AN ASSESSMENT OF THE ANTIFERTILITY PROPERTY OF *Ocimum sanctum*

BY

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THESIS

Submitted in partial fulfilment of the requirement for the degree

MASTER OF VETERINARY SCIENCE

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

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COLLEGE OF VETERINARY AND ANIMAL SCIENCES
Mannuthy - Trichur.

1979

DECLARATION

I hereby declare that this thesis entitled "AN ASSESSMENT OF THE ANTIFERTILITY PROPERTY OF Ocumum sanctum" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

Place: Mannuthy Signature:

Date : 4 8 1979 Name : K.GIRIJAN

CERTIFICATE

Certified that this thesis entitled "AN ASSESSMENT OF THE ANTIFERTILITY PROPERTY OF Coirum sanctum" is a record of research work done independently by Sri. K.Girisan under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

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Mannuthy
4-8-1979

ACKNOWLEDGEMENT

I wish to extend my sincere gratitude to Dr. Jacob V. Cheeran, Professor, Department of Pharmacology, for his valuable helps and inspiring guidance as the Major advisor.

My sincere thanks are due to Dr. A.Rajan, Professor (Project Officer), Department of Pathology, for his valuable helps and inspiring guidance as member of the Advisory Committee.

I am highly grateful to Dr. Zacharias Cherian, Associate Professor and Dr. T.L.B.Namboothiripad, Professor, as members of the Advisory Committee for their personal interest and encouragement.

The guidance and suggestions extended by Dr. P.U. Surendran, Professor of Statistics, for the statistical analysis of the data are gratefully acknowledged.

I am indebted to Dr. T.G.Nair, Dean, College of Veterinary and Animal Sciences, for all the facilities provided for the research work.

I am also grateful to the KERALA AGRICULTURAL UNIVERSITY for granting me the merit scholarship for the post-graduate studies.

I appreciate the helps and guidance extended by the staff members of the Department of Pharmacology.

With deep sense of gratitude and affection, I record my indebtedness to my beloved parents for having encouraged me to take up post-graduate studies.

I am thankful to Sri. V.T.Kurian for putting the manuscript into neat type.

K.GIRIJAY

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INTRODUCTION

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It took more than 16 centuries from the days of Christ to double the population and the next doubling of population took only 200 years and only 80 years for the third. Despite the two World Wars population multiplied and will take only 35 years to double its present strength. It is even estimated that the human population may grow seven times in the next 100 years (Gerald, 1974).

The explosive world population increase emphasises the need for the development of effective contraceptive agents and methods with minimum side effects having maximum protection for population control. It may be profitable to authentically confirm the information acquired by ancient people concerning the use of herbs and various plant materials for the control of fertility.

The most ancient descriptive literature on medicinal plants is present in one of the oldest repositories of human knowledge - The Rigveda. The Rigveda is believed to have been written between 4500 and 1600 B.C. Literature mention many plants which are reputed to possess antifertility and abortifacient properties. Some attempts have already been made to seperate the good ones from the useless and it needs a systematic investigation of the plant materials. Aiming this in view a concerted effort is being done by

for screening plants which have influence on the regulation of fertility. It is estimated that nearly 3000 plants will be screened during this phased programme of research (Soejarto et al. 1978).

Practically all major research, to date, involved with the search for new oral contraceptives has been confered to semi synthetic substances, particularly the preparations of steroid derivatives. Very little attention has been directed to the plant kingdom. Screening of plants and fodders will help to spot the incriminating feeds causing sterility in domestic animals. This may also contribute to explain the basic mechanisms of reproduction and its control, which is of great significance in human and veterinary medicine. Several plants have been found to possess antifertility property. Ocimum sanctum is one among them. However, detailed information is lacking in respect of the antifertility property of O. sanctum . It was, therefore, proposed to assess the antifertility activity of this plant.

REVIEW OF LITERATURE

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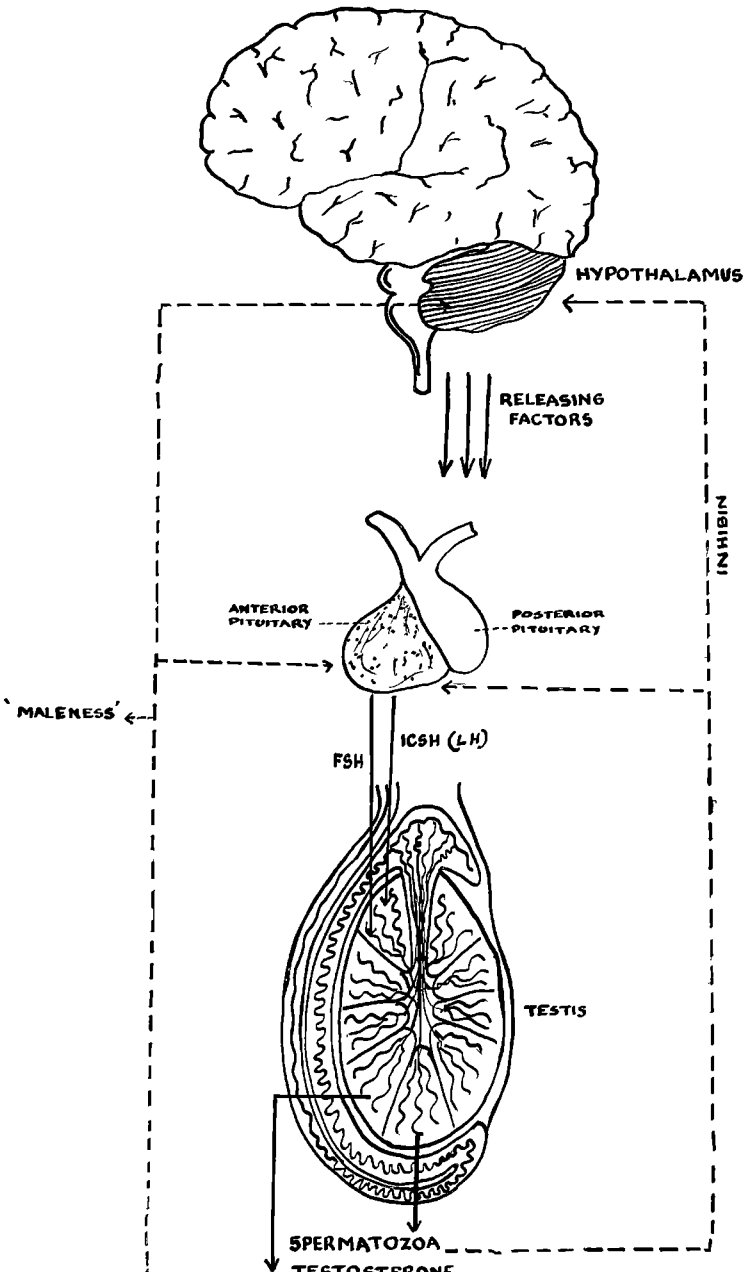
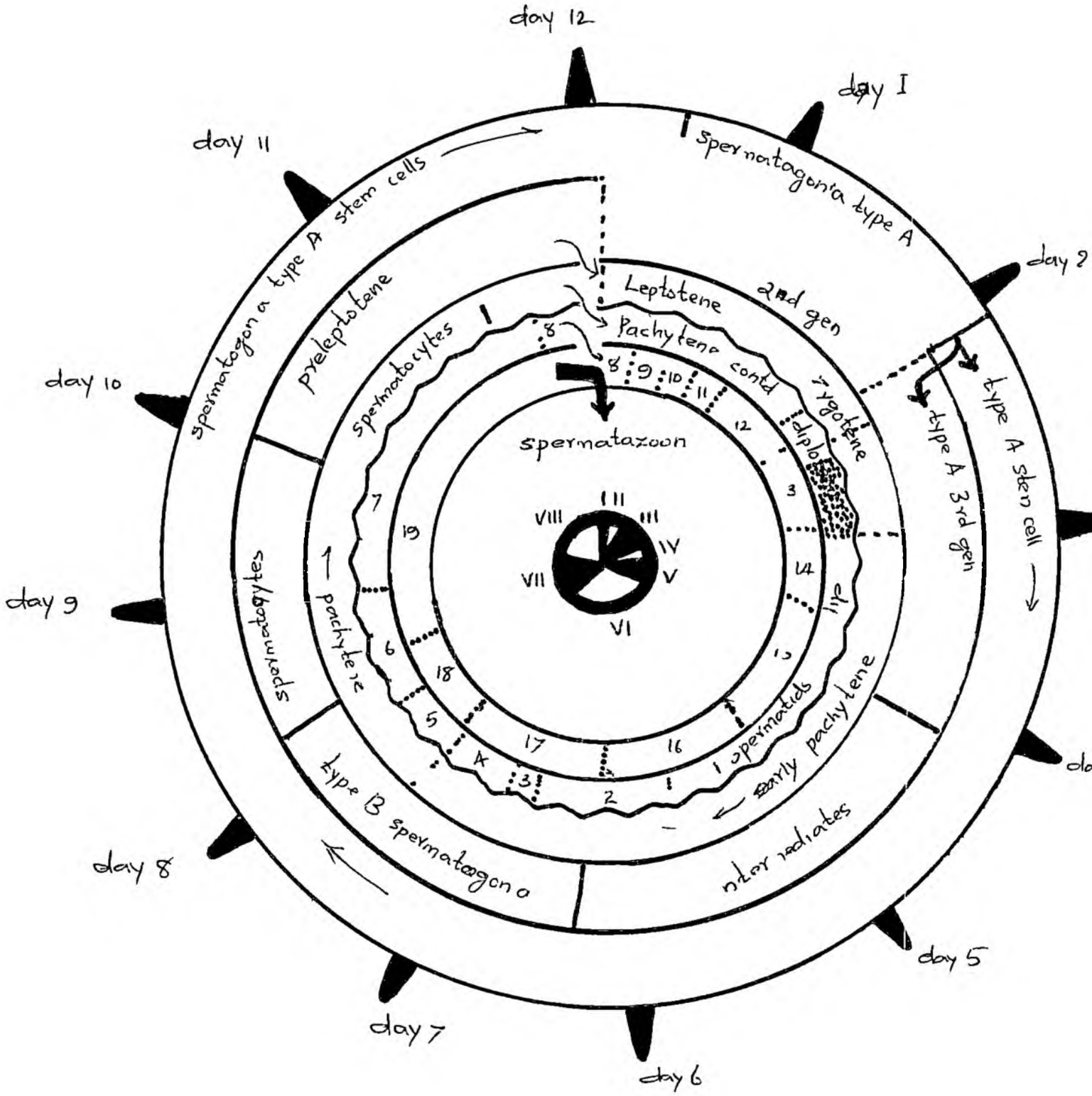
Drugs affecting fertility can influence the male as well as the female. Most of the chemicals at present used are acting on the females. The action is primarily on the gonads. The studies on female reproductive cycle are extensive and well documented. A number of drugs affecting the female reproductive cycle are available in the market. They act in more than one way and are extensively made use of in the field. But a similar drug that can be made use of in male is lacking. Probably with the possible exception of gossypol, which the Chinese has recently claimed. In order to understand the mechanism of action of antifertility drugs it is necessary to know the process of spermatogenesis and oogenesis. Therefore a brief account of the processes are described below.

Spermatogenesis

Spermatogenesis begin with the resting spermatogonia on the basement membrane. Active type A spermatogenia are formed by the division of resting type spermatogonia. The number of generations of type A spermatogonia vary with the species. One of the last generation of type A spermatogonia revert to the resting type A spermatogonium and the rest divide to form the intermediate spermatogonia. The type B spermatogonia are formed by the division of intermediate spermatogonia and this type B spermatogonia undergo the last

DIAGRAM OF NEUROENDOCRINE CONTROL OF REPRODUCTION IN MALE ANIMALS

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spermatogonia type A stem cells
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spermatocytes
pachytene
spermatids
1 spermatids
early pachytene
later radiates
type B spermatogonia
mitosis
meiotic divisions

of the mitotic divisions to form primary spermatocyte. Primary spermatocyte undergo the first of the meiotic divisions to form secondary spermatocyte which undergo the second meiotic division to form spermatids. This marks the end of spermatocytogenesis and the beginning of spermiogenesis. Spermiogenesis begin in the seminiferous tubules and is completed in the epididymis. A series of complex structural reorganisation occurs during the spermiogenesis which results in the morphological changes to form spermatozoon (McDonald, 1969). The Follicle stimulating hormone (FSH) and Luteinising hormone (LH) from the anterior pituitary control the spermatocytogenesis and spermiogenesis respectively (Roberts, 1971).

A portal circulation from the hypothalamus carries the venous blood, containing polypeptide hormones known as "releasing factors" to the anterior pituitary. Anterior pituitary hormones are liberated by these factors from their granular stores (Turner and Richens, 1973). The only exception to this is prolactin. Hypothalamus release a Prolactin release - inhibiting hormone (PRIH). Therefore, destruction of the hypothalamus can cause prolactin release by removal of the inhibitory control (Gilman and Murad, 1975).

FSH probably influence spermatogenesis indirectly, by modifying the function of the Sertoli or supporting cells of the seminiferous tubules. LH stimulate the testosterone

production by the interstitial cells. This high level of the intracellular testosterone indirectly stimulate the spermatogenesis. Thus both FSH and LH stimulate the spermatogenesis. But the rate of sperm production cannot be influenced by these hormones which remains as a biological constant for each species. Through their action on testis, FSH and LH stimulate the secretion of substances that inhibit the secretion of these hormones by the pituitary. Testosterone exert this negative feed back action for LH, directly or through conversion to estradiol 17 beta. Such a substance for FSH control remains a subject for controversy. It is suggested that a substance termed inhibin secreted by the testis synergise with testosterone to control the output of FSH from the anterior pituitary (DeKretser, 1978).

Androgenic stimulation is a substantial part of most reproductive process in male. Therefore antiandrogens like cyproterone acetate, can interfere with fertility at many stages. Though the antiandrogens possess safe antifertility effect their use as male contraceptives has been prevented because of the generalised nature of the antiandrogenic activity (Neuman and Steinbock, 1974).

From the view of the existing knowledge of endocrine control and duration of spermatogenesis, a hormonal approach to control the male fertility seems likely to be unprofitable.

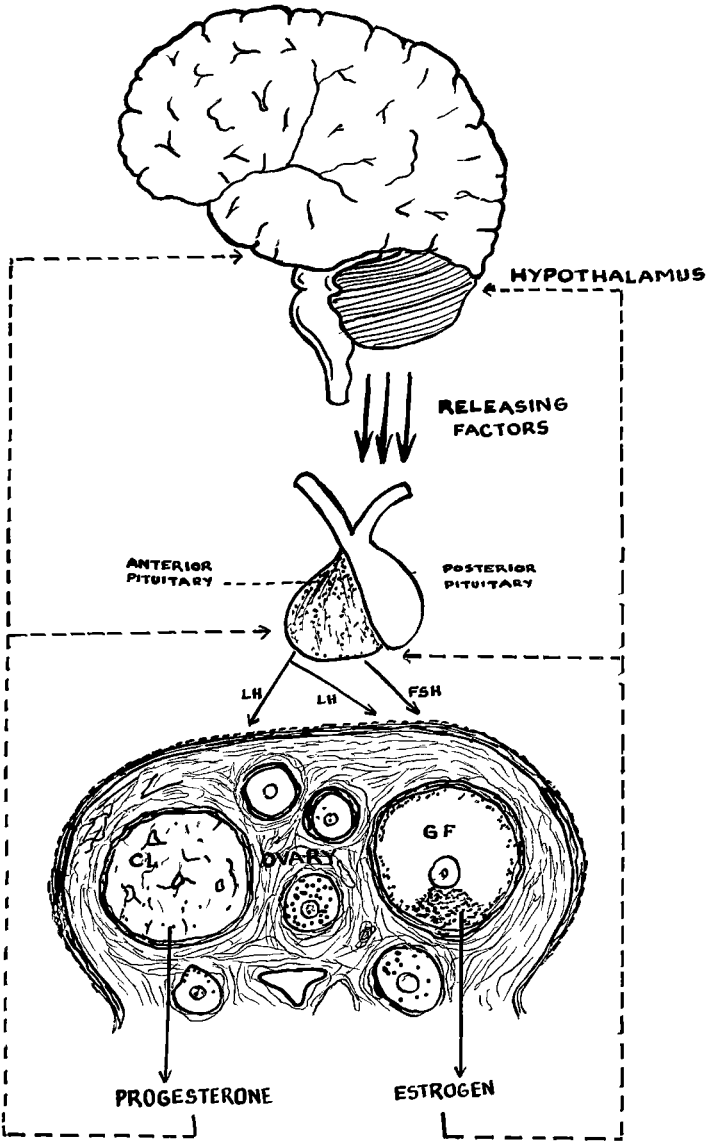
Any quick results are not likely to obtain by such means. Secondly administration of hormones and their synthetic analogues may interfere with sexual activity and are capable of producing general disturbance of the endocrine system (Jackson, 1959). Thirdly, the danger with all types of male oral contraceptives is that abnormal offspring could result from abnormal sperm (Witters and Witters, 1975).

The antifertility activity of a compound can be detected by comparing the fertility of treated and untreated males mated with normal females, preferably all of established fertility. Alterations in fertility is the primary concern, with the testicular histology as an ancillary investigation, because antifertility effects may be produced without obvious histological damage (Jackson and Mack, 1955). It is necessary to test fertility for seven weeks or more from the day one of the treatment in rats because spermatogenesis require at least six weeks. Consistent fertility patterns have been produced by seperate matings of the treated males with one female per week and it was found to be a satisfactory method to assess the antifertility property of a compound in male rats (Mack and Jackson, 1957; Jackson, 1953).

Oogenesis

The estrous cycle of domestic animals can be divided into four phases - estrum, metestrum, diestrum and proestrum.

IN FEMALE ANIMALS



Type and season of cycle	Cycle length	Ovulation	Time of ovulation	Viability of ova	No.of eggs shed	Time of fertilized ova enter uterus	Luteal phase	Luteolysis
Polyestrus any time	4-6 days	Spontaneous	8-11 hrs. from the onset of estrus	10-12 hrs.	10*	4 days post-coitus	Pseudo-pregnancy	Non pregnant uterus

Hormonal requirement for mating behavior	Pseudopregnancy		Implantation		Type of placenta	Pregnancy		
Estrogen Progesterone	Length 12-14 days	Hormonal requirement estrogen Prolactin	Days 5-6 post-coitus	Hormonal requirement Progesterone Estrogen	Hemochorial	Length 22 days	Endocrine glands required	Hormonal requirement prolactin FSH LH
							Anterior pituitary	Ovary

* Vary with strain or breed

Source: Fox and Laird (1970)
Hansel and McEntee (1970)
Farnsworth et al. (1975)

Reproductive Cycle of Rat

Sexual receptivity and ovulation occur in most species during estrum. The post-ovulatory phase is metestrum and it is this phase, in which the corpus luteum develops and begins to secrete progesterone. Diestrum is the period during which the influence of progesterone on accessory sex organs predominate. The period after the regression of corpus luteum, the progesterone level drops and this is the proestrum. Release of FSH from the pituitary stimulate the growth of the Graafian follicle. Graafian follicle secrete estrogen, this rise in level of estrogen cause estrum. Proestrum and estrum are referred to as follicular phase and metestrum and diestrum are referred to as luteal phase (McDonald, 1969).

The Graafian follicle destined for ovulation begins to enlarge rapidly and becomes turgid two to three days before the onset of estrum. There is a change in the collagen content of the follicular wall structure near the ovulation. The oozing process of ovulation itself may be dependant upon the enzymatic decomposition of this component (McDonald,1969).

The critical meiosis ie. reduction division followed by a single mitosis occurs after ovulation, while the ovum and its attendant cells are free in the oviduct. Each time one of the products of division (polar bodies) degenerate leaving the mature ovum ready for fertilization (Jackson, 1959).

Growth of the ovarian follicles and production of increased amounts of estrogenic hormones are resulted by the influence of FSH while LH cause rupture of the follicle and liberation of the ova (ovulation) and initiates the formation of corpora lutea, the temporary endocrine organ in the ovaries. Small amounts of LH may synergise with FSH in producing follicle growth and estrogen production. Prolactin can maintain corpus luteum in hypophysectomised rats (Mansel and McIntee, 1970). As in the case of males, in females also the FSH and LH secretions are under the control of "releasing factors" from the hypothalamus (Turner and Bagnara, 1973).

Interference with the reproductive process in female offers greater prospects of success. Steriods have been developed capable of preventing ovulation after oral administration. The pre-implantation and implantation phases of development also can be successfully attacked in experimental animals (Jackson, 1959).

Prostaglandins

Prostaglandins, with few exceptions, exert their effect and get inactivated principally in the tissues or organs in which they are synthesised hence they are considered as local hormones. Ovary, myometrium and menstrual fluid contain prostaglandins in concentrations that vary with the ovulatory

cycle. Human seminal fluid contain this in high concentrations. The prostaglandins type E and F are found in the uterus, menstrual fluid and in the amniotic fluid. Clinical investigation for obstetrical use has been limited almost entirely to prostaglandins E1, E2 and F2 alpha (Brazeav, 1975)

Contractions of both the pregnant and nonpregnant uterus are consistently stimulated by the PGF series. PGE1 and PGE2 cause relaxation of nonpregnant uterine tissue in vitro. But they possess more potent oxytocic action than PGF2 alpha during the last two trimesters of pregnancy (Brazeav, 1975).

Prostaglandins are much more effective than oxytocin in earlier months of pregnancy. The response of the uterus increase along with the advance in pregnancy. Intrauterine instillation by way of a cervical catheter or intra amniotic injection of PGE2 or PGF2 can cause abortion in the second trimester with high success rate and frequent but tolerable side effects (Brazeav, 1975).

In certain rodents PGF2 alpha cause luteolysis and thus eliminate the source of progesterone necessary for the maintenance of early pregnancy which result in the termination of pregnancy. The extend of action of PGF2 alpha on corpus luteum and directly on uterus has not yet been established though it is capable of terminating pregnancy in monkey

and human. Adenyl cyclase/cyclic AMP system is an intermediate in many stimulus-effector systems and many of the actions of prostaglandins may be secondary to an action on this system (Weeks, 1973). It has been shown that prostaglandin antagonists - aspirin and indomethacin - can block ovulation in experimental animals like rats. The mechanism of action of this is not clear. It is suggested that the peak level of LH accompanying ovulation is mediated under the influence of prostaglandins. Action of prostaglandin antagonists at this level prevent ovulation (Embrey, 1975).

Sensitivity of the pregnant uterus to oxytocin is increased by prostaglandins. Prostaglandin E2 with F2 alpha is administered vaginally to women who had missed their menstrual period by two to seven days as a possible birth control measure (Woodbury, 1971). Mackenzie et al. (1976) suggested that it has the advantage of not requiring hospital admission in majority of cases and it also avoid much of the physical and surgical trauma associated with the surgical termination. He also stressed the safety of the method and its potential as a self administration technique.

The semen of a number of mammalian species has been found to be devoid of prostaglandins. Though the drugs like aspirin and indomethacin profoundly depress prostaglandin synthesis and release it has not yet been reported to

influence menstruation or reproduction in patients receiving therapeutic doses. Such facts illustrate the difficulty of assessing the physiological significance of these group of autocoids (Brazeav, 1975).

Current Contraceptive Methods

There are a wide variety of mechanical and chemical contraceptive methods available. They possess varying degrees of effectiveness, advantages and disadvantages. Among them oral contraceptives are the most effective method for preventing pregnancy (Gerald, 1974). Though the steroid contraceptives possess side effects, they are the most widely used agents for such purpose (Singel and Benoit, 1973).

The oral steroid contraceptives can be classified into three. 1.combinations (estrogen and progestin combined together) 2.sequentials (estrogen alone for the first 14 to 16 days followed by progestin alone for the next five or six days).3. Minipill (Progestin alone) (Gerald, 1974; Murad and Gilman, 1975).

The combination or sequential preparations could interfere with fertility in any one of the several ways. The mixture inhibit ovulation. Estrogen inhibit secretion of FSH while LH release is blocked by the continued progesterone action. Thus ovulation is prevented by estrogen while progestin ensures the withdrawl bleeding prompt and brief in duration, similating a menstrual bleeding. The suppressants

administered will alter the endometrium in such a way providing an unfavourable media for implantation. When these drugs are taken in the proper way, ovulation by itself is prevented. Hence there is no chance to find out whether the estrogen - progestin mixtures could interfere with implantation. Altered secretory activity of the cervix is adverse for the survival of the sperm. The "minipill" has got no influence on ovulation, its action is mediated by the alteration of cervical mucus and endometrium (Murad and Gilman, 1975

Nausea, occasional vomiting, dizziness, headache, discomfort in the breast, gain in weight and higher incidence of several types of tumours are the frequent side effects noticed with the use of steroids (Murad and Gilman, 1975).

The only long acting injectable contraceptive, available commercially, is depomedroxy progesterone acetate (DMPA). which is given once in three months. DMPA is subject to controversy because it disrupt the menstrual cycle in a considerable proportion of users and also information is lacking on its metabolism in women. Norethisterone oenanthate (NET-OEN) is another three monthly injectable preparation. The high rate of pregnancy among the users showed it to be unacceptable (Kessler and Standley, 1977).

Preparations to be used in the vagina as spermicidal agents are available. The primary constituents employed in these range from organic mercurials to organic acids to sodium soaps. Surface active agents are employed in several

preparations to facilitate better spread and sperm contact (Pincus, 1965).

Possible sites of action of Antifertility Agents

Hypothalamus - pituitary. The actioning of the pituitary is under intimate control of the hypothalamus by means of hormone specific role giving factors and the question as to whether certain substances might act on the hypothalamus and/or pituitary has been controversial. For example, steroids may exert some effects directly on the pituitary. But the antifertility activity of these are suggested to be through the hypothalamus (Zanos, 1970). Hence these two are considered together.

The basic mechanisms are in the following manner. 1. Disruption of the normal humoral and hormonal function of the hypothalamus and/or pituitary respectively by steroids, nonsteroids (metmellisure) having antigonadotropic activity and by steroid antagonists. 2. Disruption of the neural input to the hypothalamus eg. from the environment and from the postulated "clock" that control the release of gonadotrophin releasing factor(s) in spontaneous ovulators (McCann, 1968; Greep, 1970).

Post-ovulatory antifertility activity can be obtained by interfering the gonadotrophin secretion. In rats and mice luteal function remain directly under the control of

the pituitary for half the length of the pregnancy (Farnsworth et al. 1975).

Sperm count was reduced to zero when normal males were treated with progestagens or androgens and full recovery was noticed on cessation of therapy. Such results were due to the depression of the gonadotrophins from the pituitary. Treatment with progestagens also caused loss of libido due to impaired testosterone production. During the spermatogenic suppression using testosterone, loss of libido was not seen because here the interstitial cell function was effectively replaced (DeKreste , 1978).

Ovary.

Inhibition of ovulation and/or steroidogenesis can cause an infertility effect. It appeared that protein synthesis is to be involved in the intra-ovarian mechanism causing ovulation. Pregnant mare serum gonadotrophin induced ovulation in hamsters was blocked by the Actinomycin D by inhibiting the DNA dependent RNA synthesis (Armstrong, 1970).

Estrogen is needed for the normal luteal function of rabbit and rat. In such species estrogen antagonists are likely to interfere luteal function (Rabhaetwar, 1971). Steroidogenesis itself, may be involved in the process of ovulation and in induction or maintenance of pregnancy (Farnsworth et al. 1975).

Oviduct.

The disturbance of tubal transport may be accompanied with failure of implantation because normal implantation is depended upon the correct timing of the arrival of the blastocyst in the uterus (Emmens, 1970). Therefore, substances having the ability to alter oviductal motility are capable of inhibiting fertility.

The oviduct, especially the region of the isthmus, possess a rich sympathetic innervation in rats, rabbits and humans. At least in these species autonomic drugs can influence the rate of ovum transport though their ability to do so may be modified by estrogens and progesterone (Armstrong, 1970).

A critical balance of estrogen and progesterone may be necessary for the normal post-ovulatory events to occur. Hence both estrogens and antiestrogens can alter ova transport and thereby impair fertility. In accelerated transport of ova, decreased fertility is thought to be due to the uterine environment and not due to the rapid transport (Emmens, 1970). Fertility can be impaired by preventing the fertilized ova from getting to the uterus, the normal site for implantation. This is called tube locking (Giannina et al. 1971). In bitches tube locking is done by injecting 0.1 to 1 mg of diethylstilbestrol within 24 to 48 hours after mismating (Roberts, 1971).

Certain compounds found to be antiestrogenic at lower doses are found to be estrogenic at higher doses. With respect to whether a given compound exerts antifertility activity, estrogenic activity and/or antiestrogenic activity can vary from species to species, at least between rats and hamsters (Farnsworth et al. 1975).

Uterus.

The naturally occuring alkaloid, agroclavine, has been reported to inhibit implantation in rats and mice (Mantle, 1969). A structurally related compound D-6-methyl-8-cyanomethylergoline, has been suggested to possess centrally mediated anti-implantation activity (Mantle and Finn, 1971).

Certain substances like quinacrine instilled locally to the uterus impaired fertility by inducing giant cell foreign body reaction in the endometrium and a consequent obstruction of the lumen (Zarem, 1970).

The compounds which can stimulate the uterine contraction cause an abortifacient type antifertility effect. Eg. oxytocin and Prostaglandins (Farnsworth et al. 1975).

A number of plant products like vinblastin from Catharanthus roseus, have been shown to elicit varying degrees of antifertility activity due to their cytotoxicity. Demecolcine derived from several colchicum species on administered to pregnant rats produced fetal death by direct

action on the fetus. The placentae and the sites of implantation remained without any damage (Morris et al. 1967; Farnsworth et al. 1975).

Vagina.

Preparations mostly containing polyethoxy derivatives are available for use in the vagina as spermicidal agents. Acrosin, an enzyme extractable from acrosome of the sperm is essential for the penetration of the zona pellucida of the ovum by the spermatozoon during the process of fertilization. Addition of acrosin inhibitors increase the spermicidal activity of the preparations (Zaneveld et al. 1972).

Testis.

Agents could be developed to disrupt seminiferous tubule function without affecting androgen production by the interstitial cells. There are agents like cyclophosphamide and chlorambucil, which can disrupt the spermatogenesis through their action on the replicating cells. Action of these agents on other tissues prevented them from wide use (DeKretser, 1978).

Epididymis.

Sperm acquire the morphology, metabolism and progressive motility during its transit through the epididymis. An amino derivative of alpha chlorohydrin (DL-1-amino-3-dichloro-2-propanol hydrochloride) showed promising results in interfering with the maturation process in the epididymis.

The high degree of toxicity prevented it from further studies (DeKrester, 1978).

Immunisation.

The possibility of the use of hormone antibodies as agents to control fertility has been reported by Moudgal et al.(1974). Human chorionic gonadotrophin is vital for the maintainance of pregnancy and it is produced only during pregnant period. Hence induced immunity to human chorionic gonadotrophin would disrupt pregnancy at very early stage. Female baboons immunised with a synthetic hCG fragment produced significant reduction in fertility (Kessler and Standley, 1977).

There are certain components in the reproductive system which are not represented in other body systems. Many of these are immunogenic. In view of such evidences efforts are being made to develop an acceptable vaccine for fertility regulation. In experiments with antigens, a lactate dehydrogenase isoenzyme (LDH-x), one of the enzymes normally present on sperm surface has reduced the fertility in rats and mice (Stevens, 1978).

Plant Materials with possible Antifertility property

The information regarding the ability of certain plants in fertility regulation are available in plenty. In most ethnomedical reports (The term 'ethnomedical' is used in preference to "folklore" because primary source of

information have been obtained through the periodical literature and scientific information, rather than from laity) the candidate plants are described simply as "uterotonic", "contraceptive", "prevents pregnancy", "abortifacient", "for amenorrhoea", "expels placenta", "an emenagogue", "antifertility agent", "cytotoxic", "ecbolic", "promotes menstrual flow", etc. Great problems are introduced in the process of selection of plants for further studies just because of the vagueness in terminology. In great many cases the results have been subject to controversy because materials found to be positive in result by one research group are later reported by another research group to have no activity and vice versa (Soejarto et al.1978).

The term contraceptive agent refer to those which prevent ovulation and/or fertilization and abortifacients are those which act after the implantation has taken place. Agents which act after the occurance of fertilization but prevent from implantation taking place are termed by some workers as interceptives (Farnsworth et al. 1975).

Wilson et al. (1947) fed 10 per cent rutin diet for 28 to 400 days for both male and female rats. He got no significant antifertility effect. But Cutting et al.(1951) got an opposite result when a diet containing 0.1 per cent of the flavanoid glycoside rutin was given for female mice.

In 1949, Cranston and Robinson and later some other workers demonstrated the antigonadotrophic activity of Lithospermic acid without producing irreversible or histological damage to pituitary. This material was obtained from the plant Lithospermum ruderale. The active ingredient was found to be concentrated in the roots and that too during the months from June to September (Graham and Noble, 1953).

Pottlerin, the antifertility principle of Mallotus philippensis, at a dose level of 10 mg per kg body weight gave 100 per cent antifertility effect for 10 days and 80 per cent effect for 20 days. Hundred per cent infertility was produced by a dose 20 mg per kg body weight (Graham and Noble, 195). Counteraction of the chorionic gonadotrophin was the cause for such an action (Varma et al. 1959).

Volatile oils derived from the plants like Tanacetum vulgare (tansy), Hedeoma pulegeioides (pennyroyal), Ruta graveolens (rue), Petroselinum sativum (apiol) and Juniperus sabina (savin) etc. were employed in the past to induce abortion (Tollman, 1967).

The plant Polygonum hydropiper administered as dry whole plant, temporarily impaired the fertility of male and female mice and produced sterility in female guinea pig. No evidence of estrogenic or androgenic activity was obtained. It seemed likely that this material interferes with

the gonadotrophic function of the pituitary (Jackson, 1959).

Colchicine a tropolone alkaloid, can arrest cell divisions at metaphase. A number of compounds with markedly less toxicity than the parent compound have been derived. These compounds retained antimitotic activity against mouse spermatozoa (Jackson, 1959).

By virtue of the cytotoxic property, Vinblastin obtained from Catharanthus roseus, demecolcine derived from several Colchicum species etc. have antifertility property (Morris et al. 1967). Vinblastin largely available from the plant Vinca rosea possess anticancer property (Goldstein et al.1974).

Hot alcoholic extract of the seeds of Butea monosperma was effective in preventing pregnancy in all the tested eight rats at a dose level of 300 mg/kg body weight given on days one to four of pregnancy. The same extract showed antiovulatory activity in rabbits. Flowers of this plant exhibited anti-implantation activity in rats at a dose level of 300 mg/kg body weight (Khanna and Choudhury, 1968).

Though the plant Ocimum sanctum has not been mentioned as an antifertility agent in literature, Vohora et al. (1963) reported that the aqueous extract of this plant at a dose of 100 mg per kg body weight when given for 14 days to one series of five rats, three had no sites of implantation on day 10 of pregnancy. In another series, the same extract at a dose 200 mg per kg body weight when given for

one to seven days of pregnancy 2/5 rats had no evidence of implantation. Only 3/10 rats delivered at term which suggested an abortifacient type of action. Jain and Tarafder (1970) suggested the O.sanctum to have abortifacient activity. Batta and Santhakumari (1971) reported the benzene extract of this plant to possess 80 per cent antifertility activity. Petroleum ether extract of the same was found to be 60 per cent effective in preventing pregnancy. Kashnathan et al. (1972) reported the antifertility effect of feeding mice with O.sanctum leaves along with normal diet. Slight impairement of the spermatogenesis was observed histologically. The seminal plasma of the treated animals were having low pH level. An increase in the reducing substances, acid and alkaline phosphatases and a decrease in mucoproteins of the seminal plasma were noticed. Though mating did take place between treated males and untreated females, fertilization did not occur. The physical changes like decrease of pH, hypertonic environment and difference in concentrations of chemical substances of biological importance like mucoproteins, alkaline phosphatase and acid phosphatase were supposed to be the reason for the sterility.

Tewari et al. (1970) attributed some antifertility effect for betel leaf stalk (Tambul patrabrint) in rats and rabbits. It exhibited no estrogenic or antiestrogenic activity in immature and spayed types of animals. The cause for the antifertility activity was supposed to be due

to the mild progestational activity of this material.

The plant Gleditsia horrida was found to contain two saponins having antifertility property (Chou et al. 1971).

The antifertility activity of various chromatographic fractions of Taxus baccata was tested in female albino rats. Fractions one and 13 inhibited pregnancy in 60 per cent of the albino rats. Partial or complete reabsorption was noticed in the animals receiving fractions one, four and eight (Garg, 1972).

Meyer et al. (1973) found that aqueous ethanol extract from the roots of Tabernaemontana heyneana prevented pregnancy in adult female rats. The active ingredient of this plant coronaridine, an indole alkaloid at a dose level 5 mg per kg body weight per day prevented pregnancy and showed high degree of estrogenic activity.

Quinine and castor oil have been used extensively in the past, alone or in combination with pituitary extract to induce abortion (Farnsworth et al. 1975).

Sparteine, an alkaloid derived from several Leguminosae species is the only clinically useful abortifacient plant product known at present. It has virtually all the properties of oxytocin and ergot alkaloids (Kamboj et al.1975).

Hundred per cent antifertility effect was obtained with the alcoholic extracts of Cuminum cyminum (seeds)

and Hyptis suaveolens (leaves) at a dose level of 150 mg/kg and 125 mg/kg body weight respectively in albino rats (Garg, 1976).

Pakrashi and Basak (1976) reported that the juice of unripe fruits and leaves of Ananas comosus to possess abortifacient activity.

Premakumari et al. (1977) showed that plumbagin obtained from Plumbago zeylanica, when given orally at a dose level of 1 mg per 100 g body weight, resulted in significant antiimplantation and abortifacient activity in albino rats. The same dose of plumbagin has antiovulatory activity in rabbits.

Perhaps the apt example of an unmodified fertility regulating agent of plant origin is m-xylohydroquinone. It is the one and the only of such agents that has been extensively studied in humans. This materials was first isolated from peas (Pisum sativum) by Lanyal in 1952. There was little evidence of side effects from its use in females as revealed by a number of publications appeared up to 1960. A review of all the articles appeared up to 196 , showed only 60 per cent effectiveness and that was the cause for the diminished interest in this agent as a fertility regulating agent (Cocjarte et al. 1970).

Literature abound with number of plants possessing antifertility activity. The number of such plants may come even upto 300 (delasslo and Henshaw, 1954; Choudhury and Vohora, 1970; Farnsworth et al.1975).

Other Pharmacological Properties of Ocimum sanctum

The plant Ocimum sanctum is an erect herbaceous, much branched, softly hairy, annual plant. Height of this vary from 30 to 75 cm. The leaves are purple coloured, elliptic oblong, acute or obtuse, entire or serrate and pubescent on both sides. The plant is propagated by seeds (The Wealth of India, 1960). Because of the great aromatic and medicinal value of Ocimum, this has been considered as one of the most important genera of Labiatae. It is represented by about 60 species of which most are tropical, chiefly Asiatic. Out of these only six species occur in India. The "Sacred Tulsi of Hindus", O.sanctum is one of the most used household remedies in India (Gupta, 1967).

Other common species of India are O.americanum, O basilicus, O.gratissimum, O.alscendens, and O.kilimandscharicum. These different species of Ocimum are primarily used in indigenous system of medicine, while almost all of them contain essential oil (Gupta, ., 1967).

Dupear (1952) reported, the O.sanctum to contain alkaloids, glycosides, tannins, saponin, fat and essential

oils. The therapeutic value was attributed to the essential oils. Chopra et al.(1953) reported the leaves to yield 0.7 per cent essential oils containing 71.3 per cent eugenol, 3.2 per cent carvacrol, 20.4 per cent methyl eugenol and 1.7 per cent caryophyllene. The leaves contain ascorbic acid (83 mg/100 g) and carotene (2.5 mg/100 g) (The Wealth of India, 1966). Twenty two macro and micro-elements were detected in six species of Lamiaceae by Zinchenko and Zinchenko (1970). Thirteen of them consistently had Ba, Ti, Mn, Cr, Ni, Zr, Cu, Fe, Mg, Si, Ca, Pb and Al. Some of the plants had the following in higher proportion Al, Mn, Cr, Zr, and V. Large amounts of Fe, Pb, Ti, and Ca are present in some species.

The plant has a pungent bitter taste and is with stomachic, cholagogue, anthelmintic, and antipyretic properties. Diseases of the heart and blood, leucoderma, asthma, bronchitis, vomitting, foul smells, lumbago, pains, hiccough, painful eye, purulent discharge of the ear etc. are certain conditions in which the plant O.sanctum is used (Kirthikar and Basu, 1935).

Nadkarni (1954) described the following properties for O.sanctum. Leaves of this plant ground with water are applied on bad boils. Infusion of the leaves can be given in malaria and as a stomachic in gastric diseases of children and in hepatic affections. The juice of the basil leaves is good if taken orally by the persons who are affected

with bad skin diseases such as itches, ring worm, leprosy, bad blood etc. Topical application of the same or preferably mixed with juice of lemon as a paste is advisable in diseases of the skin. As a domestic remedy for cough, catarrh, bronchitis and diarrhoea dried plant, in decoction (1 in 10) is employed. Decoction of the leaves with addition of little cardomon powder is a nourishing and aphrodisiac drink. Ear ache can be effectively brought down by pouring the leaf juice into the ear. It can cure chronic fever, haemorrhage, dysentry and dyspepsia. Fresh juice of this plant possess antiemetic and anthelmintic property.

Basil leaves are considered to be beneficial in snake bite and lightning strokes. It is even claimed that we ring a garland made up of small beads of the wood of basil plant trunk can generate electric current which can cure some diseases (Vaidkarni, 1954).

Bhat and Broker (1954) reported that the alcoholic and aqueous extracts of the seeds of O.sanctum to be effective in prolonging the coagulation time of plasma by staphylococagulase.

An oil obtained from O.sanctum by ether extraction of steam distillate saturated with sodium chlorides inhibited Mycobacterium tuberculosis and Micrococcus pyogenes

var aureus, in vitro at 10 and 100 γ/ml respectively (Gupta and Viswanathan, 1955).

The plant O.sanctum has been popularly used in Cuba for self treatment of diabetes. Oyda and Fortun (1964) subjected the dried leaves for clinical testing. The extract was administered to patients with long histories of increasing need for insulin and tolbutamide. It produced hypoglycemic effect and the beneficial effect continued 30 to 60 days after the treatment was discontinued. A group of non diabetics subjected to similar treatment showed a gradual lowering of blood sugar level. Dhar et al. (1968) reported the plant to possess oral hypoglycemic effect in rats.

Watery extract of O.sanctum possessed transient hypotensive effect (Singh, et al. 1970). Such action was not blocked by mepyramine and hexamethonium. Atropine partially blocked the hypotensive effect. It produced direct depression of the heart. The contractions induced by acetylcholine, carbachol and histamine on smooth muscles were inhibited by the extract. Hexaxarbitone sleeping time was potentiated by the extract.

Different factors with growth promoting property, were noticed in the leaves of O.sanctum (Malviya and Gupta, 1971).

MATERIALS AND METHODS

MATERIALS AND METHODS

Authenticated samples of the fresh leaves of Ocimum sanctum were collected from the surrounding localities of the college during the months of August and September, 1970. Leaves were dried in the sun light and powdered using a pulverizer. The drying process caused a loss of weight by 8 to 92 per cent. The dry powder was stored at room temperature.

Cold extract of the dried and powdered leaves was prepared at room temperature by passing benzene (BP 80°C) repeatedly through the powder in a percolator until the extract was colourless. The extract was filtered and the solvent was removed by vacuum distillation at temperature varying from 50 to 60°C. The residue, a semi-solid, tarry and adhesive substance was stored in vacuum desiccator at room temperature.

Five to six litters of benzene was made use of for every 100 gms of the dried powder. The residue obtained was on an average 5.5 per cent by weight of the dried powdered leaves.

Animals used for the experiment were Albino rats obtained from the Small Animal Breeding Station attached to the College. Body weight of the female rats used for the

experiment ranged from 80 to 115 grams and that of the males were 100 to 175 grams at the beginning of the treatment. Animals were selected from a colony of rats with proven fertility. All the selected animals were apparently healthy and mature. Fourteen male/female rats constituted one group of experimental animals. Ten male/female animals constituted control group.

All the female animals were assured to be cycling normally by examining the vaginal smear before subjecting to treatment. Vaginal fluid was taken from the rats by introducing half a ml of normal saline into the vagina by means of a pipette and withdrawing it after sucking back and forth a few times. The smears were made with the vaginal fluid on glass slides and were examined under the microscope. Various stages of the estrus cycle were distinguished following the criteria detailed by Eckstein and Zuckerman (1960).

Two animals were housed in one cage and were given ad libitum water and feed of the given constituents.

Bengal gram or Horse gram	- 40 g
Maize	- 30 g
Meat cum bone meal	- 30 g (46% protein)
Yeast tablets 0.3 g	- 5 nos.
Shark liver oil	- 5 drops.

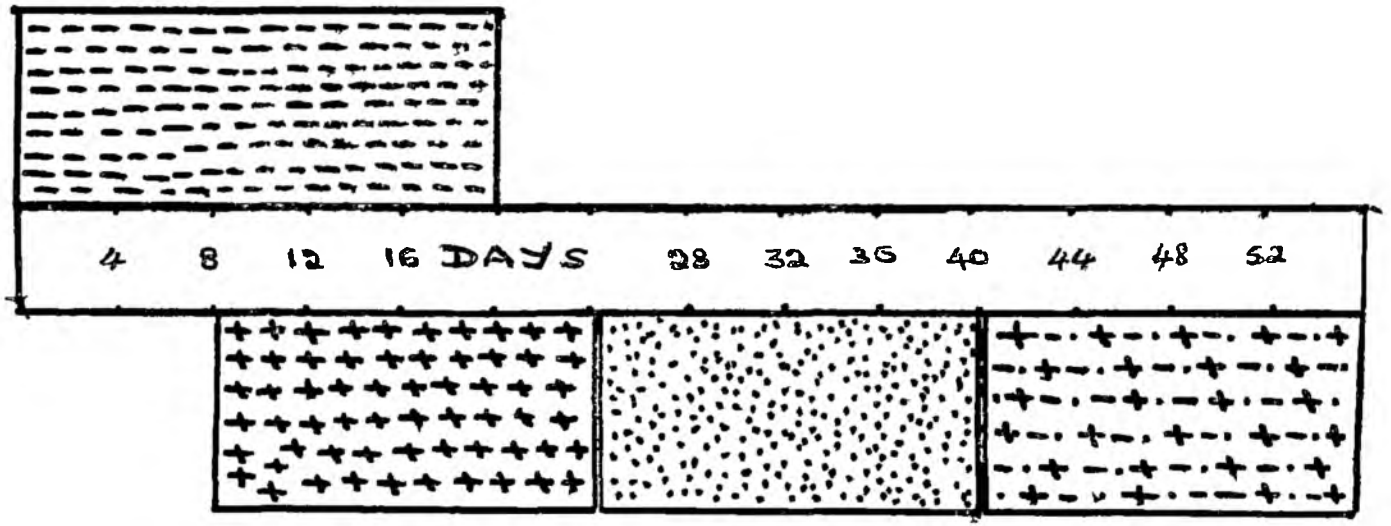
The residue was administered to the animals in the form of 5 per cent emulsion, prepared in 10 per cent solution of Tween 80 (Polysorbate) in water.

One group of female animals were treated with the emulsion at a daily dose level of 200 mg per kilogram body weight , orally using a stomach tube for eight days. After 24 hours of the last day of medication, one male rat was added for every two female rats. Male rats were rotated from cage to cage in every four to five days. Vaginal smears were examined every day, as described above and those in which the spermatozoa were found were separated. The males and females were put together upto a maximum of 24 days. On the 25th day all the males were separated from the females. Females were retained for 22 days more from the day on which spermatozoa were found in the vaginal smear or from the day of separation from the males to see whether they deliver or not. After weaning the offsprings, the delivered female animals of the treated group were again put along with males, to see whether they deliver or not in the second time. The treated animals were weighed and sacrificed by stunning and decapitation. Pituitary, liver, kidney, uterus and ovary were collected immediately after decapitation. Biometry of the collected organs, except that of the pituitary, were taken soon after removal from the body of the animal. The organs were preserved in

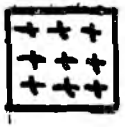
10 per cent formalin solution. Similar pattern was followed for another group of female animals treated with the same extract at a dose level of 400 mg per kilogram body weight per day. Control group of animals were run simultaneously which were dosed with the vehicle alone.

One group of male rats were treated daily at a dose level of 200 mg per kilogram body weight, orally, for a duration of 70 days. One mature female rat was added for every two males on the 9th day of the treatment. The female animals were rotated from cage to cage in every four to five days. The females were separated from the males at 16 days interval and another set was added on the next day. Thus on the 50th day of the beginning of the treatment, the third set of females were seperated. All the females were retained for 22 days more, from the day of seperation from the males, to see whether they deliver or not. Treated male animals were weighed and sacrificed by stunning and decapitation. The organs - pituitary, liver, kidney and testis - were collected soon after decapitation. Biometry of the organs, except that of the pituitary, were taken soon after removal from the animals' body. All the organs were preserved in 10 per cent formalin solution. Similar pattern was followed for a group of control male animals which were dosed with the vehicle alone.

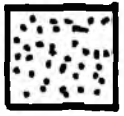
Thin pieces of the tissues fixed in 10 per cent formalin solution were washed in running water to remove the formalin. Dehydrated in ascending grades of alcohole, cleared in two changes of xylene and transferred to melted paraffin at 56°C. After three changes of paraffin embedding was done in fresh paraffin. Sections cut at 5 microns thickness were stained with Haematoxylin and eosin (Reynolds, 1973). Sections of pituitary were also stained by Mallorys method (Luna, 1968).



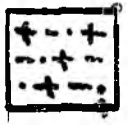
Treatment period



First group of female animals



Second group of female animals



Third group of female animals

Shedule of experiment - Male animals

RESULTS

RESULTS

Fertility rate

The frequency of mating rate in the treatment and control groups were identical. In the group of 14 females, treated at a dose of 200 mg per kilogram body weight, and mated with normal males, only two delivered at term. In another set of females, treated at a dose level of 400 mg per kilogram body weight, only two rats delivered, out of the 14 experimental animals. Thus the fertility rate was only 14 per cent in both the groups. However, the rats that delivered in both the groups, were again allowed to mate, but failed to conceive and deliver subsequently.

In the case of the group, where in males were treated with the extract at the rate of 200 mg per kilogram body weight, three sets of females were introduced at three specific intervals as described previously. In the first group of the females introduced ie. the animals introduced from 9th to 24th days, from the first day of the treatment of the males, only two delivered out of the seven females. No delivery took place in the two subsequent group ie. 25th to 40th day group and 41st to 56th day group, from the beginning of the treatment.

The control animals showed 50 per cent fertility. Therefore the experimental animals showed a reduction in fertility at the following rates.

Biometry

There was no significant difference in the length of uterus between the treatment and control groups of animals (Table XIII). Similar response was observed for the weight of the uterus + ovary also (Table XII).

On comparing the means it was found that the liver weight and kidney weight of the treatment and control groups of females, to be homogeneous. Similarly that of the treatment and control groups of males also were homogeneous. However, on comparison of the male and female groups, they were found to be significantly different (Table XIV and XV).

No significant difference was observed in the weight of the testis between the treatment and control groups (Table XVI).

The correlations of the body weight with the weight of the uterus + ovary, length of the uterus, weight of the kidney, weight of the liver and weight of the testis were analysed in each group. At 5 per cent level significant correlation was observed between the body weight and the length of the uterus in the 200 mg per kg treated group and in the control group of females. Similar result was obtained for the correlation of the weight of the uterus + ovary with the body weight in the control group. At 5 per cent level the correlation of the body weight with

the weight of the liver was significant in the treatment and control groups of males. Similar was the observation for the correlation of the body weight with the weight of the testis in the treatment group of males. At 10 per cent level significant correlation was observed between the body weight and the weight of the kidney in the control groups. Similar result was obtained for the correlation of the body weight and weight of the liver in the female control group. There were no significant correlations in the remaining observations (Table XVII).

Histopathology

Ovary showed reduced activity in both the treatment groups characterised by the poor development of the follicles. The number of developing follicles were few and in these antrum formation and follicular fluid accumulation were less compared to the control. The number and amount of luteal tissue formed were also few (Fig. 1 and 2).

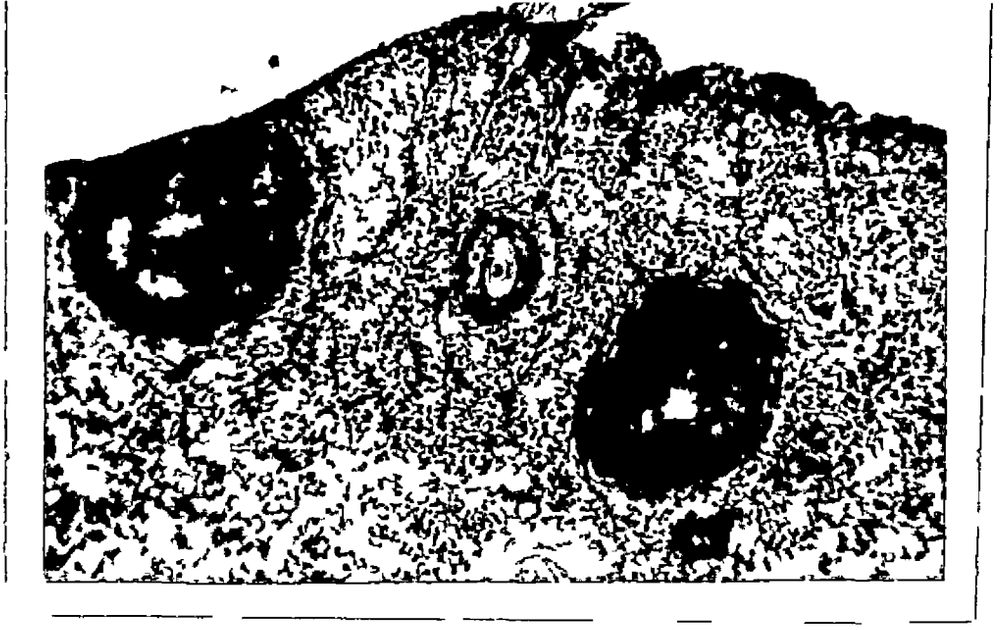
Testis of the treated male animals showed partial impairement of spermatogenesis. Many of the seminiferous tubules did not show any evidence of spermatogenesis. In these tubules there were only one or two layers of spermatogonial cells and were without showing any evidence of proliferative activity. There were no sperms in these tubules.

Certain other tubules along with collection of sperms revealed degenerated desquamated cells and hyalinised bodies of varying sizes in large numbers (Fig. 4 and 5).

Pituitary in all the three treatment groups had congestion of the vessels. Basophils showed degranulation and vacuolation of the cytoplasm (Fig.3). This was more pronounced in the animals subjected to higher dose levels.

Liver of the group of animals dosed at 200 mg per kg body weight - both male and female groups - had slight engorgement of the sinusoids. The animals subjected to higher dose level had liver with focal areas of hyperplasia of the hepatic cells.

Kidney in all the treated groups had slight congestion of the vessels.



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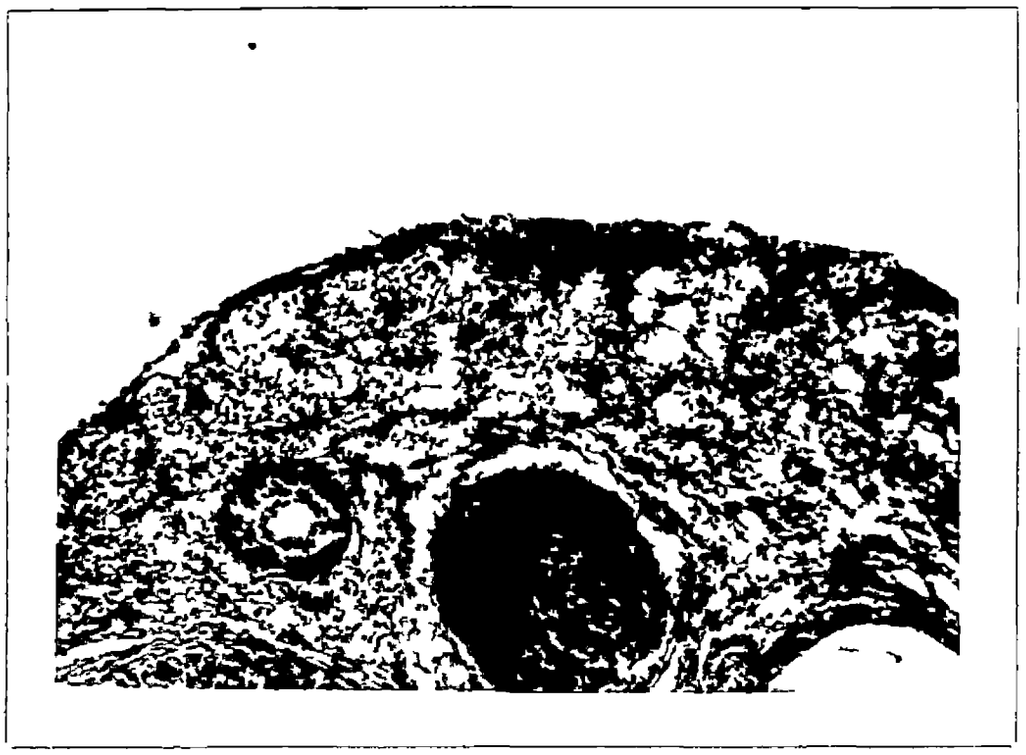
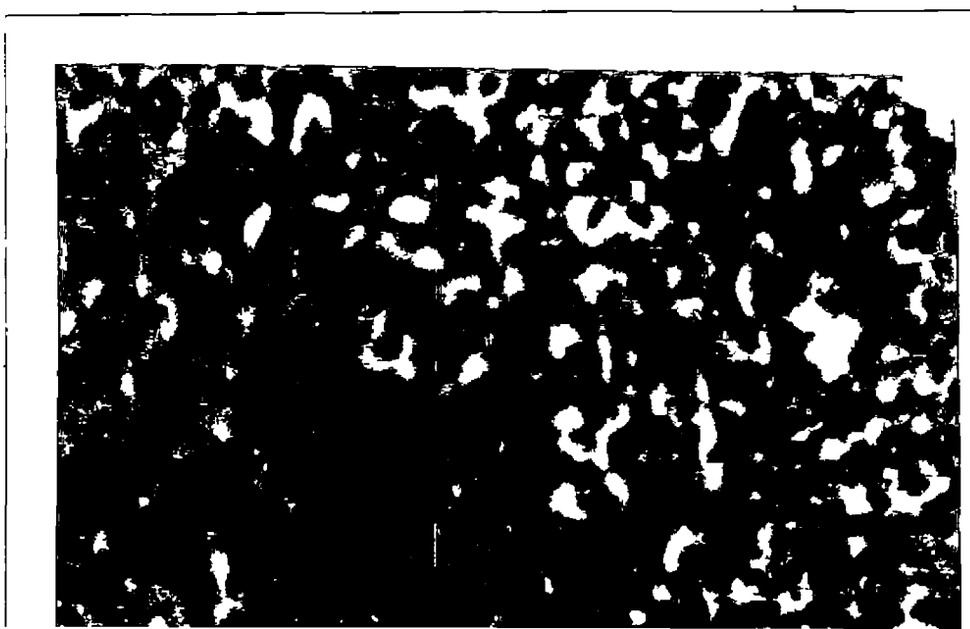


Fig



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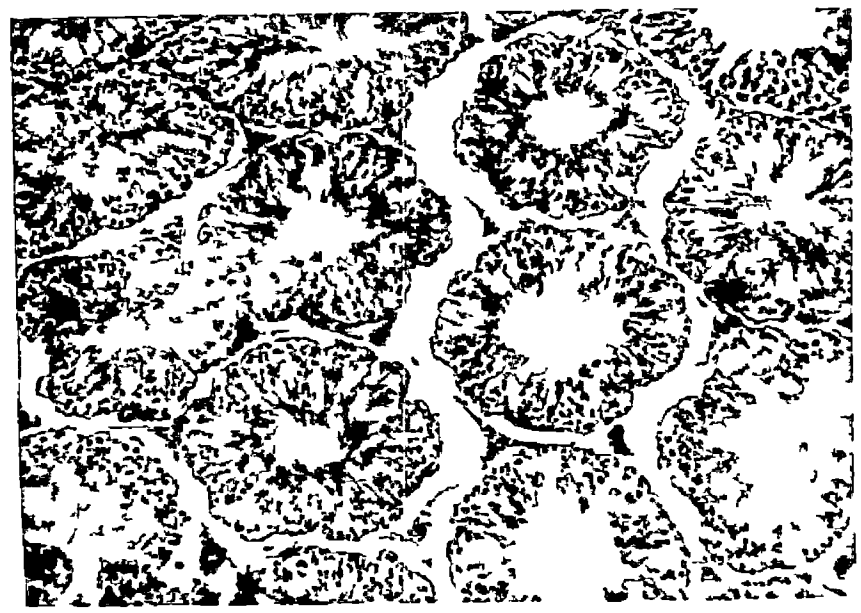

Fig 4

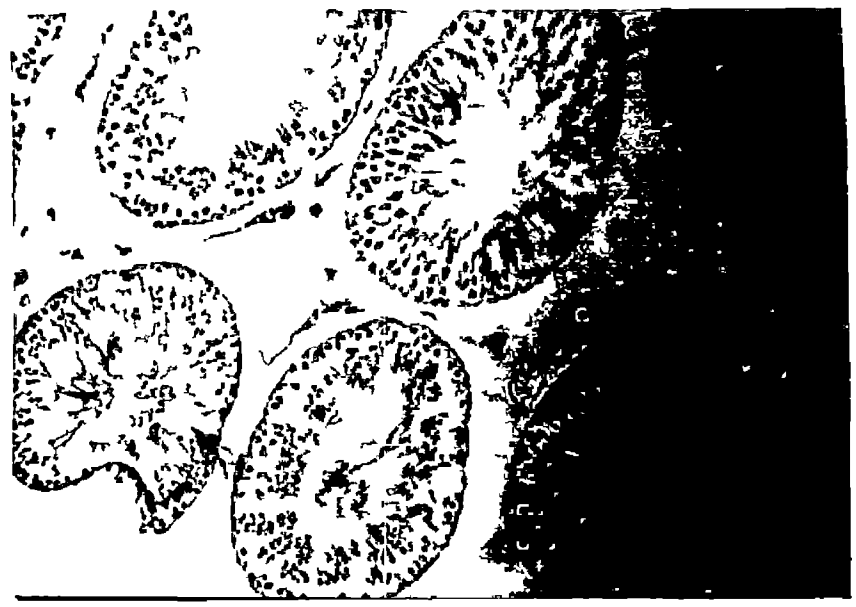

Fig 5

Fig 6

TABLES

Table I

Group I - Females 200 mg/kg body weight

Weight of the animals Grams	Dose in mg	Dose in ml
86	17.2	0.344
82	16.4	0.328
90	18.0	0.360
96	19.2	0.384
115	23.0	0.460
90	18.0	0.360
93	18.6	0.372
115	23.0	0.460
90	18.0	0.360
96	19.2	0.384
96	19.2	0.384
86	17.2	0.344
80	16.0	0.320
82	16.4	0.328

Table II

Group II - Females 400 mg/kg body weight

Weight of the animal - grams	Dose in mg	Dose in ml
86	34.4	0.688
87	34.8	0.696
88	35.2	0.704
90	36.0	0.720
90	36.0	0.720
96	38.4	0.768
100	40.0	0.800
103	41.2	0.824
103	41.2	0.824
100	40.0	0.800
90	36.0	0.720
120	48.0	0.960
130	52.0	1.040
130	52.0	1.040

Table III

Group III - Males 200 mg/kg body weight.

Weight of the animal - grams	Dose in mg	Dose in ml
90	18.0	0.360
93	18.6	0.372
103	20.6	0.412
111	22.2	0.444
102	20.4	0.408
113	22.6	0.452
128	25.6	0.512
126	25.2	0.504
86	17.2	0.344
95	19.0	0.380
140	28.0	0.560
158	31.6	0.632
155	31.0	0.620
171	34.2	0.684

Table IV

Details of the litter

	Litter size	Sex ratio Male:Female	Total weight.gms grams	Weight of males. grams	Weight of females grams	Livability	Abnormality
Females treated at a dose of 200 mg/kg body weight	6	2 : 4	25.645	9.780	15.865	Alive	Nil
	5	2 : 3	23.565	9.350	14.215	Alive	Nil
Females treated at a dose of 400 mg/kg body weight	6	3 : 3	25.102	13.005	12.097	Alive	Nil
	1	1 : 0	4.800	4.800	--	Alive	Nil
Normal females mated with males treated at a dose of 200 mg/kg body weight	7	4 : 3	25.093	13.790	11.303	Alive	Nil
	5	3 : 2	24.698	13.213	9.385	Alive	Nil
Control Animals	4	2 : 2	18.4	9.8	8.6	Alive	Nil
	5	3 : 2	24.8	15.1	9.7	Alive	Nil
	6	3 : 3	25.8	13.5	12.3	Alive	Nil
	6	4 : 2	26.8	17.84	8.96	Alive	Nil
	5	3 : 2	23.4	14.54	8.86	Alive	Nil

Table V

Biometry of the organs - Females 200 mg/kg body weight.

Weight of the animal grams	Weight of the uterus + ovary grams	Length of the uterus cms	Weight of the kidney grams	Weight of the liver grams
108	0.12	3.3	0.560	7.260
98	0.25	2.6	0.620	2.520
98	0.12	3.8	0.750	3.270
101	0.43	2.2	0.840	4.010
104	0.37	2.2	0.570	3.040
97	0.19	2.9	0.7 0	3.530
104	0.14	3.6	0.700	3.380
96	0.29	2.4	0.670	3.760
96	0.18	1.6	0.570	2.350
95	0.14	1.5	0.640	3.340
92	0.09	1.9	0.(20	3.010
95	0.16	2.7	0.650	3.150
90	0.18	2.3	0.570	2.500
92	0.13	2.4	0.660	2.630

Table VI

Biometry of the organs - Females 400 mg/kg body weight.

Weight of the animal grams	Weight of the uterus-ovary grams	Length of the uterus cms	Weight of the kidney grams	Weight of the liver grams
102	0.120	2.1	0.76	3.12
95	0.090	2.2	0.62	2.41
102	0.140	2.4	0.70	2.89
100	0.150	2.5	0.70	2.7[illegible]
113	0.160	2.4	0.73	3.15
103	0.150	2.6	0.70	2.71
110	0.140	2.4	0.[illegible]0	2.6[illegible]
108	0.190	3.1	0.8[illegible]	4.53
96	0.240	2.4	0.67	2.96
110	0.280	2.3	0.[illegible]6	3.62
130	0.176	2.8	0.71	3.44
138	0.172	2.6	0.70	3.44
142	0.176	2.7	0.72	3.4[illegible]
90	0.130	2.8	0.67	2.94

Table VII

Biometry of the organs - Males 200 mg/kg body weight.

Weight of the animal grams	Weight of the testis grams	Weight of the kidney grams	Weight of the liver grams
102	1.59	0.790	2.840
126	1.14	0.910	4.240
134	1.61	0.960	4.080
104	1.40	0.610	3.060
120	1.10	0.700	4.080
160	2.36	1.120	6.020
160	1.89	0.960	4.560
134	1.98	0.640	4.740
182	2.41	1.416	6.100
156	2.02	1.265	5.020
147	1.70	1.000	.970
176	1.91	0.928	3.880
125	1.70	0.888	3.760
98	1.32	0.952	3.620

Table VIII

Biometry of the organs - control female animals

Weight of the animal grams	Weight of the uterus + ovary grams	Length of the uterus cms	Weight of the kidney grams	Weight of the liver grams
98	0.175	2.9	0.795	3.51
90	0.091	2.6	0.815	3.10
93	0.095	2.2	0.775	3.42
94	0.105	2.9	0.720	3.46
84	0.105	2.7	0.675	3.02
88	0.057	2.2	0.635	3.05
82	0.098	1.9	0.598	2.82
103	0.150	2.8	0.700	2.71
108	0.190	3.1	0.800	4.53
96	0.140	2.4	0.670	2.96

Table IX

'iometry of the organs - control male animals.

'eight of the animal grams	'eight of the testis grams	'eight of the kidney grams	'ei ht of the liver grams
147	1.80	1.20	5.86
96	2.98	0.93	3.80
113	1.55	0.93	3.86
112	1.58	0.78	3.86
116	1.71	0.73	3.68
118	1.71	0.80	3.62
112	1.54	0.83	4.78
160	2.42	1.02	5.82
132	1.60	0.92	4.00
122	1.42	0.82	4.12

Table X

Analysis of variance table for the weight gain in Male animals

Source of variation	df	SS	MSS	F
Treatment	3	4648.77	1549.59	2.75
Error	44	24781.15	563.21	
Total	47	29429.92		

F value at 5% level = 2.82

Table XI

Analysis of variance table for the weight gain in female animals.

Source of variation	df	SS	MS	F
Treatment	5	3592.81	718.56	8.41*
Error	70	5980.29	85.44	
Total	75	9573.10		

F value at 5% level = 2.35

On comparing the mean differences of each group weight gain was found to be uniform in treated and control groups of female animals.

Table XII

Analysis of variance table for the weight of utarus + ovary

Source of variation	df	SS	MS	F
Treatment	2	0.025	0.0125	1.99
Error	35	0.220	0.00625	
Total	37	0.245		

F value at 5% level = 3.27

Table XIII

Analysis of variance table for the length of the uterus.

Source of variation	df	SS	MS	F
Treatment	2	0.0109	0.0055	0.023
Error	35	8.4391	0.241	
Total	37	8.45		

F value at 5% level = 3.27

Table XIV

Analysis of variance table for the weight of liver.

Source of variation	df	S'	M	F
Treatment	4	20.45	5.112	10.11*
Error	57	23.82	0.505	
Total	61	49.27		

F value at 5% level = 2.52.

On comparing the means it was found that the liver weight of the treatment and control groups of females are homogeneous. Similarly that of the treatment and control groups of males were also homogeneous. Further in each comparison these two groups were found to be significantly different.

Table XV

Analysis of variance table for the weight of the kidney

Source of variation	df	SS	MSS	F
Treatment	4	0.67	0.168	4.54*
Error	57	2.13	0.037	
Total	61	2.80		

F value at 5% level = 2.52

On comparing the means it was found that the kidney weight of the treatment and control groups of females were homogeneous. Similarly that of the treatment and control groups of males were also homogeneous. Further in each comparison these two groups were found to be significantly different.

Table XVI

Analysis of variance table for the weight of the testis

Source of variation	df	SS	MS	F
Treatment	1	0.0544	0.0544	0.3221
Error	22	2.7156	0.1689	
Total	23	3.77		

F value at 5% level = 4.30

Table XVII

Table of correlations.

	Body weight with the weight of uterus + ovary	Body weight with the length of the uterus	Body weight with the weight of the kidney	Body weight with the weight of the liver	Body weight with the weight of the testis
T_1	0.2921 (14)	0.62* (14)	0.0012 (14)	0.4256 (14)	---
T_2	0.171 (14)	0.0589 (14)	0.219 (14)	0.001 (14)	---
T_3	0.67* (10)	0.70* (10)	0.566 (10)	0.59 (10)	---
T_4	---	---	0.14 (14)	0.69* (14)	0.77* (14)
T_5	---	---	0.579 (10)	0.61 (10)	0.105 (10)

* Significant at 5% level
Significant at 1% level.

T1 = 200 mg/kg body weight - female group.
T2 = 400 mg/kg body weight - female group.
T3 = Control - female group.
T4 = 200 mg/kg body weight - male group.
T5 = Control - male group.

The values given within the brackets are the number of observations.

DISCUSSION

DISCUSSION

Fertility rate

The group of female animals treated with the extract at a dose level of 200 mg per kg body weight showed 72 per cent reduction in fertility from that of the control animals. Similar response was obtained from another group of female animals subjected to treatment with the extract at a dose level of 400 mg per kg body weight. Even though mating had taken place in normal frequency 12 animals out of 14 of these experimental groups remained non-pregnant. The pups of the delivered animals were weaned and the mothers were again given chance to mate with normal males. One of these female animals conceived with subsequent mating. These facts suggest that though the extract has not suppressed the estrum, it was capable of preventing normal pregnancy. Such an action may be mediated by the prevention of ovulation, fertilization, implantation or development of the embryo.

The results of the histopathological examination of the ovary revealed impairment of functioning in both the treated female groups. The development of the follicles were poor and the developing follicles were few. Such changes of the ovary were reflected in the pituitary also. In the pituitary the basophils showed degranulation and vacuolation of the cytoplasm. These observations are suggestive of the poor gonadotrophic stimulus from the pituitary. Releasing factors from the hypothalamus in addition to causing the

release of gonadotrophic hormones also stimulate its synthesis by the pituitary (Nakabayash et al. 1974). Hence the suppression of gonadotrophic stimulus may be mediated through the hypothalamus. The improper release of the gonadotrophic hormones is clearly indicated by the poor follicular development in the ovary. The fact that the treated animals manifested normal estrous cycle denoted normal or nearly normal steroid synthesis. Also the experimental animals did not reveal any atrophy of the genital tract which also testifies normal or nearly normal steroidal action on the system. However, the reduced number of maturing follicles and the predominant atretic changes in the already formed follicles suggest that proper hypophyseal stimulus for maturation and rupture of the follicle is not released from the pituitary. It therefore amounts to suggest that Ocimum sanctum has some effect primarily in the pituitary to supress gonadotrophic hormone release. This apparently explains the infertility in the treated animals.

In this study, it can be assumed that, ovulation has not taken place. This is evident from the histopathological examination of the ovary, which showed poor development of the Graafian follicles. This can be due to the poor stimulation of the ovary by the gonadotrophic hormones. The low level of FSH is probably by the poor stimulation of the pituitary from the higher centres. From this, it is evident that the extract is having anovulatory activity.

The normal female mated along with the treated males, produced only two litters. The animals conceived and delivered belonged to the group that were introduced from 9th to 24th day of the beginning of the treatment. None of the female animals of the subsequent groups ie. 25th to 40th days and 41st to 56th days became pregnant. This indicated that the extract administered was capable of causing sterility at least from 25th day of the beginning of the treatment and this effect continued at least upto 56th day of the treatment.

The results of the histopathological examination supported the reduced fertility rate. In the testis, focal areas of the seminiferous tubules did not show any evidence of spermatogenesis. Certain other tubules along with collection of sperms revealed degenerating desquamated cells and hyalinised bodies. Similar observations has been reported by Yashmathan et al.(1972). Pituitary showed degranulation and vacuolation in the cytoplasm of the basophils. This suggested the impaired gonadotrophin production. The extract might have acted primarily on the pituitary and the changes in the testis may be secondary due to this.

Examination of the vagina of females cohabitated with the treated males revealed spermatozoa, indicating normal mating rate. It could be presumed that O.sanctum did not adversely affect the libido or mating behaviour in the treated animals.

Litter

Albino rats will produce an average number of six young ones in ta litter (Parris, 1962). In this experiment both the co trol and treatment animals produced an average litter size of five. Johny (1972) obtained similar values from the same stock of animals. The birth weight of the young one i. influenced by sex, size of the litter, physical co ditions of the mother and her age (Parris, 1962). The average birth weight of the male offspring was 4.37 grams and 4.51 grams and that of the fem le was 4.21 grams and 4.41 grams in the treatment and control groups respectively. Sex ratio of the offsprings did not vary significantly between the treatme t and control groups. Similar observation was made by the Patta and Panthakumari (1972) in rats after treating with benzene extract, petroleum ether ex ract, ethanol extract, acetone extract and ether extract of O.sanctum.

Toxicity

No macroscopic teratogenecity was observed on the pups born to the experimental mothers and those born to experimental fathers. Further no significant difference was observed in the weight gain of the exp rimental and control groups of animals. On histological examination, the liver showed slight engorgement of the sinusoids in the group with 200 mg per kg body weight. In the group with 400 mg per kg body weight, liver showed focal areas of hyperpla ia

of hepatic cells. Such an action is more suggestive of an hepatotropic action. Kidney of the treatment group exhibits no toxic signs on histopathological examination.

Results of the biometry showed no significant difference in the weights of various organs examined between the treatment and control groups.

Results of the previous experiments with this plant material suggested abortifacient activity (Vohora et al. 1969; Jain and Tarafder, 1970) and anti-implantation activity (Datta and Santhakumari, 1971). In male animals Kashanthan et al. (1972) attributed sterility to the partial impairement of spermatogenesis and physical changes on the testes. In this study anovulatory activity in female animals and partial impairement of the testicular functioning in males were found to be the cause for the reduced fertility rate. The variation in the results obtained can be due to the fact that the active ingredient present in plant depend upon several factors like nature of the soil, the climate, the season, the stage of growth, cultivation practices etc. (Chopra et al. 1956).

Further studies are required to elucidate the exact mechanism of action of O.sanctum on the reproductive system.

SUMMARY

SUMMARY

A study was undertaken in albino rats to assess the antifertility property of the benzene soluble fraction of the dried leaves of the plant Ocimum sanctum. The extract was administered orally to female rats at the rate of 200 mg. per kilogram body weight for a duration of eight days. Similar pattern was followed for another group of female rats at the rate of 400 mg per kilogram body weight. One batch of male rats were treated orally at the dose level of 200 mg per kilogram body weight for 20 days. The vehicle, 10% solution of Tween 80 in water, was given for control rats. From the study the following observations were made:

1. Female rats in both the groups showed 72 per cent reduction in fertility from that of the control, when mated with untreated males.

2. Treated male rats showed 43 per cent reduction in fertility, when they were mated with untreated female rats, during the period of nine to 24 days from the beginning of the treatment.

3. Complete sterility was observed in the male rats during the period of 25th to 56th days from the beginning of the treatment.

4. The extract had no effect on litter size, sex ratio, birth weight and livability of the offspring born to the treated rats.

5. Administration of the extract had no effect on the weight gain in the treated animals.

6. Histopathological examination of the treated rats of both sexes showed degenerative changes in the basophils of the pituitary.

7. Histopathological examination revealed poor ovarian function in the treated female animals.

8. Impairement of the spermatogenesis was evident on histopathological examination of the testis of the treated animals.

From the above observations, it was concluded that:

1. Administration of the extract, at the rate of 200 and 400 mg per kilogram body weight, over a period of eight days in female rats and 200 mg per kilogram body weight in male rats for 20 days showed reduction of fertility. The higher dosage was not superior in reducing the fertility.
2. The extract had anovulatory activity on the ovary and impaired spermatogenesis on the testis.
3. Degenerative changes in the basophils of the adenohypophysis suggest the improper release of the gonadotrophic hormones.

4. Administration of the extract in both sexes produced toxic effects neither to the treated animals nor to their offspring.

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AN ASSESSMENT OF
THE ANTIFERTILITY PROPERTY OF
Ocimum sanctum

BY

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ABSTRACT OF A THESIS

Submitted in partial fulfilment of the
requirement for the degree

MASTER OF VETERINARY SCIENCE

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ABSTRACT

A study was carried out in rats, to assess the antifertility property of the benzene fraction of the leaves of the plant Ocimum sanctum. Premating treatment was done in female animals at dose levels of 200 and 400 mg per kilogram body weight for a duration of eight days. Male animals were subjected to the treatment at a dose level of 200 mg per kilogram body weight for 20 days. The experimental animals were allowed to mate with untreated animals of the opposite sex. Histopathological examination of the organs - pituitary, ovary, testis, liver and kidney were carried out.

Results of the study suggested considerable reduction of fertility in both the sexes. This can be attributed to the impaired release of gonadotrophic hormones and the resulting improper functioning of the gonads. Administration of the extract showed no toxic effects in the treated rats as well as in their offspring.