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Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Salvia miltiorrhiza* Bunge, radix et rhizoma

Final

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

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

Herbal substance(s) (binomial scientific name of the plant, including plant part)	<i>Salvia miltiorrhiza</i> Bunge, radix et rhizoma
Herbal preparation(s)	Not applicable
Pharmaceutical form(s)	Not applicable
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Table of contents

Table of contents	2
1. Introduction	4
1.1. Description of the herbal substance(s), herbal preparation(s) or combinations thereof ..	4
1.2. Search and assessment methodology	6
2. Data on medicinal use	7
2.1. Information about products on the market	7
2.1.1. Information about products on the market in the EU/EEA Member States	7
2.1.2. Information on products on the market outside the EU/EEA	7
2.2. Information on documented medicinal use and historical data from literature	9
2.3. Overall conclusions on medicinal use	13
3. Non-Clinical Data	13
3.1. Overview of available pharmacological data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof.....	13
3.1.1. Primary pharmacodynamics	13
3.1.2. Secondary pharmacodynamics	17
3.1.3. Safety pharmacology	17
3.1.4. Pharmacodynamic interactions	18
3.1.5. Conclusions	20
3.2. Overview of available pharmacokinetic data regarding the herbal substance(s), herbal preparation(s) and relevant constituents thereof.....	20
3.3. Overview of available toxicological data regarding the herbal substance(s)/herbal preparation(s) and constituents thereof	22
3.3.1. Single dose toxicity.....	22
3.3.2. Repeat dose toxicity.....	23
3.3.3. Genotoxicity	23
3.3.4. Carcinogenicity.....	23
3.3.5. Reproductive and developmental toxicity	23
3.3.6. Local tolerance	23
3.3.7. Other special studies.....	24
3.3.8. Conclusions	24
3.4. Overall conclusions on non-clinical data	24
4. Clinical Data	24
4.1. Clinical pharmacology	24
4.1.1. Overview of pharmacodynamic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents.....	24
4.1.2. Overview of pharmacokinetic data regarding the herbal substance(s)/preparation(s) including data on relevant constituents.....	24
4.2. Clinical efficacy	25
4.2.1. Dose response studies.....	25
4.2.2. Clinical studies (case studies and clinical trials)	25
4.3. Clinical studies in special populations (e.g. elderly and children)	30
4.4. Overall conclusions on clinical pharmacology and efficacy	30
5. Clinical Safety/Pharmacovigilance	30
5.1. Overview of toxicological/safety data from clinical trials in humans.....	30

5.2. Patient exposure	31
5.3. Adverse events, serious adverse events and deaths.....	31
5.4. Laboratory findings.....	32
5.5. Safety in special populations and situations	32
5.5.1. Use in children and adolescents.....	32
5.5.2. Contraindications.....	32
5.5.3. Drug interactions and other forms of interaction	32
5.5.4. Fertility, pregnancy and lactation.....	33
5.5.5. Overdose.....	33
5.5.6. Effects on ability to drive or operate machinery or impairment of mental ability	34
5.5.7. Safety in other special situations	34
5.6. Overall conclusions on clinical safety.....	34
6. Overall conclusions (benefit-risk assessment).....	34
Annex	34

1. Introduction

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

In international pharmacopoeias different herbal substances and herbal preparations from *Salvia miltiorrhiza* root and rhizome are monographed.

- Herbal substance(s):
 - "Danshen" consist of the dried, whole or fragmented rhizome and root (European Pharmacopoeia 2017; Chinese Pharmacopoeia 2015).

European Pharmacopoeia (2013, 2017)

Salvia miltiorrhiza root and rhizome is covered by the 8th edition of the European Pharmacopoeia (2013). *Salvia miltiorrhiza* root and rhizome consist of the dried, whole or fragmented rhizome and root of *Salvia miltiorrhiza* Bunge, radix et rhizoma, collected in spring or autumn, containing minimum 3.0% of salvianolic acid B (C₃₆H₃₀O₁₆; Mr 719) and minimum 0.12% of tanshinone II (C₁₉H₁₈O₃; Mr 294.3).

Chinese Pharmacopoeia (1963, 1977, 1985, 1990, 1995, 2000, 2005, 2010 and 2015)

The Chinese Pharmacopoeia: "Danshen", the dried whole or fragmented rhizome and root.

- Herbal preparation(s):
 - "Processed" baked with wine (British Herbal Pharmacopoeia 2014).

British Herbal Pharmacopoeia (2014)

According to the British Herbal Pharmacopoeia (2014) "Processed *Salvia miltiorrhiza* rhizome and root" is defined as *Salvia miltiorrhiza* rhizome and root that has been "processed". It contains not less than 0.04% of tanshinone IIA (C₁₉H₁₈O₃), not less than 0.17% of rosmarinic acid (C₁₈H₁₆O₈) and not less than 3.0% of salvianolic acid B (C₃₆H₃₆O₁₆), calculated with reference to the dried material. Production: it is collected in spring or autumn, separated from soil, washed clean, softened thoroughly, sliced longitudinally or transversely and dried. It may be stir-baked with wine.

- "Jiudanshen" roasted, fragmented root, according "jiuzhi" method (Chinese Pharmacopoeia 2015).

Chinese Pharmacopoeia (1963, 1977, 1985, 1990, 1995, 2000, 2005, 2010 and 2015)

The Chinese Pharmacopoeia: "jiudandhen" separated from soil, washed clean, softened thoroughly, sliced longitudinally or transversely and dried. It may be stir-baked with wine (according the "Jiuzhi-method").

Chinese Pharmacopoeia 2015

According to the 2015 edition of the Pharmacopoeia of the People's Republic of China, beside the monographs for the crude and processed drug, additionally, a monograph for total phenolic acids and another monograph for the tanshinones are included:

- "Salvia total phenolic acids": extraction solvent water, then precipitate with industrial ethanol to 70% (V/V), containing minimum 0.50% of rosmarinic acid (C₁₈H₁₆O₆) and minimum 5.0% of salvianolic acid B (C₃₆H₃₀O₁₆);

- “Tanshinones”: extraction solvent: ethanol, then washed with water, containing minimum 2.1% of crypto-tanshinone (C₁₉H₂₀O₃) and minimum 9.8% of tanshinone II A (C₁₉H₁₈O₃).

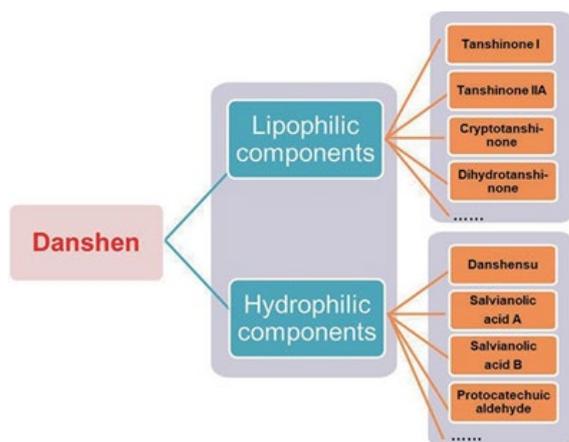
The total phenolic acids and the tanshinones are prepared by a complex (cleaning/precipitating) procedure and it is questionable if they can be still considered as an herbal preparation.

Main characteristic constituents of the herbal substance

More than 200 compounds have been identified from *Salvia miltiorrhiza* (Wang *et al.*, 2017a). They can be classified into two major groups: water-soluble (hydrophilic) phenolic compounds and nonpolar (lipid-soluble) diterpenoid compounds (Li *et al.*, 2009).

The lipophilic compounds include more than 30 diterpenes. Most of them are diterpene chinone compounds of the tanshinone type, including tanshinone I, IIA, IIB; cryptotanshinone; and other related compounds. At least 50 components have been isolated and identified from the aqueous extracts. Phenolic acids are the main type of hydrophilic components from Danshen. The main are polyphenolic acids (such as various salvianolic acids) and related compounds (such as danshensu, protocatechuic aldehyde, and protocatechuic acid). In addition to phenolic acids and diterpene compounds, other compounds include baicalin, sitosterol, ursolic acid and daucosterol isolated from alcohol extract, and dimethoxy-flavanone isolated from ethyl acetate extract. Moreover, vitamin E and tannin have also been found in certain Danshen extracts (Zhou *et al.*, 2005).

The main constituents according to Li *et al.* (2018) are:



The results of a quality control, including nuclear magnetic resonance (NMR), high performance thin layer chromatography (HPTLC) combined with a pharmacological assay showed that processing causes huge quality variation among market samples, especially in tanshinones, resulting in significant variation of the biological activity (Kum *et al.*, 2019).

Potential confounding

Potential confounding is possible, as many different definitions of the drug (not-roasted drug (Danshen), roasted drug, wine-baked/processed drug (jiudanshen), funfang Ddanshen (which is a combination of unknown content of *Salvia miltiorrhiza*, *Panax notoginseng*, and *Cinnamomum camphora*)) exist in parallel.

The European Pharmacopoeia uses a different definition of the drug as the British Herbal Pharmacopoeia (defines “processed/baked with wine”; however, not clarified what “baked” means, what wine to be used, no information about temperature, Drug to Extract Ratio (DER), duration ...).

Confounding results from the undifferentiated use of the name of “products of *Salvia miltiorrhiza*”. In China are commercially available tablets, capsules, granules, injectables, oral liquids, sprays, and dripping pills (which are made by blending the herbal extract with excipients under thermal condition followed by dripping the mixture into an insoluble cooling liquid in which the droplets are solidified to form the “dripping pill”) of either Danshen or Fufang Danshen (combination) (Zhou *et al.*, 2005). In the Chinese literature and Chinese reviews, different definitions of “dosage form” from Europa exist.

From the information in publications (*in vitro*, *in vivo* or clinical studies) and safety reports it is often not clearly defined which preparation and composition (mono or combination preparation) of these commercial preparations were used.

Accordingly, in the Chinese literature often “dosage forms” are mentioned and not the information on the specific preparation, e.g., Zhou *et al.* (2005): “Among all the available dosage forms (of *Salvia miltiorrhiza*), the Fufang Danshen Tablet and Fufang Danshen Dripping Pill are the 2 most widely used products in China”. This example shows that in praxis a combination product with unknown content is the most widely used in China.

In addition, the content of certain constituents of danshen products sold in Chinese pharmacies, changed after processing. Lack of consistency of the levels of the active ingredients in these products contributes to variations efficacy, demonstrated in clinical trials (Lin & Lu, 1999 cited in Awaad *et al.*, 2010).

Gruenwald *et al.* (2000) states mistaken identity can occur with *Salvia przewalskii* or *Salvia trijuga*. Other names can lead to confounding: red ginseng, red-rooted salvia, Chinese sage, red sage.

According Kum *et al.* (2016) in some regions Danshen substitutes including *Salvia przewalskii*, *Salvia bowleyana*, and *Salvia sinica* have been used. Such local substitutes are often erroneously called Danshen. Proton nuclear magnetic resonance spectrometry (¹H-NMR) metabolomics approach coupled with HPTLC were used to analyse the chemicals within Danshen products collected from all types of sellers including wholesalers, suppliers and manufacturers. Ninety-seven dried roots of Danshen, its substitutes and Danshen-derived final products were sampled from different companies. Seven out of 28 final products were highly likely to contain no *Salviae miltiorrhizae radix*. Six sources from online stores contained neither salvianolic acid B nor tanshinone IIA. Only six of the final products had significant salvianolic acid B and tanshinone IIA, the chemical standards for *Salvia miltiorrhiza*. The authors concluded the results illustrate the variable composition of Danshen-labelled products sold on the market.

- 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

A literature search was performed in scientific databases and medical databases of the division for Complementary and Alternative Medicines of the Federal Institute for Drugs and Medical Devices (BfArM), Medline, PubMed, Cochrane Database of Systematic Reviews, EMBASE and toxicological databases (TOXLINE). The keywords were “*Salvia miltiorrhiza*”, “Sage”, “Danshen”, “*Salvia miltiorrhiza* Bunge”. Additional hand search was performed in books on herbal medicines and plant monographs in the BfArM owned library. The bibliography of included trials and other relevant reviews was searched to identify further potential trials. The BfArM pharmacovigilance database was searched for potential adverse events reports. The search engine Google was used additionally for information on *Salvia*

miltiorrhiza preparations e.g., medical devices, food/dietary supplements, cosmetics on the European market.

Information about products on the market was received from other member states to the call for scientific data.

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

According to the information provided by the National Competent Authorities in AT, BE, CY, CZ, DE, DK, ES, FI, HR, HU, IE, LT, LV, MT, PL, SE, and SK no preparations are on the market.

AT provided information on domestic commerce, without information on the sale character (food supplement/for TCM herbal combinations, individual mixed in pharmacies).

The Netherlands provided information on one THMP product registered in 2016. The active ingredient is dry extract of *Salvia miltiorrhiza* Bunge, radix et rhizoma; extraction solvent: 90% ethanol (V/V). The indication is given with "Traditional herbal medicinal product for the alleviation of mild menstrual pain" with a posology of 2 times daily 975 mg dry extract. The national decision was based on confidential documents of the applicant. According to the information of the Netherlands, the applicant has submitted invoices over a period of 1991 to 2012 that show the import of Danshen (raw material and tablets) containing *Salvia miltiorrhiza* root and rhizome into the EU over the period of 1991-2014 which indicate that "Danshen" has been delivered to TCM centres, individual TCM therapists and pharmacies.

Assessor's comment:

Data available to the HMPC regarding the THMP in the Netherlands are not sufficient for inclusion of this herbal preparation in a European Union herbal monograph.

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

Not applicable.

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

In the Netherlands, the herbal tea from *Salvia miltiorrhiza* root and rhizome (dried comminuted) is used as food supplement at least since 1991.

The recommended daily dose is 9-15 g, and the duration of use is not specified.

Assessor's comment:

Data available to the HMPC regarding the food supplement in the Netherlands are not sufficient for inclusion of this herbal preparation in a European Union herbal monograph.

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

Table 1: Information provided by the Chengdu University of Traditional Chinese Medicine (28 June 2018) on medicinal used products in China

Name	Market time ¹	Active substance	Pharmaceutical form / Posology	Indication / Description	Reference*
Danshen Pian [丹参片]	Since 1990	Total extract with 90% ethanol of <i>Salviae miltiorrhizae radix</i> (no information on DER) and then re-extracted with water (no information on DER) (Tanshinones)	Tablet, 1 g herbal substance per tablet 3-4 tablets per time, 3 times a day (9-12 g herbal substance per day)	Angina, Shen disorders by a malnourished heart (e.g., anxiety and cardiac nervous)	Danshen Pian, 1990; 2002
Danshen Koufuye [丹参口服液]	Since 1996	Aqueous extract of <i>Salviae miltiorrhizae radix</i> (no information on DER)	Oral liquid, 10 ml per time, 3 times a day	Palpitations and chest pain caused by blood stasis, angina	Danshen Koufuye, 1996; 2005
Danshen Heji [丹参合剂]	Since 2002	Total extract of <i>Salviae miltiorrhizae radix</i> with water and then precipitate with ethanol to 80% (<i>Salvia</i> total phenolic acids)	Mixture, each ml of mixture contains 0.55 g of the herbal substance 10 ml per time, 2 times a day (11 g herbal substance per day)	Palpitations and chest pain caused by blood stasis, mild angina	Danshen Heji, 2002a; 2002b
Danshen Jiaonang [丹参胶囊]	Since 2005	Aqueous extract of <i>Salviae miltiorrhizae radix</i> (no information on DER, aqueous extract in capsules?)	Capsule, ≥13 mg salvianolic acid B per capsule 3-4 capsules per time, 3 times a day	Angina, Shen disorders by a malnourished heart (e.g., anxiety and cardiac nervous)	Danshen Jiaonang, 2005

¹ The market time is conservatively estimated by the approval number recorded in CFDA. Due to some historical reasons, the time to publish national standards is usually later than the time to market.

* References provided by the Chengdu University are not included in the List of References

In addition to the mono-component products listed, 153 combination products containing *Salviae miltiorrhizae radix* are approved in China.

Assessor's comment:

In China, in the traditional medicine, the TCM, the whole drug is used in form of decoction, normally from several drugs/drug-combinations.

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

Few European pharmacopoeias or accepted collections in the European countries have introduced the drug. They always refer to the use as TCM (the use of the whole drug as oral powder or according to the TCM as decoction/tea).

Table 2: Overview of historical data

Herbal preparation	Documented Use / Traditional Use	Pharmaceutical form Strength (where relevant) Posology Duration of use	Reference
Salvia miltiorhiza	Herbal substance recommended for colic	N/A	List <i>et al.</i> (1979)
Salviae miltiorrhizae radix, dried		9-15 g for decoction and 10-20 drops of not specified solutions ("Sol. Tonif., Conc. Hydr.")	Vangermeersch (1986)
Salviae miltiorrhizae radix, dried	Arteriosclerosis, angina pectoris, cirrhosis of the liver. Abnormal menses, insomnia, nervousness	Daily dosage (herbal tea): 3-15 g either as a single drug or within a formulation together with other drugs as tea	Paulus and Ding (1987)
Salviae miltiorrhizae radix et rhizoma, dried	For menstrual disorders, painful periods and amenorrhoea, for concretions and congelations in the abdomen, for stabbing pains in the chest and abdomen, for warm-connected joint pains, for furuncles, carbuncles and painful swellings, nervous restlessness and insomnia, for spleen and liver swelling, for angina pectoris	9-15 g (The herbal substance is washed, soaked, sliced and dried (Danshen) or slices of the root processed following the Jiuzhi method (Jiudanshen))	Stöger (1985, 2009) [translation of the Chinese Pharmacopoeia]
Salviae miltiorrhizae radix et rhizoma, dried	For menstrual disorders, painful periods and amenorrhoea, for so-called concretions and	Herbal substance: 9-15 g Herbal substance as herbal tea: 3-15 g	Hänsel <i>et al.</i> (1994) Reference to: Chinese Pharmacopoeia IX

Herbal preparation	Documented Use / Traditional Use	Pharmaceutical form Strength (where relevant) Posology Duration of use	Reference
	<p>congelations in the abdomen, for stabbing pains in the chest and abdomen, for angina pectoris, for so-called warm-connected joint pains, for furuncles, carbuncles and painful swellings, for spleen and liver swelling, for nervous restlessness and insomnia</p>		<p>Stöger EA, Friedl F (1991) Arzneibuch der Chinesischen Medizin, Monographien des Arzneibuches der Volksrepublik China 1985 und nachfolgender Ausgaben, 2. Aufl., Deutscher Apotheker Verlag, Stuttgart</p> <p>Paulus E, Ding YH (1987) Handbuch der traditionellen chinesischen Heilpflanzen, Karl F. Haug Verlag, Heidelberg, S. 221-222</p>
<p>Salviae miltiorrhizae radix</p>	<p>Promoting blood flow and stimulating menstrual discharge, used in menstrual disorders, amenorrhea, dysmenorrhea, mass formation in the abdomen, poking pain in the chest and abdomen</p> <p>Removing blood stasis and relieving pain, used in pain in acute arthritis and subcutaneous infections</p> <p>Easing the mind, used in fidgets and insomnia</p> <p>Recently used in hepatospenomegaly</p>	<p>Daily dosage: 9-15 g</p> <p>(Preparations not further described)</p>	<p>Zhu (1998)</p>

Herbal preparation	Documented Use / Traditional Use	Pharmaceutical form Strength (where relevant) Posology Duration of use	Reference
	and angina pectoris		
Salviae miltiorrhizae radix et rhizoma, dried	Metrorrhagia, pain following menstruation, amenorrhea, post-partum bleeding and pain, angina pectoris, furuncles, carbuncles, painful swellings, swelling of the liver and spleen, and joint pain	Preparation: Jiudanshen - slices of the root, to which wine has been added in accordance with the Jiuzhi method, are roasted until dry Daily dosage of herbal substance: 9-15 g Daily dosage of tea: 3-15 g of the drug	Gruenwald <i>et al.</i> (2000)
Salviae miltiorrhizae radix	N/A	Daily dosage: 9-15 g	Stöger (2009)
Salviae miltiorrhizae radix et rhizoma, dried	For menstrual disorders, painful periods and amenorrhoea, for so-called concretions and congelations in the abdomen, for stabbing pains in the chest and abdomen, for angina pectoris, for so-called calor-connected joint pains, for furuncles, carbuncles and painful swellings, for spleen and liver swelling, for nervous restlessness and insomnia	Herbal substance: 9-15 g herbal substance as herbal tea: 3-15 g	Blaschek <i>et al.</i> (2017) Reference to: Chinese Pharmacopoeia IX Stöger EA, Friedl F (1991) <i>Arzneibuch der Chinesischen Medizin, Monographien des Arzneibuches der Volksrepublik China 1985 und nachfolgender Ausgaben</i> , 2. Aufl., Deutscher Apotheker Verlag, Stuttgart Paulus E, Ding YH (1987) <i>Handbuch der traditionellen chinesischen Heilpflanzen</i> , Karl F. Haug Verlag, Heidelberg, S. 221-222

According to List *et al.* (1979), Hänsel *et al.* (1994), Blaschek *et al.* (2017), Tang and Eisenbrand (1992), Hellemans (1994), Hempen and Fischer (2009, 2018), Portert (1978), Wagner *et al.* (2011) *Salvia miltiorrhiza* is used according to the Chinese medicinal tradition.

There are a large number of publications on the drug and its ingredients, particularly in Chinese, which were accessible for the present evaluation almost only in a reduced secondary form, without the necessary information on the investigational product, dosage and effect. The following information on the most important effects therefore does not permit a conclusive evaluation but is merely intended to provide an insight into the possible potential of this drug, which has not yet been included in Western research and therapy (Blaschek *et al.*, 2017).

Paulus and Ding stated that western practitioners are not able to practice the classical syndrome diagnostic (Bian Zheng) and consider the comprehensive application instructions (Ying Yang), necessary for professional use of the Chinese drugs. An unprofessional use could lead to aggravation of the disease. Clinical studies are necessary to prove efficacy in Western indications (Paulus and Ding, 1987).

Uses outside the EU

According to information of the Chengdu University of Traditional Chinese Medicine, *Salvia miltiorrhiza* root and rhizome have been used in China, Japan, and other nations of the Far East for over two millennia. *Salviae Miltiorrhizae radix* has been officially recorded in Chinese Pharmacopoeia since 1963 and continuously been included in the edition of 1977, 1985, 1990, 1995, 2000, 2005, 2010 and 2015 of Chinese Pharmacopoeia.

The actions listed in the Chinese Pharmacopoeia (2010) are:

- to activate blood and eliminate stasis;
- unblock the meridian to relieve pain;
- clear the heart and relieve vexation;
- cool blood and disperse abscess.

Indications are chest impediment and heart pain, pain in the epigastrium and abdomen, hypochondriac pain, aggregation and accumulation, painful heat impediment, insomnia caused by vexation, menstrual irregularities, dysmenorrhea and amenorrhea, sore, ulcer, swelling and pain. The dosage is 10-15 g per day.

Salvia miltiorrhiza is subject of review articles. In a review article published in the *Acta Pharmacologica Sinica*, Li *et al.* (2018) describes Danshen as the dried root of rhizome of *Salvia miltiorrhiza* Burge, *radix et rhizoma* that has been used in Asian countries for treating cardiovascular diseases, including coronary heart disease, myocardial infarction, angina pectoris and atherosclerosis. The author states, that Danshen and its preparations (such as Fufang Danshen Dripping Pill, Fufang Danshen injection, and Danhong injection, among others) have been widely used in China. The most used preparations are combinations thereof. However, clinical applications of these Danshen preparations in other countries are still limited.

In a review article Zhou *et al.* (2005) assume that the dried root of *Salvia miltiorrhiza* is a commonly used Traditional Chinese medicine (TCM) for improving body function (e.g., promoting circulation and improving blood flow). In addition, it has been used for the treatment of cardiovascular diseases such as coronary heart disease, hyperlipidaemia, and cerebrovascular disease.

According to Binsky *et al.* (1986) the drug is used in TCM in two major combinations, depending on indication. For the use for deficient blood induced menstrual irregularity or amenorrhea with abdominal pain it is used in combination with *Radix rehmanniae glutinosa conquitae*, *Radix ligustici wallichii*, *Radix angelicae sinensis*.

Huang (1999) classifies the drug as “antianginal herb” in TCM. Traditional Chinese physicians prescribe this herb to stabilize the heart and calm nerves, “lighten” blood and remove “stagnant” blood.

Salvia miltiorrhiza has been used in China to treat neurasthenic insomnia, because of it is claimed to have tranquilizing effect. According to Awaad *et al.* (2010) it also decreases the release of norepinephrine, dopamine and serotonin during brain ischemia. Induced arterial dilation in the brain could help re-perfuse the brain better allowing a faster recovery (Awaad *et al.*, 2010).

Chen and Chen (2004) reported that liquor-fried Dan Shen (with grain-based liquor) can improve blood circulation, remove blood stasis, and relieve pain. Holmes (2002) noted that the wine-braised herb is the best for “invigorating the blood and channels”.

2.3. Overall conclusions on medicinal use

It is acknowledged that *Salvia miltiorrhiza* root and rhizome have a long tradition of medicinal use in TCM. However, according to HMPC the available data is does not fulfil the requirements of Directive, in particular demonstration of traditional use (TU) for at least 15 years within the EU. The registration in the Netherlands was partly based on confidential information that could not be disclosed and therefore nor the herbal substance, nor the herbal preparation are considered sufficiently justifying 15 years of TU within the EU.

3. Non-Clinical Data

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

3.1.1. Primary pharmacodynamics

According to Zhou *et al.* (2005), pharmacological studies have concentrated on Danshen components such as danshensu, salvianolic acid B, and tanshinone IIA. The data on other Danshen components are very limited.

- **Effect on heart diseases**

In a review article in the *Acta Phamacologica Sinica* (Li *et al.*, 2018) the pharmacological properties of the dried root or rhizome of *Salvia miltiorrhiza* Burge, radix et rhizoma for treating cardiovascular diseases, including coronary heart disease, myocardial infarction, angina pectoris and atherosclerosis were described. The authors review the anti-atherosclerotic effects and molecular mechanisms of individual major components (Table 3).

Table 3: Therapeutic benefits of bioactive components of Danshen in atherosclerotic vascular diseases (from Li *et al.*, 2018)

Compound	Animal Model	Effects and mechanisms
Tanshinone IIA	ApoE ^{-/-} mice+HFD	↓Lesion size and instability, ↓CLIC1, ↓SRA, ↓CD36, ↓LOX1, ↓PPAR γ , ↓CD68, ↓NF- κ B, ↓MMP-9
	ApoE ^{-/-} (OVX) mice+HFD	↓Lesion size, ↓NF- κ B, ↓sICAM-1 ↓AP1, ↓E-selectin, ↓p-ERK1/2, ↓HDL, ↑SOD
	Rabbits+HCD	↓Lesion size, ↓neointima, ↓CD40, ↓MMP-2/9, SOD, ↓MDA, ↓oxLDL, ↑GPx, ↓VCAM-1, ↓IL-1 β
	Rats+HFD	↓Hepatic lipid deposition
	Rats+balloon injury	↓Aortic calcification, ↓ROS, ↓MDA, ↓oxLDL, ↑Cu/Zn-SOD
	Mice+carotid artery	↓Intimal hyperplasia, ↓PCNA

	ligation	
	Rats+ elastase perfusion	↓AAA incidence, ↑elastin fibers, ↑VSMC content, ↓TLR4, ↓pNF-κB, ↓MyD-88, ↓MMP-2, ↓MMP-9, ↓MCP-1, ↓iNOS
Cryptotanshinone	ApoE ^{-/-} mice+HFD	↓Lesion size and instability, ↓IL-1β, ↓TNFα, ↓IL-6, ↓IL-17A ↓IFNγ, ↓MMP-9, ↓LOX1, ↓ROS
Dihydrotanshinone	ApoE ^{-/-} mice+HFD	↓Lesion size, ↓TLR4, ↓NF-κB, ↓MyD88, ↓ROS, ↓LOX1, ↓NOX4
Danshensu	Rats+ methionine-rich diet	↓Lesion size, ↓Hcy, ↓TNFα, ↓ICAM-1, ↓ET1, ↑NO
Salvianolic acid A	ApoE ^{-/-} mice+HFD	↓Lesion size, ↓CCL20, ↓CCR6
	ApoE ^{-/-} mice+HFD	↓Aneurysm severity, ↓MMP-2/9 ↓Elastin fragmentation, ↓Macrophage infiltration
	SHR	↑Relaxation
	Rat+STZ+HFHS	↓vWF, vasorelaxation, ↓MDA, ↓AGE
Salvianolic acid B	Rabbits+HCD	↓Lipid deposition, ↓neointimal formation, ↓LDL oxidation
	ApoE ^{-/-} mice+HFD	↓Neointimal formation, ↓foam cell, ↓MMP-2/9, ↓COX-2, ↓CD36
	Rats+balloon injury	↓Neointimal formation, ↓CXCR-4
Protocatechuic aldehyde	Rats+balloon injury	↑Re-endothelization, ↓neointima, ↑GPER1, ↑CD31, ↓VCAM-1, ↓CD40

The authors conclude, that both lipophilic and hydrophilic components may function in concert, targeting different tissues and signalling pathways to achieve the versatile cardiovascular actions of Danshen in experimental animals and humans. However, the differential pharmacokinetic and pharmacodynamics properties of individual compounds remain a hurdle to the systematic evaluation of the cardiovascular efficacy of Danshen. In particular, tanshinone IIa and cryptotanshinone have relatively low oral bioavailability. Therefore, new formulation strategies are necessary. Although research investigating the cardiovascular effects of Danshen is expanding, many questions remain unaddressed. In animal studies, danshensu has been shown to dilate coronary arteries, inhibit platelet aggregation, improve microcirculation, and protect the myocardium from reperfusion injury of the ischemic heart. The mechanism for some of its observed activity may be related to inhibition of Ca²⁺ aggregation in cardiac muscle cells and prevention of Ca²⁺ overload. In animal studies, salvianolic acid B has been shown to protect the brain from ischemia-reperfusion injury. In addition, salvianolic acid B can inhibit platelet aggregation as well as oxidative modification of low-density lipoprotein (LDL), leading to the prevention of the uptake of LDL by cultured macrophages. From *in vivo* studies, sodium tanshinone IIA sulfonate has been shown to significantly reduce myocardial infarct size. Tanshinone IIA has also been shown to inhibit LDL oxidation as well as angiotensin II activity, resulting in attenuation of cardiac cell hypertrophy (Li *et al.*, 2018).

Water-soluble extract

In aging guinea pigs fed a diet containing 75, 100, or 150 mg/kg daily of a water-soluble extract (unknown composition) of *Salvia miltiorrhiza* for 28 days, blood biochemical parameters remain unaffected, except for the fibrinogen levels of the high group decreased. At high dose a significant decrease in whole blood viscosity was observed (Hou *et al.*, 2007).

Inhibition of platelet aggregation was observed in rabbits intragastrically administered an extract of *Salvia miltiorrhiza* (dose and product not specified in English language abstract) (AHPA, 2013).

Danshensu:

The mechanism for some of observed activity of danshensu may be related to inhibition of Ca²⁺ aggregation in cardiac muscle cells and prevention of Ca²⁺ overload (Zhou *et al.*, 2008).

Inhibition of calcium channels has been suggested as the mechanism of action for the vasorelaxant activity of *Salvia miltiorrhiza* (Lam *et al.*, 2005, 2006).

- **Anticoagulant and anti-thrombotic activities**

Water-soluble extract

In aging guinea pigs fed a diet containing 75, 100, or 150 mg/kg daily of a water-soluble extract (unknown composition) of *Salvia miltiorrhiza* for 28 days, blood biochemical parameters remain unaffected, except for the fibrinogen levels of the high group decreased. At high dose a significant decrease in whole blood viscosity was observed (Hou *et al.*, 2007).

Inhibition of platelet aggregation was observed in rabbits intragastrically administered an extract of *Salvia miltiorrhiza* (dose and product not specified in English language abstract) (AHPA, 2013).

In rats with bile duct ligation, administration of 0.4 g daily of an aqueous extract of Danshen for 4 weeks, significantly reduced histological grades of fibrosis and ameliorated the portal hypertensive state as compared with control (Huang *et al.*, 2001).

Salvia miltiorrhiza increases the proteolyses of fibrinogen-to-fibrinogen degradation products (Adams *et al.*, 2006).

- **Anti-allergic activity**

Components of the drug as tanshinone I, tanshinone IIA and cryptotanshinone and dihydrotanshinone I interfere with IgE receptor-mediated tyrosine phosphorylation (Wang *et al.*, 2007).

- **Anticancer effects**

Aqueous extract (not specified)

The aqueous extract of *Salvia miltiorrhiza*, was found to inhibit the proliferation of human hepatoma HepG2 cells. It was also observed that crude extract treatment caused apoptotic cell death.

Salvianolic acids

Salvianolic acid A was claimed as an antitumor drug in a Chinese patent. It showed synergistic effects in combination with other antitumor agents (Jiang *et al.*, 2005).

Salvianolic acids are able to inhibit the formation of thrombosis and decrease the plasma endothelin and thromboxane B2 level. Salvianolic acid A showed strong anticoagulant activity (Awaad *et al.*, 2010).

Tanshinones

Tanshinone shows anticancer-specific activity. It inhibits migration, invasion and gelatinase activity on highly invasive human lung adenocarcinoma cell line, CLI-5 *in vitro* and *in vivo* (Lee *et al.*, 2008).

Tanshinone IIA expresses cytotoxic activity against many kinds of human carcinoma cell lines. This activity is mediated by inducing differentiation and apoptosis. In addition, it inhibits cancer cell invasion and metastasis on three ways: first by inhibiting DNA synthesis and proliferation of cancer cells, second by inducing proliferation, differentiation and apoptosis and third by inhibiting the telomerase activity in cancer cells and changing the expression of cellular surface antigens (Yuan *et al.*, 2004).

Tanshinone IIA selectively induces apoptosis of cancer cell through mitotic arrest. Unlike vincristine and paclitaxel, it interferes only with the mitotic spindle during the M phase, but not the microtubule structure in interphase cells (Zhou *et al.*, 2008).

Tanshinones induces apoptosis in human breast cancer cells (MCF-7 and MDA-MB-231) *in vitro*. They show potential to serve as an adjunctive reagent in the treatment of human breast cancer.

Tanshinones exert influence on cell adhesion molecules (CAM) which are mediators of carcinogenesis and cancer metastasis (Nizamutdinova *et al.*, 2008).

Chen *et al.* (2013) concluded in a systematic review on anticancer properties of *Salvia miltiorrhiza*, that evidence accumulated in the last decade has demonstrated that Danshen exhibits anticancer effects, especially the tanshinones. Tanshen IIA is one of the most investigated with both *in vitro* and *in vivo* anticancer effects comparable to those of TCM-derived natural products such as berberine, curcumine, oridonin. A series of synthesized derivatives showed anticancer effects as well. However, limited data are available. Though several molecules have been identified as potential targets of danshen or its components, the detailed mechanisms, the pharmacokinetic profiles and the potential toxicity are still unclear.

- **Anti-HIV-activity**

Water soluble extracts

Water soluble extracts of Danshen exhibit potent inhibitory effects against HIV-1 integrase activity *in vitro* and viral replication *in vivo* (Wang *et al.*, 2007).

- **Antibacterial effect**

Tanshinones

Cryptotanshinone and dihydrotanshinone I show antibacterial activity against a broad range of gram-positive bacteria (Wang *et al.*, 2007).

- **Neuropharmacological properties**

Salvia diterpenes

The effects on different pathways, including apoptosis signalling, oxidative stress phenomena, the accumulation of amyloid beta plaques, and tau phosphorylation, have been considered to be mechanisms of the anti-Alzheimer properties of *Salvia* diterpenes. Additionally, effects on the benzodiazepine and kappa opioid receptors and neuroprotective effects are noted as neuropharmacological properties of *Salvia* diterpenes, including tanshinone IIA, salvinorin A, cryptotanshinone, and miltirone (Akaberi *et al.*, 2016).

- **Effects on osteoporosis**

Lin *et al.* (2017) reviewed the experimental evidence of both *in vitro* and *in vivo* preclinical studies of Chinese single herbs and their active ingredients in postmenopausal osteoporosis. The review includes three single herbs (Herba Epimedii, Rhizoma Drynariae, and *Salvia miltiorrhiza*) and eight constituents (saikosaponins, linarin, echinacoside, sweroside, psoralen, poncirin, vanillic acid, and osthole). Aqueous extracts of *Salvia miltiorrhiza* could enhance *in vivo* bone mechanical strength and prevent trabecular bone resorption in OVX Sprague-Dawley rats and prevented OVX-induced bone loss. Individual compound tanshinone prevented a decrease in trabecular bone volume and trabecular number and an increase in osteoclast surface in vertebra, and partially prevented a decrease in trabecular bone volume and trabecular number in the tibia.

Tanshinones

Panwar *et al.* (2018) tested 31 tanshinones for their activity against CatKin enzymic and cell-based assays. The inhibitory potency against triple helical and fibrillar collagen degradation was determined in enzyme assays, by scanning electron microscopy and mechanical strength measurements. Human osteoclast assays were used to determine the effects of the inhibitors on bone resorption, its reversibility and osteoclastogenesis. Twelve compounds showed effective anti-collagenase activity and protected collagen against destruction and mechanical instability without inhibiting the hydrolysis of non-collagenous substrates. Six compounds were effective in osteoclast bone resorption assays with IC₅₀-values of <500 nM. The IC₅₀ values of these tanshinones in osteoclast-mediated bone resorption

assays were between 60 and 800 nM and thus between 4 and 50 times less potent than odanacatib with an IC₅₀ value of 14 nM shown in Panwar *et al.* (2017). None of these tanshinones had effects on cell viability, reversibility of bone re-sorption inhibition and osteoclastogenesis.

- **Effects in osteoarthritis**

Rabbits with experimentally induced osteoarthritis were given an intra-articular injection of Danshen (0.7 ml/day, no information on the extract) for 5 weeks. Danshen decreased the expression and activity of matrix metalloproteinase 9 (MMP-9) and MMP-13 and increased the expression of their inhibitor of matrix metalloproteinase 1 (TIMP-1) and TIMP-2. Apoptosis in osteoarthritic cartilage tissues was attenuated by Danshen, accompanied with increased expression of B cell lymphoma 2 (Bcl-2) and decreased levels of Bcl-2-associated X protein (Bax). Further, Danshen inhibited the nuclear accumulation of nuclear factor kappa-B (NF-κB) p65 in osteoarthritic cartilage. The therapeutic effects of Danshen *in vivo* were comparable to that of sodium hyaluronate (Xu *et al.*, 2017).

- **Pain-relieving effects**

Tanshinones

Di Cesare Mannelli *et al.* (2018) investigated the pain-relieving profile of tanshinone IIA (TIIA) and cryptotanshinone (CRY) in animal models of neuropathic pain induced by Oxaliplatin, anticancer drug characterized by a dose-limiting neurotoxicity. A single administration *per os* of CRY (30 mg/kg) significantly, in a dose dependent manner, attenuated chemotherapy-induced pain. Seven-days repeated administrations highlighted the effectiveness and potency of both CRY and TIIA (10 mg/kg). According to the authors the results demonstrated the long-lasting pain-relieving effects of Danshen and its related bioactive constituents in animal models of neuropathic pain.

Assessor's comment:

Pharmacological studies have concentrated on Danshen components. An assessment of the recommended posology of the whole drug in humans from experiments of extracted components is not possible. The pharmacology of the constituents is of limited relevance.

Further, pharmacological studies focus on effects in the indications as treating cardiovascular diseases, as coronary heart disease, myocardial infarction, angina pectoris and atherosclerosis, but also in cancer and HIV and osteoporosis. The focus of the pharmacological studies on the anticoagulant and anti-thrombotic activities complies with the TCM use "to activate blood and eliminate stasis".

In the pharmacological studies with extracts no specification (DER) is available, so the information (on the dose) is insufficient for conclusions for clinical relevance.

- **Cytotoxicity on tumor cells**

Methanolic extract

Ryu *et al.* (1997) isolated 18 constituents from a methanolic extract of *Salvia miltiorrhiza*. The antiproliferative activity of the constituents against five human tumour cells, i.e., A549 (non-small cell lung), SK-OV-3 (ovary), SK-MEL-2 (melanoma), XF498 (central nerve system), and HCT-15 (colon), was evaluated using the SRB (sulfrhodamine-B) method and the calculated IC₅₀ values against each tumour cells. All constituents exhibited a significant (IC₅₀ values ranged from 0.2 to 8.1 pg/ml) but presumably nonspecific cytotoxicity against all examined tumour cells.

3.1.2. Secondary pharmacodynamics

3.1.3. Safety pharmacology

Tanshinones IIa

Morton *et al.* (2015) determined the vasoactive role of tanshinones IIa (TS) in multiple arteries during pregnancy. Further they assessed the ability of TS to improve uterine blood flow in a rodent model of intrauterine growth restriction. Wire myography was used to assess vascular responses to the water-soluble derivative, sodium tanshinone IIA sulphonate (STS) or to the endothelium-dependent vasodilator, methylcholine. At mid-pregnancy, STS caused direct vasodilation of rat resistance (pEC₅₀ mesenteric: 4.47±0.05 and uterine: 3.65±0.10) but not conduit (carotid) arteries. In late pregnancy, human myometrial arteries responded with a similar sensitivity to STS (1 to 100 µmol/l) (pEC₅₀ myometrial: 3.26±0.13). STS treatment for the last third of pregnancy in eNOS^{-/-} mice increased uterine artery responses to methylcholine (E_{max} eNOS^{-/-}: 55.2±9.2% vs. eNOS^{-/-} treated: 75.7±8.9%, p<0.0001). Vasodilation in resistance arteries was consistent between rodent and human arteries. The vascular effects, however, did not lead to improved uterine or umbilical blood flow *in vivo*, nor to improved foetal biometrics, body weight and crown-rump length. In contrast, STS treatment increased the uterine artery resistance index and decreased offspring body weight in control mice, raising a concern for its use during pregnancy.

3.1.4. Pharmacodynamic interactions

In a review Zhou *et al.* (2005) reported on the effects of Danshen on the pharmacokinetics and pharmacodynamics of warfarin in rats. Danshen can increase the absorption rate constant, AUC, maximum concentration, and elimination half-life but decrease the clearance and apparent volume of distribution of both R- and S-warfarin in rats. Danshen aqueous extract could stimulate the metabolic activity of CYP isozymes in Danshen-pretreated rats, with a significant reduction on the maximum concentration and AUC of diazepam. Intraperitoneal treatment with Danshen decoction could induce liver microsomal CYP content in rats. In addition to warfarin, salicylate in therapeutic concentration was reported to be able to significantly decrease free Danshen concentration as measured by free-digoxin-like activity.

Interactions with warfarin

In rats administered warfarin (2 mg/kg) with or without pre-treatment with intraperitoneal administration of 5 g/kg of an aqueous extract (details unknown) of *Salvia miltiorrhiza* twice daily for 3 days, the absorption rate, volume of distribution and elimination half-life of warfarin were significantly decreased, while the maximum concentration was significantly increased (Lo *et al.*, 1992).

Interaction with diazepam

An increase in clearance and decrease in plasma levels of orally administered diazepam (15 mg/kg single dose) were observed in rats orally pretreated with a *Salvia miltiorrhiza* extract (unknown composition) at a dose of 100 mg/kg for 15 days (Jinping *et al.*, 2003).

Differing effect with digoxin

In rats administered Danshen, differencing effects on digoxin assays were observed. One assay, microparticle enzyme immunoassay, showed lower digoxin concentration, a second showed elevated levels (fluorescence polarization immunoassay), and a third showed no change (chemiluminescence assay) (Dasgupta *et al.*, 2002).

Effect on cytochrome P450 activity

An increase in cytochrome P450 activity was observed in the livers of rats that had been orally administered 20 or 100 mg/kg of an aqueous extract of *Salvia miltiorrhiza* daily for 15 days. The pharmacokinetic parameters of diazepam were significantly different between the two groups. In the danshen pretreated group, the maximum concentration of diazepam and the area under the plasma concentration–time curve were reduced to about 72.7% and 44.4%, respectively, while the total body

clearance was markedly increased by 2-fold. The cytochrome activity was primarily induction of the drug-metabolising isoenzyme CYP3A (Jinping *et al.*, 2003).

Qiu *et al.* (2008) examined the potential for the metabolism-based drug interaction arising from seven active components (danshensu, protocatechuic aldehyde, protocatechuic acid, salvianolic acid B, tanshinone I, tanshinone IIA, and cryptotanshinone) of danshen extract. Probe substrates of cytochrome P450 enzymes were incubated in human liver microsomes (HLMs) with or without each component of Danshen extract. IC₅₀ and Ki values were estimated, and the types of inhibition were determined. Among the seven components of Danshen, tanshinone I, tanshinone IIA, and cryptotanshinone were potent competitive inhibitors of CYP1A2 (Ki = 0.48, 1.0, and 0.45 µM, respectively); danshensu was a competitive inhibitor of CYP2C9 (Ki = 35 µM), and cryptotanshinone was a moderate mixed-type inhibitor of CYP2C9 (Ki = 8 µM); cryptotanshinone inhibited weakly and in mixed mode against CYP2D6 activity (Ki = 68 µM), and tanshinone I was a weak inhibitor of CYP2D6 (IC₅₀ = 120 µM); and protocatechuic aldehyde was a weak inhibitor of CYP3A4 (IC₅₀ = 130 and 160 µM for midazolam and testosterone, respectively). The data indicated that it was necessary to study the *in vivo* interactions between drugs and pharmaceuticals with Danshen extract.

Zhou *et al.* (2012) presents an overview of the effects of Danshen and its active ingredients on cytochrome P450 (CYP450) isoenzymes and drug transporters (Table 4).

Table 4: Inhibition (IC₅₀) of Danshen and its active components on different CYP450 isozymes in human liver microsomes (from Zhou *et al.*, 2012).

Components	CYP1A2	CYP2C9	CYP2D6	CYP2E1	CYP3A4
Water extract	NA	NA	NA	NA	136 µg/ml
Ethanol extract	2.91 µg/ml	NA	NA	NA	17.5 µg/ml
Salvianolic acid B	105 µM	>200 µM	>200 µM	NA	>200 µM
Danshensu	110 µM	50 µM	>200 µM	NA	>200 µM
Tanshinone IIA	0.2–1.7 µM	>200 µM	120 µM	–	>200 µM
Tanshinone I	0.75–2.01 µM	>200 µM	>200 µM	9.86 µM	>200 µM
Cryptotanshinone	0.68–3.06 µM	23.9–33.0 µM	75 µM	14.8 µM	>200 µM
Dihydrotanshinone	0.50 µM	7.48 µM	NA	0.72 µM	3.22 µM

NA, currently not available; –, cannot be determined.

Because tanshinones inhibit and induce several CYP450 isozymes, it is concluded that CYP-related herb-drug interactions may occur. Therefore, precautions should be taken when using Danshen preparations rich in tanshinones.

Xu *et al.* (2018) investigated the inhibition of Danshen components on CYP2C8 and CYP2J2. Recombinant CYP2C8 and CYP2J2 were used, and the mechanism, kinetics, and type of inhibition were determined. Taxol 6-hydroxylation and astemizole O-desmethyastemizole were determined as probe activities for CYP2C8 and CYP2J2, respectively. Metabolites were analysed using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The results demonstrated that salvianolic acid A was a competitive inhibitor of CYP2C8 (Ki = 2.5 µM) and mixed-type inhibitor of CYP2J2 (Ki = 7.44 µM). Salvianolic acid C had moderate non-competitive and mixed-type inhibitions on CYP2C8 (Ki = 4.82 µM) and CYP2J2 (Ki = 5.75 µM), respectively. Tanshinone IIA was a moderate competitive inhibitor of CYP2C8 (Ki = 1.18 µM). Dihydrotanshinone I had moderate non-competitive inhibition on CYP2J2 (Ki = 6.59 µM), but mechanism-based inhibition on CYP2C8 (KI = 0.43 µM, kinact = 0.097 min⁻¹). Tanshinone I was a moderate competitive inhibitor of CYP2C8 (Ki = 4.20 µM). Danshen preparations appear not likely to pose a significant risk of drug interactions mediated by CYP2C8 after oral administration. However, there are many injectables prepared from Danshen used in China. The inhibitory effects on intestinal CYP2J2 mediated drug metabolism should not be neglected when preparations are given orally in combination with other drugs, for example amiodarone and cyclosporin A.

3.1.5. Conclusions

The results hint to possible interactions with warfarin, diazepam and the CYP isoenzymes.

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

In animal studies for both danshensu and tanshinone IIA, the 2 major components in Danshen, were absorbed rapidly after oral administrations of either extract formulation or individual components (Tables 5 and 8). On the other hand, salvianolic acid B, another major component, was found to be poorly absorbed in animal studies (Table 6). Only limited pharmacokinetic studies on protocatechuic aldehyde and cryptotanshinone have been conducted. Protocatechuic aldehyde was found to be absorbed orally with the appearance of a double peak concentration (Table 7). Cryptotanshinone was found to be metabolized to tanshinone IIA after intravenous administration and could not be absorbed after oral administration (Zhou *et al.*, 2005).

Table 5: Pharmacokinetics (PK) of Danshensu in different Danshen formulations (from Zhou *et al.*, 2005)

Table I Pharmacokinetics (PK) of Danshensu in Different Danshen Formulations				
Species	Dose	PK Parameter	Findings	Reference
SD rat	p.o. 8 g/kg Danshen aqueous extraction (danshensu 35.7 mg/g)		Absorbable from gastrointestinal tract	38
SD rat	p.o. 0.3 mL/kg 667 g/L of Fufang Danshen Dripping Pill solution	k_e : 0.083 min ⁻¹ $t_{1/2\alpha}$: 11.9 min, $t_{1/2\beta}$: 196.8 min t_{max} : 30 min, C_{max} : 9.4 µg/mL	Two-compartment model; fast absorption and distribution; slow clearance	39
SD rat	p.o. 2.5 mL/100 g Fufang Danshen extract solution	t_{max} : 15 min, C_{max} : 703.6 µg/mL	Fast absorption; slow clearance (26.7 µg/mL at 6 h); unknown compound was found in serum	40
SD rat	p.o. 10 g/kg Danshen extract (4.25% danshensu)	MRT: 205.8 ± 4.2 min AUC: 23809.2 ± 1036.2 µg/mL•min	Double-peak phenomenon in the concentration-versus-time plot	41
Rabbit	i.v. 30 mg/kg danshensu	k : 0.046 ± 0.014 min ⁻¹ $t_{1/2}$: 16.6 ± 5.8 min	Single-compartment model	42
Rabbit	i.v. danshensu 6.25 mg/kg Danshen Injection	$t_{1/2}$: 31.7 ± 2.5 min AUC: 1996 ± 356 µg/mL•min MRT: 47.5 ± 9.6 min V_d : 15 ± 23 mL/kg Cl_r : 3.13 ± 0.97 mL/min/kg		43
Dog	Danshen aqueous extraction	C_{max} : 4.7 µg/mL t_{max} : 105 min	Rapid clearance (0.21 µg/mL at 5 h)	44
Human	Sublingual 250 mg Fufang Danshen Dripping Pill	k : 0.002 min ⁻¹ , $t_{1/2}$: 332.4 min	Single-compartment model	45, 46
Human	p.o. Fufang Danshen Granule (danshensu content: 20 mg)	k_e : 0.028 min ⁻¹ , $t_{1/2\alpha}$: 24.6 min k : 0.013 ± 0.002 min ⁻¹ $t_{1/2\beta}$: 55.2 ± 9.6 min	Absorbable from gastrointestinal tract; eliminate from kidney; no significant difference on elimination half-life of danshensu after p.o. of 2 doses	47
Human	p.o. Danshen decoction (danshensu content: 20 mg)	k : 0.013 ± 0.003 min ⁻¹ $t_{1/2\beta}$: 56.4 ± 12.6 min	Excretions of danshensu by urine after p.o. granule preparation were lower than that of decoction	
Human	p.o. Fufang Danshen Dripping Pill		Probable metabolites are danshensu sulfate and protocatechuic aldehyde glucuronide	48

AUC, area under the plasma level-time curve; Cl_r , total body clearance; C_{max} , maximum concentration of drug; k , overall drug elimination rate constant (first order); k_e , transfer rate constant from the central to the effect compartment; k_a , first-order absorption rate constant; MRT, mean residence time; $t_{1/2}$, half-life; $t_{1/2\alpha}$, absorption half-life; $t_{1/2\beta}$, elimination half-life; t_{max} , time of occurrence for maximum (peak) drug concentration; V_d , steady-state volume of distribution.

Table 6: Pharmacokinetics (PK) of salvianolic acid B (magnesium lithospermate B) (from Zhou *et al.*, 2005)

Table II Pharmacokinetics (PK) of Salvianolic Acid B (Magnesium Lithospermate B)

Species	Dose	PK Parameter	Findings	Reference
Wistar rat	i.v. 4 mg/kg magnesium lithospermate B (MLB)	AUC: 87.8 ± 10.9 µg/mL•min CL _r : 55.52 ± 7.07 mL/min/kg V _d : 7.60 ± 1.03 L/kg t _{1/2α} : 12.3 ± 2.14 min t _{1/2β} : 128 ± 4.68 min	Unchanged MLB in urine and bile was extremely low after i.v. injection	49
	i.v. 20 mg/kg MLB	AUC: 1130 ± 329 µg/mL•min CL _r : 23.5 ± 6.0 mL/min/kg V _d : 3.61 ± 1.16 L/kg t _{1/2α} : 22.7 ± 4.29 min t _{1/2β} : 176 ± 30.4 min	Nonlinear pharmacokinetics between 2 doses; suggest a saturated distribution or a saturated metabolism might occur at a high dose	
	p.o. 20 mg/kg MLB	No salvianolic acid B could be detected in plasma		
	p.o. 100 mg/kg MLB	AUC: 1.26 ± 0.36 µg/mL•min C _{max} : 0.041 ± 0.007 µg/mL t _{max} : 20 ± 5.47 min About 65% of the dose was left in the gastrointestinal tract even 4 hours after administration.	Extremely low bioavailability (0.022%); poor absorption from the rat small intestine; unchanged MLB in urine and bile was extremely low after p.o. administration.	
	In situ jejunal loop perfusion (0.1 mM, 2.5 mL MLB)	Most of the dose was retained in the loop after 20-minute infusion.	Verified the poor absorption of MLB from the small intestine	
Wistar rat	i.v. 4 mg/kg MLB	Four major metabolites—namely, 3-monomethyl-(M1), 3,3''-dimethyl-(M2), 3,3''-dimethyl-(M4), and 3,3'',3''-trimethyl-(M3) lithospermic acid B—were excreted into bile rapidly after i.v. and oral administration. The enzyme responsible for the biotransformation is catechol o-methyltransferase. Antioxidative activities of the metabolites were confirmed.		50
	p.o. 100 mg/kg MLB			
Dog	i.v. 3 mg/kg MLB	AUC: 109.3 µg/mL•min t _{1/2α} : 2.2 min t _{1/2β} : 43 min CL _r : 28 mL/min/kg	Two-compartment model; high affinity to tissues and organs; suggest a saturated distribution and metabolism might occur at a high dose	51
	i.v. 6 mg/kg MLB	AUC: 247.9 µg/mL•min t _{1/2α} : 2.7 min t _{1/2β} : 42 min CL _r : 26 mL/min/kg		
	i.v. 12 mg/kg MLB	AUC: 582.4 µg/mL•min t _{1/2α} : 2.9 min t _{1/2β} : 42 min CL _r : 21 mL/min/kg		

For definitions of abbreviations, see Table I.

Table 7: Pharmacokinetics (PK) of protocatechuic aldehyde in different Danshen formulations (from Zhou *et al.*, 2005)

Table III Pharmacokinetics (PK) of Protocatechuic Aldehyde in Different Danshen Formulations

Species	Dose	PK Parameter	Findings	Reference
SD rat	p.o. 8 g/kg Danshen aqueous extraction (protocatechuic aldehyde 6.19 mg/g)		Absorbable from gastrointestinal tract	38
SD rat	p.o. 2.5 mL/100 g Fufang Danshen extract solution		Not found in serum; unknown compound was found in serum	40
SD rat	p.o. 10 g/kg Danshen extract (1.58% protocatechuic aldehyde)	MRT: 258.3 ± 3.6 min AUC: 938.4 ± 39.6 µg/mL•min	Double-peak phenomenon in the concentration-versus-time plot	41

For definitions of abbreviations, see Table I.

Table 8: Pharmacokinetics (PK) of tanshinone IIA in different Danshen formulations (from Zhou *et al.*, 2005)

Table IV Pharmacokinetics (PK) of Tanshinone IIA in Different Danshen Formulations

Species	Dose	PK Parameter	Findings	Reference
SD rat	p.o. 15 mg/kg tanshinone IIA	t _{1/2α} : 33 min t _{1/2β} : 217.8 min k _e : 0.028 min ⁻¹ C _{max} : 5.57 µg/mL t _{max} : 51 min AUC: 1445.4 µg/mL•min	Short half-life; two-compartment model	52
Wistar rat in situ intestine perfusion	Tanshinone IIA 1008 µg/50 mL	k _e : 0.014 ± 0.010 min ⁻¹ t _{1/2} : 63.1 ± 32.6 min	The transport mechanism of tanshinone IIA perfusion is similar to active transport or facilitated diffusion.	53
	717 µg/50 mL	k _e : 0.012 ± 0.001 min ⁻¹ t _{1/2} : 58.7 ± 3.9 h		
	504 µg/50 mL	k _e : 0.017 ± 0.001 min ⁻¹ t _{1/2} : 40.7 ± 3.5 min		
Rabbit	p.o. 4 g aqueous extraction of Danshen	t _{1/2α} : 223.8 min t _{1/2β} : 1371 min t _{max} : 51.6 min	Fast absorption; slow clearance. The absorption of tanshinone IIA is faster in Fufang dosage form.	54
	p.o. 4 g aqueous extraction of Danshen	t _{1/2α} : 29.4 min t _{1/2β} : 1243.8 min t _{max} : 41.4 min	Two-compartment model	

For definitions of abbreviations, see Table I.

Assessor's comment:

From the tables it can be concluded that the pharmacokinetic profile depends on the preparation/formulations. An assessment is not possible, as the information on the used extract is limited ("fufang Danshen", "funfang danshen dripping pill solution" = combination products, "Danshen extract, 1,58 protocatechuic aldehyde" = no clear information, "Danshen injection" = no clear information).

High-performance liquid chromatography coupled with a linear ion trap-Orbitrap mass spectrometer (HPLC-LTQ-Orbitrap) was used to determine the *in vitro* and *in vivo* chemical and metabolic profiles of Danshen 50% ethanolic extract. Danshen freeze-dried powder was prepared by refluxing the extract twice with 50% (V/V) ethanol (100 g/700 ml for 3 hours the first time, and 100 g/500 ml for 2 hours the second time) after soaking in 50% ethanol for 30 min. Each extract was mixed, filtered, vacuum-evaporated, and freeze-dried. The yield of powdered extract was about 42.3% (w/w). As a result, 118 components were ambiguously or tentatively identified, including 38 original components and 80 transformative components. Among these components, 7 phenolic acids and 28 tanshinones were identified in rat plasma; 17 phenolic acids and 46 tanshinones were tentatively identified in rat urine; 25 phenolic acids and 37 tanshinones were identified in rat faeces; and 1 phenolic acid and 17 tanshinones were identified in rat bile. The metabolic pathway for phenolic acids was mainly methylation/demethylation (11 out of 33), while tanshinones mostly showed hydroxylation (31 out of 85) and methylation/demethylation (19 out of 85). Hydrogenation, sulfation, acetylation, and glutathione conjugation were found to be the possible metabolic pathways of Danshen after oral application in rats (Pang *et al.*, 2018).

Sun *et al.* (2013) studied the pharmacokinetics of different doses of Salvianolic acid A (SAA) in beagle dogs after oral administration. After single-dose oral administration of SAA, the mean peak plasma concentration (C_{max}) values for groups treated with 5, 10 and 20 mg/kg doses ranged from 14.38 to 38.18 $\mu\text{g/L}$, and the mean area under the concentration-time curve ($AUC_{(0-t)}$) values ranged from 38.77 to 130.33 ($\mu\text{g/L}\cdot\text{h}$). SAA showed lack of dose proportionality over the dose range 5-20 mg/kg, based on the power model. However, the increase in systemic exposure with dose appeared linear. The absolute bioavailability was calculated to range from 1.47% to 1.84%. The pharmacokinetic properties of SAA in beagle dogs after oral administration were characterized as rapid oral absorption, quick clearance, and poor absolute bioavailability.

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

3.3.1. Single dose toxicity

Water extract

After intraperitoneal (IP) application of 48 g/kg bw of a water extract in mice, no animals died in 48 hours. Two of 10 animals died after application of 64 g/kg bw (Blaschek *et al.*, 2017).

The LD_{50} of orally administered water-soluble extract of Danshen in mice is 25.8 g/kg (TPPG, 1998 cited in Zhou, 2005). No fatalities were reported in mice intraperitoneally administered 43 g/kg of a decoction of Danshen (Chen and Chen, 2004).

Danshensu (3-(3,4-dihydroxyphenyl) lactic acid), a natural phenolic acid, is isolated from *Salvia miltiorrhiza* root. In the acute toxicity study, danshensu intravenously administered to rats failed to induce any signs of toxicity or mortality up to a maximum practical dosage of 1500 mg/kg body weight. Test substance administered to mice caused dose-dependent general behaviour adverse effects and mortality with the medial lethal dose of 2356 mg/kg. The no observed adverse effect level and the lowest observed adverse effect level were 1835 mg/kg and 2000 mg/kg, respectively (Gao *et al.*, 2009).

3.3.2. Repeat dose toxicity

Subchronic toxicity

No toxic effects were observed in rats orally administered 2.5 g/kg of a water extract of *Salvia miltiorrhiza* daily for 90 days (Zhou *et al.*, 2005).

No adverse effects were observed in mice intraperitoneally administered 2.4 g/kg of a decoction daily for 14 days (Chen and Chen, 2004).

Isolated substances

In two weeks of oral application of daily 10 mg tanshinon in mice, or 50 mg in rat, 2.4 g/kg rabbit (IP) no toxic reaction was seen (Blaschek *et al.*, 2017).

Danshensu (3-(3,4-dihydroxyphenyl) lactic acid) is a natural phenolic acid which is isolated from *Salvia miltiorrhiza* root. In a subchronic study, rats were administered by daily intraperitoneal injection 50, 150, or 450 mg/kg danshensu for 90 days, resulting in no mortality, no changes in body weight, food consumption, hematological and serum chemistry parameters, organ weights, or gross pathology or histopathology. The only treatment-related finding was a transient writhing response observed in the 450 mg/kg group (Gao *et al.*, 2009).

Beagle dogs were treated with danshensu at doses of 17, 50, and 150 mg/kg/day, and observed for 90 days followed by recovery periods. Measurements included clinical observations, body weight, food consumption, temperature, electro-cardiography (EGC), hematology, blood chemistry, urinalysis, gross necropsy, organ weight, and histopathology. No significant adverse effects on these parameters were observed. The only treatment-related finding was a hard knot at injection site observed in the 150 mg/kg group after 2-3 weeks continuous administration. It returned to normal after 3-4 days withdrawal (Li *et al.*, 2009).

3.3.3. Genotoxicity

A not specified extract is not mutagen in the Ames-Test on *Salmonella typhimurium* TA98 and 100, with and without metabolic activation (Blaschek *et al.*, 2017).

3.3.4. Carcinogenicity

Not available.

3.3.5. Reproductive and developmental toxicity

Wang *et al.* (2017b) evaluated the cardiotoxicity and developmental malformations of Tan-IIA using zebrafish normal embryos and dechorionated embryos. After treatment with Tan-IIA in different concentrations for four-day periods, obvious pericardial oedema, spinal curvature, and even missing tails were observed in zebrafish embryos. The LC₅₀ values in the dechorionated embryo group at 72 hours post-fertilization (hpf) and 96 hpf were 18.5 µM and 12.8 µM, respectively, and the teratogenicity was manifested at a concentration of about 1 µM. The main endpoints of teratogenicity were scoliosis, malformation of tail, and pericardium oedema. According to the authors the toxicity of Tan-IIA at high concentrations on zebrafish, indicates a potential risk of its clinical application at an increased dose.

3.3.6. Local tolerance

Not available.

3.3.7. Other special studies

Not available.

3.3.8. Conclusions

Data on specific preparations (according to the declaration guideline) are not available. The available data are scarce and not sufficient for an adequate safety assessment.

3.4. Overall conclusions on non-clinical data

Specific data on pharmacokinetics and interactions are not available.

Non-clinical information on the safety is scarce. The results hint to possible interactions with warfarin, diazepam and CYP isoenzymes.

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

Tests on reproductive toxicity, genotoxicity and carcinogenicity are not available.

4. Clinical Data

4.1. Clinical pharmacology

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

Clinical pharmacological data on specific preparations are not available.

The actions listed in the Chinese Pharmacopoeia (2010) are:

- to activate blood and eliminate stasis;
- unblock the meridian to relieve pain;
- clear the heart and relieve vexation;
- cool blood and disperse abscess.

Cardiovascular and neurological conditions

Water extracts (not specified) of *Salvia miltiorrhiza* have been administered intravenously in 100 cases for the treatment of nerve deafness with together with TCM drugs that promoted blood. The standard dose was 20-30 ml in solution four times daily for two to four treatment courses of two weeks each, separated by 3-day-intervals (Hu *et al.*, 1992).

Hepatitis B

In a dosing study of *Salvia miltiorrhiza* extract injections in patients with hepatitis B, doses of 8, 16, or 24 ml were administered for 60 days. The treatment was associated with a decrease in liver enzyme levels, including alanine aminotransferase and total bilirubin (English abstract, Ye *et al.*, 2005).

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

Danshensu is the only component that has been studied in humans. Based on the available human data, danshensu is absorbed quickly. The half-life for danshensu following sublingual administration is

reported to be much longer than that after oral dosing. However, the actual dosage for danshensu in the sublingual dosage form rises questions (Zhou *et al.*, 2005).

Clinical pharmacokinetic data on specific preparations are not available.

4.2. Clinical efficacy

4.2.1. Dose response studies

Not available.

4.2.2. Clinical studies (case studies and clinical trials)

Angina pectoris

In a review article, Zhou *et al.* (2005) evaluate clinical studies performed in patients with angina pectoris. Nine reports in the Chinese literature compared a Danshen product with isosorbide dinitrate (ISDN) for the treatment of stable angina pectoris or unstable angina pectoris. At least 4 studies had reported clinical comparisons of a Danshen products (unknown preparation, unknown composition, unknown DER) with nitroglycerin. At least 2 published studies on combination products Fufang Danshen spray and 3 published studies on combination products as Fufang Danshen injection have been reported. Numerous randomised controlled trials indicate that this product is at least as effective as sublingual ISDN and comparable to sublingual nitroglycerin. The reliability of the majority of these studies, however, is low due to the small number of subjects and the lack of clearly defined endpoints. Although many studies mentioned randomisation, most of them did not specify the method of randomisation. Only two studies had used the double-blind design, but the details of double blinding were not described. Last, only one study described withdrawals and dropouts. Hence, all the studies have a very low Jadad score in general.

Assessor's comment:

The majority of the listed clinical studies in the review of Zhou et al. (2005) were performed with a combination product (Fufang Danshen) and not with a mono-preparation. The methodology of the studies is not clear and of poor methodological quality. Therefore, they are not introduced in this assessment report. No conclusion can be drawn from the available studies on the efficacy of Salvia miltiorrhiza tea as mono-preparations.

Shao *et al.* (2018) verified the efficacy of Danshen injection as adjunctive therapy in treating angina pectoris in a systematic review and meta-analysis. The major databases were systematically searched for the published randomised controlled trials on Danshen injection until April 2016. A total of 2985 potential studies were found. After scanning the titles and abstracts, 2945 studies were excluded. Finally, 10 studies were selected for quality evaluation. The ten studies were all published in Chinese journals and the publication was between the years 2000 and 2016. Meta-analysis was conducted on the primary outcomes i.e., the improvements in symptoms and electrocardiography (ECG). The dosage ranged from 20 to 30 ml as intravenous drip. Ten RCTs, including 944 anginal patients, were identified in this meta-analysis. Compared with using antianginal agents (β -blockers, calcium antagonists, nitrates, etc.) alone, a Danshen injection combined with antianginal agents had a better therapeutic effect in symptom improvement (odds ratio [OR], 3.66; 95% confidence interval [CI]: 2.50-5.36) and in ECG improvement (OR, 3.25; 95% CI: 1.74-6.08). The authors concluded a Danshen injection as adjunctive therapy seemed to be more effective than antianginal agents alone in treating angina pectoris. However, the chemical composition of the Danshen injection is not specified and more evidence is needed to accurately evaluate the efficacy of Danshen injection because of the low methodological quality of the included RCTs.

Acute ischaemic stroke - Cochrane review (Wu *et al.*, 2007)

In the Cochrane systematic review "Dan Shen agents for acute ischaemic stroke" (Wu *et al.*, 2007) 460 references through electronic searches and hand searches were identified. Of these, 449 irrelevant references were excluded. Eleven potentially eligible trials were identified, of which six trials (Cao, 1994; Mao, 2001; Min, 2004; Pan, 1992; Zhai, 2001; Zhang, 2002 all cited in Wu *et al.*, 2007) were included (494 acute ischaemic stroke patients from 894 participants). Two trials (Geng, 2000; Xu, 1995 all cited in Wu *et al.*, 2007) were excluded because they were not randomised or quasi-randomised trials. Three trials (Gao 1998; Liang 1996; Luo 1998 all cited in Wu *et al.*, 2007) are awaiting assessment because they have not reported the numbers of participants during the acute period of ischaemic stroke. The six included trials were all conducted in China. The average age of participants in the included studies ranged from 56.4 to 76 years. Each trial included more males than females. All six trials have the distinct inclusion criteria and the level of severity of acute ischaemic stroke patients. One trial (Pan, 1992 cited in Wu *et al.*, 2007) reported the numbers of patients with severe stroke.

Study medication according to Wu *et al.* (2007):

- Compound Dan Shen agents were studied in five included trials (Cao, 1994; Mao, 2001; Min, 2004; Pan, 1992; Zhang, 2002 all cited in Wu *et al.*, 2007);
- Dan Shen injection was evaluated in one trial (Zhai, 2001 cited in Wu *et al.*, 2007);
- Two trials (Cao, 1994; Pan, 1992 all cited in Wu *et al.*, 2007) combined Compound Dan Shen injection with snake venom in the treatment group and used snake venom alone in the control group;
- One trial (Zhang, 2002 cited in Wu *et al.*, 2007) combined Compound Dan Shen injection with low-molecular-weight heparin in the treatment group and used low-molecular-weight heparin alone in the control group;
- One trial (Min, 2004 cited in Wu *et al.*, 2007) used Compound Dan Shen injection 20 ml and basic treatment compared with basic treatment alone for 14 days;
- One trial (Zhai, 2001 cited in Wu *et al.*, 2007) compared Dan Shen injection and basic treatment with basic treatment alone for 14 days. Oral Compound Dan Shen dropping pill was used in one study (Mao, 2001 cited in Wu *et al.*, 2007). It was administered three times daily, with a treatment length of 28 days.

Meta-analysis of the six included trials showed that Danshen agents might improve neurological impairment after acute ischaemic stroke. The authors concluded this result should be interpreted with caution because of the poor methodological quality of the included trials and the small numbers of participants.

All the included trials were generally of poor quality. The authors could not be sure that allocation was well concealed and truly random. Three trials (Mao, 2001; Pan, 1992; Zhai, 2001 all cited in Wu *et al.*, 2007) did not mention the method of allocation and concealment. Two trials (Cao, 1994; Zhang, 2002 all cited in Wu *et al.*, 2007) were quasi-randomised controlled trials. Only one trial (Min, 2004 cited in Wu *et al.*, 2007) allocated patients by random number. Therefore, the concealment was not adequate. This could have led to selection bias. The authors concluded it is therefore plausible that Danshen is ineffective, and the apparent benefits are simply due to bias arising from the methodological weaknesses of the studies.

The duration of treatment ranged from 14 days to 28 days in these trials. The period of follow up was not enough to assess the long-term effect of the Danshen products tested. The short follow up cannot confirm the long-term effect of the intervention. Outcome assessment can usually be blinded but whether these trials used a blinded method to assess outcome or not is unclear.

Primary outcome measures should be at the level of activities. All these trials were focused on the level of neurological deficit. Impairment outcome measures were the surrogate endpoint. The most important outcome for patients is their abilities in activities of daily living rather than their neurological deficits. The authors were concerned that none of the trials reported any deaths.

Only two trials reported adverse events in this review, and they did not provide the numbers of participants presenting with these events in each group (Min, 2004; Zhang, 2002 all cited in Wu *et al.*, 2007). They could not draw a conclusion on the safety of Danshen preparation tested, because snake venom and low-molecular-weight heparin can also have an adverse action, such as thrombocytopenia. Danshen may increase the risk of bleeding; this will only be adequately quantified within large-scale clinical trials.

A definite conclusion regarding the efficacy and adverse events associated with Danshen could not be drawn from review due to the limited number of trials identified, the limited duration of treatment, and inadequate recording and reporting of adverse events.

Risk factors of atherosclerosis

Van Poppel *et al.* (2015) performed a randomised, placebo-controlled, double-blind crossover study with Danshen (water-extract of the *Salvia miltiorrhiza* root, no further information) or placebo for 4 consecutive weeks. There was a wash out period of 4 weeks. Twenty-three of the 36 initially screened subjects underwent randomisation and were enrolled in the study. Of the 20 analysed participants, 11 received placebo first. Inclusion criteria were age 40-70 years, hyperlipidemia, and hypertension.

Danshen (water extract) increased LDL and total cholesterol levels compared to placebo. LDL cholesterol levels were 3.82 ± 0.14 mmol/l after Danshen and 3.52 ± 0.16 mmol/l after placebo treatment (mean \pm SE; $p < 0.05$ for treatment effect corrected for baseline). The water extract of Danshen could theoretically increase these risks to 20.3% and 37.5% for male non-smokers and smokers respectively (calculated for Dutch population).

Danshen treatment had no effect on blood pressure. These results were further substantiated by the observation that Danshen had an effect neither on endothelial function, nor on markers of inflammation, oxidative stress, glucose metabolism, haemostasis and blood viscosity.

The selection of participants was based on evidence-based criteria of increased cardiovascular risk, i.e., increased LDL cholesterol or triglyceride levels and hypertension. According to the TCM theory, Danshen is used to treat "blood stasis". It is not known what diagnosis according to evidence-based medicine corresponds with this TCM qualification. The results of four weeks of treatment with the herbal product Danshen (water extract) do not support the use to treat risk factors of atherosclerosis.

Acute myocardial infarction (AMI) – Cochrane review (Wu *et al.*, 2008)

The Cochrane systematic review "Danshen (Chinese medicinal herb) preparations for acute myocardial infarction" (Wu *et al.*, 2008) analysed randomised controlled trials and other controlled trials lasting at least 7 days. All of the studies were published in Chinese. The initial search yielded 269 records. After scanning the title and abstract, 49 of these appeared to be comparative studies of Danshen preparations. Of the 49 published articles initially identified, 43 articles were excluded for several reasons (studies not randomised; outcome of interest was not reported; unclear description of interventions; multiple versions of studies; combination studies etc.).

Two of the remaining studies used the same interventions, where danshen was the principal ingredient of a 'kangxingeng heji' decoction and 'yiqi huoxue' injection. Within the first 6 hours of enrolment, AMI patients were treated with a 10 ml 'yiqi huoxue' injection together with a 250 ml infusion of 5% glucose infusion given by intravenous drip, which 1 week later was changed to taking 'kangxingeng heji' orally. Both the 'quyu huatan xiezhu' and 'yiqi huoxue' formulations in the next study contain the

basic elements Danshen, chishao and honghua. Another study compared huangqi injection with Danshen injection versus placebo, and shenmai injection with danshen injection versus placebo, respectively, while another study used intravenous danshen injection. The sixth study used isosorbide dinitrate in the experimental group, and isosorbide dinitrate plus Danshen injection in the control group.

Various Danshen formulations used in the clinical studies according to Wu *et al.* (2008):

- Kangxingen Heji (Danshen, Cishao, Yujin, Huangqi, Dangshen, Huangjing): Oral intake while intravenous drip both Yiqi injection and Huoxue injection;
- Yiqi injection (Huangqi, Dangshen, Huangjing): Intravenous drip with Huoxue injection;
- Huoxue injection (Cishao, Danshen, Yujin): Intravenous drip with Yiqi injection;
- Fufang Danshen injection (Danshen, Jiangxiang) Intravenous drip;
- Huangqi injection (Huangqi): Intravenous drip;
- Shenmai injection (Hongshen, Maidong): Intravenous drip;
- Quyu huatan xiezhuo fang (Jioudahuang 6-10 g, Quanguai 10-15 g, Jioubai 10 g, Zhike 10 g, Yujin 10 g): Oral the water decoction, 2 times a day in one week;
- Yiqi Huoxue fang (Huangqi 10-20 g, Dangshen 10-15 g, Danshen 15-20 g, Cishao 10-15 g, Honghua 6-10 g, Chenpi 6-10 g): Oral the water decoction, 2 times a day in one week.

Names (Latin) of the herbs of included studies:

Danshen (*Salviae miltiorrhizae radix*), Chishao (*Paeoniae radix rubra*), Yujin (*Curcumae radix*), Huangqi (*Astragali radix*), Dangshen (*Codonopsis radix*), Huangjing (*Polygonati rhizoma*), Jiangxiang (*Lignum dalbergiae odoriferae*), Hongshen (*Ginseng radix*), Maidong (*Ophiopogonis radix, tuber*), Jioudahuang (*Rhei radix et rhizoma*), Quanguai (*Trichosanthis fructus*), Xiebai (*Allii macrostemi bulbis*), Zhiqiao (*Aurantii fructus*), Chenpi (*Pericarpium citri reticulatae*).

Six studies comprised of 2368 participants were included. Only one trial reported was judged to be a genuine RCT and showed no statistically significant difference in reduction of total mortality (Peto OR 0.55, 95% CI 0.23 to 1.32), but a quasi-RCT (in Chen, 1984a cited in Wu *et al.*, 2008), reported a reduced total mortality (Peto OR 0.42, 95% CI 0.23 to 0.77). Pooling these trials yielded an approximate halving of mortality in those patients treated with Danshen preparations plus usual care compared with usual care alone (Peto OR 0.46, 95% CI 0.28 to 0.75).

Another study described reported a single death in both the shenmai injection plus Danshen injection group and the huangqi injection plus Danshen groups, with eight deaths in each control group. There was a borderline statistically significant difference in the former (Peto OR 0.25, 95% CI 0.06 to 0.97), but not the latter (Peto OR 0.32, 95% CI 0.08 to 1.34). In the retrospective study (Wang, 1994 reported in Wu *et al.*, 2008), an analysis of total mortality did not show a significant advantage for Danshen (Peto OR 0.78, 95% CI 0.59 to 1.03; data not shown). Another study reported cites the number of deaths in the study groups in the results section, although mortality was not listed as an outcome. Deaths in the isosorbide dinitrate plus Danshen group numbered six of 24 patients, and in the isosorbide dinitrate group two of 24 patients, a non-significant difference (Peto OR 3.05, 95% CI 0.71 to 13.21).

Assessor's comment:

In none of the clinical studies, the tested medication was an oral tea or another oral mono-preparation of Salvia miltiorrhiza. From the six studies included in this review, the authors concluded that, although Danshen compound may possibly have beneficial effects on AMI mortality compared to routine treatments, the evidence must be considered inconclusive. This is because of the small number of available studies and the poor quality. Many of the constituents of the pharmacologically prepared drugs used in trials cannot be clearly specified. None of the trials used concealment of allocation. None

of the included studies mentioned dropouts, performed an intention-to-treat analysis or assessed compliance. The comparator treatment or 'basal or routine treatment' was often sub-optimal. A large number of the trials used self-prepared formulation or hospital-made preparations for treating AMI and many of the constituents of the pharmacologically prepared drugs used in trials cannot be clearly specified. In addition, the safety of Danshen preparation was considered as unproven.

Awaad *et al.* (2010) conclude that although numerous clinical studies have demonstrated that specific Danshen products in China are effective and safe, most of these lack sufficient quality, because of poor design, small sample size and use of poorly defined products of uncertain composition and consistency (Hu *et al.*, 2005).

Urolithiasis

Chen *et al.* (2018) conducted a nationwide retrospective Cohort Study in Taiwan with treatment of urolithiasis with [not known] preparations containing *Salvia miltiorrhiza*. They used the LHID 2000 (Longitudinal Health Insurance Database 2000) which contains medicine information between 1996 and 2013. All cases diagnosed with calculus from January 2000 to December 2010 and aged ≥ 18 years were the study cohort population. There were 8,536 Danshen-users and non-Danshen-users in each cohort.

The proportion of baseline comorbidities in Danshen-users was higher than that in non-Danshen-users. The mean (median) follow-up period for Danshen-users and non-Danshen-users was 6.27 (5.98) years and 5.09 (4.86) years, respectively.

The incidence of calculus surgical treatment in the Danshen-users was less than that in the non-Danshen-users: 1.071% in 1,000 person-years (200 people followed up for 5 years) and 3.142% in 1,000 person-years, respectively. The incidence of any bleeding disorder in the Danshen users was less than that in the non-Danshen-users (1.708% in 1000 person-years and 2.577% in 1,000 person-years, resp.). The authors concluded that the results of the study are limited because of limited patient number, a surrogate outcome instead of recurrence and unknown stone site and number.

Assessor's comment:

It is not known, what preparations are used in the retrospective study. The Danshen dose per year was in 5838 cases less than 1096 g per year, corresponding less than 3 g per day, typical for TCM-decoctions of multi-combination preparations/herbal mixtures. The medication was used several years. The effect of the co-medication (other drugs) is not analysed in the study. From this retrospective, statistical analysis, no conclusions can be drawn for clinical use in urolithiasis.

Psoriasis

Deng *et al.* (2014) assessed in a meta-analysis the efficacy and safety of oral forms of phytotherapy in psoriasis. All 1614 clinical studies were identified. Ten randomised controlled trials that compared a plant-based intervention with placebo or a pharmacotherapy in the treatment of psoriasis vulgaris and used Psoriasis Area Severity Index (PASI) as an outcome measure were included. In total 658 participants completed the 10 studies. Superiority to placebo was found in 2 out of 3 studies. In 6 out of 7 studies, the effect of the phytotherapy was reported as comparable to the pharmacotherapy in the short term when assessed as PASI 50. The safety of the phytotherapies was discussed.

The analysed preparations in the studies were:

Single plants: Neem leaves (*Azadirachta indica*) and *Tripterygium wilfordii* extract.

The multi-ingredient formulae are: Whentonghuaya formula, Lanchun Quingre decoction, Yinxiebing formula, Huoxue Sanyu Xiaoyin decoction, Quingre Jiedu decoction, Kangjin No1 formula, Haiting decoction and compound Zeqi granule.

Clinical studies with a 50% clinical efficacy rate and above were included in the analyses. The most commonly used plants were *Oldenlandia diffusa*, *Rehmannia glutinosa* and *Salvia miltiorrhiza*. The authors concluded these three plants, and their active constituents appear to warrant further research attention in the search for future medications for psoriasis.

Assessor's comment:

No clinical studies were performed with Salvia miltiorrhiza as mono-preparations. Results regarding efficacy and safety of the different combination preparations cannot be transferred to the mono-preparations.

Osteoporosis

Guo *et al.* (2014) reviewed about 130 research papers with the aim to provide a comprehensive overview about the historical TCM interpretation of the action of *Salvia miltiorrhiza* in osteoporosis. Thirty-six clinical trials were identified which used *Salvia miltiorrhiza* in combination with other herbs and components to treat post-menopausal, senile, and secondary osteoporosis. On average the trials were characterized by high efficacy (>80%) and low toxicity problems. The quality of the studies was limited because of small patient samples, short treatment duration, frequent lack of detailed numerical data, and no clear endpoints. The authors concluded the review highlights the anti-osteoporotic potential of *Salvia miltiorrhiza* in clinical applications and the potential of the herb to provide potent compounds targeting specific pathways in bone resorption and bone formation.

Assessor's comment:

No clinical studies were performed with Salvia miltiorrhiza as mono-preparation. Results regarding efficacy and safety cannot be concluded from clinical studies of Salvia miltiorrhiza in combination with other herbs and components and with lack of sufficient quality.

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

Not available.

4.4. Overall conclusions on clinical pharmacology and efficacy

In reviews, of clinical (Chinese) studies for acute ischaemic stroke 460 references and for acute myocardial infarction 269 references were identified. Only few studies were randomised or quasi randomised. Therefore, the majority of the studies were excluded from the assessment. From the few remaining clinical studies, no plausibility or well-established use (WEU) for a herbal preparation can be concluded. Most of these studies lack sufficient quality, because of poor design, small sample size and use of poorly defined products of uncertain composition and consistency. Variation between formulations and batches of treatments are inevitable consequences of traditional Chinese medicine. This variation is a factor that may contribute to any heterogeneity between different study results. The overall treatment concept for traditional Chinese medicine is different from that used in Western medicine.

In lack of well-conducted trials in humans and less scientific evidence, no specific oral dose can be recommended for adults and no recommendations can be made for the safe use of Danshen in children (Awaad *et al.*, 2010).

5. Clinical Safety/Pharmacovigilance

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

Not available.

5.2. Patient exposure

No data available from Europe for the medicinal use.

Hsing-Yu *et al.* (2014) analysed the 57,315 Chinese Herbal Medicine prescriptions made for 20,141 TCM users during 1998–2008 for dysmenorrhea in Taiwan. A total of 647 different herbals were used. Each prescription contained 5.32 CHM on average, and more than 90% prescriptions contained more than two CHM. *Salvia miltiorrhiza* was used in 9% of the prescriptions. No information is available on the composition of the 10 percent of twice-preparations and mono-preparations (calculated 64 from the 647 different CHM).

Assessors' comment:

The clinical use as mono-preparations in the indication dysmenorrhea cannot be concluded from the data. The results comply with the known TCM TU; in TCM on average combinations of herbal teas are used. Data on patient exposure for mono-preparations for safety evaluation are not available.

The data give no validity and are not relevant for the use as mono-preparation in a European indication outside the European Union.

5.3. Adverse events, serious adverse events and deaths

Danshen may cause hypotension and dizziness. It should be used with caution in patients with known hypotension and under antihypertensive medication (Awaad *et al.*, 2010).

A meta-analysis of *Salvia miltiorrhiza* studies and case reports for the treatment of heart attack indicated that adverse events reported in association with *Salvia miltiorrhiza* and funfang Danshen (combination product) were anaphylactic reaction, abdominal discomfort, and loss of appetite, pruritus, low blood pressure, dizziness, and headache and with excessive use an increased risk of bleeding after intravenous use. Bleeding events were bleeding in the skin or mucous membranes or excessive menstrual bleeding. An increase in serum aminotransferase has also been reported. Details on doses and preparations were not included with the review (Wu *et al.*, 2008).

In a meta-analysis of intravenously administered Danshen preparations of not specified aqueous extracts in patients for acute ischaemic stroke, no differences in adverse events between treatment and control groups were reported (Wu *et al.*, 2008).

In a controlled clinical study with a water-extract of the *Salvia miltiorrhiza* root (no further information) 20 participants were analysed (Poppel *et al.*, 2015). The most reported side effects during Danshen treatment were headache (n = 5), dizziness (n = 3), change in stool frequency (n = 3) and flatulence (n = 2). One serious adverse event occurred during Danshen treatment: a peripheral facial nerve paralysis. As only 20 participants were analysed, a high number of adverse events occurs.

Case reports of adverse events:

Bensky *et al.* (2004; cited in AHPA, 2013) reported that a small percentage of persons taking *Salvia miltiorrhiza* might experience dry mouth, dizziness, nausea, weakness, shortness of breath, numbing or coldness of the hands, anxiety or tachycardia. Allergic reactions have been reported. One case of liver damage and two cases of shock have been reported after injection of preparations (details on doses and products not known).

Cases with abdominal discomfort or decreased appetite have been reported (Zhou *et al.*, 2005).

Allergic reactions such as rash, itching or shortness of breath can occur to *Salvia miltiorrhiza* or its constituents (Awaad *et al.*, 2010).

Su *et al.* (2015) stated that in recent years with its wide range of application of *Salvia miltiorrhiza* products an increased number of side effects such as abdominal discomfort, decreased appetite,

convulsions, dystonia syndrome, and allergy have been reported. However, once stopping the use of the products, the side effects are relieved.

5.4. Laboratory findings

A (unspecified) water extract of Danshen given for 4 consecutive weeks, with a wash out period of 4 weeks, had no effect on blood pressure, on endothelial function nor on markers of inflammation, oxidative stress, glucose metabolism, haemostasis and blood viscosity in a cross over clinical study with 20 analysed patients (Poppel *et al.*, 2015).

5.5. Safety in special populations and situations

Not available.

5.5.1. Use in children and adolescents

In lack of well-conducted trials in humans and less scientific evidence, no specific oral dose can be recommended for adults and no recommendations can be made for the safe use of Danshen in children (Awaad *et al.*, 2010).

5.5.2. Contraindications

Hypersensitivity to the active substance.

A reference text on traditional Chinese medicine indicates that *Salvia miltiorrhiza* is contraindicated in pregnancy (Bensky *et al.*, 1986).

Salvia miltiorrhiza should be used with caution in any situation associated with bleeding, including menstruation, nose bleedings, of blood in the urine, or if blood is expected during coughing Chen and Chen, 2004, cited in AHPA, 2013).

Assessor's comment:

There are concerns with regard to the product's clinical safety. Preparations of Salvia miltiorrhiza may be harmful by virtue of their composition and therefore these products are not eligible for a simplified registration. Important is the fact that harm can result in situations with bleeding, as menstruation.

5.5.3. Drug interactions and other forms of interaction

Clinical interactions with warfarin are reported by Chan (2001), Tam *et al.* (1995), Yu *et al.* (1997) and Izzat *et al.* (1998), Upton and Romm (2010).

In the Botanical Safety Handbook of the AHPA 2013, *Salvia miltiorrhiza* is classified in the interaction class C, herbs for which clinically relevant interactions are known to occur.

Over-anticoagulation and bleeding

Tam *et al.* (1995) reported a case of adverse interactions between Danshen and warfarin in a patient with gastric carcinoma. The absence of other precipitation factors and the temporal relationship between the administration and the associated increased INR from 2.0 to >5.5 were strongly suggestive of an adverse interaction.

Three cases of over-anticoagulation and bleeding were reported when receiving warfarin therapy with Danshen. Because of the risk of potential pharmacokinetic and pharmacodynamic interactions, Danshen should be avoided in patients taking warfarin (Zhou *et al.*, 2005).

Salvia miltiorrhiza has been shown to slow the metabolism of warfarin, increasing plasma levels of the drug (Izzat *et al.*, 1998; Lo *et al.*, 1992).

Several published case reports have suggested an interaction between *Salvia miltiorrhiza* and warfarin. In all cases, the patients were on long-term warfarin therapy and were taking other drugs and/or herbs. They all experienced increased INR, temporally related to the consumption of Chinese *salvia*. No details were provided (Chan, 2001).

Danshen may increase the risk of bleeding when taken with other drugs sharing this side effect. Examples include aspirin, anticoagulants such as warfarin or heparin, anti-platelet drugs like clopidogrel and non-steroidal anti-inflammatory drugs such as ibuprofen or naproxen (Awaad *et al.*, 2010).

Cardiac glycosides and hypotensive drugs

A review of the literature on the use of *Salvia miltiorrhiza* suggested that this herb may act synergistically with digitalis, cardiac glycosides, and hypotensive drugs and that the dosage of these drugs may need to be modified if used concurrently with *Salvia miltiorrhiza* (Wu *et al.*, 2008).

Danshen may increase toxicity from digoxin and influence blood levels of digoxin. Excessive hypotension may occur if taken with antihypertensives, such as ACE-inhibitors like captopril or lisinopril and beta-blockers like atenolol or propranolol. In combination with beta-blockers bradycardia may result (Hu *et al.*, 2005).

Effect on cytochrome P450 enzymes

An induction and/or inhibition of cytochrome P450 enzymes has been shown both *in vitro* and *in vivo*. The clinical relevance not known (Zhou *et al.*, 2005).

A sequential, open-label, two-period pharmacokinetic interaction study design was used to compare midazolam pharmacokinetic parameters before and after 14 days of administration of Danshen tablets (extract not declared). Twelve healthy volunteers received a single oral dose (15 mg) of midazolam followed by Danshen tablets (4 tablets orally, 3 times a day) for 14 days. On the last day of the study, they received four Danshen tablets with a 15 mg midazolam tablet and plasma concentrations of midazolam and its corresponding metabolite 1-hydroxymidazolam were measured prior to and after the administration of Danshen tablets periodically for 12 hours. The findings suggest that multiple dose administration of Danshen tablets may induce CYP3A4 in the gut. Accordingly, caution should be taken when Danshen products are used in combination with therapeutic drugs metabolized by CYP3A (Qiu *et al.*, 2010).

5.5.4. Fertility, pregnancy and lactation

A reference text on traditional Chinese medicine indicates that *Salvia miltiorrhiza* is contraindicated in pregnancy (Bensky *et al.*, 1986).

The use is contraindicated in pregnancy and breastfeeding because of anticoagulant activity bearing the risk of miscarriage or bleeding. Additionally, effects on the foetus or nursing infants are unknown (Awaad *et al.*, 2010).

5.5.5. Overdose

No data available for specific preparations.

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

Patients should be advised not to drive or operate machinery, as it may cause drowsiness (Awaad *et al.*, 2010).

5.5.7. Safety in other special situations

Not applicable.

5.6. Overall conclusions on clinical safety

In Europe there is one medicinal product (registered 2016). No information exists from official pharmacovigilance centres.

From TCM use anaphylactic reaction, abdominal discomfort, loss of appetite, pruritus, low blood pressure, dizziness, and headache and an increased risk of bleeding are known. Bleeding events were bleeding in the skin or mucous membranes or excessive menstrual bleeding are reported.

Interactions with warfarin, digitalis, cardiac glycosides, and hypotensive drugs are known from TCM use. Danshen may increase the risk of bleeding when taken with other drugs sharing this side effect. Examples include aspirin, anticoagulants such as warfarin or heparin, anti-platelet drugs like clopidogrel and non-steroidal anti-inflammatory drugs such as ibuprofen or naproxen.

In the Botanical Safety Handbook of the AHPA 2013, *Salvia miltiorrhiza* is classified in the interaction class C, herbs for which clinically relevant interactions are known to occur.

As in TCM usually low doses and combinations are used, from high dosed mono-preparations more extensive adverse events and interaction are expected.

There are clinical safety concerns regarding bleedings after intravenous use.

The clinical safety for high dosed mono-preparations is not established.

6. Overall conclusions (benefit-risk assessment)

From available clinical studies, no plausibility or WEU use for a herbal preparation can be concluded. Most of these lack sufficient quality, because of poor design, small sample size and use of poorly defined products of uncertain composition and consistency. The requirements for WEU are not met.

The requirements for TU, self-medication character, specified strength/posology, and appropriate route of administration, period of TU and plausibility are not met.

Annex

List of references