



Oxidative Stress and Male Infertility: Role of Herbal Drugs

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Abstract

Infertility is a universal health problem affecting 15% of couples, out of which 20–30% cases are due to male infertility. The leading causes of male infertility include hormonal defects, physical reasons, sexual problems, hazardous environment, stressful lifestyle, genetic factors, epigenetic factors, and oxidative stress. Various physiological functions involve reactive oxygen species (ROS) and nitrogen species at appropriate levels for proper smooth functioning. ROS control critical reproductive processes such as capacitation, acrosomal reaction, hyperactivation, egg penetration, and sperm head decondensation. The excessive free radicals or imbalance between ROS and endogenous antioxidant

enzymes damages sperm membrane by inducing lipid peroxidation causing mitochondrial dysfunction and DNA damage that eventually lead to male infertility. Numerous synthetic products are available in the market to treat infertility problems, largely ending in side effects and repressing symptoms. Ayurveda contains a particular group of Rasayana herbs, called *vajikarana*, that deals with nourishment and stimulation of sexual tissues, improves male reproductive vitality, and deals with oxidative stress via antioxidant mechanism. The present study aims to describe oxidative stress and the role of herbal drugs in treating male infertility.

Keywords

Oxidative stress · Vajikarana · Antioxidant · Herbal drugs · Male infertility

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9.1 Introduction

Infertility (or subfertility) is defined as the failure of couples to establish a clinical pregnancy after 1 year of consistent unprotected sexual intercourse (Zegers-Hochschild et al. 2017). According to the WHO, about 50–80 million people suffer from infertility worldwide. Globally, infertility affects approximately

13–15% of all couples, and out of all cases of infertility, the male is responsible in 20–30% of cases. Male infertility is widespread not only in developing countries but also in developed ones. The exact figure is unpredictable because majority of cases are not registered often due to the scarcity and high cost of medical resources and treatment, respectively, sociocultural phobia, humiliations, etc. (Leslie et al. 2021). The leading causes of male infertility include hormonal defects, physical reasons like blockage of the ejaculatory pathway, sexual problems like erectile dysfunction or impotence, hazardous environment and stressful lifestyle, genetic factors like chromosomal abnormalities, single-gene mutations, and epigenetic factors (Babakhanzadeh et al. 2020; Iammarrone et al. 2003). Such factors can be broadly categorized (Table 9.1) in primary gonadal and hypothalamic-pituitary disorders which can be both, congenital and acquired, and disorders of sperm transport (post-testicular).

9.2 Pathophysiological Factors of Male Infertility

9.2.1 Hormonal Defects

The right concentration of male hormones is required for the proper functioning of the testes and sexual development. They are produced by hypothalamic-pituitary-gonadal axis. Decrease or lack of release of gonadotropic-releasing hormone (GnRH) by the brain produces less testosterone and reduced sperm production, resulting in disorders like Kallmann syndrome (Monaco et al. 2015). Furthermore, the insufficient release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary gland causes failure to stimulate the testes, decrease in testosterone and sperm production, and defects in spermatogenesis (Wdowiak et al. 2014). Elevated prolactin concentrations also reduce sperm production and impotence (Marrag et al. 2015). The treatment of such disorders includes the use of

Table 9.1 Causes of men infertility

A. Primary gonadal disorders	
<i>Congenital</i>	Y-chromosome deletions, Klinefelter syndrome, cryptorchidism, congenital anorchia, Noonan syndrome, myotonic muscular dystrophy, sickle cell disease, varicocele, androgen insensitivity syndrome, 5 α reductase deficiency
<i>Acquired</i>	Orchitis (mumps pyogenic, traumatic), malignant neoplasm (germ cell, leukemia, lymphoma), trauma, torsion, castration, systemic illness (renal failure, liver cirrhosis or other hepatic disorders, cancer, etc.), retroperitoneal fibrosis, drugs (cytotoxic drugs, alkylating agents, alcohol, marijuana, anti-androgens, histamine receptor antagonists, etc.), environmental toxins (dibromochloropropane, carbon disulfide, cadmium, lead, mercury, etc.), irradiation, hyperthermia
B. Hypothalamic-pituitary disorders	
<i>Congenital</i>	Congenital GnRH deficiency (Kallmann syndrome), hemochromatosis, multiorgan genetic disorders (Prader-Willi syndrome, Laurence-Moon-Biedl syndrome, familiar cerebral ataxia)
<i>Acquired</i>	Pituitary and hypothalamic tumors and cysts, infiltrative disorders (sarcoidosis, histiocytosis, tuberculosis), trauma, postsurgical, post-irradiation, vascular (infarction, aneurysm), hormonal (hyperprolactinemia, androgen, estrogen, and cortisol excess), drugs (opioids and psychotropic drugs, GnRH agonists or antagonists, etc.)
<i>Systemic</i>	Chronic illnesses, obesity, nutritional deficiencies
C. Disorders of sperm transport (post-testicular)	
Epididymal dysfunction (due to drugs, infection, etc.), abnormalities of the vas deferens (congenital absence, Young's syndrome, infection, vasectomy), ejaculatory dysfunction (spinal cord disease, autonomic dysfunction, premature ejaculation)	

GnRH gonadotropin-releasing hormone

long-term hormonal therapy involving the use of sex steroids (testosterone injections) or gonadotropin-releasing hormone (Schagen et al. 2016). But such treatment regimens lead to other complications such as diabetes mellitus, heart diseases, etc.

9.2.2 Physical Reasons and Sexual Problems

Various physical or anatomical changes, viz., blockage of the ejaculatory pathway, enlargement of sperm vessels (varicocele), testicular torsions, impaired sperm circulation, genital tract infection, obstruction in semen flow by damaged urinary bladder sphincter (retrograde and antegrade ejaculation), and its movement along the path, also lead to infertility. Erectile dysfunction, early ejaculation, and inability to ejaculate are problems related to intercourse that led to the inability of couples to establish a clinical pregnancy or indirectly cause infertility (Babakhanzadeh et al. 2020; Sun et al. 2018).

9.2.3 Lifestyle and Environment

Changing lifestyle or more of a sedentary lifestyle has been considered as one of the major reasons for infertility. Excessive consumption of alcohol, high-fat diet, smoking, prolonged sitting, poor nutrition, repeated use of drugs, high stress (leading to anxiety, depression, and other psychological disturbances), pollution in the air, etc. causes degradation of sperm, reduce sperm count and motility, and increase the chances of impotency in men. Exposure to harmful radiation, hazardous substances, and high temperature can also lead to infertility (Katib 2015; Mustafa et al. 2019).

9.2.4 Genetic and Epigenetic Factors

Among various genetic factors, chromosomal abnormalities and single-gene mutation are one of the leading causes of infertility. Chromosomal defects like microdeletions and disarrangement in chromosome fragments can cause dysfunction in spermatogenesis. Genetic mutations like a defect in ciliary function, congenital bilateral absence of the vas deferens (CBAVD), and testicular dysgenesis syndrome are also responsible for male infertility.

Furthermore, epigenetic modifications, like acetylation, methylation, hypermethylation, etc., of the germ cells cause alteration in the expression of the genes that control spermatogenesis and other characters of sperm, leading to the deficiency in semen parameters (Boissonnas et al. 2013; Iammarrone et al. 2003; Neto et al. 2016; Stouffs et al. 2014).

9.2.5 Oxidative Stress (OS)

Free radicals, of both oxygen and nitrogen, can act as friend and foe for our reproductive system. These free radicals are generated as by-products of normal physiological effects in different cells including sperm cells. When their concentration is at appropriate levels, they act as friends and help in critical processes like capacitation, hyperactivation, egg penetration, and sperm head decondensation (Barati et al. 2020). Under normal conditions, human sperm generates free radicals (ROS and RNS) by numerous pathways, which instigate tyrosine kinases, and cyclic adenosine monophosphate (cAMP) that increases tyrosine phosphorylation levels (Wagner et al. 2018). Free radicals such as hydrogen peroxide, superoxide anion (O_2^-), nitric oxide, nicotinamide adenine dinucleotide (NADH), and nicotinamide adenine dinucleotide phosphate (NADPH) activate phosphorylation of protein tyrosine (p81 and p105). Furthermore, these phospho-tyrosine proteins are overexpressed during sperm capacitation. In the female genital tract, sperm gets hyperactive due to tyrosine phosphorylation in its tail region that further aids in the acrosome reaction which eventually helps the sperm to get attached to the zona pellucida (Fig. 9.1).

It has also been observed that apart from phosphorylation of tyrosine proteins, free radicals also induce sperm capacitation which is a compulsory process for fertilization (Tremellen 2008; Thundathil et al. 2003), while antioxidants like catalases, superoxide dismutase (SOD), and NADPH oxidase inhibit sperm capacitation (Takeshima et al. 2021). Moreover, Ca^{2+} also stimulates cyclic adenosine monophosphate

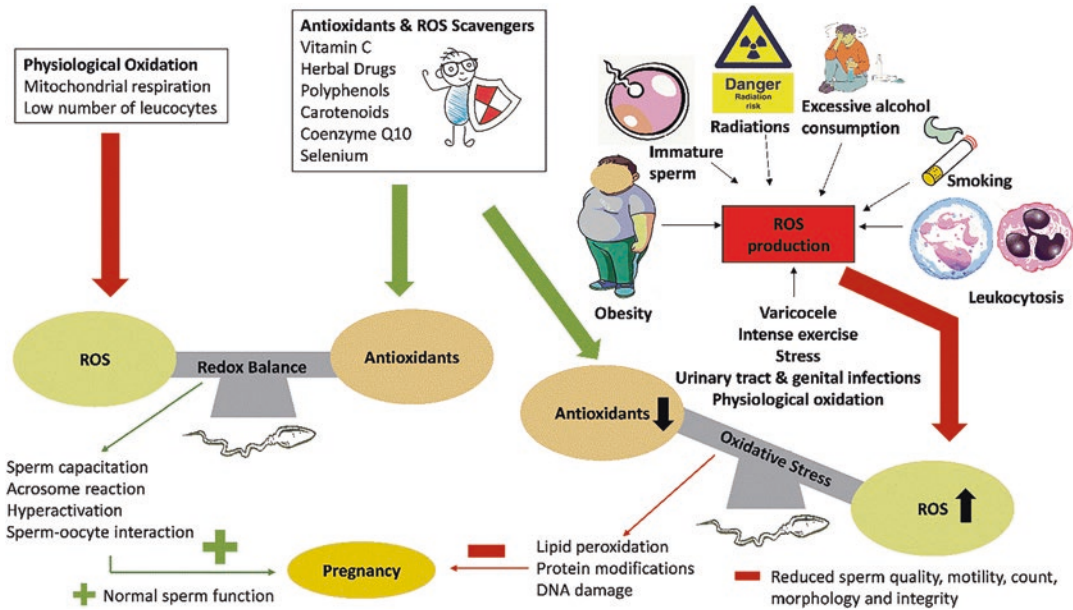


Fig. 9.1 ROS and OS in male infertility

(cAMP) during the capacitation process, which further regulates the superoxide anion generation and leads to rise in phosphorylation of p81 and p105 (Leclerc et al. 1998). Moreover, activities of adenylyl cyclase (AC) and phosphodiesterases (PDEs) also regulate the production and degradation of cAMP, thereby regulating its levels (Lefièvre et al. 2002). The above literature showed that many intricate pathways are involved during sperm capacitation and acrosomal reaction, which require ROS for functioning.

Human semen contains antioxidants, which can be categorized into enzymatic and nonenzymatic antioxidants, in the seminal plasma to protect sperm from OS. The former group includes glutathione peroxidase (GPX), SOD, glutathione S-transferase, catalase, etc., whereas the latter one includes ascorbic acid, alpha-tocopherol, coenzyme Q10, myoinositol, astaxanthin, taurine, transferrin, L-carnitine, urate, melatonin, and lactoferrin. Under normal conditions, these antioxidants scavenge free radicals and preserves redox homeostasis in the sperm so that the sperm delivers the healthy and intact DNA to the oocyte during fertilization. Disproportion amid production and scavenging or neutralization of these

free radicals heads to oxidative stress and acts as foe for our system (Fig. 9.1).

Various conditions that lead to such an oxidative stress include both endogenous [immature sperm (Sabeti et al. 2016), leukocytospermia (Fariello et al. 2009), metabolic syndromes, etc.] and exogenous [smoking (Aboulmaouahib et al. 2018), alcohol (Akang et al. 2017), radiations, environmental causes, etc.] factors. Free radicals during OS also have an indirect effect on the production of male reproductive hormones which leads to increased production of immature sperm (Barati et al. 2020). Immature sperm are the ones that have excess cytoplasmic residues, and because of this, they develop mitochondrial dysfunction which eventually leads to depletion of energy required for sperm motility and also produce excess of ROS (Sabeti et al. 2016). Leukocytes, though few, are found in normal semen, but when their number is more than 10,00,000/ml, the condition is known as leukocytospermia. Infection or inflammation of the reproductive tract is the main reason for leukocytospermia which also increase the levels of ROS in the semen leading to OS (Fariello et al. 2009). OS leads to various pathological and biochemical changes in sperm such as damage to axoneme,

decrease in ATP levels, and generation of 4-hydroxynonenal and malondialdehyde (MDA) which further initiates lipid peroxidation and DNA and mitochondrial damage (Fang and Zhong 2019).

During OS, ROS affects the double bonds present in the structure of the membrane lipids, thereby initiating lipid peroxidation of sperm membrane that changes its structure, dynamics, and fluidity (Gaschler and Stockwell 2017). ROS also cause oxidation of sulfhydryl groups, along with the change in membrane structure, which also decrease the sperm motility (Saleh and Agarwal 2002). Moreover, lipid peroxidation of membranes also initiates a series of reduction-oxidation reactions of mutagenic and genotoxic electrophiles causing sperm damage (Bui et al. 2018). Furthermore, hydrogen peroxide (H₂O₂), a non-radical ROS, easily passes through the sperm membrane and enters into its cytoplasm. It inhibits glucose-6-phosphate dehydrogenase (G6PD) enzyme, thereby reducing the levels of NADPH, a molecule required for the activity of glutathione peroxidase, an antioxidant enzyme (Said et al. 2004). Due to the reduction of NADPH, the activity of glutathione peroxidase also reduces, thus weakening the antioxidant defense against OS (Walczak-Jedrzejowska et al. 2013). All these events eventually lead to sperm damage or sperm with poor motility. High levels of ROS also cause damage to mitochondrial membranes resulting in the activation of caspases which finally initiate apoptosis in sperm (Wagner et al. 2018).

ROS also attacks the guanine base of the DNA, thereby converting it to 8-hydroxyguanine, and under stress conditions, these further get oxidized to 8-hydroxy-2-deoxyguanosine (8-OHdG) (Noblanc et al. 2013). Glycosidase enzyme present in sperm acts on 8-OHdG and releases another base compound, thereby causing further damage to DNA which can further lead to other changes like changes in single- or double-strand fragment, DNA fragmentation, base pair configuration, etc. OS is considered as one of the major reasons for sperm DNA fragmentation (Muratori et al. 2015). Such changes eventually lead to genomic instability that affects the production or genesis of sperm (Barati et al. 2020).

Numerous synthetic products are available globally to treat infertility problems, largely ending in side effects and repressing only symptoms. Thus, once again, the focus has been shifted on herbal and Ayurvedic treatments for curing male reproductive problems (Dutta and Sengupta 2018). Ayurveda contains a particular group of Rasayana herbs, called *vajikarana*, that deals with nourishment and stimulation of sexual tissues and improves male reproductive vitality. In Sanskrit, *vaji* means “horse,” and *karana* means “power,” giving the idea of horse’s strength. *Vajikarana* herbs revitalize the seven dhatus (body elements) and restore equilibrium in the body. They act on the hypothalamus and limbic system and modulate the neuroendocrine-immune system. Besides, another category of herbs known as *shukrala* increases spermatogenesis and can improve sperm count, quality, and motility (Dalal et al. 2013). The herbs can also be classified on the basis of their beneficial effects on the male reproductive health, such as herbs that (i) enhance or stimulate semen production (*Mucuna pruriens*, *Asparagus racemosus*, etc.), (ii) improve semen quality (*Vetiveria zizanioides*, *Sesamum indicum*, etc.), (iii) revitalize ejaculatory functions (*Strychnos nux-vomica*, *Cannabis sativa*, etc.), (iv) improve nourishment and ejaculatory performance (*Cinnamomum tamala*, *Asparagus racemosus*, etc.), and (v) increase libido (*Asparagus racemosus*, *Withania somnifera*, etc.) (Chauhan et al. 2014).

9.3 Some Common Plants Used to Treat Male Infertility

9.3.1 *Nigella sativa* (Family: Ranunculaceae)

Nigella sativa, also known as *black cumin*, *black seed*, *Habbatus sauda*, and *kalonji*, is a medicinal plant widely used traditionally in Ayurveda, Unani Tibb, and Siddha systems of medicine. In Islamic literature, it is considered as *Tibb-e-Nabawi* (Prophetic medicine) and is believed to be the most extraordinary form of healing medicine (Shuid et al. 2012; Yimer et al. 2019). It has

been extensively used as anti-inflammatory, spasmolytic, bronchodilator, immunomodulator, antioxidant, antidiabetic, antihypertensive, liver tonics, diuretics, digestive, and analgesics and in skin disorders (Yimer et al. 2019). The main active compounds isolated from the seeds are thymoquinone (30–48%), thymohydroquinone, dithymoquinone, p-cymene (7–15%), carvacrol (6–12%), 4-terpineol (2–7%), anethole (1–4%), longifolene (1–8%), α -pinene and thymol, and alkaloids nigellimine and nigellimine-N-oxide, nigellidine, and nigellicine (Fig. 9.2). Furthermore, the seeds also contain a water-soluble pentacyclic triterpene alpha-hederin, used in treating cancer; unsaturated fatty acids, mainly dihomolinoleic (10%), linoleic (50–60%), eicosadienoic (3%), and oleic acid (20%); saturated fatty acids mainly palmitic and stearic acid; and α -sitosterol (44–54%) followed by stigmasterol (6–20%). Most of the beneficial properties of *N. sativa* are attributed due to the presence of thymoquinone (Fig. 9.2), the major bioactive component of the essential oil (Ahmad et al. 2013).

Alcoholic extract of *N. sativa* increases the production of sperm cells, increases sperm motility, and improves epididymal sperm reservation, gonadotropin content, testosterone levels, and fertility indexes in male rats. Black cumin seeds induce a rise in spermatogenesis, levels of hormones like testosterone and luteinizing hormone (LH), and an increase in the weight of reproductive organs (Parandin et al. 2012). The treatment with seeds also increased the sperm count, fertility index, and overall characters of sperm. A randomized, double-blind, placebo-controlled clinical study conducted on 68 infertile Iranian men revealed that 2.5 mL of black seed oil twice daily for 2 months significantly improved the sperm count and motility (Kolahdooz et al. 2014). Thymoquinone (TQ) at 50 mg/kg p.o. protected the testicular tissue by alleviating inflammation and apoptosis and by restoring the average balance of sex hormones (Alyoussef and Al-Gayyar 2016). Moreover, TQ treatments significantly increase testosterone level in serum, testicular GSH, and

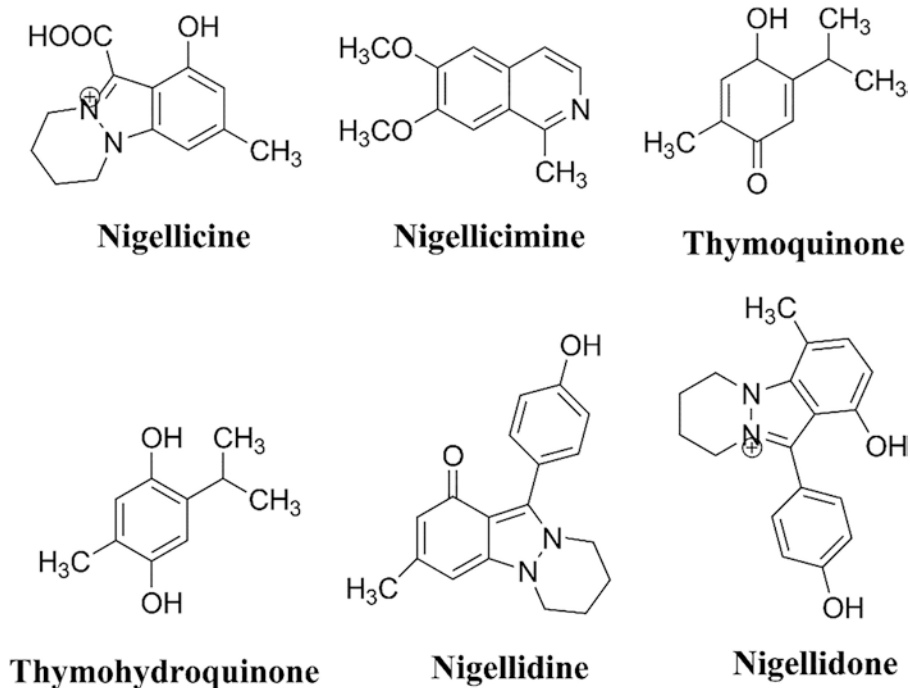


Fig. 9.2 Chemical constituents of *Nigella sativa*

SOD activity and lower MDA and nitric oxide activity when compared with the control group (Fouad et al. 2014). It also increases the mean volumes of testis and seminiferous tubules, count of spermatogenic cells, and Leydig cells (Darand et al. 2019; Yimer et al. 2019). These studies indicate that *N. sativa*/TQ can be used as an alternate source for developing natural aphrodisiac agents.

9.3.2 *Mucuna pruriens* (Family: Fabaceae)

It is also known as *velvet bean* or *Kapikacchu* or *Konch* and is an underutilized wild legume that is spread throughout the tropical and subtropical regions across the globe. It is largely utilized as fodder, forage, and green manure crop. It is mentioned in Rigveda as a *Balavardhaka aushadhi* (strength-promoting medicine) (Pandey and Lalitha 2018). The plant is used to treat impotence and diabetes mellitus, whereas the seeds are used to manage Parkinson's, diabetes, arthritis, atherosclerosis, and analgesic, antipyretic, and antioxidant activities. It is a common ingredient in itching powder due to the presence of 5-hydroxytryptamine (serotonin) in the seed pods which causes severe itching (Yadav et al. 2017).

The seeds of velvet beans contain beta-sitosterol, 3-(3,4-dihydroxyphenyl)-L-alanine (L-DOPA), gallic acid, and glutathione. It also contains alkaloids like mucunadine, mucunine, pruriendine, and pruriene, 3-methoxy-1,1-dimethyl-6,7-dihydroxy-1,2,3,4-tetrahydroquinoline, and 3-methoxy-1,1-dimethyl-7,8-dihydroxy-1,2,3,4-tetrahydroquinoline (Fig. 9.3). A β -carboline alkaloid, 6-methoxyharmaline, has also been isolated from leaves. Seeds also contain oil rich in palmitic, stearic, oleic, and linoleic acids (Sathiyarayanan and Arulmozhi 2007; Yadav et al. 2017).

Alkaloids present in seeds of *M. pruriens* are considered as main bioactive compounds as they trigger spermatogenesis and increase the weight of the testes in male rats (Saksena and Dixit 1987). The plant escalates mounting frequency, duration to ejaculate, and intromission frequency

in male rats (Amin et al. 1996; Suresh et al. 2010). It also significantly recovers the spermatogenic loss induced by ethinyl estradiol administration in male rats. The plant showed beneficial effects by reducing ROS, regulating apoptosis, and increasing germ cell number. Aphrodisiac activity of *M. pruriens* can also be due to L-DOPA, the principal constituent of the plant, which accounted for pro-spermatogenic properties (Singh et al. 2013). The L-DOPA metabolite, dopamine, may further stimulate the hypothalamus and anterior pituitary to secrete GnRH, FSH and LH, which improves testosterone synthesis in the Leydig cells of the testicles (Sriraman et al. 2003). The ethanolic extract of seeds exhibited noticeable improvement in sexual potency, libido, sperm parameters, and endocrine levels (Suresh and Prakash 2012). *M. pruriens* improves semen quality by attenuating OS-induced lipid peroxidation in the seminal vesicles and reinstating GSH, ascorbic acid, catalase, and SOD levels (Ahmad et al. 2008; Shukla et al. 2010). The plant also raises LH, adrenaline, testosterone, noradrenaline, and dopamine levels and lowers FSH and prolactin levels in infertile men. It also improves steroidogenesis and semen quality in infertile males (Ahmad et al. 2008). Many drugs like "Tentex forte," "Speman," and "Confido" by Himalaya Drug Company contain *Mucuna* for sexual well-beings (Bhagwati and Singh 2017).

9.3.3 *Asparagus racemosus* (Family: Asparagaceae)

Asparagus racemosus, commonly known as *Shatavari* or *Shatavar* or *Shatmul*, is an ingredient of *Vajikarana Rasayana* in Ayurveda for its aphrodisiac role. It acts as a vitalizer and regulates hormone imbalance. The primary chemical constituents present in the plant are steroidal saponins, shatavarin I to IV, sarsasapogenin, adscendin (A, B, C), and asparanin (A, B, C); alkaloid, asparagine; isoflavones, 8-methoxy-5,6,4'-trihydroxyisoflavone-7-O- β -D-glucopyranoside; flavonoids, quercetin and rutin (Fig. 9.4); and sterols, sitosterol, 4,6-dihydroxy-2-O-(2'-hydroxyisobutyl). Unani traditional sys-

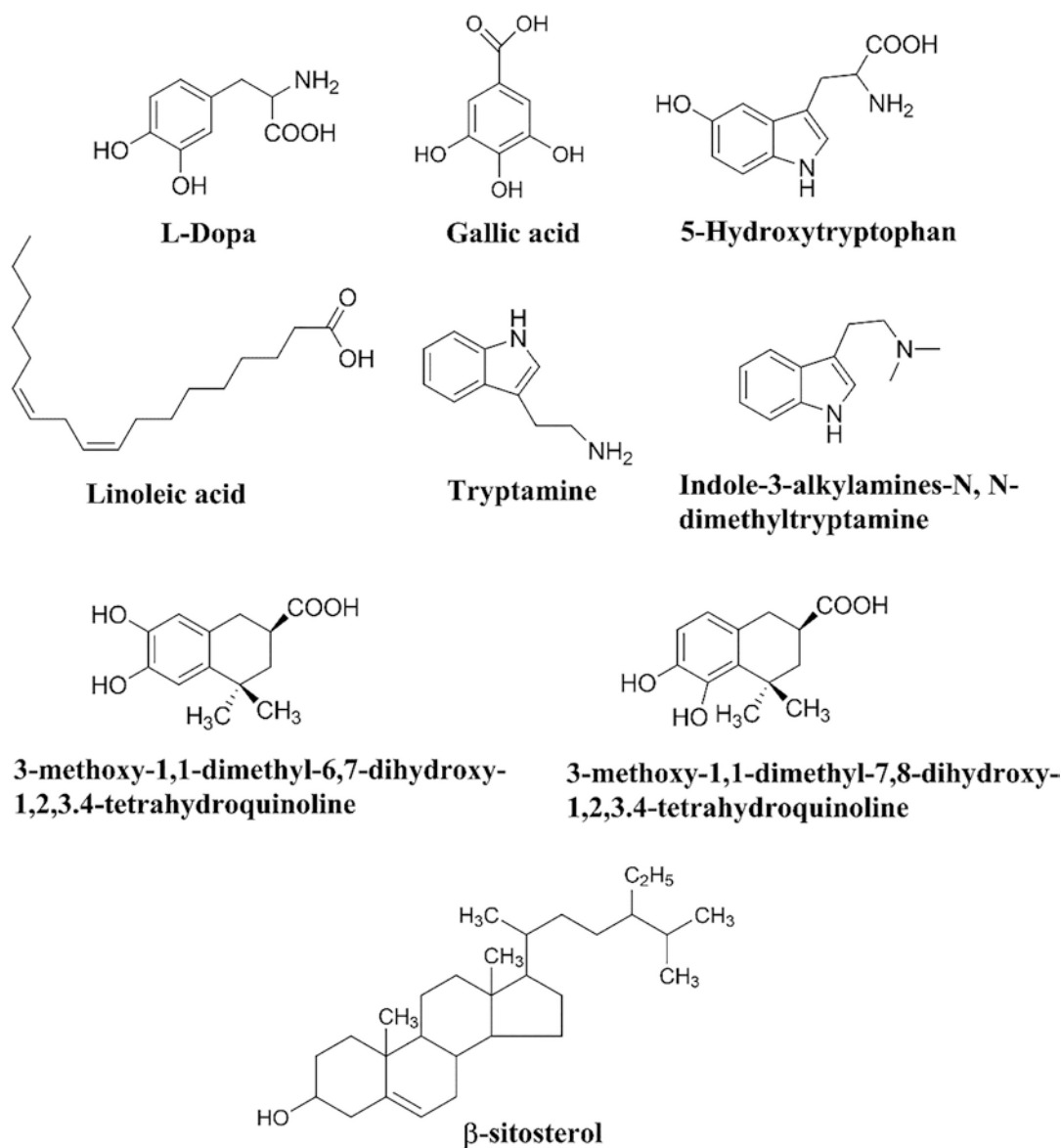
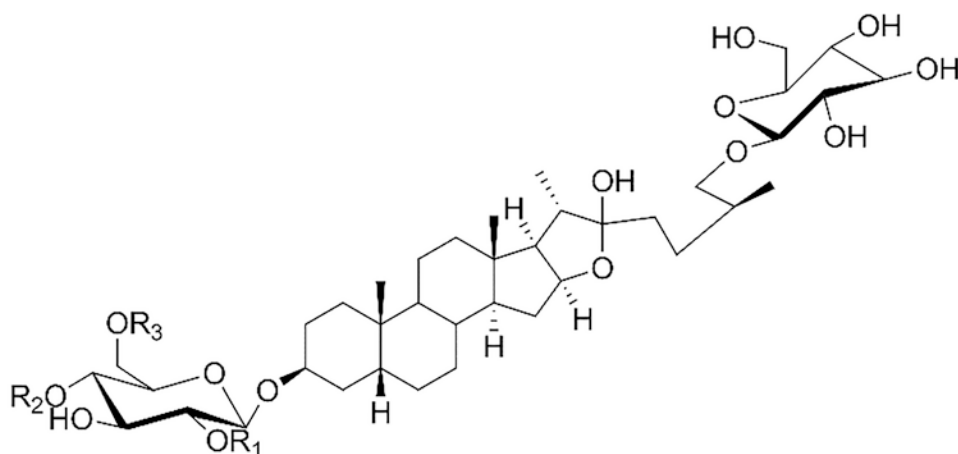


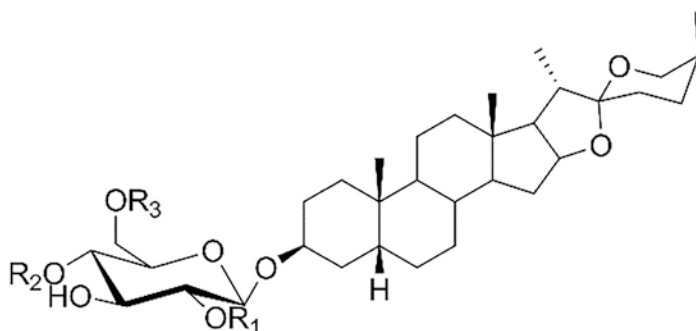
Fig. 9.3 Chemical constituents of *Mucuna pruriens*

tem of medicine used shatavari as *Mwallide mani* (ovulation-inducing), *Mwallide labn* (galactagogue), *Mugallize mani* and *Muqawwie bah* (aphrodisiac), *Dafe jiryan* (prevent spermaturia), *Dafe sailanur rehm* (prevent leucorrhoea), *Muqawwie qalb* (cardiac tonic), and *Muhallile warm* (anti-inflammatory) (Shameem and Majeedi 2020). The cooling property of the herb controls *pitta* in the small intestine and balances

the heating effect of herbs like garlic, onion, and ashwagandha, which improve sperm count. The effect of Shatavari on *pitta* also helps in preventing the sperm damage, thereby improving/maintaining the sperm count. It can also be given in combination with Brahmi (*Centella asiatica* also known as gotu kola) to boost libido and benefit in overcoming emotions like anger and irritability (Dutta and Sengupta 2018).



Shatavarin I (or Asparoside B): $R_1 = \beta\text{-D-Glc}$, $R_2 = \alpha\text{-L-Rha}$, $R_3 = \text{H}$



Shatavarin IV (or Asparinin B): $R_1 = \beta\text{-D-Glc}$, $R_2 = \alpha\text{-L-Rha}$, $R_3 = \text{H}$

Shatavarin V : $R_1 = \alpha\text{-L-Rha}$, $R_2 = \beta\text{-D-Glc}$, $R_3 = \text{H}$

Shatavarin VIII : $R_1 = \beta\text{-D-Glc}$, $R_2 = \alpha\text{-L-Ara}$, $R_3 = \beta\text{-D-Glc}$

Shatavarin IX : $R_1 = \beta\text{-D-Glc}$, $R_2 = \beta\text{-D-Glc}$, $R_3 = \text{H}$

Shatavarin X : $R_1 = \beta\text{-D-Glc}$, $R_2 = \beta\text{-D-Glc}$, $R_3 = \text{H}$

Fig. 9.4 Chemical constituents of *Asparagus racemosus*

Shatavari root extract significantly increased the number of mounts and mounting frequency as well as mating performance in adult male albino rats (Mishra et al. 2010; Wani et al. 2011). The plant has shown to enhance sexual activity and treat numerous sexual disorders like lack of sexual desire, erectile failure, and premature ejaculation in males. Moreover, the aqueous extract of *A. racemosus* rich in steroidal saponins and fructo-oligosaccharides restores the sexual functions

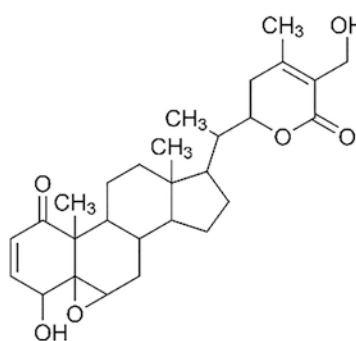
deteriorated by alloxan or streptozotocin treatment and can be used to treat sexual dysfunctions related to diabetes due to hyperglycemia (Thakur et al. 2009). Shatavari (at 100 mg/kg dose) along with three other herbs increases sperm count and nitric oxide release and improves penile erection in male albino rats (Thakur et al. 2011). A herbo-mineral preparation including Shatavari and Gokshura (50 g each) along with 5 g of Anhrka bhasma has shown to increase the production and

quality of sperm, thereby improving the fertility of a 28-year-old male (Kumar and Venkatesh 2020).

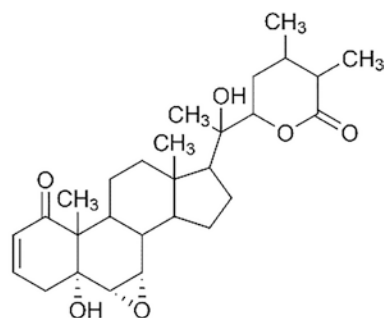
9.3.4 *Withania somnifera* (Family: Solanaceae)

Withania somnifera, also known as *Ashwagandha*, *Indian ginseng*, and *winter cherry*, is a well-known Ayurvedic *Rasayana* drug widely used for increasing energy and longevity (Winters 2006).

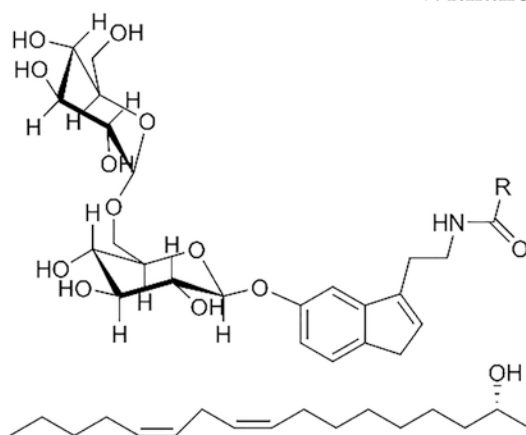
The plant has been reported for its effects against inflammation, as an antioxidant, immunomodulating, antistress, memory enhancer, and anticonvulsant activities. Phytochemical studies on *Ashwagandha* revealed the presence of various steroidal lactones (collectively known as withanolides) and alkaloids (withanine, somniferine, somnine, somniferinine). The prime withanolides isolated from the plant include withaferin A, withanone, withanolide A–Q, sitoindoside VII–X, and withanamides A–I (Fig. 9.5) (Mirjalili et al. 2009; Singh et al. 2010).



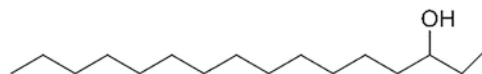
Withaferin A



Withanolide A



WA



WC

R consisting of Withanamide A (WA) and Withanamide C (WC)

Fig. 9.5 Chemical constituents of *Withania somnifera*

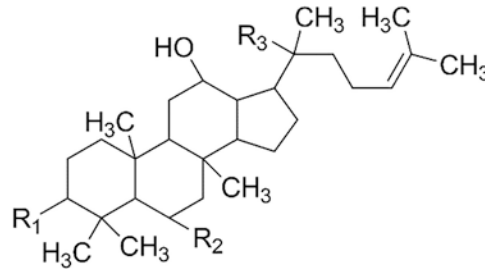
Studies exhibited that ashwagandha roots increase sperm motility, semen volume, and sperm count, stabilize testosterone production in oligospermic male, and, therefore, it can be used as a valuable treatment for infertility (Pathak et al. 2020). The plant acts via oxidative and non-oxidative process to exert its effect on male fertility. In oxidative mechanism, *W. somnifera* maintains antioxidant enzymes and the cofactors responsible for antioxidant activity. Non-oxidative mechanism includes regulation of the hypothalamus-pituitary-gonadal axis and hypothalamus-pituitary-adrenal axis for the proper functioning of reproductive organs. It regulates endocrine homeostasis, reduces the stress response, and normalizes the cortisol levels to improve male fertility (Sengupta et al. 2018). Moreover, the roots of the plants hold considerable amounts of lactate and lactate dehydrogenase (LDH) which stimulate Krebs cycle and increase ATP and cAMP levels, improving sperm concentration, quality, and motility (Gupta et al. 2013; Teixeira and de Araujo 2019).

W. somnifera exhibited enhancement in spermatozoa factors in males who have idiopathic infertility (Azgomi et al. 2018). *W. somnifera*, along with *Cynomorium coccineum*, showed testosterone-like effect and influence spermatogenesis in the seminiferous tubules of immature rats (Abdel-Magied et al. 2001). Treatment with the plant in infertile males has also improved semen quality by inhibiting lipid peroxidation, restoring antioxidant enzymes, increasing testosterone and LH levels, and reducing FSH and prolactin levels (Ahmad et al. 2010). Moreover, withanone exhibited GABA-mimetic action which regulates gonadotropin-releasing hormone (GnRH) at cellular levels which support the claims of Ashwagandha extracts in improving sexual function and testosterone production in male rats (Kataria et al. 2015). Some of the market formulations for male infertility problems, containing *W. somnifera*, by Himalaya Drug Company, are “Speman,” “Himplasia,” “Confido,” and “Tentex Forte.”

9.3.5 *Panax ginseng* (Family: Araliaceae)

Ginseng is one of the ancient herbs used in traditional Chinese medicine. The most common varieties of ginseng are Asian ginseng (*Panax ginseng*), American ginseng (*Panax quinquefolius*), and Japanese ginseng (*Panax japonicus*) (Leung and Wong 2013). Based on their structural differences, the plant consists of three tetracyclic dammarane triterpenoid saponin glycosides (called as ginsenosides): panaxadiols (e.g., Rb1-Rb3, Rc, Rd., Rg3, Rh2, and Rs1), panaxatriols (e.g., Re, Rf, Rg1–2, and Rh1), and oleanolic acid derivatives (e.g., Ro) (Fig. 9.6). Ginseng has been used mainly as a tonic to rejuvenate fragile bodies and restore proper metabolism in the body. It possesses antioxidant, anti-apoptotic, anti-inflammatory, immunostimulatory activities and showed beneficial effects on aging and neurodegenerative diseases. The plant and its various chemical constituents reduce lipid peroxidation, maintain cellular ATP levels, and inhibit excitotoxicity and Ca²⁺ over-influx into neurons (Choudhary et al. 2013).

Ample studies have exhibited the importance of ginseng in boosting of sex hormone levels, sperm numbers, and testicular antioxidants, restoring Leydig cells, and improving spermatogenesis and sperm motility (Kopalli et al. 2015; Ku et al. 2020). Many ginsenosides especially Rg1 (10 mg/kg) help in treating erectile dysfunction by inducing synthesis of nitric oxide (NO) in endothelial cells by glucocorticoid receptor-dependent, non-genomic mechanisms. NO release causes smooth muscle relaxation which allows more blood to enter into the corpus cavernosum, causing an erection (Leung and Wong 2013). Experimental models revealed that the intake of *Panax ginseng* (5%), ginsenoside Rg1 (10 mg/kg), and ginsenoside Rb1 (10 µg/kg) increases serum testosterone levels, improves copulatory behavior, and increases LH secretion (Fahim et al. 1982; Tsai et al. 2003; Wang et al.



Ginsenoside Rb₁: R₁ = OGlc(2-1)Glc, R₂ = H, R₃ = OGlc(6-1)Glc

Ginsenoside Rb₃: R₁ = OGlc(2-1)Glc, R₂ = H, R₃ = OGlc(6-1)Xyl

Ginsenoside Rc: R₁ = OGlc(2-1)Glc, R₂ = H, R₃ = OGlc(6-1)Araf

Ginsenoside Rd: R₁ = Glc(2-1)Glc, R₂ = H, R₃ = Glc

Ginsenoside Re: R₁ = OH, R₂ = OGlc(2-1)-Rha, R₃ = OGlc

Ginsenoside Rg₁: R₁ = OH, R₂ = OGlc, R₃ = OGlc

Fig. 9.6 Chemical constituents of *Panax ginseng*

2010). Apart from this, saponins from the stem and leaves of *P. ginseng* also reduce the oxidative stress linked with hyperthermia and heat stress. They also inhibit the activation of mitogen-activated protein kinase (MAPK) signaling pathways and the expression of apoptotic proteins (Liu et al. 2021). Rg3-enriched extract of Korean red ginseng attenuates heat stress-induced testicular damage and change in expression of sex hormone receptors that affect spermatogenesis (Kopalli et al. 2019). Long-term administration of its aqueous extract significantly delays the aging-induced testicular dysfunction by modulating the expression of enzymes that regulate oxidation, acetylation, and growth-related activities linked with spermatogenesis (Kopalli et al. 2017; Kopalli et al. 2015). It also prevents or treats psychological stress-induced male infertility by increasing antioxidant enzyme expression, sex hormone receptor expression, and functioning of spermatogenesis-related proteins (Lee et al. 2019). The plant also prevents oxidative stress and apoptosis linked with monobutyl phthalate in human Sertoli cells by increasing the expression of nuclear factor erythroid 2-related factor 2 (NRF-2), sirtuin (SIRT-1), and antioxidant enzymes (De Freitas et al. 2019).

9.3.6 *Trigonella foenum-graecum* (Family: Fabaceae)

Trigonella foenum-graecum Linn, also known as *fenugreek*, is an aromatic annual plant widely grown in Egypt, India, China, France, Spain, and Turkey. The plant comprises of active constituents, such as alkaloids (choline, trigonelline, carpaine), saponins (yamogenin, fenugrin, gitogenin, yuccagenin, tigonenin, diosgenin, neotigogenin, sarsasapogenin), flavonoids (luteolin, kaempferol, naringenin, quercetin, tricin 7-O-D glucopyranoside, iso-vitexin, vitexin), coumarins (methyl coumarin, trigocoumarin, trimethyl coumarin), steroids, and phenolics like gallic acid, catechin, chlorogenic acid, vanillic acid, and syringic acid (Fig. 9.7) (Dini 2018). The seeds contain diosgenin which is an essential precursor for synthesizing several sex hormones including testosterone and estrogen. Traditionally, fenugreek seeds were given to lactating females as a stimulant for milk production (El-Hak and Elrayess 2018).

Fenugreek seed aqueous extract improved the sperm damage caused by bisphenol by reducing MDA levels, decreasing the expression of apop-

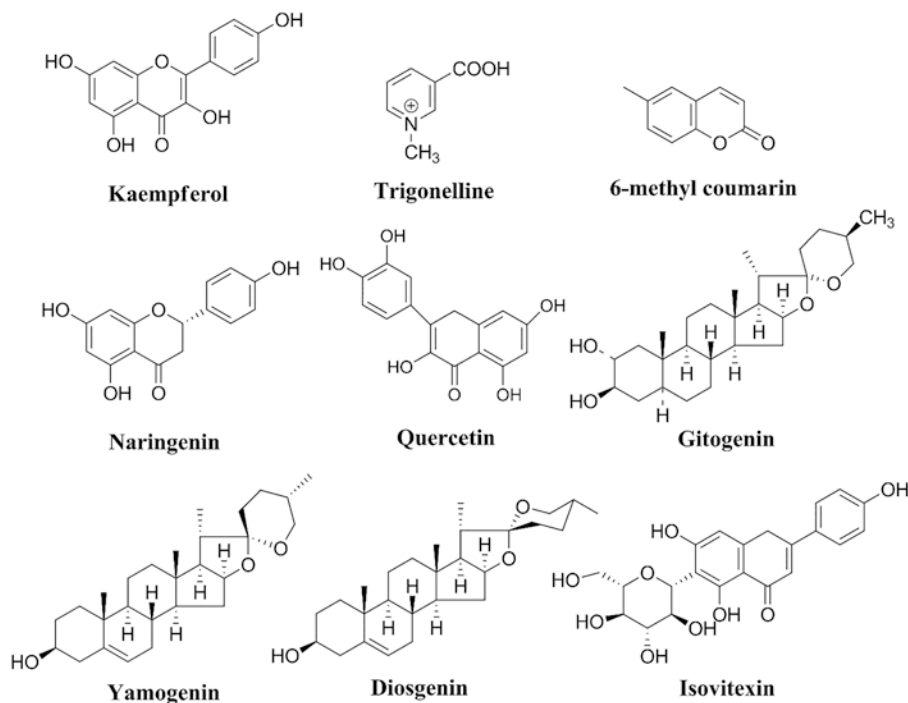


Fig. 9.7 Chemical constituents of *Trigonella foenum-graecum*

totic markers, increasing levels of antioxidant enzymes and thereby improving sperm parameters (Kaur and Sadwal 2020). Furosap™, a patented 20% protodioscin-enriched seed extract of fenugreek, increases free testosterone levels, sperm count, and sperm motility and causes significant alleviation in mood, reflex erection, and overall performance in male subjects (Maheshwari et al. 2017; Swaroop et al. 2017). Testofen®, a patented formulation by Gencor Pacific Lifestage Solutions, containing standardized extract of *Trigonella foenum-graecum* boosts male libido and maintains prolactin, testosterone, and prostate-specific antigen levels (Rao et al. 2016). A 12-week, single-site, double-blind, randomized clinical trial, on 120 male subjects, aged 43–70, showed that Testofen® effectively improved sexual health by remarkably increasing sexual desire, arousal, and testosterone levels (Rao et al. 2016). The mixed extract of fenugreek seeds and *Lespedeza cuneata* exhibited significant improvement in testosterone defi-

ciency syndrome (Park et al. 2018). Moreover, consumption of aqueous extract of fenugreek seeds improves fertility and reproductive function in male rats (Hind et al. 2017).

9.3.7 *Allium sativum* (Family: Liliaceae)

Allium sativum, also known as *garlic*, is an intensely aromatic bulb crop that is cultivated across the globe. Traditionally, garlic is used as an aphrodisiac, to relieve cough problems, prevent graying of hair, lower cholesterol, and treat eczema, rheumatism, and high blood pressure. The *Kashyapa Samhita* contains a special chapter called “Lasunkalpa Addhyaya,” which deals with the uses and pharmaceutical preparations containing garlic for treating infertility in males and females (Vaijnath and Manikrao 2018). Fresh garlic bulb contains water (65%), carbohydrates (28%), organosulfur compounds (2.3%), proteins

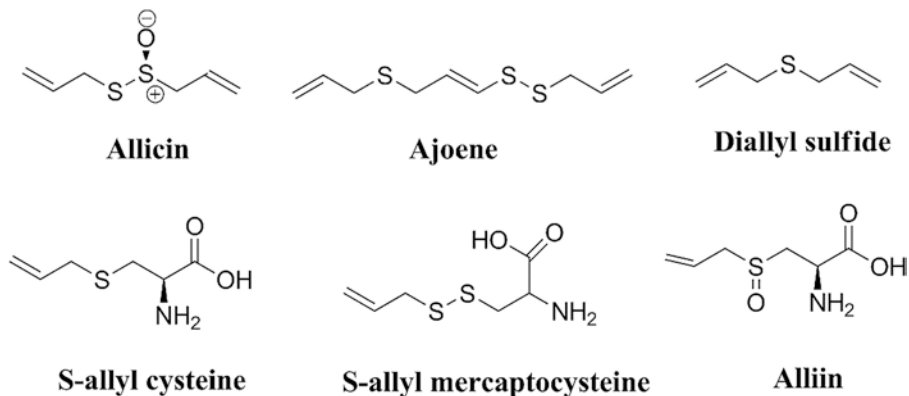


Fig. 9.8 Chemical constituents of *Allium sativum*

(2%), fiber (1.5%), amino acids (1.2%), saponins, and phenolics (Nouroz et al. 2015). Many sulfur-containing compounds include alliin, diallyl polysulfides, S-allylcysteine, diallyl sulfide, diallyl disulfide, diallyl trisulfide, S-allyl mercaptocysteine, and vinylidithiins (formed in the breakdown of allicin) (Fig. 9.8). Saponins reported from *Allium sativum* extract include proto-eruboside B; eruboside B; voghierosides A1, A2, B1, B2, C1, C2, D1, D2, E1, and E2; gitogenin 3-O-tetrasaccharide; and agigenin 3-O-trisaccharide. The phenolics isolated from garlic extract contain caffeic, ferulic, vanillic, *p*-hydroxybenzoic, and *p*-coumaric acid (Fig. 9.8) (Kuetze 2017).

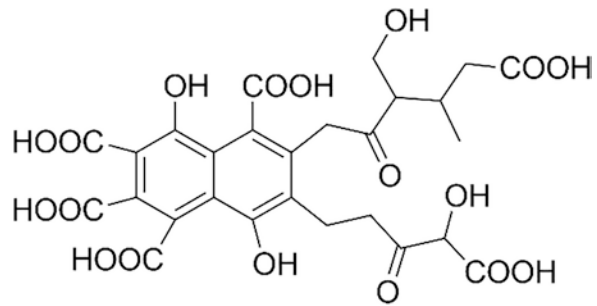
Many studies revealed the importance of garlic in the treatment of male infertility due to sulfur compounds that directly affect the uptake of CYP450 and glutathione S-transferase and protect spermatogenesis. It inhibits caspase-3 and CYP450 enzymes, which had a deleterious effect on the testicles (El-Akabawy and El-Sherif 2016). Moreover, garlic has antioxidant properties which reduce lipid peroxidation and increase fertility in men (Hammami and El May 2013). It also acts as a precursor to testosterone production, which further stimulates sexual cells and testosterone secretion from the testicles, boosts LH from the pituitary gland, and improves spermatogenesis. Garlic also inhibits reactive oxygen species and increases sperm motility and survival

(Musavi et al. 2018). It also increases the flow of blood in the testis. Fresh garlic juice (120 mg/kg) protects semen oxidation by decreasing MDA levels and increasing antioxidant activity in rat testes (Ghalehkandi 2014). The aged garlic extract (250 mg/kg, 14 days) causes an increase in sperm count, motility, testis weight, recuperation of seminiferous tubules, and decreased sperm abnormality and death via antioxidant mechanism (Nasr 2017).

Similarly, treatment with single bulb garlic (250 mg/kg) improves sperm count, sperm motility, and sperm normality in male mice with hyperlipidemia (Qadariah et al. 2020). Also, aqueous garlic extract enhances spermatogenesis and ameliorates testicular and hematological alterations induced by cadmium poisoning in male rats (Mbegbu et al. 2021). The primary protective mechanism of garlic for testicular damage is to combat oxidative stress by decreasing free radicals and increasing antioxidant parameters in the semen (Adeyemi et al. 2021; Alsenosy and El-Aziz 2019; Eric et al. 2020; Ifeoma et al. 2018; Nasr et al. 2017).

9.3.8 Shilajit (Asphaltum, Mineral Pitch)

Shilajit, also known as *salajit*, *shilajatu*, *mimie*, or *mummiyo*, is a drug of mineral origin. It occurs

Fig. 9.9 Fulvic acid**Fulvic acid**

as a blackish-brown powder or an exudate from high mountain rocks between India and Nepal. It is known as a rejuvenator and as an antiaging compound in Ayurveda. Shilajit is composed of humic substances, mainly fulvic acid (60–80%) (Fig. 9.9) plus some oligo-elements, including selenium. The composition of the phytocomplex varies from region to region (Carrasco-Gallardo et al. 2012).

The pure extract of shilajit and processed shilajit capsules improve spermatogenesis and male fertility and have beneficial effects on oligozoospermia (Biswas et al. 2010; Chouhan et al. 2018). It decreases oxidative stress in sperm and improves sperm quality parameters, such as motility, plasma membrane integrity, etc., in the semen of buffalo (Sultan et al. 2021). Furthermore, when those sperm were stored under cryogenic conditions, shilajit preserves their viability, livability, and DNA integrity, indicating its beneficial effects on sperm quality. In another study, shilajit increases the serum LH and testosterone levels and the number of seminiferous tubular cell layers in the testes and improves spermatogenesis in the treated rats (Park et al. 2006). Moreover, treatment with shilajit also increases the weight of reproductive organs and sperm production, enhances activities of testicular enzymes, and reverts the adverse effects of cadmium on motility and concentration of spermatozoa (Mishra et al. 2018). Inhibition of phosphodiesterase 5A (PDE5A) has become the first-line therapy for treatments

of erectile dysfunction. Fulvic acid, the main constituent of shilajit, binds to the catalytic site of PDE5A and blocks the degradative action of cGMP for penile erection (Bhavsar et al. 2016). Shilajit displayed a peripheral parasympathomimetic effect for endothelium-dependent relaxation of corpus cavernosum smooth muscles, which supports the traditional claims of shilajit on libido and fertility (Kaur et al. 2013).

Apart from these extensive studied plants to treat male infertility, there are numerous other plants that have exhibited beneficial effects against male infertility. Some of the plants/phytoconstituents responsible for enhancing male fertility and fertilization process are mentioned in Table 9.2.

9.4 Conclusion

OS is considered as one of the major causes of male infertility. It leads to increase in lipid peroxidation and DNA damage which disturbs sperm functions making early diagnosis of infertility essential to avoid permanent impairment in the long run. Proper diet, exercise, reduced smoking, moderate consumption of alcohol, and non-exposure to radiation have substantial effects on lowering OS levels, thus improving male fertility. Besides lifestyle changes, antioxidant therapy is also used to prevent OS in the body, but additional studies are required for modulating their doses and duration.

Table 9.2 Miscellaneous plants responsible for improving male fertility

S. no.	Plant name (family)	Part used	Extract/phytoconstituent category	Mechanism of action	Effects	References
1	<i>Ajuga iva</i> (L.) Schreb. (Lamiaceae)	Leaves	Methanol extract	Enhancing the testosterone and 17 β -estradiol levels	Protecting from oxidative stress and cellular toxicity and maintaining the number and motility of spermatozooids	Hamden et al. (2008)
2	<i>Apium graveolens</i> L. (Apiaceae)	Leaves and seeds	Phyto-estrogens	Stimulating effects on testosterone levels	Enhance spermatogenesis	Abarikwu et al. (2020)
3	<i>Camellia sinensis</i> L. (Theaceae)	Leaves	Powder	Upregulated the gene expression of SOD1, SOD3, and XRCC1 and improved the levels of SOD and GSH	Reduce the rate of sperm malformation by affecting the change of androgen levels and oxidative stress	Han et al. (2020)
4	<i>Cardiospermum halicacabum</i> L. (Sapindaceae)	Aerial parts	Saponins and flavonoids	Boosted serum testosterone level	Increase in caput and epididymal sperm count and sperm motility	Adewoyin et al. (2017)
5	<i>Chlorophytum borivilianum</i> Santapau & R.R. Fern. (Asparagaceae)	Roots	Aqueous extract/phenolic compounds	Increase SOD, CAT, and GPX, increase epididymal sperm density	Increase sperm count, motility, and viability	Giribabu et al. (2014)
6	<i>Cinnamomum zeylanicum</i> L. (Lauraceae)	Bark	Phenolic compounds and essential oil	–	Improvement of male infertility	Yüce et al. (2013)
7	<i>Cistanche tubulosa</i> (Schrenk) Wight (Orobanchaceae)	–	Echinacoside	Enhancing star, CYP11A1, 3 β -HSD, 17 β -HSD, and CYP17A1 levels	Increased sperm motility	Santiago et al. (2021)
8	<i>Crocus sativus</i> Linn. (Iridaceae)	Flowers/stigmas	Safranal	–	Improved normal morphology and total and progressive sperm motility	Heydari et al. (2008)
9	<i>Curcuma longa</i> (Zingiberaceae)	Rhizomes	Curcumin	Reduced MDA and inflammatory markers	Increased sperm count, concentration, total motility, and vitality	Alizadeh et al. (2018)
10	<i>Eruca sativa</i> Mill. (Brassicaceae)	Aerial parts	Aqueous extract/polyphenols and flavonoids	Restores the activity of antioxidant enzymes such as SOD, CAT, and GPX in rat testis	Increase sperm progressive motility and viability, mitochondrial function, and decreased immotile sperm	Grami et al. (2020), Grami et al. (2018)
11	<i>Lepidium meyenii</i> Walp. (Brassicaceae)	Roots	Ethanol extract/macamides, macaenes, and glucosinolates	Improvement of the systemic antioxidant capacity of stallions	Increase testis weight, sperm count, motility, ejaculate volume, improved erection, mounting activity, and sperm quality	Clément et al. (2010) Del Prete et al. (2018)

12	<i>Ionidium suffruticosum</i> (L.) Ging (Violaceae)	Leaves	Alkaloids, steroidal lactones, and flavonoid	Increasing testicular oxidative biomarkers, SOD, and CAT	Increased spermatogenesis, increased sperm counts, lessened sperm agglutination	Chenniappan and Murugan (2017)
13	<i>Moringa oleifera</i> Lam. (Moringaceae)	Leaves	Hydroethanolic extract/naringin	–	Increase testicular weight and volume	Santiago et al. (2021)
14	<i>Petasites japonicas</i> (Asteraceae)	Leaves	Butanol fraction of methanol extract/eremophilane-type sesquiterpenoids	Stimulate spermatogonial stem cell proliferation and through its antioxidant effects	Increase spermatogenesis	Kang et al. (2015)
15	<i>Punica granatum</i> (Lythraceae)	Fruit rind	Tannins, phenols (ellagic acid), and flavonoids	Reduced oxidative damage by suppressing extra-creation of free radical	Improved semen and biochemical parameters by scavenging ROS and preventing oxidative DNA damage	Al-Olayan et al. (2014), Türk et al. (2010)
16	<i>Sesamum indicum</i> (Pedaliaceae)	Seed, seed powder/ oil	Unsaturated fatty acids and lignans such as sesamin and sesamol, sesamin, butylated hydroxytoluene	Increasing body antioxidant activities	Increasing seminal parameters, testosterone level	Abbasi et al. (2013), Ashamu et al. (2010), Khani et al. (2013)
17	<i>Solanum lycopersicum</i> L. (Solanaceae)	Fruit	Lycopene	Normalizing the activity of antioxidant enzymes	Protect rat testis from germ cell loss, preventing testis and epididymis loss of weight and restoring the impairment of sperm motility. Improve sperm parameters in oligozoospermia	Ateşşahin et al. (2006), Santiago et al. (2021), Nouri et al. (2019)
18	<i>Tinospora cordifolia</i> (Menispermaceae)	Whole plant	–	–	Increases semen cholesterol and antioxidant parameters	Jayaganthan et al. (2013)
19	<i>Tribulus terrestris</i> (Zygophyllaceae)	Fruits	Methanol extract/4,5-di-p-coumaroylquinic acid	Increased release of testosterone, FSH, and LH and enhanced tissue antioxidant capacity	Pretreatment with methanolic extract has protective and antioxidant effects in sodium valproate intoxicated rats	Hammouda et al. (2013), Shalaby and Hammouda (2014)
20	<i>Vitis vinifera</i> (Vitaceae)	Seeds	Anthocyanin oligomers (flavonoid)	Increases intracellular vitamin C levels and scavenges ROS and free radicals	Protected sperm cell against DNA damage, reduced activities of nitric oxide synthase, and attenuated apoptosis of germ cells induced by torsion/detorsion of testicles	Bayatli et al. (2013), Hala et al. (2010), Shi et al. (2003)
21	<i>Zingiber officinale</i> (Zingiberaceae)	Roots/ rhizomes	Aqueous, ethanolic extract/zingerone, gingerdiol, zingiberene, gingerols, and shogaols	Increase total antioxidant capacity and androgenic activity	Increases sperm parameters (viability, concentration and motility), decrease sperm cell abnormality	Abo-Ghanema et al. (2012), Bordbar et al. (2013), Khaki et al. (2009), Khaki et al. (2008), Zahedi et al. (2010)

SOD superoxide dismutase, GSH glutathione, GSH-PX glutathione peroxidase, MDA malondialdehyde, ROS reactive oxygen species, T testosterone, LH luteinizing hormone, FSH follicle-stimulating hormone

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