

ANTIFERTILITY PROPERTIES OF THE NONSAPONIFIED FRACTION OF SEEDS OF *PSORALIA CORYLIFOLIA* IN THE ADULT FEMALE RATS

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Abstract. Nonsaponified fraction of seeds of *Psoralia corylifolia* when administered in the dosage of 60 and 90 mg/day shows antifertility properties in the adult rats. This is attributed to the failure of implantation and resorption in early or late stages of pregnancy.

On the basis of previous studies with nonsaponified fraction of *Psoralia corylifolia*, it was concluded that the substance has some estrogenic property which influenced the implantation in ovariectomized female rats.¹ The work was undertaken with the view that this substance might prove to be an antifertility substance in intact pregnant female rats.

Material and Methods

Mature virgin female rats 100-200 g in weight and 3-3½ months of age of Sprague Dawley strain were kept with adult males. Pregnancy was confirmed in the morning before 10.00 a.m. by the presence of vaginal plug or the presence of spermatozoa in the vaginal smears. The day on which the sperms were found, was designated as day I of pregnancy. Laparectomies were performed by the method used by Nutting and Meyer.² Autopsy was performed on 26th day of pregnancy. Ether was used as an anaesthetic. Clean but not sterile, conditions were maintained during surgical manipulations. Thirty pregnant rats were divided into 5 groups of 5 animals each, except in group D which contained 10 animals. Group A was kept as control and no treatment was given to them. The treatment with the doses of 30, 60, and 90 mg were injected subcutaneously in 0.2 ml corn oil daily per group B, C, D and E respectively. Treatment was started from day I of pregnancy and continued till autopsy. Laparectomy was done on the 9th day of pregnancy to check the implantation sites or delayed blastocysts. Autopsy was performed on 26th day of pregnancy to check the number of live foetuses, dead foetuses, resorption sites or absorption scars. Nonsaponified fraction of *Psoralia corylifolia* in petroleum ether was obtained from the Pharmacological Section of Drugs Research Institute, which was prepared by the method described earlier.³ Statistical analysis was done by the method described by Snedecor and Cochrane.

Results

Results are presented in Table 1. In the normal control group implantation sites occurred in 80% of the animals and pregnancy was maintained in all the implanted animals with normal parturition. In groups

B, C and D, the size of implantation sites were smaller (1.5-2.5 mm), than the normal one (3.00 mm). In group 'B' delayed parturition occurred in 40% animals (half of the implanted animals) and in the remaining 40% of the implanted animals reabsorption sites were observed.

In group 'C' with the dose of 60 mg, the percentage of animals with implantation sites is reduced and parturition did not occur in them. At autopsy only one (20%) of the implanted animals exhibited 5 live foetuses which were normal in weight, size and structure. And the other 20% of the implanted animals showed neither resorption sites nor placental scars. Uterus was found in +++ condition.

In group 'D' when the higher dose (90 mg) was given, neither implantation sites nor delayed blastocysts were recovered from the uterus, which is statistically significant from the control ($P < 0.05$). Uterus of all the animals were in +++ condition.

In group 'E' with the same dose, when injections were missed on the 3rd day of pregnancy, 80% of animals exhibited implantation sites. The size of implantation was smaller (1.5-2.5 mm) than the normal one (3.00 mm). At autopsy on the 26th day of pregnancy in 60% animals neither resorption sites nor placental scars were found. Uterus of those animals were in +++ condition. In 20% animals absorption scars were found.

Discussion

Group 'A' shows implantation sites in 80% sperm +ve females and *delayed blastocysts* in 20% sperm +ve females. This result shows that mating is fertile.

None of the unimplanted animals in the treated group shows the recovery of delayed blastocysts. It may be due to the oestrogenic nature of the substance which flushes the blastocyst in the treated groups.

The results on implantation shows that the number of animals with implantation sites is reduced with the increase in the dose, completely prevent the implantation and terminated the pregnancy at the earlier stage of pregnancy. There is a significant difference in the percentage of animals with implantation sites between the untreated

TABLE 1. EFFECTS OF NONSAPONIFIED FRACTION OF SEEDS *Psoralia corylifolia* ON THE MAINTENANCE OF PREGNANCY IN INTACT PREGNANT FEMALE RATS.

Group	No. of animals	*Treatment	% with C.L. of animals with			Average No. with S.E. of		
			Implant sites	Delayed blast	Parturition	Implant sites	Delayed blast	Pups born
A	5	Control nil	80.00±34.8% (4/5)	20.00±34.88% (1/5)	80.00±34.88% (4/5)	8.00±0.46	4.00±0.00	7.50±0.33
B	5	30 mg/day	80.00±34.88% (4/5)	0.00±0.00% (0/5)	†40.00±42.92% (2/5)	5.50±0.74	0.00±0.00	4.00±2.82
C	5	60 mg/day	40.00±42.92% (2/5)	0.00±0.00% (0/5)	**0.00±0.00% (0/5)	5.00±2.82	0.00±0.00	0.00±0.00
D	10	90 mg/day	‡0.00±0.00% (0/10)	0.00±0.00% (0/10)	0.00±0.00% (0/10)	0.00±0.00	0.00±0.00	0.00±0.00
E	5	90 mg/day	***80.00±34.88% (5/4)	0.00±0.00% (0/5)	0.00±0.00% (0/5)	6.25±1.27	0.00±0.00	0.00±0.00

*Treatment was given from day 1 of pregnancy till parturition.
†Parturition was delayed. **Five normal live foetuses were found at autopsy on 26th day of pregnancy.

‡Injections were given regularly from day 1 of pregnancy to the 9th day of pregnancy. ***Injections were missed on 3rd day of pregnancy.

(group 'A') and treated 90 mg/0.2 ml animals (group 'D'). This shows the treatment effects at the implantation stage. As it is known that a delicate balance of oestrogen and progesterone is required for implantation, i.e. 1:4000 in adult female rats.⁵ This substance with high dose may have disturbed the hormonal balance and prevented the implantation. In group 'D' where neither implantation sites for delayed blastocysts were observed and/or recovered, the substance may effect the preimplantation stages, i.e. on blastocysts which are flushed in all the animals of group 'D'. It is due to the oestrogenic nature of the substance. As it has been reported earlier^{6,7} that high levels of progesterone and/or estrogen accelerated the rate of transport of embryos and hence flushes the blastocyst from the uterus. The size of the implantation sites in all the treated groups are smaller than the normal. The size ranges from 1.5 to 2.5 mm. While in the normal control group the size of implantation sites are 3 mm. The results indicate that the treatment of the substance not only delay the implantation but also reduces the average number of implantation sites.

In the postimplantation stages, the results show that pregnancy cannot be maintained with the treatment of this substance. With the increase in dose the failure in pregnancy increases. The constant supply of progesterone is needed in the early period of gestation, this treatment might have effected the required supply.

In group 'E' when injections were missed on third day of pregnancy the percentage of animals with implantation sites increase. But if the injections are

given regularly (in group 'D') with the high dose of 90 mg, the optimum oestrogen-progesterone ratio needed for implantation is disturbed and implantation is completely prevented, presumably due to the high estrogenic nature of the substance. This shows that this substance is effective when given before the implantation for preventing the implantation and after for interrupting the pregnancy.

Reabsorption Sites and Absorption Scars

Reabsorption of implantation sites occur in the treated groups. It has been reported by Hishaw⁸ that estrogenic substance interferes with the decidumata formation in rat, presumably by interfering with progesterone and there are also possibilities that pregnancy is also terminated by the degeneration of placenta.

In group 'E' at autopsy in most of the implanted animals resorption sites occur and in 20% females absorption scars were found and in 60% implanted females neither absorption sites nor absorption scars were found. This showed that in 60% animals pregnancy terminated at a very early stage.

Delayed Parturition

In mammalian species withdrawal of progesterone is responsible for the onset of parturition. As long as progesterone is dominant, parturition cannot occur.⁹ The substance might prevent the progesterone withdrawal and, therefore, parturition does not occur in

substance-treated groups. In group 'B' in which parturition is delayed, injections were missed for three days (20-22 days).

By the above results it is concluded that the higher dose, i.e. 90 mg/rat/day is found sufficient to terminate pregnancy at earlier stages and with lower dosages at later stage. This was attributed to failure of implantation and resorption in early or late stages of pregnancy. The data seems to indicate that the substance contains some antifertility substance, and further substantiate our previous finding that the substance may be estrogenic in nature. Its mode of action is to be investigated.

References

1. F. Samad and Z. U. Khan, All Pakistan Urdu Science Conference Abstract, 1972, p. 9.
2. E.F. Nutting and R.K. Meyer, *J. Endocer.*, **29**, 235 (1964).
3. N.M. Din, N.U. Durrani, Z.U. Khan and Z.A. Jan, 23rd All Pakistan Science Conference Abstract, 1971, p. 10.
4. G.W. Snedecor and W.G. Cochran, *Statistical Methods* (Iowa State University Press, Iowa, 1968), p. 239.
5. R.L. Cochran and R. K. Meyer, *Soc. Exptl. Biol. Med.*, **96**, 155 (1957).
6. Z.U. Khan and R.K. Meyer, *Fertil. Steril.*, **20**, 667 (1969).
7. H.O. Burdick, R. Whitney and G. Pincus, *J. Anat. Res.*, **67**, 513 (1937).
8. F.L. Hishaw, J.T. Velardo and H.K. Ziel, *J. Clin. Endocer.*, **14**, 763 (1954) as quoted by Jackson H. (1966) in *Antifertility Compounds in the Male and Female* (Charles C. Thomas), p. 186.
9. A. Csapo, *Ann. New York Acad. Sci.*, **75**, 790 (1959).