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Antifertility activity of the aqueous leaf extract of *Cissampelos pareira* in male albino mice

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Abstract. Aqueous leaf extract of *Cissampelos pareira* (L) Hirsuta (family Menispermaceae) was evaluated for the validity of the anti-fertility effect in male mice after oral administration with the extract at dose of 0.5 and 1.0 ml/ 100 gBW daily for 14 days. The significant increment of plasma prolactin and depletion of follicle stimulating hormone (FSH) in the high dose treated group were found ($p<0.05$). Interestingly results, the depletion of seminal testicular weight was presented in both treated groups whereas seminal quality impairment including a decrease in total sperm count and a increase in percentage of abnormal sperms were shown in the high dose treated group only. The results indicated the infertility effect of aqueous *Cissampelos pareira* leaf extract in male mice occurring after high dose of extract intake by causing prolactin and FSH disturbance.
Key words: *Cissampelos pareira*, prolactin, seminal quality, antifertility.

Introduction. *Cissampelos pareira* (L) Hirsuta (family Menispermaceae) is a woody climbing vine and widespread in northeast Thailand (Smitinand & Lasen 1991). Their leaves mainly consist of alkaloids such as pareirubrines A and B (Morita et al 1993) and pectin such as galacturonic acid (Singthong et al 2004, 2005). The oral median lethal dose of methanolic *C. pareira* leaf extract was found to be 7.3 g/ KgBW in mice (Ganguly 2007ab). Interesting property of pectin in *C. pareira* leaves is gel formation within 2-3 minutes after extraction with water. This gel commonly has been consumed as dessert or cooking with spicy by northeast Thais. In India, this plant is popularly known as a medicinal herb by folk people for some treatments such as dysentery, diuretic and taumatic pain therapy (Mukerji & Bhandari 1959). Besides being use as ailment therapy, *C. pareira* is also used as birth control agent among rural people (Tiwari et al 1982). Recently, Ganguly (2007a) found the antifertility effect of *C. pareira* leaf extract after feeding female albino mice with 450 mg/ kgBW of extract daily for 21 days. It affected reproductive aspect by extending diestrus stage of oestrous cycles, altering gonadotrophin release (LH, FSH and prolactin) and significant depletion of the litter numbers. Therefore, the present study is an attempt to evaluate the claimed antifertility effect of *C. Pareira* leaf extract using similar aspects of reproductive physiology in male mice.

Material and Method

Collection and extraction. *C. pareira* (L) Hirsuta leaves were collected from the Agricultural Garden, Faculty of Agriculture, Khonkaen University and were authenticated by plant taxonomist at Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen, Thailand. They were washed and freshly squashed in distilled water (leaves:water = 1:5, w/v), then filtered with cotton mesh and subsequently used for treatment.

Animals. Adult male mice strain ICR (8-week old, weighing 35-40 grams) were obtained from the National Laboratory Animal Center of Mahidol University, Nakornprathom

province, Thailand. They were housed under a 12:12 h of light-dark cycle at 25 ± 1 °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).

Treatments. Male mice were divided into 3 groups, 6 mice for each. Group I received distilled water dose of 1 ml/100 gBW as control, Group II and III received *C. pareira* leaf extract doses of 0.5 and 1.0 ml/ 100 gBW as treated groups.

Hormone assay. After 14 days of treatment, blood samples of all groups were collected by cardiac puncture and plasma was obtained from the blood sample after centrifuged at 3,000 rpm for 10 minutes at room temperature for prolactin and follicle stimulating hormone (FSH) assay by radioimmunoassay (^{125}/IRMA kit, ICN Biomedicals, Inc. Costa Mesu, CA 926261)

Seminal evaluation. After blood sampling, epididymis and vas deferens of all groups were excised and torn with a syringe needle (No.25) in 2 ml of 0.9% NaCl and incubated at 35°C for seminal analysis. Total sperm counts and viable sperms were determined by 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 Atessahim et al (2006). Finally, sex organs including testes and seminal vesicles were weighed.

Statistical analysis. All results were expressed as mean ± standard deviation ($\bar{x}$ ± SD). Data were analyzed by one-way analysis of variance (ANOVA) and Duncan's test for multiple comparision using SPSS software version 11.0. Value of $p<0.05$ were considered to be significant.

Results

Hormone evaluation. Plasma prolactin and FSH levels of the control and treated groups with CPE at doses of 0.5 and 1.0 ml /100 gBW were presented in Table 1 and Figure 1. The high dose treated group revealed a significant increase in plasma prolactin and a significant decrease in FSH level, compared to control ($p< 0.05$). However, the low dose treated group did not show any significantly different results ($p> 0.05$).

Organ weight. Body weight, testicular and seminal vesicular weight of the control and treated groups were presented in Table 2. Body and testicular weight of all groups did not show different results. Interestingly, seminal vesicular weight were significantly decreased in both treated groups, compared to control ($p< 0.05$)

Seminal analysis. Seminal quality including total sperm count, percentage of abnormal sperms, motile sperms and viable sperms were shown in Table 3. The high dose treated group with PCE (1.0 ml/ 100 gBW) showed a significant decrease in total sperm count (10^{-6} cells /ml) and percentage of abnormal sperms ($p<0.05$). The abnormal sperms were found in many forms including medial protoplasmic droplet, bent tail and bent middle piece sperms as shown in Figure 2. But any non-significant results were observed in the low dose treated group with PCE (0.5 ml/100 gBW), compared to control ($p>0.05$).

Table 1

$\bar{x}$ ± SD of plama prolactin and FSH level in control and *C. pareira* leaf extract(CPE) treated groups

Treated group (ml/ 100 gBW N=6	prolactin level (ng/ ml)	FSH level (mIU/ml)
0	0.143 + 0.014[a]	0.796 + 0.185[a]
CPE 0.5	0.233 + 0.053[a]	0.720 + 0.113[a]
CPE 1.0	0.382 + 0.082[b]	0.330 + 0.059[b]

N-number of experimental animals, same alphabet within column- non-signifcant different ($p> 0.05$), different alphabet within column– significant different ($p< 0.05$)

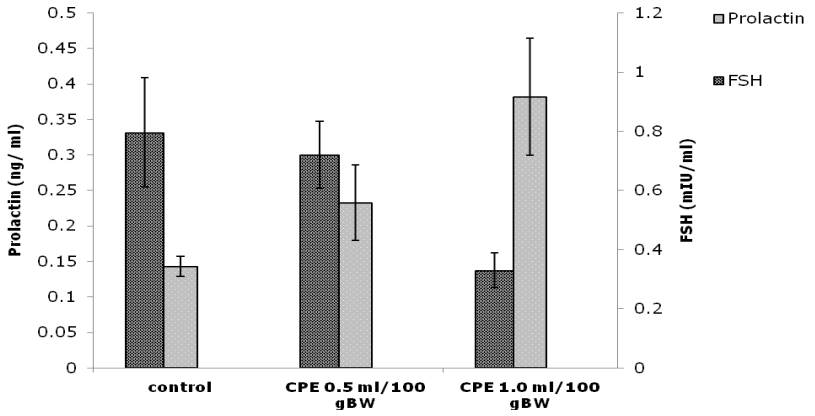

Figure 1. $\bar{x}$ + SD of plasma prolactin and FSH levels of control and treated groups with CPE for 14 days.

Table 2

$\bar{x}$ + SD of body weight, testicular and seminal vesicular weight in control and CPE treated groups

Treated group (ml/ 100 gBW N=6	body weight (g)	organ weight	
		testes (mg)	seminal vesicles (mg)
0	36.00 + 0.58	132.00 + 6.00[a]	23.80 + 1.40[a]
CPE 0.5	31.17 + 2.91	122.00 + 5.04[a]	17.10 + 1.50[b]
CPE 1.0	33.67 + 3.86	133.00 + 4.00[a]	16.70 + 0.07[b]

N-number of experimental animals, same alphabet within column- non-signifcant different ($p > 0.05$), different alphabet within column– significant different ($p < 0.05$)

Table 3

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

Treated group (ml/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	23.33 ± 4.86 [a]	88.11 ± 0.27 [a]	15.83 ± 4.86 [a]	89.25 ± 2.19 [a]
CPE 0.5	24.17 ± 4.78 [a]	89.83 ± 1.99 [a]	12.50 ± 1.28 [a]	91.93 ± 3.11 [a]
CPE 1.0	18.83 ± 3.85 [b]	72.72 ± 5.14 [a]	22.25 ± 1.76 [b]	87.25 ± 2.79 [a]

N - number of experimental animals, same alphabet within column- non-significant different ($p > 0.05$), different alphabet within column- significant different ($p < 0.05$)

Discussion. The present study revealed that aqueous leaf extract of *C. pariera* at dose of 0.5 and 1.0 ml/ 100 gBW increased the plasma prolactin level but decreased FSH level. According to Freeman et al (2000), prolactin is a polypeptide hormone which is synthesized in lactrotrophs of anterior pituitary gland. It plays a biological role in mammalian reproduction other than lactating effect including gonadotropin (follicle stimulating hormone, FSH and luteininzing hormone, LH) synthesis and secretion suppression. In man, hyperprolactinemia is an important cause of infertility (Musud et al 2007). Previous evidence of an increase in plasma prolactin after herb extract intake, thus functioning as the antifertility agents has been reported in many cases such as leaf extract of *C. paereira* in female mice (Ganguly et al 2007a), root extract of *Mimosa pudica* in female albino mice (Ganguly et al 2007b), fruit extract of *Silybum marianum* in

female rats (Capasso et al 2009) and seed extract of *Hibicuss sabdariffa* in male mice (Sookjai & Luangpirom 2010). FSH is a gonadotropin which is important for spermatogenesis and testosterone synthesis in testes (Jones 1997). Our study also showed the concordant results of the seminal quality impairment in the high dose of PCE (1.0 ml/ 100 gBW) treated group including the significant depletion of total sperm count and the significant increment in percentage of abnormal sperms, which were important effects as antifertility agent (Sarkar et al 2000). Huang et al (2000) claimed that high level of blood prolactin caused apoptosis of germ cells in male rats. Interestingly, depletion of seminal vesicular weight was also found in both treated groups, suggesting alteration of androgen production in testes (Jones 1997). Furthermore, increment percentage of abnormal sperms was caused by alteration of sperm maturation in epididymis (Jones 1997; Sarkar et al 2000). Previous similar study was reported that oral administration of methanolic *C. Pareira* leaf extract at dose of 450 mg/ kgBW daily for 21 days caused an antifertility effect in female mice by increasing blood prolactin and causing estrous cycle alteration with diestrus stage prolongation.

Conclusions. This study may be concluded that the aqueous leaf extract of *Cissampelos pareira* (L) Hirsuta had lactogenic activity by increasing blood prolactin which may be valued for milk induction during lactation of female animals. In contrast, it may cause an adverse effect on testicular function as antifertility agent by disturbance in blood prolactin secretion, which directly caused gonadotropin secreton imbalance and finally affected on testicular function.

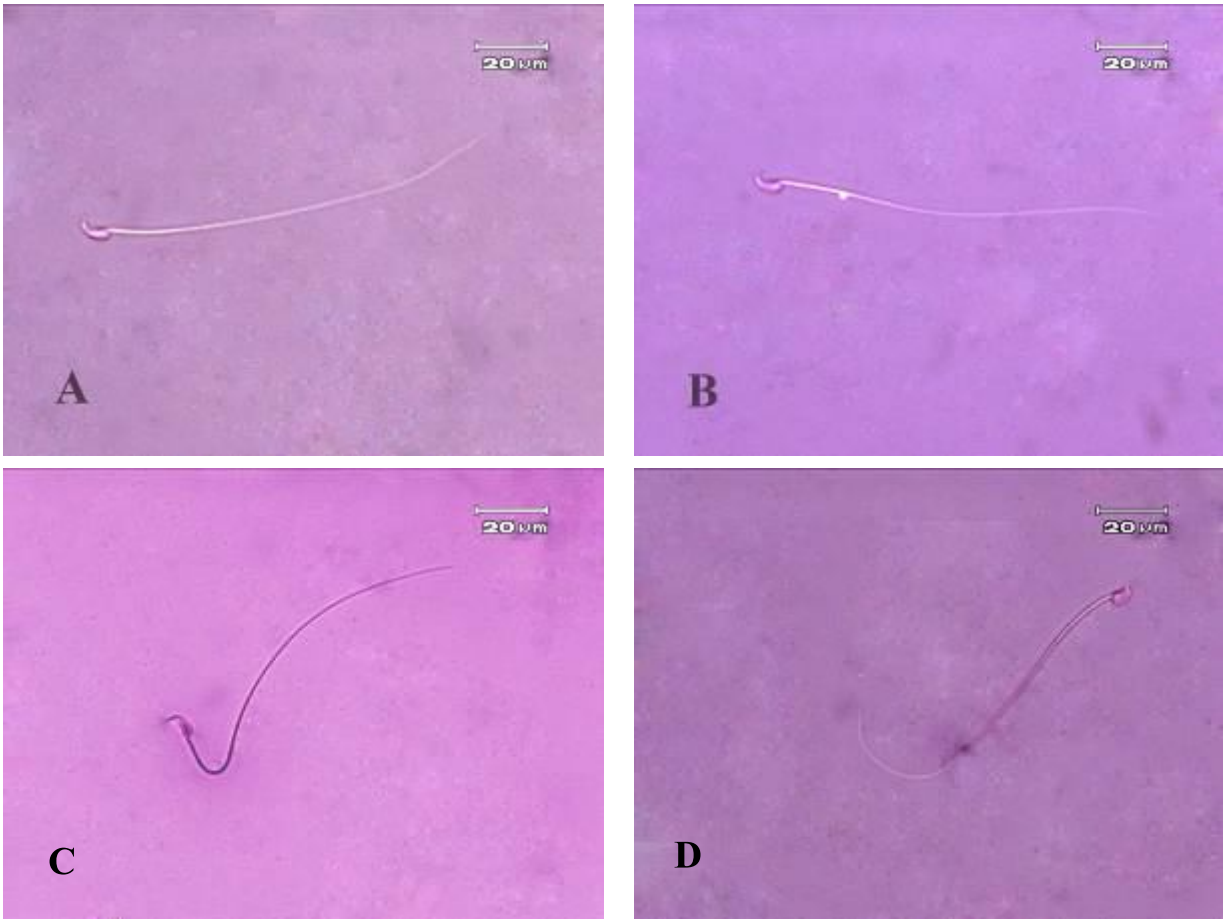

Figure 2. Showing normal and abnormal sperms of mice (Negrosin-Eosin stain), bar=20 nanometer. A, normal sperm; B, medial protoplasmic sperm; C, bent middle piece sperm and D, bent tail sperm.

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References

Atessahin A., Turk G., Karaham I., Yilmaz S., Ceribast. A. O., Bulmus O., 2006 Lycopene prevent adriamycin-induced testicular toxicity in rats. Fertil Steril **85** (suppl 1) :1216-1222.

Capasso R., Aviello G., Capasso F., Sarvino F., Izzo A. A., Lembo F., Borrelli F., 2009 Silymarin BIO-C^R an extract from *Silybun marianum* fruits induces hyperprolactinemia in intact female rats. Phytomed **16**:839-844.

Freeman M. E., Bela K., Lerant A., Nagy G., 2002 Prolactin structure, function and regulation of secretion. Physiol Rev **2**(4):1523-1631.

Ganguly M., Borthakur M. K., Devi N., Mahanta R., 2007a Antifertility activity of the methanolic leaf extract of *Cissampelos pareira* in female albino mice. J Ethnopharmacol **111**:688-691.

Ganguly M., Devi N., Mahanta R., Borthakur M. K., 2007b Effect of *Mimosa pudica* root extract on vaginal estrous and serum hormones for screening of antifertility activity in albino mice. Contraception **76**:482-485.

Huang W. J., Wang P. S., Yeh J. Y., Chang L. S., 2007 The prolactin induced germ cell apoptosis in seminiferous tubules is through an intrinsic caspase pathway. Fertil Steril **88**:384.

Jones R. E.,1997 Hormonal control of sex accessory structure: In Human Reproductive Biology. 2nd edition, New York: Academic Press, pp 98-107.

Morita H., Masumoto K., Takeya K., Itokawa H., Itaka Y., 1993 Structures and solid state tautomeric forms of two novel antileukemic tropoloisoquinoline alkaloids, pareirubrines A and B, from *Cissampelos pareira*. Chem Pharm Bull **41**:1418-1422.

Muerji B., Bhandari P. R., 1959 *Cissampelos pareira* source of a new eurariform drug. Planta Midica **7**:250-259.

Musud S., Mehboob F., Bappi M. U., 2007 Severe hyperprolactinemia directly depresses the gonadal activity causing infertility. Esculapio **2**(4):25-28.

Sarkar M., Gangopadhyay P., Basak B., Chakrabarty K., Banerji J., Adhikary P., Chatterjee A., 2000 The reversible antifertility effect of *Piper betle* Linn. on Swiss albino male mice. Contraception **62**:271-274.

Singthong J., Cui S. W., Ningsonond S., Goff H. D., 2004 Structure characterization, degree of esterification and some gelling properties of Krueo Ma Noy (*Cissampelos pareira*) pectin. Carbohydrate polymer **58**:391-400.

Singthong J., Ningsonond S., Cui S. W., Goff H. D., 2005 Extraction and physicochemical characterization of Krueo Ma Noy pectin. Food Hydrocol **19**:793-801.

Smitinand T., Larsen K., 1991 Flora of Thailand Vol.**5** Part 3 Bangkok:Forest Herbarium, Royal Forest Department, Thailand.

Sonmez M., Turk G., Yuce A., 2005 The effect of ascorbic acid supplementation on sperm quality, lipid peroxidaton and testosterone levels of male Wistar rats. Theriogenology **63**:2063-2072.

Sookjai K., Luangpirom A., 2010 Effect of aqueous *Hibiscus sabdariffa* Linn. seed extract on plasma prolactin and seminal quality in male mice. The 4th Sino-Thai International Conference, "Traditional Medicine and Research and Development in Pharmacy Related area", 11-13 July,2010, Khon Kaen, Thailand.

Tiwari K. C., Majumder R., Bhattacharjee S., 1982 Folklore information from Assam for family planning and birth control. Inter J Crude Drug Res **20**:133-137.

Yokoi K., Uthus E. O., Nielsen F. H., 2003 Nickel deficiency diminishes sperm quality and movement in rats. Biol Trace Ele Res **93**:141-153.

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