PHARMACOLOGICAL EVALUATION OF AQUEOUS ROOT EXTRACT OF *SELAYAK HITAM:* LACK OF OXYTOCIC ACTIVITY

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SUMMARY

Aqueous root extract of Selayak Hitam *a plant commonly found in Malaysian jungles and reported to have abortifacient property was screened for oxytocic effect. Results obtained from* in vitro *experiments on isolated uterus preparation from both pregnant and non pregnant rats and* in vivo *experiments on uterus contraction in rats* in situ, *showed that the extract lacks oxytocic effect. It is concluded that the alleged abortifacient property if any, is not mediated through oxytocin or oxytocic-like effect.*

INTRODUCTION

Selayak Hitam, a plant belonging to the family of *Annonaceae*, is commonly found in Malaysian jungles. The plant possesses long oval leaves and normally grows to a moderate height between four to five feet as shown in Fig. 1. Aqueous extract of the root of *Selayak Hitam* had been claimed, and used by the medicinal man to induce abortion. For this purpose, the root is usually boiled overnight in a pot and the extract taken.

Fig. 1 *Selayak Hitam*, a plant belonging to the family of *Annonaceae*.

Oxytocin, an octapeptide hormone of the neurohypophysis possesses potent and selective stimulating effect on the contractile activity of uterine smooth muscle. While immature uterus is quite resistant to oxytocin,[1] the responsiveness of mature uterus increases as gestation progresses. Oxytocin can initiate and enhance contraction of uterus as early as the twentieth week of pregnancy and if present in sufficient concentration, oxytocin can result in therapeutic abortion.[2] It is therefore of interest to screen for the oxytocic activity of aqueous extract of *Selayak Hitam* root both in non-pregnant and in pregnant rat uterus.

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MATERIALS AND METHOD

Preparation of aqueous extract

Freshly-collected *Selayak Hitam* (SH) roots were thoroughly washed with tap water to get rid of the soil and allowed to dry. The roots, ground to powder form and dried at 40 °C in an oven until constant weight achieved, was used for the extraction. To every 250 g of dried powder, 1.25 ℓ of tap water was added. The suspension was heated for 30 min at 45°C in a 3 ℓ conical flask. Crude extract was then squeezed through linen cloth and filtrate (F_I) obtained. Saturated lead acetate solution was added to filtrate F_I to precipitate tannins, proteins and starch. The solution was then filtered (F_{II}). Ammonium sulphate solution was then added to precipitate out the excess lead ions. The solution was again filtered (F_{III}). Filtrate F_{III} was then evaporated to dryness under partial vacuum at 45°C with a rotary evaporator. 300 ml of methanol was then added to F_{III} and the undissolved ammonium sulphate crystals filtered off to obtain the final filtrate F_{IV}. The final filtrate F_{IV} was evaporated to dryness and appropriate volume of water/saline was added for screening of the oxytocic activity. Approximately 10 g extract was obtained for every 250 g dry weight of SH root powder used.

Animals used

Sexually-matured (approximately three-months-old) female, albino rats weighing 200-250 g were used in the entire investigation. Both non-pregnant and pregnant rats were used. For the non-pregnant rat, each received 100 μg/kg stilboestrol subcutaneously 24 hr prior to experimentation so as to bring the rat into estrus state, for, it has been shown earlier that the responsiveness of non-pregnant uterus to oxytocin is at its peak at estrus.[3] For the pregnant rat, pregnancy was confirmed by the standard vaginal smear method after overnight mating between one male and four females in a cage. This was taken as day 1 of pregnancy.

In-vitro isolated rat uterus preparation

The rat was killed by a blow on the head and the two uterine horns were located and carefully dissected out into a petri dish containing de Jalon 's solution. The uterus was then cleared of fats and connective tissues and the two horns separated by cutting at the bifurcation. A piece of horn approximately 1.5 cm long was set up in an organ bath of 15 ml capacity in de Jalon's solution at room temperature and attached, via a thread to a kymograph (Phipps and Bird model) for recording of contraction. The organ bath was bubbled with carbogen (95% O_2 /5% CO_2) throughout the experiment. The uterus was allowed to equilibrate and settle for at least 15 min before experimentation began. During this time the tissue was washed with fresh de Jalon' s solution at five min intervals. A 45 sec drug-contact-time and a 4 min drug-cycle-time was used to contruct the dose-response relationship to submaximal doses of oxytocin both in the absence and in the presence of a fixed dose of SH extract. In the latter case, introduction of SH extract was made one min prior to the introduction of oxytocin. The experiments were performed both on isolated non-pregnant and on day 14 pregnant rat uterus.

Rat uterus *in situ* preparation

Non-pregnant female rat was anaesthetised by intraperitoneal injection of sodium pentobarbitone (Nembutal, Abbott), 40 mg/kg. Following anaesthesia, the trachea was cannulated by polythene tubing (PE 205). Two polythene catheters (PE 10) were inserted, one placed in the left jugular vein for intravenous injection, while the other placed in the left carotid artery for monitoring of systemic blood pressure. The arterial catheter was connected to Statham pressure transducer coupled to the polygraph (Grass Model) on which blood pressure was recorded. Both catheters had been previously filled with 0.9% NaCl containing heparin 50 U/ml.

Recording of the uterus contraction was made via a cannula connected to the uterus. This was achieved by first, a longitudinal incision made along the mid-line on the lower abdominal wall. The uterus was then located and cannulation made with a polythene tube (PE10) filled with heparinised saline (50U/ml) inserted into the left horn 0.5 - 1 cm from the junction between the two horns at the cervix. The tubing was connected to a syringe and heparinised saline was then connected to Statham

pressure transducer coupled to the polygraph. Contraction of the uterus is reflected through pressure changes and recorded on the polygraph. The abdominal opening was covered with cotton wool moistened with normal saline and the animal kept warm by a table lamp. The whole experimental set-up is as shown in Fig. 2.

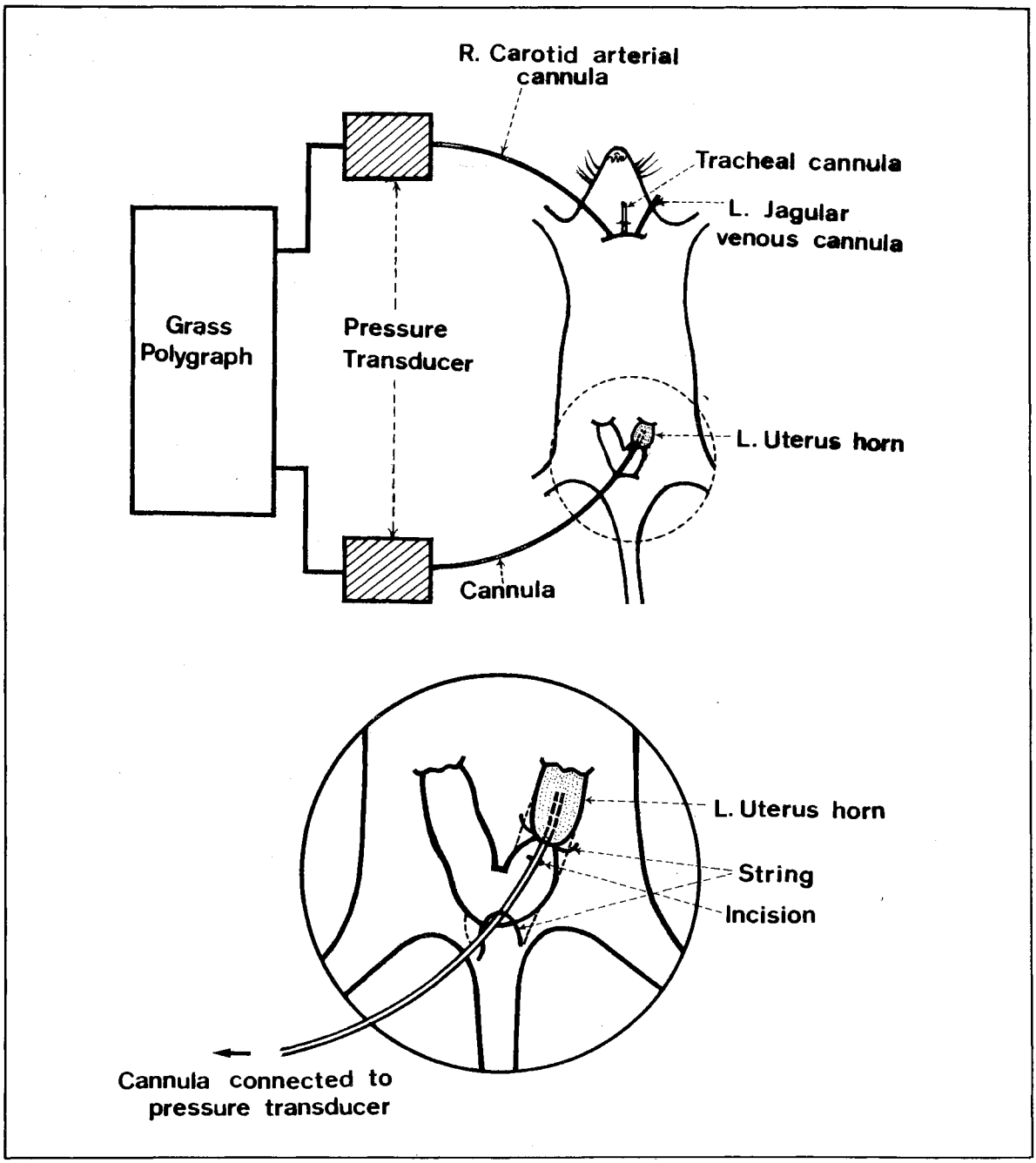

Fig. 2 Experimental set-up for the recording of uterus contraction in the rat *in situ*.

Uterine contraction was measured according to *Montevideo* unit which is the product of amplitude (rise of contraction from the basal tone to the peak) and the frequency (number of contractions per min) as described earlier.[2] In each case, contraction was measured five minutes before (serving as control) drug administration, the first five minutes and the second five minutes after administering the drug/extract. The percentage changes in *Montevideo* unit after each drug/extract treatment was calculated with respect to that five minutes before administration of drug/extract.

RESULTS

Initial studies on aqueous root extract of SH showed that the extract at concentration up to 5 mg does not initiate any contraction on the isolated uterus preparation. This is unlike oxytocin, which exerts a dose dependent relationship on the contracture of both the non-pregnant and pregnant (14 days) isolated uterus preparations as shown in Fig. 3 and Fig. 4. Moreover, in the presence of aqueous SH root extract the dose response relationship of oxytocin on both the contracture of isolated non-pregnant and pregnant uterus preparations were not significantly altered.

Fig. 5 which is obtained by plotting the increase in uterine contraction and frequency expressed as *Montevideo* unit in anaesthetised female rat *in situ* for the first five minutes and for the second five minutes following administration of oxytocin. It can be seen that the percentage change in *Montevideo*

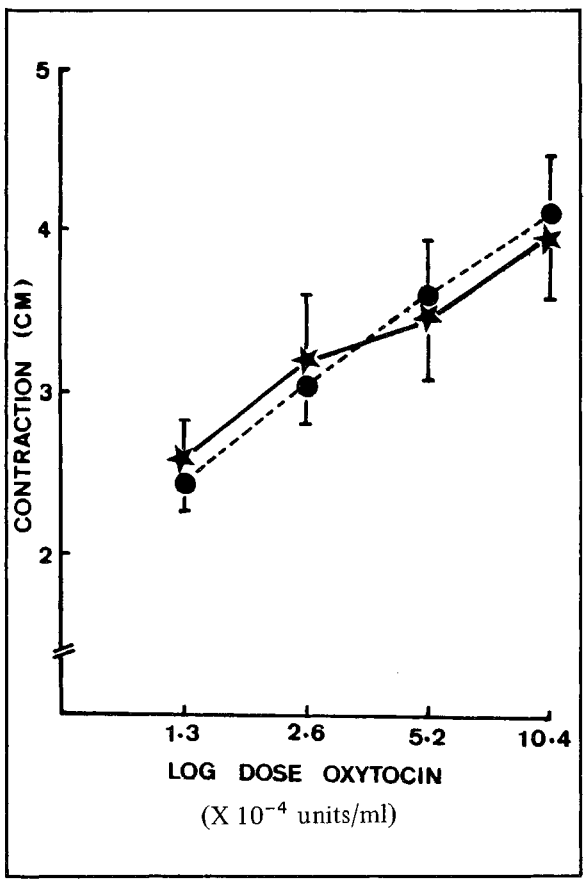

Fig. 3 Effect of oxytocin alone (★) and oxytocin in the presence of (●) PM extract on isolated non-pregnant uterus preparation.

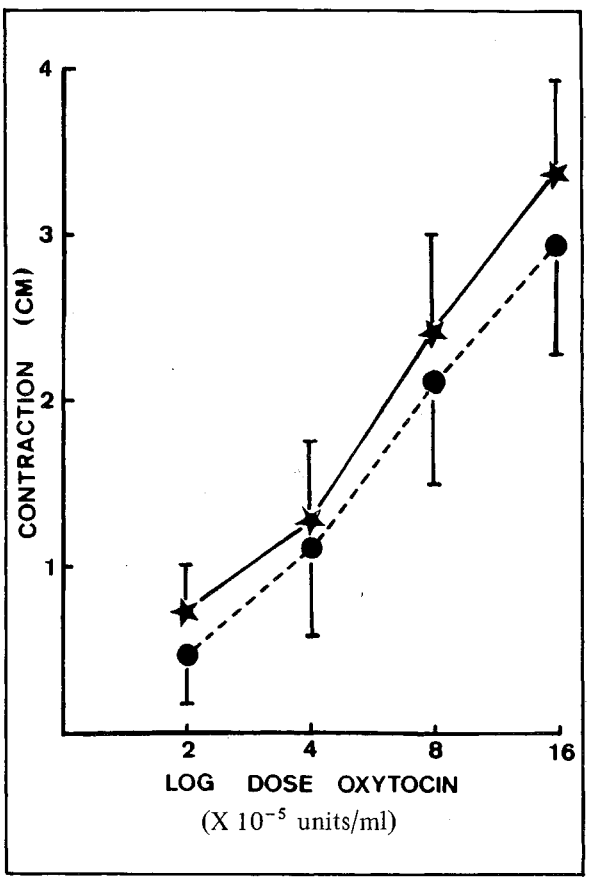

Fig. 4 Effect of oxytocin alone (★) and oxytocin in the presence of SH extract (●) on isolated pregnant uterus preparation.

unit is greatly enhanced during the first five minutes and persist for the next five minutes following intravenous administration of oxytocin. However, when similar experiments were conducted replacing oxytocin with aqueous root extract of SH (up to doses of 80 mg/animal given intravenously) there was no significant change in *Montevideo* unit both for the first five minutes and for the next five minutes following intravenous administration of the extract as shown in Fig. 6.

DISCUSSION

Aqueous root extract of SH appears to have no oxytocin or oxytocic effect. This was evidenced from results obtained both *in vitro* and *in vivo* experiments. Firstly, in *in vitro* experiments on isolated uterus preparation, SH root extract, unlike oxytocin, does not elicit any contractions. Secondly, in the presence of aqueous root extract of SH, the oxytocin-induced contracture in isolated non-pregnant and in isolated pregnant uterus preparations were not enhanced. These results are consistent with the observation made on *in vivo* experiments in which rats *in situ* under pentobarbitone general anaesthesia, aqueous root extract of SH does not elicit any uterine contraction expressed in terms of *Montevideo* unit. This is in contrast to results obtained with oxytocin where oxytocin consistently and reproducibly increases the uterine contraction expressed in terms of *Montevideo* unit both for the first five minutes and for the five minutes thereafter following intravenous administration of oxytocin.

Conclusion therefore can be made that aqueous root extract of SH lacks oxytocin or oxytocic effect on rat uterus preparations. The alleged abortifacient

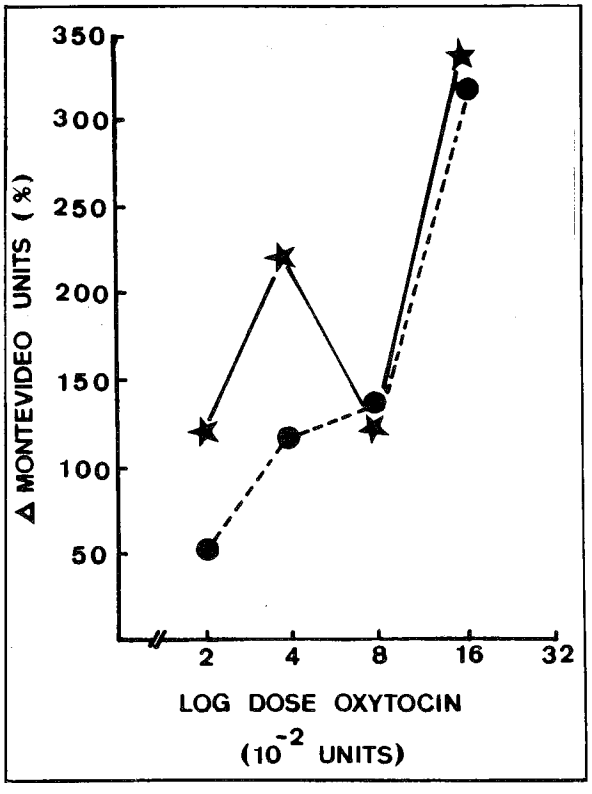

Fig. 5 Uterine contraction during the first 5 min (★) and during the second 5 min (●) following administration of oxytocin in anaesthetised rat. For explanation, see text.

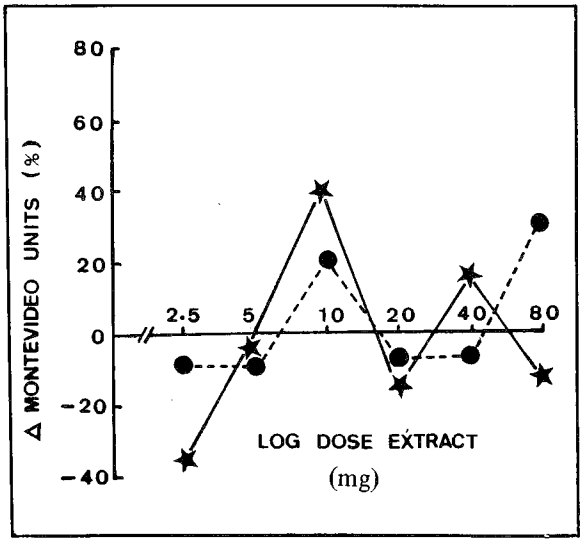

Fig. 6 Uterine contraction during the first 5 min (★) and during the second 5 min (●) follwing administration of SH extract in anaesthetised rat. For explanation, see text.

effect of aqueous root extract of SH, if any, is not likely to be acting through oxytocic effect. Other possible mechanism through which SH root extract can act as abortifacient agent may be via embryotoxic effect. And in fact some drugs that are cytotoxic used in cancer and as immunosuppressants can be embryotoxic and had been used as abortifacients.[4] Some examples of these drugs that produce a high incidence of abortion are azathioprine, chlorambucil, cyclophosphamide and mercaptopurine. The possible embryotoxic effect of aqueous SH root extract is currently being investigated.

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