PHARMACOLOGICAL EVALUATION OF AQUEOUS ROOT EXTRACT OF *SELAYAK HITAM*: TERATOGENIC AND POSSIBLE ABORTIFACIENT EFFECT

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SUMMARY

Studies made on aqueous root extract of Selayak Hitam, *a plant alleged to possess abortifacient activity in pregnant mothers established that the extract is teratogenic and did in fact cause abortion in mice. It was also observed that the aqueous root extract is relatively toxic as judged by the number of deaths occuring following administration of the extract. The mechanism by which abortion is brought about is unknown but it is possible that the abortifacient effect is due to the induced teratogenic activity, brought about by the extract.*

INTRODUCTION

Aqueous extract of the root of *Selayak Hitam*, a plant commonly found in Malaysian jungle had been reported and used as abortifacient by pregnant women to induce abortion. We had previously studied this aqueous root extract for possible oxytoxic activity, for oxytocin in sufficient concentration can initiate and contract uterus, thus resulting in therapeutic abortion.[1] However, no such effect exists,[2] as abortion can also be the result of embryotoxicity that is brought about by certain chemical agents,[3] it is therefore of interest to screen the aqueous root extract of *Selayak Hitam* for teratogenic activity and for abortifacient effect in pregnant mice.

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MATERIALS AND METHOD

Preparation of aqueous extract

Aqueous extract of *Selayak Hitam (SH)* roots was prepared according to the method described earlier.[2]

Animals used

Adult mice weighing 35-40 g were used in the present investigation. Pregnancy in the female animal was confirmed by the usual vaginal smear method after an overnight mating between one male and four females in a cage. This is taken as day 1 of pregnancy.

Experimental protocol

In attempting to establish the teratogenic effect of the extract, 35 pregnant mice were obtained for this series of experiments. The mice were divided into four groups: A, B, C, and D. The mice in group A served as control. Each mouse in group

B received 100 mg, that of group C received 200 mg and that of group D received 400 mg of SH extract. The extract was given as a single dose. Administration of SH extract was made on day 12 of pregnancy by oral route. Control animals each received an equal volume of tap water. Day 12 pregnancy was chosen because it had been shown earlier that in mice and in rats this periods is within the sensitivity period of fetal development.[4] All animals were fed on standard food pellets and tap water *ad libidum* throughout the entire period of experimentation. Daily observations were made on all animals. Since cannibalism is frequent amongst rodents especially if the fetuses are malformed, caesarean operation was performed on all pregnant mice under pentobarbitone general anaesthesia one or two days before term to obtain reliable data. Observations were then made on the number of implantation site(s), the number of fetus-dead or live and recording made on any gross abnormality.

In the second series of experiments, a total of 23 pregnant mice were obtained. Of these twenty three, three served as control while the other twenty were subjected to cumulative administration of aqueous SH extract beginning on day 15 of pregnancy. On this day, each of the twenty mice received 400 mg of SH extract orally. This dose was increased daily until the surviving mice each received a maximum dose of 1600 mg extract given not later than day 17 of pregnancy according to the schedule shown in Table I. Control mice, each received an equal volume of tap water accordingly. No attempt was made to recover the fetus through caesarean operation in this series of experiments and surviving animals were allowed to proceed to term. Close observations were daily on each animal. All animals were fed on standard food pellet and tap water *ad libidum* throughout.

RESULTS

Table II summarises the effect of SH root extract on the development of the fetuses whose mother received varying dosage of the extract. Fetuses obtained from caesarean operation showed that some of these fetuses were dead and many of the fetuses were malformed. The nature and degree of malformation is shown in Figs. 1 and 2 and they include general body defects. This teratogenic effect appears to be dose-dependent and is evident in group C where each mouse received 200 mg of SH extract given as single dose. The incidence of teratogenic effect is higher in group D where each mouse received 400 mg of SH extract.

TABLE I
CUMULATIVE DOSING SCHEDULE OF *SELAYAK HITAM* EXTRACT

	Day of Pregnancy			
Group	15	16	17 (a.m.)	17 (p.m.)
Control (3)	water (n=3)	water (n=3)	water (n=3)	water (n=3)
Experimental (20)	400 mg (n=20)	800 mg (n=17)	1600 mg (n=11)	1200 mg (n=7)

( ) denotes the number of animals used in each group

TABLE II
EFFECT OF VARYING DOSAGE OF *SELAYAK HITAM* EXTRACT ON THE DEVELOPMENT OF FETUSES

Group	Treatment	Total Fetuses	Dead Fetuses (%)	Malformed Fetuses (%)
A	Control (9) SH Extract (mg/animal)	94	0	0
B	100 (8)	84	0	0
C	200 (9)	92	11 (12%)	10 (11%)
D	400 (9)	91	20 (22%)	15 (17%)

( ) denotes the number of animals used in each group

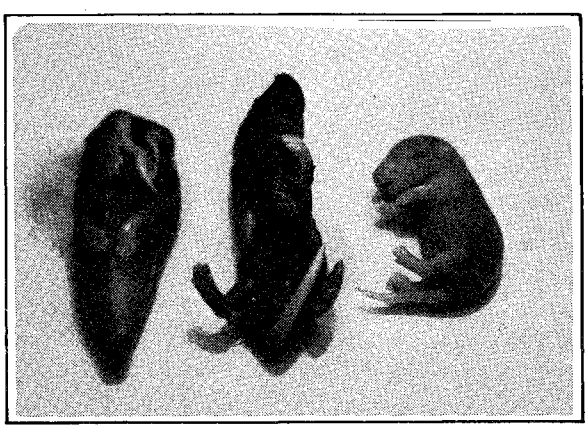

Fig. 1 Malformed fetuses (centre and left) as compared to a normal fetus (right).

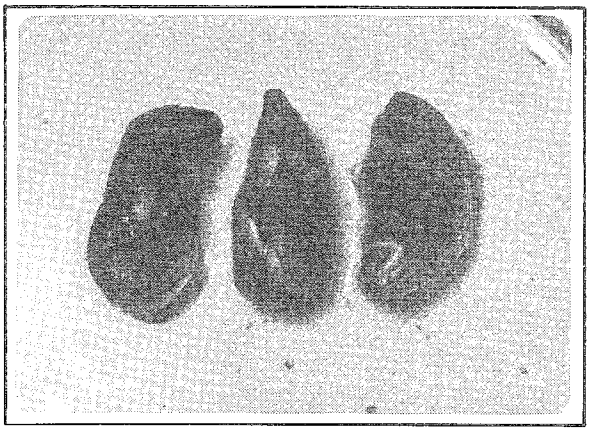

Fig. 2 Malformed fetuses. Note the general body defects in all.

Table III summarises the effect of cumulative administration of aqueous SH extract on both the mother and the fetuses. Control study showed that both the mother and the fetus were normal. No toxic symptoms were detected and the number of fetuses born averaged 10 per litter. However, when 200 mg of aqueous SH extract was given to each and every of the 20 mice on day 15 of pregnancy, one died, two mothers delivered normal fetuses and vaginal bleeding was seen in one. On day 16 of pregnancy, each of the remaining 17 mice received 400 mg of the extract making the total extract received to 800 mg; four died, two normal birth-fetuses were all premature as shown in Fig. 3. Not only the fetuses were premature but the litter size had been reduced from an average of ten to six. The mother that had vaginal bleeding before, survived and it survived until day 19 of pregnancy and when sacrificed, no fetuses were to be found although there were four implantation sites, indicating that the fetuses had been aborted earlier. In the morning of day 17 of pregnancy, each of the 11 surviving mice received a further 400 mg bringing the total extract received to 1200 mg; four more died. Another died on the same evening when each of the remaining seven mice received another 400 mg of extract bringing the total extract received to 1600 mg. No extract was given after day 17 and on day 18 of pregnancy it was observed that of the remaining six mice; 2 gave birth to premature fetuses (litter size three and five) and vaginal bleeding was observed in one that gave birth to 11 fetuses, all premature. Premature fetuses are shown in Fig. 4. Finally on

TABLE III
EFFECT OF CUMULATIVE ADMINISTRATION OF *SELA YAK HITAM* EXTRACT ON BOTH THE MOTHER AND THE FETUSES OF MICE

Treatment	n	Observations on	
		Mother	Fetuses
Control	3	normal	30(12, 8, 10) all normal
PM Extract (mg/animal) Day 15 of Pregnancy (400)	20	1 died 2 deliver normal fetuses 1 vaginal bleeding	20(10,10) – all normal
Day 16 of Pregnancy (800)	17	4 died 2 births	12(6,6) all premature
Day 17 of Pregnancy a.m. (1200)	11	4 died	
Day of Pregnancy p.m. (1600)	7	1 died	
Day 18 of Pregnancy	6	2 deliver	8(3,5) all premature
		1 vaginal bleeding	11 – all premature
Day 19 of Pregnancy	3	2 deliver	6(2 still in sac)
		1 sacrificed	0(4 implantation sites)

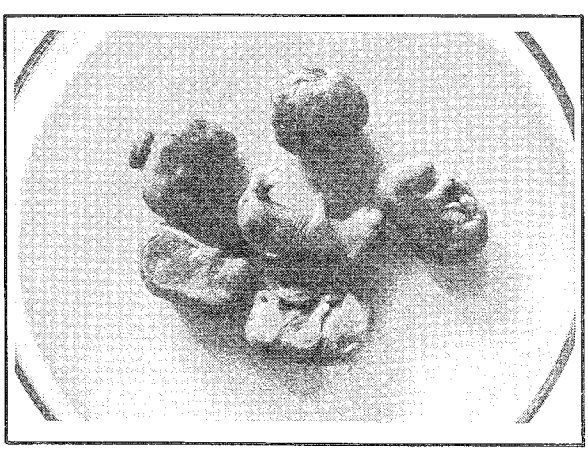

Fig. 3 Premature birth of fetuses following administration of 800 mg SH extract to the pregnant mother on day 16 of pregnancy.

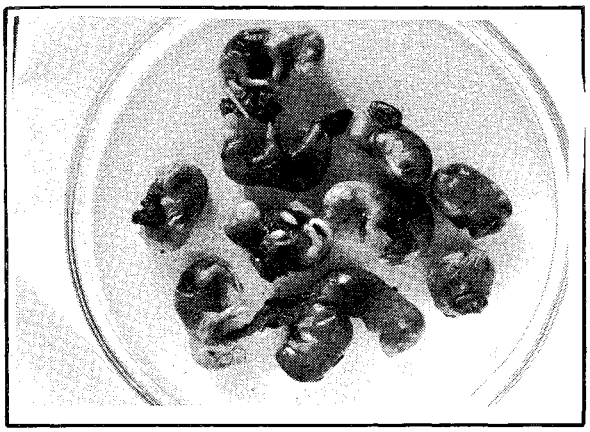

Fig. 4 More premature births of fetuses following administration of 1600 mg SH extract to the pregnant mother on day 18 of pregnancy.

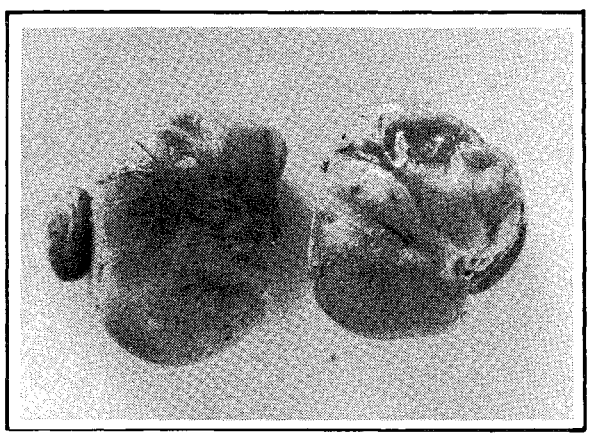

Fig. 5 Premature birth of fetuses following administration of 1600 mg of SH extract to the pregnant mother on day 19 of pregnancy. Note fetuses are still in the sac.

day 19 of pregnancy it was observed that of the remaining three mice; two gave birth to fetuses that were not only premature but these fetuses were still in the sac as shown in Fig. 5. As mentioned earlier, the one that survived up to the end had no fetus although there were four implantations sites.

DISCUSSION

Aqueous extract of *Selayak Hitam* is teratogenic in mice. This is apparent in the group of mice where each received 200 mg of the extract. The deformation is extreme in nature, externally all parts of the fetuses are affected by the extract. The extract also displays abortifacient effect, for cumulative administration of the extract consistently results in the premature birth of the fetuses. This was apparent beginning on day 16 of pregnancy where each mother received 800 mg of extract. Some of the fetuses were still in sacs when born following the administration of the extract. Abortifacient property was clearly demonstrated in one case following administration of 400 mg of SH extract on day 15 of pregnancy. In this case, vaginal bleeding was noted and at term when sacrificed there were no fetuses although there were four implantation sites on the uterus. Some deaths were recorded throughout the cumulative administration of the extract indicating that the extract is quite toxic. In conclusion, aqueous root extract of *Selayak Hitam* is relatively toxic to the mother, teratogenic to the fetuses and possesses abortifacient property in mice. The mechanism through which the extract brings about an abortion is not known but, the abortifacient effect being the consequence of the induced teratogenic activity cannot be ruled out. The active principle(s) from the extract responsible for the effects reported remains to be elucidated. Fractionation of the extract and isolation of the active principle(s) are currently in progress in our laboratory.

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