

## Effect of Watermelon (*Citrullus lanatus*) Flesh Extract on Sexual Behavior of Male Rats

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### ABSTRACT

*The effects of red watermelon (*Citrullus lanatus*) flesh extract on male sexual behavior as well as its adverse effects were investigated using animal models. The suspension of the flesh extract was administered orally at doses of 100, 500 and 1000 mg/kg to different groups of male rats (n = 5) daily for 22 days. The receptive female rats were prepared by hormonal treatment. The mating behavior was investigated and compared with the standard reference drug, sildenafil citrate. The adverse effects of the plant extract were also evaluated by observing at least once daily for any sign of toxicity, water and food intake, stress and changes in behavior. The animal procedures were conducted in accordance with the Institutional Animal Care and Use Committee, Ubon Ratchathani Rajabhat University, Thailand. The results indicated that oral administration of watermelon flesh extract caused a significant increase in Mounting Frequency, Intromission Frequency and Ejaculatory Latency in first and second series and caused a significant decrease in the Mounting Latency and Intromission Latency ( $P < 0.05$ ). The extract at a dose of 1000 mg/kg did not produce clinical signs of toxicity or mortality in any of animals during the treatment duration. The weight of body and reproductive organs showed no significant difference in the flesh extract groups compared to the control group ( $P > 0.05$ ). Based on the aphrodisiac property of watermelon in animal models observed in this present study, this plant may be useful for men with erectile dysfunction. In addition, watermelon flesh extract did not produce undesirable effects on male rats, indicating that its short-term use is apparently safe. Thus, our findings support the use of watermelon flesh for increasing potency in males.*

**Keywords:** *Citrullus lanatus*, Watermelon, Sexual behavior, Aphrodisiac, Male rats

## INTRODUCTION

Erectile dysfunction (ED) is a common disorder in older men, causing a decrease in quality of life. Multiple risk factors are associated with ED, including hypertension, diabetes, coronary artery disease, smoking and alcohol consumption (Lue, 2000). In Thailand, the prevalence of ED has increased from 37.5% in 2000 to 42.8% in 2008 (Permpongkosol et al., 2008). Sildenafil citrate or Viagra has been used to increase potency, sustain satisfactory erections and improve sexual satisfaction without affecting sexual desire in men with ED (Lue, 2000; Park et al., 2011). However, sildenafil citrate may do harm to patients with coronary ischemia or congestive heart failure, or patients taking multidrug antihypertensive regimens (Lue, 2000).

Several medicinal plants have long been used as aphrodisiacs to improve sexual health in man worldwide, because they are apparently safe and have fewer side effects (Sandroni, 2001; Drewes et al., 2003). Aphrodisiacs are substances that can increase sexual function and sexual desire (Sandroni, 2001; Soni et al., 2012). Sexual desire is modulated by the central nervous system (Soni et al., 2012). On the other hand, sexual function is not always dependent on sexual desire, but dependent on a neurovascular regulation via the hemodynamic mechanisms of penile erection (Soni et al., 2012). It is generally accepted that medicinal plants are composed of numerous chemical constituents, which usually exert their therapeutic effects through multiple mechanisms (Drewes et al., 2003; Cao et al., 2008). The nature of these actions results in either the stimulation (aphrodisiac) or inhibition (anti-fertility) of sexual behavior. Watermelon, claimed to have aphrodisiac potential (Drewes et al., 2003; Figueroa et al., 2011; Jayaprakasha et al., 2011; Munglue et al., 2012), has been receiving attention.

Watermelon (*Citrullus lanatus*) belongs to the family Cucurbitaceae and is rich in the amino acids arginine and citrulline, which play important roles in the production of the potent vasodilator, nitric oxide (NO) (Cormio et al., 2011; Munglue et al., 2012; Rimando and Perkins-Veazie, 2005). NO is a physiological signal acting to regulate the mechanisms underlying penile erection through the activation of soluble guanylate cyclase to convert guanosine triphosphate to cyclic guanosine monophosphate (cGMP) (Sandroni, 2001; Drewes et al., 2003; Cormio et al., 2011; Estrada-Reyes et al., 2013;). In addition, oral L-citrulline supplementation can improve penile erection in men with mild ED (Cormio et al., 2011). Ethnopharmacological relevance demonstrated that watermelon supplementation can improve aortic hemodynamics in patients with prehypertension, suggesting that watermelon has a potent vasodilator (Collins et al., 2007; Figueroa et al., 2011). In addition, watermelon extract induces the relaxation of smooth muscle cells through NO pathway modulation and reduction of intracellular  $Ca^{2+}$  [ $Ca^{2+}$ ]<sub>i</sub> (Jayaprakasha et al., 2011). Recently, our data indicated that watermelon extracts can exert their effects in rat isolated uterine strips by inhibiting  $Ca^{2+}$  influx and some of the  $Ca^{2+}$  signaling element involved in smooth muscle contraction (Munglue, 2011; Munglue et al., 2012). Watermelon has been reported to have tocolytic properties (Jayaprakasha et al., 2011; Munglue et al., 2012). Few studies have examined the effects of watermelon extract on female reproductive phy-

siology (Jayaprakasha et al., 2011; Munglue et al., 2012); its effect on male sexual behavior has not yet been elucidated.

To the best of our knowledge, the effect of watermelon flesh extract on male rat sexual behavior has not been examined and the safe use of this plant should be evaluated. As there is an urgent need to find better drugs with fewer undesirable side effects to improve sexual performance, and novel compounds are sought (Drewes et al., 2003), the aims of the present study were, therefore, to investigate the effects of watermelon flesh extract on sexual behavior along with its adverse effects on sexually active male rats.

## MATERIALS AND METHODS

### Plant material collection

The fruits of watermelon were collected locally in the Province of Ubon Ratchathani, Thailand, where the plant was cultivated under natural conditions. Voucher specimen was identified and deposited at the Program of Biology, Faculty of Science, Ubon Ratchathani Rajabhat University, Thailand.

### Plant extraction

The watermelon fruit was cleaned, the flesh isolated from the rind and the seeds removed. Watermelon flesh was dried in a hot air oven at 60°C for 4 days (Munglue, 2011). Dried watermelon flesh was extracted with ethanol (70%) and evaporated under vacuum to yield 45% (weight/weight [w/w] based on the dried starting weight). Watermelon flesh extract was suspended in distilled water and administered orally by intragastric tube.

### Animal preparation

The experiments conducted were in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources, National Research Council, Thailand. The animal procedures were conducted in accordance with the Institutional Animal Care and Use Committee, Ubon Ratchathani Rajabhat University, Thailand. Twenty-five 25 male (weighing 350-400 g) and 10 female (weighing 200-250 g) Wistar rats were used in this study. They were housed under standard conditions, and were fed on standard diet with water *ad libitum* (Wethangkaboworn and Munglue, 2013).

The male rats were randomly divided into five groups of five animals each:

**Group I:** received distilled water orally and served as control.

**Group II:** received the watermelon flesh extract 100 mg/kg body weight daily (WF 100).

**Group III:** received the watermelon flesh extract 500 mg/kg body weight daily (WF 500).

**Group IV:** received the watermelon flesh extract 1000 mg/kg body weight daily (WF 1000).

**Group V:** received sildenafil citrate 5 mg/kg body weight, one hour prior to the experiment and served as a standard group.

The female rats used for mating test were made receptive by hormonal treatment (Sandroni, 2001; Wethangkaboworn and Munglue, 2013). Briefly, female rats were given an oral dose of ethinyl estradiol suspension (100 µg/animal) 48 h prior to the experiment. Then, the animals were injected with progesterone subcutaneously at a dose of 1 mg/animal 6 h before the sexual behavior test (Sandroni, 2001; Wethangkaboworn and Munglue, 2013).

### **Effect on sexual behavior**

Sexual behavioral examination was observed at 22 days of drug treatment. Single male rats were gently dropped in 60×50×40 cm glass cages and acclimatized for 5 min. Then, a receptive female was presented to the male by placing it gently into the cage. The sexual parameters were recorded and calculated as follows (Sandroni, 2001; Tajuddin et al., 2005; Wethangkaboworn and Munglue, 2013):

- (a) time from the introduction of the female into the male's cage to the first mount or Mounting Latency (ML).
- (b) time from the introduction of the female to the first intromission by the male or Intromission Latency (IL).
- (c) time from the first intromission of a series to the ejaculation or Ejaculatory Latency (EL).
- (d) time from the first ejaculation to the next intromission by the male or Post Ejaculatory Interval (PEI).
- (e) number of mounts before ejaculation or Mounting Frequency (MF).
- (f) number of intromission before ejaculation or Intromission Frequency (IF).

### **Effect on body weight and reproductive organ weights**

To examine the effect of the plant extract on the body weight of the animals, treated rats were weighted on day 22 and compared to the control group. Then, the animals were sacrificed by asphyxiation with CO<sub>2</sub>. Testes, seminal vesicles, prostate gland and epididymides were carefully removed, cleared from the adipose tissue and weighted.

### **Adverse effects of the extract**

All treated animals were observed daily for signs of toxicity and stress and behavior changes. The parameters recorded were salivation, rhinorrhea, lachrymation, ptosis, writhing, convulsions and tremors. Food and water intake was also noted (Tajuddin et al., 2005; Wethangkaboworn and Munglue, 2013).

### **Statistical analysis**

Data are expressed as mean ± standard error of the mean. Significance difference was analyzed using one-way analysis of variance (ANOVA). *P value* <0.05 was considered statistically significant (Wethangkaboworn and Munglue, 2013).

## RESULTS

### Effect on sexual behavior

The results obtained with the test for general mating behavior showed that oral administration of watermelon flesh extract significantly increased MF, IF, EL<sub>1</sub> and EL<sub>2</sub> ( $P < 0.05$ ) and caused a significant decrease in ML and IL ( $P < 0.05$ ). The standard reference drug, sildenafil citrate, significantly increased MF, IF, EL<sub>1</sub>, EL<sub>2</sub> and PEI and decreased ML and IL, when compared with the control animals ( $P < 0.05$ ) (Table 1).

**Table 1.** Effect of watermelon extract on mating behavior in male rats observed at 22 days of the treatment.

| Parameters               | Treatments  |                            |                           |                           |                                    |
|--------------------------|-------------|----------------------------|---------------------------|---------------------------|------------------------------------|
|                          | Control     | WF<br>(100 mg/kg)          | WF<br>(500 mg/kg)         | WF<br>(1000 mg/kg)        | Sildenafil<br>citrate<br>(5 mg/kg) |
| MF                       | 10.18±1.47  | 13.25±4.68 <sup>ns</sup>   | 15.00±3.87*               | 16.50±2.12*               | 27.50±2.84*                        |
| IF                       | 7.50±0.28   | 11.25±0.75*                | 12.15±1.50*               | 14.25±1.45*               | 16.75±1.31*                        |
| ML (in sec)              | 45.25±1.35  | 39.75±7.15 <sup>ns</sup>   | 29.25±3.26*               | 26.75±1.45*               | 23.00±1.08*                        |
| IL (in sec)              | 57.50±3.37  | 55.79±6.57 <sup>ns</sup>   | 45.04±2.07*               | 39.08±1.09*               | 27.50±0.67*                        |
| EL <sub>1</sub> (in sec) | 216.75±4.02 | 220.30±11.20 <sup>ns</sup> | 235.24±2.35*              | 240.63±6.15*              | 313.22±4.56*                       |
| EL <sub>2</sub> (in sec) | 281.25±8.91 | 286.24±4.36 <sup>ns</sup>  | 298.75±1.64*              | 301.75±1.87*              | 385.25±4.49*                       |
| PEI (in sec)             | 370.19±2.32 | 366.23±2.90 <sup>ns</sup>  | 355.24±5.14 <sup>ns</sup> | 350.12±2.10 <sup>ns</sup> | 180.12±1.49*                       |

Note: WF = watermelon flesh extract, MF = mounting frequency, IF = intromission frequency, ML = mounting latency, IL = intromission latency, EL<sub>1</sub> = ejaculatory latency in first series, EL<sub>2</sub> = ejaculatory latency in second series, PEI = post ejaculatory interval. Values are expressed as mean ± SEM, n = 5 (number of animals in each group). One-way analysis of variance (ANOVA) was used. \* $P$  value  $< 0.05$  was considered statistically significant when compared to the control group. ns = not significant.

### Effect on body weight and relative reproductive organ weights

The animals receiving the plant extract did not show any changes in body weight or relative reproductive organ weights (Table 2) when compared to the control animal group ( $P > 0.05$ ).

**Table 2.** Effects of watermelon extract on body weight and relative reproductive organ weights of male rats observed at 22 days of the treatment.

| Parameters               | Treatments  |                           |                           |                           |                                    |
|--------------------------|-------------|---------------------------|---------------------------|---------------------------|------------------------------------|
|                          | Control     | WF<br>(100 mg/kg)         | WF<br>(500 mg/kg)         | WF<br>(1000 mg/<br>kg)    | Sildenafil<br>citrate<br>(5 mg/kg) |
| Body weight (g)          | 436.01±9.25 | 442.09±8.71 <sup>ns</sup> | 432.02±8.65 <sup>ns</sup> | 440.74±7.29 <sup>ns</sup> | 435.08±8.95 <sup>ns</sup>          |
| Prostate gland<br>(g%)   | 0.24±0.02   | 0.23±0.01 <sup>ns</sup>   | 0.24±0.01 <sup>ns</sup>   | 0.24±0.01 <sup>ns</sup>   | 0.24±0.01 <sup>ns</sup>            |
| Seminal vesicles<br>(g%) | 0.33±0.03   | 0.36±0.02 <sup>ns</sup>   | 0.33±0.04 <sup>ns</sup>   | 0.35±0.03 <sup>ns</sup>   | 0.35±0.02 <sup>ns</sup>            |
| Testes (g%)              | 0.91±0.02   | 0.93±0.04 <sup>ns</sup>   | 0.84±0.02 <sup>ns</sup>   | 0.96±0.04 <sup>ns</sup>   | 0.83±0.02 <sup>ns</sup>            |
| Epididymides<br>(g%)     | 0.26±0.01   | 0.26±0.01 <sup>ns</sup>   | 0.23±0.01 <sup>ns</sup>   | 0.27±0.01 <sup>ns</sup>   | 0.26±0.01 <sup>ns</sup>            |

Note: WF = watermelon flesh extract, Values are expressed as mean ± SEM, n = 5 (number of animals in each group). One-way analysis of variance (ANOVA) was used. \**P* value <0.05 was considered statistically significant compared to the control group. ns = not significant.

### Adverse effects of the extract

The plant extract did not produce any signs of toxicity, stress or changes in behavior. The food and water intake was similar to the control animals. In addition, the extract at a high dose, 1000 mg/kg, did not cause treatment-related signs of toxicity or mortality in any of the animals during the treatment period.

## DISCUSSION

The results indicated that the watermelon plant extract caused a significant increase in potency or sustained erection. In addition, it was also observed to be devoid of any sign of toxicity.

ML and IL are parameters of sexual arousal (Tajuddin et al., 2005; Suresh et al., 2009). Furthermore, MF and IF are indicators of libido and potency (Tajuddin et al., 2005; Suresh et al., 2009). The decreases in ML and IL and the increases in MF and IF produced by watermelon flesh extract revealed that this plant may be a useful sexual stimulant.

EL and PEI are important for evaluating prolonged duration of coitus and the rate of recovery from exhaustion after the first series of mating, respectively (Tajuddin et al., 2005; Suresh et al., 2009). Medicinal plants with an aphrodisiac property should increase EL and decrease PEI (Tajuddin et al., 2005; Suresh et al., 2009). In this present study, watermelon extract increased EL and tended to decrease PEI. Thus, our results support this suggestion.

Sildenafil citrate was able to produce very significant decreases in ML, IL and EL and increases in MF and IF, when compared to the plant extract. However, in this experiment, the standard reference drug, sildenafil citrate, was used to evaluate the quantitative value and not to compare the mechanisms of action (Tajuddin et al., 2005).

It has been reported that substances that affect potency are generally mediated through induction of vasodilators, NO and cGMP (Sandroni, 2001; Estrada-Reyes et al., 2013). Our finding on the stimulatory effect of watermelon flesh extract on male rat sexual behavior might be due to NO-cGMP relaxant pathway modulation (Drewes et al., 2003; Jayaprakasha et al., 2011; Munglue et al., 2012; Estrada-Reyes et al., 2013;). It was demonstrated that ethanolic extracts of watermelon flesh and rind produced a significant decrease in rat uterine contraction through the activation of NO production (Munglue et al., 2012). In addition, the inhibitory effects of the plant extracts on uterine smooth muscle can be a result of the addition of NO inhibitors (Munglue et al., 2012). These results indicated that watermelon is a potent tocolytic.

The major constituents found in watermelon are citrulline, arginine, lycopene and  $\beta$ -carotene (Rimando and Perkins-Veazie, 2005; Jayaprakasha et al., 2011). Previous studies have indicated that both citrulline and arginine were able to improve sexual function in patients with erectile dysfunction (Zorgniotti and Lizza, 1994; Melman, 1997; Cormio et al., 2011;). Hence, the aphrodisiac property of watermelon might be due to citrulline, arginine or such compounds found in this plant. Further research is needed to identify the active compound(s) responsible for its aphrodisiac activity and the mechanisms underlying its action.

In this present study, the plant extract did not cause any side effects or deaths during the treatment period. In addition, treated animals experienced no changes in general behavior, body weight and relative reproductive organ weight. Food and water intake were similar to those of the control animals, which suggested that watermelon could be used for a long time without producing signs of toxicity or treatment-related adverse effects.

## CONCLUSION

Based on the aphrodisiac property of watermelon in animal models observed in this present study, this plant may be useful for the treatment of erectile dysfunction. In addition, watermelon flesh extract did not produce any undesirable effects on male rats, indicating that its short-term use of this plant is apparently safe. Thus, our findings support the use of watermelon flesh for increasing potency in males.

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