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Review

Effects of plants and plant products on the testis

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Abstract

For centuries, plants and plant-based products have been used as a valuable and safe natural source of medicines for treating various ailments. The therapeutic potential of most of these plants could be ascribed to their anticancer, antidiabetic, hepatoprotective, cardioprotective, antispasmodic, analgesic and various other pharmacological properties. However, several commonly used plants have been reported to adversely affect male reproductive functions in wildlife and humans. The effects observed with most of the plant and plant-based products have been attributed to the antispermatogenic and/or antisteroidogenic properties of one or more active ingredients. This review discusses the detrimental effects of some of the commonly used plants on various target cells in the testis. A deeper insight into the molecular mechanisms of action of these natural compounds could pave the way for developing therapeutic strategies against their toxicity.

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1 Introduction

Male reproduction is a multifaceted process that involves the testes, epididymis, accessory sex glands and associated hormones. Testes perform two highly organized and intricate events, called spermatogenesis and steroidogenesis, which are vital 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 [1]. The interstitial compartment, which comprises Leydig cells, are the site of steroidogenesis [2]. Subsequent to the process of spermatogenesis, spermatozoa transits from the testis to the ejaculatory ducts, undergoing a sequence of modifications that results in the accomplishment of its ability to move, capacitate and to interact with zona pellucida of the female ovum [3]. This complex process is strictly regulated by the hypothalamo–pituitary–testicular axis, which involves the pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Apart from LH, FSH and androgens, various growth factors, hormones and estrogens are involved in regulating the testicular functions.

The testis is well equipped with powerful intrinsic defense systems that protect the spermatozoa during its spermatogenic/post-spermatogenic journey and from the injuries caused by other intrinsic or extrinsic factors. Nevertheless, the testis is one of the organs that are very vulnerable to assault, which is reflected by the adverse

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trend in male reproductive health during the past several years. The male reproductive system is extremely sensitive to various environmental factors such as life style, drugs, radiation, pollution and toxicants, the result of which could be congenital abnormalities in infants and functional alterations in adults [4]. Several natural and synthetic products are reported to target the testis at the hormonal level or spermatogenesis or both. In this review, we discuss on some of the commonly used plant products that could hamper the functionality of the testis, thereby leading to infertility.

2 Plants that impair testicular functions

Plants, since ancient times, have been used globally across varied cultures throughout the known civilizations as a valuable and safe natural source of medicines and as agents of therapeutic, industrial and environmental utilities. From the inception of civilization, humans have relied on plants that could meet their basic necessities such as food, shelter, clothing, fuel and health. Of all the uses ascribed to the plants, their curative abilities played an inevitable part in the lives of primitive societies, as plants comprised their sole source for healing ailments. The sacred knowledge about 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 [5]. The Indian and Chinese systems of traditional medicine are well established, with written records dating back to thousand of years [6]. A wide majority of herbal plants possess pharmacological principles, which has rendered them useful as curatives for numerous ailments. According to the World Health Organization (WHO) reports, 70%–80% of the world population confide in traditional medicine for primary healthcare [7].

Plants have a long folklore of use in aiding fertility. The Indian sacred text, Rig Veda, describes a 'holy brew' called soma, the intake of which is believed to bestow upon humans infinite powers, including aphrodisiacal qualities [8]. The medical historians have recorded plants that could be used as contraceptives, emmenagogues and abortifacients [5]. 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 plants. Plants are known to heal as well as hurt. Several plants are reported to enhance reproductive processes but, on the other hand, to also hinder testicular functions. The effects of plants on testicular functions could be rightly compared with those of a double-edged sword. This review is confined to the toxicological attributes of some of the commonly used plants to the functions of testis.

3 Plants that affect spermatogenesis

Spermatogenesis is a complex process by which an interdependent population of undifferentiated germ cells undergoes multiplication and maturation to form functional haploid spermatozoa. 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. The final phase, also called spermiogenesis, involves the differentiation of immature spermatids into mature spermatozoa. Spermiogenesis comprises polarization of the spermatid, formation of acrosomal cap and flagellum, cytoplasmic remodeling and elongation of the nucleus. Endocrine regulation by testosterone and the architecture of the Sertoli cells and seminiferous tubules also forms an important decisive factor in spermatogenesis [9].

Several plants and plant products are reported to impede various stages of testicular spermatogenesis in many different animal species such as dogs, rats, humans and monkeys [10–12] (Figure 1). Cannabinoids, one of the oldest narcotic drugs of plant origin, are known to impair human health since olden times [13]. Intraperitoneal injection of mice with low doses of *Cannabis* extracts (40 mg, 60 mg and 80 mg) 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. Damage to the basement membrane, shrinkage of the seminiferous tubules, scanty cytoplasm and shrunken nuclei in the germinal epithelium, and complete arrest of spermatogenesis were also reported. The effects were seen to be reversed on withdrawal of the drug for 45 days, and it was speculated that the endogenous antioxidant system along with the FSH/LH feedback loop was responsible for the observed protective effects on withdrawal [14]. A pharmacokinetic study on the plasma, brain and testis of rats was conducted, which measured the concentration of tetrahydrocannabinol in these tissues following exposure to $C^{14}\Delta$-8-labeled tetrahydrocannabinol. Impairment of spermatogenesis

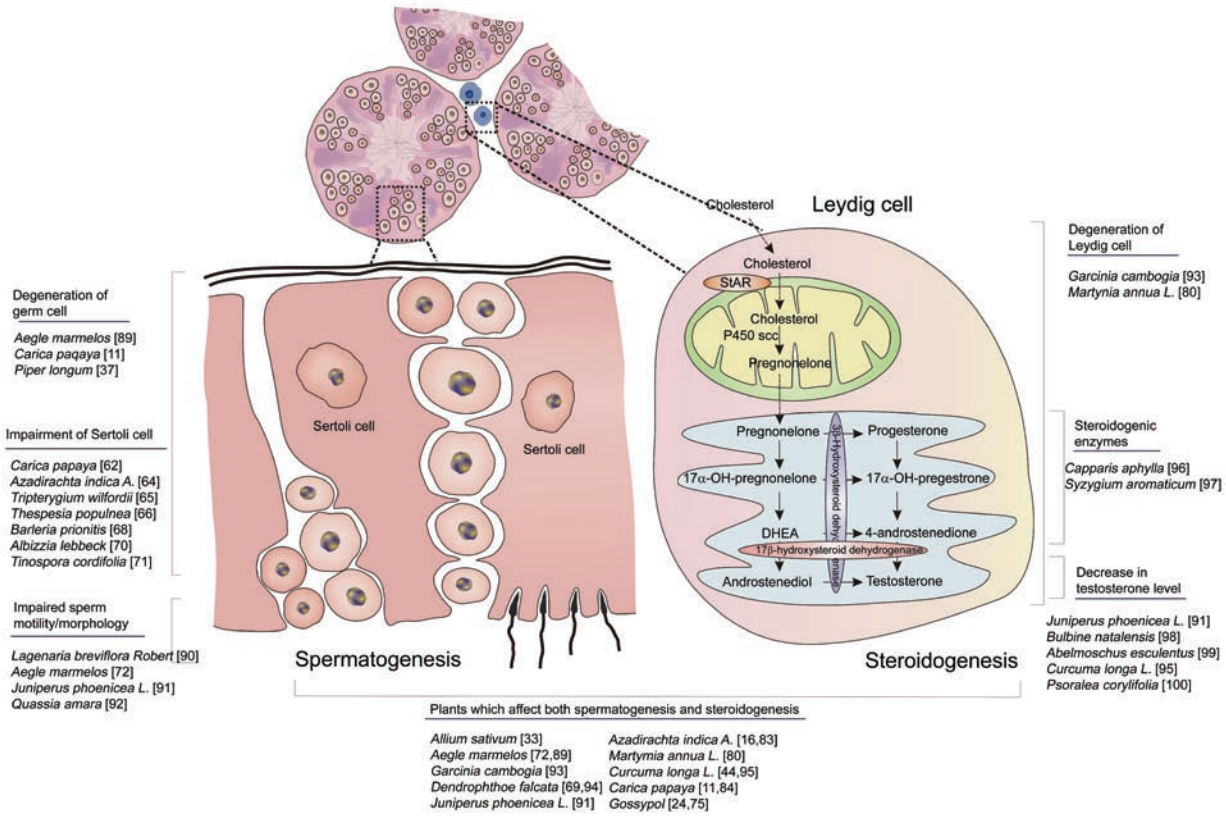

Figure 1. Target sites of plant toxicity in testis.

before the stage of meiosis and premature apoptosis along with structural and functional anomalies of sperm cells were reported. It was hypothesized that tetrahydrocannabinol, a lipid molecular signal, desynchronizes the membrane signaling and induces a death signal in the lipid bilayer, leading to early apoptosis in sperm cells [15].

Neem (*Azadirachta indica*) has long been documented to have antifertility effects [16, 17]. 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. The effects were reported to revert back to normalcy after 42 days of withdrawal of the treatment [18]. On the contrary, azadiractin, an active ingredient of neem, given at doses of 5 mg, 10 mg and 50 mg per kg body weight (b.w.) did not show any evidence of reproductive toxicity in parent rats or litters (F[1B] and F[2B]) over two generations, implicating the safe use of the compound as a biopesticide [19]. Although several studies claim the potential use of neem as a contraceptive, neem oil is reported to induce other toxic effects, such as severe metabolic acidosis [20, 21], encephalopathy [22], ventricular fibrillation [23] and nervous abnormality.

Gossypol, a polyphenolic compound present in the stem, seeds and roots of *Gossypium* species, is known to exert unique and selective effects upon reproduction in various species such as rats, mice, hamsters, rabbits, monkeys and human beings [24]. The contraceptive effect of gossypol was first discovered in China. During the times of drought in China, the cotton cake that was left over after the extraction of the oil from the fiber of the plant was consumed by animals and humans. Shortly after, the contraceptive action of cotton cake was identified and immense quantities of gossypol were extracted from the cotton plant. This discovery led to large-scale testing of gossypol as a male contraceptive in China during the 1970s [25]. Gossypol is reported to invoke antifertility effects in rats at 30 mg per kg b.w., whereas a much lesser dose, 0.3 mg per kg b.w., could incite infertility in humans, making the compound very efficient in humans than in rats [12]. Later, it was

found to reduce blood potassium (hypokalemia) [26], and the sterilization effect was found to be irreversible, although a few studies claim the effect to be reversible. Supplementation with potassium salts has been reported to bring normalcy in the gossypol-treated animals. 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 [27].

Carica papaya is recognized from ancient times for its medicinal properties and the contraceptive characteristics of papaya seed extracts have been reported in the 1970s [28, 29]. 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 b.w. [11]. 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 [30]. Administration of the chloroform extract of papaya to male rabbits for 150 days caused a decline in sperm concentration with oligospermia on the 75th 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 [31].

Alcoholic extracts of *Momordica charantia* have been reported to exhibit antispermatogenic effects in dogs [10]. Oral administration of alcohol extracts of the seeds of *Momordica charantia* to male albino rats at a dose of 25 mg per 100 g b.w. 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 [32]. The crude extract of garlic (*Allium sativum*) 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% [33]. Furthermore, induction of germ-cell apoptosis through increased expression of caspase inhibitors such as Baculoviral IAP repeat-containing protein 3 (BIRC3) and Baculoviral IAP repeat-containing protein 2 (BIRC2) and activation of caspase-3 were also reported [34]. Pepper (*Piper longum*), a commonly used spice, is reported to induce sterility in laboratory male mice [35]. 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 [36]. 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 [37, 38].

Vincristin and vinblastin, two pharmacologically active compounds isolated from *Catharanthus roseus* or *Vinca rosea*, have since long been reported to interfere with reproduction [39, 40]. A single injection of 15 μg of vincristine and 40 μg of vinblastin to adult rats was reported to cause degeneration of A4 spermatogonia, 48 h after injection. Changes in the seminiferous tubule and a decline in the percentage of primary spermatocytes, round and elongated spermatids were reported in rats when the extracts of *Vinca rosea* were administered at various doses [41]. *Ocimum sanctum* or Holy Bail or Tulsi has also been reported to hamper reproduction by targeting spermatogenesis, thereby leading to antifertility [42]. Accumulation of sperm in the lumen of seminiferous tubules was observed when rats were administered with ginger (*Zingiber officinale*) rhizome powder at doses of 50 mg and 100 mg for 20 consecutive days [43]. The rhizome extract of *Curcuma longa* at a dose of 600 mg per kg b.w. for 56 and 84 days caused a reduction in the diameter of seminiferous tubules, loosening of the germinal epithelium, intraperitoneal vacuolation and mixing of spermatids at different stages of spermatogenesis in male Wistar rats, with the effects being reversible following cessation of treatment for 56 days [44].

The extracts from the roots of *Tripterygium wilfordii*, a Chinese herb that is used in the treatment of various diseases like rheumatoid arthritis, hepatitis, spondylitis and skin disorders, has been shown to exert powerful antifertility effects in rats and human males, with the effects being observed at doses much lower than the ones used to cure rheumatoid arthritis [45]. The glycosides of *Tripterygium wilfordii* at a daily dosage of 10 mg for 7 weeks or 13 weeks and 20 mg for 4 weeks or 10 weeks significantly inhibited spermatogenesis and the turnover of basic nuclear protein synthesis in the late elongated spermatids of rat testis [46]. Several other plants are also known to hamper fertility by targeting spermatogenesis at various stages. A list of few recently published articles on plants that impair spermatogenesis is summarized in Table 1.

3.1 Plants that affect sperm motility and morphology

The spermatozoa formed during the process of

Table 1. Plants that affect spermatogenesis.

Plant name	Plant part used/extract	Dose and duration	Observed effects	References
Allium sativum	Crude extract	5%, 10%, 15% and 30%; 30 days	Dose-dependent decrease of increase in the percentage empty seminiferous tubules.	[33]
Lagenaria breviflora Robert	Whole fruit and ethanol	1 000, 2 000, 4 000 and 8 000 mg kg^{-1} day^{-1}; 14 days	Significant decrease in sperm motility and viability.	[90]
Azadirachta indica A.	Leaf and powder	0%, 5%, 10% and 15% of neem leaf meal; 16 weeks	Mild depressive effect on spermatogenesis, sperm quality and seminiferous tubule diameter.	[101]
Aegle marmelos	Leaf and ethanol extract	200 and 300 mg kg day^{-1}; 60 days	Reduces sperm motility, concentration. Morphological changes in the testis. Reduced the surface area of Sertoli and Leydig cells.	[72]
Garcinia cambogia	Seeds and ethanol extract	100 and 200 mg kg^{-1} day^{-1} ; 6 days per week for 6 weeks treatment	Distortion in the arrangement of the spermatogenic cells.	[93]
Dendrophthoe falcata	Stem and 70% methanol extract	100 mg kg^{-1} day^{-1}; 60 days	Inhibition of spermatogenesis by decreasing the weight of testis, epididymis and accessory sex organs. Reduced the Sertoli cell surface area.	[69]
Juniperus phoenicea L.	Cones and ethanol extract	400 or 800 mg $kg^{-1}day^{-1}$; 21 days	Spermatogenic arrest and decreases sperm motility, count.	[91]
Rosmarinus officinalis L.	Leaf and ethanol extract	250 and 500 mg $kg^{-1}day^{-1}$; 63 days	Decreases spermatogenesis by decreasing the primary and secondary spermatocyte in the testis.	[102]
Quassia amara	Bark and chloroform extract	12.5%, 25%, 50% and 100%; 15 days	Decreased sperm parameters, epididymal α-galactosidase activity and abnormal sperms.	[92]
Martynia annua	Root and ethanol extract	50, 100 and 200 mg kg^{-1} day^{-1}	Spermatogenic arrest by showing degeneration of spermatocytes and a dose-related reduction in sperm parameters.	[80]
Mentha arvensis	Leaf and petroleum ether extract	10 and 20 mg per animal per day; 20, 40 and 60 days	Reduced testis, epididymis weight and spermiogram with normal morphology of sperm.	[103]
Hibiscus sabdariffa	Calyx and aqueous extract	1.15, 2.30 and 4.60 g kg^{-1} day^{-1};	Distortion of seminiferous tubules.	[104]
Cestrum parqui	Leaf and filtered extract	40, 62.5, 100, 150 and 250 μg mL^{-1}	Spermicidal activity at high dose with damage to sperm membrane.	[105]
Tropaeolum tuberosum	Tubers and aqueous extract	1 g kg^{-1} day^{-1} mL^{-1}; 7, 12, 21 and 42 days	Reduces testicular functions after one spermatogenic cycle by reducing spermatid and sperm number, daily sperm production.	[106]
Curcuma longa L.	Rhizome and aqueous extract	600 mg kg^{-1} day^{-1}; 56 and 84 days	Suppresses spermatogenesis	[44]
Barleria prionitis	Root and methanolic extract	100 mg kg^{-1} day^{-1}; 60 days	Spermatogenic cells such as primary spermatocytes, secondary spermatocytes and round spermatocytes were declined.	[107]

spermiogenesis are morphologically mature but immotile and gets released into the lumen of the seminerous tubule, which then proceeds into the rete testis via the seminiferous fluid. The peristaltic movements of the adjoining myoid cells of the testis transport the immotile spermatozoa through a series of tubules known as efferent ductules, which connects the retes testis to the head of the epididymis. The passage of the sperm through three segments of the epididymis-caput, corpus and cauda is very essential for the final maturation of the sperm [47]. The synthesis and secretion of various proteins by the epididymis, as well as the attainment of various morphological, biochemical and motile properties during the passage through the epididymis are fundamental for the fully fertilizing capabilities of spermatozoa. Reduced sperm number or altered sperm morphology may be indicative of the problems encountered during spermatogenesis or spermiogenesis or the impairment of epididymal environment. Several plant products are reported to alter the morphology of the sperm or to diminish its motility.

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 1 600 mg per kg b.w. for 18 days [48]. The alcoholic seed extracts of *Abrus precatorious* at a dose of 100 mg per kg b.w. 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 male 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 [49]. 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 [50]. A significant decrease in the density and motility of the ejaculated spermatozoa were observed in patients receiving the root extracts of *Tripterygium wilfordii* as treatment for rheumatoid arthritis [45]. Increased retained proximal cytoplasmic droplets in the sperm, separation of the heads and tails of the spermatozoa and reduced sperm motility were observed when mature rams were fed with locoweed, *Astragalus lentiginosus* [51]. Incubation of guinea spermatozoa with the crude aqueous extract of *Echeveria gibbiflora* caused a hypotonic-like effect, which included distention of the plasma membrane over the acrosomal region and formation of a huge head bubble [52]. Electron microscopic observation of human spermatozoa revealed the presence of a sticky dense material intercalated along the plasma membrane on exposure to a purified fraction from the crude aqueous extract of *Echeveria gibbiflora* [53].

Oral administration of ethanolic extracts of neem to adult male mice at 0.5 mg, 1 mg or 2 mg per kg b.w. 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 s of exposure to 3 mg dose [54. 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 [55]. In our laboratory, we have demonstrated a reduction in rat sperm motility, viability and count on exposure to piperine at 10 mg and 100 mg per kg b.w. [38]. Graded doses of the *mormodica* seed extract induced abnormalities in the size and shape of rat sperm along with dorsoventral constrictions in the middle region of the sperm head, which was proposed to be due to alterations in cauda epididymal milieu and androgen deficiency [56]. 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 [57]. *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 0.5 g per mL, respectively [58]. The benzene extract of *Ocimum sanctum* leaves when administered to male rats at a dose of 250 mg per kg b.w. 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 [59]. Although several studies have demonstrated the noxious effects of various plants and/or their products on sperm motility and morphology, the mechanism(s) involved in contributing these effects are poorly understood. Plants may induce

deterioration of sperm functions either due to the direct action of the active ingredients of plants on sperm cells and/or by targeting Leydig cells or Sertoli cells and the associated functions. The later part of this review will discuss about the plants that are reported to target Sertoli cell/Leydig cell functions.

3.2 Plants that affect Sertoli cells

The somatic Sertoli cells have a very important role in controlling the process of spermatogenesis throughout the adult life. They foster the developing germ cells by regulating the flow of vital nutrients and growth factors through the tight junctions [60]. In addition, the rate and quality of spermatogenesis are determined by the number of Sertoli cells present [61]. Therefore, any agent that damages the viability and function of Sertoli cells may have profound effects on spermatogenesis. Chloroform extracts of the seeds of *Carica papaya* when administered to male albino rats and langur monkeys at a dose of 50 mg per kg b.w. 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 [62, 63]. Intra-epithelial vacuoles of varying sizes in the cytoplasm of Sertoli cells and disturbances in the co-existence of Sertoli–Sertoli/ Sertoli–germ cell were observed when *Azadirachta* leaf powder was administered to albino rats for 48 days [64]. An *in vitro* study on the effects of mutiglycosides of *Tripterygium wilfordii* and gossypol acetate at a dose of 3.0 or 30 μg mL^{-1} on primary cultures of Leydig and Sertoli cells resulted in the complete death of both the cell types within 24 h of exposure. It was concluded that Sertoli cells are more sensitive than Leydig cells to both the compounds [65]. Enlargement of the Sertoli cells was observed when 400 mg of the leaf extract of *Thespesia populnea* was administered to male Swiss mice for 15 days [66]. Pure theobromine 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, signifying Sertoli cells as primary targets for theobromine toxicity [67].

Root extracts of *Barleria prionitis* (100 mg per kg b.w.), methanolic extracts of *Dendrophthoe falcata* (100 mg per kg b.w.), methanolic extracts of *Albizzia lebbeck* bark (100 mg per kg b.w.), methanolic extracts of *Tinospora cordifolia* (100 mg per kg b.w.) and ethanolic extracts of *Aegle marmelos* leaves (300 mg per kg b.w.) are reported to reduce the cross-sectional surface area of Sertoli cells when administered orally to male Wistar rats for 60 days [68–72]. There are very few studies that elucidate the molecular mechanism of action by which plants impede the functions of Sertoli cells. Sertoli cells express androgen receptors and require intratesticular testosterone for their normal development and function. It is possible that plants impair Sertoli cell functions by targeting the intratesticular testosterone production by Leydig cells and/or at the level of the hypothalamo–pituitary axis.

4 Plants that affect Leydig cells and steroidogenesis

Apart from spermatogenesis, the testis performs another important function, the synthesis of androgens that are vital in maintaining spermatogenesis. The hormonal regulation of spermatogenesis is well organized, with a feed-back mechanism involving the hypothalamus, pituitary gland and testis [73]. 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 [74]. 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.

Several studies affirm the undisputable role of gossypol in impairing testicular spermatogeneis [75, 76]. Gossypol acetic acid, a polyphenolic compound isolated from the seeds of cotton plant 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 [77]. Reduction in the levels of testosterone, LH and follicle-stimulating hormone was reported when the crude methanol extract of *Quassia amara* was administered to male albino rats [78]. 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 [79]. Ethanolic extracts of the roots of *Martynia annua* to male rats at doses of 100 and 200 mg per kg b.w. for 60 days caused Leydig cell atrophy and a significant reduction in the serum concentration of LH and testosterone [80]. 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 [71]. *Mentha piperita labiatae* (20 g L^{-1}) and *Mentha spicata labiatae* (20 g L^{-1}) herbal teas when fed to Wistar rats increased the FSH and LH levels and decreased total testosterone levels [81]. 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* [82]. The leaves of *Azadirachta indica* when administered orally at a dose of 500 mg per kg b.w. exhibited a regression and decrease in the number of Leydig cells and their nuclear diameter, indicating androgen deficiency [83]. *Carica papaya* seed extracts when administered orally at doses of 50 and 100 mg per kg b.w. for 8 weeks to sexually mature Wistar rats caused pronounced hypertrophy of pituitary gonadotrophs and degeneration of Leydig cells [84]. 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 [85].

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 [33]. 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 [86]. 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 [87]. 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 [83, 88]. Table 2 summarizes a few recent articles on plants that are reported to impair Leydig cell 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. Additional studies are warranted to understand intensely the molecular mechanisms by which plants or their active ingredients hamper steroidogenesis in various species.

5 Conclusion

The toxic effects of most of the plants on reproduction were identified while administering them for therapeutic use and/or during contraceptive research. Although a few plants have reached clinical trials, most of them failed the trails due to their toxicity or due to the irreversibility of the effects. Several plants that are reported to have beneficial effects against various ailments were later found to have harmful effects on reproduction. Future research should be directed towards studying the toxic effects of all the commonly used plants. The detailed mechanism of action of natural products in inducing reproductive toxicity should be elucidated.

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Table 2. Plants that affects steroidogenesis.

Plant name	Plant part used/extract	Dose and duration	Observed effects	References
Allium sativum	Crude extract	5%, 10%, 15% and 30%; 30 days	Reduces testosterone secretion	[33]
Aegle marmelos	Leaf and ethanol extract	200 and 300 mg $kg^{-1}day^{-1}$; 60 days	Reduces testosterone levels	[72]
Aegle marmelos	Leaf and aqueous extract	50 mg per 100 g b.w. per day; 28 days	Steroidogenesis was reduced with a reduction in germ cells in testis	[89]
Capparis aphylla	Whole plant and ethanol extract	50, 100 and 200 mg kg^{-1} day^{-1}; 18 days	Reduces steroidogenic enzymes	[96]
Garcinia cambogia	Seeds and ethanol extract	100 and 200 mg $kg^{-1}day^{-1}$; 6 days a week for 6 weeks treatment	Degeneration of the Leydig cells	[93]
Dendrophthoe falcate	Stem methanol extract	50, 100 and 200 mg kg^{-1} day^{-1}; 60 days	Decreases serum testosterone levels	[94]
Juniperus phoenicea L.	Cones and ethanol extract	400 or 800 mg kg^{-1} day^{-1}; 21 days	Decreases testosterone levels	[91]
Martynia annua	Root and ethanol extract	50, 100 and 200 mg kg^{-1} day^{-1}; 60 days	Degeneration of Leydig cells	[80]
Syzygium aromaticum L.	Flower buds and hexane	15, 30 and 60 mg kg^{-1} day^{-1}; 35 days	Reduction in the steroidogenic enzymes and testosterone levels at higher dose	[97]
Bulbine natalensis	Stem and aqueous extract	25, 50 and 100 mg kg^{-1} day^{-1}; 7 days	Decreases testosterone and progesterone at high dose	[98]
Abelmoschus esculentus	Fruit and methanolic extract	70 mg kg^{-1} day^{-1}; 28 days	Decreases serum testosterone levels	[99]
Albizia. lebbeck L	Bark and methanolic extract	100 mg kg^{-1} day^{-1}; 60 days	Decrease in Leydig cells nuclear area and number of mature Leydig cells Decreases serum testosterone levels	[70]
Curcuma longa L.	Crude alcoholic extract	500 mg kg^{-1} day^{-1}; 60 days	Decreases serum testosterone levels	[95]
Psoralea corylifolia	Crude extract	10 g kg^{-1}—single dose; 3, 7 days after treatment	Decreases serum testosterone levels	[100]
Chromolaena odoratum L.	Leaves and aqueous extract	250 and 500 mg kg^{-1} day^{-1}; 14 days	Decreases serum testosterone levels	[108]

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