ANDROGENIC EFFECTS OF ALCOHOLIC EXTRACT FROM *CNIDIUM MONNIERI* (L.) CUSS FRUITS IN EXPERIMENTAL ANIMALS

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Traditional medicine used the fruits of Cnidium monnieri (L.) Cuss to treat male hypogonadism and male infertility. This study was carried out to investigate the potential androgenic properties of the alcoholic extract of Cnidium monnieri (L.) Cuss fruits (CFE) in peripubertal castrated male rats. Rats were randomly divided into 5 groups and castrated 7 days before being administered 0.5% CMC, testosterone and CFE at 50 mg/kg/day, 150 mg/kg/day and 250 mg/kg/day, respectively, for 10 days. The relative weight of five target androgen-dependent organs (including seminal vesicles (SV), ventral prostate (VP), paired Cowper's glands (COW), glans penis (GP) and levator ani-bulbocavernosus (LABC) muscle and blood testosterone level were evaluated. Our results showed that CFE at 150 mg/kg/day and 250 mg/kg/day increased the weights of two or more target organs and blood testosterone level. In conclusion, CFE at both doses of 150 mg/kg/day and 250 mg/kg/day has the androgenic activity in male rats.

Keywords: *Cnidium monnieri* (L.) Cuss fruit, target androgen-dependent organs, testosterone, *Wistar* rats.

I. INTRODUCTION

Androgens, produced mostly by the testes, play a vital role in male reproductive and sexual functions. Androgens are crucial for the development of male reproductive organs, such as the epididymis, vas deferens, seminal vesicle, prostate and penis.[1] Male hypogonadism is a clinical syndrome caused by deficient androgen production, which can negatively affect the functions of many organs and the patient's quality of life.[1,2] This condition can manifest at any time in life but increases with age and contributes to male infertility.[2] It is diagnosed on the basis of clinical signs and symptoms related to androgen deficiency.[1]

Male hypogonadism is associated with several physical and psychological symptoms that requires long term testosterone treatment. The goal of testosterone treatment is to restore the symptoms, improves the sexual functions and maintains well-being. However, systemic testosterone replacement therapy can produce adverse effects, including effects on prostate glands, mammary glands, liver and the cardiovascular system.[1,2]

Herbal plants have been used for a long time to improve the male sexual and reproductive function in countries with long-standing traditional medicine. Many herbal products are also marketed with the treatment goal of increasing testosterone levels, including *Allium*

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cepa L., *Zingiber officinale*, *Trigonella foenum-graecum*, *Tribulus Terrestris*, and *Nigella sativa*…[3]

Traditional medicine in some Asian countries, including China, Vietnam indicates that dried fruits of *Cnidium monnieri (L.)* Cuss are used to treat male hypogonadism and male infertility. According to Do Tat Loi, the fruit of *Cnidium monnieri (L.)* Cuss has a bitter and spicy taste, enters the kidney and triple burner meridians. It has the effect of strengthening yang, benefitting the kidneys, eliminating wind and dampness, and is used to treat impotence.[4]

Previous results from several researchers have shown that osthole (a compound in the fruit of *Cnidium monnieri (L.)* Cuss) has the effect of relaxing the smooth muscle of the corpus cavernosum, increased the levels of testosterone, LH, FSH and NOS activity, increases the expression of androgen receptors in the testes. [5,6,7] There have been limited studies to identify the efficacy of *Cnidium monnieri (L.)* Cuss fruits harvested in Vietnam. Therefore, to provide evidence of the androgenic activity of *Cnidium monnieri (L.)* Cuss fruit extract, as a basis for further studies, this study was conducted to investigate the potential androgenic properties of the alcoholic extract of *Cnidium monnieri* (L.) Cuss fruits in peripubertal castrated male rats.

II. MATERIALS AND METHODS

1. Subjects

The investigational product

The alcoholic extraction of *Cnidium monnieri* (L.) Cuss fruits (CFE) was performed at the Institute of Natural Products Chemistry - Vietnam Academy of Science and Technology. It was mixed with 0.5% carboxymethyl cellulose (CMC) before being orally given to the animals.

Experimental animals

Healthy male *Wistar* rats were provided by Vietnam Military Medical University to ensure that the rats were 42 to 50 days of age when they were enrolled in the study. Rats were housed at the Pharmacology Department, Hanoi Medical University. They were acclimatized for seven days prior to the research and maintained in specific standard conditions.

2. Methods

Androgenic effects of CFE in castrated male rats were evaluated according to Hershberger method in castrated male rats.[8]

Immature male rats (42 to 50 days of age) were anesthetized by intraperitoneal injection of diazepam (10 mg/kg/b.w) and ketamine (50 mg/kg b.w). The testicles were removed on both sides. A single dose of amikacin (100 mg/kg b.w) was injected to rats to prevent infection and a single dose of piroxicam (3 mg/kg b.w) was injected to rats to reduce postoperative pain right after being castrated. The castrated animals were used for the experiments seven days later. Rats were randomly divided into 5 groups:

- Group 1: were administered 0.5% CMC.
- Group 2: were administered testosterone propionate 0.4 mg/kg b.w/day.
- Group 3: were administered CFE 50 mg/kg b.w/day.
- Group 4: were administered CFE 150 mg/kg b.w/day.
- Group 5: were administered CFE 250 mg/kg b.w/day.

Rats were administered once a day in ten days. Twenty-four hours after the last dose, rats were weighed and sacrificed. Blood samples were collected and centrifuged to separate serum for testosterone concentration test. Five target androgen-dependent organs (including seminal vesicles (SV), ventral prostate (VP),

paired Cowper's glands (COW), glans penis (GP) and levator ani-bulbocavernosus (LABC) muscle were dissected and weighed.

Research parameters included rat body weight (g), relative organ weights (mg/100 g b.w), blood testosterone concentration (nmol/l).

III. RESULTS

Table 1. Effects of CFE on rat's body weight

Group	Body weight (g)		% weight gain
	Before treatment	After treatment	
Group 1: untreated group	76.53 ± 3.16	126.84 ± 6.32	65.70 ± 4.80
Group 2: testosterone-treated group	75.92 ± 3.12	121.22 ± 4.44	60.06 ± 2.81
Group 3: 50 mg/kg CFE-treated group	77.14 ± 3.88	126.84 ± 5.26	65.14 ± 4.25
Group 4: 150 mg/kg CFE-treated group	78.57 ± 5.20	125.41 ± 4.64	61.99 ± 7.00
Group 5: 250 mg/kg CFE-treated group	76.84 ± 4.93	119.39 ± 7.44	56.37 ± 7.43

**p < 0.05: compared with group 1, **p < 0.01: compared with group 1, ***p < 0.001: compared with group 1*

#p < 0.05: compared with group 2, ##p < 0.01: compared with group 2, ###p < 0.001: compared with group 2

§p < 0.05: compared with group 3, §§p < 0.01: compared with group 3, §§§p < 0.001: compared with group 3

¥p < 0.05: compared with 150 mg/kg group 4, ¥¥p < 0.01: compared with group 4, ¥¥¥p < 0.001: compared with group 4

The results in Table 1 showed that the body weight as well as the body weight gain of rats in all groups did not have statistically significant differences (p > 0.05).

Table 2. Effects of CFE on the weights of target androgen-dependent organs

Group	Weights of target androgen-dependent organs (mg/100g b.w)				
	SV	VP	GP	COW	LABC
Group 1: untreated group	11.30 ± 1.18	7.00 ± 1.55	20.07 ± 1.29	1.97 ± 0.30	20.74 ± 2.77
Group 2: testosterone-treated group	290.11 ± 26.25***	97.30 ± 8.96***	41.74 ± 1.76***	21.02 ± 2.20***	205.70 ± 13.85***
Group 3: 50 mg/kg CFE-treated group	11.01 ± 1.13###	7.58 ± 1.43###	21.28 ± 1.64#	2.37 ± 0.26###	29.51 ± 2.17*,###

Group	Weights of target androgen-dependent organs (mg/100g b.w)				
	SV	VP	GP	COW	LABC
Group 4: 150 mg/kg CFE-treated group	11.14 ± 1.20$^{\#\#\#}$	4.97 ± 0.72$^{\#\#\#}$	28.10 ± 2.89$^{*,\#,\S}$	2.95 ± 0.73$^{\#\#\#}$	34.16 ± 4.88$^{*,\#\#\#}$
Group 5: 250 mg/kg CFE-treated group	17.97 ± 2.35$^{*,\#\#\#,\S,¥}$	6.44 ± 1.07$^{\#\#\#}$	28.33 ± 1.73$^{**,\#,\S}$	2.83 ± 0.24$^{\#\#\#}$	34.00 ± 2.80$^{**,\#\#\#}$

$^{*}p < 0.05$: compared with group 1, $^{**}p < 0.01$: compared with group 1, $^{***}p < 0.001$: compared with group 1

$^{\#}p < 0.05$: compared with group 2, $^{\#\#}p < 0.01$: compared group 2, $^{\#\#\#}p < 0.001$: compared with group 2

$^{\S}p < 0.05$: compared with group 3, $^{\S\S}p < 0.01$: compared with group 3, $^{\S\S\S}p < 0.001$: compared with group 3

$^{¥}p < 0.05$: compared with group 4, $^{¥¥}p < 0.01$: compared with group 4, $^{¥¥¥}p < 0.001$: compared with group 4

The results in Table 2 showed that:

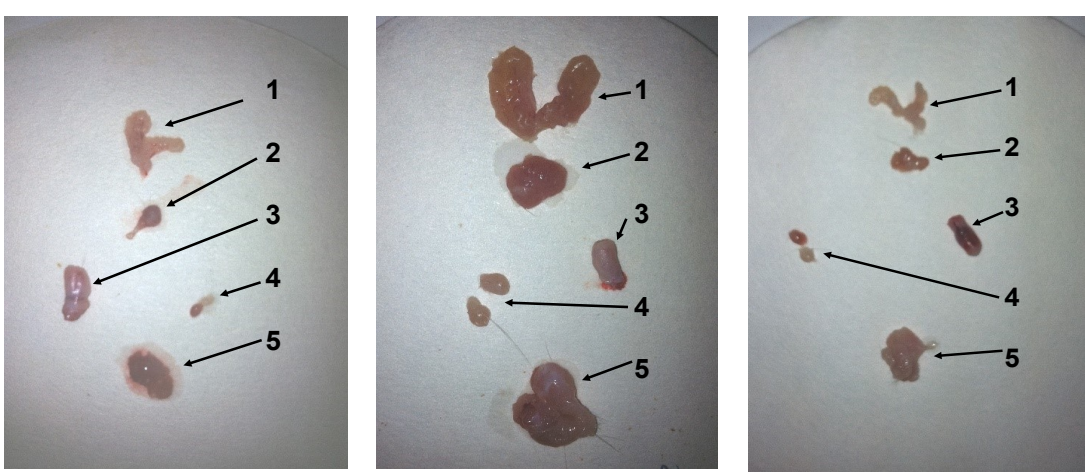
- In testosterone-treated rats, the weights of target androgen-dependent organs increased significantly compared with untreated rats ($p < 0.001$).

- In CFE-treated rats:

+ At 50 mg/kg/day, the weight of LABC muscle significantly increased compared with untreated rats ($p < 0.05$), the weights of other organs was not significantly different ($p > 0.05$).

+ At 150 mg/kg/day, the weights of the GP and LABC muscle significantly increased compared with untreated rats ($p < 0.05$), the weights of other organs was not significantly different ($p > 0.05$). The weight of GP significantly increased compared with group 3 ($p < 0.05$).

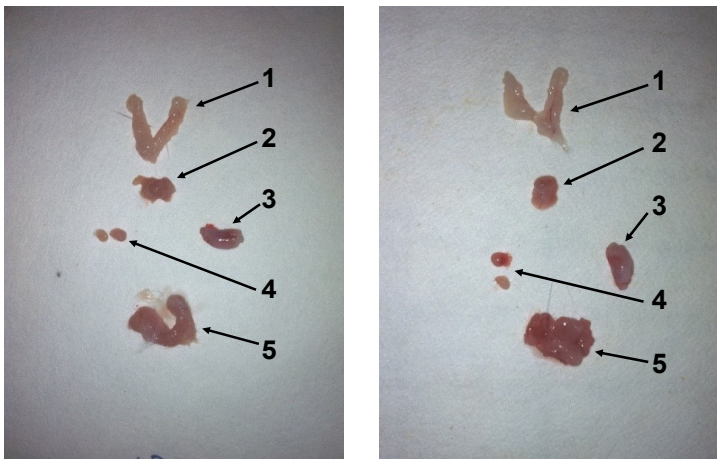
+ At 250 mg/kg/day, the weights of SV, GP and LABC muscle significantly increased compared with untreated rats ($p < 0.05$), the weights of other organs was not significantly different ($p > 0.05$). The weight of GP significantly increased compared with group 3 ($p < 0.05$) and the weight of SV significantly increased compared with group 3 and group 4 ($p < 0.05$).



Group 1 Group 2 Group 3

Figure 1. Images of target androgen-dependent organs

SV; 2. VP; 3. GP; 4. COW; 5. LABC muscle



Group 4 Group 5

Figure 1. Images of target androgen-dependent organs (cont.)

SV; 2. VP; 3. GP; 4. COW; 5. LABC muscle

Table 3. Effects of CFE on blood testosterone concentration

Group	Testosterone concentration (nmol/l)
Group 1: untreated group	0.14 ± 0.04
Group 2: testosterone-treated group	31.73 ± 7.91**
Group 3: 50 mg/kg CFE-treated group	0.17 ± 0.04$^{\#\#}$
Group 4: 150 mg/kg CFE-treated group	0.62 ± 0.17$^{*,\#\#,\S}$
Group 5: 250 mg/kg CFE-treated group	3.69 ± 1.04$^{*,\#\#,\S}$

** p < 0.05: compared with group 1, ** p < 0.01: compared with group 1, *** p < 0.001: compared with group 1*

$^{\#}$ p < 0.05: compared with group 2, $^{\#\#}$ p < 0.01: compared with group 2, $^{\#\#\#}$ p < 0.001: compared with group 2

§p < 0.05: compared with group 3, §§p < 0.01: compared with group 3, §§§p < 0.001: compared with group 3

$^{¥}$p < 0.05: compared with group 4, $^{¥¥}$p < 0.01: compared with group 4, $^{¥¥¥}$p < 0.001: compared with group 4

The results in Table 3 showed that:

- In CFE-treated rats at 50 mg/kg/day, testosterone concentration was not significantly different compared with untreated rats (p > 0.05).

- In CFE-treated rats at 150 mg/kg/day and 250 mg/kg/day, testosterone concentration significantly increased compared with untreated rats and 50 mg/kg CFE-treated rats (p < 0.05).

IV. DISCUSSION

The model for evaluating the androgenic activity of drugs or substances based on the comparison of the relative weights of target androgen-dependent organs was described by Hershberger.[8] Currently, this model is considered as one of the most valuable

experimental model and is commonly used to demonstrate the androgenic activity of drugs or substances. The assays can be carried out in rats or mice. However, rats serve as preferred species used because mice's target organs are small and do not have clear anatomical borders that leads to difficulty in identifying and dissections the organs.[8]

Hershberger bioassays can be carried out in peripubertal castrated male rats according to OECD 441 or weanling uncastrated male rats according to OECD 115.[8,9] Hershberger bioassay in peripubertal castrated male rats achieves a high sensitivity by using male rats with minimal endogenous androgen production, eliminating the role of endogenous testosterone and eliminating the feedback mechanism of the hypothalamus-pituitary-testis axis when the target androgen-dependent organs are not fully developed. In addition, castration removes the main testosterone-producing organ and eliminates large differences in testosterone concentrations between rats. Therefore, the number of rats for androgenic activity screening is reduced. According to OECD 441, the number of young castrated rats per each dose group is at least 6 rats; while when conducting the experiment on adult or intact pubertal male rats, the number of rats for each batch is higher.[8,9] Therefore, Hershberger bioassay in peripubertal castrated male rats was used in this study to evaluate the androgenic activity of our investigational product. After being castrated, rats were not treated immediately but after an appropriate period so that the target organs reduced to the lowest levels and reached the stable baseline weights, the endogenous androgen concentration in the blood was minimal, the hypothalamus-pituitary-testis axis was unable to compensate through the feedback mechanism; and the tissue response was highest.[8,10]

This bioassay was based on the changes in weights of five target androgen-dependent organs in peripubertal castrated male rats, including: the ventral prostate (VP), seminal vesicle (SV) (plus fluids and coagulating glands), levator ani-bulbocavernosus (LABC) muscle, paired Cowper's glands (COW) and the glans penis (GP). According to OECD, a statistically significant increase in any two or more of the five target androgen-dependent organ weights is considered a positive androgen agonist result.[8,9]

Testosterone was used as a positive control drug in our study. As expected, in testosterone-treated rats, the weights of all five target organs increased significantly compared with untreated rats. Androgens play a crucial role in the development of male reproductive organs and the major androgen is testosterone secreted from testes. The androgen receptor is found in all male reproductive organs and can be stimulated by either testosterone or its metabolite dihydrotestosterone.[11,12] Testosterone is converted to dihydrotestosterone, then dihydrotestosterone binds to the receptors in the target cell membrane and stimulates a series of protein synthesis reactions in the target cells, increasing the cell proliferation leading to the increase in these organs' weights.[11,12]

This study evaluated the potential androgenic activity of CFE at three doses of 50 mg/kg b.w/day, 150 mg/kg b.w/day and 250 mg/kg b.w/day. Our results showed that CFE 50 mg/kg b.w/day increased the weight of one target organ significantly while CFE 150 mg/kg b.w/day and 250 mg/kg b.w/day increased significantly the weights of two or three target organs. According to OECD, CFE at two doses of 150 mg/kg b.w/day and 250 mg/kg b.w/day possessed androgenic effects. The bioassay evaluating

the androgenic effects based on the changes in target organ's weights has high sensitivity and does not require complicated techniques. Thus, it is suitable for initially screening the effects of drugs or substances and orienting the selection of doses for subsequent studies. In Vietnam, some medicinal herbs have been demonstrated their androgen activities by this bioassay such as *Morinda officinalis*, *Cordyceps militaris*.[13,14]

Another research index recommended in OECD guidelines is the testosterone concentration in the blood.[8,9] Our results showed that CFE at doses of 150 mg/kg/day and 250 mg/kg/day caused a statistically significant increase in blood testosterone level in rats. This result was consistent with the effects of CFE on target androgen-dependent organ weights mentioned above.

Substances with androgenic potentials can be classified into three categories: those that stimulate the biosynthesis of testosterone through the central pathway; those that stimulate the biosynthesis of testosterone through the testicular pathway; those that act as an exogenous testosterone. A previous study evaluated the effects of osthole, an active compound of *Cnidium monnieri* (L.) Cuss fruits on androgen levels in male rats and showed that osthole increased testosterone, LH, FSH levels.[6] Another study demonstrated that osthole increased blood testosterone levels and increased the expression of androgen receptors in the testes of cyclophosphamide-induced reproductive dysfunction mice.[7] Our study was carried out in young castrated male rats and could not demonstrated whether CFE could stimulate the testes to produce testosterone. Was CFE itself an exogenous testosterone? Scientists have found a sterol in the ethyl acetate extract of *Cnidium monnieri* (L.) Cuss fruits i.e daucosterol.[15] This is a natural sterol-like compound. Therefore, it is not yet possible to rule out the possibility that CFE acts as an exogenous testosterone. Further experimental studies needed to be carried out to elucidate the mechanism of CFE.

V. CONCLUSION

Our findings demonstrated that the alcoholic extract of *Cnidium monnieri* (L.) Cuss fruits at 150 mg/kg b.w/day and 250 mg/kg b.w/day had androgenic effects in rats.

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