SPERM DENSITY AND ULTRASTRUCTURE OF SERTOLI CELLS IN MALE RATS TREATED WITH *KAEMPFERIA PARVIFLORA* WALL. EX BAKER EXTRACT

Paiwan Sudwan[1,2], Kanokporn Saenphet[1], Supap Saenphet[1] and Chalobol Wongsawad[1]

[1]Department of Biology, Faculty of Science; [2]Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand

Abstract. The purpose of this study was to determine the effects of *Kaempferia parviflora* Wall. Ex Baker on the sperm density and ultrastructure of Sertoli cells in male albino rats. Three treated groups were orally administered with the rhizome extract of *K. parviflora* at various doses (60, 120, and 240 mg/kg bw/day) for 60 days. A control group received distilled water of 1 ml/day. At the end of the treatment period, the epididymal sperm density was determined and small pieces of testis were processed for ultrastructure study of Sertoli cells under the transmission electron microscope (TEM). The results showed that male rats treated with *K. parviflora* extract at the dose of 60 mg/kg bw/day had significantly higher sperm density than that of the control group. Electron micrographs demonstrated that all *K. parviflora* treated groups had prominent granules in the Sertoli cells, which secreted their substances to spermatogonia in the basal compartment of the seminiferous epithelium. Male rats treated with *K. parviflora* extract at the dose of 60 mg/kg bw/day also had highly condensed lysosome in the basal part of Sertoli cells. It was concluded that the ethanolic extract of *K. parviflora* involves sperm density and ultrastructure of Sertoli cells in male rats.

INTRODUCTION

Kaempferia parviflora Wall. ex Baker is a traditional herb of Thailand, known in Thai as *Krachaidum*. It belongs to the Zingiberaceae family. According to Thai traditional medicine, their rhizomes are believed to have sexual enhancing properties (Churdboonchart, 2000; Wutythamawech, 2000). This plant is sometimes referred to as "Thai ginseng" and has long been used among Thai men for sexual enhancement (Wutythamawech, 2000; Chaophaya Aphibhubejhr Hospital, 2006). They become a popular product and have been promoted commercially as *Krachai dum* wine, tea, and supplementary food, as well as being fermented in honey and white whisky. *K. parviflora* tea showed positive effects on the seminal vesicle and spermatogenesis at the dose of 60 and 120 mg/kg for 30 days (Jitjaingam *et al*, 2005) and the light microscope showed this plant extract increased granules of Sertoli cells and decreased serum estradiol levels (Sudwan and Saenphet, 2006). However, reports of the ethanolic extract of *K. parviflora* at 60, 120, and 240 mg/kg BW for 60 days showed a morphological change in the liver of all treated groups and reduced the time in the first 10 minutes of rat courtship behavior (Sudwan *et al*, 2006). Therefore, the effects of the ethanolic extract of *K. parviflora* on sperm density and ultrastructure of Sertoli cell need to determine. The aim of this research was to investigate the effects of ethanolic extract of *K. parviflora* rhizomes on sperm density and ultrastructure of Sertoli cell in male albino rats.

Correspondence: Paiwan Sudwan, Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200 Thailand.
Tel: 66-053-945312, Fax: 66-053-945304
E-mail: pasudwan@mail.med.cmu.ac.th

MATERIALS AND METHODS

Plant and preparation

The rhizomes of *K. parviflora* were collected from Loei Province, Thailand, and authenticated at the Botany Section, Queen Sirikit Botanic Garden, Ministry of Natural Resources and Environment, Mae Rim, Chiang Mai (voucher specimen no. 06-051717). The rhizomes were sliced, dried at 60 ˚C, ground to a fine powder, extracted with 50% ethanol in a soxhlet apparatus, and evaporated by rotary evaporation. Three

doses of the extract (60, 120, and 240 mg/kg bw) were prepared by dissolving in distilled water to the desired concentrations.

Animal and treatment

Twenty-eight adult male Wistar rats were used. The rats were purchased from the National Laboratory Animal Center, Thailand. They were housed under standard animal housing conditions at the Department of Biology, Faculty of Science, Chiang Mai University with free access to food and tap water. The animals were randomly assigned into four groups of seven rats each. Rats of the three treatment groups were orally administered with 60, 120, and 240 mg/kg bw (1 ml) of the crude extract for 60 days. The fourth group served as controls and was treated with 1 ml of the distilled water in a similar manner.

Sperm density

At the end of the treatment period, all rats were anesthetized, sacrificed, and examined for sperm density and the ultrastructure of Sertoli cells. The right cauda epididymis was cut into small pieces, and homogenized in 10 ml of 0.9% NaCl, and the sperm number was estimated in duplicate using an improved Neubauer hemocytometer. The testes were processed for histological study under the transmission electron microscope.

Ultrastructural study of Sertoli cells

Pieces of right testis were cut in 1 x 1 x 1 mm^3 and fixed in glutaraldehyde plus paraformaldehyde for conventional transmission electron microscopic procedure (Bazzola and Russell, 1998). The plastic blocks were sectioned at 500-900 nanometers, contrasted with uranyl acetate and lead citrate, and examined under the transmission electron microscopes with the cooperation of the Electron Microscopy Research and Service Center, Faculty of Science and the Medical Science Research Equipment Center, Faculty of Medicine, Chiang Mai University.

Statistical analysis

Sperm density was expressed as mean ± SE. Statistical evaluations were made using one-way ANOVA, followed by least significant difference (LSD) multiple comparisons test. Significance was set at $p \leq 0.05$.

RESULTS

The results showed that male rats treated with *K. parviflora* extract at the dose of 60 mg/kg bw/day had significantly higher sperm density than that of the control group (Fig 1). Transmission electron micrographs demonstrated that there were spermatogonia resting on the basal layer of the seminiferous epithelium in all groups (Fig 2A, 3A, 4A, 5A). During the developmental process, the cells of the spermatogenic series were supported by Sertoli cells. The Sertoli cells were bound to one another and within the tubule they divided into basal and adluminal compartments (Fig 2A, 3A, 5A). The cytoplasm

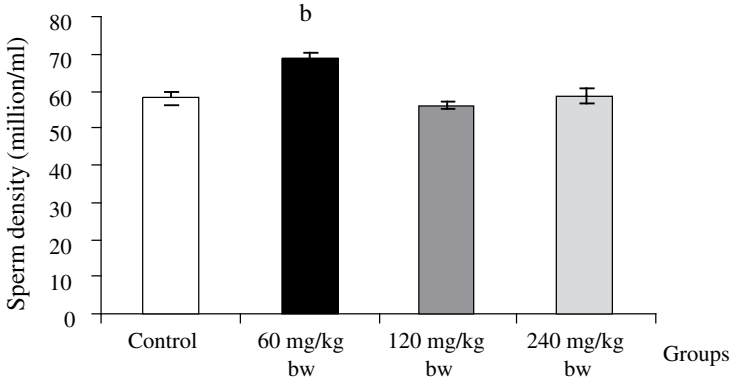

Fig 1- Sperm density in the control and treated groups for 60 days (n = 7). The values are expressed as mean ± SE. [b]Significant difference ($p < 0.5$) from control.

of the Sertoli cells of the control animals showed evidence of normal composition (Fig 2B), while all *K. parviflora* treated groups had prominent granules in their cytoplasm (Fig 3A, 3C, 4A-B, 5A). Sertoli cells secreted their substances to spermatogonia in the basal compartment of the seminiferous epithelium. Male rats treated with *K. parviflora* extract at the dose of 60 mg/kg bw/day also had highly condensed lysosome in the basal part of Sertoli cells (Fig 3B). These lysosomes contained dense particulate material and amorphous granular material. However, electron micrographs presented the normal morphology of spermatozoa in the control and treated groups (Fig 2C-D, 3D, 5B-C).

DISCUSSION

In this experiment, *K. parviflora* was given to male rats to study the sperm density and ultrastructure of Sertoli cells. Male rats treated with *K. parviflora* had more secretory granules in the cytoplasm of Sertoli cells compared with those of the control group. Electron micrographs demonstrated some granules were dropping into the intercellular spaces, which were situated beneath the cytoplasmic bridge of the Sertoli cells. These spaces supported and enclosed the spermatogonia as in a previous study using light microscope (Sudwan and Saenphet, 2006). In this manner, they were postulated to act as nutritional and metabolic supports for the developing spermatogenic cells (Fawcett, 1994; Burkitt, *et al*, 1998; Fawcett and Jensh, 2002).

Administration of *K. parviflora* extract at the dose of 60 mg/kg bw/day for 60 days in male rats produced significantly higher sperm density compared with that of the control group. It could then be assumed that the plant extract might have had an androgenic effect on increased sperm density (Watcho *et al,* 2004). Additionally, Sudwan and Saenphet (2006) observed that this plant had an effect on other steroid hormones, such as decreasing serum estradiol levels in male rats after a treatment period. Because estradiol is an androgen-antagonist hormone, it is possible that it plays a role in the improvement of the response of Sertoli cells and consequently gives a benefit to spermatogenesis.

There was an increase in the spermatogenesis in the male rats treated with *K. parviflora* extract at the dose of 60 mg/kg bw/day group. Thus, this group had more residual cytoplasm of the spermatid, which is ingested by lysosome in the Sertoli cells. The lysosome enzymes of the Sertoli cells had a role to degrade the residual bodies in the seminiferous tubule (Fawcett, 1994; Fawcett and Jensh, 2002). The ultrastructure micrographs also had plenty of lysosome and irregularly shaped conglomerates in the basal part of Sertoli cells.

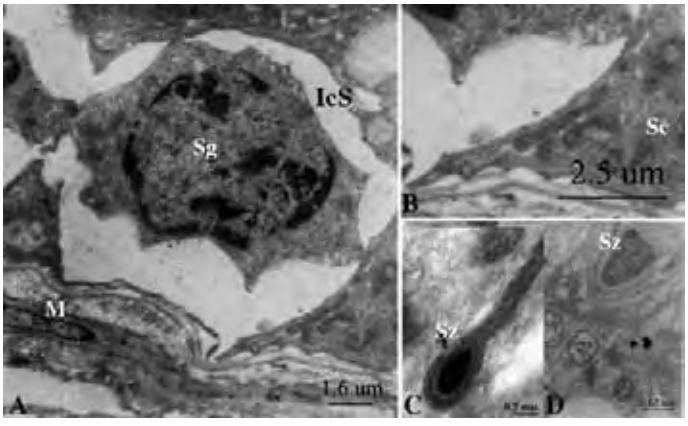

Fig 2- Electron micrographs of seminiferous tubule in the control male rats: A) Spermatogonia (Sg) resting on the basal layer of the seminiferous epithelium beneath which is a slender smooth muscle like (myoid) cells (M). It placed into basal compartment beneath the cytoplasmic bridge of Sertoli cells (Se). 4,000× (IcS = intercellular space). B) Sertoli cell cytoplasmic organelles. 4,000×. C & D) Normal morphology of spermatozoa (Sz). 6,000× and 16,000×, respectively.

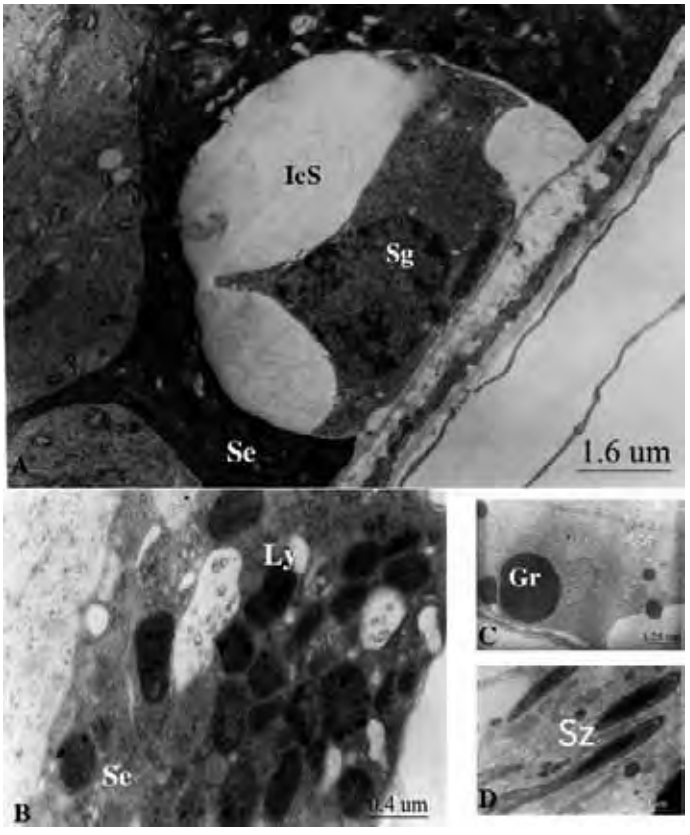

Fig 3- Electron micrographs of seminiferous tubule in the male rats treated with *K. parviflora* extract at the dose of 60 mg/kg bw/day: A) Spermatogonia (Sg) resting on the basement membrane of the seminiferous epithelium. The Sertoli cells (Se) are bound to one another and within the tubule divided into basal and adluminal compartments. 6,300×. B) Highly condensed lysosome (Ly) in the basal part of Sertoli cells shown at higher magnification. They contained electron dense particulate material and amorphous granular material. 25,000×. C) Prominent granules (Gr) in the cytoplasm of Sertoli cells. The Sertoli cells secreted their substances into the intercellular space (IcS) in the basal compartment of the seminiferous epithelium to spermatogonia. 8,000×. D) The normal morphology of spermatozoa (Sz). 8,000×.

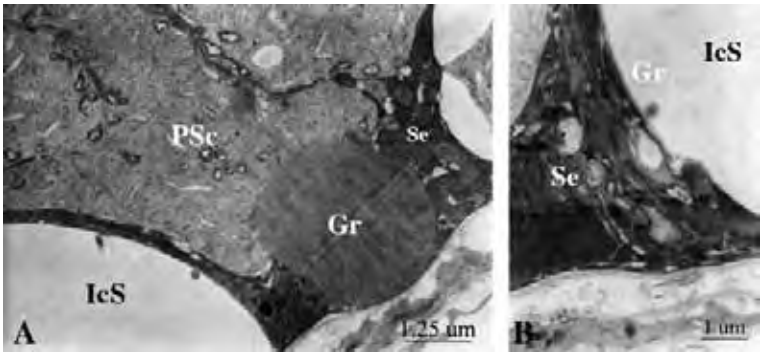

Fig 4- Electron micrographs of seminiferous tubule in the male rats treated with *K. parviflora* extract at the dose of 120 g/kg bw/day.; A) Sertoli cells (Se) resting upon the basement membrane of seminiferous tubule and their prominent granules (Gr); PSc = Primary spermatocyte. 8,000×. B) Sertoli cells having secreted their substances into the intercellular space (IcS). 10,000×.

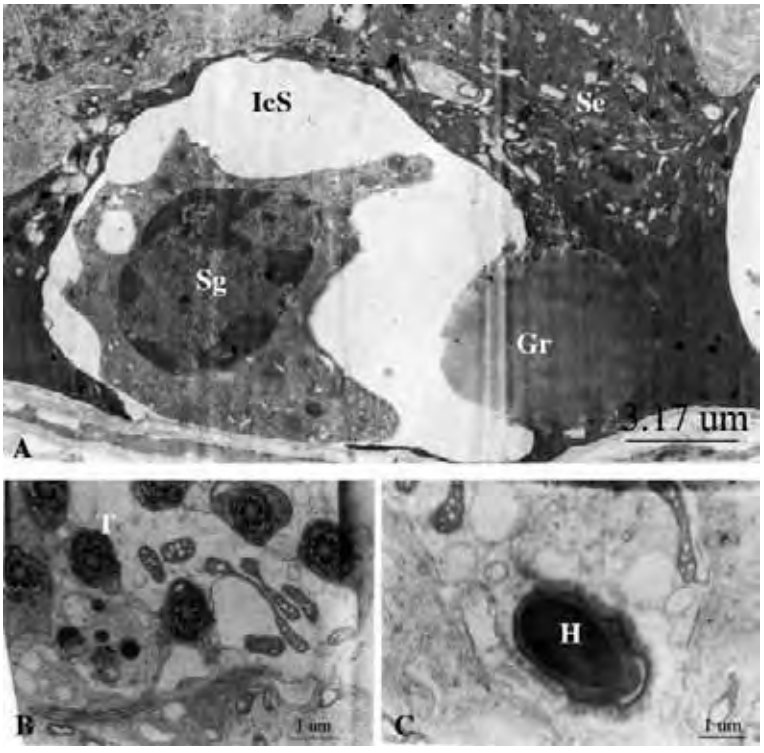

Fig 5- Electron micrographs of seminiferous tubule in the male rats treated with *K. parviflora* extract at the dose of 240 g/kg bw/day. A) Spermatogonia (Sg) resting upon the basement membrane of the seminiferous epithelium. The Sertoli cells (Se) are bound to one another and within the tubule divided into basal and adluminal compartments as the control group. One Sertoli cell has prominent granules (Gr) in its cytoplasm, which are adjacent to the intercellular space (IcS). 3,150×. B) Normal morphology of tail (T), and C) head (H) of spermatozoa (Sz). 10,000×.

This evidence demonstrated that *K. parviflora* extract induced secretory granules, which affected an increase of spermatogenesis and lead to increased amounts of lysosomes. Thus, the Sertoli cells played an important role in germ cell maturation and were highly susceptible to extraneous promotion or damage to the cytoplasmic organelles or inclusions (McCall and Eroschenko, 1988; Kuromaru *et al*, 1989; Lohiya *et al*, 2002; Sudwan and Saenphet, 2006). In conclusion, the ethanolic extract of *K. parviflora* involved in spermatogenesis, possibly mediated by Sertoli cells, leads to an increase of sperm density and secretory granules in Sertoli cells.

ACKNOWLEDGEMENTS

The authors are grateful to Ms Budsabong Kuntalue for her technical assistance. Special thanks are given to the Faculty of Science and Faculty of Medicine, Chiang Mai University for their support.

REFERENCES

Burkitt HG, Young B, Heath JW. Wheater's functional histology. 3rd ed. Edinburgh: Churchill Livingstone,1998:323-34.

Bazzola JJ, Russell LD. Electron microscopy: principles and techniques for biologists. 2nd ed. Boston: Jones and Bartlett, 1998.

Churdboonchart V. Thai vegetables; food and drug. Bangkok: Amarin Printing, 2000 (in Thai).

Chaophaya Aphibhubejhr Hospital. Medicinal plants, 2006. [Cited 2006 Oct 31]. Available from: URL: http://www.abhaiherb.com/herb/index.asp?HID=15.

Fawcett DW, ed. Bloom and Fawcett: a textbook of histology. 12th ed. New York: Chapman and Hall, 1994:768-99.

Fawcett DW, Jensh RP. Bloom and Fawcett: concise histology. 2nd ed. London: Oxford University, 2002:269-81.

Jitjaingam A, Kakaew A, Saenphet K, Seanphet S, Aritajat S. Effects of *Kaempferia parviflora* Wall. Ex. Baker on reproductive organs, hematology and kidney function of male rats [Abstract]. Bangkok: The 31th Congress on Science and Technology of Thailand, 2005:85.

Kurohmaru M, Nishida T, Hayashi Y, Yamashiro S. Three-dimensional structure of some Sertoli cell organelles in the cotton rat and Shiba goat. *J Submicrosc Cytol Pathol* 1989; 21:653-8.

Lohiya NK, Manivannan B, Mishra PK, *et al*. Chloroform extract of Carica papaya seeds induces long-term reversible azoospermia in langur monkey. *Asian J Androl* 2002;4: 17-26.

McCall RD, Eroschenko VP. Ultrastructural abnormalities in the germinal epithelium of adult quail testes following insecticide chlordecone exposure. *Toxicology* 1988;48: 177-89.

Sudwan P, Saenphet K. *Kaempferia parviflora* Wall. ex Baker involves testicular histology and estradiol in male rats[Abstract]. Chiang Rai: 10th International Congress of Ethnobiology, 4-9 November 2006:154-5.

Sudwan P, Saenphet K, Saenphet S, Suwansirikul S. Effects of *Kaempferia parviflora* Wall. ex Baker on sexual activity in male rats and its toxicity. *Southeast Asian J Trop Med Public Health* 2006;37 (suppl 3):210-5.

Watcho P, Kamtchouing P, Sokeng, *et al*. Androgenic effect of *Mondia whitei* roots in male rats. *Asian J Androl* 2004;6:269-72.

Wutythamawech W. Encyclopedia of Thai herbs I. Bangkok: Phet 69 Printing, 2000 (in Thai).