THE EFFECTS OF RED KWAO KRUE (*BUTEA SUPERBA* ROXB.) EXTRACT ON SPERM QUALITY AND TESTOSTERONE LEVEL IN MICE

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INTRODUCTION

Butea superba Roxb. has been claimed to use for aphrodisiac[1)]. Male infertility refers to the inability of a male to achieve a pregnancy in a fertile female that low quantity or poor quality of the sperm and lack of testosterone are the most common causes[2)]. Approximately 30% of infertile couples experience infertility due to male factor i.e. reduction in sperm quality and production. Clinical andrologists have therefore tried to improve the fertility potential of the sperm cells to achieve better results in spontaneous fertilization or IVF/ICSI[3)]. Moreover, lower testosterone level may increase risk of Alzheimer's disease in older men[4)]. Previous finding showed that sildenafil significantly increased total testosterone level, sperm motility, sperm concentration, sperm–oocyte binding, resulting in stimulation of spermatogenesis, promotion of the physical and functional maturation of spermatozoa, and maintaining the accessory organs of the male reproductive tract[5-6)]. However, the results of these studies are controversial. Some of these studies demonstrated no significant effects of sildenafil on these parameters[7)]. Therefore, new generation of phytopharmaceuticals that increase spermatogenetic and androgenic effects to treat these diseases instead of synthetic drugs alone are research objectives of far reaching importance. The objective of this study was to investigate the effects of *Butea superba* extract and sildenafil on the sperm quality and testosterone level in male mice.

MATERIALS AND METHODS

Plant materials and preparation of extracts Fresh tuberous roots of *Butea superba* Roxb. were collected from Chiang Rai province, Thailand. The plant specimens was authenticated and compared to herbarium specimen (BCU 11046) at the herbarium of the Department of Botany, Faculty of Science, Chulalongkorn University.

Extraction Dried, powdered roots of *Buteasuperba* Roxb. (25 kg) were extracted continuously with ethanol by Soxhlet extractor, filtered through filter paper, and evaporated to remove the solvent. The ethanol extract (375 g) was separated by silica gel column chromatography and high performance liquid chromatography (HPLC). The spectral analyses, including UV, IR, LC-MS, ^{1}H and ^{13}C NMR were compared with previously reported values and values obtained from standard compounds.

Animals Sixty adult male mice, aged about 130 days, weighing 30-40 g, were obtained from the Animal Care Building, Suranaree University of Technology, Nakhon Ratchasima, Thailand. The experimental protocol was approved in accordance with guideline for the care and use of laboratory animal by animal care and use committee (ACUC). The animals were housed at room temperature (25 ± 0.5 ºC) on a reverse day-night cycle.

Experimental procedures Mice were divided into 6 groups with 10 animals each. Before treatment (pre-treatment), blood and sperm of all mice in these groups were collected for comparison to those of the post-treatment. During treatment period, control (Con), *Butea superba* Roxb. crude extract (Cru), sildenafil (Sil), fraction B (FrB), fraction C (FrC), and fraction E (FrE) mice, were fed with either 0.5 ml of distilled water, 1, 250, 10, 40, 50, and 150 mg/kg BW/day, respectively. The animal's weight was recorded every day throughout 14 consecutive days. At the end of treatment period, all mice were sacrificed and subjected to necropsy. The heart, liver, spleen, kidney, stomach and the reproductive organs (testis, seminal vesicle and prostate glands) were examined. The blood and sperm were collected to compare sperm motility, sperm count, haematology, testosterone level and blood chemistry with pre-treatment in each mouse and between groups.

Statistical analysis All data are presented as the mean ± S.E.M. Significant differences between the relative selected organ weight and body weight of control and treatment groups were analyzed by ANOVA. The difference between pre- and post- treatment groups were calculated by paired student's *t-test*. Then, significant difference between each group was compared using ANCOVA. The Tukey HSD post hoc test at $p<0.05$ and $p<0.01$ were considered statistically significant difference between each group.

RESULTS

The quality and quantity of crude extract and each fraction from Butea superba The major components of each fraction were identified and orally administered to male mice. The 1.5% (w/w) yield from dried powder of Cru at the dose of 1,250 mg /kg BW/day consisted of 53.57 μg of genistein plus 312.95 μg of biochanin A and 396.59 μg of unknown compound 1 (Un1). FrB (0.022% yield) at the dose of 40 mg/Kg BW/day consisted of 1,260.00 μg of unknown compound 1 (Un1). FrC (0.030% w/w yield) at the dose of 50 mg/Kg BW/day consisted of 6.78 μg of genistein plus 31.59 μg of unknown compound 1 (Un1). Also, FrE (0.054% w/w yield) at the dose 150 mg/Kg BW/day consisted of 66.92 μg of biochanin A as the main compound plus 9 unkown compounds.

Sperm motility The effects of BS extracts and sildenafil on sperm motility (%) of mice are presented in Figure 1. The results exhibited that there were significant increases in sperm motility of all post-treated groups compared to pre-treatment ($p<0.01$) except for control. Apart from this, the highest motility level was found in the fractions C and E compared to others including sildenafil ($p<0.01$).

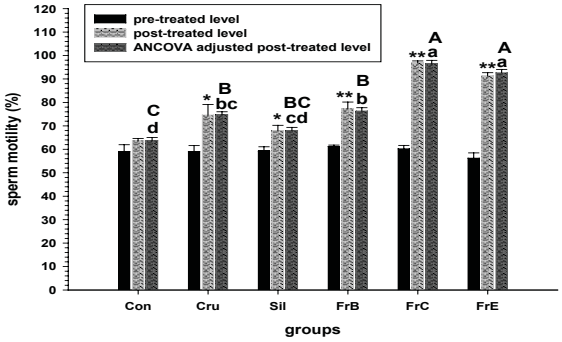

Figure 1 Effects of BS extracts and sildenafil on sperm motility (%) of mice. Significant difference between pre- and post-test in each group was compared using paired student t-test at * p <0.05, ** p <0.01. Significant difference between ANCOVA adjusted post-treated level in each group was compared using ANCOVA and Tukey HSD post hoc test at; a p<0.05, A p<0.01.

Sperm count and morphology Figure 2 shows the effects of BS extracts and sildenafil on sperm number (x100,000 n/ml) of mice. The results exhibited significant increase in sperm number of all post-treated groups compared to pre-treatment ($p<0.01$) except for control. Also, fraction C showed the highest sperm number. Besides, the sperm number of crude extract, sildenafil, fractions B and E treated groups were significantly higher than control group ($p<0.01$). The morphology of these sperms was normal compared with control. In fact, the result of sperm count was almost the same as that of sperm motility.

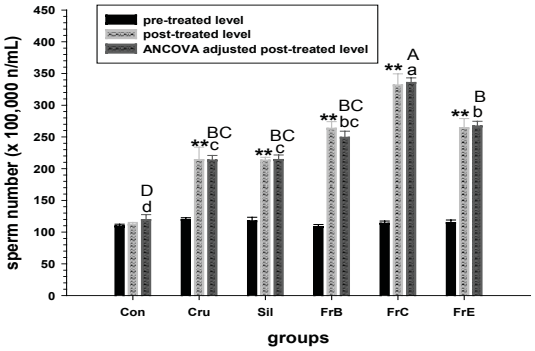

Figure 2 Effects of BS extracts and sildenafil on sperm number (x100,000 n/ml) of mice. Significant difference between pre- and post-test in each group was compared using paired student t-test at * p <0.05, ** p <0.01. Significant difference between ANCOVA adjusted post-treated level in each group was compared using ANCOVA and Tukey HSD post hoc test at; a p<0.05, A p<0.01.

Haematology and blood chemistry The cholesterol level of post-treated groups of fractions C, E, B and sildenafil were significantly higher than the baseline level. The cholesterol level of fractions C, E and sildenafil were significantly higher than those of control ($p<0.05$), whereas the hemoglobin level of sildenafil and crude extract post-treated groups were significantly lower than the baseline value at $p<0.01$ and 0.05, respectively. Although the hemoglobin level of fraction E treated group was significantly higher than those of control and crude extract groups ($p<0.05$). Obviously, only post-treated sildenafil group exhibited significantly lower hematocrit level than baseline ($p<0.01$). Furthermore, the PMN level of fractions B and C post-treated group were significantly higher than the baseline value at $p<0.05$ and 0.01,

respectively. However, these levels were not significantly different from that of control. Similarly, the MCHC level of fraction C post-treated group was significantly higher than pre-treated level ($p<0.05$); however, the value was not significant compared to those of control. In addition, the MPV level of crude extract and sildenafil treated groups were significantly lower than control ($p<0.01$).

Histology of testis It can be seen from Figure 3 that the spermatogenesis of all treated groups yielded more mature spermatids than that of control. These spermatogenesis processes were higher in both spermatogenesis levels and spermatid numbers. These findings lend support to assumption that compounds isolated from BS can increase both sperm number and motility.

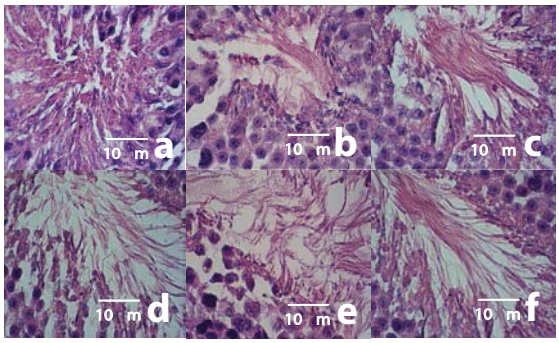

Figure 3 Micrographs of testicular (seminiferous tubules) section of mice; a = control DW 0.5 ml/kg BW/day, b = crude extract 1,250 mg/kg BW/day, c = Sildenafil 10 mg/kg BW/day, d = fraction B 40 mg/Kg BW/day, e = Fraction C 50 mg/Kg BW/day, f = Fraction E 150 mg/kg BW/day. All groups were treated for 14 days. All micrographs displayed at X100 magnification.

Testosterone Level Figure 4 shows the effects of BS extracts and sildenafil on testosterone level of mice. There were significant increases in testosterone level of all post-treated groups compared to pre-treatment ($p<0.01$) except for the control. The highest testosterone level was observed in fraction C treated groups. Serum testosterone level of both fractions C and E treated groups were significantly higher than other groups ($p<0.01$). This level in the crude extract, fraction B and sildenafil treated groups was also significantly higher than control ($p<0.01$).

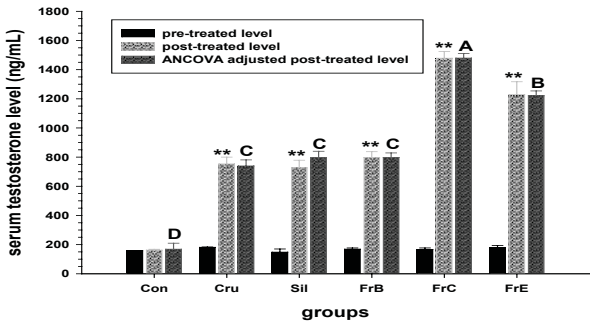

Figure 4 Effects of BS extracts and sildenafil on testosterone level (ng/ml) of mice. Significant difference between pre- and post-test in each group was compared using paired student t-test at * p<0.05, ** p<0.01. Significant difference between ANCOVA adjusted post-treated level in each group was compared using ANCOVA and Tukey HSD post hoc test at; a p<0.05, A p<0.01.

Body weight There was no significant difference in the relative growth rate measured by living body weight of male mice treated with BS extract compounds and sildenafil groups when compared to the control ($p<0.01$).

Selected reproductive and vital organs The testes weights of crude extract and sildenafil treated groups were significantly higher than those of control and other groups ($p<0.01$). Also, the epididymis weight of fraction E was significantly higher than others, whereas those of the crude extract, fraction B and sildenafil treated groups were significant lower than control ($p<0.01$). Furthermore, the seminal vesicle weights of fractions B, C and E treated groups were significantly higher than the remainder of these groups ($p<0.01$). The prostate gland weights were significant higher in the crude extract and sildenafil groups ($p<0.01$). However, the spleen weights of fractions B, C and E treated groups were significantly higher than control ($p<0.01$). On the contrary, the stomach weight of fraction E treated group was lower than control ($p<0.01$).

The histopathology of the heart, liver, spleen, kidney, and stomach of all treated groups revealed normal appearance compared to control group.

DISCUSSION

This investigation provides evidence that *Butea superba* extract could increase testosterone level, sperm number and motility in mice. This extract might therefore be developed to increase testosterone, sperm number and motility in men.

The results exhibited that there were significant increases in testosterone level of all post-treated groups compared to pre-treatment ($p<0.01$) except for the control. In addition, the increase in testosterone level of fraction C treated groups was the highest and this level of both fractions C and E treated groups were significantly higher than those of other groups ($p<0.01$). Apart from this, the results showed significant increase in sperm number of all post-treated groups compared to pre-treatment ($p<0.01$) except for control. Fraction C also showed the highest sperm number. Besides, the sperm number of crude extract, sildenafil, fractions B and E treated groups were significantly higher than control group ($p<0.01$). These results were confirmed by sperm morphology micrographs. Similarly, there were significant increases in sperm motility of all post-treated groups compared to pre-treatment ($p<0.01$) except for control. The highest motility level was found in groups treated with fractions C and E ($p<0.01$). These findings provide evidence that genistein, Un1 and biochanin A may play an important role in increasing the sperm number, motility and testosterone level. These results support previous finding that that sildenafil-treated mice showed significantly increased total testosterone level, as well as noteworthy ultrastructural alterations of Leydig cells, such as a vesicular smooth endoplasmic reticulum, large vacuoles, enlarged discontinue cristaes of mitochondria and vesicles of whorle membranes at the periphery typical of an activated steroid-secreting cell[5]. Previous findings on the stimulation of Leydig cells by sildenafil can be explained by the function of Leydig cells in producing testosterone, the male sex hormone, which stimulates spermatogenesis, promotes the physical and functional maturation of spermatozoa, maintains the accessory organs of the male reproductive tract, etc. Therefore, the increase in testosterone level would result in higher sperm number and motility. Recent studies have also revealed that blood testosterone concentrations were lower in male patients suffering from Alzheimer's disease (AD)[2,4]. These *Butea superba* extracts might be useful to treat AD in men in order to increase testosterone level in these patients. Blood analysis showed that the cholesterol level of fractions C, E and sildenafil treated groups were significantly higher than control ($p<0.05$). One reason could be that cholesterol, a precursor of testosterone, is prepared to synthesize testosterone[8-9].

CONCLUSION

These findings provide evidence that *Butea superba* extract in fractions C and E and sildenafil can increase testosterone level, sperm number and motility of mice compared to control group. These results can be explained by assuming that genistein, unknown compound 1 and biochanin A may play important role. These findings may provide evidence that these BS extracts may be developed as treatment to increase testosterone level, sperm number, sperm motility and infertility or Alzheimer's disease in men after safety level is investigated.

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