Journal of Medicinal Plants Research Vol. 5(16), pp. 3861-3867, 18 August, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

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

Sudawadee Yotarlai[1], Vipavadee Chaisuksunt[1], Kanokporn Saenphet[2] and Paiwan Sudwan[1]*

[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.

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, Nakhorn 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. 082) 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 micro-scope 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 ± SD).

Group	Number of motile sperm			Number of non-motile sperm
	Progressive	Non-progressive	Circle	
Control	15.50 ± 7.66	33.17 ± 11.99	2.17 ± 2.48	149.17 ± 6.85
B. rotunda juice (60 mg/kg.bw)	20.86 ± 11.72	27.00 ± 4.55	4.14 ± 5.67	148.00 ± 16.31
B. rotunda juice (120 mg/kg.bw)	16.62 ± 11.26	24.12 ± 9.01	4.87 ± 6.60	155.12 ± 22.53
B. rotunda juice (600 mg/kg.bw)	13.75 ± 9.22	25.25 ± 12.74	4.37 ± 5.50	157.00 ± 14.73

There were no significantly 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 ± SD).

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

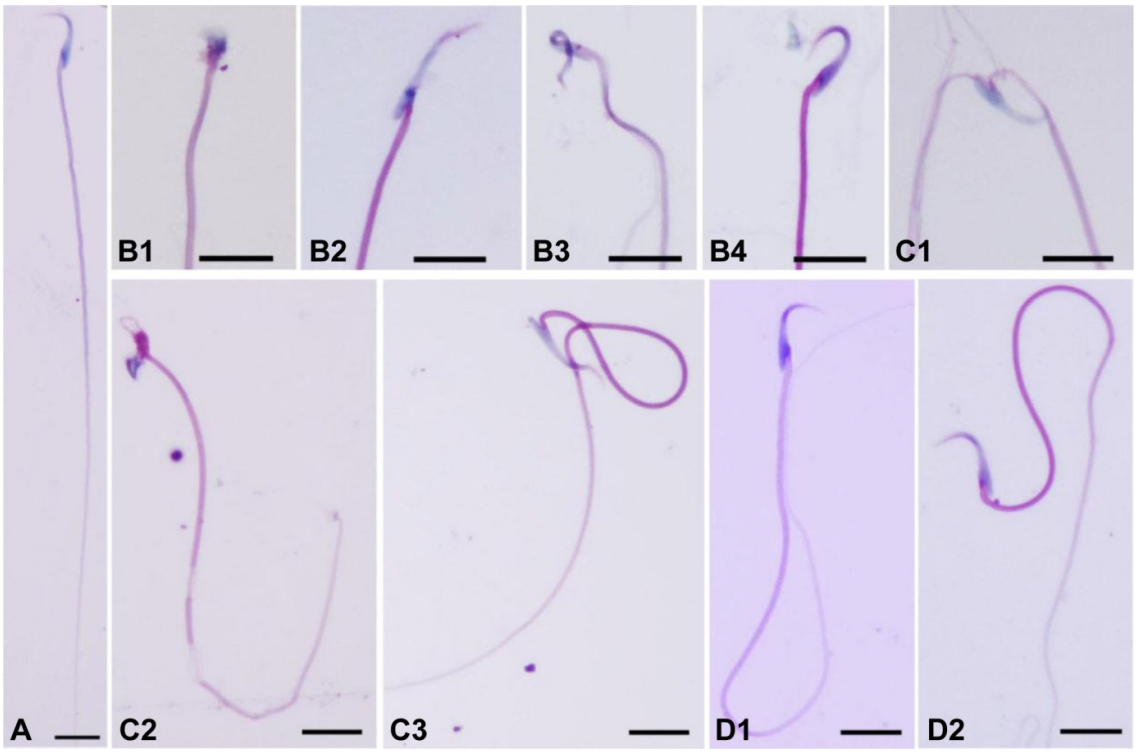

Figure 1. Morphological classification of rat epididymal sperm. Normal sperm (A); sperm with different patterns of abnormal heads (B1-4); sperm with both abnormal head and tail (C1-3); sperm with different patterns of abnormal tails (D1-2). The scale bar shown in the figure represents 10 µm.

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 ± SD).

Group	Number of normal sperm	Number of abnormal sperm		
		Head only	Head and tail	Tail only
Control	76.07 ± 8.97	3.00 ± 1.56	6.79 ± 4.08	113.50 ± 7.28
B. rotunda juice (60 mg/kg.bw)	77.67 ± 10.19	2.83 ± 0.96	5.38 ± 3.77	114.12 ± 7.41
B. rotunda juice (120 mg/kg.bw)	84.00 ± 10.15	3.21 ± 1.77	4.00 ± 2.34	108.79 ± 10.48
B. rotunda juice (600 mg/kg.bw)	76.83 ± 12.74	2.80 ± 0.98	5.29 ± 4.54	115.08 ± 9.43

There were no significantly 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 ± SD).

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

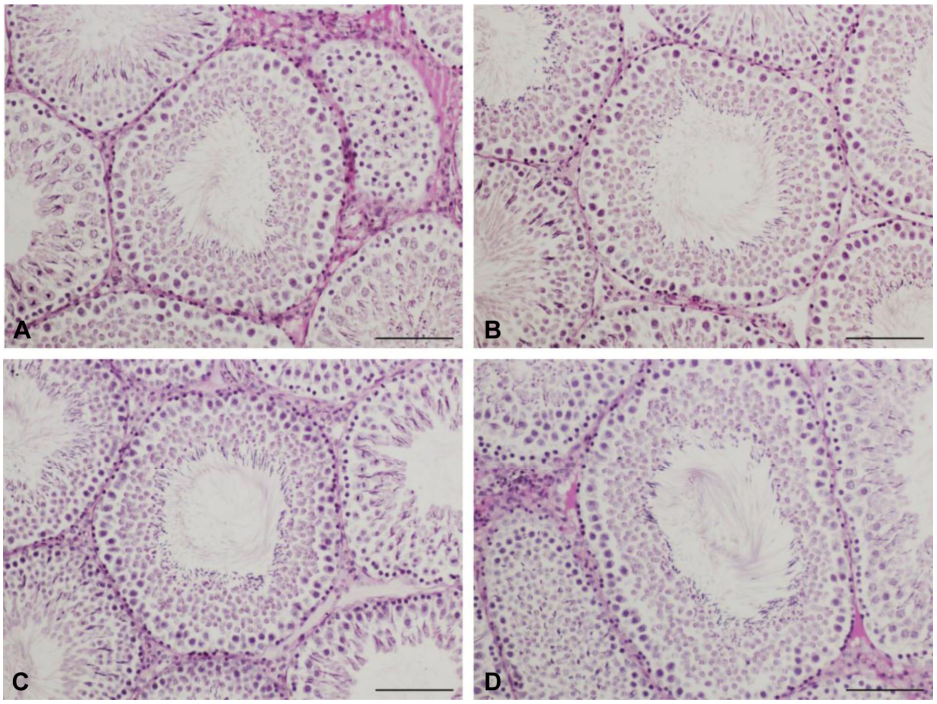

Figure 2. Histological feature of the stage VII to VIII of seminiferous epithelium of the pre-mature rats compared between the control group (A) and *B. rotunda* juice treated groups at 60 mg/kg.bw (B), 120 mg/kg.bw (C), and 600 mg/kg.bw (D). The bar shown in each figure represents 100 µm.

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

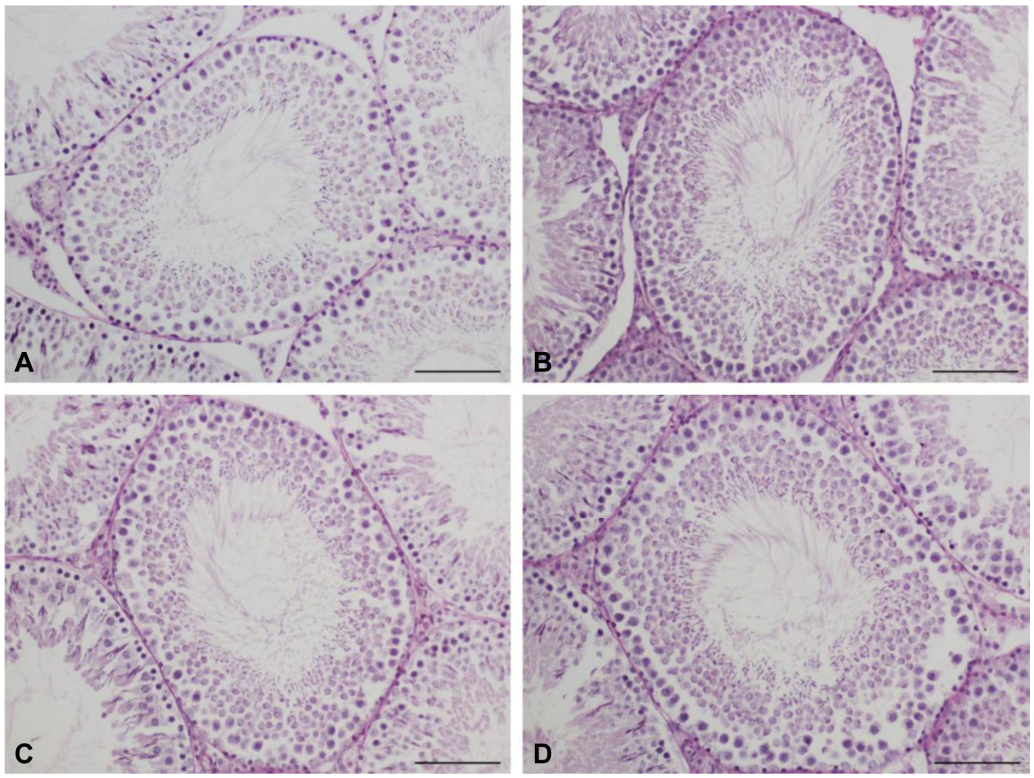

Figure 3. Histological features of the stage VII to VIII of seminiferous epithelium of the mature rats compared between the control group (A) and *B. rotunda* juice treated groups at 60 mg/kg.bw (B), 120 mg/kg.bw (C), and 600 mg/kg.bw (D). The bar shown in each figure represents 100 µm.

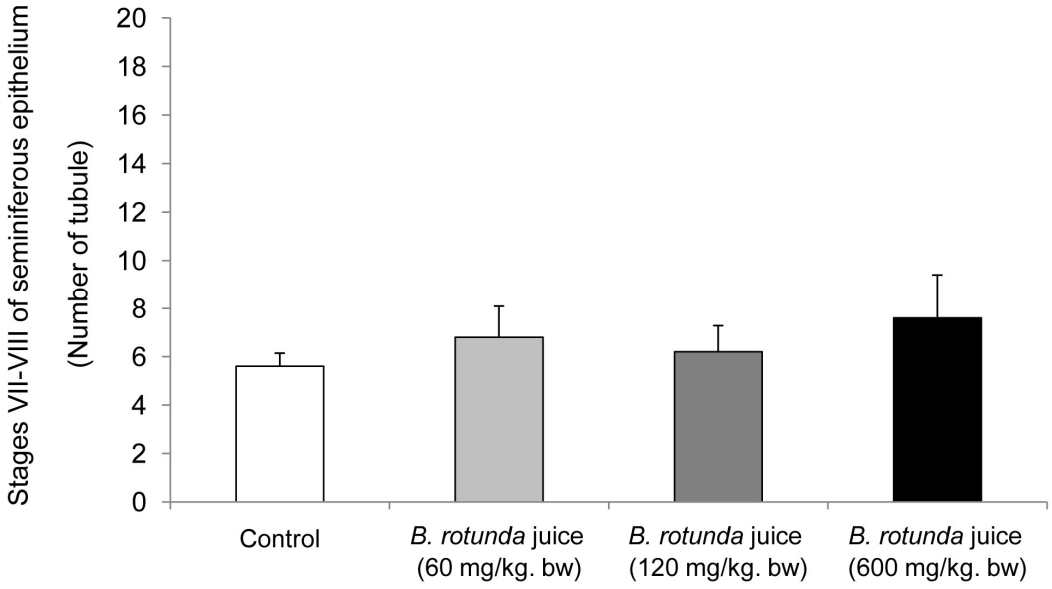

Figure 4. Numbers of stages VII toVIII of seminiferous epithelium of the pre-mature rats administered with varying doses of *B. rotunda* juice for 30 days compared with control (mean ± SD).

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

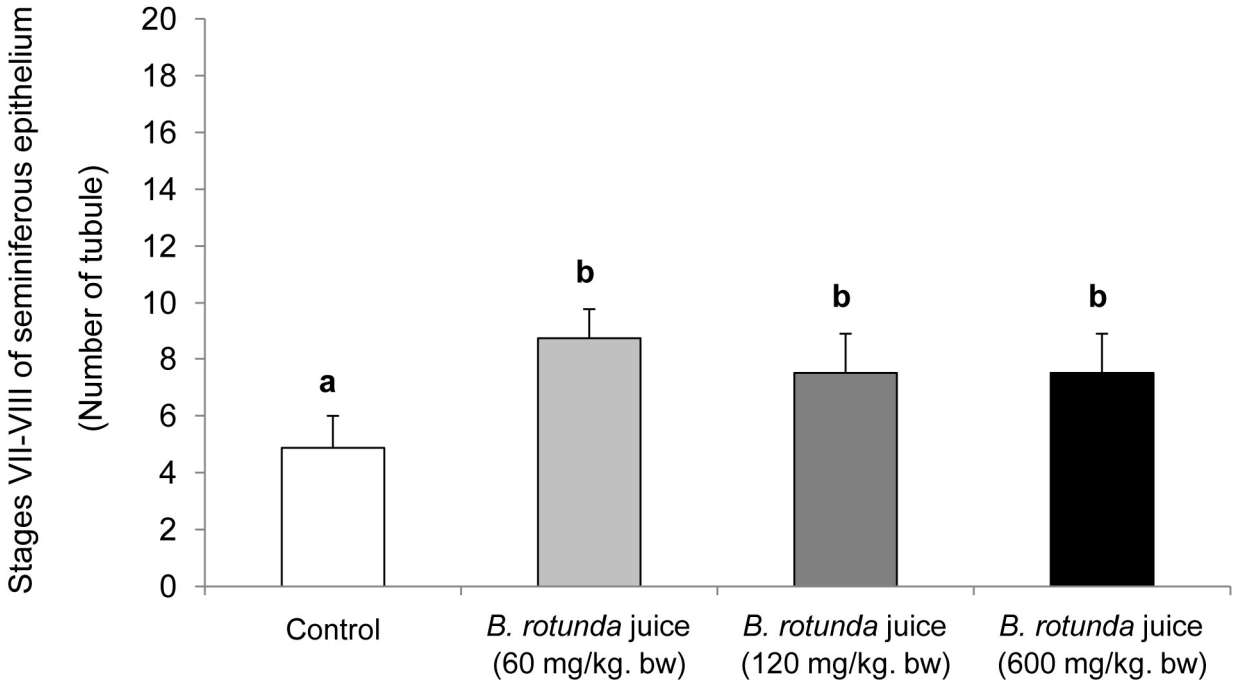

Figure 5. Numbers of stages VII to VIII of seminiferous epithelium of the mature rats administered with varying doses of *B. rotunda* juice for 30 days compared with control (mean ± SD). [a,b] Different letters indicate significant differences between groups. The mean differences are significant at the 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 energenation 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, Hamza AA (2006). Effects of Roselle and Ginger on cisplatin-induced reproductive toxicity in rats. Asian J. Androl., 8(5): 607-612.

Auger J, Kunstmann JM, Czyglik 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, Juthapruth S, Mahachai W, Mahady G (2006). Antibacterial activity of *Boesenbergia rotunda* (L.) Mansf. and *Myristica fragrans* Houtt. against *Helicobacter pylori*. Songklanakarin J. Sci. Technol., 28: 157-163.

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

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

Cederroth CR, Vinciguerra M, Gjinovci A, Kuhne F, Klein M, Cederroth M, Caille D, Suter M, Neumann D, James RW, Doerge DR, Wallimann T, Meda P, Foti M, Rohner-Jeanrenaud 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, Chantrasmi V (2006). Amazing Thai medicinal plants. Royal Flora Ractchphruek 2006, Horticultural Research Institute and Horticultural Science Society of Thailand, Bangkok. Pp. 10–11.

Clarke R, Leonessa 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, Mellstrom 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, 360(9327): 151-159.

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

Hassun FPA, Cedenho 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: 236-242.

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.

Kiat TS, Pippen 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. Bioorg. Med. Chem. Lett., 16(12): 3337-3340.

Park WS, Shin 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, Bundschu K, Liebermann J, Williams JC, Armesilla AL, Emerson M, Oceandy D, Knobeloch KP, Neyses L (2004). Plasma membrane Ca^{2+} 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, Aritajat S, Sitasuwan N (2007). Effects of *Boesenbergia rotunda* (L.) Mansf. 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): 133-138.