Review Article

Indian J Med Res 148 (Supplement), December 2018, pp 107-114
DOI: 10.4103/ijmr.IJMR_1968_17

Quick Response Code:

Natural products in regulation of male fertility

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Received December 12, 2017

Medicinal plants may prove useful in developing plant-based strategies for regulation of male fertility. The present review describes the antifertility potential of certain medicinal plants, *viz. Azadirachta indica*, *Curcuma longa, Allamanda cathartica* and *Bacopa monnieri* in Parkes (P) male mice. The results suggested that treatment with the aqueous extracts of these plants caused reversible suppression of spermatogenesis and fertility in P mice and that there were no signs of detectable toxicity in treated mice. Further research needs to be done to develop plant-based strategies for control of male fertility.

Key words Fertility -indigenous plants - mice - seminiferous tubules - spermatogenesis - spermatozoa

Introduction

According to the World Population Prospects (2017), the world population is around 7.6 billion, and with the present trend, it is anticipated to rise to 8.6 billion by 2030, 9.8 billion by 2050 and 11.2 billion by 2100[1]. The population of our country has increased by >181 million during 2001-2011[2]. Both government and non-government organizations are making all efforts to control the human population, but the outcome has not been very satisfactory. One of the possible reasons could be the limited availability of contraceptive choices[3]. Women are the main users of the contraceptives. Contraceptives developed for females are effective in preventing unplanned pregnancy; however, because of side effects, some women cannot use these contraceptives on health ground[4,5]. Therefore, the development of male contraceptive will help in planning family[6].

The male contraceptives act by blocking meeting of sperm to the egg either by physical barriers (condoms, vasectomy and experimental vas occlusion methods) or by inhibiting spermatogenesis (hormonal and non-hormonal methods)[6]. Approximately 30 per cent of couples currently depend on condom and vasectomy as male methods of fertility regulation, although both of these methods have their own limitations[6]. The major drawbacks of condom and vasectomy are their high failure rates and lack of complete reversibility after the reversal operation, respectively[7]. A contraceptive that is safe, effective, reversible and rapid in action should be considered acceptable for use in men. Besides, it should not affect other androgen-dependent functions. In addition, the application mode should be easy and price considerably low.

In Indian traditional medical system of Ayurveda, many herbal extracts have been used for the treatment of a variety of ailments and that these extracts have also been used in regulating as well as improving fertility[8]. Several compounds derived from natural herbs in different phases of clinical development

signify natural products as sources of new drug candidates[9].

Several studies were conducted to develop herbal contraceptives[10-13]. The success in search of a plant-based male contraceptive is best illustrated with the discovery of gossypol by Chinese scientists, which is regarded as a major breakthrough in male contraception[14,15]. This gave a great impetus to researches on gossypol and considerable amount of work has been carried out on the antifertility properties of this compound in both animals and humans[16-22]. In clinical studies, however, gossypol produced two major side effects: occasional occurrence of hypokalaemia and variable differences in reversibility of male fertility[23]. The potential of low dose of gossypol together with steroid hormones has also been investigated for use in male contraception[24,25]. Efforts are being continued to explore a suitable plant product for use in the regulation of male fertility (Table). The present review describes the antifertility potential of four plants, *viz*. *Azadirachta indica*, *Curcuma longa, Allamanda cathartica* and *Bacopa monnieri* in Parkes (P) strain male laboratory mouse, which has been used as an animal model in our laboratory[32,37,42,43].

Azadirachta indica

A. indica L. (family, *Meliaceae*), known commonly as *neem*, is a medicinal plant and this is found in semi-tropical and tropical climates in countries such as India, Pakistan and Bangladesh[44,45]. Extracts of different parts of this tree have been found useful in the treatment of ulcer, malaria, liver disease, cancer, high blood sugar, dermatological disease, intestinal worms, fever, eye problem, urinary disorder, *etc*[46,47]. Azadirachtin is the most important and active constituent of *neem*, while others include nimbolinin, nimbin, nimbidin, nimbidol, sodium nimbinate, gedunin, salannin and quercetin[48].

***Antifertility studies*:** Upadhyay *et al*[49] reported that a single administration (50 µl) of *neem* oil into the lumen of the vas deferens on each side in male rats induced a long-term inhibition of fertility, without affecting the libido. *Neem* oil impaired spermatogenesis up to nine months after the treatment, while the serum level of testosterone was not affected and also there was no increase in the anti-sperm antibodies. Joshi *et al*[50] reported that *Azadirachta* treatment in rats caused a decrease in diameter of the seminiferous tubules, with atrophy of the spermatogenic elements and the Leydig cells. They suggested that the cessation of the spermatogenic process after *neem* treatment was caused probably because of the deficiency in androgen production. There was a gradual recovery in anti-androgenic action of the *A. indica* in male albino rats 8, 16 and 24 days after withdrawal therapy. Parshad *et al*[51] showed no effect of aqueous extract of *neem* leaf on spermatogenesis and on the litter size and fertility index.

In P mice, neem treatment (50, 100 and 200 mg/kg body weight/day for 28 days) did not affect body weight and also the weights of testis, epididymis and seminal vesicle[42]. The testis of control mice showed normal histological features. By contrast, testes in *neem*-treated mice exhibited both affected and normal seminiferous tubules in the same sections. The affected seminiferous tubules showed degenerative changes such as presence of vacuoles in the germinal epithelium, loosening of epithelium, marginal condensation of chromatin in round spermatids, formation of giant cells and mixing of germ cell types in stages of spermatogenesis. Further, the *neem* treatment also affected sperm parameters in the epididymis and fructose level in the seminal vesicle. The litter size in females impregnated by *neem*-treated males was also affected. By six weeks of treatment withdrawal, however, the changes caused in the reproductive organs recovered to control levels[42].

The mechanism by which *A. indica* causes impairment in spermatogenesis is not properly understood. It is reported that the antifertility effect of *neem* is not associated with alterations in the serum level of testosterone[49,51]. In P mice, vacuoles were often noticed in the epithelium in affected seminiferous tubules in the testis after *neem* treatment. Intraepithelial vacuoles have also been noticed in affected seminiferous tubules in rat testis after gossypol treatment, and such vacuoles are reported to occur primarily in the Sertoli cells[52]. Thus, it is probable that in P mice, *neem* treatment causes suppression of spermatogenesis by acting through Sertoli cells[42].

Curcuma longa

C. longa L. (family, *Zingiberaceae*) a perennial herb, is grown throughout India. Curcumin is the active ingredient in turmeric and this exhibits protective and preventive properties against several diseases such as cancer and autoimmune, neurological, metabolic, lung, liver and cardiovascular diseases[53,54]. Besides curcumin, turmeric also contains sesquiterpenes (turmerone, atlantone, zingiberone, turmeronol, germacrone and bisabolene), carbohydrates, protein, resins and caffeic acid[55].

Antifertility studies: Aqueous rhizhome extract of *C. longa* in a dose of 500 mg/kg body weight for 60 days

Table. List of plants exhibiting antifertility properties in male rats and mice

Plant	Type of extract and part of plant	Route of administration, dose and duration	Animal model	Effects	References
Abrus precatorius	Ethanolic extract of seed	Intraperitoneal; 20, 40 and 60 mg/kg BW for 20 day	Mouse	Suppression of spermatogenesis; decreased serum testosterone and decreased sperm count	26
Aegle marmelos	Aqueous extract of leaves	Oral; 100, 200 and 300 mg/kg BW for 60 days	Rat	Decreased reproductive organs weight; decreased serum testosterone; and anti-spermatogenic and antifertility effects	27
Allium sativum	Crude extract of bulb	Feed; 5, 10 and 15 per cent for 30 day	Rat	Germ cell apoptosis; and inhibition of Leydig cell steroidogenesis	28
	Aqueous extract of bulb	Oral; 500 and 1000 mg/kg BW for 28 days	Rat	Increased morphologically abnormal spermatozoa and decreased sperm concentration	29
Andrographis paniculata	Alcoholic extract of leaf	Oral; 250 and 500 mg/kg BW for 30 and 60 days	Rat	Decreased weights of testis and epididymis; reduced size of seminiferous tubules; and degeneration of spermatozoa	30
Citrus limon	Ethanolic extract of leaf	Oral; 500 and 1000 mg/kg BW for 35 days	Mouse	Anti-spermatogenic and antifertility effects; reduced serum testosterone; and reversibility after 56 days of treatment withdrawal	31
Coccinia indica	Ethanolic extract of leaf	Oral; 200 and 500 mg/kg BW for 35 days	Mouse	Anti-spermatogenic and antifertility effects; reduced serum testosterone; and reversibility 56 days after treatment withdrawal	32
Dalbergia sissoo	Aqueous extract of leaf	Oral; 50 and 100 mg/kg BW for 35 days	Mouse	Anti-spermatogenic and antifertility effects; reduced serum testosterone; and reversibility after 56 days of treatment withdrawal	33
Enicostemma axillare	Ethanolic extract of leaf	Intragastric; 375 and 750 mg/kg BW for 55 days	Rat	Inhibited spermatogenesis and steroidogenesis; and reversibility 55 days after treatment withdrawal	34
Juniperus phoenicea	Ethanolic extract of cones	Intraperitoneal; 400 or 800 mg/kg BW for 21 days	Rat	Anti-spermatogenic and antifertility effects	35
Madhuca indica	Alcoholic extract of leaves	Oral; 200 mg/kg BW for 20 days	Rat	Decreased body weight; decreased reproductive organs weight; regressed seminiferous tubules; and decreased serum testosterone	36
Mimusops elengi	Aqueous extract of fruit	Oral; 200, 400 and 600 mg/kg BW for 35 days	Mouse	Anti-spermatogenic and antifertility effects; and reversibility 56 days after treatment withdrawal	37
Opuntia dillenii	Methanolic extract of phylloclade	Oral; 50 mg/kg BW for 30 days	Rat	Reduced serum testosterone level; decreased sperm count and motility; and reduced fertility	3
Tabernaemontana divaricata	Ethanolic extract of leaf	Oral; 50, 100 and 200 mg/kg BW for 60 days	Rat	Decreased reproductive organs weight; decreased sperm count and motility; spermatogenic arrest; and reduced serum testosterone and fertility	38

Contd...

Plant	Type of extract and part of plant	Route of administration, dose and duration	Animal model	Effects	References
Taraxacum officinale	Aqueous extract of whole plant	Oral; 1.065 and 2.130 g/kg BW for 60 days	Rat	Decreased testis weight; decreased sperm count and motility; spermatogenic arrest and reduced fertility	39
Terminalia chebula	Aqueous-ethanolic (1:1 v/v) extract of fruits	Oral; 60 mg/0.5 ml distilled water for 28 days	Rat	Affected spermatogenesis; decreased activities of androgenic key enzymes and decreased plasma testosterone	40
Trachyspermum ammi	Ethanolic extract of fruit	Oral, 100, 200 and 400 mg/kg BW for 60 days	Rat	Reduced testis weight; decreased sperm number and motility; increased production of abnormal sperm; and reversibility after 120 days of treatment withdrawal	41
Urena lobata	Ethanolic extract of root	Intragastric; 300 and 600 mg/kg BW for 55 days	Rat	Inhibition of spermatogenesis and steroidogenesis and reversibility 55 days after treatment withdrawal	34

BW, body weight

caused a decrease in the weight of the epididymis, seminal vesicle, ventral prostate and testis[56]. The treatment also caused a reduction in sperm count and motility and a reduction in the number of germ cells and hence decreased fertility; the Leydig cells were also adversely affected. These effects of the treatment were attributed to the anti-androgenic nature of the extract. These authors further reported return of sperm count and motility in the epididymis of *Curcuma*-treated rats two months after withdrawal of treatment[56].

In our study in P mice, *Curcuma* treatment (600 mg/kg body weight/day, for 56 and 84 days) had no effect on body weight but caused a marked depression in weights of the testis, epididymis and seminal vesicle[43]. The treatment also had adverse effects on sperm parameters in the cauda epididymidis, on levels of sialic acid and fructose in the epididymis and seminal vesicle, respectively, and on serum level of testosterone. Further, fertility of *Curcuma*-treated males was also affected. Histologically, testes in *Curcuma*-treated mice exhibited degenerative changes in the seminiferous tubules although normal tubules were also seen in sections. The diameter of the seminiferous tubules and height of the germinal epithelium in testes of *Curcuma*-treated mice were also decreased. By 56 days of withdrawal therapy, however, the changes noted in the reproductive indices recovered to control levels[43].

The mechanism by which *Curcuma* treatment induces anti-spermatogenic effects in mice testis is not properly understood. In immature male rat, it is suggested that *Curcuma comosa* (an another species of *Curcuma*) acts directly on the testis or indirectly inhibits gonadotropin secretion, thereby lowers testosterone production, or acts at both the levels[57]. It is known that testosterone is essential for sustenance of spermatogenesis[58]. The observation in P mice that *Curcuma* treatment caused reduction in the serum level of testosterone suggested that the *Curcuma*-induced suppression of spermatogenesis in mice testes was probably caused because of the deficiency of testosterone. The curcumin analogues are also shown to interfere with 17β-hydroxysteroid dehydrogenase isoform 3 activity and that this enzyme plays an important role in testosterone biosynthesis in the Leydig cells[59].

Allamanda cathartica

A. cathartica L. (family, *Apocynaceae*) known commonly as the golden trumpet, yellow bell or the buttercup flower, exhibits various pharmacological properties such as anticancer, anti-inflammatory, antimicrobial, antifungal, anti-leukaemic, wound healing, antibiotic, anti-dermatophytic and anti-hypertensive[60]. The roots of *A.cathartica* contain iridoid lactone, allamandin and two other iridoids, allamandicin and allamdin; leaves and stem contain sesquiterpenes, ursolic acid, β-amyrin and β-sitosterol andursolic acid, β-amyrin and β-sitosterol, respectively, and flowers contain kaempferol, quercetin and hesperitin[61].

Antifertility studies: In P mice, *A. cathartica* treatment (150 mg/kg body weight/day for 14, 28 and 42 days) did not affect the body weight or the weights of testis and seminal vesicle, although epididymal weight was markedly decreased in treated mice[62]. Sperm parameters (motility, viability and number) in the cauda epididymidis and fertility were also affected in treated males[62]. Fructose level in the seminal vesicle and level of serum testosterone were not affected. In *Allamanda*-treated mice, marked histological changes were observed in the testis, and both affected and normal seminiferous tubules were seen in the same section. In testes of mice dosed with *A. cathartica* leaf extract, the affected seminiferous tubules exhibited diverse degenerative changes[62]. Germinal epithelial height and tubular diameter were also decreased in treated mice. Testis in treated mice showed high percentage of affected seminiferous tubules than in controls. By 56 days of withdrawal therapy, however, the changes caused in the reproductive organs recovered to control levels[62].

In P mice, treatment with *A. cathartica* caused inhibition of spermatogenesis and this inhibition did not appear to be mediated via Leydig cells as no differences could be detected in serum testosterone level between treated mice and controls[62]. It is, therefore, likely that the treatment may act directly on the seminiferous tubules, resulting into the suppression of spermatogenesis. The observation that the intraepithelial vacuoles were frequently noticed in the seminiferous tubules showing degenerative changes in testes of *Allamanda*-treated mice support the above contention[52,62].

Bacopa monnieri

The plant *B. monnieri* (L.) Wettst. (family, *Plantagenaceae*) known as *Brahmi* grows in damp soils and marshes throughout the subcontinent[63,64]. Pharmacological effects of *Brahmi* have also been evaluated in laboratory studies and results show many beneficial actions/properties including memory boosting, anti-Parkinson, anti-stroke, anticonvulsant, antidepressant, anti-anxiety, antioxidant, gastrointestinal and hepatoprotective, antimicrobial and anti-inflammatory activities[65]. *Bacopa* contains brahmine, nicotinine, herpestine, des-saponin glycosides-triterpenoid saponins such as bacosides A and B[66].

Antifertility study: In P mice, *Bacopa* treatment (250 mg/kg body weight/day for 28 and 56 days) did not affect the body weight or the weights of the testis, epididymis and seminal vesicle, but weight of epididymis was significantly decreased in mice treated with the plant for 28 days compared to controls[67]. Sperm parameters (motility, viability and number) in the cauda epididymidis and fructose level in the seminal vesicle were also adversely affected by the treatment; further, fertility of males was also affected as females mated to *Bacopa*-treated males did not show live implants[67]. The serum level of testosterone, however, remained unaltered in treated mice. Histologically, alterations were noticed in testes of *Bacopa*-treated mice, while testes in controls showed normal features. The testis in treated mice showed both affected and normal seminiferous tubules in the same section. The affected seminiferous tubules in both the dosage groups showed exfoliation of germ cells, loosening of germinal epithelium, presence of vacuoles in the epithelium and formation of giant cells[67]. Further, height of the germinal epithelium and diameter of the seminiferous tubules were also decreased in testes of mice treated with *Bacopa* compared to controls. By 56 days of treatment withdrawal, however, the changes induced in the reproductive organs returned to control levels[67].

In P mice, *Bacopa* treatment did not influence testosterone secretion by the Leydig cells as no differences could be noted in serum testosterone level between treated mice and controls[67]. It may, therefore, be hypothesized that the *Bacopa* acts directly on the seminiferous tubules. Sertoli cells are well known to play an important role in maintenance of spermatogenesis and that any damage to these cells would cause suppression of spermatogenesis. The occurrence of intraepithelial vacuoles in the affected seminiferous tubules in testes of treated mice suggested that the anti-spermatogenic action of *Bacopa* in P mice was mediated via Sertoli cells[52,67].

Conclusion

Treatment with *A. indica*, *C. longa, A. cathartica* and *B. monnieri* in P mice produced reversible inhibition of the spermatogenic process and fertility, thereby advocating viability of these plants in male contraception. One of the major issues associated with plant-based research for fertility regulation is that the results show much variation, with 0-100 per cent activity with the same plant, and further, a herbal contraceptive practiced by humans may not be effective in animal models[11]. Hence, a better approach would be to assess the efficacy of the plants in humans themselves, after evaluation of

their safety in animal models. Further, time and place of collection, proper identification, standard protocol for extraction and schedule of administration should also be considered while interpreting the results. It should also be remembered that many phytomedicines are extracts of the whole plants and synergistic interactions between components of the plants are essential for their efficacies; in many cases, it has been found that a total herb extract has exhibited a better outcome than an isolated compound[68].

***Financial support & sponsorship*:** This work was supported by grants (3/1/2/13/01-RHN; 3/1/2/15/04-RHN) from the Indian Council of Medical Research, New Delhi, and the University Grants Commission, New Delhi, to the Centre of Advanced Study in Zoology, Banaras Hindu University, Varanasi.

***Conflicts of Interest*:** None.

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