BIOLOGICAL ACTIVITY OF AN INDIGENOUS PLANT PREPARATION*

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Summary : ROC 101, is an indigenous herbal preparation accredited with antifertility properties. Consumption of diets containing ROC 101, impaired the fertility of female mice and rats and produced sterility in male mice. These effects were reversible in the females but not in the males. Administered to male mice, ROC 101 inhibited spermatogenesis. The plant preparation failed to show any estrogenic, androgenic or anti-androgenic activity.

Key words : indigenous plants anti-fertility activity ROC 101

INTRODUCTION

In our programme to screen plants for possible antifertility activity, we have come across an indigenous preparation which impairs the fertility of female rodents and produces sterility in male rodents. The plant preparation designated as ROC 101 was referred for testing by the Indian Council of Medical Research. Since the clinical trials are still in progress, it has not been possible to decode the plant preparation for this presentation.

The plant preparation, ROC 101 has been reported to prevent conception in women when administered twice a day for three days during menstruation (1). In the laboratory we have screened it for its antifertility activity in mice and rats and studied its hormonal activity in an attempt to elucidate its mode of action in the inhibition of fertility.

MATERIALS AND METHODS

The studies were carried out in Swiss mice and Wistar rats, bred and maintained in the Animal House of the Institute. The powdered plant preparation was incorporated in the diet of mice and rats at 1.0, 2.5, 5.0 and 10.0% dose levels. The approximate daily consumption of normal diet by mice and rats in our colony is 6 *gms* and 15 *gms* respectively. Thus the daily intake of ROC-101 in mice was 0.06, 0.15, 0.3 and 0.6 *gms* and 0.15, 0.4, 0.75 and 1.5 *gms* in rats.

The effect of ROC 101 on the reproductive performance was studied at 4 dose levels in mice and at 3 dose levels in rats. Animals of both sexes were fed the experimental diets for a

*Presented at the symposium held during XVII Annual Conference of the Association of Physiologists and Pharmacologists of India, Trivandrum, March 5-7, 1972.

period of 28 days, then paired until mating occurred. The males were then removed and tested for fertility with normal fertile females. The treated females were paired with normal males to establish when pseudopregnancy, resorption or abortion occurred. Normal partners were changed regularly to avoid consumption of the experimental diet, but the treated animals were exclusively fed the experimental diet. Any treated female that failed to mate was transferred to the stock diet and mated to a normal partner. At the end of the experiment, the treated males were transferred to normal diet and allowed to mate with normal females.

RESULTS

The reproductive performance of both male and female mice was severally affected by the administration of ROC 101 in the diet (Table I). There was a 100% inhibition of fertile matings

TABLE I : Effect of ROC-101 on the fertility of male and female mice

Dose	*No. of treated pairs*	*Fertile* matings*	*Infertile** matings*	*Sterile*** matings*
1.0%	12	10 (83.3%)	2 (16.6%)	0
2.5%	12	0	12 (100%)	0
5.0%	12	0	10 (83.3%)	2 (16.6%)
10.0%	12	0	9 (75%)	3 (25%)

*Those which resulted in the birth of young ones.

**Those which did not result in the birth of young ones and included those that terminated in pseudopregnancy, resorption and abortion.

***Those in which female did not accept the male as confirmed by a vaginal plug or presence of sperms in the vaginal smear.

when both the partners had been administered diets containing 2.5% or more of ROC 101 for 28 days prior to mating.

If only the female partner was administered the experimental diet (Table II), a 100% inhibition of fertile matings was observed at the 5 and 10% dose levels. There was an increase in the number of sterile matings with increasing dosage. The treated females mated readily with normal partners after the withdrawal of ROC 101 from the diet and became pregnant.

However, if the male partner received the experimental diet (Table III), inhibition of fertile matings was evident from the lowest dose. The number of sterile matings had increased considerably at the 10% dose. Although the treated males mated readily with normal partners

after withdrawal of ROC 101 from the diet, the proportion of infertile matings, predominantly pseudopregnancies, remained abnormally high. The sterility induced in the males was not reversible.

TABLE II : Effect of ROC-101 on the fertility of female mice

Dose	*No. of treated females*	*No. of normal males used for mating*	*Fertile matings*	*Infertile matings*	*Sterile matings*
1.0%	12	12	12(100%)	0	0
2.5%	12	12	6(50%)	6(50%)	0
5.0%	12	12	1(8.3%)	8(66.6%)	3(25%)
10.0%	12	12	0	7(58.3%)	5(41.6%)
After discontinuation of treatment					
2.5%	6	6	6(100%)	0	0
5.0%	11	11	4(36.3%)	7(63.6%)	0
10.0%	12(1 died)	12	5(45.4%)	6(54.5%)	0

TABLE III : Effect of ROC-101 on the fertility of male mice

Dose	*No. of treated males*	*No. of normal females inseminated*	*Fertile matings*	*Infertile matings*	*Sterile matings*
During treatment					
1.0%	12	12	6(50%)	6(50%)	0
2.5%	12	12	0	12(100%)	0
5.0%	12	28	0	25(89.2%)	3 (10.7%)
10.0%	12	27	0	16(59.2%)	11 (40.7%)
After discontinuation of treatment					
1.0%	6	6	6 (100%)	0	0
2.5%	12	12	0	12(100%)	0
5.0%	12	21	0	21(100%)	0
10.0%	12	29	0	23(79.3%)	6 (20.6%)

Table 4 summarizes the data on the effect of ROC 101 on the fertility of female rats. A 100% inhibition of fertility was observed at the 2.5 and 5.0% dose levels, with the treated females recovering fertility on discontinuation of the experimental diet.

TABLE IV : Effect of ROC-101 on the fertility of female rats

Dose	*No. of treated females*	*No. of normal males used for mating*	*Fertile matings*	*Infertile matings*	*Sterile matings*
1.0%	12	12	12(100%)	0	0
2.5%	8	8	0	8 (100%)	0
5.0%	8	8	0	8 (100%)	0
After discontinuation of treatment :					
2.5%	8	8	3 (37.5%)	5 (62.5%)	0
5.0%	8	8	4 (50%)	4 (50%)	0

The effect of chronic treatment with ROC 101 on the organ weights of female mice is given in the histogram (Fig. 1). Mice were administered the experimental diet for 30 days and

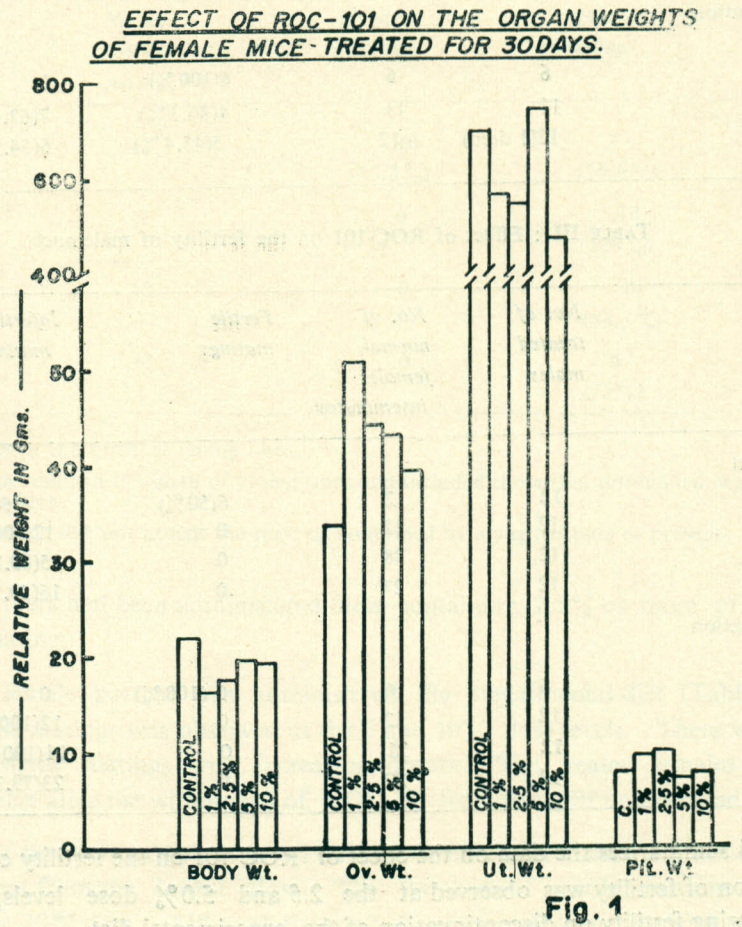
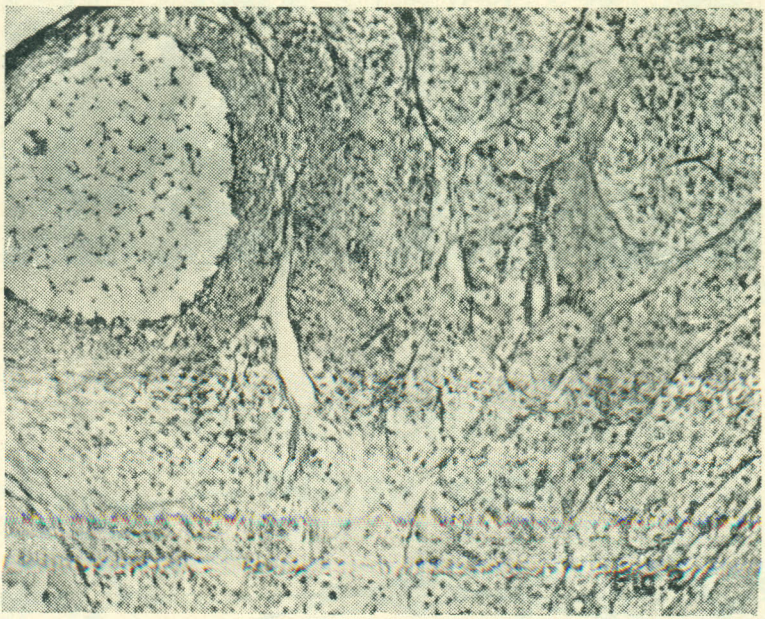


Fig. 1

then killed. As can be observed from the histogram, administration of ROC 101 caused a reduction in the body weight of all the animals. As compared to normal controls, ovarian weights were increased whilst the uterine weights were decreased, except at the 5.0% dose. The pituitary weights which were increased at the two lower doses were within control levels at the high doses.

The plant preparation does not appear to be antiovulatory since newly formed corpora lutea were seen in the ovaries of all treated mice. At the two higher doses, the ovarian stroma was filled with luteal type of tissue (Fig. 2) and there was increased follicular atresia. Thus the



possibility of the ovum being destroyed after ovulation is greater and may be the cause of inhibition of fertility. The uterii of all treated mice showed proliferation of the endometrial glands (Fig. 3) indicating estrogenic stimulation.

In the experiments with male mice, ROC 101 was administered at the 2.5, 5.0, and 10.0% doses. Mice were sacrificed after 1, 3, 5, 7, 14, 21 and 28 days of treatment. Male mice failed to gain weight at the same rate as the controls (Graph 1). The weight of the testes (Graph 2) were reduced at all dose levels, the effect more pronounced at the 5 and 10.0% dose levels after 21 days of treatment. There was no change in the ventral prostate weights of mice administered ROC 101 at the 2.5 and 5.0% dose levels (Graph 3). The reduction in the ventral prostate weights after 7 days of administration of 10% dose of ROC 101 is difficult to explain, since these weights were comparable to normal controls after 28 days of treatment.

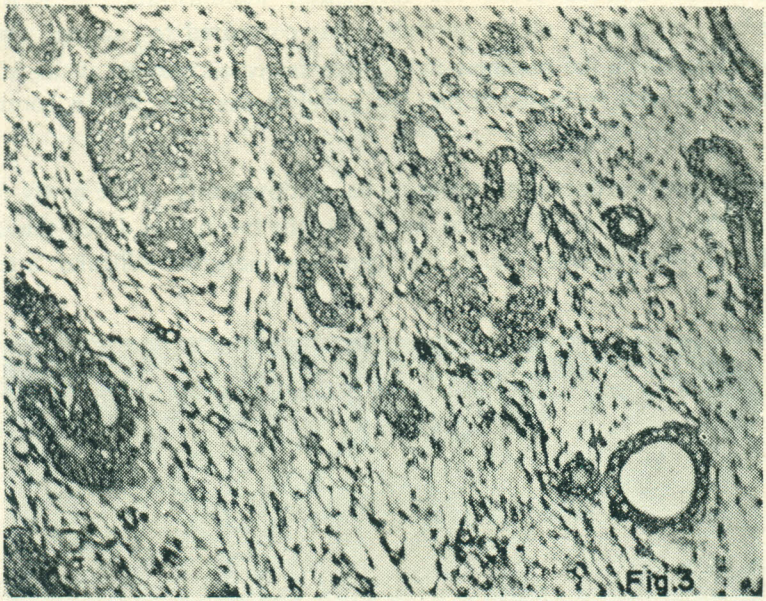
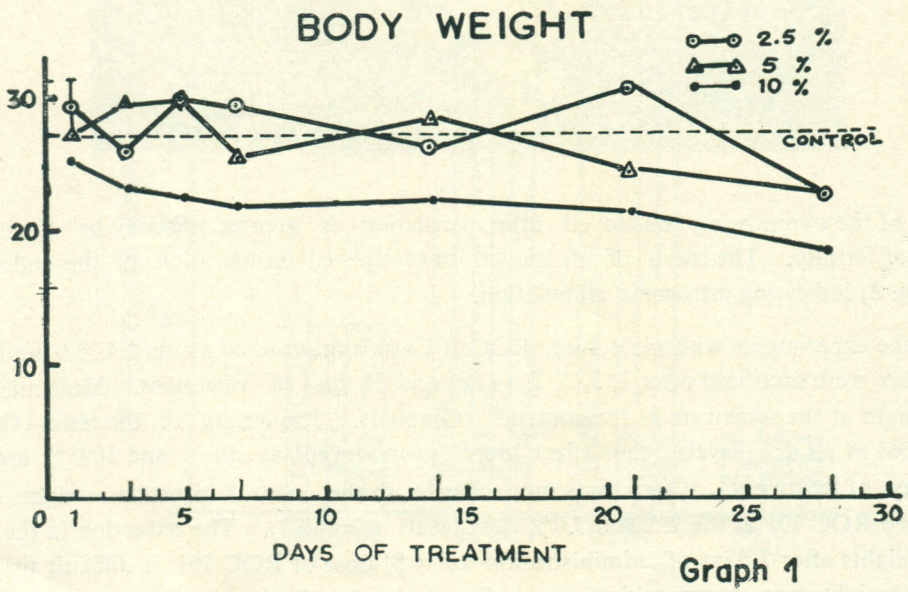
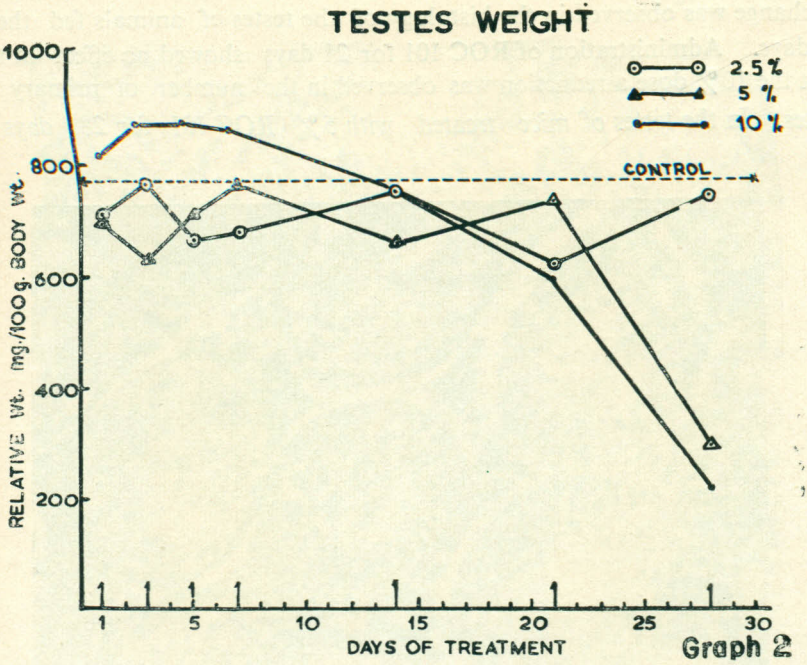


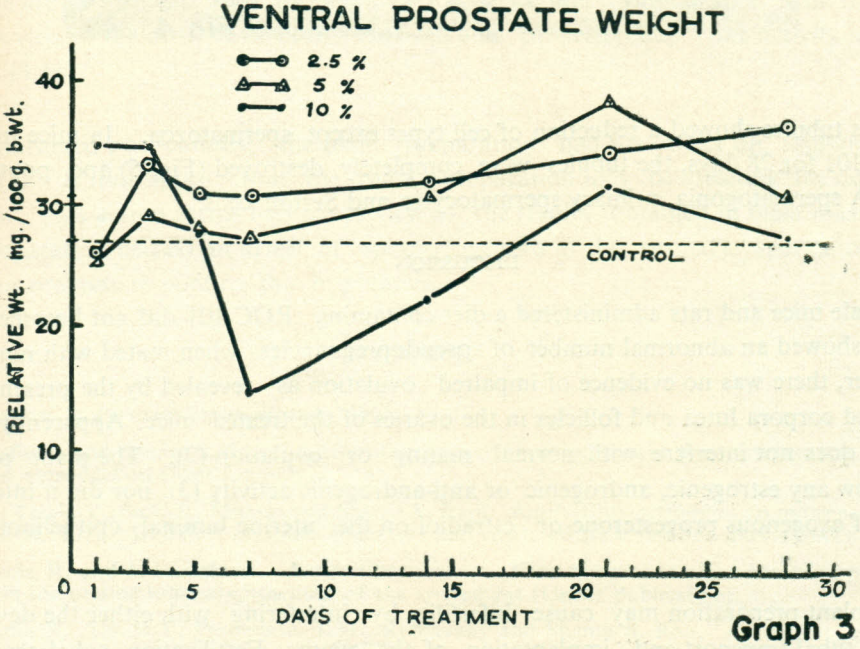
Fig. 3


BODY WEIGHT
2.5 %
5 %
10 %
CONTROL
30
20
10
0
1
5
10
15
20
25
30
DAYS OF TREATMENT

Graph 1



Graph 2



Graph 3

No change was observed in the histology of the testes of animals fed the plant prepa tion for 14 days. Administration of ROC 101 for 21 days showed no effect at the 5.0% dos However, at the 10% dose a reduction was observed in the number of primary and seconda spermatocytes. In the testes of mice treated with 5% ROC 101 for 28 days (Fig. 4), th

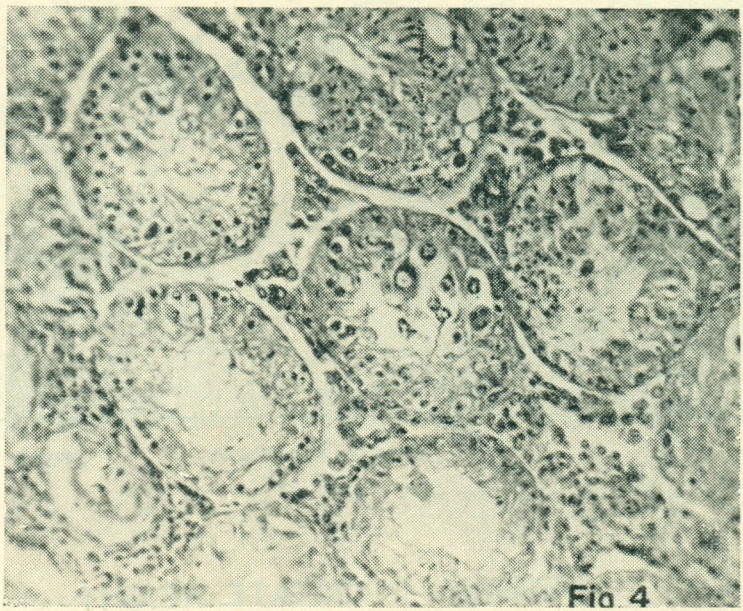


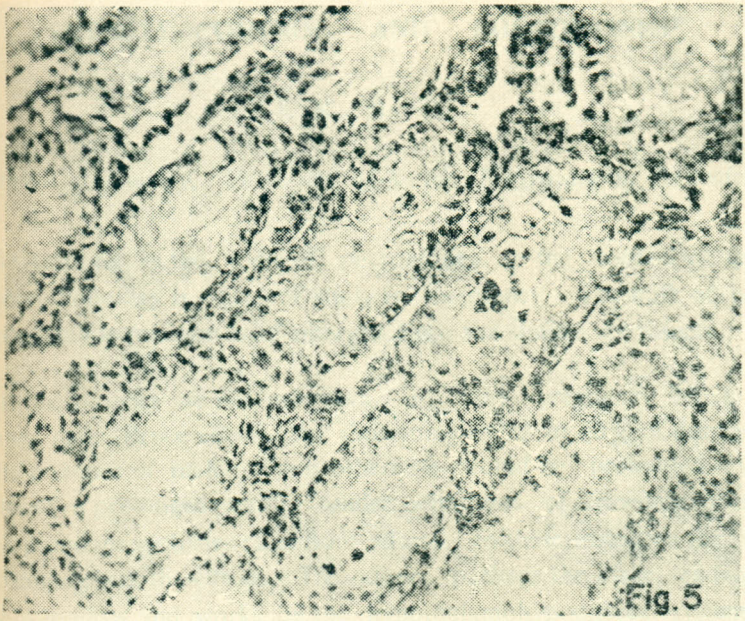
Fig. 4

seminiferous tubules showed a reduction of cell types except spermatozoa. In mice treated with 10% ROC 101 for 28 days, the tubules were completely destroyed (Fig. 5) and populated only with Type A spermatogonia, primary spermatocytes and Sertoli cells.

DISCUSSION

Female mice and rats administered a diet containing ROC 101 did not become pregnant, but instead showed an abnormal number of pseudopregnancies when mated with normal males (2). Further, there was no evidence of impaired ovulation as revealed by the presence of both newly formed corpora lutea and follicles in the ovaries of the treated mice. Apparently the plant preparation does not interfere with normal mating or ovulation (2). The plant preparation failed to show any estrogenic, androgenic or anti-androgenic activity (3), nor did it interfere with the action of exogenous progesterone or estradiol on the uterine luminal epithelium of spayed rats (4).

The plant preparation may cause infertility by interfering with either the development, fertilization, tubal transport and implantation of the ovum. Fertilization, tubal transport and



implantation are all influenced by estrogen or progesterone. Interferance with them can be ruled out in view of the absence of both estrogen and progesterone – like activity in the plant preparation (3,4).

Administered post-coitally, the plant preparation had no effect on implantation and pregnancy (2). It thus appears that the plant preparation may be affecting the development of the ovum. This action seems feasible in view of the results obtained in male mice (5) where the plant preparation caused an arrest of spermatogenesis at the primary spermatocyte stage. Studies are in progress to confirm this hypothesis.

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