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Mitigation of cimetidine induced testicular toxicity in mice by *Kaempferia parviflora* Wall. Ex Baker rhizome extract

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Abstract. *Kaempferia parviflora* Wall. Ex Baker is a herb belonging to the family Zingiberaceae, its rhizomes were used as tea or wine and well known as Thai ginseng. This present study is to evaluate the mitigative effect of aqueous rhizome extract of *K. parviflora* (KPE) against testicular toxicity induced by subcutaneous injection of cimetidine 2 mg / 100 gBW for 14 days in male mice. The extracts at oral doses of 5, 10 and 20 mg / 100 gBW were pretreated for 7 days and co-treated with cimetidine for 14 days exhibited the significant results of mitigation by elevating seminal quality, higher than the cimetidine treated group ($P < 0.05$). This incidence was supported by the concordant results of testicular histological studies, showing the recovery of spermatogenic cells in seminiferous tubules in the group pretreated with KPE before co-treated with cimetidine by presenting a significant increase in spermatid layers. This present results suggested a possible use of aqueous *K. parviflora* rhizome extract as mitigative agent against testicular toxicity induced by cimetidine.
Key words: *Kaempferia parviflora*, cimetidine, testicular toxicity, mitigative effect.

Introduction. Cimetidine is a medicinal use in the treatment of stomach ulcer and acid reflux, which is avaliable without prescription. It belongs a class of drugs known as H_2 blockers (histamine blocker) (Ronald & Asbly 2003) and inhibits histamine evoked gastric acid secretion in a dose dependent manner (Anonymous 1999). Cimetidine acts as non-steroid anti-androgen by inhibiting testosterone transformation to dihydrotestosterone (Baba et al 1981) and inhibiting dihydrotestosterone-receptor binding in prostate gland (Winters et al 1979). Cimetidine regularly causes hypospermia in man (Fody & Walker 1985) which affected by the increase of blood prolactin (Wang et al 1982). Previous studies showed that cimetidine is a testicular toxicant in rats by exhibiting severe damage of seminiferous tubules, affecting peritubular myoid cell apoptosis and thereby causing abnormal spermatogenesis (Franca et al 2000; Sasso-Cerri et al 2001; Sasso-Cerri & Sasso Cerri 2008).

Kampferia parviflora Wall. Ex Baker (family Zingiberaceae) is a Thai medicinal herb. Its rhizomes contain many kinds of flavonoids including hydroxyflavones (Daodee et al 2003; Yenjai et al 2004) and anthocyanins (Vichitphan et al 2007). They were used for anti-inflammation and anti-febris (Herunsalee et al 1994), anti-HIV *in vitro* (Sookkongwaree et al 2006), anti-cancer (Patanasethanont et al 2007) and anti-allergic activities (Tewtrakul & Subhadhirasakul 2008). Recently, *K. parviflora* rhizomes were well known as Thai ginseng. Thai folks in the northeast of Thailand used *K. parviflora* rhizomes which were extracted by boiling in water or fermenting in 45% ethanol, for increasing male libido or impotent improvement. There are many studies to prove this issue. For example, rats orally received alcoholic extract exhibited on increase in sexual behavior including mount frequency and intromission frequency (Sudwan et al 2006) and increased intra-testicular blood flow as extract dose dependent (Chaturapanich et al 2008). Blood testosterone level of male rats were also significantly elevated after

feeding with *K. parviflora* rhizome powder at dose of 1,000 mg/ kgBW for 5 days (Trisomboon et al 2007) and *K. Parviflora* powder showed a significant increasment of prostate gland weight after 45 day treatment (Trisomboon et al 2008).

Therefore, it is interesting to evaluate an mitigative effect of aqueous *K. parviflora* rhizome extract against cimetidine induced testicular toxicity in mice. Testicular toxicity was induced by subcutaneous injection with cimetidine dose of 2 mg/100 gBW for 14 days (Komnont & Luangpirom 2008). The mitigative effect of the extract against cimetidine toxicity was assessed by blood testosterone determination, seminal analysis and testicular histopathological studies.

Material and Method

Preparation of rhizome extract. The rhizomes of *Kampferia parviflora* were purchased from the gardener in Loei province, northeast of Thailand and were authenticated by plant taxonomist from Department of Biology, Faculty of Science, Khon Kaen University, Thailand. They were cleaned, sliced and then dried at 50^{o} C for 1-2 days. The dried samples were extracted in boiling water for 1 hour, then filtered and evaporated at 50^{o} C until 5.4 g amber solid extract/ 100 g rhizome dry weight was obtained (modified method of Patanasethanont et al 2006).

Cimetidine solution. Cimetidine in ampoule (2 mL) concentration of 100 mg/ mL obtained from Pharmaland Ltd., Thailand, was diluted with normal saline (0.9% NaCl) to prepare the dose of 2 mg/ mL for experiment by subcutaneous injecton.

Animals. Adult male mice strain ICR (8-week old, weighed of 35-40 g) were obtained from the National Laboratory Animal Center of Mahidol University, Salaya district, Nakornprathom province, Thailand. They were housed under a 12:12 h of light-dark cycle at 25 $\pm$ 1^{o} C and were fed on standard pellet diet with water *ad libitum*. The experiments were performed after the experimental protocols have been approved by the Institutional Animal Ethics Committee, Khon Kaen University, Thailand. (Reference No. 0514.1.12.2 / 17)

Experiment. Male mice were divided into 6 groups, 6 mice for each. Group I received distilled water dose of 1 mL/100 gBW by oral administration for 14 days as a negative control, Group II received *K. parviflora* rhizome extract (KPE) dose of 20 mg/100 gBW by oral administration for 21 days as extract toxicity test, Group III received cimetidine dose of 2 mg/100 gBW by subcutaneous injection for 14 days as a positive control, Groups IV, V and VI were pretreated by oral administration with KPE doses of 5, 10 and 20 mg/100 gBW, respectively for 7 days and subsequently co-treated with cimetidine by subcutaneous injection for 14 days as treated groups.

Testosterone determination. At the end of the treatment, body weights of all treated groups were recorded. Blood samples of all groups were collected by cardiac puncture and plasma was obtained from the blood after centrifugation at 3,000 rpm for 10 minutes at room temperature for testosterone determination by radioimmunoassay (The DSL-400 ACTIVE$^{(R)}$ Testosterone Coated-Tube RIA Kits, Diagnostic System Laboratories, Inc.)

Seminal analysis. After blood sampling, epididymis and vas deferens of all groups were excised and tore with syringe needle (No.25) in 2 mL of 0.9% NaCl and incubated at 35^{o} C for seminal evaluation. Total sperm counts and viable sperms were determined by the modified method of Yokoi et al (2003), motile sperms were evaluated by the method described by Sonmez et al (2005) and abnormal sperms were investigated by the method described by Atessahin et al (2006).

Histopathological studies. After seminal sampling, testes were removed and weighed, then fixed in Bouin's solution and subjected to a paraffin method process. They were sectioned at the thickness of 5 μm and the sections were stained with Haematoxylin and Eosin(H&E). The histopathological occurrences of spermatogenic cells in seminiferous tubules of testes were observed under a light microscope.

Statistical analysis. All results were expressed as mean $\pm$ standard deviation ($\bar{X} \pm$ SD). Data were analysed by One-way analysis of variance (ANOVA) and Duncan's test for multiple comparisons using SPSS software. Values of $P < 0.05$ were considered significant.

Results and Discussion

Testicular weight and plasma testosterone determination. Testicular weight and plasma testosterone (T) level of control and all treated groups were shown in Table 1. All groups did not show any significantly different results. However, T level of *K. parviflora* rhizome extract treated group showed a non-significant increment while a non-significant depletion of T level was also found in cimetidine treated group, compared to control ($P > 0.05$). Similarly, previous results showed that Sprague Dawley rats receiving cimetidine at dose of 50 mg/ kgBW decreased blood T level and cimetidine acted as non- steroid androgen by inhibing T transforming to dihydrotestosterone in prostate gland (Baba et al 1981). This present study also showed that all groups which were pre-treatment with KPE and subsequently co-treated with cimetidine exhibited non-significant increment of T level, compared to the cimetidine treated group ($P > 0.05$). Likewise, previous studies were reported that oral administration of *K. parviflora* rhizome powder in male at dose of 1000 mg/ kgBW for 5 days, affected testicular function by increasing T synthesis and secretion (Trisomboon et al 2007), increased sexual behavior after 60 day treatment (Sudwan et al 2006), increased prostate gland weight after 45 day treatment (Trisomboon et al 2008). Besides, the alcoholic *K. parviflora* rhizome extract also increased intra-testicular blood flow of male rats after receiving the extract at dose of 10-40 mg/ kgBW as extract doses dependent (Chaturapanich et al 2008).

Table 1

$\bar{X} \pm$ SD of plasma testosterone level and testicular weight / body weight in the control group and the treated groups

Treated groups (mg / 100 gBW) N=6	Testosterone level (ng / mL)	Testicular weight / body weight
0	1.02 ± 1.10^a	0.71 ± 0.05^a
KPE 20	1.48 ± 0.70^a	0.72 ± 0.13^a
C 2	0.99 ± 0.54^a	0.71 ± 0.06^a
C 2 + KPE 5	1.38 ± 0.02^a	0.74 ± 0.04^a
C 2 + KPE 10	1.05 ± 0.45^a	0.75 ± 0.04^a
C 2 + KPE 20	1.04 ± 0.20^a	0.74 ± 0.31^a

N = number of experimental animals, same alphabet in column-non significant different ($P > 0.05$)

Seminal analysis. The KPE treated group showed non-toxicity on testicular function while the cimetidine treated group had adverse effect on spermatogenesis by revealing a significant increase in percentage of abnormal sperms ($P < 0.05$) and a significant depletion in total sperm count, percentage of motile sperms and viable sperms, compared to control ($P < 0.05$) as shown in Table 2. The abnormal sperms were found many forms such as medial protoplasmic droplet, coiled tail and bent middle piece sperms which were suggested causing alteration of sperm maturation in epididymis (Jones 1997; Sarkar et al 2000). Fody & Walker (1985) claimed that cimetidine regularly causes hypospermia in man. Previous study was reported that depletion of total sperm count and number of motile sperms were found in Charles River rats after receiving cimetidine by intra-peritoneal injection for 1-6 weeks (Kazerouni & Nayeri Kaman 2000). The present study also showed a mitigation of cimetidine testicular toxicity in all groups, which were pretreated with KPE and co-treated with cimetidine by presenting a significant improvement of seminal quality, compared to the cimetidine treated group ($P < 0.05$). *K. parviflora* rhizomes are rich in flavonoids (Yenji et al 2004), which act as a potent antioxidant (Vichitphan et al 2007), since they are capable of reducing drug toxicity. For example, *Hibiscus sabdariffa* calyx extract exhibited a protective effect on tetracycline induced testicular toxicity in mice (Luangpirom & Taweebot 2010).

Table 2

$\bar{X} \pm$ SD of total sperm count, percentage of motile sperms, abnormal sperms and viable sperms in the control group and the treated groups

Treated groups (mg/100gBW) N = 6	Total sperm counts $\bar{X} \pm$ SD ($x10^6$) cells/mL	Motile sperms ($\bar{X} \pm$ SD, %)	Abnormal sperms ($\bar{X} \pm$ SD, %)	Viable sperms ($\bar{X} \pm$ SD, %)
0	27.12 ± 1.72^a	77.99 ± 1.56^a	14.37 ± 3.27^a	82.83 ± 1.55^{ac}
KPE 20	25.50 ± 2.88^{ab}	81.16 ± 3.86^a	13.31 ± 2.90^a	82.38 ± 1.84^a
C 2	$17.67 \pm$	61.22 ± 13.16^b	18.91 ± 2.90^b	71.33 ± 3.01^b
C 2 + KPE 5	1.63^c	82.44 ± 2.28^a	10.62 ± 6.52^a	$83.94 + 2.79^a$
C 2 + KPE 10	25.50 ± 2.07^{ab}	81.11 ± 2.75^a	18.68 ± 1.87^b	79.38 ± 1.81^c
C 2 + KPE 20	23.16 ± 4.07^b	58.72 ± 20.83^b	12.38 ± 1.68^b	71.05 ± 3.41^b
	22.50 ± 5.89^b			

N = number of experimental animals, same alphabet in column-non significant different ($P > 0.05$) and different alphabet in column- significant different ($P < 0.05$)

Histopathological studies. The results of spermatogenic cell layers, which were observed in cross -section of seminiferous tubules in the testes of the control and treated groups were shown in Table 3. Testicular histological architecture of the KPE treated group was similar to at of control group. However, the cimetidine treated group presented a significant depletion in spermatid layers ($P < 0.05$). This result may indicate that cimetidine had an effect on the second meiosis stage of spermatogenesis. Similarly, the previous study was reported that cimetidine at dose of 50 mg/ kgBW caused alteration of seminiferous tubules after 59 days of intraperitoneal injection including partial devoid of spermatgenic cells, reduction of peritubular tissues and death of peritubular myoid cells by apoptosis (Franca et al 2000) and detachment of Sertoli cells from lamina densa (Sasso Cerri & Sasso Cerri 2008). It could be inferred that cimetidine directly affected testicular structure and resulted in abnormal spermatogenesis. This present study also showed a mitigation of cimetidine toxicity in the groups which were pretreated with KPE and co-treated with cimetidine by showing recovery of spermatogenic cells in seminiferous tubules such as an increase in spermatid layers as shown in Fig. 1.

Table 3

$\bar{X} \pm$ SD of spermatogenic layers in seminiferous tubules of the control group and the treated groups

Treated groups (mg/100gBW) N = 6	spermatocyte layer ($\bar{X} \pm$ SD)	spermatid layer ($\bar{X} \pm$ SD)
0	1.37 ± 0.21^a	3.26 ± 0.14^a
KPE 20	1.35 ± 0.19^a	$3.80 + 0.16^c$
C 20	1.33 ± 0.12^a	1.61 ± 0.40^b
C 20 + KPE 5	1.34 ± 0.13^a	2.82 ± 0.25^a
C 20 + KPE 10	1.42 ± 0.16^a	3.23 ± 0.25^a
C 20 + KPE 20	1.45 ± 0.14^a	2.92 ± 0.24^a

N = number of experimental animals, same alphabet in column-non significant different ($P > 0.05$) and different alphabet in column- significant different ($P < 0.05$)

Conclusions. Cimetidine at dose of 2 mg/ 100 gBW had an adverse effect on testicular function of male mice by depleting of blood testosterone level, altering spermatogenic cells in semineferous tubules and finally affected spermatogenesis by showing low quality of seminal analysis after 14 days of subcutaneous injecton. The pre-treament with *K. parviflora* rhizome extract for at least 7 days before co-treatment with cimetidine was capable of reducing cimetidine induced-testicular toxicity.

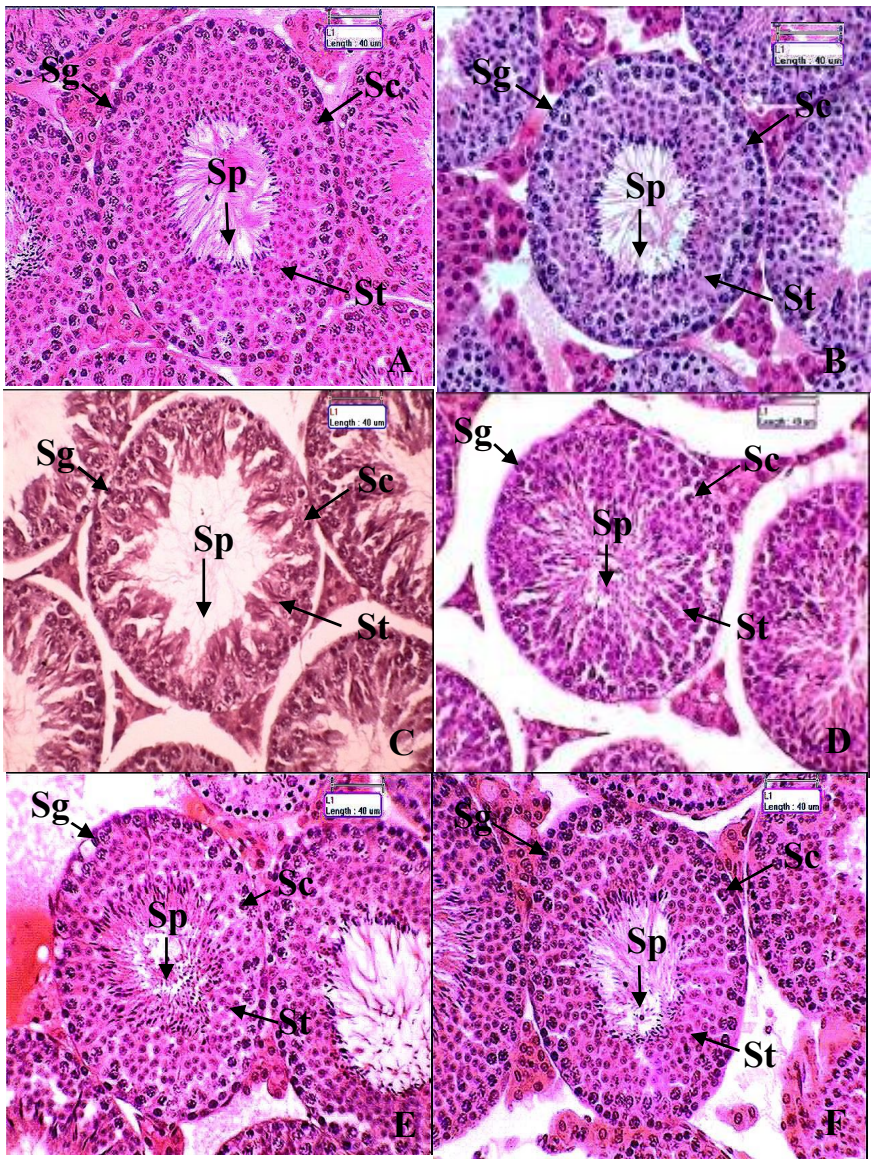

Figure 1. Cross-section of testes (H&E, bar = 40 µm) showing histological changes of seminiferous tubules; A,control group and B, group received *K. pariflora* rhizome extract (KPE) exhibiting normal arrangement of spermatogenic cells from spermatogonium(Sg), spermatocyte(Sc), spermatid(St) to spermatozoa(Sp);C, group received cimetidine (C) 2 mg exhibiting a depletion of spermatid layers; D, group received C +KPE 5 mg and E, group received C + KPE 10 mg. presenting recovery of spermatid layers and F, group received C + KPE 20 mg showing normal architecture similar as control group.

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