

## REVIEW

*This paper is dedicated to the 70<sup>th</sup> anniversary of the founding of Physiologia Bohemoslovaca (currently Physiological Research)*

## Puncture Vine (*Tribulus Terrestris* L.) in Control of Health and Reproduction

Alexander V. SIROTKIN<sup>1</sup>, Adriana KOLESAROVA<sup>2</sup>

<sup>1</sup>Constantine the Philosopher University in Nitra, Nitra, Slovak Republic, <sup>2</sup>Slovak University of Agriculture in Nitra, Nitra, Slovak Republic

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### Summary

*Tribulus terrestris*, L. (puncture vine) have been used as a folk medicine for five thousands of years, but its targets, effects, their mechanisms and application requires further studies. This paper reviews the provenance, constituents and properties of *Tribulus terrestris*, L., its general physiological and health effects, as well as the currently available knowledge concerning its influence on male and female reproductive processes and their dysfunctions. Analysis of the available publications demonstrated the influence of *Tribulus terrestris* on a wide spectrum of targets and physiological processes and disorders. In particular, *Tribulus terrestris* can be a stimulator of male and female reproductive processes at the level of central nervous system, sexual behaviour, pituitary and gonadal hormones and their receptors, gonadal functions (including ovarian folliculogenesis and spermatogenesis), improvement of the quality and quantity of gametes (at least of sperm) and fecundity. This ability of puncture vine is applicable for the improvement of man's sexual desire and sperm quality *in vivo* and *in vitro*, as well as of women's libido, activation of women's reproductive organs, fecundity, and treatment of infertility, especially that related to the polycystic ovarian syndrome.

### Key words

Nutrition • Health • Testis • Ovary • Hormone • Signalling

### Corresponding author

A. V. Sirotkin, Dept. Zoology and Anthropology, Constantine the Philosopher University in Nitra, Tr. A. Hlinku 1, 949 74 Nitra, Slovak Republic. E-mail: [asirotkin@ukf.sk](mailto:asirotkin@ukf.sk)

### Introduction

Puncture vine (*Tribulus terrestris* L.) is a popular medicinal plant, which is considered as a traditional stimulator of masculine sexual desire. The available reviews concerning this plant do not reflect the recent information (Ukani *et al.* 1997, Chhatre *et al.* 2014, Shahid *et al.* 2016, Zhu *et al.* 2017), or they are focused on puncture vine's influence and use in man's reproduction (Neychev and Mitev, 2016, GamalEl Din, 2018, Sanagoo *et al.* 2019, Santos *et al.* 2019, Abarikwu *et al.* 2020). Current reviews concerning *Tribulus terrestris* effect on female reproduction, as well as on non-reproductive physiological and therapeutical actions of puncture vine, are practically absent in the available literature. This review summarizes briefly the available information concerning *Tribulus terrestris* provenance, constituents, properties, its action, and application in the control and treatment of male and female reproductive and non-reproductive processes and their disorders.

### Provenance and properties

The genus *Tribulus*, belonging to the family *Zygophyllaceae*, comprises about 20 species in the world, of which *Tribulus terrestris*, L. is the most common and popular as medicinal and food herb of this genus (Ukani *et al.* 1997, Chhatre *et al.* 2014, Parham *et al.* 2020).

The phytochemical study of *Tribulus terrestris* revealed the presence of steroidal saponins, flavonoids,

flavanol glycosides, alkaloids, and tannins (Chatre *et al.* 2014, Saiyed *et al.* 2016, Shahid *et al.* 2016, Zhu *et al.* 2017). The saponins included furostanol and spirostanol saponins of tigogenin, neotigogenin, gitogenin, neogitogenin, hecogenin, neohecogenin, diosgenin, chlorogenin, ruscogenin, and sarsasapogenin. In addition, four sulfated saponins of tigogenin and diosgenin type were also isolated. Saponins show variation in type, structure and composition of their aglycone moiety and oligosaccharide chains, but many saponins have surface active properties, as well as ability to reduce the risk of many chronic diseases and to exerts cytotoxic effects on cancer (Singh *et al.* 2017) (see below). *Tribulus terrestris* is characterized by high concentration and number (more than 18) kinds of flavonoids. The main flavonoids isolated from leaves and fruits of puncture vine were kaempferol, kaempferol-3-glucoside, kaempferol-3-rutinoside, and tribuloside [kaempferol-3- $\beta$ -d-(6''-p-coumaroyl) glucoside], caffeoyl derivatives, quercetin glycosides, including rutin and kaempferol glycosides (quercetin 3-O-rutinoside, quercetin 3-O-glycoside and kaempferol 3-O-glycoside) with anti-oxidant and phytoestrogenic properties (Chhatre *et al.* 2014, Kuchakulla *et al.* 2020, Verma *et al.* 2020). The plant also contains a mixture of beta-carboline alkaloids: harmane, norharmane, tetrahydroharmane, harmine, harmaline, harmol, harmalol, ruin and dihydroruin (Al-Bayati *et al.* 2008). The plant size, number of seeds, and the contents of furostanol saponins are very variable in dependance on the location and conditions of plant growth (Šalamon *et al.* 2006). Therefore, *Tribulus terrestris* contains a number of phytochemicals with potential biological activity.

### Physiological actions

The fruits and roots of *Tribulus terrestris*, alone or in combination with other medicinal plants, have been used as a folk medicine for five thousands of years in various Asiatic countries (Cchatre *et al.* 2014, Shahid *et al.* 2016, Zhu *et al.* 2017, Kamrani *et al.* 2019). In the ayurvedic medicine its application is recommended for the treatment of urinary affection, urinary calculi, polyuria, dyspnoea, cough, piles dysuria, heart disease and as a gastric stimulant (Ukani *et al.* 1997). In native Chinese medicine, the leaves of *Tribulus terrestris* are used for treatment of stomach problems, bladder stones, male reproductive disorders (Kumari and Singh 2015), and ocular diseases (Yuan *et al.* 2020).

Some of the *Tribulus terrestris* effects listed above were confirmed by modern Western medicine. The current scientific literature describes the following physiological, protective and therapeutical activities of puncture vine (Chhatre *et al.* 2014, Shahid *et al.* 2016): diuretic, antiurolithic, immunomodulatory, antidiabetic, hypolipidemic, cardiogenic, on central nervous system, hepatoprotective, anti-inflammatory, analgesic, antispasmodic, antibacterial, anthelmintic, larvicidal, and anticariogenic and anticancer activities (Chhatre *et al.* 2014, Parham *et al.* 2020, Verma *et al.* 2020). It is noteworthy that the toxic influence of *Tribulus* on cancer cells was more pronounced than that on healthy fibroblasts, suggesting the possibility of selective application of this plant against cells with malignant transformation (Neychev *et al.* 2007). In-vitro studies showed the protective effect of puncture vine against ocular retina injury (Yuan *et al.* 2020). *Tribulus* phytochemicals can be a promising replacement for routine antibiotics and antivirals including anti-HIV treatment (Shaheen *et al.* 2019, Parham *et al.* 2020). Animal studies revealed its applicability for protection against bone loss induced by estrogen deficit during age-related osteoporosis (Marques *et al.* 2019). Clinical studies indicated its applicability for weight loss (Salgado *et al.* 2017), prevention and treatment of hypertenzia, coronary hearth disease, cerebral arteriosclerosis, myocardial infarction, thrombosis (Verma *et al.* 2021), urinary tract infections, urolithiasis, dysmenorrhea, edema, hypertension, hypercholesterolemia (Shahid *et al.* 2016, Zhu *et al.* 2017, Shaheen *et al.* 2019) and physical fitness and muscular performance (Al-Bayati *et al.* 2008, Ma *et al.* 2017, Wu *et al.* 2017, Sellami *et al.* 2018). On the other hand, some studies did not reveal *Tribulus terrestris* action on physical performance in athletes (Pokrywka *et al.* 2014), sportsmen's muscle mass (Ma *et al.* 2017), body composition and muscular endurance in resistance-trained men (Sellami *et al.* 2018).

The variability in the targets of *Tribulus terrestris* can be due to the variability in its biological active constituents. The constituents of *Tribulus terrestris*, which are responsible for the particular effects of this plant, are however not fully determined. One group of possible puncture vine constituents could be phytoestrogenic saponins diosgenin, dioscin and protodioscin. Their anti-oxidant, anti-inflammatory, anti-diabetic, anti-cancer, cardioprotective and neuroprotective properties, as well as the ability to promote libido and physical fitness are similar to

properties of *Tribulus terrestris* (Parama *et al.* 2020). The next candidate constituent of puncture vine could be phytosterols, in particular beta-sitosterols, which, like puncture vine, can be beneficial for the prostate function, the urinary system, and the cardiovascular system (Sellami *et al.* 2018). Nevertheless, the only similarity in action of *Tribulus* and its molecules cannot be direct evidence, that just these molecules and their amounts are defining the action of this plant and explain the variability in its effect.

Some evidence indicates the adverse effect of puncture vine overconsumption: it can induce sleeping disorder, burnout and fatigue, hypertension, high heart rate (Sellami *et al.* 2018), photosensitisation and the resulted injury in both humans and animals (Chen *et al.* 2019). One case of intoxication with *Tribulus* has been reported (Pokrywka *et al.* 2014). On the other hand, clinical trials did not report serious adverse events induced in women by long *Tribulus terrestris* treatment (Martimbianco *et al.* 2020).

Therefore, *Tribulus terrestris* can target a number of variable physiological processes. Therefore, it can be a promising tool for treatment of a wide array of illnesses.

## Mechanisms of action

The number of processes affected by puncture vine indicates the possible variability in its mechanisms of action, which are sometimes not properly discovered. Nevertheless, the mediators of plant action on some selected processes and illnesses have been currently outlined.

Some *Tribulus* effects could be mediated by changes in hormones, cytokines and growth factors release, binding and reception. For example, in rats undergoing exercise *Tribulus terrestris* extract promoted muscle gain and physical performance, which was associated with an increase in plasma level of both IGF-I and its receptor (Wu *et al.* 2017). In boxers, the ability of *Tribulus terrestris* to alleviate muscle damage and to promote anaerobic performance was associated with a decrease in plasma insulin-like growth factor binding protein-3 (IGFBP-3), but not in its target – IGF-I (Ma *et al.* 2017). These reports indicate, that physical performance (and maybe activity, proliferation and reparation of cells other than myocytes) could be promoted in puncture vine by two ways - *via* reduction in IGFBP-3, which binds/inactivates the IGF-I and *via* up-

regulation of IGF-I receptors. Under the influence of puncture vine, the increased amount of free IGF-I binds and activates increased the amounts of IGF-I receptors.

The bone-protective effect of *Tribulus terrestris* observed in rats is explained by its ability to increase bone mineral density. This activity may be at least partially attributable to an increase in the serum level of dehydroepiandrosterone (but not of testosterone or estradiol) and a Ca<sup>2+</sup>-sparing effect (Marques *et al.* 2019).

The anti-inflammatory action of puncture vine can be mediated by prostaglandin. For example, addition of *Tribulus terrestris* to mouse macrophages inhibited their cyclooxygenase 2 (COX-2), an enzyme promoting prostaglandin E2, which is in turn involved in promotion of inflammation. It suggests that prostaglandin E can be a mediator of anti-inflammatory action of *Tribulus terrestris* (Hong *et al.* 2002).

The anti-inflammatory action of *Tribulus* can be mediated also by the intracellular promoter of inflammation – transcription factor nuclear factor kappa-B (NF-κB). For example, *Tribulus* can protect on rat brain from inflammation induced by formalin and carrageenan by down-regulation of NF-κB (Ranjithkumar *et al.* 2019). On the contrary, derivatives of *Tribulus terrestris* alkaloids can destroy cancer cells (Jurkat E6-1) *via* up-regulation of this transcription factor (Basaiyye *et al.* 2017). Gautam and Ramanathan (2019) demonstrated that the ability to puncture vine saponins to prevent brain inflammation and to relieve pain can be attributed to attenuation of both pro-inflammatory cytokines, Tumor Necrosis Factor Alpha (TNF-α), Interleukin 1 beta (IL-1β), and interleukin 6 (IL-6) and brain neurotransmitters, glutamate and aspartate.

Moreover, the neuroprotective action of *Tribulus* on rat brain was associated with activation of anti-apoptotic and pro-proliferating MAP kinase pathway (Ranjithkumar *et al.* 2019, Reshma *et al.* 2019) and neuronal survival pathway (BDNF), by changes in apoptosis-related protein kinases JNK, GSK3β/βcatenin (Ranjithkumar *et al.* 2019) and activation of promoters of apoptosis caspase-3 and AIF (Ranjithkumar *et al.* 2019, Yuan *et al.* 2020). The toxic effect of *Tribulus* on human fibroblasts was also associated with suppression of their proliferation, activation of their apoptosis and changes in cellular polyamines' homeostasis (Neychev *et al.* 2007).

A number of *Tribulus* phytochemicals including flavonoid, tannin, and phenolic acids have antioxidant properties, which can prevent oxidative stress, inflammation, apoptosis and the development of

a number of illnesses mentioned above (Parham *et al.* 2020). For example, *Tribulus terrestris* can protect ocular retina cells from oxidative stress-induced injury and apoptosis and increase their resistance and viability through activating numerous antioxidant enzymes and PI3K/Akt-Nrf2 signaling pathway (Yuan *et al.* 2020).

Studies on rats indicated that the anti-hypertensive effect of puncture vine can be due to its action on membrane hyperpolarization and relaxation of arterial smooth muscle, to boost up the discharge of nitric oxide from the nitrergic nerve endings and endothelium and/or by inhibitory action on angiotensin converting enzyme (Kamrani *et al.* 2019, Verma *et al.* 2020).

The ability of *Tribulus terrestris* to relieve painful urination or dysuria, hematuria, urinary urgency, burning micturition, frequent urination, nausea, and vomiting can be explained by its bacteriostatic and bacteriocidal action on a wide spectrum of urogenital bacteria, viruses and fungi (Al-Bayati *et al.* 2008, Shaheen *et al.* 2019, Parham *et al.* 2020). This action could be explained by the ability of *Tribulus* saponins to induce lysis of bacterial membranes (Al-Bayati *et al.* 2008).

These observations demonstrate the variability of the mechanisms mediated by *Tribulus terrestris* on various target organs and processes (and sometimes on the same target).

## Effect on male reproductive process

The best-known feature and area of *Tribulus terrestris* application in folk and official medicine is its ability to treat the loss of libido and infertility in animals (Haghmorad *et al.* 2019) and man (Sahin *et al.* 2016, Shahid *et al.* 2016, GamalEl Din 2019). It is proposed that saponins like dioscoreine, diosgenin, and the protodioscin can have beneficial effects on libido, whilst phytosterols, in particular beta-sitosterols, can be beneficial for the prostate function, and, therefore, for male fecundity (Sellami *et al.* 2018).

Analysis of phytochemical and pharmacological studies on animals and humans revealed an important role of *Tribulus* in treating erectile dysfunction and sexual desire problems (Neychev and Mitev, 2016) and fertility (Kumari *et al.* 2018). On the other hand, the performed clinical studies provided controversial and inconclusive results concerning the applicability of this plant for treatment of male infertility. Such results could be due to clinical trials, which were imperfect from the viewpoint

of methodology and patient number, as well as the differences in causes of male infertility (Gamal El Din 2018).

In contrast to *Tribulus terrestris* action on libido and erectile function, the ability of this plant to improve on male sperm parameters, namely number, motility and morphology, has been demonstrated in the majority of performed clinical studies (Roaiah *et al.* 2017, Salgado *et al.* 2017, Sanagoo *et al.* 2019). Animal and in-vitro studies also demonstrated that *Tribulus terrestris* can increase sperm quantity, quality in rats (Martino-Andrade *et al.* 2010, Kumari and Singh 2015, Sahin *et al.* 2016, de Souza *et al.* 2019, Salahshoor *et al.* 2020), rams (Kistanova *et al.* 2005), livestock (Clément *et al.* 2012) and humans (Asadmobini *et al.* 2017, Khaleghi *et al.* 2017). In rams *Tribulus terrestris* increased fertility (Kistanova *et al.* 2005).

The positive influence of *Tribulus* on sperm has been associated with signs of activation of male reproductive organs. At least, *Tribulus terrestris* increased mouse testis weight and recovered the spermatogenic cycle (Kumari and Singh, 2015). Puncture vine enhanced the number of Leydig, spermatogonia and spermatid cells in rat testis (Haghmorad *et al.* 2019). Similarly, protodioscin, the main phytochemical agent of the *Tribulus*, can promote proliferation of Sertoli and germ cell proliferation, which promotes the growth and function of seminiferous tubules in men (Salgado *et al.* 2017). On the other hand, no influence of *Tribulus terrestris* extract on weight and histostructure of rat prostate, seminal vesicles, and height of germinal layer of seminiferous tubule has been found (Martino-Andrade *et al.* 2010, Sahin *et al.* 2016, Salahshoor *et al.* 2020).

Therefore, the ability of *Tribulus terrestris* to improve sperm characteristics and therefore male fertility can be evident. On the other hand, *Tribulus* action on the state of reproductive organs has been examined only in rats, whilst the obtained results were sometimes contradictory. *Tribulus terrestris* applicability to treat psychological and physical impotence remains an open question too, which should be addressed by strong clinical studies.

## Mechanisms of effects on male reproduction

Results of the basic studies indicate that *Tribulus terrestris* can affect male reproductive processes *via* the same extra- and intracellular mediators as the non-reproductive processes (see above).

Some (but not all) studies indicated that puncture vine can affect male reproductive processes through the up-regulation of release of reproductive hormones - gonadotropins and androgens. Treatment of rats with *Tribulus terrestris* increased level of LH (but not FSH) and testosterone in their plasma (Ghosian Moghaddam *et al.* 2013, Haghmorad *et al.* 2019, Salahshoor *et al.* 2020). Sahin *et al.* (2016) observed the stimulatory action of *Tribulus* extract on rat plasma testosterone, but not LH or FSH. Some studies showed the ability of *Tribulus terrestris* to increase dihydrotestosterone (Selgado *et al.* 2017) and testosterone (Sahin *et al.* 2016, Roaiah *et al.* 2017, Sellami *et al.* 2018, GamalEl Din 2019) levels in men's plasma. Other studies however did not detect *Tribulus terrestris* influence on androgen release in rat (Martino-Andriade *et al.* 2010, Ghosian Moghaddam *et al.* 2013) or humans (Pokrywka *et al.* 2014, Neychev and Mitev, 2016, Ma *et al.* 2017, Santos *et al.* 2019, Kuchakulla *et al.* 2021), although the puncture vine influence on libido and fecundity occurred. These observations suggest that „empirical evidence to support the hypothesis that this desirable effects are due to androgen enhancing properties of *Tribulus terrestris* is, at best, inconclusive“ (Neychev and Mitev, 2016), and androgens are not (or not always) mediators of male reproduction-stimulating activity of *Tribulus terrestris*. More probable appears the hypothesis explaining *Tribulus terrestris* effect on brain structures. At least, treatments with puncture vine extract promoted nicotinamide adenine dinucleotide phosphate-diaphorase activity and androgen receptor immunoreactivity in rat brain (Gauthman and Adaikan, 2005). These structures could be central mediators of puncture vine action on sexual behavior. Therefore, this plant could promote androgen-dependent events not (or not only) *via* androgen production, but also through androgen reception and effects in the brain.

Puncture vine can improve sperm production through activation of proliferation of testicular cells and spermatogenesis, which has been reported after *Tribulus terrestris* or its constituent protodioscin in mice (Kumari and Singh, 2015), rat (Haghmorad *et al.* 2019) and man (Salgado *et al.* 2017).

*Tribulus terrestris*-induced stimulation of rat reproductive behavior and testosterone release were associated with reduction of transcription factor NF- $\kappa$ B and increased the levels of NF-E2-related factor 2 (Nrf2) and heme-oxygenase-1 (HO-1) accumulation in their male reproductive organs (Sahin *et al.* 2016). It suggests

that these signaling substances could be intracellular mediators of puncture vine on libido or androgen release. Furthermore, there is emerging compelling evidence from experimental studies in animals for possible endothelium and nitric oxide-dependent mechanisms underlying *tribulus terrestris* aphrodisiac and pro-erectile activities (Neychev and Mitev, 2016). It is still to be examined whether intracellular signaling mechanisms mediate puncture vine action on human male reproductive processes.

The stimulatory action of *Tribulus terrestris* on male sperm quality could be due to antioxidative properties of some plant molecules (see above). Addition of antioxidants usually increases sperm quality. *Tribulus terrestris* increased the concentration of antioxidative enzymes and the anti-oxidant activity in mice testis, as well as the sperm resistance to oxidative stress (Kumari and Singh, 2015, Salahshoor *et al.* 2020). Clément *et al.* (2012) explained the benefits of *Tribulus terrestris* extract for livestock sperm by antioxidant properties of puncture vine constituents.

Therefore, *Tribulus terrestris* can improve various male reproductive parameters *via* brain structures and through direct action on the testis and sperm. This effect can be mediated by stimulatory action of puncture vine action on ovarian gonadotropins, androgens, androgen receptors, several intracellular signaling pathways and by the ability of *Tribulus terrestris* to scavenger reactive oxygen species. The possible targets and mechanisms of *Tribulus* action on male and female reproductive system are present in Fig.1.

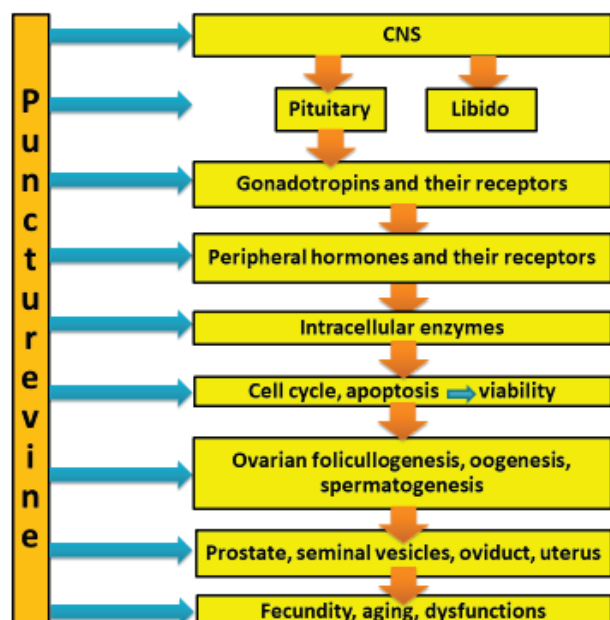
## Effects on female reproductive processes

The effect of *Tribulus terrestris* on female reproductive processes and its application for treatment of female reproductive disorders are studied much less than this plant in relation to male reproduction. Nevertheless, the few available publications indicate the influence of puncture vine on female reproductive hormones, behavior, ovarian, oviductal and uterus state, ovarian cycle, ovulation and fecundity.

Clinical studies demonstrated the ability of *Tribulus terrestris* to promote libido and other signs of sexual behavior and to prevent their decline in pre- and postmenopausal women (Mazaro-Costa *et al.* 2010, Martimbianco *et al.* 2020).

Abadieva *et al.* (2016) reported that feeding with *Tribulus terrestris* altered the expression of bone

morphogenetic protein (BMP) 15 and growth differentiation factor (GDF) 9 in rabbit ovarian structures. *Tribulus terrestris* caused a decrease in the BMP15 mRNA level in the oocytes and an increase in the cumulus cells. The GDF9 mRNA level increased significantly in both oocytes and cumulus cells.



**Fig. 1.** The possible targets of puncturevine (*Tribulus terrestris*, L.) in male and female reproductive systems (simplified). The detailed explanations are in the text.

An *in-vitro* study showed the ability of *Tribulus terrestris* extract to promote accumulation of both proliferation and apoptosis markers in cultured porcine ovarian granulosa cells. The stimulatory action of puncture vine extract on both proliferation and apoptosis could indicate the ability of this plant to boost ovarian cell turnover. Furthermore, in these experiments, *Tribulus terrestris* prevented and even reversed the stimulatory action of metabolic hormone ghrelin on apoptosis marker (Sirotkin *et al.* 2020).

Experiments on rats with polycystic ovarian cysts (Dehghan *et al.* 2012) and with ovarian syndrome (PCOS) (Sandeep *et al.* 2015, Sayed *et al.* 2016), as well as on women with oligo/anovular infertility (Arentz *et al.* 2014) demonstrated the ability of *Tribulus terrestris* to induce their ovarian follicle development, ovulations, and fertility. *Tribulus terrestris* given together with *Withania somnifera*, prevented the signs of polycystic ovarian syndrome in rats – increase in estradiol and testosterone (but not in LH) release, increase in ovarian (but not uterus) weight and prolongation of the estrous cycle

(Sayed *et al.* 2016). Experiments of Dehghan *et al.* (2012) on rats demonstrated the applicability of *Tribulus terrestris* for luteinization and treatment of ovarian cysts.

Some animal studies demonstrated the ability of *Tribulus terrestris* extract to promote the development of uterine and vaginal tissue in rats (Esfandiari *et al.* 2011), but other studies did not show this action in rats (Martino-Andrade *et al.* 2010, Sayed *et al.* 2016) or women (Arentz *et al.* 2014).

Therefore, the available publications suggest the ability of *Tribulus terrestris* to promote female reproductive functions on various levels. It can promote female sexual desire, to alter release of pituitary gonadotropins and ovarian steroid hormones, BMP15 and GDF9, to promote ovarian cell proliferation and apoptosis, to promote ovarian cycle, development of uterus and vagina, ovulation and fecundity, as well as to mitigate the signs of PCOS and oligo/anovular infertility. Nevertheless, the available information concerning *Tribulus terrestris* action on animal ovary and uterus is conflicting. Furthermore, the ability of *Tribulus terrestris* to prevent signs of PCOS was more pronounced in animal experiments, than in clinical studies. The performed clinical trials were methodologically heterogenic, and they were performed on a relatively small number of patients, therefore they could not be considered as conclusive yet (Arentz *et al.* 2014).

## Mechanisms of effects on female reproduction

There is evidence that *Tribulus terrestris* stimulates ovulation and fecundity and relieves symptoms of PCOS *via* promotion of release of pituitary and ovarian hormones – the known stimulators of reproductive processes. At least treatment of rats with *Tribulus terrestris* prevented the signs of induced PCOS - decrease in plasma FSH and increase in LH, testosterone and estradiol level, increase in ovarian weight and decrease in weight of uterus (Sayed *et al.* 2016). Puncture vine treatment in women was associated with an increase in FSH and estradiol (which is characteristic for healthy women), but not of LH and testosterone (which is characteristic for women suffering from PCOS) (Arentz *et al.* 2014). Other studies however did not note a decrease, but increase in testosterone release in premenopausal women treated with *Tribulus terrestris* (Martimbianco *et al.* 2020).

The yeast androgen bioassay demonstrated that

extract of *Tribulus terrestris* has an anti-androgen activity, whilst other plants (including *Whithania somnifera*) exhibited the androgenic properties (Sandeep *et al.* 2015). This anti-androgen action of puncture vine could explain its medicinal effect against PCOS associated with women androgenisation.

In contrast to animals, women's sexual desire is promoted by both estrogens and androgens, which activate sexual centers in CNS (Cappelletti and Wallen, 2016). The ability of *Tribulus terrestris* to promote women's sexual behavior can be explained by increased production of androgen (Martimbianco *et al.* 2020) and/or estrogen (Arentz *et al.* 2014). Estrogen, whose production can be boosted by puncture vine, is also a known regulator of ovarian cell proliferation and apoptosis, as well as a promoter of ovarian follicle development, survival and oogenesis (Sirotkin 2014). These processes can be promoted not only by estrogen, but also by BMP15 and GDF9 (Sanfins *et al.* 2018), whose expression in the ovary has been affected (mainly increased) by *Tribulus terrestris* (Abadieva *et al.* 2016).

Steroid hormones can regulate ovarian folliculogenesis and the resulting fecundity through an effect on ovarian cell proliferation and apoptosis. Their changes under direct influence of *Tribulus terrestris*, have been documented (Sirotkin *et al.* 2020). Therefore, steroid hormones might be considered as mediators of puncture vine action on ovarian state too.

The similarity of stimulatory action of *Tribulus terrestris* and estrogen on uterine and vaginal tissue's growth and development (Esfandiari *et al.* 2011) indicates that puncture vine contains phytoestrogens, activating estrogen receptors, which could be one of the possible mediators of *Tribulus terrestris* action on female reproductive system.

The involvement of other mechanisms in mediating puncture vine on female reproduction cannot be excluded either. It is very probable that brain structures, antioxidants a. o. can mediate *Tribulus terrestris* action not only on male (see above), but also on female reproduction.

The available data enables us to hypothesise several interrelated signaling pathways mediating *Tribulus terrestris* action on female reproductive functions and their disorders. They can include CNS structures, pituitary gonadotropins, steroid hormones, BMP15, GDF9 and their target processes including ovarian cell proliferation, apoptosis, ovarian folliculogenesis, oogenesis, ovulation and fecundity.

These hypothetical mechanisms of *Tribulus terrestris* action on female reproductive system are presented in Fig.1. The intervention of *Tribulus terrestris* to several regulatory pathways at once is highly probable.

## Application in reproductive biology and medicine

The results of pre-clinical and clinical studies demonstrated the applicability of *Tribulus terrestris* for improvement of sperm quality and therefore of fertility in man and farm animals. It is not to be excluded that it could be useful also for promotion man's libido and erectile functions, although such application requires further validation yet.

The available information suggests that *Tribulus terrestris* could be a potent biostimulators of female reproductive processes – of sexual desire, ovarian cell turnover and follicular development, ovulation and fecundity. Its stimulatory influence on oogenesis, uterus and vagina growth are possible too (see Fig.1 for review), although this influence has not been verified on women. Furthermore, a limited number of clinical trials suggested that *Tribulus terrestris* can be successfully used for treatment of PCOS and related infertility.

## Conclusions and possible direction of future studies

Analysis of the available publications demonstrated the influence of *Tribulus terrestris* on a wide spectrum of targets and physiological processes. This effect can be mediated by multiple extra- and intracellular signalling pathways. Some performed clinical studies demonstrated the applicability of this plant and its polyphenols for treatment of a number of various disorders according to the principles of traditional oriental and modern medicine. In particular, *Tribulus terrestris* can be a stimulator of male and female reproductive processes at the level of central nervous system, sexual behaviour, pituitary and gonadal hormones and their receptors, gonadal functions (including ovarian folliculogenesis and spermatogenesis), improvement of the quality and quantity of gametes (at least of sperm) and fecundity (Fig.1). This ability of puncture vine is applicable for improvement of man's sexual desire and sperm quality *in vivo* and *in vitro*, as well as of women's libido, activation in women reproductive organs, fecundity, and treatment

of infertility, especially related to PCOS.

Nevertheless, understanding the biological role and application of puncture vine requires more profound studies. Although the main studies of this plant were aimed at its medical application, the physiological and medicinal effects of *Tribulus terrestris* were expressed better in animal experiments than in clinical trials, whose protocols and results are variable and therefore less conclusive. In the main cases, it remained unknown which plant constituent was responsible for a particular effect. Often the mediators of puncture vine effects have been only hypothesised on the basis of their changes after treatment with this plant, but their mediatory role remains to be demonstrated. A wide variety of possible factors mediating puncture vine effect on various targets and their possible interrelationships complicate understanding the mechanism of action of this plant and the causes of variability in its observed effects. Finally, the medicinal

application of puncture vine requires more clinical tests on a larger number of patients with an adequate protocol and control groups. Nevertheless, the available data listed here demonstrate the biological and medicinal potency of this plant, the study and application of which is worth further efforts.

### Conflict of Interest

There is no conflict of interest.

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