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A COMPREHENSIVE REVIEW ON ANTIFERTILITY AGENTS AND THEIR IN VITRO AND IN VIVO ANIMAL MODELS

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ABSTRACT

This review explores on herbal extracts which possess antifertility activity and focusing on their evaluation through both in vitro and in vivo models. the study delves into the mechanism and efficacy of these agents in preventing fertility, shedding light on potential advancements in reproductive medicine.by analyzing data from diverse models. The review aims to contribute valuable insights for the development and refinement of antifertility drugs. The examination of antifertility drugs utilizing both in vitro and in vivo animal models is the main topic of this review. In order to offer light on possible developments in reproductive medicine, the study explores the processes and effectiveness of these drugs in limiting fertility. The goal of the review is to provide important insights for the creation and improvement of antifertility medications by examining data from various models.

INTRODUCTION

In emerging nations, the population boom is a major contributor to pollution and poverty. The population's exponential growth has had a negative impact on humankind's social, economic, and technical advancement. As a result, reducing or controlling priorities. There are a lot of synthetic contraceptives on the market, but they're all either not very effective or have negative side effects. It established a population control program that

included research on conventional medical procedures. Plants have been used as a source of medicine since antiquity, although herbal therapy is often overlooked in scientific medicine. The World Health Organization recommended using locally accessible, potent plants as medication replacements **[1]**.

Evaluation of anti-fertility agents

Clinical trials may come after an experimental evaluation of a drug's medicinal usefulness, efficacy, and toxicity in animals. To evaluate anti-fertility activities in experimental animals such as mice and rats, in-vivo animal models are used.

Parameters used to evaluate anti-fertility agents

For the study of anti-fertility activity many in-vivo models have been used. Estimation of sex hormones Blood samples were collected from rats for estimations of serum levels of sex hormones. Within 12 hours of preparation, sera were divided into sterile vials, frozen, and utilized for the measurement of prolactin, testosterone, estrogen, Follicle Stimulating Hormone (FSH), and Luteinizing hormone (LH) **[5]**.

Assessment of sperm motility and count Progressive motility was tested immediately. Semen was pressed onto a slide that had been warmed up before the right cauda epididymis was cut. A cover-slip was used to mix the semen after two drops of warm 2.9% sodium citrate were introduced. A 400× magnification was used to visually assess the proportion of increasing sperm motility. For every sample, many fields were used to assess the motility. The ultimate motility score was determined by averaging the three estimates that came before it. To count sperm, the left cauda epididymis was cut, and any dripping semen was immediately drawn into a red blood pipette until it reached the 0.5

point. The resulting mixture was then diluted with warm normal saline until it reached the 101 marks. A droplet containing the semen combination was positioned on the Neubauer counting chamber and examined with a ×40 magnification.

Assessment of sperm viability and morphology

Using eosin/nigrosin stain, a viability study (% of living spermatozoa) was conducted. Two drops of the dye were applied to a drop of semen that had been squeezed onto a microscope slide. Thin smears were then made and examined under a 400-magnification light microscope. Non-viable sperm stained red whereas viable sperm remained colorless. Using 40 microscope objectives, the stained and unstained sperm cells were counted, and an average value for each was recorded, from which % viability was computed. The slides stained with eosin-nigrosin (5 slides/rat) were inspected under a light microscope at 400 magnifications to estimate the proportion of morphologically aberrant spermatozoa. On each slide, 300 sperm cells were inspected (1 500 cells for each rat), and the head, tail, and total.

Mating trial test Mating trial test of male rats was done, 5d before the termination of the experiment. Every male rat was kept in a solitary cage and cohabitated overnight at a 1:2 ratio with proestrous females. The presence of sperm and vaginal plug in the vaginal smear the next morning indicated positive mating. Every female who tested positive for sperm was monitored closely, and the resulting pregnancies were recorded after the dam gave birth. Next, the reproductive parameters listed below were calculated: The formula for mating success percentage is number mated/number paired × 10; for fertility success percentage, it is number pregnant/number paired × 100; and for fertility index, it is number pregnant/number mated × 100 **[5]**.

Body and sex organ weights

The animals' initial and ultimate body weights were noted. After being removed from adhering tissues and blood, the testes, epididymides, seminal vesicle, and ventral prostate were weighed to the closest milligram. Organ weights were expressed as relative weights (body weight × 100) for each organ **[5]**.

Quantification of fructose in seminal vesicle: Seminal vesicle homogenate was made at a tissue concentration of 50 mg/mL in order to quantify fructose. Seminal plasma, the supernatant, was deproteinized by centrifuging it for 15 minutes at 2,500 r/min after adding 50 mL of zinc sulphate and sodium hydroxide to create a total dilution of seminal plasma 1:16. 200 milliliters of clear seminal plasma were utilized, and at 470 nm, the optical densities of the standard and samples were compared to the blank to determine the fructose content. Plotting the value in the standard curve against the value

stated in mmol/mL of seminal plasma allowed for the determination of the fructose content **[5]**.

Abortifacient activity (Anti-implantation activity): The abortifacient effect of the plant extracts was evaluated in female albino rats. Every day, the vaginal smears of female rats in cages with known fertility were checked. Material that was not stained was examined using a light microscope. The phases of the estrous cycle were ascertained by utilizing the observed cell proportion. In the evening of proestrous, female rats were housed in a 2:1 ratio with males who had demonstrated fertility, and the next day, the evidence of copulation was checked. The day when the rats that showed large clusters of spermatozoa in their vaginal smears were separated was marked as the first day of pregnancy. Four groups (one control group, three experimental groups of six animals each, and one group of these rats) were randomly assigned to these rats. Group I acted as control and was given the single vehicle. Different extracts were given to Groups II, III, and IV. The animals were laparotomized on the tenth day of pregnancy while under light ether anesthesia in sterile settings. The implantation locations were identified by examining the two uterine horns. The abdominal wound was then stitched up in layers. Care was taken after surgery to prevent infection. After that, the extract for testing was given to pregnant rats that had surgery, monitored by an intragastric soft rubber catheter, from day 11 to day 15. The animals were permitted to mature to term. Following delivery, the number of pups was ascertained and the extract's antifertility efficacy assessed. Litters were inspected to look for any deformities **[1, 6, 7]**. quantity of resorptions divided by quantity of corpus luteum × 100 equals percentage abortifacient activity **[8]**.

Post-coital antifertility activity (Pre-implantation activity)

The percentage of animals with no implantations in their uteri upon laparotomy on day 10 of pregnancy is known as the anti-implantation activity. Every day, vaginal smears from the rats were observed, and those with a regular estrous cycle were chosen. Rats in the proestrus phase of the cycle were housed in a 2:1 ratio with males that had been shown to be fertile, and the next morning, the rats were checked for signs of copulation. The day that the rats showed large clusters of spermatozoa in their vaginal smears was marked as the first day of pregnancy. The rats were then sorted into five groups, each with six rats. The extract was given between days one and seven of pregnancy. Additionally, the medicine in powder form was given from day 1 to day 7 of pregnancy. The vehicle was given to control rats (distilled water). Day 25 saw the completion of a laparotomy under semi-sterile circumstances and light ether

anesthesia. The number of implantation sites and presence or absence of corpora luteagraviditis were assessed by examining the uteri **[9–12]**.

Pre-implantation loss frequency = number of missed implants (corpora lutea implants)/number of corpora lutea x 100 176

Effect on estrous cycle

The female animals were artificially induced into the estrus phase (heat) by either giving an oral suspension of ethinyl estradiol at a dose of 100 mg/animal 48 hours before pairing and subcutaneous injections of progesterone at a dose of 1 mg/animal 6 hours before the experiment, or by sequentially giving subcutaneous injections of progesterone (0.5 mg/100 g body weight) and estradiol benzoate (10 mg/100 g body weight) 48 and 4 hours before the experiment, respectively. The vaginal smear method was used between 8 and 10 am to assess the estrous cycle. Using a plastic pipette filled with 10 mL of regular saline, vaginal secretion was collected. After using the pipette to cleanse the vagina three times, the vaginal fluid was transferred to a glass slide. Every animal was represented by a different slide. A light microscope was used to view the unstained secretion. Following confirmation of a consistent four-day cycle lasting two weeks, the study animals were chosen, split into six groups, and given test medication. For 28 days, the impact of test medications on the estrous cycle was observed **[9, 13]**.

Antiestrogenic activity: Between 8 and 10 am, the vaginal smear method was utilized to evaluate the estrous cycle. Vaginal secretion was collected using a plastic pipette that was loaded with 10 mL of ordinary saline. The vaginal fluid was moved to a glass slide following three rounds of vaginal cleansing with the pipette. Each animal was shown on a separate slide. The unstained secretion was examined using a light microscope. Once a regular four-day cycle lasting two weeks had been confirmed, the study animals were divided into six groups and given test drugs. The effect of test medicines on the estrous cycle was monitored for 28 days [9,13]. On the eleventh day, all of the rats' surviving right-sided ovaries were removed after they were put to sleep with ketamine (60 mg/kg, intraperitoneally). after being thoroughly cleaned, dried, and their weights were noted. Weight changes in the ovaries before and after extract therapy were computed. The ovarian weight percentage inhibition was computed using the following formula: Ovarian weight percentage inhibition is equal to $[1 - (XE - C)]/E - C \times 100$. In this case, XE represents the mean ovarian weight of rats treated with extract and estradiol, C represents the mean ovarian weight of rats treated with vehicle, and E represents estradiol **[9]**.

Antigonadotrophic effect

The following formula was used to calculate the ovarian weight % inhibition: [1 − (XE − C)]/E − C × 100. The mean ovarian weight of rats treated with extract and estradiol is represented by XE in this instance, while the mean ovarian weight of rats treated with vehicle is represented by C, and estradiol is represented by E **[9]**. The ovariectomized rats were divided into six groups and treated. On 12th day after treatment, the remaining right ovaries of all rats properly dissected out using same anesthetic condition. Cleaned, dried and their respective weights were recorded and percentage increase in ovarian weight compared with weight of the left ovaries were calculated. Percentage increase in the weights of ovary was calculated using the formula **[9]**. Percentage increase in ovarian weight = (weight of right ovary − weight of left ovary)/weight of left ovary × 100.

Histological analysis

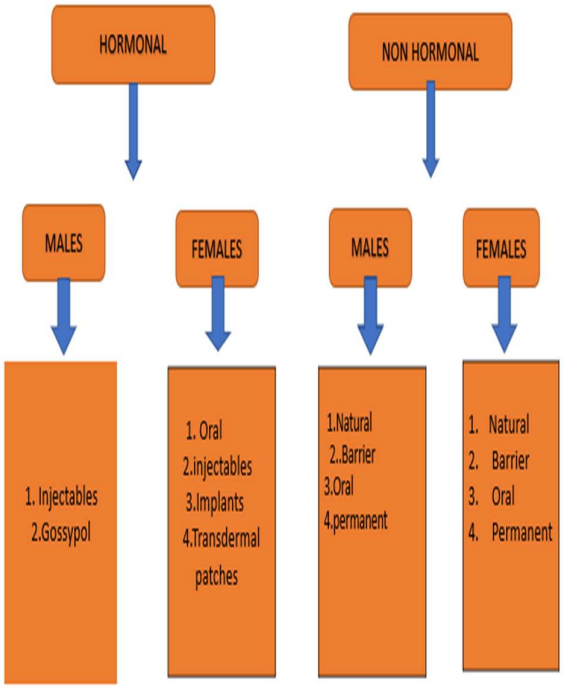
After male and female rats' abdomens were cut open, the testes and uteri were carefully removed, preserved in 10% normal saline, and then processed as usual for paraffin embedding. Hematoxylin and Eosin Stalin (H/E) were used to obtain 5 mm sections from each using a rotary microtome, which were then examined under a light microscope **[5, 14]**.

Measurement of some biochemical and blood parameters

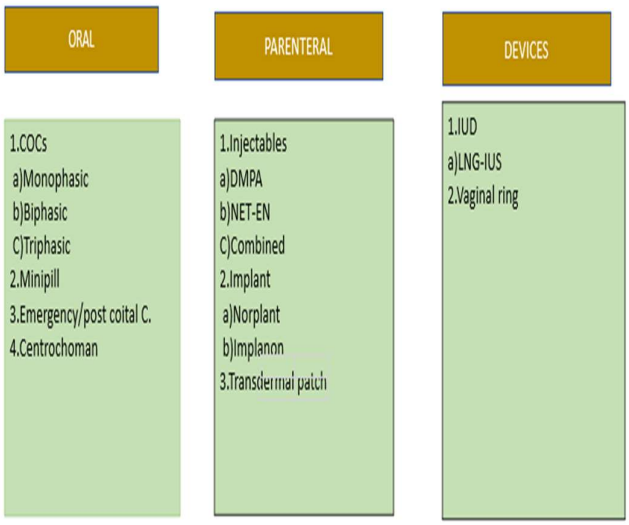
At the moment of scarification, blood samples were taken from each rat's heart and placed into both heparinized and non-heparinized tubes. Standard procedures were used to measure serum levels of hemoglobin (Hb) concentration, packed cell volume (PCV), cholesterol, total proteins, red blood cell count, total leucocytic count (TLC), alanine aminotransferase (ALT), creatinine, and urea **[5, 15]**. Determination of testicular and serum cholesterol (Chod PAP method)

As the building block for numerous physiologically significant steroids, including bile acids, steroid hormones, and vitamin D, cholesterol is known to be necessary for healthy sexual function. The Chod-PAP method can be used to measure the amounts of serum and testicular cholesterol. In short, 0.02 cm3 of the working reagent is added, and after five minutes, the mixture's absorbance is measured at 546 nm **[13]**.

CLASSIFICATION:



S. No.	PLANT NAME	Mg/kg DOSE/BODY WEIGHT	ACTIVITY
1	*Aloe barbedensis*	100	Anti-implantation
2	*Abroma augusta*	50	Anti-implantation
3	*Abutilon indicum*	500	Anti-implantation
4	*Artemisia vulgaris*	300&600	Anti-implantation
5	*Cichorium intybus*	50	Anti-implantation
6	*Cuscuta reflexa*	800	Anti-implantation
7	*Curcuma longa*	200	Anti-implantation
8	*Ocimum sanctum*	300	Antifertility
9	*Quassia amara*	Single daily i.m injections of the extract for 15 days	Antifertility
10	*Rubia cordifolia*	250	Anti-implantation
11	*Syzygium aromaticum*	15,13&60	Degenerative changes in the seminiferous tubules
12	*Terminalia bellirica*	50	Antifertility
13	*Urtica diocia*	250	Anti-implantation



List of some herbal anti-fertility agents [35]

Sperm motility and count:

Testing for progressive motility was done right away. Seminal fluid was extracted from the right cauda epididymis and placed on a heated slide. Semen was combined with two drops of warm 2.9% sodium citrate using a cover slip. At 400x magnification, the percentage of increasing sperm motility was assessed visually. Three distinct fields were used to measure the motility of each sample Because the final motility score was determined by taking the mean of the three successive assessments. The left cauda epididymis was cut, and any dripping semen was immediately sucked into a red blood pipette until it reached the 0.5 mark. Warm normal saline was then added to dilute the semen to the 101 level for the spermatozoan count. Under a ×40 magnification, a drop of the semen mixture was observed on the Neubauer counting chamber. A total of 106 spermatozoan cells were counted and expressed per milliliter **[11]**. Vaginal opening: This apoptosis-mediated process is utilized as an external marker to indicate the onset of puberty **[12]**. It happens as a result of elevated secretion of estradiol and may be induced by injecting immature rats or mice with estradiol **[13]**. In mice, vaginal opening can happen up to 10 days before to the first vaginal cornification and, thus, the start of the estrus cycle, whereas in rats, it happens concurrently with the first ovulation **[14]**. Watching mice every morning from the age of twenty-four to thirty days allows one to determine the age at which they open their vagina. Sometimes the vulva can be easily inspected to identify the opening. In mice, the vaginal opening happens at approximately 26 days of age **[15]**.

Chick oviduct method:

Estrogenic compound screening is done using this technique. The weight of the chick oviduct increases as a function of dose with the estrogenic chemicals. For six days, subcutaneous injections of the test medication or standard (estradiol) are given twice a day at different doses to pullet chicks. Twenty-four hours following the final injection, the animals are slaughtered, and the body and oviduct weights are recorded. The test group's oviduct's increased weight indicates that it may have estrogenic potential **[17]**.

Four- day Uterine Weight Assay:

The idea behind this test is that estrogenic drugs raise the uterine weight by increasing protein synthesis. The test medication (estradiol) or the standard (ovariectomy) was administered intramuscularly to female albino rats for three days. The uterus was removed from the abdomen and the animals were slaughtered on the fourth day. After the uterus was empty, the weight of the moist uterus was promptly measured. The uterus was dehydrated by heating it to 100 °C in an

oven. To determine the increase in dry weight, the weight was measured once more. An increase in uterine weight is a sign that the test substance has estrogenic properties **[18]**.

Abortifacient activity:

The plant extracts were tested in female albino rats for abortification activity. The vaginal smears of caged female rats of glorious fertility were monitored daily. Unstained material was observed under a light microscope. The proportion among the cells observed was used for the determination of the estrous cycle phases. Female rats were caged with males of proved fertility within the ratio of 2:1, in the evening of proestrous and examined the following day for the proof of sexual activity. Rats exhibiting thick clumps of spermatozoa in their vaginal smears were separated and which day was selected day 1 of pregnancy. These rats were indiscriminately distributed into 4 groups, a control group and 3 experimental groups of 6 animals every. Group I received vehicle only and served as control. Groups II, III, and IV were given extracts that were entirely different. The animals were laparotomized under light ether anesthesia under sterile conditions on the tenth day of pregnancy. The implantation locations were identified by examining the two uterine horns. The abdominal wound was then stitched up in layers. Care was taken after surgery to prevent infection. Following surgery, the extract under test was given to pregnant rats by an intragastric soft rubber tube from day 11 to day 15. It was permitted for the animals to travel to term. Following delivery, the pups were counted, allowing for an evaluation of the extract's antifertility properties. Litters were inspected to look for any deformities **[19, 20, 21]**.

Antigonadotrophic effect:

Using the vaginal smear technique, five successive normal estrus cycles in female rats were investigated. The rats had a left-side ovariectomy after receiving a pretreatment of atropine (1 mg/mL) and ketamine (60 mg/kg) for anesthesia. The left ovary was carefully removed from the surrounding adipose tissue, dried by soaking on paper, and then weighed. Six groups of ovariectomized rats received treatment. After treatment, on the twelfth day, all rats' surviving right ovaries appropriately compound out the same anesthesia state. Ovaries were cleaned, dried, and their weights were noted. The percentage increase in ovarian weight above the weight of the left ovaries was then computed. % Rise in the ovarian weights was computed using the formula **[23]**. Weight of right ovary - weight of left ovary = weight of left ovary x 100 = percentage increase in ovarian weight.

Mating trial test:

Five days before to the experiment's conclusion, the male rats' mating trial test was completed. All male rats were kept in an extremely solitary cage and cohabitated

overnight at a ratio of around 1:2 with proestrous females. The presence of spermatozoa and vaginal plug in the vaginal smear the next morning indicated positive mating. After the dam gave birth, all spermatozoon-positive females were monitored and the resulting pregnancies were recorded. Next, the reproductive parameters that followed were calculated: Mating success % = number mated/number paired × 10; Fertility success % = number pregnant/number paired × 100; Fertility index = number pregnant/number mated × 100 **[11]**.

A The ovaries of each rat were removed, and the organs' weight was recorded. These ovariectomized rats were divided into thirty groups. All animals receive the two doses of estradiol (0.1 mg/rat and 1.0 mg/rat) and test chemicals for four consecutive days, with the exception of the rats in the control group. The rats were given ketamine (60 mg/kg intraperitoneally) on day eleven to put them to sleep, and each animal's remaining right-sided ovaries were removed. After giving the animals a thorough bath and drying, their varied weights have been recorded. The ovaries' pre- and post-extract treatment weight changes were calculated. To calculate the percentage inhibition of ovarian weight, the following formula was used: Ovarian weight inhibition % = [1-(XE-C)]/E-C × 100.

Antiestrogenic activity:

anti-ovulatory behavior HCG Induced Ovulation in Rats: The theory behind this test is that human chorionic gonadotropin (HCG) injection causes ovulation to occur in two days, even though immature female albino rats do not ovulate spontaneously. The test medication is given in different dosages to immature female albino rats, and then HCG is given. Following two days of HCG injection, the animals were slaughtered, and their ovaries were removed and histologically analyzed. Ovulation triggered by HCG is inhibited by compounds having antiovulatory action. Rabbits with sexual maturity that receive intravenous infusion of substances such as cupric acetate will ovulate a few hours later. To ensure they are not pregnant, mature rabbits are kept separately (separated) for a total of twenty-one days. Following the administration of the test medication, cupric acetate is injected intravenously 24 hours later. The animals are killed and their ovaries are removed 24 hours after the cupric acetate was administered. To determine the total number of ovulation sites, a histopathological examination is performed on both ovaries. In comparison to the animals in the control group, the number of ovulation sites is lower in drugs with antiovulatory effect **[18]**.

Gestational activity Pregnancy maintenance test:

This test is based on the progesterone hypothesis. When an ovariectomy is

performed in the early part of a pregnancy due to inadequate progesterone, the pregnancy ends. Nevertheless, an ovariectomy carried out in the latter stages of pregnancy does not result in an abortion since the placenta produces the progesterone needed to sustain the pregnancy. Ovariectomies are performed on female Sprague-Dawley rats on the eighth day of pregnancy. Starting the day before the ovariectomy, the test drug is injected beneath the skin once a day for 13 days. On day 21 of gestation, the animals are put to death, and the number of viable embryos and implantation sites is then tallied. A normal pregnant rat has eleven implantation sites and about ten viable embryos **[17]**.

Carbonic anhydrase activity in Rabbits:

It has been discovered that the amount of progesterone generated from the corpus luteum influences the amount of carbonic anhydrase in the uterine endometrium of rabbits. Estradiol is used to set immature female albino rabbits; therefore, the test medication or standard medication is administered as in the Clauberg test. The uteri are dissected and the animals are slaughtered 24 hours after the last drug injection. The endometrial extract's carbonic anhydrase activity is measured. The endometrium's carbonic anhydrase activity is elevated by exogenous progestins **[17]**.

EXPERIMENTAL IN VITRO ANIMAL MODELS

Spermicidal activity:

This is a simple in vitro process that may be applied directly to human seminal fluid. To test sperm motility, a fresh sample of human seminal fluid is put on a slide, two drops of an extract from a herbal medicine are added, and the slide is then viewed under a microscope. When spermicidal.

Estrogen receptor binding assay:

The test chemicals' capacity to bind to estrogen receptors indicates their estrogenic activity. Estradiol is employed as the reference, and sources of estrogen receptors include the human endometrium and mouse uteri.

Gestagen Receptor Binding Assay:

The test substances' progesterone activity is ascertained by their capacity to bind to progesterone receptors. Progesterone receptors may be extracted from human uteri collected following a hysterectomy as well as from the uteri of rabbits primed with estrogen. The norm is to utilize progesterone **[17]**.

Androgen receptor binding assay:

The androgenic activity of the test compounds is shown by their ability to bind to androgen receptors. Testosterone, the androgenogenic hormone, is employed as the reference, and androgen receptors are obtained from the kidney of mice or the ventral prostate of rats. Compounds that decrease the activity of 5α reductase have an antiandrogenic effect since this enzyme is

involved in the conversion of testosterone to dihydro-testosterone. The prostate of rats or people with benign prostatic hyperplasia is the source of the enzyme 5α-reductase **[17]**.

CONCLUSION

Agents that can induce pregnancy termination and stop ovulation or fertilization are known as antifertility agents. The current review has included comprehensive details on all the *in vitro* and *in vivo* techniques available for inducing antifertility activity in animal models.

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