

ภาคผนวก 1

Full Length Research Paper

Effects of *Boesenbergia rotunda* juice on sperm qualities in male rats

Sudawadee Yotarlai¹, Vipavadee Chaisuksunt¹, Kanokporn Saenphet² and Paiwan Sudwan^{1*}

¹Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand.

²Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand.

Accepted 20 May, 2011

Boesenbergia rotunda (L.) Mansf. is one of Thai medicinal plants locally known for its male sexual enhancing effect. However, the study of other impacts of this plant on the male reproductive system is still very rare. To investigate the effects of *B. rotunda* on sperm qualities, the fresh juice of this plant was tested on both pre-mature and mature male rats by oral administration at the doses of 60, 120 and 600 mg/kg.bw for 30 days. The results showed that *B. rotunda* juice significantly progressively increased the motility of sperm at the doses of 60 and 120 mg/kg.bw and enhanced the number of normal sperm at all doses in the mature rats. Additionally, significant prominent stages VII to VIII of seminiferous epithelium was found in treated mature rats at all doses. There was no effect of *B. rotunda* on the pre-mature rats. These findings suggest that the *B. rotunda* juices could enhance fertility by improving the quality of sperm and its effect is age dependable.

Key word: *Boesenbergia rotunda*, sperm morphology, sperm motility, seminiferous epithelium, pre-mature rat, mature rat.

INTRODUCTION

The World Health Organization (WHO) has recognized infertility as an important public health issue (Vayena et al., 2001). A study reported that approximately 15% of couples had had the experience of infertility at least once in their lifetime (Evers, 2002). Hassun et al. (2005) reviewed that this problem effected on male factors of 51.2% of conjugal infertility and the males in 39% of these couples had idiopathic reasons with abnormal semen analyses. Furthermore, the reduction of sperm qualities, closely related to increasing age has been reported in humans (Auger et al., 1995).

Many medicinal plants are widely used to treat or relieve different aspects of male infertility for long times. Evidently, several studies in animals have shown that the sperm qualities of males' reproduction could be improved by various medicinal plants such as *Lepidium meyenii*, *Hibiscus sabdariffa*, *Zingiber officinale* and Korean ginseng (Bustos-Obregón et al., 2005; Amin and Hamza, 2006; Park et al., 2007). In Thailand, *Boesenbergia*

rotunda (L.) Mansf., commonly known as "Krachai", belongs to the Zingiberaceae family and is widely distributed as commercial cultivation in the provinces of Kanchanaburi, Nakhon Pathom, Nakhon Sawan and Ratchaburi (Chomchalow et al., 2006). Fresh rhizomes have a characteristic aroma and slightly pungent taste that are used for cooking in traditional medicine for health-promotion. The rhizomes of *B. rotunda* were found to contain a variety of antioxidant active compounds such as panduratin A, cardamonin, 2',6'-dihydroxy-4'-methoxychalcone, 2',4'-dihydroxy-6'-methoxychalcone, 4'-hydroxypanduratin A (Shindo et al., 2006). Moreover, there have been reported that some derivatives isolated from *B. rotunda* rhizomes have anti-dengue-2 virus NS3 protease (Kiat et al., 2006), anti-*Helicobacter pylori* activity (Bhamarapravati et al., 2006) and has anti-inflammatory properties (Boonjaraspinyo et al., 2010). It had also been reported to remedy many diseases such as anti-flatulent, stomach discomfort, diuretic, leucorrhea, anti-dysenteric and treatment of oral disease (Chomchalow et al., 2006). Interestingly, a recent study of *B. rotunda* extract in male rats found that it could increase the diameter of seminiferous tubules and the testicular and seminal vesicle weights (Sudwan et al., 2007).

*Corresponding author. E-mail: pasudwan@med.cmu.ac.th. Tel: +66-53-945-312 ext. 208, +66-081-366-1526. Fax: +66-53-945-304

Even though there have been a number of studies investigating the actions of *B. rotunda*'s derivatives, scientific information about the effects of this plant on reproductive properties is still very few. The present study is aimed to investigate the effects of *B. rotunda* on the sperm qualities in both pre-mature and mature rats by using two sperm parameters, that is, sperm motility and sperm morphology as the assessment. The histological morphology of seminiferous epithelium was also evaluated.

MATERIALS AND METHODS

Preparation of *B. rotunda* juice

The fresh *B. rotunda* rhizomes from Chiang Mai Province, Thailand were weighed before being washed several times and then air-dried. These rhizomes were sliced into small pieces, blended with a fruit extractor and then filtered. The *B. rotunda* juice was prepared every 3 days and kept at 4°C in a refrigerator.

Animals and treatment

Sixty-four male Wistar rats (*Rattus norvegicus*) were purchased from the National Laboratory Animal Centre, Salaya, Nakhon Pathom, Thailand. Pre-mature rats, aged 4 weeks and mature rats, aged 6 weeks (n = 32 each) were housed (3 rats / cage) under standard conditions, controlled temperature at 25±2°C with 12/12 h light / darkness regimen and were fed commercial diet (CP, Mice feed No. 682) and water.

They were then acclimatized for one week before starting the experiments. Each of the animals' age groups were divided into 4 batches (n = 8 each) and were fed by needle-feeding into the esophagus with *B. rotunda* juice at the doses of 60, 120 and 600 mg/kg.bw for 30 days, respectively. The control group only received distilled water at 1 ml/ day. After 30 days, the animals were sacrificed to remove the reproductive organs. The experimental procedure is in accordance with the institutional guides for the Animal Care and Use (No. 11/2551) and approval obtained from the Animal Ethics committee, Faculty of Medicine, Chiang Mai University.

Sperm motility analysis

The sperm were collected from the right caudal epididymis which was dissected to release the sperm into 10 ml of normal saline (0.9% NaCl). Then, the sperm were placed on the slide and covered with a cover slip for motility analysis under a light microscope using x40 objective lens. Sperm motility classification was divided into four patterns; the progressive, the non-progressive, the circle, and the non-motile sperm. A total of 200 sperm were counted per animal.

Sperm morphology analysis

Sperm in normal saline was smeared on a clean slide. The slide was air-dried and fixed in methanol. Subsequently, it was stained with methylene blue and basic fuchsin on a hot plate. Then, sperm morphology was assessed under the light microscope using x40 objective lens. The morphological features of individual spermatozoa were classified into four patterns; the normal, the sperm with abnormal head and normal tail, the sperm with

abnormal head and tail, and the sperm with normal head and abnormal tail. A total of 600 sperm were identified per animal.

Seminiferous epithelium examination

The left testes were dissected and then fixed with 4% paraformaldehyde, dehydrated in a graded series of ethanol, and finally embedded in paraffin wax. Paraffin blocks were cut at 5 µm thick and stained with periodic acid-Schiff's reaction (PAS) and counter-stained with hematoxylin. The sampling cycles of seminiferous epithelium 20 tubule profiles for each section were identified according to Hess (1990) and then the stages VII to VIII were counted. The histological appearances of testicular tissue were also observed.

Data analysis

The sperm motility and morphology data were expressed by mean ± standard deviation (SD) and analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni test. In case of the homogeneity of variances showing significant differences, the Kruskal-Wallis test followed by Mann-Whitney test were used. The SPSS version 17.0 was employed for all statistical analysis. The differences were considered statistically significant when the probability was less than 5%.

RESULTS

Effect on sperm motility of male rats

During the experiment with *B. rotunda* juice, no clinically abnormal signs or death were observed in any group of the animals. There were no significant changes in the sperm motility of pre-mature rats treated with *B. rotunda* juice when compared to the control (Table 1). The progressive movement of sperm was significantly increased in mature groups receiving at the doses of 60 and 120 mg/kg.bw ($p < 0.05$) when compared with the control group (Table 2).

Effect on sperm morphology of male rats

Types of sperm abnormality

Abnormal head, hairpin neck or bent tail was normally found in all of rats treated with *B. rotunda* juice including the control groups (Figure 1). In all pre-mature groups, the sperm morphology was not affected by the administration of *B. rotunda* juice at any dose (Table 3). On the contrary, the mature rats treated with *B. rotunda* juice at the all doses showed a significant increase in the number of normal sperm and decrease ($p < 0.05$) in that of the abnormal sperm tails when compared to the control (Table 4).

Histological appearance of the seminiferous epithelium

Generally, normal histological characteristics of the

Table 1. Numbers of the motile and non-motile sperm of the pre-mature rats administered with varying doses of *B. rotunda* juice for 30 days, compared with control (mean \pm SD).

Group	Number of motile sperm			Number of non-motile sperm
	Progressive	Non-progressive	Circle	
Control	15.50 \pm 7.66	33.17 \pm 11.99	2.17 \pm 2.48	149.17 \pm 6.85
<i>B. rotunda</i> juice (60 mg/kg.bw)	20.86 \pm 11.72	27.00 \pm 4.55	4.14 \pm 5.67	149.00 \pm 16.31
<i>B. rotunda</i> juice (120 mg/kg.bw)	16.62 \pm 11.26	24.12 \pm 9.01	4.87 \pm 6.60	155.12 \pm 22.53
<i>B. rotunda</i> juice (600 mg/kg.bw)	13.75 \pm 9.22	25.25 \pm 12.74	4.37 \pm 5.50	157.00 \pm 14.73

There were no significant differences between groups.

Table 2. Numbers of the motile and non-motile sperm of the mature rats administered with varying doses of *B. rotunda* juice for 30 days, compared with control (mean \pm SD).

Group	Number of motile sperm			Number of non-motile sperm
	Progressive	Non-progressive	Circle	
Control	27.43 \pm 10.13 ^a	27.42 \pm 11.63	4.14 \pm 5.49	141.00 \pm 16.20
<i>B. rotunda</i> juice (60 mg/kg.bw)	40.62 \pm 13.28 ^b	20.50 \pm 9.71	4.62 \pm 8.45	134.50 \pm 18.31
<i>B. rotunda</i> juice (120 mg/kg.bw)	54.87 \pm 11.68 ^c	19.25 \pm 9.85	4.37 \pm 7.73	116.50 \pm 14.06
<i>B. rotunda</i> juice (600 mg/kg.bw)	44.00 \pm 25.18 ^{abc}	20.00 \pm 13.62	5.37 \pm 5.53	130.62 \pm 23.84

^{a,b,c} Different letters indicate significant differences between groups within each column. The mean differences are significant at the 0.05 level.

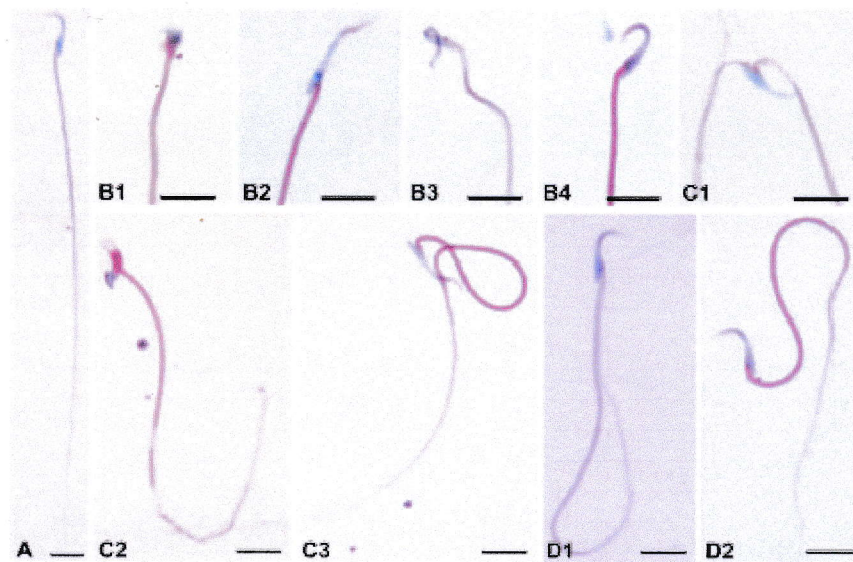


Figure 1. Morphological classification of rat epididymal sperm. Normal sperm (A); sperm with different patterns of abnormal heads (B1-4); sperm with both abnormal head and tail (C1-3); sperm with different patterns of abnormal tails (D1-2). The scale bar shown in the figure represents 10 μ m.

seminiferous epithelium were observed in both pre-mature and mature rats of the treated and control groups.

The morphologies of seminiferous epithelium were in normal arrangement (Figures 2 and 3). The number of

Table 3. Numbers of the normal and abnormal sperm of the pre-mature rats administered with varying doses of *B. rotunda* juice for 30 days, compared with control (mean \pm SD).

Group	Number of normal sperm	Number of abnormal sperm		
		Head only	Head and tail	Tail only
Control	76.07 \pm 8.97	3.00 \pm 1.56	6.79 \pm 4.08	113.50 \pm 7.28
<i>B. rotunda</i> juice (60 mg/kg.bw)	77.67 \pm 10.19	2.83 \pm 0.96	5.38 \pm 3.77	114.12 \pm 7.41
<i>B. rotunda</i> juice (120 mg/kg.bw)	84.00 \pm 10.15	3.21 \pm 1.77	4.00 \pm 2.34	108.79 \pm 10.48
<i>B. rotunda</i> juice (600 mg/kg.bw)	76.83 \pm 12.74	2.80 \pm 0.98	5.23 \pm 4.54	115.08 \pm 9.43

There were no significant differences between groups.

Table 4. Numbers of the normal and abnormal sperm of the mature rats administered with varying doses of *B. rotunda* juice for 30 days, compared with control (mean \pm SD).

Group	Number of normal sperm	Number of abnormal sperm		
		Head only	Head and tail	Tail only
Control	57.83 \pm 5.24 ^a	2.31 \pm 1.27	4.10 \pm 2.23	135.75 \pm 4.34 ^a
<i>B. rotunda</i> juice (60 mg/kg.bw)	82.24 \pm 14.29 ^b	1.79 \pm 1.20	2.33 \pm 1.55	113.62 \pm 13.47 ^b
<i>B. rotunda</i> juice (120 mg/kg.bw)	76.04 \pm 14.45 ^b	1.71 \pm 0.92	3.33 \pm 1.15	118.92 \pm 13.78 ^b
<i>B. rotunda</i> juice (600 mg/kg.bw)	81.33 \pm 17.51 ^b	1.18 \pm 0.56	2.79 \pm 2.00	114.71 \pm 15.55 ^b

^{a,b} Different letters indicate significant differences between groups within each column. The mean differences are significant at the 0.05 level.

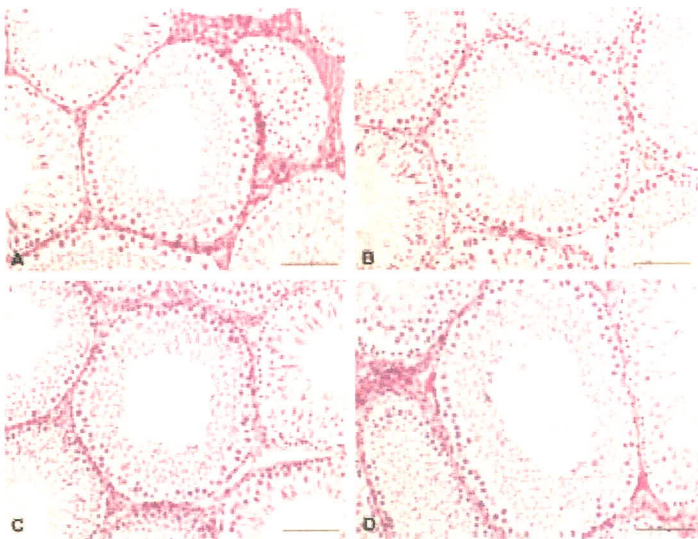


Figure 2. Histological feature of the stage VII to VIII of seminiferous epithelium of the pre-mature rats compared between the control group (A) and *B. rotunda* juice treated groups at 60 mg/kg.bw (B), 120 mg/kg.bw (C), and 600 mg/kg.bw (D). The bar shown in each figure represents 100 μ m.

the stages VII to VIII of seminiferous epithelium were not significantly different in the treated pre-mature rats at

all doses ($p < 0.05$) when compared to the control (Figure 4). However, the mature rats treated with all doses of

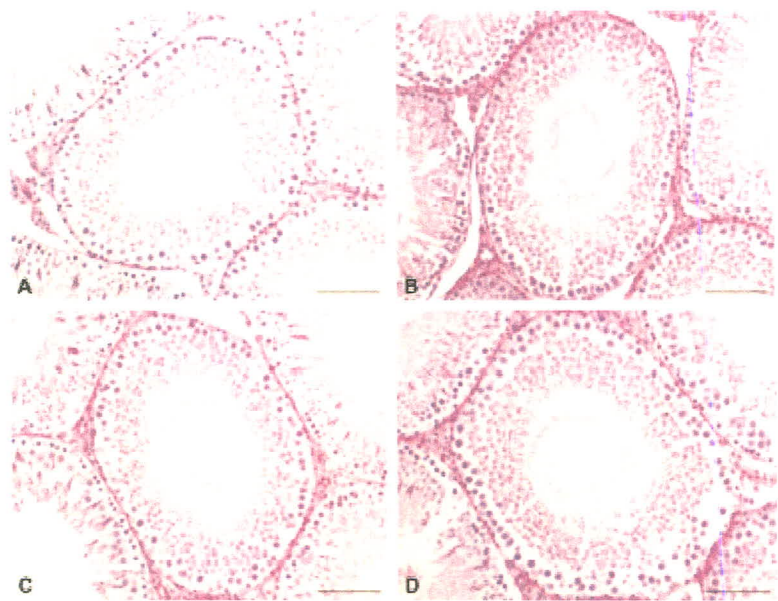


Figure 3. Histological features of the stage VII to VIII of seminiferous epithelium of the mature rats compared between the control group (A) and *B. rotunda* juice treated groups at 60 mg/kg bw (B), 120 mg/kg bw (C), and 600 mg/kg bw (D). The bar shown in each figure represents 100 μ m.

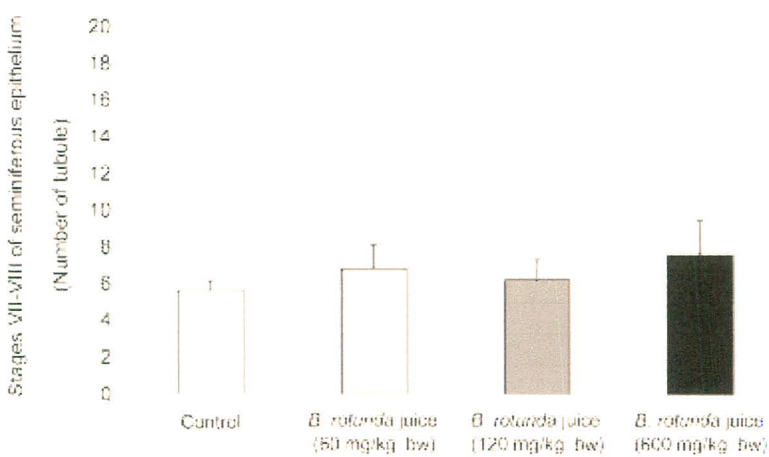


Figure 4. Numbers of stages VII to VIII of seminiferous epithelium of the pre-mature rats administered with varying doses of *B. rotunda* juice for 30 days compared with control (mean \pm SD).

B. rotunda juice showed a significant increase ($p<0.05$) in the number of the stages VII to VIII of seminiferous

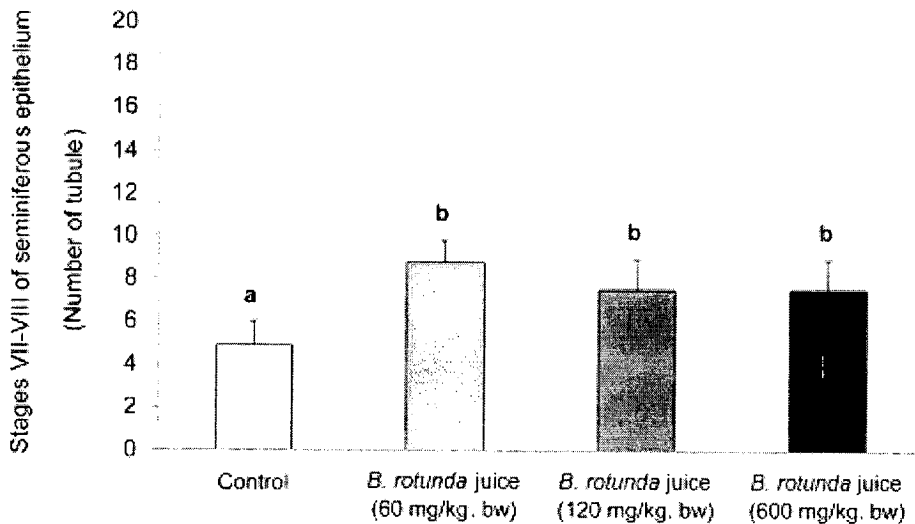


Figure 5. Numbers of stages VII to VIII of seminiferous epithelium of the mature rats administered with varying doses of *B. rotunda* juice for 30 days compared with control (mean \pm SD). ^{a,b} Different letters indicate significant differences between groups. The mean differences are significant at the 0.05 level.

epithelium (Figure 5).

DISCUSSION

Many native medicinal plants have been used for prevention, relief and remedy of many aspects of male reproduction. In the present study, *B. rotunda* juice obviously showed positive effects on sperm qualities in mature male rats. The results may be promoted by the antioxidant activity of flavonoid ingredients which has been found in the rhizomes of *B. rotunda* (Shindo et al., 2006). Similarly, antioxidant activity of *Hibiscus sabdariffa* and *Zingiber officinale* exhibited the protective effect of testicular tissue and sperm quality by encouraging scavengers of free radicals from oxidative stress and lipid peroxidation (Amin and Hamza, 2006). Evidently, antioxidant activity results in preserving and enhancing the process of spermatogenesis in mice as reported by D'cruz and Mathur (2005). In light of this previous evidence, antioxidant property of *B. rotunda* may be responsible for better quality of sperm and therefore, could improve fertility.

The motility and maturation of sperm are associated with the spermatogenesis and are also dependent on many factors. A transcription factor exclusively cAMP-responsive element modulator (CREM), is one of the factors involved in the regulation of gene expression by cAMP, which is an important role in germ cell

differentiation that effects round spermatids. In addition, CREM activator proteins found in the germ cells of the testes of mature animals were abundantly expressed in pachytene spermatocytes and round spermatids. These proteins had the highest exhibit in spermatogenic stages VII to VIII of seminiferous epithelium (Delmas et al., 1993; Walker and Habener, 1996; Behr and Weinbauer, 2001). Moreover, Park et al. (2007) reported that the increase of sperm motility in rats may have been caused CREM activation. Taken together, it is possible that *B. rotunda* juice could improve the sperm parameters via CREM activation in mature rats, but not in the pre-mature ones. Furthermore, the motility of sperm needed the energization of adenosine triphosphate (ATP), which is synthesized by the mitochondria in the body of the tail (Guyton and Hall, 2006) as well as plasma membrane Ca^{2+} -dependent ATPase 4 (PMCA4), highly enriched in the sperm tail, is important for male fertility by implicating calcium signal transduction in sperm motility (Schuh et al., 2004). Although, *B. rotunda* juice may enhance the mitochondrial activity and improve the PMCA signal pathway and results in increasing the progressivity movement in mature rats at the doses of 60 and 120 mg/kg.bw. at the dose of 600 mg/kg.bw. there was no effect. The results suggest that the highest concentration of *B. rotunda* juice were caused by the excess phytoestrogen in the rats and the estrogen which induce a biphasic response by inhibiting protein kinase that effected the decrease of ATP synthesis (Clarke et al.,

2001; Cederroth et al., 2008) of mitochondria activity in sperm. In contrast, mature rats receive *B. rotunda* juice at the doses of 60 and 120 mg/kg. bw showing a dose-dependent response of sperm progressive movement; this may be due to concentrations been appropriated and not because of excess estrogen. However, further study should be done to verify if *B. rotunda* could activate CREM and sperm energy.

The present study demonstrated that the number of the stages VII to VIII of seminiferous epithelium in the mature testes rats treated with *B. rotunda* juice was higher than that of the control group. Since the stages VII to VIII in the cycle composed of step 7 of the round spermatids and step 19 of the elongated spermatids (Hess, 1990), the more number of the stages VII to VIII of seminiferous epithelium in the mature rats could result in increasing the number of mature sperm released from the testes. Consistently, Bustos-Obregón et al. (2005) reported that the increase in length of stages VII to VIII seminiferous epithelium in mice resulted from improvement in the late stages of spermatogenesis.

In conclusion, *B. rotunda* juice could significantly improve sperm production and qualities in mature male rats. It is possible that *B. rotunda* juice could promote this effect via anti-oxidant property and may stimulate CREM activation, but the precise mechanism needs to be further investigated.

ACKNOWLEDGEMENTS

This work was supported by The Thailand Research Fund (TRF) MRG5180115 and Faculty of Medicine, Chiang Mai University, Thailand. The authors would like to thank The Animal Laboratory Building, Faculty of Science, Chiang Mai University, Thailand for providing the animal experimental venue and Dr. Supap Saenphet and Miss Lamaiporn Lueinta for their helpful technical assistance.

REFERENCES

Amin A, Hantza AA (2006). Effects of Roselle and Ginger on displatein-induced reproductive toxicity in rats. *Asian J. Androl.*, 8(5): 607-612.

Auger J, Kunstmann JMT, Czaplik F, Jouannet P (1995). Decline in semen quality among fertile men in Paris during the past 20 years. *N. Engl. J. Med.*, 332(5): 281-285.

Behr R, Weinbauer GF (2001). cAMP response element modulator (CREM): an essential factor for spermatogenesis in primates? *Int. J. Androl.*, 24(3): 126-135.

Bhamarapravati S, Juthapitru S, Mahachai W, Mahady G (2006). Antibacterial activity of *Boesenbergia rotunda* (L.) Manst. and *Myristica fragrans* Houtt. against *Helicobacter pylori*. *Songklanakarin J. Sci. Technol.*, 28: 157-163.

Boonjarasirong S, Boonmars T, Aromdee C, Kaewsamut B (2010). Effect of fingerroot on reducing inflammatory cells in hamster infected with *Coxsackievirus* and N-nitrosodimethylamine administration. *Parasitol. Res.*, 106(6): 1485-1489.

Bustos-Obregón E, Yucra S, González GF (2005). *Lepidium meyeri* (Maca) reduces spermatogenic damage induced by a single dose of malathion in mice. *Asian J. Androl.*, 7(1): 71-76.

Cederroth CR, Vinciguerra M, Gjinová A, Kühne F, Klem M, Cederroth M, Calle D, Siler M, Neumann D, James RW, Doerge DP, Walzmann T, Meda P, Foti M, Rohrer-Jeanraud F, Vassalli JD, Nef S (2008). Dietary phytoestrogens activate AMP-activated protein kinase with improvement in lipid and glucose metabolism. *Diabetes*, 57(5): 1176-1185.

Chomchalow N, Bansiddhi J, Chantrasm V (2006). Amazing Thai medicinal plants. Royal Flora Ratchaphruek 2006, Horticultural Research Institute and Horticultural Science Society of Thailand. Bangkok. Pp. 10-11.

Clarke R, Lecnessa F, Welch JN, Skaar TC (2001). Cellular and molecular pharmacology of antiestrogen action and resistance. *Pharmacol. Rev.*, 53(1): 25-71.

D'Cruz SC, Mathur PP (2005). Effect of piperine on the epididymis of adult male rats. *Asian J. Androl.*, 7(4): 363-368.

Delmas V, Vander HF, Melstrom B, Jegou B, Sassone-Corsi P (1993). Induction of CREM activator proteins in spermatids: down-stream targets and implications for haploid germ cell differentiation. *Mol. Endocrinol.*, 7(11): 1502-1514.

Evers JL (2002). Female subfertility. *Lancet*, 350(9327): 151-159.

Guyton AC, Hall JE (2006). Textbook of medical physiology. 11th ed. WB Saunders, Philadelphia. Pp. 997-999.

Hassun FPA, Cadenho AP, Lima SB, Ortiz V, Srougi M (2005). Single nucleotide polymorphisms of the heat shock protein 90 gene in varicocele associated infertility. *International Braz. J. Urol.*, 31: 226-242.

Hess RA (1990). Quantitative and qualitative characteristics of the stages and transitions in the cycle of the rat seminiferous epithelium: light microscopic observations of perfusion-fixed and plastic-embedded testes. *Biol. Reprod.*, 43(3): 525-542.

Khal TS, Pappen R, Yusof R, Ibrahim H, Khalid N, Rahman NA (2006). Inhibitory activity of cyclohexenyl chalcone derivatives and flavonoids of fingerroot, *Boesenbergia rotunda* (L.), towards dengue 2 virus NS3 protease. *Borg. Med. Chem. Lett.*, 16(12): 3337-3340.

Park WS, Shim DY, Kim Do R, Yang WM, Chang MS, Park SK (2007). Korean ginseng induces spermatogenesis in rats through the activation of cAMP-responsive element modulator (CREM). *Fertil. Steril.*, 88(4): 1000-1002.

Schuh K, Cartwright EJ, Jankevics E, Bindschü K, Liebermann J, Williams JC, Arnesilla AL, Emerson M, Coeandy D, Knobloch KP, Neyres L (2004). Plasma membrane Ca²⁺ ATPase 4 is required for sperm motility and male fertility. *J. Biol. Chem.*, 279(27): 28220-28226.

Shindo K, Kato M, Kinoshita A, Kobayashi A, Koike Y (2006). Analysis of antioxidant activities contained in the *Boesenbergia pandurata* Schult. Rhizome. *Biosci. Biotechnol. Biochem.*, 70(9): 2281-2284.

Sudwan P, Saenphet K, Anjalai S, Silasitwan N (2007). Effects of *Boesenbergia rotunda* (L.) Manst. on sexual behaviour of male rats. *Asian J. Androl.*, 9(6): 849-855.

Vayena E, Rowe PJ, Griffin PD (2001). Medical, ethical and social aspects of assisted reproduction. Current practices and controversies in assisted reproduction: Report of a WHO meeting. Geneva, Switzerland.

Walker WH, Habener JF (1996). Role of transcription factors CREB and CREM in cAMP-regulated transcription during spermatogenesis. *Trends Endocrinol. Metab.*, 7(4): 133-139.

ภาคผนวก 2

Asian Journal of Andrology

Volume 11, Issue 5, Supplement 1, October 2009

亚洲男性科学杂志
CN 31-1795/P
ISSN 1008-882X (Print)
ISSN 1745-7262 (Online)

The Proceedings of the Third Asia-Pacific Forum on Andrology

10-13 October 2009, Nanjing, China



Shanghai Institute of Materia Medica
Chinese Academy of Sciences
Shanghai Jiao Tong University
Asian Society of Andrology
www.asiaandro.com
www.nature.com/aja



pelvic endometriosis and altered basal body temperature (BBT). **Methods:** This study population consisted of infertile couples who have been diagnosed as having endometriosis. **Results:** A significant association was found between the presence of pelvic endometriosis and the appearance of a late decline in BBT during the early follicular phase of the menstrual cycle. A temperature of 37.80°C on the first 3 days of the menses is associated with pelvic endometriosis. **Conclusion:** A relatively common problem in women is endometriosis. The association between endometriosis and infertility is clearly established. It is proposed that endometriosis has the potential to produce pathology in two ways: 1) Hypothalamic-pituitary ovarian axis and luteal phase abnormalities. 2) Peritoneal inflammatory response. The findings of this study support the clinical diagnosis of endometriosis in infertile women. The BBT chart analysis may be useful as a clinical adjunct when endometriosis is suspected.

E-mail: mabashed@btb.net.bd / bashed23@dhaka.net

Pt8-15

Effect of Uremia and kidney transplantation on testicular volume and male reproductive ability

Long-Gen Xu^{1*}, Hui-Ming Xu², Xiao-Feng Zhu³, Ying Wu²

¹117th Hospital of PLA, Hangzhou 310013, China

²Zhejiang Family Planning Science and Technology Institute, Hangzhou 310012, China

Aim: To investigate the effect of Uremia and kidney transplantation on testicular volume and male reproductive ability. **Methods:** 40 Uremia patients' semen samples and 40 kidney transplantation patients' semen samples were tested. And calculate as: Fertility index (FI) = sperm concentration (10^6 mL^{-1}) \times sperm viability \times sperm normal morphology rate. Thirty of these Uremia patients were monitored for testicular volume by color ultrasound before and after kidney transplantation. **Results:** FI in normal fertility people was 13.03 (14.26), FI in kidney transplantation patients was 7.19 (10.18), and FI in Uremia patients was 0.23 (0.76). Testicular volume of Uremia patients: which on the left side before surgery was $6.82 \pm 1.49 \text{ mL}$, and on the right side before surgery $7.46 \pm 1.89 \text{ mL}$. At 1 month, 3 months, and 1 year after kidney transplantation, the left side of Testicular volume rose to 8.25 ± 1.67 , 9.31 ± 1.56 , 9.80 ± 1.51 ; and the right side of testicular volume to 9.18 ± 1.76 , 10.41 ± 1.43 , 11.09 ± 1.45 . The FI and testicular volume had statistics differences ($P < 0.01$) between Uremia patients and after kidney transplantation groups. **Conclusion:** Uremia reduces Male testicular volume and impairs reproductive ability, but they can be improved by successful kidney transplantation.

E-mail: xulonggen@sina.com

Pt8-16

The suitable time for male renal transplant recipients to father pregnancy and childbirth

Long-Gen Xu^{*}, Shu Han, Yong Liu, Hong-Wei Wang, Yi-Rong Yang, Feng Qiu, Wan-Ling Peng, Li-Gong Tang, Jing Fu, Xiao-Feng Zhu, Qi-Zhe Song, Xian-Fan Ding, You-Hua Zhu

Center for Renal Transplantation, the 117th Hospital, Hangzhou

310013, China

Aim: To determine the appropriate time for male renal transplant recipients to father pregnancy after transplantation. **Methods:** A total of 212 cases of male renal transplant recipients who fathered 216 children after transplantation, including four cases of the two-child birth, among eight organ transplant centers in China between December 1981 and August 2007, were retrospectively analyzed and their children's growth and development status were investigated. **Results:** The 212 renal transplant recipients fathered 216 children 15–204 (46.91 ± 26.19) months after transplantation, including 115 boys and 101 girls. Among them, 20 recipients fathered 20 offspring, including three preterm birth (15.0%), 15–24 (21.00 ± 2.94) months after transplantation; 192 recipients fathered 196 offspring, including six preterm birth (3.1%), 25–204 (49.56 ± 26.06) months after transplantation. The newborn babies weighed $1\,930\text{--}4\,600$ ($3\,253 \pm 379$) g. One boy suffered from a pair of soft toe. All other children were normally developed as indicated in physical examination. **Conclusion:** It would be appropriate for male renal transplant recipients with normal renal function to father pregnancy and have offspring two years after transplantation.

E-mail: xulonggen@sina.com

Pt8-17

Boesenbergia rotunda (L.) Mansf. juice did not affect androgenic and estradiol levels in premature male rats.

Paiwan Sudwan^{1*}, Kanokporn Saenphier², Supap Saenphier²

¹Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand

²Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

Aim: To determine if Krachai, *Boesenbergia rotunda* (L.) Mansf. juice could promote an increase of the androgenic and estradiol hormones, reproductive organ weights and epididymal sperm density, and at same time, produce toxicity in premature male rats. **Methods:** Thirty-two premature male Wistar rats (4 weeks old) were equally divided into a control and three *B. rotunda* juice-treated groups. *B. rotunda* juice was orally administered at the doses of 60, 120 and 600 mg kg⁻¹ body weight, to treated groups of male rats ($n = 8$), daily for 30 days and the controls received a similar amount of distilled water. After the treatment periods, all animals were anesthetized on day 31. Their blood was collected for hematological analysis. Serum was prepared for the electrochemiluminescence immunoassay (ECLIA) to determine testosterone and estradiol levels and the radioimmunoassay (RIA) to determine androstenedione (ADD) levels. The reproductive organs were dissected and weighed and the epididymal sperm density was evaluated. **Results:** There were no significant differences in serum testosterone, androstenedione and estradiol levels, the relative weight of the reproductive organs (testis, caudal epididymis, seminal vesicle and prostate gland) and sperm density between the control and treated groups. Toxicological study revealed no significant difference of hematocrit, WBC or differential cell count. **Conclusion:** *B. rotunda* juice did not modify the testosterone.



ADD and estradiol levels, the sexual organ weights, or the epididymal sperm density during the 30 days of treatment, and high concentration of juice could not harm premature male rats.

E-mail: pasudwan@med.cmu.ac.th

Pt8-18

Long toxicity of Shen Yan Ling tablet and its effect on the reproductive function in rats

Yu-Gui Cui¹, Xing-Hai Wang^{2*}, Ding-Zhi Ma², Yue Jia¹, Rui-Fen Cai², Li Gao¹, Ming-De Yang¹, Jian-Sun Tong²

¹Center of Clinical Reproductive Medicine, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China

²Jiangsu Province Family Planning Institute, Nanjing 210036, China

Background: Shen Yan Ling is an innovative Chinese traditional medicine composed with Li Gong Teng, Huang Qi, and others. This compound has conclusively curative effect on renal diseases with low side effect. **Aim:** To investigate the long toxicity of Shen Yan Ling and its effect on the reproductive function. **Methods:** Shen Yan Ling was provided by Jiangsu Kangyuan Pharmaceutical Company and the experiment was approved by the Chinese Regulation of New Drug Research before Clinical Application. Adult SD rats were divided into four equal groups (20 rats in each group with male to female ratio 10:10) as follows: low dose of Shen Yan Ling (1.25 g kg⁻¹), middle dose (2.50 g kg⁻¹), high dose (5.00 g kg⁻¹) and control. The standard test of long toxicity was designed to observe the asked parameters, and to observe serum hormones and testicular and epididymis sperm. **Results:** Compared with controls, general status and body weight were normal after 3 months of treatment with Shen Yan Ling. Parameters of blood cytology and biochemistry fluctuated in the normal range, without any significant changes ($P > 0.05$). Compared with control, the mass coefficient of main organs did not change significantly and a slight change in hepatic and pulmonary pathology in high dose group was found. Although serum sexual hormones did not change significantly, sperm counts in testis and epididymis reduced significantly in high dose group ($P < 0.05$). **Conclusion:** Shen Yan Ling has no significant long-accumulated toxicity on rats after 3 months of treatment with the designed doses (1.25 to 5.00 g kg⁻¹) but it exerts negative effect on the reproductive function if treated with high dose of long term.

E-mail: njxhw@online.com

Pt8-19

Application of the continuous measurement of urine β -FSH excretion in patients with pubertal disorders

Yu-Gui Cui^{1*}, Lin Song², Xiao-Fang Yang², Ting Feng¹, Qin-Qin Pan¹, Fu-Song Di¹

¹Center of Reproductive Medicine, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China

²Department of Applicative Toxicology, Nanjing Medical University, Nanjing 210029, China

Aim: To evaluate the significance of urine β -FSH excretion

in the clinical practice and pathophysiological study by continuously measuring the urine β -FSH excretion in the patients with puberty disorders including precocious and delayed puberty. **Methods:** Five male volunteers (aged 5, 19, 22, 27 and 33 years) and four female volunteers (aged 5, 28, 28 and 33 years) were selected as control. Four patients with the hypogonadotropic hypogonadism (Kallmann's syndrome or IHH aged 17, 17, 19, 24 years), five patients with hypergonadotropic hypogonadism (Klinefelter's syndrome, aged 16, 16, 17, 20, 22 years), four patients with the central precocious puberty (aged 3, 5, 5, 7 years) and one patient with isosexual peripheral precocious puberty (breast development, aged 5 years) were involved to collect their early-morning urine samples for 30 to 32 days. One normal men and one normal woman collected urine samples for 63 to 64 days. The urine β -FSH was assayed with the method of EIA, then corrected by creatinine (Cr) concentration. **Results:** The urine β -FSH level of normal adult men was $1.16 \pm 0.20 \mu\text{g mg}^{-1} \text{Cr}$ with small peak variation in their curves, while normal adult women have higher baseline ($3.12 \pm 0.68 \mu\text{g mg}^{-1} \text{Cr}$) and very sharp peak variation in curves corresponding with their cycles. Patients with the hypogonadotropic hypogonadism had lower levels of urine β -FSH, and patients with idiopathic hypogonadism had higher levels with irregular fluctuation, than matching men. Meanwhile, patients with the central precocious puberty had much higher levels of urine β -FSH with irregular peaks, and patients with isosexual peripheral precocious puberty had almost normal levels, than their cotemporary children. The patterns were coincident with the clinical characteristics and serum FSH levels. **Conclusion:** The urine β -FSH excretion was a useful parameter for the clinical classify diagnoses and pathophysiological study on puberty disorders.

E-mail: cuiyguj@njmu.edu.cn

Pt8-20

No needle no scalpel vasectomy (NNSV): an Indian experience

RCM Kaza^{1*}, Bajjit Kaur², Jasvinder Singh³, Sachin Patel¹

¹Department of Surgery, Maulana Azad Medical College, New Delhi, India

²Male Family Planning Center, Civil Hospital, Amritsar, India

³Maulana Azad Medical College, New Delhi, India

Aim: To test the feasibility and effectiveness of jet injector anesthesia technique i.e. "no needle no scalpel vasectomy" (NNSV) over traditional needle anesthesia technique in no scalpel vasectomy (NSV). **Methods:** This study was conducted in: 1) Department of Surgery and Department of Family Planning, Maulana Azad Medical College and Lok Nayak Hospital, New Delhi, India. 2) Civil Hospital, Amritsar, Punjab. Inclusion criteria and exclusion criteria were set according to our current practice guidelines and national standards. After informed consent 700 clients were randomized in to two groups: group A underwent NSV with jet injector anesthesia and group B underwent NSV with traditional needle injection anesthesia. For group A Medajet XL® jet injector and for group B 26" gauge needle with 5 mL syringe were used for giving local anesthesia. Medajet XL® was used as explained by Ronald S Weiss and Philip S Li. Both the groups were compared in terms of time

¹Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand
²Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
 Correspondence to: Dr Paitwan Sudwan (pasudwan@med.cmu.ac.th)

Aims: To determine if *Krachai, Boschnbergia rotunda* (L.) Mansf. juice could promote an increase of the androgenic and estradiol hormones, reproductive organ weights and epididymal sperm density, and at same time, produce toxicity in premature male rats. **Methods:** Thirty-two premature male *Wistar* rats (4 weeks old) were equally divided into a control and three *B. rotunda* juice-treated groups. *B. rotunda* juice was orally administered at the doses of 60, 120 and 600 mg/kg body weight, to treated groups of male rats ($n = 8$), daily for 30 days and the controls received a similar amount of distilled water. After the treatment periods, all animals were anesthetized on day 31. Their blood was collected for hematological analysis. Serum was prepared for the electrochemoluminescence immunoassay (ECLIA) to determine testosterone and estradiol levels and the radioimmunoassay (RIA) to determine androstenedione (ADD) levels. The reproductive organs were dissected and weighed and the epididymal sperm density was evaluated. **Results:** There were no significant differences in serum testosterone, androstenedione and estradiol levels, the relative weight of the reproductive organs (testis, caudal epididymis, seminal vesicle and prostate gland) and sperm density between the control and treated groups. Toxicological study revealed no significant difference of hematocrit, hemoglobin, hemoglobin concentration, hemoglobin concentration, hemoglobin concentration, hemoglobin concentration, or differential cell count. **Conclusion:** *B. rotunda* juice did not modify the testosterone, ADD and estradiol levels, the sexual organ weights, or the epididymal sperm density during the 30 days of treatment and high concentration of juice could not harm premature male rats.

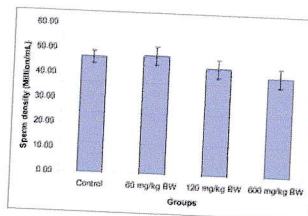


Figure 3. Sperm density of male rats treated with *B. rotunda* juice for 30 days (one-way ANOVA; there were no significant differences). The data were expressed as mean \pm SD

Toxicological study revealed no significant difference of hematocrit, WBC or differential cell count (Table 1) or body weight of the control and the treated groups (Fig 4). All groups had a significant increase in body weight (Fig 4).

Baccharis rotunda L. (Mansé) (Knecht) is belonging to the Zingiberaceae family. Its rhizomes have been used for health-promoting, antifungal, stomach discomfort, leucorrhoea, diuretic, antidiarrhetic and as an ingredient of traditional remedies for impotency (4). The ethanol extract of this plant, known as "Ta ginseng" (3) and is recorded as an aphrodisiac and the seminal vesicles and seminiferous tubular diameter, while the testes and epididymal sperm density, in the estradiol-treated male rats. The testosterone levels did not change in male rats (5). However, the investigation was determined if *B. rotunda* juice could promote an increase of the androgenic and estradiol hormone, affective organ weights and epididymal sperm density, and at same time, produce premature male rats.

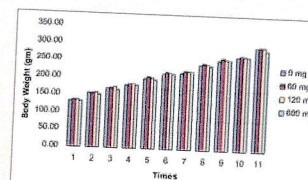


Figure 4. Average body weight of male rats during 30 days. Each point represents average body weight at every 3 days.

```

graph TD
    A[40 rats] --> B[10 rats per group]
    B --> C[Control]
    B --> D[HR 60 mg/kg]
    B --> E[HR 120 mg/kg]
    B --> F[HR 600 mg/kg]
    C --> G[Day 31]
    D --> G
    E --> G
    F --> G
    G --> H[Sexual organs, Sperm density, Testosterone, ADD, Estradiol, Blood chemistry and hematological tests]
    H --> I[Data analysis]
  
```

40 rats

10 rats per group

Control

HR 60 mg/kg

HR 120 mg/kg

HR 600 mg/kg

Day 31

Sexual organs, Sperm density, Testosterone, ADD, Estradiol, Blood chemistry and hematological tests

Data analysis

The blood was collected for hematological analysis. Serum was prepared for the electrochemiluminescence immunoassay (ECLIA) to determine testosterone and estradiol levels and the radioimmunoassay (RIA) to determine androstenedione (ADD) levels. The reproductive organs were dissected and weighed and the epididymal sperm density was evaluated. The SPSS was employed for all statistical analyses. Significance was inferred when $p < 0.05$.

There were no significant differences in serum testosterone, androstenedione and estradiol levels (Fig 1), the relative weight of the reproductive organs (Fig 2), and sperm density (Fig 3), between the control and treated groups.

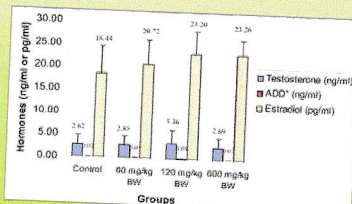


Figure 1. Testosterone, androstenedione (ADD) and estradiol levels of male rats treated with *B. ramulosa* extract for 30 days (one-way ANOVA and Kruskal-Wallis Test (*), there were no significant differences). The data were expressed as mean \pm SD.

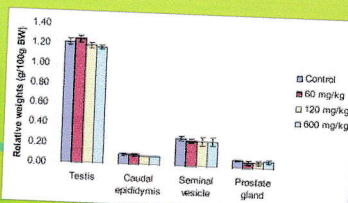


Figure 2. Relative reproductive organ weights (g/100 g body weight) of male rats treated with *B. rotunda* juice for 30 days (one-way ANOVA; there were no significant differences). The data were expressed as mean \pm SD.

Toxicological study

The results showed no significant difference between body weight of the male rats receiving *B. rotunda* juice at doses of 60, 120, and 600 mg/kg BW and the controls (Fig 4). All groups had a significant increase in body weight.

Discussion

The present study, *B. rotundifolia* juice could not promote the androgenic and estradiol hormones, reproductive organs weight and epididymal sperm density. However, the previous research demonstrated that *B. rotundifolia* juice at the dose of 0.5 g/kg bw affected the rats in mount frequency by consistent behavior throughout the 3-hour 10-min periods (12). It was assumed to maintain the time for copulation organ weight in the longer period. Moreover, the ethanolic extract of *B. rotundifolia* caused an increase in the sexual organ weights and semimembranous tubular diameter (5), and secretory granules (Sertoli cell) (13).

Our results showed no significant differences between body weight of the male rats. All groups had a significant increase in body weight. There were no histopathological changes of liver and kidney or no significant difference of hematocrit, WBC or differential cell counts in all rats as the works on premature and mature rats (12, 14). All values were at a normal level (15). Thus, continuous high concentration of *B. rotundifolia* juice could not harm premature male rats, which could support the use of *B. rotundifolia* as a traditional medicine for primary health care without toxicity (2, 12, 13, 14).

Conclusion: *B. rotunda* juice did not modify the testosterone, ADD and estradiol levels, the sexual organ weights, or the epididymal sperm density during the 30 days of treatment, and high concentration of juice could not harm premature male rats.

Acknowledgements

This work was supported by The Thailand Research Fund (TRF; MRG5180115) and Faculty of Medicine, Chiang Mai University, Thailand. Special thanks to the Animal Facility Unit, Department of Biology, Faculty of Science and Reproductive Medicine Laboratory, Department of Obstetrics and Gynecology, Faculty of Medicine, Chiang Mai University for providing necessary research facilities, Mr Apirij Jitjanom for their practical assistance.

References

- [illegible]

ภาคผนวก 3



Joint International Tropical Medicine
Meeting 2008

"Tropical Medicine in the -omics Era"

Abstracts

13 - 14 October 2008

Imperial Queen's Park Hotel, Bangkok, Thailand

JITMM 2008

EVALUATION OF EPIDIDYMAL SPERM MORPHOLOGY AND MOTILITY IN PRE-PUBERTY MALE RATS GIVEN *BOESENBERGIA ROTUNDA* JUICE

Sudawadee Phante¹, Paiwan Sudwan¹,
Kanokporn Saenphet²

¹ Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand

² Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

Boesenbergia rotunda (L.) Mansf. is the medicinal plant widely used for sexual potential promotion. The purpose of this study was to test the ability of this plant on pre-puberty male rat's reproduction by evaluating morphology and motility of their sperm. Oral administration of *B. rotunda* root juice at the doses of 60, 120 and 600 mg/kg. bw was conducted in pre-puberty male rats for 30 days. The control group only received distilled water at 1 ml/day. After treatment period, the caudal epididymis was dissected and the fresh epididymal sperm samples were prepared to assess sperm morphology and motility parameters. It was found that neither the morphology nor the motility of sperm in all treated groups was different from those of control group. Therefore, the treatment did not affect spermatogenesis and morphology of caudal epididymis. It could be concluded that *B. rotunda* juice at all doses used in this study did not promote rat's sperm quality. ♦

Keywords: *Boesenbergia rotunda*, pre-puberty rat, sperm, morphology, motility

SEXUAL BEHAVIOR IN PREMATURE MALE RATS TREATED WITH *BOESENBERGIA ROTUNDA* (L.) MANSF JUICE

Paiwan Sudwan¹, Kanokporn Saenphet²,
Songkiet Suwansirikul¹

¹ Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand

² Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

The present study was designed to investigate whether the *Boesenbergia rotunda* (Krachai), a medicinal plant recommended for sexual enhancing property, could promote the sexual behavior of premature male rats or not. Safety evaluation of this plant was also conducted with the emphasis of the histopathology of liver and kidney. Thirty-two 4-week-old male rats were equally divided into four groups. Three experimental groups were orally administered with *B. rotunda* rhizome juice at the doses of 60, 120 and 600 mg/kg bw for 30 days, whereas controls received distilled water 1 ml/day. The results showed that only two treated rats (1 from 60 and 1 from 600 mg/kg bw) presented their intromission behavior. In the whole 30 min period of observation, all sexual parameters (courtship behavior; mount frequency, MF; mount latency, ML; intromission frequency, IF; and intromission latency, IL) of treated rats were not significantly different from those of controls. However, when the observation was conducted at three 10-min intervals over a 30 min period, the MF of rats treated with 600 mg/kg bw of the juice was found consistent throughout the three 10-min periods, while that of control and other treated group was higher in the 1st 10-min period than in the 2nd and 3rd 10-min periods. This consistent frequency of mounting may reflect the better copulating potency of rats. The histopathological changes of liver and kidney were not observed in treated rats. These results indicated that the high concentration of *B. rotunda* juice could be safely consumed.

Keywords: *Boesenbergia rotunda*, sexual behavior, premature rat, histopathology, medicinal plant

² Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand.

The present study was designed to investigate whether the *Boswellia* resin (Koshiwa), a medicinal plant recommended for sexual enhancing property, could promote the sexual behavior of premature male rats or not. Safety evaluation of this plant was also conducted with the emphasis of the histopathology of liver and kidney. Thirty-two 4-week old male rats were equally divided into four groups. Three experimental groups were orally administered with *B. retunda* rhizome juice at the doses of 60, 120 and 600 mg/kg bw for 36 days, whereas controls received distilled water 1 mL/day. At the end of the study, only treated rats were sacrificed and from 600 mg/kg bw presented their behavior, body weight, food intake, water intake, MF, MI, IF, and IIF. The histopathology of liver and kidney behavior, mouth frequency, MF; mouth Intake, MI; Intermittence frequency, IF; and Intermittence Intake, IIF of treated rats were not significantly different from those of controls. However, when the observation was conducted at three 10-min intervals over a 30-min period, the MF of rats treated with 600 mg/kg bw of the juice was found consistent throughout the three 10-min periods, while that of control and other treated group was highest in the first 10-min period. This consistent frequency of mounting may reflect the sexual arousing potency of this resin. The histopathological changes of liver and kidney were not observed in treated rats. These results indicated that the high concentration of *B. retunda* juice could be safely consumed.

Boswellia serrata (L.) Miq. (Kanchai), a medicinal plant recommended for sexual enhancing property. It is belonging to the Zingiberaceae family (Chondrochul et al., 2006; Hongsong, 1998). It is sometimes referred to as "Thai ginseng" and has been long used among Thai men (Chaopha-abaihuabhuang hospital, 2005; Wutyantamwong, 2000). Its rhizomes have been used as an antitumor, for stomach discomfort, leukorrhea, diuretic and antidysenteric, treatment of oral diseases (Chondrochul et al., 2006). We previously reported that the ethanolic extract from *B. serrata* in the doses of 60, 120 and 240 mg/kg for 60 days significantly increased the relative testicular weight and the diameter of the seminiferous tubules in the castrated rats (Wachot et al., 2001; Wachot et al., 2004; Wachot et al., 2005) (Sudwan et al., 2007). Moreover, the electron microscopy showed this plant extract increased granules of Sertoli cells meanwhile they presented the normal morphology of spermatozoa (Sudwan & Saengphet, 2007). Because, the ethanolic extracts from *B. serrata* tended to positive effects on reproductive male rats and the safety used was reported (Saengphet et al., 2007). The plant may have an opportunity to make itself fully applied in various doses and times (Wachot et al., 2001; Wachot et al., 2004), in various solvent extraction or method of extraction (Ang and Ngai, 2001), or in condition of male rats (Ang and Ngai, 2001; Chuttham et al., 2002; Carro-Juarez et al., 2004). It is very remarkable to continuous study on safety and efficacy of *B. serrata* in various doses and times.

The aim of this research was to investigate the effects of the *B. serrata* juice on sexual activity and safety evaluations with the emphasis of the histopathology of liver and kidney of premature male rats.

Preparation of *B. rotunda* Juice.

The fresh *B. rotunda* rhizomes were collected from Chiang Mai province

Animals and housing

The experiments were carried out with 32 premature male and 20 female Wistar rats (4 weeks old) body weight approximately 80–120 and 75–110 grams, respectively. They were housed in groups of three per cage under a standard environmental condition. All experiments in this study were approved for ethical conduct on the Animal Care and Use (No. 11/2551), Faculty of Medicine, Chiang Mai University, Thailand.

Male rats were dia-

Male rats were divided into four groups of 8 rats each. Group I was a control group and received only distilled water 1 ml/day. Group II, III and IV were administered orally with 60, 120 and 600 mg/kg bw of the *B. rotunda* juice each day for 30 days, respectively. The procedure of sexual behavior testing in each of male rats between day 26-30 of the *B. rotunda* juice application was described previously (Sudwan *et al.*, 2006; Sudwan *et al.*, 2007).

Histopathological Study

The liver and kidney of each animal were studied. The changes of histological structures were investigated by medical pathologist under a light microscope.

Data were expressed as mean and standard deviation (SD), median and interquartile range. The statistical analysis was carried out using SPSS. Significance was considered to be significant at $P < 0.05$.

Present in Table 1-4

Table 1. Percentages of sexual behavior of male rats treated with *B. rattus* juice (60, 120 and 600 mg/kg) and control groups between day 25-30. AI = mount frequency, IF = intromission frequency

Parameter	Groups			
	Control (n=8)	60 mg/kg (n=8)	120 mg/kg (n=8)	600 mg/kg (n=8)
Counting (%)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)
(median; range)	(0.75-8.0)	(0.75-8.0)	(0.02-8.0)	(0.05-8.0)
MP (%)	423.50-724.50	449.50-729.25	584.50-761.00	430.25-742.25
(median; range)	100 (0.0)	100 (0.0)	100 (0.0)	100 (0.0)
IF (%)	12 (0.0)	11 (0.0)	12 (0.0)	11 (0.0)
(median; range)	5.80 (35-80)	3.90 (23-63)	0.75 (16-23)	6.00 (35-100)
IF (%)	0 (0.0)	22 (0.0)	0 (0.0)	12 (0.1)
(median; range)	0 (0-1)	0 (0-1)	0 (0-1)	0 (0-6)
		0 (0.0)		0 (0.0)

There were no significant differences.
Data expressed as median and interquartile range.

Table 2 Sexual behavior parameters of male rats treated with *B. rotundifolia* juice (60, 120 and 600 mg/kg) and control groups. AM = mating frequency; IF = intruders/mating frequency; AI = Mating latency; ANOVA = F(3,15)

Parameter	Groups (n=6 each)			
	Control	60 mg/kg	150 mg/kg	600 mg/kg
Cholesterol	600.85±154.59	611.12±164.50	699.85±144.65	637.00±157.57
MF	15.62±13.52	15.83±13.82	14.42±16.09	14.08±12.33
IF	0.00±0.00	0.30±1.06	0.00±0.00	0.50±1.41
ML	280.25±525.76	75.83±102.59	17.25±15.02	29.68±22.21
Cumulative efficiency	0.00±0.00	0.31±0.62	0.00±0.00	0.61±0.84

There were no significant differences, one-way ANOVA. Data expressed as Mean (s.d.) standard deviation (s.d.).

Table 3. Times of courtship behavior (seconds) of male rats receiving *B. rotundifolia* juice at different doses for 26–30 days, in three 10 minute observations over a 30 minute period: 1st 10 min = 1st 10 minutes observation, 2nd 10 min = 2nd 10 minutes observation, 3rd 10 min = 3rd 10 minutes observation.

Groups (n=8 each)	Day 26-30		
	1 st 10 min	2 nd 10 min	3 rd 10 min
Control	313.12±72.13 ^a	160.88±70.74 ^a	128.50±33.16 ^a
60 mg/kg	318.84±61.86 ^a	162.12±63.33 ^a	126.12±62.67 ^a
120 mg/kg	359.38±113.80 ^a	163.58±55.04 ^a	147.25±55.88 ^a
600 mg/kg	315.62±43.78 ^a	168.50±75.43 ^a	142.88±60.14 ^a

a,b The mean differences are significant at the 0.05 level; Two-way ANOVA followed by one-way ANOVA and LSD. In general, the doses of *B. reusenda* had no significant differences, while three 10 minute intervals of *B. reusenda* treatment had significant effects.

Table 4. MIF (numbers) of male rats receiving *B. rotundifolia* juice at different doses for 26-30 days, in three 10 minute observations over a 30 minute period, 1st 10 min = 1st 10 minutes observation, 2nd 10 min = 2nd 10 minutes observation, 3rd 10 min = 3rd 10 minutes observation.

Groups (n=6 each)	Day 26-30		
	1 st 10 min	2 nd 10 min	3 rd 10 min
Control	10.88±11.00 ^{ab}	2.00±1.85 ^c	2.75±2.19 ^{cd}
60 mg/kg	9.50±7.50 ^{ac}	2.12±2.10 ^{cd}	3.58±4.38 ^{cd}
120 mg/kg	9.75±4.12 ^b	1.50±1.31 ^c	3.12±4.55 ^{cd}
600 mg/kg	7.25±5.70 ^{cd}	3.62±4.63 ^{ab}	3.50±4.50 ^{cd}

available. The mean differences are significant at the 0.05 level. Two-way ANOVA followed by one-way ANOVA (LSD). In general, the doses of *B. revivida* had no significant differences, while three 10 minute intervals of *B. revivida* treatment had significant effects.

The histopathological changes of liver and kidney revealed no association with consuming *B. rotundifolia* juice for 30 days.

Results of the experiments indicate that all doses of *B. rotunda* juice produced no significant changes in the courtship behavior, MF, IF, MI, and IL in the whole 30 min period as in mature male rats treated with *B. rotunda* extract (Sudwan et al. 2007) and *K. parviflora* extract (Sudwan et al. 2006). Here, *B. rotunda* juice at the dose of 600 mg/kg bw affected the rats in MF by consistent behavior throughout the three 10-min periods, while that of control and other treated group was highest in the 1st 10-min period, is a sign of sexual potentment of male, reflecting its ability to copulate the female (Pfaus et al. 2001). Because mount is one type of stereotyped copulatory response, it can be assumed that *B. rotunda* juice is necessary to maintain the time for copulation quality in the longer period.

As the recent works in mature rat (Saenphet *et al.* 2007), this present study found that there were no histopathological changes of liver and kidney in treated animals. So, the safety consuming and continuing high concentration of *B. rotunda* juice although the animals are in premature age and supported this plant were used as a medicinal plant for primary health care without toxicity (Hemhongs, 1998; Chomchalow *et al.* 2006; Saenphet *et al.* 2007).

It was concluded that *B. rotunda* juice at 600 mg/kg bw consistent MF of rat throughout the three 10-min periods, and the high concentration of *B. rotunda* juice could be safely consumed.

This work was supported by The Thailand Research Fund (TRF: MRG5180115) and Faculty of Medicine, Chiang Mai University, Thailand. Special thanks to the Animal Facility Unit, Department of Biology, Faculty of Science, Chiang Mai University for providing necessary research facilities, Mr Apirrit Jitjirngam, Ms. Sudawadee Phante and Ms. Lanniporn Lueinla for their practical assistance.

[illegible]

ภาคผนวก 4

บทความ การเสนอผลงานแบบโปสเตอร์

การประชุมนักวิจัยรุ่นใหม่ พว เมธีวิจัยอาวุโส สกว. ครั้งที่ 9

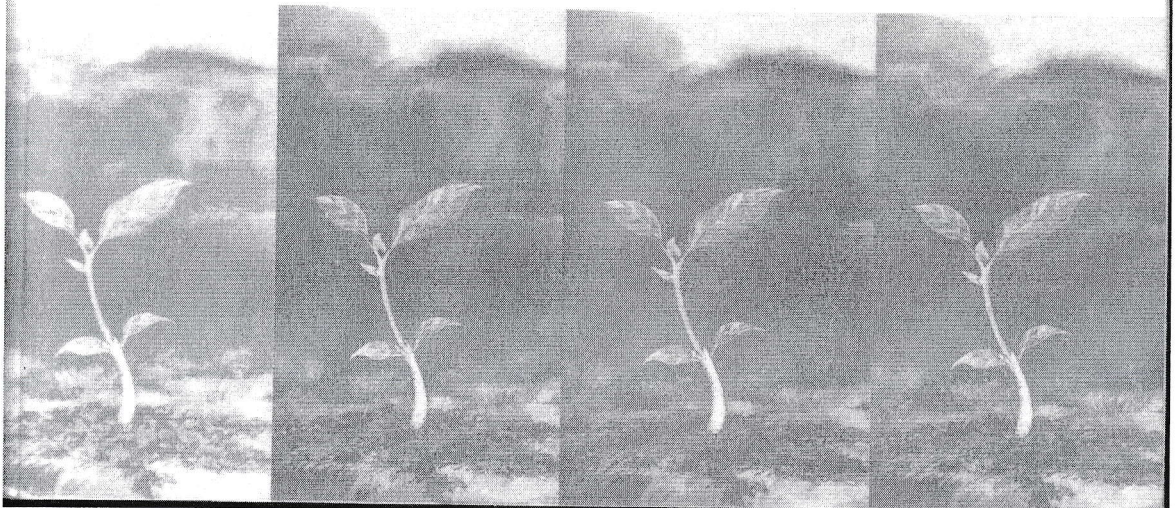
วันที่ 15-17 ตุลาคม 2552

โรงแรมฮอติเดย์อินน์ รีสอร์ท รีเจนท์ บีช ชะอำ
จังหวัดเพชรบุรี

สำนักงานกองทุนสนับสนุนการวิจัย (สกว.)



สำนักงานคณะกรรมการการอุดมศึกษา (สกอ.)



Sexual Activity in Premature Male Rats Treated with *Boesenbergia Rotunda* (L.) MANSF. Juice

Sudwan, P.^{1*}, Saenphet, K.², Suwansirikul, S.¹

¹Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand

²Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand

BIO : Biological Sciences

Abstract

The present study was designed to investigate whether the *Boesenbergia rotunda* medicinal plant recommended for sexual enhancing property, could promote the sexual behavior of premature male rats. Safety evaluation of this plant was also conducted with emphasis on the histopathology of liver and kidney. Thirty-two 4-week-old male rats were equally divided into four groups. Three experimental groups were orally administered *rotunda* rhizome juice at the doses of 60, 120 and 600 mg/kg bw for 30 days, whereas controls received distilled water 1 ml/day. The results showed that only two treated rats (1 from 600 mg/kg bw) presented their intromission behavior. In the whole 30 min period of observation, all sexual parameters (courtship behavior; mount frequency, MF; mount latency, ML; intromission frequency, IF; and intromission latency, IL) of treated rats were significantly different from those of controls. However, when the observation was conducted in three 10-min intervals over a 30 min period, the MF of rats treated with 600 mg/kg bw juice was found consistent throughout the three 10-min periods, while that of control and other treated group was highest in the 1st 10-min observation. This consistent frequency of intromission may reflect the better copulating potency of rats. The histopathological changes of liver and kidney were not observed in treated rats. These results indicated that the high concentration of *B. rotunda* juice could be safely consumed.

Keywords: *Boesenbergia rotunda*, sexual behavior, premature rat, histopathology, medicinal plant

*Corresponding author.

Tel.: 0-5394-5312 ext. 208, 08-1366-1526; Fax: 0-5394-5304

E-mail: pasudwan@med.cmu.ac.th

บทคัดย่อ

การเสนอผลงานแบบโปสเตอร์

ทุนพัฒนาศักยภาพในการทำงานวิจัย

ของอาจารย์รุ่นใหม่ และทุนส่งเสริมนักวิจัยรุ่นใหม่

การประชุมนักวิจัยรุ่นใหม่ WU เมาท์วิชัยอาวุโส สกว.
ครั้งที่ 10

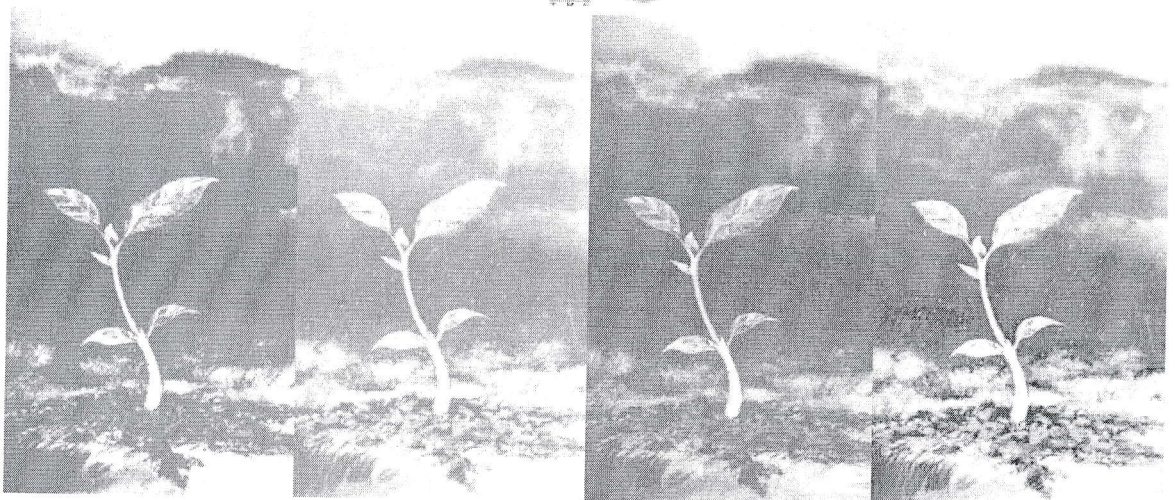
วันที่ 14-16 ตุลาคม 2553

โรงแรมฮอเลียดอินน์ รีสอร์ท รีเจนท์ บีช ชะอำ
จังหวัดเพชรบุรี

สำนักงานกองทุนสนับสนุนการวิจัย (สกว.)



สำนักงานคณะกรรมการการอุดมศึกษา (สกอ.)



***Boesenbergia rotunda* (L.) Mansf. Juice did not Affect Androgenic and Estradiol Levels in Premature Male Rats**Sudwan, P.^{1*}, Saenphet, K.², Saenphet, S.²¹*Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand*
²*Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand***Abstract**

Aim: To determine if Krachai, *Boesenbergia rotunda* (L.) Mansf., juice could promote an increase of the androgenic and estradiol hormones, reproductive organ weights and epididymal sperm density, and at same time, produce toxicity in premature male rats. **Methods:** Thirty-two premature male Wistar rats (4 weeks old) were equally divided into a control and three *B. rotunda* juice-treated groups. *B. rotunda* juice was orally administered at the doses of 60, 120 and 600 mg kg⁻¹ body weight, to treated groups of male rats ($n = 8$), daily for 30 days and the controls received a similar amount of distilled water. After the treatment periods, all animals were anesthetized on day 31. Their blood was collected for hematological analysis. Serum was prepared for the electrochemiluminescence immunoassay (ECLIA) to determine testosterone and estradiol levels and the radioimmunoassay (RIA) to determine androstenedione (ADD) levels. The reproductive organs were dissected and weighed and the epididymal sperm density was evaluated. **Results:** There were no significant differences in serum testosterone, androstenedione and estradiol levels, the relative weight of the reproductive organs (testis, caudal epididymis, seminal vesicle and prostate gland) and sperm density between the control and treated groups. Toxicological study revealed no significant difference of hematocrit, WBC or differential cell count. **Conclusion:** *B. rotunda* juice did not modify the testosterone, ADD and estradiol levels, the sexual organ weights, or the epididymal sperm density during the 30 days of treatment, and high concentration of juice could not harm premature male rats.

Keywords: *Boesenbergia rotunda*, androgenic hormones, estradiol, premature rat, toxicity

Outputs

1. Sudwan P, Saenphet K, Saenphet S. *Boesenbergia rotunda* (L.) Mansf. juice did not affect androgenic and estradiol levels in premature male rats. Asian J Androl 2009; 11(5 Suppl 1): 164-65.

*Corresponding author.

Tel.: 08-1366-1526, 0-5394-5312 ext. 208; Fax: 0-5394-5304

E-mail: pasudwan@med.cmu.ac.th



Sexual Activity in Premature Male Rats Treated with *Boesenbergia Rotunda* (L.) MANSF. Juice

Paiwan Sudwan^{1*}, Kanokporn Saenphet², Songkiet Suwansirikul¹

¹ Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand.

² Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand.

*Correspondence: Paiwan Sudwan, Department of Anatomy, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand. Tel 66-53-94-5312 ext 208, 66-81-366-1526 Fax 66-53-945304 E-mail: pasudwan@med.cmu.ac.th

Aim

The present study was designed to investigate whether the *Boesenbergia rotunda* (Krachai), a medicinal plant recommended for sexual enhancing property, could promote the sexual behavior of premature male rats. Safety evaluation of this plant was also conducted with the emphasis on the histopathology of liver and kidney.

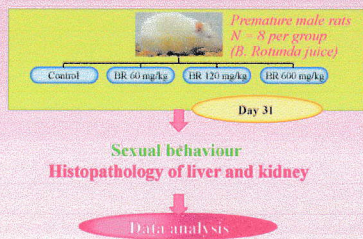
Keyword: *Boesenbergia rotunda*, sexual behavior, premature rat, histopathology, medicinal plant

Introduction

Boesenbergia rotunda (L.) Mansf. (Krachai) is a medicinal plant recommended for sexual enhancing property and belongs to the Zingiberaceae family (1, 2). It is sometimes referred to as "Thai ginseng" and has long been used among Thai men (3, 4). Its rhizomes have been used as an antitumor, for stomach discomfort, leukorrhea, diuretic and antisyndromic and treatment of oral diseases (2). It was previously reported that the ethanol extract from *B. rotunda* at the doses of 60, 120 and 240 mg/kg for 60 days significantly increased the relative testicular weight and the diameter of the seminiferous tubules and at the dose of 60 mg/kg also significantly increased the relative weight of the seminal vesicle (5). Moreover, the electron micrographs showed this plant extract increased granules of Sertoli cells while they presented the normal morphology of spermatozoa (6). Ethanol extract from *B. rotunda* tended to have positive effects on reproductive male rats and were evaluated to be safe (7). This study will make the plant fully apparent in various doses and times (8, 9) in various solvent extraction or method of extraction (10), or in condition of male rats (10-12). It is recommended to continue study on the effects of the *B. rotunda* on sexual activity.

The aim of this research was to investigate the effects of the *B. rotunda* juice on sexual activity of premature male rats with safety evaluations regarding the histopathology of liver and kidney.

Material and methods



Results

The results showed that only two treated rats (1 from 60 and 1 from 600 mg/kg bw) presented their intromission behavior (Table 1).

Table 1. Percentages of sexual behavior of male rats treated with *B. rotunda* juice (60, 120 and 600 mg/kg) and control groups between day 26-30. MF = mount frequency, IF = intromission frequency.

Parameter	Control (n=8)	60 mg/kg (n=8)	120 mg/kg (n=8)	600 mg/kg (n=8)
Courtship (%)	100 (12.5)	100 (12.5)	100 (12.5)	100 (12.5)
Intromission	425 (53.125)	405 (50.625)	565 (70.625)	490 (61.25)
MF (%)	100 (12.5)	100 (12.5)	100 (12.5)	100 (12.5)
Intromission	425 (53.125)	405 (50.625)	565 (70.625)	490 (61.25)
IF (%)	40 (5)	12 (1.5)	40 (5)	12 (1.5)
Intromission	425 (53.125)	405 (50.625)	565 (70.625)	490 (61.25)

There were no significant differences. Data expressed as median and interquartile range.

In the whole 30 min period of observation, all sexual parameters (courtship behavior; mount frequency, MF; intromission latency, IL; intromission frequency, IF, and intromission latency, IL) of treated rats were not significantly different from those of controls (Table 2).

Table 2. Sexual behavior parameters of male rats treated with *B. rotunda* juice (60, 120 and 600 mg/kg) and control groups. MF = mount frequency, IF = intromission frequency, ML = Mount latency.

Parameter	Control (Mean (SD))	60 mg/kg	120 mg/kg	600 mg/kg
Courtship	400 (61.54)	441 (12.64)	400 (61.54)	427 (61.57)
MF	15.6 (2.12)	15.0 (1.82)	14.6 (2.09)	14.0 (2.23)
IF	0.20 (0.08)	0.26 (0.10)	0.00 (0.00)	0.30 (0.41)
ML	20.2 (2.02)	17.8 (1.02)	17.2 (1.14)	20.0 (2.21)
Intromission	4.20 (0.08)	5.01 (0.42)	4.00 (0.00)	4.01 (0.01)

There were no significant difference one-way ANOVA. Data expressed as mean and standard deviation (SD).

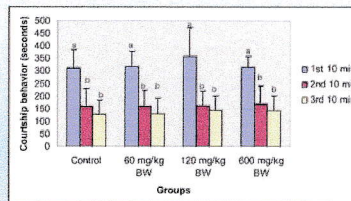


Figure 1. Times of courtship behavior (seconds) of male rats receiving *B. rotunda* juice at different doses for 26-30 days, in three 10-min observations over a 30 min period. 1st 10 min = 1st 10 min observation, 2nd 10 min = 2nd 10 min observation, 3rd 10 min = 3rd 10 min observation.

ab. The mean differences are significant at the 0.05 level. Two-way ANOVA followed by one-way ANOVA and LSD.

In general, the doses of *B. rotunda* juice had no significant differences, while three 10-min intervals of *B. rotunda* treatment had significant effects.

However, when the observation was conducted at three 10-min intervals over a 30 min period, the MF of rats treated with 600 mg/kg bw of the juice was found consistent throughout the three 10-min periods, while that of control and other treated group was highest in the first 10-min observation (Figure 2).

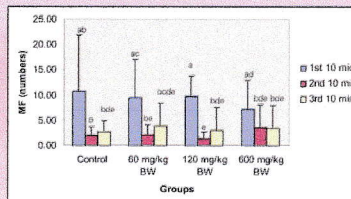


Figure 2. MF (numbers) of male rats receiving *B. rotunda* juice at different doses for 26-30 days, in three 10 minute observations over a 30 min period. 1st 10 min = 1st 10 min observation, 2nd 10 min = 2nd 10 min observation, 3rd 10 min = 3rd 10 min observation.

ab.cde. The mean differences are significant at the 0.05 level. Two-way ANOVA followed by one-way ANOVA and LSD.

In general, the doses of *B. rotunda* juice had no significant differences, while three 10-min intervals of *B. rotunda* treatment had significant effects.

Additional result, there were no the histopathological changes of liver and kidney in male rats with consuming *B. rotunda* juice for 30 days.

Discussion

Results of the experiments indicate that all doses of *B. rotunda* juice produced no significant changes in the courtship behavior, MF, IF, ML, and IL in the whole 30 min period of observation between treated and control groups. We found similar patterns of courtship behavior in three 10-min intervals over a 30 min period of all groups as in mature male rats treated with *K. parviflora* (Sudwan et al. 2006) and *B. rotunda* extracts (Sudwan et al. 2007). Here, *B. rotunda* juice at the dose of 600 mg/kg bw affected the rats in MF by consistent behavior throughout the three 10-min periods, while that of control and other treated group was highest in the 1st 10-min observation, being a sign of sexual potential of male, reflecting its ability to copulate the female. Because mount is one type of stereotyped copulatory responses (15), it can be assumed that *B. rotunda* juice is necessary to maintain the time for copulation quality in the longer period. Also in recent works on mature rats (7), the histopathological changes of liver and kidney in treated groups were not observed. Continuous high concentration of *B. rotunda* juice were safely consumed, even though the animals were of premature age. This supported the assumption that this plant can be used as a medicine for primary health care without toxicity (1, 2, 7). Further studies are necessary to demonstrate the effects of this plant on reproductive organs, histology evaluation of sex glands or sex hormones.

In conclusion, *B. rotunda* juice at 600 mg/kg bw consistent MF of rat throughout the three 10-min periods, and the high concentration of *B. rotunda* juice could be safely consumed.

Acknowledgements

This work was supported by The Thailand Research Fund (TRF; MRG5180115) and Faculty of Medicine, Chiang Mai University, Thailand. Special thanks to the Animal Facility Unit, Department of Biology, Faculty of Science, Chiang Mai University for providing necessary research facilities, Mr Apirrit Jitjirangam, Ms. Sudavadee Phant and Ms. Laniaporn Lucinta for their practical assistance.

References

- Henshaw P. Thai Herbal Health. 1998 (in Thai).
- Chenakul N, Rattakul S, Chantana V. Using Thai Medicinal Plant. 2006.
- Wattayachew W. Encyclopedia of Thai Herbs. 2000 (in Thai).
- Chongkiet-achakulchai Hospital. *Boesenbergia rotunda* (Krachai) Herbal Tea. 2008.
- Sudwan P, Saenphet K, Saenphet S, Suwansirikul S. Effects of *Boesenbergia rotunda* (L.) Mansf. on sexual behavior of male rats. 2007.
- Sudwan P, Saenphet K. Effects of *Boesenbergia rotunda* (L.) Mansf. on Uterine weight of Sertoli cells and Spermatozoa in Male Albino Rats. 2007.
- Saenphet K, Sudwan P, Saenphet K, Wongsakulpanich P, Thongkiet K. Safety evaluation of ethanol extract from *Boesenbergia rotunda* (L.) root on male rats. 2007.
- Wachon P, Kanchanachai P, Kanchanachai P, et al. Reversible antiproliferative and antifertility activities of *Boesenbergia rotunda* in male albino rats. 2001.
- Wachon P, Kanchanachai P, Kanchanachai P, et al. Antiproliferative effect of *Boesenbergia rotunda* in male rats. 2004.
- Ang H, Ng H, Ng H. Aphrodisiac effect of *Boesenbergia rotunda* in male rats. 2004.
- Chongkiet-achakulchai Hospital. *Boesenbergia rotunda* (Krachai) Herbal Tea. 2008.
- Chongkiet-achakulchai Hospital. *Boesenbergia rotunda* (Krachai) Herbal Tea. 2008.
- Chongkiet-achakulchai Hospital. *Boesenbergia rotunda* (Krachai) Herbal Tea. 2008.
- Chongkiet-achakulchai Hospital. *Boesenbergia rotunda* (Krachai) Herbal Tea. 2008.
- Chongkiet-achakulchai Hospital. *Boesenbergia rotunda* (Krachai) Herbal Tea. 2008.

Outputs

Sudwan P, Saenphet K, Suwansirikul S. Sexual Activity in Premature Male Rats Treated with *Boesenbergia rotunda* (L.) Mansf. Juice. Poster presentation at the Joint International Tropical Medicine Meeting 2008, Bangkok, Thailand, 13-14 October, 2008.

