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ANTIFERTILITY ACTIVITIES OF THE RAPHANUS SATIVUS: A PRECLINICAL STUDY

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ABSTRACT

Introduction- The present study was carried out to evaluate the antifertility activity of *Raphanus sativus* on male Wistar albino rats. The effect on sperm count, sperm motility, abnormalities in sperm, weight of testis and epididymis was studied using the fresh juice of *Raphanus sativus route*. Methods- The fresh juice of *Raphanus sativus*(FJRS) was introduced by oral route at various doses (1ml/100gm, 2ml/100gm, 5ml/100gm) to evaluate the antifertility activity. Normal Saline (1ml/100gm) was used as control agent. Depending on the methods used, the outcome measures were sperm count(millions/ml ±SEM), sperm motility(%±SEM) weight of testis and epidydimis (mg) and total percent sperm abnormalities. The data was analysed using one-way ANOVA followed by Student T test. Results- *Raphanus sativus* had significantly decreased the sperm count and sperm motility. A decrease in individual weight of testis and epidydimis was found. The percent sperm abnormalities was found to be increased compared to the control which also suggests the antifertility effect of *Raphanus sativus*. It also has dose dependent anti fertility effects and the results are expressed as mean ± SEM. Conclusions- The present study have shown that *Raphanus sativus* has an antifertility property.The encouraging results obtained from this plant, may be utilized as an antifertility agent. Further studies on isolation of specific active phytoconstituents and a search into its possible mechanism of action is needed.

KEYWORDS: Antifertility; *Raphanus sativus*; Testis; Epidydimis; Sperm motility.

INTRODUCTION

Birth control is extremely important in order to improve the life of the future generations. In developing countries like India, population explosion is a curse and is damaging to the development of the country and society. The developing countries are already facing a lack in their resources, and with the rapidly increasing population, the resources available per person is reduced further, leading to increased poverty, malnutrition. In fact it is extremely important in terms of better availability of resources and a clean world. It is imperative that we control population explosion. So the size of the family should be reduced by adopting any suitable method of family planning. One of the method is the use of the available contraceptives means which affects the different phases of the reproductive physiology.[1] Radishes have had a long relationship with man belonging for the family Brassicaceae.The annual growing hermaphrodite plant is native to South Asia, Middle east and India.[2,3,4] Raphanus sativus is under cultivation as vegetable crop in Chhattisgarh. The natives consume it for its delicious and pungent taste. For the traditional healers of Chhattisgarh, Raphanus sativus is a valuable medicinal herb. They use it both internally and externally in treatment of many common as well as complicated troubles. The traditional healers specialized in treatment of hypertension suggest the patients to take Raphanus sativus roots in good quantity in order to maintain the blood pressure to normal.[5] Several studies have been carried out to study its pharmacological effects. Phytochemicals such as Beta sitosterol isolated from seeds, phytosterols, glucobrassicin among others are present.[6,7,8,9] The plant contains raphanin, which is antibacterial and antifungal.[10,11] It has been extensively used and studied in the Indian system of medicine for treatment of various ailments.

MATERIAL AND METHODS

Animals

Male Wistar albino rats weighing 175-250g were used for the experiment. They were maintained under standard husbandry conditions with a temperature of 250± 10C, RH 45 to 55% and was kept under 12: 12 light/dark cycle. One- week time was provided to the animals to acclimatize with the laboratory environment. 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 male rats with normal spermatogenesis were taken for the study. Each group contained 6 rats. One group was kept as control and administered normal saline by oral route. The other 3 groups received 1ml/100gm, 2ml/100gm and 5ml/100gm fresh juice of *Raphanus sativus* by oral route respectively for 30 days. All treatments were given orally 1 hour before the experiment by oral gavage. Doses were determined based on the acute toxicity studies as per OECD guidelines.[12] The rats were allowed access to standard pellet feed and water ad libitum.

Preparation of fresh juice of Raphanus sativus

Taxonomic identification of the plant was done by the botanist. Roots of *Raphanus sativus* was purchased from the local market. Fresh roots were chopped in to small pieces after washing properly in running water. Adequate washing was done to remove soil and other contaminants. The juice was extracted out by mashing the root in a mixer. It was stained and then filtered. This technique was employed to extract the fresh juice of *Raphanus sativus* that had to be administered for a period of 30 days.

Observation of Sperm count and motility

Six healthy adult male albino rats were used in the study. The rats were anaesthetized using light ether anaesthesia. The skin of the scrotum was cut to open tunica vaginalis. Small incision was then made so that epididymis can come out. After pulling out the testis, contents of cauda epididymis were removed and flushed in 1 ml. of previously warmed buffer saline at 37°C to make suspension of spermatozoa.[13] The testes were returned back and the wound was sutured properly.[14] At least 85% initial normal sperm motility and 2.5x107 /cc sperm count was considered for the selection of samples. Wet drop technique was applied to study the motility of spermatozoa.[15,16]

Statistical analysis

Results are expressed as mean ± Standard Error of Mean(SEM). Statistical differences between means were analyzed using one-way ANOVA followed by Student T test.

RESULTS

Spermatogenesis Parameters

The data for the result of sperm count and motility are presented in the Table number 1.The test groups have shown the decreased sperm count that was 52.3±0.06, 42 ± 0.05($p<0.02$) &

27.5± 0.07($p<0.01$) for 1ml, 2ml and 5ml/100g body weight of rat respectively. And motility 51.1% ± 0.06, 38% ± 0.05 & 28.5% ± 0.05($p<0.001$) respectively in the FJRS treated rats when compared to control that was 68.3 ± 0.06 & 81.4% ± 0.05 respectively.

The changes in the individual weight of testis and cauda epididymis are presented in Table number 2. The group 1, 2 & 3 have shown the decreased weights of testis and epididymis in the FJRS treated rats were1010±.06 ($p<0.05$), 1001±.06 ($p<0.02$) & 800±.06 ($p<0.001$) and 480±.06, 430±.06 & 320±.06 ($p<0.05$) when compare to control that was 1220±.06 and 520±.06 respectively.

The results for the percent sperm abnormalities are summarized in Table 3. In the FJRS treated rats the percent sperm abnormalities were increasing significantly in the group of the doses 1, 2 and 5 ml/100g of body weight that was 25%, 27%&32% respectively when compare to the group of normal saline that was 10%.

Table 1: Effect on sperm count and sperm motility of rat treated with fresh juice of Raphanus sativus for 30 days.

Treatment	Route Of administration	No. of animals per group	Dose	Treatment duration	Sperm count (millions per ml) ±SEM	% of sperm Motility ±SEM
Control (saline)	oral	6	-	30 days	68.3 ± 0.06	81.4% ± 0.05
FJRS	oral	6	1ml/100gm	30 days	52.3± 0.06	51.1% ± 0.06
FJRS	oral	6	2ml/100gm	30 days	42 ± 0.05#	38% ± 0.05
FJRS	oral	6	3ml/100gm	30 days	27.5 ± 0.07**	28.5 ± 0.05 *

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

Table 2: Effect on weight of Testis and Epididymis of rat treated with fresh juice of Raphanus sativus for 30 days.

Organs	Control Normal saline(5ml/kg)	Group 1 FJRS (1ml/100gm)	Group 2 FJRS (2ml/100gm)	Group 3 FJRS (5ml/100gm)
Testes(mg)	1220±.06	1010±.06##	1001±.06#	800±.06*
Epididymis(mg)	520±.06	480±.06	430±.06	320±.06##
Route of administration	Oral	Oral	Oral	Oral
Epididymis(mg)	520±.06	480±.06	430±.06	320±.06##

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

Table 3: Effect on % abnormality of sperm of rat treated with fresh juice of Raphanus sativus for 30 days.

Drug	Dose mg/kg	Route of administration	No of animals	Total sperm count	Hook less	Amorphous	Folded	Two tailed/headed	Banana shaped	Total Abnormal sperm	%Total Abnormal Sperm
Control	Normal Saline 1ml/ 100gm	oral	6	3000	90	60	60	30	60	300	10
FJRS	1ml/ 100gm	oral	6	3000	390	150	60	60	90	750	25
FJRS	2ml/ 100gm	oral	6	3000	360	90	150	180	30	810	27
FJRS	5ml/ 100gm	oral	6	3000	360	210	150	150	90	960	32

DISCUSSION

The normal range of the sperm count in rats is 71 millions/ ml of semen. A sperm cell consists of three structures highly adopted for reaching and penetrating a secondary oocyte: a head, mid piece and a tail. Enzymes within the acrosome includes hyaluronidase and protease, which aids penetration of the sperm cell in to a secondary oocyte. In the mid piece are many mitochondria, which provides ATP for locomotion. The tail, a typical flagellum, propels the sperm cell along its way.[17]

If sperm count decreases the fertility of that rat will be affected, so it is important to know the count of sperm of that rat.The normal range of the sperm motility in rats is 76%. If there is reduction in the sperm motility, the fertility of that rat will be affected, hence it is an important parameter to be analysed for antifertility activity of *Raphanus sativus.*

The sperm Motility and sperm count was decreased in rats treated with 1, 2 & 5 ml of fresh juice of *Raphanus sativus* and were statistically significant. The reduction in the number of spermatozoa may be due to the altered androgenic synthesis.[18,19,20]

This is indicated from preliminary studies involving the reduction in weight of testis and accessory reproductive organs, especially Cauda epididymis, which may be due to the reduction in the number of spermatogenic elements and spermatozoa. Spermatogenesis begins in the spermatogonia, which contain the diploid chromosome number and differentiates into a primary spermatocytes. Each primary spermatocyte enlarges and then begins meiosis. The four haploid cells resulting from meiosis II are called spermatids. The final stage of spermatogenesis, is the maturation of haploid spermatids in to sperm. Because

no cell division occurs in spermatogenesis, each spermatid develops into a single sperm cell. During the process, spherical spermatids transform into elongated, slender sperm[21].This proposition was further strengthened by our studies on the principal steroid precursor cholesterol, involved in the biogenic pathway of androgen in both adrenals testes. Certain hypothalamic neurosecretory cells increase their secretion of gonadotropin –releasing hormone (GnRH). This hormone, in turn, stimulates gonadotrophs in the anterior pituitary to increase their secretion of the two gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone. LH stimulates leydig cells, which are located between seminiferous tubules, to secrete the hormone testosterone. This steroid hormone is synthesized from cholesterol in the testes and is the principal androgen. It is lipid soluble and readily diffuses out of Leydig cells in to the interstitial fluid and then into blood. Testosterone acts in a negative feedback manner to suppress secretion of LH by anterior pituitary gonadotrophs and to suppress secretion of GnRH by hypothalamic neurosecretory cells. In some target cells, such as those in the prostate and seminal vesicle, the enzyme 5-α reductase converts testosterone to other androgens called dihydrotestosterone (DHT).

FSH acts indirectly to stimulate spermatogenesis. FSH and testosterone act synergistically on the sertoli cells to stimulates secretions of androgen- binding protein (ABP) into the lumen of the somniferous tubules and in to the interstitial fluid around the spermatogenic cells. ABP binds to the testosterone, thereby keeping the concentration of testosterone high near the seminiferous tubules. Testosterone stimulates the final steps of spermatogenesis. Once the degree of spermatogenesis required for male reproductive function has been achieved, Sertoli cells release inhibin, a protein hormone named for its inhibition of FSH secretion by the anterior pituitary. Inhibin thus inhibits the secretion of hormone needed for spermatogenesis. If spermatogenesis is proceeding too slowly, less inhibin is released which permits more FSH secretion and an increased rate of spermatogenesis.[22] Various steps in mammalian reproduction such as spermatogenesis, ovulation, fertilization, ovum transport in the tube and the uterus, blastocyst development and implantation, and fetal maintenance are known to be regulated to a greater or lesser degree by the endogenous steroid hormones.[23] So for the steroidal hormone synthesis the availability of cholesterol and the G6PD is a very important factor. The important role of cholesterol and the G6PD and the protein in steroidogenesis and the synthesis of steroid hormones are well established.

LDL is present in blood and it contains heavy amount of cholesterol. This cholesterol is utilized for the synthesis of testosterone. The Leydig cells in the testes contain LDL receptors that use the cholesterol contained in LDL.[24] Accumulation of cholesterol in testis and adrenal clearly indicated less utilization of it in androgen biosynthesis. Less synthesis of androgen possibly resulted in the reduction of fertility rate.[25] The increase in cholesterol contents could be due to non-utilization of substances for androgen biosynthesis by fewer Leydig cells present or due to damage.

Reduction in the weight of sex accessory structure indicate the androgen ablation which results in decrease in overall cellular activities and increase in cell death, leading to regression of these organs[26,27,28] Sperm counts are considered to be one of the important factors that affects fertility. When the sperm count decreases the male is considered to be infertile. Sperm motility and fertilizing capacity of spermatozoa was decreased by Raphanus sativus treatment could be due to the androgen deficiency.[29] Treatment with Raphanus sativus alters the biochemical milliew. Reduced testicular glycogen level was correlated with diminished post meiotic germ cells a site of glucose metabolism.[30] Reduced protein content and protein in testes and sex accessories could be correlated with the absence of spermatozoa in the lumen.[31,32]

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.Further detailed investigations are necessary to document the active constituents.

CONCLUSION

The present study was done for the investigation of anti fertility studies of *Raphanus sativus*. The findings in the study confirm the anti fertility activity. The results obtained from the present study have shown that *Raphanus sativus* causes decrease in sperm count, motility and the weight of testis and epididymis in male rats along with percent increase in the sperm abnormalities.

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.

REFERENCE

1. Ashok G K. Handbook of Health Education and Community Pharmacy. CBS Publishers and Distributors, New Delhi.
2. Park J E and Park K. Textbook of Preventive and social medicine; 13 Ed.
3. www.fertiletext.org.
4. http://www.blackwell-synergy.com/doi/abs/10.1111/j.1472-8206.2005.
5. RHS. *The Garden. Volume 111*. Royal Horticultural Society, 1986.
6. Duke J A and Ayensu E S. *Medicinal Plants of China* Reference Publications, Inc. 1985 ISBN 0-917256-20-4.
7. Facciola. S. *Cornucopia - A Source Book of Edible Plants.* Kampong Publications 1990 ISBN 0-962 8087-0-9.
8. Rastogi R P and Mehrotra B N. Compendium of Indian Medicinal Plants.Vol III Centeral Drug Research Institute, Lucknow. Publication and Information Directorate , New Delhi, 94-95.
9. Plant Physiol, 1984 March; 74(3): 487–493.
10. Rastogi R P and Mehrotra B N. Compendium of Indian Medicinal Plants. Vol III Central Drug Research Institute, Lucknow. Publication and Information Directorate, New Delhi, 545-546
11. Bown D. *Encyclopedia of Herbs and their uses.* Dorling Kindersley, London, 1995 ISBN 0-7513-020-31
12. Organization for Economic Cooperation and Development. OECD Guidelines for the Testing of Chemicals. Acute Oral Toxicity -Up-AndDown-Procedure. UDP, 425. France: OECD Publishing, 2008.
13. Sur T K, Pandit S, Pramanik T, Bhattacharya D; Effect of Aegle marmelos leaf on rat sperm motility; Indian Journal of Pharmacology, 2002; 34: 276-77.
14. Bhargava V, Prakash AO. Spermicidal activity in vitro of bark extract of Azadirachta indica in rats. Ancient Sci Life, 2000; 20: 77-81.
15. Sander F V Carmer S D. A Practical method for testing the Spermicidal action of Chemical Contraceptive; Human Fertility, 1941; 6: 134-7.
16. W.H.O Laboratory Manual for the Examination of Human Semen and Semen Cervical mucus interaction Singapore Press Concern, 1980.
17. Waugh A, Grant A. Ross and Wilson, Anatomy and Physiology, 2001; 9: 449.
18. Sur T K, Pandit S, Pramanik T. Antispermatogenic activity of leaves of Aegle mermelos, Corr.in albino rats: A preliminary report. Biomedicine, 1999; 19: 199-202.

19. Guin A K, Das S Datta C, Dey S and Sengupta D. Biochemical effect of non antiovulatory doses of different progestins on cycling rat uterus. Indian Journal of Experimental Biology, 1980; 18: 355-57.
20. Sur T K, Pandit S, Pramanik T, Bhattacharya D. Effect of Aegle Marmelos Leaf on rat sperm motility. Indian journal of Pharmacology, 2002; 34: 278.
21. Gerard J Tortora,Sandra Reynolds Grabowski.Principles of anatomy and physiology,10th ed.John Wiley & Sons, Inc, 1018-21.
22. Gerard J Tortora, Sandra Reynolds Grabowski. Principles of anatomy and physiology, 10th ed. John Wiley & Sons, Inc, 1021-23.
23. Roy S. Control of fertility by Steroidal Contraceptives. Indian J Pharmac, 1970; 2(1): 7-21.
24. Chaudhuri K Sujit, Concise medical physiology, Fourth ed, 310.
25. Sherins R J and Hawards S S. Male infertility In: Campbell's urology 4th Ed. J H Harison R F, Giltes A D, Perimulter T A, Stamey and P C Walsh (Eds) Co. Vol. 1. Sect. II, Chap. 2 WB sounders Philadelphia: 715.
26. Adhikary P, Banerji J, Choudhary D, A K Das, Deb CC, Mukherjee S R and Chatterjee A. Effect of Piper betle Linn (Stalk) extract on male rat fertility. Indian J Pharmac, 1998; 22: 145-49.
27. Coffey D S. Androgen action and sex accessory tissue in 'The physiology of reproduction' Knobil E and Neill J D (Eds). Raven Press, New York, 1988; 1081-1119.
28. Huang H F S, Li M T, Hagen S V, Zhary H F and Irwin R J. Androgen modulation of messenger ribonucleic acid of retinoic acid receptors in the prostrate, seminal vesicle and kidney in the rat. Endocrinology, 1997; 138(2): 559-93.
29. Joshi S C, Verma P, Sharma M and Chaudhari N. Effect of benomyl on testicular cell population dynamics and fertility of male albino rat. Bullet Env Sci, 2002; XIX: 17-21.
30. Gupta G, Srivastava A K and Setty B S. Androgen regulation of glycolytic and HMP pathway in epididymis and vasa deferens of rhesus monkey. J Exp Biol, 1993; 31: 350.
31. Gunaga K P, Rao M S, Sheth A R and Rao S S. The role of glycogen during the development of rat testes and prostrate. J Repord fertil, 1972; 29: 157-62.
32. Wright W W, Parvinen M, Musto N A, Gonsalus G I, Philip D M, Mathur J P and Bardin C W. Identification on stage protein synthesized by rat seminiferous tubules. Biol Reprod, 1983; 29: 257-70.