Chiang Mai J. Sci. 2018; 45(2) : 811-820
http://epg.science.cmu.ac.th/ejournal/
Contributed Paper

Evaluation of Long-term Administration of Aqueous Extract from *Pseuderanthemum palatiferum* (Nees) Radlk. Leaves on the Reproductive System of Male Albino Rats

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Received: 27 March 2015
Accepted: 1 April 2016

ABSTRACT

The aim of this study was to evaluate the effects of aqueous extract from the leaves of *Pseuderanthemum palatiferum* (Nees) Radlk. on sperm parameters and histology of reproductive organs of male rats. This study was conducted by oral administering the rats with aqueous extracts from the leaves of *P. palatiferum* at the doses of 5, 10, 15 and 20 mg/kg body weight for 60 days. The control group received only distilled water. The results showed the reductions in semen quality of epididymal spermatozoa such as density, motility and morphology of all treated rats. The decrease in seminiferous tubule diameter and height of prostatic epithelium was significantly observed in all treated rats ($p < 0.05$) while the apoptosis in prostatic epithelium cells were slightly seen in rats treated with highest dose of *P. palatiferum*. Nevertheless, the extract had no effect on weight and histoarchitectures of testes and seminal vesicle. It can be concluded that aqueous extract from the leaves of *P. palatiferum* caused an adverse effects on some male reproductive parameters. However, further study was required to obtain more conclusive result of *P. palatiferum* through an antifertility activity.

Keywords: extract, rats, reproductive system, *Pseuderantheumum palatiferum* (Nees) Radlk.

1. INTRODUCTION

The medicinal plants are extensively used for curing various ailments and maintaining human health since the ancient time. One of the largely used plants in folk medicine is *Pseuderanthemum palatiferum* (Nees) Radlk. of the Acanthacea family, commonly found in South East Asia, especially, in Vietnam and Thailand. It is also known as *xuan-hoa* or *hoan-ngoc* in Vietnamese and *phaya wanon* in Thai. Traditionally, the plant is used in the treatment of human diseases; diarrhea, wound, trauma, stomachache, high blood pressure, colitis and nephritis [1-3]. Phytochemical constituents of *P. palatiferum* leaf extracts have been proved to have triterpenoid, saponin, β-sitosterol, salicylic acid, palmitic acid, phytol, stigmasterol, pseuderantin, kaempferol and apigenin [4-5]. These substances are clearly known to show physiological activities [6-8]. Although, the broad spectrum of

therapeutic properties of *P. palatiferum* was documented, its effect on male reproductive system has never been studies. Furthermore, scientific report demonstrated that a large number of medicinal plants that were generally used for treating various illnesses were linked to male infertility. Intragastric administration of hexane extract from the rhizome of *Curcuma comosa* Roxb. at the dose of 500 mg/kg body weight for 7 consecutive days caused significantly decreased in testicular, ventral prostate and seminal vesicle weight of rats. Moreover, the notable decrease in density and motility of rat's cauda epididymal sperm was found [9]. Though, green tea is known to have various therapeutic efficacies, oral administration of green tea extract (*Camellia sinensis* L.) at 2.5 and 5 % (w/v) for 26 days significantly reduced serum testosterone level, epididymal sperm density and inhibited the spermatogenesis in testes of male rats [10]. Similarly, a large number of plants such as *Achilea mellefolium*, *Aegle marmelos*, *Capparis aphylla* (Roth.), *Ocimum sanctum* and *Ximenia americana* have been reported to have antifertility effects on male experimental animals [11-15]. The present study was, thus, designed to evaluate the effect of administration of the aqueous extract from the leaves of *P. palatiferum* on the male reproductive function including sperm parameters, reproductive organ weight, and histopathology of sex and accessory organs.

2. MATERIALS AND METHODS

2.1 Plant Materials

The leaves of *P. palatiferum* were harvested from Chiang Mai Province, Thailand. The plant materials were authenticated by botanist of the Queen Sirikit Botanic Garden, Mae Rim, Chiang Mai Province, Thailand, where a voucher specimen registered under the number WP2615 is deposited.

2.2 Plant Extraction

Fresh leaves of *P. palatiferum* were washed with tap water and cut into small pieces, then soaked in distilled water (1:10 w/v) for 4 hours [16]. The residue was removed by filtration. The aqueous extract was filtered through Whatman No. 1, concentrated by vacuum rotary evaporator and lyophilized by freeze drying process to obtain dry matter. The percentage yield of the extract was 6.00 (w/w). The powder extract was subsequently reconstituted in distilled water at the appropriate concentration for the further experiment.

2.3 Phytochemical Analysis

The presence of various phytochemical constituents in *P. palatiferum* leaves extract such as tannins, alkaloids, terpenoids, flavonoids, saponins, coumarins, phlobatannins, anthraquinones, phenolics, cardiac glycosides, sterols and reducing sugar was analyzed using the standard methods [17-18].

2.4 Animals

Forty male Wistar rats (*Rattus norvegicus*), 6 weeks old and weighing 200-240 g, were purchased from the National Laboratory Animals Center, Salaya Campus, Nakhon Prathom, Thailand. The animals were housed in sanitary cages and had access to tap water and a standard diet (C. P. 082). The room temperature was controlled at 24-26 °C in a 12 hrs light/dark cycle. All procedures involving the animals were conducted with strict adherence to guidelines and procedures reviewed and approved by the Institutional Animal Care and Use Committee of the Biology Department, Faculty of Sciences, Chiang Mai University (Re. 002/10).

2.5 Animal Treatment

Rats were randomly divided into five groups. Each group consisted of eight rats.

Animals in groups I, II, III and IV orally received a single daily dose of aqueous extract from the leaves of *P. palatiferum* at the doses of 5, 10, 15 and 20 mg/kg body weight, respectively, for 60 consecutive days. Group V orally received only the distilled water and was considered as control. Doses of the extract administration and duration time used in this study based on the use of the plant in the traditional treatment of human diseases [1, 19]. Body weights of rat were recorded on the first day before the treatment of *P. palatiferum* and the day of anesthesia. Moreover, signs of toxicity and mortality were observed.

At the end of treatment period, all rats were anesthetized with ether. Blood samples were collected using cardiac puncture technique. Testes and accessory sex organs (prostate gland and seminal vesicle) were dissected out, cleared of connective tissue and fat, weighed and fixed in bouin's solution for histopathological examination. The right epididymis was then dissected out for analysis of sperm parameters.

2.6 Investigation of Sperm Parameters

The cauda epididymal spermatozoa suspension was used for sperm analysis with a modification of the standard method [20]. Right cauda epididymis was finely minced in 10 ml of isotonic solution pre-warmed to 37 °C and allowed to incubate for 5 minutes. The sperm suspension (10 µl) was then filled in the counting chamber and allowed to stand for 5 minutes. The total sperm density was counted, calculated and expressed as the number of sperms per milliliter. One drop of sperm suspension was placed in cleaned grass slide. Sperm motility such as progressive movement, non-progressive movement and no movement were counted [21]. The abnormal sperms were examined by smear preparation. The numbers of abnormal sperms were calculated according to a modification of the method described previously [22].

2.7 Histopathological Examination

Fixed testes, seminal vesicle and prostate gland were dehydrated by increasing alcohol concentration, cleared in xylene and embedded in paraffin wax. The 6 µm thick of paraffin section was performed by rotary microtome. The sections were stained with hematoxylin and eosin dye. The histoarchitectures of testes and accessory sex organs were examined and photographed under the light microscope.

2.8 Statistical Analysis

All data were expressed as mean ± standard error. Statistical analysis was performed using the Statistical Package for the Social Science (SPSS) for windows. The $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1 Phytochemical Analysis

Qualitative investigation of aqueous extract from the leaves of *P. palatiferum* revealed the presence of various phytochemical constituents. Flavonoids, tannins, terpenoids, saponins, phenolics, cardiac glycosides, sterols and reducing sugar were found while alkaloids, coumarins, phlobatannins and anthraquinones were absent.

3.2 Body and Reproductive Organ Weights

Administration of aqueous extract from the *P. palatiferum* leaves at the doses of 5, 10, 15 and 20 mg/kg body weight for 60 days did not significantly affect the change in body and relative proportion of reproductive organs (testes, seminal vesicle and prostate

gland) weights of rats when compared to the controls (Table 1).

3.3 Sperm Density, Motility and Abnormality

Although, reduced density and progressive motility of epididymal spermatozoa were slightly observed in rats treated with aqueous extract from the leaves of *P. palatiferum* at all doses, there were not significant when compared to those of the control. The increase in numbers of sperm abnormality was observed in rats treated with *P. palatiferum* at all doses when compared to the control group ($p > 0.05$). Increased abnormal morphology of sperm was noted in all treated groups. The most common abnormality of epididymal spermatozoa encountered in the treated rats was the coiled tail, looped tail and cytoplasmic droplet, while the occurrence of acephali of sperm was slightly found (Figure 1).

Table 1. Body and relative organ weights of rats treated with aqueous extract from the leaves of *P. palatiferum* at the doses of 5, 10, 15 and 20 mg/kg body weight for 60 days compared to the control group.

treatments	body weight (g)			relative organ weights (g/100 g body weight)		
	initial	final	body weight gain	testes	seminal vesicle	prostate gland
control	212.50±9.89	434.17±10.46 [a]	221.67±8.62 [a]	1.96±0.03 [a]	1.15±0.08 [a]	0.38±0.03 [a]
P5 mg/kg BW	216.25±14.82	447.50±11.26 [a]	231.25±12.31 [a]	1.95±0.06 [a]	1.19±0.07 [a]	0.37±0.04 [a]
P10 mg/kg BW	223.13±18.31	465.00±9.50 [a]	241.88±9.49 [a]	1.90±0.08 [a]	1.13±0.08 [a]	0.41±0.04 [a]
P15 mg/kg BW	230.63±21.95	447.50±6.20 [a]	216.88±10.85 [a]	2.02±0.04 [a]	1.19±0.12 [a]	0.34±0.04 [a]
P20 mg/kg BW	229.38±20.08	440.62±6.71[a]	211.25±5.73 [a]	1.93±0.04 [a]	1.16±0.14 [a]	0.33±0.05 [a]

A same superscript letters within each column are not significantly different at $p > 0.05$. Values are mean ± SE of 8 animals in each group.

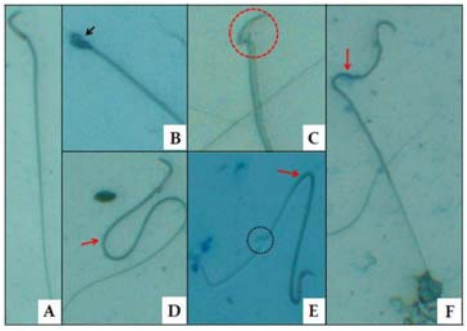

Figure 1. Morphology of abnormal spermatozoa collected from epididymis of rats treated with aqueous extract from the leaves of *P. palatiferum* compared to the normal sperm. A: normal sperm, B and C: acephali, D: looped tail; E: cure tail, cytoplasmic droplet (dark circle), F: curved tail.

3.4 Histopathological Examination

The effect of *P. palatiferum* extract on diameters of seminiferous tubule and height of prostate and seminal vesicle was shown in Table 3. There was significant decrease in epithelial thickness of prostate gland and diameter of seminiferous tubule of all *P. palatiferum*-treated rats when compared to control rats ($p < 0.05$). Testes section of treated and control rats showed no pathological features (Figure 2-4). Seminiferous tubule of all rats showed normal histological characteristic and abundant of spermatogenic cells such as spermatogonia, spermatocyte, spermatid and free spermatozoa was seen in lumen (Figure 2). Normal histological features of

prostate and seminal vesicle were visible. Normal columnar epithelium and distribution of seminal fluid within lumen were seen (Figure 3). Prostate section consisted of various prostatic alveoli lining with columnar epithelium with variable diameters. Irregular lumens were filled with prostatic fluid and distribution of stroma was normal (Figure 4). However, the apoptotic cells were slightly seen in prostatic section of rats treated with *P. palatiferum* at the the dose of 20 mg/kg body weight (figure 5).

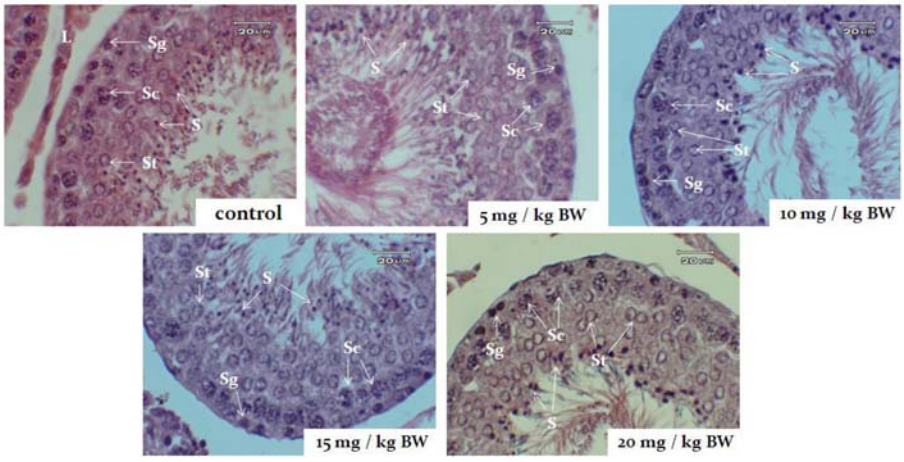

Figure 2. Photomicrograph of testes of rats treated with aqueous extract from the leaves of *P. palatiferum* at the doses of 5, 10, 15 and 20 mg/kg body weight for 60 consecutive days compared to the control group. (H&E, 40x) Sg; spermatogonia, Sc; sertoli cell, St; spermatid, Sz; spermatozoa, L; Leydig's cell.

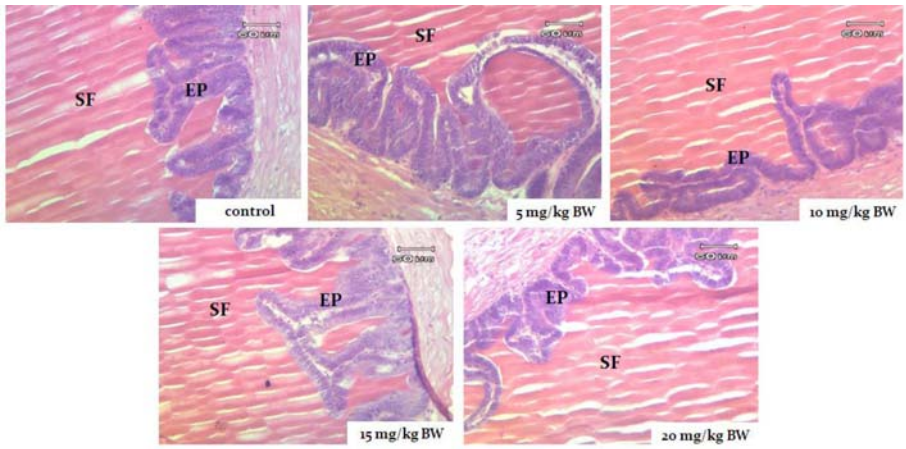

Figure 3. Photomicrograph of seminal vesicle of rats treated with aqueous extract from the leaves of *P. palatiferum* at the doses of 5, 10, 15 and 20 mg/kg body weight for 60 days compared to the control group. (H&E, 10x) **EP**; seminal epithelium, **SF**; seminal fluid.

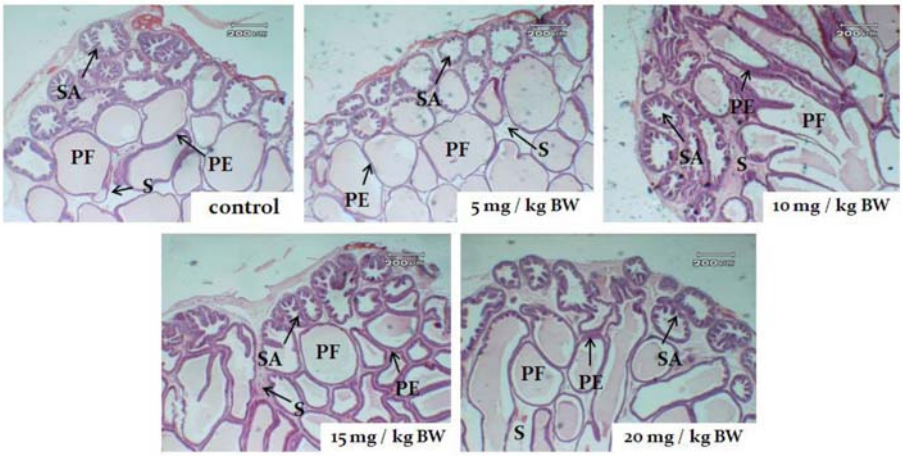

Figure 4. Photomicrograph of prostate section of rats treated with aqueous extract from the leaves of *P. palatiferum* at the doses of 5, 10, 15 and 20 mg/kg body weight for 60 days compared to the control group. (H&E, 40x) **SA**; secretory alveoli, **PF**; prostatic fluid, **S**; stroma; **PE**; prostatic epithelium.

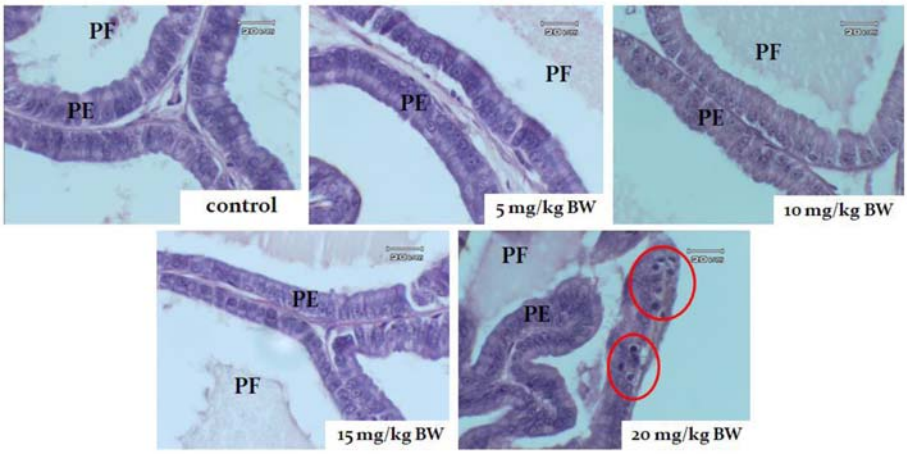

Figure 5. Photomicrograph of prostate section of rats treated with aqueous extract from the leaves of *P. palatiferum* at the doses of 5, 10, 15 and 20 mg/kg body weight for 60 days compared to the control group. (H&E, 40x) **EP**; epithelium, **PF**; prostatic fluid, circle; apoptotic cell

4. DISCUSSION

Many plants are extensively used to manage various ailments because they are readily and cheaply alternative. Phytochemical constituents contained in plants possess a broad-spectrum physiological activity. Kaempferol and apigenin glycosides, for example, possess hypoglycemic activity. Kaempferitrin upregulated glucose uptake in rat's soleus muscle [8] and activated the insulin signaling pathway, resulting in the reduction of blood glucose level [7]. Apigenin fucopyranoside also showed significant stimulation of insulin secretion and glycogen synthesis [6]. Nevertheless, bioactive phytoconstituent from plants may affect male fertility. To prove the safety use of the medicinal plants in traditional

treatment on male reproduction, this study designed to investigate the effect of aqueous extract from the leaves of *P. palatiferum* on some parameters of male reproductive system.

Phytochemical investigation of *P. palatiferum* aqueous extract represented flavonoids, tannins, terpenoids, saponins, phenolics, cardiac glycosides, steroids and reducing sugar. Chronic administration of *P. palatiferum* extract in male rats revealed no clinical sign of toxicity. There were no differences in body weight gain and weight of reproductive organs between the treated and control groups (Table 1). Nevertheless, aqueous extract at all doses tend to reduce epididymal sperm density and progressive motility. Similarly, an increase in numbers of abnormal and immotile sperms was higher in *P. palatiferum* treated rats when compared to control rats (Table 2). Sperm parameters such as density, motility, morphology are key markers for the spermatogenesis and maturation in seminiferous tubule and epididymis, respectively [23], alteration in these indices indicates the loss of male fertility. Since androgen is necessary for spermatogenesis in seminiferous tubule and maturation and motility of epididymal spermatozoa [12, 24]), androgen deficiency could lead to sperm morbidity and impaired fertility. From our results, it is possible that aqueous extract of *P. palatiferum* may cause adverse effect on gonadotrophic hormone, leading to androgen depletion and consequently impair the physiological maturation of sperm. Previous study reported the fertility suppression of various plant extracts in experimental animals [9-10, 12-15, 25]). Moreover, flavonoids, a bioactive chemical exhibited in plant materials, were found to have antiandrogenic activity in male rat and dog [26]. This compound acts by inhibiting aromatase and 17 β-hydroxysteroid dehydrogenase activity [27], leading to androgen depletion. Therefore, the reduction fertility indices observed in this study may be due to the antiandrogenic activity of flavonoid compounds represented in *P. palatiferum* leaves extract. Since the cauda epididymis of mammals is responsible for the storage of mature spermatozoa [28], the other possibility of sperm abnormality is that *P. palatiferum* extract may act specifically at cauda epididymis. This hypothesis was supported by an increase in immotile and abnormal of epididymal spermatozoa evidenced from this study. Furthermore, *P. palatiferum* extract may interfere oxidative phosphorylation, the enzymatic reaction require for ATP which is necessary for sperm motility. In addition, male infertility caused by the reduction in ATP was previously documented [29]. Another notable change in sperm morphology was coiled and looped tail and acephali (Figure 1) and they are unable to fertilize mature eggs. Similarly, morphological changes of spermatozoa have been reported in rats treated with andrographolide, a major constituent in plant, namely, *Andrographis paniculata* [25]. The deleterious effect on head and tail morphology of sperm was showed in rats treated with the extract from the leaves, stem bark and root of *Ximenia Americana* [1]. In addition, we suggested that abnormality in motility of sperm recorded in this study may be due to the direct effect of *P. palatiferum* extract in oxidizing fatty acid of phospholipids, leading to lipid peroxidation of mitochondrial sheath. Mitochondrial damage can decrease the motility of sperm. Furthermore, the loss of sperm capacity in motility caused by lipid peroxidation has been clearly documented [30-31]. Our results suggested that the aqueous extract from the leaves of *P. palatiferum* at the doses and treatment period in this study did not alter

body weight gain and relative weight of reproductive organs in male rats. Nevertheless, impairment in some sperm function might be associated with inhibitory influence on androgenic status after long-term administration of *P. palatiferum* aqueous extracts.

Histological observation revealed that *P. palatiferum* extract did not alter testicular morphology as well as seminal vesicle histoarchitecture (Figure 2 and 3). However, the reduction in diameter of seminiferous tubule and height of epithelial cells of prostate was clearly noted (Table 3). These changes might be a result of the reduction in testosterone level of treated rats. Prostate gland secretes alkaline fluid that helps neutralize the acidity in male and female genital tract and provides the sufficient power to carries sperm out of the body. *P. palatiferum* extract at dose of 20 mg/kg body weight could induce apoptosis in prostatic epithelium of rats (Figure 4). This finding may be due to the bioactive constituents consisted in this aqueous extract which affects the tissues through various mechanisms. It has been reported that apigenin; a bioactive flavonoids, contained in various plants has potential to induce apoptosis in human promyelocytic leukemia cells (HL-60 cell line) [32]. Therefore, it is probable that epithelial cell apoptosis observed in prostate gland were a result of the action of the flavonoids compound contained in the leaves of this plant.

Table 2. Effect of administration of aqueous extract from the leaves of *P. palatiferum* at the doses of 5, 10, 15 and 20 mg/kg body weight for 60 days on some parameters of epididymal sperm of rats compared to the control group.

sperm parameters	treatment groups (mg/kg body weight)				
	control	P5	P10	P15	P20
sperm density ($\times 10^6$ cell/ml)	55.32±2.31 [a]	52.64±3.97 [a]	50.61±7.00 [a]	47.44±3.90 [a]	45.30±2.92 [a]
progressive sperm (%)	28.23±4.95 [a]	19.17±4.50 [a]	17.67±3.67 [a]	16.13±4.77 [a]	21.50±2.36 [a]
non-progressive movement (%)	39.09±6.80 [a]	37.42±8.99 [a]	30.75±7.75 [a]	39.25±7.92 [a]	34.17±7.52 [a]
immotile sperm (%)	32.50±5.36 [a]	49.36±8.90 [a]	46.81±6.61 [a]	42.00±6.73 [a]	43.33±11.36 [a]
abnormal sperm (%)	28.11±2.46 [a]	34.00±2.15 [a]	32.31±2.90 [a]	32.41±1.93 [a]	32.94±2.32 [a]

A same superscript letters within each row are not significantly different at $p > 0.05$. Values are mean ± SE of 8 animals in each group.

Table 3. Reproductive histology of rats treated with *P. palatiferum* extracts at the doses of 5, 10, 15 and 20 mg/kg body weight for 60 days compared to the control group.

treatments	seminiferous tubule diameter (mm)	epithelial cell height (μm)	
		prostate gland	seminal vesicle
control	0.239±0.006 [a]	24.93±0.55 [a]	19.56±1.25 [a]
P5 mg/kg BW	0.225±0.006 [b]	20.28±3.62 [b]	21.25±1.66 [a]
P10 mg/kg BW	0.228±0.004 [b]	16.46±1.38 [b]	19.50±0.37 [a]
P15 mg/kg BW	0.226±0.003 [b]	15.33±1.35 [b]	17.95±1.51 [a]
P20 mg/kg BW	0.227±0.008 [b]	16.30±0.93 [b]	18.05±1.27 [a]

A different superscript letters within each column are significantly different at $p < 0.05$. Values are mean ± SE of 8 animals in each group.

5. CONCLUSIONS

It can be concluded that administration of *P. palatiferum* aqueous extract at duration and doses used in this study could suppress fertility parameters of male rats. This effect may have an inhibitory influence on androgen synthesis, leading to alteration in spermatogenesis and sperm maturation. The use of *P. palatiferum* in folk medicine in long duration time may cause infertility in male. However, these conclusion are based on the preliminary investigation, the further study is required for investigation of testosterone concentration, the fractionation of *P. palatiferum* extract and identify their effects on androgen dependent targets to obtain more conclusive result of *P. palatiferum* through an antifertility activity.

ACKNOWLEDGEMENT

The authors would like to thank the Research and Researchers for Industries, Thailand Research Fund (No. PHD57I0046) for financial support. We are grateful to Mr. Suluk Wutteerapol for his assistance through the consultation in laboratory techniques. Our sincere thanks also extend to Graduate School, Chiang Mai University for supporting this research.

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