ANTIFERTILITY EFFECT AND SOME PHARMACOLOGICAL ACTIONS OF *BUTEA FRONDOSA* SEED EXTRACTS*

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Butea frondosa (palash) also known as *Butea monosperma*, widely occurs in India and Burma. Various parts of this tree are used in the Ayurvedic System of Medicine (14). Vagbhata, the classic Ayurvedic text, does not mention the antifertility action and use of this plant (7). Its antifertility effect has been alluded to by Kirtikar and Basu (13). Antifertility effect in experimental animals has been observed by using the crude extracts of its seed and, crude and purified extracts of the petals (6,11). Work on the antifertility effect of the seeds extract and its mechanism of action are reported here.

MATERIALS AND METHODS

Extraction procedure

Fresh samples of the dry seeds of *Butea frondosa* (obtained from Messrs Hamdard Dawakhana, New Delhi) were extracted thus—

(*a*) *Alcoholic extract*—The seeds were powdered and kept in rectified spirit at room temperature (26-28°C) for 1 week. The extract was filtered and dried at room temperature. For oral administration the dried extract was triturated with gum acacia to which distilled water was then slowly added to get a homogeneous suspension ; the volume of suspension was so adjusted that it contained 10% W/V gum acacia. For subcutaneous injection, 1 *gm* of the dried extract was triturated with 1 *ml.* groundnut oil and 0.4 *ml.* of polysorbate 80 (Tween 80) and distilled water was then slowly added to obtain an emulsion of 40 *ml.* volume.

(*b*) *Chloroform extract*—It was obtained by following the same procedure as in (*a*).

(*c*) *Aqueous extract*—The seed powder was boiled in distilled water for 30 minutes and then left for 24 hours at room temperature. The extract was filtered and boiled to obtain dry residue which was then used after dissolving in water.

These extracts were preserved at 8°C.

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As positive control for antifertillity study, Noracyclin (CIBA) was used. It is one of the oral contraceptives available in this country and each tablet contains 2.5 *mg* lynestrenol, a progestin and 0.075 *mg* mestranol, an oestrogen. Three tablets of Noracyclin were triturated with 1 *ml.* groundnut oil and 0.4 *ml.* polysorbate 80. Distilled water was then slowly added to make 37.5 *ml.* It was preserved at -4°C.

As negative control, the following were used—

(*a*) suspension of 10% W/V gum acacia in distilled water,

(*b*) emulsion of groundnut oil (1*ml.*) and polysorbate 80 (0.4 *ml.*) in distilled water (37.5 *ml.*).

Antifertility study

(*For convenience the dried alcoholic extract of Butea frondosa seeds is hereafter mentioned as the extract*)

Mice—Six female mice of proved fertility were kept with one male mouse in each cage and every morning the mice were checked for vaginal plug. Pregnancy period was counted from the day the plug was found, and the pregnant animals were isolated. The drugs were administered orally, daily, for 5 days after mating. On the 10th day of pregnancy laparotomy was performed under ether anaesthesia and uterine horns were observed for number and size of implants. On the 20th day the foetuses were delivered by Caesarian operation. They were counted andthen checked for gross teratogenic effects. After stitching uterine and abdominal walls the mothers were allowed to recover and nurse the litters.

Rats—Female rats of proved fertility and regular oestrus cycle were mated with males that had sired litters before. Presence of sperms in vaginal smears (which were preparede very morning) indicated mating and commencement of pregnancy. They were further treated like the pregnant mice as described above.

The room temperature was maintained between 25-28°C. When left for mating with individual males, care was taken not to introduce another male in those cages (3).

Oestrus cycle and mating behaviour — Twenty female rats were divided into 4 equal groups. Daily vaginal smears were taken to study the nature of epithelium. Three groups received the extract, respectively, in the doses 10 *mg*, 100 *mg*, and 1 *gm* (per *Kg* body weight) orally, daily, for 15 days ; the fourth group received gum acacia in water and served as a control. After 15 days, the animals were allowed to mate. Mated animals were treated like the rats in the antifertility study described above.

Ovulation — The method of Bennett *et al.* (1) was used. The extract was administered orally , daily, for 15 days to mature, normally cycling female rats. On days 5, 6, 7 and 8 of this period, 3 *ml.* of 1% filtered solution of Dianil Blue '2R' was injected intraperitoneally. The

animals were killed on the 16th day and colourless or pink corpora lutea on the blue coloured ovarian surfaces were counted. As positive controls ethinyl oestradiol and Noracyclin were given for 15 days. Animals of the negative control group received gum acacia in water for the same period.

Benett *et al.* (1) have reported occurrence of severe diarrhoea and its control by tetracycline in rats receiving Dianil Blue '2R'. Therefore, in the experiment described above, 0.05 *mg* oxytetracycline was added per *ml.* of the drinking water of the rats.

Anti-oestrogenic activity — Forty immature female mice (about 24 days old) were divided into 5 equal groups. The control group received gum acacia in water orally. Oestradiol dipropionate (0.05 μg) in groundnut oil was injected subcutaneously every day for 5 days to the individual animals of the second group. Remaining groups received, daily, for 5 day, 0.05 μg oestradiol subcutaneously and different doses of the extract orally. On the 6th days, after sacrificing the animals, the uteri were taken out, dissected clean, quickly dried between filter papers and then weighed.

Androgenic activity—The technique of Harper and Walpole (8) was used with slight modification. Fortynine immature male mice (about 28 days old) were divided into 7 equal groups. Control group received gum acacia in water and the remaining groups were given different doses of the extract orally, daily, for 5 days. The animals were sacrificed on the 6th day and, testes, seminal vesicles and adrenals were dissected clean and weighed.

Decidua cell reaction—The method of Shelesnyak and Kraicer (16) was used. For inducing pseudopregnancy, electric current (volts 9, frequency 300/second, total duration 10 seconds) was applied to the cervix of normally cycling mature female rats. The day of giving the stimulus was taken as day 1 of pseudopregnancy and the animals were given different doses of the extract orally, daily, for 5 days. The vaginal smear was studied daily for 4 days and the animals which showed vaginal cytology of oestrus phase were discarded; those which continued to show dioestrus phase were considered to have gone into pseudopregnancy and received intraperitoneally, on the 4th day, 1 *ml.* aqueous solution containing 20 *mg* pyrathiazine (Pyrrolazote, Upjohn) per animal. The animals were killed on the 8th day of pseudopregnancy and uteri were examined and graded for decidua cell reaction (16).

Abortifacient and teratogenic actions—During 12 through 15 days of pregnancy, mated female mice received orally, daily, 0.5, 1, 1.5, 2 and 3 *gm/kg* of the extract and the animals were observed for the evidence of abortion. On the 20th day of pregnancy, the foetuses were delivered by Caesarian operation and checked for signs of gross malformation.

Other pharmacological actions

Anti-inflammatory study—Cotton pellet technique of D' Arcy *et al.* (5) was followed. Pellets of 10 *mg* weight were dissected out (together with surrounding granulation capsule) on the 8th day of insertion. The extract was given orally, daily, for the first 5 days in the dose of 0.5,

1.0 or 1.5 *gm/kg*. Hydrocortisone (a single intraperitoneal dose of 5 *mg*/animal on the day of insertion of pellets) and 1.5 *ml.* gum acacia in water (daily, orally, for 5 days) were used as positive and negative controls respectively.

Isolated tissues—The effect of the extract was studied on the isolated preparations of guinea-pig ileum, rat uterus and frog rectus (4).

Blood pressure and respiration—Dogs anaesthetised with pentobarbitone were used. The extract was injected through the canulated femoral vein. ECG was recorded in 2 dogs.

Diuretic action—It was studied in the anaesthetised dogs by the method described by Bhide *et al.* (2). The extract was given intravenously in the dose of 50 *mg* or 100 *mg/kg* through the cannulated femoral vein.

Acute toxicity—Sixty adult mice (about 30 *gm*) were divided into 6 groups; five groups received orally, as a single dose, 0.5, 1,3, 5 or 10 *gm* of the extract per *kg* body weight. Sixth group received only the vehicle (gum acacia suspension). The animals were observed for 30 hours. Post-mortem dissections were conducted.

RESULTS

Antifertility Study

The results in Table I show that the antifertility activity occurs in the seeds of *Butea frondosa* and in alcoholic extract but not in the water or chloroform extract. When extracted in alcohol for 8 days at room temperature, the residual seed powder was found to be devoid of antifertility effect; which indicates adequate extraction of the active principle by the said procedure.

TABLE I

The effect of Butea frondosa seed preparations on rat pregnancy. Each preparation was given to 5 rats.

	Daily dose per rat	% rats having no *implants on the 20th day of pregnancy*	*Average no. of implants/rat of the group on the 20th day of pergnancy*
Control	1.5 *ml.* gum acacia in water	0	7.5
Seed powder	200 *mg*	60	4.2
Seed powder from which alcohol soluble part has been extracted	200 *mg*	0	9.8
Dried alcoholic extract	10 *mg*	60	2.6
Water soluble fraction of the dried alcoholic extract	50 *mg*	0	7.8
Dried aqueous extract of seed powder	100 *mg*	0	8.2
Chloroform extract of seed powder	100 *mg*	0	7.5

TABLE II

Effect of the extract on mice and rat pregnancy

Daily dose (mg/kg)	*Total No. of animals in the group*		*% animals having no implant on 20th day*		*Average no. of implants/rat of the group on the 20th day of pregnancy*	
	Mice	*Rats*	*Mice*	*Rats*	*Mice*	*Rats*
Control 1 *ml.* (gum acacia in water)	54	10	11	0	6.4	7.8
10	9	10	78	20	1.6	6.0
50	10	10	40	40	1.5	5.2
100	..	10	..	30	..	4.2
100 (subcutaneous)	..	5	..	60	..	4.2
250	10	10	60	40	3.6	3.6
500	10	9	80	33	1.3	4.5
1000	10	7	70	57	1.4	1.6
1500	9	4	44	25	7.2	5.5

Results in Table II indicate that the extract has distinct antifertility activity in rats and mice. However, it does not show clearcut dose-response relationship. At 100 *mg/kg* dose,

TABLE III

Effect of Noracyclin on mice and rat pregnancy. In these experiments the foetuses were counted at laparotomy on the 10th day but several animals were allowed to be delivered by the natural parturition without 20th day laparotomy

**Fraction of the tablet/kg (comparison with human dose)*	*No. of animals in the group*		*%animals having no implants on 20th day of pregnancy*		*Average No. of implants per animal of the group*	
	Mice	*Rats*	*Mice*	*Rats*	*Mice*	*Rats*
Control (1 *ml.* oil and polysorbate 80 emulsion per animal)	20	5	10	0	7.1	8.0
1/50 (human dose)	15	4	47	50	4.2	1.75
1/25 (twice human dose)	..	4	..	50	..	4.00
2/25 (4 times human dose)	..	5	..	60	..	2.8
1/5 (10 times human dose)	28	12***	82	59**	1.1	2.3**
3/5 (30 times human dose)	8	7***	100	100	0	0
1/25 (twice human dose) + *Butea frondosa* extract (500 *mg/kg*)	..	5	..	20	..	6.4

*Human dose taken as 1 tablet of Noracyclin per day for a 50 *kg* woman.

**Every rat of this group had implants on the 10th day and their average number was 7.3 per animal. For all remaining groups and for all animals mentioned in other tables, 10th and 20th day values were identical.

***To be published by Kapila *et al.*, reference 11.

parenteral administration of the extract did not produce complete suppression of fertility. In these experiments, the average number of off-springs produced by the extract-treated groups of animals was less than the control one. The full-term new born off-springs of both mice and rats did not manifest gross malformations. The results of Noracyclin are presented in Table III.

Noracyclin at 1 to 10 times human dose reduced fertility in rats and mice; however, complete antifertility effect could be seen only at 30 times human dose. Animals receiving Noracyclin were not subjected to Caesarian operation and were allowed to deliver by the natural process. They delivered on about 30th day (instead of on about 21st day) of pregnancy; this prolongation of gestation is known to be due to the progestational compound (lynestrenol) in Noracyclin. Effective doses of the extract and Noracyclin when given together were, if at all, less effective than when given separately (Table III).

Oestrus cycle and mating behaviour—Control animals had regular oestrus cycle of about 4 days. The extract when given for 15 days did not produce any effect on the oestrus cycle. Noracyclin prolonged oestrus phase, in about 50% of the rats, from about 1 day to 4 days.

Out of 15 animals which received the extract for 15 days, 7 showed delayed mating. This was seen particularly in the group which received 1 *gm/kg* dose. That group also showed significant reduction in off-springs (average 2.4 compared to the control value 6.8).

Ovulation—The results are given in Table IV.

TABLE IV

Effect of feeding the extract and Noracyclin for 15 days on ovulation in the rat. Each group consists of 4 normally cycling rats

Drug and daily dose	*%rats that had no corpora lutea on ovaries*	*Average No. of corpora lutea per rat of the group*
Control 1.5 *ml.* gum acacia in water/rat	25	3.5
Control 1.5 *ml.* of oil and polysorbate 80 emulsion/rat	25	5.0
The extract		
50 *mg/kg*	25	4.0
500 *mg/kg*	25	2.2
1000 *mg/kg*	50	1.7
Noracyclin (Fraction of the tablet/kg)		
1/50 (human dose)	0	5.3
1/5 (10 times human dose)	25	3.3
3/5 (30 times human dose)	50	1.0
Ethynyl oestradiol, 1 *mg/kg*	100	0

Both the extract and Noracyclin produced some inhibition of ovulation, although, even at the highest doses, they did not completely suppress it.

Anti-oestrogenic activity—The average weight of uteri in the group which received 0.05 μg of oestradiol was 50 *mg* (S.E.$\pm$1.13). In the remaining 3 groups which received, besides oestradiol, 0.1, 1 or 1.5 *gm/kg* of the extract, the average weights (*mg*$\pm$S.E.) of uteri were 48.7$\pm$2.30, 47.5$\pm$0.80 and 48.5$\pm$1.13 respectively. By using the t-test, the difference between the control and extract-treated groups was found to be insignificant ($p>0.1$).

Androgenic activity—The weights of adrenals, testes and seminal vesicles in the groups which received the extract in the doses of 0.05, 0.1, 0.25, 0.5, 1.0 and 1.5 *gm/kg* did not differ significantly ($P>0.1$, calculated by the t-test) from the control values (which were as average *mg* weights$\pm$S.E.-adrenals-2.83$\pm$0.42; testes-75.6$\pm$8.4 and seminal vesicles 2.70$\pm$0.58).

Decidua cell reaction (DCR)—The extract blocked the DCR. This blocking effect though impressive, was partial, in that, complete prevention of DCR could not be obtained even with 1 *gm/kg* dose. Also, there was no clearcut dose-response relationship (Table V).

TABLE V

Number of uterine horns showing DCR following intraperitoneal injection of pyrathiazine. Individual horns of rats were separately assessed on the eighth day. The grades are from 0 *to* 4. *Each group consists of* 5 *animals.*

Group and dose	*No. of individual horns showing the undermentioned grades of response*					*Average grade of DCR/animal*
	0	1	2	3	4	
Control 1.5 *ml.* gum acacia in water	3	1	1	0	5	4.6
The extract (10 *mg/kg*)	9	1	0	0	0	0.2
The extract (100 *mg/kg*)	8	0	2	0	0	0.8
The extract 1 *gm/kg*	8	0	0	2	0	1.2

Abortifacient and teratogenic actions : Complete resorption of implants resulted in 4 out of 5 mice when it was given daily in the dose 1 *gm/kg* during 12 through 15 days of pregnancy. A single dose of 2 *gm/kg* of the extract resulted in the death of pregnant mice which, on post-mortem examination, showed presence of implants in the uteri. Incidentally, this dose, when administered to adult male mice and non-pregnant female mice, did not produce any mortality in the acute toxicity study. No obvious teratogenic effect was seen in mice or rats when the extract was given orally during this period of organogenesis.

Other pharmacological actions—The extract did not show any statistically siginificant anti-inflammatory action in rats. It had noaction on the isolated rectus abdominis muscle of the frog nor did it alter its response to acetylcholine, carbachol, suxethonium or potassium chloride. It did not alter the action of 5-HT on the isolated uterus of oestrogenized rats. The extract inhibited the action of acetylcholine and histamine on the guinea-pig ileum. Inhibition of the former was complete at the dose of 0.5 *mg* of the extract per *ml.* bath fluid while complete inhibition of histamine could not be obtained even by 10 *mg/ml.* concentration. The inhibition of both the agonists was short-lived and the tissues regained their sensitivity within 2-3 minutes.

The extract did not show any significant effect on the carotid blood pressure, ECG and respiration of the anaesthetised dogs nor had it any diuretic action. The LD_{50} in mice was 7.5 *gm/kg*.

DISCUSSION

Butea frondosa seed extract has partial but distinct antifertility action in rats and mice. The active principle in the seeds can be extracted by alcohol but not by water or chloroform. Therefore, it is probably different from the ether-soluble anthelmintic principle which occurs in the seeds and which is poorly soluble in alcohol (10). The chemical nature of the antifertility principle present in the seeds is not known. However, in this connection, it is interesting to note that petals of *Butea frondosa* flowers contain a flavonoid called butrin which has been found to possess clear antifertility activity in rats (11). It is not known whether the seeds of *Butea frondosa* also contain butrin or related substances.

The extract did not show clearcut dose-response relationship in its antifertility action in rats and mice (Table II). Also, in every group of animals that received individual doses of the extract, there were always some animals which produced normal number of foetuses. Since the subcutaneous administration of the extract could not substantially increase the antifertility effect, the possibility of poor gastro-intestinal absorbtion of the extract causing partial antifertility effect can be excluded.

After the present work was submitted as a thesis, a report appeared on the antifertility action of *Butea frondosa* seed extract in rats (12). In that report complete supression of pregnancy is reported in rats receiving 300 *mg/kg* of the hot alcoholic extract of the seeds. However, at 500 *mg/kg*. dose (which killed 5 out of 8 rats), one of their 3 surviving animals showed 9 implants on the 10th day. This fact again indicates lack of dose-response relationship and partial antifertility action.

In this work, besides showing lack of clearcut dose-response relationship, *Butea frondosa* extract has manifested a paradoxical phenomenon in both rats and mice, namely, at the highest dose used (1.5 *gm/kg*), it showed less antifertility action than at the smaller doses. At present no explanation can be offered for this paradox.

Noracyclin was used in this work as a positive control. In mice and rats its 20th day antifertility effect was proportional to the dose. With the exception of ten times human dose, in all these experiments (Table III) the antifertility effect on the 10th and 20th days was identical. With ten times human dose, however, there was a paradoxical finding, namely, all the 12 animals showed normal number of implants on the 10th day, although most of them underwent resorption by the 20th day. It is not clear why only at this particular dose level such effect was seen.

Although the extract and Noracyclin individually reduced number of implants in the rats, when given together there was no additive antifertility effect (Table III).

Even at higher doses, the extract did not have any effect on the oestrus cycle of the rat. This suggests that the extract has no oestrogenic or anti-oestrogenic action. Noracycline produced prolongation of the oestrus due to oestrogenic property of lynestrenol and mestranol (15).

Only at higher doses the extract reduced number of ova shed by the ovaries (Table IV). Complete inhibition of ovulation could not be obtained even at the dose of 1.0 *gm/kg*. Even at thirty times human dose, Noracyclin inhibited but did not completely suppress the ovulation (Table IV), although, this dose could produce complete suppression of implantation when given in early pregnancy (Table III).

When the extract was given before mating, the female rats took longer time to mate than the control animals and had less number of implants.

Decidua cell reaction (DCR) is important for nidation of the zygote. This reaction was markedly inhibited by the extract although no dose-response relationship was seen. The antifertility effect of the extract could be mainly due to its anti-DCR action.

For studying DCR a more convenient and easier method is recently described (9).

In moderate doses, the extract did not produce any teratogenic effect in the rats when given during the period of organogenesis (12-15 days of pregnancy); larger doses (2-3 *gm/kg*) had lethal effect on the foetus probably due to direct toxic action.

The extract does not appear to have gross pharmacodynamic actions; it had only weak antihistamine and anti-acetylcholine action on the isolated guinea-pig ileum. In the doses used during the present antifertility study it was apparently well-tolerated by the animals and its LD_{50} is comparatively high. Thus, the antifertility action of the extract reported here can not be due to gross pharmacodynamic or toxic effects.

SUMMARY

Dried alcoholic extract of *Butea frondosa* seeds has distinct antifertility activity in mice and rats when given orally, daily, during the first five days of pregnancy. The antifertility activity occurs in the seed powder and its alcohol extract but not in the aqueous or chloroform

extract. The antifertility effect did not show dose-response relation and was incomplete; which indicates low efficacy. The extract did not have oestrogenic, anti-oestrogenic or androgenic actions. It partially inhibited ovulation. It significantly suppressed the decidua cell reaction in rats, which could be an important mechanism of its antifertility action.

Noracyclin, the positive control in the present study, is a much more efficacious antifertility agent. Complete suppression of pregnancy was achieved by 30 times human dose, although this dose only partially inhibited ovulation.

The extract is devoid of anti-inflammatory action. It has no effect on the isolated frog's rectus abdominis muscle or rat uterus but has weak and short-lived antihistamine and antiacetylcholine actions on the isolated guinea-pig ileum. It does not have any action on blood pressure, ECG or respiration in the dog. On oral administration its LD_{50} in mice was 7.5 *gm/kg*. Its antifertility action is probably not due to any pharmacodynamic or toxic effects.

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