



## Effect of Ethanolic Seed Extract of *Nelumbo nucifera* on Male Rat Sexual Behavior

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### Abstract

The seeds of *Nelumbo nucifera* have been used as an antifertility agent in traditional medicine since ancient times. However, the effect of ethanolic seed extract of *Nelumbo nucifera* on male rat sexual behavior has not yet been investigated and the safe use of this plant should be evaluated. Thus, the aim of this present study was therefore to determine the effect of *Nelumbo nucifera* seed extract on sexual behavior as well as its adverse effects using animal models. *Nelumbo nucifera* seeds were ethanolic extracted. The suspension of the extract was administered by oral gavage at the dose of 100, 500, and 1000 mg/kg, to different groups of male rats (n = 5) for 21 consecutive days. Preparation of sexually receptive female rats was performed by hormonal treatment. The general 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 results indicated that oral administration of *Nelumbo nucifera* seed extract produced a significant increase in the Mounting Latency and Intromission Latency and caused a significant decrease in the Mounting Frequency, Intromission Frequency and Ejaculatory Frequency in Second Series. The extract at a dose of 1000 mg/kg did not produce treatment-related signs of toxicity or mortality in any of animals during the treatment period. Thus, the resultant significant and sustained decrease in the sexual behavior of male rats without any side-effects suggest that the ethanolic seed extract of *Nelumbo nucifera* has an antifertility property, which might be due to the suppression of the drug in the testicular androgen levels. The present study supports the traditional use of *Nelumbo nucifera* seed for fertility regulation in male.

**Keywords:** *Nelumbo nucifera* seed, antifertility, sexual behavior, male rats

## 1. Introduction

The world population is growing rapidly and this problem causes the need for effective birth control methods (1, 2). Contraceptive agents are generally used to control human fertility both in male and female worldwide (1, 2). It has been reported that male contraceptives are less effective than female contraceptives because of this dependence on a complex hormonal modulation in spermatogenesis (2). In addition, male contraceptives should be complete and reversible fertility and low toxicity as the female birth control pill (2). Most contraceptive agents are the synthetic drugs and have undesirable side effects like hypertension and increased risk of cancer (1, 2). Recent works have been examined and analyzed the nonhormonal compounds that can inhibit sperm production in several types of animals (1, 2). However, long term use of nonhormonal agents may be toxic to liver and kidney (2). Therefore, development of contraceptive agents from natural products with fewer side effects is required (2, 3). Many medicinal plants have been used in alternative medicine for fertility regulation in male since ancient times (1, 3). *Nelumbo nucifera*, belongs to the family of Nelumbonaceae, is a perennial aquatic plant of China, Japan and Thailand. This plant is widely cultivated for food and drink and generally used as a medicinal plant (1, 4, 5). Most research has focused on its antioxidant, anti-inflammatory, anti-obesity, anti-pyretic and anti-cancer properties (1, 4, 5). In addition, *Nelumbo nucifera* seed extract has an anti-steroidogenic effect as it decreased the weight of ovaries and testis of rat (4, 5). It also demonstrated that *Nelumbo nucifera* seed extract caused an inhibitory effect on estrogen production in female rats (6). The result supports the antiestrogenic property of *Nelumbo nucifera* seed extract in female (4, 5, 6).

Phytochemical studies indicated that *Nelumbo nucifera* contains a high level of alkaloids (neferine, nuciferine, liensinine, isoliensinine and remrefidine),

flavonoids, triterpenoids and tannins (1, 4, 5).

To the best of our knowledge, the effect of *Nelumbo nucifera* seed 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 that help to control the birth rate, and novel compounds are sought (1), the aims of the present study were therefore, to investigate the effect of ethanolic seed extract of *Nelumbo nucifera* on sexual behavior along with its adverse effects on sexually male rats.

## 2. Material and methods

### 2.1 Plant material

The seeds of *Nelumbo nucifera* were collected locally from Ubon Ratchathani, Thailand, during October to November 2012. The plant was identified by herbal specimen at Program in Biology, Ubon Ratchathani Rajabhat University, Thailand.

### 2.2 Plant extraction

The seeds of *Nelumbo nucifera* were manually collected. They were dried in a hot air oven at 60°C for 7 days. Dried seeds (50 g) were macerated with 75% ethanol (500 mL) for 14 days. The extract was filtered through Whatman paper No. 1 and dried by rotary evaporator. The yield was expressed as 4 mg/g based on dried seed weight.

### 2.3 Animal preparation

The animal procedures were conducted in accordance with the Institutional Animal Care and Use Committee, Ubon Ratchathani Rajabhat University, Thailand. A total of 25 male (weighing 350-400 g) and 25 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*.

The male rats were randomly divided into 5 groups of 5 animals:

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

**Group II:** received the *Nelumbo nucifera* seed extract at the dose of 100 mg/kg body weight daily (NSE 100).

**Group III:** received the *Nelumbo nucifera* seed extract at the dose of 500 mg/kg body weight daily (NSE 500).

**Group IV:** received the *Nelumbo nucifera* seed extract at the dose of 1000 mg/kg body weight daily (NSE 1000).

**Group V:** received sildenafil citrate 5 mg/kg body weight, one hour before the experiment and served as a standard reference group (8).

The female rats used for mating test were made receptive by hormonal treatment (8, 9). Briefly, female rats were received the suspension of ethinyl estradiol orally at the dose of 100 µg/animal 48 h prior to the experiment. Then, the animals were injected with progesterone subcutaneously at the dose of 1 mg/animal 6 h before sexual behavior test (9).

#### 2.4 Study on sexual behavior

Male sexual behavioral examination was carried out daily for 22 days of drug treatment. Single male rats were gently dropped in 60x50x40 cm glass cages and acclimatized for 5 min. Then, a receptive female was presented to male by placing it gently into the cage. The standard sexual parameters were recorded and calculated as followed (8, 9):

**Mounting Latency (ML)** is defined as the time from the introduction of female into the cage of the male up to the first mount.

**Intromission Latency (IL)** is defined as the time from the introduction of the female up to the first intromission by the male.

**Ejaculatory Latency (EL)** is defined as the time from the first intromission of a series up to the ejaculation.

**Post Ejaculatory Interval (PEI)** is defined as the time from the first ejaculation up to the next intromission by the male.

**Mounting Frequency (MF)** is defined as the number of mounts before ejaculation.

**Intromission Frequency (IF)** is defined as the number of intromission before ejaculation.

#### 2.5 Study on adverse effects of the extract

All treated animals were observed daily for signs of toxicity and stress and behavior changes. The parameters were salivation, rhinorrhea, lachrymation, ptosis, writhing, convulsions and tremors. Food and water intake was also recorded (8).

#### 2.6 Statistical analysis

Data are expressed as mean  $\pm$  standard error of the mean. The significance of difference was analyzed using one-way analysis of variance (ANOVA). *P* value  $<0.05$  was considered statistically significant.

### 3. Results

#### 3.1 Effect of the extract on sexual behavior

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

#### 3.2 Adverse effects of the extract

The plant extract did not produce any signs of toxicity, stress and 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 animals during the treatment period.

**Table 1.** Effect of *Nelumbo nucifera* seed extract on mating behavior in male rats.

Parameters	Mean ± SEM				
	Control	NSE (100 mg/kg)	NSE (500 mg/kg)	NSE (1000 mg/kg)	Sildenafil citrate (5 mg/kg)
MF	10.18±1.47	9.25±2.68 <sup>ns</sup>	7.00±0.87*	6.50±0.32*	27.50±2.84*
IF	7.50±0.28	5.25±0.75*	3.25±0.50*	2.75±0.47*	16.75±1.31*
ML (in sec)	45.25±5.55	56.75±1.65 <sup>ns</sup>	80.25±3.56*	100.75±6.75*	23.00±1.08*
IL (in sec)	57.50±3.37	59.49±5.23 <sup>ns</sup>	68.04±1.71*	79.78±1.59*	27.50±0.67*
EL <sub>1</sub> (in sec)	216.75±7.02	231.30±12.02 <sup>ns</sup>	237.24±11.95 <sup>ns</sup>	247.63±16.35 <sup>ns</sup>	313.22±4.56*
EL <sub>2</sub> (in sec)	281.25±8.91	293.24±3.70 <sup>ns</sup>	333.75±8.64*	330.75±5.87*	385.25±4.49*
PEI (in sec)	375.19±2.32	376.23±4.95 <sup>ns</sup>	378.24±8.19 <sup>ns</sup>	379.02±8.10 <sup>ns</sup>	180.12±1.49*

NSE = *Nelumbo nucifera* seed extract, MF = mounting frequency, IF = intromission frequency, ML = mounting latency, IL = intromission latency, EL<sub>1</sub> = ejaculatory frequency in first series, EL<sub>2</sub> = ejaculatory frequency 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 with the control group. ns = not significant.

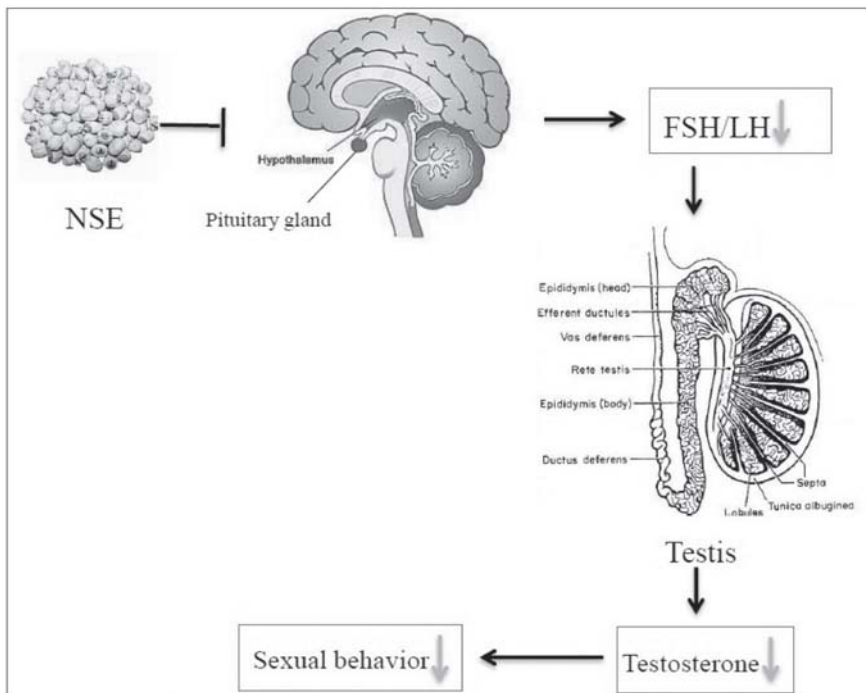
### 4. Discussion

*Nelumbo nucifera* seeds have been reported to have an antifertility property (1, 4, 5, 6, 10). Few studies have been examined the effects of *Nelumbo nucifera* seed on male reproductive physiology, its effect on male sexual behavior has not yet been elucidated. Therefore, this present study was aimed to investigate the effect of *Nelumbo nucifera* seed extract on sexual behavior of male rats. The results indicated that the plant extract caused a significant decrease in sexual performance parameters. Furthermore, the extract did not produce any signs of toxicity or mortality in any of animals during the treatment period.

There is evidence that substances that affect the libido may act at the level of the central nervous by activating a specific neurotransmitter or specific sex hormone concentration (2, 6, 7, 8, 9, 10). Our finding on the inhibitory effect of *Nelumbo nucifera* seed extract on male rat sexual behavior supported its antifertility activity might be due to the inhibition of (see Figure 1): 1) gonadotropin

releasing hormone production at the hypothalamic level or; 2) gonadotropic hormone production at the pituitary level, including follicle stimulating hormone (FSH) and luteinizing hormone (LH) or; 3) testosterone production at the testicular level (6, 7, 8, 9, 10). It was demonstrated that petroleum ether extract of *Nelumbo nucifera* seeds produced a significant decrease in glucose-6-phosphate dehydrogenase (G6PD) activity in the testis of male rats (5). Much has been reported that G6PD is an enzyme that plays an important role in the regulation of testicular androgen biosynthesis in the testis (5). Thus, low G6PD contents produce a decrease in testosterone level, leading to impairment of sexual performance (7, 11). This could be the reason why *Nelumbo nucifera* extract caused major disruptions in sexual motivation/arousal (5).

In this experiment, the standard reference drug, Sildenafil citrate, was used to evaluate the quantitative value and not to compare the mechanisms of action. There is evidence that medicinal plants with antifertility property should be produced a significant increase in the ML and IL (12). Thus, our findings support to these suggestions.



**Figure 1.** Effect of *Nelumbo nucifera* seed extract on male rat sexual behavior. NSE = *Nelumbo nucifera* seed extract, FSH = follicle stimulating hormone, LH = luteinizing hormone. See the text for details.

The major constituents found in *Nelumbo nucifera* were alkaloids, flavonoids, triterpenoids, carbohydrate, unsaturated fatty acids and amino acids (1, 4, 5, 10).

It was revealed that alkaloids possess anti-androgenic and anti-spermatic activities (11). The mechanisms of action are thought to be due to  $\alpha$ -adrenoceptor antagonistic and antimuscarinic properties. (11).  $\alpha$ -adrenoceptor antagonistic substances produced a significant decrease the contraction of vas deferens and caudal epididymis induced by neural stimulation, resulting in a decrease in sperm emission from caudal epididymis to vas deferens (11, 13). In addition, antimuscarinic agents can reverse fertility in infertile men by unknown mechanism (14). Therefore, alkaloids might cause inhibitory effect of the *Nelumbo nucifera* on sexual behavior in this present study. Our results also show that the plant extract did not cause any side-effects or deaths during the treatment

period. In addition, treated animals did not change in general behavior. The food and water intake was similar to those of the control animals, which suggested that *Nelumbo nucifera* can be used for longer time without producing any signs of toxicity or treatment-related adverse effects.

Taken together, based on the inhibitory effect of *Nelumbo nucifera* on male sexual behavior observed in the present study, its mechanism of action could be due to alkaloids or other compounds found in the extract. Further research is needed for the identification of its active compound(s) responsible for antifertility activity and the mechanisms of actions. In addition, *Nelumbo nucifera* seed extract did not produce any undesirable effects on male rats, indicating that its short term use is apparently safe. Thus, our findings support the traditional use of *Nelumbo nucifera* seed for male fertility regulation.

## 5. Acknowledgements

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