ORAL CONTRACEPTIVE-PART IV
HORMONAL AND ANTIHORMONAL EFFECTS OF ROTTLERIN

By

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The effect of rottlerin on the oestrus behaviour of rats, on fertility when administered during various stages of the oestrus cycle and the effect on isolated uterus has been reported in part III of this study (Varma *et al.*, 1959). Tne present investigation includes the hormonal and the anti-hormonal effects of rottlerin with an idea to elucidate the mechanism involved in producing inhibition in fertility rate of rats.

METHODS AND MATERIALS

Albino rats obtained from Central Drug Research Institute, Lucknow, were used in this experiment. The colony was maintained in our laboratory and animals were given standard diet reported previously (Gujral *et al.*, 1959). Drug was fed by an oral cannula.

Androgenic effect.—Male rats weighing between 80 to 100 gm. were divided in four groups containing four rats each, in such a way that each group had rats belonging to the common litters. All rats except those belonging to Group I were castrated. One month after castration, treatment with rottlerin was started and one animal was sacrificed from each group one, two, three and four weeks after the commencement of the treatment. The prostate and seminal vesicle were removed and kept in Bouin's fluid for 24 hours. Tissues were later dried and weighed.

Group	I	– Uncastrated untreated.
Group	II	– Castrated and treated with 25 mg./kg. of rottlerin daily.
Group	III	– Castrated and treated with 0.3 mg. testosterone propionate I. M. daily.
Group	IV	– Castrated untreated.

Oestrogenic effect.—Groups each of five adult female rats weighing between 80 to 100 gm. and paired in litter mate groups were selected for the experiment. Ovariectomy was performed by the usual procedure. Drug was started in doses of 25 mg./kg. 15 days after the operation and continued for

10 days. Vaginal smear were taken daily. One group served as control. Presence of cornified cells in the vaginal smear in place of the usual leucocytic picture of castrated rats was taken as characteristic of oestrogenic activity.

Anti-oestrogenic effect.—Three groups each of five female rats weighing between 80 to 100 gm. were selected for the experiment. They were ovariectomised and treated in the following way.

Group I – No treatment.
Group II – Oestrogen 50 mg./kg. daily for 5 days I. M.
Group III – Oestrogen 50 mg./kg. daily I. M. and rottlerin 25 mg./kg. daily orally for 5 days.

After the treatment animals were killed, their uteri removed, kept for 24 hours in Bouin's fluid, dried and weighed and prepared for histological examination.

Anti progestrational effect.—Female rats weighing between 80 to 100 gm. were primed with oestrogen (10 mg./kg. I. M. daily for five days) seven days after ovariectomy and then divided in batches of five rats each. One batch was given progesterone alone (5 mg./kg. I. M. daily for five days) and another progesterone in the same dosage schedule alongwith rottlerin (25 mg./kg. daily for five days). Animals were sacrificed after treatment, their uteri fixed in Bouin's fluid for 24 hours, dried and weighed, and micro-sections cut for histological examination.

Anti-gonadotropin effect.

(a) Twelve immature female rats weighing between 45 to 60 gm. were selected for the experiment. They were divided in three groups each containing four rats. The treatment was given for five days after which all the animals were sacrificed, their ovaries and uteri examined and weighed after preliminary fixing in Bouin's fluid for 24 hours. Sections were prepared for histological examination. Chorionic gonadotropin (CGT) was used in these experiments.

Group I —No treatment.
Group II —CGT 50 I. U. daily I. P.
Group III —CGT 50 I. U. daily I. P. and rottlerin 25 mg./kg. daily.

(b) Twenty immature male rats weighing between 44 to 48 gm. were divided in two groups of ten rats each. First group was treated with chorionic gonadotropin (4 units daily) for three days while the second group received rottlerin (25 mg./kg. daily) for the same period along with gonadotropin.

Animals were sacrificed on the fourth day, prostate and seminal vesicles were removed, fixed in Bouin's fluid for 24 hours and prostate carefuly isolated and weighed.

RESULTS AND DISCUSSION

A resume of Table 1 shows that rottlerin possesses no androgenic effect. The average weights of prostate and seminal vesicles of the uncastrated untreated group is similar to that of castrated and testosterone reated group and is higher than that of castrated groups treated or not treated with rottlerin.

Rottlerin does not induce the appearance of cornified cells in the vaginal smear of ovariectomised rats thus showing absence of any oestrogenic effect. Investigation into the anti-oestrogenic nature of the drug shows that cornified cell-containing smear of ovariectomised oestrogen treated animals is not reversed by rottlerin. Autopsy did not show any sign of active inflammation. Uteri were swollen and filled with clear fluid. Histological examination of the uteri of the three groups shows that proliferation and increase in thickness of the endometrium produced by oestrogen in ovariectomised rat is not affected by rottlerin. There are no progestrational changes. Table 2 shows that the drug does not adversely affect the average weights of the uteri of oestrogen treated rats. The increase in weight of the uteri of the drug-treated group seems to be consequent on reduction in body weight because uterus weight has been considered as weight per 100 gm. body weight. The results obtained indicate absence of progestrational and anti-oestrogenic effect in the drug.

Table 3 shows that drug treatment does not reduce the average uterine weight when given in progesterone-receiving ovariectomised rats primed with oestrogen. On the contrary there is an increase in the uterine weight which seems to be consequent to reduction in body weight. Autopsy of the animals in both groups revealed no signs of any active inflammation. Histological examination of uteri of both the groups presented similar picture with progestrational changes indicating absence of anti-progestrational activity in the drug.

Table 4 shows statistically significant difference in the weight of uteri and ovaries of gonadotropin treated animals compared to those of the group treated with rottlerin as well as gonadotropin. Macroscopic examination revealed big, swollen, reddish ovaries in the group receiving only gonadotropin as against small, whitish and shrunken ones in the control group as well as gonadotropin plus rottlerin treated group. In Table 5 one finds significant difference in the average weight of prostates of gonadotropin and gonadotropin plus rottlerin-treated group.

TABLE I

Effect of rottlerin (25 mg./kg. orally per day) on weight of prostate and seminal vesicles of rats.

Treatment.		No. of rats.	Period of treatment (in wks.) starting 4 weeks after castration.	Body weight in Gm.		Prostate weight in mgm. per 100 Gm. body weight	Seminal vesicles weight in mgm. per 100 Gm. body weight
				Initial	Final		
1. Uncastrated untreated		1	1	85	125	14.5	29.5
		1	2	80	105	13.5	21.0
		1	3	82	110	15.0	11.0
		1	4	80	110	16.0	27.0
	Mean			81.75	112.5	14.75	22.12
2. Castrated, rottlerin		1	1	100	118	10.5	12 0
		1	2	85	90	10.0	5.5
		1	3	80	100	8.0	2.2
	Mean	1	4	90	100	10.0	3.0
				88.75	102	9.63	5.67
3. Castrated, 0.3 mg. Testosterone propionate per day.		1	1	80	120	17.5	15.0
		1	2	80	100	18.0	32.0
		1	3	85	135	32.0	48.0
		1	4	87.5	125	27.8	45.5
	Mean			83.1	120.0	23.8	35.1

TABLE 2

Effect of rottlerin (25 mg./kg. orally) on the weight of uteri of ovariectomised rats.

Treatment	No. of animals	Mean body weight in Gm.		Average % gain in body weight	Average weight of uteri in mg. per 100 Gm. body weight
		Initial	Final		
Control	5	96.7	108.7	12.4	112.4
Oestrogen 5 mg. I.M. x 5.	5	98.5	105	6.3	227.8
Oestrogen 5 mg. I.M. x 5 + rottlerin x 5.	5	100.0	99.6	—0.4	251.6

TABLE 3

Effect of rottlerin (25 mg./kg. daily orally) on ovariectomised rats treated with oestrogen and progesterone. five rats were used in each group.

Group	Average body weight in Gm.		Average weight of uteri per 100 Gm. body weight.
	Initial	Final	
Oestrogen 1 mg. I.M. x 5 + progesteone 0.5 mg. I.M. x 5.	68.5	75.0	217·5
Oestrogen 1 Mg. I.M. x 5 + (progesterone 0.5 mg. I.M x 5 + rottlerin x 5.	87	78.3	258.6

TABLE 4

Effect of rotterin (25 mg. / kg. orally) on ovaries of immature rats treated with chorionic gonadotropin (CGT.)

Treatment	No. of rats.	Mean body weight in Gm.		Gain in body weight %	Average weight of uteri mgm. per 100 Gm.	Average weight of overies. mgm. per 100 Gm.	T.
		Initial	Final				
1, CGT 50 IU (IP) x 5	4	58.0	70.0	20.7	41.6@ ±12.36	19.6* ± 0.3	*8.8 P∠0.01
2. CGT 50 IU (IP) x 5 – rottlerin x 5	4	46.2	51.2	10.8	23.5@ ±6.45	11.6* ±1.8	@4.06 P∠0.01
3. Control.	4	59.5	70.0	17.6	25.9 ±9.99	13.5 ±2.1	

TABLE 5

Effect of rottlerin (25 mg./kg. orally) on the weight of prostate of immature rats treated with CGT.

Treatment	No. of animals	Average body weight in Gm.	Average weight of prostate mg./ 100 Gm. body weight	S.D.	T.
1. CGT 4 IU (IP) x 3	10	45.1	24.31	± 3.26	3.93 P<0.01
2. CGT 4 IU (IP) x 3 –rottlerin x 3.	10	44.6	19.56	± 2.07	

Figures 1 and 2 show the difference between the ovaries of immature rats treated with gonadotropin and gonadotropin plus rottlerin.

Fig. 1.

Antifertility effect of rottlerin has been reported previously (Gujral *et al*, 1959). The underlying mechanism of this effect is not certain. The drug

increases the period of oestrus cycle by prolonging the dioestrus phase. The present investigation rules out androgenic, oestrogenic, progestrational, anti-oestrogenic and anti-progestrational effect in the drug.

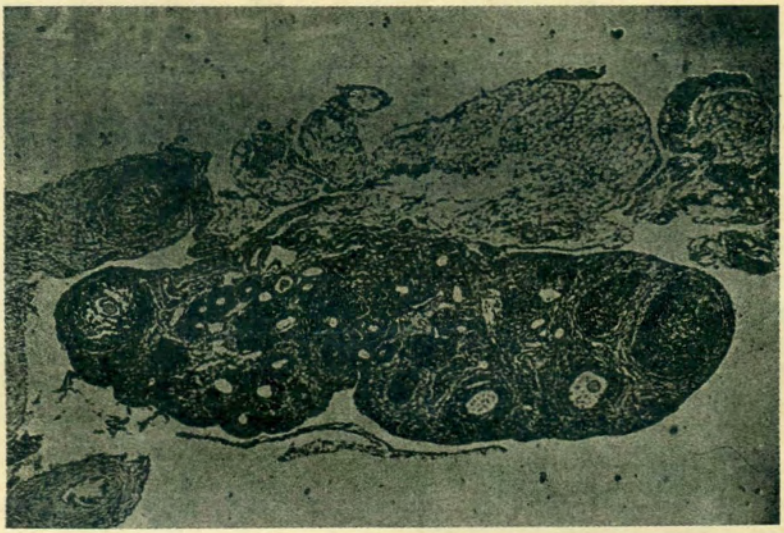

Fig. 2.

Obviously the mechanism of antifertility effect must reside elsewhere. The only positive finding is antagonism of the drug to lutenising as well as folliculoid actions of chorionic gonadotropins. It is very difficult to say whether the drug antagonises only the chorionic gonadotropins or other gonadotropins. The nuclear degeneration observed in the mature graffian follicles of gonadotropin plus drug treated rats may even be due to selective toxicity of the drug for maturing ova.

It is likely that drug antagonises pituitary gonadotropins peripherally leading to a disturbance in the oestrus cycle in such a way that dioestrus phase is plolonged. Antifertility produced by the drug seems to depend on this effect of the drug.

SUMMARY

1. Rottlerin possesses no androgenic, oestrogenic, progestrational, anti-oestrogenic and antiprogestrational effect.

2. It antagonises the peripheral effects of chorionic gonadotropin.

REFERENCES

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