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*Original Article*

# Effects of *Cnidium monnieri* (L.) Cuss. fruit (*Fructus Cnidii*) extract on male erectile function in experimental animals

[Efectos del extracto de fruto de *Cnidium monnieri* (L.) Cuss. (*Fructus Cnidii*) sobre la función eréctil masculina en animales de experimentación]

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**Abstract**

*Context*: Epidemiological data have shown a high prevalence and incidence of erectile dysfunction (ED) worldwide. There is a tendency to use traditional medicines to treat ED. Among them, *Fructus cnidii* (the mature fruit of *Cnidium monnieri* (L.) Cuss.) is often included in products for ED management.

*Aims*: To evaluate the potential effects of an alcoholic extract of *Fructus Cnidii* on erectile function in conscious rabbits and on intracavernous pressure (ICP) in anesthetized rats.

*Methods*: Male New Zealand White rabbits were randomized to receive a single dose of sildenafil 4.5 mg/kg b.w, or *Fructus Cnidii* extract 60 mg/kg b.w. Erection was then evaluated by measuring the length of uncovered penile mucosa and total erection time. Male Wistar rats were randomized to receive a single dose of sildenafil 10 mg/kg b.w or *Fructus Cnidii* extract 150 mg/kg b.w. ICP and mean arterial pressure (MAP) were determined by cavernous nerve electrostimulation before and after treatment with *Fructus Cnidii* extract.

*Results*: Our results showed that no significant difference was detected in penis length at various time points and total erection time between rabbits receiving sildenafil and *Fructus Cnidii* extract. *Fructus Cnidii* extract increased basal ICP, maximal ICP, response time, and ICP/MAP index but did not affect MAP in rats.

*Conclusions*: *Fructus Cnidii* extract showed positive effects on erectile function in experimental male animals.

*Keywords*: erectile function; *Fructus Cnidii*; intracavernous pressure; osthole.

**Resumen**

*Contexto*: Los datos epidemiológicos han mostrado una alta prevalencia e incidencia de la disfunción eréctil (DE) a nivel mundial. Existe una tendencia a utilizar medicamentos tradicionales para tratar la DE. Entre ellos, el *Fructus Cnidii* (el fruto maduro de *Cnidium monnieri* (L.) Cuss.) se incluye a menudo en productos para el manejo de la DE.

*Objetivos*: Evaluar los posibles efectos de un extracto alcohólico de *Fructus Cnidii* sobre la función eréctil en conejos conscientes y sobre la presión intracavernosa (PIC) en ratas anestesiadas.

*Métodos:* Se aleatorizaron conejos machos blancos de Nueva Zelanda para recibir una dosis única de sildenafilo 4,5 mg/kg de peso corporal o extracto de *Fructus Cnidii* 60 mg/kg de peso corporal. Posteriormente, se evaluó la erección midiendo la longitud de la mucosa peneana descubierta y el tiempo total de erección. Ratas Wistar macho fueron aleatorizadas para recibir una dosis única de sildenafilo 10 mg/kg de peso corporal o extracto de *Fructus Cnidii* 150 mg/kg de peso corporal. La PIC y la presión arterial media (PAM) se determinaron mediante electroestimulación del nervio cavernoso antes y después del tratamiento con extracto de *Fructus Cnidii*.

*Resultados*: Nuestros resultados mostraron que no se detectaron diferencias significativas en la longitud del pene en diversos momentos ni en el tiempo total de erección entre los conejos que recibieron sildenafilo y extracto de *Fructus Cnidii*. El extracto de *Fructus Cnidii* aumentó la PIC basal, la PIC máxima, el tiempo de respuesta y el índice PIC/PAM, pero no afectó a la PAM en ratas.

*Conclusiones*: El extracto de *Fructus Cnidii* mostró efectos positivos sobre la función eréctil en animales machos de experimentación.

*Palabras Clave*: *Fructus Cnidii*; función eréctil; osthole; presión intracavernosa.

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**Abbreviations:** ARRIVE: Animal Research: Reporting of In Vivo Experiments; BP: Blood pressure; cAMP: Cyclic adenosine monophosphate; CMC: Carboxyl methyl cellulose; ED: Erectile dysfunction; eNOS: Endothelial nitric oxide synthetase; GMP: Guanosine monophosphate; ICP: Intracavernous pressure; L-NAME: L- nitro arginin methyl ester; MAP: Mean arterial pressure; NANC: Non-adrenergic non-cholinergic; nNOS: Neuronal nitric oxide synthetase; NO: Nitric oxide; NOS: Nitric oxide synthetase; ODQ: Oxadiazolo quinoxalin; PDE-5: Phosphodiesterase-5.

## INTRODUCTION

Erectile dysfunction is a phrase used by the World Association of Andrology since 1997, replacing the word "impotence" (Kandeel et al., 2001). The male sexual response cycle is a sequential series of psycho-physiological phases, including desire, arousal, orgasm, and resolution. Erectile dysfunction (ED), considered an impairment in the arousal phase, is defined as the consistent or recurrent inability to attain and/or maintain penile erection sufficient for sexual satisfaction, including satisfactory sexual performance (Burnett et al., 2018; Salonia et al., 2024).

Epidemiological data have shown a high prevalence and incidence of ED worldwide (Leslie and Sooriyamoorthy, 2025; Salonia et al., 2024). The available data indicate that 52% of men in the US between 40 and 70 years of age have ED (Muneer et al., 2014; Salonia et al., 2024). In a study of men aged 30-80 years, the prevalence of ED was 19.2% and closely related to age, with an age-driven increase from 2.3% to 53.4% (Braun et al., 2000). It is estimated that ED will affect 322 million men worldwide by 2025 (Ayta et al., 1999). Previously, it was thought that ED was due to mental causes. The discovery of prostaglandins and phosphodiesterase-5 (PDE-5) inhibitors led to an understanding of vasculogenic ED mechanisms. With achievements in physiology and pathophysiology, scientists have profoundly understood the causes and pathogenesis of ED. The pathophysiology of ED may be vasculogenic, neurogenic, anatomical, hormonal, drug-induced, and/or psychogenic. In most ED cases, numerous pathophysiological pathways co-exist and negatively impact erectile function (Gratzke et al., 2010; Salonia et al., 2024). ED is not only frequently associated with urological conditions and procedures but also related to numerous risk factors, including age, diabetes mellitus, dyslipidemia, hypertension, cardiovascular diseases, obesity, lack of exercise, smoking and drug use, and other comorbidities (Gratzke et al., 2010; Salonia et al., 2024).

Insights into the pathogenesis of ED have led to advances in the diagnosis and treatment of ED. Treatment strategies include lifestyle changes, management of comorbidities, and invasive or noninvasive ED treatment methods. Oral PDE-5 inhibitors, such as sildenafil and tadalafil, are usually the first-line treatment for ED. These drugs inhibit the outflow of blood from the penis to induce erection, and are generally well tolerated (Kim et al., 2021). However, there is a discontinuation rate due to adverse effects, such as headaches, dyspepsia, nasal congestion, flushing, syncope, vision loss, priapism, and myocardial infarction (Anand Ganapathy et al., 2021). More invasive options for ED management include intracavernosal injection therapy of vaso-active substances, vacuum erection devices, and penile prosthesis implants (Burnett et al., 2018; Salonia et al., 2024). The results of ED treatment may depend on the invasiveness, efficacy, safety, and costs of each method, as well as patient preference and adherence (Montorsi et al., 2010).

In Asian countries, male sexual dysfunction, including ED, often remains undiagnosed and untreated because of conservative cultural and religious beliefs, socioeconomic conditions, and lack of awareness (Irfan et al., 2020). Many patients fail to seek modern healthcare, and many physicians are reluctant to ask patients about their sexual health. Nowadays, the popularity of traditional medicines is increasing all over the world. There is a tendency to use medicinal herbs to control ED, especially in countries with longstanding traditional medicine, under the impression that they are natural and safe (Hafizi Fard and Akhgari, 2018). In recent years, there has been exponential growth in the market of medicinal herbs for the treatment of ED (Salonia et al., 2024). Various medicinal herbs are used as aphrodisiacs, compounds that can increase sexual arousal, libido, potency (erection), and/or sexual pleasure (Malviya et al., 2011; Sin et al., 2021). Many studies have been conducted to investigate the effect and mechanism of traditional medicine in treating ED. Some herbal plants exhibited satisfying outcomes and might improve erectile function (Li et al., 2017).

Among these herbal plants, *Fructus Cnidii* (the mature fruit of *Cnidium monnieri* (L.) Cuss.) is often included in products for ED management (Li et al., 2017). It is a popular herbal medicine in Vietnam, annually harvested from June to August (Do, 2019). Excerpt from traditional medicine's literature, *Fructus cnidii* has a bitter and spicy taste and has the effect of strengthening yang, benefiting the kidneys, eliminating wind and dampness, and is used alone or in combination with other herbs to treat male impotence and infertility (Do, 2019; Hoang and Tran, 2010).

Despite its diverse uses in traditional medicine, there is limited information regarding how *Fructus Cnidii* could improve ED. The effects may partly be

attributed to osthole, an active ingredient found in *Fructus Cnidii* (Li et al., 2017). Osthole has been reported to relax rabbit corpus cavernosum tissue, through increasing nitric oxide released from endothelium and inhibiting phosphodiesterase. The above factors play an important role in erectile function. However, male sexual behavior (including erection) comprises a complex pattern of genital and somatomotor responses, elicited, directed, and maintained by multiple external and internal elements (Hull and Rodríguez-Manzo, 2009). There has been no comprehensive study on the effects of *Fructus Cnidii* on male sexual function. In our previous study, we evaluated the effects of *Fructus Cnidii* extract on male rat sexual behaviors. It was demonstrated that *Cnidium monnieri* (L.) Cuss. fruit extract promoted sexual behaviors in male rats, including mounting, vaginal intromission, and ejaculation (Dau et al., 2020). Among male sexual disorders, ED is the most common and is also a health problem of great concern today due to its great impact on men's life quality and the possibility related to accompanying diseases. Thus, this study was carried out to evaluate the potential effects of the alcoholic extract of *Fructus Cnidii* on erectile function in conscious rabbits and on intracavernous pressure (ICP) in anesthetized rats.

The aim of this study is consistent with the ethnomedical use of *Fructus Cnidii*. Ethnomedical use guides scientific studies to evaluate the effects of medicinal herbs. Conversely, experimental studies on animal models would contribute to providing scientific evidence for the ethnomedical use of *Fructus Cnidii*, clarifying and suggesting its mechanism of action. It promises to develop products from available medicinal herbs in the treatment of ED, overcoming the disadvantages of chemical drugs and providing more treatment options for patients.

## MATERIAL AND METHODS

### Chemicals

Sildenafil tablets (Viagra, Pfizer, Australia) were used as positive controls in this study. Ketamine injection solution (Pantapharma GmbH, Germany) was used as an anesthetic drug. Heparin injection solution (Pantapharma GmbH, Germany) was used as an anticoagulant drug.

### Plant material

Fresh plant materials were collected from Lang Son, Viet Nam (21°51'N, 104°45'E) and identified by the Department of Botany, Hanoi University of Pharmacy, based on the Vietnamese Pharmacopoeia 5th edition. The plant extract was prepared and stored at the Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology.

### Extraction of *Fructus Cnidii*

The alcoholic extraction of *Fructus Cnidii* was performed at the Institute of Natural Products Chemistry – Vietnam Academy of Science and Technology. The extract contained 35% of osthole, with an extraction coefficient of 3%. It was mixed with the suspending agent 0.5% carboxymethyl cellulose (CMC) before being orally given to the animals. The experimental doses in this study were selected mainly based on findings from our previous studies of toxicities and effects on sexual behavior in male rats. The dose of 150 mg/kg b.w in rats was according to Dau et al. (2020). The dose on rabbits was converted based on extrapolation doses between species, from rats to rabbits, in compliance with US-FDA guidance (2005).

### Research subjects

Healthy male New Zealand white rabbits (2.3 ± 0.2 kg) and male Wistar rats (300 ± 20 g) were purchased from Vietnam Military Medical University and housed at the Department of Pharmacology, Hanoi Medical University. Throughout the study, animals were kept in standard laboratory conditions (12-hour light/dark cycle, constant temperature of 23 ± 1°C) and fed appropriate diets, with *ad libitum* access to water. All animals were closely monitored every day for signs of distress and sickness. Only animals that remained alive at the end of the study were included in the final analysis.

All procedures were approved by Hanoi Medical University's Institutional Ethical Review Board (Reference number: IRB00003121) prior to starting. The authors followed the ARRIVE guidelines 2.0 and the relevant national guidelines/regulations for caring and handling laboratory animals.

### Experimental protocol

*Assessing erectile function in conscious male rabbits*

The assessment was conducted in a quiet room to minimize any external stimulation. Twelve rabbits were allowed 30 minutes of acclimatization in the testing environment before starting. Animals were randomized to receive a single dose of sildenafil 4.5 mg/kg b.w (as positive control) or *Fructus Cnidii* extract 60 mg/kg b.w. The erection was evaluated by measuring the length (cm) of uncovered penile mucosa every five minutes after the agent administration until the penis returned to normal. Rabbits did not require anesthesia during the test. This experiment was carried out based on the method of a conscious rabbit model (Vogel, 2008).

*Measuring ICP in anesthetized male rats*

Before the investigation, sixteen male rats were intraperitoneally injected with ketamine at a dose of 25 mg/kg for anesthesia. Each rat was then placed in the supine position on an operating table. Anesthetized animals were randomized to receive a single dose of sildenafil 10 mg/kg b.w or *Fructus Cnidii* extract 150 mg/kg b.w after measuring the ICP (with and without nerve stimulation) at pre-treatment. This experiment was carried out based on the method of monitoring ICP in an anesthetized rat model (Mehta et al., 2008; Rehman et al., 1998).

The measurement of ICP was performed as follows:

1. A catheter (polyethylene-50 tube) was inserted into the rat's carotid artery to monitor the arterial blood pressure (BP).
2. A midline incision of the abdomen was used to expose the prostate. The cavernosal nerve was accessed and carefully dissected, allowing for further stimulation.
3. The penis was dissected up to the penile crura, exposing the corpus cavernosal muscles surrounding the crura.
4. A 26-gauge needle, connected to a polyethylene-50 tube pretreated with heparin 100 IU/mL, was installed in the corpus cavernosum to measure ICP.
5. The BP and ICP were evaluated using a BP manometer transducer and recorder (PowerLab, ADInstruments, Colorado, USA). During the measurement, the cavernous nerve was electrically stimulated for durations of 60 seconds (with a rhythmic stimulator connected to bipolar electrodes at a voltage of 5 V, a frequency of 20 Hz) at different time points: before treatment; 15 minutes, 30 minutes, and 45 minutes after the administration of sildenafil or the extract. Pressure was measured before, during, and after stimulation to examine the hemodynamic changes in response to autonomic nerve stimulation.

In brief, the following indices were measured in anesthetized male rats at pre-treatment, 15 minutes, 30 minutes, and 45 minutes following treatment: Basal ICP (before cavernous nerve stimulation - mmHg); maximal ICP after cavernous nerve stimulation (mmHg); stimulus-response period (seconds – s); mean arterial BP (MAP - mmHg); maximal ICP/MAP ratio. The data were recorded using Labchart Pro software (ADInstruments, Colorado, USA).

**Statistical analysis**

All collected data were analyzed using STATA 3.0 (StataCorp, Texas, USA). Researchers were blinded to the treatment regimens during every analysis step. Results were expressed as mean ± standard deviation (SD). The differences between groups were analyzed using the Mann-Whitney U and Proportion tests, which were considered statistically significant at $p \leq 0.05$.

## RESULTS

**Effects of the *Fructus Cnidii* extract on the penile erection of adult male rabbits**

Fig. 1 shows that no significant difference was detected in penis length at various time points following treatment (5 minutes, 10 minutes, 15 minutes, 20 minutes, 25 minutes, and 30 minutes) between rabbits receiving sildenafil and *Fructus Cnidii* extract (Fig. 1A). Animals of both groups also displayed similar total erection time, as seen in Fig. 1B.

**Effects of the *Fructus Cnidii* extract on the ICP of adult male rats**

First, we assessed the ICP measurements before and after stimulating the cavernous nerve. Results in Table 1 demonstrate that at all time points, no significant difference in basal pressure (before nerve stimulation) was observed between animals given sildenafil and *Fructus Cnidii* extract. Compared to pre-treatment, the basal ICP of sildenafil-treated rats significantly increased at every post-treatment time point (15, 30, and 45 minutes post-treatment). Meanwhile, this *Fructus Cnidii* extract group parameter was remarkably higher than pre-treatment when assessed at 30 and 45 minutes following administration.

We assessed the maximal ICP after nerve stimulation and found that this parameter did not differ between rats given sildenafil and the *Fructus Cnidii* extract group (Table 2). However, compared to pre-treatment, the maximal ICP of both groups was significantly increased at every post-treatment assessment (15, 30, and 45 minutes after treatment).

Fig. 2 demonstrates significant increases in the maximal ICP following nerve stimulation at pre-treatment, compared to the baseline measurements in both groups receiving sildenafil and *Frustucs Cnidii* extract ($p<0.05$). Similarly, after treatment, rats treated with sildenafil or *Frustucs Cnidii* extract also had the maximal ICP remarkably higher than the basal pressure ($p<0.01$) at every assessed time point.

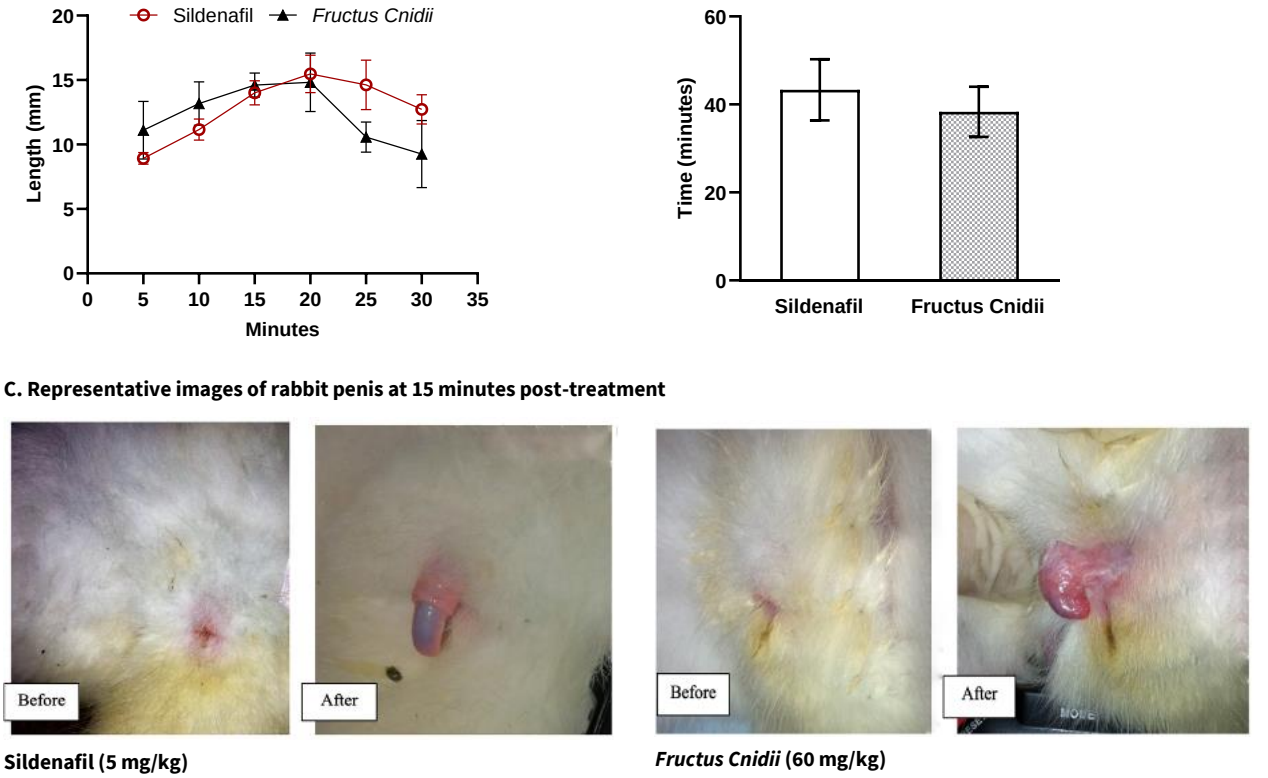


**Figure 1.** Effects of the *Fructus Cnidii* extract on the erectile function of adult male rabbits.

**(A)** The length of the penis during erection; **(B)** Total erectile time in rabbits; **(C)** Representative images of rabbit penis at 15 minutes post-treatment. Results in **(A)** and **(B)** are expressed as mean ± SD (n = 6). No statistically significant difference was observed between groups.

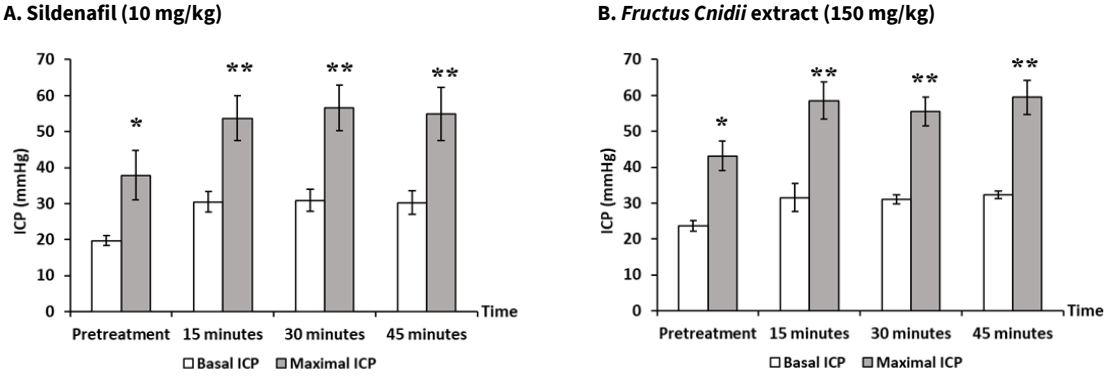


**Figure 2.** Comparisons between the basal and maximal intracavernous pressures of rats given sildenafil and *Fructus Cnidii* extract at different time points.

**(A)** Sildenafil group; **(B)** *Fructus Cnidii* extract group. Results in **(A)** and **(B)** are expressed as mean ± SD (n = 8). *p<0.05, indicating statistically significant differences, compared to the basal intracavernous pressure at the same time point; **p<0.01, indicating statistically significant differences, compared to the basal intracavernous pressure at the same time point. ICP: Intracavernous pressure.

Next, we continued to measure the duration of response when stimulating the cavernous nerve in rats of both groups. Results from Table 3 show that at all time points, no significant difference in response period was detected between animals given sildenafil and *Fructus Cnidii* extract. In sildenafil-treated rats, the response durations at every post-treatment measure (15, 30, and 45 minutes) were significantly longer compared to pre-treatment.

For the *Fructus Cnidii* extract group, the responding period at 30 minutes post-treatment significantly increased compared to pre-treatment. Meanwhile, the measures at 15 and 45 minutes following treatment tended to be longer than those at pre-treatment.

**Table 1.** Effects of *Fructus Cnidii* extract on the basal ICP before cavernous nerve stimulation in rats.

| Group | Basal ICP (mmHg) | | | |
|---|---|---|---|---|
| | Pre-treatment | 15 min post-treatment | 30 min post-treatment | 45 min post-treatment |
| Sildenafil (10 mg/kg) | 19.70 ± 1.36 | 30.47 ± 2.87 $^{\alpha}$ (↑54.7%) | 30.90 ± 3.01 $^{\alpha}$ (↑56.9%) | 30.22 ± 3.24 $^{\alpha}$ (↑53.3%) |
| *Fructus Cnidii* extract (150 mg/kg) | 23.63 ± 1.54 | 31.51 ± 3.87 | 31.02 ± 1.32 $^{\alpha}$ (↑31.6%) | 32.31 ± 1.09 $^{\alpha}$ (↑36.7%) |

Data are expressed as mean ± SD (n = 8). $^{\alpha}$p<0.05, indicating a statistically significant difference compared to pre-treatment. ICP: Intracavernous pressure.

**Table 2.** Effects of *Fructus Cnidii* extract on the maximal ICP after cavernous nerve stimulation in rats.

| Group (n=8) | Maximal ICP (mmHg) | | | |
|---|---|---|---|---|
| | Pre-treatment | 15 min post-treatment | 30 min post-treatment | 45 min post-treatment |
| Sildenafil (10 mg/kg) | 37.89 ± 6.78 | 53.69 ± 6.19 $^{\beta}$ (↑41.7%) | 56.60 ± 6.26 $^{\alpha}$ (↑49.4%) | 54.86 ± 7.40 $^{\beta}$ (↑44.8%) |
| *Fructus Cnidii* extract (150 mg/kg) | 43.14 ± 4.10 | 58.52 ± 5.22 $^{\beta}$ (↑35.7%) | 55.57 ± 3.97 $^{\beta}$ (↑28.8%) | 59.43 ± 4.72 $^{\beta}$ (↑37.8%) |

Data are expressed as mean ± SD (n = 8). $^{\alpha}$p < 0.05, indicating a statistically significant difference compared to pre-treatment. $^{\beta}$p<0.01, indicating a statistically significant difference compared to pre-treatment. ICP: Intracavernous pressure.

**Table 3.** Effects of *Fructus Cnidii* extract on the response time to cavernous nerve stimulation in rats.

| Group | Response time to nerve stimulation (s) | | | |
|---|---|---|---|---|
| | Pre-treatment | 15 min post-treatment | 30 min post-treatment | 45 min post-treatment |
| Sildenafil (10 mg/kg) | 90.81 ± 21.41 | 157.26 ± 22.28 $^{\alpha}$ (↑73.2%) | 179.74 ± 24.32 $^{\alpha}$ (↑97.9%) | 124.23 ± 19.52 $^{\alpha}$ (↑36.8%) |
| *Fructus Cnidii* extract (150 mg/kg) | 129.08 ± 16.36 | 172.97 ± 40.09 | 151.46 ± 18.65 $^{\alpha}$ (↑17.3%) | 119.32 ± 20.03 |

Data are expressed as mean ± SD (n = 8). $^{\alpha}$p<0.05, indicating a statistically significant difference compared to pre-treatment.

Lastly, we assessed the hemodynamic changes in response to nerve stimulation during ICP evaluation. **Fig. 3A** shows that at every measured time point following treatment, the MAP of both studied groups did not differ from that of pre-treatment. Additionally, no significant difference in MAP was observed between animals given sildenafil and *Fructus Cnidii* extract during this assessment.

We further investigated the maximal ICP/MAP ratio. Results in Fig. 3B demonstrated that the pre-treatment ratios of both groups were not significantly different. However, the ratios evaluated at 15, 30, and 45 minutes post-treatment of rats administered sildenafil and *Fructus Cnidii* extract were remarkably higher than the respective pre-treatment ratios. No significant difference was found when comparing the two groups at the same post-treatment time points.

## DISCUSSION

The fruit *of Cnidium monneier*i (L.) Cuss (*Fructus Cnidii*) has a long history of being used as a traditional remedy for male impotence, which is "erectile dysfunction" in modern medical terms. Experimental animal models evaluating the effect of different interventions on erectile function have been developed for many years, including studies on conscious and anesthetized animals.

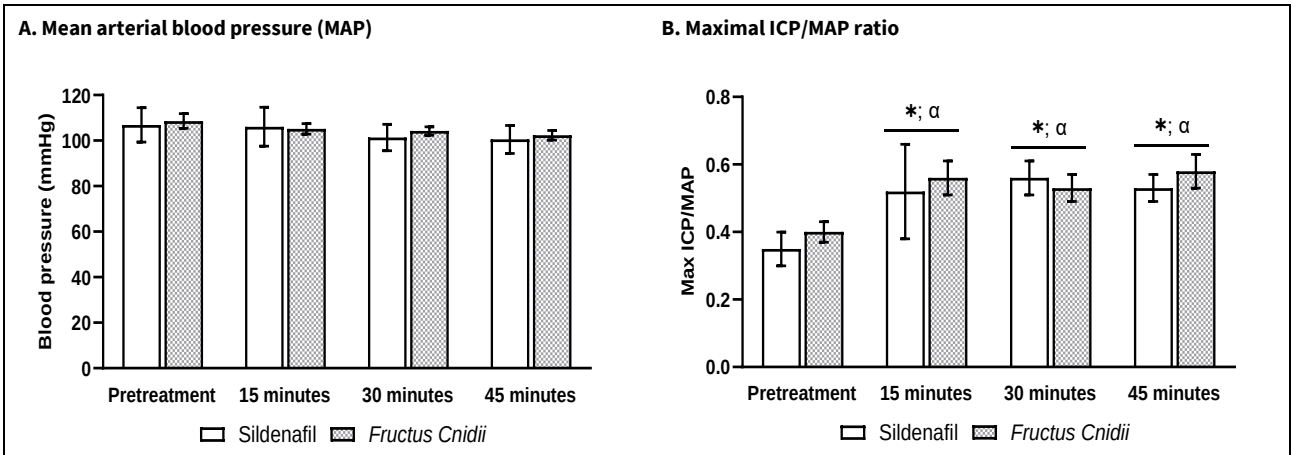


**Figure 3.** Effects of the *Fructus Cnidii* extract on the blood pressure of adult male rats during the assessment of intracavernous pressure.

**(A)** Mean arterial blood pressure; **(B)** The ratio of maximal intracavernous pressure and mean arterial pressure. Results in **(A)** and **(B)** are expressed as mean ± SD (n = 8). *p < 0.05, indicating statistically significant difference, compared to the sildenafil group at pre-treatment; αp < 0.05, indicating statistically significant difference, compared to the *Fructus Cnidii* extract group at pre-treatment. ICP: Intracavernous pressure; MAP: Mean arterial blood pressure.

Conscious rabbits can be used as a simple and quantitative model for assessing compounds that show potential for treating ED (Bischoff and Schneider, 2001). The similarity of cavernosal smooth muscle contraction/relaxation in rabbits and humans has been demonstrated (Bischoff and Schneider, 2001; Vogel, 2008). Moreover, in conscious rabbits, no sexual or direct stimulation, such as estrous females, apomorphine or metal penis ring, is required to induce erection. Therefore, any agent that promotes penile erection in the absence of sexual stimulation could be identified using this animal model. Taken together, we used conscious rabbits to investigate the potential of an agent for ED treatment.

However, this model does not reflect the quality of erections (ICP) or hemodynamic changes associated with an erectile response (Mehta et al., 2008; Singh et al., 2013). Therefore, evaluating ICP is important and necessary following the investigation on conscious rabbit models. Our study was conducted on anesthetized male rats to elucidate the effect on erection based on ICP measured after electro-stimulating the cavernous nerve. Under normal physiological conditions, neurotransmitter balance between sympathetic and parasympathetic nerves maintains cavernosal smooth muscle tone. Electrostimulation of the cavernous nerve in rodents causes a rapid increase in eNOS to achieve physical erection. This stimulation may induce a sustained increase in ICP even after the stimulation is terminated (Rehman et al., 1998). The mechanism is similar to that of sexual stimulation.

*Effects of sildenafil as a positive control drug*

As shown in our results, sildenafil caused penile erection in rabbits and increased the baseline ICP value (when there was no electrical stimulation of the cavernous nerve). A previous study evaluated the effects of sildenafil in anesthetized rabbits with direct injection of it into the corpus cavernosum and increased ICP in the absence of electrical stimulation of the cavernous nerve (McAuley et al., 2001). Choi et al. (2002) also obtained similar results when evaluating the effects of vardenafil and sildenafil on ICP in rats. This effect of sildenafil on increasing basal ICP in the absence of sexual stimulation can only be explained by the scientists' observation that, in addition to the long-proven PDE5 inhibition mechanism, sildenafil also has the ability to cause cavernous smooth muscle relaxation by a mechanism independent of the cyclic NO/GMP pathway (McAuley et al., 2001; Stief et al., 2000).

The significant effect of sildenafil in this study is to increase maximal ICP and the response duration in rats. It can easily be explained by the fact that sildenafil is a potent and selective inhibitor of cGMP-specific PDE5 in the corpus cavernosum, where PDE5 is responsible for the degradation of cGMP. When the NO/cGMP pathway is activated, as occurs with sexual stimulation, the inhibition of PDE5 by sildenafil increases corpus cavernosum levels of cGMP. This is an important physiological process that improves blood flow to specific areas, especially in the treatment of ED (McAuley et al., 2001; Stief et al., 2000). Moreover, sildenafil at the dose of 10 mg/kg b.w did not affect mean arterial pressure (MAP), thereby increasing the maximal ICP/MAP ratio in rats.

*Effects of Fructus Cnidii extract*

Our results revealed that *Fructus Cnidii* extract induced penile erection in rabbits. Additionally, the

extract increased the basal ICP, maximal ICP, and response time in anesthetized rats. A compound derived from *Fructus Cnidii*, osthole, has been shown to possess potent relaxant properties. In a previous study (Chen et al., 2000), osthole induced a relaxant effect on isolated rabbit cavernous smooth muscle. This effect was not inhibited by tetrodotoxin and was primarily driven by an increase in endothelium-derived NO. This suggests that this effect was the direct action on the corpus cavernosum rather than via a neuronal mechanism (Chen et al., 2000). According to another study (Chen et al., 2000), endothelium deprivation, along with the presence of L-NAME (a NOS inhibitor) or ODQ (a guanylate cyclase inhibitor), significantly reduced the osthole-induced relaxation. However, it did not completely block the effect, as a consistent portion of the osthole relaxant effect remained in the endothelium-deprived tissue (Chen et al., 2000). This suggests osthole does not solely act through the endothelium-NO pathway, but also acts directly on the cavernous smooth muscle.

One adverse effect of pharmaceutical agents, increasing NO release or cGMP/cAMP, is hypotension. Thus, in addition to evaluating the effects of *Fructus Cnidii* extract on ICP, our study evaluated its effects on MAP and ICP max/MAP ratio. Results showed that sildenafil and *Fructus Cnidii* did not influence MAP, leading to increased ICP max/MAP ratios. Therefore, *Fructus Cnidii* extract at the dose of 150 mg/kg/day did not affect BP in rats. However, in our previous study of acute toxicity, symptoms of vasodilation were observed when administered a much higher dose (Dau et al., 2014). Therefore, if *Fructus Cnidii* extract is used in humans, attention should be paid to its undesirable vasodilating effects, especially the possibility of causing hypotension.

Here, our experimental models using rabbits and rats have provided valuable and reproducible investigation regarding the effect of *Fructus Cnidii* extract on ED management. The advantages of measuring ICP in anesthetized animals are the accurate assessments of ICP, BP, and response to nerve stimulation. However, this method could not assess ICP in the conscious state and ICP changes during sexual activity. Recording of ICP in conscious rats via telemetry, especially with male sexual behaviors, might solve this drawback. Moreover, penile erection is a complex physiological process composed of multiple neurologic, vascular, and humoral events. Further studies are needed to fully understand the mechanisms of this extract on various factors that could affect erectile function.

## CONCLUSION

*Fructus Cnidii* extract positively affected the erectile function in experimental male animals. Our findings support the clinical use of *Fructus Cnidii* extract for the treatment of ED.

## CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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|---|---|---|---|---|---|---|---|---|
| Concepts or ideas | x | x | x | x | x | x | | x |
| Design | x | x | x | x | x | x | | x |
| Definition of intellectual content | x | x | x | x | x | x | | x |
| Literature search | x | x | x | x | x | | x | x |
| Experimental studies | x | x | x | x | x | x | x | x |
| Data acquisition | x | | x | x | x | | x | x |
| Data analysis | x | | | x | x | | x | x |
| Statistical analysis | x | | | x | x | | x | x |
| Manuscript preparation | x | | x | x | x | x | x | x |
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