Review Article

The potentiality of medicinal plants as the source of new contraceptive principles in males

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Abstract

Rising human population throughout the world especially in developing and underdeveloped countries has detrimental effects on life supporting system on earth. Traditionally, plants have been used to treat different kinds of ailments. The growing importance of phytochemicals in males has been reported. Contraceptive ability of plants has been reported in several animal models. The reversibility of the anti-fertility effects of plants and its active compounds are of potential clinical relevance in the development of male contraceptive. This review attempts to discuss the latest reports on the potentiality of medicinal plants as the source of new contraceptive principles in males.

Keywords: Contraceptive, testis, steroidogenesis, spermatogenesis, medicinal plants.

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Introduction

Plants have been used globally across varied cultures as a safe natural source of medicines. From time immemorial, humans have relied on plants that could meet their basic necessities such as food, shelter, fuel and health. Of all the numerous uses attached to plants, their therapeutic abilities played an inevitable part in the lives of primitive societies, as they relied on plants for healing ailments. The knowledge of the healing powers of plants was initially passed down orally through generations, and as civilizations grew written records were prepared for the benefit of the population [1]. A wide majority of herbal plants possess pharmacological principles, which has rendered them useful as curatives for numerous diseases. World Health Organization reports that 70% – 80% of the world population confide in traditional medicine for primary health care. Several plants are reported to enhance reproductive processes but, on the other hand, may also hinder testicular functions.

Male reproduction is a complex process that involves the testes, epididymis, accessory sex glands and associated hormones. Testes perform two highly organized and intricate functions, called spermatogenesis and steroidogenesis, which are crucial for the perpetuation of life. Spermatogenesis, a highly dynamic and synchronized process, takes place within the seminiferous tubules of the testis with the support of somatic sertoli cells, leading to the formation of mature spermatozoa from undifferentiated stem cells [2]. The interstitial compartment, which comprises Leydig cells, is the site of steroidogenesis in the testis [3].

Reduction in the levels of testosterone, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) was reported when the crude methanol extract of medicinal plant was administered to male albino rats [4, 5]. Administration of the methanol extract of *Sarcostemma acidum* at a dose of 100 mg to male albino rats for 60 days caused a decrease in the number of mature Leydig cells and an increase in the degeneration of Leydig cell population [6]. Ethanolic extracts of the roots of *Martynia annua* to male rats at doses of 100 and 200 mg per kg body weight for 60 days caused Leydig cell atrophy and a significant reduction in the serum

concentration of LH and testosterone [7]. Aqueous extract of leaf stem *Leptadenia hastata* Decne has been reported to reduce the progressive velocity, linearity and sperm motility of male Wistar rats [8]. Leydig cell nuclear area and mature Leydig cell numbers were significantly reduced on oral administration of 70% methanolic extract of *Tinospora cordifolia* stem to male rats at the dose level of 100 mg per rat per day for 60 days [9]. *Mentha piperita labiatae* (20 g L^{-1}) and *Mentha spicata labiatae* (20 g per litre) herbal teas when fed to Wistar rats increased the FSH and LH levels and decreased total testosterone levels [10]. Ethanolic extracts of *Colebrookea oppositifolia* (200 mg) when administered orally for 8–10 weeks was reported to cause a decrease in the nuclear and cytoplasmic surface area of Leydig cells [11].

The medical historians have recorded plants that could be used as contraceptives and abortifacients [1]. The safety of many of the herbal drugs is only relative, but the population feels more assured because of their long and widespread usage and their familiarity with these plants. Conventional drugs used as male contraceptive are often inadequate [12] therefore; any efforts to explore antifertility effect of any natural product in males carry a great clinical significance as this can help males also to participate significantly in population control programs. Thus, the current review attempts to discuss some of the commonly used plants that could be used as male contraceptive.

Spermatogenesis

Spermatogenesis is a complex process by which an interdependent population of undifferentiated germ cells undergoes multiplication and maturation to form functional haploid spermatozoa [13]. Spermatogenesis consists of three phases: (a) the spermatogonial phase; (b) the spermatocyte phase; and (c) the spermatid phase. During the spermatogonial phase, the diploid spermatogonium undergoes mitosis to form stem cells and primary spermatocytes. This is followed by the spermatocyte phase, in which the primary spermatocytes undergo two rounds of meiosis to form haploid spermatids [13]. The final phase, also called spermiogenesis, involves the differentiation of spermatids into mature spermatozoa. Spermiogenesis comprises polarization of the spermatid, formation of acrosomal cap and flagellum, cytoplasmic remodeling and elongation of the nucleus [13].

Steroidogenesis

A steroid is a type of organic compound that contains a specific arrangement of four rings that are joined to each other. Examples of steroids include cholesterol, the sex hormones estradiol and testosterone, and the anti-inflammatory drug dexamethasone. The sterane core of steroids is composed of seventeen carbon atoms bonded together to form four fused rings: three cyclohexane rings (designated as rings A, B, and C in the figure to the right) and one cyclopentane ring (the D ring). The steroids vary by the functional groups attached to these rings and by the oxidation state of the rings. Sterols are special forms of steroids, with a hydroxyl group at position-3 and a skeleton derived from cholestane [14]. Hundreds of distinct steroids are found in plants, animals, and fungi. All steroids are made in cells either from the sterols lanosterol (animals and fungi) or from cycloartenol (plants). Both lanosterol and cycloartenol are derived from the cyclization of the triterpene squalene [15]. Steroidogenesis is the biological process by which steroids are generated from cholesterol and transformed into other steroids. The pathways of steroidogenesis differ between different species. Several medicinal plants have been reported to affect various stages of spermatogenesis [16-18] and steroidogenesis [19, 20] in many different animal species such as rabbits, goats, sheep, dogs, rats, humans and monkeys.

Steroidogenesis and Medicinal Plants

Several studies affirm the undisputable role of plant products in impairing testicular steroidogenesis [21-26]. In males, LH plays a role in reproductive function by modulating testicular Leydig cell differentiation and steroidogenesis. Testosterone secreted by Leydig cells, in turn, promotes male sexual differentiation, pubertal androgenization, and fertility. In the testis, functional LH receptors are expressed in Leydig cells during fetal development, transiently in early postnatal life, and from puberty to adult life [27]. Although primarily expressed in gonads, LH receptors are also found in several extra-gonadal sex organs, including the prostate [28, 29], epididymis [30], and seminal vesicles [31], but the physiological significance of these extra testicular receptors remains unclear.

The hormonal regulation of spermatogenesis is well organized, with a feed-back mechanism involving the hypothalamus, pituitary gland and testis [32]. The neurons of the hypothalamus synthesize and secrete gonadotropin-releasing hormone, which induces the production and release of LH and FSH from the pituitary gland. LH causes the synthesis of testosterone in the Leydig cells of the testis, which exerts a negative feedback on hormone release from the hypothalamus and pituitary [33]. FSH acts on sertoli cells, resulting in the production of androgen-binding protein, which helps in the passage of testosterone through sertoli–sertoli junctional complexes. Any factor that could perturb the LH-stimulated Leydig cell steroidogenesis could have an enormous impact on endocrine regulation of spermatogenesis and could lead to infertility. Numerous plant products are known to target Leydig cells and hinder their functions. Most of the plants impair steroidogenesis by targeting the enzymes involved in the process at the level of Leydig cells and / or at the level of the hypothalamo–pituitary–gonadal loop (Table 1).

Table 1 Effect of medicinal plants on steroidogenesis.

Medicinal Plant	Observed effects	Reference
Bulbine natalensis	Decreases testosterone and progesterone at high dose	34
Juniperus phoenicea L.	Decreases testosterone levels	35
Capparis aphylla	Reduces steroidogenic enzymes	36
Psoralea corylifolia	Decreases serum testosterone levels	37
Aegle marmelos	Reduced testosterone levels	38
Garcinia cambogia	Degeneration of the Leydig cells	36
Dendrophthoe falcate	Decreases serum testosterone levels	39
Capparis aphylla	Reduces steroidogenic enzymes	36
Abelmoschus esculentus	Decreases serum testosterone levels	40
Albizia. lebbeck L	Decrease in serum testosterone levels	41
Chromolaena odoratum	Decreases serum testosterone levels	42
Allium sativum	Reduces testosterone secretion	43
Syzygium aromaticum L.	Reduction in the steroidogenic enzymes and testosterone levels at higher dose	44

Table 2 Plants that affects spermatogenesis

Plant name	Phytochemical elements	Observed effects	Reference
Acacia auriculiformis	triterpenoid saponins	Immobilization of sperm at lowest concentration	52
Acacia concinna	saponin	Spermicidal and semen coagulating activities	53
Albizzia lebbek	Lebbekanin-E	Spermicidal activity	54
Anagallis arvensis	-	Spermicidal and semen coagulating activities	53
Andrographis paniculata	-	Antispermatogenic activity	55
Azadirachta indica	azadirachtin	Antispermatogenic activities and histological changes in testes and epididymides	56
Balanites roxburghii	-	Mass atrophy of spermatogenic elements due to secondary effects of hyperglycemia in dogs	57
Barleria prionitis	-	Antifertility effect on male rats	58
Berberis chitria	Palmitine hydroxide	Impairment of germ cells	59
Bursera fagaroides	Glycosides	Human spermatozoa and those obtained from mouse epididymis became agglutinated and immobilized	60
Calotropis procera	Calotropin	Antispermatogenic effect and leydig cell atrophy	61
Carica papaya	-	Antispermatogenic effect	62
Moringa oleifera	-	antifertility activity	63
Ocimum sanctum	-	Atrophy of Leydig Cells	64

Suppression of the activities of steroidogenic enzymes including the P450 side-chain cleavage enzyme, 3 β-hydroxysteroid dehydrogenase, 17 α-hydroxylase, 20 α-hydroxylase and 17 β-hydroxysteroid dehydrogenase, was observed when primary mouse Leydig cells were incubated with varying concentrations of crude *Toona sinensis* [45]. The leaves of *Azadirachta indica* when administered orally at a dose of 500 mg per kg body weight exhibited a regression and decrease in the number of Leydig cells and their nuclear diameter, indicating androgen deficiency [46]. The aqueous extracts *leptadenia hastata* when administered orally at varying doses per kg body weight exhibited anti-androgenic property [47]. *Carica papaya* seed extracts when administered orally at doses of 50 and 100 mg per kg body weight for 8 weeks to sexually mature Wistar rats caused pronounced hypertrophy of pituitary gonadotrophs and degeneration of Leydig cells [48]. Palmitine hydrochloride isolated from the roots of *Berberis chitria* at a dose of 30 mg per kg per day when administered orally to dogs for 30 days resulted in 66% and 27% reduction, respectively, in mature and immature Leydig cells [49].

Intraperitoneal injection of mice with low doses of *Cannabis* extracts was reported to induce increased lipid peroxidation in the testis, along with concomitant decrease in the levels of antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase [13]. Endocrine regulation by testosterone produced by sertoli cells and seminiferous tubules also forms an integral part of spermatogenesis [50]. Additional studies are warranted to understand intensely the molecular mechanisms by which plants or their active ingredients hamper steroidogenesis in various species.

Spermatogenesis and Medicinal plants

Plants are the source of medication for preventive, curative and protective purposes. Use of medicinal plants such as mint, ginger, garlic, onions and sacred basil can be observed in daily life. The ethnomedicinal characteristics of different plant parts used as drugs have been reported [51]. The pharmacological activity and the phytochemical compositions that confirm the traditional use of some of these medicinal plants has been validated [51]. The spermatocidal effects of these plants discussed below could be as a result of their numerous phytochemical elements (Table 2).

Neem (Azadirachta indica)

Neem has long been documented to have antifertility effects [25, 65-67]. Oral administration of ethanolic

extracts of neem to adult male mice at 0.5 mg, 1.0 mg or 2.0 mg per kg body weight for 6 weeks interfered with sperm DNA and caused chromosome strand breakage, spindle disturbances and deregulation of genes responsible for sperm morphology. A linear decrease in the percentage of sperm motility was observed with various concentrations (1–50 mg per 1 million sperm) of neem leaf extract, with motility falling to absolute zero within 20 seconds of exposure to 3 mg dose [68]. Atrophy of the Leydig cells was observed when the leaf extracts of *Azadirachta indica* and flower extract of *Malvaviscus conzattii* were administered to male albino rats [46, 69, 70]. The aqueous leaf extract of neem when administered to male mice at a dose of 200 mg kg^{-1} for 28 days damaged the seminiferous tubules, resulting in the slackening of germinal epithelium, marginal condensation of chromatin in round spermatids, degeneration of germ cells and the derangement of germ cell types from their orderly arrangement in spermatogenesis [71]. Their effects were reported to revert back to normalcy after 42 days of withdrawal of the treatment [71]. On the contrary, *azadirachtin*, an active ingredient of neem, given at doses of 5 mg, 10 mg and 50 mg per kg body weight did not show any evidence of reproductive toxicity in adult rats or their litters over two generations, implicating the safe use of the compound as a biopesticide [67, 72].

Gossypol herbaceum Linn

Gossypol, a yellow polyphenolic compound present in the stem, seeds and roots of *Gossypium* species. It is known to exert unique and selective effects upon reproduction in various species such as rats, mice, hamsters, rabbits, monkeys and human beings [73]. The contraceptive effect of gossypol was first discovered in China. Gossypol was reported to invoke antifertility effects in rats at 30 mg per kg body weight, whereas a much lesser dose, 0.3 mg per kg body weight, could incite infertility in humans, making the compound very efficient in humans than in rats [18]. The WHO investigators claim that gossypol has a slow recovery pattern and irreversible effect, and the safety and efficacy of gossypol as a contraceptive continue to be controversial [74]. Several studies affirm that gossypol treatment reduced the level of serum testosterone and luteinizing hormones in dose and duration dependent manner [22, 75]. Gossypol acts directly on testes and induces azoospermia or oligospermia [75] Gossypol acetic acid, when incubated with isolated rat interstitial cells at a dose of 50 μg mL^{-1} caused a dramatic decrease in histochemical stain for 3-β-HSD, proving the direct inhibitory effect of the compound [76]. Gossypol blocked cAMP formation in sperm, which resulted into inhibition of sperm motility [73].

Carica papaya

Carica papaya is recognized from ancient times for its medicinal properties. The contraceptive characteristics of papaya seed extracts have been reported in the 1970s [77, 78]. Degeneration of germ cells and germinal epithelium, reduction in the number of Leydig cells and presence of vacuoles in the seminiferous tubules were observed when crude ripe seeds of papaya were administered orally to male Wistar rats at a dose of 100 mg per kg body weight [17]. The crude chloroform extract of papaya seeds at a dose of 5 mg per animal per day for 40 – 60 days reduced the fertility potential to 0%, with the suppression of cauda epididymal sperm motility [79]. This suggest that contraceptive effects of chloroform extract of papaya seeds are mainly post testicular in nature without influencing toxicological profiles and libido. Administration of the chloroform extract of papaya to male rabbits for 150 days caused a decline in sperm concentration with oligospermia on the 75^{th} day and azoospermia after 120 days. Membrane damage in the acrosome, bent mid piece, coiled tail, detached head and arrest of spermatogenesis beyond the level of spermatocytes were also observed [80]. Chloroform extracts of the seeds of paw paw when administered to male albino rats and monkeys at a dose of 50 mg per kg body weight for 360 days caused reduction in nuclear and cytoplasmic volume and vacuolization of the sertoli cells, with the effects being reversible 60-120 days after withdrawal of the treatment [81, 82].

Momordica charantia

Alcoholic extracts of *Momordica charantia* have been reported to exhibit anti-spermatogenic effects in dogs [83]. Oral administration of alcohol extracts of the seeds of *Momordica charantia* to male albino rats at a dose of 25 mg per 100 g body weight for 35 days caused a decrease in the number of spermatocytes and spermatids, with the effects being more significant when administered through the intraperitoneal route [84].

Abrus precatorious

Testicular degeneration characterized by reduced number of cells in the epithelium along with reduction in the number of sperm cells was observed when the aqueous extract of *Abrus precatorious* was administered to male rats at doses of 400 mg, 800 mg and 1600 mg per kg body weight for 18 days [85]. The alcoholic seed extracts of *Abrus precatorious* at a dose of 100 mg per kg body weight for 60 days significantly lowered cauda epididymal sperm motility and brought about a decrease in the levels of succinate dehydrogenase and ATPase in the sperm of albino rats. Scanning electron microscopic studies on sperm morphology revealed decapitation, acrosomal damage and formation of bulges on the midpiece region of sperms following exposure to *Abrus precatorious* seed extracts [86]. Irreversible impairment of the motility of human spermatozoa at a concentration of 20 mg per mL of the methanol extract of *Abrus precatorious* seed extracts was reported, which may be due to the decline in cAMP and enhanced generation of reactive oxygen species [87]. Dose-dependent decrease in the enzyme activity of 3α, 3β, 17β-hydroxysteroid dehydrogenases and degeneration of Leydig cells were reported when *Abrus precatorius* was administered to male rats [88].

Garlic (Allium sativum)

The crude extract of garlic when administered to male rats at varying concentrations (5%, 10%, 15% and 30%) for 30

days caused an increase in the percentage of empty seminiferous tubules and brought about a decrease in serum testosterone levels, with the effects being invoked at a dose as low as 10% [89, 90]. Graded doses of the *mormodica* seed extract induced abnormalities in the size and shape of rat sperm along with dorso-ventral constrictions in the middle region of the sperm head, which was proposed to be due to alterations in cauda epididymal milieu and androgen deficiency [91]. An *in vitro* study on the effects of allitridum, an active principle from garlic, has been reported to inhibit sperm motility and complete immobilization of rat, hamster and human spermatozoa at a dose of 7.5 mg mL^{-1} of allitridum treatment [92]. A significant reduction in the levels of serum testosterone and LH was reported when crude extracts of garlic were administered to male rats for 30 days [89]. *In vitro* studies on the crude aqueous extract of *Allium sativum* have been reported to reduce sperm viability, membrane disintegration of sperm and irreversible immobilization of ram epididymal and human ejaculated sperm at doses of 0.25 g and 0.50 g per mL, respectively [93].

Pepper (Piper longum)

Pepper, a commonly used spice, is reported to induce sterility in laboratory male mice [94]. Piperine, an alkaloid extracted from the fruits and roots of black pepper, has been shown to cause damage to the germ cells and seminiferous tubules when administered orally for 30 days [95]. Suppression in the levels of antioxidant enzymes, and increase in lipid peroxidation in testis and epididymis along with activation of caspase 3 and Fas apoptotic proteins in testicular germ cells were reported when piperine was administered to male Wistar rats at doses of 10 mg and 100 mg for 30 days [96, 97]. An *in vitro* study on hamster sperm showed that piperine interferes with acrosome reaction through the inhibition of calcium influx by stimulation of efflux, thereby impairing fertility [98]. Laboratory studies have demonstrated a reduction in rat sperm motility, viability and count on exposure to piperine at 10 mg and 100 mg per kg body weight [97].

Ocimum sanctum

The benzene extract of *Ocimum sanctum* leaves when administered to male rats at a dose of 250 mg per kg body weight for 48 days was reported to decrease sperm count, motility and the forward velocity of the sperm. The effects were found to be reversible upon withdrawal of treatment for 2 weeks [99]. Several studies have demonstrated the detrimental effects of *Ocimum sanctum* and other commonly used medicinal plants on the ultra structure of the testis [81, 82].

Thespesia populnea

Krishnamoorthy and Vaithinathan [100] observed enlargement of the sertoli cells when 400 mg of the leaf extract of *Thespesia populnea* was administered to male Swiss mice for 15 days. Wang and Waller [101] reported that pure the bromine when administered to male rats at a dose of 500 mg for 7 days inhibited the binding ability of androgen-binding protein and reduced the androgen concentration in seminiferous tubule fluid, implicating sertoli cells as primary targets for theobromine toxicity. Several studies have reported reduction in the cross-sectional surface area of Sertoli cells of male Wistar rats administered some plant extracts for 60 days [9, 102-105].

Conclusion

Pharmacological effects of many plants have been studied. However, there are many limitations regarding safety and efficacy of these preparations. Knowledge about the active principles of plant preparations is not well defined and information on toxicity and adverse effects of these plant preparations are lacking. Information regarding pharmacokinetics and bioavailability is not available. Assurance of safety, quality and efficacy of medicinal plants and plant preparations are key issues, which need to be addressed. Selection of plant should be based quality, standardization of methods of preparation, enforcement of regulation regarding appropriate labels are measures, which will improve the quality and acceptability of herbal preparations. Ecotype pharmacological evaluation is very essential when the plants are used in crude form. The relative proportion of phytochemical present in medicinal plants can vary in different ecotypes. There is need also a need for documentation of research and publication of results in peer reviewed journals. Most of the information on pharmacological study of the plants is incomplete since they are published as abstract presented at conferences. In conclusion future research effort should be directed towards the safety, quality and efficacy of medicinal of plants and plant preparations used as natural contraceptive.

References

1. Kritiker KR, Basu BD. Indian Medicinal Plants. New Canaught Place, Dehradun, India. Bishen Singh, Mahendra Pal Singh ed. 1975; 785-788.
2. Hess RA, Renato de Franca L. Spermatogenesis and cycle of the seminiferous epithelium. Adv Exp Med Biol 2008; 636: 1-15.
3. Osinowo OA. Introduction to Animal Reproduction. Sophie Academic Services Limited, Abeokuta, Nigeria 1ST edition 2006; 91.
4. Raji Y, Bolarinwa AF. Antifertility activity of Quassia amara in male rats – in vivo study. Life Sci 1997; 61: 1067- 1074.
5. Bazrafkan M, Panahi M, Saki G, Ahangarpour A, Zaeimzadeh N. Effect of aqueous extract of Ruta graveolens on spermatogenesis of adult rats. Int J Pharmacol 2010; 6: 926-929.
6. Venma PK, Sharma A, Mathur A, Sharma P, Gupta RS. Effect of Sarcostemma acidum stem extract on spermatogenesis in male albino rats. Asian J Androl 2002; 4: 43-47.
7. Mali PC, Ansari AS, Chaturvedi M. Antifertility effect of chronically administered Martynia annua root extract on male rats. J Ethnopharmacol 2002; 82: 61-67.

8. Bayala B, Telefo PB, Bassole IHN, Tamboura HH, Belemtougri RG, Sawadogo L, Malpaux B, Dacheux JL. Anti-spermatogenic activity leptadenia hastata leaf stems aqueous extracts in male Wistar rats. J Pharmacol Toxicol 2011b; 1-9.
9. Gupta RS, Sharma A. Antifertility effect of Tinospora cordifolia (Wild) stem extract in male rats. Indian J Exp Biol 2003; 41: 885-889.
10. Akdogan M, Ozguner M, Kocak A, Oncu M, Cicek E. Effects of peppermint teas on plasma testosterone, follicle-stimulating hormone, and luteinizing hormone levels and testicular tissue in rats. Urology 2004; 64: 394- 398.
11. Gupta RS, Yadav RK, Dixit VP, Dobhal MP. Antifertility studies of Colebrookia oppositifolia leaf extract in male rats with special reference to testicular cell population dynamics. Fitoterapia 2001; 72: 236-245.
12. Ringheim K. Male involvement and contraceptive methods for men: Present and future. Social Change 1996; 26: 88-99.
13. D'Cruz SC, Vaithinathan S, Jubendradass' R, Mathur PP. Effects of plants and plant products on the testis. Asian J Andro 2010; 12: 468–479.
14. Moss GP. Nomenclature of Steroids. Pure and Applied Chem 1989; 61 (10): 1783-1822.
15. Lichtenthaler H. The 1-Dideoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. Annu Rev Plant Physiol Plant Mol Biol 1999; 50: 47-65.
16. Ogbuewu IP, Omede AA, Uchegbu MC, Okoli IC, Iloeje MU. Clinical significance of dietary soy-isoflavones: A review. Proceedings of the International Conference on Global Food Crisis. Federal University of Technology, Owerri, April 19- 24, 2009a; 113-117.
17. Ogbuewu IP, Agoh C, Etuk IF, Okoli IC, Iloeje, MU. Evaluation of the cholesterol characteristics of the liver of Dutch Belt rabbit bucks fed toasted commercial poultry diets supplemented with soy milk. Int J Agric Res Dev 2010a; 2(1):77-81.
18. Rahim F, Saki G, Bazrafkan M. Effect of alcohol extracts of the Ruta graveolens L. on the count, motility and in vitro fertilization capacity of rat's sperm. Asian J Plant Sci 2010; 9: 63-66.
19. Udoh P, Kehinde A. Studies on antifertility effect of pawpaw seeds (Carica papaya) on the gonads of male albino rats. Phytother Res 1999; 13: 226- 228.
20. Liu GZ, Lyle KC, Cao J. Clinical trial of gossypol as a male contraceptive drug. Part I. Efficacy study. Fertil Steril 1987; 48: 459-461.
21. Mathur N, Jain GC, Pandey G. Effect of Tecoma stans leaves on the reproductive system of male albino rats. Int J Pharmacol 2010; 6: 152-156.
22. Udoh P, Patil DR, Deshpande MK. Histopathological and biochemical effects of gossypol acetate on pituitary-gonadal axis of male albino rats. Contraception 1992; 45: 493- 509.
23. Malihezaman M, Sara P. Effects of aqueous extract of Anethum graveolens on male reproductive system of rats. J Biol Sci 2007; 7: 815-818.
24. Ogbuewu IP. Physiological responses of rabbits fed graded levels of neem (Azadirachta indica) leaf meal. MSc. Thesis, Federal University of Technology, Owerri, 2009; 253.
25. Ogbuewu IP, Okoli IC, Iloeje MU. Semen quality characteristics, reaction time, testis weight and seminiferous tubule diameter of buck rabbits fed neem (Azadirachta indica A Juss) leaf meal based diets. Iran J Reproduct Med 2009; 7: 23-28.
26. Ogbuewu IP, Ogundu UE, Opara MN, Okoli IC, Umesiobi DO, Herbert U, Iloeje MU. Spermatozoa manipulation techniques: A current reproductive technology tool kit in reproductive physiology. A review, J Med Sci 2010b; 10(5): 110-123.
27. Saez JM. Leydig cells: endocrine, paracrine, and autocrine regulation. Endocr Rev 1994; 15: 574-626.
28. Tao YX, Lei ZM, Woodworth SH, Rao CV. Novel expression of luteinizing hormone/chorionic gonadotropin receptor gene in rat prostate. Mol Cell Endocrinol 1995; 111: R9-R12.
29. Reiter E, McNamara M, Closset J, Hennen G. Expression and functionality of luteinizing hormone/chorionic gonadotropin receptor in the rat prostate. Endocrinology 1995; 136: 917-923.
30. Zhou HM, Zhang T, Liu YX. Rat epididymis expresses luteinizing hormone receptor (LHR). Chin Sci Bull 1996; 41: 1608-1610.
31. Tao YX, Lei ZM, Rao CV. Seminal vesicles are novel sites of luteinizing hormone/human chorionic gonadotropin receptor gene expression. J Androl 1998; 19:343-347.
32. Sofikitis N, Giotitsas N, Tsounapi P, Baltogiannis D, Giannakis D, Pardalidis N. Hormonal regulation of spermatogenesis and spermiogenesis. J Steroid Biochem Mol Biol 2008; 109: 323-330.
33. Dufau ML, Winters CA, Hattori M, Aquilano D, Baranao JL. Hormonal regulation of androgen production by the Leydig cell. J Steroid Biochem 1984; 20: 161-73.
34. Yakubu MT, Afolayan AJ. Reproductive toxicologic evaluations of Bulbine natalensis Baker stem extract in albino rats. Theriogenology 2009; 72: 322-332.
35. Shkukani H, Salhab A, Disi A, Shomaf M, Al Quadan F. Antifertility effect of ethanolic extract of Juniperus phoenica in male albino rats. J Herbal Pharmacother 2007; 7: 179-189.
36. Sarathchandran I, Manavalan R, Akbarsha MA, Kadalmani B, Karar PK. Effect of ethanolic extract of Capparis aphylla (Roth) on testicular steroidogenesis in rat. J Biol Sci 2007; 7: 582-584.
37. Takizawa T, Mitsumori K, Takagi H, Nasu M, Yasuhara K, et al. Sequential analysis of testicular lesions and serum hormone levels in rats treated with a Psoralea corylifolia extract. Food Chem Toxicol 2004; 42: 1-7.

38. Chauhan A, Agarwal M. Reversible changes in the antifertility induced by Aegle marmelos in male albino rats. Syst Biol Reprod Med 2008; 54: 240-246.
39. Gupta RS, Kachhawa JB. Evaluation of contraceptive activity of methanol extract of Dendrophthoe falcata stem in male albino rats. J Ethnopharmacol 2007; 112: 215-218.
40. Olatunji-Bello II, Ijiwole T, Awobajo FO. Evaluation of the deleterious effects of aqueous fruit extract of Abelmoschus esculentus (Okro fruit) and some male reproductive parameters in Sprague Dawley rats. J Phytol 2009; 1: 461-468.
41. Gupta RS, Kachhawa JB, Chaudhary R. Antispermatogenic, antiandrogenic activities of Albizia lebbeck (L.) Benth bark extract in male albino rats. Phytomedicine 2006; 13: 277-283.
42. Yakubu MT, Akanji MA, Oladiji AT. Evaluation of antiandrogenic potentials of aqueous extract of Chromolaena odoratum (L.) K. R. leaves in male rats. Andrologia 2007; 39: 235-243.
43. Hammami I, Nahdi A, Mauduit C, Benahmed M, Amri M, et al. The inhibitory effects on adult male reproductive functions of crude garlic (Allium sativum) feeding. Asian J Androl 2008; 10: 593-601.
44. Mishra RK, Singh SK. Safety assessment of Syzygium aromaticum flower bud (clove) extract with respect to testicular function in mice. Food Chem Toxicol 2008; 46: 3333-3338.
45. Poon SL, Leu SF, Hsu HK, Liu MY, Huang BM. Regulatory mechanism of Toona sinensis on mouse Leydig cell steroidogenesis. Life Sci 2005; 76: 1473-1487.
46. Aladakatti RH, Ahamed RN. Ultrastructural changes in Leydig cells and cauda epididymal spermatozoa induced by Azadirachta indica leaves in albino rats. Phytother Res 2005a; 19: 756-766.
47. Bayala B, Pellicer-Rubio MT, Bassole IHN, Belemtougri R, Tamboura HH, Malpaux B. Effects of aqueous extracts of Leptadenia hastate (pers.) decne. (asclepediaceae) on male reproductive functions using castrated immature rats. Res J Med Plant 2011a; 5: 180-188.
48. Udoh P, Essie I, Udoh F. Effects of Carica papaya (paw paw) seeds extract on the morphology of pituitary-gonadal axis of male Wistar rats. Phytother Res 2005; 19: 1065-1068.
49. Gupta RS, Dixit VP. Testicular cell population dynamics following palmitine hydroxide treatment in male dogs. J Ethnopharmacol 1989; 25: 151-157.
50. Saez JM, Avallet O, Lejeune H, Chatelain PG. Cell-cell communication in the testis. Horm Res 1991; 36: 104-115.
51. N'guessan K, Tiébré M, Aké-Assi E, Zirihi GN. Ethnobotanical Study of Plants Used to Treat Arterial Hypertension, in Traditional Medicine, by Abbey and Krobou Populations of Agboville (Côte-d'Ivoire). European J Scient Res 2009; 35(1): 85-98.
52. Prakash AO, Pathak S, Mathur R. Post coital contraceptive action in rats of a hexane extract of the aerial parta of ferula jaeschkeana. J Ethnopharmac 1991; 34: 221-234.
53. Kamboj VP, Dhawan BN. Research on plants for fertility regulation in India. J Ethnopharmac 1982; 6:191-226.
54. Varshney IP, Vyas P, Srivastava HC, Singh PP. Study of Albizzia lebbek Benth wood saponin, Lebbekanin-E. Natl Acad Sci Lett (India) 1979; 2: 135-136.
55. Akbarsha MP, Manivannan B, Hamid KS, Vijayan B. Antifertility effect of Andrographis paniculata (Nees) in male albino rats. Indian J Exper Biol 1990; 28: 421-426.
56. Awasthy KS. Genotoxicity of a crude leaf extract of Neem in male germ cells of mice. Cytobios 2001; 106 Suppl 2: 151-164.
57. Dixit VP. Effect of Cannabis sativa extract on testicular function of Presbytis entellus entellus. Planta Medica 1981; 41: 288-294.
58. Gupta RS, Kumar P, Dixit VP, Dobhal MP. Antifertility studies of the root extract of Barleria prionitis Linn. in male albino rats with special reference to testicular cell population dynamics. J Ethnopharmac 2000; 70:111-117.
59. Gupta RS, Tank R, Dixit VP. Quantitative analysis of germ cells and leydig cells in rat and dog made infertile with solasodine. Adv Contr Deliv Syst U.S.A. V 1989; 389-397.
60. Huacuja RL, Delgado NM, Carranco LA, Reyes LR, Rosado GA. Agglutinating and immobilizing activity of an ethanol extract of Bursera fagaroides on human and mammalian spermatozoa. Arch Invest Med (Mex.) 1990; 21: 393-398.
61. Gupta RS, Sharma N, Dixit VP. Calotropin - A novel compound for fertility control. Ancient Sci Life 1990; 9: 224-230.
62. Stokes T. Papaya male contraceptive. Trends Plant Sci 2001; 6: 143.
63. Hukla B, Sha H, Wang C, Yu D, Wu X, Yu X. Proton NMR identification of Yuanhuatin--an antifertility significant component. Bopuxue Zazhi 1984; 1: 477-480.
64. Singh SP. Male contraception through an indigenous plant Ocimum sanctum linn. Int J Androl 1997; 20: 14-18.
65. Choudhary DN, Singh JN, Verma SK, Singh BP. Antifertility effects of leaf extracts of some plants in male rats. Indian J Exp Biol 1990; 28: 714-716.
66. Joshi AR, Ahamed RN, Pathan KM, Manivannan B. Effect of Azadirachta indica leaves on testis and its recovery in albino rats. Indian J Exp Biol 1996; 34:1091-1094.
67. Ogbuewu IP, Odoemenam VU, Obikaonu HO, Opara MN, Emenalom OO, Uchegbu MC, Okoli IC, Esonu BO, Iloeje MU. The growing importance of neem (Azadirachta indica A. Juss) in agriculture, industry, medicine and environment: A review. Research J Med Plant 2011; 5(3): 230-245.

68. Awasthy KS. Genotoxicity of a crude leaf extract of neem in male germ cells of mice. Cytobios 2001; 106 (Suppl 2): 151-164.
69. Aladakatti RH, Ahamed RN. Changes in Sertoli cells of albino rats induced by Azadirachta indica A. Juss leaves. J Basic Clin Physiol Pharmacol 2005b; 16: 67-80.
70. Chakraborty S, Pakrashi A. Antifertility effect of chronically administered Malviscus conzattii flower extract on fertility of male rats. Contraception 1991; 43: 273-285.
71. Mishra RK, Singh SK. Effect of aqueous leaf extract of Azadirachta indica on the reproductive organs in male mice. Indian J Exp Biol 2005; 43: 1093-1103.
72. Srivastava MK, Raizada RB. Lack of toxic effect of technical azadirachtin during postnatal development of rats. Food Chem Toxicol 2007; 45: 465-471.
73. Coutinho EM. Gossypol: a contraceptive for men. Contraception 2002; 65: 259-263.
74. Waites GM, Wang C, Griffin PD. Gossypol: reasons for its failure to be accepted as a safe, reversible male antifertility drug. Int J Androl 1998; 21: 8-12.
75. Zhuang LZ, Gu ZP, Chang CC. Comparison of sensitivities of rat spermatozoa, Sertoli and Leydig cells to gossypol acetic acid in vitro by the LD50. Zhongguo Yao Li Xue Bao 1986; 7: 563-567.
76. Paz GF, Homonnai ZT. Effect of the antifertility agent, gossypol acetic acid, on the metabolism and testosterone secretion of isolated rat interstitial cells in vitro. Contraception 1984; 29: 543-552.
77. Bodhankar SL, Garg SK, Mathur VS. Antifertility screening of plants. Part IX. Effect of five indigenous plants on early pregnancy in female albino rats. Indian J Med Res 1974; 62: 831-837.
78. Chinoy NJ, Ranga Geetha M. Effects of Carica papaya seed extracts on the physiology of the vas deferens of albino rats. Acta Eur Fertil 1984; 15: 59-65.
79. Lohiya NK, Goyal RB. Antifertility investigations on the crude chloroform extract of Carica papaya Linn. seeds in male albino rats. Indian J Exp Biol 1992; 30: 1051-1055.
80. Lohiya NK, Mishra PK, Pathak N, Manivannan B, Jain SC. Reversible azoospermia by oral administration of the benzene chromatographic fraction of the chloroform extract of the seeds of Carica papaya in rabbits. Adv Contracept 1999; 15: 141-161.
81. Lohiya NK, Manivannan B, Goyal S, Ansari AS. Sperm motility inhibitory effect of the benzene chromatographic fraction of the chloroform extract of the seeds of Carica papaya in langur monkey, Presbytis entellus entellus. Asian J Androl 2008; 10: 298- 306.
82. Manivannan B, Mittal R, Goyal S, Ansari AS, Lohiya NK. Sperm characteristics and ultrastructure of testes of rats after long-term treatment with the methanol subfraction of Carica papaya seeds. Asian J Androl 2009; 11: 583- 599.
83. Dixit VP, Khanna P, Bhargava SK. Effects of Momordica charantia L. fruit extract on the testicular function of dog. Planta Med 1978; 34: 280–286.
84. Naseem MZ, Patil SR, Ravindra A, Patil RS. Antispermatogenic and androgenic activities of Momordica charantia (Karela) in albino rats. J Ethnopharmacol 1998; 61: 9-16.
85. Adedapo AA, Omoloye OA, Ohore OG. Studies on the toxicity of an aqueous extract of the leaves of Abrus precatorius in rats. Onderstepoort. J Vet Res 2007; 74: 31-36.
86. Rao MV. Antifertility effects of alcoholic seed extract of Abrus precatorius Linn. in male albino rats. Acta Eur Fertil 1987; 18: 217-220.
87. Ratnasooriya WD, Amarasekera AS, Perera NS, Premakumara GA. Sperm antimotility properties of a seed extract of Abrus precatorius. J Ethnopharmacol 1991; 33: 85-90.
88. Sinha S, Mathur RS. Effect of steroidal fraction of seeds of Abrus precatorius Linn. on rat testis. Indian J Exp Biol 1990; 28: 752-756.
89. Hammami I, Nahdi A, Mauduit C, Benahmed M, Amri M. The inhibitory effects on adult male reproductive functions of crude garlic (Allium sativum) feeding. Asian J Androl 2008; 10: 593–601.
90. Hammami I, Amara S, Benahmed M, El May MV, Mauduit C. Chronic crude garlic-feeding modified adult male rat testicular markers: mechanisms of action. Reprod Biol Endocrinol 2009; 7: 65.
91. Girini MM, Ahamed RN, Aladakatti RH. Effect of graded doses of Momordica charantia seed extract on rat sperm: scanning electron microscope study. J Basic Clin Physiol Pharmacol 2005; 16: 53-66.
92. Qian YX, Shen PJ, Xu RY, Liu GM, Yang HQ. Spermicidal effect in vitro by the active principle of garlic. Contraception 1986; 34: 295-302.
93. Chakrabarti K, Pal S, Bhattacharyya AK. Sperm immobilization activity of Allium sativum L. and other plant extracts. Asian J Androl 2003; 5: 131-135.
94. Munshi SR, Rao SS. Antifertility activity of an indigenous plant preparation I. Effect on reproduction. Indian J Med Res 1972; 60: 1054-1060.
95. Malini T, Manimaran RR, Arunakaran J, Aruldhas MM, Govindarajulu P. Effects of piperine on testis of albino rats. J Ethnopharmacol 1999; 64: 219-225.
96. D'Cruz SC, Vaithinathan S, Saradha B, Mathur PP. Piperine activates testicular apoptosis in adult rats. J Biochem Mol Toxicol 2008; 22: 382-388.
97. D'Cruz SC. Mathur PP. Effect of piperine on the epididymis of adult male rats. Asian J Androl 2005; 7: 363-368.
98. Piyachaturawat P, Sriwattana W, Damrongphol P, Pholpramool C. Effects of piperine on hamster sperm capacitation and fertilization in vitro. Int J Androl 1991; 14: 283-290.

99. Ahmed M, Ahamed RN, Aladakatti RH, Ghosesawar MG. Reversible anti-fertility effect of benzene extract of Ocimum sanctum leaves on sperm parameters and fructose content in rats. J Basic Clin Physiol Pharmacol 2002; 13: 51-59.
100. Krishnamoorthy P, Vaithinathan S. Effect of the extract of Thespesia populnea leaves on mice testis. J Environ Biol 2003; 24: 327-30.
101. Wang Y, Waller DP. The bromine toxicity on Sertoli cells and comparison with cocoa extract in male rats. Toxicol Lett 1994; 70: 155-164.
102. Gupta RS, Kumar P, Dixit VP, Dobhal MP. Antifertility studies of the root extract of the Barleria prionitis Linn in male albino rats with special reference to testicular cell population dynamics. J Ethnopharmacol 2000; 70: 111-117.
103. Gupta RS, Kachhawa JB, Sharma A. Effect of methanolic extract of Dendrophthoe falcata stem on reproductive function of male albino rats. J Herbal Pharmacother 2007; 7: 1–13.
104. Gupta RS, Kachhawa JB, Chaudhary R. Antispermatogenic, antiandrogenic activities of Albizia lebbeck (L.) Benth bark extract in male albino rats. Phytomedicine 2006; 13: 277-283.
105. Chauhan A, Agarwal M. Reversible changes in the antifertility induced by Aegle marmelos in male albino rats. Syst Biol Reprod Med 2008; 54: 240-246.