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Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Tribulus terrestris* L., herba

Final

Based on Article 10a of Directive 2001/83/EC (well-established use)

Based on Article 16d(1), Article 16f and Article 16h of Directive 2001/83/EC (traditional use)

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Tribulus terrestris* L., herba
Herbal preparation(s)	Dry extract (DER 35-45:1), extraction solvent methanol 80% V/V, containing not less than 45% of furostanolic saponins calculated as protodioscin
Pharmaceutical form(s)	Herbal preparations in solid dosage forms for oral use
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1. Introduction

1.1. Description of the herbal substance(s), herbal preparation(s) or combinations thereof

- Herbal substance(s)

Tribulus terrestris L., herba

Definition: dried, whole, or fragmented flowering and fruit bearing aerial parts of *Tribulus terrestris* L.

Tribulus terrestris L., herba is not subject of Ph. Eur., pharmacopoeia of any EU Member State or any other pharmacopoeia.

While the herb is not subject of any official pharmacopoeia, *Tribulus terrestris* fruit is subject of British Pharmacopoeia (2018) and of Japanese Pharmacopoeia, Korean Pharmacopoeia, Pharmacopoeia of China and Siddha Pharmacopoeia of India, and Ayurvedic Pharmacopoeia of India (Ștefănescu *et al*., 2020).

For fruit, WHO monograph (2009) Fructus tribuli exists. The fruits and roots of *Tribulus terrestris* can be found also among Natural Health Products published by Health Canada in 2019 (Natural Health Products Ingredients Database).

The whole plant is subject of Homeopathic Pharmacopoeia of India (Homeopathic Pharmacopoeia of India, 2016).

Tribulus terrestris is widespread in Mediterranean, subtropical and desert climates worldwide. The plant grows commonly throughout India. It is an aggressive colonizer in all continents except Antarctica. (Adaikan *et al*., 2001, Chhatre *et al.*, 2014).

T. terrestris is an annual (sometimes perennial in warm climates) herb with a long, slender, branched taproot. The greenish-red stems are up to 2 m long, branched, radiating from a central axis and covered with fine hairs. Leaves, 3-7 cm long, are in opposite pairs with one of the pair slightly smaller than the other. Each leaf consists of three to eight pairs of opposite, oblong-lanceolate leaflets, each leaflet being 5 to 15 mm long and 3 to 5 mm wide, base obliquely rounded, upper surface green, sparsely pubescent, lower surface whitish pubescent, stipules linear, up to 10 mm long, pedicel 2-4 cm long. The flowers are yellow, 5-petalled, 7 to 15 mm in diameter, sepals 3-5 mm long, petals 3-12 mm long, stamens about as long as the petals, solitary and borne on short stalks in the axils of the smaller of each pair of leaves. Fruit globose, consisting of (usually) 5 hairy or nearly glabrous, often muriculate, woody cocci, each with 2 pairs of hard, sharp spines, one pair longer than the other. Seeds are yellow, variable in shape but more or less ovoid and 2-5 mm long (Invasive Species Compendium, *Tribulus terrestris* (puncture vine); Homeopathic Pharmacopoeia of India, 2016; Chhatre *et al.*, 2014; Adaikan *et al*., 2001; Hashim *et al.*, 2014; Das *et al.*, 2017, van Valkenburg and Bunyapraphatsara, 2001, WHO 2009).

Variation in burr morphology, chromosomes number and isozymes has been reported by Morrison and Scott (Scott and Morrison, 1996, Morrison and Scott, 1996, 1996a).

Preliminary phytochemical study of TT revealed the presence of steroidal saponins, flavonoids, tannins, terpenoids, polyphenol carboxylic acids, and alkaloids.

The substances of potential therapeutic interest are steroidal saponins. Steroidal saponins from different parts of *Tribulus terrestris* L. are the subject of many published studies (e.g., Cai *et al.,* 2001, De Combarieu *et al.*, 2003, Conrad *et al*., 2004, Huang *et al.*, 2003; Deepak *et al.*, 2002, Dinchev *et*

al., 2008, Hashim *et al.*, 2014, Joshi and Uniyal, 2008, Kang *et al.*, 2014, Kostova and Dinchev, 2005, Su *et al.*, 2009, 2009a, Liu *et al.*, 2010, Sun *et al.*, 2002, Chen *et al.*, 2012, Vasait, 2017, Wang *et al.*, 2016, Xu *et al.*, 2000, Qi *et al.*, 2018, Semerdjieva and Zheljazkov, 2019 (review), Chhatre *et al.*, 2014 (review), Ștefănescu *et al.*, 2020 (review), Umadevi and Srinath, 2017 (review), Zhu *et al.*, 2017 (review), Zheng *et al.*, 2017, Lazarova *et al.*, 2011, Sarvin *et al.*, 2017, Tian *et al.*, 2019).

Steroidal saponins are presented in glycosidic form. The oligosaccharides chains are commonly composed of galactose, glucose, rhamnose and xylose, which could have units of long and various linkage types to the hydroxyl group at C-3 and/or C-26 (Qi *et al.*, 2018, Kostova and Dinchev, 2005).

The highest content of saponins in the aerial parts was met during the pre-flowering and flowering periods (Dinchev *et al.*, 2008).

The following furostanol saponins - protodioscin, neoprotodioscin and their respective sulphates, methylprotodioscin, prototribestin, methylprototribestin, tribol (lack of sugar moiety at C-26), terrestrinins A, B and spirostanol saponins diosgenin, tigogenin, neotigogenin, gitogenin, neogitogenin, hecogenin, neohecogenin, chlorogenin, ruscogenin, sarsapogenin, tribulosin; four sulphated saponins of tigogenin and diosgenin type are reported (Kostova and Dinchev, 2005).

A different distribution of the steroidal saponins in the different parts of the plant has been reported by Qui *et al.* While stems and leaves have similar saponins distribution behaviour, the fruits are quite different with them. Spirostanolic nucleus showed a high expression in leaves. In case of furostanol saponins, the distribution is depending on the length of oligosaccharides. The saponins with lower molecular weight (< 1100 Da), i.e. with short sugar chain, can be preferably found in fruits, and the saponins with higher molecular weight (>1100 Da, longer sugar chain) are situated in leaves and stems (Qi *et al.*, 2018).

Survey of the literature data reveals some differences in the saponin content and the saponin composition of *T. terrestris* growing in different geographic regions of the world (Kostova and Dinchev, 2005, Joshi and Uniyal, 2008, Ganzera *et al.,* 2001).

Phytochemically, currently two chemotypes were recognised:

- chemotype containing protodioscin as a dominant saponin and sulphur containing saponins prototribestin and tribestinin in samples from East South Europe and West Asian regions and
- chemotype containing tribulosin as a dominant saponin in samples from India and Vietnam (Dinchev *et al.*, 2008).

The saponins analysed from samples from India and China contained sugar units such as galactose, glucose, rhamnose, and xylose, while those from Bulgaria had only glucose and rhamnose (Ganzera *et al.*, 2001).

In addition, presence of flavonoids is reported. These include mainly derivatives of kaempferol, quercetin and isorhamnetin; kaempferol, kaempferol-3-glucoside (astragalin), kaempferol-3- rutinoside and tribuloside [kaempferol-3-β-d-(6″-p-coumaroyl) glucoside], rutin, hyperoside, quercetin, quercetrin, naringenin, naringin, hesperetin, apigenin, caffeoyl derivatives (Bhutani *et al.*, 1969, Louveaux *et al.*, 1997, Das *et al.*, 2017, Ammar *et al.*, 2018, Ștefănescu *et al.*, 2020).

Content of main flavonoids is 1.5 times higher that of main saponins (Das *et al.*, 2017, Chhatre *et al.*, 2014).

The plant contains sterols such as β-sitosterol, β-sitosterol-D-glucoside, stigmasterol, cholesterol, campesterol (Deepak *et al.*, 2002, Li *et al.*, 1998, Das *et al.*, 2017, Ammar *et al.*, 2018, Ștefănescu *et al.*, 2020).

β-carboline alkaloids i.e. harmalol, harmaline and neoharmane have been isolated from stems and harmane from the fruits of *Tribulus terrestris* (Kadam *et al.*, 2019). Paper chromatography of herb alkaloid fraction has shown a presence of 5 alkaloid compounds, one of the alkaloids gave the same spot and fluorescence as harmane. Presence of the harmine and harmol were not confirmed. Generally, the authors found that the amount of alkaloids is below the level of classification of the herbal substances as alkaloid herbs (Borkowski and Lutomski, 1960). Tribulusterin and perlolyrin are new alkaloids isolated from the fruit of *Tribulus terrestris* (Bremner *et al.*, 2005).

Eleven phenolic amides (N-trans-cinnamoyltyramine, N-trans-feruloyltyramine, N-(2-(4-hydroxyphenyl) - 2-methoxyethyl) cinnamamide, N-trans-feruloyloctopamine, N-trans-caffeoyltyramine, N-trans- coumaroyltyramine, terrestriamide ferulamide, N-cis-feruloyltyramine, N-cis-caffeoyltyramine, cis-terrestriamide) were isolated and characterized from the fruits of *T. terrestris* from South Korea (Kim *et al.*, 2017). Additionally, tribulusamides A and B and terrestriamide have been isolated from fruits collected from the north-western part of China (Li *et al.*, 1998). Terrestribisamide has been isolated from fruits by Wu *et al.* (1999).

Other substances found in the plant are carbohydrates in a form of free sugars, e.g. raffinose, arabinose, galacturonic acids and stachyose; the main polysaccharide is inulin (Ammar *et al.*, 2018).

In addition, terpenoids – mainly oxygenated compounds monoterpenes (mainly dill ether), sesquiterpenes (mainly hydroxytoluene and dihydroagarofuran (Ammar *et al.*, 2018, Ibrahim and Kadhim, 2015; Vasait, 2017) have been found in the plant. The plant contains peptides and amino acids, total protein content in the aerial part is 16.63%. Essential amino acids are phenylalanine, threonine, valine, leucine and lysine and non-essential amino acids aspartic acid, serine, glutamic acid, glycine, alanine, tyrosine and arginine are present as non-essential amino acids (Ammar *et al.*, 2018, Vasait, 2017). Presence of phenolic derivatives (other than flavonoids) such as pyrogallol, gallic acid, protocatechuic acid, catechin, catechol, chlorogenic acid, p-hydroxybenzoic acid, caffeic acid, vanillic acid, ferulic acid, salicylic acid, ellagic acid, coumaric acid and cinnamic acid (Ammar *et al.*, 2018), tannins (Vasait, 2017, Ibrahim and Kadhim, 2015) is reported. Additionally fatty acids – saturated and unsaturated (major unsaturated fatty acid linolenic acid and major saturated was heptadecanoic acid) have been reported by Ammar *et al.*, 2018. Macro elements found in the plant are Na, Mg, K, Ca, Fe and trace elements Mn, Cu, Zn, Mo, Cr, Al, Ba, V, Se, Ni, Sr, Cd. There is an indication that the plant can accumulate Zn, Cd, Sr, Ba, Se (Tkachenko *et al.*, 2020).

- Herbal preparation(s)

  The following herbal preparation has been reported as constituent of medicinal products on the market in the EU/EEA Member States (for further information see section 2 "Data on medicinal use"):

  Dry extract (DER 35-45:1), extraction solvent methanol 80% V/V, containing not less than 45% of furostanolic saponins calculated as protodioscin.

- Combinations of herbal substance(s) and/or herbal preparation(s) including a description of vitamin(s) and/or mineral(s) as ingredients of traditional combination herbal medicinal products assessed, where applicable.

  Not applicable

1.2. Search and assessment methodology

Book chapters, articles and letters in Journals, Medical press reviews, Acts of law and regulations (see the List of references in Annex)

Search engines used: Google, Google Scholars

Scientific databases: PubMed (Using the Mesh term '*Tribulus terrestris'*, 501 hits; 'Puncture vine' 507 hits; 'caltrop' 542 hits. Search date: February 2022)

Medical databases: Cochrane Database of Systematic Reviews (Using the search terms '*Tribulus terrestris,*' 'Gokshura', 'caltrop')

Toxicological databases: ToxNet

Pharmacovigilance resources: EudraVigilance 15.2.2022, active substance *Tribulus terrestris*

Data from EU and non-EU regulatory authorities: AEMPS, Health Canada, WHO monographs, EFSA

2. Data on medicinal use

2.1. Information about products on the market

2.1.1. Information about products on the market in the EU/EEA Member States

Information on medicinal products marketed in the EU/EEA

Table 1: Overview of data obtained from marketed medicinal products

Active substance	Indication	Pharmaceutical form	Regulatory Status
Tribuli terrestris herbae extractum siccum (35-45:1) extraction solvent methanol 80% containing not less than 45% of furostanolic saponins	Impotentia coeundi, male sterility, when due to oligospermia or reduced spermatozoa motility; status of sexual depression and exhaustion.	Film-coated tablets containing 0.25 g of the extract in one tablet Dosage and therapy duration are determined by the severity of the case. Most frequently used doses are 1-2 tablets 3 times daily during meals.	Bulgaria 1981 - 2002
Tribuli terrestris herbae extractum siccum (35-45:1) extraction solvent methanol 80% containing not less than 45 % of furostanol saponins calculated as protodioscin	In the complex therapy of decreased libido, impotence, infertility; dyslipoproteinemia; menopausal and post castration syndrome	Film- coated tablets containing 250 mg of the extract in one tablet, corresponding to 112.5mg of furostanolic saponins calculated as protodioscin <u>Posology</u> 1.*Decreased libido, impotence and infertility –* *In men with decreased libido, impotence and infertility* 1-2 tablets x 3 day; duration of use – 90 days In women with endocrine infertility 1-2 tablets x 3 day	Bulgaria Marketing authorisation in 2002

Active substance	Indication	Pharmaceutical form	Regulatory Status
		applied from the 1st to the 12th day of the menstrual cycle 2. *Dyslipoproteinemia* 2 tablets x 3 day; duration of use – 3 months 3. *Menopausal and post castration syndrome* 1-2 tablets x 3 day; duration of use – 60-90 days; supporting dose-2 tablets/day and duration of use 1-2 years	
Tribuli terrestris herbae extractum siccum 35-45 : 1, extracted with methanol 80% (V/V)	Traditional herbal medicinal product used for treatment of reduced libido and impotence in men.	Film coated tablets 1 film coated tablet contains 250 mg of the extract corresponding to 125 mg of furostanol saponins calculated as protodioscin 1-2 tablets (corresponding to 250 – 500 mg of the extract) 3x daily, duration of use maximum 3 months	Czech Republic TUR, 2015

This overview is not exhaustive. It is provided for information only and reflects the situation at the time when it was established.

Information on relevant combination medicinal products marketed in the EU/EEA

Not applicable

Information on other products marketed in the EU/EEA (where relevant)

Food supplements

The products containing extract from *Tribulus terrestris* are widely used in Europe as food supplements in case of impotence and libido disorders and for optimizing health, as well as by athletes to keep themselves fit and in good physical shape (Evstatieva and Tchorbanov, 2011).

However, some countries consider using parts of the *Tribulus terrestris* plant to produce food supplements to be unsafe, so they do not allow such supplements to be sold. This is reported through the European Rapid Alert System for Food and Feed (RASFF). Its use in food supplements is authorised in some European Union member states, such as Italy, without any comments or restrictions. However, certain restrictions do exist in other countries. For instance, its use is authorised in Belgium as long as no toxicity is found in regard to the daily recommended dose. In Germany, reference is made to the dose established by the WHO, and in France it is required that the parts of the plant be boiled. In Denmark, it is considered that according to available information it is not possible to establish a threshold below which use of *Tribulus terrestris* plant parts in food supplements is considered safe for health. Supplements containing said plant are thus classified as non-authorised products, thereby prohibiting its placing on the market. In Spain, on the request of the Management

Board of the Spanish Agency for Consumer Affairs, Food Safety and Nutrition, a risk assessment was conducted by Section of Food Safety and Nutrition of the Scientific Committee in order to determine whether or not the consumption of fruits, aerial parts and the extracts of *Tribulus terrestris* in food supplements is safe. The Scientific committee concluded that there is not enough toxicological data available to assess the safety of the use of *Tribulus terrestris* plant parts in food supplements. In any case, the maximum daily quantity of *Tribulus terrestris* in food supplements should not exceed the dose used for pharmacological purposes. The part of the plant used, whether it was extracted or prepared in some other way, as well as its saponin content should all be made clear on the supplement itself (Report of Spanish Agency, 2015).

The Health Products Regulatory Authority (HPRA) of Ireland considers products containing the herbal substance *Tribulus terrestris* L. as medicinal products. This herbal substance is included in the prescription regulations SI 540/2003, first schedule (S.I. No. 540/2003 - Medicinal Products (Prescription and Control of Supply) Regulations 2003 (irishstatutebook.ie).

Tribulus terrestris (whole plant) is listed in the EFSA "Compendium of botanicals reported to contain naturally occurring substances of possible concern for human health when used in food and food supplements". The reason is the presence of β-carboline alkaloids (40-80 mg/kg dry matter), e.g. harmane and norharmane, lithogenic steroidal saponins, e.g. protodioscin and mycotoxin sporidesmin and toxic effects on the central nervous system observed in sheep, hepatotoxicity observed in male rats after oral administration of fruits and reported effect on testosterone levels and prostate weight following administration of a fruit extract with high protodioscin level to castrated male rats (EFSA Journal, 2012).

2.1.2. Information on products on the market outside the EU/EEA

Medicinal products

The medicinal product containing the same extract as the product authorised in Bulgaria is registered in Serbia with the following indication: traditional medicinal product used in men with reduced libido and impotence (SmPC - 515-01-02913-16-001.pdf (alims.gov.rs) - accessed 10/01/2022).

In Russia, the same product is used as lipid-lowering agent and for treatment of erectile dysfunction (Instruction for use - https://medside.ru/tribestan- accessed 10/01/2022) and in Ukraine for the prevention and treatment (monotherapy and complex) disorders of the reproductive system in men and women (Instruction for use - https://compendium.com.ua/info/6523/tribestan/- accessed 10/01/2022).

In Brazil, a product containing 250 mg of dry extract of *Tribulus terrestris* L. (containing minimum 45 % of steroidal saponins calculated as protodioscin, no details on plant part, DER and extraction solvent) is used to improve symptoms of hormonal imbalance and for increase of sperm production (Product information – https://consultaremedios.com.br/androsten/bula - accessed 28/02/2022).

For traditional use of the plant in India and China, see section 2.2.

2.2. Information on documented medicinal use and historical data from literature

Tribulus terrestris is commonly known as Gokshur (Sanskrit); puncture vine, land (or small) caltrops, yellow vine, goat head and devil's horn (English); Croix de chevalier (French), Gokharu (Hindi); Bethagokharu or Nanagokharu (Gujarathi); Nerinjil (Tamil); and Khar-e-khusak khurd (Urdu) (Chhatre *et al.*, 2014, Hashim *et al.*, 2014, van Valkenburg and Bunyapraphatsara, 2001) and Bai Ji Li (China).

Tribulus terrestris (TT) is used in folk medicines as a tonic, aphrodisiac, palliative, astringent, stomachic, antihypertensive, diuretic, lithotriptic, and urinary disinfectant (dried fruit). It is referred to as a remedy for liver, kidney, urinary and cardiovascular systems. TT has been used for centuries in Ayurveda (known as Gokhru in Hindi) to treat impotence, genital diseases and sexual frailty and in TCM (known as Bai Ji Li) for similar indications (Das *et al.*, 2017; Sharma, 2012).

Its popularity grew in Eastern Europe in the 1970s once its properties were discovered by the Bulgarian Olympic weightlifting team and has continued to flourish as it is regarded as a safe, legal, and herbal alternative to anabolic steroids. It is mostly used in extract form, which is largely composed of the aerial portions of the plant or the fruits (Qureshi *et al.*, 2014).

No historical use of *Tribulus terrestris* L., herb (exclusively) has been found in the searched literature.

Therapeutic actions traditionally attributed to Gokhru (*Tribulus terrestris*) in Ayurvedic tradition:

- Historically Gokhru is used in the treatment of urinary affection (urinary calculi, polyuria, piles dysuria), as a gastric stimulant, aphrodisiac, nutritive, also in case of heart diseases, dyspnoea, cough. It can be concluded from Ayurvedic point of view that Gokhru pacifies deranged Vata dosha (Ukani *et al*., 1997, Zhu *et al.*, 2017).
- Gokhru has been used for boosting hormone production in men and women (in 5,000-year-old history). The used parts are whole plant, fruit and root as well. Plants and fruits are also used in in the treatment of spermatorrhoea, impotence, infertility, phosphaturia, dysuria, gonorrhoea, gleet, chronic cystitis, renal calculi, incontinence, gout, menstrual disorders, and postpartum haemorrhage (in form of decoctions or infusion) (Sivapalan, 2016, Das *et al.*, 2017, Chhatre *et al*., 2014).
- Gokhru has a long-standing use of being a revitalizer and energizer (it increases energy) helping in sexual and kidney disfunctions as well as colic pains, hypertension and hypercholesterolemia (Sharma, 2012).

Therapeutic actions traditionally attributed to Bai Ji Li (*Tribulus terrestris*) in Traditional Chinese Medicine (TCM):

- *Tribulus terrestris* is used in TCM for liver in restoring the depressed liver, for treatment of fullness in the chest, mastitis, flatulence, kidney and urinary tract disease and skin disorders (treatment of skin pruritus) and is also used for treatment of headache, vertigo and dizziness, also as a cough expectorant. It can be found in the Shen-Nong Pharmacopoeia, the oldest known pharmacological work in China (Sivapalan, 2016, Zhu *et al.*, 2017; Cheng, Traditional Chinese Medicine Basis, 2020), where it is considered a highly valuable drug.
- The fruits of *Tribulus terrestris* were used for treatment of eye trouble (acute conjunctivitis, it improves eyesight), oedema, abdominal distension, morbid leukorrhea, and sexual dysfunction (Umadevi and Srinathrao, 2017, Zhu *et al.*, 2017).

Use of *Tribulus terrestris* in the world (including EU):

- USA – as food supplements with claim of general stimulating action on motor activity (De Combarieau *et al.*, 2003).
- Iraq – as a tonic, aphrodisiac, analgesic, astringent, stomachic, anti-hypertensive, diuretic, lithon- triptic and urinary anti- infective (Ibrahim and Kadhim, 2015).

- South Africa - as a tonic for diarrhoea and diseases of the throat and the eyes (Umadevi and Srinathrao, 2017).
- South and North America, Europe and Africa - as a medicine with astringent, abortifacient, emmenagogue, galactagogue, aphrodisiac, diuretic, anthelmintic, tonic and haemostatic properties, against abscesses and ulcers, nosebleed, dysentery, sore throat, painful urination, calculous affections and aphthae (van Valkenburg and Bunyapraphatsara, 2001).
- Vietnam and Argentina - externally in ophthalmia, by exposing the eyes and for ulcers of the mouth (van Valkenburg and Bunyapraphatsara, 2001).
- Pakistan – in problems with urination, impotence and haemorrhages (van Valkenburg and Bunyapraphatsara, 2001).
- Thailand - as diuretic for treatment of dysuria with bladder stones, leucorrhoea, kidney dysfunction and abnormal urination (van Valkenburg and Bunyapraphatsara, 2001).
- Bulgaria – for treatment of infertility, impotence and decreased libido, for purification of blood, and to treat haemorrhoids (Chhatre *et al.*, 2014, Umadevi and Srinathrao, 2017).
- Greece – for treatment of infertility, impotence, erectile dysfunction, and low libido (Pokrywka et al., 2014, Sivapalan, 2016), in Greek history to treat ailments such as headache, nervous disruption, constipation, and sexual dysfunction (Sharma, 2012).

Table 2: Overview of historical data

Herbal preparation	**Documented use / Traditional use**	**Pharmaceutical form** **Strength (where relevant)** **Posology** **Duration of use**	**Reference**
Natural product obtained from overground part of *Tribulus terrestris*, containing not less than 45% furostanol saponins with prevailing content of protodioscin	Impotentia coeundi, male sterility, when due to oligospermia or reduced spermatozoa motility; status of sexual depression and exhaustion.	Film-coated tablets containing 0.25 g of the extract in one tablet Dosage and therapy duration are determined by the severity of the case. Most frequently used doses are 1-2 tablets 3 times daily during meals.	Dimitrov *et al*., 1982

2.3. Overall conclusions on medicinal use

Table 3: Overview of evidence on period of medicinal use

Herbal preparation **Pharmaceutical form**	**Indication**	**Posology, Strength**	**Period of medicinal use**
Traditional use			
Natural product obtained from overground part of *Tribulus terrestris*, containing not less than 45% furostanol saponins	Impotentia coeundi, male sterility, when due to oligospermia or reduced spermatozoa	Dosage and therapy duration are determined by the severity of the case. Most frequently used doses are 1-2 tablets (corresponding to 250 – 500	Bulgaria registered 1981 - 2002

Herbal preparation Pharmaceutical form	Indication	Posology, Strength	Period of medicinal use
with prevailing content of protodioscin	motility; status of sexual depression and exhaustion	mg of the extract) 3 times daily during meals	Dimitrov *et al*., 1982
Well established use			
Tribuli terrestris herbae extractum siccum (35-45:1) Extraction solvent methanol 80% Containing not less than 45 % of furostanol saponins calculated as protodioscin	In the complex therapy of decreased libido, impotence, infertility; dyslipoproteinemia; menopausal and post castration syndrome	Film- coated tablets containing 250 mg of the extract in one tablet, corresponding to 112.5mg of furostanolic saponins calculated as protodioscin <u>Posology</u> 1.*Decreased libido, impotence and infertility –* *In men with decreased libido, impotence and infertility* 1-2 tablets x 3 day; duration of use – 90 days In women with endocrine infertility 1-2 tablets x 3 day applied from the 1st to the 12th day of the menstrual cycle 2. *Dyslipoproteinemia* 2 tablets x 3 day; duration of use – 3 months 3. *Menopausal and post castration syndrome* -1-2 tablets x 3 day; duration of use – 60-90 days; supporting dose- 2 tablets/day and duration of use 1-2 years	Bulgaria Marketing authorisation in 2002

The only preparation on the European market is dry extract from *Tribulus terrestris* L., herba (DER 35-45:1), extraction solvent methanol 80% V/V, containing not less than 45% of furostanol saponins calculated as protodioscin.

The medicinal product containing this extract has been approved in Bulgaria in 1981 (and reported in the article by Dimitrov *et al*., 1982) with indications exclusively for men. Requirement of 30 years on the market is fulfilled, nevertheless the extract is adjusted to high amount of furostanol saponins and is obtained by in-house developed extraction technology. Taking into consideration this fact and the fact that no information on traditional use of *Tribulus terrestris* herb has been found in the searched literature, the traditional EU monograph cannot be established.

The medicinal product with the same extract has been authorised in 2002 in Bulgaria based on company own data (unpublished). Although period of 10 years for WEU elapsed, the WEU monograph cannot be established as publicly available clinical data are considered not sufficient to confirm WEU. For further details, see section 4.4.

3. Non-Clinical Data

3.1. Overview of available pharmacological data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

Assessor´s comment:

Non-clinical data that can be unequivocally attributed to the herbal substance defined in chapter 1.1 and extracts thereof are limited. For the sake of completeness, studies with fruit extracts and extracts whose description is incomplete but which may be considered useful in assessing the efficacy and safety of the herbal substance are also mentioned here.

3.1.1. Primary pharmacodynamics

Effect on sexual organs

Studies with male animals

Tribulus terrestris extract from the herb/aerial parts of the plant

No data available

Tribulus terrestris extract from the fruits

Sharawy *et al*., 2015

The objective of the experiment was to compare the effects of *Tribulus terrestris extract* (dry ethanol 70% extract from fruits; TT) and probiotics on scrotal circumference, testicular volume, reaction time, semen characteristics, and testosterone hormone levels of rams. The study was carried out on eight sexually mature Rahmani rams. The eight rams were used as control group for one month before treatment (weekly assessment for all the parameters). Rams were divided into two groups, each group included 4 animals. The first group received TT (1.5 g/ head /day, orally in 100 ml water suspension) once daily for two months. The second group received probiotics (10 m. / head/ day, orally in the form of 100 ml water suspension) for two months. In the second, third and fourth months from administration of TT and probiotics; scrotal circumference, testicular volume, reaction time, semen characteristics, and testosterone hormone levels were assessed weekly in all the studied rams. Results showed that scrotal circumference, sexual desire, hydrogen ion concentration, sperm morphology (total and secondary abnormalities percent), and total sperm number per ejaculate increased significantly ($P<0.01$) in TT group as compared to probiotics and control groups. Ejaculate volume, mass motility, individual motility, alive sperm percentage, sperm morphology, sperm cell concentration and acrosome integrity percentage increased significantly ($P<0.01$) in TT and probiotics groups as compared to control one. Serum testosterone level exhibited a significant increase ($P<0.05$) in TT group as compared to control and probiotics groups. It was concluded that administration of TT resulted in an improvement in the sexual performance, semen quality, and testosterone level of rams.
Singh *et al*., 2012

The aim of the study was to evaluate the effect of acute and repeated dose administration of lyophilized aqueous extract of the dried fruits of *Tribulus terrestris* (LAET) on sexual function in sexually sluggish male albino rats. To assess the effect of chronic *T. terrestris* exposure on the hypothalamus--pituitary--gonadal axis, testosterone level estimation and sperm count were carried out. Twenty-eight-day oral toxicity studies were carried out to evaluate the long-term effects of the LAET administration on different body systems. A dose-dependent improvement in sexual behaviour was observed with the LAET treatment as characterized by an increase in mount frequency,

intromission frequency, and penile erection index, as well as a decrease in mount latency, intromission latency, and ejaculatory latency. The enhancement of sexual behaviour was more prominent on chronic administration of LAET. Chronic administration of LAET produced a significant increase in serum testosterone levels and the sperm count. No overt body system dysfunctions were observed in 28-day oral toxicity study. The authors concluded that findings of the study validate the traditional use of *T. terrestris* as a sexual enhancer in the management of sexual dysfunction in males.

Assessor´s comment: *In the different parts of the article, different information on origin of the extract (lyophilised aqueous extract of the dried fruit versus standardised whole plant extract containing ≥20% w/w total saponins) is reported. This fact reduces the plausibility of the study.*

In vitro (ex vivo)/in vivo

Do *et al*., 2013

In the study effect of a *Tribulus terrestris* extract and the mechanism of action of the extract on relaxation of the corpus cavernosum (CC) were investigated. The erectogenic effects of an oral preparation of the extract were also assessed. The relaxation effects and mechanism of action of the TT on rabbit CC were investigated in an organ bath. The intracavernous pressure (ICP) was tested on rats after oral administration of the extract for 1 month (2,5, 5, 10, 50 and 100 mg/kg) to evaluate whether the relaxation response of the CC shown in the organ bath occurred in vivo. Additionally, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) were measured in the CC by immunoassay. Smooth muscle relaxation was expressed as the percentage decrease in precontraction induced by phenylephrine. The ICP was also assessed in rats after oral administration of the extract for 1 month, and changes in concentrations of cGMP and cAMP were monitored. Concentration-dependent relaxation effects of the extract on the CC were detected in the organ bath study. Relaxation of the CC by the TT was inhibited in both an endothelium-removed group and an L-arginine methyl ester pre-treatment group. The ICP measured after oral administration of the TT for 1 month was higher than that measured in the control group, and a significant increase in cAMP was observed in the TT group. The authors concluded that TT showed a concentration-dependent relaxation effect on the CC in an organ bath. The mechanism involved NO/NOS signalling in the CC endothelium. Moreover, an in vivo study after 1 month of oral administration of the TT showed a significant concentration-dependent increase in ICP compared with that in the control group.

Tribulus terrestris extract in which plant part used is not specified

In vivo

Martino-Andrade *et al*., 2010

The study has been performed to investigate the possible effects of *Tribulus terrestris* dry ethanolic extract (no details on plant part used, extraction solvent and DER) containing 16.43 % of protodioscin (TT) on endocrine sensitive organs in intact and castrated male rats. Three different dose levels of TT (11, 42 and 110 mg/kg/day) were administered to castrated males for 7 days and to intact males for 28 days. In addition to TT treatment, all experiments also included a group of rats treated with dehydroepiandrosterone (DHEA). In experiments using castrated males the authors also used testosterone and 17α-ethynyl oestradiol, respectively, as positive controls for androgenicity and oestrogenicity. Neither DHEA nor TT was able to stimulate androgen sensitive tissues like the prostate and seminal vesicle in both intact and castrated male rats. In addition, administration of TT to intact male rats for 28 days did not change serum testosterone levels as well as did not produce any quantitative change in the faecal excretion of androgenic metabolites. However, a slight increase in the number of homogenization-resistant spermatids was observed in rats treated with 11 mg/kg/day of TT

extract. In conclusion, *Tribulus terrestris* was not able to stimulate endocrine sensitive tissues such as the prostate and seminal vesicle in Wistar rats, indicating lack of androgenic and estrogenic activity in vivo. The authors also showed a positive effect of TT administration on rat sperm production, associated with unchanged levels of circulating androgens.

Bashir *et al*., 2009

The study was designed to investigate the effects of extract of *Tribulus terrestris* on body weight and testicular development of prepubertal rats. Two-week old rats were divided into two groups of 10 pups each (A: control and B: experimental). Group B was given *Tribulus terrestris* extract (no details on part plant used, DER, extraction solvent and saponins content) in an oral dose of 70mg/kg daily for 20 days. Pups were weighed and sacrificed on 34th day post-natal; their testes were removed for gross and microscopic studies using. Pups received *Tribulus terrestris* extract showed statistically significant increase in mean body ($p<0.05$) and paired testes weight ($p<0.05$) without significant effect on the mean relative tissue body weight index ($p>0.05$). Histological slides of the testes showed a significant increase in seminiferous tubules containing early spermatids in the treated group when compared to that of control ($p<0.05$). The mean diameter of seminiferous tubules in the treated group was also noticeably increased ($p<0.05$). Spermatids of the experimental group were at acrosomal phase of spermogenesis, whereas those of control group were at Golgi phase, implying thereby that spermatogenesis was present at an advanced stage in the experimental as compared to the control group of animals.

Esfandiari and Dehghani, 2010

The aim of the study was to determine the histological and histomorphometrical change of seminiferous tubule in mature and immature Wistar rats after using *Tribulus terresteris* (TT) (no details on the extract). Twenty male Wistar rats were selected and randomly divided into four groups: 1) Mature control group (MCG). 2) Mature experimental group (MEG) (orally received 75 mg/kg TT daily for 14 days). 3) Immature control group (ICG). 4) Immature experimental group (IEG) (orally received 75 mg/kg TT daily for 14 days). The number of leydig cells had increment in experimental group when compared with ICG. Result showed that the thickness of the wall of seminiferous tubule in experimental group significantly increased ($P < 0.05$). Also, TT treatment groups resulted the accumulation of spermatogenic cells were increased in the seminiferous tubule when compared with control group. In addition, sperm was not observed in ICG but sperm were also observed to increase in the treatment groups. It is concluded that TT may improve the sexual activity, increased testestrone by intensification of leydig cells and may cause early puberty in immature rat.

Adaikan *et al*., 2000

The objective of the study was to investigate the effect of oral treatment of *Tribulus terrestris* (TT) extract on the isolated corpus cavernosal tissue of New Zealand white (NZW) rabbits and to determine the mechanism by which protodioscin (PTN), a constituent of the TT, exerts its pharmacological effects. Twenty-four NZW rabbits were randomly assigned to 4 experimental groups of 6 each, Group I served as control, Groups II to IV were treated with the extract at different dose levels, i.e. 2. 5 mg/kg, 5mg/kg and 10 mg/kg body weight, respectively. The TT extract (no details on plant part used, extraction solvent, DER and saponins content) was administered orally, once daily, for a period of 8 weeks. The rabbits were then sacrificed, and their penile tissue isolated to evaluate the responses to both contracting and relaxing pharmacological agents and electrical field stimulation (EFS). PTN on its own had no effect on the isolated corpus cavernosal strips. The relaxant responses to EfS, acetylcholine and nitroglycerin in noradrenaline precontracted tissues from the treated group showed an increase in relaxation of a concentration dependent nature compared to that of the tissues from control group. However, the contractile, anti-erectile response of corpus cavernosal tissue to

noradrenaline and histamine showed no significant change between the treatment and the control group. The relaxant responses to acetylcholine. nitroglycerin and EFS by more than 10%, 24% and 10% respectively compared to their control values and the lack of such effect on the contractile response to noradrenaline and histamine indicate that PTN has a proerectile activity. The enhanced relaxant effect observed is probably due to increase in the release of nitric oxide from the endothelium and nitrergic nerve endings, which may account for its claims as an aphrodisiac. However, further study is needed to clarify the precise mechanism of its action.

Gauthaman and Adaikan, 2005

Administration of *Tribulus terrestris* extract (TT, plant part used, extraction solvent, DER and saponins content not specified) increased sexual behaviour and intracavernous pressure both in normal and castrated rats and these effects were probably due to the androgen increasing property of TT. The objective of the study was to evaluate the effect of TT on nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH-d) activity and androgen receptor (AR) immunoreactivity in rat brain. Twenty-four adult male Sprague-Dawley rats were divided into two groups of twelve each. Group I was treated with distilled water and Group II was treated with TT at the dose of 5mg/kg body weight orally, once daily for 8 weeks. Following treatment transcardiac perfusion was done with Ringer lactate, 4% paraformaldehyde and 30% sucrose. The brain tissue was removed and sections of the paraventricular (PVN) area of hypothalamus were taken for NADPH-d and AR immunostaining. There was an increase in both NADPH-d (67%) and AR immunoreactivity (58%) in TT treated group and these results were statistically significant compared to the control. Chronic treatment of TT in rats increases the NADPH-d positive neurons and AR immunoreactivity in the PVN region. Androgens are known to increase both AR and NADPH-d positive neurons either directly or by its conversion to oestrogen. The mechanism for the observed increase in AR and NADPH-d positive neurons in the present study is probably due to the androgen increasing property of TT. The findings from this study add further support to the aphrodisiac claims of TT and its androgen releasing property. The aphrodisiac effects of TT are probably mediated by increase in both androgen receptors and enzyme nitric oxidase synthase containing neurons. Further studies to identify the mRNA and protein level of androgen and oestrogen receptors may provide more conclusive evidence as regards to the central effects of TT.

Gauthaman and Adaikan, 2008

Hormonal effects of *Tribulus terrestris extract* (TT, no details on the plant part, DER, extractions solvent and saponins content) were evaluated in primates, rabbit and rat to identify its usefulness in the management of erectile dysfunction (ED). TT extract was administered intravenously, as a bolus dose of 7.5, 15 and 30 mg/kg, in primates for acute study. Rabbits and normal rats were treated with 2.5, 5 and 10 mg/kg of TT extract orally for 8 weeks, for chronic study. In addition, castrated rats were treated either with testosterone cypionate (10 mg/kg, subcutaneously: biweekly for 8 weeks) or TT orally (5 mg/kg daily for 8 weeks). Blood samples were analysed for testosterone (T), dihydrotestosterone (DHT) and dehydroepiandrosterone sulphate (DHEAS) levels using radioimmunoassay. In primates, the increases in T (52%), DHT (31%) and DHEAS (29%) at 7.5 mg/kg were statistically significant. In rabbits, both T and DHT were increased compared to control, however, only the increases in DHT (by 30% and 32% at 5 and 10 mg/kg) were statistically significant. In castrated rats, increases in T levels by 51% and 25% were observed with T and TT extract respectively that were statistically significant. TT increases some of the sex hormones, possibly due to the presence of protodioscin in the extract. TT may be useful in mild to moderate cases of ED.

Gauthaman *et al*., 2002

Sexual behaviour and intracavernous pressure (ICP) were studied in both, normal and castrated rats, to further understand the role of *Tribulus terrestris* (TT) containing protodioscin (PTN) as an

aphrodisiac. Adult Sprague-Dawley rats were divided into five groups of 8 each that included distilled water treated (normal and castrated), testosterone treated (normal and castrated, 10 mg/ kg body weight, subcutaneously, bi-weekly) and TT (no details on the part plant used, extraction solvent, DER and saponins content) treated (castrated, 5 mg/kg body weight, orally once daily). Decreases in body weight, prostate weight and ICP were observed among the castrated groups of rats compared to the intact group. There was an overall reduction in the sexual behaviour parameters in the castrated groups of rats as reflected by decrease in mount and intromission frequencies (MF and IF) and increase in mount, intromission, ejaculation latencies (ML, IL, EL) as well as post-ejaculatory interval (PEI). Compared to the castrated control, treatment of castrated rats (with either testosterone or TT extract) showed increase in prostate weight and ICP that were statistically significant. There was also a mild to moderate improvement of the sexual behaviour parameters as evidenced by increase in MF and IF; decrease in ML, IL and PEI. These results were statistically significant. It is concluded that TT extract appears to possess aphrodisiac activity probably due to androgen increasing property of TT.

Gauthaman *et al*., 2003

Apart from its claims for improvement of sexual functions in men, the puncturevine plant (*Tribulus terrestris*: TT) has long been considered as an energizer and vitalizer in the indigenous system of medicine. Sexual behaviour and intracavernous pressure (ICP) measurements were taken in rats to scientifically validate the claim of TT [containing protodioscin (PTN)] as an aphrodisiac. Forty sexually mature male Sprague–Dawley rats were randomly divided into four groups of 10 each. Group I served as a control group and groups II, III, and IV were treated with three different doses of TT extract (no details on the plant part, DER, extraction solvent and saponins content) (2.5, 5 and 10 mg/kg body weight, respectively), orally, once daily for 8 weeks. Weight was recorded and the rats from all four groups were subjected to sexual behaviour studies with primed females and various parameters namely mount and intromission frequencies (MF and IF, respectively), mount, intromission and ejaculation latencies (ML, IL, and EL, respectively) as well as postejaculatory interval (PEI) were recorded. In addition, blood pressure and ICP were recorded for all rats at the end of study. Increases in body weight (by 9, 23, and 18% for groups II, III & IV) and ICP (by 43% and 26% for groups III and IV) were statistically significant compared to the control group. Increases in MF (by 27% and 24%) and IF (by 19% and 22%) for the groups III and IV were statistically significant. Decreases in ML (by 16%, 23%, and 22% for groups II, III, and IV) and PEI (by 20% for group III) were statistically significant compared to the control. The weight gain and improvement in sexual behaviour parameters observed in rats could be secondary to the androgen increasing property of TT (PTN). The increase in ICP which confirms the pro-erectile aphrodisiac property of TT could possibly be the result of an increase in androgen and subsequent release of nitric oxide from the nerve endings innervating the corpus cavernosum.

Grigorova *et al*., 2008

Tribulus terrestris extract (no details on the extract) was added to the water of 10 cocks from the population White Plymouth Rock – mini cocks once daily in dose 10 mg/kg body weight for a period of 11weeks. The trial lasted 20 weeks-1week preparatory and 19 weeks experimental period. Eight weeks of the experimental period were intended to measure the aftereffect of the tested product. It was found, that *Tribulus terrestris* extract improves cock´s semen quality: volume of ejaculate, spermatozoids concentration and motility, and shortened the time of methylene blue decolouration.

Kistanova *et al.*, 2005

Tribulus terrestris extract (no details on the extract) was added to the forage of 8 rams of Pleven Blackhead and Abaci breed once daily in dose 1.5 g per head for a period of 40 days. Semen parameters and sexual behaviour during semen collection were evaluated. It was found that *Tribulus*

terrestris extract improves semen quality of rams: the count of spermatozoids, time of viability and motility of sperms increased. The great number of born lambs after the use of treated rams for insemination confirmed high fertility of their semen. All experimental rams manifested a good libido and active sexual behaviour.

Ștefănescu *et al.*, 2021

Testosterone, LH and FSH levels and sperm morphology after oral administration 25 mg/body weight of TT extract (plant part and extraction solvent were not specified) with low protodioscin content group (below limit of quantification) (TT-LPC) and TT extract with high protodioscin content (162.42 µg/g) (TT-HPC) in diabetic rats were studied. After twelve weeks of treatment LH, FSH and testosterone levels were measured. Luteinizing hormone levels were not significantly different compared with the control group; however, the FSH and testosterone levels were significantly higher in the in the TT-HPC group compared with the diabetic control group. The testosterone level is correlated in part with the protodioscin concentration in extracts and according to the authors is probably mediated through an FSH-linked pathway. In the TT-HPC and TT-LPC groups, a reduction (not statistically significant) of abnormal shape germ cells was observed compared with the diabetic control group.

Substances isolated from *Tribulus terrestris*

Gross saponins extracted from *Tribulus terrestris* herb

In vivo

The study by Zhang *et al.* (2019) investigated the effect of gross saponins of *Tribulus terrestris* herb (GSTT, no details on composition) on erectile function in rats resulting from type 2 diabetes mellitus (T2DMED). The animals were divided into 4 groups: control group, GSTT group receiving GSTT 40 mg/kg/d, sildenafil group receiving sildenafil citrate 5 mg/kg/d and mixed group receiving equal amounts of saponin solution at 40 mg/kg/d and sildenafil citrate suspension at 5 mg/kg/d. The animals were treated for 4 weeks. After 4 weeks treatment intracavernous pressure (ICP) and mean arterial pressure (MAP) values of the GSTT were significantly higher than those of the T2DMED group ($P<0.05$). Unlike the T2DMED group, the GSTT group showed significantly increased nitric oxide (NO) levels in the cavernous tissue($P<0.05$) and decreased reactive oxygen species (ROS) levels ($P<0.05$). There was no significant difference between the GSTT group and the sildenafil group in increasing cyclic adenosin monophosphate (cGMP) levels ($P>0.05$), and the mixed group had higher levels than these two groups ($P<0.05$). Immunohistochemistry and Western blotting showed that the expression of endothelial nitric oxide synthase (eNOS) in the GSTT was significantly higher than that in the T2DMED groups ($P<0.05$). The smooth muscle/collagen ratio of the GSTT group was significantly higher than that of the T2DMED group ($P<0.05$), the expression of TIMP-1 was lower than that of T2DMED group ($P<0.05$), the apoptotic index and cleaved caspase 3 and cleaved caspase 9 expression level of GSTT group were lower than that of the T2DMED group ($P<0.05$). The authors concluded that GSTT can protect T2DMED rats' erectile function by improving penile endothelial function and inhibiting cavernosum fibrosis, inhibiting apoptosis, and is synergistic with sildenafil.

Studies with female animals

Tribulus terrestris extract from the herb/aerial parts of the plant

No data available

Tribulus terrestris extract in which plant part used is not specified

In vivo

Martino-Andrade *et al*., 2010

The study has been performed to investigate the possible effects of *Tribulus terrestris* dry ethanolic extract (no details on plant part used, extraction solvent and DER) containing 16.43 % of protodioscin (TT) on endocrine sensitive organs in in a post-menopausal rat model using ovariectomized females. Three different dose levels of TT (11, 42 and 110 mg/kg/day) were administered to castrated females for 28 days. In experiments using castrated females the authors also used testosterone and 17α-ethynyl oestradiol, respectively, as positive controls for oestrogenicity. In ovariectomized females, TT did not produce any stimulatory effects in uterine and vaginal epithelia. In conclusion, *Tribulus terrestris* was not able to stimulate endocrine sensitive tissues such as the uterus and vagina in Wistar rats, indicating lack of estrogenic activity in vivo. The authors also showed a positive effect of TT administration on rat sperm production, associated with unchanged levels of circulating androgens.

The study by Abadjieva and Kistanova (2016) investigated the effect of *Tribulus terrestris* on the Growth Differentiation Factor 9 (GDF9) and Bone Morphogenetic Protein 15 (BMP15) expression in the oocytes and cumulus cells at mRNA and protein levels during folliculogenesis in two generations of female rabbits. The experiment was conducted with 28 New Zealand rabbits. Only the diet of the experimental mother's group was supplemented with a dry extract of *T. terrestris* (containing 60% furostanol saponins determined as protodioscin, max. 10% of flavonoids and max. 10 % of tannins; plant part, extraction solvent, DER not specified) in daily doses of 3.5 mg/kg body weight for 45 days prior to insemination. The BMP15 and GDF9 transcripts were detected in the oocytes and cumulus cells of rabbits from all groups. *T. 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. The downregulated expression of BMP15 in the treated mothers' oocytes was inherited in the F1 female offspring born to treated mothers. BMP15 and GDF9 showed sensitivity to the bioactive compounds of *T. terrestris*.

In vitro

Sirotkin *et al.,* 2019

The objective of the study was to examine the direct effects of the medicinal plant *Tribulus terrestris* L. (puncturevine) on the basic functions of ovarian cells (from pubertal gilts), including their proliferation, apoptosis, and response to the physiological hormonal stimulator ghrelin. In the first series of experiments, porcine ovarian granulosa cells were cultured with or without puncturevine extracts (95% extract, no other details) at concentrations of 0, 1, 10, or 100 µg/ml. In the second series of experiments, these cells were cultured with ghrelin at concentrations of 0, 1, 10, or 100 ng/ml, either alone or in combination with puncturevine (10 µg/ml). The expression levels of the proliferation marker (PCNA) and the apoptosis marker (bax) were analysed via quantitative immunocytochemical methods. Puncturevine was found to stimulate the accumulation of both proliferation and apoptotic markers. Additionally, ghrelin alone could promote the proliferation and apoptosis of ovarian cells. The presence of puncturevine reversed ghrelin stimulated apoptosis and instead induced apoptotic inhibition. However, puncturevine did not modify the proliferation-inducing effect of ghrelin. These observations demonstrated that (1) puncturevine directly promotes cell proliferation and apoptosis, turnover, of ovarian cells; (2) ghrelin is involved in the regulation of ovarian cell apoptosis and proliferation, consistent with existing evidence; (3) puncturevine antagonises and even reverses the effects of the hormonal regulator, ghrelin, on ovarian cell apoptosis, but not proliferation; and (4) puncturevine affects not only the basic functions of ovarian cells but also their responses to upstream hormonal regulators.

Hypolipidemic effect

Tribulus terrestris extract from aerial parts of the plant

In vivo

The methanolic (70%) extract from aerial part (with fruits) of *Tribulus terrestris* L. caused a significant decrease in the levels of total cholesterol, triglycerides and LDL-cholesterol in streptozocin induced diabetic rats. (El-Tantawy and Hassanin, 2007)

Tribulus terrestris extract from fruits

Sharawy *et al.,* 2015

The objective of the experiment was to compare between the effects of *Tribulus terrestris* extract (TT, dry ethanol 70% extract from fruits) and probiotics on serum total cholesterol levels of rams. The eight rams were used as control group for one month before treatment (weekly assessment for all the parameters). Rams were divided into two groups, each group included 4 animals. The first group received TT (1.5 g/ head /day, orally in 100 ml water suspension) once daily for two months. The second group received probiotics (10 m. / head/ day, orally in the form of 100 ml water suspension) for two months. In the second, third and fourth months from administration of TT and probiotics; serum total cholesterol was assessed weekly in all the studied rams. Results showed that Serum total cholesterol level decreased significantly ($P<0.01$) in TT group as compared to the control group.

Tribulus terrestris extract in which plant part used is not specified

Grigorova *et al*., 2008

Tribulus terrestris extract (no details on the extract) was added to the water of 10 cocks from the population White Plymouth Rock – mini cocks once daily in dose 10 mg/kg body weight for a period of 11weeks. The trial lasted 20 weeks-1week preparatory and 19 weeks experimental period. Eight weeks of the experimental period were intended to measure the aftereffect of the tested product. Serum total cholesterol content in the experimental cocks was 9.24% lower ($p>0.05$) in comparison with the control group. The aftereffect of *Tribulus terrestris* on the studied parameters was maintained for 8 weeks.

Tuncer *et al.*, 2009

The aim of the study was to investigate the pleotropic effects of an extract of a traditional herb, *Tribulus terrestris* (TT, no details on the extract), on the lipid profile and vascular endothelium of the abdominal aorta in New Zealand rabbits fed a cholesterol-rich diet. Eighteen rabbits were randomly divided into three groups (n= 6 for each). One experimental group (EG-I) was given a cholesterol-rich diet, a second experimental group (EG-II) was treated with TT following a cholesterol-rich diet, and a control group (CG) was fed a standard diet. Blood samples were collected on day 0 and then at weeks 4 and 12 to determine total serum cholesterol (TC), high density lipid- cholesterol (HDL-C), low density lipid-cholesterol (LDL-C) and triglyceride (TG) levels. Tissues were collected from the abdominal aorta for immunohistochemistry and transmission and scanning electron microscopy. In EGII, the serum lipid profile was significantly lower than that of EG-I at week12 with a reduction of TC: 65%; LDL-C: 66%; HDL-C: 64%; TG: 55%. Ultrastructural analysis revealed that endothelial damage was more prominent in EG-I compared to EG-II. The ruptured endothelial linings and damaged cellular surfaces increased in EG-I compared to EG-II. The data indicate that dietary intake of TT can significantly lower serum lipid profiles, decrease endothelial cellular surface damage and rupture and may partially repair the endothelial dysfunction resulting from hyperlipidaemia.

Substances isolated from *Tribulus terrestris*

Tribulus terrestris saponins (no further information on composition)

In vivo

Guo *et al.*, 2007

This experiment was designed to determine whether Tribulus saponins extracted (ethanol/water, no further details on composition) from fruits (TS) relieve left ventricular remodelling (VR) after myocardial infarction (MI) in a murine hyperlipemia (HL) model. MI and HL models were induced, and high and low doses of TS and simvastatin were administrated to the rats. Four weeks later, echocardiographic observation was performed, and the left and right ventricular weight index (LVWI, RVWI) was calculated. Echocardiographic results showed that both high dose of TS and simvastatin had a beneficial effect on increasing fractional shortening (FS) and ejection fraction (EF), reducing left ventricular end diastolic volume (LVEDV), systolic volume (LVESV), left ventricular dimension end diastole (LVDd) and systole (LVDs), and decreasing LVWI, as compared to those in the HL-MI model group ($p < 0.05$, 0.01). Both medicines had little impact on thickness of the anterior and posterior wall. No significant difference was observed between each treatment group ($p > 0.05$). In conclusion, TS not only lowered serum lipidaemia, but also relieved left ventricular remodelling, and improved cardiac function in the early stage after MI.

Misiakiewicz-Has *et al.*, 2021

The aim of the present study was to investigate the influence of TT saponins (without further details on composition) and TT saponins plus inulin on the plasma lipid profile and liver fatty acids of rats with induced diabetes mellitus type 2 (T2DM). The study was performed on 36 male Sprague–Dawley rats divided into two main groups: control and diabetic. Animals of the diabetic (DM) group were fed a high-fat diet and injected with streptozotocin (low doses). Animals of the control group (nDM) were on a regular diet and were injected with buffer. The animals of both groups (diabetic and non-diabetic) were then treated with saponins (100 mg/kg) or saponins (no details on saponins origin) (100 mg/kg) and inulin (100 mg/kg) per os for 30 days. The comparison of plasma lipid profile has indicated a statistically significant decrease in plasma HDL cholesterol in the control diabetic (c-DM) group compared to the control non-diabetic (c-C) group and an increase (on the border of a statistically significant difference) of the TC/HDL rate in control diabetic compared to control non-diabetic. There were no significant changes in the level of total cholesterol, LDL, nor triglycerides between c-DM and c-C groups. Supplementation with saponins or saponins + inulin did not cause significant changes in the diabetic nor in the non-diabetic group. One tendency is visible (although not significant): the level of plasma triglycerides is increased in non-diabetic group treated with saponins + inulin, while the level of triglycerides is decreased in the diabetic group treated with saponins + inulin.

Table 4: Overview of the main non-clinical data/conclusions

Herbal preparation tested	**Posology**	**Experimental model**	**Reference**	**Main non-clinical conclusions**
Effect on sexual organs				
comparable/similar preparations to preparations of the monograph				
No data available				
other preparations				
extracts from fruits				
Lyophilized aqueous extract of the dried fruits of *Tribulus terrestris* (LAET) or Standardised whole plant extract	Oral administration 50 and 100 mg/kg/day	*In vivo* Rats – male animals	Singh *et al.,* 2012	Acute study: dose dependent increase in sexual behaviour Chronic study: greater dose dependent increase in sexual behaviour,

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
containing ⩾20% w/w total saponins *Assessor´s comment: in different parts of the article there is different information on the extract*				increase of testosterone level and sperm count
Dry ethanolic (90%) extract from fruits	Organ bath study 0.25, 0.5, 1, 2 and 4 mg/ml Oral administration 2.5, 5, 10, 50 and 100 mg/kg/day	*In vitro/ex vivo* Rabbit corpus cavernosum *In vivo* Rats – male animals	Do *et al.*, 2013	A concentration-dependent relaxation effect on the CC in an organ bath. Significant dose dependent increase of intracavernous pressure and increase of cyclic adenosine monophosphate level *in vivo*
Extracts in which plant part used is not specified				
Tribulus terrestris extract (plant part and extraction solvent were not specified) with low protodioscin content group (below limit of quantification) and TT extract with high protodioscin content (162.42 µg/g)	Oral administration 25 mg/bw	*In vivo* Rats – male animals	Ștefănescu *et al.*, 2021	No significant change in luteinizing hormone level, FSH and testosterone levels significant higher compared with control group
Tribulus terrestris dry ethanolic extract (no details on plant part used, extraction solvent and DER) containing 16.43 % of protodioscin (TT)	Oral administration 11, 42 and 110 mg/kg/day	*In vivo* Rats – male and female animals	Martino-Andrade *et al.*, 2010	slight increase in the number of homogenization-resistant spermatids observed in rats treated only with low dose 11 mg/kg/day of TT extract
Tribulus terrestris extract (no details on part plant used, DER, extraction solvent and saponins content)	Oral administration 70mg/kg daily	*In vivo* Rats – male animals	Bashir *et al.*, 2009	spermatogenesis was present at an advanced stage
Tribulus terrestris extract (no details on part plant used, DER, extraction solvent and saponins content)	Oral administration 75 mg/kg daily	*In vivo* Rats – male animals	Esfandiari and Dehghani, 2010	thickness of the wall of seminiferous tubule significantly increased increment number of Leydig cells

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
Tribulus terrestris extract (no details on plant part used, extraction solvent, DER and saponins content)	Oral administration 2.5, 5 and 10 mg/kg/day	*In vivo* Rabbits – male animals	Adaikan *et al.,* 2000	proerectile activity, the enhanced relaxant effect on corpora cavernosa
Tribulus terrestris extract (no details on plant part used, extraction solvent, DER and saponins content)	Oral administration 5 mg/kg/day	*In vivo* Rats – male animals	Gauthaman and Adaikan, 2005	increased sexual behaviour and intracavernous pressure
Tribulus terrestris extract (no details on plant part used, extraction solvent, DER and saponins content)	Intravenous administration 7.5, 15 and 30 mg/kg/day (primates) 2.5, 5 and 10 mg/kg/day (rabbits and rats) Oral administration 5 mg/kg/day (rats)	*In vivo* Primates Rabbits Rats male animals	Gauthaman and Adaikan, 2008	In primates increase of testosterone, dihydrotestosterone and dehydroepiandosterone sulphate levels In rabbits increase of dihydrotestosterone level at 5 and 10 mg/kg In rats increase of testosterone level
Tribulus terrestris extract (no details on plant part used, extraction solvent, DER and saponins content)	Oral administration 5 mg/kg/day	*In vivo* Rats – male animals	Gauthaman *et al.,* 2002, 2003	Increase of prostate weight and intracavernous pressure Mild to moderate improvement of the sexual behaviour parameters
Isolated substances				
Gross saponins extracted from *Tribulus terrestris* herb (GSTT)	Oral administration 40 mg/kg/day	*In vivo* Rats – male animals	Zhang *et al.,* 2019	Increase of intracavernous pressure and mean arterial pressure values significantly increased nitric oxide levels in the cavernous tissue and decreased reactive oxygen species levels no significant difference in increasing cyclic adenosine

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
				monophosphate levels when compared with sildenafil group
Hypolipidemic effect				
Comparable/similar preparations to preparations of the monograph				
Methanolic (70 %) extract from aerial part (with fruits) of *Tribulus terrestris L.*	Oral administration 50 mg/kg/day	*In vivo* Rats	El-Tantawy and Hassanin, 2007	significant decrease in serum triglycerides, total cholesterol and LDL-c cholesterols
Other preparations				
Extracts in which plant part used is not specified				
Tribulus terrestris extract (no details on plant part used, extraction solvent, DER and saponins content)	Oral administration 5 mg/kg/day	*In vivo* Rabbits	Tuncer *et al.*, 2008	Reduction of total serum cholesterol, high density lipid-cholesterol, low density lipid-cholesterol and triglyceride levels
Isolated substances				
Tribulus terrestris saponins (no further information origin or composition)	Oral administration 100 mg/kg/day	*In vivo* Rats	Misiakiewicz-Has *et al.*, 2021	Significant decrease in plasma high-density lipoprotein cholesterol in the control diabetic group compared to the control non-diabetic group and increase (on the border of a statistically significant difference) of the total cholesterol/high-density lipoprotein rate in control diabetic compared to control non-diabetic. No significant changes in the level of total cholesterol, low-density lipoprotein, nor triglycerides between control diabetic and control non-diabetic groups
Saponins from *Tribulus terrestris,* fruit (no details on composition)	Oral administration 12.15 and 24.3 mg/kg/day	*In vivo* Rats	Guo *et al.*, 2007	lowered serum total cholesterol, low-density cholesterol and total

Herbal preparation tested	Posology	Experimental model	Reference	Main non-clinical conclusions
				cholesterol/high-density cholesterol rate observed in animals with high cholesterol levels

Note: The following data are considered supportive and were not included in Table 4 due to unreliable/unconvincing results for interpretation to clinical use (e.g., exploratory study designs, irrelevant species tested, i.e., other than general laboratory animals with available historical control data:

Effect on sexual organs - Sirotkin *et al.* (2019), Abadjieva and Kistanova (2016), Grigorova *et al.* (2008), Kistanova *et al.* (2005), Sharawy *et al.* (2015).

Hypolipidemic effect - Grigorova *et al.* (2008), Sharawy *et al.* (2015).

3.1.2. Secondary pharmacodynamics

Effect on muscles

Tribulus terrestris extract (no details on plant part used)

In vivo

In the study by Wu *et al.* (2017) indicates that the effect of *Tribulus terrestris* extract (containing 71.35 % of tigogenin and diosgenin (no details on plant part, extraction solvent, DER) on the performance of rats undergoing high intensity exercise may be attributed to the induced increases of circulating testosterone and insulin growth factor-1 (ICF-1), as well as increases of androgen receptor and IGF-1 receptor protein expression levels in the gastrocnemius, resulting in increased muscle weight and myosin content in gastrocnemius.

Hypoglycaemic activity

Methanolic extract from aerial parts of the plant

In vivo

The study has been performed to investigate the anti-hyperglycaemic activity of methanol extract from aerial parts of *Tribulus terrestris L.* in glucose-loaded normal rabbits. The animals were randomly assigned to 4 groups (n = 5) and treated with a single oral dose. Group 1 served as normal control group and received distilled water; group 2 served as hyperglycaemic control; group 3 was treated with glibenclamide (5 mg/kg, aqueous suspension) and served as reference standard; group 4 received methanol extract of *Tribulus terrestris* L. (from aerial parts of the plant, no details on DER and saponins content) (250 mg/kg). Groups 3 and 4 were orally treated with glucose (5 g/kg) after 1 h of drug and extract administration, respectively. Fasting blood glucose (FBG) was determined prior to (0 h) and at 30 min, 1, 2 and 3 h after dosing for acute toxicity study. On comparing within groups, a single dose of the methanol extract of *Tribulus terrestris* L. lowered FBG to levels comparable to that of glibenclamide (36 vs. 55 %) and reaching the initial level (0 h) at 2 h. FBG were significantly ($p < 0.05$) lowered at 2 and 3 h in both glibenclamide (45.5 and 56.9 %) and extract (45.7 and 52.7 %) groups as compared with their respective glucose levels at 0.5 h. On the other hand, on comparing between groups, both glibenclamide and methanol extract significantly ($p < 0.05$, $p < 0.001$) lowered the rise in blood glucose at 1 h (33.9 and 22.5 %), 2 h (62.8 and 59.16 %), and 3 h (64.6 and 57.1

%) with respect to the hyperglycaemic control group. The authors concluded that the methanol extract of the aerial parts of *Tribulus terrestris* L. possesses potential antihyperglycaemic activity in glucose-loaded normal rabbits. (El-Shaibany *et al.*, 2015).

The extract of *T. terrestris* significantly decrease fasting glucose level in diabetic rats. After 3 weeks of treatment with *T. terrestris* methanolic extract from aerial part of the plant with fruits (no details on DER and saponins content) in dose of 50 mg/kg body weight, glucose level was significantly decreased to the normal level. (El-Tantawy and Hassanin, 2007).

Tribulus terrestris extract with low (under LoQ) and high (162.42 µg/g) protodioscin content (plant part used is not specified)

In vivo

TT extract (plant part and extraction solvent were not specified) with low protodioscin content group (below limit of quantification) (TT-LPC) and TT extract with high protodioscin content group (162.42 µg/g) (TT-HPC) were orally administered to diabetic rats at the dose of 25 mf/kg b.w.. After twelve weeks of treatment, fasting blood glucose and insulin levels were measured. Both TT preparations reduced elevated blood glucose level. Insulin level was not significantly different compared with the control group. (Ștefănescu *et al.*, 2021)

Cinnamic acid amides from *Tribulus terrestris*

In vitro

The study attempted to isolate from *Tribulus terrestris* extract the responsible metabolites and elucidate their inhibition mechanism of α-glucosidase. By fractionating *T. terrestris* extracts, three cinnamic acid amide derivatives were ascertained to be active components against α-glucosidase. The lead structure, N-trans-coumaroyltyramine, showed significant inhibition of α-glucosidase (IC_{50} = 0.42 µM). Moreover, all active compounds displayed uncompetitive inhibition mechanisms that have rarely been reported for α-glucosidase inhibitors. This kinetic behaviour was fully demonstrated by showing a decrease of both K_m and V_{max}, and K_{ik}/K_{iv} ratio ranging between 1.029 and 1.053. The molecular modelling study revealed that the inhibitory activities are tightly related to π-π interaction as well as hydrogen bond interaction between enzyme and inhibitors. (Song *et al.*, 2016).

Neuroprotective activity

Tribulus terrestris extract standardised to 45% of saponins (plant part used is not specified)

In vivo

In the study by Alzahrani *et al.* (2018) *Tribulus terrestris* extract (TTE) standardised to minimum 45 % of saponins (plant part and extraction solvent were not specified) was tested for its ability to improve motor dysfunction and alleviate rotenone induced oxidative DNA damage and neurotoxicity in mice in doses 5 and 10 mg/kg. The results demonstrated that TTE ameliorated the motor dysfunctions induced by rotenone as well as markers of inflammation and DNA damage (8-OHdG and MTH1 expression). Indicators of oxidative stress and upregulation of the microglia marker (CD11b) were suppressed by the higher dose of TTE (10 mg per kg). Finally, the higher dose of TTE improved the Cresyl violet staining and tyrosine hydroxylase immunostaining in the substantia nigra. In summary, TTE ameliorated the locomotor dysfunction and dampened the DNA damage and oxido-inflammatory stress in rotenone-parkinsonian mice.

Methanolic extract from *Tribulus terrestris* fruits

In vitro and in vivo

To test the hypothesis that *Tribulus terrestris* methanolic extract (TTME, plant part used fruit, no details on DER) possessed antioxidant potential and can ameliorate Parkinson's disease (PD) via modulation of α-synuclein, acetylcholinesterase (AChE), TNF-α, and IL-1β, in silico and in vivo studies were performed. The PD model in rats was prepared by giving haloperidol, 1 mg/kg, i.p. Rats were divided into six groups: control, disease control, standard, and treatment groups receiving TTME orally at 100, 300, and 1000 mg/kg dose levels for 21 days. Behavioural observations and biochemical analyses were done. The TTME modulatory effect on mRNA expression of α-synuclein, AChE, TNF-α, and interleukins in the brain homogenate was estimated by RT-PCR. Compounds detected in HPLC analysis disrupted the catalytic triad of AChE in in silico studies. Behavioral observations showed significant ($p < 0.05$) improvement in a reversal of catatonia, muscular strength, locomotor functions, stride length, and exploration in a dose-dependent manner (1000 >300 >100 mg/kg) of PD rats. Endogenous antioxidant enzyme levels CAT, SOD, GSH, and GPx were significantly restored at a high dose ($p < 0.05$) with a notable ($p < 0.05$) decrease in the MDA level in TTME treatedgroups. TTME at a high dose significantly ($p < 0.05$) decreased the level of acetylcholinesterase. RT-PCR results are showing down-regulation in the mRNA expression levels of IL-1β, α -synuclein, TNF-α, and AChE in TTME-treated groups compared to the disease control group, indicating neuroprotection. (Saleem *et al.*, 2020).

Anti-inflammatory activity

Tribulus terrestris extract – saponins rich (plant part used is not specified)

In vivo

Ameliorative effect of a commercial formula of a saponin rich extract of TTS (containing not less than 45% saponins, no details on plant part use, extraction solvent and DER) in a model of dietary obesity in female rats focusing on their ability to control the inflammatory burden, insulin resistance (IR), adipokine expression and the related reproductive system pathologies was examined. Female rats were fed with high fat diet (HFD) for 14 weeks to launch diet-induced obesity; they were assigned as: the obese control female rats (OFR) which received no treatment and TTS (5 and 10 mg/kg/day) treated rats; they were compared to a normal rat group. The IR index, serum/tissue inflammatory cytokines, and adipose tissue adipokine expression ere determined and the secondary ovarian pathologies examined. Body weight gain, serum triglycerides and IR (>5-fold) in the OFR group were greater than the normal group; TTS lessened these parameters compared with the OFR group. TTS, at 10 mg/kg dose, ameliorated mRNA expression of leptin and visfatin genes in addition to serum inflammatory cytokine levels. Moreover, TTS corrected the hyperprolactinemia and other hormonal disturbances and ameliorated the ovarian pathologies. The study highlighted that the anti-inflammatory properties of TTS helped in alleviation of IR and body weight gain in OFR. Upon correction of obesity manifestations, the gonadal hormone dysregulations and ovarian pathologies were subsequently ameliorated. (Abdel-Mottaleb *et al.*, 2022).

Tribulus terrestris extract (plant part used is not specified)

In vitro/in vivo

In the study, the anti-inflammatory effect of *Tribulus terrestris* L. extract (BJL – no further details on plant part used, extraction solvent and DER) in both transgenic zebrafish line Tg (MPO:GFP) in vivo and mice macrophage RAW 264.7 cells in vitro were evaluated, and the action mechanisms underlying the anti-inflammatory activity of BJL were also studied. The production of nitric oxide (NO) was measured by Griess reagent. The mRNA expression levels of inflammatory cytokines and inducible nitric oxide synthase (iNOS) were measured by real-time PCR, and the intracellular total or phosphorylated protein levels of NF-κB (Nuclear factor kappa-light-chain-enhancer of activated B cells), Akt (Protein kinase B), and MAPKs (Mitogen-activated protein kinases) including MEK, ERK, p38,

and JNK were detected by western blot. It has been found that BJL significantly inhibited fin transection or lipopolysaccharide- (LPS-) induced neutrophil migration and aggregation in zebrafish in vivo. In mice macrophage RAW 264.7 cells, BJL ameliorated LPS-triggered excessive release of NO and transcription of inflammatory cytokine genes including tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β). BJL also reduced the LPS-induced elevations of intracellular iNOS and nuclear factor kappa B (NF-κB) which mediate the cellular NO and inflammatory cytokine productions, respectively. Moreover, LPS dramatically increased the phosphorylation of Akt and MAPKs including MEK, ERK, p38, and JNK in RAW 264.7 cells, while cotreatment BJL with LPS suppressed their phosphorylation. Taken together, our data suggested that BJL presented potent anti-inflammatory effect, and the underlying mechanism was closely related to the inhibition of Akt/MAPKs and NF-κB/iNOS-NO signalling pathways. (Zhao *et al*., 2021).

Flavonoid fraction from leaves

In vivo

To investigate the anti-inflammatory activities of flavonoids fraction from *Tribulus terrestris* L. leaves in vivo, the ear swelling was selected as inflammatory model induced by xylene in mice. The ear swelling degree (mg) decreased very significantly ($p \leq 0.01$) in the aspirin group (0.2 g/kg) compared to the control group. Moreover, 12.5, 25 and 50 g RMM (raw medicinal material)/kg of flavonoids fraction can reduce the swelling degree in a dose-dependent manner. The anti-inflammatory activities of 12.5 and 50 g RMM/kg of flavonoids fraction exhibited very significantly ($p \leq 0.01$) and significantly ($p \leq 0.05$) comparing to the aspirin group, and there was no significant difference between 25 g RMM/kg of flavonoids fraction and significant aspirin group ($p > 0.05$). 25 g RMM/kg of flavonoids fraction exhibited similar anti-inflammatory effect to aspirin, and 50 g RMM/kg of flavonoids fraction revealed stronger anti-inflammatory activity. In addition, there was a similar trend in inhibition ratio (%). (Tian *et al.*, 2019).

In vitro

Cytotoxic effect of flavonoids fraction from *Tribulus terrestris* L. leaves on RAW 264.7 cells was measured by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay. Cells were incubated with flavonoids fraction at the concentrations of 50, 100 and 200 μg/ml after pre-treated with LPS or medium. The results indicated that cell viability was not significantly ($p > 0.05$) affected by flavonoids fraction in the experimental concentration range from 50 to 200 μg/ml, namely, the experimental concentration of flavonoids fraction is safe for RAW 264.7 cells. Cells were pre-treated with 50, 100 and 200 μg/ml of flavonoids fraction for 1 h, followed by LPS (1 μg/ml) stimulation for 18 h, and then incubated with 100 μl of neutral red solution (100 mg/ml) for 30 min. As shown in Fig. 9, it was found that phagocytosis increased significantly ($p < 0.01$) after the LPS-induced comparing to the control group, which was revealed that the experiment model was established successfully. Moreover, flavonoids fraction can reduce phagocytosis significantly ($p< 0.01$) compared with the LPS treatment group in a dose-dependent manner. In addition, the 100 and 200 μg/ml of flavonoids fraction exhibited much better inhibition of phagocytic activity than dexamethasone. (Tian *et al.*, 2019).

Terrestrosin D

In vivo

The co-administration of TED (terrestrosin D- in the dose of 10 mg/kg applied intraperitoneally) with BLM (bleomycin) in the pulmonary tissues of mice significantly suppressed the inflammatory and fibrotic changes observed in the classic BLM models. The inflammatory response was suppressed from the initial stage (reduction of IL-8 levels), with no 'bypassing' effects to initiate the downstream process of inflammation. Additionally, the fibrotic process was also suppressed in terms of fibrotic

marker quantification and morphological analysis, providing evidence to support our conclusion that TED is capable of suppressing the entire development process of BLM-induced pulmonary fibrosis in mice. (Qiu *et al.*, 2019).

N-trans-ρ-caffeoyl tyramine

In vitro

In the study, the effects of N-trans-ρ-caffeoyl tyramine (CT) isolated from fruits of *T. terrestris* on the production of nitric oxide (NO), and the expression of pro-inflammatory cytokines and COX-2 in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells were investigated. It was found that the ethanolic extract of *T. terrestris* (EETT) and CT inhibited the production of nitric oxide (NO), tumour necrosis factor-α (TNF-α), interleukin (IL)-6 and IL-10 in the lipopolysaccharide (LPS)-stimulated RAW 264.7 cells in a dose-dependent manner. In addition, CT markedly suppressed the expression of COX-2 and the production of prostaglandin E2 (PGE2) in response to LPS stimulation. Furthermore, CT markedly decreased p-c-Jun N-terminal kinase (p-JNK) protein expression in LPS-stimulated RAW 264.7 cells. COX-2 and p-JNK were measured by western blot analysis. (Ko *et al.*, 2015).

Tribulusamide D

In vitro

The study by Lee *et al*. (2017) investigated the anti-inflammatory effect of tribulusamide D (isolated from ethanolic extract from *Tribulus terrestris* fruit) on lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. Tribulusamide D inhibited the production of LPS-induced nitric oxide and prostaglandin E2, by reducing the expression of inducible nitric oxide synthase and cyclooxygenase-2 expression, respectively. The expression of these genes associated with inflammation was determined using reverse transcription-polymerase chain reaction and western blot analysis. Furthermore, tribulusamide D reduced the expression of LPS-induced inflammatory cytokines, including interleukin (IL)-6, IL-10 and tumour necrosis factor-α. They were quantified using an enzyme-linked immunosorbent assay. In addition, the present study confirmed that the inhibitory effects of tribulusamide D on the inflammatory response were mediated through inactivation of mitogen-activated protein kinase p38 and inhibition of nuclear localization of nuclear factor-B, which were also determined by western blot analysis.

Antihypertensive activity

Methanolic and aqueous fraction of the extract from whole plant

In vivo/in vitro

The effects of methanolic and aqueous extracts from the whole plant of *Tribulus terrestris* on rat blood pressure (BP) and the perfused mesenteric vascular bed were investigated. The extracts dose-dependently reduced BP in spontaneously hypertensive rats (SHRs) with the aqueous fraction being more potent than the methanolic fraction at all doses tested. In vitro, the methanolic but not aqueous extract produced a dose-dependent increase in perfusion pressure of the mesenteric vascular bed. When perfusion pressure was raised with phenylephrine (10−5 M), the aqueous extract produced a dose-dependent reduction in perfusion pressure at all doses. A low dose of the methanolic extract produced a vasoconstrictor effect while higher doses produced dose-dependent reduction in perfusion pressure. l-NAME (N(G)-Nitro-L-arginine methyl ester) (10−4 M) significantly reduced but did not abolish vasodilation induced by the extracts. Vasodilator responses to aqueous and methanolic fractions were significantly reduced in preparations where perfusion pressure was raised with KCl (60 mM). A combination of KCl and L-NAME abolished the vasodilator responses induced by the extracts. It was concluded that methanolic and aqueous extracts of *Tribulus terrestris* possess significant

antihypertensive activity in spontaneously hypertensive rats. The antihypertensive effects appeared to result from a direct arterial smooth muscle relaxation possibly involving nitric oxide release and membrane hyperpolarization. (Phillips *et al.*, 2006).

Aqueous extract from fruit

In vivo

The study investigated the antihypertensive mechanism of *Tribulus* extract (plant part used fruits, extraction solvent water) in 2K1C hypertensive rats by measurement of circulatory and local ACE (angiotensin-converting enzyme) activity in aorta, heart, kidney and lung. Four groups of rats were selected; control, sham, operated or hypertensive and *Tribulus* treated hypertensive group. Hypertension was induced using silver clip on renal artery by surgery. Four weeks after surgery, a single daily dose of 10 mg/kg of lyophilized aqueous extract of *Tribulus* fruit were given orally to 2K1C rats for four weeks. ACE activity was determined by high performance liquid chromatography (HPLC). Blood pressure was measured by the tail-cuff method. The systolic blood pressure (SBP) was significantly increased in 2K1C rats compared to control rats. The SBP of *Tribulus* fed hypertensive rats was significantly decreased compared to hypertensive rats. The ACE activity in all tissues of 2K1C rats including aorta, heart, kidney, lung as well as serum were significantly increased compared to normal rats. The ACE activity in all tissues of *Tribulus* fed hypertensive rats was significantly lower than that of hypertensive rats, which was more pronounced in kidney. These results indicated that there is a negative correlation between consumption of *Tribulus* and ACE activity in serum and different tissues in 2K1C rats. (Sharifi *et al.*, 2003).

Anti-atherosclerotic activity

Total saponins extracted from fruits

In vitro

Total saponin extracted from *Tribulus terrestris* fruit (TSETT) has been reported to protect against atherosclerosis. In the study the cellular and molecular mechanisms of TSETT underlying protection against atherosclerosis was studied. Methods: Cell proliferation was measured with Methyl thiazolyl tetrazolium (MTT); Intracellular H_2O_2 was measured with DCFHDA, a fluorescent dye; Intracellular free Ca2+ was measured with a confocal laser scanning microscopy; Genes expression was measured with gene array and real-time quantitative polymerase chain reaction (RT-PCR); Phosphorylation of extracellular signal-regulated kinase 1/2 (phospho-ERK1/2) was measured with cell-based enzyme-linked immunosorbent assay (ELISA) and western blotting. Results: TSETT significantly suppressed the increase in cells proliferation induced by angiotensin II, significantly suppressed the increase in the intracellular production of H2O2 induced by angiotensin II, significantly inhibited the increase in intracellular free Ca^{2+} induced by H_2O_2, significantly inhibited the increase in phospho-ERK1/2 induced by angiotensin II; significantly inhibited the increase in mRNA expression of *c-fos, c-jun* and *pkc-a* induced by angiotensin II. Conclusion: These findings provide a new insight into the anti-atherosclerotic properties of TSETT and provide a pharmacological basis for the clinical application of TSETT in anti-atherosclerosis. (Li *et al*., 2013).

Cardioprotective activity

Hydroalcoholic extract (50%) from the whole plant

In vivo

The study evaluates the cardioprotective potential of hydro-alcoholic extract of *Tribulus terrestris* L. (total saponins content 43.77 % w/w). Wistar male albino rats weighing 150-200 g were randomly divided into three main experimental groups; sham (saline treated only), isoproterenol (ISP) control

(saline and ISP) and *Tribulus terrestris* treatment groups (*T. terrestris* and ISP). Saline or *T. terrestris* extract 250 mg kg-1 once daily were orally administered for 30 days. Isoproterenol was administered in rats to induce myocardial infarction. On days 29 and 30, the animals of ISP control and *T. terrestris* treatment group were administered ISP (85 mg kg^{-1}, subcutaneously) at an interval of 24 h. On the day 31, 48 h after first dose of ISP, hemodynamic parameters were recorded. After sacrificing the animals, the hearts were excised and subjected to biochemical, histopathological, and ultrastructural studies. ISP-administration produced a significant decrease in the activities of endogenous antioxidant defence enzymes viz. superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx) and tissue antioxidant, reduced glutathione (GSH) along with a concomitant increase in the lipid peroxidation product malonaldehyde (1.1DA). In addition, a significant decrease in the activities of myocardial injury markers i.e., creatine phosphokinase-MB (CK-J\1B isoenzyme) and lactate dehydrogenase (LDH) was also observed in the heart of ISP control group as compared to sham control. Cardiac dysfunction was observed as a decrease in mean arterial pressure (1.1AP), heart rate (HR), left ventricular rate of peak positive and negative pressure change {(+) and (-) LV dP/and elevated left ventricular end diastolic pressure (LVEDP) following ISP administration. These functional alterations were supported by severe modifications in histopathological and ultrastructural assessment. Pre-treatment with *T. terrestris* resulted in the increased activities of SOD, CAT, GSHPx and prevention of depletion of tissue glutathione along with inhibition of lipid peroxidation. In addition, treatment with *T. terrestris* decreased the leakage of CK-J\1B and LDH enzymes from myocardium, there was a significant improvement in cardiac function as evidenced by correction of J\1AP, HR, LVEDP and contractility and relaxation. The possible underlying mechanism of the cardioprotective effect of *T. terrestris* could be due to restoration of endogenous myocardial antioxidant status or free radical scavenging activity along with correction of the altered hemodynamic parameters and preservation of histoarchitectural and ultrastructural alterations. (Ojha *et al.*, 2008).

Tribulosin and Gross saponins from *Tribulus terrestris*

In vitro (ex vivo)

The aim of the study was to investigate the protective effect of tribulosin, a monomer of the gross saponins from *Tribulus terrestris*, against cardiac ischemia/reperfusion injury and the underlying mechanism in rats. Isolated rat hearts were subjected to 30 min of ischemia followed by 120 min of reperfusion using Langendorff's technique. The hearts were assigned to seven groups: control, ischemia/reperfusion (I/R), treatment with gross saponins from *Tribulus terrestris* (GSTT, no further details on composition) 100 mg/L, treatment with tribulosin (100, 10, and 1 nmol/L) and treatment with a PKC inhibitor (chelerythrine) (1 μmol/L). Infarct size was assessed by triphenyltetrazolium chloride staining. Malondialdehyde (MDA), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) contents as well as superoxide dismutase (SOD) and creatine kinase (CK) activities were determined after the treatment. Histopathological changes in the myocardium were observed using hematoxylin-eosin (H&E) staining. Apoptosis was detected with terminal deoxynucleotidyl transferase nick-end labelling (TUNEL) assay. Bcl-2 (B-cell lymphoma 2), Bax, caspase-3, and PKCε protein expression were examined using western blotting. Tribulosin treatment significantly reduced MDA, AST, CK and LDH contents, and increased the activity of SOD. The infarct size of I/R group was 40.21% of the total area. GSTT and various concentrations of tribulosin treatment decreased the infarct size to 24.33%, 20.24%, 23.19%, and 30.32% ($P<0.01$). Tribulosin treatment reduced the myocardial apoptosis rate in a concentration dependent manner. Bcl-2 and PKCε protein expression was increased after tribulosin preconditioning, whereas Bax and caspase-3 expression was decreased. In the chelerythrine group, Bcl-2 and PKCε expression was decreased, whereas Bax and caspase-3 expression was increased. (Zhang *et al.*, 2010).

Concentrated preparation, kaempferol, ruscogenin, protodioscin, protogracillin and harmine

In vitro

Tribulus terrestris (in a form of concentrated preparation, no details on the preparation) and its active ingredients including kaempferol, ruscogenin, protodioscin, protogracillin and harmine were used in the study to examine their effect on porcine coronary artery. The results showed that *T. terrestris* can induce the immediate relaxation of porcine coronary in dose-dependent manner. However, the main compounds of *T. terrestris* in the study cannot cause relaxation of porcine coronary artery compared with corresponding solvent. (Tey *et al.*, 2021).

Hepatoprotective activity

Extract from aerial parts of the plant

In vivo

The effects of the *T. terrestris* extract (aerial parts of plant, extraction solvent methanol : acetone : water 2:2:1, 1 kg of the plant extracted with 3 litters of the solvent, dried and redissolved in methanol) in the attenuation of hepatotoxicity induced by α-cypermethrin in rats are investigated by Ali *et al*. (2018). Forty mature rats were randomly assigned into four equal groups group1 (Control), rats were kept as control group; group 2 (TT), rats were orally administered (100 mg of T. terrestris extract/kg BW); groups 3 (CYP), rats were orally administered 1/20 LD50 of alpha-cypermethrin (0.533 mg/kg, BW); and group 4 (TT+CYP), rats were orally administered alpha-cypermethrin at a dose level of 0.533 mg/kg BW along with 100 mg of the *T. terrestris* extract /kg BW. Gastric intubation was used for the administration of both alpha-cypermethrin and the *T. terrestris* extract for sixty-five days The administration of the *T. terrestris* extract decreased liver enzymes: alanine amino transferase (ALT), and aspartate amino transferase (AST). Also, it increased antioxidant; glutathione (GSH) and paraoxnase-1(POX-1) enzyme, and decreased oxidant; malondialdehyde (MDA) and nitric oxide (NO). Furthermore, it decreased liver inflammatory markers; tumour necrosis factor alpha (TNF - α), adiponectin and lipocalin. (Ali *et al*., 2018).

Extract from fruits

In vivo

The study has been performed to assess the useful impacts of *Tribulus terrestris* (TT – fruit extract, extraction solvent and DER were not specified) and silymarin (SLM) against carbon tetrachloride (CCl_4)-induced hepatotoxicity. Forty-two male rats were partitioned into six groups: group I: received 0.3% Sodium carboxymethyl cellulose (CMC-Na) in distilled water, group II: TT (500 mg/kg BW, orally), group III: SLM (200 mg/kg, orally) for 14 consecutive days (on days 11 and 12 intraperitoneal corn oil), group IV: CCl_4, group V: TT (500 mg/kg BW) plus CCl_4, and group VI: SLM (200mg/kg orally) plus CCl_4. The CCl_4 was administered (2.0 ml/kg BW) intraperitoneal on days 11 and 12. Sera were collected for assessment of hepatic injury markers and proinflammatory cytokines. Additionally, liver tissue oxidative stress, lipid peroxidation, histopathological examination, and immunohistochemical analysis (Bax and bcl-2) were done. CCl_4 injection induced significant reductions in hepatic antioxidants while increased hepatic lipid peroxidation as well as serum hepatic injury biomarkers and pro-inflammatory cytokines. The histopathological examination showed necrotic and degenerative changes in the hepatic tissue, while immunohistochemical analysis revealed marked hepatic expression of activated Bax, and bcl-2, following CCl_4 injection. TT pre-treatment significantly improved all examined parameters and restored the hepatic architecture. The study illustrated that TT effectively alleviates hepatic oxidative damage, apoptosis, and inflammation, induced by acute CCl_4 intoxication. (Kilany *et al.*, 2020).

Nephroprotective activity

Ethanolic extract (70%) from aerial part of *Tribulus terrestris* freeze dried

In vivo

Ten male Sprague-Dawley rats in the acute kidney injury (AKI) and 10 in the *Tribulus terrestris* groups received the extract solvent and extract of the plant (aerial part, extraction solvent ethanol 70%, DER not specified) (11 mg/kg), respectively, for 13 days (oral administration). On day 14, ischemia for 30 minutes and reperfusion for 24 hours were induced on the rats. In the last 6 hours of the reperfusion period (24 hours), urine samples were collected. At the end of this period, blood samples were also taken to determine plasma urea nitrogen, creatinine, and electrolyte concentrations. The kidney tissues were collected for measuring the level of oxidative stress and histological studies. In the *Tribulus terrestris* group, the increase in plasma creatinine and urea nitrogen concentrations was significantly less following reperfusion, and their values reached the same level as that in the sham group. Creatinine clearance and urine osmolarity in the *Tribulus terrestris* group was higher in comparison with the AKI group, whereas sodium absolute excretion, fractional excretion of potassium, oxidative stress, and cellular damages were less. It has been concluded that oral administration of *Tribulus terrestris* extract for 2 weeks can decrease kidney functional disturbance, oxidative stress, and cellular damages following reperfusion injury in rats. (Najafi *et al*., 2014).

Extract from *Tribulus terrestris* (no further details on the plant part used, extraction solvent and DER)

In vivo

The aim of the study was to investigate the immune histochemical and ultrastructural alterations in the cerebral cortex of experimental rabbits on a cholesterol rich diet treated with TT. The rabbits were divided into three groups and followed for 12 weeks as control group (CG); experimental group I (EG-I), fed with a cholesterol-rich diet; experimental group II (EG-II), treated with an extract of TT (5mg/kg/day) after a cholesterol-rich diet of 4 weeks. In EG-I there were ultrastructural changes, including mitochondrial degeneration, increased lipofuscin pigments, myelin sheath damage with axoplasmic shrinkage and electron dense granules in the neurovascular unit. The number of synapses apparently decreased in both experimental groups. Administration of TT extract in EG-II led to marked ultrastructural alterations in neurons, including decreased mitochondrial degeneration ($P<0.001$) and extensive oedematous areas in the neurovascular unit. However, in EG-II, lamellar myelin, axonal structures and mitochondria were well protected. The authors concluded that alterations possibly indicate that saponins have an effect on the neurons either directly or by its conversion to steroidal saponins. Therefore, these findings add further evidence supporting the protective claims of TT in cerebral architecture in dietary induced hyperlipidaemia. (Berkman *et al.*, 2009).

Ethyl acetate/menthol extract from fruits

In vitro

In the study, reno protective effects of *Tribulus terrestris* extracts from fruits (TT) obtained with ethyl acetate combined with 50% methanol on obesity-related glomerulopathy (ORG) were evaluated. In vitro, IC_{50} of TT was 912.01 mg/L, and the appropriate concentration of TT against oxidized-low density lipoprotein (ox-LDL) induced human renal glomerular endothelial cells (HRGECs) was 4 mg/L. TT significantly increased the viability (63.2%) and migration (2.33-fold increase) of HRGECs. ORG (obesity-related glomerulopathy) model rats were induced by a chronic high-fat diet (45%) for 20 weeks and were then treated with TT extract (2.8 g/kg/d) for 8 weeks. Subsequently, the kidneys were removed, and their differentially expressed protein profile was identified using two-dimensional electrophoresis coupled with matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF)-TOF MS. Molecular categorization and functional analysis of bioinformatic annotation suggested that excessive energy metabolism, decreased response to stress and low immunity were the potential

aetiologies of ORG. After TT administration for 8 weeks, body weight, blood pressure, serum cystatin C and cholesterol were decreased. Additionally, TT significantly enhanced the resistance of rats to ORG, decreased energy consumption and the haemorrhagic tendency, and improved the response to acute phase reactants and immunity. According to authors TT may play a protective role against ORG in rats. (Jiang *et al.*, 2018).

Cytotoxic activity

Ethanol, ethyl acetate and methanol extracts from aerial parts of the plant

In vitro

The cytotoxic effects of the ethanol, ethyl acetate and methanol extracts from aerial parts of the plant (extraction method is described) on MCF-7 breast cancer cells in the concentration range of 10-1000 ppm at 24 h of exposure were investigated by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) method. *T. terrestris* L. ethanol extracts at 1000 ppm caused a moderate cytotoxic effect on MCF-7 breast cancer cells. It was determined that ethanolic extracts in the concentration range of 10-500 ppm and methanol and ethyl acetate extracts in the concentration of 10-1000 ppm caused cell proliferation. (Akbaba *et al*., 2021).

Methanol extract and saponins extracted from seeds and leaves

In vitro

The study was undertaken to elucidate the anticancer mechanism of TT on MCF-7 breast cancer cells. Cytotoxic effect of the herb was assessed by 3-(4,5-diethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Apoptotic potential was assessed through DNA fragmentation, TUNEL and caspase 3 activity assays. Expressions of genes regulating the apoptotic pathway were examined by RT-PCR and expression of proteins was analysed by immunocytochemistry. The result of MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay revealed that methanolic and saponin extracts from leaves and seeds of TT were cytotoxic to MCF-7 cells. Cytotoxicity studies on peripheral blood mononuclear cells (PBMC) proved that TT extracts (methanol and acetone, no further details were non-toxic to non-malignant cells. Treatment of human breast cancer MCF-7 cells with seed and leaf methanol and saponin extracts of TT resulted in fragmentation of DNA and induction of apoptosis. This was evident by agarose gel electrophoresis of DNA and TUNNEL assay. The extracts of TT also caused a significant increase in caspase 3 activity in MCF-7 cells. TT extracts caused an induction of 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 were also upregulated indicating the possible induction of extrinsic apoptotic pathway. According to the authors, the results suggest that the *Tribulus terrestris* (TT) extracts may exert their anticancer activity by both extrinsic and intrinsic apoptotic pathways. (Patel *et al*., 2019).

Ethanolic extract from fruits

In vivo

The study was performed to confirm hypothesis that the treatment with TT dry extract (hydro-ethanolic extract from the fruits containing minimum 40% of steroid saponins) would protect the male reproductive system against cyclophosphamide (CP) toxicity. Mice received dry extract of TT (11 mg/kg) or vehicle by gavage for 14 days. Saline or CP was injected intraperitoneally at a single dose (100 mg/kg) on the 14th day. Animals were euthanized 24 h after CP administration, and testes and epididymis were removed for biochemical and histopathological analysis and sperm evaluation. The dry extract of TT was evaluated by HPLC analysis and demonstrated the presence of protodioscin (1.48%, w/w). CP exposure increased lipid peroxidation, reactive species, and protein carbonylation and altered

antioxidant enzymes (SOD, CAT, GPx, GST, and GR). Moreover, acute exposure to CP caused a reduction on 17 β-HSD activity, which may be related to the reduction in serum testosterone levels, histopathological changes observed in the testes, and the quality of the semen. The study highlighted the role of TT dry extract to ameliorate the alterations induced by CP administration in mice testes, probably due to the presence of protodioscin. (Pavin *et al*., 2018).

In vitro

Aqueous extract from fruits

The antitumor activity of aqueous TT fruit extract (extraction method is described) was analysed by testing the cytotoxicity and the effect on clonogenicity in HepG2 cells. Apoptosis and cell cycle arrest induced by TT were dissected by flow cytometry and its inhibitory effect on NF-κB activity was determined by analysing the expression levels of NF-κB/IκB subunit proteins. The suppression of NF-κB-regulated gene expression by TT was assessed by RT-PCR. TT extract repressed clonogenicity and proliferation, induced apoptosis, and enhanced accumulation in the G0/G1 phase of liver cancer cells. It also turned out that TT extract inhibited NF-κB-dependent reporter gene expression and NF-κB subunit p50 expression, while it enhanced the cellular level of IκBα by inhibiting the phosphorylation and degradation of IκBα. In addition, IKK activity was inhibited in a dose-dependent manner. Furthermore, TT extract suppressed the transcription of genes associated with cell cycle regulation, anti-apoptosis, and invasion. The data showed that TT extract blocks proliferation and induces apoptosis in human liver cancer cells through the inhibition of NF-κB signalling. (Kim *et al.*, 2011).

Tribulus saponins

In vitro

The focus of the review is to provide an updated overview of various aspects of the biological properties of the saponins extracts of the TT plant (no details on the composition), to highlight a photoprotective effect against UVB-induced skin carcinogenesis, and to demonstrate the molecular mechanisms through which TT saponins regulate cell death. The recent research demonstrated that TT saponins resulted to have a dual efficacy to prevent malignant transformation of epithelial cells caused by UVB exposure. In fact, TT saponins, on one hand, protect healthy keratinocytes by UVB irradiation-dependent damage and, on the other, enhance UVB-caused apoptosis in squamous cell carcinoma, thus suggesting that TT saponins possibly work as UVB-damage sensors to exert their biological action. Together, these findings encourage further mechanistic and in vivo studies for developing TT saponins as a promising chemo preventive and/or chemotherapeutic agent against UVB-caused skin alteration and tumours in humans. (Sisto and Lisi, 2019, review).

The study examined the role of saponins derived from *Tribulus terrestris* L. (TT) on the modulation of apoptosis in normal human keratinocytes (NHEK) exposed to physiological doses of UVB and to evaluate their antitumoral properties. In NHEK, TT saponins (no details plant part used and composition) attenuate UVB-induced programmed cell death through inhibition of intrinsic apoptotic pathway. In squamous cell carcinomas (SCC) TT saponins do not make the malignant keratinocytes more resistant to UVB and determine an enhanced apoptotic response. The photoprotective effect of TT saponins is tightly correlated to the enhancement of NER genes expression and the block of UVB mediated NF-jB activation. Collectively, our study shows experimental evidence that TT has a preventive efficacy against UVB-induced carcinogenesis and the molecular knowledge on the mechanisms through which TT saponins regulate cell death suggests great potential for TT to be developed into a new medicine for cancer patients. (Sisto *et al.*, 2012).

Saponins from aerial part of the plant

In vitro

The study aimed to analyse the impact of saponin extract from TT on cell processes in breast carcinoma cell lines. The variations in expression of a group of 32 selected genes were examined by real-time PCR after saponin treatment of MCF7 and MCF10A cell lines. Only three genes – CXCR4, CCR7 and BCL2, showed changes in their mRNA levels after the application of the herb extract. While CXCR4 expression was reduced in both cell lines, CCR7 and BCL2 levels decreased only in tumorigenic MCF7 cells, implying cell-specificity of the saponin action. The results suggested that TT extract containing saponins (ethanol/butanol extract from aerial part of the plant, saponins content more than 99%) was likely to affect the processes of apoptosis and metastasizing of cancer cells. Further in vivo studies will show its applicability as an anticancer therapeutic agent. (Goranova *et al.*, 2015).

Standardised and saponins enriched extracts from *Tribulus terrestris*

In vitro

The aim of the study was investigation of the antiglycation and antitumoral potential of standardized (TtSE) and saponins-enriched extracts (TtEE) of *Tribulus terrestris* herbal medicine. The procedures for the evaluation of the antiglycation activity of the standardized (TtSE -standardised to 43.21 % of total saponins) and saponins-enriched extracts of *T. terrestris* (**(**TtEE, 72.8 % of total saponins, plant part used is not specified) were: determination of relative mobility in electrophoresis (RME), free amino groups using OPA method and advanced glycation end-products (AGEs) fluorescence. Antioxidant activity was determined by DPPH radical scavenging test. In vitro antitumor activity of TtSE and TtEE was evaluated in human tumour cell lines. The results were obtained by antiglycation tests (RME, OPA method and AGEs fluorescence determination), using BSA as protein and ribose as glycation agent, and antioxidant assay (DPPH test); it was verified that both extracts of *T. terrestris* have antiglycation and antioxidant activity. In addition, the extracts were able to induce death of more than 50% of human tumour cell lines. In conclusion, the study showed that standardized and saponins-enriched extracts of *T. terrestris* herbal medicine present antiglycation and antioxidant and antiproliferative action in human tumour cells lines. The saponins-enriched extract proved a greater antiglycation and antioxidant activity in comparison to the standardized type. (Figueiredo *et al.*, 2021).

Natural molecules structurally close to diosgenin (five saponins: diosgenin, hecogenin, tigogenin, sarsasapogenin, smilagenin; two steroidal alkaloids: solasodine, solanidine; one sterol: stigmasterol)

Trouillas *et al*. (2005) reported that the saponins, structurally similar to diosgenin, present in *T. terrestris* extracts, might block the cell cycle, suppress proliferation, and induce apoptosis in human sarcoma cell lines.

Terrestrosin D

In vitro and in vivo

In vitro, terrestrosin D (TED) strongly suppressed the growth of prostate cancer cells and endothelial cells in a dose-dependent manner. TED induced cell cycle arrest and apoptosis in PC-3 cells and human umbilical vascular endothelial cells (HUVECs). TED-induced apoptosis did not involve the caspase pathway. TED also decreased ΔΨm in PC-3 cells and HUVECs. In vivo, TED significantly suppressed tumour growth in nude mice bearing PC-3 cells, without any overt toxicity. Immunohistochemical analysis showed TED induced apoptotic cell death and inhibited angiogenesis in xenograft tumour cells. (Wei *et al.*, 2014).

Alkaloid extract from fruits

In vitro

The study demonstrates apoptosis-inducing potential and mechanism of action of *Tribulus terrestris* alkaloid extract in Jurkat E6-1 cancer cell line. Liquid Chromatography-Mass Spectrometry and High

Resolution-Mass Spectrometry analysis identified the presence of four N-feruloyltyramine derivatives, namely trans-N-feruloyl-3-hydroxytyramine (1), trans-N-coumaroyltyramine (2), trans-N feruloyltyramine (3) and trans-N-feruloyl-3-ethoxytyramine (4) in the alkaloid extract. Compounds 2 and 3 have not been yet reported in the alkaloid extract of *T. terrestris*. In silico analysis revealed therapeutic potential of N-feruloyltyramine derivatives and strong binding efficiency to both chains of Tumour Necrosis Factor Receptor 1. Treatment of alkaloids extract to Jurkat E6-1 clone induced dose-dependent cytotoxicity (LC50 140.4 mg mL1). Jurkat cells treated with alkaloids extract at sub-lethal concentration showed DNA fragmentation, enhancement in caspase-3 activity and phosphatidylserine translocation (apoptosis indicator) compared to control cells. Gene expression analysis using Human Apoptosis RT2 Profiler PCR Array analysis upon alkaloid treatment was found to significantly alter expression of critical genes such as TNFR1, FADD, AIFM, CASP8, TP53, DFFA and NFKB1. These genes are predicted to mediate apoptotic cell death via both intrinsic and extrinsic apoptosis pathway. In summary, the identification of new N-feruloyltyramine derivatives from alkaloid extract of *T. terrestris* fruit with probable anti-leukemic and pharmacological potential is reported by the authors. (Basaiyye *et al.*, 2018).

Diuretic activity

Extract from fruits and leaves

In vivo /in vitro

Al-Ali *et al*., 2003

Tribulus terrestris L. (Zygophyllaceae) which is called Al-Gutub (in Iraqi dialect) or Qutˆıba (in classical Arabic medicine), and Zea mays were both used alone or in combination by Iraqi herbalists to propel urinary stones. Aqueous extract of the leaves and fruits of *T. terrestris* were studied to determine their diuretic activity and the contractile effect. The aqueous extract was filtered, and the solvent was evaporated to produce a dry crude extract. The dry extract was then dissolved in physiological saline to make the required concentrations. Wistar male rats were used for the diuresis test and strips of isolated Guinea pig ileum were used for the contractility test. The aqueous extract of *T. terrestris*, in oral dose of 5 g/kg elicited a positive diuresis, which was slightly more than that of furosemide at the dose of 120 mg/kg. Z. mays aqueous extract did not result in significant diuresis when given alone in oral dose of 5 g/kg. Na^+, K^+ and Cl^+ concentrations in the urine had also much increased. In addition to its diuretic activity *T. terrestris* had evoked a contractile activity on Guinea pig ileum.

Antiurolithiatic activity

Water extract from fruits

In vitro

Aggarwal et al.*, 2010*

The study attempted to evaluate the antilithiatic properties of Tribulus terrestris commonly called as "gokhru" which is often used in ayurveda to treat various urinary diseases including urolithiasis. The activity of Tribulus terrestris was investigated on nucleation and the growth of the calcium oxalate (CaOx) crystals as well as on oxalate induced cell injury of NRK 52E renal epithelial cells. Tribulus terrestris extract (TT, water extract from fruits) exhibited a concentration dependent inhibition of nucleation and the growth of CaOx crystals. When NRK-52E cells were injured by exposure to oxalate for 72 h, Tribulus terrestris extract prevented the injury in a dose-dependent manner. On treatment with the different concentrations of the plant, the cell viability increased, and lactate dehydrogenase release decreased in a concentration dependent manner. The current data suggests that TT not only has a potential to inhibit nucleation and the growth of the CaOx crystals but also has a cytoprotective

role. Results of the study indicate that it could be a potential candidate for phytotherapy against urolithiasis.

Kaushik et al., 2017

The assessment of antilithiatic activity of 4 selected aqueous extracts from fruit of Tribulus terrestris (AE 1-4, with different extraction temperature and time and solid: liquid ratio; AE1 - temp. 23.50 °C, time 19.50 h and S:L 1g/12 mL; AE2 - temp. 4.16 °C, time 19.50 h and S:L 1g/12 mL, AE3 - temp. 35 °C, time 36 h and S:L 1g/20 mL; AE4 - temp. 35 °C, time 3 h and S:L 1g/20 mL) revealed enhanced nucleation and aggregation inhibition of calcium oxalate crystals with AE1 and AE2, which in addition significantly altered the size and morphology of calcium oxalate monohydrate (COM) crystals compared to AE3 and AE4. In vitro cell culture-based studies on renal epithelial cells (MDCK, NRK-52E and PK 15) proved that the AE1 showed higher cytoprotective potency by increasing cell viability as compared to the oxalate treated group. The free radical scavenging activity of aqueous extract lowered the reactive oxygen specie's induced damage and potentially reduced the signals of programmed cell death due to oxalate injury. In addition, modulation of the COM crystal morphology was enhanced by AE1 as compared to AE2. The FTIR and GC-MS analysis of AE1, showed the presence of biomolecules which could aid in the attenuation of lithiatic process.

In vivo

Kaushik et al., 2019

The preventive and curative urolithiatic efficacy in experimentally induced nephrolithiatic Wistar rats, along with preclinical toxicity was evaluated following oral administration of statistically optimized aqueous extract from fruits of T. terrestris. Treatment showed augmented renal function, restoration of normal renal architecture and increase in body weight. Microscopic analysis of urine revealed excretion of small sized urinary crystals, demonstrating that treatment potentially modulated the morphology of renal stones. Tissue enzymatic estimation affirmed the antioxidant efficacy of treatment with reduced free radical generation. Significant upregulation of p38MAPK at both the gene and protein level was noted in hyperoxaluric group and interestingly treatment reversed it. Acute oral toxicity study established the Median Lethal Dose (LD50) to be greater than 2000 mg/kg body weight (b.wt.) No observed adverse effect level (NOAEL) by repeated oral toxicity for 28 days at 750 mg/kg b.wt. was noted. This study lends scientific evidence to the safe, preventive and curative potential of statistically optimized aqueous extract of T. terrestris at a dose of 750 mg/kg b.wt. and suggests that the extract shows promise as a therapeutic antiurolithic agent.

The effect of an aqueous extract from fruits of Tribulus terrestris administered orally at a dose of 5 g/kg body weight was studied in six male adult rats in whom hyperoxaluria was induced and maintained by hydroxyproline and sodium glycolate respectively. Twenty-four-hour urinary oxalate excretion reversed to normal, from 1.97±0.314 to 0.144±0.004 mg/mg creatinine ($p<0.01$) within 21 days of administration of T. terrestris extract and remained so until 15 days after withdrawal of extract and sodium glycolate. (Sangeeta et al., 1993).

Antibacterial and antifungal activity

Extracts from different parts of the plant

In vitro

Antimicrobial activity of organic and aqueous, ethanolic and chloroform extracts from fruits, leaves and roots of Tribulus terrestris L., an Iraqi medicinal plant used as urinary anti-infective in folk medicine, was examined against 11 species of pathogenic and non-pathogenic microorganisms: Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Corynebacterium diphtheriae, Escherichia coli, Proteus

vulgaris, Serratia marcescens, Salmonella typhimurium, Klebsiella pneumoniae, Pseudomonas aeruginosa and Candida albicans using microdilution method in 96 multiwell microtiter plates. All the extracts from the different parts of the plant showed antimicrobial activity against most tested microorganisms. The most active extract against both Gram-negative and Gram-positive bacteria was ethanol extract from the fruits with a minimal inhibitory concentration (MIC) value of 0.15 mg/ml against B. subtilis, B. cereus, P. vulgaris and C. diphtheriae. In addition, the same extract from the same plant part demonstrated the strongest antifungal activity against C. albicans with an MIC value of 0.15 mg/ml. (Al-Bayati and Al-Mola, 2008).

Flavonoid fraction from T. terrestris leaves

The antibacterial activities of flavonoid fraction from Tribulus terrestris L. leaves against Escherichia coli (ATCC 25922), Salmonella (ATCC 51812), Staphylococcus aureus (ATCC 25923) and Streptococcus (ATCC 49619), were tested and gentamicin was used as a positive control for ensuring the accuracy and reliability of assay method. The inhibition effect of gentamicin was measured with MIC values ranged from 0.5 μg/ml to 2.0 μg/ml against Escherichia coli, Salmonella, Staphylococcus aureus and Streptococcus. flavonoids fraction exhibited a stronger antibacterial effect on gram-positive bacteria (MIC, 0.25 g RMM/ml) than gram-negative bacteria (MIC, 1.0 g RMM/ml). (Tian et al., 2019).

Spasmolytic activity

Saponins mixture from aerial parts of the plant

In vitro

The study investigated the effects of lyophilised saponin mixture of this plant on several smooth muscle preparations in vitro. The lyophilised material was obtained from dried and powdered aerial parts of T. terrestris L. by specific extraction method for saponins. Saponin mixture has caused a significant decrease on peristaltic movements of isolated sheep ureter and rabbit jejunum preparations in a dose dependent manner (p<1l.05). However, it has been observed no effect on isolated rabbit aorta and its contractile response to KCI or noradrenaline (p>O.05). According to these results it has been suggested that T. terrestris L. or its saponin mixture may be useful on some smooth muscle spasms or colic pains. (Arcasoy et al., 1998).

Analgetic activity

Flavonoid fraction from T. terrestris leaves

In vivo

The analgesic activity of flavonoids fraction (12.5, 25 and 50 g / kg) from Tribulus terrestris L. leaves was evaluated with writhing response of mice induced by 0.6% acetic acid, and the writhing times were evaluation index. The writhing times decreased very significantly ($p \leq 0.01$) in the aspirin (200 mg/kg) group compared to the control group, which was indicating that the pain model was established successfully and can be used for evaluation the analgesic effect of flavonoids fraction. In addition, 12.5, 25 and 50 g /kg of flavonoids fraction decreased writhing times with a dose-dependent manner. Compared with the aspirin group, 25 g RMM/kg of flavonoids fraction exhibited similar analgesic action of 12.5 g /kg of flavonoids fraction shown very significantly ($p \leq 0.01$) comparing to the aspirin group, namely, 12.5 g /kg of flavonoids fraction exhibited a weaker analgesic action. Moreover, the effects of different concentrations of flavonoids fraction from Tribulus terrestris L. leaves on inhibition ratio (%) were the same with the writhing times. (Tian et al., 2019).

Methanolic extract from fruit

In vivo

In the study the analgesic effect of methanolic (80%) extract from fruits of Tribulus terrestris on male albino mice was evaluated by formalin and tail flick test. Extraction of the fruits of the plant was done by two different methods (soxhlation and percolation) with methanol 80%. The percolated extract was injected intraperitoneally in mice at 50, 100, 200, 400, and 800 mg/kg. The results showed that a dose of 100 mg/kg of percolated extract had the highest significant analgesic effect compared to the control group ($P < 0.01$) in formalin and tail flick test. The analgesic effect of the extract was lower than morphine, 2.5 mg/kg in both tests, and higher than acetylsalicylic acid (ASA) 300 mg/kg in chronic phase of pain in formalin test ($P < 0.05$). Pre-treatment of animal with naloxone did not change the analgesia induced by the plant extract in both tests, therefore the involvement of opioid receptor in the analgesic effect of this plant was excluded. The results of ulcerogenic studies indicate that the gastric ulcerogeneicity of plant extract is lower than the indomethacin in the rat's stomach. It can therefore be concluded that T. terrestris extract has a suitable analgesic effect and further studies are required to produce a more effective product of this plant to substitute for conventional analgesic drugs. (Heidari et al., 2007).

Effect on cognitive functions

Aqueous extract from fruits

In vivo

The study was designed to evaluate the effect of aqueous extract from fruits of Tribulus terrestris on learning and memory in rodents. Thirty Wistar rats were divided in 5 groups of 6 rats each. Baseline values for the time taken to reach reward chamber (TRC) in the Hebb William Maze and transfer latency (TL) in the T-maze were recorded on Day 1. Mean of 5 sessions was calculated for each rat. Group I was normal control, group II piracetam standard, group III, IV and V received Tribulus terrestris orally at 100mg/kg, 200mg/kg and 400mg/kg respectively for 14 days. At the end of 14 days, each rat was tested for TRC and TL and compared with the control group. Results: Group IV showed a significant decrease in TRC when compared to group I in Hebb William Maze ($p<0.0001$). Group IV also showed a significant decrease in TL when compared to group I in T-maze ($p<0.0001$). Group III showed a significant decrease in TL when compared to group I in the T-maze ($p=0.035$), however there was no decrease in TRC in this group. Conclusions: The aqueous extract of fruits of Tribulus terrestris showed a dose dependent beneficial effect in learning and memory models in rats, with 200mg/kg being most beneficial. (Prabhu et al., 2014).

Assessor's comment:

Extensive literature data are available on secondary pharmacodynamic effects of Tribulus terrestris (e.g. extracts from aerial part of the plant or from fruits, or its constituents such as saponins, terrestrosin D, flavonoid and alkaloid fractions) tested in vitro or in rodents (mice, rats). However, in majority of publications, details on extracts preparation or specifics of the compounds tested are not available. Moreover, most of studies were conducted only at one dose level, didn't demonstrate statistical significance in comparison to control groups or were designed to follow only limited number of parameters not allowing firm conclusions on mechanism of action correlating to dose/concentration or constituents tested.

3.1.3. Safety pharmacology

Hepatorenal toxicity

Tribulus terrestris herb

In the study by Aslani *et al.* (2003) seven, 1–2-year-old native goats were fed dried *Tribulus terrestris* from Sabzevar district of Khorasan province for 8 weeks. Two goats showed clinical signs of toxicity including weight loss, depression, ruminal stasis, icterus and elevation of body temperature. Haematological and biochemical trails revealed a declining of packed cell volume (PCV) and plasma total protein and elevation of total and direct bilirubin, blood urea nitrogen (BUN), creatinin and potassium concentrations and serum aspartate amino transferase (AST) activity. At necropsy, the affected goats showed gross pathological changes and marked microscopic lesions in liver and kidneys including generalized icterus, hepatocellular degeneration and necrosis, biliary fibrosis and proliferation, renal tubular necrosis and crystalloid materials in bile ducts and renal tubules. Focal degeneration and necrosis of ventricular muscle of the heart were observed in one goat.

Tribulus terrestris seeds and fruits

Tribulus terrestris (seeds and fruits) was orally administered via gavage in dose 5 mg, once day for 60 days to rats. After 60 days the animals were sacrified and the livers were subjected to histopatological analysis. The histopatological analysis revealed periportal, midzonal and pericentrilobular regions with parenchymal cells with varying degrees of vacuolation. Many hepatocytes were ballooned. The centrilobular vein was dilated and congested. Dilated sinusoids containing erythrocytes were observed. Focal inflammatory lesions occurred in treated animals parenchyma and portal area. The portal area showed fibrosis, biliar duct proliferation with small cells. Histometric study showed smaller cytoplasm and cell volumes, and higher values for numer of hepatocytes per mm^3. The hepatocytes and cholangiocytes nuclei were less voluminous. (Paula-Lopez *et al.*, 2006).

Terrestrosin D

Both the in vitro and in vivo studies showed that the spirostanol saponin terrestrosin D (TED) had potential hepatorenal toxicity. Nonetheless, hepatorenal toxicity induced by oral treatment with TED at a dosage range of 5 – 15 mg/kg daily for 28 consecutive days to Sprague-Dawley (SD) rats was reversible after 14 days of TED withdrawal. (Sun *et al.*, 2021).

Nefrotoxicity

Hydroalcoholic extract from *Tribulus terrestris*

The study investigated the potential effects of the hydroalcoholic extract of *Tribulus terrestris* (details on plant part, DER and concenteation of ethanol are not specified containing 45.84 % w/w of saponins) on the renal complications in streptozotocin (STZ)-induced diabetic rats. Diabetes was induced by administering STZ (90 mg/kg) to the 2-days old neonates. After 6 weeks of induction, diabetic rats were treated with 50 mg/kg hydroalcoholic extract of *T. terrestris* for 8 weeks. The anti-hyperglycaemic nature was confirmed by reduction in blood glucose and improvement in insulin levels. Diabetic renal injury associated with decrease in total proteins and albumin levels was observed to be improved by *T. terrestris* extract. Glomerular filtration rate along with inflammatory and growth factors, adiponectin and erythropoietin were also improved by the treatment, though the findings were not significant. However, the beneficial antidiabetic effects of *T. terrestris* extract in plasma were not observed in kidney histopathology. This was confirmed by the quantitative estimation of unhydrolyzed fraction of saponins (major component: protodioscin) in plasma and kidney samples of normal and diabetic rats. Hence, it can be concluded that 8 weeks treatment with *T. terrestris* extract produces potential toxic effects in kidney, which are independent of its anti-diabetic action. (Gandhi *et al.*, 2013).

3.1.4. Pharmacodynamic interactions

Tribulus terrestris herb

No data available

Gross saponins extracted from *Tribulus terrestris* herb

In the study by Zhang et al. (2019) synergistic effect with sildenafil in the improvement of erectile function has been reported.

Saponins, due to their amphiphilic molecule, have membrane permeabilizing properties, thus, they could increase the absorption of other compounds. (Ștefănescu *et al.*, 2020).

3.1.5. Conclusions

The data on primary and secondary pharmacodynamic exclusively for *Tribulus terrestris* L., herb and methanolic extract thereof are very limited. In most of the studies information on plant part used, extraction solvent and DER is missing. Some of the data were obtained with extract from fruits. Nevertheless, these data are included in the Assessment report for information, as the fruits form an integral part of the aerial part of the plant.

Safety pharmacology studies as defined by ICH S7 guidelines are not available. In some of published studies adverse effects on major organ system such as CNS (in sheep) or organs such as liver and kidney (sheep, goats, rats) have been reported after chronic consumption of large amount of the herb. Neither metabolic nor pharmacokinetic/toxicokinetic data are available for ovine studies and thus similarities or species specific toxicities in comparison to human are unknown.

Saponins have membrane permeabilizing properties, thus, they could increase the absorption of other compounds. However, no tests on humans or animals were performed to confirm this increased absorption.

3.2. Overview of available pharmacokinetic data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof

No data available for *Tribulus terrestris* L., herba

Crude extract of the steroidal saponins from *Tribulus terrestris*

Ovine metabolism of *Tribulus terrestris* saponins during geeldikkop experimentally induced by dosing seep orally with a crude extract of the steroidal saponins from *Tribulus terrestris*. GC-MS analysis of the sheep's ruminal contents, bile, faeces and urine for free and conjugated sapogenins, revealed the general features of the metabolic pathway by which diosgenin and yamogenin glycosides were converted into the glucuronides of epismilagenin and episarsasapogenin. (Miles *et al.*, 1994).

Protodioscin

Protodioscin (PG, isolated from the rhizomes of *Dioscorea zingiberensis* C.H.) was tested in a pharmacokinetic study on rats at the different doses of 50, 100, and 200 mg kg^{-1}, the blood sample was obtained at different time points of 0 h (prior to administration), 1 h, 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, 24 h, 36 h, and 48 h after PG administration. The primary pharmacokinetic parameters were: the peak plasma concentration (C_{max}); the time to peak concentration (T_{max}); the elimination half-life ($t_{1/2}$); the terminal elimination rate constant (k_e); mean residence time (MRT); clearance (CL); the area under the plasma concentration-time curve from time 0 to the time of the last measurable concentration (AU_{C0-t}); and from time 0 to infinity ($AU_{C0-\infty}$). PG could reach its C_{max} of 16.14, 29.74, and 50.62 ng mL-1 in a relatively long T_{max} of 10.43 ± 2.15, 10.87 ± 1.43, and 11.25 ± 3.15 corresponding to three determined concentrations, respectively. These results indicated that PG was slowly absorbed into the circulatory blood system with low oral absolute bioavailability in vivo. Other

pharmacokinetic parameters like $t_{1/2}$ (over 11.47 ± 2.13), k_e (within 0.07 ± 0.01), MRT (within 19.23 ± 1.45), and CL (within 0.21 ± 0.02) were consisted with poor elimination and excretion characteristics of PG. Considering C_{max}, AUC_{0-t} and $AU_{C0-\infty,}$ they increased and showed a perfect proportion within the dose range, demonstrating that these parameters of PG displayed a dose-dependent relationship and might exhibit linear pharmacokinetics. According to the authors more detailed information should be further carried out to investigate the different mechanisms such as limited poor bioavailability and some other phenomena. (Zhang *et al.*, 2015).

Terrestrosin D

The toxicokinetic study demonstrated that the systematic exposure of SD rats to terrestrosin D (TED) had an accumulation phenomenon and a dose-dependent trend after a 28-day repeated-dose oral administration. The tissue distribution study revealed that TED had a targeted distribution in the liver and kidneys accompanied by a phenomenon of accumulation in SD rats. Network pharmacology combined with molecular docking methods was used to screen for the key targets (HSP90AA1, CNR1, and DRD2) and the key pathways of TED-induced hepatorenal toxicity. It has been conluded that Terrestrosin D had potential hepatorenal toxicty. (Sun *et al.*, 2021).

Assessor's comment:

Pharmacokinetic studies for Tribulus terrestris, herba and extracts thereof are not available. There are limited pharmacokinetic studies data for saponins, including protodioscin. In sheep the saponin glycosides are converted to the glucuronides. Protodioscin was slowly absorbed into the circulatory blood system with low oral absolute bioavailability in vivo.

3.3. Overview of available toxicological data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof

3.3.1. Single dose toxicity

Dry methanolic extract from aerial parts of the plant

According to Tanev and Zarkova, 1981 (in Adaikan *et al.*, 2001) *Tribulus terrestris* is considered practically safe in high doses (lethal dose in 50% of the population [LD50] was 1942 mg/kg body mass with intraperitoneal application to mice and over 10 000 mg/kg body mass with oral application) and no untoward effects are reported with its use both in animal studies and clinical trials.

Ten mice were used and randomly assigned to control or treatment group (5 animals per group). Animals were deprived of food but given water 16 h prior to dosing. Methanol extract at a dose of 2 g/kg were then given orally to test group, while the control group received water at the same volume. Body weight, signs of toxicity (general behaviour, motor activities, aggressiveness, reaction to noise, reaction to pinch, state of tail and state of excrement) and mortality were observed after administration at the third hour on the first day, and throughout the following 48 h and then daily thereafter for 14 days. There were no toxic symptoms or mortality observed in any animals, which lived up to 14 days, following the administration of the methanol extract of the aerial parts of *T. terrestris* at a single dose level of 2 g/kg body weight. Moreover, the observed behavioural changes and toxicological signs showed no obvious differences between the treated and control animals. The behavioural patterns of animals as well as breathing were also normal with no disturbance in food intake, water consumption or sleep. (El-Shaibany *et al.*, 2015).

Saponins mixture from aerial parts of the plant

Median lethal dose (LD_{50}) of saponin mixture on Swiss albino mice was calculated according to Litchfield·Wilcoxon method via ip route. LD_{50} and its 95% confidence limits were 813 and 739·894 mg.kg·' respectively. (Arcasoy *et al.*, 1998).

Hydroalcoholic extract of Tribulus terrestris *fruits*

The present study investigated the toxicological effects extract of Tribulus terrestris in Albino mice. The herbal extract was prepared by maceration of the fruits of plant. The extract was administered by oral route as per study design (OECD guidelines) with single dose of 2000 or 5000 mg/kg. The animals were observed for 24 hours, and no toxic effects were observed. None of the animals revealed any signs or features of toxicity. There was zero percent mortality in the group of animals. It can be concluded that maximum non-lethal dose is beyond 5000 mg/kg in the albino mice model. No serious detrimental effects were observed during the acute toxicity study of the medicinal plant even at very high dosage of 5000 mg/kg. Hence the LD_{50} (Lethal dose 50) is above 5000 mg/kg and cannot be precisely calculated. (Vikram and Kaushal, 2015).

Aqueous extract of Tribulus terrestris *fruits*

In the safety study by Kaushik et al. the preclinical toxicity was evaluated following oral administration of aqueous extract from *T. terrestris*, fruits to a group of five male and five female rats. Acute oral toxicity study established the Median Lethal Dose (LD_{50}) to be greater than 2000 mg/kg body weight (b.wt.) No observed adverse effect level (NOAEL) by repeated oral toxicity for 28 days at 750 mg/kg b.wt. was noted. (Kaushik *et al*., 2019).

Saponin fraction from fruits of *Tribulus terrestris*

Acute oral toxicity was conducted with SFTT extract in Wister albino rats according to OECD. The saponin fraction of *Tribulus terrestris* (SFTT) was suspended in 2% (V/V) carboxymethyl cellulose (CMC) solution. Healthy rats of either sex were divided in groups of 6 (3 male + 3 female). The extract was administered by gavages at doses of 0.05, 0.1, 0.25. 0.5, 1.0 and 2.0 g/kg body weightin single doses to both, male and female rats. The animals were observed for general behavioral changes, signs of toxicity and mortality continuosly for 1h after treatment, then intermittently for 4h, and there after over a period of 24 h. The rats were further observed for up to 14 days following treatment for behavioral changes and signs of toxicity/death and the latency of death. Mortality rate was recorded after 24 h. The animals survived throughout the experimantal period and did not show any sign of toxic symptoms or any kind of behavioral changes immediately after dosing and during the period of 14 days. From this study it is revealed that the LD_{50} dose of SFTT is above 2.0 g/kg body weight indicating the low oral toxic nature of the extract. (Hemalatha and Hari, 2014).

3.3.2. Repeat dose toxicity

There are no studies on repeat dose toxicity available for preparations from *T. terrestris* L., herba.

Aqueous extract from fruits

Aqueous extract of *T. terrestris* was administered orally to 48 rats (24 female + 24 male) daily at the dose of 75, 225 and 750 mg/kg for the period of 28 days. Animals were examined daily for signs of toxicity and mortality. Further, rats were subjected to detailed clinical examination as per the OECD guidelines, in which any changes in fur, eyes, mucous membranes, occurrence of unusual secretions and excretions and alterations in autonomic activity such as lacrimation, piloerection, pupil size, respiration or gait were noted. This exercise was done both before initiation of the treatment and weekly thereafter during the treatment period. Body weights of all animals were recorded before initiation of treatment (day 0), once in a week (on days 7, 14, 21 and 28) and at necropsy (day 29). Food consumption was also recorded weekly. Blood and plasma samples of all animals were subjected to clinical haematology and clinical chemistry evaluations at the end of the treatment. Analysis of urine

was performed in the last week of treatment. At the end of treatment, all animals were sacrificed for detailed necropsy. Organ weights (kidney, liver, heart, adrenals spleen, testis, uterus, and brain) were also recorded for all animals and histopathological evaluation performed for each group. The authors concluded at the end of the treatment period (28 days), oral administration of aqueous extract of *T. terrestris* up to the dose of 750 mg/kg b.wt. did not induce any alterations in body weight, food consumption or haematological parameters. However, slight, non-significant lowering of platelet count at the dose of 225 mg/kg b.wt., mean corpuscular and haemoglobin concentration in male rats at the dose of 225 mg/kg b.wt. and 750 mg/kg b.wt. were incidental because they were comparable to control. Similarly, clinical chemistry parameters, few instances differing significantly ($p < 0.05$) from the vehicle control group values occurred during the study. These included increased alanine aminotransferase (ALT), triglycerides and phosphorus in the female rats at a dose of 225 mg/kg b.wt. and lowering of ALT in male rats treated at 750 mg/kg b.wt. and Aspartate aminotransferase in male rats treated at 75 mg/kg and 750 mg/kg b.wt, but these alterations were considered to be incidental in nature or non-adverse, i.e no toxicological significance, and the values were within the historical control range of Wistar rats in the test facility. Urinalysis parameters for all dose group animals were found comparable to control at the end of the treatment period. Further, absolute, and relative (% body weight) values of liver, kidneys, lungs, adrenals, testes, uterus, brain, spleen and heart did not depict any alterations up-to the dose of 750 mg/kg b.wt. The necropsy examinations did not reveal incidence of any remarkable pathological alterations. Microscopic evaluation of all tissues/organs categorically proved that no incidence of histopathological alterations was seen. According to the authors the NOAEL was found to be 750 mg/kg b.w. (Kaushik *et al*., 2019).

Saponin fraction from fruits of *Tribulus terrestris*

The sub-acute oral toxicity study was conducted with Saponin rich butanol fraction of *Tribulus terrestris* fruits (SFTT) extract in Wister albino rats according to OECD guideline. Twenty-four animals were randomly divided into four groups of six animals each. Group I served as control and received a suspension of 2 ml/kg CMC p.o. (2% V/V of CMC). Group II- Animals received 100 mg/ kg body weight of SFTT in 2 ml/kg of 2% CMC p.o. daily for 28 days. Group III: Animals received 200 mg/kg body weight of SFTT in 2 ml/kg of 2% CMC p.o. daily for 28 days. Group -IV: Animals received 400mg/kg body weight of SFTT in 2 ml/kg of 2% CMC p.o. daily for 28 days. Food and water intake was recorded daily; body weight was recorded once in a week throughout the study period. At the end of experimental period, the animals were euthanized, blood samples collected and used for biochemical estimations.

No significant differences in body weighs were recorded in the 28-day treatment period. Further there was no alteration in food and water intake in all the treated groups as compared to control. The animals that survive cannot lose more than 10 % of the initial body weight. In sub-acute toxicity study rats treated with 100, 200 and 400 mg/kg doses of SFTT extract had a progressive gain in the body weight during the study period of 28 days and this increase in weight was not significantly different from that of the control. This gradual weight increase may be due to the improvement in the nutritional state of the animals due to the increased food and water intake. In the investigation SFTT did not have any significant effect on the haematological parameters measured, suggesting the nontoxic nature of the drug. The saponin rich butanol fraction of *Tribulus terrestris* (SFTT) extract treatment for 28 days did not cause any disturbance in hepatic functions as compared to control rats. In the entire drug treated animals the biochemical parameters and the liver marker enzymes were within normal biological and laboratory limits. However, the protein level is found to be elevated in animals' group which received 100mg/kg and 200 mg/kg dose, and the enzyme aspartate amino transferase levels is slightly decreased in animals' group that received 400mg/kg dose. There were no significant changes in the serum levels of AST, ALT and ALP and bilirubin demonstrating that liver function was preserved in animals exposed to SFTT extract over the 28 days period. Kidney function

was evaluated by means of serum urea, creatinine and blood electrolyte concentrations. Repeated oral administration of SFTT extract for 90 days did not cause significant changes in renal function. From biochemical parameters it is evident that the kidney function was well maintained as there was no increase in the values of urea creatinine and electrolytes levels in serum when compared to the normal rats. The level of total and LDL Cholesterol was found to be decreased in the group III and Group IV rats which received the drug at the dose level of 200mg/kg and 400mg/kg body weight. However, no significant changes were observed in the HDL and VLDL fractions of the cholesterol as comparable to that of the control rats. However, significant decrease in the triglycerides level is observed in group IV animals when compared to the control, group II and group III animals. The detailed histopathological examination of the liver and Kidney did not reveal any observable cellular damage. Liver and kidney cells appear normal architecture on comparison with the control group. The authors concluded that oral administration of SFTT extract to rats at doses of 100, 200 and 400 mg/kg/day for 28 consecutive days caused no significant toxicological effects. (Hemalatha and Hari, 2014).

3.3.3. Genotoxicity

There are no studies on genotoxicity available for preparations from *T. terrestris* herba.

The only genotoxicity study available is the study with the extracts from the whole plant which is included for information only:

Water, methanol and chloroform extracts from the whole plant

In vitro

The whole *T. terrestris* plant was extracted with water, methanol, and chloroform. The genotoxic potential of *T. terrestris* extracts was assessed by Comet assay in a rat kidney cell line (NRK-52E) and by Ames assay in *Salmonella typhimurium TA98 and TA100* strains.

The methanol and chloroform extracts of *T. terrestris* showed no evidence of mutagenic activity against *S. typhimurium TA98 and TA100* at concentrations of 30, 60, 300, 600, 1200, and 2400 mg/ml in the Ames assay. However, the water extracts of *T. terrestris* at the concentrations studied were mutagenic to the TA100 strain when metabolically activated (≥2.11-fold) and induced frame shift mutations. The results obtained from the Comet assay showed that DNA damage was not induced in NRK-52E cells exposed to extracts of *T. terrestris* for 24 h. Significant differences were not found in tail intensity in NRK-52E cells treated with the plant extracts at 3, 6, 30, 60, 120, and 240 mg/ml compared with the control group ($p>0.05$). Mean tail intensity was 1.76–11.43 in cells exposed to the highest concentration of *T. terrestris* extracts, which was approximately 5.51-fold and less than the positive control (25 µM H_2O_2) (tDNA%: 62.24).

The authors concluded that *T. terrestris* extracts did not exhibit genotoxic effects on DNA on NRK-52E cells while in the Ames test the water extract might induce frame shift mutations when metabolically activated. (Abudayyak *et al*., 2014).

3.3.4. Carcinogenicity

No data available.

3.3.5. Reproductive and developmental toxicity

No data available

3.3.6. Local tolerance

No data available

3.3.7. Other special studies

Hepatogenous photosensitisation (Geeldikkop)

Tribulus terrestris herb

Four farms in the eastern wheat belt of western Australia recorded caltrop (*Tribulus terrestris*) poisoning in March 1986; caltrop had been grazed on 3 of the farms; 2 weeks after the last episode, spores of *Pithomyces chartarum* were not found in caltrop and soil from 2 of the farms. The daily maximum temperature and relative humidity on one farm varied from 21°C to 39°C and 21% to 83%, respectively. Morbidity was 5-37% and mortality 30-100%. Symptoms included anorexia, photophobia, dehydration, icterus, and serous exudation around eyes, ears, nares and tails. Kidneys were swollen and green, and livers orange. Hepatocytes and Kupffer cells contained acicular clefts. Caltrop poisoning may become more prevalent in western Australia. (Jacob and Peet, 1987, Australian Veterinary Journal 1987, Vol. 64 No 9, pp.288-289 abstract in Invasive Species Compendium).

Two outbreaks of photosensitivity disease occurred in weaner sheep in south western New South Wales during early autumn 1982. In each instance there was a history of access to the annual herb*, Tribulus terrestris* and both the clinical and pathological findings were consistent with geeldikkop, a major disease in the Republic of South Africa. The prevalence rates of clinical cases were 21 and 37%, while the case fatality rates approached 70%. Clinical signs were dominated by jaundice and photosensitisation. Ochre and khaki discolouration were present in the liver and kidneys, respectively. Histopathologically, the most characteristic lesion was the presence of acicular, cholesterol-like clefts in the lumens of bile ducts and in the cytoplasm of hepatocytes and Kupffer cells. Similar structures were also evident in the lumens of nephrons in association with segmental hyperplasia of the neighbouring tubular epithelium. The possible pathogenesis of the hepatogenous photosensitisation and its resemblance to geeldikkop are discussed in the article. (Glastonbury *et al.*, 1984).

In the review by Chen *et al*. (2019) *Tribulus terrestris* is listed among the plants causing hepatogenous photosensitisation in sheeps. The morbidity reported was 17.1 – 100 % and mortality 5.7 – 24.2 %. The highest number of outbreaks was observed in Australia and Iran, several were reported in Turkey.

Photosensitization was induced in sheep by dosing them with cultures of a *Pithomyces chartarum* isolate obtained from *Tribulus terrestris* plants collected during an outbreak of geeldikkop in the Karoo. Thus for the first time a mechanism whereby *T. terrestris* plants can contribute to the causation of ovine hepatogenous photosensitivity was demonstrated. In the latter experiment geeldikkop was induced in the sheep on *T. terrestris* pastures, while those receiving identical doses on veld with little *T. terrestris* developed facial aczema. Geeldikkop, therefore, can be brought about by the ingestion of *T. terrestris* plants together with toxic cultures of *P. chartarum*. The plant appears not only to act as a vehicle for ingestion of spores, but also to interact with sporidesmin to induce lesions typical of geeldikkop, whereas sporidesmin alone results in facial eczema. Indications are that it can enhance the ability of sporidesmin to cause photosensitivity or, possibly, vice versa. (Kellerman *et al.*, 1980).

An outbreak of hepatogenous photosensitization in a flock of sheep in the Central Valley of California, attributed to ingestion of *Tribulus terrestris*, is reported by McDonough *et al*. The intoxication was attributed to *Tribulus terrestris* as no other plants known to cause hepatogenous photosensitisation were found in the animals environment. Physical examination of the animal revealed bright yellow mucous membranes with swelling, erythema, and crusted exudate of the skin over the face, muzzle, and ears. The ewe was recently shorn and had diffuse reddening of the skin along the dorsum.

Laboratory data confirmed hepatic involvement. Elevated aspartate aminotransferase (AST) (767 IU/liter) and Sorbitol dehydrogenase (168 IU/liter) activities were consistent with hepatocellular disease, and increased total bilirubin (1.2 mg/dl), alkaline phosphatase (380 IU/liter), and -glutamyltransferase (45 1 IU/liter) indicated cholestasis. Mildly elevated serum urea nitrogen (48 mg/dl) and high normal total protein (8.6 g/dl) were probably due to dehydration. A total white blood cell count (20,800/µl) was characterized by a moderate neutrophilic leukocytosis (13,312/µl). Copper concentrations determined from serum and a liver biopsy were within the normal ranges. Histologic changes in the liver consisted of periductal concentric lamellar fibrosis, which was mildly edematous and diffusely infiltrated by small numbers of plasma cells and rare macrophages. Bile ducts frequently were distorted by clear acicular clefts, which contained pale yellow-green birefringent crystals visible in frozen sections. The large bile ducts had similar clefts lined by eosinophilic globules along with foci of epithelial hyperplasia interspersed with necrotic cells that were occasionally sloughed into the lumen, Apoptotic hepatocytes were randomly scattered throughout the parenchyma (Fig. 3). Kupffer cells were prominent with large amounts of cytoplasmic pale yellowgreen pigment. Histologically, skin lesions consisted of marked irregular acanthosis, a thick serocellular crust over the epidermis, and replacement of the superficial dermis by granulation tissue. The walls of blood vessels in the dermis from affected portions of skin were widened by homogenous eosinophilic material. Small aggregates of plasma cells and a few macrophages were present between myocytes of the papillary muscle of the left ventricle. No other histologic changes were found and the pathology findings were interpreted to represent hepatogenous photosensitization based on association of hepatic obstructive disease and characteristic skin lesions. (McDonough *et al.*, 1994).

Consumption of *Tribulus terrestris*, particularly by the grazing of young wilted plants or sometimes hay, causes cholangitis and photosensitization in sheep. This plant has caused enormous loss of sheep in South Africa, where the toxicosis is known as geeldikkop ("yellow bighead," because of icterus and marked edema of ears and face). The disease has been reproduced by oral administration of crude extracts of steroidal sapogenins or their glycosides, saponins, of *T. terrestris*, but which of the various saponins in the plant are responsible for the disease is unknown. The disease has also been reproduced experimentally by co-administration of sporidesmin (a mycotoxin produced by *Pithomyces chartratum*) and *T. terrestris*, but the liver lesions are histologically distinct from those produced by sporidesmin alone (facial eczema). In geeldikkop, the most characteristic gross finding is the presence of a white, semifluid accumulation of fine, crystalline material that can be expressed from the cystic duct and larger intrahepatic ducts. The gallbladder mucosa is also partly covered with a fine, crystalline deposit. The gross lesions of geeldikkop are similar to those of facial eczema in that there is generalized icterus, and the liver is discolored by bile pigment and either slightly swollen or distorted, according to the duration of the disease. In poisoning by sporidesmin alone, however, there is more obvious edema and fibrosis of bile ducts, and bile infarcts in the parenchyma are more common. The acute lesion consists of swelling and feathery vacuolation of hepatocytes, and marked hyperplasia of Kupffer cells that may show similar cytoplasmic changes. In these acute cases, the presence of acicular crystals may be very difficult to detect. With more chronic intoxication, the most consistent histologic abnormality is the presence in bile ducts of various amounts of crystalline material. The crystals are fine, flat, and are deposited in affected ducts, and, less commonly, in hepatocytes themselves and in renal tubules. There may be associated bile ductular proliferation in severe cases, but often the degree of histologic hepatocellular damage is mild compared to the severity of the photosensitization. The cholestasis is not solely the result of mechanical obstruction by the crystals, because photosensitization can occur in such outbreaks in animals whose livers show very little cholangitis and contain very few crystals. The severity of peribiliary fibrosis is more variable and probably depends on the relative contribution of sporidesmin to the intoxication. There is some hepatocellular degeneration and apoptosis, and there is fairly uniform swelling of cytoplasm. Bile pigment accumulates in Kupffer cells and hepatocyte cytoplasm, but not to any marked extent in canaliculi. Focal necrosis of the gallbladder

mucosa is often present. The severity of the hepatic lesion increases with the duration of exposure. The biliary crystals from sheep with geeldikkop have been determined to be composed principally of calcium salts of steroidal sapogenins present in *T. terrestris*. Plant saponins are metabolized in the rumen and liver to episapogenin glucuronides, which in the presence of calcium may precipitate, forming the characteristic biliary crystals. Cholestasis is likely related to reduced bile acid secretion into the lumen of the canaliculi, rather than biliary occlusion by these plant sapogenins. This explains the evidence of cholestasis before crystals can be seen histologically. Other unidentified plant components may also play a role in hepatocellular and biliary injury. It has also been suggested that the toxins responsible may act primarily on the membranes of the bile canaliculi. (Cullen and Stalker, 2015).

Neurotoxicity

Tribulus terrestris herb

Ingestion of large amounts of the weed *Tribulus terrestris* (cathead, caltrops), over many months, can result in an irreversible nervous disorder in sheep. The most characteristic clinical presentation is that of profound hind limb paresis. A unique aspect of this limb paresis is that it is much more prominent on one body side, that is, it is expressed asymmetrically. This makes the animal lean to one side in the hindquarters and consequently, to walk or run diagonally rather than straight ahead. Although predominantly a hind limb paresis, eventually there is involvement of the fore limbs as well. By this stage the animal finds great difficulty in getting up and protracted recumbency is commonly observed. Because the paresis is much greater on one body side, this becomes the side on which the animal will lie down, or lean against fence posts and consequently becomes obviously rubbed and abraided. In an affected sheep the weaker body side remains constant. In half of the affected flock the left side will be affected and in the other half the right side. In other pelvic limb paresis syndromes of sheep an affected individual may sway, or lean, both to the left and to the right, at different times. (Bourke, 1995).

Betacarboline alkaloids

The betacarbolines harmane, norharmane, tetrahydronorharmane, harmine, harmaline and harmol were administered to sheep to assess their effects on upper motor neurone function. Harmane at a dose rate of 54 mglkg induced hypomotility, head tremors, pelvic limb paresis, hypermetria and a wide based stance. A range of similar effects were observed with norharmane at the same dose rate. Tetrahydronorharmane at a dose rate of 54 mglkg induced hypermotility followed by hypomotility, asymmetrical pelvic limb paresis, hypermetria, a wide based stance, and stereotyped eating behaviour. Harmine and harmaline at 6 mg/kg induced mild head and body tremors, and at 18 mglkg induced hypomotility, intense head and body tremors, pelvic limb paresis, crossing over of limbs, neck extension and head swaying. Harmol was not effective at 54 mglkg by either the subcutaneous or intraperitoneal routes, but at an intravenous dose of 27 mglkg it induced hypermotility followed by hypomotility, body tremors, limb paresis, muscle asynergy, a wide based stance and jumping behaviour. Harmane, tetrahydronorharmane, harmaline and harmol were convulsive in some sheep at high dose rates.(Bourke *et al.*, 1990).

Alkaloid fraction obtained from the methanolic extract of fresh, mature, aerial part of *Tribulus terrestris* (harvested in Australia) and containing harmane and norharman (44 mg/kg dry matter). Synthetic harman and norharman were administrated as solutions of their hydrochlorides at concentrations 5 mg of free base per ml. The alkaloids were admlnlstered subcutaneously to sheep at a dose rate of 54 mg/kg. Both compounds caused slmllar nervous effects. The main effect observed was limb paresls, which in some sheep was body side blased. The clinical signs observed in the experimental sheep were consistent wlth those described for naturally occurring cases of Trlbulus terrestrls staggers. It was proposed that harmane and norharmane accumulate in tryptamine-associated neurones of the central

nervous system, during months of *tribulus* ingestion, and gradually interact irreversibly wlth a specific neuronal gene DNA sequence. (Bourke *et al.*, 1992).

3.3.8. Conclusions

There are no toxicity studies available for preparations from *T. terrestris* herba.

Testing preparations from aerial parts (including fruits), fruits or saponin fractions thereof, no serious effects were observed in rodents in single dose or sub-chronic repeat-dose toxicity studies.

Preliminary in-vitro genotoxicity data of preparations from the whole plant suggest positive effects for the aqueous extract in the AMES-test after metabolic activation.

3.4. Overall conclusions on non-clinical data

Results from relevant experimental studies on treatment of reduced libido and impotence in males to support the proposed indications are very limited. The reported pharmacological effects are not considered contradictory to the traditional uses.

Specific data on pharmacokinetics and interactions are not available.

Non-clinical information on the safety of *Tribulus terrestris* herb and extracts thereof is scarce.

Tests on reproductive toxicity and carcinogenicity have not been performed.

As there is no information on reproductive and developmental toxicity, the use during pregnancy and lactation cannot be recommended.

The adequate tests on genotoxicity have not been performed.

4. Clinical Data

Erectile dysfunction

Erectile dysfunction is defined as the persistent inability to attain and maintain an erection sufficient to permit satisfactory sexual performance. The pathophysiology of ED may be organic (vasculogenic, neurogenic, anatomical, hormonal, drug-induced) and/or psychogenic. Treatment options include pharmacotherapy for oral use, topical/intraurethral use and intracavernous administration. Oral phosphodiesterase-5 inhibitors (PDE5i) are recommended as first-line therapy, with a response rate in about 75% of patients (Salonia et al., 2023). Erectile dysfunction may affect psychosocial health and have a significant impact on the quality of life (QoL) of patients and their partner's. Also, the cultural background or context should be considered. Expectations about sexuality and sexual performance result from norms and stereotypes, shared by a given culture.

4.1. Clinical pharmacology

4.1.1. Overview of pharmacodynamic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

Erectile dysfunction, sperm quality

Milanov *et al.* (1985) studied the effect of *Tribulus terrestris* (methanolic extract from the herb, DER 35-45:1, containing not less than 45% of furostanolic saponins calculated as protodioscin) upon the serum concentration of the hypophyseal hormones ACTH, STH. LH. FSH, the adrenal hormones aldosterone and cortisol, and the sex hormones testosterone and oestradiol. The experiments were carried out in 8 men and 8 women, aged between 28 and 45 years. The extract was administered

orally at a single dose of 250 mg for five days, three times daily. The serum samples were taken at 8 and 12 h a.m., before and after treatment. It was established that *Tribulus* extract did not alter the concentrations of the adrenal hormones and ACTH essentially. The hypophyseal-gonadal axis, however, was affected significantly - the concentration of FSH and oestradiol increased mainly in the women, and in the men - LH and testosterone. It is assumed that the mechanism of this action is complex, and it is realized both by a direct influence on the gonadal apparatus and by the tropic hormones.

Adimoelja and Adaikan (1997) discovered a correlation of dehydroepiandrosterone-sulphate (DHEA-S) level with the incident of low sex drive and higher occurrence of impotence in studies with patients diagnosed with diabetes mellitus. To test further the relationship between DHEA-S and erectile dysfunction (ED) they conducted a clinical trial of 30 non-diabetic men with ED, 30 non-diabetic men without ED and 15 diabetic men with ED. The men received 250 mg of *Tribulus terrestris* extract (plant part, DER and extraction solvent is not specified) 3 times daily for 3 months. A significant increase of DHEA-S levels in diabetic and non-diabetic subjects after treatment, and a significant increase in the frequency of successful intercourse by 60 % in both, the diabetic and non-diabetic groups with or without ED was found. The authors concluded that the improvement in the sex drive of these patients was linked to the conversion of protodioscin into DHEA-S. Further research of the direct mechanism of action of DHEA-S is needed.

Neychev and Mitev (2005) tested TT extract (dry extract containing steroidal saponins in concentration 60% of the dry matter, at the dose 200 mg) activity on 21 healthy men divided into 3 groups as well as its impact on erectile function that was evaluated by International index of erectile function -5 (IIEF-5) Questionnaire for those patients. Results showed statistically significant difference in the level of testosterone (Total & Free) & IIEF-5, but no statistically significant difference in the level of LH before & after treatment. Also, it showed statistically significant correlation between testosterone (Total & Free) & IIEF-5, but no statistically significant correlation between the level of LH & IIEF-5 before & after treatment. They monitored the levels of testosterone and luteinizing hormone. They found considerable differences in the levels of the tested hormones and concluded that TT had no steroidal effect.

Khaleghi *et al.* (2017) investigated the effects of the direct addition of *Tribulus terrestris* extract (water extract, DER and plant part used is not specified) on human sperm parameters. Semen specimens from 40 healthy men volunteers were divided into 4 groups: one group received no treatment (control group) while the others were incubated with 20, 40, and 50 mg/mL of *T terrestris* extract (experimental groups). Motility, viability, and DNA fragmentation were assessed in all groups. The incubation of human semen with 40 and 50 mg/ml of *T terrestris* extract significantly enhanced total sperm motility, number of progressive motile spermatozoa, and curvilinear velocity over 60 to 120 minutes' holding time ($P < .05$ or $P < < .01$). Furthermore, viability was significantly enhanced by using *T terrestris* extract ($P < .01$). The authors concluded that in vitro addition of *T terrestris* extract to human sperm could affect male fertility capacity.

4.1.2. Overview of pharmacokinetic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents

No data available.

4.2. Clinical efficacy

4.2.1. Dose response studies

No data available

4.2.2. Clinical studies (case studies and clinical trials)

Assessor´s comment:

Clinical studies performed with extract from fruits and with combination products as well as unpublished studies were not included in this assessment report. Only studies with extracts from aerial parts of the plant and studies without specified plant part were assessed.

Erectile dysfunction, sexual problems, hormonal problems, infertility

For assessment of efficacy in the clinical studies, mainly the International Index of Erectile Function (IIEF), a widely used, multi-dimensional self-report instrument for the evaluation of male sexual function, has been used. It has been recommended as a primary endpoint for clinical trials of erectile dysfunction (ED) and for diagnostic evaluation of ED severity. It includes 15 items that were divided into five domains of sexual function: erectile function (six items), orgasmic function (two items), sexual desire (two items), intercourse satisfaction (three items), and overall satisfaction (two items). A scoring key for each of the sexual function domains was developed and validated. Two of the items (questions 3 and 4) from the erectile function (EF) domain were specifically designed to assess key components of ED, that is, ability to achieve penetration (question 3) and ability to maintain erection (question 4) sufficient for satisfactory sexual performance (Rosen *et al*., 2002). An abridged five-item version of the 15-item International Index of Erectile Function (IIEF) was developed (IIEF-5) to diagnose the presence and severity of erectile dysfunction (ED).

Placebo controlled studies

European Study:

Kamenov *et al.*, 2017

The primary objectives were to compare the efficacy of extract of the plant *Tribulus terrestris* (TT), in comparison with placebo, for the treatment of men with erectile dysfunction (ED) and with or without hypoactive sexual desire disorder (HSDD), as well as to monitor the safety profile of the drug. The secondary objective was to evaluate the level of lipids in blood during treatment. In the study, 180 males were included aged between 18 and 65 years with mild or moderate erectile dysfunction (ED) and with or without hypoactive sexual desire disorder (HSDD): 90 were randomized to *Tribulus terrestris* (TT) and 90 to placebo. Patients with ED and hypertension, diabetes mellitus, and metabolic syndrome were included in the study. In the trial, an herbal medicine intervention of Bulgarian origin was used containing *Tribulus terrestris*, dry methanolic extract (35–45:1) 250 mg adjusted to furostanol saponins not less than 112.5 mg. Each patient received orally 3 × 2 film-coated tablets daily after meals corresponding to 1500 mg of the extract during the 12-week treatment period. At the end of each month, participants' sexual function, including ED, was assessed by International Index of Erectile Function (IIEF) Questionnaire and Global Efficacy Question (GEQ). Several biochemical parameters were also determined. The primary outcome measure was the change in IIEF score after 12 weeks of treatment. Complete randomization (random sorting using maximum allowable % deviation) with an equal number of patients in each sequence was used. Patients, investigational staff, and data collectors were blinded to treatment. All outcome assessors were also blinded to group allocation.

Efficacy: 86 patients in each group completed the study. The IIEF score improved significantly in the TT group compared with the placebo group (P < 0.0001). For intention-to-treat (ITT) there was a statistically significant difference in change from baseline of IIEF scores. The difference between TT and placebo was 2.70 (95% CI 1.40, 4.01) for the ITT population. A statistically significant difference between TT and placebo was found for Intercourse Satisfaction (p = 0.0005), Orgasmic Function (p = 0.0325), Sexual Desire (p = 0.0038), Overall Satisfaction (p = 0.0028) as well as in GEQ responses (p < 0.0001), in favour of TT.

Laboratory findings: Lipid profile - Total cholesterol, LDL, HDL, triglycerides at baseline and at 12weeks were measured. Results for parameters (total cholesterol, LDL, HDL and triglycerides) did not differ between the two treatment arms, and the p-values were greater than the pre-fixed significance level of 5%. Hormones - the results for all parameters (total testosterone, free testosterone, DHEA-S, and SHBG) did not differ between the two treatment arms and p-values were greater than level of significance fixed at 5%.

Blood pressure: The difference between mean values of blood pressure in TT and placebo groups at all visits was less than 5 mmHg (the maximal difference was 2.11 mmHg). The changes were not of clinical significance. Blood pressure remained nearly unchanged over time.

Safety: There were no differences in the incidence of adverse events (AEs) between the two groups and the therapy was well tolerated. There were no drug-related serious AEs.

Significant improvement of the IIEF score in the *Tribulus terrestris* group compared with the placebo group (P < 0.0001) was observed, with a statistically significant difference in change from baseline of IIEF scores for ITT population (2.70 (95% CI 1.40, 4.01)). A statistically significant difference between TT group and placebo was observed also for Intercourse Satisfaction, Orgasmic Function, Sexual Desire, and Overall Satisfaction, in favour of TT group.

No difference in total cholesterol, LDL, HDL and triglycerides has been observed in both groups.

Assessor's comment:

In this prospective, placebo-controlled study, a statistically significant difference for the primary outcome measure (change in IIEF score after 12 weeks of treatment) was found in the TT group compared with the placebo group (p < 0.0001). The difference was 2.70 (95% CI 1.40, 4.01) for the ITT population. A discussion on the clinical relevance, however, is missing. A prior determination of what is to be assessed as response would have been useful. Furthermore, in the sildenafil clinical trial program, for which the IIEF score originally has been developed, two of the items (questions 3 and 4) from the IIEF were specifically designed to assess key components of ED—that is, ability to achieve penetration (question 3) and ability to maintain erection (question 4) sufficient for satisfactory sexual performance. These two items were used as primary endpoints in the clinical studies of sildenafil (Rosen et al., 2002), whereas here the change in mean IIEF score was the primary outcome measure. According to the EAU (European Association of Urology) Sexual and Reproductive Health Guidelines (Salonia et al., 2023), the partner should be involved in consultation of diagnosis and treatment of ED. A partner questionnaire is missing. In addition, quality of live has not been considered. Moreover, the extract used in the study as herbal medicine intervention is adjusted to a high amount of furostanol saponins and is manufactured by a specific extraction technology that is not publicly available. The authors conclude that the study results can therefore not be extrapolated to other Tribulus terrestris preparations. In consideration of all the issues mentioned above, the study is not sufficient as basis for a well-established use monograph.

Non-European Studies:

GamalEl Din *et al*., 2018

A total of 70 randomized patients of 40 to 70 years of age with erectile dysfunction and partial androgen deficiency (total testosterone < 3.5 ng/mL) and lower urinary tract symptoms were included in the study. 35 patients (group A) received 250 mg of *Tribulus terrestris* extract containing not more than 45% of steroidal saponins three times daily (corresponding to 334.5 mg of protodioscin per day, no information on plant part used, extraction solvent and DER) for 3 months and the other 35 patients (group B) received placebo. Patients who suffered from diabetes mellitus, hypertension, hepatic failure, chronic renal failure, or neurogenic illness such as hemiplegia were excluded from the study. Moreover, smoking, alcohol or drugs abuse, or any other drugs that may affect erectile function and patients with Peyronie's disease were also excluded from the study. Initial and post-treatment levels of liver aminotransferases, PSA-t and total testosterone were investigated, as well as the score of the validated Arabic index of erectile function (5-item version of the International Index of Erectile Function) to determine the level of erection of the participants and International Prostatic Symptom Score (IPSS) to determine the presence and the severity of lower urinary tract symptoms (LUTS). The mean of aspartate transaminase was elevated in group A after 3 months of receiving *Tribulus terrestris* (26.5 (before), 27.8 (after), respectively, $p = 0.03$). There were significant elevations in the means of both total testosterone together with the score of the validated Arabic version of the IIEF-5 (2.2, 10.7 (before), 2.7, 16.1 (after), $p < 0.001$, $p < 0.001$, respectively). The mean of the total prostate-specific antigen was elevated in the treatment group (1.4 (before), 1.7 (after), $p = 0.007$, respectively). There was no worsening of the lower urinary tract symptoms in group A as there was no change in the mean score of the international prostate symptom score, which was used to assess these symptoms before and after treatment (mean 14.4 (before), 14.6 (after), $p = 0.67$, respectively). The presented randomized prospective trial demonstrated that *Tribulus terrestris* resulted in significant elevation in the mean total testosterone level as well as the mean score of the validated Arabic version of the IIEF-5 in the participants of group A who received *Tribulus terrestris* three times daily for 3 months compared with placebo group with insignificant changes in the mean total testosterone as well as in the mean score of the validated Arabic version of the IIEF-5.

Assessor´s comment:

In this prospective placebo-controlled study, a dietary supplement containing 250 mg of extract from Tribulus terrestris plant, corresponding to 111.5 mg of protodioscin, was used as herbal intervention. The type of the extract, plant part used, and extraction solvent was not specified. Mainly this fact decreases the validity of the study in the relationship to the needs of the monograph. Another limitation is the relatively small number of participants involved in the study.

Santos *et al*., 2014

Objectives of the study were to evaluate the possible effects of *Tribulus terrestris* herbal medicine in the ED treatment and to quantify its potential impact on serum testosterone levels. The prospective, randomized, double-blind and placebo-controlled study included 30 men selected from 100 patients who presented themselves spontaneously complaining of erectile dysfunction, 40 years of age or more, non-smokers, not undergoing treatment for prostate cancer or erectile dysfunction, no dyslipidaemia, no phosphodiesterase inhibitor use, no hormonal manipulation and, if present hypertension and/or diabetes mellitus that should be controlled. IIEF-5 and serum testosterone were obtained before randomization and after 30 days of study. Patients were randomized into two groups of 15 subjects each. The study group received 800 mg of *Tribulus terrestris* extract (part plant used, extraction solvent, DER and content of saponins were not specified), divided into two doses per day for thirty days and the control group received placebo administered in the same way. The groups were

statistically equivalent in all aspects evaluated. The mean (SD) age was 60 (9.4) and 62.9 (7.9), P = 0.36 for intervention and placebo groups, respectively. Before treatment, the intervention group showed mean IIEF-5 of 13.2 (5-21) and mean total testosterone 417.1 ng/dl (270.7-548.4 ng/dl); the placebo group showed mean IIEF-5 of 11.6 (6-21) and mean total testosterone 442.7 ng/dl (301-609.1 ng/dl). After treatment, the intervention group showed mean IIEF-5 of 15.3 (5-21) and mean total testosterone 409.3 ng/dl (216.9–760.8 ng/dl); the placebo group showed mean IIEF-5 of 13.7 (6-21) and mean total testosterone 466.3 ng/dl (264.3-934.3 ng/dl). The time factor caused statistically significant changes in both groups for IIEF-5 only (p=0.0004), however, there was no difference between the two groups (p=0.7914). In conclusion, at the dose and interval studied, *Tribulus terrestris* was not more effective than placebo on improving symptoms of ED or serum total testosterone.

Assessor's comment:

The extract is not specified in the article. The authors state "400 mg of Tribulus terrestris dry extract" only. Another limitation is the small number of patients (15 per each group) that participated in this study.

Open/uncontrolled studies

European study:

Stanislavov and Nikolova, 2000

A clinical trial with a Bulgarian commercial product containing 250 mg of the dry methanolic extract from *Tribulus terrestris* herb in 100 male patients with immunologic infertility and their wives was performed within the period from January 1993 to December 31, 1998. The main group of patients consisted of males positive for sperm antibodies. The recommended dosage was 1 film coated tablet 3 times daily (i.e. 750 mg of the extract per day for 60 days). Where necessary, the course was repeated or continued until conceiving of the wife. The wives were also given 1 tablet three times daily for 7 days from day 21 of their cycle. Until the 12th month of the treatment, pregnancy was registered in 44% of the female partners of 71 patients completely followed-up. The sperm quality was determined prior and after the treatment. The months after the initiation of treatment versus the data of the presumable ovulation were recorded in case of conceptions. The outcome of each pregnancy was also recorded. Statistically significant improvement in spermatozoa motility in 30% of the cases and improvement in their ability to penetrate in the preovulatory cervical mucus was observed. Stimulation of sexual libido was reported. The authors noted that administration of the *Tribulus terrestris* extract led to reduction in the levels of locally released immunoglobulins. Adverse reactions were observed in 2 patients after a treatment for more than 6 months as gastrointestinal complaints which were eliminated with antacid administration, and pain in the knees which abated spontaneously after the discontinuation of the treatment and the realization of conception.

Assessor´s comment:

This study suggests a positive influence of Tribulus terrestris extract on immunologically conditioned infertility. Because of a missing placebo control group, efficacy cannot be assessed objectively.

Non-European studies:

Roaiah *et al.*, 2015

The study was conducted on 30 male patients aged 40 to 70 years complaining of manifestations of Partial Androgen Deficiency in Aging Males (PADAM). The patients were complaining mainly of erectile

dysfunction and low libido. 750 mg/day of the dry extract from *Tribulus terrestris* (collected during the flowering period, plant part used not specified, extraction solvents ethanol 70%, or methanol or acetonitrile or hexane, DER and saponins content were not specified) divided in 3 doses (i.e. 250 mg/ per dose) as an endogenous testosterone enhancer were administered for 3 months. The evaluation of its effect had been monitored for each patient concerning its effect on serum testosterone (total & free) and luteinizing hormone (LH), as well as its impact on erectile function that was evaluated by IIEF-5 for those patients. Results showed statistically significant difference in the level of testosterone (total & free) and IIEF-5, but no statistically significant difference in the level of LH before and after treatment. In addition, it showed statistically significant correlation between testosterone (total & free) and IIEF-5, but no statistically significant correlation between the level of LH and IIEF-5 before and after treatment.

Assessor´s comment:

In this study, the use of 750 mg of Tribulus terrestris extract daily for 3 months led to statistically significant increase of testosterone (total and free) and IIEF-5. No correlation with (potential) improvement of sexual functions (i.e., libido, erection) is described in the study published and, the study is uncontrolled. Regarding the efficacy in the treatment of decreased libido and impotence in men and related indication, this study is not of clinical significance. Moreover, 3 different extraction solvents are mentioned in the article. It is not clear which extract (extraction solvent) was used in the study or whether all patients received the same extract.

Salgado *et al*., 2016

The aim of the study was to evaluate the effects of *Tribulus terrestris* on semen quality and physiological parameters. 65 men with abnormal semen evaluation were included in this study, in which they were prescribed with oral administration of commercially prepared pills containing 250 mg of *Tribulus terrestris* dried extract per capsule (corresponding to 37.5 mg of steroidal saponins expressed as protodioscin, plant part used is probably aerial part of the plant, extraction solvent and DER were not specified) three times daily for 84 days (12 weeks). Initial semen analysis of patients enrolled in this trial revealed that the predominant alteration observed was oligozoospermia (32%), followed by azoospermia and oligoasthenozoospermia (14%). Of 65 men enrolled in the study, 43 completed the study in its entirety with all physical examinations conducted, and only values from participants fully represented for that parameter were used for analysis of physiological parameters. Blood serum concentrations of dihydrotestosterone (DHT) increased significantly between days 0 and 84 ($p = 0.023$); however, there were no significant differences in dehydroepiandrosterone (DHEA), free testosterone, follicle-stimulating hormone (FSH), luteinising hormone (LH) and prolactin concentrations between days 0 and 84. Complete semen analysis evaluated at the end of treatment showed significant enhancement in sperm concentration, motility, and liquefaction time.

Moreover, all participants were evaluated for weight, body fat index and lean muscle mass. There were no significant differences in weight and in amount of fat tissue or total body mass. However, there was a significant increase in amount of lean tissue and a significant decrease in body fat percentage between day 0 and day 84.

Assessor´s comment:

In this study on 65 men with abnormal semen evaluation, daily dose of 750 mg Tribulus terrestris extract of Brazilian origin for 12 weeks led to increase of dihydrotestosterone levels, and enhancement in sperm concentration, motility, and liquefaction time. These findings support the use of Tribulus terrestris extract as potentially beneficial in case of low DHT levels and impaired sperm quality. The results demonstrated significant decrease of body fat and increase of lean mass. Study

design, low number of study patients and not further specified extract do not allow any conclusions on efficacy.

Conclusion on clinical efficacy in the treatment of decreased libido, hormonal disbalance, infertility and erectile dysfunction in men

Regarding the use of *Tribulus terrestris* extract in the treatment of decreased libido and erectile dysfunction in men, 3 published placebo-controlled studies and 3 published open studies were identified. From the assessment of the published studies, it can be concluded that the evidence of clinical efficacy is insufficient to substantiate the use of *Tribulus terrestris* extract in the treatment of decreased libido and/or erectile dysfunction in men as "well-established use indications". Either information on the extracts used is missing or the extract manufacturing is not publicly available. Other shortcomings are e.g. the small number of patients involved and inconsistencies in the study designs or study results (see above).

Hypoactive sexual disorder in women, sexual functions in women

Hypoactive Sexual Desire Disorder (HSDD) is the most common form of FSD (Female Sexual Dysfunction) and the most common sexual disorder in women in clinical practice. HSDD is defined as a deficiency or absence of sexual fantasies and of the desire for sexual activity with a great impact on interpersonal relationships, which cannot be attributed to other causes such as psychiatric or medical illnesses, or the effect of particular drugs. Both pre- and postmenopausal women may be affected by HSDD. The pathophysiology of HSDD is not known in any detail; the current scientific opinion is that it may be due to a neurobiological imbalance in the inhibitory and excitatory factors which control sexual desire in the brain (Naumann *et al*., 2010). Dopamine, norepinephrine, testosterone, oestrogen, and progesterone are excitatory, whereas serotonin, prolactin, and opioids are inhibitory of sexual desire and response. Pharmacological treatments for HSDD target some of the neurotransmitters and hormones involved in these pathways. Other factors may also play a role, including psychosocial variables (satisfaction with the relationship, self-image, past sexual experiences among others), aging, menopause, comorbid medical conditions, as well as substances and medications (Pachano Pesantez and Clayton, 2021). The great diversity of the instruments used in the study of FSD may reflect the lack of consensus or even the lack of a complete method that allows for a full evaluation of sexual function in all its areas, applicable to all cultures (Postigo *et al.*, 2016).

Non-European studies:

<u>Placebo controlled studies</u>

De Souza *et al*., 2016

The objective of this study was to evaluate the efficacy of *Tribulus terrestris* for the treatment of HSDD in postmenopausal women and evaluate its effect on the serum levels of testosterone. In the study, a Brazilian medicinal product containing *Tribulus terrestris* dry extract from aerial parts of the plant (no further details on extraction solvent, DER or saponins content) was used. A prospective randomized, double-blinded, placebo-controlled study lasting 18 months was performed. A total of 45 healthy sexually active postmenopausal women reporting diminished libido were selected to participate in the study and were randomly assigned to receive either 750 mg/d of *Tribulus terrestris* (n = 25) or placebo (n = 20) for 120 days. All participants answered the Female Sexual Function Index (FSFI) and the Sexual Quotient—female version (QS-F) questionnaires and had their serum levels of prolactin, thyroid-stimulating hormone, total testosterone, and sex hormone-binding globulin measured. A total of 36 participants completed the study (20 in the study group and 16 in the placebo group), because six (3 from each group) of them were excluded due to side effects (nausea) and 3 dropped out due to personal reasons. FSFI questionnaire results demonstrated an improvement in all domains in both

groups (P<0.05) except for lubrication which was improved only in the study group. QS-F results showed a significant improvement in the domains of desire (P<0.01), arousal/lubrication (P=0.02), pain (P=0.02), and anorgasmia (P<0.01) in women who used *T. terrestris*, whereas no improvement was observed in the placebo group (P>0.05). Moreover, free and bioavailable testosterone levels showed a significant increase in the *Tribulus terrestris* group (P<0.05).

Postigo *et al*., 2016

The prospective, randomized, double blind, placebo-controlled clinical trial included 60 postmenopausal women with sexual dysfunction. The women were divided into two groups of 30, placebo group and *Tribulus terrestris* group, and evaluated by using the Sexual Quotient—female version (SQ-F) and Female Intervention Efficacy Index (FIEI) questionnaires. The study participants received *Tribulus terrestris* tablets (containing 250 mg of the dry extract from aerial part of the plant, no further details on extraction solvent, DER or saponins content) 3 times daily or placebo for 90 days. There was no significant difference between the groups in age, age at menopause, civil status, race, and religion. In the evaluation with the SQ-F questionnaire, there were significant differences between the placebo (7.6 ± 3.2) and *Tribulus terrestris* (10.2 ±3.2) groups in the domains of desire and sexual interest ($p < 0.001$), foreplay (3.3 ± 1.5 versus 4.2 ± 1.0) ($p < 0.01$), arousal and harmonious interaction with the partner (5.7 ± 2.1 versus 7.2 ± 2.6) ($p < 0.01$), and comfort in sexual intercourse (6.5 ± 2.4 versus 8.0 ± 1.9) ($p < 0.01$). There was no significant difference between the placebo and *Tribulus terrestris* groups in the domains of orgasm and sexual satisfaction ($p = 0.28$). In the FIEI questionnaire, there was a significant improvement ($p < 0.001$) in the domains of vaginal lubrication during coitus and/or foreplay (20 versus 83.3%), sensation in the genitalia during sexual intercourse or other stimuli (16.7 versus 76.7%), sensation in the genital region (20 versus 70%), sexual intercourse and/or other sexual stimulations (13.3 versus 43.3%), and the ability to reach orgasm (20% versus 73.3%). In relation to adverse effects, a greater incidence in the *Tribulus terrestris* group than in the placebo group was observed; the most frequent adverse effects were diarrhoea (13.3%), nervousness (13.3%), dizziness (10%), and nausea (10%) in the *Tribulus terrestris* group, and nervousness (13.3%), facial flushing (13.3%), dizziness (10%), and nausea (10%) in the placebo group. However, there was no significant difference in relation to the general reference and to each one of the adverse effects, by overlap of the significance index.

Akhtari *et al*., 2014

This study was designed as a randomized double-blind placebo-controlled trial to assess the safety and efficacy of *Tribulus terrestris* in women with HSDD during their fertile years. 67 women with HSDD were randomly assigned to syrup of *Tribulus terrestris* extract (vaporised ethanol extract, plant part used leaves, no further details on concentration of extraction solvent, DER or saponins content) in a form of syrup (containing 3.5 gram of the extract in each 5 ml of the syrup). The participants used 7.5 ml of the *Tribulus terrestris* or placebo syrup twice daily for 4 weeks. Desire, arousal, lubrication, orgasm, satisfaction, and pain were measured at baseline and after 4 weeks after the end of the treatment by using the Female Sexual Function Index (FSFI). Thirty women in placebo group and thirty women in drug group completed the study. At the end of the fourth week, patients in the *Tribulus terrestris* group had experienced significant improvement in their total FSFI ($p < 0.001$), desire ($p < 0.001$), arousal ($p = 0.037$), lubrication ($p < 0.001$), satisfaction ($p < 0.001$) and pain ($p = 0.041$) domains of FSFI. Only one patient (not specified from which group) reported grade 1 abdominal pain.

Assessor's comment:

In the three studies described above, which have been conducted in non-European countries such as Brazil and Iran, information on the extracts used is missing. Furthermore, it is questionable if the results could be transferred to Europe considering the cultural differences in the perception of sexual problems.

Open/uncontrolled study

Vale *et al*., 2021

In a prospective, randomized, open study efficacy of *Tribulus terrestris* in two different dosage regimes for the treatment of sexual dysfunction in pre and postmenopausal women and its effect on the vascular resistance of the clitoral artery using Power Doppler was evaluated. A total of 104 women (52 premenopausal and 52 postmenopausal) were randomly assigned to receive 94 mg, three times/day (TT3) or 280 mg once/day for 90 days (TT1). Evaluation was performed using FSFI and QS-F questionnaires, serum levels of prolactin, TSH, total testosterone and SHBG, and clitoral artery assessment with Power Doppler ultrasound. FSFI results demonstrated an improvement in all domains in both groups ($P < 0.05$) except for the "Satisfaction" in the TT3 premenopausal group. QS-F results showed a significant improvement in the mean total score in women of both reproductive phases, for both groups. Postmenopausal patients improved in all sexual domains, except for "orgasm" in the TT1 group. PI of the clitoral artery showed no difference in both reproductive phases, in both groups. The authors conclude that *T. terrestris* can be a safe alternative for the treatment of sexual dysfunction in pre and postmenopausal women as it is effective in reducing the symptoms with no side effects. Moreover, its use increased total, free and bioavailable testosterone.

Assessor´s comment:

The limitation of this study conducted in Brazil is the low number of the participants. The extract used in the study is not sufficiently described. The study is not placebo controlled.

Conclusion on improvement of sexual satisfaction and functions in adult women

Three placebo-controlled studies and one open study (all non-European) were identified and related to pre-menopausal, menopausal and postmenopausal women with hypoactive sexual disorder or decreased libido and sexual functions (lubrication, sexual satisfaction, pain during the sexual intercourse) and treatment with *T. terrestris* extract medication. The findings from these studies are considered insufficient to substantiate the use of *Tribulus terrestris* L. extract in the treatment of menopausal and post castration syndrome to confirm "well-established use". Information on the extracts used is missing. Other shortcomings are e.g. the small number of patients involved and inconsistencies in the study designs or study results. Furthermore, it is questionable if the study results from non-European countries can be transferred to Europe considering the cultural differences in the perception of sexual problems (see above).

Conclusion on studies on non-sexual use

Published studies on blood glucose level and on body mass effect (Samani *et al*., 2016; Antonio *et al*., 2000; Rogerson *et al*., 2007; Fernández-Lázaro *et al*., 2021) are not presented and are not included in the tabular part. The extracts used in the studies are not sufficiently described and there was only a small number of patients included. Moreover, there is no medicinal product with these indications on the European market.

Studies related to hypolipidemic effect are not publicly available. In the study by Kamenov *et al.* lipid profile (Total cholesterol, LDL, HDL, triglycerides) was measured as a secondary outcome. The results for the parameters did not differ between the two treatment arms, and the p-values were greater than the pre-fixed significance level of 5%. The evidence of clinical efficacy is insufficient to substantiate the use of *Tribulus terrestris* extract in the treatment of hyperlipidemia/dyslipidemia as "well-established use indication".

Table 5: Clinical studies on humans, in sexual dysfunction in men

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
Kamenov *et al*., 2017 Efficacy and safety of *T. terrestris* in male sexual dysfunction	Prospective, randomized, double-blind, placebo-controlled trial	*T. terrestris*, dry methanolic extract (35–45:1) 250 mg/tbl corresponding to 112.5 mg of furostanol saponins expressed as protodioscin 2 tbl 3x daily Oral administration Duration: 12 weeks	180 males (90 TT+90 placebo; 86 patients in each group completed the study) 18-65 years	Mild or moderate erectile dysfunction for at least 6 months and/or secondary hypoactive sexual desire disorder	Change in mean IIEF score after 12 weeks of treatment – TT more effective than placebo secondary outcomes: changes in the scores on the individual IIEF domains, the safety assessment, and the changes in lipid levels, sex hormone levels, and blood pressure – no change	ITT yes CI 95%	Discussion on clinical relevance of the 2.70 difference in IIEF between TT and placebo groups missing; Extract manu-facturing not publicly available
GamalEl Din *et al*., 2018 Treatment of erectile dysfunction and lower urinary tract symptoms	Prospective, randomized placebo-controlled study	*T. terrestris* extract containing not more than 45% of steroidal saponins 250 mg 3x daily (corresponding to 334.5 mg of protodioscin per day, no information on plant part used, extraction solvent and	70 males (35 TT+35 placebo) 40–70 years	Erectile dysfunction and partial androgen deficiency (total testosterone – TT < 3.5 ng/mL) and lower urinary tract symptoms	Significant elevation in the mean total testosterone level and the mean score of the validated Arabic version of the IIEF-5, no worsening of the lower urinary tract symptoms	SPSS, Mann-Whitney test, Wilcoxon test	Small number of patients, unspecified extract, non-EU study

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
		DER) Duration: 3 months Oral administration					
Santos *et al*., 2014 Effect of *T. terrestris* in the erectile dysfunction and its impact on serum testosterone levels	Prospective, randomized, double-blind and placebo-controlled study	*T. terrestris* dry extract (plant part, DER, saponins content, extraction solvent not specified), 400 mg 2x daily Oral administration Duration: 30 days	30 healthy males (15 TT+15 placebo) ≥40 years	Erectile dysfunction	Improvement of symptoms of erectile dysfunction (IIEF-5), total testosterone level- not more effective than placebo	Not specified	Small number of patients, unknown method of statistical analysis, unspecified extract, non-EU study
Roaiah *et al*., 2015 Effect of *T. terrestris* on serum testosterone level and erectile function in aging males with partial androgen deficiency	Open/un-controlled study	*T. terrestris* dry extract (plant part used probably herb collected during the flowering period, extraction solvents ethanol 70%, or methanol or acetonitrile or hexane, DER and saponins content were not specified) 250 mg 3x daily Oral administration	30 males	Partial androgen deficiency – erectile dysfunction and low libido	Statistically significant difference in level of testosterone (total and free) but not statistically significant difference in level of LH before and after treatment Statistically significant correlation between testosterone (total and free) and IIEF-5 but not statistically significant correlation between level of LH and IIEF-5	Spearman's Correlation Significance test	Uncontrolle dstudy, small number of patients, unspecified extract, non-EU study

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
		Duration: 30 days			before and after treatment		
Salgado *et al.*, 2016 Effects of *T. terrestris* on semen quality and physiological parameters	Open/uncontrolled study	*Tribulus terrestris* dried extract from aerial parts of the plant (extraction solvent and DER not specified) 250 mg corresponding to 37.5 mg of steroidal saponins expressed as protodioscin 3x daily Oral administration Duration: 12 weeks	65 males (43 completed the study)	Men with abnormal semen evaluation (oligozoospermia, azoospermia and oligoasthenozoospermia	Blood serum concentrations of dihydrotestosterone (DHT) increased significantly; however, there were no significant differences in dehydroepiandrostenedione (DHEA), free testosterone, follicle-stimulating hormone (FSH), luteinising hormone (LH) and prolactin concentrations. Complete semen analysis evaluated at the end of treatment showed significant enhancement in sperm concentration, motility, and liquefaction time.	SPSS Statistics for Windows Shapiro–Wilk test Friedman test Wilcoxon–Mann–Whitney test	Uncontrolled study, small number of patients, extract not sufficiently specified, non-EU study
Stanislavov and Nikolova, 2000 *T. terrestris*	Open study	*T. terrestris*, dry methanolic extract (35–45:1) 250 mg/tbl corresponding to 112.5 mg of	100 males and 100 females	Males with sperm agglutinating antibodies and females with abnormal and normal post-coital	Statistically significant improvement in spermatozoa motility in 30% of the cases and improvement in their	Not specified	Uncontrolled study, small number of patients,

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
and human male fertility – immunological aspects		furostanol saponins expressed as protodioscin Males: 1 tbl 3x daily Wives: 1 tbl 3x daily for 7 days initiating from 21st day of cycle Oral administration Duration: 60 days (1 treatment course, which can be repeated till conceiving of the wife)		test	ability to penetrate in the preovulatory cervical mucus was observed. Stimulation of sexual libido was reported.		unknown method of statistical analysis

Table 6: Clinical studies on humans, in sexual dysfunction in women

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
De Souza *et al*., 2016 *Efficacy of T. terrestris* in the treatment of hypoactive sexual desire disorder in postmenopaus	randomized, double-blinded, placebo-controlled trial	*T. terrestris* extract from aerial parts of the plant (no further d details on extraction solvent, DER or saponins content) 250 mg 3x daily Oral administration Duration: 120 days	45 healthy postmeno-pausal women 36 participants completed the study (25 TT + 20 placebo)	healthy sexually active postmenopausal women between 1 and 10 years since their last menstrual period reporting diminished libido FSH >30 IU/L, oestradiol <40 pg/mL	In the FSFI questionnaire an improvement in all domains in both groups except for lubrication which was improved only in the study group. QS-F questionnaire significant improvement in the domains of desire,	Mann-Whitney U test Wilcoxon test ANOVA test Kolmogorov-Smirnov and Levene tests McNemar	Low number of participants, the extract used not sufficiently described, non-EU study

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
al women					arousal/lubrication, pain, and anorgasmia in the study group, no improvement in the placebo group. Significant increase of free and bioavailable testosterone levels in the study group	test	
Postigo *et al*., 2015 Assessment on the effects of *T. terrestris* in postmeno-pausal women	Prospective, randomized, double-blind, placebo-controlled study	*T. terrestris* dry extract from aerial parts of the plant (no further details on extraction solvent, DER or saponins content) 250 mg 3x daily Oral administration Duration: 90 days	60 females (30 TT + 30 placebo) No significant difference between the groups in age, age at menopause	Postmenopausal women with full at least 1 year of amenorrhea and FSH > 30 mUI/mL; sexually active; had a stable partner and no former sexual difficulty and experienced sexual dysfunction after menopause.	In the SQ-F questionnaire, significant differences between placebo and *Tribulus* groups in the domains of desire and sexual interest, foreplay, arousal and harmonious interaction with the partner, and comfort in sexual intercourse, no significant difference between placebo and *Tribulus* groups in the domains of orgasm and sexual satisfaction. In the FIEI questionnaire significant improvement in the domains of vaginal	Student's t-test chi-square test with Yates correction, the chi-square test, Mann-Whitney U test	Low number of participants, the extract used not sufficiently described, non-EU study

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
					lubrication during coitus and/or foreplay, sensation in the genitalia during sexual intercourse or other stimuli, sensation in the genital region, sexual intercourse and/or other sexual stimulations, and the ability to reach orgasm.		
Akhtari *et al*., 2014 *T. terrestris* for treatment of sexual dysfunction in women	Randomized double-blind, placebo-controlled study	*Tribulus terrestris* extract (vaporised ethanol extract, plant part used leaves, no further details on concentration of extraction solvent, DER or saponins content) 5.25 g twice daily Oral administration Duration: 4 weeks	60 women (30 TT + 30 placebo) childbearing age	women with hypoactive sexual desire disorder during their fertile years	significant improvement in their total FSFI, desire, arousal, lubrication, satisfaction and pain	ANOVA test Mauchley's Sphericity test	Low number of participants, the extract used was not sufficiently described, non-EU study
Vale *et al*., 2021 Effect of *Tribulus Terrestris* in	Prospective, randomized, open study	*Tribulus terrestris* (no further details on plant part used, extraction solvent, DER and saponin	104 women with FSD 52 premenopausal (26 + 26)	Premenopausal + postmenopausal women with more than 12 months of amenorrhoea and FSH	FSFI results demonstrated an improvement in all domains in both groups ($P < 0.05$) except for	Student´s t-test Fisher´s exact test Kolmogorov-	Low number of participants, the extract used was not

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Outcomes	Statistical analysis	Clinical relevance
the Treatment of Female Sexual Dysfunction and Clitoral Vasculari-sation. Two Different Dosage Regimes		content) 94 mg 3 x daily or 280 mg once daily Oral administration Duration: 90 days	52 postmeno-pausal (26 + 26) 85 (42 premenopausal and 43 postmeno-pausal completed the study Mean age premenopausal 38.9±7.4 years Mean age of postmeno-pausal 56±5.3 years	above 30 IU/l, oestradiol below 40 pg/ml	the "Satisfaction" in the TT3 premenopausal group. QS-F results showed a significant improvement in the mean total score in women of both reproductive phases, for both groups. Postmenopausal patients improved in all sexual domains, except for "orgasm" in the TT1 group. PI of the clitoral artery showed no difference in both reproductive phases, in both groups.	Smirnov test Levene test Mann-Whitney test Wilcoxon test	described, non-EU study

4.3. Clinical studies in special populations (e.g. elderly and children)

No data available.

4.4. Overall conclusions on clinical pharmacology and efficacy

Results of several clinical studies investigating the effects of *Tribulus terrestris* extracts in sexual dysfunction in men have been published. From the assessment of the published studies, it can be concluded that the evidence of clinical efficacy is insufficient to substantiate the use of *Tribulus terrestris* extract in the treatment of decreased libido and/or erectile dysfunction in men as "well-established use indications". Either information on the extracts used is missing or the extract manufacturing is not publicly available. Other shortcomings are e.g. the small number of patients involved and inconsistencies in the study designs or study results (see 4.2.2.).

Three placebo-controlled studies (all non-European) were identified and related to pre-menopausal, menopausal and postmenopausal women with hypoactive sexual disorder or decreased libido and sexual functions (lubrication, sexual satisfaction, pain during the sexual intercourse) and treatment with *T. terrestris* extract medication. The findings from these studies are considered insufficient to substantiate the use of *Tribulus terrestris* L. extract in the treatment of menopausal and post castration syndrome to confirm "well-established use". Information on the extracts used is missing. Other shortcomings are e.g. the small number of patients involved and inconsistencies in the study designs or study results. Furthermore, it is questionable if the study results from non-European countries can be transferred to the Europe considering the cultural differences in the perception of sexual problems (4.2.2.).

Studies related to hypolipidemic effect are not publicly available. There is no evidence of clinical efficacy to substantiate the use of *Tribulus terrestris* extract in the treatment of hyperlipidemia/ dyslipidemia as "well-established use indication".

5. Clinical Safety/Pharmacovigilance

5.1. Overview of toxicological/safety data from clinical trials in humans

Table 7: Clinical safety data from clinical trials – sexual dysfunction in men

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
Kamenov *et al.*, 2017 Efficacy and safety of *T. terrestris* in male sexual dysfunction	Prospective, randomized, double-blind, placebo-controlled trial	*T. terrestris*, dry methanolic extract (35–45:1) 250 mg/tbl corresponding to 112.5 mg of furostanol saponins expressed as protodioscin 2 tbl 3x daily Oral administration Duration: 12 weeks	180 males (90 TT+90 placebo; 86 patients in each group completed the study) 18-65 years	Mild or moderate erectile dysfunction for at least 6 months and/or secondary hypoactive sexual desire disorder	One serious AE (bronchopneumonia) was reported in the placebo arm (judged not related to the study medication). Two non-serious AEs (gastrointestinal disorders) were reported – abdominal pain in the TT arm and gastroesophageal reflux in the placebo arm. The investigational drug had no influence on vital signs and blood pressure values. No clinically significant laboratory changes or trends were observed	Extract manufacturing not publicly available
GamalEl Din *et al*., 2018 Treatment of erectile dysfunction and lower urinary tract	Prospective, randomized placebo-controlled study	*T. terrestris* extract containing not more than 45% of steroidal saponins 250 mg 3x daily (corresponding to 334.5 mg of protodioscin per day,	70 males (35 TT+35 placebo) 40–70 years	Erectile dysfunction and partial androgen deficiency (total testosterone - TT < 3.5 ng/mL) and lower urinary tract symptoms	Minimal but statistically significant elevations in the mean of AST and PSA-t after 3 months of supplementation in TT group	Detailed information on the extract is missing

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
symptoms		no information on plant part used, extraction solvent and DER) Oral administration Duration: 3 months				

Table 8: Clinical safety data from clinical trials – sexual dysfunction in women

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
De Souza *et al*., 2016 *Efficacy of T. terrestris* in the treatment of hypoactive sexual desire disorder in postmenopausal women	randomized, double-blinded, placebo-controlled trial	*T. terrestris* extract from aerial parts of the plant (no further d details on extraction solvent, DER or saponins content) 250 mg 3x daily Oral administration Duration: 120 days	45 healthy postmenopausal women 36 participants completed the study (25 TT + 20 placebo)	Healthy sexually active postmenopausal women between 1 and 10 years since their last menstrual period reporting diminished libido FSH >30 IU/L, oestradiol <40 pg/mL	Nausea; 3 from TT group and 3 from placebo group	Low number of study participants Unspecified extract.
Postigo *et al*., 2015 Assessment on the effects of *T.*	Prospective, randomized, double-blind, placebo-controlled study	*T. terrestris* dry extract from aerial parts of the plant (no further d details on extraction solvent, DER or saponins	60 females (30 TT + 30 placebo) No significant difference between the groups in age, age at	Postmenopausal women, with full autonomy, and at least 1 year of amenorrhea and FSH > 30 mUI/mL; sexually active; had a	TT group: diarrhoea (13.3%), nervousness (13.3%), dizziness (10%), and nausea (10%) Placebo group: nervousness (13.3%),	Low number of patients Extraction solvent not known

Type	Study	Test Product(s)	Number of subjects	Type of subjects	Adverse reactions	Comments
terrestris of postmenopausal women		content) 250 mg 3x daily Oral administration Duration: 90 days	menopause	stable partner and no sexual difficulty; and experienced sexual dysfunction after menopause.	facial flushing (13.3%), dizziness (10%), and nausea (10%)	
Akhtari *et al.*, 2014 *T. terrestris* for treatment of sexual dysfunction in women	Randomized double-blind, placebo-controlled study	*Tribulus terrestris* extract (vaporised ethanol extract, plant part used leaves, no further details on concentration of extraction solvent, DER or saponins content) 5.25 g twice daily Oral administration Duration: 4 weeks	60 women (30 TT + 30 placebo) childbearing age	Women with hypoactive sexual desire disorder during their fertile years	one patient reported grade 1 abdominal cramp	Ethanolic extract from leaves without further information on DER and/or saponins content

5.2. Patient exposure

Aside from market presence and data from studies, there are no concrete data concerning patient exposure.

5.3. Adverse events, serious adverse events and deaths

In the clinical studies, use of *Tribulus terrestris* extract led mostly to gastrointestinal disorders such as nausea with similar incidence as in placebo group (see section 5.2.).

Pharmacovigilance, post-marketing experience

Eudravigilance database. In a period from 2007 to 2021, 13 spontaneous case reports related to mostly unspecified use of various *Tribulus terrestris* products including herbal combination products were obtained from European pharmacovigilance system. None from the reported cases was identified as directly linked to the *Tribulus terrestris* use.

Vigilyze database. 20 cases of undesirable effect have been reported from which 2 cases were fatal. In the 2 fatal cases, Tribulus terrestris was used in combination with other 21, resp. 9 other drugs, therefore no conclusion can be drawn. The other single cases of undesirable effects are swelling, urticaria, constipation, myalgia, hyperhidrosis, malaise, lethargy, liver function test increased, blood creatine phosphokinase increased, insomnia, agitation, hypertension and acne. Causality of these cases was assessed as probable or possible.

Case reports

A 36-year-old Caucasian man was diagnosed with a 72-h-lasting priapism that occurred after the assumption of a herbal supplement based on '*Tribulus terrestris'* (brand name of a non-prescription remedy for erectile dysfunction). He referred to a dose of two tablets per day for 15 days up to the evening of intercourse; furthermore, it was determined that the patient had never been treated with a 5-phosphodiesterase inhibitor before and that he was taking no other medications at the time of admission. The patient underwent a cavernoglandular shunt (Ebbehoj shunt) in order to obtain complete detumescence, from which derived negative post-episode outcomes on sexual function (Campanelli *et al*., 2015).

Assessor´s comment:

The preparation used and the exact dose used are unknown. A possibility of overdose cannot be excluded.

A herbal combination including *Tribulus terrestris* (TT), Avena sativa (AS), and Panax Ginseng (PG), which may be effective in treatment of atherosclerosis and thrombosis, is used by patients with coronary artery disease. In this paper, three cases with coronary stents who were diagnosed with acute coronary syndrome while using this herbal combination of TT, AS and PG together with antiaggregant medications were reported.

A 45-year-old man presented with chest pain and coronary angiography confirmed a total occluded stent in left anterior descending artery which was implanted a year ago. Balloon dilation was performed to dilate the stent, resulting in full opening of the vessel.

The second case, a 53-year-old woman, was admitted to the hospital with chest pain. Coronary angiography confirmed a total occluded stent, which had been implanted three months ago. A balloon was performed to dilate the stent, and it was fully opened.

The third case, a 62-year-old man, presented with chest pain. Coronary angiography was performed and there was a 98% stenosis of the cirkumflex stent, which was implanted three months ago. A balloon was performed to dilate the stent, and it was fully opened.

It was found that all three patients had used the same herbal combination (TT, AS and PG) with antiaggregant therapy for three months ago to presentation in the clinic. Patients were discharged with the suggestion not to use this herbal combination with antiaggregant therapy. There were no problems during the four-month follow-up period. Stent thrombosis may be caused by interactions between herbal combination (TT, AS and PG) and clopidogrel in these patients under dual aggregation (Vatankulu *et al*., 2012 - in Turkish with English abstract).

Assessor´s comment:

This report describes three cases of use of a herbal combination of Tribulus terrestris with Avena sativa and Panax ginseng. The relationship of the TT itself to the described cases of stent thrombosis is thus unclear.

A case of *Tribulus terrestris* toxicity in a young healthy male, presenting with severe hyperbilirubinemia followed by acute renal failure and bile containing casts in the tubules is described. The patient had no prior history of acute or chronic liver disease. He drank alcohol on occasion. He denied using illicit drugs but had recently started to take *Tribulus terrestris* extract tablets (one tablet a day for "a few months") as part of his body-building program.

He occasionally also supplemented his diet with "protein shakes". Two weeks prior to admission he reported use of non-steroidal anti-inflammatory medication (naproxen/Aleve) in a dose of 1,000 – 1,500 mg per day for headaches.

Elevation of liver transaminases was mild, but there was profound hyperbilirubinemia followed by acute renal failure 2 – 3 weeks after the onset of hyperbilirubinemia. Kidney biopsy was performed and showed acute tubular necrosis (ATN) with bile containing tubular casts and bile droplets in the tubular epithelial cells, which is an uncommon finding in renal pathology (Ryan *et al*., 2015).

Assessor´s comment:

The dose and the extract are not specified in this case, as well as the duration of the use of the tablets ("a few months"). The use was combined with naproxen and non-specified dietary supplements. There is thus no direct relationship between use of TT tablets and acute kidney injury and hyperbilirubinemia.

A case of T. terrestris-induced hepatotoxicity, nephrotoxicity and neurotoxicity in an Iranian 28-years old male patient who used the plant's extract (T. terrestris water bought from a grocery) to prevent kidney stone formation. The patient presented with seizure and very high serum aminotransferases and creatinine after consuming 2 litres of the herbal water for 2 days. Discontinuation of the herbal remedy resulted in improvement in symptoms and normalization of his liver enzymes and improving of renal functions (Talasaz *et al*., 2010).

Assessor´s comment:

The extract used in this case including dose of TT and used part of the herb is not specified in the article. A possibility of overdosage cannot be excluded.

A 31-year-old Iranian woman was admitted to hospital due to epigastric pain radiating to back and shoulders, and weakness, malaise, nausea, and icterus. Upon admission, her vital signs were normal. She had been consuming *Tribulus terrestris* as an herbal tea several times a day for 2-3 months, in order to lose weight. Upon physical examination, the patient had generalized icterus and laboratory tests showed elevated transaminases, PT, and INR. Various causes of hepatic failure, such as viral hepatitis and autoimmune hepatitis, were ruled out and the only probable diagnosis was toxin-induced liver failure (Ataee and Dadpour, 2020).

Assessor´s comment:

The extract used in this case including dose of TT and used part of the herb is not specified in the article. A possibility of overdosage cannot be excluded. Moreover, use of other drugs or plants is not specified in the article.

A 21-year-old man was referred by his general practitioner to the breast clinic with a 5-month history of a lump in his left breast, which was occasionally painful. Seven years previously, he reported a similar swelling on the right side that had settled spontaneously. On examination, there was a well-defined nodule in the sub-areolar region on the left side consistent with gynaecomastia. This was causing him considerable discomfort especially while playing sports and he requested surgical removal. In view of the patient's symptoms and wishes, this was excised using a minimally invasive technique (mammotome probe and liposuction). Two weeks later at clinic follow-up, his wound had completely healed. The histology was reported as atypical ductal hyperplasia (ADH). Therefore, he was not discharged but kept under surveillance. When reviewed in the clinic 3 months later, he presented with another nodule again in the left sub-areolar region. On clinical evaluation and ultrasound imaging, this nodule appeared benign. Core-biopsy was reported as normal breast tissue with a possibility of gynaecomastia difficult to exclude. A complete sex hormonal profile was requested. This revealed a markedly decreased follicle-stimulating hormone (FSH), luteinising hormone (LH) and testosterone. FSH 0.59 IU/l (normal: 1.0–7.0), LH 0.26 IU/l (normal: 1.0–8.0), testosterone 1.3 nmol/l (normal: 10–50). Prolactin, oestradiol and progesterone were within normal limits. On closer questioning at this stage, the patient said that he had been taking a non-hormonal preparation derived from a plant called *Tribulus terrestris*, in the form of tablets as a steroid alternative to supplement his weight-training. On the assumption that this substance had caused the hormonal imbalance and hence gynaecomastia, he was advised to discontinue taking them. Two months later his sex-hormones were re-checked, and they had improved, FSH 11 IU/l, LH 6.1 IU/l, testosterone 15 nmol/l. The swelling in his left breast had also completely resolved (Jameel *et al.*, 2003).

Assessor´s comment:

The extract used in this case including dose of TT and used part of the herb is not specified in the article. A possibility of overdosage cannot be excluded.

5.4. Laboratory findings

See section 4.2.2

5.5. Safety in special populations and situations

No data available.

5.5.1. Use in children and adolescents

Not relevant.

5.5.2. Contraindications

Hypersensitivity to the active substance.

5.5.3. Special warnings and precautions for use

No special warning or precaution for use was identified in the literature published.

If the symptoms worsen during the use of the medicinal product, a doctor or a qualified health care practitioner should be consulted.

5.5.4. Drug interactions and other forms of interaction

One interaction case report was identified:

In 2019, a patient diagnosed with chronic myeloid leukaemia (CML), male, 57 years of age, was directed to pharmaceutical care by the medical team suspected of not being able to access treatment effect by non-accomplishment of the major molecular response (MMR) after 19 months of dasatinib treatment. In consultation with the pharmacist, it was found that he had high levels of pharmacotherapy. To this end, the pharmacist has used instruments for assessing compliance - Moriky-Green test and Haynes-Sackette test - to calculate the rate of possession of medicinal product from the pharmaceutical dispensation records. The pharmacist also investigated the patient's use of other medicinal products and identified that the patient was using *Tribulus terrestris* in the form of pellets, half a spoon every day, as a self-medication, to improve his physical disposition. In addition, the patient stored the medicine in a shelf under an electric oven, exposing the product to high temperatures. In view of the above, the possible hypothesis of therapeutic failure has been raised because of loss of quality of the medicinal product due to inadequate storage. The patient was directed to discontinue the use of *Tribulus terrestris* and store the medicine in a dry, cold place. In September 2019, the patient went through a new pharmaceutical consultation when an improvement in the therapeutic response was observed (Silva *et al*., 2021).

Assessor´s comment:

Interaction of dasatinib with Tribulus terrestris powder was not proven as the dasatinib was at the respective time stored improperly and this fact by itself could have led to the dasatinib treatment failure.

No other case of interaction has been reported.

5.5.5. Fertility, pregnancy and lactation

No clinical information related to lactation was identified, but *Tribulus terrestris* is not intended for use in lactation.

Safety during pregnancy and lactation has not been established. In the absence of sufficient data, the use during pregnancy and lactation is not recommended.

No fertility data available.

5.5.6. Overdose

No case of overdose has been reported.

5.5.7. Effects on ability to drive or operate machinery or impairment of mental ability

No studies on the effect on the ability to drive and use machines have been performed.

5.5.8. Safety in other special situations

No data available.

5.6. Overall conclusions on clinical safety

Data from clinical trials with not further specified extracts show either no adverse effect or nausea/gastrointestinal complaints, with similar incidence as in placebo groups.

The case reports published show anecdotal occurrence of mainly hepatic and renal adverse effects, not directly linked to *Tribulus terrestris* use, without closer specification of used part of the herb or extract, and without closer specification of the dose. In cases of priapism and gynaecomastia, an association with the use of not further specified *Tribulus terrestris* preparations cannot be excluded; however, the possibility of overdose cannot be excluded.

Taking into consideration data from the clinical studies and pharmacovigilance databases, no signals have been detected.

From the available data, it is not possible to conclude on clinical safety of *Tribulus terrestris* L., herba and its preparations.

6. Overall conclusions (benefit-risk assessment)

Data on historical (traditional) medicinal use of *Tribulus terrestris* L., herba and its preparations are limited in the searched literature. Historical data are available mostly for *Tribulus terrestris* L., fructus, but information on authorised/registered medicinal products containing *Tribulus terrestris* L. fructus or extracts thereof on the EU market are lacking. Documentation on a safe medicinal use for a period of 30 years in accordance with a specified therapeutic indication, strength and posology could not be found so far from products derived from *Tribulus terrestris* L., fructus.

The only preparation on the EU market derived from *Tribulus terrestris* L., herba is a dry extract (DER 35-45:1), extraction solvent methanol 80% V/V, containing not less than 45% of furostanol saponins calculated as protodioscin, approved in Bulgaria since 1981.

Although a period of 10 years medicinal use of the product has elapsed, data supporting an acceptable level of safety and recognized efficacy are not available as published data.

Although the requirement of 30 years on the market is fulfilled, the extract is adjusted to a high amount of furostanol saponins and is manufactured by a specific extraction technology that is not publicly available. Therefore, the extract is considered specific and not appropriate to support the establishing of a EU herbal monograph based on traditional use. Sufficient information on traditional use of other *Tribulus terrestris* L., herba preparations has not been found in the searched literature.

The HMPC concluded that the following requirements for the establishment of a European Union herbal monograph on traditional and/or well-established herbal medicinal products containing *Tribulus terrestris* L., herba are not fulfilled:

- the requirement laid down in Article 10a of Directive 2001/83/EC that the active substance has a recognised efficacy and an acceptable level of safety and that the period of well-established medicinal use has elapsed

- the requirement laid down in Article 16a(1)(d) of Directive 2001/83/EC that "the period of traditional use as laid down on Article 16c(1)(c) has elapsed".

Based on the above-mentioned information, the HMPC is of the opinion that a European Union herbal monograph on *Tribulus terrestris* L., herba based on traditional use and/or well-established use cannot be established at present.

Annex

List of references