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Antiepileptic "Antifertility properties of *Cissampelos pareira* Linn. leaf gel in male and female mice"

Thanaree Junaimuang[1], Ampa Luangpirom[2*], Pichet Somsapt[3]

***Corresponding author:**

Thanaree Junaimuang

[1]Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen, 40002. Thailand,
[2]Applied Taxonomic Research Center, Faculty of Science, Khon Kaen University, Khon Kaen, 40002, Thailand,
[3]Department of Radiology, Faculty of Medicine, Khon Kaen University, Khon Kaen, 40002, Thailand,

Abstract

This study aimed to evaluate the antifertility properties of *C. pareira* Linn. leaf gel in male mice by evaluation of blood prolactin, testosterone and seminal quality. In females mice, estrous index, fertility index and prolactin level were determined. The results for males revealed that the leaf gel at a dose of 30 mg/100 gBW significantly increased blood prolactin, while the leaf gel at 20 and 30 mg/100 gBW decreased testosterone levels in a dose dependent manner ($P<0.05$). Interestingly, all doses (10, 20 and 30 mg/100 gBW) of leaf gel caused significant impairement of sperm quality collected from epididymis and vas deferens after 21 days of oral adminitration. The results for females showed that the leaf gel at 40 and 60 mg/100 gBW caused irregular estrous cycle and inhibited ovulation by decrease in estrous index during the 21 days of treatment. Meanwhile, it also revealed significant increases in blood prolactin and decreased embryo implantation in pregnant mice after 10 days of treatment. It may be concluded that *C. pareira* leaf gel has antifertility properties in male by disturbance of blood prolactin and testosterone levels, consequently affecting spermatogenesis and impaired sperm quality. Meanwhile, it inhibits ovulation in females and inhibits embryo implantation or decreases fertility index by increasing prolactin in pregnant mice. Therefore, the use of *C. pareira* leaf gel as food or for therapy in traditional medicines should be avoided because of this adverse effect.

Keywords: Cissampelos pareira Linn., antifertility, prolactin, testosterone, fertility index

Introduction

Cissampelos pareira Linn. is a potential medicinal plant in family Menispermaceae [1]. It is found in subtropical part of India, Asia, East Africa and America. It is also widespread throughout northeast Thailand [2]. Its leaves had been reported to be a rich source of alkaloids and pectin [3]. Pectin from leaves forms a gel within 2-3 minutes after mashing with water [4] and local Thais in northeast Thailand cooked them as vegetables and dessert. This plant leaf gel is also used in Thai traditional medicine as diuretic agent and for traumatic wound treatment [5]. In the Ayurvedic system of traditional Indian medicine, its leaves are used in treatment of urinary tract infection [6] and diarrhea [7] because of its antiseptic properties. Interestingly, local women in Assam used this plant as an antifertility agent [8]. Recently, the antifertility properties of *C. pareira* methanolic leaf extract were found in female mice; the estrous cycles were prolonged and ovulation was inhibited after feeding with methanolic extract at 250 and 450 mg/kgBW for 21 days [9]. In male mice, decrement of blood testosterone and sperm quality impairment were also found after gavage with ethanolic extract of *C. pareira* at 200-600 mg/kgBW for 45 days [10]. However, there is little scientific information about this plant. The aim of this study was to evaluate the antifertility properties of *C. pareira* leaf gel in male and female mice using an aqueous extract, similar to that consumed by local Thai in the northeast of Thailand.

Materials and Methods

Experimental animals

Adult mice (strain ICR), males 8 week old and females 6 week old were obtained from the National Laboratory Animal Center, Nakornpathom province, Thailand. They were caged in an animal room at under 25±1 ºC and with light:dark of 12:12 h.. A standard pellet diet (Chareanpogapan Ltd., Thailand) and water *ad libitum* were freely available. The experiments were conducted after an approval by the Institutional Animal Ethics Committee, Khon Kaen University,Thailand (Reference No. 0514.12.2/42).

Leaf gel preparation

C. pareira were collected from the cultivated garden, Khon Kaen University (KKU) in March 2012 and identified by a plant taxonomist. Leaves were cleaned with distilled water, shade dried, minced with distilled water, then filtered through a cotton mesh and

dried in a hot air oven at 40 ºC. The dried sheet was obtained and was minced to powder and reconstituted with distilled water to reform as gel before treatment.

Experimental design

Experiment I- Antifertility of *C. pareira* Linn. leaf extract in male mice.

The experiments were performed on 5 groups of 6 males each. Group I, negative control, received distilled water at 0.5 ml/100gBW. Group II, positive control, received metoclopramide (a commercial hyperprolactinemia-inducing agent, Pharma supply Co.,Ltd., Thailand) as reference drug at dose of 2.0 mg/100gBW. Groups III, IV and V, treated groups, received *C. pareira* leaf gel at doses of 10, 20 and 30 mg/100 gBW daily for 21 days.

At the end of treatment, all groups were individually weighed, then blood was collected by cardiac puncture and centrifuged at 1,700 rpm for 5 min at room temperature. Plasma were kept for prolactin and testosterone assays. Prolactin assay was measured by Prolactin ^{125}I IRMA kit (for in vitro use), ICN Pharmaceuticals, Inc, USA, assay sensitivity = 0.25 ng/ml. Testosterone assay was measured with testosterone radioimmunoassay kit (The DSL-400 ACTIVE® Testosterone Coated –Tube RIA Kits, Diagnostic system Laboratories, Inc., USA), assay sensitivity = 0.14 ng/ml. After blood sampling, epididymis and vas deferens of all groups were excised and torn with needles (No. 25) in 2 ml of NaCl (0.9%) and then incubated at 35 ºC for sperm quality evaluation including total sperm counts, percentage of viable sperms. Motile sperms and abnormal sperms were investigated [11] Both testes were weighed and the results expressed as the gonadal index (gonad weight / body weight).

Experiment II- Antifertility effect of *C. pareira* Linn. leaf gel in female mice.

II.1-The experiments were performed on 5 groups of 6 females (having regular estrous cycles) each. Group I, negative control, received distilled water at 0.5 ml/100gBW. Group II, positive control, received metoclopramide as reference drug at dose of 2.0 mg/100gBW. Groups III and IV, treated groups, received *C. pareira* leaf gel at doses of 40 and 60 mg/100 gBW daily respectively for 21 days. All groups had estrous cycles determined by vaginal smear [12] from day 1 - day 21 daily and results were expressed as the estrous index (length of estrous x 100/ days of treatment [13].

II.2- The experiments were performed on 5 groups of 6 pregnant mice each. All groups were fertilized on the first day. Group I, negative control, received distilled water at 0.5 ml/100gBW. Group II, positive control, received metoclopramide as reference drug at dose of 2.0 mg/100gBW. Groups III and IV, treated groups, received *C. pareira* leaf gel at doses of 40 and 60 mg/100 gBW daily respectively for 10 days. On day 11, all groups were sacrified by neck dislocation and plasma immediately collected for prolactin determination. After blood sampling, the abdominal cavity was opened and the number of corpora lutea in ovaries and number of embryos implanted in the uterus determined, and then these were expressed as fertility index (number of embryos implanted x 100 / number of corpora lutea [14]. All groups had mammary glands collected and immediately fixed in Bouin's solution for 48 hours; then they were processed by the paraffin method. They were sectioned at 5 µm thickness and stained with periodic acid schiff (PAS) for milk sac/ alveoli detection. Sections were examined under light microscope for histological study. The results were expressed as percentage of alveoli branching in mammary glands.

Statistical analysis

Data were expressed as mean±standard deviatin (SD) and analyzed by one-way analysis of variance (ANOVA). The differences among groups were evaluated by Duncan's test. A value of $P<0.05$ was considered as statistically significant [15].

Results

The results reveal that the leaf gel of *C. pareira* altered the reproductive hormones of male mice after oral administration for 21 days. The group that received 30 mg/100 gBW of the leaf gel had significantly increased blood prolactin, while the extract at doses of 20 and 30 mg/100 gBW significantly decreased blood testosterone level in a dose dependent manner ($P<0.05$) (Table 1). It is noticed that the highest dose treated group revealed prolactin level and testosterone levels the same as the group treated with the reference drug (metoclopramide 2 mg/100 gBW). This implies that the potency of the leaf gel at the highest dose was almost the same as metoclopramide. However, the gonadal indices of all groups were not different. The effect of *C. pareira* leaf gel on sperm quality is shown in Figure. 1. All treated groups exhibited impairment of sperm quality in all parameters including a significant decrease in total sperm counts (Figure. 1A), percentage of motile sperms (Figure. 1B) and percentage of viable sperms (Figure. 1C), which contrasted with a significant increase in the percentage of abnormal morphology sperms (Figure. 1D). There were many forms of abnormal sperms such as bent middle piece sperms (Figure. 2B), detached head sperms (Figure. 2C) and medial protoplasmic droplet sperms (Figure. 2D).

The antifertility effect of *C. pareira* leaf gel on female groups is shown in Table 2 & 3. The results of vaginal smear determination during 21 days of treatment revealed that irregular estrous cycles and significant decrease in estrous index were found in groups that received metoclopramide, a hyperprolactinemia-inducing agent (2 mg/100 gBW). Meanwhile, a non-significant decrease in estrous index was presented in groups receiving *C. pareira* leaf gel (40 and 60 mg/100 gBW) (Table 2). This implies that ovulation was inhibited. Furthermore, the decrease in fertility index, increase in prolactin level and branching of alveoli in mammary glands were found in the pregnant groups receiving metoclopramide and *C. pareira* leaf gel (Table 3 and Figure. 3).

Table 1 Effect of *C. pareira* leaf gel (CPG)on gonadal index and reproductive hormones in male mice after 21 days of treatment

Treatment (mg/100 gBW), N=6	Gonadal index ($\overline{X}$ ±SD, x10^{-2})	Hormonal level ($\overline{X}$ ±SD, ng/ml)	
		Prolactin	Testosterone
0	0.38 ± 0.05^{a}	0.09 ± 0.08^{a}	1.22 ± 0.77^{a}
Metoclopramide 2	0.34 ± 0.05^{a}	0.37 ± 0.22^{b}	0.45 ± 0.18^{b}
CPG 10	0.38 ± 0.05^{a}	0.09 ± 0.06^{a}	0.77 ± 0.45^{ab}
CPG 20	0.38 ± 0.04^{a}	0.16 ± 0.12^{a}	0.60 ± 0.32^{b}
CPG 30	0.39 ± 0.05^{a}	0.35 ± 0.14^{b}	0.44 ± 0.28^{b}

N= number of animals per group
Same letter within column means non - significant difference (P>0.05)
Different letter within column means significant difference (P<0.05)

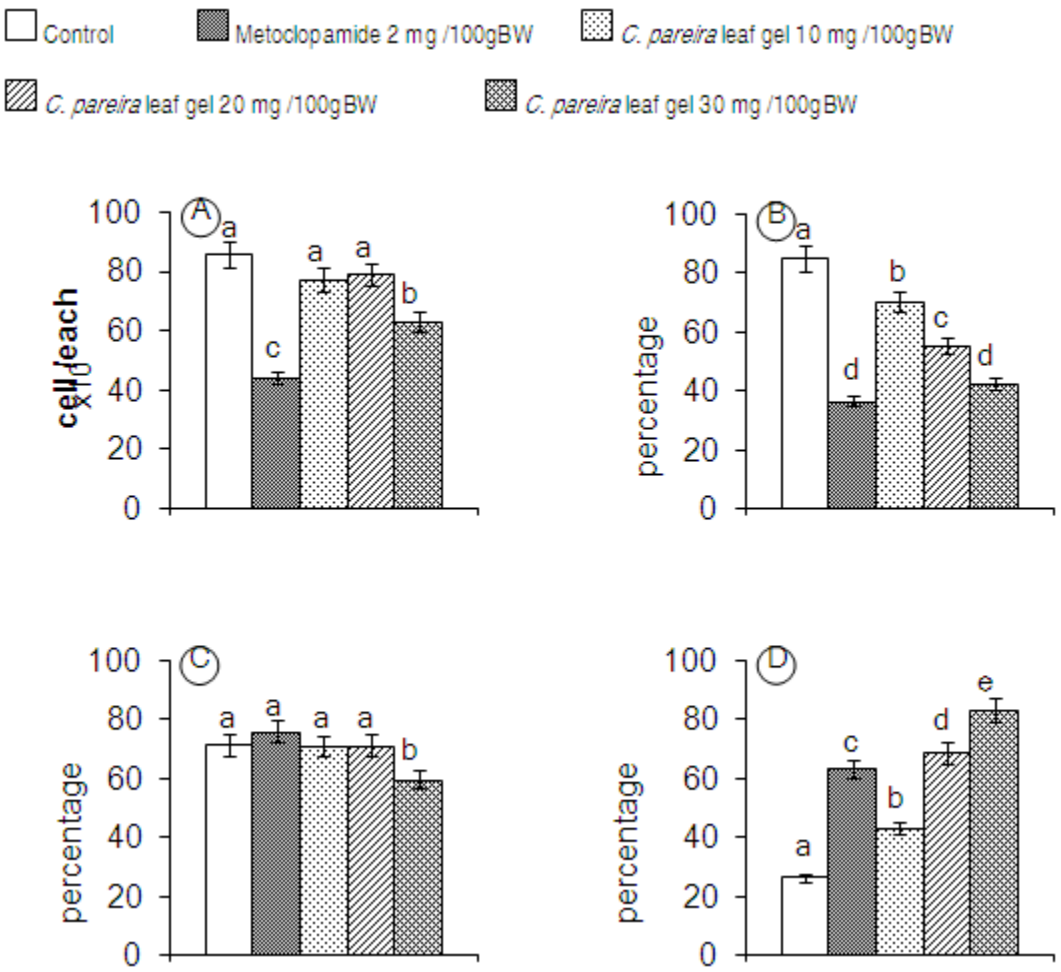

Figure 1 Effect of *C. pareira* leaf gel on sperm quality in male mice; A, total sperm count;B, motile sperms; C, viable sperms; D, abnormal morphology perms.Same letter within figure means non - significant difference (P>0.05)Different letter within figure means significant difference (P< 0.05)

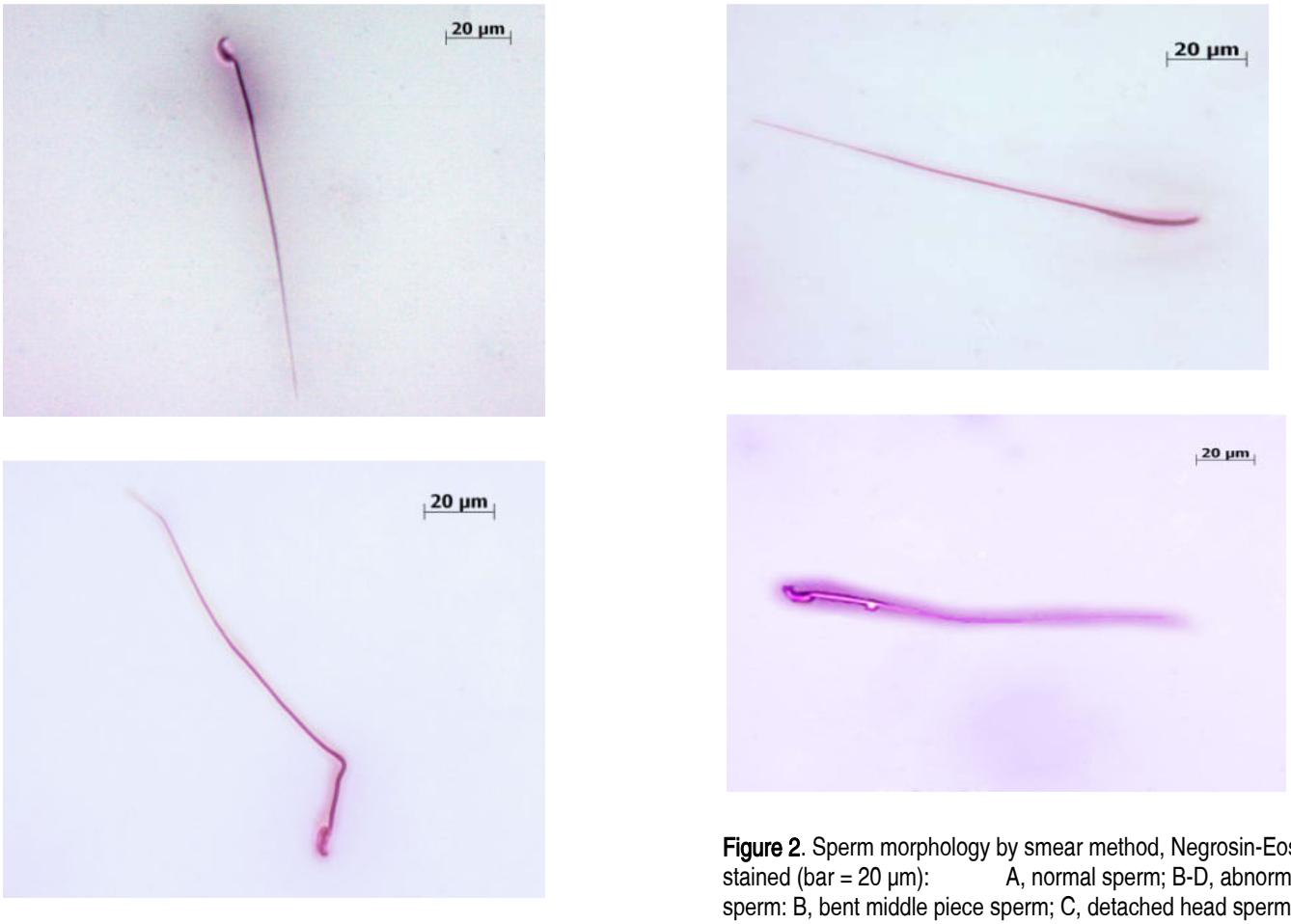

Figure 2. Sperm morphology by smear method, Negrosin-Eosin stained (bar = 20 µm): A, normal sperm; B-D, abnormal sperm: B, bent middle piece sperm; C, detached head sperm and D, medial protoplasmic droplet sperm.

Table 2 Effect of *C. pareira* leaf gel (CGP) on estrous cycle during 21 days of treatment

Treatment (mg/100 gBW) N=6	each stage duration of 21 days , $\overline{X}$ ±SD (days/estrous cycle)			Estrus index (%)
	proestrous	estrous	metestrous & diestrous	
0	1.00 ± 0.00 [a] (0.24)	7.83 ± 3.60 [a] (1.86)	12.17 ± 3.60 [a] (2.90)	37.30 [a]
Metoclopramide 2	2.20 ± 0.92 [b] (0.53)	4.00 ± 1.56 [b] (0.95)	14.80 ± 2.30 [a] (3.52)	19.05 [b]
CPG 40	1.70 ± 0.95 [a,b] (0.41)	7.00 ± 3.13 [a] (1.67)	12.30 ± 3.56 [a] (2.93)	33.33 [a]
CPG 60	1.38 ± 0.52 [a] (0.33)	6.88 ± 2.53 [a] (1.64)	12.75 ± 2.55 [a] (3.04)	32.74 [a]

N= number of animals per group
Same letter within column means non - significant difference (P>0.05),
Different letter within column means significant difference (P<0.05)

Table 3 Fertility index, prolactin level and branching of alveoli of pregnanted mice after 10 days of *C. pareira* leaf gel (CPG) treatment

Treatment (mg/100 gBW) N=6	Copora lutea ($\overline{X}$ ± SD, sites/each)	embryo implantation ($\overline{X}$ ± SD, sites/each)	Fertility index ($\overline{X}$ ± SD, %)	Prolactin ($\overline{X}$ ± SD, ng/ml)	Branching of alveoli in mammary gl. ($\overline{X}$ ± SD, %)
0	23.80 ± 1.64 [a]	14.20 ± 2.05 [a]	60.00 ± 10.67 [a]	0.14 ± 0.03 [a]	5.04 ± 2.02 [a]
Metoclopramide 2	28.00 ± 1.87 [b]	13.00 ± 3.16 [a,b]	47.07 ± 10.46 [a,b]	0.27 ± 0.04 [b]	8.59 ± 1.83 [b]
CPG 40	23.00 ± 4.24 [a]	9.50 ± 2.08 [b]	43.32 ± 7.98 [b]	0.15 ± 0.03 [a]	7.52 ± 2.04 [a,b]
CPG 60	23.20 ± 3.11 [a]	13.00 ± 2.92 [a,b]	56.94 ± 10.63 [a,b]	0.24 ± 0.09 [b]	8.34 ± 1.51 [b]

N= number of animals per group
Same letter within column means non - significant difference (P>0.05)
Different letter within column means significant difference (P<0.05)

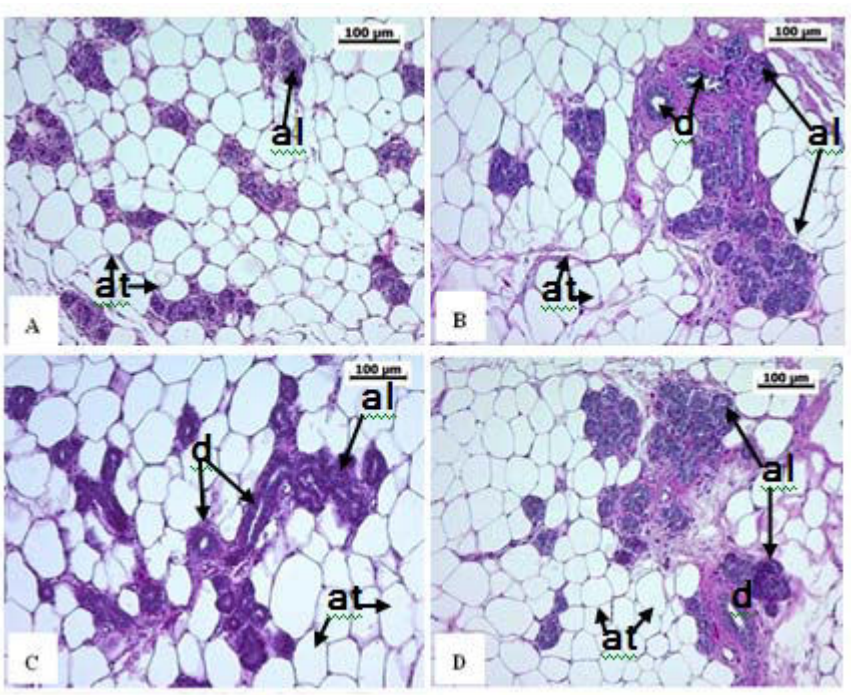

Figure 3. X-section of mammary glands (PAS stained, bar 100 µm): A, negative control group; B, positive control group received metoclopramide 2 mg/ 100 gBW; C & D, treated groups received *C. pareira* leaf gel 40 and 60 mg/ 100 gBW. (at= adipose tissue, d= duct, al=alveoli /milk sac)

Discussion

Prolactin (PRL) is well-known as a galactopoietic hormone in mammalian reproduction [16], while other functions are neglected. In animal physiology, PRL secretion is required to support testosterone synthesis; on the other hand, high PRL concentration has an inhibitory effect on reproductive function in both men and animals [17]. In man, hyperprolactinemia directly depresses gonadal function causing infertility [18]. This present study reveals that the leaf gel of *C. pareira* could be responsible for the antifertility activities in male mice after 21 days of treatment by increasing PRL level similar to metoclopramide, (a commcercial drug for stimulating prolactin release) [19]. Meanwhile, all treated groups showed a significant decrease in testosterone levels in a reverse dose depemdent manner. Testosterone is an important hormone affecting spermatogenesis and sperm maturation in epididymis and vas deferens [20]. Therefore, this hormonal disturbance may affect spermatogenesis and sperm maturation. However, the gonadal index of all groups did not show any change in this study. This implies that the disturbance testosterone level did not affect testicular weight. It is well-known that testicular weight does not determine sperm production [21], while blood PRL and testosterone levels are markers in the diagnosis of male infertility [22].

This study also evaluated the role of *C. pareira* leaf gel on spermatogenesis. The results exhibited that all doses of the leaf

gel caused adverse effect on seminal quality after 21 days of treatments, including a reduction in total sperm count, percentage of motile sperms and viable sperms. The degree of impairment was directly related to the decrease of blood testosterone. Furthermore, the abnormal sperms had secondary abnormality which occurred during sperm maturation in epididymis [23]. Spermatogenesis and sperm maturation are strictly correlated to the testosterone synthesis from Leydig cells in seminiferous tubules [20]. The high PRL condition could inhibit gonadotropin secretion, consequently inhibiting testosterone synthesis [17]. This occurrence leads to an affect on spermatogenesis and sperm maturation, causing impairment of sperm quality.

In females, groups treated with metoclopramide (2 mg/100 gBW), a commercial hyperprolactinemia-inducing agent) and groups treated with *C. pareira* leaf gel (40 and 60 mg/100 gBW) could be responsible for the antifertility effect causing irregular cycles and ovulation inhibition. However, the antifertility information of this plant is scarce. This result is incompatible to the methanolic leaf extract of *C. pareira* (25 and 45 mg/100 gBW) which caused an antifertility effect by prolonging the estrous cycle and elongation of the estrous stage [9]. However, the methanolic leaf extract of *C. pareira* (45 mg/ 100 gBW) altered reproductive hormones by increasing prolactin level in the proestrus stage and decreasing FSH of proestrous and estrous stages [9, 24], which implies that follicle growth was inhibited causing inability to ovulate. In the present study we also found that the number of embryo implantations was significantly decreased in the early pregnanted groups (1- 10 days of gestation) that received metoclopramide and *C. pareira* leaf gel, which is related to the increase of blood prolactin. This implies that *C. pareira* leaf gel increases prolactin in the pregnant group by metoclopramide activity. This result is concistent with the results of *C. pareira* methanolic leaf extract (45 mg/ 100 gBW) which decreased the litter number in albino mice after 21 days of treatment [9] This study also revealed that an increase in branching of alveoli in the mammary glands was found in pregnant groups treated with metoclopramide and *C. pareira* leaf gel. This finding could suggest that *C. pareira* leaf gel acts as an antifertility agent in females and a galactopoietics agent in pregnancy by prolactin stimulating properties.

Conclusion

C. pareira Linn.leaf gel had antifertility properties in male mice by increasing blood prolactin and decreasing testosterone levels after 21 days of oral administration. These occurrences may affect spermatogenesis and impair seminal quality. Meanwhile, the antifertility properties of *C. pareira* Linn. leaf gel were also found in females by alteration of estrous cycle, inhibition of ovulation and anti-implantation. Therefore, those using of *C. pareira* leaves as food or for therapy in traditional medicine should consider this adverse effect.

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References

[1]. Manu A, Tanvi S, Anu D, Neeraj B & Ahmad SA, An inside reviewof *Cissampelos pareira* Linn.: A potential of medicinal plant of India, Inter Res J Pharm, 2012, 3: 38-41.

[2]. Smitinand T & Larsen K, Flora of Thailand Vol. 5 Part 3. The Forest Herbarium Royal, Forest Department, Bangkok, 1991.

[3]. Singh A, Duggal S, Singh J & Katekhaye S, An inside preview of ethnopharmacology of *Cissampelos pareira* Linn., Inter J Biol Tech, 2010, 1: 114-120.

[4]. Singthong J, Cui SW, Ningsanond S & Goff HD, Structural characterization degree of esterification and some gelling properties of Krueo Ma Noy (*Cissampelos pareira*) pectin, Carbohyd Polym, 2004, 59: 391-400.

[5]. Mokkasamit M, Ngarmwathana W, Sawasdimongkol K & Permphiphat U, Pharmacological evaluation of Thai medicinal plants, J Med Ass Thailand, 1971, 54: 490- 503.

[6]. Dandiya PC & Chopra YM, CNS-active drugs from plants indigenous to India. Indian J Pharma. 1970, 2: 67–90.

[7]. Amresh G, Rao V, Mehrotra S & Shirwaiker A, Standardization and ethnopharmacological evaluation of antidiarrhoeal herbal formulation Manipal Academy of Higher Education ,Manipal (Dissertation), Karnataka, India, pp: 24, 2003.

[8]. Maurya R, Srivastava S, Kulshreshta DK & Gupta CM, Traditional remedies for fertility regulation, Curr Med Chem, 2004, 11: 1431-1450.

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

[10]. Harisha B, Antifertility effect of hydro-alcoholic extract of *Cissampelos*

pareira Linn.on male albino rats, Int J Pharml Res& Dev, 2012, 3: 87-93.

[11]. Luangpirom A, Sirisan W & Pontaisong J, Antifertility activity of the aqueous leaf extract of *Cissampelos pareira* in male albino mice, ABAH bioflux, 2010, 2: 59-64.

[12]. Cooper RL, Goldman JM & Vandenbergh JG, Mornitoring of the estrous cycle in the laboratory rodent by vaginal lavage. In method of toxicology in female reproductive toxicology. JJ Heindel and RE Chapin (Eds) Vol 3 pp57-58, San Diego, Academic press,USA, 2010, 189-248.

[13]. Baligar PN & Kaliwal BB, Induction of gonadal toxicity to female rats after chronic exposure to mancozeb, IND Health, 2001, 39: 235-243.

[14]. Masson JM, & Kang YJ, Test method for assessing female reproductive and developmental Toxicology, In principle and method of toxicology. Hayes, Raven Press. New York, 1994.

[15]. Zar JH, Biostatistical analysis. 5th edition, Pearson Prentice Hall, Inc. New Jersey, USA, 2010, 189-248.

[16]. Freeman ME, Kanyicska B, Lerant A & Nagy G, Prolactin: structure, function, and regulation of secretion, Physiol Rev, 2000, 80: 1523-1528..

[17]. De Rosa M, Zarrilli S, Di Sarno A, Milano N, Gacione M, Boggia B, Lombardi G & Colao A, Hyperprolactinemia in men, Endocrine, 2003, 20: 75.-82.

[18]. McCallum RW, Sowers JR, Hershman JM & Sturdevant RA, Metoclopramide stimulates prolactin secretion in man, J Clin Endocrineol Metab, 1976, 42: 1148-1152.

[19]. Masud S, Mehboob F & Bappi MU, Severe hyperprolactinemia directly depresses the gonadal activity causing infertility, Esculapio, 2007, 2: 25-27.

[20]. Jones RE, Hormonal control of sex accessory structure: In Human Reproductive Biology, 2nd edition, Academic press, New York. USA, 107-109.

[21]. Chandra AK, Sengupta P, Goswami H & Sarkar M, Excessive dietary calcium in the disruption of structural and functional status of adult male reproductive system in rat with possible mechanism, Mol Cell Biochem, 2012, 364: 181-191.

[22]. Chandra AK, Sengupta P, Goswami H & Sarkar M, Effect of dietary magnesium on testicular histology, steroidogenesis, spermatogenesis and oxidative marker in adult rats, Indian J Exper Biol, 2012, 51: 37-47.

[23]. Sorensen AMJ, Animal reproduction: principles and practice, Graw-Hill, USA, 1979.

[24]. Sharma RK, Goyal AK & Bhat RA, Antifertility of plant extracts on female reproduction: A review, Int J Pharm Bio Sci, 2013, 3: 493-514.