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Effect of White *Nelumbo nucifera* on Rat Sperm Motility and Sperm Viability

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ABSTRACT

Nelumbo nucifera Gaertn., also known as Indian lotus, is a large aquatic perennial plant with a long history of use in traditional medicine. According to reports, white *N. nucifera* petal extract has higher phytochemical compounds with the potential to improve sperm viability and to reduce oxidative stress compared with the red petal extract. Studies of the white *N. nucifera* petals on the male reproductive system are still not fully explored. This study aimed to determine how white *N. nucifera* petal tea affects the motility and viability of sperm in adult male rats. Four groups of eight male rats were created (n=8). The experimental groups 2-4 received various concentrations of white *N. nucifera* tea, while the control group (1) received distilled water. To determine sperm motility and viability, sperm suspension taken from the right caudal epididymis was examined under a light microscope. The results showed the number of motile sperm was significantly increased and the number of non-motile sperm was significantly lower in male rats given white *N. nucifera* tea at a dose of 0.55 mg/kg BW, when compared to the control group. However, the number of viable sperm did not differ significantly among groups. The indication of enhanced sperm motility can support the usage of white *N. nucifera* petal tea for improving sperm quality.

INTRODUCTION

Nelumbo nucifera Gaertn. (*N. nucifera*) is well-known as a large aquatic perennial plant and it has been reported that most parts of this plant can be used in traditional medicine [1]. *N. nucifera*, also known as «Bualuang» in Thai, is a member of the Nelumbonaceae plant family and has been widely grown in all parts of Thailand [2]. The stamen of *N. nucifera* is listed as an herb the Thai herbal pharmacopoeia, 2019 [3], and the red flower of *N. nucifera* has been reported as a popular aquatic perennial plant [4]. Additionally, *N. nucifera* is a blossoming plant containing elevated degrees of natural antioxidants [1]. While the white *N. nucifera* flower is not widely used, a previous study reported that the white *N. nucifera* petal extract had rich phytochemical contents including quercetin, gallic acid, catechin, and p-hydroxybenzoic acid and had more potential to scavenge free radicals and enhance sperm viability than red petals [5]. Sperm motility and viability parameters refer to the capabilities of sperm involved in male reproductive function [6]. One of the causes of male reproductive disability is abnormal sperm function, which can result from the oxidation of sperm membrane proteins and lipids [7, 8]. Male infertility is an issue for men that puts them under emotional stress [9]. Consuming antioxidants, particularly natural antioxidants from plants that contain phenols and flavonoids, may help prevent male infertility [10]. As a result, natural antioxidants extracted from plants have been utilized to stop male reproductive abnormalities [6].

Although *N. nucifera* has previously been reported to enhance sperm quality in *in vitro* models [11], white *N. nucifera* petal tea has not been shown to affect sperm motility and viability in male rats. Moreover, the aqueous extract from white *N. nucifera* petals is similar to the method used in Thai recipes and often used in traditional medicine. Therefore, the purpose of this study was to determine whether white *N. nucifera* petal tea could affected the motility and viability of sperm in adult male rats.

METHODOLOGY

Plant materials and extraction procedure

In September 2019, white *N. nucifera* petals were procured from the Thung Yang subdistrict, Laplae district, Uttaradit Province, Thailand. The specimens were deposited and authenticated at the Herbarium, Faculty of Pharmacy, Chiang Mai University (voucher specimen number 023248–2). The dried petals were extracted using hot distilled water at 7580- ˚C [11] for 35- min [12]. The petal tea was dried by lyophilization with 12›5٪ yield of crude powder and stored at –20 ˚C before experimentation.

Animals and experimental design

Thirty-two mature male Wistar rats (*Rattus norvegicus*) aged 68-weeks with weights of 220-240 g were purchased from Nomura Siam

International CO., LTD (n = 32). Two to three rats were housed in each cage under standard environment conditions, controlled temperature at 25±2 °C, 12 h dark/ 12 h light cycle and fed standard diet and filtered water. After one week for acclimatization, the rats were divided into four groups (n = 8 each). Group 1 (control) was administered 1 mL of distilled water, additionally the animals in group 24- were administered 1mL of 0.55, 1.10, and 2.20 mg/kg BW of white *N. nucifera* aqueous extract (WNAE), respectively for 30 days. The dose of 1.10 mg/kg BW is consistent with the daily recommended intake of tea for humans [13, 14]. At the end of the treatment period, the animals were sacrificed to remove the right caudal epididymis. The experimental protocol was approved by the Animal Ethics Committee, Faculty of Medicine, Chiang Mai University (No. 62564/).

Sperm motility

The sperm suspension was prepared from each right caudal epididymis of the male rats and homogenized in 10 mL of a Krebs–Henseleit solution. The analysis of sperm motility was performed by using 20 µL of each rat's sperm suspension, which were sucked up by micro pipette and placed into double sides of an improved Neubauer hemocytometer. The sperm movement were recorded by digital video under light microscope at a magnification of 400x (Olympus CH2). The sperm motility in the central compartment of counting chamber was analyzed and classified by a simple random sampling method until a total number of sperm counted reached 200 sperm per each rat [11].

Sperm viability

Trypan blue (TB) was added to the 20 µL of sperm suspension. Ten µL of this blend was spread on a slide. After being air-dried, the slides were fixed for four minutes in a fixative solution consisting of 86 mL of 1N HCl, 14 mL of 37% formaldehyde solution, and 0.2 g neutral red. They were then air-dried after being stained with a Giemsa solution containing 7.5% Giemsa at 40 °C for four hours. Sperm that were stained blue of trypan blue considered dead, and those unstained blue were considered viable. Under a light microscope, the viability of the sperm was examined and photographed at 1000x magnification. One hundred sperm of each rat were counted and categorized by digital photograph [11], which taken from the smear slides starting from the left to the right direction by Image View.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). The mean of sperm motility and viability were analyzed by one-way ANOVA for analysis of variance and followed by Tukey's test. Significance was set at $p \leq 0.05$.

RESULTS

Sperm motility

The male rats receiving white *N. nucifera* petal tea at the doses of 0.55 mg/kg BW ($p<0.05$) had a significantly increased number of motile sperm when compared with the control group. The experimental groups at the doses of 1.10 and 2.20 mg/kg BW showed no significant changes in comparison to the number of motile sperm in the control group. Additionally, the number of non-motile sperm was significantly lower in male rats given white *N. nucifera* tea at a dose of 0.55 mg/kg BW, when compared to the control group *(***Figure 1**).

Sperm viability

The sperm viability of all groups of male rats treated with white *N. nucifera* petal tea did not differ significantly from the control group. Figure 2 showed the data regarding viable and dead sperm, and the investigation of rat epididymal sperm is demonstrated in Figure 3.

DISCUSSION

N. nucifera is used in Thai recipes and traditional medicine. However, there is little scientific data regarding the use of this plant and its effects on the male reproductive system. Thai people are accustomed to drinking this kind of tea, so they are familiar with this method of making white *N. nucifera* tea extract with hot water. It contains essential phytochemical compounds, especially total phenols and flavonoids [5]. In this study, the effect of white *N. nucifera* petal tea on the male reproductive system was evaluated by studying sperm

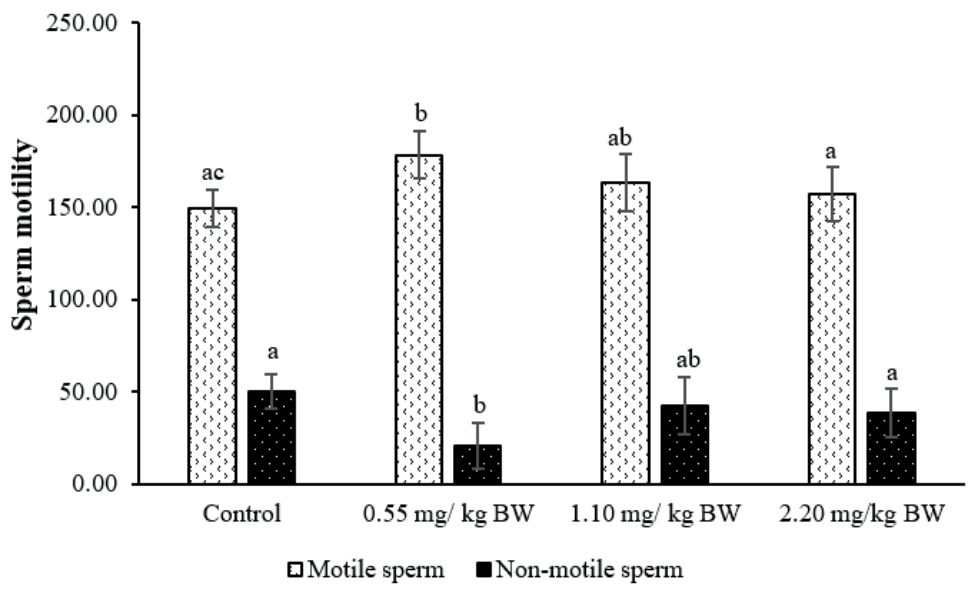

Figure 1. Sperm motility of male rats administrated with various concentrations of white *N. nucifera* petal tea and the control group. Different letters indicate significant differences other groups at $p \leq 0.05$ (number of sperm motility and sperm viability analyzed by One-way ANOVA subsequently Tukey's test). Data are mean values ± standard deviation (error bars).

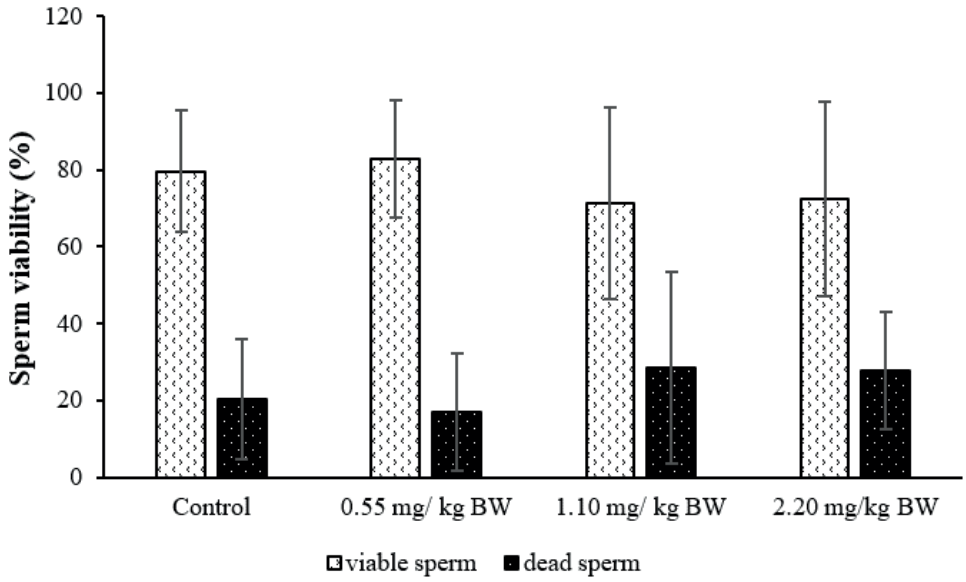

Figure 2. Sperm viability of male rats administrated with various concentrations of white *N. nucifera* petal tea and the control group. There were no significant differences (One-way ANOVA). Data are mean values ± standard deviation (error bars).

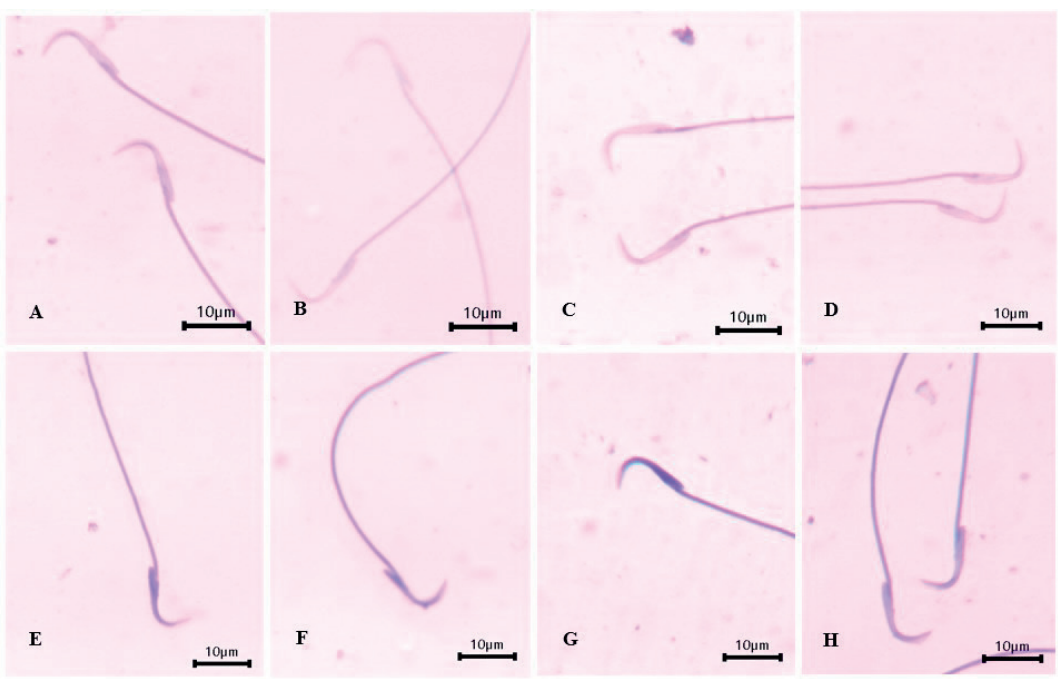

Figure 3. Rats' sperm stained with trypan blue and Giemsa demonstrate viable and dead sperm. Viable sperm of male rat treated with distilled water, white *N. nucifera* petal tea at 0.55, 1.10, and 2.20 mg/kg BW (A-D) were unstained blue, whist dead sperm (E-H) were stained blue of trypan blue, respectively. Photographed at a magnification of 1000x under light microscope.

motility and viability. Administration of white *N. nucifera* petal tea at the dose of 0›55 mg/kg BW in male rats for 30 days significantly increased the number of motile sperm when compared with the control group (P<0›05). Additionally, the number of non-motile sperm was significantly lower in male rats given white *N. nucifera* petal tea at a dose of 0›55 mg/kg BW, when compared to the control group. The present results indicated that consumption of white *N. nucifera* petal tea has a positive effect on sperm motility and viability in rats. This is similar to *B. rotunda* juice at low and moderate concentrations, which showed significantly higher numbers of progressive sperm [15]. These results are consistent with cattle sperm induced with mancozeb and co-administrated white *N. nucifera* aqueous extract (WNAE) to improve the sperm motility, sperm viability, acrosome integrity, and normal sperm morphology [11]. In another study, Desferol, an iron chelating agent, showed increased sperm motility and decreased abnormal sperm morphology [16]. In addition, WNAE also showed potential to improve

the rat sperm viability from $FeSO_4$ exposure [5]. Sperm motility requires ATP from mitochondria [17], and thus the white *N. nucifera* petal tea may have increased the number of motile sperm via enhanced ATP levels in the mitochondria. It can be concluded that WNAE can have positive effects on sperm quality in both *in vitro* [15] and the *in vivo* models. It has been shown that white *N. nucifera* petal tea contains bioactive compounds including (+)-delta-Tocopherol; kaempferitrin; ouabain; convallatoxin; salasodine; isorhamnetin-3-O-rutinoside; 2′, 3, 3′4, 4′-pentahydroxy-4′-glucosulchalcone; 4, 8′-Bi ((+)-epicatechin); and quercetin-3-Oarabinoglycoside. These compounds are flavonoids and alkaloids [11], which are strong natural antioxidants [18–19] with the potential for free radical scavenging, which may be possible that WNAE in the 30 days' period results in increased energy preservation and sperm motility but not affecting sperm viability. This result showed enhanced male reproductive parameters. More specifically, white *N. nucifera* petal tea at the dose of 0،55 mg/kg showed the highest potential benefits for the reproductive function in male rats, while the higher doses were consistently to the control in the sperm motility and viability. In further study may be addressed the concentration of this extract according to the present finding and the duration of experiment should be considered for more clearly results in other parameters. Therefore, white *N. nucifera* petal tea has the potential to enhance male reproductive function in male rats.

CONCLUSION

In conclusion, the white *N. nucifera* tea increased the number of motile sperm, possibly by increasing antioxidants and radical scavenging. This indicates that usage of white *N. nucifera* tea has positive effects on sperm motility and may be of benefit for improving sperm quality. Other parameters that related to sperm quality such as the detail of sperm morphology should be considered in the further study.

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