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Acute and subchronic oral toxicity studies of *Nelumbo nucifera* stamens extract in rats

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ABSTRACT

Ethnopharmacological relevance: Since the use of *Nelumbo nucifera* stamens in herbal medicines as well as in cosmetic products are highly prevalent in Thailand and increasing worldwide, acute and subchronic toxicity studies to confirm the safe use of *Nelumbo nucifera* stamens are warranted.
Aim of the study: Acute and subchronic oral toxicity studies of *Nelumbo nucifera* stamens extract in rats were performed in the present study in order to evaluate its safety.
Materials and methods: In acute toxicity study, *Nelumbo nucifera* stamens extract was administered by oral gavage to Sprague–Dawley rats (5 males and 5 females) at a dose of 5000 mg/kg. In subchronic toxicity study, the extract at doses of 50, 100, and 200 mg/kg/day were given orally to groups of rats (6 rats/dose/sex) for 90 consecutive days.
Results: The extract at a dose of 5000 mg/kg produced no treatment-related signs of toxicity or mortality in any of the animals tested during 14 days of the study. In the repeated dose 90-day oral toxicity study, there was no significant difference in body weight between the control and all treatment groups with the exception of the body weight of the female group treated with 200 mg/kg/day of the extract which was statistically significantly less than that of its control counterpart on day 90 but the percent weight changes of both groups were almost similar. Some statistically significant differences in hematological and biochemical parameters as well as in some internal organ weights of both male and female rats treated with the extract at the highest dose were observed. However, no abnormality of internal organs was observed in both gross and histopathological examinations.
Conclusions: These results suggest that the oral lethal dose of *Nelumbo nucifera* stamens extract for male and female rats is in excess of 5000 mg/kg and the no-observed-adverse-effect level (NOAEL) of the extract for both male and female rats is considered to be 200 mg/kg/day.

1. Introduction

Nelumbo nucifera Gaertn., also known as "Bua Luang", "sacred lotus", or simply "lotus" in Thai, is a plant in the Nelumbonaceae family. This plant is an aquatic perennial, and cultivated in tropical regions worldwide particularly in Asia. It is a well known medicinal plant in Asia. A review on this plant by Mukherjee et al. (2009) stated that there are several therapeutic potential of various parts of this plant, such as hypoglycemic, antidiarrheal, antimicrobial, diuretic, antipyretic as well as anti-inflammatory activities of its rhizomes extracts (Mukherjee et al., 1995a,b,c,d, 1996a,b, 1997). Hepatoprotective (Sohn et al., 2003), antiproliferative (Liu et al., 2004), anti-inflammatory (Lin et al., 2006) as well as antioxidant (Rai et al., 2006) activities of its seed extracts have also been reported. In Thai and Chinese traditional medicine, dried *Nelumbo nucifera* stamens are combined with other herbs to treat fever and allergy, or used as a neurotonic and cardiotonic as a fragrant herbal tea (Bunyapraphatsara and Chokecharoenporn, 1998; Picheansoonthon et al., 1999; Phonkot et al., 2008; La-ongsri et al., 2009). The methanol and ethyl acetate extracts of *Nelumbo nucifera* stamens exhibit strong antioxidant activity in scavenging peroxynitriles ($ONOO^-$) system, 1,1-diphenyl-2-picrylhydrazyl (DPPH) system and total reactive oxygen species (ROS) system (Jung et al., 2003). The methanol extract of *Nelumbo nucifera* stamens has also been shown to exert an inhibitory effect on rat lens aldose reductase, an enzyme that has been shown to play

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an important role in the complications associated with diabetes (Lim et al., 2006). Recently, *Nelumbo nucifera* stamens derived compounds have been reported to exert their anti-Alzheimer effects as acetylcholinesterase inhibitors (Jung et al., 2010).

The main chemical compositions of *Nelumbo nucifera* stamens include flavonoids, alkaloids, and carotenoids (Chinese-Thai Expert Committee on Technology Transfer of Herbal Medicines, 2004). Various flavonoids with antioxidant activity were isolated including kaempferol, kaempferol 3-O-beta-D-glucuronopyranosyl methylester, kaempferol 3-O-beta-D-glucopyranoside, kaempferol 3-O-beta-D-galactopyranoside, myricetin 3′,5′-dimethylether 3-O-beta-D-glucopyranoside, kaempferol 3-O-alpha-L-rhamnopyranosyl-(1→6)-beta-D-glucopyranoside, kaempferol 3-O-beta-D-glucuronopyranoside (Jung et al., 2003), and 4 isorhamnetin glycosides (Hyun et al., 2006). Some of these flavonoids also show rat lens aldose reductase inhibitory activity *in vitro* (Lim et al., 2006).

Since the use of *Nelumbo nucifera* stamens in herbal medicines (as an alternative treatment to conventional drugs) as well as in cosmetic products (as anti-oxidant and skin-whitening agents) are highly prevalent in Thailand and increasing worldwide, toxicity studies to confirm the safe use of *Nelumbo nucifera* stamens are warranted. Herein, two toxicity studies were performed to evaluate the safety of the ethanol extract of *Nelumbo nucifera* stamens. The acute oral toxicity study was carried out at a very high dose, whereas the repeated dose 90-day oral toxicity study was performed to establish the no-observed-adverse-effect level (NOAEL) of the extract.

2. Materials and methods

2.1. Preparation of test material

Nelumbo nucifera stamens were purchased from a local market in Bangkok and were authenticated. A voucher specimen (BKF 137083) was deposited at the Forest Herbarium, Royal Forest Department, Bangkok. Dry powder of the stamens (5.0 kg) was extracted with 95% ethanol (4 × 4 L) by percolation at room temperature over a period of two weeks. The extract was filtered and the combined filtrate was evaporated to remove ethanol under reduced pressure at 55 °C and lyophilized to give the *Nelumbo nucifera* stamens extract (541 g). The extract-polyvinylpyrrolidone-10 (PVP-10) complex was prepared by dissolving the ethanol extract and PVP-10 in the ratio of 1:4 in methanol. The solvent was removed under reduced pressure and then freeze-dried to give a powder which was used in the study. The powder was subsequently reconstituted in distilled water to the final concentrations required for the experiments. Eighty percent PVP-10 (w/w) in distilled water served as control vehicle in all experiments.

2.2. Experimental animals

Male and female Sprague–Dawley rats, aged 7–8 weeks, were purchased from the National Laboratory Animal Center Mahidol University, Nakorn Pathom, Thailand. In the acute oral toxicity study, 10 rats of each sex were used, whereas in the repeated dose 90-day oral toxicity study, 36 rats of each sex were used. All rats were labeled by earmark and housed in groups (5 per cage for acute toxicity study and 3 per cage for 90-day toxicity study) of the same gender in stainless steel, open-mesh cages in a room maintained under environmentally controlled conditions of 23 ± 2 °C and a 12 h light-dark cycle. All rats were acclimatized at least one week before starting the experiments, and had free access to water and food, except for the withdrawal of food the night before dosing and 3–4 h after dosing. All procedures were approved and conducted in accordance with the Animal Ethics Committee of the Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand (Protocol number 3/2550).

2.3. Acute oral toxicity study

The acute oral toxicity study was conducted in compliance with the Organization for Economic Cooperation and Development (OECD) Guideline for the Testing of Chemicals No. 420, adopted December 17, 2001 (OECD, 2001) with slight modification. After acclimatization, 10 rats of each sex were randomly divided into two groups of 5 males and 5 females. The control group received the vehicle in a volume of 1 mL/100 g body weight by gavage, whereas the treatment group received the extract-PVP complex at the dose of 25 g/kg which was equivalent to the extract at the dose of 5000 mg/kg. The first rat of each sex in each group were dosed and observed, then a further four rats of each sex in each group were subsequently dosed when no mortality occurred within 48 h.

All animals were observed individually for clinical signs of toxicity immediately after dosing and at 1, 2, 4, and 8 h after dosing. Observations were focused on changes in skin, fur, eyes, mucous membranes, respiratory and circulatory systems, autonomic and central nervous systems as well as somatomotor activity and behavioral pattern. The number of survivors were noted after 24 h and then maintained for a further 14 days with a once daily observation. Animals were weighed on day 0, and on days 7 and 14. At the end of the study, all surviving animals were sacrificed with diethyl ether. Gross pathological examinations of all major internal organs such as heart, lungs, livers, kidneys, spleen, adrenal glands, and sex organs (and histopathological examination if necessary) were then performed.

2.4. Repeated dose 90-day oral toxicity study

This study was conducted in compliance with the OECD Guideline for the Testing of Chemicals No. 408; Repeated Dose 90-Day Oral Toxicity Study in Rodents, adopted September 21, 1998 (OECD, 1998) with slight modification. Based on its recommended dose of 1 teaspoon of lotus stamens (approximately 120 mg) per cup of tea for cardiotonic effect, and the assumption of mean body weight of patients of 60 kg, therefore the dose used would be 2 mg/kg. Percent yield of the extract was 10.82, thus the dose of lotus stamens extract recommended was assumed to be approximately 0.2 mg/kg. However, traditionally, the usual uncertainty factor of 100 should have been applied to account for both inter-species and inter-individual variations (IPCS, 2005), the final dose of the extract in rats would then be 20 mg/kg. Therefore, the doses of 50, 100, and 200 mg/kg/day were selected as tested doses. After acclimatization, 36 rats of each sex were randomly divided into six groups of 6 males and 6 females. The control group received the vehicle. The three treatment groups received the extract-PVP complex at doses that equivalent to 50, 100, and 200 mg/kg/day of the extract, respectively. The animals in the control satellite group (received the vehicle) and the treatment satellite group (received the extract-PVP complex at the highest dose) were kept for further 28 days post-dosing in order to observe for reversibility, persistence, or delayed occurrence of any toxicity. Both the vehicle and the extract-PVP complex were given in a volume of 1 mL/100 g body weight by gavage once daily for 90 consecutive days.

The body weights of all animals were recorded on day 0 (before dosing), day 90 and on the day of autopsy. Clinical observations were made once daily to detect signs of toxicity. The focus of the observations was the same as described above for the acute toxicity study. Abnormal findings were recorded with the times of onset and disappearance. Any rat which died during the study was examined pathologically.