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Uterotrophic, Fetotoxic and Abortifacient Effect of a Malaysian Variety of *Plumbago rosea* L. on Isolated Rat Uterus and Pregnant Mice

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Abstract: Traditionally *Plumbago rosea* L. is used as an abortifacient in the Southeast Asian region. Methanolic root extract of a local species of *Plumbago rosea* L. was studied to evaluate its traditional antifertility claim. Interestingly, it was found to possess dose related inhibitory effect on uterine contractile responses elicited by oxytocic agents on isolated uteri of pregnant and pseudo-pregnant rats. Furthermore, it was found to possess significant ($p<0.05$) fetotoxic activity along with mild abortive potential in pregnant mice when given orally at high doses (400 and 800 mg kg^{-1}) once daily for ten days starting from day 10 of gestation. The results derived indicated possible presence of utero-active compound (s) in this plant that inhibited oxytocic agents induced uterine motility. Moreover, pronounced fetotoxic and mild abortifacient potentials observed at higher doses in pregnant mice might support its accredited traditional use to avoid unwanted pregnancy.

Key words: Abortifacient activity, fetotoxicity, uterotrophic activity

INTRODUCTION

Ethnomedical literature recorded in several ancient folkloric medicinal systems contains thousands of references on the use of plants for a variety of reproduction related purposes (Badami *et al*., 2003). Recently continuous efforts are on going to develop antifertility products from plants. Plant based contraceptive measures such as crude plant extract or composite preparation with scientifically proven efficacy could be of beneficial and appreciable to the poor population of some of the developing countries with population burden, as the cost of such contraceptive choices would be within their affordability. Indeed, extensive researches are being carried out to evaluate the putative abortifacient and other antifertility activities of different plants as well as traditionally used folk contraceptives all over the world. During the last few decades many medicinal plants have been tested for their traditionally claimed antifertility related activities by employing modern pharmacological approaches and some of them have gained scientific supports for their abortifacient, anti-implantation and other antifertility activities (Kamath and Rana, 2002; Elbetieha *et al*., 2000; Mukherjee *et al*., 1996; Lemonica and Alvorenga, 1994).

This study sets out to evaluate the traditionally claimed antifertility activity of local variety of *Plumbago rosea* L. root. In the ancient Sanskrit medicinal literature, the abortifacient activity of this plant has been recorded (Burkhil, 1966). In Malaysia it is known as Cheraka merah and has several folk medicinal uses in the Southeast Asian countries. Locally it is used as an abortifacient by chewing the roots for sometimes. Beside its antifertility activity, other folk medicinal uses of this plant include uses in rheumatism, leprosy, stimulation of digestion and as emmenagogue (Burkil, 1966; Padua *et al*., 1999).

Scientific research of antifertility activity along with other activities of this plant as single preparation or as part of composite preparation has also been done in some parts of the world (Sharma and Mahanta, 2000; Devi *et al*., 1998; Solomon *et al*., 1993; Lal *et al*., 1983). However, to the best of our knowledge and based on literature survey in Medline and other scientific resource database, no scientific research has yet been done on the uterotrophic, fetotoxicity and abortifacient activity of the local Malaysian variety of *Plumbago rosea* L. To this end, in this study an attempt has been made to evaluate the traditionally claimed antifertility activity of this plant using animal model through *in vitro* and *in vivo* approaches.

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MATERIALS AND METHODS

Plant material: Plant *Plumbago rosea* L. was collected from local source at Penang, Malaysia. Identification of the plant was done in the School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia and a voucher specimen has been kept in our laboratory for future reference.

Preparation of extract: Roots of the plants were cut into pieces (2-3 inches long), dried in oven at 40°C for 48 h and milled to powder. The powdered plant materials was then soaked in distilled water in a water bath preheated to 40°C for 24 h, filtered through a linen cloth followed by filter paper (Whatman, Maidstone, UK). Starch, proteins and tannins were then precipitated from the filtrate using excess lead acetate solution followed by filtration with filter paper (Whatman, Maidstone, UK). Saturated ammonium sulfate solution was then added to the obtained yellowish filtrate and again filtered using filter paper. The final filtrate was then dried in a rotary evaporator at 70°C and the dried material was then dissolved in methanol to separate the ammonium sulfate and to dissolve the active compounds. The suspension was filtered again and dried in a rotory evaporator at 40°C until a concentrated solution was obtained which was finally kept at 4°C until further use. The total yield of the methanolic root extract was approximately 15% in terms of dry weight of the roots.

***In vitro* study:** *In vitro* study was done using uteri of pregnant and pseudo-pregnant albino rats obtained from the animal house facility of Universiti Sains Malaysia, Penang, Malaysia. Adult female albino rats were given silbesterol (0.1 mg kg^{-1}) sub-cutenenously 24 h prior to the study to achieve the estrous phase of the vaginal cycle as it is considered that response to uterine stimulants like oxytocin or acetylcholine is greater in this phase. Routine vaginal swabs were done to confirm the onset of pregnancy. Pregnant and pseudo-pregnant rats were then sacrificed by cervical dislocation under light ether anesthesia and uteri were collected for *in vitro* study. The collected uteri from both pregnant and pseudo-pregnant rats were washed with ringer's solution. Approximately 1.5 cm of the uterus was cut and suspended in an organ bath filled with ringer's solution (20 mL) and the bath was continuously aerated with a mixture of 95% O_2 and 5% CO_2. The bath temperature was maintained at 37°C using a thermostat. The tissue was attached to a pressure transducer that was coupled to a polygraph (Grass model 79E, Grass Instrument Co., Quincy, Mass. USA). The tissues were stabilized until no spontaneous contraction was observed. During the stabilization period the tissue was washed with ringer's solution every 15 min.

In the initial steps of the *in vitro* study, sub-maximal doses of acetylcholine and oxytocin were established using dose response curves of oxytocin and acetylcholine. To determine the sub-maximal doses, a sub-maximum contraction range of 30-70% of the maximum contraction elicited by the maximum doses of either oxytocic agent was considered. Graded doses of *Plumbago rosea* L. extract were used to study its possible inhibitory effect on the responses elicited by these oxytocic agents.

Acute toxicity study: Acute toxicity study in mice (n = 12) revealed that the LD_{50} of the root extract of *Plumbago rosea* L. is ≥1000 mg kg^{-1} (data not shown).

***In vivo* study:** Female and male albino mice were used in this study. Female and male animals were housed separately in a ratio of 3:1 for copulation. Routine vaginal swabs were done to determine the onset of pregnancy. Pregnant mice were then randomly grouped (n = 10) into four treatment groups and one control group. Treatment group animals were dosed once daily with graded doses (100, 200, 400 and 800 mg kg^{-1}) of the extract and control group animals received distilled water through oral gavage starting from day 10 of pregnancy. The animals were kept in the animal house facility of Universiti Sains Malaysia, Penang, Malaysia in proper condition legitimized by animal ethics committee with free access to water and food. Animals were monitored for any occurrence of abortion till day 19 of pregnancy. Animals were then sacrificed by cervical dislocation under light ether anesthesia and autopsy was done to examine the number of pregnant mice, viable and dead fetuses. Further, the fetuses were also examined for any external deformities.

Statistical analysis was done using student's t-test and the results were considered significant at $p<0.05$.

RESULTS

Uterotrophic effect of *Plumbago rosea* L. extract: In the initial experiments dose responses of acetylcholine and oxytocin were determined in an attempt to establish the sub-maximal dose used in the subsequent experiments with the plant extract. It was determined that the minimum dose of acetylcholine to produce response to uteri of pseudo-pregnant rats was 1.0×10^{-5} M, while the sub-maximal and maximal doses were 4.0×10^{-5} and 6.4×10^{-4} M, respectively. Whereas, on the same tissue sample the minimum, sub-maximal and maximal doses of oxytocin were 1.0×10^{-3} M, 2.0×10^{-3} M and 6.4×10^{-2} M, respectively (Table 1).

Table 1: Dose responses of acetylcholine and oxytocin on uteri of pregnant and pseudo-pregnant rat. Five (n = 5) different observations were made for each dose of either oxytocic agent used

Acetylcholine			Oxytocin		
Response on uterus (Developed tension in g) (mean±SEM)			Response on uterus (Developed tension in g) (mean±SEM)		
Doses (M)	Pregnant rat	Pseudo-pregnant rat	Doses (M)	Pregnant rat	Pseudo-pregnant rat
1.0×10^{-5}	5.0±0.97	4.67±1.61	1.0×10^{-3}	11.5±1.14	6.83±0.46*
2.0×10^{-5}	10.33±1.52	11.5±2.96	2.0×10^{-3}	17.0±1.29	14.67±2.33
4.0×10^{-5}	16.33±1.63	16.16±3.06	4.0×10^{-3}	21.67±1.02	19.17±2.36
8.0×10^{-5}	20.50±1.41	20.67±3.09	8.0×10^{-3}	26.0±1.61	23.5±2.57
1.6×10^{-4}	24.17±1.38	24.5±2.99	1.6×10^{-2}	30.0±1.13	27.67±2.21
3.2×10^{-4}	29.17±2.89	27.67±3.42	3.2×10^{-2}	33.3±1.26	30.5±2.11*
6.4×10^{-4}	29.83±2.68	29.83±4.17	6.4×10^{-2}	33.3±1.36	31.5±2.51

*Significant at $p<0.05$

Table 2: Effect of methanolic extract of *Plumbago rosea* L. root on the response of acetylcholine and oxytocin on uteri of pregnant and pseudo-pregnant rats. Five (n = 5) different observations were made for each dose of the extract used

Pregnant rat uterus					Pseudo-pregnant rat uterus				
Dose of extract (mg)	Dose of acetylcholine (M)	Inhibition of response* (%)	Dose of Oxytocin (M)	Inhibition of response* (%)	Dose of extract (mg)	Dose of acetylcholine (M)	Inhibition of response* (%)	Dose of Oxytocin (M)	Inhibition of response* (%)
6.4×10^{-2}	4.0×10^{-5}	3.5±1.1	2.0×10^{-3}	9.3±3.4	6.4×10^{-2}	4.0×10^{-5}	4.2±1.7	2.0×10^{-3}	6.93±4.9
12.8×10^{-1}	4.0×10^{-5}	16.6±4.3	2.0×10^{-3}	17.9±4.8	12.8×10^{-1}	4.0×10^{-5}	13.6±3.3	2.0×10^{-3}	15.63±6.1
25.6×10^{-1}	4.0×10^{-5}	28.3±8.8	2.0×10^{-3}	18.8±5.0	25.6×10^{-1}	4.0×10^{-5}	29.4±3.5	2.0×10^{-3}	21.18±3.0
51.2×10^{-1}	4.0×10^{-5}	58.3±12.3	2.0×10^{-3}	68.9±5.1	51.2×10^{-1}	4.0×10^{-5}	55.4±5.5	2.0×10^{-3}	50.18±12.0
103.0×10^{-1}	4.0×10^{-5}	95.5±2.6	2.0×10^{-3}	97.1±2.0	103.0×10^{-1}	4.0×10^{-5}	97.1±1.9	2.0×10^{-3}	97.11±1.4

*Data of % inhibition of oxytocic agents induced responses are presented here as mean±SEM None of the observations were significant ($p<0.05$) when % inhibitory responses to either oxytocic agents were compared between pregnant and pseudo-pragnant animals

Table 3: Effect of methanolic extract of *Plumbago rosea* L. root on pregnant mice

P. rosea L. extract dose (mg/kg i.p.)	No. of pregnant mice	No. of live fetus	No. of dead fetus	Mortality of pregnant mice	No. of mice suffered from abortion	Average of dead fetus/mouse (%) (mean±SD)
100	10	84	9	-	-	8.63±9.10
200	10	77	13	-	-	15.00±8.83
400	10	68	26	1	1	28.15±26.22*
800	10	-	65	6	4	100.00±0.00*
Control	10	78	7	-	-	7.80±8.02

*Significant, $p<0.05$ (Student t-test). The LD_{50} of the root extract of *Plumbago rosea* L. was ≥ 1000 mg kg^{-1}

Moreover, the dose response of actetylcholine and oxytocin was also studied on the uteri of pregnant rat. For acetylcholine the minimum, sub-maximal and maximal doses were 1.0×10^{-5} M, 4.0×10^{-5} M and 6.4×10^{-4} M, respectively. On the same tissue type these doses for oxytocin were 1.0×10^{-3} M, 2.0×10^{-3} M and 3.2×10^{-2} M, respectively (Table 1).

In the subsequent experiments the uterotrophic effect of methanolic extract of *Plumbago rosea* L. root was determined using the sub-maximal doses of acetylcholine and oxytocin established in experiments with uteri from pregnant and pseudo-pregnant rats. Five independent observations were made for each doses of the extract used. It was observed that the extract exhibited a dose related inhibitory effect on the acetylcholine and oxytocin elicited uterine contractile activity. The data presented are mean±SEM (Table 2).

***In vivo* study on antifertility effect:** In this study, groups of mice were dosed with graded doses (100, 200, 400 and 800 mg kg^{-1}) of methanolic root extract of *Plumbago rosea* L. once daily for ten days starting from day 10 of gestation. It was observed that lower doses, 100 and 200 mg kg^{-1} did not produce any significant adverse effect in terms of fetotoxicity, abortion and death of animals. However, fetuses were smaller in size when compared with those of control group animals. Whereas, moderate to severe fetotoxicity was observed at higher doses with 100% fetal mortality in the group treated with 800 mg kg^{-1}. Moreover, in some treatment groups, surviving fetuses were found stunted when compared with those of control animals but were without any external malformations. Death of pregnant mice and occurrence of abortion also occurred in these dose groups although none of these observations were significant (Table 3).

DISCUSSION

In this study, uterotrophic, fetotoxic and abortifacient activity of a local variety of *Plumbago rosea* L. were studied. In the *in vitro* study, an attempt was made to see

the effect of the *Plumbago rosea* L. root extract on contraction produced by acetylcholine and oxytocin on isolated rat uteri. Acetylcholine is known to increase the uterine contraction and its action is proportional to its concentration and a similar mode of utero-stimulatory action of oxytocin is also known. It is also known that the effect of oxytocin depends on the level of estrogen and a decreased effect was observed in tissues with low estrogen level (Chan, 1980; 1963). The data obtained in this study further strengthen this view as uterine contractile activity of oxytocin was greater in uteri of pregnant animal as compared to that of pseudo-pregnant animals (Table 1). In the subsequent experiments with the extract, it was observed that the methanolic root extract of *Plumbago rosea* L. exhibited dose dependent inhibitory effect on the acetylcholine and oxytocin (Table 2) induced uterine contractions and hence indicated the possible presence of utero-active compound (s) that might be involved in such inhibitory effect. Further, it can be suggested that uterotrophic activity of *Plumbago rosea* L. extract might have similarity with effects elicited by adrenaline, histamine or prostaglandin antagonists as these agents are well know to inhibit uterine contraction (Datte *et al*., 1996; Vulliemoz, 1981). The findings of *in vitro* study led us to speculate that any possible antifertility potential of methanolic root extract of *Plumbago rosea* L. might not be due to its direct effect on uterus of animal.

However, in the *in vivo* study, fetotoxicity and a mild abortifacient activity of *Plumbago rosea* L. root extract was observed. It was observed that mice treated with higher doses had abortion on day 18 and day 19 of pregnancy and this might be due to the possible expansion and evacuation of uterus as these events can cause abortion. In relation to this, based on the *in vitro* results, other possibility might be a putative utero-active activity of the extract, which probably caused uterine contraction at these doses.

Death of fetuses was also observed in animals dosed with higher doses of extract. Moreover, it was observed that the highest dose, 800 mg kg^{-1} caused 100% mortality in fetuses (Table 3). This might be due to maternal toxicity caused by the extract as such toxicity may cause late fetal death (Khera, 1985). Other possibility might be inhibition of mitotic division of the fetus as the animals were treated during stage of organogenesis, which usually occur between day 6-15 of gestation in rodent (Goonasekera *et al*., 1995). Death of pregnant mice was also observed in this study, which might be due to toxic effect of the dose of extract used (Table 3). However, in toxicity study it was observed that the 24 h LD_{50} of *Plumbago rosea* L. in normal mice were 239.88 and 1250 mg kg^{-1} for intraperitonial and oral administration, respectively. As reported earlier, in sub-acute toxicity study, although no mortality was observed, some changes were observed in terms of organ weight along with changes in biochemical parameters (Solomon *et al*., 1993). These findings might be implicated in explaining a possible low level toxic effect of this plant extract in mice.

In conclusion, data obtained in both *in vitro* and *in vivo* study suggested that this local Malaysian variety of *Plumbago rosea* L. might have uterotrophic activity but not oxytocic activity. The plant also exhibited pronounced fetotoxicity and mild abortifacient activity. Hence, the present study provides some supports for its accredited traditional use as abortifacient that has been in practice in this region. However, further in-depth studies are required to substantiate these results and isolation of compounds with possible antifertility potentials should be pursued to develop this plant based contraceptive measures.

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