EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

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Committee on Herbal Medicinal Products (HMPC)

Assessment report on *Paeonia lactiflora* Pall. and/or *Paeonia veitchii* Lynch, radix (Paeoniae radix rubra)

Final

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

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

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Paeonia lactiflora* Pall. and/or *Paeonia veitchii* Lynch or a mixture, radix (Paeoniae radix rubra)
Herbal preparation(s)	Not applicable
Pharmaceutical form(s)	Not applicable
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1. Introduction

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

- Herbal substance(s)

Peony red root is not yet covered by the current European Pharmacopoeia 8th edition (2015). A draft monograph of the EUROPEAN PHARMACOPOEIA COMMISSION Pharmeuropa 27.1 defines the herbal substance. The herbal substance is described only in the Pharmacopoeia of the People´s Republic of China (2010) and the Taiwan Herbal Pharmacopoeia. *Paeonia veitchii* Lynch is not found in the Japanese Pharmacopoeia and not in the WHO monographs.

Pharmeuropa 27.1 (draft): PEONY ROOT, RED; Paeoniae radix rubra; Chishao; 赤芍 is the whole or fragmented, dried root of *Paeonia lactiflora* Pall. or *Paeonia veitchii* Lynch or a mixture of the two, with rhizome and rootlets removed. Content: minimum 1.8% of paeoniflorin (C23H28O11; Mr 480.5) (dried herbal substance). The absence of Paeonol is part of the identification.

Pharmacopoeia of the People´s Republic of China (2010): Paeonia radix rubra; Red peony root is the dried root of *Paeonia lactiflora* Pall. or *Paeonia veitchii* Lynch (Ranunculaceae). The herbal substance is collected in spring and autumn, removed from rhizome, rootlet and dried in the sun.

The pharmacopoeia of Japan XVI (2011): Peony Root is the root of *Paeonia lactiflora* Pallas (Paeonaceae).

The Taiwan Herbal Pharmacopoeia: PAEONIAE RUBRA RADIX 赤芍 Chih Shao / Chi Shao/ Red peony root is the dried root of *Paeonia lactiflora* Pall. or *Paeonia veitchii* Lynch (Fam. Ranunculaceae).

WHO Monographs on Selected Medicinal Plants 1999: Radix Paeoniae is the dried root of *Paeonia lactiflora* Pallas (Paeonaceae) with reference to the Pharmacopoeia of Japan and the Pharmacopoeia of the People´s republic of China.

Main characteristic constituents of the herbal substance

According to Blaschek *et al*. (2013) the herbal substance contains monoterpenes and monoterpenes glycosides (as paeoniflorin, albiflorin, oxipaeoniflorin), gallotannines, triterpenoids as ß-sitosterol, daucosterol, benzoic acid, carbohydrates.

There is a great variation in the content of constituents depending on the harvest period and usage of peeled or unpeeled herbal substance.

Paeonols are found in the root bark of *P. suffruticosa*, which is not part of this herbal substance.

Liu *et al*. (2009) separated and characterized the major constituents in Paeoniae radix rubra by fast high-performance liquid chromatography coupled with diode-array detection and time-of-flight mass spectrometry. A total of 26 components were screened and identified including 11 monoterpene glycosides, 11 galloyl glucoses and 4 other phenolic compounds.

Potential confounding materials

Paeonia lactiflora var. *trichocarpa* Stern; *Paeonia japonica* (Makino) Miyabe & H. Takeda; *Paeonia suffruticosa* Andrews syn.; *Paeonia moutan* Sims (Herbal Medicines Compendium, 2013).

The Pharmeuropa 27.1 contains a test of thin-layer chromatography for identification of the herb and specification of the absence of paeonol.

White peony is distinguished from red peony by the process of peeling, cooking in water and drying of the root of *Paeonia lactiflora*. (Bruneton, 1995).

- Herbal preparation(s)

Comminuted herbal substance (Pharmeuropa 27.1)

Assessors 'comment:
Potential confounding is possible as a lot of different definitions of "peony radix" exist in parallel and the published non clinical and clinical literature does not always gives exact information. For example the pharmacopoeia of Japan XVI (2011) and the WHO does not distinguish red and white peony.

In conclusion, in cases where publications refer to "white" ore "red" peony they are only cited in the respective monograph and supporting documents. In cases where the publications refers to "Paeonia lactiflora" or "peony root" in general, the literature is considered in both monographs and supporting documents.

- 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 using the DIMDI (Deutsches Institut für Medizinische Dokumentation und Information)-database information system (in ZT00; CC00; CCTR93; CDSR93; DAHTA; CDAR94; AR96; GA03; GM03; INAHTA; MK77; NHSEED; ED93; ME60; CV72; CB85; AZ72; IA70; BA70; EM47; DH64; EA08; DD83; II78; IS74), the database of the division for Complementary and Alternative Medicines of the Federal Institute for Drugs and Medical Devices (BfArM) and information received from other member states or submitted as response to the call for scientific data by EMA. Additional hand searches were performed in books on herbal medicines and plant monographs in the BfArM own library. The bibliographies of included trials and other relevant reviews were searched to identify further potential trials.

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 Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Italy, Latvia, Lichtenstein, Lithuania, Luxemburg, Malta, The Netherlands, Norway, Poland, Portugal, Romania, Slovak Republic, Slovenia, Spain and Sweden no authorised or registered medicinal products are on the market.

In the United Kingdom, one traditional herbal medicinal product containing an ethanolic tincture of red peony root was registered in 2011. The national decision took into account usage of herbal

preparations by herbal practitioners in the United Kingdom. Data available to the HMPC are not appropriate to include the herbal preparation in a European Union monograph.

Additionally, this ethanolic tincture is not a preparation reported to be used in TCM in China, where only aqueous extracts or the powder (decoction) are used. As it can be seen by the comparison of the indications of red and white peony root in Table 2, herbal practitioners used the tincture of peony root in an indication that is not mentioned in the Pharmacopoeia of the People´s Republic of China (2010).

The herbal substance is used in Traditional Chinese Medicine (TCM) combinations and homeopathic medicinal products, and, from the data available, they are not sufficient to fulfil the legal requirements of Directive 2004/24/EC as regards single active substances in traditional herbal medicinal products and according to the principles applied for the establishment of EU monographs.

Table 1: Overview of data obtained from marketed medicinal products

Active substance	Indication	Pharmaceutical form	Regulatory Status
Red peony root tincture (1:3), extraction solvent ethanol 25% V/V	A traditional herbal medicinal product used for the symptomatic relief of hot flushes associated with the menopause	3 times daily 1 ml (20 drops) Duration of use: no restriction (long term use in menopause)	THMP 2011, UK

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

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

Not applicable

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

Not applicable

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

Red peony root is traditionally used in various TCM combination products in China and Taiwan (up to 12 different substances). Table 2 shows that whole ore fragmented, dried root of *Paeonia lactiflora* (Paeoniae radix rubra) has a different use in the TCM compared with peeled, boiled and dried root (Paeoniae radix alba).

Klein *et al.* (2010) analysed prescription patterns of Chinese Medicinal Herbs in Switzerland from the database of Lian Chinaherb AG. Baishao (Paeoniae radix alba) was one of the most commonly used herbs and was used in 41.4% of the TCM prescriptions. Paeoniae radix rubra was reported to be used less frequently.

Table 2: Comparison of the TCM-use of white and red peony root

Pharmacopoeia of the People´s Republic of China (2010): indication for **peony root, white**	Pharmacopoeia of the People´s Republic of China (2010): indication for **peony root, red**
Indications (TCM)	Indications (TCM)
Blood deficiency and sallow complexion	Heat entering nutrient-blood aspects
Menstrual irregularities	Macula and papula caused by warm toxin
Spontaneous sweating	Hematemesis and epistaxis
Night sweating	Red painful swelling eyes
Hypochondriac pain	Liver depression with hypochondriac pain
Abdominal pain	Amenorrhea and dysmenorrhea
Spasm and pain of limbs	Abdominal pain caused by aggregation and accumulation
Headache and dizziness	Injuries from falls
	Swelling abscess
	Sore and ulcer

Feng et al. (2010) and Zhang JJ (2013a) reported, Paeoniae radix rubra and Paeoniae radix alba are both TCMs commonly used in China. Although being of similar origins, the clinical efficacies of the two medicines are regarded different. The two TCMs are regarded as two independent medicines.

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

Red peony root is used in Chinese herbal medicines. Few European pharmacopoeias or accepted collections in the European countries have introduced red peony root. Referring to the medicinal use they hint to references on the uses as TCM or give a public statement for the European medicinal use in the EU.

Table 3: Overview of historical data

Herbal preparation	**Documented use / Traditional use**	**Pharmaceutical form / posology**	**Reference**
Comminuted herbal substance	Actions (TCM): to clear heat and cool blood, dissipate stasis and relieve pain Indications (TCM): • Heat entering nutrient-blood aspects • Macula and papula caused by warm toxin • Hematemesis and epistaxis • Red painful swelling eyes • Liver depression with hypochondriac pain • Amenorrhea and dysmenorrhea • Abdominal pain caused by aggregation and accumulation • Injuries from falls • Swelling abscess • Sore and ulcer	Daily dose: 6-12 g	Pharmacopoeia of the People´s Republic of China (2010)
Comminuted herbal substance	Actions (TCM) Chih Shao / Chi Shao: Blood-regulating medicinal (Blood-activating and stasis-dispelling medicinal)	Daily dose: 3-12 g	Taiwan Herbal Pharmacopeia

Herbal preparation	Documented use / Traditional use	Pharmaceutical form / posology	Reference
Dried root of *Paeonia lactiflora* P. but not *Paeonia veitchii* Lynch.			JP XVI
Comminuted herbal substance and preparations thereof	Negative-monograph for paeonia radix (public statement)		Kommission E (1988)
Comminuted herbal substance	The use according ChinP IX is not recommended as the efficacy is not proved		Blaschek *et al*. (2013)
Dried root of *Paeonia lactiflora P.* but not *Paeonia veitchii* Lynch.	Uses supported by clinical data: None Uses described in pharmacopoeias and in traditional systems of medicine: As analgesic, anti-inflammatory and antispasmodic in the treatment of amenorrhoea Dysmenorrhoea Pain in the chest and abdomen Dementia Headache Vertigo Spasm of the calf muscles Liver disease Allergies Anticoagulant	Daily dose: 6-15 g rude plant material, powder, decoction	WHO monographs (1999)
P. officinalis L., Radix paeoniae	Antispasmodicum Use in folk medicine for asthma, cramps and gout	No information	Hoppe (1981)
P. officinalis L., Radix paeoniae	Antispasmodicum, cramps of the stomach, antiepilepticum	tincture of wine (1:5) Single dose: 5-10 drops	Madaus (1976)
Paeoniae radix of *P. rubra* Hort.	No actual use, earlier as antiepilepticum	No information	Frerichs (editor) Hager (1938); List (editor) Hager (1977)

2.3. Overall conclusions on medicinal use

No herbal medicinal product or traditional herbal medicinal product with red peony root could be identified on the marked in the EU before 2011.

In conclusion, there is no single herbal preparation for which 15 years of medicinal use in the EU could be confirmed from literature or based on the regulatory status overview. The requirement laid down in Article 16a(1)(d) of Directive 2001/83/EC that “the period of traditional use as laid down on Article 16c(1)(c) has elapsed”, is not considered fulfilled according to available data and the principles applied for the establishment of European Union monographs.

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

Antispasmodic activity

In-vitro

Paeoniflorigenone

Kimura *et al*. (1984) found blocking effects of paeoniflorigenone, isolated from cold aqueous extracts of peony root, on neuromuscular junctions of frogs and mice. The suppressing effect by paeoniflorigenone (300 µg/ml) was not reversed by neostigmine. Paeoniflorigenone (100 µg/ml) inhibited weakly acetylcholine (5 µg/ml) induced slow contractions. Paeoniflorigenone decompressed on heating, therefore, the authors concluded that it may not necessarily play a predominant role in the clinical therapy of muscle pain.

Assessor's comments
For in-vitro data, a physiological correlation is not possible. The tested substance paeoniflorigenone is only one component of the root. The results do not highlight any specific activity for the aqueous extract of the whole root.

Anti-inflammatory activity and arthritis activity

In–vitro

Total glycosides of *Paeonia lactiflora* Pall. root (TGP)

(ethanol reflux, ethylacetat extraction, dried powder, consists of 90% paeoniflorin)

Chang Y *et al*. (2009) showed that TGP (312.5 µg/ml) significantly inhibited the proliferation of synoviocytes, decreased the production of IL-1, TNF-α, PGE2, and elevated the level of cAMP. The authors concluded the results indicated that TGP might exert its anti-inflammatory effects through inhibiting the production of pro-inflammatory mediators in synoviocytes of rats with collagen induced arthritis (CIA), which might be associated with its ability to regulate cAMP-dependent EP2/EP4-mediated pathway.

Paeoniflorin

Chen JY *et al*. (2012) showed that paeoniflorin inhibited proliferation of fibroblast-like synoviocytes through suppressing g-protein-coupled receptor kinase 2. As compared with the normal rats, the expression or g-protein-coupled receptor kinase 2 increased significantly in fibroblast-like synoviocytes from CIA-rats. After treatment with paeoniflorin ($10^{5\text{ -}8}$ mol/l) the expression of g-protein-coupled receptor kinase 2 in fibroblast-like synoviocytes (*in-vitro*) decreased evidently.

Jiang B *et al*. (2012) examined the inhibitory effect of paeoniflorin on the inflammatory vicious cycle between adipocytes and macrophages. The authors concluded, paeoniflorin exhibits anti-inflammatory properties by inhibiting the vicious cycle between 3T3-Li-adipocytes and RAW 264.7-macrophages.

In-vivo

Paeoniflorin

Li *et al*. (2012) treated collagen-induced arthritis rats with paeoniflorin (25, 100 mg/kg) intragastrically from day 18 to 38 after immunization. In CIA-rats the levels of anti-CII antibody, IgA, IgG and IgM in serum enhanced, BAFF/BAFF-R, PI3K, p-Akt and m-TOR were highly expressed. Paeoniflorin (100 mg/kg) decreased arthritis score, relieved ankle and paw swelling, improved spleen histopathology and decreased the levels of IgA, IgM, IgG and anti-CII antibody.

Wu *et al*. (2014) determinated paeoniflorin in rat plasma, following the oral administration of Paeoniae radix alba aqueous decoction, showing to be absorbed. In the pharmacological experiment paeoniflorin was administered for 4 weeks in a low, middle and high dose (0.5; 1 and 3 mg/kg per day) in rats with collagen-induced arthritis. Paeoniflorin significantly improved the disease resistant ability of RA rats and reduced the levels of the inflammatory cytokines IL-1ß and TNF-α.

Zheng YQ *et al*. (2007) studied the effects and mechanisms of paeoniflorin, a bioactive glucoside from peony root, on adjuvant arthritis in rats. Rats were treated with paeoniflorin (5, 10, 20 mg/kg) orally from day 14 to 20 after inducing adjuvant arthritis. Paeoniflorin an 10, 20 mg/kg per day suppressed rat AA at least partly by inhibiting abnormal proliferation of synoviocytes and the production of IL-1, PGE2, IL-6, VEGF and GM-CSF by synoviocytes and reducing Gi and Cox-2 expression.

Total glucosides of peony (TGP)

Zheng YQ and Wei (2005) found that TGP suppressed adjuvant arthritis in rats and intervenes cytokine-signalling between different types of synoviocytes. Intragastric administration of TGP (25, 50, 100 mg/kg bw, days 14-21) significantly decreased secondary inflammatory reaction, bone destruction and ultrastructure change of synoviocytes in in adjuvant arthritis rats The results suggested, TGP possesses anti-inflammatory effects by modulating the pro-inflammatory mediators production from MLS and phosphorylation of macrophage like synoviocytes (MAPKs) from fibroblast-like-synoviocytes (FLS).

Zhu L *et al*. (2005) analysed the effect and mechanisms of TGP on joint damage in rat collagen-induced arthritis. TGP (25, 50, 100 mg/kg/d) was orally administered to rats from day 14 to 28 after immunisation. The therapeutic effect of TGP could be associated with its ability to ameliorate the secretion and metabolism of synoviocytes and to inhibit the abnormal proliferation and VEGF, bFGF, MMP-1 and MMP-3 production by FLS.

Zhu L *et al*. (2006) found that TGP had inhibitory effect on hyper functional synoviocytes from rats with collagen-induced arthritis. The authors suggested, its mechanism may be related with the inhibition of abnormal proliferation and secretion of synoviocytes.

Xu HM *et al*. (2007) analysed the effect and mechanisms of TGP on adjuvant arthritis in rats. Intragastric administration of TGP (50, 100 mg/kg bw) significantly decreased secondary inflammatory reaction in adjuvant arthritis rats. Suppressing the activity of IL-1 and TNFα, decreased PGE2 and increased cAMP levels in synoviocytes of AA rats were observed after TGP administration. In the immunoblot analysis TGP could up-regulate the expression EP2 and EP4.

Lin J *et al*. (2012) found that TGP inhibits Th1/Th17 cells via decreasing dendritic cells activation in rheumatoid arthritis. TGP treatment (150 mg/kg i.p.) significantly decreased percentage and number of Th1 and Th17 cells in CIA in mice. TGP treatment inhibited dendritic cells (DCs) maturation and reduced production of IL-12 and IL-6. Moreover, TGP treatment in RA patients showed shank population of matured DCs and IFN-γ-, IL-17-production cells.

Wei *et al.* (2013) administered orally TGP at 60 mg/kg bw for 3 months to New Zeeland rabbits with antigen-induced arthritis. The authors concluded that TGP prevent juxta-articular bone loss and prevent subchondral bone destruction in experimental arthritis.

Assessor's comments
The tested substance paeoniflorin is only one component of the root. The results do not highlight any specific activity for the aqueous extract of the whole root. For in-vitro data with paeoniflorin a physiological correlation is not possible. The in-vivo data with orally administered TGP hint an anti-inflammatory activity. The tested dose is unclear, as no information on the DER of the extracts is given and the extraction solvent is not described clearly. The used alcoholic extract (TGP) is not part of the preparations to draw conclusions on for inclusion into the monograph based on missing information on the period of traditional use (see section 2) .

Analgesic activity

Paeoniflorin

The results of the study Lee KK *et al*. (2011) showed Paeoniae radix and Gentianae radix extracts orally administered, did not induce significant antinociception in non-diabetic mice. However peony root (250, 500 mg/kg) dose-dependently increased the nociceptive threshold in diabetic mice between 0.5 and 2 h after administration. Both paeoniflorin (30 mg/kg) and albiflorin (10 mg/kg) significantly elevated the nociceptive threshold between 0.5 and 3 h and between 0.5 and 1 h after oral administration, respectively.

Zhang XJ *et al*. (2009) found that the analgesic effect of paeoniflorin in rats with neonatal maternal separation-induced visceral hyperalgesia was mediated through adenosine A (1) receptor by inhibiting the extracellular signal-regulated protein kinase (ERK) pathway. Paeoniflorin was administered by i.p. injection (180 mg/kg bw) and morphine (5 mg/kg bw) was chosen as positive control.

Assessor's comments
The results for peony root extract (no further information) are contradictory regarding anti-nociception. The tested substance paeoniflorin is only one component of the root. Results are not relevant for establishing a monograph.

Antipyretic activity

In-vivo

Aqueous extract

Rawat and Malviya (2010) tested the antipyretic activity of aqueous root extract of Paeoniae radix (100 and 200 mg/kg orally, the percentage yield of the prepared extract was 12%±0.51 (w/w)) in rats. The root extract showed significant reduction in normal body temperature and yeast-provoked elevated temperature comparable to that of paracetamol. The antipyretic effect started after 1 hour and lasted for at least 4 hours after administration.

Assessor's comments
The tested extract concentrations 100 and 200 mg/kg orally correspond to approximately 7-14 g extract/58-116 g herbal substance in a 70 kg person. The tested concentrations are higher as the used human doses in TCM. Results are not relevant for establishing a monograph.

Anti-allergic-activity

In-vivo

Ethanolic extract, Paeonol, Paeoniflorin

Lee B (2008) analysed the anti-allergic effect of the root of *Paeonia lactiflora* (80% ethanolic extract) and its constituents paeoniflorin and paeonol in the model of passive cutaneous anaphylaxis in mice. The test agents were orally administered for 5 days. *Paeonia lactiflora* extract, paeoniflorin and paeonol potently inhibited passive cutaneous anaphylaxis reaction and scratching behaviours in mice. Paeoniflorin exhibited the most potent inhibition against scratching behaviours and the acetic acid-induced writhing syndrome in mice. Paeonol most potently inhibited passive cutaneous anaphylaxis reaction and mast cells degranulation.

Assessor's comments
The results hint an anti-allergic effect, but are of limited relevance for establishing a monograph as the tested dose is unclear and the DER of the extracts and the dosages are not described clearly. Paeoniflorin is only one component of the root.

Immuno-modulatory activity

In-vivo

Paeoniflorin

Chen Y *et al.* (2007) analysed the effects of paeoniflorin on the level of antibodies and cAMP produced by splenocytes in rats with adjuvant arthritis. Paeoniflorin 25, 50, and 100 mg/kg bw) were given by intragastric administration for 7 days from the 17 days after immunization. Paeoniflorin (50 and 100 mg/kg bw) reduced the levels of circulating immune complexes, anti-C II antibody and anti-TB antibody in serum in rats with adjuvant arthritis. The inhibition ratios were dosage dependent. Paeoniflorin (12.5, 62.5 and 312.5 mg/l) decreased the elevated levels of cAMP in splenocytes *in-vitro*.

Total glycosides of peony (TGP)

Li CL *et al*. (2013) examined the effects of total glucosides (100 mg /kg per day intragastrically) of peony for delaying onset of Sjogren's syndrome in an animal study using non-obese diabetic mice model. Compared to normal saline group, in TGP group saliva flow rate, submandibular glands index and the ration of regulatory T cells were significant higher, while anti-SSA/SSB and lymphocytic foci were significant lower. TGP demonstrated a similar effectiveness as hydroxychloroquine in delaying the onset of Sjogren´s like disease in mice.

In-vitro

Total glycosides of peony (TGP)

Jia *et al.* (2012) showed, that TGP in 1562.5 µg/ml regulates cytokines production in lupus CD4+ T cells from systemic lupus erythematosus patients. IL-10 mRNA and protein levels were increased, in contrast the mRNA levels of IL-6 and IL-17A significantly decreased.

Zhao M *et al*. (2012) examined the molecular mechanism of TGP in prevention of autoimmune response. They found, TGP (1562.5 µg/ml) induces regulatory CD4(+)CD25(+) T cells by increasing Foxp3 demethylation in lupus CD4(+) T cells. The IFN-γ andIL-2 expression was enhanced in TGP treated lupus CD4 T cells.

Methanolic extract

Leem *et al*. (2004) tested *Paeonia lactiflora* root extracts on the secretions of chemokines as monocyte chemotactic protein-1 and -3 in human nasal fibroblasts. The extract significantly decreased the secretion of MCP-1 and MCP-3. The authors concluded *Paeonia lactiflora* may play a role in nasal inflammation.

Paeoniflorin

Tsuboi *et al*. (2004) investigated whether paeoniflorin induces DNA fragmentation in murine T lymphocytes and human T-cell leukemia Jurkat cells. Paeoniflorin induces fragmentation of DNA in murine thymocytes, spleen cells, splenic T-cells and Jurkat cells in a concentration dependent manner. DNA fragmentation was also induced in some but not all non-T-cell lines. The results suggest that sensitivity on paeoniflorin depends on cell type and that T-lineage cells are included in the highly sensitive group.

Yang L *et al*. (2013) conducted a comparative study on bioactivities of Paeoniae radix alba and Paeoniae radix rubra. The authors concluded Paeoniae radix alba and its major compounds possess more significant bioactivities on J774 macrophage cells than Paeoniae radix rubra and its major compounds, and the herbal substances should be classified into different category in TCM.

	Paeoniae radix alba	**Paeoniae radix rubra**
LPS induced inflammation	Cell viability enhaced:1 mg/ml	Cell viability inhibited: 1 mg/ml
Cell proliferation	Activated by 1 mg/ml	Inhibited by 1 mg/ml
Early cell apoptosis	Higher increase	increase
LPS production in J774 cells	0.1 and 1 mg/ml reduce NO production	0.1 and 1 mg/ml reduce NO production

Assessor's comments
No experimental studies were conducted with the aqueous extract or tea preparations. The in-vitro experiments were conducted with concentrations of 1562.5 µg/ml TGP. For in-vitro data it should be kept in mind that for clear effects observed at concentrations of >100 µg/ml a physiological correlation is not plausible. Dose dependent effects of paeoniflorin on the level of antibodies could be shown in-vivo. The tested substance paeoniflorin is only one component of the root. The results have limitations and are not relevant for establishing a monograph.

Anticoagulant and antiplatelet activity

In-vivo

Aqueous extract of *Paeonia lactiflora* Pall.

Ishida *et al*. (1987) measured the plasma re-calcification time in mice, what was find to be useful for following the anticoagulative activity and isolated the active principles as paeoniflorin and benzoylpaeoniflorin.

Different fractions of a methanolic extract

Kang *et al*. (1991) analysed platelet anti-aggregation of peony root. The ethylacetate soluble fraction showed strong inhibition at the concentration of 1 mg/ml against collagen or arachidonic acid induced aggregation and at 3 mg against ADP induced aggregation. The aqueous fraction did not inhibit the platelet aggregation.

Paeoniflorin

Ye J *et al*. (2001) evaluated the anti-thrombosis effect of paeoniflorin in a photochemical reaction thrombosis model *in-vivo* in rats. The time of the beginning of the irradiation to the occlusion of the vein was defined as the thrombosis time. Paeoniflorin injected in the mesenteric venue, could significantly prolong thrombosis time (no information about the used concentration). Ten, 50, and 250 µg/ml increased tissue-type plasminogen activator (t-PA) activities in HUVEC. However, no significant effect was observed in plasminogen activator inhibitor (PAI) activity.

In-vitro

Koo *et al*. (2010) analysed the platelet anti-aggregatory and blood anti-coagulant effects of compounds isolated from *Paeonia lactiflora* and *Paeonia suffruticosa* by the turbidimetric method and measurement of coagulation time. Paeonol, paeoniflorin and benzoylpaeoniflorin were found to be the major common active constituents and they would collectively contribute to improving blood circulation.

Assessor's comments
In-vitro studies with unknown concentration of the substances paeoniflorin and benzoylpaeoniflorin hint to an anticoagulant and antiplatelet activity. As in-vivo paeoniflorin was injected in the mesenteric venue, or missing information, no conclusion can be drawn for oral use and clinical effects. No preclinical studies were conducted with the aqueous plant extract.

Atherosclerosis effects

In-vitro

Paeoniflorin

Li JZ *et al*. (2013) found inhibitory effects of paeoniflorin on lysophosphatidylcholine-induced inflammatory factor production in human umbilical vein endothelial cells (HUVECs). The results suggest that PEP suppresses LPC-induced inflammatory factor production through inhibition of the HMGB1 RAGE/TLR-2/TLR-4-NF-κB pathway.

Aqueous extracts

Zhu Y *et al*. (2013) compared the chemical components between Baishao and Chishao aqueous extracts and their effects on proliferation of rat thoracic aorta smooth muscle cells. The results indicate the extracts have different chemical components and produce different biological effects. (Chinese, only English abstract)

In-vivo

Total glycosides of peony (TGP)

Li J *et al*. (2011) analysed the effects of total glucosides from peony (*Paeonia lactiflora* Pall.) roots (120 mg/kg, 240 mg/kg) intragastrically administered for 15 weeks on experimental atherosclerosis in rats. Compared to control, TGP significant lowered the serum level of total cholesterol, triglyceride, LDL-C, TNF-alpha, IL-6, C-reactive protein increased the ratios of HDL-C/LDL-C and ApoA1/ApoB, decreased the intima media-thickness of abdominal aortal wall and improved the morphological change of the aorta. The authors concluded the beneficial effects are associated with lowering blood lipids and inhibiting the expression of inflammatory cytokines.

Zhu HM *et al*. (2004) conducted an experimental study on preventive effect of Paeoniae radix rubra (no further information) to restenosis after carotid balloon injury in high fat-diet rabbits. It could decrease the proliferating cell nuclear antigen positive expression and inhibit the proliferation of collagen type I and reduce the generating of new intima. Paeoniae radix rubra had a preventive effect

on the restenosis after carotenoid bollon injury in high fat-diet induced atherosclerotic rabbits. (Chinese, only English abstract)

Assessor's comments
In-vivo studies with oral administered TGP hint atherosclerosis effects. No preclinical studies were conducted with the aqueous plant extract.

Antiviral activity

In-vitro

Aqueous extract from *Paeonia veitchii* Lynch and *Paeonia lactiflora* Pall.

Guo *et al*. (2006) analysed in an *in-vitro* screening 16 aqueous extracts (20 g herbal substance boiled in 1000 ml water) of traditionally used medicinal plants in China against enteroviruses. Both Paeonia herbal preparations exhibited the strongest anti-Coxackie virus B3 activity on viral replication. *Paeonia veitchii* possessed an EC_{50} of >500.0 (mg/L).

Aqueous extract

Lin TJ *et al.* (2013) tested the anti-viral activity of decoct of *Paeonia lactiflora* pallas (100 g herbal substance was decocted with 1000 ml water three times, decoctions collected, mixed, concentrated) against human respiratory syncytial virus in human respiratory tract cell lines. The final concentration was at 10, 30, 100 and 300 mg/ml for bioactivity assay and up to 3000 µg/ml for cytotoxicity test. *Paeonia lactiflora* decoct was time-dependently and dose-dependently effective against HRSV in Hep-2 and A549 cells. 10µg/ml had a comparable anti-HRSV activity with 10 µg/ml ribavirin. *Paeonia lactiflora* decoct was effective against viral penetration and stimulated IFN-ß secretion.

Assessor's comments
In-vitro studies hint an antiviral activity of aqueous extracts. As the extracts were concentrated (no information on the DER) the data are not relevant for establishing a monograph.

Antibacterial activity

In-vitro

Methanolic extract from Paeoniae radix and its fractions

Im *et al*. (2012) evaluated the nitric oxide production inhibitory effect and antibacterial activity of the methanol extract and fractions from Paeoniae radix. As a result the ethyl acetate fraction of the methanol extract showed the highest antibacterial activity and the ethyl acetate fraction in the highest nitric oxide production inhibitory effect. The aqueous fraction showed no activity in all the tests performed.

Steam distillate

Ngan *et al*. (2012a) examined the inhibiting effects of *Paeonia lactiflora* root steam distillate constituents and structurally related compounds on human intestinal bacteria. The yield of the oil was 0.01% on dry weight basis of the root. *Paeonia lactiflora* root steam distillate was proven to have high growth-inhibiting activity against *B. fragilis, B. thetaiotomicron, C. paraputrificum, S. typhimurium,* and *B. breve*. Seven pure compounds were used in the study. The four major constituents were paeonol, myrtenol, myrtenal and (Z)-myrtenol. Paeonol, the major compound exhibited moderate to low growth-inhibiting activity against the test bacteria.

Ngan *et al*. (2012b) found growth-inhibiting, bactericidal, and urease inhibitory effects of *Paeonia lactiflora* root constituents and related compounds on antibiotic-susceptible and -resistant strains of *Helicobacter pylori.*

Assessor's comments
No preclinical studies were conducted with the aqueous plant extract. In-vitro studies with different lipophilic fractions and constituents of unknown concentrations hint possible antibacterial effects. Results are not relevant for establishing a monograph.

Antifungal activity

Ethanolic extract of Paeoniae radix

Kim *et al*. (2009) analysed the antifungal activity of seven herbal plant extracts against five different Candida species by agar diffusion assay. The ethanolic extract (100% ethanol) of Paeoniae radix showed no antifungal activity.

Anti-hyperlipidaemic effects

In-vivo* and *in-vitro

Aqueous extract of *Paeonia lactiflora* Pallas radix

Goto *et al*. (1999) examined the effect of PRE on endothelial function. The extract of Paeoniae radix was prepared by boiling in water. The yield was 11.0 g Paeoniae radix extract from 100 g of herbal substance. Male springue rats were divided in four groups: control group, high cholesterol diet group, low PRE group (high colesterol plus 60 mg /d PRE) and high Paeoniae radix extract group (high cholesterol plus 360 mg per day extract). After 10 weeks of treatment, the body weights in high cholesterol, low Paeoniae radix extract and high Paeoniae radix extract groups were significantly lower compared with that of the control group. Plasma total cholesterol level was significantly increased by about 9-10 fold in the high cholesterol, low Paeoniae radix extract from and high Paeoniae radix extract from groups compared with the control group after 2 weeks. There were no differences in body weight, plasma total cholesterol and triglyceride levels among the high cholesterol low Paeoniae radix extract and high Paeoniae radix extract groups. In endothelium-dependent relaxation to acetylcholine in aorta of rats, the relaxation of the high Paeoniae radix extract group was greater than the high cholesterol and low Paeoniae radix extract group.

In-vivo

Paeoniflorin

Yang HO *et al*. (2004) isolated paeoniflorin from *Paeonia lactiflora* and analysed the antihyperlipidemic effect in experimentally induced hyperlipidemic rats. Paeoniflorin showed a lowering effect of total cholesterol, LDL and triglyceride levels compared with the control group at the dose of 200 and 400 mg/kg p.o. once a day for 4 weeks.

In-vitro

Paeoniflorin

Lin HR (2013) evaluated the activity of paeoniflorin against LXR by the mammalian one-hybrid and transient transfection reporter assays. Paeoniflorin (20-100 µM) transactivated GAL4 rat cholesterol 7 α-hydroxylase, phospholipid transferprotein and ATP-binding cassette A1 gene promotors in dose-dependent manner. The authors suggested paeoniflorin acts as a liver X receptor agonist.

Assessor's comments
In-vivo studies with oral administered paeoniflorin hint a lowering effect of total cholesterol, LDL and triglyceride levels. Oral administered aqueous extract showed contrary results (*Goto H et al., 1999). There were no differences in body weight, plasma total cholesterol and triglyceride levels among the high cholesterol group and peony root extract groups. The yield was 11.0 g Paeoniae radix extract from (PRE) 100 g of herbal substance. The daily dose of 60 mg /d PRE and 360 mg /d PRE corresponds to 0.5 g/3.3 g herbal substance per rat of 350-380 g. The dose corresponds approximately 95 g (low dose)/632 g herbal substance (high dose) in a 70 kg human person and is higher than the doses used in humans in TCM.*

Cardiovascular disorders

In-vitro

Aqueous extract from *Paeonia lactiflora*

Goto *et al*. (1996) showed that an extract from Paeoniae radix relaxed prostaglandin F2a pre-contracted aortic ring preparations of isolated rat aorta that contained endothelium. The active component was gallotannin. Paeoniflorin and paeonol lacked a vasodilator effect.

Ethanol extract of Paeoniae radix rubra

Jin *et al*. (2012) analysed the vasodilatory effects of ethanol extract (70% aqueous ethanol, DER unknown) of Paeoniae radix rubrae and its mechanism of action in the rat aorta. The extract induced relaxation of the phenylephrine-precontracted aortic rings in a concentration dependent manner. The possible mechanism of vasorelexation of EPL involve enhacement of the NO-cGMP system via the Akt- and SOCE-cNOS-sGC signalling pathway through activation of K_{Ca} and K_{ATP} channels and inhibition of L-type CA^2 channels.

In-vivo

Total glycosides of peony (TGP)

Long *et al*. (2012) analysed the cardio protective effect of total peony glycosides against isoprenaline-induced myocardial ischemia in rats. Compared with model rats, TGP treatment (269.4 mg/kg intragastralic administration for 3 days before ISO administration and 449 mg/kg for 3 days after ISO administration) exhibited significantly reduced activities of GOT, LDH and CK, increased activity of SOD and lower levels of MDA. The protective effect of TGP treatment was even better than that of propranolol.

Paeonol and Paeoniflorin

Nizamutdinova *et al*. (2008) investigated the effect of paeonol and paeoniflorin, the postulated main active principles of *Paeonia albiflora*, on myocardial ischemia/reperfusion injury in rats. Administration of
10 mg/kg paeoniflorin or paeonol i.p. 1 h prior to reperfusion injury resulted in a significant improvement of the hemodynamic parameters. The administration of paeoniflorin or paeonol reduced myocardial infarct size, through inhibition of apoptosis.

Assessor's comments
No preclinical studies were conducted with the aqueous plant extract. The experiments of Long J et al. (2012) were performed with the isolated TGP in high doses (269.4 mg/kg intragastralic administration corresponds to 19 g TGP in a 70 kg person). Results are not relevant for establishing a monograph.

Anti-diabetic activity

In-vitro

Other plant extract: Fractions of methanolic extract of *Paeonia lactiflora*, radix rubra

Baumgartner *et al*. (2010) tested 11 fractions of a methanolic Paeoniae radix extract (no DER information) on their pharmacological activity in an enzyme based assey. Pentagalloylglucose was able to enhance insulin receptor phosphorylation at a concentration of 10 µmol, which could account to the anti-diabetic activity.

Ethanolic extract of the root of *Paeonia lactiflora* Pallas

Juan *et al*. (2010) examined the anti-hyperglycaemic effect of Paeoniae radix extract (95% ethanol, no DER information) via the transcriptional suppression of phosphoenopyruvate carboxykinase (PEPCK) in H4IIE cells of rats with streptozotozin-induced diabetes. The suppressive effects on hyperglycaemia and phosphoenolpyruvate carboxylase were dose and time-dependent. Paeonol and paeoniflorin did not suppress phosphoenolpyruvate carboxylase expression at testing concentration.

Methanolic fraction of an Ethanolic extract of Paeoniae radix rubra

Juan *et al*. (2011) evaluated the insulin mimetic novel suppressors of phosphoenolpyruvate carboxykinase (PEPCK) gene transcription from Paeoniae radix rubra extract (80% ethanol). The extract of Paeonia radix rubra suppressed PEPCK expression in H4IIE cells in an insulin receptor independent manner, in the presence of an insulin receptor antagonist (HNMPA-AM). The potent suppressive activity could be attributed to at least two distinct components (1,2,3,4,6-penta-O-galloyl-beta-D-glucose (PGG) and the non-PGG fraction (NPF).

Paeoniflorin

The results of Kong *et al*. (2013) showed effects of paeoniflorin on tumour necrosis factor-alpha-induced insulin resistance and changes of adipokines in 3T3-L1 adipocytes. Potential mechanism for the anti-inflammatory actions were suppression of the activation of IKK/NF-κB and JNK/SAPK which interrupt insulin signalling pathway and induce inflammatory factors expression, and enhancement of PPARγ expression, which improves insulin signalling pathway and increases adiponectin expression.

Assessor's comments
No preclinical studies were conducted with the aqueous plant extract and no in-vivo studies are available.

Antitumor activity

In-vitro

Paeoniae radix extract (no information on plant species, DER, extraction solvent)

Abdelhamed *et al*. (2013) screened 138 medicinal plant extracts in a concentration of 1 µg/ml against TRAIL-sensitive and insensitive TNBC cell lines MDA-MB-231 and MDA-MB 468. Among them only five plants did not cause apparent cytotoxicity as a single regimen but showed significant synergistic effects against both cell-lines. The Paeonia plant extract did not meet the criteria.

Paeoniflorin

Fang S *et al*. (2012) reported that paeoniflorin may effectively modulate multidrug resistance of the human gastric cancer cell line SGC7901/vincristine via the inhibition of NF-κB activation and down

regulation its target genes MDRI1, BCL-XL and BCL-2. It is suggested, that NF-κB activation may play a role in the development of chemotherapy resistance in carcinoma cells.

According Kwon *et al*. (2007) a lot of examples (Paeonia radix alba) of the induction of apoptosis by nutritional supplements and herbs through the generation of reactive oxygen species exist. The authors concluded, however nutritional supplements and herbs must be used with caution, as few research studies have actually evaluated their safety and efficacy and no guidelines for dose and usage are available.

Aqueous extract of *Paeonia lactiflora* Pallas

Lee SM *et al*. (2002) examined the cell growth of HepG2 and Hep 3 B cells in the presence of various concentrations of Paeoniae radix extract (PRE). The Paeoniae radix extract inhibited hepatoma cells growth by inducing apoptosis in a p53 independent pathway at a concentration of 4.6 and 4.4 mg/ml. The PRE could induce the internucleosomal DNA fragmentation of HepG2 and Hep3B cells. The authors concluded it may be a promising anticancer agent for inhibiting the growth of p53-deficient tumours.

Monoterpene glycosides

Washida *et al*. (2009) isolated a new monoterpenes glycoside 3-O-galloylpaeoniflorin and four known compounds from *Paeonia lactiflora* roots, which showed androgen receptor binding activity. The activity of pentagalloylglucose was equivalent to flutamide (IC_{50} 5.0 µmol). The authors proposed that pentagalloylglucose inhibits prostate cancer cell growth by acting as an androgen receptor antagonist.

In-vivo

Total glycosides of peony (TGP)

Xu HY *et al.* (2012) analysed the antitumor activity of total peony glycoside against human chronic myelocytic leukaemia K562 cell lines *in-vitro* and *in-vivo* in nude mice. TGP (1.5 g/kg) or CTX (25 mg/kg) was administered via intraperitoneal injection every 4 days. At 32 days TGP showed a significant decreased tumour volume and tumour weight in nude mice inoculated with k562 cells.

Xu W *et al*. (2013) studied the anti-tumour effect of total glycosides from radix paeoniae rubra in S180 tumour-bearing mice. Total glycosides of peony group was intragastrically administered 120 mg/kg/d TGC. CTX group was intraperitoneally injected with 100 mg/kg CTX. TGP significantly inhibited the growth of tumour cells in tumour-bearing organisms, enhanced the cytotoxic activity of NK cells, and increased the serum IL-2 and IL-4 levels.

Assessor's comments
For in-vitro data it should be kept in mind that for clear effects seen at concentrations of >100 µg/ml a physiological correlation is not plausible, so results with concentrations of 4.6 and 4.4 mg/ml are not convincing. In other in-vitro experiments the Paeonia plant extract did not meet the criteria for effectiveness. No in-vivo experiments were conducted with aqueous extracts. From in-vivo experiments via intraperitoneal injection no conclusions can be drawn for oral use.

Cerebral ischaemia and cognitive effects

In-vivo

Paeoniflorin-derivates

Abdel-Hafez *et al*. (1998) evaluated 8 paeoniflorin-derivates on their effect on the memory deficits induced by scopolamine using a step-down type of a passive avoidance task in mice at concentrations of 0.002-0.02 mmol/kg. The results indicated that full methylation in the chemical structure

significantly enhanced the ameliorating effect. No correlation to the natural concentration of the derivates in the plant was given. The authors assumed further clarification is necessary for (chemical) design of more potent candidates.

Paeoniflorin

Xiao *et al*. (2005) examined the effects of paeoniflorin on the cerebral infarction, behavioural and cognitive impairments at the chronic stage of transient middle cerebral artery occlusion in rats. One day (10 mg/kg twice s.c.) or seven-day (2.5 times 10 mg/kg, twice a day, s.c) injection of paeoniflorin significantly reduced the infarct volume as well as ameliorated the deficits in neurological symptoms caused by transient middle cerebral artery occlusion at chronic stage.

Tang *et al*. (2010) tested the anti-inflammatory effect of paeoniflorin (10 mg/kg intravenously) on cerebral infarction induced by ischemia-reperfusion injury in Sprague-Dawley rats. The results indicated that both pre-treatment and post-treatment with paeoniflorin reduced the ratio of cerebral infarction area. Pre-treatment also reduce the neurological deficit score.

Total glycosides of peony (TGP)

Liu *et al*. (2004) analysed the protective effect of TGP on complete cerebral ischemia reperfusion injury in rats. TGP (20 mg/kg) possessed protective effects on the histopathological changes of ischemia cerebral tissues in rats. (Chinese only English abstract)

Zhang AP *et al*. (1993) showed that TGP, (50 mg/kg 7d, ig) could enhance the episode duration of slow-wave sleep in normal rats and restore the sleep parameters in the insomniac rats induced by caffeine (12.5 mg/kg 7 days, i.p.). (Chinese, only English abstract)

Aqueous extract of peony root, paeoniflorin

Ohta *et al*. (1993) analysed the effect of four herbs Japanese angelica root, cnidium rhizome, peony root and rhemmania root on the scopolamine induced spatial working memory disruption in rats. Among the component herbs, peony root extract (0.25 and 1 g dried her/kg, orally) exhibited the most antagonizing effect on the scopolamine disruption of the choice accuracy. Paeoniflorin (0.01-1 mg/kg, peroral) dose-dependent attenuated the scopolamine-induced impairment in the choice accuracy.

Aqueous extract of peony root

Tsuda *et al*. (1997) evaluated the protective effects of peony root extract and its components on neuron damage in the hippocampus induced by the cobalt focus epilepsy model in rats. Neuron damage in the CA1 area of the hippocampus and frequent spike discharges induced by application of metallic cobalt to the cerebral cortex of rats were completely prevented when the extract was continuously administered orally at 1 g/kg per day for 30 days prior to cobalt application. The use of paeoniflorin alone had no effect. The gallotannin fraction had incomplete protective action. A combination of the gallotannin and paeoniflorin fraction showed complete action in the same way as peony root extract.

Total glycosides of peony (TGP)

Yang J *et al*. (2000a) analysed the protective effect of TGP against cerebral ischemia-reperfusion injury model in mice. TGP could improve the learning and memory capacity of model mice in step down test. (Chinese only English abstract)

Yang J *et al*. (2001) analysed the protective effects of TGP on cerebral ischemia mice. TGP could prolong gasp time of decapatative mice, lessen cerebral water content and decrease permeability of cerebral capillary significantly. (Chinese only English abstract)

Yang J *et al*. (2000b) analysed the improvement effects of TGP on ability of learning and memory in model mice induced by drugs. TGP could reduce irritated time, prolonged duration of safe staying, lessen the times of error and adjust latent duration. In model mice of spatial resolution TGP increased ratio of successful landing, reduced times of mistake and lessoned the time of landing. (Chinese only English abstract)

Assessor's comments
Neuron damage in the CA1 area of the hippocampus and frequent spike discharges induced by application of metallic cobalt to the cerebral cortex of rats were prevented when an aqueous extract was continuously administered orally. In disruption of the choice accuracy, peony root extract exhibited an antagonizing effect on the scopolamine. The doses correspond to 17.5-70 g dried herb for a 70 kg person and hint to possible effects.

Gastrointestinal activity

In-vitro

Pentagalloylglucose

Ono *et al*. (2000) found that Pentagalloylglucose, an antisecretory component of Paeoniae radix, inhibits gastric H+, K±ATPase with an IC50 of 166 nmol/l and may be responsible for inhibition of acid secretion by Paeoniae radix.

Liver protective activity

In-vivo

Total glucosides of peony (TGP)

Results of a study in rats by Dai LM *et al.* (1993) showed that pre-treatment with TGP (10, 20, mg/kg per day times 7 days, i.p.) diminished the hepato-cellular degeneration and necrosis induced by D-Galactosamine or CCl_4. (Chinese, only English abstract).

Wang H *et al*. (2005) evaluated effects of TGP (60 and 120 mg/kg, oral) on immunological hepatic fibrosis in rats. Histological results showed that TGP improved the human albumin-induced alterations in the liver structure, alleviate lobular necrosis and significantly lowered collagen content.

Zheng LY *et al*. (2008) concluded by their study results, TGP (0.15 g/kg bw, 0.05 g/kg bw) may protect liver function and modulate serum lipid for the fatty liver rats caused by insulin resistance, and its action mechanism may be concerned with enhancing sensitivity and antioxidative ability, decreasing serum lipid.

Aqueous extract of Paeoniae radix rubra

Li R *et al*. (2011) studied the hepatoprotective action of Paeoniae radix rubra aqueous extract against CCl_4-induced hepatic damage in Wistar rats. The extract was given (100, 200, or 300 mg/kg bw) by gavage to the animals on 28 consecutive days. At all doses the extract reduced the fatty degeneration and necrosis hepatic injury score. The increased levels of the serum enzymes AST, ALT and ALP produced by CCl_4 were attenuated at three different dose levels almost like bifendate treatment.

Paeonia radix rubra and Paeonia radix alba alcoholic extract

Wang R *et al*. (2012) found, that oral administered Paeonia radix rubra and Paeonia radix alba extract (12 g/kg for 6 days) attenuate CCl_4 -induced acute liver injury in rats. The extracts reduced the serum levels of several bile acids while they increased the serum levels of choline and 5-methylenetetrahydrofolate.

Paeoniflorin

Li X *et al*. (2009, 2010) investigated the effects of paeoniflorin on hepatic fibrosis of mice infected with S. japonicum. Paeoniflorin was administrated orally on the 12th day (30 days of successive administration) after infection. Paeoniflorin (30 mg/kg per day) ameliorates schistosomiasis liver fibrosis through regulating IL-13 and its signalling molecules in mice.

In-vitro

Ethanolic extract

The results of Kuo *et al*. (2012) in the *in-vitro* experiment in rat HSCs and LX-2, a human HSC cell line, show Paeoniae radix extract (80% ethanol) and its active components paeonol reduces PDGF-stimulated hepatic stellate cell migration. Hepatic stellate cells play a key role in the pathogenesis of liver fibroses.

Lu *et al*. (2012) performed a screening for identifying of hepatoprotective compounds in Paeoniae radix rubra. Fluorescein diacetate labelled and MTT assay were applied for screening the hepatoprotective fractions on HepG2 cells exposed to galactosamine. Three hepatoprotective fractions were founded, in which three compounds were identified as paeoniflorin, ethyl palmitate and ethyl linoleate.

Assessor's comments
The experiments of Wang R et al. (2012) were performed with ethanolic extracts in high doses (12 g/kg corresponding to 840 g in a 70 kg person). TGP, what are isolated substances of peony, may protect liver function and modulate serum lipids in-vivo. No preclinical studies were conducted with the aqueous plant extract. Results are not relevant for establishing a monograph.

Lung protective activity

In-vivo

Paeoniae radix rubra (no further information on the plant extract)

Chen C *et al*. (2005) investigated the effect of Paeoniae radix rubra on expression of iNOS and eNOS in lipopolisacharide-induced acute lung injury in rats. Compared with the saline group, expression of iNOS and eNOS was markedly increased in the lipopolysaccharide group. Paeoniae radix rubra for treatment group and for prevention group the expression of iNOS and eNOS were significant lower than in the lipopolysaccharide group. (Chinese, only English abstract)

Chen C *et al*. (2008) investigated the effect of pretreatment (i.v. injection of 30 mg/kg bw) of Paeoniae radix rubra (no further information on the plant extract) on acute lung injury induced by intestinal ischemia/reperfusion in rats and its protective mechanism. Under light microscope, the pathologic changes induced by ischemia/perfusion group were significantly attenuated by Paeoniae radix rubra. The protective effect in acute lung injury was related to its property of inducing hemeoxygenase-1 (HO-1) expression and inhibition lipid peroxidation.

Zhang F *et al*. (2005) analysed the protective effects of pre-treatment of Paeoniae radix rubra (parenteral 30 mg/kg, no further information) on the acute lung injury induced by intestinal ischemia/reperfusion in rats. Arterial blood analysis shows that the partial pressure of oxygen and

partial pressure of carbon dioxide in the Paeoniae radix rubra pre-treatment group were obviously higher than those in the injury group. The malondialdehyde was lower in the Paeoniae radix rubra group. The morphological changes and the injured severity was relieved compared with that of the injury group. (Chinese, only English abstract)

Paeoniflorin

Ji *et al*. (2013) found paeoniflorin attenuated bleomycin-induced pulmonary fibrosis in mice by suppressing the synthesis of type I collagen via inhibiting the activation of TGF-ß/Smad pathway and increasing the expression of IFN-γ. In mice orally treated with bleomycin consecutive 21 days, paeoniflorin (50 mg/kg) significantly prolonged the survival periods.

Assessor's comments
The experiments have methodological deficiencies as they were conducted with preparations of unknown composition /DER, i.v. injection or only from isolated substances. Results are not relevant for establishing a monograph.

Neuroprotective activity

In-vitro

Paeoniflorin

Cao BY *et al*. (2010) evaluate a neuroprotective effect of paeoniflorin and found that paeoniflorin (50 µM) protects PC12 cells from MPP and acidic damage via autophagic pathway.

Methanolic extract

Lee SM *et al*. (2008) examined the protective effects of *Paeonia lactiflora* Pall. on hydrogen peroxide-induced apoptosis in PC12 cells. The total phenolic content was 89.65 mg per g of extract. After 2 h of cell exposure to 0.5 mM H_2O_2 a marked reduction in cell survival was observed in the MTT reduction assay and LDH release assay.

In-vivo

Aqueous extract

Chen T *et al*. (2011) found that Paeoniae alba radix extract (prepared of decoction) caused enhancement of the nerve growth factor-mediated neurite outgrowth from PC12 cells as well as their expression of growth associated protein and synapsin I *in-vitro*. In the *in-vivo* study silicon rubber chamber filled with PR aqueous extract were used to bridge a 10 mm sciatic nerve defect in rats. At the conclusion of 8 weeks, regenerated nerves in the PR groups had a higher rate of successful regeneration across the wide gap, relatively larger mean values of total nerve area, myelinated axon, count and blood vessel number and significantly larger nerve conductive velocity compared to the control group.

Paeoniflorin

Liu DZ *et al*. (2005) examined the neuroprotective effect of paeoniflorin on transient ischemic model in rat by activating adenosine A1 receptor in a manner different from its classical agonists. The administration of paeoniflorin (2.5 and 5 mg/kg bw s.c.) produced a dose-dependent decrease in both neurological impairment and the histologically measured infarction volume. Similar results were obtained when paeoniflorin was given in permanent ischemia model.

Assessor's comments
No in-vivo experiments were conducted with orally administered extracts.

Uterine activity

In-vivo

Methanolic extract of peony root

Harada *et al*. (1984) analysed the effects of Japanese Angelica root and peony root extracts (3 g/kg) on uterine contraction in the rabbit in situ. Intra-duodenal administration increased uterine activities. In some animal preparations inhibiting effect of the extracts on uterine contraction was noted after the uterotonic effect terminated.

"Crude alcoholic extract"

Madari and Jacobs (2004) reported *Paeonia officinalis* was used in traditional medicine of ancient Persia as abortificient. A crude alcohol extract of the root of *Paeonia officinalis* produced uterine stimulation in rat confirming the traditional use as abortificant.

Assessor's comments
There is no information on the dosages used for abortification in the report of Madari (2004). The in-vivo experiments of Harada (1984) were conducted with very high doses 3 g/kg corresponding to 210 g herb for a 70 kg person, so no conclusions for clinical relevance can be drawn. The possible effect should be considered for safety reasons.

Antioxidant activity

In-vitro

Paeoniflorin

Chen T *et al*. (2011) analysed the protective effects of paeoniflorin extracted by *Paeonia lactiflora* Pall. against hydrogen peroxide-induced oxidative stress in human umbilical vein endothelial cells (HUVECs). Paeoniflorin increased significantly the percent cell viability of HUVECs injured by H_2O_2 using the MTT assay. By flow cytometric analyses, paeoniflorin attenuated H_2O_2 induced apoptosis and intracellular reactive oxygen species production.

Methanol extract

Im and Lee (2011) analysed the tyrosinase inhibitory activity and melanin production inhibitory activity of the extract and fractions from Paeoniae radix. The total polyphenol content of the extract was 73.45 mg/g. The tyrosinase inhibitory activity of the ethyl acetate fraction showed higher activity than arbutin used as a positive control. In nontoxic concentration range, the ethylacetate fraction showed strong melanin production inhibitory effect and the authors considered it could be applicable to functional materials for skin-whitening agents.

In-vivo* and *in-vitro

Ethanolic extract

The results of the experiments of Lee SC *et al.* (2005) showed, the oral administration of 50% ethanol extract of peony root (no DER information), gallic acid and methyl gallate potently inhibited the formation of micronucleated reticulocytes in the mouse peripheral blood induced by a $KBrO_3$ treatment *in-vivo*. The ethanolic extract exhibited a significant free radical scavenging effect against DPPH radical generation and had an inhibitory effect on lipid peroxidation as measured by the level of malondialdehyde formation.

Effect on renal tubulointerstitium in rats

In-vivo

Total glucosides of peony (TGP)

Fang F *et al*. (2008) administered TGP 50, 100, 200 mg/kg per day orally once a day for 8 weeks in diabetic rats. Increased indices for tubulointerstitial injury were significantly ameliorated by TGP treatment with 100 and 200 mg/kg bw. Elevated expression of osteopontin protein was inhibited with 100 and 200 mg/kg bw, and increased expression of α-smooth muscle actin with 50, 100, and 200 mg/kg bw.

Zhang C-Q *et al*. (2014) examined the effect of TGP on TLR signal pathway in the kidney from diabetic rats. TGP 50, 100, 200 mg/kg per day was administered orally once a day for 8 weeks in diabetic rats. Western blot analysis showed, that the increased levels of TLR2, TLR4, MyD88, p-IRAK1, p-IRF3 and NF-κB p65 in the kidneys of diabetic rat were significantly suppressed by TGP treatment.

Zhang P (2009) analysed the effect of TGP on the expression of nephrin in the kidneys from diabetic rats. TGP 50, 100, 200 mg/kg per day was administered orally once a day for 8 weeks in diabetic rats. Western blot analysis showed that the expression of nephrin protein was reduced in the kidneys of diabetic rats but significantly increased in the TGP treatment group. The expression of TNF-α, NF-κB p65 and 3-NT protein were inhibited by TGP treatment.

Wu *et al*. (2009) analysed the renoprotective effect of TGP and its mechanism in streptozotocin-induced diabetic rats. The rats were treated orally with TGP for 8 weeks. Treatment at 50, 100 and 200 mg/kg significantly lowered 24 h urinary albumin excretion rate. 100 and 200 mg/kg reduced indices for tubolointerstitial injury in diabetic rats.

Assessor's comments
No in-vivo experiments were conducted with orally administered extracts. The in-vivo experiments with orally administered TGP may hint a renoprotective effect for very high doses of isolated substances of peony root. Results are not relevant for establishing a monograph.

Wound healing effect

In-vivo

Aqueous extract

Malviya and Jain (2009) examined the wound healing activity of aqueous extract (no further information) of Paeoniae radix by incision, incision and dead space wound models on Wistar rats. The results indicated that radix Paeoniae extract accelerates the wound healing process by decreasing the surface area of the wound and increasing the tensile strength. The histological examination of the granulation tissue of treated group showed increased cross-linking of collagen fibres and absence of monocytes.

Anti-depressant-like effect

In-vivo

Total glycosides of peony (TGP)

Mao *et al*. (2009) examined the effects of peony glycosides on mice exposed to chronic unpredictable stress. Chronic unpredictable stress induced depression, as indicated by increase in immobility time in the tail suspension test, was alleviated by s.c. administered TGP (160 mg/kg). The authors suggest the effect is probably mediated by inhibition of monoamine oxidation.

Mao *et al*. (2012a) found that peony glycosides (160 mg/kg, s.c.) reverse the effects of corticosterone on rats. Depression-like behavior was suppressed and brain–derived neurotrophic factor (BDNF) expression was increased in the rat model.

In a mini-review Mao *et al*. (2012b) summarised the results of literature regarding the anti-depressant-like effect. The peony extract was active in the mouse forced swim test and tail suspension test, and produced anti-depressant effects in chronic unpredictable mild stress-induced depression model in mice and rats. The mechanisms of peony were likely mediated by the inhibition of monoamine oxidase activity, neuroprotection, modulation of the function of hypothalamic-pituitary-adrenal axis, inhibition of oxidative stress and the up-regulation of neurotrophins.

Assessor's comments
The experiments were performed with applied s.c. TGP. No in-vivo experiments were conducted with orally administered traditional preparations (aqueous extracts, decoction).

Blood enriching

In-vivo

Zhang JJ *et al.* (2013b) conducted a comparative study on effects of blood enriching on mouse model of blood deficiency syndrome induced by compound method of bleeding, starved feeding and exhausting of Paeoniae radix alba and Paeoniae radix rubra (2 g/kg bw, no information about the extract) paeoniflorin and albiflorin. At the 7th day Paeoniae radix alba has a better effect of blood enriching than Paeoniae radix rubra. Albiflorin was more effective than paeoniflorin.

Assessor's comments
It is unclear which herbal preparation or whether the crude herbal substance was administered. The dose of 2 g/kg corresponds to 140 g for a 70 kg person and is higher as traditionally (TCM) used dosage ranges.

Effect on colitis

In-vivo

Paeoniflorin

Zhou *et al*. (2010) found that paeoniflorin increases beta-defensin expression and attenuates lesion in the colonic mucosa from mice with oxazolone-induced colitis through regulating the expression of HBD-2, IL-6 and IL-10. (English abstract only).

3.1.2. Secondary pharmacodynamics

Not available

3.1.3. Safety pharmacology

Not available

3.1.4. Pharmacodynamic interactions

Not available

3.1.5. Conclusions

The preclinical studies were often performed with isolated substances as paeoniflorin or TGP. Wang C *et al.* (2012) reported, when administered orally, paeoniflorin was absorbed poorly in gastrointestinal

tract, leading to a very low bioavailability of 3-4%. Therefore experiments with paeoniflorin are considered of minor relevance. Aqueous extracts were often administered over the doses ranges known from the TCM. Often no information on the DER of the final administered extracts was available.

No conclusions can be drawn from preclinical experiments and pharmacological data. No therapeutic posology in humans is known for the use in Europe. In summary, available data are not adequate to support establishment of a European Union monograph.

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

Absorption

***In-vitro:* rat everted gut sacs**

Paeoniflorin

Dong *et al.* (2009) showed that the absorption of paeoniflorin was linear at different intestine segments and dosages. The square of degrees correlation coefficient exceed 0.9, which is consistent with a zero order rate process. The K_a of paeoniflorin showed a dose-dependent increase along with the raised dose of extractive Paeoniae radix alba, indicated it was a mechanism of passive absorption. (English abstract only)

***In-vivo*: rats**

Pharmacokinetic comparison between Paeoniae radix rubra and Paeoniae radix alba

Wang CH *et al.* (2008) conducted a comparative pharmacokinetic study of paeoniflorin after oral administration of decoction of Paeoniae radix rubra and Paeoniae radix alba in rats. There was a significant difference between the pharmacokinetic characteristics of Paeoniflorin alone, Paeoniae radix rubra and Paeoniae radix alba (3.24%, 4.26% and 1.82%). The results suggested that the bioavailability of paeoniflorin in rats after oral administration was very low, about 3%. The T_{max}, AUC $_{0-\infty}$, C_{max} were increased after oral consumption of Paeonia radix rubra compared to Paeoniae radix alba.

Feng *et al*. (2010) reported a significant difference between the pharmacokinetic characteristics of Paeoniae radix rubra and Paeoniae radix alba, by the HPLC-ESI-MS method. Aqueous solutions of the herbal substances were given at a dose of containing 0.2 g/g crude herbal substance to rats.

	Paeoniae radix rubra	**Paeoniae radix alba**
C_{max} paeoniflorin	185.24±26.24 ng/ml	34.44±13.43 ng/ml
AUC $_{0-\infty}$ paeoniflorin	52.274.73±489.32 ng h/ml	315.08±85.44 ng h/ml
T_{max} paeoniflorin	0.67±0.07 ng/ml	0.33±0.02
$T_{1/2}$ paeoniflorin	1.86±0.27	0.85±0.11

The increase of AUC suggested, paeoniflorin was absorbed better from the decoction of Paeoniae radix rubra than of Paeoniae radix alba. The longer T_{max} and $T_{1/2}$ implied the delay of absorption and slower of elimination of paeoniflorin.

Hsiu *et al*. (2003) investigated the metabolism and pharmacokinetics of paeoniflorin by oral administration in rats. The paeoniflorin levels in serum were below the detection limit through the study. In conclusion paeoniflorin was not absorbed *per se*, whereas its aglycone paeoniflorigenin was absorbable and circulating in the bloodstream.

Tang and Eisenbrand reported in the Handbook of Chinese Medicinal Plants, Chemistry, Pharmacology, Toxicology (2011) that paeoniflorin seemed to be poorly absorbed from the gastrointestinal tract, resulting in an extremely low bioavailability.

Wang C *et al*. (2012) reported, according to previous studies, when administered orally, paeoniflorin was absorbed poorly in gastrointestinal tract, leading to a very low bioavailability of 3-4%. The causes of paeoniflorin´s poor bioavailability are poor permeation efflux via P-glycoprotein and hydrolytic degradation by esterase in the intestine. Therefore they compared the pharmacokinetics of paeoniflorin microemulsion with paeoniflorin saline after repeated dosing in rats with adjuvant arthritis. The plasma concentration of 25, 50 mg/kg were undetectable by the HPLC assay. The AUC_{0-1}, the mean retention time and the C_{max} were increased in the 100 mg/kg microemulsion group, indicating a better absorption of the formula.

Distribution

Paeoniflorin

Chen LC (1999) administered orally peony root extracts to mice (24.6 g dry extract obtained from 200 g crude material, extraction solvent aqua) at a dose of 10 mg/kg paeoniflorin for quantification of paeoniflorin. The calibration curve for paeoniflorin was linear over the concentration range 10-200 ng/ml. The recoveries of paeoniflorin from mice plasma were found to be 74.49, 76.83, 80.38, and 80.56% for concentrations of 30, 80, 120, and 160ng/ml respectively. The plasma concentration-time curves fitted with mean terminal half-lives of 94.16 minutes.

Cao C *et al*. (2006) determined the paeoniflorin concentration in the cortex of normal and cerebral ischemia-reperfusion rats and plotted the time course profile in rat cortex. Paeoniflorin could quickly penetrate through the blood-brain barrier after intravenous administration in a dose of 60 mg/kg bw. The cortex concentration in ischemia-reperfusion rats were lower 5 minutes after dosing and declined more slowly than in normal control.

Liang *et al*. (2013) analysed the profiling and identification of the absorbed constituents and metabolites of Paeoniae radix rubra (PRR) decoction in rat plasma and urine by the HPLC-DAD-ESI-IT-TOF-MS(n) technique. The results indicated that (epi)catechin-related compounds, gallic acid-related compounds and paeoniflorin were the main precursors of the 90 found metabolites. Phase I reactions (dehydroxylation, decarboxylation, dehydrogenation) and phase II reactions (sulfation, glucuronidation, and methylation) were observed as the main metabolic pathways or PRR.

Sun *et al.* (1996) reported glycosidases, produced in *E. coli* as a cytoplasmic protein, convert natural glycosides to bioactive compounds. Paeoniflorin was biotransformed to paeonimetabolins I and II. While paeoniflorin shows depressant, antispasmodic and anti-inflammatory effects, the paeonometabolin I showed anticonvulsive effects, what was interpreted as increased pharmacological activity.

Elimination

***In-vivo* in rats**

Decoct of Paeoniae radix rubra

Jiang F *et al*. (2012) conducted a comparative pharmacokinetic study of paeoniflorin and albiflorin after oral administration of Paeoniae radix rubra decoct in normal rats and the acute cholestasis hepatitis rats. Particularly at the high dose group, paeoniflorin and albiflorin eliminated much more slowly in the cholestasis hepatitis rats group and the concentration increased in the plasma (higher $AUC_{0-\infty}$, T_{max} longer). The results showed that acute liver injury could alter the pharmacokinetic parameters.

Pharmacokinetic interactions

In-vivo

Aqueous extracts

Chen LC *et al*. (2002) analysed the pharmacokinetic interactions between carbamazepine and Paeoniae radix (*Paeonia lactiflora*) extracts (300 mg/kg; 24.6 g extract weighting from 200 g of raw material by decoct and drying) in rats. The significant decrease of T_{max} indicated that simultaneous oral administration of PR contributes to more rapid absorption of carbamazepine. A significant decrease in protein binding rate was found (62.7 and 68.1%), what was considered probably free from clinical consequences because the wide fluctuations in carbamazepine concentrations between doses. There were no changes in AUC, $T_{1/2}$, C_{max}.

Chen LC *et al.* (2001) analysed the pharmacokinetic interactions between phenytoin and Paeoniae radix (*Paeonia lactiflora*) extracts (300 mg/kg; 24.6 g extract weighting from 200 g of raw material by decoct and drying) in rats. The significant increase of T_{max} indicated that simultaneous oral administration of PR delayed the absorption of phenytoin. No significant decrease in protein binding rate was found. There were no changes in AUC, $T_{1/2}$, $C_{max, MRT, CL/F}$ of phenytoin.

In-vitro

Paeoniflorin

Dong *et al*. (2009) showed, paeoniflorin is a substrate of P-glycoprotein, and extractive Paeoniae radix alba could induce expression of the P-glycoprotein *in-vitro* in the rat everted gut sac model. (Chinese, abstract in English)

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

3.3.1. Single dose toxicity

Not available

3.3.2. Repeat dose toxicity

Not available

3.3.3. Genotoxicity

Ethanolic extracts of *Paeonia lactiflora* Pallas and Paeoniae radix rubra (*P. albiflora* var. *hortensis* Makino): Chang *et al.* (1989) examined 40 Chinese herbal drugs for mutagenicity in the SOS chromotest (E. coli PQ37) and in the SOS umu test. The ethanolic herbal extracts in 4 mg/ml showed no mutagenicity.

Aqueous extracts of *Paeonia lactiflora* Pallas and Paeoniae radix rubra (*P. albiflora* var. *hortensis* Makino): Chang *et al.* (1989) examined 40 Chinese herbal drugs for mutagenicity in the SOS chromotest (*E. coli* PQ37) and in the SOS umu test.

The results of the aqueous extracts of *Paeonia lactiflora* were not presented. The authors assumed, no of the 40 Chinese herbal drugs seem to exhibit mutagenic activities under the experimental conditions. The aqueous extracts of Paeoniae radix rubra (*P. albiflora* var. *hortensis* Makino) in 4 mg/ml and 50 mg/ml showed no mutagenicity in the SOS umu test (-S9: 82; +S9: 69 versus–S9: 38; +S9:72; mitomycin: 566 ß-Galactosidase activity/unit). Aqueous extracts of Paeoniae radix rubra (*P. albiflora*

var. *hortensis* Makino) exhibited an antimutagenic activity against mitomycin C and aflatoxin B1 in SOS umu test.

Yu *et al*. (2004) examined the toxicological safety and stability of the components of an irradiated Korean medical herb, Paeoniae radix. The mutagenicity of the aqueous extract was examined triplicatedly in *Salmonella* reversion assay at 5 dosages from 62 to 5000µg/plate. The number of revertant colonies of each strain in test group did not increased comparing with negative control group. The increase of the colony formation by irradiate sample was not appreciated in both direct non-activate and indirect activated tests. The extract did not show cytogenetic toxicity in the culture of the Chinese hamster ovary cells. The authors concluded, chronic toxicity tests are needed for public acceptance in the application of irradiation for the hygienic technology of the herbs.

3.3.4. Carcinogenicity

Not available

3.3.5. Reproductive and developmental toxicity

Not available

3.3.6. Local tolerance

Not available

3.3.7. Other special studies

Harada *et al*. (1984) analysed the effects of Japanese Angelica root and Peony root extracts (3 g/kg, no DER information) on uterine contraction in the rabbit *in situ*. Intra-duodenal administration increased uterine activities. Madari H and Robert S. Jacobs (2004) reported, *Paeonia* officinalis was used in traditional medicine of ancient Persia as abortificient. A crude alcohol extract of the root of *Paeonia officinalis* produced uterine stimulation in rat confirming the traditional use as abortifacient.

3.3.8. Conclusions

Only limited toxicological data are available from literature. Adequate tests on genotoxicity, carcinogenicity, reproductive and developmental toxicity and local tolerance are not available. There are some hints for abortificient effects. Consequently, the use in pregnants should be contraindicated (also in the combination preparations of the TCM) for safety reasons.

3.4. Overall conclusions on non-clinical data

Non-clinical information on the safety of peony root is limited. The preclinical pharmacological data is not appropriate to document clear pharmacological actions for the aqueous extracts used in TCM. The limited data available on single and repeated dose toxicity reveal no suspicion of safety concerns regarding human therapeutic TCM doses. There are some hints for abortifacient effects which should be considered for safety reasons. Test on carcinogenicity, reproductive and developmental toxicity and local tolerance are not available. Specific data on pharmacokinetics and interactions are not available.

Numerous publications on Paeoniae radix or constituents exist, however, often an appropriate description of herbal substance or herbal preparations used are missing. Available data are not sufficient to establish a European Union monograph.

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

Total glucosides of peony (TGP)

Lin J *et al.* (2012) treated 10 patients with diagnosis of rheumatoid arthritis (RA) with TGP by oral administration of 15 mg/kg per day more than 3 months, while another 10 patients did not take TGP. Peripheral blood mononuclear cells were obtained from the patients by Ficoll-Hypaque separation. TGP treatment led to decreased percentage of matured DC and Th1/Th17 cells in peripheral blood of RA patients. The results were in accordance with the *in-vivo* tests in the model or with collagen induced arthritis (CIA) in mice. The authors conclude the results may explain the functions of TGP in reducing inflammation and immune response in autoimmune disease.

Wang ZJ (1994) reported improvements in immune function by total glycosides of peony in rheumatoid arthritis patients. (No further information, Chinese, only English abstract).

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

Wang C *et al*. (2012) reported TGP contain more than 90% of paeoniflorin. Paeoniflorin was absorbed poorly in gastrointestinal tract, leading to a very low bioavailability (3-4%). TGPs slow effect seriously limited its application to RA patients (Liu DZ *et al*, 2005) und must be improved by novel formulations as microemulsions.

4.2. Clinical efficacy

According the review article “Peony” in Herbal Medicines (2013), there is limited reliable clinical information available for the pharmacological effects and its main constituents. Many studies are available where peony is combined with other herbs in TCM or Traditional Japanese Medicine (TJM) or given with conventional treatment, so that clinical information on the action of peony alone is generally not available and therefore the effects found cannot always be attributed to peony.

4.2.1. Dose response studies

Not available

4.2.2. Clinical studies (case studies and clinical trials)

Patients with active rheumatoid arthritis

Total glucosides of peony (TGP) of *Paeonia lactiflora* Pallas

Zhao YX and Liu (2006) conducted a clinical observation on effects of leflunomide (no information on dose) and TGP on patients with rheumatoid arthritis. Forty persons were treated with TGP and leflunomide and the control group of 40 persons was treated with leflunomide alone for 12 weeks. The total effective rate was higher in the treatment group than in the control group (97.5% vs 85.0%) without significant difference in adverse reactions.

Chen Z *et al.* (2013) analysed in a 24-week, open label, randomised clinical trial the efficacy of TGP and the protective effect on hepatotoxicity in the combination treatment with methotrexate and leflunomide in 204 patients with active rheumatoid arthritis. No statistically significant differences were found.

Du and Dong (2005) analysed the clinical efficacy of methotrexate combined with TGP on rheumatoid arthritis for 3 months. 30 patients were only administered orally methotrexate, while 31 patients were treated with methotrexate plus TGP (no dose information). The total effective rate was 90%, 94% 100% in the methotrexate plus TGP group and 87%, 90% 94% in the methotrexate group at 4, 8 and 12 weeks. (Chinese, only English abstract)

Wang Y and Xing (2007) conducted a clinical observation on the effect of TGP combined with methotrexate on rheumatoid arthritis for 24 weeks. Two hundred and sixty patients were assigned to two groups; the treated group (180 cases) was orally administered with methotrexate and TGP (no information on dose) and the control group (80 cases) with methotrexate plus sulfasalazine. The total effective rate at the 4th, 8th, 12th,24th week of the treatment was 70%, 81%, 94%, 98% in the treated group and 60%, 85%, 93% and 94% respectively. The authors concluded TGP combined methotrexate treatment shows a quicker initiation with less side effects. (Chinese, only English abstract).

Zhang W and Dai (2012) reported, a preparation of TGP was approved by State Food and Drug Administration of China to enter market as a disease-modifying drug for RA in 1998. However, there was no report available to show whether TGP treatment can retard the radiographic progression of the disease.

Patients with psoriatic arthritis

Total glucosides of peony (TGP)

Wang YN *et al.* (2014) analysed the beneficial effect of TGP on psoriatic arthritis links to circulating Tregs and Th1 cell function in clinical observation study. Nineteen patients received in a 12-week-treatment 3 times 600 mg TGP per day. The response was evaluated at 12 weeks, by determining whether at least a 25% reduction of disease activity score (DAS28) was met. Six patients (32%) exhibited ≥25% decrease of DAS28 and were defined as responder. All responders displayed a continuous decrease in Treg and Th1 numbers, concomitant with decrease in Th1-type cytokine levels and decrease in serum IL-6 levels. Non-responders lacked these alterations.

Patients with chronic urticaria

Long *et al*. (2010) conducted a clinical observation on the treatment of chronic urticaria with TGP capsules combined with cetirizine to assess the effect and adverse reaction. A total of 120 patients were assigned to two groups by lottery, 65 in the treated group and 55 in the control group. They all were orally treated with cetirizine tablet 10 mg per day, but to the treated group, additional 0.2 g TGP was given three times per day. The therapeutic course for both groups was 4 weeks. The effectiveness of treatment was observed, and the changes of total symptom score, serum levels of interleukin-4 (IL-4), and immunoglobulin E (IgE) were measured before and after treatment. Moreover, a follow-up was carried out one month after ending the treatment.

RESULTS

There were two dropout cases in the treated group and seven in the control group. Thus the study was accomplished on 63 patients in the treated group and 48 patients in the control group. The total effective rate was assessed at 73.02% (46/63) in the treated group, which was significantly higher than 47.92% (23/48) in the control group ($P<0.01$). After treatment, the total symptom score decreased in both groups, but the decrement in the treated group was more significant ($P<0.05$).

Serum levels of IL-4 and IgE in the treated group lowered significantly, while the changes in the control group were insignificant, so statistical significant differences were shown between groups ($P<0.01$). A follow-up study showed that the relapse rate in the treated group was 30.00% (6/20), while that in the control group was 90.00% (9/10), and the former was lower than the latter ($P<0.01$). Adverse reactions, revealed as drowsiness, dizziness, and weakness, were seen in eight cases and seven cases in the two groups, respectively. Besides, mild diarrhea occurred in two cases of the treated group. No toxicity on liver, kidney and nerve system was found.

The authors recommended more than 4 weeks medication, as through the observation in the first two weeks no relevant differences were between the groups. The symptom improving began in the latter two weeks of the treatment.

Patients with alopecia areata

Total glycosides of peony (TGP)

Yang DQ *et al*. (2012) conducted a randomised controlled trial comparing TGP capsule and a compound glycyrrhizin tablet for alopecia areata 3 months long. A total of 86 outpatients were randomised into two groups. The TGP group of 44 patients received 10 mg Vitamin B2 and three times 600 mg TGP daily. The control group received 3 times daily 50 mg glycyrrhizin. In the treatment group the cured and markedly effective rate was 36.36%, 50.00% and 68.18% at the end of the first, second and third month of treatment, versus 38.10%, 57.14% and 71.43% respectively. There was no statistic difference between the groups.

In the TGP group occurred 12 adverse reactions: 2 times abdominal pain, 6 times loose stool and 4 times increase of stool frequency. In the glycyrrhizin group occurred 14 adverse reactions: 2 times hypokalemia, 3 times increased blood pressure, 5 times edema, 2 times increased weight, 1 decrease in muscle strength.

Patients with Non-systemic Involved Sjögren Syndrome

Total glycosides of peony (TGP)

Zhang HF *et al*. (2007) conducted a retrospective clinical observation on effect of TGP in treating patients with non-systemic involved Sjögren syndrome. Twenty seven patients received TGP and 20 patients received hydroxychoroquine (HCQs) for over 2 years. In the TGP group saliva secretion was significant increased from the 6th month, as in the HCQ group. Y-globulin decreased significantly from the 6^{th} month in the TGP group and in the HCQ group from the 3^{rd} month. Mild diarrhea occurred in 4 cases in the TGP group, one case with severe diarrhea was dropped. In the HCQ group 2 patients were dropped because of sever adverse reactions. The authors concluded TGP has an equivalent efficacy to HCQ but a better safety. (Chinese, only English abstract)

Patients with systemic lupus erythematosus

Zhang HF, Xiao and Hou (2011) conducted a clinical study of TGP in patients with systemic lupus erythematosus. Twenty nine patients of the TGP1 group took orally TGP for 5 or more years. 47 patients took TGP for more than one year (TGP2 group). 20 Patients served as control. The average daily dose of prednisone, total CTX dose and SLE disease activity index were lower in the TGP1 group than in the TGP2 group. The average daily dose of prednisone, total CTX dose were lower in the TGP2 group than in the control group. The authors concluded TGP could reduce the average daily dose of prednisone and the total CTX dose especially for the medication of more than five years.

Acute pancreatitis

Red peony root decoction

Zhang M *et al*. (2008) analysed red peony root decoction in treatment of severe acute pancreatitis in a randomised controlled trial. The treatment group (48 cases) received red peony root decoction 1-2 times a day versus rhubarb in the control group (48 cases). The duration of abdominal tenderness, fever and abdominal distension were less in the treatment group. The time length for antibiotics use, nasojejunal feeding start, nasojejunal feeding, hospital stays were decreased in the treatment group compared with the rhubarb group.

Table 4: Clinical studies on humans, in in rheumatoid arthritis

Type	Study	Test Product(s)	Number of subjects	Subjects	Outcomes	Statistical analysis	Clinical relevance
Zhao YX, Liu Y (2006) effects of leflunomid (LEF) (no information on dose) and total glucosides of peony on rheumatoid arthritis	clinical observation 12 weeks	1. leflunomid (LEF) (no information on dose) and total glucosides of peony 2. leflunomid	Test group: 40 persons TGP and leflunomide control group: 40 persons LEF alone	Patients with rheumatoid arthritis	The total effective rate was higher in the treatment group than in the control group (97.5% vs 85.0%) without significant difference in adverse reactions	No information about methods available from publication	Only TGP and not the whole plant (decoct) was analysed
Efficacy of total glucosides of peony (TGP) and the protective effect on hepatotoxicity in the combination treatment with methotrexate (MTX) and leflunomide (LEF)	24-week, open label, randomised clinical trial	TGP group: MTX: 10 mg per week LEF: 29 mg per day TGP: 3x0.6 g daily Control group: MTX: 10 mg per week LEF: 29 mg per day	204 patients 18 to 75 years, 10 withdrew the study 105: TGP group 89: control group	Patients with rheumatoid arthritis, disease active score in 28 joints (DAS28)>3.2 TGP group: 5.98±1.14 Control group (CG): 6.28±1.34	Protective effect on hepatotoxicity ALT>1.5 to≤2 times= 1.9% vs 10.1% TGP vs CG; AST>2 to≤3 times= 2.9% vs 12.4% TGP vs CG; Elevated liver enzymes 3 times the ULN: 4.8% (TGP) and 12.4% (CG) No statistically significant difference Efficacy Reduction of the DAS28 values: TGP: 2.07±1.3 CG: 2.01 ±1.11 Patients with good or moderate response at week 12: TGP: 81.9%; CG:74.6% No statistically significant difference	Mean standard deviation, median, t-test, Mann-Whitney U-Test, Kruskal-Wallis-test	Only TGP and not the whole plant (decoct) was analysed; No statistically significant differences; No placebo group; No conclusion on efficacy in patients with RA is possible

Type	Study	Test Product(s)	Number of subjects	Subjects	Outcomes	Statistical analysis	Clinical relevance
Du J and Dong B (2005) efficacy of total glucosides of peony (TGP) in the combination treatment with methotrexate (MTX)	3-month, open label, randomised clinical trial	No information	31 patients TGP+MTX 30 Patients MTX	Patients with rheumatoid arthritis	The total effective rate was 90%, 94% 100% in the MTX plus TGP group and 87%, 90% 94% in the MTX group at 4, 8 and 12 weeks Insignificant difference (p>0.05)	No information about methods available from publication	No placebo group; No conclusion on efficacy in patients with RA is possible
Long JW *et al.* (2010): treatment of chronic urticaria with total glucosides of peony capsule combined with cetirizine to assess the effect and adverse reaction	Clinical observation	TGP group: 1x daily10 mg cetirizine 3x 0,2 g TGP Cetirizin group 1x daily 10 mg cetirizine 4 weeks treatment, 4-weeks follow up	120 patients, 65 treatment group 55 control group	Chronic urticarial for over 3 months, 16-60 years old, no other treatment	The total effective rate: 73.02% (46/63) in the treated group, 47.92% (23/48) in the control group (P<0.01) relapse rate treated group 30.00% (6/20), control group 90.00% (9/10), IL-4 (ng/ml) treated group: Pre-treat.:151.23±31.00 Post-treat: 105.97±9.52 IL-4 control group: Pre-treat.:155.92±24.22 Post-treat: 150.96±30.79 IgE (IU/ml) treatment group Pre-treat.:1012.91±217.73 Post-treat: 300.00±169.19 control group: Pre-treat.:997.54±252.40	No information about methods available from publication	Only TGP and not the whole plant (decoct) was analysed; no placebo group. Significant differences in relapse rate hint to possible clinical benefit

Type	Study	Test Product(s)	Number of subjects	Subjects	Outcomes	Statistical analysis	Clinical relevance
					Post-treat:943.35±240.86		
Wang Y and Xing HY (2007): efficacy of total glucosides of peony (TGP) in the combination treatment with methotrexate (MTX) on rheumatoid arthritis	Clinical observation	TGP group: MTX TGP Control group: MTX Sulfasalazine	TGP group: 180 patients Control group: 80 patients	Patients with rheumatoid arthritis	total effective rate at the 4th, 8th, 12th,24th week of the treatment TGP group 70%, 81%, 94%, 98% Control group 60%, 85%, 93% and 94%	No information about methods available from publication	Only TGP and not the whole plant (decoct) was analysed; no placebo group. Significant differences hint possible low clinical benefit
Wang YN *et al.* (2014) beneficial effect of TGP on psoriatic arthritis	Observational study	12 weeks, 3 times daily 600 mg TGP	19 patients	Patients with active, psoriatic arthritis, no anti-inflammatory medicinal products, DMARD, immunsuppressants 4 weeks before the study; no topical treatment 2	disease activity score (DAS28) Six patients (32%) exhibited ≥25% decrease of DAS28	No information about methods available from publication	Low number of patients, no control, only supportive

Type	Study	Test Product(s)	Number of subjects	Subjects	Outcomes	Statistical analysis	Clinical relevance
				weeks before			
Yang DQ *et al.* (2012) Efficacy of TGP on Alopecia areata	Randomised controlled trial	3 month TGP group 3 times daily 600 mg TGP 10 mg Vit B2 Glycyrrhizin group 3 times daily 50 mg glycyrrhizin 10 mg Vit B2	TGP group 44 cases Glycyrrhizin group 42 cases	Patients from 18-65 years with alopecia areata≤S3 (the area of hair loss is not greater than 50-75%)	Four grades of cured 1. Cured: 100% hairs grew again 2. markedly effective: 70% 3. effective: 30% 4. failed In the TGP group cured and markedly effective rate was 36.36%, 50.00% and 68.18% at the end of the first, second and third month of treatment, versus 38.10%, 57.14% and 71.43%. No significant difference between groups	No information about methods available from publication	No placebo control, concomitant use of vitamin B2; only TGP and not the whole plant (decoct) was analysed
Zhang HF (2007) efficacy and safety of TGP in non-systemic involved Sjögren syndrome	retrospective clinical observation	2 years TGP group hydroxychoroquine (HCQs) group	non-systemic involved Sjögren syndrome 27 patients TGP 20 patients hydroxychloroquine (HCQs)		Increase of saliva secretion TGP group from the 6th month, as in the HCQ group. Decrease of γ-globulin TGP group from the 6th month in the HCQ group from the 3th month.	No information about methods available from publication	Only TGP and not the whole plant (decoct) was analysed
Zhang HF, Xiao WG, Hou P (2011) efficacy and adverse reaction of TGP	retrospective clinical observation	Duration: More than 5 years 1. TGP1 group: 5 years or more years. 2. TGP2 group: one to	TGP1: 29 cases TGP2:47 cases Control group: 20 cases	patients with systemic lupus erythematosus	average daily dose of prednisone:TG1<TG2<control total CTX dose: TG1<TG2<control SLE disease activity index: TG1<TG2	No information about methods available	Only TGP and not the whole plant (decoct) was analysed

Type	Study	Test Product(s)	Number of subjects	Subjects	Outcomes	Statistical analysis	Clinical relevance
in patients with systemic lupus erythematosus		5 years 3. Control group All groups: Cyclophosphamide, prednisone			No adverse reaction correlated to TGP	from publication	
Zhang M (2008) treatment of severe acute pancreatitis	randomised controlled trial	Red peony root decoction versus Rhubarb decoction	Treatment group: 48 cases Control group 48 cases	Patients with severe acute pancreatitis	The duration of abdominal tenderness, fever and abdominal distension were less in the treatment group. The time length for antibiotics use, nasojejunal feeding start, nasojejunal feeding, hospital stays were decreased in the treatment group compared with the rhubarb group.	No information about methods available from publication	

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

Not available

4.4. Overall conclusions on clinical pharmacology and efficacy

Clinical pharmacological data of peony root decoction/preparations are not available. The published studies were often conducted with total glycosides of peony (TGP, which contains approximately 90% paeoniflorin). No placebo controlled studies were available. The prospective controlled clinical studies were often conducted with a low number of patients and comedication was used.

In summary, the available clinical data are not considered sufficient to fulfil the requirements of a well-established medicinal use with recognised efficacy and to be eligible for a marketing authorisation according to Art. 10a of Directive 2001/83/EC. .

5. Clinical Safety/Pharmacovigilance

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

Chen Z *et al.* (2013) analysed in a 24-week, open label, randomised clinical trial the efficacy of TGP and the protective effect on hepatotoxicity in the combination treatment with methotrexate and leflunomide in 204 patients with active rheumatoid arthritis. The incidence of gastrointestinal tract disturbances, mostly diarrhoea, were higher in the TGP group (TGP plus methotrexate plus leflunomide) than in the control group (methotrexate plus leflunomide), (12.4% vs 1.1%, p=0.002). The authors concluded, although most of these events were mild and self-resolved in 1 to 2 weeks, more attention is needed in clinical praxis.

He and Dai (2011) reported in a review article the first prospective double blind trial of TGP was conducted 1993 in 450 patients with rheumatoid arthritis. They were randomized to receive TGP 1.8 g per day or methotrexate 810 mg per week) for 12 weeks. Therapeutic response was achieved in 71.7 of TGP-treated patients and 81.7 of methotrexate *in-vitro.* The adverse events occurred in 13.3% patients and most of them were gastrointestinal tract disturbances, mostly mild diarrhoea. Most of them were self-resolved in 1 to 2 weeks.

Yang DQ *et al.* (2012) conducted a randomised controlled trial comparing TGP capsule and compound glycyrrhizin tablet for alopecia areata 3 month long. A total of 86 outpatients were randomised into two groups. The TGP group of 44 patients received 10 mg Vitamin B2 and three times 600 mg TGP daily. The control group received 3 times daily 50 mg glycyrrhizin. In the TGP group occurred 12 adverse reactions: 2x abdominal pain, 6x loose stool and 4x increase of stool frequency. In the glycyrrhizin group occurred 14 adverse reactions: 2x hypokalemia, 3x increased blood pressure, 5x edema, 2x increased weight, 1 decrease in muscle strength.

5.2. Patient exposure

Not available

5.3. Adverse events, serious adverse events and deaths

Timmermans *et al*. (2009) reported a case of contact dermatitis due to peony in a 56-old nursery man presented a 1 year history of facial eczema. A positive patch test reaction was interpreted as allergic and not as irritant reaction as patch tests with the extracts in 20 controls were negative.

Ward (2005) reported in “The Essential Guide to Clinical Safety” that a statement, bai shao or chi shao were found to be hepatotoxic, was based on one reference that had been incorrectly interpreted and no further evidence supported this. The Traditional Remedies Surveillance Team has repeatedly stated that no single ingredient has been implicated in the cases of hepatic reactions to herbs. There is still a lack of incidence data to fully define this problem, which is not unique to Chinese herbal medicine.

Ahmad *et al*. (2012) reported from the traditional TCM use adverse reactions as blistering of mouth and throat, diarrhoea, dizziness, fainting, gastroenteritis, haematuria, nausea, salivation, stomach pain, vomiting and/or possible death were known.

5.4. Laboratory findings

Not available

5.5. Safety in special populations and situations

No data available.

5.5.1. Use in children and adolescents

No data available.

5.5.2. Contraindications

No data available.

5.5.3. Special warnings and precautions for use

No data available.

5.5.4. Drug interactions and other forms of interaction

According to Landmark (2008) antiepileptic drugs have a high propensity to interact with concomitant medications. The administration of phenytoin in combination with Paeoniae Radix increased phenytoin T_{max} 3-fold in rat model and this was attributed to a delay of phenytoin absorption. In a clinical experimental study with valproate no changes in biochemistry analyses were observed.

Chen LC *et al*. (2000) evaluated clinical interactions between the antiepileptic medicinal product valproic acid and Paeoniae Radix. The pharmacokinetics of VPA were investigated in a randomised, open-label, two-way crossover study. Six healthy volunteers received the following treatments in a crossover design:

(i) 1-2 g extract powder of Paeoniae radix once daily for 7 days and one 200 mg VPA on day 7 or

(ii) one 200 mg VPA tabl*et al*one on day 7

The mean maximum plasma concentration of VPA was attained at within 6 h after oral administration VPA alone and 3-4 h after oral administration in combination with PR. The plasma level of VPA declined with a half-life of 11.71 and 11.91 h respectively. No statistically significant difference was obtained in the pharmacokinetic parameters T_{max} C_{max}, AUC, $t_{1/2}$, MRT, CL/F and V_d/F and the protein binding rates of VPA. No concomitant treatment during 7 days was analysed, as VPA was given only one tablet on day 7.

In the Alternative medicine Review (2001) it was assumed, peony may not be combined with antibiotics. Damage to the gut flora by antibiotics might interfere with the process for cleaving the aglycons of peony and theoretically decreasing peony´s efficacy.

5.5.5. Fertility, pregnancy and lactation

Zhang and Dai (2012) report, that traditional use of peony root in TCM indicated that Radix Paeoniae may have abortifacient activity, so the use is contraindicated in pregnancy for safety reasons.

5.5.6. Overdose

No data available.

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

No data available.

5.5.8. Safety in other special situations

No data available.

5.6. Overall conclusions on clinical safety

Available data are not sufficient to establish a European Union monograph.

6. Overall conclusions (benefit-risk assessment)

No clinical studies support the well-established use of peony root in any (western medicine) indication.

The requirements for TU (self-medication character, specified strength/posology, appropriate route of administration, period of traditional use, plausibility and safety) are not met. No constituent with known therapeutic activity or active marker can be recognised by the HMPC.

A public statement is suggested. In conclusion, the following requirements for the establishment of a European Union herbal monograph on traditional or well-established herbal medicinal products containing *Paeonia lactiflora* Pall. or *Paeonia veitchii* Lynch or a mixture, radix rubrae are not fulfilled:

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

- the requirement laid down in Article 16a(1)(b) of Directive 2001/83/EC that the herbal substance or herbal preparation is “exclusively for administration in accordance with a specified strength and posology”

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

- the requirement laid down in Article 16a(1)(e) of Directive 2001/83/EC that “the data on the traditional use of the medicinal product are sufficient; in particular the product proves not to be harmful in the specified conditions of use and the pharmacological effects or efficacy of the medicinal product are plausible on the basis of long-standing use and experience”

- the HMPC acknowledges the existence of numerous publications on Paeoniae radix or constituents; however, often an appropriate description of herbal substance or herbal preparations used are missing. Available data are not sufficient to establish a European Union monograph.

Annex

List of references