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Original Article

Tribulus terrestris and female reproductive system health: A comprehensive review

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ABSTRACT

Background: *Tribulus terrestris* L. (*T. terrestris*) positive performance on the male sexual system has been confirmed, but little is known about its effects on the female reproductive system.
Purpose: This review discussed in detail the beneficial impact of *T. terrestris* and its secondary metabolites on the female reproductive system.
Study design and methods: In this review, the scientific Databases of Science direct, Pubmed, Web of Science, Google, Google Scholar, Researchgate, EMBASE, Scientific Information (SID), and Elsevier were searched profoundly. Studies about the pharmacological activities of *T. terrestris* on the female reproductive system in each aspect of investigations: human, *in vivo*, and *in vitro* studies, in the period from 1998 to 2020 were admitted. Our study was not limited by the language of publications.
Results: 23 articles about the effects of *T. terrestris* on the female reproductive system were found. These studies approved the *T. terrestris* efficacy on improvements in histological features of the ovary and uterus of polycystic ovary syndrome patients as well as the well-working of normal ovaries, enhancements in the sexual desire of postmenopausal syndrome, improve ovarian and breast cancers.
Conclusion: These studies showed that the positive effect of *T. terrestris* on the female reproductive system was due to the presence of a secondary metabolite called protodioscin; a steroidal saponin compound, as the dominant active component of this plant.

Abbreviations: BCL2, B cell CLL/lymphoma-2; BMI, body mass index; BMP15, Bone morphogenetic protein 15; CDKs, cyclin-dependent kinases; CCR7, CC chemokine receptor 7; CXCR4, CXC chemokine receptor 4; CYP, cyclophosphamide; DHEA, Dehydroepiandrosterone; ERK1/2, extracellular signal-regulated kinases 1 and 2; FSH, Follicle-stimulating hormone; GDF9, Growth differentiation factor 9; GnRH, gonadotropin-releasing hormone; HSDD, Hypoactive Sexual Desire Disorder; IC_{50}, half-maximal inhibitory concentration; LH, Luteinizing hormone; mTOR, mammalian Target of Rapamycin; PCOS, Polycystic ovary syndrome; RT-PCR, real-time polymerase chain reaction; *T. terrestris*, *Tribulus terrestris* L.

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1. Introduction

Tribulus terrestris L. (a member of Zygophyllaceae family), commonly known as Tribulus, Hard thorns, and goat head in China, is a perennial plant, widespread in the Mediterranean area, subtropical and deserts around the world (Adaikan et al., 2001; Chhatre et al., 2014). It is popular as sexual activity enhancer and it is widely used in traditional herbal medicine. Investigations on its pharmacological activities show that *T. terrestris* could improve the reproductive systems of both genders. It could increase the concentration of the hormones (such as estradiol, and very slightly of testosterone), could enhance male and female libido, sperm production and spermatogenesis, and ovulation (Adaikan et al., 2000; Gauthaman et al., 2002; Martino-Andrade et al., 2010; Fatima et al., 2014; Keshtmand et al., 2014; Ghanbari et al., 2016). Many studies have reported the fundamental role of secondary metabolites from *T. terrestris* (Dixon, 2001; Zhu et al., 2017). Moreover, *T. terrestris* was detected as an alternative/complementary treatment to hormone substitution therapy in elderly individuals (Martino-Andrade et al., 2010). The presentment of steroidal saponins could be responsible for natural hormonal efficacy by directly stimulating female endocrine-sensitive tissues (such as the uterus and the vagina) (Nian et al., 2006).

Much evidence has been gathered on the utilization of *T. terrestris* with the production of pharmaceutical and nutritional supplements. In this respect, some steroidal saponins have been previously isolated from *T. terrestris*. Tribestane and vitanone are food supplements and pharmaceutical preparations containing saponins, as the active compounds that have been commercially existing and used to treat impotency (Su et al., 2009). Mazaro-Costa et al. (2010) introduced *T. terrestris* fruits as an herbal supplement effective in a sexual performance in women. In addition, it is a nutritional supplement highly debated regarding its physiological and actual effects on the organism. The main highlighted efficacy of *T. terrestris* is to increase the anabolic and androgenic action of testosterone by activating the production of endogenous testosterone. However, a case report of two urine samples from female athletes indicates that treatment with *T. terrestris* in the short term does not affect the endogenous metabolism of testosterone (Saudan et al., 2008). Moreover, several reports confirmed an improvement in egg fertility in hens receiving *T. terrestris* (Surdjiiska et al., 2005; Kashamov, 2007; Nikolova et al., 2010).

The present study is a comprehensive review of collected and summarized studies on the uses of *T. terrestris* in the female reproductive system. It could be used as an applicable gathered document to accelerate future related researches. Our study contributes by commenting on the quality of current studies, pointing out gaps in our knowledge, presenting rigorous research requirements to adequately address the efficacy issues of *T. terrestris* on female reproductive system disorders.

2. Pharmacology

The fruits and roots of *T. terrestris* have been used as a folk medicine for thousands of years in China, India, Sudan, and Pakistan (Table 1). Modern researches demonstrated that the chemical constituents such as steroidal saponins and flavonoids from *T. terrestris* were the main contributors to the traditional pharmacological activities such as antioxidant, antiaging, antiurolithic, immunomodulatory, absorption enhancing, hypolipidemic, hepatoprotective, anti-inflammatory, analgesic, antispasmodic, anticancer, antibacterial, antifungal, anthelmintic, and larvicidal (Anand et al., 1994; Adaikan et al., 2001; Al-Bayati et al., 2008; Chhatre et al., 2014; Hashim et al., 2014; Sivapalan, 2016; Zhu et al., 2017). In addition, in the therapeutic protocols, it has been reported that high doses of the aqueous extracts of the *T. terrestris* fruits had genotoxic efficacy (Qari and El-Assouli, 2019).

3. Search strategy and terms used

A comprehensive, electronic search was conducted for studies published between 1998 and 2020 using Science direct, Pubmed, Web of Science, Google, Google Scholar, Researchgate, EMBASE, Scientific Information Database (SID), and Elsevier Databases. Keywords related to 'Female reproductive system', 'Female reproductive organ', 'Uterus', 'Ovary', 'Ovarian cancer', 'Breast cancer', 'Uterus cancer', 'Vaginal cancer', 'Polycystic ovarian syndrome', 'Menopausal syndrome', 'Female sexual dysfunction', 'Female sexual desire', 'Estrogen', 'Progesterone', 'Follicle-stimulating hormone', 'Luteinizing Hormone', 'Female fertility', 'Testosterone' combined with *T. terrestris* were used. There was no language restriction. Ethical approval was not required because of the literature study. Research articles aimed at investigating the effects of *T. terrestris* on the female reproductive system were included in the study. We evaluated all published articles, abstracts presented at meetings, congress, as well as case reports, and reports. The impact of manuscripts and their quality assessment was considered.

4. Effects on the female reproductive system

This review was conducted according to the PRISMA protocol (Moher et al., 2009). We found 33 articles about the effects of *T. terrestris* on the female reproductive system. However, 10 articles containing papers related to the mix of herbs were deleted together with non-original ones. In general, 23 original papers related to only the effects of this species on the female reproductive system were selected (Fig. 1, Tables 2 and 3). Some of them include original data presented in scientific events.

In this study the main inclusion criteria were investigations

Table 1
Traditional pharmacological uses of *T. terrestris*.

Pharmacopeia	Part of *T. terrestris*	Therapeutic application	Reference
China	Roots and Fruits	▪ Tonifying the kidneys ▪ Diuretic ▪ Cough expectorant ▪ Improves eyesight ▪ Treatment of skin ▪ Improving headache, vertigo, ▪ Treatment of mammary duct blockage ▪ Improving cardiotonic properties ▪ treatment of sexual dysfunction ▪ Improving diabetes	China Pharmacopoeia Commission (2015) Su et al. (2009) Zhu et al. (2017)
India	Roots and Fruits	▪ Improving cardiotonic properties ▪ Treatment of sexual problems in both men and women (such as infertility, impotence, erectile dysfunction, and low libido) ▪ Improving diabetes	Mohammed et al. (2014) Zhu et al. (2017)
Sudan	*T. terrestris* plant	▪ Demulcent and in nephritis ▪ Treatment of inflammatory disorders	Mohammed et al. (2014)
Pakistan	*T. terrestris* plant	▪ Diuretic and uricosuric	Akram et al. (2011)

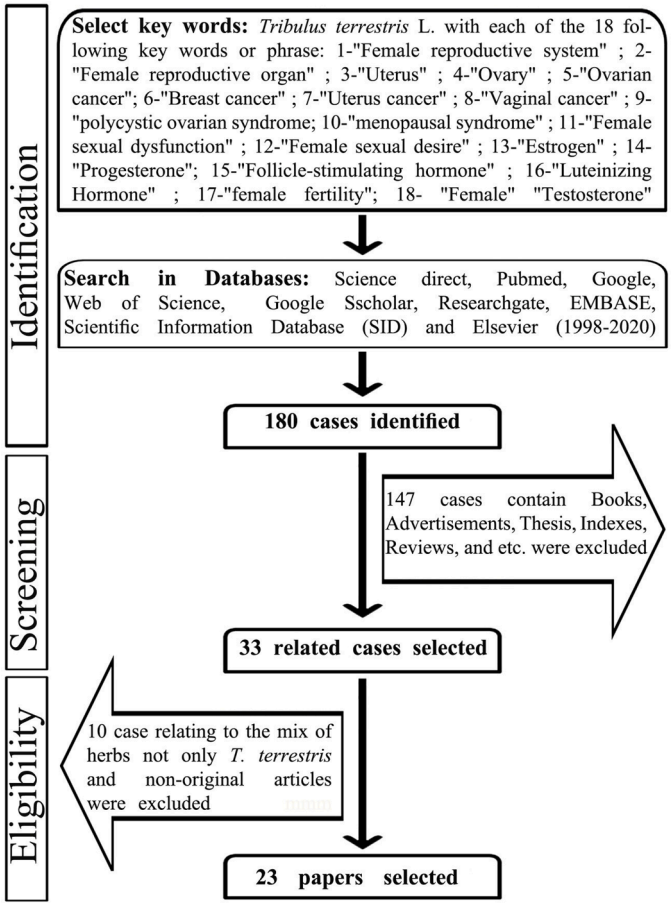

Fig. 1. Demonstrating the steps of choosing appropriate articles, like a flowchart, according to inclusion and exclusion criteria for this review.

regarding the impact of *T. terrestris* on i) uterus and ovary, ii) menopausal syndrome and female sexual dysfunction and desire, and iii) breast and ovarian cancers. These studies demonstrated the efficacy of *T. terrestris* on the modulation of female gonad hormones in all cases, histological features of ovary and uterus of normal and polycystic ovary syndrome (PCOS) patients, improving libido, the sexual desire of postmenopausal individuals, and toxicity on ovarian and breast cancers (Table 2).

5. Effects on uterine tonic activity

Results of the effects of *T. terrestris* on uterine tonic activity are described in Tables 2 and 4.

A study on the effect of 2% *T. terrestris* seed powder dissolved in water on female Wistar rats, confirmed the effectiveness of this herbal supplement on the uterine tonic, increasing both serum level of progesterone, and the number of pups. Related information is presented in Tables 2, 4, and 6 (Rajurkar et al., 2019). In addition, Adaay, and Mosa (2012) have been reported the effectiveness of *T. terrestris* on the uterine parameters (Tables 2 and 4–6). Furthermore, it has been reported that Exher (an herbal mixture of 10 plants, one of which is *T. terrestris*) was effective for uterine tonic activity on rats and was most helpful for the treatment of uterine disorders. This herbal drug was the impressive utility of the tonic on uterine defects like bleeding, fibroids and disability, abortion, and oligomenorrhea (Hanumantharayappa et al., 2014). Thus, it seems that Exher could improve the living condition of millions of women by lowering the side effects. Hence, its therapeutic effectiveness must be revealed on long-term treatment with this formulation. In addition, Martino-Andrade et al. (2010) reported that under *in vivo* conditions, *T. terrestris* was not capable to stimulate the uterus and vagina as endocrine sensitive tissues in Wistar rats, indicating an absence of androgenic and estrogenic activity.

6. Effects on ovary

Our searches about the impacts of *T. terrestris* on ovaries are categorized into four parts: i) normal ovaries subjects, ii) polycystic ovary syndrome (PCOS), iii) follicular development and ovulation, iv) ovary protection against alkylating agents (Tables 3 and 6). Moreover, the article data related to the effects of *T. terrestris* on female sex hormones are presented in Table 6.

The positive effect of *T. terrestris* on normal ovaries has been demonstrated by different studies (Esfandiari et al., 2011; Adaay and Mosa, 2012; Sirotkin et al., 2020). These studies showed that the effects of *T. terrestris* on normal ovaries appear to vary depending on the dose as well as the number of days of administration. Luteinizing hormone (LH), 17β-estradiol, and follicle-stimulating hormone (FSH) plasma levels significantly decreased with higher doses and the number of administrated days. Nevertheless, in both studies, the researchers did not evaluate testosterone and other male sexual hormones (Esfandiari et al., 2011; Adaay and Mosa, 2012). Investigation on the effect of *T. terrestris* extract on ovarian activity in immature Wistar rat showed that the number of corpus luteum, diameters of the follicle and theca internal layer significantly increased in the groups receiving *T. terrestris* extract orally for 1 and 2 weeks at a dose of 10 mg/kg/day. In addition, according to ovarian histologic studies, corpus luteum was observed in groups receiving *T. terrestris* for 1 and 2 weeks, and hyperemia in ovarian medulla was observed only in the group receiving this dose for 1 week. The authors concluded that *T. terrestris* has a LH-like activity that induces corpus luteum formation and thus starting puberty. Moreover, the observed enhancement in corpus luteum diameter may relate to the increase in progesterone production and it needs more studies (Esfandiari et al., 2011). Evidence of a significant increment in endometrial lining cells height and that the growing follicles may reach maturity, may lead to accelerating hormonal action especially FSH and LH (Adaay and Mosa, 2012). Sirotkin et al. (2020) examined the *T. terrestris*' effects on the basic functions of ovarian cells; their proliferation, apoptosis, and response to the physiological hormonal stimulator ghrelin, which is described in Table 2.

7. Effects on folliculogenesis

Although scientists have addressed probable effects of *T. terrestris* on folliculogenesis, there are indefinably limitations in the information in this area, the mechanisms involving the activity of *T. terrestris* for this cellular event are unclear yet. Nikolova et al. (2010), Adaay and Mosa (2012) have shown the efficacy of *T. terrestris* on folliculogenesis of treated mothers on Guinea fowls and mice, respectively, without declaring any molecular related mechanism. Based on our best knowledge regarding *T. terrestris* and folliculogenesis, only one study was accompanied on mammals (rabbits), and screened the molecular pathways of folliculogenesis in rodents, with the consideration of inhibin serum levels in each menstrual phase, gives us light for achieving more profound results from case-control clinical trials, monitoring folliculogenesis, endometriosis, PCOS. The data of the effects of *T. terrestris* on follicular development and ovulation has been approved by one study of Abadjieva and Kistanova (2016) on 28 rabbits. Growth differentiation factor 9 (GDF9) secrets from cumulus cells and bone morphogenetic protein 15 (BMP15) secrets from both ovary and cumulus cells. GDF9 and BMP15 are considered to be two oocyte-specific genes, and play a key role in the regulation of folliculogenesis in many species, demonstrating the effect of *T. terrestris* on GnRH BCL2, B cell CLL/lymphoma-2 in both ovary and follicular cells for puberty of follicles. It is because that these two proteins are responsible for the development and function of the oocyte-cumulus cell complex targeted to clarify the molecular events

Table 2
Summary of Original articles about *T. terrestris impact* on female reproductive system.

	Study subject	Type of study		*T. terrestris* preparation (Days of treatment)	Efficacy of *T. terrestris* on female reproductive performance	Predicted effective secondary metabolite	References
1	Uterine tonic activity	*In vivo*	16 Wistar Rats	Seed powder added with feed (63)	▪ Increased uterus weight and estrogen concentration in the serum ▪ Preventing and curing uterine fibroids and abnormal uterine bleeding only in the functional ovary presence	Not mentioned	Rajurkar et al., 2019
2	Uterine & ovarian parameters	*In vivo*	60 Mice	Aqueous extract (14 & 28)	▪ Treatment for 14 days had obvious effects on both ovarian and uterine parameters ▪ Treatment for 28 days was more effective on the uterine parameters	Steroidal saponins	Adaay and Mosa, 2012
3	Endocrine sensitive organs	*In vivo*	Wistar Rats	Water extract (28)	▪ Is not able to stimulate endocrine sensitive tissues (such as the uterus and vagina), indicating a lack of estrogenic activity *in vivo*	Protodioscin	Martino-Andrade et al., 2010
4	Normal Ovary	*In vivo*	20 Immature Wistar Rats	Pure extract (7 & 14)	▪ 14 days treatment was more effective than 7 days ▪ Induces corpus luteum formation, growth, and beginning puberty	Steroidal saponins (protodioscin)	Esfandiari et al., 2011
5	Normal Ovary	*In vitro*	Ovarian granulosa cells	Extract	▪ Directly promotes proliferation and apoptosis, that is, turnover, of ovarian cells ▪ Antagonizes and even reverses the effects of the hormonal regulator ghrelin on ovarian cell ▪ Affects not only the basic functions of ovarian cells but also their responses to upstream hormonal regulators	Saponins, flavonoids, glycoside alkaloids, acids, and tannins	Sirotkin et al., 2020
6	GDF9 & BMP15 expression during folliculogenesis	*In vitro*	28 Rabbits oocytes & cumulus cells	Dry extract (45)	▪ A clearly expressed sensitivity by BMP15 and GDF9 to the bioactive compounds of *T. terrestris*	Steroidal saponins (protodioscin)	Abadjieva and Kistanova, 2016
7	Polycystic Ovary Syndrome	Clinical	122 Women	Tablet (90)	▪ Effectiveness and safety of the combined herbal medicine and lifestyle	Not mentioned	Arentz et al., 2017
8	Polycystic Ovary Syndrome	*In vivo* & *In vitro*	Rats & RIKILTYeast cell	Methanolic extract (72)	▪ *In vitro* antiandrogen activity of plant extracts ▪ *In vivo* positive effects on different parameters of PCOS	Not mentioned	Sandeep et al., 2015
9	Polycystic Ovary Syndrome	*In vivo*	50 Wistar Rats	Hydroethanolic extract (62)	▪ A luteinizing effect on ovarian cysts ▪ Remove ovarian cysts and resume ovarian activity	Saponins, diosgenins, alkaloids, amides	Dehghan et al., 2012
10	Cyclophosphamide toxicity in the ovary	*In vivo*	48 Rats	Hydroalcoholic extract (70)	▪ Improve the side effects of CYP toxicity in the rats' ovary	Steroidal saponins	Rezaie et al., 2013
11	Menopausal syndrome	Clinical	60 Women	Powder (56)	▪ Effective in the alleviation of menopausal transition symptoms ▪ Be a safer alternative to hormone replacement therapy	Protodioscin	Fatima and Sultana, 2017
12	Postmenopausal symptoms	Clinical	60 Women	Hydroalcoholic extract (56)	▪ Increased postmenopausal women's sexual satisfaction	Protodioscin	Tadayon et al., 2018
13	Premenopausal women with HSDD	Clinical	40 Women	Pills (120)	▪ A safe alternative for the treatment of HSDD by reducing the symptoms, with no side effects	Steroidal saponins	Vale et al., 2017
14	HSDD in postmenopausal women	Clinical	45 Women	Pills (120)	▪ A safe alternative for the treatment of HSDD by reducing the symptoms, with few side effects	Protodioscin	De Souza et al., 2016
15	Sexual function of menopausal women	Clinical	60 women	Tablet (90)	▪ Effective in treating sexual problems among menopausal women	Steroidal saponins	Postigo et al., 2016
16	Patients with sexual dysfunction	Clinical	144 Women	Tablet (90)	▪ Safety and effectiveness in the treatment of female sexual dysfunction	Protodioscin	Gama et al., 2014
17	Women with sexual dysfunction	Clinical	67 Women	Syrup containing ethanolic extract (28)	▪ Safely and effectively improve desire in women with HSDD	Not mentioned	Akhtari et al., 2014
18	Postmenopausal women with sexual desire dysfunction	Clinical	66 Women	Tablet (90)	▪ A good option for the treatment of postmenopausal women with sexual desire dysfunction	Saponins	Guazzelli et al., 2014
19	Ovary tumor cell lines	*In vitro*	Human cell line	Extract	▪ Inhibitory activities against ovary cancer cell lines	Saponins	Bouabdallah et al., 2015

(*continued on next page*)

Table 2 (*continued*)

	Study subject	Type of study		*T. terrestris* preparation (Days of treatment)	Efficacy of *T. terrestris* on female reproductive performance	Predicted effective secondary metabolite	References
20	Breast cancer	*In vitro*	Bcap-37 cell line	Extract	▪ Potent inhibitory effect in a concentration-dependent manner	Saponins	Sun et al., 2003
21	Breast cancer	*In vitro*	MCF7 cell line	Hydroalcoholic extract	▪ Selective antitumor activity on human cancer cells	Protodioscin	Angelova et al., 2013 (Original)
22	Breast cancer	*In vitro*	MCF7 & MCF10A cell line	Hydroalcoholic extract	▪ Effect on the processes of apoptosis and metastasizing of cancer cells	Protodioscin	Goranova et al., 2015 (Original)
23	Breast cancer	*In vitro*	MCF7 cell line	Methanol and saponin extracts	▪ Significant increase in caspase 3 activity in MCF-7 cells ▪ Caused an induction of the intrinsic apoptotic pathway, which was evident by the upregulation in the expression of Bax and p53 genes and downregulation in the expression of Bcl-2. FADD, AIF, and caspase 8 genes ▪ Upregulated indicating the possible induction of extrinsic apoptotic pathway	Saponins	Patel et al., 2019 (Original)

regarding *T. terrestris* effects on folliculogenesis (Abadjieva and Kistanova, 2016). GDF-9 is mostly expressed by oocytes, although to a lesser extent in primary granulosa cells. GDF-9 has different functions on granulosa cells and theca cells, depending on the species, and works by interacting with active receptors (ALK) −5 (TGF-βRI) and BMP receptors type 2 (BMPR) −2). BMP15 is a member of the TGF-β superfamily produced by oocytes. The results have obviously shown the relevant up-regulated expression of *BMP15* and *GDF9* in both mothers who were *T. terrestris* administrated and F_1 female offspring using real-time polymerase chain reaction (RT-PCR).

8. Effects on Pcos

The aerial part and the fruit of *T. terrestris* promote regular ovulation and could reduce ovarian cysts in women with PCOS. In addition, one review article studied the characteristics of many medicinal drugs, including *T. terrestris*, for the treatment of PCOS. The authors mentioned the Gokshura drug, derived from the fruit and root of *T. terrestris*, which is commercially supplied as a tonic for increasing female fertility of suffered PCOS patients. However, there was not any reference related to the claim of the developed Gokshura drug in their review paper (Sonal and Sanjay, 2017). According to the investigations about the effect of *T. terrestris* on PCOS, only one study was based on human research and three on animal research (Tables 3 and 6). Arentz et al. (2017) studied the efficacy and the safety of a lifestyle intervention coupled with herbal medicine against regular lifestyle by applying MediHerb Tribulus Forte (Integria Healthcare Pty Ltd, Australia) examined on overweight 122 kg women selected from communities of Australia. Tribulus Forte (Integria Healthcare Pty Ltd, Australia) as herbal medicine containing *T. terrestris* extract equivalent to 13.5 g of aerial parts. The primary outcomes were significant improvement of oligomenorrhoea/amenorrhoea, regardless of weekly regular exercises, up to 150 min per week. Secondary outcomes were the recovery in hormone secretion, body mass index (BMI), quality of life, reduction in depression, anxiety, and stress, increase in the prevalence of pregnancy, and birth, all concerned the safety of this herbal intervention. Their outcomes showed that the follicular phase was increased in the test group (MD 68.9 pmol/l, 95% CI 5.5 to 132.3, $p = 0.03$), and LH was lowered (MD 1.82 IU/l, 95% CI 3.5 to 0.1, $p = 0.04$). The estimated medium effect size was found for both oestradiol and LH ($\eta 2$ $p = 0.08$ and 0.06, respectively). There were no significant differences between groups for sex hormone-binding globulin (SHBG), or free androgen index (FAI) and testosterone. Moreover, women assigned to the group of herbal medicine plus lifestyle showed significantly improved HRQoL compared with controls for the total PCOSQ score (MD 31.1, 95% CI 41.4 to 20.7, $p < 0.01$) and for the domains of concerns about body hair (MD 3.0, 95% CI 4.8 to 1.1, $p = 0.01$), body weight (MD 5.24, 95% CI 7.8 to 2.7, $p < 0.01$), menstrual problems (MD 3.9, 95% CI 5.3 to 2.4, $p < 0.01$) infertility (MD 3.9, 95% CI 5.7 to 2.1, $p < 0.01$), and emotions (MD 8.4, 95% CI 11.4 to 5.4, $p < 0.01$). Participants to the group of herbal medicine tablets plus lifestyle recorded a significantly greater reduction in depression, anxiety, and stress scores compared with controls. Mean scores in the test group at the endpoint were 4.3 (95% CI 5.9 to 2.7, $p < 0.01$), 4.0 (95% CI 5.4 to 2.6, $p < 0.01$) and 5.0 (95% CI 6.5 to 3.5, $p < 0.01$), respectively, lower with an estimated large magnitude of effect for all three domains. In this regard, we suggested that further investigations are needed to explain the specific clinical effects of each component of *T. terrestris* extract.

Dehghan et al. (2012) emphasized the effectivity of *T. terrestris* directly or indirectly on luteinization of follicular cysts. They reported that the number of rats with corpus luteum were 0,1 and 3 in control, treatment group 5 mg and 10 mg, respectively. Moreover, the number of rats with ovarian cyst received from 5 in the control group to 4 and 1 in the treated groups with 5 and 10 mg, respectively. In their research, effects of *T. terrestris* on cornified vaginal cells in different experimental groups revealed a significant decrease in 57 days (control group: 64 ± 10.3; treatment with 5 mg/kg: 23 ± 8; treatment with 10 mg/kg: 22 ± 10.8). Another study suggested the existence of phytoestrogens within *T. terrestris* to influence both the brain for the secretion of sexual hormones and its antioxidant capacity (Saiyed et al., 2016). Furthermore, Sandeep et al. (2015) only showed normalization of *T. terrestris* on the estrous cycle of PCOS-induced rats by reduction of steroid hormone levels. They observed in their study that the extracts of *T. terrestris* decreased the dihydrotestosterone-induced fluorescence response from 326 ± 8 to 220 ± 7 at a plant extract concentration of 5.1 ng/ml. The groups treated with *T. terrestris* showed different percentages in various phases. The animals from the *T. terrestris* (10 mg) group showed a stressed incidence of the estrous phase with a percentage of 63.9, an increase in the metestrous phase was observed with a percentage of 16.7. In addition, the weight gain of PCO-induced rats was 6.50 ± 0.55 and 4.05 ± 0.48 in control and *T. terrestris* (10 mg) groups, respectively. Thus, the extract-treated rats improved their weight gain significantly. Moreover, serum testosterone concentration was 42.66 ± 4.76 in the control group, which significantly decreased in the *T. terrestris* receiving group at doses of 5 mg (28 ± 1.3) and 10 mg (28.2 ± 0.85). Regarding animal studies, oral and intraperitoneal administration of *T. terrestris* in rats had a significant and varied effect on PCOS, which may be due to the part of the plant used and to the extraction method. Furthermore, the intraperitoneal administration of *T. terrestris* affects PCOS in a

Table 3
Summary of deleted articles related to the mix of herbs not only *T. terrestris* or non-original articles.

	Study subject	Type of study	Study Model	*T. terrestris* preparation (Days of treatment)	Efficacy of *T. terrestris* on female reproductive performance	Predicted effective secondary metabolite	References
1	Uterine tonic activity	*In vivo*	Rats	Aqueous extract of a polyherbal mixture of 10 plants containing *T. terrestris* that named Exher (21)	▪ Doubtful in treating dysmenorrhea and the conditions like threatened abortion ▪ Improved progesterone level	Not mentioned	Hanumantharayappa et al., 2014 (Original)
2	Polycystic Ovary Syndrome	*In vivo*	24 Rats	*T. terrestris* + *Withania somnifera* hydroalcoholic extract (28)	▪ Significant recovery of follicle-stimulating hormone; Luteinizing hormone, estradiol, and testosterone levels in serum ▪ Significant antiandrogen effects by reducing increased testosterone level and preventing ovarian dysfunction	Phytoestrogens (such as alkaloids, flavonoids, saponins)	Saiyed et al., 2016 (Original)
3,4,	Menopausal symptoms	Clinical	80 Women	A capsule of *T. terrestris* + 3 other plants (28)	▪ Effective in reducing menopausal symptoms	Not mentioned	Taavoni et al., 2016 & S. 2017 (Congress)
5	Female sexual dysfunction	*In vivo*	Rats	Ethanolic extract in polyherbal formulation (11 & 21 & 28)	▪ Possess aphrodisiac activity ▪ Effective in female sexual dysfunction	Steroidal saponins	Kaspate et al., 2015 (Original)
6,7	Postmenopausal women with HSDD	Clinical	66 Women	Tablet (90)	▪ It may be evaluated as a good therapeutic option for post menopause women with HSDD/low sexual desire	Not mentioned	Rolim-Lima et al., 2014; Silva et al., 2015 (Meeting)
8,9	Sexual function of menopausal women	Clinical	60 women	Tablet (90)	▪ Proved effective for treating sexual disorders in post-menopausal women.	Not mentioned	Postigo et al., 2012, & S. 2017 (Congress)
10	Sexual dysfunction in women	Clinical	67 Women	Tablet (28)	▪ May safely and effectively improve some sexual problems including desire, arousal, and orgasm	Not mentioned	Raisi et al., 2014 (PODIUM SESSION – ESSM)

Table 4
Summary of articles data about *T. terrestris impact* on uterine tonic activity.

Reference Parameter	Reproductive organs weight (mg/100 gm.B.W)		Uterus weight (g)		Endometrial lining cells height (μm)		Endometrial Glands diameter (μm)		Uterine luminal epithelium cell height (μm)	
	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment
Rajurkar et al., 2019	–	–	1.62±0.02	2.81±0.05*	–	–	–	–	–	–
Adaay and Mosa, 2012 (2 weeks' treatment with 100 mg/kg/day)	274.49±48.63	273.50 ± 48.77	–	–	13.78±0.67	27.5 ± 1.96*	38.85±1.27	52.91±2.15*	–	–
Adaay and Mosa, 2012 (4 weeks' treatment with 100 mg/kg/day)	109.79±26.90	237.35±60.18*	–	–	12±1.03	17.55±1.19*	45.07±4	36.73±1.48	–	–
Adaay and Mosa, 2012 (2 weeks' treatment with 200 mg/kg/day)	274.49±48.63	246.17±75.47	–	–	13.78±0.67	22±2.96*	38.85±1.27	51.41±2.88*	–	–
Adaay and Mosa, 2012 (4 weeks' treatment with 200 mg/kg/day)	109.79±26.90	269.92±45.40*	–	–	12±1.03	31.14±1.84*	45.07±4	51.17±2.70	–	–
Martino-Andrade et al., 2010 (4 weeks' treatment with 110 mg/kg/day)	–	–	94±4.28	85 ± 4.31	–	–	–	–	9.85±0.1	9 ± 0.08

Values are presented as mean ± SEM
* significantly different than control group (Before Treatment) ($p < 0.05$); (-): not mentioned

dose-dependent manner, but did not change the LH and FSH values in any of the prescriptive models.

In summary, we suggest that although *T. terrestris* is more effective in normal ovaries, the recovery of PCOS could achieve in prolonged usage. This controversy can be explained by unresponsive theca internal cells of PCOS patients to *T. terrestris*. Hence, in the case of PCOS, co-administration of *T. terrestris* with LH raising agents would be helpful for decreasing time course and increasing the effectiveness of *T. terrestris* to avoid unwanted results on the endometrium. Moreover, it has adhered that in PCOS, the best time of using *T. terrestris* is on days 5 to 14 of the menstrual cycle to restore menstrual regularity, although the optimal dose has not been established. Overall, these insufficient studies demonstrated the potential effects of *T. terrestris* on the treatment of PCOS and we conclude that the dosage and the targeted menstrual cycle would affect FSH or LH properties of *T. terrestris* on PCOS. However, more *in vivo* (animal models) and clinical trial studies are needed to confirm this adherence, as we suggested about endometrium and folliculogenesis.

9. Protective effects against alkylating agents on ovary

The protective effects of *T. terrestris* on toxicity induced by cyclophosphamide (CYP; an alkylating agent) in the rat ovary have been determined by one article, written in Persian (Rezaie et al., 2013). In this research the rats were administrated with only normal saline, CYP (30 mg/kg, 2 times/week for 7 weeks), *T. terrestris* extract (10 mg/kg, 2 times/week for 10 weeks), and vitamin C (80 mg/kg, 2 times/week for 7 weeks). The results indicated that *T. terrestris* extract had a similar recovery effect as vitamin C and it is suitable for alleviating ovarian toxicity induced by CYP.

10. The effect on the menopausal syndrome, hypoactive sexual desire disorder (HSDD), and sexual dysfunction

Menopause – a natural phase of life, is determined as the time following the final menstrual period, and it is generally confirmed when a woman has lost her periods for 12 continuous months in the absence of other obvious causes. Menopause can cause physical problems for women (NAMS, 2010). Fatigue, headache, loss of libido, irritability, insomnia, depression, nervousness, joint and muscle pain, and palpitations are Menopausal syndrome symptoms (Beck et al., 1993; Taavoni et al., 2011). A study on sexual dysfunction showed an association between menopause, aging, and sexual disorders prevalence (Ishak et al., 2010). HSDD, a common sexual complaint affecting nearly 1 in 10 women in the USA, Europe, and Australia, is a multifaceted and multifactorial disorder with psychological, biological, and interpersonal relationships all being potential contributing agents. It seems that alternative and complementary medical therapies might be helpful for older adults' symptoms management. This could be a contribution to the strategies available for improving postmenopausal women's life quality and increasing the family and the community health levels. In this regard, *T. terrestris* has long been attributed to impressing boosting sex drive and libido in humans in traditional herbal medicine texts (Tester, 2014). In general, the results of the *T. terrestris* effects on the menopausal syndrome, HSDD, and sexual dysfunction showed that it is an effective drug for them, and the papers addressed it as a reliable supplement that came out of the high-end experiments. The summary of papers related to the effect of *T. terrestris* on the menopausal syndrome, HSDD, and sexual dysfunction are presented in Tables 2, 7, 8. Based on these articles, *T. terrestris* is effective in reducing the symptoms by increasing the serum levels of testosterone (Gama et al., 2014; De Souza et al., 2016; Vale et al., 2017), the co-occurrence of enhanced female sexual function and increased DHEA levels (Gama et al., 2014) and possible synergy between *T. terrestris* and FSH-LH mechanism (Akhtari et al., 2014). The mean DHEA levels in pretreatment and post-treatment were 57.83 ng/ml and 67.18 ng/ml, respectively, which showed significant differences (Gama et al., 2014).

In one study, the *T. terrestris* receiving group displayed a higher reduction compare to the placebo group in the total score of the Menopause Rating Scale (MRS) total mean score (10.43 ± 5.19 *vs.* 16.86 ± 4.9). In addition, the *T. terrestris* receiving group showed comparatively higher reduction than the placebo group in somatic (4.03 ± 2.38

Table 5
Summary of articles data about *T. terrestris* impact on ovaries.

Reference Parameter	Number of corpora lutea		The diameter of corpora lutea		Number of secondary follicles		The diameter of mature follicles (μm)		The thickness of theca intema layers (μm)	
	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment
Adaay and Mosa, 2012 **(2 weeks' treatment with 100 mg/kg/day)**	15.20±1.38	13.30±0.37	–	–	10.25±0.85	12±1.53	195.65±8.65	286±6*	–	–
Adaay and Mosa, 2012 **(4 weeks' treatment with 100 mg/kg/day)**	17.40±1.79	15.70±1.42	–	–	16 ± 0.58	17 ± 1.39	289.76±23.32	323.29±38.13	–	–
Adaay and Mosa, 2012 **(2 weeks' treatment with 200 mg/kg/day)**	15.20±1.38	17.20±1.29	–	–	10.25±0.85	15.50±1.19*	195.65±8.65	241.74±8.72*	–	–
Adaay and Mosa, 2012 **(4 weeks' treatment with 200 mg/kg/day)**	17.40±1.79	19.40±3.76	–	–	16 ± 0.58	16.43±1.95	289.76±23.32	300.50±38.79	–	–
Esfandiari et al., 2011 **(1 week's treatment)**	15.5 ± 0.29	16±0.8	0	407.2 ± 7.22* μm	0	4.5 ± 1.29*	–	–	12.12±0.17	29.65±0.43*
Esfandiari et al., 2011 **(2 week's treatment)**	17±0.8	18±0.8	380.2 ± 8.36	605.2 ± 10.39* μm	1.25±0.95	5.25±1.7*	–	–	17.5 ± 0.55	70.83±0.59*
Dehghan et al., 2012 **(treatment with 5 mg)**	–	–	0 ± 0	0.24±0.54mm	2.60±1.4	8.80±3.1*	–	–	1.08±0.39	0.61±0.04*
Dehghan et al., 2012 **(treatment with 10 mg)**	–	–	0 ± 0	0.65±0.65mm	2.60±1.4	20.60±2.7*	–	–	1.08±0.39	0.82±0.27*
Rezaie et al., 2013	–	–	–	–	7.08±0.2	8.08±0.16	–	–	–	–

Values are presented as mean ± SEM.
* significantly different than control group (Before Treatment) ($p < 0.05$).

Table 6
Summary of articles data about *T. terrestris* impact on female sexual hormones.

Reference Parameter	Serum Progesterone level (ng/ml)		FSH (mIU/ml)		LH (mIU/ml)		Serum Estrogen level (ng/ml)		Estradiol level	
	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment
Rajurkar et al., 2019 (14 days treatmen)	78.38±3.77	110.75±6.36	–	–	–	–	–	–	–	–
Rajurkar et al., 2019 (63 days treatmen)	93.24±1.75	356.13±31.11*	–	–	–	–	–	–	–	–
Adaay and Mosa, 2012 (2 weeks' treatment with 100 mg/kg/day)	–	–	0.42±0.10 (mIU/ml)	0.88±0.24 (mIU/ml)	0.52±0.15	0.7 ± 0.31	–	–	–	–
Adaay and Mosa, 2012 (4 weeks' treatment with 100 mg/kg/day)	–	–	1.08±0.20	1.3 ± 0.28	0.9 ± 0.13	1.24±0.18	–	–	–	–
Adaay and Mosa, 2012 (2 weeks' treatment with 200 mg/kg/day)	–	–	0.42±0.10	0.74±0.12	0.52±0.15	1.00±0.20	–	–	–	–
Adaay and Mosa, 2012 (4 weeks' treatment with 200 mg/kg/day)	–	–	1.08±0.20	1.03±0.17	0.9 ± 0.13	0.87±0.11	–	–	–	–
Sandeep et al., 2015 (5 mg)	17.95±4.37	10.2 ± 0.5*	–	–	–	–	–	–	42.66±8	47.97±1.01
Sandeep et al., 2015 (10 mg)	17.95±4.37	13±3.97					–	–	42.66±8	40.1 ± 3
Rezaie et al., 2013	–	–	–	–	–	–	3520±110	3510±80	–	–

Values are presented as mean ± SEM.
* significantly different than control group (Before Treatment) ($p < 0.05$); -: not mentioned.

Table 7
Summary of articles data about *T. terrestris* impact on mean serum testosterone levels.

Reference Parameter	Free testosterone level		Bioavailable testosterone		Total testosterone	
	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment
Vale et al., 2017	0.24 ± 0.2	0.26 ± 0.1*	5.58 ± 3.5	6.02 ± 2.6*	18.1 ± 8.4	20.5 ± 9.7
De Souza et al., 2016	0.2 ± 0.1	0.2 ± 0.2*	4.7 ± 3.3	5.4 ± 4.3*	12 ± 5.7	14.2 ± 6.9
Gama et al., 2014	7.83 ± 4.67	7.74 ± 3.9	–	–	24.21 ± 13.17	22.65 ± 11.58

Values are presented as mean ± SEM.
* significantly different than control group (Before Treatment) ($p < 0.05$); -: not mentioned.

vs. 6.72 ± 2.5), urogenital (2.3 ± 1.60 *vs.* 3.93 ± 1.72), and psychological (4.1 ± 2.43 *vs.* 6.33 ± 2.24) composite subscale scores with a statistically extremely significant difference after treatment (Fatima and Sultana, 2017). Tadayon et al. (2018) observed that evaluation of sexual satisfaction before the intervention and 8 weeks after the intervention in *T. terrestris* receiving group significantly increased from 25.4 ± 8.34 to 37.56 ± 6.65, respectively.

The level of libido was evaluated only in one study and in the low desire disorder; women had a significant recovery (Raisi et al., 2014). Moreover, a clinical study about the effects of *T. terrestris* on 50 menopausal women reported that most (if not all) of the symptoms were relieved in 49 women (Tabakova et al., 1984–1987). However, these results were questionable due to the absence of reporting regarding the instrument of assessment and a possible conflict of interest. In addition, *T. terrestris* fruit was effective in sexual performance in women (Mazaro-Costa et al., 2010). Given the inadequacy of studies on the effects of *T. terrestris* on the menopausal syndrome, HSDD, and sexual dysfunction, more comprehensive investigations are needed on all the symptoms that occur in postmenopausal women. Furthermore, the dosage used, and the involving hormonal/cellular axis should be considered.

11. Effects on ovarian and breast cancers

Many natural sources from medicinal plants such as silvestrol (*Aglaia foveolata*), vinblastine and vincristine (*Catharanthus roseus*), paclitaxel (*Taxus brevifolia*), eliptinium (*Bleekeria vitensis*), artemisinin (*Artemisia annua*), chrysin (*Passiflora incarnata*), and others had anticancer properties and were utilized in chemotherapy drugs. Moreover, their highly efficient antitumor activities have been precisely approved (Taraphdar et al., 2001; Newman et al., 2003). A vast number of studies demonstrated high cytotoxicity of *T. terrestris* (Bedir and Khan, 2000; Bedir et al., 2002; Hu and Yao, 2003; Sun et al., 2003, 2004; Wang et al., 2009; Neychev et al., 2007; Ivanova et al., 2009; Angelova et al., 2013; Bouabdallah et al., 2015; Patel et al., 2019; Sadeghi Dinani et al., 2019).

T. terrestris, was also shown to be effective on ovarian and breast cancers, mainly because the existence of saponins constituents such as protodioscin (Bouabdallah et al., 2015; Sun et al., 2003; Angelova et al., 2013; Goranova et al., 2015; Patel et al., 2019) (Table 2). In addition, Neychev et al. (2007) found that saponin fraction from *T. terrestris* is less toxic to normal human fibroblasts. The cell cycle arrest and apoptosis in human sarcoma cell lines were also reported by the application of saponin, which is structurally similar to diosgenin and present in *T. terrestris* extracts (Trouillas et al., 2005). In another study, the

Table 8
Summary of articles data about *T. terrestris* impact on the menopausal syndrome, HSDD, and sexual dysfunction.

Domains	Reference							
	Vale et al., 2017		De Souza et al., 2016		Postigo et al., 2016		Gama et al., 2014	
	QS-F	FSFI	QS-F	FSFI	QS-F	FSFI	QS-F	FSFI
Desire (Pretreatment)	(+): 15 (-): 5	1.65	(+): 14 (-): 6	2.04	20%	7.4	–	2.51
Desire (Post-treatment)	(+): 6 (-): 14	3.24*	(+): 5 (-): 15	3.66*	83.3%*	10.2*	–	4.35*
Arousal (Pretreatment)	(+): 13 (-): 7	2.01	(+): 11 (-): 9	1.98	20%	4.9	–	2.43
Arousal (Post-treatment)	(+): 3 (-): 17	3.27*	(+): 4 (-): 16	3.74*	70%*	7.2*	–	3.09*
Lubrication (Pretreatment)	(+): 9 (-): 11	2.60	(+): 11 (-): 9	2.76	–	–	–	3.16
Lubrication (Post-treatment)	(+): 3 (-): 17	3.98*	(+): 3 (-): 17	4.62*	–	–	–	3.18
Orgasm (Pretreatment)	(+): 17 (-): 3	2.32	(+): 18 (-): 2	2.16	26.7	3.6	–	2.75
Orgasm (Post-treatment)	(+): 8 (-): 12	3.84*	(+): 10 (-):	4.12*	73.3*	2.8	–	3.08*
Satisfaction (Pretreatment)	(+): 18 (-): 2	3.02	(+): 16 (-): 4	2.88	–	–	–	2.90
Satisfaction (Post-treatment)	(+): 7 (-): 13	4.36*	(+): 10 (-): 10	4.66*	–	–	–	3.45*
Pain (Pretreatment)	–	3.84	–	3.8	–	–	–	2.82
Pain (Post-treatment)	–	4.58*	–	5.00*	–	–	–	2.77
Overall (Pretreatment)	–	15.44	–	15.62	–	15.9	–	16.57
Overall (Post-treatment)	–	23.27*	–	25.8*	–	20.2*	–	19.93*

Domains	Reference			
	Akhtari et al., 2014		Guazzelli et al., 2014	
	QS-F	FSFI	QS-F	FSFI
Desire (Pretreatment)	–	3.66±0.69	1	–
Desire (Post-treatment)	–	3.90±0.71*	3*	–
Arousal (Pretreatment)	–	3.61±0.92	3	–
Arousal (Post-treatment)	–	4.21±0.67*	4*	–
Lubrication (Pretreatment)	–	4.15±1.13	–	–
Lubrication (Post-treatment)	–	4.66±0.87*	–	–
Orgasm (Pretreatment)	–	3.21±0.98	1	–
Orgasm (Post-treatment)	–	4.20±0.72*	2.5*	–
Satisfaction (Pretreatment)	–	3.44±1.15	1	–
Satisfaction (Post-treatment)	–	4.61±0.93*	3*	–
Pain (Pretreatment)	–	4.19±1.56	2	–
Pain (Post-treatment)	–	5.07±1.01*	1.5*	–
Overall (Pretreatment)	–	22.41±2.87	8	–
Overall (Post-treatment)	–	26.80±3.03	14*	–

FSFI, Female Sexual Function Index, SQ-F, Sexual Quotient Female Version; (+), presence of sexual problem related to the domain; (-), absence of sexual problem related to the domain; -: Not mentioned.
* significantly different than control group (Before Treatment) ($p < 0.05$)(+), presence of sexual problem related to the domain; (-), absence of sexual problem related to the domain; -: Not mentioned, * significantly different.

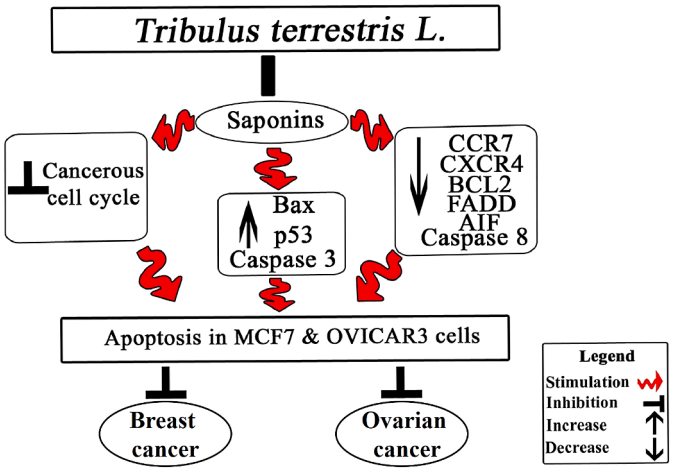

Fig. 2. The suggested mechanism of ovarian and breast cancer improvement by *T. terrestris*.

Table 9
Main phytochemicals in *T. terrestris* (Zhu et al., 2017).

Phytochemical compounds	Kinds of Phytochemical compounds
Steroidal saponins	▪ 58 kinds of spirostanol saponins (such as protodioscin and protogracillin) ▪ 50 kinds of Furostanol saponins
Flavonoids	▪ 7 kinds of Flavonoids with quercetin as the basic parent structure ▪ 9 kinds of flavonoids with isorhamnetin as the basic parent structure ▪ 6 kinds of flavonoids with kaempferol as the basic parent structure
Alkaloids	■ 11 kinds of alkaloids containing Tribulusamide C, tribulusterine, tribulusin A, harmine, harman, harmmol, tribulusimide C, terrestriamide, N-trans-coumaroyl tyramine, N-trans caffeoylyramine, terrestribisamide
Others	■ glycosides ■ tannins ■ terpenoids ■ phytosterols ■ amide derivatives ■ amino acids (alanine, threonine) ■ organic acids (benzoic acid, vanillic acid, 2-methyl benzoic acid, ferulic acid, succinic acid, palmitic acid monoglyceride, succinic acid, docosanoic acid, *Tribulus* acid, and others) ■ other substances (such as 4-ketopinoresinol, Uracil nucleic acid, Coumarin, Emodin, Physcion, proteins)

T. terrestris IC_{50} against OVCAR-3 (ovarian carcinoma cell line) was calculated to be 157 μg/ml (Sadeghi Dinani et al., 2019). Sirotkin et al. (2020) reported efficacy of *T. terrestris* extract based on Bax level – a marker of cytoplasmic apoptosis, which indicated its effect on fundamental ovarian functions.

The studies showed that under *in vitro* conditions, *T. terrestris*' saponins decrease metastasis in breast cancer cell lines by lowering CCR7 (CC chemokine receptor 7), CXCR4 (CXC chemokine receptor 4) levels. They also induce apoptosis in breast cancer cells by down-regulating the expression of *Bcl-2* (B cell CLL/lymphoma-2) and up-regulation of *FADD* (fas associate with death domain), *AIF* (apoptosis-inducing factor), and *Caspase 8* (cysteine-aspartate protease 8) genes. It means that *T. terrestris* can induce both the external pathway of apoptosis in breast cancers by regulating *FADD* and the internal pathway by downregulating *Bcl-2*. In addition, *T. terrestris* caused an increase in activity of Caspase 3, which is essential for the cleavage of DNA in cancer cells, an up-regulation of the pro-apoptotic molecule of Bcl-2 associate x protein (*Bax*), and *p53* (protein p53 or police of genome) gene expression that indicate activation of internal apoptosis pathway (Goranova et al., 2015; Patel et al., 2019). Newly p53 status found by immunohistochemistry (IHC) or by DNA sequencing and the IHC expression of antiapoptotic *Bcl-2* has been proposed as an alternative prognostic marker in breast cancer. However, their correlation with conventional parameters and patient prognosis remains unknown. Only limited information exists about the distribution of *p53* and *Bcl-2* within the molecular breast cancer subtypes. The p53 protein, encoded by the *p53* gene and mutated in almost 30% of breast cancer, is a transcription factor. When *p53* is activated as part of the cellular stress response, it regulatesmany genes involved in cellular processes including cell cycle, senescence, and apoptosis. Its mutations are often associated with the construction of a stable protein that is readily traceable by IHC (Fabi et al., 2020). It has been reported that *Bcl-2* is a gene regulated by the HMGA1-p53-HIPK2 complex and HMGA1 is able to abolish the repression promoted by *p53* on *Bcl-2* expression (Esposito et al., 2010). Furthermore, caspase activation occurred, it means that the process of apoptosis is completed (caspase are located in the terminal chain of apoptosis molecular cascade) and this process is caspase-dependent. *T. terrestris*' leaves and seeds are cytotoxic to MCF7 cells. MCF-7 cells are well characterized and have been widely used as a model for checking the pathogenesis of breast cancer. Moreover, it may offer a pro-apoptotic tendency induced by *T. terrestris* extracts in MCF7 cells (Kulsoom et al., 2018). Furthermore, studies on saponins from other plants have shown that they exert a cytotoxic effect on cancer cell lines by regulating proliferation mediators (cyclins and cyclin-dependent kinases (CDKs)), modulating signaling pathways (Mammalian Target of Rapamycin (mTOR)), extracellular signal-regulated kinases 1 and 2 (ERK1/2), and elevating pro-apoptotic molecules levels (Law et al., 2012; Podolak et al., 2010; Nag et al., 2012; Xiao et al., 2012). However, with regard to *in vitro* studies on the effect of different steroid spirostanol and furostanol saponin compounds of *T. terrestris* extracts, there is neither an insufficient number of publications nor displaying their target molecules in cancer cell lines (Bedir et al., 2002; Hu and Yao, 2003; Ivanova et al., 2009). In addition, alkaloids of *T. terrestris* would be effective for the induction of the apoptosis pathway by regulating both intrinsic and extrinsic pathways in cancer cells (Basaiyye et al., 2017). In summary, it seems that *T. terrestris* would be a choice in the case of combination therapy for ovarian and breast cancers, specifically for hormone-resistant cells. Thus, we suggest that the study of apoptosis, angiogenesis, and metastases brings us an interesting area for further *in vitro* and *in vivo* researches. The suggested mechanism of ovarian and breast cancer improvement by *T. terrestris* is illustrated in Fig. 2.

12. Phytochemical molecular mechanism

Many studies demonstrated the fundamental role of secondary metabolites of *T. terrestris* and further considered each specific medicinal property of the plant to its individual secondary metabolites. In this respect, the main secondary metabolites of *T. terrestris* (including steroidal saponins, flavonoids, glycosides, phytosterols, tannins, terpenoids, amide derivatives, amino acids, and proteins) are summarized in Table 9 (Dixon, 2001; Zhu et al., 2017). Among the various kinds of constituents, steroidal saponins and flavonoids are considered to be the main secondary metabolites with different biological activities. There are insufficient data about *T. terrestris* and its secondary metabolites on the female reproductive system. Nevertheless, the actions of *T. terrestris* on sexual response have been attributed mainly to its steroidal properties (Wu et al., 1996), and mostly to protodioscin (Fig. 3) as a steroidal saponin (Rowland and Tai, 2003; Moghaddam et al., 2013). The exact mechanism of aphrodisiac effects of *T. terrestris* and its long-term activity are not fully understood. Further studies are needed to elucidate the pathophysiological mechanisms of action of *T. terrestris* as well as its efficacy and safety. However, it seems that the steroidal saponin (especially protodioscin) is the main compound responsible for the effects of *T. terrestris* on the female reproductive system. Several

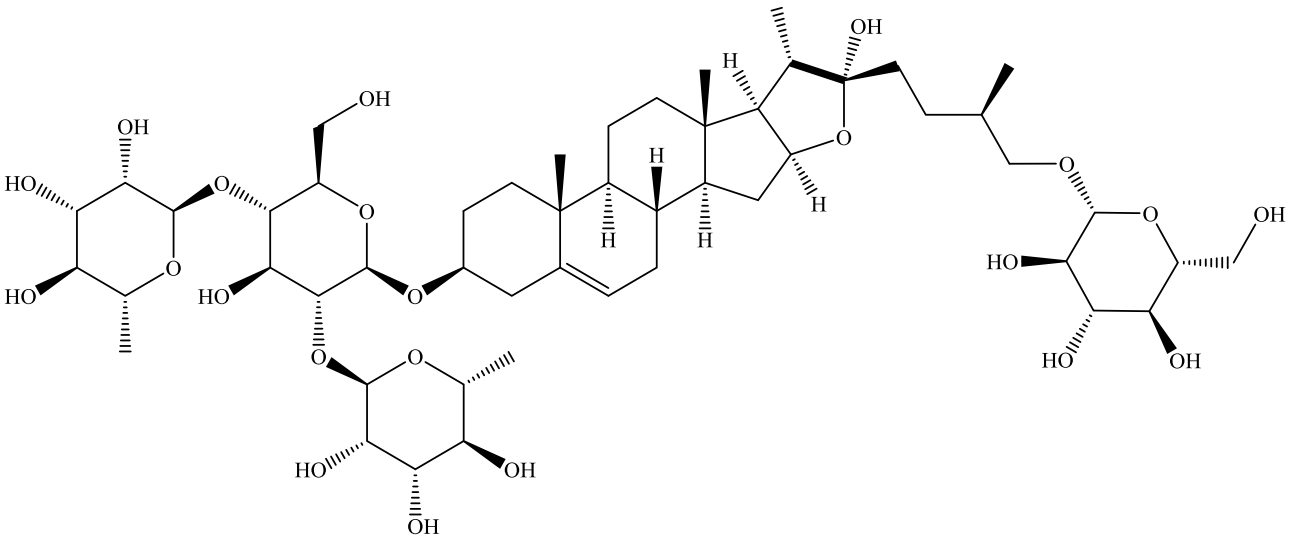

Fig. 3. Chemical structure of protodioscin.

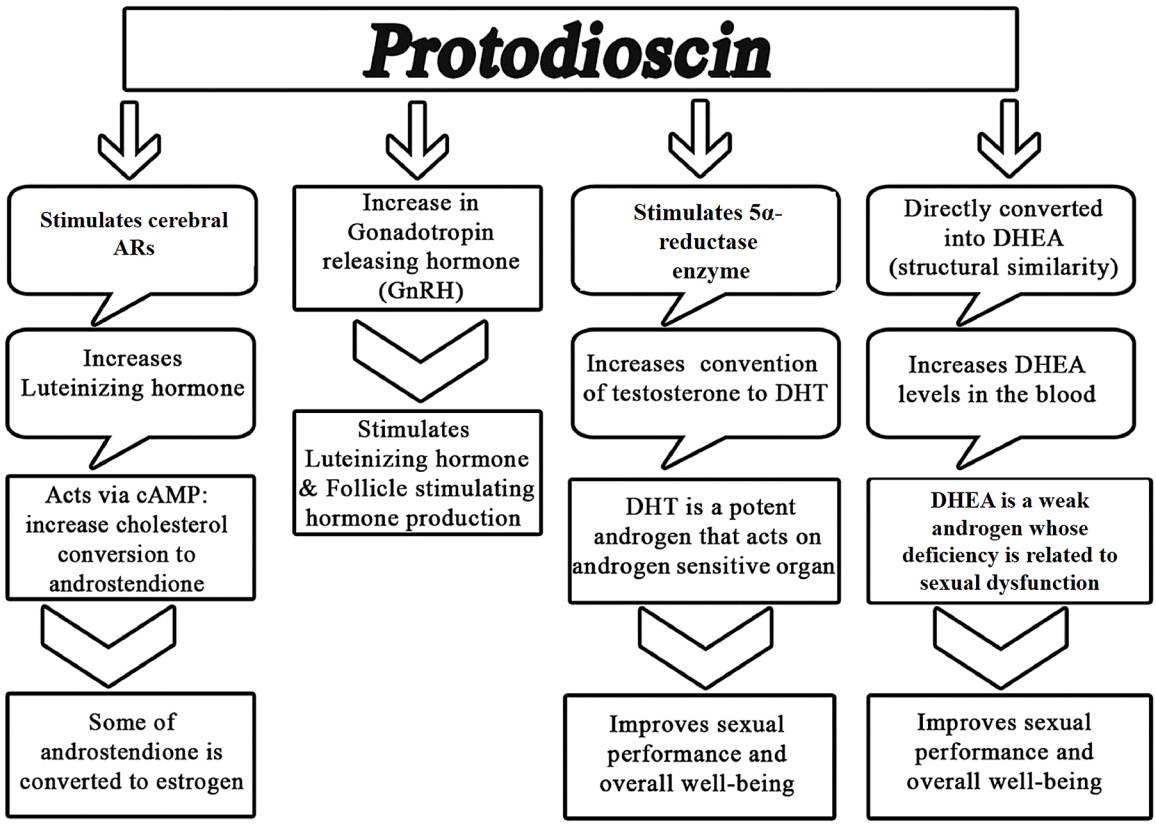

Fig. 4. Mechanisms involved for female reproductive system improvement by *T. terrestris*.
AR: androgen receptors; DHT: dihydrotestosterone; DHEA: dehydroepiandrosterone.

mechanisms have been proposed to explain this activity of *T. terrestris*: i) Protodioscin could convert into the androgen DHEA, naturally occurring sex steroid precursors to androgens and estrogens. Evidence showed that improvement in sexual symptoms was accompanied by a significant increase in DHEA levels, without causing an increase in testosterone levels, ii) Protodioscin leads to a direct increase in LH. This hormone has many receptors in *theca interna* cells of ovarian follicles and acts via cAMP to increase conversion of cholesterol to androstenedione. Some of androstenedione is converted to estrogen, which enters the circulation. Thus *T. terrestris* increases some sex hormones, possibly due to the presence of protodioscin in the extract which varies, iii) It is claimed that *T. terrestris* increased gonadotropin-releasing hormone (GnRH) from the hypothalamus which in turn stimulates the production of LH and FSH. Since studies have shown that *T. terrestris* consumption is associated with increased male testosterone levels, and Leydig cells are responsible for the secretion of testosterone that is independent of the secretion of LH. The *theca interna* cells are sensitive to LH and responded to the secretion of testosterone. Therefore, the hypothesis that *T. terrestris* acts like LH can be applied and confirmed to both sexes (Fig. 4).

Since the growth of the endometrium depends on the FSH in the first

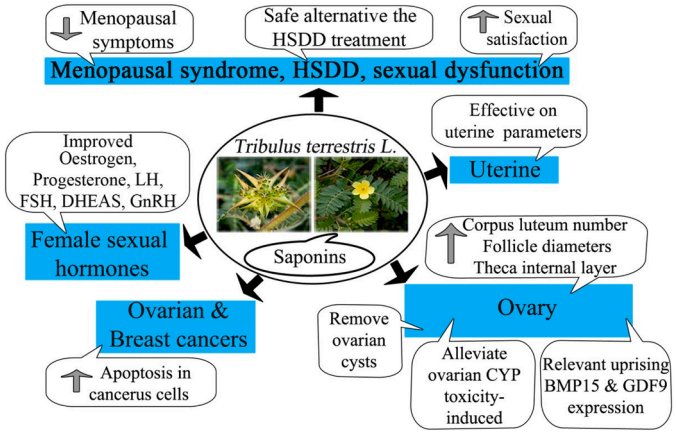

Fig. 5. *T. terrestris* beneficial effects on female reproductive system health.

stage of the monthly period, women should be asked about the status of the women's monthly cycle. In this review, we have seen, at high doses, *T. terrestris* can help to grow endometrial glands, which are FSH, dependent. As endometriosis is another cause of infertility in women; one of the main reasons for this is the increase in the amount of FSH, it is suggested that the use of low dose *T. terrestris* in the second half of the monthly cycle, secretary phase and dependent on LH would be prescribed, to these patients. Nevertheless, according to the best of our knowledge, we did not find any document related to the effect of *T. terrestris* on endometriosis and clinical trials that refer to the administration of *T. terrestris* in both proliferative (FSH dependent) phase and secretary (LH dependent) would bright the dark edges of this issue. Evaluating the serum level of inhibin, a negative feedback protein on the secretion of gonadotropins would be promising for confirming these endometriosis-related researches. Moreover, the assessment of serum inhibin level, which is secreted by follicular cells, in these two menstrual phases, may explain why both FSH and LH can promote the effect of *T. terrestris*.

13. Limitations and discussion on future research directions

However, the importance of the current findings about the effects of *T. terrestris* on the female reproductive system, the limitations of these studies, and directions for future researches included: i) There are many considerations when reviewing research related to the effects of *T. terrestris* on the female reproductive system. It is difficult to conduct a meta-analysis on these studies not only due to heterogeneity of the studies themselves but also the preparation methods and dosages; ii) the single-blind, short duration of the treatment. Long-term effects were not tested in these studies. It is suggested to do the same research on sexual satisfaction and function with more month intervention; iii) Small sample size was used in these studies, thus the results cannot be generalized to a wider population. Hence, trials with longer duration in larger sample size are recommended; iv) The majority of studies available were relate to the vasomotor symptoms of menopause, rather than sexual symptoms specifically. Varieties of symptom rating scales were used, and an aspect of sexual function was usually included as a small subsection in these scales. Therefore, future studies should make use of standardized scales to determine sexual function, such as the Female Sexual Function Index (FSFI); v) Almost all the studies emphasized that the actions of its components are steroid saponins of furostanol, being identified as protodioscin. However, further analysis of the impact of saponins at *in vitro* and *in vivo* models should be performed to elucidate this issue; vi) The future studies should be more than ever seen in some societies, where women are moderate and shy because of their cultural beliefs about sexual concepts; vii) Although the efficacy of *T. terrestris* on cancerous cells has been established, there is insufficient data about affected molecules by *T. terrestris* on ovarian and breast cancer cells. Thus, further researchers are suggested to consider apoptosis, angiogenesis, and metastasis pathways, three mechanisms involved in tumorigenesis, which would be affected by *T. terrestris* therapy for these two types of cancers.

14. Conventional doses and side effects

Contractual doses of this plant range 250–750 mg/day taken in equal proportions various times a day. Side effects of *T. terrestris* commonly have not been reported, but this may stem as much from a loss of systematic data as from a true absence of side effects. In the lack of well-controlled, long-term studies, its side effects are questionable. Because of the lack of studies on *T. terrestris*, we could not suggest a verdict on the trace or safety of this herb. Nevertheless, *T. terrestris* consumption in a young woman could cause hepatic failure (Ataee and Dadpour, 2020). In addition, it caused minor gastrointestinal side effects and acts as a diuretic. Furthermore, it may elevate testosterone, blood pressure, and growth hormone production. Moreover, patients usually are instructed to use *T. terrestris* intermittently in several-week cycles, because extended continuous usage may reduce the body's sensitivity. The World Health Organization (2004) recommended patients using *T. terrestris* extract to avoid excessive exposure to sunlight due to the possibility of phototoxic reactions. The use of sunscreen with a high sun protection factor (> 30) during treatment is recommended. The safety of *T. terrestris* extract for use in pregnancy and during breastfeeding has not been established.

15. Summary of evidence

The existence of medical influencing components of the plants has turn on a light as a treatment trend; based on traditional medicine, for the treatment of diseases. *T. terrestris*' aphrodisiac properties via enhanced steroid production is acceptable, but an effect on arousal or orgasmic function in humans has yet to be illustrated. Its popularity among athletes and its enduring usage in Indian medicine offered that undocumented pro-sexual effects might yet to be revealed. In this paper, we updated a comprehensive review of the medical beneficiary of the *T. terrestris* plant and its secondary metabolites on the female reproductive system and then categorized them to summarize their results to provide evidence for other researches. This review tries to lighten the efficacy of each main constituent of *T. terrestris* on the female reproductive systemas an applicable gathered document to accelerate future related researches. Based on our researches, preclinical and clinical studies provided evidence that *T. terrestris*, as an herbal medicine, may have beneficial effects on female reproductive system health (Fig. 5). These studies have reported influence of *T. terrestris* on women's sex drive and animal studies have reported effects of *T. terrestris* on LH, FSH, and developing follicles. Although, our search outcome illustrated that there is limited information about the effects of *T. terrestris* on the female reproductive system. Thus we emphasize that further researches on this subject should be addressed.

Our search showed that the effects of *T. terrestris* on PCOS were bound and only 1 case was examined on the human subjects. Also studies on effects of *T. terrestris* on other aspects of ovary and uterine were limited. The effect of *T. terrestris* on normal ovaries suggests that low doses of *T. terrestris* over a short period also have to impress significant effects on the uterine endometrium. Because many of the fertility problems of women are due to endometrial shortness and subsequently implantation failure, these conclusions could be effective for these individuals. However, to prove this claim, it is necessary to conduct multi-central double-blinded researches. The proposed mechanism for these modifications in *T. terrestris* recipient groups at a low dose of *T. terrestris* would be the LH-like activity of *T. terrestris*. Furthermore, the mechanism for higher doses with prolonged usage is still not clear. Moreover, *T. terrestris* was showed to be effective in

treating sexual complications among menopausal women. In addition, studies showed positive effects of *T. terrestris* on ovarian and breast cancers. Finally, in the studied articles, the positive effects of *T. terrestris* on the female reproductive system were reported to be mainly caused by the presence of protodioscin (a steroidal saponin) in this plant.

Author contributions

AG, NA, and JE contributed as the senior author and the principal investigator of this study, refined the study, and wrote the first draft of the manuscript. S-EN, AM, IYA, YQ and PL critically revised the manuscript. M-HF and JX identified and reviewed the studies for eligibility. All authors read, critically reviewed, and approved the final manuscript. All data were generated in-house, and no paper mill was used. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Competing Interest

The authors declare that there is no conflict of interest associated with this work.

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