, sloughing of seminiferous epithelium and its degeneration, thickening of tunica propria or degenerative changes of Leydig cells (Figure 1A). In Group II, there were degenerations of seminiferous epithelial cells and decreased numbers and layers of spermatogenic cells (Figure 1B). The testicular histopathology of the other groups had a similar appearance to that of the control group (Figures 1C, D, E and F).

Discussion and Conclusion

In the hyperlipidaemic rats with high serum cholesterol and low density lipoprotein, ED, prostatic enlargement and bladder overactivity were suggested (15). Therefore, balancing blood cholesterol levels or keeping them at a certain level is an important aspect in the treatment of ED. Herbal remedies have been a common treatment. In the treatment of ED, TT (5), AS (12) and WG (10) extracts and ayurvedic combinations (2) are used.

A study carried out on male Wistar albino rats fed a high-cholesterol diet (4% cholesterol and 1% cholic acid) for five months found no significant changes in the serum HDL or triglyceride levels but a significant increase in the serum total cholesterol and LDL levels in the hypercholesterolemic group compared to the control group. In addition, no significant changes were found in the plasma total testosterone and estriol levels, but decreased free testosterone and sex hormone-binding globulin levels were observed (14). In this study, the serum total cholesterol levels were significantly increased in Group II compared to Group I, but there were no significant changes in the free testosterone levels between groups. In addition, the non-significant decrease in the free testosterone level in Group II of the current study might be attributed to the lower diet cholesterol levels and the shorter study period.

Table 1. The effects of TT, AS, WG and triple combination (TC) on total cholesterol, free testosterone and Johnsen testicular biopsy score in rats fed a high-cholesterol diet.

Tablo 1. Yüksek kolesterollü diyetle beslenen ratlarda total kolesterol, serbest testosteron ve Johnsen testis biyopsi skoru üzerine TT, AS, WG ve üçlü kombinasyonun (TC) etkileri.

Parameters	Group I	Group II	Group III	Group IV	Group V	Group VI
	Control	2% Chol	2% Chol + TT	2% Chol + AS	2% Chol + WG	2% Chol + TC
Total cholesterol (mg/dl)	71.00 (56-85)	89.00 (84-107)[a]	74.00 (68-112)	74.00 (67-80)	68.00 (57-89)[b]	71.00 (57-75)[c]
Free testosterone (pg/mL)	22.80 (13.40-30.15)	13.65 (11.80-21.30)	18.40 (13.45-30.25)	21.30 (14.40-29.90)	18.10 (14.15-30.10)	21.45 (16.90-31.80)
Johnsen testicular biopsy score	9.50 (8.95-9.80)	7.55 (6.95-8.15)[d]	8.60 (7.15-8.65)	8.45 (8.15-8.60)	8.45 (8.15-8.60)	9.50 (8.50-9.80)[e]

Notes: Group I: Control group; Group II: Cholesterol-only group; Group III: 2% cholesterol + TT-supplied group; Group IV: 2% cholesterol + AS-supplied group; Group V: 2% cholesterol + WG-supplied group; Group VI: 2 % cholesterol + TC-supplied group. Each group consisted of seven rats. All data were given as: median (minimum-maximum).

[a] P<0.05 compared to Group I; [b] P<0.05 compared to Group II; [c] P<0.05 compared to Group II; [d] P<0.05 compared to Group I; [e] P<0.05 compared to Group II.

Notlar: Grup I: Kontrol grubu; Grup II: Sadece kolesterol grubu; Grup III: %2 kolesterol + TT-uygulanan grup; Grup IV: %2 kolesterol + AS-uygulanan grup; Grup V: %2 kolesterol + WG-uygulanan grup; Grup VI: %2 kolesterol + TC uygulanan grup.

Her bir grup 7 rattan oluştu. Bütün veriler ortanca (minimum-maksimum) olarak verildi.

[a] P<0.05, Grup I'e kıyasla; [b] P<0.05, Grup II'ye kıyasla; [c] P<0.05, Grup II'ye kıyasla; [d] P<0.05, Grup I'e kıyasla; [e] P<0.05, Grup II'ye kıyasla.

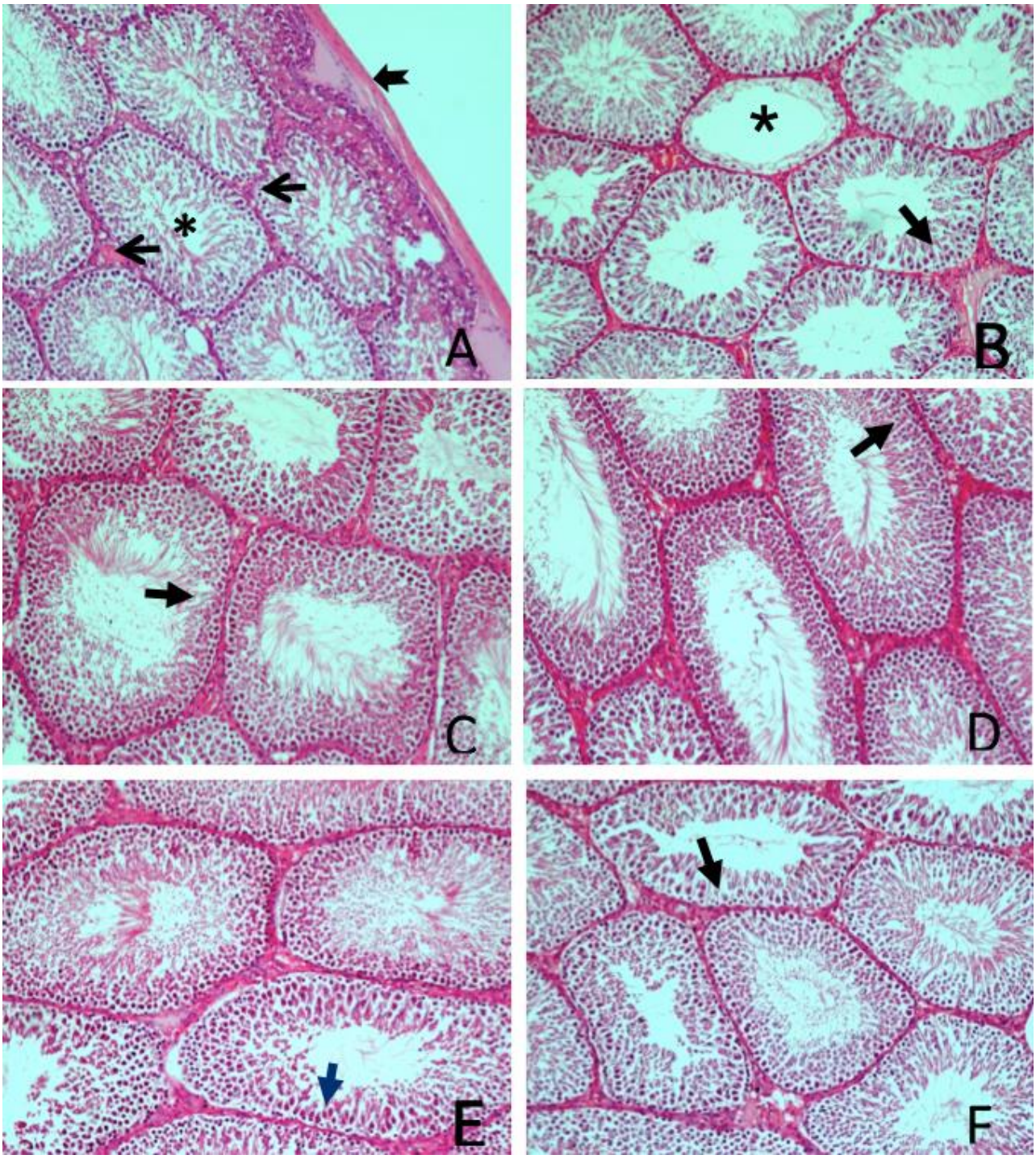

Figure 1. A- The testis of Group I was surrounded with tunica albuginea (thick arrow) with normal seminiferous tubules (asterisk) and interstitial areas (thin arrow). B- In Group II, there were degenerations of seminiferous epithelial cells (asterisk). C, D, E and F- The testicular histopathology of Group III, IV, V and VI had a similar appearance to that of the control group (thin arrow). (H&E, X20).
Şekil 1. A- Grup I'in testisi normal seminifer tübüller (yıldız) ve interstisyel alanlar (ince ok) ile tunika albuginea (kalın ok) ile çevriliydi. B- Grup II'de seminifer epitel hücrelerinde dejenerasyonlar (yıldız) vardı. C, D, E ve F – Grup III, IV, V ve VI'nın testis histopatolojisi kontrol grubunun testis histopatolojisine benzerdi (ince ok). (H&E, X20).

TT extract exhibited enhancement of dihydrotestosterone levels, sperm concentration and motility in infertile men in a clinical trial (20). Similarly, in patients with partial androgen deficiency, TT showed significant increase on serum total and free testosterone and erectile function (17). However, TT was ineffective in the treatment of idiopathic infertility via non-significant changes on serum total and free testosterone, LH and semen parameters (18). Gauthaman and Ganesan (5) have stated that a TT extract of 5 mg/kg taken orally for eight

weeks enhances intracavernous pressure and sexual behaviours in castrated rats and has an aphrodisiac effect due to increased androgen levels. In this study, there were no significant changes in the free testosterone levels between the groups, but a histopathological examination in Group III showed the remedial effects of TT extract against the testicular damage induced by a high-cholesterol diet in rats.

Hypercholesterolemia leads to significant erectile dysfunction (15). In this study, AS prevented hypercholesterolemia induced by the high-cholesterol diet in consistent with the study that dietary oat proteins were revealed to significantly decrease plasma and liver total cholesterol levels (23). In addition, AS revealed positive effects against the testicular damage induced by the high-cholesterol diet as found in Group IV. Thus, AS may exhibit positive effects in ED by hypocholesterolemic effects.

Ginseng is considered a promising agent to enhance general well-being. Also, it is used to treat male sexual dysfunction and to increase sexual behaviour in traditional Chinese medical practice (11). Salvati et al. (21) have stated that an increase in the numbers of total spermatozoa and spermatozoa with forward progression in mL, an increase in plasma total and free testosterone, DHT, FSH and LH levels and a decrease in PRL levels are found in patients treated with *Panax ginseng* CA Meyer extract compared to healthy individuals. Thus, ginsenosides may be effective on a hypothalamus-pituitary-testis axis. In addition, wild ginseng root extracts were found to increase testosterone concentrations and spermatid populations. Accordingly, it has possible positive effects on male reproductive functions by decreasing reactive oxygen species production (24). In the current study, the total cholesterol levels were significantly decreased in Group V compared to Group II, but the free testosterone levels and JTBS were not significantly increased. This suggests that the addition of WG to rat feed may provide a beneficial effect on the improvement of MSD.

In addition, in this study, a triple combination (TC) powder (7.5% TT, 3.75% AS and 2.5% WG) was used in Group VI because it was cheaper and more easily supplied. It was determined that TC most effectively prevented a decrease in JTBS due to hypercholesterolemia in rats fed a high-cholesterol diet. As a result, it was found that TC, compared to only TT, AS or WG supplied in feed, was more effective in increasing JTBS in male Wistar albino rats fed a high-cholesterol diet. Thus, it was concluded that TC might be effective in the improvement of MSD in rats fed a high-cholesterol diet. Also, a further clinical trial similar to this study should be carried out to determine if it would be beneficial to obtain such treatment under the control of a doctor.

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