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A REVIEW ON MEDICINAL PLANTS WITH ANTI-FERTILITY ACTIVITY

Taqiuddin

Department of Pharmacology Lovely Professional University, Phagwara Jalandhar - 144411, Punjab, India.

Keywords:

Contraceptives, Anti-fertility, Medicinal plants, Synthetic steroidal drugs

Correspondence to Author:
Mr. Taqiuddin

Associate Professor,
Department of Pharmacology,
Lovely Professional University,
Phagwara Jalandhar - 144411,
Punjab, India.

E-mail: taqicology@gmail.com

ABSTRACT: Objectives: Along with increasing availability and utilization of contraception, it is also important to confirm that the effects of contraception use on resumption of fertility after discontinuation and serious adverse effects produced by synthetic steroidal contraceptives remained a major problem. However, current evidence on resumption of fertility after contraception use is inconclusive, and practically fertility after termination of contraception remains a big concern for women who are using contraception. This fear poses a negative impact on utilization and continuation of contraception. Therefore, the development of new fertility regulating drug from medicinal plants is an attractive proposition. So available reports and identifying the associating factors are important for designing a strategy to overcome the problem. **Methods:** The review is conducted through a systematic literature search of research articles. Few bibliographic databases and libraries: Pub Med/Medline, Global Health Database, Science direct, Embase, the Cochrane Library were used. **Results:** The review of different database studies that enrolled profiles of various medicinal plants used for contraception was analyzed. **Conclusion and Recommendation:** Contraceptive use, regardless of its duration and type, does not have a (negative) effect on the ability of women to conceive following termination of use, and it does not significantly delay fertility, but the serious adverse effects associated with synthetic steroidal contraceptives are definite and significant. So, it can be recommended to develop contraceptives from herbal medicinal plants with the least toxicity and more efficacy.

INTRODUCTION: Anti-fertility agents are drugs that control fertility [1] and are also called oral contraceptives. These drugs affect and are involved in the menstrual cycle and ovulation in females. Estrogen and progesterone in combined form are given as birth control pills. The anti-fertility substance is deemed to be active in females when it prevents fertilization, prevents ovulation, implantation, and destroys the zygote or causes abortion.

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In males, it prevents spermatogenesis, inhibits testosterone, or affects the gonadotrophin of the organs or the mortality of sperm. Currently, population size is being controlled in many developing countries [2]. Thus the control of human fertility in the sense of its limitation is the most important and urgent of all biosocial and medical

problems confronting mankind today. The development of new fertility regulating drugs from medicinal plants is an attractive proposition. Man, since time immemorial, has been using herbs or plant products as medicine for abortifacient and anti-fertility activities. Although in recent times, synthetic drugs are used extensively in modern medicine systems. However, many modern medicines are developed through the clues obtained from phytochemicals. Moreover, phytochemicals are even today are important resources for medicinal uses. Plant products are becoming more popular than synthetic drugs due to their low toxicity and long-standing experience of exposure to these drugs in ethnic medicine systems like Ayurveda. Family planning has been promoted through several methods of contraception. Still, due to serious adverse effects produced by synthetic steroidal contraceptives, attention has now been focused on indigenous plants for possible contraceptive effects. Hence, there is a need to search for suitable products from indigenous plants that could be effectively used in the place of pills.

Literature Survey:

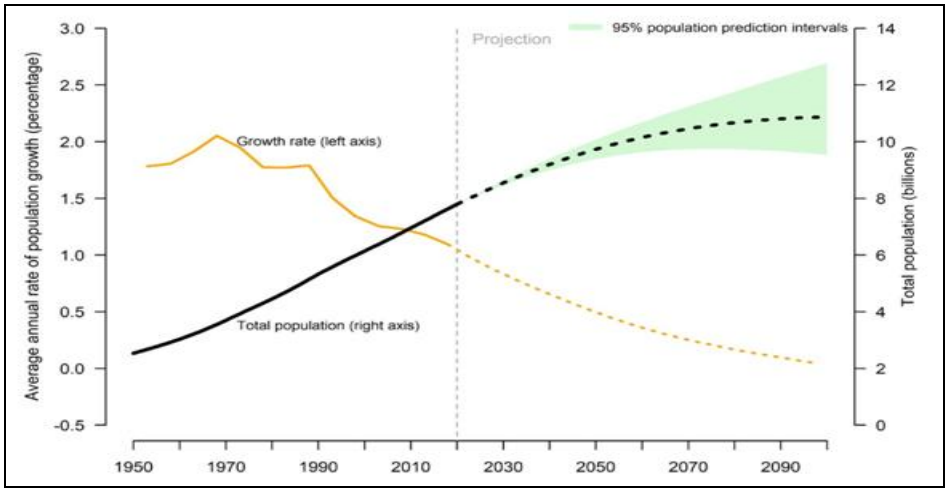

FIG. 1: POPULATION SIZE AND ANNUAL GROWTH RATE FOR THE WORLD: ESTIMATES 1950-2020 AND MEDIUM-VARIANT PROJECTION WITH 95 PERCENT PREDICTION INTERVALS 2020-2100.

Data Source: United Nations, Department of Economic and Social Affairs, Population Division (2019). World Population Prospects 2019. Population growth continues at the global level, but the rate of increase is slowing, and the world's population could cease to grow around the end of the century. According to the medium-variant projection, of the eight SDG regions, only sub-Saharan Africa is projected to sustain rapid population growth through the end of the century.

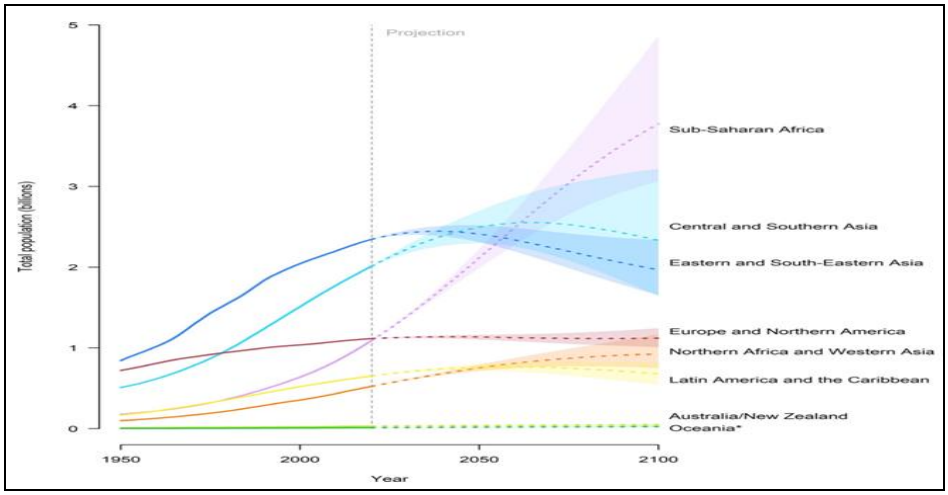

FIG. 2: POPULATION BY SDG REGION: ESTIMATES, 1950-2020, AND MEDIUM-VARIANT PROJECTION WITH 95 PERCENT PREDICTION INTERVALS, 2020-2100.

Data Source: United nations department of economic and social affairs. Population Division (2019). World population prospects 2019. (Excluding Australia and New Zealand [3].

The Physiology of Female Reproductive System[4]: Reproductive activity in the human female and subhuman primates depends on the regular cyclic activity of the hypothalamus, pituitary, ovaries, and uterus. The regular monthly vaginal bleeding observed in most primates is directly dependent upon the cyclic release of the steroid hormones, estrogen, and progesterone, from the ovaries. Ovarian steroid synthesis is in turn, coordinated in a cyclic manner by the central nervous system through the secretion of pituitary follicle-stimulating hormone (FSH) and luteinizing hormone (LH).

The release of LH and FSH depends on the hypothalamic peptide luteinizing hormone-releasing hormone (LHRH). During an ovarian cycle, a cohort of follicles develops in the ovaries. Growth of germ cells and estrogen synthesis occurs in the follicles during the first half of the cycle. A single follicle gains dominance and matures to release an ovum, whereas all other follicles belonging to the same cohort undergo atresia. At the site of ovulation, a corpus luteum is formed from the ruptured follicle and is responsible for progesterone and estrogen secretion during the later part of the cycle. The corpus luteum is maintained for a fixed period and as its function decreases, a new set of follicles begins to develop, and a new ovarian cycle begins. When pregnancy occurs, the ordinary ovarian cycle is suspended and the hormone relaxin secreted by corpus lutea, and placenta ensures uterine quiescence and prevents early abortion of the pregnancy.

During the ovarian cycle, the endometrium proliferates under the influence of ovarian estrogen and progestins. With regression of the corpus luteum, the endometrium is sloughed, and a menstrual period occurs. The period of time between the beginning of one menstrual period and the beginning of the next is termed the menstrual cycle. Although in the strict sense the ovarian cycle and the menstrual cycle refer to cyclic changes in the ovary and endometrium, respectively, these terms are often used synonymously with "reproductive cycle", which implies the cyclic, morphologic, and hormonal changes which occur in the central nervous system, anterior pituitary, ovary, and uterus.

Female Hormonal System[5]:

- A hypothalamic-releasing hormone, luteinizing hormone-releasing hormone (LHRH).
- The anterior pituitary hormones, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), both of which are secreted in response to the releasing hormone from the hypothalamus.
- The ovarian hormones estrogen and progesterone are secreted by the ovaries in response to the two hormones from the anterior pituitary gland.
- The various hormones are not secreted in constant amounts throughout the female monthly sexual cycle but instead are secreted at drastically different rates during different parts of the cycle.

Estrogens [6]: Estrogens are a class of steroid hormones linked principally with the control of female sex organ responsiveness and of reproduction. The important endogenous estrogens are 7 β-estradiol, estrone and estriol. The most potent biogenic form is 17 β-estradiol. Estrogens are biosynthesized in the ovary [6]. Phytoestrogens are naturally occurring plant estrogens with structural similarity to estrogens and have the capacity to bind to estrogen receptors. Phytoestrogens seem to possess estrogenic and antiestrogenic activity depending on an individual's circulating endogenous estrogen and the number and types of estrogen receptors [7]. The major chemical groups of phytoestrogens are classified as flavonoids, (flavones, flavanones and iso-flavanoids) coumastrans, lignans and myco-estrogens. These compounds interacting with the estrogenic receptor exert estrogenic activity, such as uterotropic effect, sterility, or disruption of normal reproductive processes in farm animals grazing in pastures with high plant sources in phytoestrogens. In humans' cancer-protective properties have been associated with phytoestrogens [6].

Physiology of Estrogens [8]: The estrogens are largely responsible for the changes that take place at puberty in girls and account for the secondary sexual characteristics of females. By direct action, estrogens cause growth and development of the vagina, uterus, and fallopian tubes.

Estrogen act to cause enlargement of the breasts through the promotion of ductal growth, stromal development, and the accretion of fat. They also contribute to the molding of the body contours, shaping the skeleton, and bringing about the pubertal growth spurt of the long bones and its culmination by fusion of epiphyses. Growth of axillary and pubic hairs and pigmentation of the genital regions are effects of estrogens.

The ovaries' cyclic changes in estrogens and progesterone production regulate corresponding events in the fallopian tubes, uterus, cervix, and vagina. These changes prepare the uterus for implantation if the ovum is fertilized and proper timing of events in these tissues is essential for a successful pregnancy. If pregnancy does not occur, the endometrium is shed and is visible externally AS the menstrual discharge.

Estrogens have a positive effect on bone mass, bone is continuously remolded at sites called bone-remodeling units by the resorptive action of osteoclasts and the bone-forming action of osteoblasts. Estrogen directly acts on osteoclasts to increase the rate of apoptosis which leads to a reduced number of osteoclasts. Estrogens have many effects on lipid metabolism that is it slightly elevates serum triglycerides and slightly reduce total serum cholesterol level.

However, more important actions are an increase in HDL levels and a decrease in LDL values and lipoprotein. Estrogens have effects on many serum proteins, particularly those involved in hormone binding and clotting cascades. In general, estrogens tend to increase plasma levels of cortisol-binding globulin (CBG or transcortin), thyroxin-binding globulin (TBG), and sex steroid-binding globulin (SSBG), which binds both androgens and estrogens. They also cause a small increase in coagulation factors VII and XII and a decrease in the anticoagulation factors, protein C, Protein S and antithrombin III.

Mechanism of Action of Estrogen [9]: Estrogen may act on the hypothalamic center to inhibit the secretion of gonadotropin-releasing factor, thereby preventing pituitary gonadotropin secretion and the resultant ovulation. The transport of ova through the tubules is either accelerated or inhibited so that the ova enter the unprepared uterus where it is degenerated or expelled.

Administered estrogens also alter the delicate balance of estrogen and progesterone required for implantation of the blastocyte in the endometrium. Estrogens also induce the formation of hormonal receptors necessary for the interaction of the different hormones. Most of the actions of estrogens are mediated by the activation of intracellular receptors. Intracellular receptors play a dominant role in actions of the steroids hormones.

Estrogens after entering into the cell form a complex with specific cytoplasmic binding proteins (receptors). The estrogens-receptor complex undergoes a conformational change and is thereby activated. This activated complex then enters the nucleus and releases the free estrogen. The estrogen molecule then binds to the acceptor sites on nuclear chromatin. The drug binding to the chromatin material results in either an increase or decrease in the production rate of certain types of RNAs that is reflected into alterations in the rate of synthesis of corresponding enzymes and other proteins.

These alterations are thus ultimately projected into the biological response. Literature survey reveals the Medicinal plants and plant products possessing anti-fertility effect. In the 21st century a search has been made for plants used as anti-fertility and fertility-inducing plants. It has been considered that herbal drugs are cheaper and safer as compared to synthetic drugs and may be used without any side effects [10].

Research on the anti-fertility activity of Indian medicinal plants has been reviewed exhaustively by Choudhury and Haq [11, 12], Kamboj and Dhawan [13, 14,] R.G. Mali [15]. In this regard, it is decided to collect information on such medicinal plants possessing anti-fertility effects. So, a bit of attempt is made, and a brief account of plants possessing an anti-fertility effect is listed below.

TABLE 1: MEDICINAL PLANTS POSSESSING ANTI-FERTILITY ACTIVITY

S. no.	Name	Parts used	Family
1	*Woodfordia fruticosa* [16]	(flower)	*Lythraceae*
2	*Balanites roxburghii* [17]	(fruits)	*Balanitaceae*
3	*Saraca indica* [18]	(bark)	*Fabaceae*
4	*Achyranthes aspera* [19]	(root)	*Amaranthaceae*
5	*Asparagus africanus* [20]	(leaves & roots)	*Liliaceae*
6	*Artabotrysodoratissimus* &*Couroupita guianensis* [21]	(leaves & flowers)	*Annonaceae* & *Lecythidaceae*
7	*Fenugreek* seeds [22]	(seeds)	*Papilionaceae*
8	*Leonotis ocymifolia* [23]	(leaves & roots)	*Labiatae*
9	*Rumex steudelii* [24]	(root)	*Polygonaceae*
10	*Stephania hernandifolia* [25]	(leaves)	*Menispermaceae*
11	*Cyclea burmanni* [26]	(plant)	*Menispermaceae*
12	*Biophytum sensitivum* [27]	(plant)	*Oxalidaceae*
13	*Jayanti & Tekar* [28]	(flowers)	*Fabaceae &Verbanaceae*
14	*Derris brevipes* [29]	(roots)	*Papillionaceae*
15	*Calotropis procera* [30]	(roots)	*Asclepiadaceae*
16	*Marsilea minuta* [31]	(plant)	*Marsileaceae*
17	*Alangium salviifolium* [32]	(Stem bark)	*Alangiaceae*
18	*Striga orobanchioides* [33]		*Scrophulariaceae*
19	*Trans-anethole* [34]	(Fruits of *Illiciumaniusatum*)	*Magnoliaceae*
20	*Vicola indica* [35]	Vicolide D	*Compositae*
21	*Jatropha curcas* [36]	(seeds)	*Euphorbaceae*
22	*Cassia fistula* [37]	(flowers)	*Fabaceae*
23	*Azadirachta indica* [38]	(leaves)	*Meliaceae*
25	*Striga lutea* [39]		*Scrophulariaceae*
26	*Cassia occidentalis* [40]		*Fabaceae*
27	*Derris brevipes*		*Fabaceae*
28	*Justicia simplex*		*Acanthaceae*
29	*Achyranthus aspera* [41]	Leaves	*Amaranthaceae*
30	*Pouzolzia mixta* [42]		*Urticaceae*
31	*Caesalpina pulcherrima*	Leaves	*Fabaceae*
32	Linn [43,44]	Leaves flowers	*Apocynaceae*
33	*Tabernaemontana divaricate* [45,46]		*Euphorbiaceae*
34	*Jatropha gossypifolia* [47]	Leaves	*Apocynaceae*
35	*Alstonia scholaris* [48]	Rhizome	*Zingiberaceae*
36	*Curcuma longa* [49]	Petiole	*Piperaceae*
37	*Piper betel* [50]	Leaves	*Boraginaceae*
38	*Cordia dichotoma* [51]	Stem bark	*Phyllanthaceae*
39	*Hymenocardia acida* [52]	leaves	*Boraginaceae*
	Heliotropium indicum [53]		

TABLE 2: MEDICINAL PLANTS POSSESSING ANTI-OVULATORY OR ANTIESTROGENIC ACTIVITY

S. no.	Name	Parts used	Family
1	*Momordica charantia* [54]	(seeds)	*Cucurbitaceae*
2	*Momordica cymbalaria* [55]	(roots)	*Cucurbitaceae*
3	*Calotropis procera* [56]	(roots)	*Asclepiadaceae*
4	*Afrormosia laxiflora* [57]	(plant)	*Papillionaceae*
5	*Ammania baccifera* [58]	(Whole plant)	*Lytharaceae*
6	*Corchorus olitorius* [59]	(plant)	*Tiliaceae*
7	*Salvia fruticosa* [60]	Stem	*Labiatae*
8	*Musa paradisiaca* [61]	Bark	*Musaceae*
9	*Thespesia populnea* [62]		*Malvaceae*

TABLE 3: MEDICINAL PLANTS POSSESSING ESTROGENIC ACTIVITY

S. no.	Name	Parts used	Family
1	*Epimedium brevicornum* [63]	(leaves)	*Berberidaceae*
2	*Bupleurum marginatum* [64]	(Whole plant)	*Apiaceae*
3	Azadirachta india [65]	Bark	*Meliaceae*

TABLE 4: MEDICINAL PLANTS POSSESSING ESTROGENIC ACTIVITY

S. no.	Name	Parts used	Family
1	*Epimedium brevicornum* [63]	(leaves)	*Berberidaceae*
2	*Bupleurum marginatum* [64]	(Whole plant)	*Apiaceae*
3	*Azadirachta india* [65]	Bark	*Meliaceae*

TABLE 5: MEDICINAL PLANTS POSSESSING ANTI-IMPLANTATION ACTIVITY

S. no.	Name	Parts used	Family
1	*Melia azedarach* [66]	(leaves)	*Meliaceae*
2	*Cardiospermum halicacabum* [67]	(Whole plant)	*Sapindaceae*
3	*Thespesia populnea* [68]	(seeds)	*Malvaceae*
4	*Mimosa pudica* [69]	(leaf)	*Mimosaceae*
5	*Inula viscosa* [70]	(leaf)	*Compositae*
6	*Pueraria tuberosa* [71]		*Faboideae*
7	*Nigella sativa* [72]	(seeds)	*Rananculaceae*
8	*Marine algae* [73]		*Culevapaceae*
9	*Carica papaya* [74]	(seeds)	*Caricaceae*
10	*Mecheliachampaka* & *Centrathrum anthelminticum* [75]	(anthers & seeds)	*Magnoliaceae*
11	*Neem* [76]	(bark)	*Meliaceae*
12	*Rivea hypocratiriformis* [77]		*Convolvulaceae*
13	Asparagus africanus [78]	Roots	*Asparagaceae*

TABLE 6: MEDICINAL PLANTS POSSESSING ABORTIFACIENT ACTIVITY

S. no.	Name	Parts used	Family
1	*Momordica charantia*[79]		Cucurbitaceae
2	*Adhatoda vasica*[80]		Acanthaceae

TABLE 7: HERBAL ABORTIFACIENT USED IN NORTH MAHARASHTRA15

S. no.	Botanical Name/Family	Local Name	Parts used	Method of use
1.	*Abrusprecatorius* Linn. Fabaceae	Gunj	Seeds	A thin paste obtained by grinding seeds with water is applied on external genitalia
2.	*Achyranthes aspera* Linn. Amaranthaceae	Aghada	Roots	A paste prepared from roots is applied on the abdomen.
3.	*Amaranthus spinosus* Linn. Amaranthaceae	Mutla	Leaves	30ml decoction of whole plant is given orally thrice a day for 5-8 days.
4.	*Annona squamosa* Linn. Annonacea	Sitaphal	Seeds	Seed powder is given empty stomach for five days.
5.	*Aristolochiabracteolata* Lam. Aristolochiaceae	Gindhan	Whole plant	A thin paste prepared is given orally (10g) twice a day for 3 days.
6.	*Bauhinia racemosa* Lam. Caesalpiniaceae	Apta	Stem bark	15g paste prepared by pounding the bark with water is given twice a day orally for 7 days.
7.	*Calotropis gigantea* (Linn.) R.Br./Asclepiadaceae	Rui	Roots	Root paste (20g) is given orally once a 2 days for 3 days.
8.	*Calotropis procera* (Ait.) R.Br.	Tambadi Rui	Juice	15g of root paste is given orally once a day for 3 days
9.	*Carica papaya* Linn. Caricaceae	Papai	Latex of raw fruit	20ml of latex of raw fruit is given orally once a day for 3 days.
10.	*Celosia argentea* Linn. Amaranthaceae	Kurdu	Roots	Root paste (10g) is given orally once a day for 6 days.
11.	*Cynodondactylon* pers. Poaceae	Durva	Entire plant	20-30ml extract of whole plant is given once a day for 5 days.
12.	*Gloriosa superba* Linn. Liliaceae	Bachnag	Roots	25ml of root extract is given twice a day orally for 6 days.
13.	*Hibiscus rosa-sinensis* Linn. Malvaceae	Laswand	Stem bark	25ml of stem bark extract given twice a day orally for 3 days.
14.	*Lawsoniainermis* Linn. Malvaceae	Mechandi	Leaves	A paste prepared from leaves is given orally once a day for 5 days.
15.	*Moringa oleifera* Lam.	Shewaga	Flowers	10g churna of dried flowers is given orally

	Moringaceae			thrice a day for 5 days.
16.	*Nerium indicum* Mill. Apocynaceae	Kancher	Leaves	20ml of decoction of leaves is given twice a day orally for 4 days.
17.	*Rhynchosia minima* (Linn.) DC. Fabaceae	Turvel, Dhaktaranghevda	Leaves	20ml of decoction is given twice a day for 7 days.
18.	*Sesbania sesban* (Linn.) Merr. Fabaceae	Shevari	Seeds	15g seed paste is given orally thrice a day for 5 days.
19.	*Smithiaconferta* J.E. Sm. Fabaceae	Bhaji	Leaves	Leaves extract (20-25ml) is given twice a day orally for 7 days.
20.	*Tephrosia purpurea* (Linn.) Pers. Fabaceae	Unhali	Leaves	10ml of leaf extract is given orally thrice a day for 7 days.

TABLE 8: MEDICINAL PLANTS AND PRODUCTS POSSESSING REPRODUCTIVE TOXICITY ACTIVITY

S. no.	Name	Parts used	Family
1	*Labisia pumila* [81]		Myrsinaceae
2	*Asparagus racemosus* [82]	(roots)	Liliaceae
3	*Monocrotaline* [83]	(Crotalaria spectabilis)	Leguminoceae
4	*Monocrotophos* [84]	(Organophosphate pesticide)	Aleurodidae
5	*Azadirachta indica* [85]	(seeds)	Meliaceae

TABLE 9: MEDICINAL PLANTS POSSESSING MALE ANTI-FERTILITY ACTIVITY

S. no.	Name	Parts used	Family
1	*Cananga odorata* [86]	(Root bark)	Annonaceae
2	*Barleria prionitis* [87]	(roots)	Acanthaceae
3	*Albizia lebbek* [88]		Mimosaceae
4	*Azadirachta indica* [89]	Leaves	Meliaceae
5	*Carica papaya* [90]	Seeds	Caricaceae
6	*Tabernaemontana divaricate* [91]	Leaves	Apocynaceae
7	*Carica papaya* (pawpaw) Linn [92]	Roots	Caricaceae
8	*Cassia siamea* [93]	Stem bark	Fabaceae

TABLE 10: TABLE 2: SUMMARY OF MEDICINAL PLANTS EXHIBITING ANTI-FERTILITY ACTIVITY IN MALES 94.

S. no	Name of the plant	Common/ English Name	Part used	Type of plant extract/active principle	Activities
1.	*Acacia concinna* DC.	Shikakai	Stem bark	Acacic acid	Spermicidal and semen coagulating activities
2.	*Acacia auriculae formis* A. Cunn	-	-	Triterpene, saponins	Sperm immobilizing effect
3.	*Acacia caesia* wight &Arn.	Aila	Fruit	Saponins	Immobilization of spermatozoa
4.	*Achillea millefolium* Linn.	Gandna yarrow	Flowers	Ethanol and hydroalcoholic extract	Antispermatogenic effect
5.	*Achyranthes aspera* Linn.	Kadaladi	Root	50% Ethanol extract	Spermicidal action
6.	*Balantiesroxburghii* Linn.	Hingan	Fruit	Pulp extract	Antispermatogenic activity
7.	*Bambusaarundinaceae* Willd.	Baans	Shoots (render)	Ethanol extract	Impaired the structural and functional activity of epididymis
8.	*Berberis chitria* Buch. Ham. Ex Lindl.	-	Root	Palmitine hydroxide	Antispermatogenic action
9.	*Cannabis sativa* Linn.	Ganja	Leaves	Butin	Testicular lesions and atrophy of Leydig cells
10.	*Carica papaya* Linn.	Papita	Fruit	Dry powder	Antispermatogenic activity
11.	*Diploclisiaechinatus* Linn.		Stem	Ecdysterone	Spermicidal activity

12.	*Embeliaribes* Burn. F.	Vidang	Berry	Embelin	Reduced testosterone level anti-fertility activity
13.	*Foeniculum vulgare* Mill.	Saunf	Whole plant	Alcoholic extract	Antiandrogenic activity
14.	*Gloriosa superba* Linn.	Shakarpus phi	Tuber	Ethanol extract	Shrinkage of seminiferous tubules and leydig cells
15.	*Hibiscus rosasinensis* Linn.	Gudhal	Flower	50% Ethanol extract	Antispermatogenic and antiandrogenic activity

Compounds Possessing Spermicidal Activity

- Amphotericin B & Nystatin [95].
- 2, 4-dichlorobenzamil [96].
- Magainins [97].

Miscellaneous Methods Used In Anti-fertility Activity:

- Nitrogen/Oxygen, Sulfur donor heterocyclic compounds [98].
- Centochroman [99].
- Vaccines [100].
- Polyherbal formulation with allopathic drugs [101].
- Levonorgestrol releasing intrauterine system [102].
- Rat uterine peroxidase assay method [103].

Evaluation of Anti-fertility Agents: The therapeutic value, efficacy, and toxicity of the drug may be evaluated in animals experimentally, followed by clinical trials. *In-vivo* animal models are employed to assess anti-fertility activity in experimental animals like rats and mice. 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. Sera were separated into clean bottles, stored frozen, and used within 12 h of preparation for the estimation of testosterone, estrogen level, prolactin, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) [104].

Assessment of Sperm Motility and Count: Progressive motility was tested immediately. The right cauda epididymis was incised, and semen was squeezed on a pre-warmed slide. Two drops of warm 2.9% sodium citrate were added to semen and mixed by a coverslip. The percentage sperm motility was evaluated visually at 400 × magnification. Motility estimates were performed from three different fields in each sample. The mean of the three successive estimations was used as the final motility score. For sperm count, the left cauda epididymis was incised, and semen that oozed was quickly sucked into a red blood pipette to the 0.5 marks and then diluted with warm normal saline up to mark [101]. A drop of the semen mixture was placed on the Neubauer counting chamber and viewed under the magnification of × 40. The total numbers of sperm cells were counted and expressed as 106/mL [104].

Assessment of Sperm Viability and Morphology: A viability study (percentage of live spermatozoa) was done using eosin/nigrosine stain. A drop of semen was squeezed onto a microscope slide, and two drops of the stain were added. Thin smears were then prepared and observed under a light micro- scope at 400 × magnification. Viable sperm remained colorless while non-viable sperm-stained red. The stained and the unstained sperm cells were counted using 40 × microscope objectives, and an average value for each was recorded from which percentage viability was calculated. To determine the percentage of morphologically abnormal spermatozoa, the slides stained with eosin nigrosine (5 slides/rat) were viewed under a light microscope at 400 × magnifications. A total of 300 sperm cells was examined on each slide (1500 cells for each rat) and the head, tail, and total abnormality rates of spermatozoa were expressed as a percent [104].

Mating Trial Test: A mating trial test of male rats was done 5 d before the termination of the experiment. Each male rat was cohabitated overnight with proestrus females in a ratio of 1:2 and housed in a single cage. Positive mating was confirmed by sperm and vaginal plug in the vaginal smear the following morning. Each sperm-positive

female was kept under observation and the resultant pregnancies were noted when dam gave birth. The following reproductive parameters were then computed [104]:

Mating success % = number mated/number paired × 10

Fertility success % = number pregnant/number paired × 100

Fertility index = number pregnant/number mated × 100

Body and Sex Organ Weights: The initial and final body weights of the animals were recorded. The testes, epididymitis, seminal vesicle, and ventral prostate were dissected out, freed from adherent tissues and blood, and weighed to the nearest milligram. Organ weights were reported as relative weights (organ weight/bodyweight × 100) [104].

Quantification of Fructose In Seminal Vesicle: For fructose quantification, the seminal vesicular homogenate was prepared at a tissue concentration of 50 mg/ml. The supernatant (seminal plasma) was deproteinized by adding 50 mL of zinc sulfate and sodium hydroxide to make a total dilution of seminal plasma 1:16, followed by centrifugation at 2500 r/min for 15 min. For fructose measurement, 200 m L of clear seminal plasma was used and the optical density of standard and samples were measured against blank at 470 nm. The concentration of fructose was obtained by plotting the value in standard curve and the value expressed in the unit of m mol/mL of seminal plasma [104].

Post-Coital Anti-fertility Activity (Pre-Implantation Activity: The anti-implantation activity is expressed as the percentage of animals showing absence of implantations in uteri when laparotomies on day 10 of pregnancy. Vaginal smears from each rat were monitored daily, and the rats with normal estrous cycles were selected. Rats found in the proestrus phase of the cycle were caged with males of proven fertility, in the ratio 2:1, and examined the following morning for evidence of copulation. Rats exhibiting thick clumps of spermatozoa in their vaginal smears were separated, and that day was designated as day 1 of pregnancy, and those rats were divided into five groups containing six rats in each group. The extract was administered from day 1-7 of pregnancy. The powdered drug was also administered from day 1–7 of pregnancy. Control rats received the vehicle (distilled water). On day 25, laparotomy was performed under light ether anesthesia and semi sterile conditions. The uteri were examined to determine the number of implantation sites and no of corpora lutea graviditis [105, 106, 107, 108].

Frequency of pre-implantation losses = missing no. of implants (corpora lutea implants)/ no of corpora lutea × 100

Effect on Estrous Cycle: The female animals were artificially brought into the estrus phase (heat) by administering either suspension of ethinyl estradiol orally at the dose of 100 mg/animal 48 h prior to the pairing and subcutaneous administration of progesterone at the dose of 1 mg/animal 6 h before the experiment or alternatively by the sequential administration of estradiol benzoate (10 mg/100 g body weight) and progesterone (0.5 mg/100 g body weight) through subcutaneous injections, 48 and 4 h respectively. Estrous cycle was determined between 8 am and 10 am using vaginal smear method. Vaginal secretion was collected with a plastic pipette with 10 mL of normal saline. The vagina was flushed three times with the pipette and the vaginal fluid was placed on a glass slide. Different slides were used for each animal. The unstained secretion was observed under a light microscope. After confirmation of regular four-day cycle for 2 weeks the animals were selected for study and divided into six groups and treated with test drugs. The effect of test drugs on the estrous cycle was monitored for 28 d [104, 109].

Antiestrogenic Activity: All the rats were ovariectomized by the same methods described in previous procedure and the weight of the ovaries were recorded. The ovariectomized rats were randomly taken and divided in thirteen groups. Except control, other groups were administered with different doses of estradiol (0.1 mg/rat and 1.0 mg/rat) and followed as by test compounds respectively for 4 consecutive days. On the eleventh day, the rats were anesthetized using ketamine (60 mg/kg, I.P.) and the remaining right-sided ovaries were dissected out from all the animals. Properly cleaned, dried and their respective weights were recorded. The ovaries' weight variations prior to and after treatment with extracts were calculated.

Percentage inhibition of ovarian weight was calculated using the following equation:

Percentage inhibition in ovarian weight = [1-(XE−C)]/E-C × 100

Where, C indicates mean ovarian weight from rats treated with vehicle, E for estradiol and XE indicates the mean ovarian weight of rats treated with extract and estradiol [104].

Antigonadotrophic Effect: Female rats were studied for 5 consecutive normal estrus cycles by vaginal smear method. The rats were anesthetized using ketamine (60 mg/kg) pretreated with atropine (1 mg/mL) and left side ovariectomy was performed. Left ovary was dissected out carefully from surrounding fatty tissue and dried by soaking on filter paper and weighed. The ovariectomized rats were divided into six groups and treated. On 12^{th} day after treatment, the remaining right ovaries of all rats were properly dissected out using the same anesthetic condition. Cleaned, dried, and their respective weights were recorded A percentage increase in ovarian weight compared with weight of the left ovaries was calculated. Percentage increase in the weights of ovary was calculated using the formula [104].

Percentage increase in ovarian weight = (weight of right ovary - weight of left ovary)/weight of left ovary × 100

Histological Analysis: Testes and uteri were carefully dissected after abdominal incision from male and female rats, respectively, fixed in 10% normal saline, and processed routinely for paraffin embedding. Sections of 5mm from both were obtained with a rotary microtome, stained with Hematoxylin and Eosin Stalin (H/E) respectively, and observed under a light microscope [110].

Measurement of Some Biochemical and Blood Parameters: Blood samples were collected from the heart of each rat at the time of scarification into non-heparinized and heparinized tubes.

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphates, creatinine, and urea in addition to red blood cell (RBC) count, total leucocytic count (TLC). hemoglobin (Hb) concentration, packed cell volume (PCV), cholesterol, and total proteins were determined by standard methods [111].

Determination of Testicular and Serum Cholesterol (Chod PAP Method): Cholesterol is the precursor in the synthesis of many physiologically important steroids such as bile acids, steroid hormones, and vitamin D, and its requirement for normal sexual activity has been well established. Testicular and serum cholesterol concentrations may be determined by the Chod–PAP method as briefly,0.02 cm3of the working reagent, and the absorbance of the resulting mixture is read after 5 min at 546 nm [109].

Determination of Total Protein: A timed rate biuret method was used to measure total protein concentration in serum or plasma. Proteins in the sample combined with the reagent producing alkaline copper-protein chelate. A detector monitored the rate change in absorbance at 545 nm. The observed rate of chelate formation is directly proportional to the total protein concentration in the sample.

CONCLUSION: In conclusion, this review aims to summarize anti-fertility and evaluation of anti-fertility activities. Already, medicinal plants have proven efficacy as anti-fertility agents. The mechanism of action of traditional drugs reported having anti-fertility activity needs to assess further for toxicity studies.

Further research is required to ascertain the anti-fertility activity of novel Herbal and traditional drugs to exploit their activity as anti-fertility agents. Comprehensive Assessment utilizing in vivo animal models using rats and mice shall further strengthen their roles.

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