ORAL CONTRACEPTIVE—PART III
FURTHER OBSERVATIONS ON THE ANTIFERTILITY EFFECT OF ROTTLERIN

By

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The antifertility effect of Mallotus philippinensis in rats and guineapigs has been reported by Gujral and coworkers (1959 a). In a further study Gujral *et al.* (1959 b) reported the effects of various chemical constituents and fractions of this plant on the fertility rate of female rats. As the antifertility effect of the plant was shown by these workers to be due to the presence of rottlerin in it, it was thought desirable to study this active principle in greater detail. The present communication includes investigations of certain other effects of rottlerin.

METHODS AND MATERIALS

The experiments were conducted in albino rats belonging to the colony of Central Drug Research Institute, Lucknow. The animals were given standard diet as reported previously (Gujral *et al.*, 1959 a). Rottlerin was made into an emulsion and fed in the required doses by oral cannula.

Oestrus cycle.—Ten adult female rats showing regular oestrus cycle were selected for the experiment. The vaginal smears were taken daily for 15 days preceeding the drug treatment and for the subsequent period of 15 days of the drug administration. Thus the same rats served as their control. The smears were stained with Wright's stain and criterion used for determining the stages of oestrus cycle was the same as described by Allen (1922).

Effect on fertility when given in various stages of the oestrus cycle.—Previously tested fertile rats were used in this set of experiments. Animals were divided in four groups each containing five rats.

Group I—The drug was given during the stage of oestrus only and the rat was cohabitated with a male.

Group II—The drug was given on the day of proestrus only and co-habitated.

Group III—The drug was given for a period of 6 days after the mating.

Group IV—The drug was given during dioestrus only but in no case for more than 6 days and rats were put along with male partners thereafter.

Estimation of approximate lethal dose (ALD).—Rats of either sex weighing 100 ± 10 gms. were used in the experiment. Single increasing doses of rottlerin starting from 62.5 mg./kg. were given in a single rat and the general behaviour was observed. An intermediate dose between one which did not kill and one which killed was given to 3 rats and their weights recorded for 3 days.

Effect on isolated uterus.—A pregnant guinea pig was killed by a blow on the head, the abdomen opened and the uterus removed. The uterine muscle was kept in Dale's solution and one piece was mounted in an isolated organ bath maintained at 37-38°C and aerated artificially. Recordings were taken on a drum. Four such experiments were done.

TABLE I

Showing effect of rottlerin (25 mg./kg. per day given orally) on oestrus behaviour of rats observed for 15 days.

No. of animals		No. of cycles	Mean length of cycle in days.	SD ±	t
10	Before drug treatment	29	5.2	0.42	5.57
	During drug treatment	14	12.0	3.87	$P<0.001$

TABLE 2

Showing effect of rottlerin (25 mg./kg. per day) on fertility of rats when given in various stages of oestrus cycle.

	Drug given during oestrus	Drug given during proestrus	Drug given after mating	Drug given during dioestrus
No. of pairs	5*	5	5	5
No. of matings	4	3	5	0
No. of sterile matings	0	0	0	0
No. of fertile pairs	4	3	5	0
No. of young ones born	27	23	40	0
Sterile rats %	0	40	0	100
Fertile rats %	100	60	100	0
100% fertility recorded within days after the cessation of drug	..	25	..	40

*One rat died during the experiment.

TABLE 3

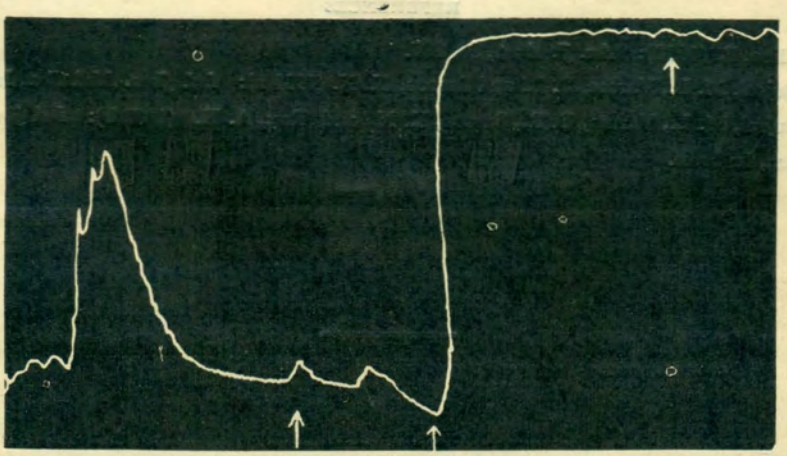
Showing effect of a single dose of rottlerin on weight and survival of rats.

Dose in mg./kg.	No. of rats	Time in hours between drug administration and death.	Initial body weight in Gms.	Body weight after 3 days in Gms.	Loss in body weight in Gms.
62.5	1	Not dead	95.0	94.0	1
125	1	Not dead	110.0	108 0	2
250	1	Not dead	103.0	101.0	2
375	1	Not dead	102.0	99.0	3
500	1	Not dead	96.5	90.0	6.5
625	3	Not dead	101.3	96.0	5.3
750	1	4 hours	93.0	..	..
1000	1	2 hours	97.0	..	..

RESULTS AND DISCUSSION

Table I shows that rottlerin prolongs the duration of oestrus cycle to a statistically significant degree. This is the result of prolongation of the dioestrus phase of the oestrus cycle. There is an obvious disturbance in the normal oestrogen-progestrone-balance as regulated by pituitary gonadotropins. The nature of the disturbance, however, is yet uncertain.

Table 2 shows that so long as the drug is administered during oestrus or after mating, it does not affect the fertility of female rats. However, when the drug is given during proestrus, fertility is reduced to 60 per cent whereas administration of the drug during dioestrus brings down the fertility to 0 per cent. The drug therefore has to be given before ovulation in order to prevent fertility. It is for this reason that the drug treatment on the day of oestrus and after mating has no effect on fertility. There is variable effect when the drug is given on the day of proestrus and complete infertility when administered during dioestrus. All the sterile rats during the experiment mate successfully 20 to 40 days after the cessation of the drug. Non-interference with the normal gestation when treatment is started after mating strongly suggests that the drug is neither abortifacient nor harmful to the developing foetus. This is further supported by lack of effect on the isolated uterine movements of guinea pig as shown in Figure 1. Antifertility effect of the drug is not permanent but certainly lasts for sometime after the drug is stopped.



Rottlerin 1. 25 mg. Pitocin 2. 5 I.U. Rottlerin 1. 25 mg.

Fig. 1.

Effect of rottlerin on isolated gravid uterus. There is no effect on the spontaneous or pitocin - induced contractions of uterine muscle.

A study of Table 3 shows that approximate lethal dose (ALD) is 750 mg./kg. and animal dies 4 hours after the drug feeding. There is a gradual loss of body weight for 3 days after a single dose and the animals start gaining weight from the fourth day. This shows that the drug is absorbed slowly and is excreted slowly. This is compatible with the finding that the antifertility effect of the drug overruns the period of drug treatment.

SUMMARY

1. The antifertility effect of rottlerin seems to be due to a significant prolongation of the duration of oestrus cycle mainly because of the lengthening of the period of dioestrus during drug administration.

2. The drug has to be given before ovulation to prevent fertility.

3. The drug has no effect on the contraction of isolated gravid guinea pig uterus.

4. ALD of the drug in rats is quite high (750 mg./kg.)

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