EFFECTS OF FU PLUS TABLETS ON INTRACAVERNOSAL PRESSURE IN ANIMAL EXPERIMENTS

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Erectile dysfunction is defined as the persistent inability to attain and maintain an erection sufficient to permit satisfactory sexual performance, that may affect physical and psychosocial health, and the patients quality of life. A popular trend is to discover and research therapeutic drugs derived from medicinal herbs. FU Plus tablets including Epimedium extract, Tribulus Terrestris Extract, L-Arginine HCl, Red Ginseng extract, Zinc gluconate, Selenium yeast and Yohimbine HCl is a product expected to treat erectile dysfunction. The study was carried out to evaluate the effects of FU Plus tablets on the erectile function through intracavernosal pressure (ICP) in experimental animals. Wistar rats were divided into 3 groups of ten that were given normal saline, sildenafil and FU Plus tablets, respectively. ICP response after cavernous nerve stimulation was recorded. Sildenafil-treated rats and FU Plus-treated rats had shorter response latency, higher peak ICP and longer response duration than untreated control rats. In conclusion, FU Plus tablets have the potential for treating erectile dysfunction.

Keywords: Erectile dysfunction, FU Plus tablets, intracavernosal pressure, *Wistar* rats.

I. INTRODUCTION

Erectile dysfunction (ED) is defined as the persistent inability to attain and maintain an erection sufficient to permit satisfactory sexual performance.[1] ED can cause poor physical and mental health-related quality of life.[1,2] Moreover, the partners of ED men often experienced difficulties in relationship, sexual activities and sexual satisfaction.[3] Thus, the burden of ED can affect both the sufferers and their partners. Furthermore, ED prevalence can be underestimated in developing countries.

The etiology of ED consists of various factors, including neurological, vascular, hormonal, and psychological factors.[1,3] ED has been significantly associated with general health status. ED treatment therapies include lifestyle modifications, noninvasive and invasive treatment options such as phosphodiesterase-5 (PDE5) inhibitors, alprostadil, hormonal replacement therapy, and penile prosthesis surgery.[4] However, such therapies have been reported to have mild to severe side effects. According to a diversity of medicinal plant species, a popular trend in ED treatment is to discover and research therapeutic drugs derived from medicinal herbs in the combination of active compounds. FU Plus tablet including *Epimedium* extract, *Tribulus Terrestris* Extract, L-Arginine HCl, Red Ginseng extract, Zinc gluconate, Selenium yeast and Yohimbine HCl is a product expected to treat ED. Before conducting clinical trials, it is necessary to carry out the experimental studies to evaluate the efficacy and safety of the product. To evaluate the efficacy in nonclinical studies, rat models have been employed for investigating erectile function. Rats are usually used for the study of male erectile function due to exhibiting

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several benefits. Firstly, the morphological and functional sexual characteristics of humans are recapitulated in rodents. Secondly, compared with larger animals used in ED studies, rodents are more economical to purchase, house, and maintain. Thirdly, rodent models provide advantages in reproducible and subsequent behavioral as well as neurophysiological studies. Therefore, rodents have quickly become the primary animals used in the study of male erectile dysfunction.[5]

This study was carried out to evaluate the effects of FU Plus tablets on erectile function through intracavernosal pressure (ICP) in adult male rats.

II. MATERIALS AND METHODS

1. Subjects

The investigational product

FU Plus tablets were supplied by Mediplantex National Pharmaceutical Joint Stock Company. The ingredients of each tablet are Epimedium extract 50mg, Tribulus Terrestris extract 50mg, L-Arginine HCl 150mg, Red Gingseng extract 50mg, Zinc gluconate 35mg, Selen yeast 2000 ppm 2.5mg, Yohimbine HCl 1.5mg. The intended indication of FU Plus tablets are male erectile dysfunction with the intended dose of 1 - 2 tablets in adults.

Experimental animals

Male, adult *Wistar* rats (200 ± 20 grams) anesthetized with chloral hydrate (250 mg./kg., i.p.) were selected. Rats were raised for 7 days before conducting research to adapt to the environment and breeding conditions and during the experiment at the laboratory of the Department of Pharmacology, Hanoi Medical University. The temperature in the experimental animal room was 22°C ± 3°C. The relative humidity was at a minimum of 30% and preferably would not exceed a maximum of 70%, other than during room cleaning. The daily artificial lighting sequence would be 12 hours light /dark. Food and water was provided *ad libitum*.

2. Methods

Rats were divided into three groups and given the reagent 2 hours prior to ICP recording: group 1 consisted of control rats (n = 10), group 2 consisted of 10mg/kg sildenafil-treated rats (n = 10), group 3 consisted of 0.24 tablets/kg FU Plus treated rats (n = 10). The experiment was conducted according to Mehta's method.[6]

Rats were anesthetized with an intraperitoneal injection of chloral hydrate at 250 mg/kg body weight, then placed on the operating table, and disinfected. The Powerlab system, stimulator, pressure probes and Labchart software were connected and set up.

ICP monitoring procedure

The ICP monitoring procedure includes the following steps:

(1) Step 1: The cavernous nerve was exposed.

(2) Step 2: A 26 gauge needle connected to a polyethylene tube (PE-50) containing physiological saline with 100 IU/mL of heparin was inserted into one side of the corpora cavernosa for ICP measurement.

(3) Step 3: Basal ICP was recorded before stimulating the cavernous nerve.

(4) Step 4: The cavernous nerve was stimulated by using a square pulse stimulator connected to a platinum bipolar electrode positioned on the cavernous nerve using five volts with a frequency of 20 Hertz and duration of 1 min. The intracavernosal pressure after stimulation of the cavernous nerve was recorded.

(5) Step 5: Offline data was analyzed using Labchart pro software. Data were expressed as mean ± standard deviation (M ± SD). Differences

are considered statistically significant when $p < 0.05$.

Research indicators

- Basal ICP (mmHg): intracavernosal pressure before cavernous nerve stimulation: basal ICP (mmHg)

- Response latency (seconds): time from cavernous nerve stimulation until intracavernosal pressure began to increase

- Peak ICP (mmHg): maximum intracavernosal pressure after cavernous nerve stimulation

- Response duration (seconds): time from cavernous nerve stimulation until ICP returned to normal.

III. RESULTS

1. Effects of FU Plus tablets on basal ICP

Table 1. Effects of FU Plus tablets on basal ICP

Groups	Basal ICP (mmHg) (Mean ± SD)
Group 1 (control group)	38.08 ± 9.93
Group 2 (sildenafil)	43.88 ± 9.16
Group 3 (FU plus)	37.77 ± 4.62

As shown in Table 1, the basal ICP of sildenafil-treated group and FU plus-treated group were not statistically different from the basal ICP of the normal control group ($p > 0.05$).

2. Effects of FU Plus tablets on ICP response

As shown in Chart 1 and 2, FU Plus and sildenafil both have the effect of shortening response latency, increasing peak ICP compared with the control group ($p < 0.05$, $p < 0.01$). The response latency of FU Plus-treated rats was not statistically different from the response latency of sildenafil-treated rats ($p > 0.05$).

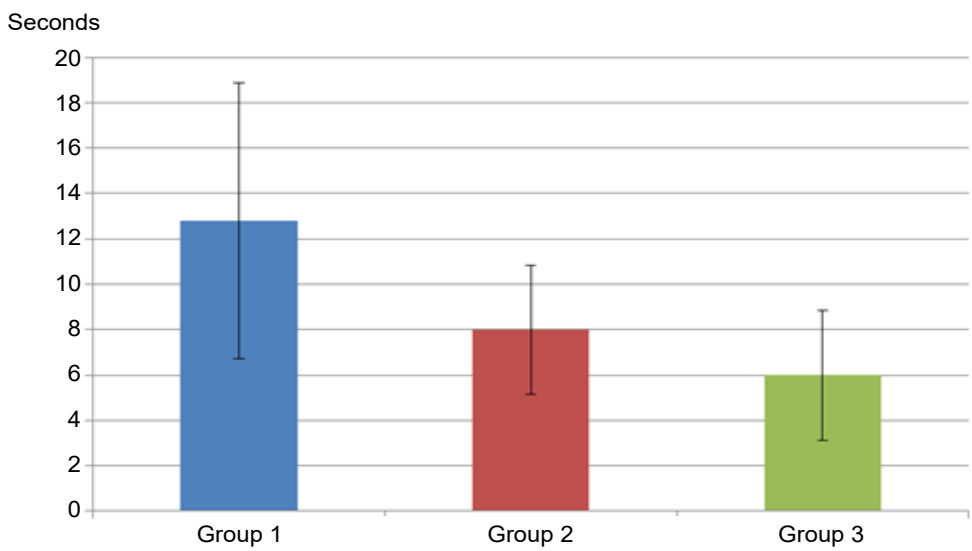

Chart 1. Effects of FU Plus tablets on response latency

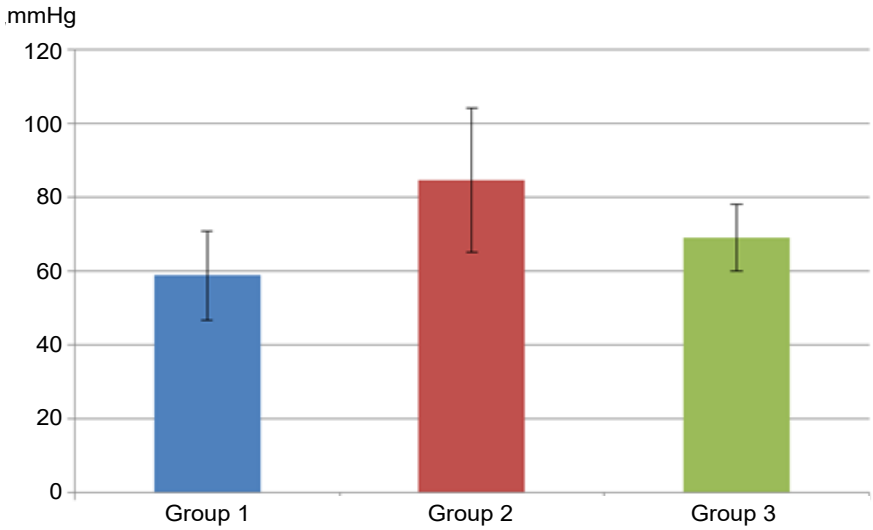

Chart 2. Effects of FU Plus tablets on peak ICP

Table 2. Effects of FU Plus tablets on response duration

Groups	Response duration (seconds) (Mean ± SD)
Group 1 (control group)	62.30 ± 12.94
Group 2 (sildenafil)	114.90 ± 52.00**
Group 3 (FU plus)	88.26 ± 28.57*

$*p_{2-1} < 0.05$; $**p_{2-1} < 0.01$

As shown in Table 2, FU Plus and sildenafil both have the effect of increasing response duration compared with the control group ($p < 0.05$, $p < 0.01$). The response duration of FU Plus-treated rats were not statistically different from the response duration of sildenafil-treated rats ($p > 0.05$).

IV. DISCUSSION

FU Plus tablets, a combination of several ingredients, are intended to treat male erectile dysfunction. This study evaluated the effects of FU Plus tablets on ICP in adult male rats. The principle of this method allows to evaluate the erectile ability by recording ICP after electrical stimulation of the cavernous nerve.[6]

In normal state, the balance between neurotransmitters from sympathetic and parasympathetic nerves maintains cavernosal smooth muscle tone. When there is no sexual stimulation, a small amount of nitric oxide (NO) is still released from nonadrenergic noncholinergic (NANC) nerve terminals and vascular endothelium so the amount of cyclic GMP is low. The sympathetic system prevails over the parasympathetic system to maintain the penis in a normal state with basal ICP values.[7] Our results showed that basal ICP values of sildenafil and FU Plus tablets-treated rats were not statistically different from the normal control rats' values.

After electric cavernous nerve stimulation, peak ICP values increased nearly 2 times compared with the basal ICP values in all

groups. The mechanism of erection caused by electrical cavernous nerve stimulation is similar to sexual stimulation, NO released from NANC neurons and the endothelium is probably the principal neurotransmitter for penile erection. Within the myocyte, NO activates a soluble guanylyl cyclase raising intracellular concentrations of cGMP. cGMP in turn activates a specific protein kinase (i.e., protein kinase G [PKG]), which phosphorylates certain proteins and ion channels, resulting in opening of the potassium channels and hyperpolarization, sequestration of intracellular calcium by the endoplasmic reticulum and inhibition of calcium channels, diminishing calcium influx. The consequence is a drop in the free cytosolic calcium concentration and smooth muscle relaxation. The importance of the NO/guanylate cyclase/cGMP/PKG pathway to erection is unequivocal, so any agent that inhibits the hydrolysis or increases the synthesis of cyclic cGMP can be used to treat ED.[6,7]

In our study, sildenafil-treated rats had shorter response latency, higher peak ICP, and longer response duration than untreated control rats. This can be easily explained by the mechanism of action of sildenafil, a PDE5-selective cyclic nucleotide phosphodiesterase inhibitor. Its actions result from its inhibition of cGMP-hydrolytic activity in vascular smooth muscle myocytes and the consequent increases in intracellular cGMP content and the potentiation of protein kinase G-mediated vasodilatory response.[8]

Our results also showed that FU Plus-treated rats also had shorter response latency, higher peak ICP, and longer response duration than untreated control rats; and the response latency, response duration of FU Plus-treated rats were not significantly significant compared with sildenafil-treated rats. This result might due to the mechanism of the ingredients in FU Plus tablets, including *Epimedium* extract, *Tribulus Terrestris* Extract, L-Arginine HCl, Red Ginseng extract, Zinc gluconate, Selenium yeast and Yohimbine HCl.

Epimedium has rejuvenation and energetic functions and has been commonly used in many traditional formulas for centuries via “nourishing the kidney and reinforcing the Yang”. Studies have shown that Epimedium possesses multispectral therapeutic activities, including treating ED.[9] *Epimedium* may serve as a potential inhibitor of PDE5.[10]

Tribulus terrestris and Red ginseng were demonstrated to be helpful in treating ED by inducing relaxation of the smooth muscles of the corpus cavernosum via the nitric oxide (NO) pathway.[11,12] Moreover, L-arginine, a natural precursor of nitric oxide, was demonstrated to show the additive effect, indicating the potential synergetic effect of arginine with the current first-line treatment (PDE inhibitors).[13]

Zinc co-administration was shown to significantly improve absolute and relative penile weights, depressed oxidative stress, inhibited NO depletion and inactivated AchE, thus enhancing NO/cGMP signaling and cholinergic-dependent penile erection.[14]

It was not until the demonstration of expression of functional alpha-2-adrenergic receptors in human corpus cavernosum that a credible mechanism was provided for the improvement in erectile function in patients treated with yohimbine. Postsynaptic alpha-2-adrenergic receptors localized distally to adrenergic nerve terminals may be activated by circulating catecholamines and induce contractility of the corporeal tissue. Adrenergic blockade with yohimbine would reverse the process.[15]

For selenium yeast, although the mechanism

in treating ED remains unclear, a cross-sectional investigation revealed that selenium deficiency was associated with a prevalence of ED and there was an association between dietary selenium intake and ED.[16]

Our findings revealed that FU plus tablets had the effect of improving erectile function as shown by increasing the response of ICP to electrical cavernous nerve stimulation in adult male rats. This effect may be due to various mechanisms of action. This is also one of the advantages of a product that combines medicinal herbs and active compounds. Further studies might be needed to evaluate the effect of FU plus tablets on the sexual behavior of experimental animals.

V. CONCLUSION

FU Plus tablets have the effects of shortening response latency, increasing peak ICP, and response duration to electrical cavernous nerve stimulation in adult male rats. Our results suggest that FU Plus tablets have the potentials for treating male erectile dysfunction.

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