POTENTIAL ANDROGENIC ACTIVITY OF FU PLUS TABLET IN CASTRATED MALE RATS

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FU Plus tablet is a multi-component herbal and micronutrient formulation. This study aimed to evaluate the androgenic activity of FU Plus tablets in castrated male Wistar rats using the Hershberger bioassay in accordance with OECD Test Guideline 441. Peripubertal male Wistar rats were bilaterally orchiectomized, except for a group of intact biological controls. Animals were randomly allocated to five groups (n = 10 per group): biological control, model control, positive control (testosterone, 19.2 mg/kg/day), FU Plus low dose (0.24 tablet/kg b.w/day), and FU Plus high dose (0.72 tablet/kg b.w/day). Treatments were given orally for 10 days, and then androgen-responsive tissues were excised and weighed to assess androgenic activity. Our results showed that FU Plus significantly increased the weights of the ventral prostate and glans penis at both doses ($p < 0.05$ vs. model) and significantly improved seminal vesicle weight at the higher dose ($p < 0.05$). In conclusion, FU Plus tablets were demonstrated to have androgenic activity in castrated male rats, as evidenced by their stimulatory effects on select androgen-responsive tissues.

Keywords: FU Plus tablets, androgenic activity, Hershberger bioassay, *Wistar* rats.

I. INTRODUCTION

Androgens, primarily testosterone and dihydrotestosterone, are pivotal in the development and maintenance of male reproductive tissues, including the prostate, seminal vesicles, and skeletal muscles.[1] These hormones exert their effects through androgen receptors, influencing various physiological processes essential for male health. Androgen deficiency, whether due to aging, disease, or surgical castration, is associated with diminished sexual function, reduced muscle mass, and decreased quality of life.[2] While testosterone replacement therapy (TRT) remains the standard treatment for hypogonadism, concerns about potential adverse effects-such as erythrocytosis, prostate hypertrophy, and cardiovascular risks-have prompted interest in alternative or adjunctive therapies.[3]

FU Plus tablet is a polyherbal and micronutrient-based formulation that contains extracts known or suggested to influence androgenic activity. Specifically, *Epimedium brevicornum* (Horny Goat Weed) and *Tribulus terrestris* have been demonstrated to have androgen-mimetic properties in preclinical models, potentially through stimulation of luteinizing hormone or direct androgen receptor modulation.[4,5] L-Arginine and Red ginseng may

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support nitric oxide synthesis and endocrine balance. In contrast, micronutrients such as zinc, selenium, and yohimbine hydrochloride have been implicated in male reproductive health and hormonal regulation.[6,7]

The Hershberger bioassay, as outlined in OECD Test Guideline 441, is a validated *in vivo* screening method used to detect androgenic or antiandrogenic activity of an investigational product based on its effects on accessory sex organs in castrated male rats. A compound is considered androgenic if it significantly increases the weight of at least two of five androgen-responsive tissues (i.e., ventral prostate, seminal vesicles, glans penis, Cowper's glands, and levator ani–bulbocavernosus muscle) relative to the castrated control.[8]

Given the composition of FU Plus tablets and the potential for synergistic androgenic effects, this study was carried out to assess its androgenic activity using the OECD 441-compliant Hershberger assay. By evaluating tissue-specific responses in castrated male rats, this study provides foundational evidence for the endocrine-modulating potential of this novel formulation.

II. MATERIALS AND METHODS

1. Subjects

Investigational product

FU Plus tablets were produced by Mediplantex National Pharmaceutical Joint Stock Company and supplied by International Western Europe Chemical Technology Pharma Joint Stock Company (Hanoi, Vietnam). Each FU Plus tablet contains the following active ingredients: Epimedium extract (50mg), *Tribulus terrestris* extract (50mg), L-Arginine hydrochloride (150mg), Red ginseng extract (50mg), Zinc gluconate (35mg), Selenium yeast (2000ppm, equivalent to 2.5mg), and Yohimbine hydrochloride (1.5mg).

The tablets were crushed and mixed with distilled water immediately before each administration to the experimental animals.

Experimental animals

Male peripubertal Wistar rats (postnatal day 42 - 50) were acclimatized under standard laboratory conditions for 7 days prior to surgical procedures. Animals were housed under standard housing conditions (temperature of 22 ± 3°C, relative humidity of 50 – 60%, and a 12-hour light/dark cycle). Standard rodent chow and tap water were provided *ad libitum*. All animal procedures were conducted in accordance with institutional animal care and use guidelines and followed the National Guideline (Decision number: 141/QD-K2DT) at the Laboratory of the Department of Pharmacology, Hanoi Medical University.

2. Methods

Experimental procedure

The study was conducted using the Hershberger bioassay model in accordance with the OECD Test Guideline 441.[8] Animals were randomly assigned to experimental groups (n = 10 per group). Rats in Groups 2, 3, 4, and 5 underwent bilateral orchiectomy under general anesthesia using chloralhydrate 250 mg/kg b.w to remove both testes and epididymides, thereby eliminating endogenous androgen production. Rats in Group 1 remained intact and served as the normal control group. Following surgery, all animals were allowed to recover under standard housing conditions for a period of 7 days to ensure stabilization and regression of androgen-dependent tissues.

Beginning on Day 8 post-castration, rats were administered either distilled water, testosterone undecanoate, or FU Plus orally once daily in the morning for a consecutive 10-

day treatment period. The treatment regimen was as follows:

• Group 1 (Normal control group): Intact rats received distilled water at a volume of 10 mL/kg body weight/day.

• Group 2 (Model control group): Castrated rats received distilled water at a volume of 10 mL/kg body weight/day.

• Group 3 (Positive control group): Castrated rats received testosterone undecanoate at a dose of 19.2 mg/kg body weight/day.

• Group 4 (FU Plus low dose group): Castrated rats received FU Plus at a dose of 0.24 tablet/kg body weight/day, equivalent to the human daily dose of 2 tablets.

• Group 5 (FU Plus high dose group): Castrated rats received FU Plus at a dose of 0.72 tablet/kg body weight/day, equivalent to the human daily dose of 6 tablets.

Clinical Observations and Body Weight Monitoring

Animals were observed daily for clinical signs and general health. Body weights were recorded from the day of randomization through the final day of dosing.

Necropsy and Tissue Collection

Twenty-four hours after the final dose, animals were weighed and euthanized. A complete necropsy was performed, and the following androgen-dependent tissues were carefully dissected, cleaned of adherent fat and fascia, and weighed fresh to the nearest 0.1mg: ventral prostate (VP), seminal vesicles with coagulating glands (SVCG), levator ani-bulbocavernosus muscle complex (LABC), paired Cowper's glands (COW), and glans penis (GP). Each tissue was weighed immediately on an analytical balance. Results were expressed as tissue weight (mg) per 100 g of body weight.

Data analysis

Data were expressed as mean ± standard deviation (SD). Statistical comparisons among groups were performed using one-way analysis of variance (ANOVA) using SPSS software version 16.0 (SPSS Inc., Chicago, IL, USA). A p-value ≤ 0.05 was considered statistically significant.

III. RESULTS

Effects of FU Plus Tablets on Seminal Vesicle Weight

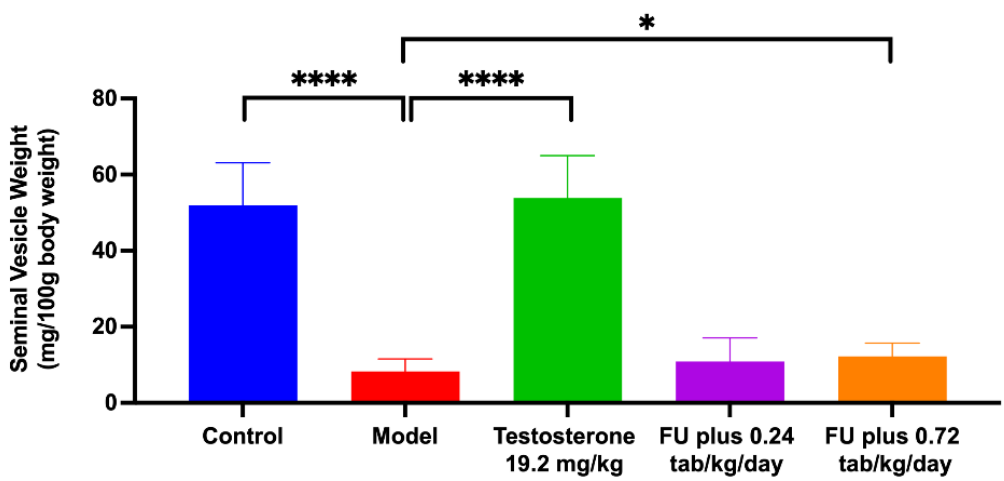

Chart 1. Effects of FU Plus Tablets on Seminal Vesicle Weight

*The results were expressed as mean ± SD (n = 10). *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001*

In castrated rats, seminal vesicle weight significantly decreased compared to intact controls (8.30 ± 3.22 vs. 51.95 ± 11.18 mg/100 g body weight, $p < 0.0001$). Treatment with testosterone undecanoate markedly restored this parameter (53.87 ± 11.13 mg/100g, $p < 0.0001$ vs. model). FU Plus at 0.24 tablets/kg/day caused a non-significant increase (10.87 ± 6.18 mg/100g), while the higher dose of 0.72 tablets/kg/day resulted in a significant increase in seminal vesicle weight compared to the model group ($p < 0.05$), indicating a dose-dependent androgenic effect (Chart 1).

Effects of FU Plus Tablets on Prostate Weight

Castration led to a marked reduction in prostate weight (6.78 ± 1.75 mg/100g) compared to intact animals (34.91 ± 9.69 mg/100g, $p < 0.0001$). Testosterone treatment significantly increased prostate weight ($p < 0.001$). FU Plus administration at both 0.24 and 0.72 tablets/kg/day significantly increased prostate weight compared to the model group (9.12 ± 2.95 and 9.18 ± 2.97 mg/100g, respectively; $p < 0.05$), demonstrating consistent androgenic activity in this tissue (Chart 2).

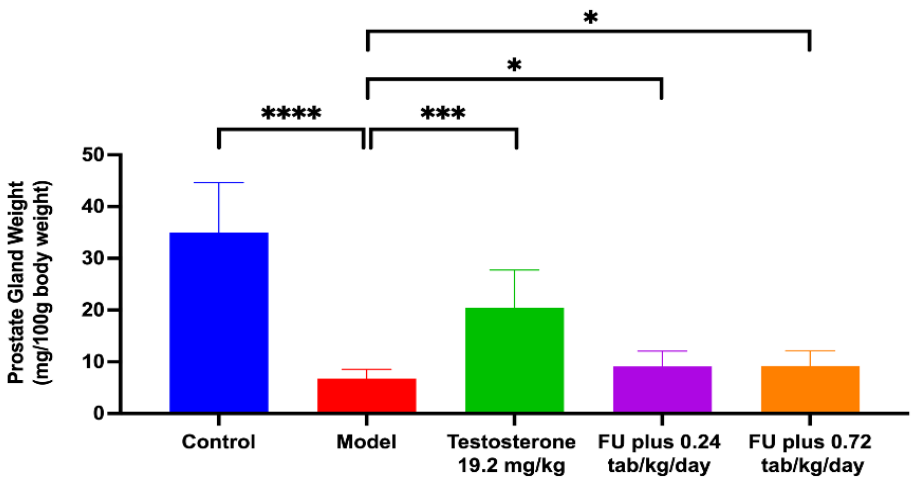

Chart 2. Effects of FU Plus Tablets on Prostate Weight

*The results were expressed as mean ± SD (n = 10). *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001*

Effects of FU Plus Tablets on Glans Penis Weight

The weight of the glans penis in castrated rats significantly declined relative to intact controls (19.30 ± 5.78 vs. 50.24 ± 6.98 mg/100g, $p < 0.0001$). Testosterone administration significantly restored this parameter (38.54 ± 7.85 mg/100g, $p < 0.0001$). FU Plus at both tested doses significantly increased glans penis weight (28.41 ± 11.32 and 27.80 ± 6.56 mg/100g for 0.24 and 0.72 tablets/kg/day, respectively; $p < 0.05$ vs. model), supporting its androgenic potential (Chart 3).

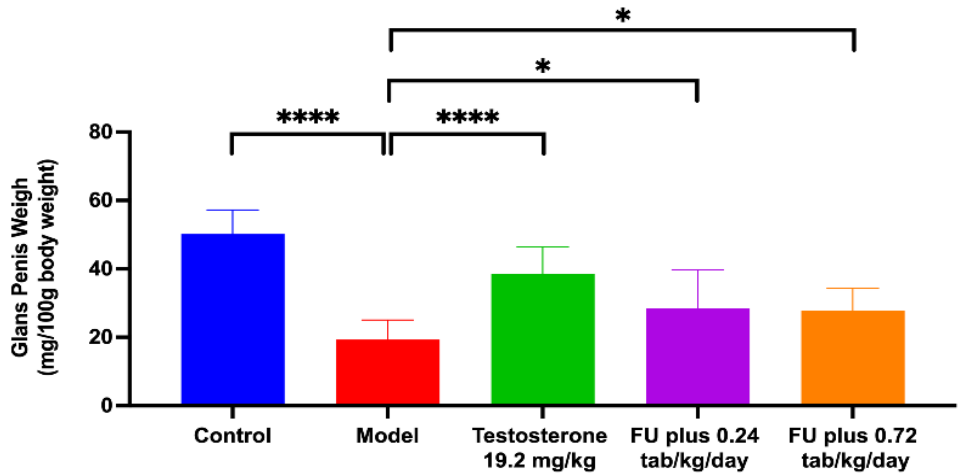

Chart 3. Effects of FU Plus Tablets on Glans Penis Weight

*The results were expressed as mean ± SD (n = 10). *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001*

Effects of FU Plus Tablets on Cowper's Gland Weight

Castration caused a marked decrease in Cowper's gland weight (2.93 ± 1.44 mg/100g) compared to intact controls (11.68 ± 1.95 mg/100g, p < 0.0001). Testosterone significantly increased Cowper's gland weight (6.26 ± 2.78 mg/100g, p < 0.001 vs. model). However, FU Plus did not produce a statistically significant change in Cowper's gland weight at either tested dose (2.95 ± 1.18 and 2.98 ± 0.98 mg/100g, p > 0.05 vs. model), suggesting no observable androgenic effect on this tissue (Chart 4).

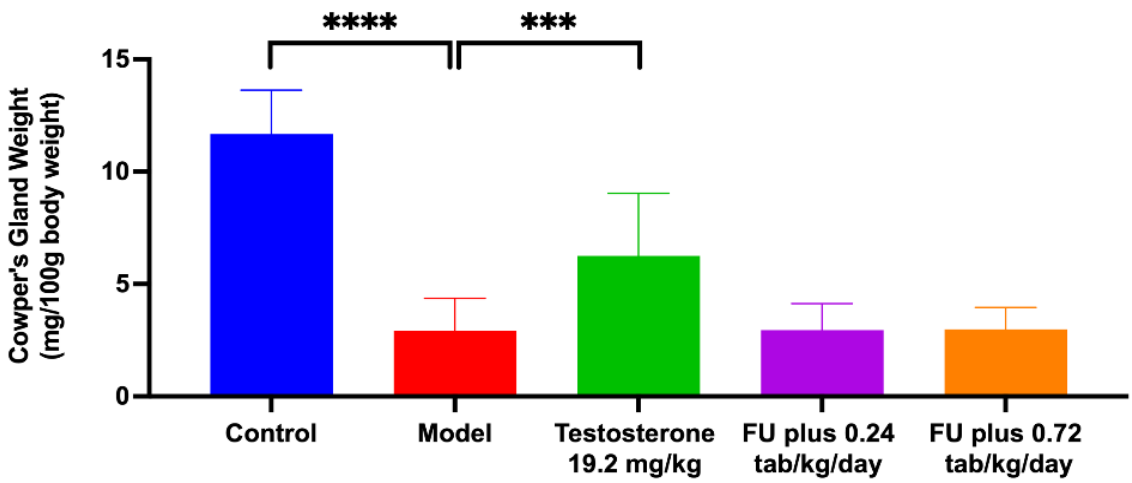

Chart 4. Effects of FU Plus Tablets on Cowper's Gland Weight

*The results were expressed as mean ± SD (n = 10). *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001*

Effects of FU Plus Tablets on Levator Ani–Bulbocavernosus Muscle Weight

The weight of the levator ani–bulbocavernosus muscle was significantly reduced by castration (65.11 ± 13.96 vs. 128.56 ± 31.52 mg/100g in intact rats, $p < 0.0001$). Testosterone treatment increased muscle mass to 116.50 ± 35.25 mg/100 g ($p < 0.001$ vs. model). FU Plus at both 0.24 and 0.72 tablets/kg/day failed to induce a significant change in this muscle's weight compared to the model group ($p > 0.05$), indicating limited androgenic influence on muscle tissue (Chart 5).

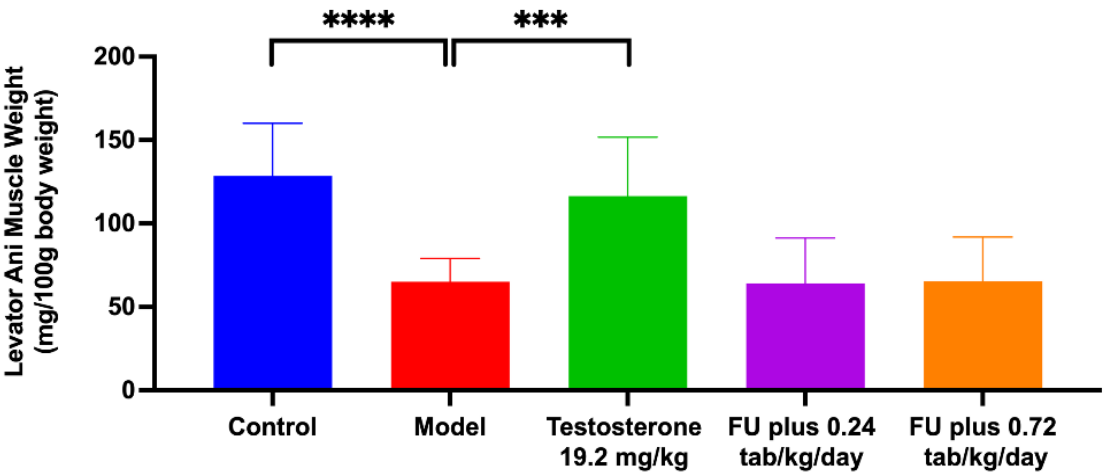

Chart 5. Effects of FU Plus Tablets on Levator Ani–Bulbocavernosus Muscle Weight

*The results were expressed as mean ± SD (n = 10). *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001*

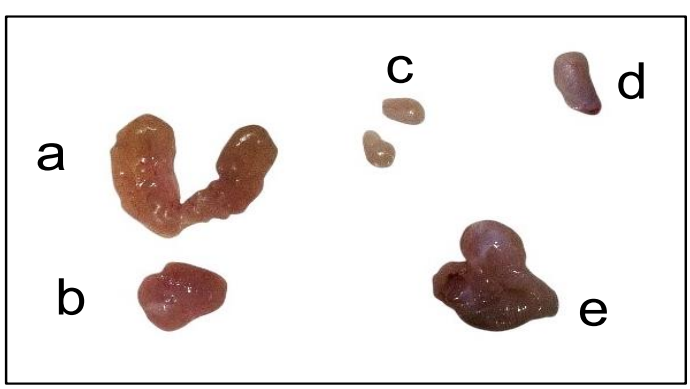

Figure 1. Isolated androgen-dependent reproductive organs

a) Seminal Vesicle, b) Prostate, c) Cowper's Glands, d) Glans Penis

e) Levator Ani–Bulbocavernosus Muscle

IV. DISCUSSION

The evaluation of androgenic properties is essential in drug development and endocrine disruptor screening, particularly for identifying substances that mimic or interfere with endogenous androgens. In vitro assays, such as androgen receptor (AR) binding assays and AR-responsive reporter gene assays, are commonly employed for their high-throughput

capacity and mechanistic insight.[9] These methods measure direct ligand-receptor interactions or transcriptional activation of AR target genes, yet they often lack the ability to assess systemic pharmacokinetics, bioactivation, or tissue-specific responses. Consequently, in vivo assays remain critical for a more integrative assessment of androgenic effects. The Hershberger bioassay, standardized under OECD Test Guideline 441, is a short-term *in vivo* screening assay that uses castrated peripubertal male rats to measure weight changes in five androgen-dependent tissues.[10] Unlike in vitro tests, the Hershberger assay captures endocrine interactions in the whole organism, including absorption, metabolism, and tissue-specific effects.[11] Furthermore, it allows for the detection of both androgen agonists and antagonists, making it a versatile tool for preclinical screening and regulatory toxicology.

Testosterone is the principal endogenous androgen responsible for the development and functional maintenance of male reproductive organs and androgen-responsive tissues. It exerts its biological effects primarily through intracellular androgen receptors (ARs), which, upon ligand binding, translocate to the nucleus and modulate transcription of target genes involved in tissue growth and differentiation.[12] In the context of the Hershberger bioassay, exogenous testosterone is administered to castrated male rats to compensate for the removal of endogenous androgen sources and to validate the assay's responsiveness. Testosterone stimulates hypertrophy and hyperplasia of androgen-dependent tissues, including the ventral prostate, seminal vesicles, glans penis, Cowper's glands, and levator ani–bulbocavernosus (LABC) muscle.[10,11] In this study, testosterone undecanoate at 19.2 mg/kg b.w/day significantly increased the absolute weights of all five target tissues compared to the castrated vehicle control group ($p < 0.001$), demonstrating its robust androgenic and anabolic activity. These findings are consistent with the established role of testosterone in upregulating AR expression and growth factor signaling pathways in reproductive tissues. The strong and consistent tissue responses observed further validate the Hershberger model and provide a reference point for evaluating the androgenic potential of novel test compounds such as FU Plus Tablet.

In the present study, FU Plus tablets produced significant androgenic effects on the ventral prostate, glans penis, and seminal vesicles in castrated male rats. In accordance with OECD 441 Guideline, a test compound was defined as positive for androgenic or antiandrogenic activity if it produced a statistically significant increase or decrease, respectively, in the absolute weights of at least two out of the five androgen-responsive target tissues compared to the appropriate control group. The observed androgenic effects of FU Plus tablets can be attributed to the combined action of its herbal and micronutrient constituents, which influence androgen production, receptor activation, and vasodilatory pathways. *Epimedium brevicornum*, standardized for icariin, acts as a natural selective PDE5 inhibitor and also enhances steroidogenesis by activating the PI3K/Akt pathway in Leydig cells, resulting in increased testosterone synthesis.[13] Icariin also promotes androgen receptor (AR) expression in target tissues such as the prostate and accessory glands, supporting tissue proliferation and secretory activity.[14] *Tribulus terrestris* has been shown to upregulate LH and enhance testosterone biosynthesis through hypothalamic–pituitary–gonadal (HPG)

axis stimulation, contributing to the growth of seminal vesicles and penile tissues.[15] Furthermore, Panax ginseng improves blood flow to genital organs and induces endothelial nitric oxide synthase (eNOS) activity, which supports erectile tissue function and penile development.[16]

Zinc plays a critical role in male reproductive physiology by modulating 5α-reductase activity, enhancing dihydrotestosterone (DHT) production in the prostate and seminal vesicles.[6] Zinc also stabilizes AR conformation and facilitates DNA binding, increasing the transcriptional activity of androgen-responsive genes.[17] Selenium, another essential trace element present in FU Plus, supports antioxidant protection in androgen-dependent tissues and may reduce oxidative stress-induced inhibition of testosterone biosynthesis.[18] Yohimbine, an α2-adrenergic receptor antagonist, augments sympathetic outflow and penile blood flow, which indirectly enhances penile tissue responsiveness to androgenic stimuli.[19] Collectively, the pharmacological synergy among these components leads to enhanced androgenic signaling, resulting in significant increases in the weights of the ventral prostate, seminal vesicles, and glans penis. These findings support the androgenic potential of FU Plus as a functional nutraceutical intervention targeting male reproductive health.

The androgenic response of seminal vesicles appears to be dose-dependent, as only the high dose of FU Plus Tablets significantly increased their weight. Seminal vesicles are particularly sensitive to circulating androgens, especially dihydrotestosterone (DHT), which binds androgen receptors (ARs) to drive glandular growth and secretory function.[20] FU Plus contains icariin and *Tribulus terrestris*, which enhance testosterone biosynthesis and AR expression; however, these effects may require higher systemic levels to exert measurable changes in accessory sex organs.[13,15] Additionally, micronutrients like zinc and selenium act synergistically to enhance AR binding and protect steroidogenic tissues from oxidative damage, further supporting androgen responsiveness.[6,18] The lack of effect at the lower dose suggests that androgenic signaling thresholds specific to seminal vesicles were not sufficiently activated.

This study provides the first preclinical evidence of the androgenic potential of FU Plus Tablets using the validated Hershberger bioassay, a popular model for assessing androgen-responsive tissues. Future studies should incorporate hormonal profiling, AR expression analysis, and fertility endpoints to elucidate systemic and molecular effects. Extended dosing and comparison with synthetic androgens could also better define the pharmacodynamic profile of FU Plus Tablets.

V. CONCLUSION

In conclusion, FU Plus Tablets had androgenic activity in castrated male rats, as evidenced by significant increases in the weights of the ventral prostate and glans penis at both doses of 0.24 tablets/kg b.w/day and 0.72 tablets/kg b.w/day, and the weights of seminal vesicles at the higher dose. These effects are likely attributed to the synergistic actions of herbal extracts and micronutrients that modulate androgen biosynthesis and receptor activity. These findings support its potential as a supplement for androgen-deficient conditions.

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The authors declare no conflict of interest.

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