Research Paper

Evaluation of the female reproductive toxicity of aqueous extract of *Labisia pumila var. alata* in rats

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Received: 11.8.2005
Revised: 22.9.2005
Accepted: 29.1.2006

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ABSTRACT

Objective: To detect potential adverse effects of aqueous extract of *Labisia pumila var. alata* (LPE) or `Kacip Fatimah' on the estrous cycle, reproductive performance, post-natal growth and offspring survival of rats.

Materials and Methods: Forty eight (48) female Sprague Dawley rats with consecutive 4 to 6 days estrous cycle were given distilled water (as control) or LPE at 2, 20, 200, 400 or 800 mg/kg daily by gavaging ten days prior to mating, mating (a maximum period of ten days), gestation and lactation periods of seven days. Dams and fetuses were sacrificed on day seven postnatal.

Results: *Labisia pumila* extracts did not alter the estrous cycle and general health of all rats. All the animals proceed towards successful mating and pregnancies. There was no significant difference in the duration of pregnancy and all pregnant rats delivered normally. Statistically no test agent-related changes in the maternal body weight, number of implantations, litter size and pup body weights were observed. Other parameters measured include pup sex ratio, live birth index, pup viability index and percentage of implantation death which also showed no significant difference.

Conclusion: The present findings indicate that water based extracts of *Labisia pumila var. alata* do not pose any significant reproductive toxicity or complication in pregnancy and delivery in rats. The extract did not significantly alter the duration of pregnancy in rats, however the duration of delivery was not evaluated in this study.

KEY WORDS: Herb, adverse effect, estrous cycle, pregnancy, lactation.

Labisia pumila var. alata (LPA) or popularly known in Malaysia as Kacip Fatimah (KF), is a very popular herb amongst the local womenfolk. Traditionally, the water decoction of the root or whole plant of KF are consumed by the Malay women for induction and facilitation of labour.[1] Currently there are many commercial products containing this herb have emerged in Malaysian market for the purpose of enhancing vitality and libido, however, there is no scientific data on their quality, safety, and efficacy to substantiate claims. Studies supported by the Government of Malaysia, involving this herb conducted at various universities and institutes are in various stages, like extract preparation-standardization (undergoing patenting), authentication, and evaluation of safety and efficacy. Reports have shown that the LPA displayed a non-significant response to *in vitro* estrogen activity[2] and had appreciable amount of iron.[3] In addition, LPA root and leaves were found to contain two novel benzoquinoid compounds 1, 2 as major components.[3] More information regarding this herb is expected to be available in the near future.

The objectives of the present study are to evaluate the female reproductive toxicity and potential effect of KF in inducing labor in rats. Standardized aqueous extract of *Labisia pumila var. alata* (LPE) at doses of 2-800 mg/kg/day were administered to determine the safety and efficacy of this herb. The general acute and sub-acute (28 days) toxicity studies of the same extract in rats were already performed by a team in Herbal Medicine Research Center of Institute for Medical Research, Kuala Lumpur, Malaysia. The results of the study revealed that the estimated LD_{50} of the extract is more than 5 g/kg BW and the extract produced no significant adverse effects (personal communication). A chronic toxicity study is on going and at present, no deleterious effects is observed in rats. A phase 2 clinical trial conducted in post-menopausal women by a research team at School of Medical Sciences, Universiti Sains, Malaysia concluded that the therapeutic dose of the extract is 2.5 mg/kg/day (personal communication).

About 48 female Sprague Dawley rats were used in this Segment I (female reproductive toxicity) study. Rats with

regular 4-6 days estrous cycle were given vehicle (distilled water) as control or LPE at 2, 20, 200, 400, or 800 mg/kg daily, by gavage, 10 days prior to mating, during mating (a maximum period of 10 days), throughout gestation and lactation periods of 7 days. Dams were permitted to deliver their litters naturally. At birth (day 1), pups were individually counted, weighed, and examined for external malformation and sexed. Dams and fetuses were sacrificed on day seven postpartum.[4] The parameters measured are presented in Table 1. Data were analyzed using SPSS version 11.0. Data on maternal body weight throughout the study was analyzed by General Linear Model Repeated Measures. Mean days of estrous cycle, length of pregnancy (days), pregnancy index, number of pups both at birth and on D7 lactation (litter size), pups body weight during lactation and number of implantation sites per litter were analyzed by one-way ANOVA, followed by Scheffe test if differences were found. Additionally, Kruskal Wallis test (non-parametric) followed by Mann-Whitney test (when appropriate) were used to assess the live birth index, viability index and percentage of post-implantation death. The

Table 1

Parameters measured in rats treated with *Labisia pumila* aqueous extract prior to mating, mating period, gestation, and lactation periods of 7 days

Maternal	*Pregnancy*	*Fetal*
1. General observation and behavior (sleepy, lethargy, withdrawn, excitement, abnormal posture, respiratory, changes of excrement, and abnormal coat condition)	1. Length of pregnancy (days).	1. Number of pups at birth (litter size)
2. Fates of females (survivability or death)	2. Pregnancy index = (No. of females delivering live young/ No. of females with evidence of pregnancy) × 100	2. Sex distribution (at birth and day 7 post-natal)
3. Body weight throughout study		3. Live birth index = (No. of live offspring/ No. of offspring delivered) × 100
4. Estrous cycle (duration and cyclicity)		4. Viability index = (No. of live offspring at D7 lactation/No. of live offspring born) × 100
5. Mating index = (No. of females mated/ No. of females cohabited) × 100		5. Growth rate of offspring during lactation (D1–D7 postnatal)
6. Maternal visceral changes (day 7. post-partum).		6. Body weight of male and female pups (D1–D7 postnatal)
		7. Gross malformation
		8. Pups development (movement, spontaneous righting reflexes and forelimbs grasp)
		9. Number of implantation sites at autopsy
		10. Percentage of post implantation death = (No. implantation – No. live fetuses/ No. implantation × 100)

Table 2

Pregnancy and fetal parameters of rats treated with *Labisia pumila* aqueous extract prior to mating, mating period, gestation and lactation periods of 7 days

	Group 1 0 mg/ kg/day	*Group 2 2 mg/ kg/day*	*Group 3 20 mg/ kg/day*	*Group 4 200 mg/ kg/day*	*Group 5 400 mg/ kg/day*	*Group 6 800 mg/ kg/day*	*P-value*
No. of rats examined	8	8	8	8	8	8	–
Pregnancy index (%)[a]	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	100 (0)	n/s
Length of pregnancy (days)[a]	22.00 ± 0.19	22.00 ± 0	21.63 ± 0.18	21.88 ± 0.23	21.75 ± 0.31	21.38 ± 0.42	n/s
No. of pups at birth / litter[a]	7.63 ± 1.31	8.13 ± 0.93	8.63 ± 0.86	8.25 ± 0.70	8.50 ± 0.93	7.38 ± 0.60	n/s
Live birth index (%)[b]	100 (7.50)	100 (0)	100 (0)	100 (9.38)	100 (0)	100 (0)	n/s
No. of pups on day 7 post natal[a]	6.63 ± 1.24	7.75 ± 0.92	8.13 ± 0.97	7.50 ± 0.78	7.50 ± 1.15	7.38 ± 0.60	n/s
Viability index (%)[b]	100 (10.42)	100 (8.33)	100 (6.82)	100 (18.75)	100 (0)	100 (0)	n/s
Implantation sites / litter[a]	8.88 ± 1.08	10.25 ± 0.96	9.88 ± 0.67	9.13 ± 0.55	10.25 ± 0.53	9.25 ± 0.59	n/s
Post implantation death (%)[b]	10.56 (39.40)	19.45 (27.04)	4.17 (24.31)	9.17 (21.53)	13.64 (39.65)	20.20 (27.68)	n/s
Sex ratio M: F (at birth)	1.33: 1	1.37: 1	1.09: 1	1.06: 1	1.26: 1	1.57: 1	n/s
Sex ratio M: F (at D7 post natal)	1.35: 1	1.35: 1	1.10: 1	1.07: 1	1.31: 1	1.57: 1	n/s
Malformations of pups	Nil	Nil	Nil	Nil	Nil	Nil	-

Values are [a]mean ± SEM; [b]median (Interquartile range); $P>0.05$ between all groups: Statistically not significant

level of significance was set at 5%. The parametric data were expressed as Mean ± Standard error of mean (SEM) or ratio while the non-parametric data were expressed as median (Inter quartile Range) (IQR).

Results indicated that LPA extracts did not alter the general health or estrous cycle of rats. All studied animals proceed toward successful mating and pregnancies. The mean duration of pregnancy (in days) was shortened to 21 days in animals that received the herbal extract at a dose of 20 mg/kg/day and above however, it was not statistically significant. All pregnant rats were delivered normally with no evidence of prematurity or abortion suggesting that the extract is not an abortifacient or having prostaglandin like activity when consumed orally. None of the rats exhibited significant amount of fetal resorption indicating that the herb was nontoxic to the fetuses. Statistically no test agent-related changes in the maternal body weight, number of implantations, litter size, and pup body weights were observed. No significant difference in pup sex ratio, live birth index, pup viability index and percentage of post implantation death were noted in this study.

The present findings indicated that water based extracts of *Labisia pumila var. alata* do not pose any significant reproductive toxicity or complication in pregnancy, delivery and early pup growth in rats. The no observable adverse effect level (NOAEL) of the extract in this study is 800 mg/kg/day. A closer observation on the duration of pregnancy (in hours) and parturition time were not evaluated in this study to support the traditional claim of this herb.

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