

INVESTIGATION ON ANTIFERTILITY ACTIVITIES OF THE FRESH JUICE OF RAPHANUS SATIVUS ON EXPERIMENTAL ANIMALS.

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Summary

Antifertility activity of fresh juice of *Raphanus sativus* was studied in female rats. Fresh juice of *Raphanus sativus* was introduced by oral route at various dose levels. It has shown prolongation of each of the estrous phases in estrous cycle. A decrease in the number of implantation, average number of pups delivered, average weight of the pups, number of corpora lutea, weight of ovary, the number of estrous cycle per day and average number of estrous days increased in the treated animals compared to control. The result of the biochemical estimation of cholesterol, G6PD and protein in blood also supports the result of estrous cycle and indicates the antifertility effect of the *Raphanus sativus*.

Key words: Antifertility; *Raphanus sativus*; estrous cycle; corpora lutea; implantation.

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Raphanus sativus has utility both as a vegetable and medicine. It has been extensively used in the Indian system of medicine for treatment of various ailments. *Raphanus sativus* has emmunogouge, laxative, diuretic and anti-spasmodic activity¹.

In the rural areas of Uttar Pradesh, the ayurvedic practitioners discourage the use of *Raphanus sativus* in patients suffering from the fertility problems. However its implication on pregnancy, cholesterol and G6PD is unknown. Therefore, the current study was undertaken to probe in to it.

Material and Methods

Animals

Female Wistar albino rats (175-250g) were maintained under standard husbandry conditions (temperature of 250 ± 10 °C; RH 45 to 55% and 12: 12 light/dark cycle). The experiments were performed after prior approval of the study protocol by the institutional animal ethics committee of Sree Siddaganga college of Pharmacy, Tumkur, Karnataka, India. The study was conducted in accordance with the guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Experimental design

Totally four groups of female rats with normal estrous cycle were taken for the study. Each group contained 6 rats. One group was kept as a control and administered normal saline in a dose of 1ml/100gm by the oral route. The other 3 groups received 1ml/100gm, 2ml/100gm and 5ml/100gm fresh juice of *Raphanus sativus* by oral route respectively for 15 days.

Preparation of fresh juice of *Raphanus sativus*

Roots of *Raphanus sativus* were chopped in to pieces after washing properly in running water and the juice was extracted out of mashing the root in mixer.

Observation of estrous cycle

The vaginal smear of each female rat was examined daily. Only rats with normal estrous cycle² were selected for the experiment. Anti-fertility activity was determined as described by Khanna and Chaudhary 1968³.

The female rats in the proestrous phase of estrous cycle were caged with male rats of proven fertility in the ratio of 3:1. The evidence of copulation was confirmed by observing the lumps of spermatozoa in the vaginal smear and this day was taken as day one of pregnancy. Mated rats were randomly distributed into 4 groups of 6 animals each. The animals were laprotomised under mild ether anesthesia on day 16 and the number of implantation and corpora lutea were counted. All the animals were sutured and allowed for full term and the numbers of litters born were noted after delivery.

The semi autoanalyzer (Galaxo SmithKline Pharmaceuticals Ltd.) was used for the estimation of total cholesterol, total protein and G6PD by the kits from Erba Company.

Statistical analysis

Results are expressed as mean \pm SEM. Statistical differences between means were analyzed using one-way ANOVA followed by Student T test.

Results

Pregnancy parameters

The data on estrous cycle are shown in Table 1 and 2. It is evident that the estrous cycle became irregular after administration of the fresh juice of *Raphanus sativus*. In the first week of treatment group 1 (1ml/100gm) showed the extension for two days in proestrous and estrous phase, group 2 (2ml/10gm) and group 3 (5ml/100gm) has shown the extension in the estrous phase for two days. In the second week observation of group 1 has shown an extension for four days in estrous phase. Group 2 and group 3 have shown the extension of estrous phase for two days but group 2 has also shown the extension of metaestrous for 2 days. In both weeks of study the control has shown the regular pattern of estrous phases in estrous cycle four 4 days as proestrous-estrous-metaestrous-diostrous.

The data on weight of ovary, vaginal cornification and corpora lutea are shown in Table 3. The weight of the ovary in the control female rats is 6.0 ± 0.04 ($p < 0.001$). However in group 1, it is 5.6 ± 0.25 ($p < 0.001$), group 2 is 4.9 ± 0.09 ($p < 0.001$) and in group 3 is 4.8 ± 0.08 ($p < 0.001$). The weight of the ovary has decreased as the dose increased. Examination of vaginal cornification has revealed that there was percentage increase in the number of cornified cells. As the dose was increased the percentage of cornified cells increased. In group 2 and 3, the cornification was 100% ($p < 0.02$) and in group 1 it was 90% as compared to the control. The number of corpora lutea are decreased in the fresh juice of *Raphanus sativus* treated groups. Group received 1, 2 and 5ml/100gm body weight dose have shown a decreased number of corpora lutea of 9, 10 and 8 respectively as compared to control.

The effects of the fresh juice of *Raphanus sativus* on implantation, number of litters delivered and on weight of litters are presented in Table 4. The fresh juice of *Raphanus sativus* also showed anti implantation activity. Group 1, 2 and 3 have shown decreased number of implantation 7 ($p < 0.05$), 8 ($p < 0.05$) and 6 ($p < 0.05$) respectively as compared to control. The number of litters born were significantly reduced in all the groups treated with *Raphanus sativus*. In the doses of 2ml/100gm and 5ml/100gm, it is observed that 50% rats did not deliver any litters. The lapotomy of these rats on 15th day showed the resorption of the implantation sites. The control group has shown the number of pups as 10 ($p < 0.001$) and in groups 1, 2 and 3 were 6 ($p < 0.01$), 6 ($p < 0.01$) and 5 ($p < 0.01$) respectively.

Biochemical studies

The data for biochemical parameters are summarized in Table 5. The levels of total cholesterol and total protein in the treated rats were elevated significantly in the group 1, 2 and 3 compared to control. Cholesterol level in group 1, 2 and 3 was 135.3 ± 0.03 , 135.5 ± 0.04 ($p < 0.01$) and 143 ± 0.03 ($p < 0.05$) respectively and in control group it was 100.6 ± 0.006 . The protein level in group 1, 2 and 3 was 8.88 ± 0.006 , 8.90 ± 0.06 and 9.02 ± 0.008 respectively and in the control was 8.47 ± 0.006 . But the levels of G6PD in the treated rats were decreased significantly in group 1, 2 and 3 compared to control. G6PD level in group 1, 2 and 3 was 58.4 ± 0.06 , 51.6 ± 0.06 and 43 ± 0.06 respectively and in the control was 74.8 ± 0.002 ($p < 0.02$).

Table 1: Effect on pattern of different phases of estrous cycle of rats treated with fresh juice of *Raphanus sativus* for 15 days.

Estrous days	Control Normal saline(5ml/kg)	Group1 FJRS(1ml/100gm)	Group2 FJRS(2ml/100gm)	Group3 FJRS(5ml/100gm)
First week	P/O/M/D	P/P/O/O	O/O/M/D	O/P/O/O
Second week	P/O/M/D	O/O/O/O	O/O/M/M	O/O/D/P

P=Proestrous phase, O=estrous phase, D=Diestrous phase, M=Metaestrous phase

Table 2: Effect on prolongation of estrous days of different phases of estrous cycle on rats treated with fresh juice of *Raphanus sativus* for 15 days.

Group/dose	Diestrus	Proestrus	estrous	Metaestrus
Control	1 (57hr.)	1 (12hr.)	1 (12hr.)	1 (21hr.)
1/1ml/100gm	4 (228hr.)	4 (48 hr.)	7 (84hr.)	0
2/2ml/100gm	3 (171hr.)	3 (36hr.)	6 (72hr.)	5 (105hr.)
3/5ml/100gm	6 (342hr.)	5 (60hr.)	8 (96hr.)	4 (84hr.)

Table 3: Effect on weight of ovary, vaginal cornification (%), number of corpora lutea on rats treated with fresh juice of *Raphanus sativus* for 15 days.

Parameter	Control Normal saline (5ml/kg)	Group 1 FJRS(1ml/100gm)	Group 2 FJRS(2ml/100gm)	Group 3 FJRS(5ml/100gm)
Weight of ovary (mg)	6.0 ± 0.04*	5.6 ± 0.25*	4.9 ± 0.09*	4.8 ± 0.08*
Vaginal cornification(%)	0	90	100#	100#
No. of corpora lutea	13	9##	10#	8**

FJRS= fresh juice of *Raphanus sativus* *p<0.001, **p<0.01, #p<0.02, ##p<0.05

Table 4: Effect on number of implantation, average number of pups and average weight of pups of rats treated with fresh juice of *Raphanus sativus* for 15 days.

Parameter	Control Normal saline (5ml/kg)	Group 1 FJRS(1ml/100gm)	Group 2 FJRS(2ml/100gm)	Group 3 FJRS(5ml/100gm)
No of implantation	11	7##	8##	6##
Average no. of pups	10*	6*	6*	5*
Average weight of pups	2.0±.05	1.9±.05	1.9±.05	1.6±.05

FJRS= fresh juice of *Raphanus sativus* *p<0.001, **p<0.01, #p<0.02, ##p<0.05

Table 5: Effect of fresh juice of *Raphanus sativus* on biochemical studies of rats.

Parameters	Control Normal saline(5ml/kg) ±SEM	Group 1 FJRS(1ml/100gm) ±SEM	Group 2 FJRS(2ml/100gm) ±SEM	Group 3 FJRS(5ml/100gm) ±SEM
Cholesterol (mg/dl)	100.6 ± 0.06	135.3 ± 0.03	135.5± 0.04**	143 ± 0.03##
Total protein (mg/dl)	8.47 ± 0.006	8.88 ± 0.006	8.90 ± 0.06	9.02 ± 0.008
G6PD (g/dl)	74.8 ± 0.002	58.4 ± 0.06	51.6 ± 0.05#	43 ± 0.06

*p<0.001, **p<0.01, #p<0.02, ##p<0.05 G6PD=Glucose 6-Phosphate Dehydrogenase.

Discussion

During the estrous cycle the maturation and ovulation of preovulatory follicles takes place under the combined and balanced influence of ovarian and extra ovarian hormones⁴. Any imbalance in these hormones leads to irregularity in the function of the ovary and irregular changes in the duration of estrous cycle⁵. It is generally accepted that the sequential changes of vaginal smear in the different phases of the estrous cycle are closely associated with gonadal steroidal secretion⁶.

In the normal case the timing of cycles is estrous cycle-12 hours, metaestrous cycle-21 hours, diestrous cycle-57 hours, proestrous cycle-12 hours⁷. In the group 1, 2 & 3 have shown the irregular estrous cycle as compare to control; there was continuous prolongation of each of the estrous phase in estrous cycle. The prolongation of estrous phase of vaginal smear reflects the estrogenic nature of the extracts.

The fertilization is dependent on the reproductive hormones like LH, FSH, Estrogen and Progesterone. At the hormonal level, estrogen and progesterone are the main factors influencing the process of implantation^{8, 9}. The result of the present study indicates that the anti implantation effect of the *Raphanus sativus* during the early days of pregnancy, may be due to the lowering of the serum progesterone level¹⁰. E.ribes fruit¹¹ was reported to alter levels of estrogen and progesterone leading to improper implantation. Similar observation has been reported with D.carota seeds¹² to exhibit antiestrogenic and antigonadotropic activity. It is well known that for implantation, exact equilibrium of estrogen and progesterone is essential and the disturbance in the level of these hormones may cause infertility¹³. The compound of hormonal values usually disturbs the hormonal milieu in the uterus and provokes an infertility effect. Therefore the anti implantation activity may be due to estrogenic activity causing expulsion of ova from the tube, disrupting the luteotrophic activity of the blastocyst¹⁴. Progesterone is essential for blastocyst implantation^{15, 16} and the maintenance of pregnancy in all phases^{17, 18}. Hence the antifertility activity of *Raphanus sativus* may be due to the antiprogesterone activity, which may be acting either as inhibitor in the biosynthesis of progesterone or as blocker in the receptor binding. Decreased number of implantation against increased the doses of *Raphanus sativus* have been observed compared to control. The weights of the stroma, the follicle and the corpus luteum constitute the net weight of the ovary. During the estrous cycle the weight of the ovarian tissues increases under the influence of gonadotrophic and steroidal hormones. The decrease in the weight of ovaries in treated rats indicates the decrease in the activity of stroma, follicle and corpus luteum in the ovary. This decrease is due to a nonavailability of either gonadotrophic or steroidal hormones or both¹⁹. The accumulation of cholesterol and decrease the content of G6PD along with the remarkable fall in the ovarian weight lead to suggest impairment in the synthesis of ovarian steroid hormones resulting in hypofunctioning of steroidogenic activity in the *Raphanus sativus* treated rats²⁰.

The cornification in the vagina is mainly due to the level of stimulation of estrogen which acts directly on the vaginal epithelium²¹. For the vaginal cornification the observation has said that there was percent increase in the number of cornified cells. As the dose increased the percentage of cornified cells increased significantly. The cornification in the vagina is mainly due to the level of stimulation of estrogen which acts directly on the vaginal epithelium²².

After the ovulation, what remains in the graaffian follicle, is called the corpus luteum. LH is required for ovulation and for the transformation of the graaffian follicle in to a functioning corpus luteum. If there is disturbance in development and functioning of corpus luteum means the drug is having the effect on hormones²³. As corpora lutea are the major source of progesterone hormone, which is responsible for

maintaining the uterus during early pregnancy. Their decrease in number or absence, in treated rats clearly leads to the reduced progesterone in the ovaries of treated rats during the estrous cycle²⁴. The decrease in the number of corpora lutea and graafian follicles and the increase in the number of atretic follicle in treated rats indicate that the development of preovulatory follicles and their conversion into corpora lutea is completely inhibited.

The considerable increase in total cholesterol content in the rat suggests impaired utilization of cholesterol in the synthesis of the steroid hormones resulting in decreased steroidogenesis because cholesterol is the precursor for steroidogenesis^{25, 26}. Protein content of uterus is correlated with suppressed action of estrogen²⁷. Evidence have been accumulated that estrogen can induce mitosis but progesterone suppresses estrogen induce mitosis in rat uterine epithelium. Since DNA component of the cell undergoes appreciable metabolic turnover during mitosis and synthesis of DNA takes place during the estrous cycle particularly after diestrous²⁵, the decreased level of DNA of rat uterus may indicate the suppression of mitosis²⁸. The levels of G6PD in the fresh juice of *Raphanus sativus* treated rats were elevated significantly in the group 1, 2 & 3 when compared to control. This enzyme is related to the steroidal hormone synthesis. The decrease in G6PD activity means a decrease in the ovarian hormone production, as gonadotropins through the activation of G6PD metabolism (enzyme involved is G6PD) increase the rate of production of gonadal hormone^{21, 22}. Similar reports have been collected for *C.papaya* seed²⁹ when it exhibits changes in the biochemical profile of the ovary and uterus involving alteration in the level of cholesterol, protein & G6PD and it is proved to be an antifertility agent.

The accumulation of cholesterol and decrease the content of G6PD along with the remarkable fall in the ovarian weight lead to suggest impairment in the synthesis of ovarian steroid hormones resulting in hypo functioning of steroidogenic activity in the *Raphanus sativus* treated rats. Though it is not possible to delineate the exact mechanism of its action and the phytoconstituents responsible for the effects of *Raphanus sativus* in this study, the results suggest some antifertility potential and a possible therapeutic use of this plant.

Conclusion

The present study was done for the investigation of fertility studies of *Raphanus sativus*. The results obtained from the present study have shown that *Raphanus sativus* possess antifertility activity. In female rats *Raphanus sativus* disturbed the estrous cycle along with increase in cholesterol & protein content and decreased the G6PD level.

Raphanus sativus was found to decrease the number of implantation, average number of pups delivered, average weight of the pups, number of corpora lutea, and weight of ovary.

Keeping in view the encouraging result obtained from this plant, it may be utilized as an antifertility agent. The wide spread availability of this plant makes it attractive candidate for further preclinical and clinical research.

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