

ANDROGENIC PROPERTIES OF “DONG TRUNG HA THAO SAPA” HARD CAPSULES ON EXPERIMENTAL ANIMALS

Pham Thi Van Anh¹, Vu Viet Hang¹, Dau Thuy Duong¹, Trinh Vinh Quang¹
Le Quan², Nguyen Huy Van², Nguyen Thi Vinh Hue², Do Tien Sy²
Nguyen Phu Tri², Nguyen Thi Van Anh², Dang Thi Phuong Hao³
Nguyen Thu Minh⁴ and Do Dieu Linh^{1,✉}

¹Hanoi Medical University

²Traphacosapa One member Co - Ltd

³Biopharm Hoa Binh JSC

⁴Bach Mai Hospital

“Dong trung ha thao Sapa” (DTHT) hard capsules prepared from *Cordyceps militaris* powder are intended to treat male hypogonadism. The aim of the current study was to evaluate the androgenic properties of this product on experimental animals. Weanling male rats and castrated peripubertal male rats were used for the study. Weanling male rats were divided into 5 groups: group I (saline-treated); group II (testosterone undecanoate-treated); group III (low dose DTHT capsules); and group IV (high dose DTHT capsules). Castrated peripubertal male rats were also divided into 5 groups: group I without castration (saline-treated); groups II to V were surgically castrated, group II (saline-treated); group III (testosterone undecanoate-treated), group IV (low dose DTHT capsules) and group V (high dose DTHT capsules). The potential effect was evaluated on five androgen-dependent tissue weight (seminal vesicles, ventral prostate, Cowper’s gland, glans penis, levator ani-bulbocavernosus - LABC muscle and the serum testosterone level in castrated rats. Five tissue weights and testes, epididymis weights were examined in weanling rats. A positive androgen agonist result should be a statistically significant increase ($p \leq 0.05$) in any two or more of the five targets’ androgen-dependent tissue. The results suggest that capsules show androgenic properties in castrated male rats by increasing weight of 3 tissues (seminal vesicles, ventral prostate, LABC muscle). DTHT hard capsules appear to be an effective therapeutic drug for treating hypogonadism.

Keywords: *Cordyceps militaris*, hard capsules, hypogonadism, weanling rats, castration, testosterone undecanoate.

I. INTRODUCTION

Male hypogonadism is a disorder associated with decreased functional activity of the testes, with reduced production of androgens and/or impaired sperm production. This is caused by poor testicular function or inadequate stimulation of the testes by the hypothalamic-pituitary axis.¹

Corresponding author: Do Dieu Linh

Hanoi Medical University

Email: dieulinhdo234@gmail.com

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The prevalence of hypogonadism increases with age and the major causes are central obesity, co-morbidities (e.g., diabetes) and overall poor health.² In healthy aging men there is only a small gradual decline in testosterone; up to the age of 80 years, age accounts for a relatively low percentage of hypogonadism.² In men aged between 40 and 79 years the incidence of symptomatic hypogonadism varies between 2.1 - 5.7%. The incidence of hypogonadism has been reported to be 12.3 and 11.7 cases per 1,000 people per year.³

Testosterone replacement therapy (TRT) is used in male patients with a deficiency or low testosterone level identified by clinical and biochemical symptoms. However, before prescribing TRT, one must be conscientious of its adverse effects linked to prostate cancer, benign prostatic hyperplasia, polycythemia and obstructive sleep apnea. Thus, nowadays, a popular trend is discovering and studying medicinal products derived from traditional herbs.

Cordyceps species, including *Cordyceps Sinensis* (CS), *Cordyceps militaris*, and others, are valuable traditional medicinal materials from *Ascomycetes* fungus parasitic to *Lepidoptera larvae*. CS has been traditionally used to enhance sexual performance and restate impaired sexual function in Chinese society.⁴ Due to the overexploitation process, CS is now in danger of extinction. Besides, *Cordyceps militaris* is considered as a species containing the same nutritional and medicinal ingredients as CS. However, no study has provided reliable evidence of their effects on sexual and reproductive functions. Therefore, the purposes of this study were to evaluate the androgenic properties of *Cordyceps militaris* in castrated male rats and weanling male rats.

II. MATERIALS AND METHODS

1. Research sample

DTHT hard capsules were provided by Traphaco Sapa Company. Each capsule contained 350 mg of *Cordyceps militaris* powder combined with several excipients including PVP, Amidon and stearate. Cordycepin and adenosine are effective components isolated from *Cordyceps militaris*. Adenosine consists of adenine attached to a ribose via a β -N9-glycosidic bond. Cordycepin, 3'-deoxyadenosine, is also known as an adenosine analog. Cordycepin from the *Cordyceps militaris* has been reported to have

acute anti-inflammatory, anti-nociceptive, anti-angiogenesis, and immunoregulatory activities.⁵ These two components in a DTHT hard capsule are 4.68 and 0.67 mg, respectively.

Andriol Testocaps, manufactured by Catalent France Beinheim have 3 blister each containing 10 capsules. For oral use, 1 capsule of Andriol Testocaps contains 40 mg testosterone undecanoate.

Research location: The study was conducted at the Laboratory of the Department of Pharmacology, Hanoi Medical University.

2. Experimental animals

Wistar male rats 42 - 50 days old, were used in our castrated rats study. The animals are castrated under anesthesia by placing an incision in the scrotum and removing both testes and epididymides with ligation of blood vessels and seminal ducts.⁶

Wistar male rats of 20-34 days old were used in our weanling rats study.⁷

All procedures conformed to all local standards of laboratory animal care. The temperature in the experimental animal room should be $22^{\circ}\text{C} \pm 3^{\circ}\text{C}$. The relative humidity should be within 30% a to 70%, other than during room cleaning. The aim should be relative humidity of 50- 60%. Lighting should be artificial. The daily lighting sequence should be 12 hours light and 12 hours dark. Water was provided *ad libitum*.

Before the experiment was carried out, rats were adapted to their laboratory condition within ten days.

3. Experimental design

3.1. Androgenic properties of DTHT capsules in castrated rats

Fifty male Wistar rats were fed in the laboratory according to standard criteria for 10 days, then divided into five groups. Group I was not

castrated. Group II to V was castrated, removing two testicles on both sides. The animals continue acclimation to the laboratory conditions to allow for the regression in the target tissue weights for seven days following castration.⁶

Group I: Control - The male rats were given orally distilled water (10 ml/kg b.wt.).

Group II: The rats were given orally distilled water (10 ml/kg b.wt.).

Group III: Positive control - The rats were given testosterone undecanoate orally of 19.2 mg/kg b.wt./day.

Group IV: DTHT capsules were given orally at 252 mg/kg b.wt./day.

Group V: DTHT capsules were given orally at 756 mg/kg b.wt./day.

Rats were treated orally for ten days. On day 11 (24 hours after the last dose), the rats were weighed then autopsied. Blood samples from the carotid artery were collected and kept standing for 15 min to clot, then centrifuged at 3000 rpm for 10 min to separate the serum, which was kept frozen at -70°C . The serum was used to estimate the testosterone levels. Five androgen-dependent tissues (seminal vesicles, ventral prostate, Cowper's gland, glans penis, LABC muscle) were dissected and weighed.

3.2. Androgenic properties of DTHT capsules in weanling rats

Forty male Wistar rats were fed in the laboratory according to standard criteria for ten days, then divided into four groups.⁷

Group I: Control - The male rats were given orally distilled water (10 ml/kg b.wt.) for 10 days.

Group II: Positive control - The animals were given testosterone undecanoate orally 19.2 mg/kg b.wt./day for 10 days.

Group III: DTHT capsules were given orally at 252 mg/kg b.wt./day for 10 days.

Group IV: DTHT capsules were given orally at 756 mg/kg b.wt./day for 10 days.

On day 11 (24 hours after the last dose), the rats were weighed and then necropsied. Blood samples from the carotid artery were collected and kept standing for 15 min to clot, then centrifuged at 10,000 rpm for 10 min to separate the serum, which was kept frozen at -70°C . The serum was used for the estimation of testosterone levels. Seven androgen-dependent tissues (testes, epididymis, seminal vesicles, ventral prostate, Cowper's gland, glans penis, LABC muscle) were dissected and weighed.

4. Statistical analysis

Data were analyzed by the T-test using Microsoft Excel software version 2010. Data were presented as a mean \pm standard deviation. A p-value of less than 0.05 is statically significant.

III. RESULTS

1. Androgenic properties of DTHT capsules in castrated rats

1.1. Secondary sex organs weight

Table 1. Effect of DTHT capsules on weights of sexual organs in castrated rats

Group	Weight (mg/100g b.wt)				
	Seminal vesicles	Ventral prostate	Glans penis	Cowper's glands	LABC
Group I	73.66 ± 16.96	57.06 ± 7.36	52.23 ± 7.98	15.93 ± 3.55	221.80 ± 56.33
Group II	16.78 ± 4.20	3.53 ± 1.68	27.29 ± 7.34	2.54 ± 0.96	59.64 ± 10.62
p ₂₋₁	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Group III	73.27 ± 14,54	36.24 ± 13.74	54.09 ± 10.16	14.28 ± 4.51	171.94 ± 63.91
p ₃₋₂	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
p ₃₋₁	> 0.05	< 0.001	> 0.05	> 0.05	> 0.05
Group IV	22.06 ± 4.02	9.38 ± 3.48	27.32 ± 7.55	3.24 ± 1.12	71.91 ± 12.42
p ₄₋₂	< 0.05	< 0.001	> 0.05	> 0.05	< 0.05
Group V	21.52 ± 5.68	8.72 ± 3.29	25.33 ± 6.86	3.18 ± 1.39	74.50 ± 18.94
p ₅₋₂	< 0.05	< 0.001	> 0.05	> 0.05	< 0.05

The sexual organs weight in group 2 (castrated rats) decreased significantly compared to group I (non-castrated rats) ($p < 0.0001$). There was a dramatic increase in the the sexual organs weight in the group using testosterone compared to group II ($p < 0.0001$).

Castrated rats using *Cordyceps militaris* in two doses for ten days had increased weight of seminal vesicles ($p < 0.05$), ventral prostate ($p < 0.001$) and LABC muscle ($p < 0.05$) compared to group II (Table 1).

1.2. Serum testosterone

Table 2. Effect of DTHT capsules on serum testosterone in castrated rats

Group	Testosterone (nmol/L)
Group I	5.392 ± 2.302
Group II	0.734 ± 0.595
p ₂₋₁	< 0.001
Group III: Testosterone	7.055 ± 5.545
p ₃₋₂	< 0.001
Group IV: DTHT 252 mg/kg b.wt./day	0.757 ± 0.674
p ₄₋₂	> 0.05

Group	Testosterone (nmol/L)
Group V: DTHT 756 mg/kg b.wt./day	0.806 ± 0.661
p ₅₋₂	> 0.05

The serum testosterone of male castrated rats in group II decreased significantly compared to the control group I (non-castrated rats) (p < 0.001). Castrated rats using testosterone increased serum testosterone

considerably compared to group II (p < 0.001). The serum testosterone of castrated rats in the groups using *Cordyceps militaris* increased as compared with group II but no significant action was observed (p > 0.05) (Table 2).

2. Androgenic properties in weanling rats

2.1. Testes and epididymis weight

Table 3. Effect of DTHT capsules on weights of testes, epididymis on weanling rats

Group	Weight (mg/100g b.wt)	
	Testes	Epididymis
Group I	1385.65 ± 296.15	235.55 ± 63.85
Group II: Testosterone	1781.76 ± 484.60	289.71 ± 59.23
p ₂₋₁	< 0.05	< 0.05
Group III: DTHT 252 mg/kg b.wt./day	1275.18 ± 254.44	228.68 ± 66.04
p ₃₋₁	> 0.05	> 0.05
Group V: DTHT 756 mg/kg b.wt./day	1340.83 ± 331.68	224.41 ± 64.45
p ₄₋₁	> 0.05	> 0.05

Group II using testosterone, testes and epididymis weights increased significantly compared to the control group I (p < 0.05). Testes and epididymis weights in weanling

rats using *Cordyceps militaris* in two doses did not have a statistical difference compared with group I (p > 0.05) (Table 3).

2.2. Secondary sex organs

Table 4. Effect of DTHT capsules on weights of sexual organs in weanling rats

Group	Weight (mg/100g b.wt)				
	Seminal vesicles	Ventral prostate	Glans penis	Cowper's glands	LABC
Group I	49.94 ± 8.87	32.64 ± 5.33	40.21 ± 9.78	8.81 ± 2.24	102.99 ± 14.89
Group II	114.04 ± 33.99	50.59 ± 14.70	61.49 ± 13.17	16.70 ± 4.06	201.10 ± 43.35
p ₂₋₁	< 0.001	< 0.01	< 0.001	< 0.001	< 0.001

Group	Weight (mg/100g b.wt)				
	Seminal vesicles	Ventral prostate	Glans penis	Cowper's glands	LABC
Group III	47.77 ± 9.45	32.15 ± 4.84	40.99 ± 5.70	8.01 ± 2.16	101.00 ± 17.46
p ₃₋₁	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
Group IV	47.18 ± 11.03	32.83 ± 6.82	41.27 ± 9.23	8.71 ± 2.53	101.74 ± 30.98
p ₄₋₁	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

The weight of sexual organs in group II increased significantly compared to group I ($p < 0.01$; $p < 0.001$). In the groups using *Cordyceps militaris*, sexual organs weight did not have a statistical difference compared with group I ($p > 0.05$) (Table 4).

2.3. Serum testosterone

Table 5. Effect of DTHT capsules on serum testosterone in weanling rats

Group	Testosterone (nmol/L)
Group I	5.11 ± 4.32
Group II	10.43 ± 5.00
p ₂₋₁	< 0.05
Group III: DTHT 252 mg/kg b.wt./day	6.26 ± 4.88
p ₃₋₂	> 0.05
Group IV: DTHT 756 mg/kg b.wt./day	5.58 ± 3.52
p ₄₋₂	> 0.05

In group II, the serum testosterone of weanling rats increased significantly compared to the control group I ($p < 0.05$). The serum testosterone in the groups using *Cordyceps militaris* did not have a statistical difference compared with group I ($p > 0.05$) (Table 5).

IV. DISCUSSION

The study was carried out according to the Hershberger model, which is considered the most valuable and widely used experimental model to demonstrate the androgenic properties of drugs. Models were carried out in male experimental animals with minimal endogenous androgen production, including the castrated peripubertal male rats according to OECD 441 and weanling male rats according to OECD 115.^{6,7} In OECD 441, castrated peripubertal male rats with removed testes and epididymides, eliminate endogenous

testosterone and the feedback mechanism of the hypothalamic-pituitary-testicular axis while secondary sex organs are not fully developed, eliminating the difference in testosterone levels between rats, thereby detecting active substances with weak androgenic properties.⁶ The Hershberger Bioassay in weanling male rats according to OECD 115 detect only substances with moderate or potent androgenic properties.⁷

The study used testosterone undecanoate orally at 19.2 mg/kg b.wt./day as a positive control for ten days. The study results

showed that testosterone at this dose showed androgenic properties in both castrated male rats and weanling male rats compared to untreated rats.

Seminal vesicles, ventral prostate, Cowper's gland, penis and LABC muscle were studied. These are five androgen-dependent tissues. Testes and epididymides were added for the weaning male rat model study. According to OECD, when the weight of at least two of the five organs above is statistically significant compared with the groups of untreated rats, the drug is considered to have androgenic properties.^{6,7}

In castrated rats model, capsules at 252 and 756 mg/kg b.wt./day for 10 days increased the weight of 3 secondary sex organs (seminal vesicles, ventral prostate, LABC muscle). Thus, the two doses exhibited androgenic properties in castrated male rats.

Based on the above results, the selected dose of capsules in rats for further studies was 252 mg/kg. The recommended human dose of DTHT capsules for adult male patients is six capsules of 500 mg per day.

Another indicator in the study of androgenic properties is the serum testosterone in rats.^{6,7} The study results showed that the group that used testosterone undecanoate orally had significantly higher serum testosterone levels. In both castrated and weanling rats models, DTHT capsules in two doses for ten days showed increased of serum testosterone but did not statistically differ. This showed that DTHT hard capsules did not increase or only slightly increase serum testosterone in both models.

What mechanism does the DTHT capsule increase testosterone in the rat's serum?

The hypothalamic-pituitary-gonadal axis is mainly regulated through LH (released from the pituitary gland) and LH receptors on the

testicular membrane-bound surface. LH induces the release of cyclic AMP (cAMP), which leads to the activation of protein kinase A (PKA) and the expression of steroidogenic enzymes. According to Kasuma (2021), *Cordyceps militaris* increased the release of LH, which in turn resulted in increased testosterone levels.¹¹ In the castrated rat model, whether the DTHT capsule can stimulate the testes to produce testosterone was not proven. Meanwhile, in the weaning male rat model, the hypothalamic-pituitary-gonadal axis remained intact. Therefore, the possibility that DTHT increases testosterone according to this mechanism cannot be excluded. With the second mechanism, is DTHT capsule a foreign testosterone or not? Sterols are important components with many physiological functions that have been found in *Cordyceps* mushrooms including ergosterol, H1-A and several other sterols. Y. H. Li and X. L. Li (2015) determined the ergosterol content in *Cordyceps sinensis* by HPLC method and obtained high yields. Ergosterol is an important raw material in the production of steroid hormone drugs. H1-A is another sterol found in *Cordyceps* mushrooms, and its structure is similar to testosterone and dehydroepiandrosterone.¹² Therefore, DTHT capsules can also have these components.

Finally, the pathway leading to androstenedione and androstenediol formation in the adrenal gland is the same as in the testis. Four enzymes play an important role in the biosynthesis of testosterone in the testes and the adrenal glands: CYP11A1, 3 β -HSD, CYP17A1 and 17 β -HSD. According to a study by Jian Wang (2016) on the protective effect of *Cordyceps militaris* against bisphenol A-induced reproductive damage, bisphenol A is considered a potent endocrine disruptor that can inhibit testosterone production by inhibiting CYP11A1, 3 β -HSD, CYP17A1 and 17 β -HSD. This study suggests that *C. militaris* can directly modulate

CYP11A1, 3 β -HSD and CYP17A1 expression, thereby attenuating the BPA-induced decline in testosterone synthesis.¹³ DTHT capsules can also cause an increase in testosterone through this mechanism.

As indicated earlier, *Cordyceps militaris* is a traditional herbal medicine with many benefits such as acute anti-inflammatory, anti-nociceptive, anti-angiogenesis, and immunoregulatory activities. This study is about the androgenic properties of *Cordyceps militaris* on experimental animals, and the results showed a good effect. In future clinical studies, extensive studies on the mechanism of DTHT capsules will strengthen the basis for its use in male hypogonadism treatment.

V. CONCLUSION

DTHT hard capsules at 252 mg/kg b.wt./day and 756 mg/kg b.wt./day for ten days show androgen properties on castrated rats proven by increased weight of 3 sexual organs (seminal vesicles, ventral prostate, LABC muscle). The result from this study would suggest further study on treating male hypogonadism .

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