Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of *Capsicum frutescens* L., fructus

Final

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

Herbal substance(s) (binomial scientific name of the plant, including plant part)	*Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of *Capsicum frutescens* L., fructus
Herbal preparation(s)	a) Soft extract (DER 4-7:1), standardised to 2–2.78% total capsaicinoids, extraction solvent ethanol 80% (V/V) b) Soft extract (DER 1.5–2.5:1), extraction solvent ethanol 96% (V/V) c) Soft extract (DER 11-30:1), extraction solvent propan-2-ol
Pharmaceutical form(s)	Herbal preparation in a medicated plaster or in semi-solid dosage forms for cutaneous use
Rapporteur(s)	R. Länger
Peer-reviewer	M. Heroutová

30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom

Telephone +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5555
Send a question via our website www.ema.europa.eu/contact

An agency of the European Union

Table of contents

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of *Capsicum frutescens* L., fructus
EMA/HMPC/674138/2013

Page 2/67

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of
Capsicum frutescens L., fructus
EMA/HMPC/674138/2013   Page 3/67

1. Introduction

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

Herbal substance(s)

Definition in the European Pharmacopoeia (monograph 1859)

Dried ripe fruits of *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small-fruited varieties of *Capsicum frutescens* L. (Capsici fructus)

Content: minimum 0.4% of total capsaicinoids, expressed as capsaicin ($C_{18}H_{27}NO_3$; M_r 305.4) (dried drug).

Constituents (Blaschek *et al.* 2012):

Capsaicinoids: 0.3 – more than 1% of total capsaicinoids, consisting of 63-77% capsaicin, 20-32% dihydrocapsaicin, 1-8% nordihydrocapsaicin, and undefined amounts of homodihydrocapsaicin I and II, vanillylamides of caprylic acid and nonylic acid.

Other constituents: fatty oil, carotenoids, ascorbic acid, volatile compounds.

Herbal preparation(s)

Herbal preparations in the European Pharmacopoeia:

Capsicum oleoresin, refined and standardised (Capsici oleoresina raffinata et normata), monograph 2336

> Extraction solvent ethanol (minimum 90% V/V), extract standardised to a content of 12 to 18% m/m of total capsaicinoids, expressed as capsaicin ($C_{18}H_{27}NO_3$; M_r 305.4)

Capsicum soft extract, standardised (Capsici extractum spissum normatum), monograph 2529

> Extraction solvent ethanol (80% V/V), extract standardised to a content of 2 to 2.4% m/m of total capsaicinoids, expressed as capsaicin ($C_{18}H_{27}NO_3$; M_r 305.4)

Capsicum tincture, standardised (Capsici tinctura normata), monograph 2337

> Extraction solvent ethanol (70% to 85% V/V), extract standardised to a defined content between 0.020 and 0.060% m/m of total capsaicinoids, expressed as capsaicin ($C_{18}H_{27}NO_3$; M_r 305.4)

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. Information about products on the market in the Member States

Regulatory status overview

Member State	Regulatory Status				Comments
Austria	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	
Belgium	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	
Bulgaria	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	
Cyprus	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	
Czech Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	
Denmark	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	
Estonia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	No products
Finland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	
France	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	
Germany	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	
Greece	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	
Hungary	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	
Iceland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	
Ireland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	
Italy	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	
Latvia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	Only combinations
Liechtenstein	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	
Lithuania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	No products
Luxemburg	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	
Malta	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	
The Netherlands	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	Only combination
Norway	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	
Poland	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	Only combinations
Portugal	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	
Romania	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	
Slovak Republic	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	
Slovenia	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	
Spain	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	
Sweden	☒ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	
United Kingdom	☐ MA	☐ TRAD	☐ Other TRAD	☐ Other Specify	

MA: Marketing Authorisation

TRAD: Traditional Use Registration

Other TRAD: Other national Traditional systems of registration

This regulatory overview is not legally binding and does not necessarily reflect the legal status of the products in the MSs concerned.

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of
Capsicum frutescens L., fructus
EMA/HMPC/674138/2013 Page 5/67

1.3. Search and assessment methodology

Databases assessed: PubMed, Scopus

Search terms: Capsicum, Capsaicin

Exclusion criteria: Botany, agriculture, biotechnology, use and misuse as spray for defence

Inclusion criteria: clinical trial, pharmacology, safety

2. Data on medicinal use

2.1. Information on period of medicinal use in the European Union

Medicinal products

Austria

	Herbal preparation	Since
1	Capsicum soft extract (DER 4-7:1), standardised to 2 –2.78% total capsaicinoids, extraction solvent ethanol (80% V/V) Medicated plaster 22 x 14 cm containing 11 mg capsaicinoids, corresponding to 35 µg capsaicinoids per cm^2	2001

Denmark

	Herbal preparation	Since
1	Capsicum soft extract (DER 4-7:1), standardised to 2 –2.78% total capsaicinoids, extraction solvent ethanol (80% V/V) Medicated plaster 22 x 14 cm containing 11 mg capsaicinoids, corresponding to 35 µg capsaicinoids per cm^2	2001

Germany

	Herbal preparation	Since
1	Capsicum soft extract (DER 4-7:1), standardised to 2 –2.78% total capsaicinoids, extraction solvent ethanol (80% V/V) Medicated plaster 22 x 14 cm containing 11 mg capsaicinoids, corresponding to 35 µg capsaicinoids per cm^2	2000
2	Capsicum soft extract (DER 4-7:1), standardised, extraction solvent ethanol (80% V/V) Medicated plaster 12 x 18 cm containing 4.8 mg capsaicinoids corresponding to 22 µg capsaicinoids per cm^2	2003
3	100 g ointment contain 0.8-1.6 g soft extract (DER 4-7:1) corresponding to 40 mg capsaicinoids calculated as capsaicin, extraction solvent: ethanol 80% (V/V)	At least since 1976
4	100 g cream contain 0.93-2.9 g soft extract (DER 1.5–2.5:1) corresponding to 50 mg capsaicinoids calculated as capsaicin, extraction solvent: ethanol 96% (V/V)	At least since 1976
5	100 g ointment contain 0.24-1.02 g soft extract (DER 11-30:1) corresponding to 53 mg capsaicinoids calculated as capsaicin, extraction solvent: propan-2-ol	At least since 1976
6	100 g cream contain 0.6627-1.8292 g soft extract (DER 4-7:1) corresponding to 53 mg capsaicinoids calculated as capsaicin, extraction solvent: ethanol 80% (V/V)	2005

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of *Capsicum frutescens* L., fructus
EMA/HMPC/674138/2013

Page 6/67

Spain

	Herbal preparation	Since
1	Capsicum soft extract (DER 4-7:1), standardised to 2 –2.78% total capsaicinoids, extraction solvent ethanol (80% V/V) Medicated plaster 22 x 14 cm containing 11 mg capsaicinoids, corresponding to 35 µg capsaicinoids per cm^2	2004
2	Oleoresin of *Capsicum annuum* L. (equivalent to 0.025 g of capsaicin). Cream (0.025%)	1996
3	Oleoresin of *Capsicum annuum* L. (312-625) mg equivalent to 75 mg of capsaicin. Cream (0.075%)	2007
4	Soft extract of Capsicum (4-7:1) corresponding to 1.9 mg of capsaicinoids, expressed as capsaicin. Extraction solvent: Ethanol 80% (V/V). Medicated plaster	2009

Sweden

	Herbal preparation	Since
1	Capsicum soft extract (DER 4-7:1), standardised to 2 –2.78% total capsaicinoids, extraction solvent ethanol (80% V/V) Medicated plaster 22 x 14 cm containing 11 mg capsaicinoids, corresponding to 35 µg capsaicinoids per cm^2	2002

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

Consolidated list of herbal preparations

Considered in the monograph:

1. Herbal preparation a) Soft extract (DER 4-7:1), standardised to 2–2.78% total capsaicinoids, extraction solvent ethanol (80% V/V)

    Medicated plaster 22 x 14 cm containing 11 mg capsaicinoids, corresponding to 35 µg capsaicinoids per cm^2 (Austria #1, Denmark #1, Germany #1, Spain #1, Sweden #1, at least since 2000)

    Medicated plaster 12 x 18 cm containing 4.8 mg capsaicinoids, corresponding to 22 µg capsaicinoids per cm^2 (Germany #2, since 2003)

    Semi-solid dosage forms corresponding to 40-53 mg capsaicinoids/100 g (Germany #3 at least since 1976, Germany #6 since 2005)

2. Herbal preparation b) Soft extract (DER 1.5–2.5:1), extraction solvent: ethanol 96% (V/V)

    Cream corresponding to 50 mg capsaicinoids calculated as capsaicin/100 g (Germany #4, at least since 1976)

3. Herbal preparation c) Soft extract (DER 11-30:1), extraction solvent: propan-2-ol

    Ointment corresponding to 53 mg capsaicinoids calculated as capsaicin/100 g (Germany #5, at least since 1976)

Not considered in the monograph:

Spain #2: insufficient data on clinical efficacy, less than 30 years of medicinal use.

Spain #3, 4: less than 10 years in medicinal use, therefore neither suitable for well-established use nor for traditional use.

General remark regarding a potential well-established use for medicinal products which act locally and are locally applied:

Following the general regulatory discussions on capsaicin containing medicinal products there is a common understanding that applications for new medicinal products cannot be based on a bibliographic or generic approach alone. The clinical efficacy of locally applied capsaicin (semi-solid dosage forms, medicated plasters) depends in a high degree on the excipients used. Therefore a bridging of clinical data from a certain medicinal product to another one without supporting data may not be acceptable from a regulatory point of view. Data on pharmaceutical as well as clinical equivalence might be necessary in order to show the relevance of the literature data for the concerned product.

Contact with the responsible national agencies is advisable prior to an application (National scientific advice).

Synthetic capsaicin:

Centrally authorised medicinal product: Cutaneous patch (14 x 20 cm) containing 179 mg capsaicin per patch or 640 µg capsaicin per cm^2.

2.2. Information on traditional/current indications and specified substances/preparations

Medicinal products

Austria

1	Medicated plaster: *Adults and adolescents*: Cutaneous treatment for the relief of muscle pain, e.g. pain in the lower back. The use in children below 12 years of age is not recommended because of the lack of data for efficacy and safety.

Denmark

1	Medicated plaster: *Adults and adolescents*: Cutaneous treatment for the relief of muscle pain, e.g. pain in the lower back. The use in children below 12 years of age is not recommended because of the lack of data for efficacy and safety.

Germany

1	Medicated plaster: *Adults and adolescents*: Cutaneous treatment for the relief of muscle pain, e.g. pain in the lower back. The use in children below 12 years of age is not recommended because of the lack of data for efficacy and safety.
2	Medicated plaster: *Adults and adolescents*: Cutaneous treatment for the relief of muscle pain, e.g. pain in the lower back.
3-4-5	Painful muscle tension in shoulder, arm and spine
6	Painful muscle tension in shoulder, arm and spine Or For the relief of muscle pain related to soft-tissue rheumatism and muscle tension in the areas of shoulder, neck and lower back

Spain

1	Symptomatic relief of muscular pain
2	Symptomatic relief of muscular pain
3	Painful diabetic neuropathy
4	Symptomatic relief of muscular pain

Sweden

1	Medicated plaster: *Adults and adolescents*: Cutaneous treatment for the relief of muscle pain, e.g. pain in the lower back. The use in children below 12 years of age is not recommended because of the lack of data for efficacy and safety.

Based on the indications of the authorised products the indication for the monograph is proposed as:

Herbal medicinal product for the relief of muscle pain, such as low back pain.

2.3. Specified strength/posology/route of administration/duration of use for relevant preparations and indications

Austria

1	Medicated plaster 22 x 14 cm containing 11 mg capsaicinoids, corresponding to 35 µg capsaicinoids per cm^2 Duration of use: maximum 1 patch per day, use over 4-12 hours. Before use of next patch interval of at least 12 hours. Use should be continued if necessary up to 3 weeks.

Denmark

1	Medicated plaster 22 x 14 cm containing 11 mg capsaicinoids, corresponding to 35 µg capsaicinoids per cm^2 Duration of use: maximum 1 patch per day, use over 4-12 hours. Before use of next patch interval of at least 12 hours. Use should be continued if necessary up to 3 weeks.

Germany

1	Medicated plaster 22 x 14 cm containing 11 mg capsaicinoids, corresponding to 35 µg capsaicinoids per cm^2 Duration of use: maximum 1 patch per day, use over 4-12 hours. Before use of next patch interval of at least 12 hours. Use should be continued if necessary up to 3 weeks.
2	Duration of use: maximum 1 patch per day, use over 4-8 hours. Before use of next patch interval of at least 12 hours. Use should be continued if necessary up to 3 weeks.
3-4	Up to 4 times daily, not longer than 2 days
5	2-4 times daily, not longer than 2 days; for an area of 15 x 15 cm 2.5-4 g ointment
6	3 times daily, not longer than 2 days (indication muscle tension) Or 3 times daily, not longer than 3 weeks (indication muscle pain related to soft-tissue rheumatism)

Spain

1	Medicated plaster, 1 per day
2	Cream 0.025%, 3-4 times daily
3	Cream 0.075%, 3-4 times daily
4	Medicated plaster, 1 per day

Sweden

1	Medicated plaster 22 x 14 cm containing 11 mg capsaicinoids, corresponding to 35 µg capsaicinoids per cm^2 Duration of use: maximum 1 patch per day, use over 4-12 hours. Before use of next patch interval of at least 12 hours. Use should be continued if necessary up to 3 weeks.

Based on the posologies of the authorised products and the data from clinical trials the posologies in the monograph are as follows:

Medicated plaster

Herbal preparation a)

Adults and elderly
One medicated plaster (22 x 14 cm) contains soft extract of Capsici fructus, corresponding to 11 mg capsaicinoids expressed as capsaicin (= 35 µg/cm^2).
One medicated plaster (12 x 18 cm) contains soft extract of Capsici fructus, corresponding to 4.8 mg capsaicinoids expressed as capsaicin (= 22 µg/cm^2).

Daily dosage: A maximum of 1 plaster per day should be applied for at least 4 and up to 12 hours. There should be an interval of at least 12 hours before a new plaster is applied at the same application area.

Semi-solid dosage forms

Herbal preparation a), b), c)

Adults and elderly
Semi-solid dosage forms corresponding to 40-53 mg capsaicinoids/100 g. Apply 2-4 times daily. To be applied in a thin layer on the affected area.

The use in children and adolescents is not considered as safety and efficacy in this age group is not supported by published data from clinical trials.

3. Non-Clinical Data

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

Many pharmacological studies have been conducted mainly with capsaicinoids/capsaicin. A systematic review of all of these studies will not be attempted here; rather a selection of studies is presented.

3.1.1. Primary pharmacology

Published data refer to isolated or synthetic capsaicin. No pharmacological data regarding herbal preparations are available.

In vitro, summary:

Compilation of Caterina *et al.* 1997; Biro *et al.* 1997; Sasamura & Kuraishi, 1999; Jordt *et al.* 2003; Szolcsányi 2004; Hayman & Kam, 2008; Hänsel & Sticher, 2010

Capsaicinoids are agonists of the vanilloid receptor (VR) (transient receptor potential vanilloid TRPV), which is located primarily on the ends of axons containing Substance P.

Such neurons are also responsible for the perception of pain. TRPVs are sensitive to elevated temperature, acids and in case of TRPV1 also to capsaicin. TRPV are ion channels selective for calcium, magnesium and sodium.

In vivo, relevant publications:

Gamse 1982 (only abstract available): The effect of systemic or intrathecal treatment of capsaicin (no more details regarding concentrations available) on thermonociception, chemonociception, content and release of immunoreactive substance P (I-SP) was investigated in newborn or adult mice. Mice treated on the 2nd day of life had normal reaction times on the hot plate and a small and inconsistent prolongation of the tail withdrawal latency. In contrast, mice treated on day 7, 10 or as adults had greatly prolonged latencies in both tests for at least 3 months. The changes in latencies were not affected by naloxone or methysergide. Responses to noxious chemical stimuli were moderately inhibited in mice treated on the 2nd day of life, but almost abolished in mice treated on day 7, 10 or as adults. Treatment of mice on day 2 caused a similar decrease of the I-SP content in spinal cord and of the capsaicin-evoked I-SP release (88%) as treatment on day 4 or 7 although behavioural changes were different. After treatment of adult mice, release of I-SP was reduced by 93%.

Wall 1987 (only abstract available): After application of capsaicin to peripheral nerves in rats (no more details regarding concentrations available), a raised threshold to the response to chemical, thermal and mechanical stimuli was noted in the area subserved by the treated nerve.

McMahon *et al.* 1991: Capsaicin cream (0.075% or 0.75%) or a vehicle cream was applied twice daily to the hind paws of rats for a continuous period of 10 weeks. The hind paws treated with 0.75% capsaicin cream became transiently hyperalgesic, which was not observed with the 0.075% concentration. After 10 weeks of capsaicin treatment the ability of C fibres to produce neurogenic extravasation was markedly reduced. After 4 weeks of recovery this ability returned to normal in 0.075% capsaicin treated animals, but remained impaired in the 0.75% group. This latter group showed a partial recovery 12 weeks after the end of treatment. The levels of substance P and Calcitonin gene-related peptide in the sural nerve supplying the treated skin area were unchanged in both treatment groups. According to the authors, these results suggest that the topical application of capsaicin at low concentrations causes a reversible impairment of the terminals of C fibres in the skin without greatly exciting those fibres and without affecting the properties of the cell soma.

Lynn *et al.* 1992 (only abstract available): Application of capsaicin solutions onto the rat skin caused a slow, dose-dependent increase in skin blood flow (rise in the laser-Doppler flux signal, with maximum flux usually occurring at 10-30 min). After application of a 33 mM solution, blood flow recovered to baseline in 2-4 hours (4 preparations). Capsaicin at 1 mM caused an average increase of 60% ($\pm$25%, n=6), a rise that was just significant (p<0.05). The average increase with 33 mM capsaicin was 171% ($\pm$23%, n=7) and increases of over 300% were seen at some locations.

Hiura 2000: Capsaicin caused neuronal damage. The treatment of neonatal rats and mice with capsaicin (50 mg/kg, s.c.) caused a loss of dorsal root ganglion cells. The cell death was regarded to be by necrosis rather than apoptosis. Despite the loss of C-fibres the withdrawal responses to noxious heat remained normal in mice treated neonatally with capsaicin.

Yoshimura & Yonehara, 2001: The authors investigated the effect of topical application of capsaicin cream on withdrawal latency in the hind foot of rat in response to radiant heat in an experimental model of neuropathic pain. A neuropathic state was induced by loose ligation of the sciatic nerve with chromic gut suture.

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of
Capsicum frutescens L., fructus
EMA/HMPC/674138/2013   Page 11/67

A marked thermal hyperalgesia was observed in response to heat stimulus applied to the operated side from 3 days through 2 weeks, followed by a gradual return to the control level by 35 days after surgery. Capsaicin cream applied to both the bilateral hind instep and sole once a day for a continuous period of 2 weeks or 4 weeks alleviated thermal hyperalgesia in a dose-dependent manner (0.1% and 1% capsaicin). A remarkable effect was observed 2 weeks after the start of the application and this effect proved to be reversible. On the other hand, in sham-operated animals when capsaicin cream was applied once daily from day 7 after the sham operation, from 1 day through 3 weeks following capsaicin application, withdrawal latency of the sham-operated paws of the capsaicin-treated group was significantly increased as compared to that of the vehicle cream-treated group. The effects of antagonists of glutamate receptor and tachykinin receptors were investigated 7 days post-surgery. Pre-treatment with MK-801 (Dizocilpine, 0.5 mg/kg, i.p.), but not with CNQX (6-cyano-7-nitroquinoxaline-2.3-dione, 0.5 mg/kg, i.p.), reversed the thermal hyperalgesia following nerve injury. Neither [2-(1-imino-2-(2-methoxyphenyl)ethyl)-7,7 diphenyl-4perhydro-iso-indolone-(3aR, 7aR)] (1–10 mg/kg, i.p.) nor (S)-N-methyl-N[4-(4-acetylamino-4-phenyl-piperidino)-2-(3.4-dichlorophenyl)butyl]benzamide)(1–10 mg/kg, i.p.) had any effect on the withdrawal latency in the injured and non-injured hind paw. The authors concluded, that these results suggested that although the manifestation of effectiveness may be delayed by changes in networks of neurotransmitters related to the nociceptive pathways following nerve injury, longer-term repetitive application of capsaicin cream has a therapeutic effect on subjects with painful peripheral neuropathy.

Sluka 2002: Pain and hyperalgesia from deep somatic tissue (i.e., muscle and joint) are processed differently from that from skin. Capsaicin (50 µl of a 0.2% solution) was injected into the plantar aspect of the skin, plantar muscles of the paw, or ankle joint, and responses to mechanical and heat stimuli were assessed until allodynia resolved. Capsaicin injected into skin resulted in a secondary mechanical allodynia and heat hypoalgesia lasting approximately 3 hours. In contrast, capsaicin injection into muscle or joint resulted in a long-lasting bilateral (1-4 weeks) mechanical allodynia with a simultaneous unilateral heat hypoalgesia. The pattern and degree of inflammation were similar when capsaicin was injected into skin, muscle, or joint, with peak increases 24 hours after injection. Heat hypoalgesia, that occurs after injection into deep tissue was reversed by spinal blockade of adenylate cyclase or protein kinase-A. Thus, injection of capsaicin into deep tissues resulted in a longer-lasting mechanical allodynia and heat hypoalgesia compared with injection of capsaicin into skin. The mechanical allodynia depended on early activation of the cyclic adenosine monophosphate (cAMP) pathway during the first 24 hours but was independent of the cAMP pathway by 1 week after injection of capsaicin.

Menéndez *et al.* 2004: In another study, capsaicin (10 µg; intra-plantar) induced an analgesic effect in control mice lasting for 2 days. In contrast, in carrageenan-treated mice, the analgesic effect of capsaicin lasted for 6 days and in complete Freund's adjuvant-treated mice for 30 days. This prolongation of capsaicin-induced analgesia during inflammation was mediated through VR1 since it was completely blocked by co-administration of capsazepine (CZP) (10 µg).

Bölcskei *et al.* 2010 (only abstract available): Agonists of the TRPV1 receptor excited TRPV1-expressing polymodal nociceptors that was followed after higher doses by a state of diminished responsiveness called desensitisation which ensued at two levels: (i) diminished responsiveness of the ion channel (TRPV1 receptor desensitisation); (ii) diminished responsiveness of the nerve endings to all stimuli including noxious heat. A comparison of the desensitising actions of TRPV1 agonists in the rat by measuring with an incremental hot/cold plate the noxious heat and cold thresholds, i.e. the lowest hot and highest cold plate temperatures respectively, that evokes nocifensive behaviour was performed.

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of
Capsicum frutescens L., fructus
EMA/HMPC/674138/2013
Page 12/67

Capsaicin (3.3-1000 nmol) applied intraplantarly evoked a sustained dose-dependent elevation of the noxious heat threshold lasting for 2-11 days. The noxious cold threshold was decreased by capsaicin with a recovery within 2-4 days. The authors concluded, using measurement of threshold temperatures eliciting nocifensive reactions in rats both in the hot and cold range revealed that capsaicin impair thermosensation in both noxious ranges due to a functional desensitisation of peripheral terminals of TRPV1-expressing sensory neurons responsible for noxious heat and cold responsiveness.

3.1.2. Secondary pharmacodynamics

Cardiovascular system

In vitro, herbal preparations:

Chularojmontri *et al.* 2010 (only abstract available): The authors tried to examine the effect of Capsicum spp. extract (CEX, no more information regarding extraction solvent and DER) and capsaicin on endothelial nitric oxide release and protection against lipopolysaccharide (LPS)-induced cellular apoptosis. Human umbilical vein endothelial cells (HUVEC) were isolated from newborn cords. The evaluation of cytotoxicity was performed by MTT assay. Endothelial nitric oxide (NO) production was evaluated by Griess reaction. Alteration in endothelial nitric oxide synthase (eNOS) expression was detected by western blot analysis. To induce oxidative stress and apoptosis, LPS was co-incubated with HUVEC in the presence or absence of CEX or capsaicin, and the VR blocker CZP. Hoechst nuclear staining was used to determine percent apoptotic nuclei. The highest concentrations of CEX (1000 µg/ml) and capsaicin (25 µM) used in the study did not induce cytotoxicity in HUVEC. Significant increase in NO release was observed when cells were incubated with CEX (100 µg/ml) and capsaicin (25 µM) and this effect was inhibited by CZP only in the capsaicin treated group. Despite the observed enhanced NO generation, western blot analysis indicated no change in eNOS expression. Interestingly, endothelial cells incubated with L-arginine (L-ARG, 1000 µg/ml) alone showed significantly increased NO production while L-ARG co-incubation abrogated CEX or capsaicin effects on endothelial NO generation. CEX (10 µg/ml) and capsaicin (1 µM) decreased apoptotic nuclei in HUVEC treated with LPS. CEX and capsaicin improved endothelial function and protected against LPS-induced apoptosis. The authors concluded that regular consumption of Capsicum spp. may promote endothelial health and reduce cardiovascular disease risk.

In vitro, capsaicin:

Min *et al.* 2004: *In vitro*, capsaicin inhibited vascular endothelial growth factor (VEGF)-induced proliferation, DNA synthesis, chemotactic motility, and capillary-like tube formation of primary cultured human endothelial cells. Capsaicin inhibited both VEGF-induced vessel sprouting in rat aortic ring assay and VEGF-induced vessel formation in the mouse Matrigel plug assay. Moreover, capsaicin was able to suppress tumour-induced angiogenesis in chick chorioallantoic membrane assay. Capsaicin caused G1 arrest in endothelial cells. This effect correlated with the down-regulation of the expression of cyclin D1 that led to inhibition of cyclin-dependent kinase 4-mediated phosphorylation of retinoblastoma protein. Signalling experiments showed that capsaicin inhibited VEGF-induced p38 mitogen-activated protein kinase, the focal adhesion kinase p125FAK, and of the gene AKT activation, but its molecular target is distinct from the VEGF receptor KDR/Flk-1.

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of
Capsicum frutescens L., fructus
EMA/HMPC/674138/2013   Page 13/67

In vivo, herbal preparations:

Nelson *et al.* 2004: The authors examined the effects of capsicum oleoresin (4.95% in ointment base, no further details available) application on pressor responses evoked by muscle contraction (MC), which are mediated by group III and IV muscle afferents in cats (n=12). Changes in peak mean arterial pressure (MAP) induced by static ipsilateral MC were significantly attenuated at 20 min and tended to approach baseline levels at 40 min after capsaicin application. The mean ($\pm$SEM) of the peak MAP for the ipsilateral side just before application (T=0), at 20 min (T+20), and 40 min (T+40) were 28.3 mm Hg$\pm$6.4, 13.8 mm Hg$\pm$2.9, and 22.6 mm Hg$\pm$5.2, respectively. There were no significant changes in heart rate. Therefore the authors concluded that cardiovascular effects due to activation of group III and IV afferent fibres were significantly attenuated by the application of capsaicin. The time course of the effects appeared to support the need for repeated capsaicin application for pain relief.

In vivo, capsaicin:

Castle 1992: The aim of the study was to examine the effects of capsaicin on cardiac K^+ currents. Ionic currents and action potentials were examined in isolated adult rat ventricular myocytes using the whole cell variant of the patch clamp technique at 25°C. Capsaicin (10 μM) increased the action potential duration (APD_{50}) from 45 ms to 166 ms. This effect was associated with an inhibition of three distinct K^+ currents. The decreasing rank order of potency was: transient outward K^+ current (I_{TO}, IC_{50}=6.4 μM), a voltage dependent non-inactivating outward current (I_K, IC_{50}=11.5 μM), and the inward rectifier K^+ current (I_{K1}, IC_{50}=46.9 μM). Capsaicin induced block of I_{TO} was characterised by a decrease in the peak current amplitude and an increase in the rate of inactivation. The inactivation of I_{TO} in the absence of capsaicin was well described by a single exponential [τ=77 (SEM 2) ms at +40 mV, n=10]. However, in the presence of 10 μM capsaicin inactivation was best described by the sum of two exponentials [τ_{FAST}=4.4(0.5) ms; τ_{SLOW}=92.4(3.0) ms, n=10] with the fast component contributing 46(2)% of the total decay. A small but consistent hyperpolarising shift (~3 mV) in the steady state voltage dependence of inactivation of I_{TO} was induced by 10 μM capsaicin. Capsaicin had no effect on the rate of I_{TO} recovery from inactivation (τ=49 ms and 48 ms for control and drug, respectively). The capsaicin analogue, resiniferatoxin, which as an irritant is up to 104-fold more potent than capsaicin, had no effect on any of the K^+ currents when presented at concentrations of up to 10 μM. In contrast, another capsaicin analogue, zingerone (30 μM) blocked I_{TO} by 52(12)% and I_K by 35%. The authors concluded, that capsaicin produced a prolongation of the rat ventricular action potential, an effect which is associated with inhibition of potassium currents.

Cancer cells, induced cancer

In vitro, herbal preparations:

Dou *et al.* 2011: Extracts prepared with 100% ethanol from a number of varieties of *Capsicum annuum* (from bell pepper lacking capsaicin to very hot varieties, DER 1:1) were tested and were found to induce significant growth arrest and apoptosis in human breast (MDA-MB-231, MCF-7) and leukaemia cancer cell lines (Jurkat T cells) *in vitro* with no significant effect on normal breast epithelial cells in a concentration of 0.1 g Capsicum/ml final solution). Furthermore, cell growth inhibition and cell death induction were positively correlated with the capsaicin content (based on the Scoville scale), and the hydroxyl radical scavenger thiourea significantly inhibited the activity of the extracts, suggesting the involvement of free radicals in mediating the biological activity of the Capsicum extracts.

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of
Capsicum frutescens L., fructus
EMA/HMPC/674138/2013   Page 14/67

Dwivedi *et al.* 2011: The objective of the study was to compare the *in vitro* anticancer activities of aqueous and ethanolic extracts (DER 1:4) of commercially available *Capsicum annuum* against the TE-13 (oesophageal squamous cell carcinoma) cell line. The extracts (concentrations 100 µl/ml to 300 µl/ml) showed cytotoxic activity but the aqueous extract was found to be more potent. Morphological analysis, DAPI (4´, 6-diamidino-2-phenylindole di-hydrochloride) staining and DNA fragmentation assays showed maximum cell death and apoptotic cell demise (88%) to occur within 24 hours with an aqueous extract of chili pepper at 300 µl/ml.

In vitro, capsaicin:

Chou *et al.* 2009: Treatment with capsaicin for 24 hours resulted in dose-dependent apoptosis in Michigan Cancer Foundation (MCF)-7 cells, which does not express caspase-3. After the addition of capsaicin, the levels of reactive oxygen species (ROS) were reduced slightly in the earlier stage of treatment. An elevation of intracellular calcium ion concentration was detected in the MCF-7 cells. In time course and dosage studies, the mitochondrial membrane potential of MCF-7 cells decreased. However, the change was not significant. The apoptosis-inducing factor translocated into the cytosol and nucleus from the mitochondria. According to the authors these results suggest that capsaicin induces cellular apoptosis through a caspase-independent pathway in MCF-7 cells, and that ROS and intracellular calcium ion fluctuation has a minimal role in the process.

Kim *et al.* 2004: The potential of capsaicin to induce apoptotic cell death in human colon cancer cells and the association of peroxisome proliferator-activated receptor γ (PPARγ) in the capsaicin action was investigated. Cell viability was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide assay. PPARγ and VR-1 expressions at the protein or mRNA levels were detected by western blot analysis and reverse transcription-polymerase chain reaction. Apoptotic cell death was determined by DNA fragmentation and quantified by enzyme-linked immunosorbent assay. HT-29 human colon cancer cells expressed PPARγ and VR-1. Treatment with capsaicin (100 – 300 µM) or the PPARγ ligand troglitazone induced apoptotic cell death in a dose-dependent manner in HT-29 human colon cancer cells. Capsaicin-induced cell death was completely blocked by bisphenol A diglycidyl ether, a specific PPARγ antagonist. Capsazepine, a specific antagonist for VR, did not inhibit capsaicin-induced apoptosis. In the opinion of the authors, the results suggested, that capsaicin-induced apoptotic cell death in HT-29 human colon cancer cells could be associated with the PPARγ pathway without the involvement of the VR.

Kyung *et al.* 2009: It was investigated whether capsaicin induces apoptosis in colon cancer cell lines. Capsaicin (0.1 – 0.5 mM) decreased cell viability in a dose-dependent manner in Colo320DM (human colon cancer cells) and LoVo (human colon metastatic adenocarcinoma) cells. In addition, capsaicin produced cell morphology changes and DNA fragmentation, decreased the DNA contents, and induced phosphatidylserine translocation. It was shown that capsaicin-induced apoptosis is associated with an increase in ROS generation and a disruption of the mitochondrial transmembrane potential. Treatment with capsaicin induced a dramatic increase in caspase 3 activity.

Lee *et al.* 2012: The authors investigated whether capsaicin alters β-catenin-dependent signallings in human colorectal cancer cells *in vitro*. Exposure of SW480 (human colon primary adenocarcinoma), LoVo (human colon metastatic adenocarcinoma) and HCT-116 (human colon carcinoma) cells to capsaicin suppressed cell proliferation. Transient transfection with a β-catenin/T-cell factor (TCF)-responsive reporter indicated that capsaicin suppressed the transcriptional activity of β-catenin/TCF. Capsaicin treatment (50 and 100 µM) resulted in a decrease of intracellular β-catenin levels and a reduction of transcripts from the β-catenin gene (CTNNB1).

These results were confirmed by a reduced luciferase reporter activity driven by promoter-reporter construct, containing the promoter region of the β-catenin gene. In addition, capsaicin destabilised β-catenin, through enhancement of proteosomal-dependent degradation. Western blot and immunoprecipitation studies indicated that capsaicin treatment suppressed TCF-4 expression and disrupted the interaction of TCF-4 and β-catenin.

Lin *et al.* 2013: The viability, cell cycle progression, and factors associated with apoptosis in human KB cancer cells treated with capsaicin were investigated. The results indicated that treatment of KB cells with capsaicin significantly reduced cell proliferation/viability and induced cell death in a dose-dependent manner compared with that in the untreated control. Cell cycle analysis indicated that exposure of KB cells to capsaicin (1-250 μM) resulted in cell cycle arrest at G2/M phase. Capsaicin-induced growth inhibition of KB cells appeared to be associated with induction of apoptosis. Moreover, capsaicin induced disruption of the mitochondrial membrane potential as well as activation of caspase 9, 3 and poly-(ADP-ribose) polymerase in KB cells.

Malagarie-Cazenave *et al.* 2011: The effects of capsaicin on the production of the cytokine interleukin (IL)-6 by PC-3 (human prostate metastatic cancer) cells at both protein and mRNA levels which were evaluated by ELISA and real-time PCR, respectively were investigated. Capsaicin-treated PC-3 cells (1-20 μM) increased the synthesis and secretion of IL-6 which was abrogated by TRPV1 antagonist CZP, as well as by inhibitors of protein kinase C alpha (PKC-α), phosphoinositol-3 phosphate kinase (PI-3K), Akt and extracellular signal-regulated protein kinase (ERK). Incubation of PC-3 cells with an anti-TNF-α antibody blocked the capsaicin-induced IL-6 secretion. According to the authors these results suggested that capsaicin-mediated IL-6 increase in prostate cancer PC-3 cells is regulated at least in part by TNF-α secretion and signalling pathway involving Akt, protein kinase ERK and PKC-α activation.

Zhang *et al.* 2003: Human T-cell leukaemia virus type 1 (HTLV-1)-associated adult T-cell leukaemia (ATL) is resistant to conventional chemotherapy. The authors examined the *in vitro* effects of capsaicin on three ATL cell lines. Capsaicin treatment (100 μM) inhibited the growth of ATL cells both in dose- and time-dependent manner. The inhibitory effect was mainly due to the induction of cell cycle arrest and apoptosis. Capsaicin treatment also induced the degradation of Tax (a HTLV-1 transcriptional transactivator protein) and up-regulation of the inhibitory subunit of NF-κB Iκ-Bα, resulting in the decrease of nuclear factor NF-κB/p65 DNA binding activity. In addition, the level of the protein Bcl-2 was found to be decreased.

Zhang *et al.* 2008: The authors investigated the mechanism of capsaicin in inducing apoptosis in pancreatic cancer cells. Treatment of AsPC-1 and BxPC-3 cells with capsaicin resulted in a dose-dependent inhibition of cell-viability and induction of apoptosis which was associated with the generation of ROS and persistent disruption of mitochondrial membrane potential. These effects were significantly blocked when the cells were pretreated with a general antioxidant *N*-acetyl cysteine (NAC). Exposure of AsPC-1 and BxPC-3 cells to capsaicin was also associated with increased expression of the protein Bax (a co-factor of the tumour suppressor protein p53), down-regulation of Bcl-2, survivin and significant release of cytochrome c and apoptosis-inducing factor (AIF) in the cytosol. On the contrary, above-mentioned effects were not observed in the normal acinar cells in response to capsaicin-treatment. Capsaicin-treatment resulted in the activation of c-Jun N-terminal kinases (JNK) and JNK inhibitor SP600125 afforded protection against capsaicin-induced apoptosis. Furthermore, capsaicin, when given orally, markedly suppressed the growth of AsPC-1 pancreatic tumour xenografts in athymic nude mice, without side effects. Tumours from capsaicin treated mice demonstrated increased apoptosis, which was related to the activation of JNK and increased cytosolic protein expression of Bax, cytochrome c, AIF and cleaved caspase-3, as compared with controls.

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of
Capsicum frutescens L., fructus
EMA/HMPC/674138/2013
Page 16/67

In vivo, capsaicin:

Han *et al.* 2001: In this study, topical application of capsaicin (1, 10 and 50 µmol) onto dorsal skin of female ICR mice strongly suppressed phorbol ester-stimulated activation of NF-κB via blockade of the inhibitory protein IκB-α degradation with subsequent inhibition of nuclear translocation of the functionally active NF-κB subunit, p65. Likewise, phorbol ester-induced activation of activator protein-1 (AP-1) was abolished by capsaicin pre-treatment. According to the authors, since altered transactivation of NF-κB and AP-1 has been implicated for neoplastic transformation and progression, the suppression of these transcription factors by capsaicin may account for its reported chemopreventive effects on mouse skin tumorigenesis as well as inflammation.

Yoshitani *et al.* 2001 (only abstract available): The modifying effects of dietary administration of capsaicin and rotenone, which is a naturally occurring pesticide derived from *Derris* and *Lonchorcarpus* species, on azoxymethane (AOM)-induced colon tumorigenesis were investigated in male F344 rats. Gavage with capsaicin and rotenone significantly elevated phase II enzymes, glutathione S-transferase (GST) and quinone reductase, in the liver and colon. In an aberrant crypt foci (ACF) bioassay, feeding of capsaicin and rotenone at a dose of 500 ppm for 4 weeks significantly inhibited ACF formation induced by AOM (20 mg/kg body weight, once a week for 2 weeks). In a subsequent long-term study designed to confirm the protective effects of both compounds on ACF development, one group was treated with AOM alone and four other groups received the carcinogen treatment plus diets containing 500 ppm test compounds for 4 weeks (initiation phase) and for 34 weeks (post-initiation phase). Two groups were treated with capsaicin or rotenone alone (500 ppm in diet) and one group was maintained on the basal diet. At the termination of the study, dietary exposure of capsaicin during the initiation phase was found to significantly reduce the incidence of colonic adenocarcinoma (60% vs. 24%, 60% reduction, p=0.0407). Rotenone feeding during the post-initiation phase also reduced the frequency of colonic adenocarcinoma (60% vs. 19%, 68% reduction, p=0.0226).

Anti-mutagenicity

In vitro, capsaicin:

Huynh & Teel, 2005: The authors investigated whether capsaicin exhibits anti-mutagenic effects toward heterocyclic amines (HCA)-induced mutagenesis in *Salmonella typhimurium* TA98 when incubated with 0.5 mg liver S9 protein from rat, hamster and human. The HCAs used were Trp-P-2, Glu-P-1 and PhIP. Capsaicin, at non-toxic amounts of 0.25 and 0.5 µmol/plate, expressed a dose-dependent inhibition of the mutagenicity of Glu-P-1 and PhIP when they are metabolically activated by rat, hamster and human liver S9 and of Trp-P-2 when activated by rat and hamster liver S9. In contrast, capsaicin enhanced the mutagenicity of Trp-P-2 in TA98 when incubated with human liver S9. The lack of consistency in the anti-mutagenic action of capsaicin toward HCAs remained unresolved.

Respiratory system

In vivo, herbal preparations:

Jang *et al.* 2011: The effect of a methanolic *Capsicum annuum* L. extract (CAE, exhaustive extraction with methanol, DER approximately 6.6:1) in a mouse model of ovalbumin-induced allergic airway inflammation was investigated. Animals were treated with CAE by oral gavage (10 mg/kg, 30 mg/kg) before ovalbumin challenge. After ovalbumin challenge, airway responsiveness to methacholine, influx of inflammatory cells into the lung, cytokine levels in broncho-alveolar lavage fluid and lung, nuclear factor-κB (NF-κB) activity in lungs, and lung histopathology were assessed. Oral treatment with CAE significantly reduced the pathophysiological signs of allergic airway disease, including increased inflammatory cell recruitment to the airways, airway hyperresponsiveness, and increased levels of

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of
Capsicum frutescens L., fructus
EMA/HMPC/674138/2013   Page 17/67

T-helper type 2 cytokines. Reactive oxygen species were also decreased in cells from broncho-alveolar lavage fluid. In addition, administration of CAE attenuated ovalbumin-induced increases in NF-κB activity in lungs.

In vivo, capsaicin:

De & Ghosh, 1988, (only abstract available): Capsaicin, isolated from *Capsicum frutescens* was given intraperitoneally to rats daily (no information on dosage) for either 3 days (acute) or 12 days (chronic), respectively. Acute treatment groups showed significant decrease in lung phospholipid and cholesterol content and increase in lung total lipid with no significant changes in lipid peroxidation and total protein content, as compared to the vehicle treated group. On the other hand, chronic treatment groups showed no significant alteration in any of the above parameters. The results suggest that acute capsaicin treatment has a profound effect on the lung membrane lipid system which may account for its reported broncho-constrictive and other associated effects.

Skin

In vivo, capsaicin:

Harada & Okajima, 2007: The topical application of 0.01% capsaicin in mice significantly increased dermal levels of insulin-like growth factor-1 between 30 and 180 min after application, while other capsaicinoids (dihydrocapsaicin, nordihydrocapsaicin), capsinoids (capsiate and analogues), anandamide and nonylic acid vanillylamide showed an effect only 30 min after application.

Gastro-intestinal tract

In vitro, herbal preparations:

Jensen-Jarolim *et al.* 1998: Using HCT-8 cells, a cell line from a human ileocoecal carcinoma, the authors studied the effects of spices on transepithelial electrical resistance (TER), permeability for fluorescein isothiocyanate labelled dextrans with graded molecular weight, and morphological alterations of tight junctions by immunofluorescence, using an anti-ZO-1 antibody, a marker for tight junction integrity. Two different reactivity patterns were observed: paprika and cayenne pepper (fruit powders extracted with Dulbeccos's modified Eagle's Medium, 150 g/l; 50 μl final extracts applied, corresponding to 7.5 mg herbal substance) significantly decreased the TER and increased permeability for 10-, 20- and 40-kDa dextrans but not for -70 kDa dextrans. Simultaneously, tight junctions exhibited a discontinuous pattern. Applying extracts from black or green pepper, bay leaf or nutmeg increased the TER and macromolecular permeability remained low. Immunofluorescence ZO-1 staining was preserved. In accordance with the above findings, capsaicin transiently reduced resistance and piperine increased resistance, making them candidates for causing the effects seen with crude spice extracts. The observation that *Solanaceae* spices (paprika, cayenne pepper) increase permeability for ions and macromolecules might be of pathophysiological importance, particularly with respect to food allergy and intolerance.

In vitro, capsaicin:

Lee *et al.* 2007b: Gastric epithelial cells (AGS or MKN45 cells) were pre-treated with various concentrations of capsaicin and infected with *Helicobacter pylori* (Vac A+, CagA+ wild-type *H. pylori* strain ATCC 49503) for different periods of time to determine IL-8 concentrations in culture supernatant by an ELISA assay. The IL-8 mRNA transcripts in H. pylori-infected gastric epithelial cells co-treated with capsaicin were measured by reverse transcriptase-polymerase chain reaction analysis. Electrophoretic mobility shift assay was used to examine the NF-κB DNA binding activity with capsaicin and immunofluorescence microscopy to examine nuclear staining of p65.

Capsaicin inhibited *H. pylori*-induced IL-8 production by gastric epithelial cells in dose- and time-dependent manner. Capsaicin as low as 100 μmol/l significantly inhibited IL-8 production in *H. pylori*-infected MKN45 cells (43.2% of control) at 24 hours incubation, whereas inhibited IL-8 production in *H. pylori*-infected AGS cells (70% of control). The authors concluded, that capsaicin inhibited IL-8 mRNA expression after infection of gastric epithelial cells with *H. pylori* for 6 hours. The addition of capsaicin (100 μmol/l) suppressed *H. pylori*-induced NF-κB activation in gastric epithelial cells at 1 hour post-infection. The degradation of the inhibitory subunit of NF-κB IκB and IKK activation (a central regulator of NF-κB activation) were inhibited by capsaicin. The authors concluded, that nontoxic dose of capsaicin inhibited *H. pylori*-induced IL-8 production by gastric epithelial cells through the modulation of IκB-, NF-κB-, and IL-8 pathways.

In vivo, herbal preparations:

Islam *et al.* 1973 (only abstract available): the application of chillies to albino rats resulted in a significant rise of free hydrochloric acid in the gastric juice. No change was found regarding volume, peptic activity and dissolved mucoproteins in the gastric juice.

In vivo, herbal preparations and capsaicin:

Kang *et al.* 1993 (only abstract available): The effect of chilli powder and its pungent ingredient capsaicin on gastrointestinal (GI) transit in the rat was studied. Fasted unanaesthetised male Sprague-Dawley rats (n=144) received by gavage a test meal containing charcoal and cellulose in water or capsaicin solvent plus ^{51}Cr as a radioactive marker. Either 100 or 200 mg of chilli powder (containing 0.13 and 0.26 mg of capsaicin, respectively) or 0.5 or 1 mg of capsaicin was added, the final volume of each meal being 1.5 ml. At 10 and 20 min, animals were killed and the amount of isotope that had left in the stomach was measured, together with the distance the charcoal column had travelled along the small intestine. Compared to controls, animals given chilli powder emptied less of their gastric content at 10 and 20 min, an effect partly reproduced by capsaicin. However, overall gastric-small intestinal transit was unaffected by chilli powder or capsaicin. Another 12 male Sprague-Dawley rats received, under light ether anaesthesia, on six occasions at 1-2 week intervals, the same six test meals as used in the previous experiment except that charcoal was not used. Total gut transit as measured by the amount of radioactive marker excreted in the stools at 18 and 24 hours was unaffected by the use of chilli or capsaicin.

Prakash & Srinivasan, 2010 (only abstract available): The protective effect of dietary spices with respect to activities of antioxidant enzymes in gastric and intestinal mucosa was examined. Groups of Wistar rats were fed for 8 weeks with diets containing black pepper (0.5%), piperine (0.02%), red pepper (3%), capsaicin (0.01%), and ginger (0.05%). All these spices significantly enhanced the activities of antioxidant enzymes - superoxide dismutase, catalase, glutathione reductase, and glutathione-5-transferase - in both gastric and intestinal mucosa, suggesting a GI protective role for these spices. In a separate study, these dietary spices were found to alleviate the diminished activities of antioxidant enzymes in gastric and intestinal mucosa under conditions of ethanol-induced oxidative stress. The gastro protective effect of the spices was also reflected in their positive effect on mucosal glycoproteins, thereby lowering mucosal injury.

In vivo, capsaicin:

Kang *et alet al.* 2011: In this study it was assessed whether dietary capsaicin attenuated the metabolic dysregulation in genetically obese diabetic KKAy mice, which have severe diabetic phenotypes. Male KKAy mice fed a high-fat diet for 2 weeks received a 0.015% capsaicin supplement for a further 3 weeks and were compared with non-supplemented controls.

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of
Capsicum frutescens L., fructus
EMA/HMPC/674138/2013   Page 19/67

Dietary capsaicin markedly decreased fasting glucose/insulin and triglyceride levels in the plasma and/or liver, as well as expression of inflammatory adipocytokine genes (e.g., monocyte chemoattractant protein-1 and interleukin-6) and macrophage infiltration. At the same time expression of the adiponectin gene/protein and its receptor, AdipoR2, increased in adipose tissue and/or plasma, accompanied by increased activation of hepatic AMP-activated protein kinase, a marker of fatty acid oxidation. The authors conclude that these findings suggest that dietary capsaicin reduces metabolic dysregulation in obese/diabetic KKAy mice by enhancing expression of adiponectin and its receptor.

Okumi *et al.* 2012: The acute effects of peroral administration of capsaicin and 6-gingerol on gastric acid secretion in conscious mice were investigated. These agents were given p.o. 30 min before the pylorus was ligated. Oral administration of capsaicin (1.0-100 mg/kg) or 6-gingerol (1.5-50 mg/kg) significantly and dose-dependently inhibited basal acid secretion. Pre-treatment with BCTC, a TRPV 1 antagonist, significantly reversed the reduced basal acid secretion by capsaicin or 6-gingerol. The combination of the lowest doses of capsaicin and 6-gingerol markedly inhibited basal acid secretion in conscious mice and this was also significantly reversed by BCTC. Moreover, the combination of the maximal dose of capsaicin and 6-gingerol inhibited basal acid secretion only to the level of a single administration of the maximal dose of capsaicin. In separate experiments, intraduodenal administration of either capsaicin (30 mg/kg) or 6-gingerol (15 mg/kg), whose doses were observed to have a significant inhibitory effect by oral administration, tended to inhibit basal acid secretion compared with the vehicle. According to the authors, these results suggest that the combination of capsaicin and 6-gingerol has an additive effect on inhibition of gastric acid secretion through activation of transient receptor potential vanilloid-1, and oral administration of transient receptor potential vanilloid-1 agonists directly stimulates transient receptor potential vanilloid-1 in the gastric lumen, resulting in a potent reduction of gastric acid secretion.

Other data

In vitro, herbal preparations:

Yang *et al.* 2012: The anti-diabetic properties and mechanisms underlying the actions of an extract of Korean red pepper (*Capsicum sp.*, extraction solvent acetonitrile, content of capsaicinoids approximately 0.54 mg/100 g) using C2C12 myotube (a mouse myoblast cell line) were evaluated. The extract (Ekrp) markedly increased 2-[N-(7-nitrobenz-2-oxa-1.3-diazol-4-yl)amino]-2-deoxy-α-glucose uptake in a concentration-dependent manner. To examine the mechanism by which Ekrp increased glucose uptake in C2C12 myotube, the phosphorylation levels of AMP-activated protein kinase (AMPK) and Acetyl-CoA carboxylase (ACC), a marker of AMPK activity were investigated using western blot analysis. Ekrp significantly increased AMPK and ACC phosphorylations in a dose dependent manner. Ekrp increased the transactivation of PPAR-γ in a concentration-dependent manner. Ekrp did not inhibit adipocyte differentiation.

In vitro, capsaicin:

Luqman *et al.* 2011: The effect of vanilloids on nuclear factor κ-light-chain-enhancer of activated B cells (NFκB) activation using stably transfected 293/NFκB-Luc human embryonic kidney cells induced by treatment with tumour necrosis factor-α (TNFα) and on aromatase activity was investigated. Capsaicin and CZP blocked TNFα-induced NFκB activation in a dose-dependent manner with 50% inhibitory concentration (IC_{50}) values of 0.68 and 4.2 μM, respectively. No significant cytotoxicity was observed at the highest concentrations tested (53.1 μM for CZP and 65.5 μM for capsaicin). In addition, these vanilloids inhibited aromatase activity with IC_{50} values of 13.6 and 8.8 μM, respectively. Computer-aided molecular docking studies showed docking scores indicative of good binding affinity of vanilloids with aromatase and NFκB.

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of
Capsicum frutescens L., fructus
EMA/HMPC/674138/2013   Page 20/67

The highly conserved residues for capsaicin and CZP binding with NFκB p50 were Ser299 and Ile278 (H-bond 2.81Å) and with NFκB p100 were Ser6, Arg82, Val86 , Arg90 (H-bond 2.89 Å), Gly4, and Ser2 (H-bond 2.81Å). The amino acids Trp224, Arg435, and
Val373 (H-bond 2.80 Å) were found to be important for the binding of capsaicin and CZP with aromatase. Based on these findings, aromatase and NFκB are suggested by the authors as valid targets for these compounds.

Santos *et al.* 2012: The inhibitory effects of the ethyl acetate extract and capsaicin (1) and dihydrocapsaicin (2) isolated from fruits of *Capsicum annuum* chili pepper type, and synthetic capsaicinoid derivatives (N-(4-hydroxyphenylethyl)decamide (3), (E)-N-(4-hydroxy-3-methoxybenzyl)-3,7-dimethylocta-2,6-dienamide (4), 4-hydroxy-3-methoxy-N-((E)-3, 7-dimethylocta-2,6-dienyl)benzamide (5) and N-(4-hydroxy-3-methoxybenzyl)decamide (6) at different concentrations were evaluated against *Streptococcus mutans*. The minimum inhibitory concentration at which the ethyl acetate extract prevented the growth of S. mutans was 2.5 mg/ml; those of the isolated compounds 1 and 2 were 1.25 µg/ml, while 3 was 5.0 µg/ml, and 4, 5 and 6 were 2.5 µg/ml, respectively.

Singh *et al.* 2001: The authors tried to show that capsaicin is capable of causing strand scission in calf thymus and plasmid DNA in the presence of Cu(II) and that this breakage is mediated by reactive oxygen species, especially the hydroxyl radical. The results further showed that capsaicin can directly generate hydroxyl radicals in the presence of Cu(II). To explore the chemical basis of the DNA breakage reaction by capsaicin, these properties of capsaicin were compared with its saturated structural analogue dihydrocapsaicin (DHC). The rate of DNA degradation, as well as hydroxyl radical formation, was found to be greater in the case of capsaicin. Both capsaicin and DHC are able to reduce Cu(II) to Cu(I), which was shown to be an essential intermediate in the DNA cleavage reaction. Stoichiometric analysis indicated that whereas 1 mol of capsaicin reduced 3 mol of Cu(II), 1 mol of DHC reduced only 2 mol of Cu(II).

In vivo, herbal preparations:

Roghani *et al.* 2004 (only abstract available): The hypoglycaemic and hypolipidemic effect of *Capsicum frutescens* was investigated in an experimental model of insulin-dependent diabetes mellitus. For this purpose, male Wistar rats (n=36) were randomly divided into 4 groups, i.e. control, pepper-treated control, diabetic, and pepper-treated diabetic groups. For induction of diabetes, streptozotocin (STZ; 60 mg/kg; i.p.) was used at a single dose. A serum glucose level higher than 250 mg/dl was considered as the presence of diabetic state. The treatment groups received oral administration of pepper-mixed pelleted food at a ratio of 1/15. Statistical analysis of the data showed that serum glucose level in diabetic group increased 2 and 4 weeks after the experiment as compared to data one week before the experiment ($p<0.001$), while this parameter was only significantly lower 2 weeks after the experiment in pepper-treated diabetic group as compared to untreated-diabetic group ($p<0.01$). In addition, there was no significant difference between pepper-treated control and untreated control groups regarding serum glucose level. In addition, triglyceride level was higher in diabetic group and there was a reduction in this parameter in pepper-treated diabetic group as compared to diabetic group at fourth week after the experiment ($p<0.05$). On the other hand, cholesterol level showed no significant reduction in pepper-treated diabetic group in comparison with untreated diabetic group.

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of
Capsicum frutescens L., fructus
EMA/HMPC/674138/2013   Page 21/67

In vivo, herbal preparations and capsaicin:

Yamaguchi *et al.* 2010: The authors investigated the influence of red chili peppers (*Capsicum annuum* L.) on intestinal B cell-dependent immune responses. Production of two isotype immunoglobulins, immunoglobulin-A and G1 (IgA and IgG1, respectively) was measured in Peyer's patch (PP) cells after treatment with Capsicum extract, capsaicin, or carotenoids. PP cells isolated from mice that had been orally injected with Capsicum extract or capsaicin secreted significant amounts of IgA and IgG1, irrespective of lipopolysaccharide-stimulation. In contrast, oral injection of β-carotene, β-cryptoxanthin or capsanthin significantly reduced the production of antibodies. Flow cytometric analysis revealed that Capsicum and capsaicin caused a small increase in the number of CD19 + B cells and a decrease in CD3 + T cells in PP, while carotenoids did not affect either population.

In vivo, capsaicin:

Nevius *et al.* 2012: VR1 is expressed on immune cells as well as on sensory neurons. The authors reported that VR1 could regulate immunological events in the gut in response to its ligand capsaicin. Oral administration of capsaicin attenuated the proliferation and activation of autoreactive T cells in pancreatic lymph nodes (PLNs) but not in other lymph nodes, and protected mice from development of type 1 diabetes (T1D). Engagement of VR1 enhanced a discreet population of CD11b /F4/80 macrophages in PLN, which express anti-inflammatory factors interleukin (IL)-10 and PD-L1. This population was essential for capsaicin-mediated attenuation of T-cell proliferation in an IL-10-dependent manner. Lack of VR1 expression failed to inhibit proliferation of autoreactive T cells, which was partially reversed in (VR1 +/+ → VR1 -/-) bone marrow chimeric mice, implying the role of VR1 in crosstalk between neuronal and immunological responses *in vivo*. In the opinion of the authors these findings implied, that endogenous ligands of VR1 could have profound effect on gut-mediated immune tolerance and autoimmunity by influencing the nutrient-immune interactions.

Liang *et al.* 2013: The effect of capsaicinoids on plasma lipids, functionality of aorta including atherosclerotic plaque development, cholesterol absorption biomarker, faecal sterol excretion, and gene expression of major receptors, enzymes, and transporters involved in cholesterol metabolism was investigated. Hamsters were divided into five groups and fed a high-cholesterol diet containing 0% to 0.03% capsaicinoids, for 6 weeks. Capsaicinoids reduced plasma total cholesterol, non-high-density lipoprotein cholesterol, and triacylglycerols with high-density lipoprotein cholesterol being unaffected. All four experimental groups had a decrease in the atherosclerotic plaque compared with CON. Dietary capsaicinoids increased the faecal excretion of total acidic sterols possibly mediated by up-regulation of cholesterol 7α-hydroxylase and down-regulation of liver X receptor alpha. Plasma sterol analysis demonstrated that capsaicinoids decreased the ratio of plasma campesterol/cholesterol, suggesting they decreased cholesterol absorption. Capsaicinoids could improve the endothelium-dependent relaxations and reduce the endothelium-dependent contractions by inhibiting the gene expression of cycloxygenase-2. However, no dose-dependent effect of capsaicinoids on these parameters was seen.

3.1.3. Safety pharmacology

Neurotoxicity

Capsaicin has been demonstrated to selectively affect primary afferent nociceptors in a way which is described as 'defunctionalisation' (review in Anand & Bley, 2011). These effects seem to depend on factors such as age of animal at the time of treatment, route of administration and dose of capsaicin used.

3.1.4. Pharmacodynamic interactions

Colvin *et al.* (2011) studied the interaction between amitriptyline and capsaicin. Transdermal patches containing amitriptyline (0.3 ml of a 2.5% solution) and capsaicin (0.05-8%) were administered to shaved backs of male Sprague-Dawly rats. Amitriptyline alone caused a complete block to pinprick for 4.5 hours, the time to full recovery was 96 hours. Combined with 8% capsaicin the complete block lasted 9 hours, the time to full recovery increased to 216 hours.

Effects seen after injection of capsaicin (e.g. Gerner *et al.* (2008) reporting a selective block of nociceptors when 10 min after application of tertiary amine local anaesthetics capsaicin 0.05% is injected) are not relevant for the use as described in the monograph.

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

Data are only available for capsaicin and not for herbal preparations.

Absorption

Cutaneous administration:

Fang *et al.* 1996: In this study, *in vivo* systemic drug plasma data of capsaicin, nonivamide (NVA) and non-pungent sodium nonivamide acetate (SNA) following transdermal ointment base administration in rabbits were performed to establish the pharmacokinetic analysis of these analogues. After the percutaneous administration (ointments containing 0.25% or 0.35% of capsaicin, NVA or SNA), the plasma profiles between capsaicin and NVA were quite different although these two analogues showed similar physicochemical properties and intravenous pharmacokinetic parameters. The bioavailability of capsaicin was 34.22% (0.25% ointment) and 27.33% (0.35% ointment).

Tsai *et al.* 1994: NVA, SNA and sodium nonivamide propionate are analogues of Capsaicin. The structure and pungent property of NVA are similar to capsaicin. The solubilities of SNA in different pH value buffer solution were higher than that of NVA and capsaicin. For the NVA and SNA, the noctanol/buffer partition coefficients decreased with increasing pH value. The fluxes of capsaicin and its analogues were determined using excised rat skin and the effect of pH was also investigated. The flux of NVA and SNA mixture was higher than individually NVA or SNA, and the ratio of 70:30 was a better choice. Sodium lauryl sulfate (SLS), an anionic surfactant, had significant effect on SNA skin permeation.

According to the published SmPCs of authorised medicinal products containing herbal preparations mentioned in the monograph capsaicin is absorbed percutaneously. Animal data suggest a systemic bioavailability of topically applied capsaicin ranging from 27 to 34%. Original data substantiating these statements are not publicly available.

Oral administration:

Donnerer *et al.* 1990 (only abstract available): [³H]-dihydrocapsaicin ([³H]-DHC) and unlabelled capsaicin (no more information available) were readily absorbed from the GI tract in rats but were almost completely metabolised before reaching the general circulation. A certain degree of biotransformation already took place in the intestinal lumen. Unchanged compounds (identified by chromatography) were present in portal vein blood. There seems to be a saturable absorption and degradation process in the GI tract and a very effective metabolism in the liver.

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of
Capsicum frutescens L., fructus
EMA/HMPC/674138/2013   Page 23/67

Less than 5% of the total amount of extracted radioactivity consisted of unchanged [³H]-DHC in trunk blood and brain, 15 min after GI application. On the other hand, approximately 50% unchanged [³H]-DHC was detected in these tissues 3 min after i.v. or 90 min after s.c. application of the capsaicinoids. Dihydrocapsaicin (DHC) or [³H]-DHC were metabolised when incubated *in vitro* with liver tissue but not with brain tissue. The metabolic product(s) did not show capsaicin-like biological activity. It can be concluded that rapid hepatic metabolisation limits systemic pharmacological effects of enterally absorbed capsaicin.

Suresh & Srinivasan, 2010: Tissue distribution and elimination of curcumin, capsaicin and piperine was examined following their oral intake in rats. Separate sets of animals (150-160 g) were orally administered the three compounds at dosages of 30 mg (capsaicin), 170 mg (piperine) and 500 mg (curcumin)/kg body weight. The tissue concentrations of administered spice compounds were determined by HPLC. Maximum distribution of 24.4% of administered capsaicin was seen at 1 hour, while no intact capsaicin was detectable after 4 days. Absorption of capsaicin was about 94% and very rapid relative to other two compounds.

Intravenous administration:

Fang *et al.* 1996: After application of 2 ml/kg capsaicin to rabbits the half-life of capsaicin was determined: 12.44 min.

Metabolism

Metabolism of capsaicin in microsomes of human origin, rats and dogs appeared to be similar (Chanda *et al.* 2008). Three major metabolites were detected: 16-hydroxycapsaicin, 17-hydroxycapsaicin and 16, 17-dehydrocapsaicin. In human skin biotransformation was slow *in vitro*, with the majority of the applied capsaicin remaining unchanged.

According to the published SmPCs of authorised medicinal products containing herbal preparations mentioned in the monograph capsaicin is metabolised mainly in the liver and eliminated in the form of metabolites in the urine and faeces. Original data substantiating these statements are not publicly available.

Pharmacokinetic interactions

In vitro, capsaicin:

Babbar *et al.* (2010, only abstract available) investigated *in vitro* the potential interaction of capsaicin against seven P450 enzymes. At concentrations occurring after ingestion of chili peppers or topical administration of a high-concentration patch, capsaicin did not cause direct inhibition of any CYP enzyme. Direct inhibition was only observed at much higher concentrations; the lowest IC_{50} value was 2 µM. For CYP2E1, the IC_{50} value was too high to calculate. With pre-incubation, inhibition decreased for CYP1A2, 2C9, 2C19 and 3A4/5, whereas inhibition of CYP2B6 increased and moderately increased for CYP2D6. Induction of CYP activity was evaluated in microsomes from hepatocyte primary cultures. Capsaicin did not induce CYP1A2, 2B6, 2C9, 2C19, 2E1 or 3A4/5. 10 µM capsaicin caused a statistically significant increase in CYP1A2 activity (8.6% of the positive control). Inhibition of drug metabolism by capsaicin should be minimal, as the ratio of $[I]/K_i$ for direct inhibition was < 0.1. It was concluded, that although pre-incubation did enhance the potency for CYP2B6 inhibition to 5.1 µM, but given that exposure to capsaicin from either food or a topical medicine is very low (≤58 nM) and transient, effects on CYPs appear unlikely.

In vivo, herbal preparations:

Bouraoui *et al.* 1995 (only abstract available): Pharmacokinetics and metabolism of theophylline were studied in three groups of male rabbits, after intravenous administration (12 mg/kg) with and without oral ground Capsicum fruit suspension. Compared with control values, plasma theophylline half-life of distribution and of elimination, areas under plasma curves, clearance and volume of distribution did not show any significant difference. On the contrary, the elimination rate constant (k(I,0)) is significantly different (0.01<p<0.05) after a single dose of capsicum and remained unchanged after a repeated dose. Concerning the metabolism of theophylline in rabbits, the results showed that the oral administration of a single dose of Capsicum fruit suspension does not significantly affect the urinary excretion of theophylline and its metabolites – 1.3 -dimethyluric acid (1.3 - DMU) and 1-methyluric acid (1-MU). On the other hand, after a repeated dose of Capsicum fruit for 7 days, the quantity of 1-MU was significantly reduced (0.01<p<0.05). In conclusion, it was found that a single dose of Capsicum fruit could affect pharmacokinetic parameters of theophylline (k(I,0), while a repeated dose affected the metabolic pathway of xanthine oxidase.

Cruz *et al.* 1999 (only abstract available): The bioavailabilities of aspirin (acetylsalicylic acid) and of salicylic acid were studied in male Wistar rats after acute and chronic oral administration of a *Capsicum annuum* extract, containing 100 mg of capsaicin per gram. With a single administration of 100 mg/kg of the extract, aspirin blood levels remained unchanged, but salicylic acid bioavailability was reduced in 44% compared with control animals. With a single administration of 300 mg/kg of the extract, aspirin blood levels were undetectable while salicylic acid bioavailability was reduced in 59%. Chronic administration once daily for 4 weeks of 100 and 300 mg/kg of the extract resulted in undetectable aspirin blood levels, while salicylic acid bioavailability was reduced in 63 and 76%, respectively, compared with controls. Results show that Capsicum ingestion reduces oral drug bioavailability, likely as a result of the GI effects of capsaicin.

Conclusions

Pharmacokinetic data, obtained from animal experiments as well as from *in vitro* studies, demonstrated that topically administered capsaicin is absorbed to a marked extent and may be detected in the blood. From the data available no direct influence on any CYP enzyme could be seen.

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

3.3.1. Single dose toxicity

Herbal substance, herbal preparations: No data available

Capsaicin:

Saito & Yamamoto, 1996: Oral LD_{50} values of capsaicin were 118.8 mg/kg for male and 97.4 mg/kg for female mice, and 161.2 mg/kg for male and 148.1 mg/kg for female rats. Major toxic symptoms in mice were salivation, erythema of skin, staggering gait, bradypnoea and cyanosis. Some animals showed tremor, clonic convulsion, dyspnoea and lateral or prone position and then died 4 to 26 min after dosing. Survivors recovered within 6 hours in mice and 24 hours in rats. Rats showed a higher incidence of cyanosis, clonic or tonic convulsion, dyspnoea or lateral position.

The acute toxicity of capsaicin dissolved in dimethyl sulfoxide in male mice was in the order intravenous > intraperitoneal> subcutaneous > oral> dermal indicating that systemic absorption and toxicity following dermal application were lower than after an oral dose (Glinsukon *et al.* 1980).

Extrapolation to a 50 kg human shows that the lethal oral and dermal doses (as LD_{50}), respectively, are 25 g and 250 g of capsaicin. The possible maximum dose of according to the monograph is far below.

3.3.2. Repeat dose toxicity

Herbal substance, herbal preparations:

Jang *et al.* 1992 (only abstract available): The toxicity of red chilli was examined in male B6C3F1 mice fed a commercial meal diet mixed with ground *Capsicum annuum* (L.) at levels of 0.5, 1.0, 2.5, 5.0, 7.5 and 10% by weight. Mice were offered control or test diets ad libitum starting at 6 weeks of age. Food consumption was measured daily and individual body weight recorded weekly for the 4-weeks feeding period. General health, body weight and food intake were apparently not adversely affected at any level of pepper consumption. Histopathological evaluation revealed slight glycogen depletion and anisocytosis of hepatocytes in the 10% group. However, other organs did not reveal any lesions attributable to the chilli exposure. It appeared, that red chilli is relatively non-toxic at the doses tested in male B6C3F1 mice.

Dkhil & Al-Quraishy, 2010: Rabbits were orally ingested 2 g/kg hot red pepper every day for 10 days. Hot red pepper induced a significant increase in temperature of rabbits after oral ingestion of each dose. Hepatic tissue damage was recorded through examination of the stained paraffin embedded sections. Inflammatory cellular infiltration and hepatocytic vacuolation were marked in rabbits, ingested the hot red pepper. Histochemical studies revealed a decrease in both of carbohydrates and protein contents in the liver. Serum alanine aminotransferase, aspartate aminotransferase, cholesterol, triglycerides and glucose were decreased in rabbit due to oral ingestion of hot red pepper.

Al-Dahmesh *et al.* 2011 (only abstract available): Oral administration of chili pepper was carried out every day for 10 days at a dose of 2 g/kg rabbit. Chili pepper induced significant increase in spleen weight as well as the number of leucocytes. Spleen architecture was altered as indicated by the histological score. White and red pulps were enlarged and the splenic capsule became thinner after administration of chili pepper to rabbits. Histochemical studies revealed a decrease in both carbohydrates and protein contents in the spleen.

Capsaicin:

Akagi *et al.* 1998: A mixture consisting of 64.5% capsaicin and 32.6% dihydrocapsaicin was administered orally in concentrations between 0% and 1% in the diet to mice for 13 weeks. Due to the pungency of the diet a reduced food intake resulted in an inhibition of the body weight gain in females. Renal toxicity was observed in all male mice treated with the 1% diet.

The objective of a study by Chanda *et al.* (2007, only abstract available) was to assess the oncogenic potential of *trans*-capsaicin when administered weekly via topical application to the dorsal skin of Tg.AC mice for 26 weeks. Male and female Tg.AC mice (25 mice/sex/group) received dose formulations containing *trans*-capsaicin dissolved in diethylene glycol monoethyl ether (DGME). The positive control was tetradecanoylphorbol-13-acetate (TPA) dissolved in DGME. Appropriate controls, including a topical lidocaine local anesthetic pretreatment (4% w/w), were maintained. All groups were dosed once weekly, except for the TPA group, which was dosed twice per week. Analysis of the macroscopic observations after the final sacrifice revealed no noteworthy treatment-related findings, with the exception of dermal masses that were randomly dispersed throughout all treatment groups for both

males and females. The frequency of dermal masses in the capsaicin-treated groups (at a dose level of up to 102 mg/kg and an application rate of 25.6 mg/cm^2/kg/week) was not elevated in comparison to either concurrent vehicle or untreated controls. Dermal application of capsaicin resulted in no increased incidence of preneoplastic or neoplastic skin lesions. Capsaicin-related non-neoplastic microscopic findings were seen sporadically in both genders and included acanthosis, hyperkeratosis/parakeratosis (primarily females), epidermal crusts, subepidermal fibrosis, epidermal ulcerations/erosions, and chronic-active inflammation. There was no evidence of a dose response in either the incidence or severity of these findings.

Rapporteur´s comment:

Repeat-dose toxicity studies were performed in a variety of animal models over a different period of time, dosage and ways of administration. Histological changes were observed after oral administration of very high doses of capsaicin. No changes were observed after cutaneous administration.

3.3.3. Genotoxicity

Herbal preparations: No data on genotoxicity for the extracts included in the monograph are available.

Herbal substance and other extracts:

Villasenor & De Ocampo, 1994: Extracts (no more information on extraction solvent or DER available) from the fruits of *Capsicum frutescens* L. were tested for their clastogenicity using the mouse-bone-marrow micronucleus (mouse-MN) assay. The results indicated that the isolate CF-1 is clastogenic at the maximum tolerated dose of 1.22 mg/kg mouse. Statistical analysis using the Wilcoxon two-sample test showed that the null hypothesis, μ (tetracycline) = μ (CF-1), is acceptable at 0.05 and 0.01 degrees of significance. Hence, the clastogenicity of CF-1 is statistically similar to that of tetracycline, a known clastogen, at the 5% and 1% levels of significance.

Tsuchiya *et al.* 2011: The authors aimed to investigate the mutagenicity and mutagens in Chilean red chili pepper (no data regarding capsaicin content published) in the AMES test using *Salmonella typhimurium* strains TA98, TA1537, TA100, and TA1535 with and without metabolic activation (S9 mix). Pure capsaicin was tested for mutagenicity using strain TA98. The presence of aflatoxins was evaluated by two-dimensional thin layer chromatography, and then the concentrations of aflatoxins B1, B2, G1, and G2 were measured by an HPLC system. In strain TA98, the mean numbers of revertant colonies with and without the S9 mix were 2.5- and 2.2-fold higher than those of each negative control, respectively. However, pure capsaicin did not show mutagenic activity in strain TA98. Aflatoxin contamination of red chili pepper was confirmed, and the concentrations of aflatoxins B1 and G1 were 4.4 ng/g and 0.5 ng/g, respectively.

Capsaicin:

In vitro:

Chanda *et al.* 2004: performed a series of tests regarding the potential genotoxicity of pure capsaicin. In the AMES test (*Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, TA 100, *E. coli* WP2uvrA), pure *trans*-capsaicin was not mutagenic to *Salmonella typhimurium* or *Escherichia coli* when dissolved in dimethylsulfoxide and tested at concentrations extending into the toxic range (up to 5000 μg per plate). *trans*-Capsaicin was weakly mutagenic in mouse lymphoma L5178Y cells, in the presence of S9 mix, when dissolved in dimethylsulfoxide and tested at concentrations extending into the toxic range. Limited evidence for very weak activity was also obtained in the absence of S9 mix.

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of
Capsicum frutescens L., fructus
EMA/HMPC/674138/2013   Page 27/67

Proudlock *et al.* 2004: An AMES test with *S. typhimurium* strains TA1535, TA1537, TA98, TA100, *E. coli* WP2 uvrA with and without metabolic activation by S9 mix was performed. Concentration of capsaicin: up to 5,000 µg/plate. In the chromosome aberration test duplicated cultures at each experimental point were treated with a volume of 10 µl/ml. A twofold dose interval was used up to the level that produced visible precipitation in the culture medium (512 µg capsaicin/ml).

The results confirmed the absence of genotoxic activity of high-purity capsaicin in the bacterial mutation and chromosome aberration tests. The authors concluded, that pure capsaicin was not active in the standard battery of *in vitro* genotoxicity assays recommended by the ICH; earlier reported *in vitro* genotoxic activity is probably associated with mutagenic impurities in commercial grades of the material.

In vivo:

Díaz *et al.* 1995: Capsaicin was administered intraperitoneally to mice in dosages of 1.46 mg/kg and 1.94 mg/kg. The administration schedule during 32 days included 3 consecutive daily injections followed by a resting period of 24 hours. The higher dose caused an increase of micronucleated normochromatic erythrocytes from day 16, in the lower dose this effect was seen after 32 days. At the end of the study the higher dosage caused also a significant increase in polychromatic erythorcytes and frequency of sister chromatid exchange in mouse bone marrow cells. The authors conclude that these results indicated that capsaicin is a genotoxicant.

Trans-Capsaicin did not induce micronuclei in bone marrow cells when tested to the maximum tolerated dose of 800 mg/kg per day in male and 200 mg/kg per day in female CD-1 mice using a 0 hour plus 24 hours oral dosing and 48 hours sampling regimen. Finally, *trans*-capsaicin did not induce structural or numerical chromosomal aberrations when evaluated for its ability to induce clastogenicity in blood lymphocytes (Chanda *et al.* 2004).

Proudlock *et al.* 2004: A rat bone marrow micronucleus test was performed up to 500 mg capsaicin/kg body weight. Systemic exposure to pure capsaicin was achieved using subcutaneous route and a rising dose toleration protocol. No evidence of cytotoxicity or genotoxicity was seen.

Lawson & Gannett, 1989: A crude mixture of capsaicinoids from *Capsicum frutescens*, capsaicin and dihydrocapsaicin (DHC) but not 4-methyl-dihydrocapsaicin (MC), a synthetic homolog of DHC, were mutagenic in the V79 assay. Oxidative metabolism of capsaicin and DHC by microsomes or ferricyanide yielded a dimer of capsaicin or DHC. Based on the lack of the mutagenicity of MC, the mutagenicities of capsaicin and DHC and the supporting chemical data, a mechanism of action involving an intermediate phenoxy radical is proposed.

Conclusion on genotoxicity data:

From the available data relating to capsaicin or chilli extracts (not further specified) it is evident that *in vitro* tests produced inconclusive results regarding mutagenic effects. A potential for mutagenicity cannot be excluded.

In the *in vivo* studies the mutagenic potential was depending on the dose and the treatment period. Lower doses were non-mutagenic when given for several days (i.p.). Longer treatment periods and higher dosages increased the occurrence of mutagenicity. Comparing such data with the daily consumption (up to 1 mg/kg body weight capsaicin consumed for years) with food in humans it may be concluded that the mutagenic potential of capsaicin is low although not completely absent. It can be assumed that concentrations in topically administered herbal preparations are far below the dosages where mutagenic effects were seen under *in vivo* conditions.

3.3.4. Carcinogenicity

Extracts: No data available

Capsaicinoids, capsaicin:

Park & Surh, 1997, Park *et al.*1998 (only abstract available): The authors assessed the tumour promoting potential of capsaicin using a two stage mouse skin carcinogenesis model. Repeated applications of capsaicin (10 µmol) onto the shaven backs of female ICR mice following a single-initiation dose of 7,12-dimethylbenz[a]anthracene or a twice-weekly application of 12-*O*-tetradecanoylphorbol-13-acetate did not cause any significant increase in papilloma formation and abnormal hyperplastic or inflammatory skin lesions, compared with the solvent control. Furthermore, the topical application of capsaicin did not induce the epidermal ornithine decarboxylase activity, suggesting that it lack tumour-promotional activity. On the contrary, the compound ameliorated the mouse skin carcinogenesis when given simultaneously with the tumour promoter, 12-*O*-tetradecanoylphorbol-13-acetate.

Akagi *et al.* 1998: A mixture consisting of 64.5% capsaicin and 32.6% dihydrocapsaicin was administered orally in concentrations of 0.025%, 0.083%, and 0.25% in the diet to mice for 79 weeks. The occurrence of tumours was either negatively correlated with the capsaicin content in the diet or similar in the treated and control groups.

Bley *et al.* 2012: summarised in their review that the postulated ability of capsaicin to damage DNA and promote carcinogenesis remained unsupported.

Conclusion on carcinogenicity data:

No data on carcinogenicity for the extracts included in the monograph is available.

From the data available it can be concluded, that capsaicin has tumorigenic as well as anti-tumorigenic properties. From very high doses a putative tumorigenic potential of capsaicin may be concluded. Under the conditions described in the monograph (topical administration, lower dosages) capsaicin can be considered not to be carcinogenic.

3.3.5. Reproductive and developmental toxicity

Fertility

Extracts: No data available

Capsaicin:

Mizrak *et al.* 2008: The rat spermatogonial stem cell lines Gc-5spg and Gc-6spg were used to study the effects of different concentrations of capsaicin during 24 and 48 hours. Initial morphological observations indicated that capsaicin at concentrations ranging from 150 µM to 250 µM and after 24 and 48 hours of exposure, had deleterious apoptotic-like effects on both cell lines: A large population of the capsaicin treated cell cultures showed signs of DNA fragmentation and caspase 3 activation. Quantification of the effect demonstrated a significant effect of capsaicin with doses of 150 µM in the Gc-5spg cell line and 200 µM in the Gc-6spg cell line, after 24 hours of exposure. The effect was dose and time dependent in both cell lines. TRPV1, the receptor for capsaicin, was found to be expressed by the spermatogonial stem cells *in vitro* and also by premeiotic germ cells *in situ*. The authors concluded that capsaicin adversely affected spermatogonial survival *in vitro* by inducing apoptosis to those cells.

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of
Capsicum frutescens L., fructus
EMA/HMPC/674138/2013   Page 29/67

Zik *et al*. 2010 and Tütüncü & Özfiliz, 2011: investigated the effect of the subcutaneous administration of capsaicin in a dose of 0.5 mg/kg/day to pre-pubertal rats. The results indicated that capsaicin interfered in the ovarian follicular development by partially inhibiting atresia-coupled apoptosis and promoting cell proliferation after 15 days of treatment.

Embryo toxicity, teratogenicity, peri-postnatal toxicity, lactation

Extracts and capsaicin:

Pellicer *et al*. 1996: The authors used the hot plate model (53±0.5°C) to study the effect of acute thermonociceptive stimulus on escape response latency in the offspring of rats that were treated during gestation, either with an aqueous red pepper solution (*Capsicum frutescens*, approximately 2.75 mg of capsaicin in 1 ml/day, by gavage during the second week), or with capsaicin (0.5 mg/ day subcutaneously, during the second week). These groups were compared with their respective controls. The difference between the control group which has been analogously manipulated to the treatment group and the one given the aqueous red pepper solution (treatment group) was 41.33%, and between the vehicle control and the one treated with capsaicin was 30.59%. These increments on the escape response latency were statistically significant.

Pellicer *et al*. 2001: Oral administration of aqueous extracts of *Capsicum frutescens* and of capsaicin during gestation produced an increase in the latency of the thermonociceptive escape response of the rat offspring. According to the authors, this indicated that capsaicin was absorbed and crossed the placenta. The effect in rat offspring was reversible.

The publicly available summaries of product characteristics of capsaicin containing medicated plasters (e.g., SmPC Elastoplast Heat Plaster 11 mg medicated plaster 2012) or cutaneous patches (SmPC Qutenza 179 mg cutaneous patch 2009) indicate that capsaicinoids may be excreted into breast milk. Therefore a risk for newborns/infants cannot be excluded.

Capsaicin:

Atkinson & Chaggar, 1983 (only abstract available): After subcutaneous administration of capsaicin to pregnant mice the authors observed that capsaicin crossed the placenta and depleted substance P from the primary afferent terminal field in the spinal cord of the foetuses.

Perfumi & Sparapassi, 1999: The study evaluated, whether some of the irreversible effects induced by neonatally administered capsaicin were present in offspring prenatally treated with the neurotoxin as well, and investigated its foetal toxicity. Capsaicin was administered subcutaneously at doses of 50, 100 and 200 mg/kg in five injections every other day between gestational days 7-15, the period of major organogenesis, or in a single subcutaneous injection of 50 mg/kg only on day 15 of gestation. In one-month old rats (prenatally capsaicin-treated) the response to noxious stimulation (hot plate and wiping tests) and the urinary excretion in response to oral water load were evaluated. Parallel experiments were conducted in one-month old rats treated with capsaicin (50 mg/kg) on the 2nd day of life. Prenatal capsaicin induced no evident treatment-related signs of toxicity in dams and offspring, nor did it influence the body growth of the pups or induce cutaneous lesions. Unlike neonatal treatment, prenatal administration of neurotoxin did not raise the threshold to thermal and chemical pain, and did not modify diuresis induced by oral load. Since researchers have proposed the existence of more than one population of capsaicin-sensitive afferent neurones which differ in their age-dependent sensitivity to capsaicin, the authors hypothesised that failure of prenatal treatment might be due either to reduced foetal availability, not capable of selectively destroying capsaicin-sensitive neurones, or to incomplete rearrangement and maturation of developing primary sensory neurons.

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of
Capsicum frutescens L., fructus
EMA/HMPC/674138/2013   Page 30/67

In fact, the existence is well known of more than one population of capsaicin-sensitive afferent neurons which differ in their age-dependent sensitivity to capsaicin.

Chanda *et al.* (2006) administered pure capsaicin to pregnant Sprague-Dawley rats via a medicated patch (640 µg/cm^2, sizes 25-50 cm^2) and to pregnant New Zealand white rabbits in a liquid formulation (10% capsaicin) at dosages of 3, 6.5 and 13 µl/cm^2 at the back of the animals. In rats the maternal no-observable-effect was less than 25 cm^2, but no litter parameters were affected even with the largest patch size. In the group with the largest patch size delayed foetal skeletal ossification was observed. In rabbits no foetal alterations were observed at all concentrations.

Milk samples analysed on day 14 of lactation showed measurable levels of capsaicin in rat`s milk at all dose levels when pregnant rats were exposed to capsaicin during gestation and lactation (Hazardous Substances Data Bank (HSDB), "Capsaicin", database of the National Library of Medicine's TOXNET system, (http://toxnet.nlm.nih.gov)).

Assessor's comment:

The vanniloid receptor type 1 is expressed in various organs of male and female reproductive system. Data from *in vitro* and *in vivo* animal studies point toward possible effects of capsaicin on fertility. However, the concentrations which can be reached when herbal preparations are administered cutaneously according to the monograph do not give reason for any concern.

Capsaicin seems not to be teratogenic in animals. However, it has been reported that capsaicin crosses the placenta and exerts a toxic effect on the peripheral nerves of foetuses. Furthermore, it was reported that capsaicin may pass into breast milk in an animal model. The herbal preparations described in the monograph should only be used during pregnancy and lactation after a careful risk-benefit assessment.

3.3.6. Local tolerance

Herbal preparations proposed for the monograph: No data available

3.3.7. Other herbal preparations, capsaicin

Capsaicin and herbal preparations of chili pepper have mild to moderate skin irritant properties (NN 2007) and causes severe irritations when coming into contact with mucous membranes like in the eye. Pepper spray as an agent for self-defence causes tears, pain in the eye and temporary blindness.

3.3.8. Rapporteur's conclusion

Due to the irritating properties of capsaicin on mucous membranes medicinal products containing herbal preparations with capsaicin should not be applied near the eyes or to mucous membranes or to injured skin, wounds and eczemas. Hands should be washed with soap and water after touching or handling the plaster/ointment.

3.4. Overall conclusions on non-clinical data

The published pharmacological data on capsaicin support the cutaneous medicinal use of herbal preparations of *Capsicum*. The evidence is supported by data generated with isolated or synthetic pure capsaicin. Capsaicin acts as an agonist at the vanilloid receptor. It desensitises the sensory nerves by depletion of substance P in the neurons.

Capsaicin induces, when applied in therapeutic doses, reversible damage of the membranes of sensory neurons. Low doses of topically administered capsaicin are not likely to produce any systemic effect, except reflectory actions.

Pharmacological investigations related to other indications are incomplete and do not support the use of Capsicum preparations in other indications.

Capsaicin has synergistic effects with other drugs like lidocaine and amitriptyline when applied locally at the same time. Therefore it is recommended not to use capsicum preparations concomitantly with other topical formulations.

Pharmacokinetic data demonstrated that topically administered capsaicin is absorbed to a marked extent and may be detected in the blood. At concentrations occurring after topical administration of a high-concentration patch, capsaicin did not cause direct inhibition of CYP enzymes.

The published toxicity data do not indicate a special risk for the cutaneous use of herbal preparations of *Capsicum*. Adequate genotoxicity tests for the herbal preparations are missing. Capsaicin seems not to be teratogenic in animals. Since capsaicin crosses the placenta and exerts a toxic effect on the peripheral nerves of foetuses and since it may pass into breast milk the medicinal products containing herbal preparations of *Capsicum* should only be used during pregnancy and lactation after a careful risk-benefit assessment. Data on carcinogenicity of the herbal preparations are not available. Capsaicin has mild to moderate skin, eye and mucous membrane irritant properties.

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

Summary:

Compilation of Caterina *et al.* 1997, Biro *et al*. 1997, Sasamura & Kuraishi, 1999; Jordt *et al.* 2003; Szolcsányi 2004; Hayman & Kam, 2008; Hänsel & Sticher, 2010

After topical application of capsaicins the initial excitation of the peripheral nociceptors results in local erythema, burning and itching. The depolarisation of the neurons by capsaicinoids results in the feeling of heat. This initial phase is followed by a desensitisation phase with an anti-nociceptive effect. The neurones loose the potential to refill the emptied depot of Substance P, moreover morphological damages on the neuron could contribute to the antinociceptive effect.

Primary pharmacodynamics

Magnusson & Koskinen, 2000: The aim of the study was to explore the *in vivo* sensation responses of capsaicin and to compare the results with the *in vitro* percutaneous absorption of the substance. The overall objectives were to determining an *in vitro–in vivo* correlation for capsaicin. Capsaicin was applied in a chamber on the volar forearm of twelve volunteers and in a flow-through diffusion chamber on excised human epidermal membranes. Topical administration of capsaicin produced a complex cutaneous sensation that changed in intensity and quality as a function of time and was characterised by sting, prick, burn and pain. Percutaneous steady-state penetrations of capsaicin with a receptor fluid consisting either of 4% bovine serum albumin in phosphate buffered saline or 50% ethanol in water were 28.2 ± 2.7 and 29.6 ± 2.9 mg/cm^2 per h, respectively.

The corresponding cumulative penetrated amounts of capsaicin after 30 min were 14.7±1.7 and 19.2 ±2.1 mg/cm^2, respectively. In the opinion of the authors the results indicate that there is a good correlation between *in vivo* physiological responses and *in vitro* percutaneous penetration of topically applied capsaicin.

Del Bianco *et al.* 1996: The effect of repeated capsaicin application on the skin of the volar surface of the forearm on the pain sensation and on the increase in blood flow induced by intradermal injection of low pH media or hypertonic solutions was investigated in 13 healthy volunteers. Capsaicin (1% in 50% ethanol) was painted on the volar skin of one forearm, chosen at random, for 7 days. The contralateral forearm was treated with the capsaicin vehicle. Pain was assessed by a visual analogue scale (VAS) and skin blood flow by a laser Doppler flowmeter. Pain sensation and increase in blood flow (both peak and area under the curve) induced by low pH media were markedly reduced in the capsaicin pretreated side. Capsaicin pre-treatment also reduced the increase in blood flow, but did not affect the pain response induced by hypertonic saline solutions.

Gibbons *et al.* 2010: 32 healthy subjects underwent occlusive application of 0.1% capsaicin cream (or placebo) for 48 hours in order to determine the effects of topical application of capsaicin on cutaneous autonomic nerves. Subjects were followed for 6 months with serial assessments of sudomotor, vasomotor, pilomotor, and sensory function with simultaneous assessment of innervation through skin biopsies. There were reductions in sudomotor, vasomotor, pilomotor, and sensory function in capsaicin-treated subjects (p<0.01 vs. placebo). Sensory function declined more rapidly than autonomic function, reaching a nadir by Day 6, whereas autonomic function reached a nadir by Day 16. There were reductions in sudomotor, vasomotor, pilomotor, and sensory nerve fibre densities in capsaicin-treated subjects (p<0.01 vs. placebo). Intraepidermal nerve fibre density declined maximally by 6 days, whereas autonomic nerve fibre densities reached maximal degeneration by Day 16. Conversely, autonomic nerves generally regenerated more rapidly than sensory nerves, requiring 40-50 days to return to baseline levels, whereas sensory fibres required 140-150 days to return to baseline. The data suggest caution should be taken when topical capsaicin is applied to skin surfaces at risk for ulceration, particularly in neuropathic conditions characterised by sensory and autonomic impairment.

Lee *et al.* 2007a: This study investigated the influence of topical capsaicin on various types of sensations including pain in the facial areas innervated by the mental nerve, and also evaluated whether the measurement of cutaneous current perception threshold (CPT) is reliable for the quantification of sensory change following capsaicin application. Twenty healthy subjects were given topical capsaicin cream (0.075%), which was applied to the mental area unilaterally, four times daily for 2 weeks. Burning sensation after capsaicin application gradually decreased with repeated applications. Repeated topical capsaicin resulted in reduced sensation to mechanical, heat and cold pain without changing non-painful tactile sensation. It also resulted in increased CPTs at 5 Hz and 250 Hz stimuli but no change in the CPTs at 2000 Hz from the first evaluation after capsaicin treatment and throughout the treatment period. According to the authors, this study demonstrated that topical capsaicin treatment for the management of chronic localised pain can be safely applied to the face without affecting non-painful normal sensations, and that CPT testing is a clinically useful tool for the quantification of sensory changes following capsaicin application.

Mohammadian *et al.* 1998: The aim of the study was to investigate local vascular and sensory changes and their correlation in order to obtain a better understanding of the mechanisms of allodynia, hyperalgesia and vascular changes following tissue inflammation induced by repetitive application of capsaicin cream. This type of application was utilised as a controlled model of inflammation which was altered in intensity due to its repetitive applicability.

Ten healthy volunteers participated in two experiments separated by at least five days. Each experiment consisted of a baseline session followed by five additional sessions. Before these sessions either 1.5 g capsaicin cream (1% capsaicin) or placebo cream was applied to the volar site of the forearm for 15 min. The areas of stroking allodynia and pin-prick hyperalgesia were mapped and the intensity of spontaneous pain (VAS) was assessed after each application of the cream. In addition, the visible flare, temperature (IR-Thermography), and blood-flow (Laser-Doppler) were measured. The first application of capsaicin was perceived as painful; it induced both secondary hyperalgesia and allodynia. Compared to placebo, the first application of capsaicin cream also resulted in an increased blood-flow, elevated temperature and visible flare. The highest values of these sensory and vascular parameters were reached after the third application. A direct correlation between visible flare, secondary mechanical hyperalgesia and allodynia following repetitive application of capsaicin indicated that both common central and peripheral mechanisms were involved in these changes.

Moller *et al.* 2006: The aim of this study was to evaluate a small-fibre dysfunction in female Fabry patients by using capsaicin, applied topically. The response to capsaicin was evaluated by laser Doppler imaging. The authors found that the female Fabry patients had a significantly smaller increase in blood flow ($p=0.0003$) after capsaicin application (100 µl, diluted to 5% in ethanol). The area of static mechanical allodynia and dynamic mechanical hyperalgesia was also significantly smaller ($p=0.006$) in female Fabry patients. This indicated that female Fabry patients have a significant loss of small-fibre function and demonstrates that it is possible to evaluate this by a non-invasive method.

Morris *et al.* 1995: The aim of the study was to investigate the role of primary afferent fibres with polymodal nociceptors in the various pain symptoms and signs associated with post-herpetic neuralgia (PHN). Forty-four patients with PHN affecting thoracic dermatomes were examined clinically for evidence of sensory disturbances to touch and pinprick and compared to 14 normal subjects and 9 subjects with evidence of past herpes zoster infection but no pain. The patients were divided into 3 groups on the basis of their clinical symptoms (steady burning discomfort n=12; burning discomfort, allodynia and hyperalgesia to pinprick n=17; burning discomfort, allodynia and hypalgesia to pinprick n=15). The groups with allodynia had significantly decreased neurogenic flare responses compared to PHN subjects without allodynia.

Nolano *et al.* 1999: The present study was performed to determine if morphological changes of intracutaneous nerve fibres contribute to desensitisation and hypalgesia. Capsaicin (0.075%) was applied topically to the volar forearm four times daily for 3 weeks to 10 healthy volunteers. At various time intervals tactile, cold, mechanical and heat pain sensations were assessed in the treated and in contralateral untreated areas. Skin blisters and skin biopsies were collected and immunostained for protein gene product (PGP) 9.5 to assess the morphology of cutaneous nerves and to quantify the number of epidermal nerve fibres (ENFs). Capsaicin resulted in reduced sensitivity to all cutaneous stimuli, particularly to noxious heat and mechanical stimuli. This hypalgesia was accompanied by degeneration of ENFs as evidenced by loss of PGP 9.5 immunoreactivity. As early as 3 days following capsaicin application, there was a 74% decrease in the number of nerve fibres in blister specimens. After 3 weeks of capsaicin treatment, the reduction was 79% in blisters and 82% in biopsies. Discontinuation of capsaicin was followed by reinnervation of the epidermis over a 6-week period with a return of all sensations, except cold, to normal levels. The authors concluded that degeneration of ENFs contributed to the analgesia accredited to capsaicin.

Reilly & Green, 1999: The authors assessed the effects of physical and chemical irritants on a profile of acute inflammatory mediators in normal human skin on 48 healthy volunteers. Skin damage in both cases was accompanied by a flux of inflammatory processes and repair mechanisms, which remained imprecisely understood.

Ten sequential cellotape strips or topical application of 0.075% capsaicin as skin irritants were used and the subsequent production and/or release of inflammatory mediators in suction blister fluids from human skin *in vivo* was characterised. In tape stripped skin, levels of prostaglandin E2 and interleukin-1alpha were increased 3.4-fold and 3.3-fold, respectively ($p< 0.0001$; $p< 0.02$), levels of tumour necrosis factor-alpha were decreased 3-fold ($p<0.01$), whereas levels of interleukin-6 and leukotriene B4 in blister fluids remained relatively unchanged. For the capsaicin-treated skin, levels of mediators showed only minor differences when compared with matched controls. However, a correlation was observed between levels of prostaglandin E2 and interleukin-1alpha in capsaicin pre-treated blister fluids ($r=0.58$, $p<0.01$, $n=19$). These data were consistent with the key roles of prostaglandin E2 and interleukin-1alpha in acute skin responses to mild irritants.

Simone & Ochoa, 1991: Cutaneous sensibility and neurogenic vasodilatation (flare) were measured before, during and after long-term topical application of capsaicin in 10 healthy volunteers. Each subject applied a vehicle cream containing 0.075% capsaicin to a 4 cm^2 area of skin on one volar forearm and vehicle alone to an identical treatment area on the other forearm, according to a double-blind procedure. Each substance was applied 4 times/day for 6 weeks. Psychophysical measurements of sensory detection thresholds, magnitude of supra-threshold heat pain, magnitude and duration of histamine-induced itch and flare area were obtained before, at 1, 3 and 7 days after the first application, and once a week thereafter for a total of 8 weeks. Capsaicin produced mild burning in all subjects which diminished in magnitude and duration over several weeks. Capsaicin significantly altered detection thresholds for heat pain and the magnitude of pain produced by supra-threshold painful stimuli. Mean detection threshold for heat pain was lowered 1.6 °C following 1 day of capsaicin application but subsequently increased to become elevated 3.5 °C after 6 weeks of application. In addition, mean magnitude of supra-threshold heat pain diminished progressively after 1 week. Heat pain thresholds returned to or near pre-treatment values within 2 weeks after discontinuing application. Detection thresholds for touch, cold sensation and pain induced by low temperature and by mechanical stimulation were not altered by capsaicin. Similarly, capsaicin did not alter the magnitude or duration of itch produced by intradermal injection of 1 microgram histamine. However, the area of flare produced by histamine was significantly reduced in capsaicin-treated skin. According to the authors, these studies demonstrated that prolonged application of capsaicin at low concentration selectively diminishes sensations of heat pain and neurogenic vasodilatation, presumably via desensitisation of heat-sensitive nociceptors. It was also shown that the decrease in heat pain is temporary and was maintained with repeated capsaicin application.

Tandan *et al.* 1992a: The authors examined the effects of topical 0.075% capsaicin cream on thermal and vibration thresholds in 22 subjects with painful diabetic neuropathy (PDN) who participated in a double-blind vehicle-controlled therapeutic trial. After 8 weeks of use, there was no significant change in warm and vibration thresholds, but the cold threshold was significantly reduced by capsaicin and vehicle creams to an equal degree. In fewer subjects who used capsaicin cream in an open-label study, there was no significant effect on sensory thresholds after up to 32 weeks of use.

Walker & McCleane, 2002: The aim of this study was to determine whether topical application of capsaicin cream causes thermal allodynia and the extent to which this is attenuated by the addition of glyceryl-trinitrate (GTN). This was a double blind placebo controlled study of 40 consenting adult subjects. Each of four creams (GTN, capsaicin, GTN/capsaicin and vehicle) was applied to the subjects with at least a 1 day interval between each application. Water at a known temperature was applied to the standard area of skin where cream had been applied. Subjects rated the resulting thermal allodynia using a 0-10 Likhert score. Thermal allodynia is usually apparent when warm water is applied to skin containing capsaicin.

The thermal allodynia caused by the topical application of capsaicin was significantly reduced by the addition of GTN. The addition of GTN to capsaicin cream significantly reduced the thermal allodynia associated with the application of capsaicin cream alone.

Kennedy *et al.* 2010: This study evaluated the pharmacodynamic effects of a single 60 min application of NGX-4010, a high-concentration (8% w/w) capsaicin patch, on both thighs of healthy volunteers. Epidermal nerve fibre (ENF) density and quantitative sensory testing (QST) using thermal, tactile, and sharp mechanical-pain (pinprick) stimuli were evaluated 1, 12 and 24 weeks after capsaicin exposure. After 1 week, there was about an 80% reduction of ENF density compared to unexposed sites. In addition, there was about an 8% increase in tactile thresholds compared to baseline and the proportion of stimuli reported as sharp mechanical pain decreased by about 15%. Twelve weeks after exposure to capsaicin, ENF regeneration was evident, but not complete, and sharp mechanical-pain sensation and tactile thresholds did not differ from unexposed sites. Nearly full (93%) ENF recovery was observed at 24 weeks. No statistically significant changes in heat- or cold-detection thresholds were observed at any time point. NGX-4010 was generally well tolerated. Transient, mild warming or burning sensations at the site of application were common adverse effects.

Secondary pharmacodynamics

Horowitz *et al.* 1992 (only abstract available): The effects of chilli on GI transit (gastric emptying, orocaecal transit, whole gut transit) were evaluated in eight healthy volunteers. In each subject, GI transit of a standard test meal was measured on two separate days. Either on the first or on the second day, 20 g of chilli powder was added to the meal. Gastric emptying was quantified with a radio isotopic technique, orocaecal transit by measurement of breath hydrogen concentrations and whole gut transit by counting the number of radio-opaque markers in the stool. The rate of gastric emptying was slower ($p<0.05$) and whole gut transit was faster ($p<0.02$) after the meal containing chilli, compared with the other meal. There was no significant difference in orocaecal transit. These results showed that ingestion of chilli was associated with significant effects on gastric emptying and intestinal transit.

Ericson *et al.* 2009: The aim of this study was to identify VR1-expressing endocrine-like cells in human antral glands and to examine whether stimulation with capsaicin causes release of gastrin, somatostatin or serotonin. Further, to investigate the effects of a chilli-rich diet, gastroscopic biopsies were received from 11 volunteers. Seven of the 11 subjects agreed to donor gastric biopsies a second time after a 3-week chilli-rich diet containing 1.4-4.2 mg capsaicin/day. VR1-immunoreactive cells were identified by double-staining immunohistochemistry against gastrin, somatostatin, and serotonin. For the stimulation studies, an *in vitro* method where antral glands in suspension were stimulated with 0.01 mM capsaicin and physiological buffer was added to the control vials was used. The concentrations of secreted hormones were detected and calculated with radioimmunoassay. The light microscopic examination revealed that VR1 was localised in gastrin cells. The secretory studies showed an increase in release of gastrin and somatostatin compared to the control vials ($p=0.003$; $p=0.013$). Capsaicin-stimulation caused a consistent raise of the gastrin concentrations in the gland preparations from all subjects. A chilli-rich diet had an inhibitory effect on gastrin release upon stimulation compared to the results that were obtained before the start of the diet. The authors concluded, that that capsaicin stimulated gastrin secretion from isolated human antral glands, and that a chilli-rich diet decreases this secretion.

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of
Capsicum frutescens L., fructus
EMA/HMPC/674138/2013   Page 36/67

Debreceni *et al.* 1999: In this study, the authors investigated the effect of 400 μg capsaicin given intragastrically on gastric emptying measured by ^{13}C-octanoic acid breath test in ten healthy human subjects. Four parameters of gastric emptying curves were taken into consideration: 1) maximum value of the curve, 2) time belonging to this maximum, 3) slope of the rising part of the curve and 4) time belonging to the 50% of the area under the curve. Administration of 400 μg capsaicin significantly increased the slope of gastric emptying curve (from 0.1 ± 0.01 to 0.139 ± 0.014 U/min, $p < 0.05$) and significantly decreased the time belonging to the maximum value of emptying curve (from 150 ± 18 to 75 ± 12 min, $p < 0.05$) and the time belonging to the 50% of the area under the curve (from 112 ± 15 to 99 ± 14 min, $p < 0.05$).

Krogstad *et al.* 1999: To determine whether neurogenic factors may be of importance in the regulation of histamine release and blood flow in psoriatic plaque, the effect of capsaicin was studied in 22psoriatic patients with active, untreated psoriatic lesions. In each of 12 patients, one microdialysis fibre was placed in non-lesional skin and one was placed in lesional skin at depths of 0.7 and 0.9 mm, respectively. Dialysates were collected for the analysis of histamine in the resting state and after 60 min of repetitive epicutaneous application of 1% capsaicin above the microdialysis catheter. In 10 patients, topical capsaicin and placebo were applied for 24 hours to lesional/lesion-free skin. Skin blood flow and perfusion (evaluated using the ^{133}xenon clearance technique and scanning laser Doppler, respectively) were measured before the application of capsaicin and after removal. After 60 min of capsaicin treatment, both the perfusion and interstitial concentration of histamine, as well as the net release of histamine, were significantly increased in affected (from 38 ± 6 to 45 ± 6 nmol/l, mean$\pm$SEM) and unaffected (from 15 ± 2 to 19 ± 2 nmol/l) skin. Compared with placebo, 24 hours of treatment with capsaicin caused a 15% decrease in perfusion in lesional skin. According to the authors the results are compatible with the hypothesis that capsaicin-sensitive nerves may induce histamine release in non-lesional and lesional skin and that afferent unmyelinated nerve fibres may contribute to the high blood flow in psoriatic plaques.

Lotti *et al.* 1994: Five patients with aquagenic pruritus were treated with capsaicin cream 0.025%, 0.5% or 1% three times daily for 4 weeks. Direct immunofluorescence (DIF) was performed before and after treatment to evaluate the storage of neuropeptides in the A delta and C type cutaneous nerve fibres. Before treatment (when by DIF the neuropeptidergic fibres appeared filled with neuropeptides), contact with water consistently provoked itching. After capsaicin treatment (when by DIF the neuropeptidergic fibres were depleted of neuropeptides), contact with water did not evoke pruritus. Areas of skin treated with the vehicle alone showed no clinical improvement or change in neuropeptide content.

Mózsik *et al.* 2005: The authors tried to evaluate the gastro-protective effect of capsaicin against the ethanol- and indomethacin (IND)-induced gastric mucosal damage in healthy human subjects. The effects of small doses (1-8 μg/ml, 100 ml) of capsaicin on the gastric acid secretion basal acid output (BAO) and its electrolyte concentration, gastric transmucosal potential difference (GTPD), ethanol- (5 ml 300 ml/l i.g.) and IND- (3 times 25 mg/day) induced gastric mucosal damage were tested in a randomised, prospective study of 84 healthy human subjects. The possible role of desensitisation of capsaicin-sensitive afferents was tested by repeated exposures and during a prolonged treatment. Intragastric application of capsaicin decreased the BAO and enhanced "non-parietal" component, GTPD in a dose-dependent manner. The decrease of GTPD evoked by ethanol was inhibited by the capsaicin application, which was reproducible. Gastric microbleeding induced by IND was inhibited by co-administration with capsaicin, but was not influenced by two weeks pre-treatment with a daily capsaicin dose of 3 times 400 μg i.g.

Szabó *et al.* 2013: The effects of capsaicinoids, atropine, cimetidine, omeprazole, famotidine and ranitidine were studied on gastric basal acid output, whereas the gastric mucosal preventive effects of capsaicinoids (capsaicin), atropine and cimetidine were tested on the IND-induced gastric mucosal microbleedings in human healthy subjects. Results were presented by molecular pharmacological method; affinity (pD) and intrinsic activity (α-values) were calculated. Intrinsic activity curves were based on comparison to atropine effect ($α_{atropine}$=1). For evaluation of physiological and pharmacological effects of compounds molar doses of pD_2 (necessary doses to produce 50% inhibition) and pA_2 (50% inhibition on intrinsic activity) were calculated from affinity and intrinsic activity curves. The pD_2 values for compounds were as follows: 5.88 for capsaicinoids, 5.4 for atropine, 2.23 for cimetidine, 3.33 for ranitidine, 3.77 for famotidine and 3.97 for omeprazole. α - value results for compounds were: 0.76 for capsaicinoids and 1 for atropine, cimetidine, ranitidine, famotidine and omeprazole all equal to 1 on gastric acid basal secretion. The pD_2 values on IND-induced gastric mucosal microbleeding were found as follows: 6 for capsaicinoids, 5.5 for atropine, and 3.5 for cimetidine, meanwhile α-values resulted 0.76 for capsaicinoids, 1 for atropine and cimetidine. The authors concluded, that atropine and capsaicinoids acted in about the same molar concentration which suggested a significant physiological role for capsaicin sensitive afferent nerves in the regulation of gastric basal acid secretion and in the prevention of chemically- induced gastric mucosal protection in human healthy subjects, suggesting a novel physiological pathway in regulation.

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

Magnusson & Koskinen, 2000: The aim of the study was to explore the *in vivo* sensation responses of capsaicin and to compare the results with the *in vitro* percutaneous absorption of the substance. The overall objectives were to determining an *in vitro-in vivo* correlation for capsaicin. Capsaicin was applied in a chamber on the volar forearm of twelve volunteers and in a flow-through diffusion chamber on excised human epidermal membranes. Topical administration of capsaicin produced a complex cutaneous sensation that changed in intensity and quality as a function of time and was characterised by sting, prick, burn and pain. Percutaneous steady-state penetrations of capsaicin with a receptor fluid consisting either of 4% bovine serum albumin in phosphate buffered saline or 50% ethanol in water were 28.2±2.7 and 29.6±2.9 µg/cm^2 per h, respectively. The corresponding cumulative penetrated amounts of capsaicin after 30 min were 14. 7±1.7 and 19.2±2.1 µg/cm^2, respectively. In the opinion of the authors the results indicate that there is a good correlation between *in vivo* physiological responses and *in vitro* percutaneous penetration of topically applied capsaicin.

Pershing *et al.* 2004: This study evaluated the uptake and elimination kinetics of capsaicinoids in human stratum corneum following a single topical exposure to 3% solutions containing 55% capsaicin, 35% dihydrocapsaicin (DHC, and 10% other analogues prepared in three vehicles: mineral oil (MO), propylene glycol (PG), and isopropyl alcohol (IPA). Capsaicinoid solutions were evaluated simultaneously in a random application pattern on the volar forearms of 12 subjects using a small, single 150 µg dose. Capsaicin and DHC were recovered from human skin using commercial adhesive discs to harvest stratum corneum from treated sites. Capsaicinoids were extracted from the stratum corneum-adhesive discs and quantified by liquid chromatography/mass spectroscopy. Both capsaicinoids were detected in stratum corneum 1 min after application with all vehicles and achieved a pseudo-steady state shortly thereafter. IPA delivered 3 times greater capsaicin and DHC into the human stratum corneum than PG or MO at all time points investigated. The C_{max} of capsaicin in IPA, PG, and MO was 16.1, 6.2, and 6.5 µg, respectively. The DHC content was 60% of capsaicin with all vehicles. The estimated T (half) of capsaicin and DHC in the three vehicles was similar (24 h).

Thus, maximal cutaneous capsaicinoid concentrations were achieved quickly in the human stratum corneum and were concentration and vehicle dependent. In contrast, capsaicinoid half-life was long and vehicle independent.

Stücker *et al.* 1999: The aim of the study was to investigate the influence of the type of preparation on the time course of action of the capsaicinoid nonivamide monitored by the axon-reflex-induced hyperaemic action and area of erythema. Sixteen healthy subjects were included in the study. The hyperaemic responses after application of nonivamide in an oil-in-water emulsion and in a water-free ointment were assessed both by laser Doppler perfusion imaging and planimetry after 0, 15, 30, 45, 60, 120 and 240 min. They were compared with the reaction after application of the nicotinic ester nicoboxil and a combination of nonivamide and nicoboxil in the same preparations. Applied as a water-free ointment, nonivamide showed a slow onset of hyperaemic action, reaching its maximum 45 min after application. When applied as an oil-in-water emulsion, however, the increase in effect was high, reaching its maximum already after 30 min. Application of the nicoboxil preparations revealed a clearly lesser influence of the base regarding the onset of maximum effect. The combination of both substances showed an additive effect for both bases, and a maximum effect was found already after 15 min with both the water-free ointment and the oil-in-water emulsion base.

Babbar *et al.* 2009: To determine systemic capsaicin exposure after single 60 or 90 min NGX-4010 (a high-concentration (640 µg/cm^2) capsaicin patch) applications, plasma samples were collected from 173 patients with postherpetic neuralgia (PHN), painful human immunodeficiency virus-associated neuropathy (HIV-AN), and painful diabetic neuropathy (PDN). The percentages of patients with quantifiable levels of capsaicin at any time point were 31% for PHN (30 of 96), 7% for HIV-AN (3 of 44), and 3% for PDN (1 of 33). The maximum plasma concentration observed in any patient was 17.8 ng/ml. Due to the limited number of quantifiable levels, a population analysis was performed to characterise the pharmacokinetics (PK) of capsaicin. Plasma concentrations were fitted adequately using a 1-compartment model with first-order absorption and linear elimination. Capsaicin levels declined very rapidly, with a mean population elimination half-life of 1.64 hours. Mean area under the curve and C_{max} values after a 60 min application were 7.42 ng×h/ml and 1.86 ng/ml, respectively. Only a few correlations between calculated PK parameters and patient characteristics were observed. Duration and area of application of the patch were detected as significant covariates explaining the PK of capsaicin. Ninety-min applications of NGX-4010 resulted in capsaicin area under the curve and C_{max} values approximately 1.78- and 2.15-fold higher than those observed in patients treated for 60 minutes. Treatment on the feet (patients with HIV-AN and PDN) produced far lower systemic exposure than treatment on the trunk (patients with PHN). Finally, larger treatment areas were associated with statistically higher Vc/F values. The low systemic exposure and very rapid elimination half-life of capsaicin after NGX-4010 administration are unlikely to result in systemic effects.

4.2. Clinical Efficacy

4.2.1. Dose response studies

No relevant data published

4.2.2. Clinical studies (case studies and clinical trials)

Clinical trials with herbal preparations proposed for the monograph

Chrubasik *et al.* 2010: soft extract (DER 4-7:1), extraction solvent ethanol 80% V/V

In a randomised double-blind multi-centre study, 281 patients (18-65 years of age) suffering from chronic soft tissue pain were treated either with a cream containing capsaicin 0.05% (n=140) or placebo (n=141). Of these, 151 were excluded from the ITT analysis, as they had in addition to their soft-tissue pain, pain of other origin. The primary outcome measure was a positive treatment response, defined as a pain sum score reduction of 30% or more. After 3 weeks of treatment, the median pain sum score had decreased by 49% (capsicum group) and 23% (placebo group) (ITT analysis, p=0.0006). The odds ratio of the responders in favour for capsaicin was 4.3 (CI 97.5% lower limit 1.9, p<0.0001). Improvements in the secondary efficacy measures confirmed the results. Likewise, all outcome measures had significantly more improved in the capsaicin-treated compared with the placebo-treated chronic back pain sufferers. All patients were included in the safety assessments. More adverse events occurred in the capsicum group (n=13) than in the placebo group (n=6). The capsaicin cream was generally well tolerated.

Keitel *et al.* 2001: Capsicum soft extract (DER 4-7:1), standardised, extraction solvent ethanol (80% V/V). Medicated plaster 22 x 14 cm containing 11 mg capsaicinoids, corresponding to 35 µg capsaicinoids per cm^2.

In a double-blind, randomised parallel-group study a capsicum plaster was compared with a placebo for 3 weeks in 154 patients (18-75 years of age) with non-specific back pain. Inclusion criteria were a history of back pain for a minimum period of 3 months and a degree of pain of 5 or more on an eleven grade VAS. The principal target variable consisted of the score of 3 combined pain scales. Secondary efficacy measures were tests of mobility, a disability index (in the context of Arhus low back rating scale) and global assessments by physicians and patients. For patients to be rated as responders their total pain score at the final examination after 3 weeks of treatment had to show a reduction by at least 30% of the baseline value. The study unequivocally achieved the target criterion with a rate of responders in the capsicum group of 60.8% against 42.1% in the placebo group (p=0.0219). The sum of the 3 separate pain scales decreased more markedly in the capsicum group than in the placebo group (38.5% compared to 28%; p=0.002). Relatively slight improvements of the impaired mobility and the functional status are explained by the characteristics of the disorder treated. The efficacy ratings by observers and patients were in favour of capsicum. Adverse effects - mostly harmless and resolving spontaneously - were reported by 15 patients in the capsicum group and by 9 in the placebo group. The tolerance ratings by investigators and patients were superior to the placebo product. This, however, partly is due to the local pharmacological actions of the drug.

Frerick *et al.* 2003: 12 x 18 cm plaster containing ethanolic soft extract standardised to 22 µg/cm^2 (no further details on the extract). Reference is made to the study by Keitel *et al.* (2001), in which a 'comparable' plaster has been used.

The efficacy and tolerance of a capsicum plaster in non-specific low back pain was investigated in a double-blind, randomised, placebo-controlled multicentre parallel group study. A total of 320 patients were randomly assigned to two groups of n=160 subjects (18-75 years of age) treated by the active or the placebo plaster. The main outcome measures used were a compound pain subscore of the Arhus low back rating scale (continuous variable), and a response criterion of a reduction in pain subscore=30% from baseline to final assessment (secondary, non-continuous variable).

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of *Capsicum frutescens* L., fructus
EMA/HMPC/674138/2013

Page 40/67

In addition, the partial pain scores, disability and mobility restriction subscores, the total score of the Arhus low back rating scale, the global evaluation of efficacy by investigator and patient, adverse events, a patient questionnaire on use of the plaster, and an evaluation of tolerance by investigator and patient were obtained. After 3 weeks treatment with capsicum and placebo plaster respectively, the compound pain subscore was reduced by 42% (capsicum) and 31% (placebo) from values on entry. Responder rate was 67% versus 49% (p=0.002). The investigators rated efficacy as "excellent" or "good" by 74% and 36%; the patient's efficacy rating "symptom free" or "improved" reached 82% and 50% . Adverse local drug reactions were found in 12 patients (7.5%) on capsicum and 5 (3.1%) on placebo.

No systemic side-effects were observed. The superiority of the treatment of chronic non-specific low back pain with capsicum plaster compared to placebo was clinically relevant and highly statistically significant.

Clinical trials with the herbal substance and other herbal preparations or pharmaceutical forms

Ahuja & Ball, 2006: The study investigated the effects of regular consumption of chilli on *in vitro* serum lipoprotein oxidation and total antioxidant status (TAS) in healthy adult men and women. In a randomised cross-over study, twenty-seven participants (thirteen men and fourteen women) ate 'freshly chopped chilli' blend (30 g/day; 55% cayenne chilli) and no chilli (bland) diets, for 4 weeks each. Use of other spices, such as cinnamon, ginger, garlic and mustard, was restricted to minimum amounts. At the end of each dietary period serum samples were analysed for lipids, lipoproteins, TAS and Cu-induced lipoprotein oxidation. Lag time (before initiation of oxidation) and rate of oxidation (slope of propagation phase) were calculated. There was no difference in the serum lipid, lipoproteins and TAS at the end of the two dietary periods. In the whole group, the rate of oxidation was significantly lower (mean difference – 0.23 absorbance × 103/min; p=0.04) after the chilli diet, compared with the bland diet. In women, lag time was higher (mean difference 9.61 min; p<0.001) after the chilli diet, compared with the bland diet. In conclusion, regular consumption of chilli for 4 weeks increases the resistance of serum lipoproteins to oxidation.

Ahuja *et al.* 2007: The objective was to investigate the effects of regular chilli ingestion on some indicators of metabolic and vascular function in a randomised cross-over dietary intervention study in healthy free-living individuals. Thirty-six participants (22 women and 14 men), aged 46±12 (mean±SD) years; BMI 26.4±4.8kg/m^2, consumed 30g/day of a chilli blend (55% cayenne chilli) with their normal diet (chilli diet), and a bland diet (chilli-free) for 4 weeks each. Metabolic and vascular parameters, including plasma glucose, serum lipids and lipoproteins, insulin, basal metabolic rate, blood pressure, heart rate, augmentation index (Aix; an indicator of arterial stiffness), and subendocardial-viability ratio (SEVR; a measure of myocardial perfusion), were measured at the end of each diet. In a sub-study, during week 3 of each dietary period, the vascular responses of 15 subjects to glyceryl-trinitrate (GTN) and salbutamol were also studied. For the whole group, there were no significant differences between any of the measured parameters when compared at the end of the two dietary periods. When analysed separately, men had a lower resting heart rate (p=0.02) and higher SEVR (p=0.05) at the end of the chilli diet than the bland diet. In the sub-study, baseline Aix on the chilli diet was lower (p<0.001) than on the bland diet, but there was no difference in the effects of GTN and salbutamol between the two diets.

Altomare *et al.* 2006: In this double-blind, randomised, placebo-controlled, crossover trial, the effects of a single dose of red hot chili pepper on the haemorrhoidal symptoms were studied. Fifty patients with second-degree and third-degree symptomatic haemorrhoids were randomly assigned to take a capsule containing red hot chili powder or placebo during lunch, scoring five haemorrhoidal symptoms (bleeding, swelling, pain, itching, and burning) on a VAS. After one week, crossover treatment was administered according to the same methodology. Other treatments and foods potentially related with anorectal symptoms were discontinued during the study periods. Patients assigned low scores to their haemorrhoidal symptoms before the study and the scores remained unchanged during the 48 hours after both placebo and chili pepper treatment, the latter showing no statistically significant effects.

Bortolotti *et al.* 2002: The aim of the study was to decrease the intensity of dyspeptic symptoms by impairing the visceral nociceptive C-type fibres with capsaicin, contained in red pepper powder. The study was performed on 30 patients with functional dyspepsia and without gastro-oesophageal reflux disease and irritable bowel syndrome (IBS). After a 2-week washout period, 15 patients received, before meals randomly and in a double-blind manner, 2.5 g/day of red pepper powder for 5 weeks, and 15 patients received placebo. A diary sheet was given to each patient to record, each day, the scores of individual and overall symptom intensity, which subsequently were averaged weekly and over the entire treatment duration. The overall symptom score and the epigastric pain, fullness and nausea scores of the red pepper group were significantly lower than those of the placebo group, starting from the third week of treatment. The decrease reached about 60% at the end of treatment in the red pepper group, whilst placebo scores decreased by less than 30%.

Bortolotti & Porta, 2011: As capsaicin contained in red pepper is able to desensitise the TRPV1 fibres, the authors evaluated whether the red pepper oral administration can decrease the symptoms of visceral hypersensitivity in IBS patients. The study was performed on 50 patients with IBS diagnosed following Rome II criteria. After a 2-week washout period, 23 patients were planned to receive 4 pills/day, for 6 weeks randomly and in a double blind manner, each containing 150 mg of red pepper powder with a coat that dissolves in the colon, and 27 patients placebo. The patients scored each day in a diary the abdominal pain and bloating intensities following the 5-point Likert scale. The weekly symptom mean scores and the final patient subjective evaluation on treatment effectiveness were statistically compared among groups and intra-groups with appropriate tests. Eight patients dropped from the study: 6 in the red pepper group for abdominal pain and 2 in the placebo group. In 8 patients, the pills were reduced to 2/day, because of the abdominal pain at the onset of treatment. The intra-group comparisons showed that in patients taking red pepper the abdominal pain and bloating mean score values of the last weeks of treatment were significantly improved with respect to pre-treatment values, unlike patients taking placebo. The final patient subjective evaluation on the treatment effectiveness showed that red pepper group scored significantly better than placebo.

Gonlachanvit *et al.* 2009: The aim of study was to investigate the effect of chili-containing foods on postprandial GI symptoms in diarrhoea-predominant IBS (IBS-D). Twenty IBS-D patients underwent GI symptoms and postprandial colonic transit evaluations after ingesting three different meals: (i) a standard meal, (ii) a spicy meal (a standard meal mixed with 2 g chili), and (iii) a standard meal with 2 g chili in capsules, in a randomised crossover fashion. Postprandial GI symptoms were scored every 15 min for 2 hours using VAS. Thirty-eight healthy volunteers were used as controls. In healthy volunteers, the spicy meals and meals with chili capsules induced only mild abdominal burning relative to the standard meals ($p < 0.05$), whereas it induced significant levels of abdominal pain and burning in IBS-D patients ($p < 0.05$). Other GI symptoms and postprandial colonic transit after spicy meals and meals with chili capsules did not differ from standard meals in IBS-D and controls ($p > 0.05$). IBS-D patients and controls reported similar oral burning symptoms when eating spicy meals ($p > 0.05$).

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of
Capsicum frutescens L., fructus
EMA/HMPC/674138/2013   Page 42/67

Both the spicy meal and the standard meal with chili capsules led to similar severity of GI symptoms (p>0.05). IBS-D patients exhibited gut hypersensitivity to chili. Chili ingestion produced more abdominal pain and burning in IBS-D patients than in healthy volunteers, but was associated with similar oral burning symptoms.

Gonlachanvit *et al.* 2007: The aim of the study was to test the hypothesis that capsaicin-containing red chili induces rectal hypersensitivity in healthy humans and 5HT-3 receptors participate in this effect. Eighteen healthy volunteers, each underwent three rectal barostat studies under three conditions: (i) oral placebo; (ii) oral chili (5 g daily × 3 days); and (iii) oral chili with 1-mg intravenous (i.v.) granisetron, in randomised, double-blinded, cross-over fashions. Rectal sensation was evaluated by using a 5-point Likert scale. Chili ingestion significantly decreased rectal threshold for first, moderate and severe urgency (18±0.9, 24±1.2, and 38±1.5 mmHg, respectively) compared with placebo (22±0.9, 31±1.3, and 45±1.4 mmHg, respectively, p<0.01). The threshold for first, moderate and severe urgency after chili with i.v. granisetron was 20±0.9, 28±1.2 and 44±1.3 mmHg, respectively. This is a significant increase compared with chili ingestion without gransisetron (p<0.05). After placebo ingestion, i.v. granisetron produced no effect on rectal sensation compared with i.v. placebo in 10 healthy volunteers (p>0.05). The authors concluded that low-dose granisetron, a 5HT-3 receptor antagonist, partially reversed chili-induced rectal hypersensitivity but had no effect on rectal perception induced only by mechanical balloon distention.

Graham *et al.* 1999: The authors investigated whether garlic or capsaicin-containing peppers would actually inhibit *Helicobacter pylori in vivo*. They performed a prospective crossover study in healthy *H. pylori*-infected adults. The urea breath test was used to assess the status of the *H. pylori* infection. On separate days subjects received three test meals consisting of beef, tortillas, and salad with one of the following: fresh garlic (10 sliced cloves), capsaicin (six sliced fresh jalapenos), two tablets of bismuth subsalicylate (positive control), or nothing added (negative control). Breath testing was done before the first meal, the evening meal, and the following morning. At least 2 days elapsed between the test substances. Twelve subjects participated (seven men, five women), with an average age of 41.4 years, range 27-51 years. Ten subjects received garlic, six received jalapenos, and 11 received bismuth. Neither garlic nor capsaicin had any *in vivo* effect on *H. pylori* (median urease activity 28.5 vs. 39.8 and 43.7 vs. 46.6 before and after garlic and jalapenos, respectively) (p>0.8). Bismuth had a marked inhibitory effect (median 55.8 vs. 14.3 before and after bismuth) (p<0.001) , respectively. This study did not support a role for either garlic or jalapenos in the treatment of *H. pylori* infection.

Gupta 2007a: This study was aimed to determine whether there was any relationship between consumption of chillies and postoperative symptoms after closed anal sphincterotomy in patients with chronic anal fissure. Patients were randomly assigned to receive analgesics and fibre supplement alone (control patients) or consumption of 1.5 g chilli powder twice daily along with identical fibre and analgesics (chilli group). The evaluation of symptoms (pain, anal burning, and pruritus) during the postoperative period was assessed by means of patients' self-questionnaires. The amount of analgesic tablets consumed and the frequency of stool during the study period were also noted. Twenty-eight patients were recruited in each arm. Postoperative symptoms were higher in the group consuming chillies during the first postoperative week. The global scores for postoperative pain (7.6 in chilli group and 2.95 in control group, p<0.001) and for anal burning (8.85 for the chilli group vs. 4.21 for the control group, p<0.0001) were significant. The authors concluded that this study showed that consumption of red chillies after anal fissure surgery should be forbidden to avoid postoperative symptoms.

Gupta 2007b: The aim of another study was to determine whether there was any relation between consumption of chilies and postoperative symptoms after haemorrhoidectomy in patients with grade III or IV haemorrhoidal disease. A total of 60 patients were randomly assigned to receive antibiotics and analgesics alone (control patients) or daily consumption of 3 g of chili powder along with identical antibiotics and analgesics (chili group). The evaluation of symptoms-pain, anal burning, pruritus, bleeding-during the postoperative period was assessed by means of patients' self-questionnaires. A global score for evaluating each postoperative symptom was compared between the two groups at the 1-week follow-up. No significant difference in age, sex distribution, or grade of disease was noted between the two groups at baseline. The incidence of post-haemorrhoidectomy symptoms was higher in the group consuming chilies during the first postoperative week. The global score for postoperative pain (14.6 for the chili group vs. 7.97 for the control group, $p<0.001$) and for anal burning (12.9 for the chili group vs. 7.82 for the control group, $p<0.0001$) were significant. Although bleeding (6.95 in the control group and 7.57 in the chili group, $p<0.81$) and pruritus (8.06 in the control group and 8.75 in the chili group, $p<0.69$) were more common in the chili group, the difference did not achieve statistical significance. This study showed that consumption of 3 g of red chilies per day during the postoperative period after haemorrhoidectomy increases the intensity of typical postoperative symptoms, stool frequency, and the consumption of analgesics.

Gupta 2008: Another study was aimed to determine if consumption of chilies increases symptoms of acute anal fissures. Individual patients were randomised to receive capsules containing chili or placebo for one week in addition to analgesics and fibre supplement. Patients were asked to note score for symptoms like pain, anal burning, and pruritus during the study period. After one week, cross over treatment was administered to the same group of patients with the same methodology and results were noted at the end of two weeks. Fifty subjects were recruited for this study. Forty-three of them completed the trial (22 in the chili group and 21 in the placebo group). The daily mean pain score was significantly lower in the placebo group in the study period. [Score 2.05 in chili group and 0.97 in placebo group, $p<0.001$]. There was a significant burning sensation experienced by the patients in the chili group (score 1.85 for the chili group vs. 0.71 for the placebo group, $p<0.001$). Patient's mean recorded improvement score was significantly higher after taking placebo. 81.3% patients preferred placebo while 13.9% preferred chilies. Two patients had no preference. The authors conclude that consumption of chili does increase the symptoms of acute anal fissure and reduces patient compliance.

Kang *et al.* 1995 (only abstract available): The aim of the present study was to determine the frequency and amount of chili taken by peptic ulcer patients and control subjects. One hundred-three Chinese patients with peptic ulcer and 87 control patients were interviewed using a standard questionnaire. Those subjects who deliberately avoided chili use because of symptoms or advice from friends or medical practitioners were excluded. The median number of times of chili use per month was eight in the ulcer group. (25-75% quartiles 1-30) compared to 24 (8-56) in the control group ($p<0.001$). The median amount of chili used per month was 312 units (25-75% quartiles 38-899) in the ulcer group compared to 834 units (274-1892) in the control group ($p<0.001$). The odds ratio of having peptic ulcer disease, adjusted for age, sex, analgesic use, and smoking by multiple logistic regression, was 0.47 (95% confidence intervals (CIs): 0.25-0.89) for subjects who had a higher intake of chili both in terms of frequency as well as amount used compared to those who took less chili. The authors concluded that the data supported the hypothesis that chili use has a protective effect against peptic ulcer disease.

Kim *et al.* 2002: The authors studied a non-pharmacological therapy of postoperative nausea and vomiting (PONV)-capsicum plaster (PAS)-at either the Korean hand acupuncture point K-D2 or the Chinese acupuncture point Pericardium 6 (P6) of both hands.

Medication: plaster 12.2 x 16.4 cm, containing 345.8 mg capsicum powder and 34.58 mg capsicum tincture (no more details), no information regarding to the content of total capsaicinoids. One hundred-sixty healthy patients were included in a randomised, double-blinded study: 60 patients were in the control group, 50 patients were in the K-D2 group, and 50 patients were in the P6 group. PAS was applied at the K-D2 point in the K-D2 group and at the P6 point in the P6 group, whereas in the control group, an inactive tape was fixed at the K-D2 point of both hands. The PAS was applied before the induction of anaesthesia and removed at 8 hours after surgery. The incidence of PONV and the need for rescue medication were evaluated at predetermined time intervals. In the treatment group, the incidence of vomiting was significantly less (22% for the K-D2 group and 26% for the P6 group) than in the control group (56.7%) at 24 hours after surgery ($p < 0.001$). The need for rescue antiemetics was significantly less in the treatment groups compared with the control group ($p < 0.001$).

Kim & Nam, 2006: The authors designed this double-blind, sham-controlled study to assess the effectiveness of capsicum plaster (PAS) at Zusanli (ST-36) acupoints on postoperative opioid analgesic requirement, side effects, and recovery profile. Medication: plaster 12.2 x 16.4 cm, containing 345.8 mg capsicum powder and 34.58 mg capsicum tincture (no more details), no information regarding to the content of total capsaicinoids. Ninety women undergoing total abdominal hysterectomy were randomly assigned to 3 treatment regimens (n=30 each): group Zusanli = PAS at Zusanli acupoints, group sham = PAS at the nonacupoints on the shoulders, and group control = placebo tape at Zusanli acupoints. The PAS was applied before induction of anaesthesia and maintained for 8 hours per day for 3 postoperative days. The total amount of morphine administered in the first 24 hours after the operation was significantly decreased in group Zusanli (31.5 ± 6.8 ml) compared with groups control (44.3 ± 10.1 ml) and sham (44.6 ± 10.4 ml) ($p < 0.01$). The incidence of postoperative side effects and the use of rescue antiemetics during the 72 hours after surgery were significantly reduced in group Zusanli compared with other groups ($p < 0.01$).

Misra *et al.* 2005: The clinical study was performed in order to compare the efficacy of stimulation of P6 acupoint with capsicum plaster in comparison with iv ondansetron for the prevention of postoperative nausea and vomiting (PONV). One hundred-twenty patients of either sex, ASA I-II, undergoing elective middle ear surgeries under general anaesthesia were included in this randomised, prospective, double-blinded and placebo-controlled study. The anaesthetic technique was standardised. Patients were divided into three groups. Group I was the control group. Capsicum plaster (1 × 1 cm, no further information available) was affixed at the P6 acupoint on both forearms 30 min before induction of anaesthesia in patients of Group II. Patients of Groups I and III received an inactive adhesive plaster at the same site. Ondansetron 4 mg iv was given to patients of Group III at the end of surgery and the rest of the patients received a placebo. The plasters were removed 6 hours after transferring the patients to the postoperative unit. Criteria were fixed for the administration of rescue antiemetics (ondansetron 4 mg iv). PONV and the requirement for rescue antiemetics were recorded by a blinded observer. The incidence of PONV and the requirement for rescue antiemetics were significantly lower in both the acustimulation and ondansetron groups at 6 hours. At 24 hours there was a reduction in the requirement for rescue medication in the ondansetron group.

Kosuwon *et al.* 2010: Capsicum tincture in a gel, corresponding to 0.0125% capsaicins per 100 g gel.

The authors aimed to evaluate the efficacy of 0.0125% capsaicin gel containing a capsicum tincture (no information regarding extraction solvent and DER) compared to a placebo (vehicle gel) in patients with symptomatic knee osteoarthritis (OA). This was a cross-over, double blinded, randomised, controlled trial of 100 patients with mild to moderate knee OA. All of the patients received either capsaicin gel or placebo gel applied to the affected knee, 3 times daily for 4 weeks with 1 week washout period after which the treatment switched to either capsaicin gel or placebo gel for the next 4

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of
Capsicum frutescens L., fructus
EMA/HMPC/674138/2013   Page 45/67

weeks. A blinded examiner used the VAS and WOMAC score (Western Ontario and McMaster Universities Arthritis Index) to do weekly assessments. Subjects averaged 61 years of age (range 44 to 82). During the enrolment phase, only female farmers presented. Mean body weight and height was 62.97 ± 10.25 kg and 1.54 ± 0.053 m, respectively the respective baseline VAS and WOMAC score was 6.4 ± 1.64 and 51.65 ± 13.3. The severity of OA, according to the Kellgren-Lawrence Classification (KL criteria) was: 83 patients with grade 2 and 16 with grade 3. The respective mean difference of VAS and total WOMAC score in the capsaicin group vs. the placebo group was statistically significant ($p<0.05$). The mean difference of the WOMAC pain, stiffness and functional subscales in the capsaicin vs. the placebo group was also significant ($p<0.05$). The only adverse event reported was a burning sensation. During the 4-week treatment with capsaicin, approximately 67% of patients had a burning sensation but none withdrew for this reason. The authors concluded that 0.0125% capsaicin gel was an effective treatment in mildly to moderately painful OA knees.

Loeser *et al.* 2012: The authors report the case of a 39-years-old female patient who suffered from trigeminal neuralgia of the left lingual nerve for 6 years. The previous therapy according to the guidelines including a Jannetta operation was unsuccessful. Only after beginning with daily mastication and consumption of very hot chilli peppers has the patient become reliably pain-free.

Park *et al.* 2004: In a randomised, double-blind, sham-controlled study, the authors compared the efficacy of capsicum plaster (PAS) applied at the Korean hand acupuncture point for the prevention of postoperative sore throat in 150 patients scheduled to undergo abdominal hysterectomy. Medication: plaster 12.2 x 16.4 cm, containing 345.8 mg capsicum powder and 34.58 mg capsicum tincture (no more details), no information regarding to the content of total capsaicinoids. Patients were assigned to a verum group (K group), a sham group and a placebo group. The K group had PAS applied at the K-A20 of both hands and placebo tape at non-acupoints at both lateral thighs, the sham group placebo tapes at the hand acupuncture points and capsicum plaster at non-acupoints at both lateral thigh. Patients in the placebo group received placebo tapes only. The PAS was applied prior to induction of anaesthesia and removed 8 hours postoperatively. The sore throat scores of Group K were significantly lower than those of other groups at 24 hours following surgery ($p=0.00027$). The prevalence of moderate to severe sore throat at 24 hours was lower for Group K (0%) than for sham and placebo controls (16% [$p=0.038$] and 19% [$p=0.032$], respectively). There were no differences in the recovery room of the sore throat scores for all groups.

Yeoh *et al.* 1995 (only abstract available): Eighteen healthy volunteers with normal index endoscopies underwent two studies four weeks apart. Each subject took 20 g chili orally with 200 ml water in one study and 200 ml water in another study. In each case this was followed half an hour later by 600 mg aspirin BP with 200 ml water. Endoscopy was repeated 6 hours later. Gastroduodenal mucosal damage was assessed by a previously validated scoring system. The median gastric injury score after chili was 1.5 compared to 4 in the control group ($p<0.05$), suggesting a gastroprotective effect of chili in human subjects.

Clinical trials with isolated capsaicin (in part)

Pain-related indications

Altman *et al.* 1994: 113 patients received either topical capsaicin (0.025%) or vehicle in a 12-week, double-blind, randomised multicentre study. Patients included suffered on primary or secondary osteoarthritis of the knee, ankle, elbow, wrist or shoulder. According to visual analogue scales capsaicin was superior compared to placebo.

Aasvang *et al.* 2008: A single-centre, randomised, double-blind, placebo-controlled study of the analgesic efficacy of a single intraoperative wound instillation of 1000 μg ultra-purified capsaicin after open mesh groin hernia repair in 41 adult male patients was performed. The primary end-point was average daily visual analogue scale (VAS) pain scores during the first week after surgery assessed as area under the curve (AUC). Pain was recorded twice daily in a pain diary for 4 weeks. Physical examination and laboratory tests were done before and 1 week after surgery, together with recordings of adverse events up to 28 days. AEs were recorded. Data were also analysed using a mixed-effects analysis with NONMEM. VAS AUC was significantly lower during the first 3 days postoperatively (p<0.05), but not for the whole 1 or 4 weeks postoperatively. Mixed-effects analysis with NONMEM revealed that pain scores were significantly lower (p<0.05) in the capsaicin group during the first 4 days. No clinically significant serious adverse events were observed, although a mild transient increase in liver enzymes was seen more often in the capsaicin-treated group.

Bernstein *et al.* 1989: In a double-blind study 32 elderly patients with chronic postherpetic neuralgia were treated with either capsaicin cream (0.075%) or vehicle for a 6-week period. Response to treatment was evaluated by VAS of pain and of pain relief, together with changes in a categorical pain scale and in a physician's global evaluation. Significantly greater relief in the capsaicin-treated group compared with vehicle was observed for all efficacy variables.

Biesbroeck *et al.* 1995: An 8-week double-blind, multicentre, parallel study compared the safety and efficacy of topical capsaicin (0.075%) and oral amitriptyline in patients with painful diabetic neuropathy involving the feet. Two-hundred-thirty-five patients were randomised to treatment with either capsaicin cream or amitriptyline capsules. Capsaicin-treated patients received inactive capsules, and amitriptyline-treated patients applied vehicle cream. A visual analogue scale of pain (VAS) intensity and measurements of interference by pain with functional activities were recorded at onset and at 2-week intervals. A VAS relief and physician's global evaluation assessed changes in pain status from baseline. Topical capsaicin and oral amitriptyline produced equal and statistically significant improvements in pain over the course of the study. By the end of week 8, 76% of patients in each group experienced less pain, with a mean reduction in intensity of more than 40%. By the end of the study, the interference with daily activities by pain had diminished significantly (p=0.001) in both groups, including improvements in sleeping and walking. No systemic side effects were observed in patients treated with topical capsaicin. Most patients receiving amitriptyline experienced at least one systemic side effect, ranging from somnolence (46%) to neuromuscular (23%) and cardiovascular (9%) adverse effects.

Capsaicin Study group 1991: A multicentre study was conducted to establish the efficacy of topical 0.075% capsaicin cream in relieving the pain associated with diabetic neuropathy. Capsaicin or vehicle cream was applied to painful areas four times per day for 8 weeks in patients randomly assigned to one of two groups. Pain intensity and relief were recorded at 2-week intervals using physician's global evaluation and VAS. Analysis at final visit for 252 patients showed statistical significance favouring capsaicin compared with vehicle for the following: 69.5% vs. 53.4% pain improvement by the physician's global evaluation scale, 38.1% vs. 27.4% decrease in pain intensity, and 58.4% vs. 45.3% improvement in pain relief. With the exception of transient burning, sneezing, and coughing, capsaicin was well tolerated. Study results suggested - in the opinion of the authors - that topical capsaicin cream is safe and effective in treating painful diabetic neuropathy.

Capsaicin Study group 1992: The authors tried to establish the effects of topically applied capsaicin on daily activities in patients with painful diabetic neuropathy (PDN). Investigators at 12 sites enrolled 277 men and women with painful peripheral polyneuropathy and/or radiculopathy in an 8-weeks double-blind vehicle-controlled study with parallel randomised treatment assignments.

Participants were unresponsive or intolerant to conventional therapy and were experiencing pain that interfered with functional activities and/or sleep. Either 0.075% capsaicin cream or vehicle cream was applied to the painful areas 4 times/day. A VAS of pain intensity and baseline measurements of the pain's interference with the ability to walk, work, participate in recreational activities, use shoes and socks, sleep, and eat were recorded at onset and at 2-weeks intervals. A physician's global evaluation scale assessed changes in pain status from baseline. Statistically significant differences are percentage of patients with improvement in favour of capsaicin versus vehicle: 69.5 vs. 53.4% with clinical improvement in pain status (p=0.012), 26.1 vs. 14.6% with improvement in walking (p=0.029), 18.3 vs. 9.2% with improvement in working (p=0.019), 29.5 vs. 20.3% with improvement in sleeping (p=0.036), and 22.8 vs. 12.1% with improvement in participating in recreational activities (p=0.037). The results from this study suggested - in the opinion of the authors - that topical 0.075% capsaicin is effective for reducing pain in patients with PDN with subsequent improvement in daily activities, enhancing the quality of the patient's life.

Cho *et al.* 2012: The objective of this study was to evaluate the efficacy of a hydrogel patch containing capsaicin 0.1% compared with a placebo hydrogel patch without capsaicin to treat chronic myofascial neck pain. The study was designed as a double-blinded randomised controlled trial. Sixty-one participants between 18 and 65 years with at least 3 months duration of neck pain and a clinical presentation of myofascial pain syndrome were enrolled in the study from September 1 to November 20, 2010. Participants received capsaicin 0.1% hydrogel patches or control hydrogel patches without capsaicin according to the randomisation scheme. All participants were instructed to apply one patch to each side of the neck and shoulder girdle overlying the point of maximal pain for 12 hours daily during the duration of the 4-week study. Each participant completed five surveys at baseline, at 2 weeks after the start of treatments, and at the conclusion of the 4-week study. The primary outcome measure was VAS. Other outcome measures included the Neck Disability Index (NDI), Beck's Depression inventory (BDI), Short Form 36 Korean version, and Euroqol 5-D. Fifty-seven patients completed the study. The mean VAS, NDI, and BDI scores were significantly decreased at 2 and 4 weeks after the start of the intervention in both groups. There was no significant difference between the two groups in any of the outcome measures.

Deal *et al.* 1991: In this double-blind randomised study, 70 patients with osteoarthritis (OA) and 31 with rheumatoid arthritis (RA) received capsaicin or placebo for four weeks. The patients were instructed to apply 0.025% capsaicin cream or its vehicle (placebo) to painful knees four times daily. Pain relief was assessed using VAS for pain and relief, a categorical pain scale, and physicians' global evaluations. Most of the patients continued to receive concomitant arthritis medications. Significantly more relief of pain was reported by the capsaicin-treated patients than the placebo patients throughout the study; after four weeks of capsaicin treatment, RA and OA patients demonstrated mean reductions in pain of 57% and 33%, respectively. These reductions in pain were statistically significant compared with those reported with placebo (p=0.003 and p=0.033, respectively). According to the global evaluations, 80% of the capsaicin-treated patients experienced a reduction in pain after two weeks of treatment. Transient burning was felt at the sites of drug application by 23 of the 52 capsaicin-treated patients; two patients withdrew from treatment because of this side effect.

Dini *et al.* 1993: Twenty-one patients with post-mastectomy pain syndrome (PMPS) were entered in an open-label trial of topical 0.025% capsaicin treatment (3 daily applications for 2 months). Out of 19 evaluable patients, 2 (10.5%) reported complete disappearance of all symptoms, and 11 (57.9%) had a reduction of pain which was never worse than mild at the end of treatment. Three months after cessation of treatment, 11 of 13 of the responding patients continued to have good pain relief, with only 1 continuing to use capsaicin. Treatment was well tolerated with no drop-out due to side effects.

Ellison *et al.* 1997: Ninety-nine assessable cancer patients with postsurgical neuropathic pain were entered into this study. After stratification, patients were to receive 8 weeks of a 0.075% capsaicin cream followed by 8 weeks of an identical-appearing placebo cream, or vice versa. A capsaicin/placebo cream was to be applied to the painful site four times daily. Treatment evaluation was performed by patient-completed weekly questionnaires. During the first 8-week study period, the capsaicin-cream arm was associated with substantially more skin burning, skin redness, and coughing (p<0.0001 for each. The capsaicin cream arm had substantially more pain relief (p=0.01) after the first 8 weeks, with an average pain reduction of 53% versus 17%. On completion of the 16-week study period, patients were asked which treatment period was most beneficial. Of the responding patients, 60% chose the capsaicin arm, 18% chose the placebo arm, and 22% chose neither (p=0.001).

Forst *et al.* 2002: The aim of this pilot study was to investigate the impact of topical capsaicin application on small nerve fibre function and neurovascular control. Capsaicin cream was applied to the feet of 13 patients with symptomatic diabetic neuropathy over a period of 8 weeks. Before and during the treatment period, the total symptoms score, the vibration, thermal (heat and cold) and pain perception thresholds, and the neurovascular responses to heat and acetylcholine stimuli were investigated. In addition, the serum plasma levels of substance P, a neurotransmitter of nociceptor C-fibres, were measured. A significant improvement in total symptoms score was observed during topical capsaicin treatment (18.3±3.2 vs. 14.3±3.3; p<0.05). An improvement in the heat perception threshold was also found (12.7±0.4 degrees C vs. 11.4 ±0.7 degrees C: p<0.05), while other sensory nerve fibre functions remained unchanged. No significant change in neurovascular control was observed, either after mild thermal injury or after stimulation with acetylcholine. Serum substance P levels increased after initiation of topical capsaicin treatment (72.9±5.8 pg/ml vs. 81.7±5.0 pg/ml; p<0.05), but returned to baseline levels during further treatment (77.4±8.3 pg/ml: n.s.).

Fusco & Alessandri, 1992: Twelve informed and consenting patients were studied to determine the influence of capsaicin on trigeminal neuralgia. All of these patients had idiopathic trigeminal neuralgia. These patients were followed up for 1 year after the topical application over the painful area of 1 g of a cream containing 0.05% capsaicin three times a day for several days. Six patients had complete and four patients had partial relief of pain; the remaining two patients had no relief of pain. Of the 10 patients who were responsive to therapy, four had relapses of pain in 95-149 days. There were no relapses following the second therapy for the remainder of the year.

Lazzeri *et al.* 1996: A randomised placebo controlled study was done to evaluate intravesical capsaicin for severe bladder pain. Follow up was 6 months. A total of 36 patients were prospectively randomised into those receiving 10 µM intravesical capsaicin twice weekly for 1 month (group 1) or placebo (group 2). All patients had pelvic pain for at least 6 months, and had no urinary tract infection within the last 3 months, functional disorders of the lower urinary tract, or other vesical or urethral pathology. Pre-treatment voiding pattern and pain score were recorded. Patients were evaluated immediately at the end of treatment (primary end point) and 6 months later (secondary end point). Both groups were adequately homogeneous with regard to age, sex ratio, duration of disease, voiding pattern and pain score. At both end points group 1 had significant improvement in frequency and nocturia but no improvement in urgency. No change was noted in group 2. A significant decrease in pain score was found in group 1 at the primary (mean plus or minus standard deviation 3.22±0.42, p<0.01) and secondary (3.83±0.47, p<0.01) end points compared to before treatment (5.61±0.40, chi-square with 2 degrees of freedom 29.25, p<0.0001). A significant improvement was also observed in the placebo group, in which the pre-treatment pain score (5.47±0.37) was decreased at the primary (4.47±0.36, p<0.01) and secondary (4.48±0.34, p<0.01, chi-square with 2 degrees of freedom 12.71, p<0.002) end points. There were no statistically significant differences between the 2 groups.

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of
Capsicum frutescens L., fructus
EMA/HMPC/674138/2013   Page 49/67

Low *et al.* 1995: The authors performed a 12-week double-blind, placebo-controlled randomised study on the efficacy of the application of capsaicin cream (0.075%) in the treatment of chronic distal painful polyneuropathy. Forty patients were enrolled and 39 completed the study. The 2 limbs were randomly assigned to capsaicin or placebo. The cream was applied 4 times a day. The first tube contained the active placebo, methyl nicotinate. In the final 4 weeks (single-blind wash-out phase), placebo was administered bilaterally. Efficacy was evaluated using the following scales: (1) investigator global, (2) patient global, (3) visual analogue (VAS) of pain severity, (4) VAS of pain relief, (5) activities of daily living, and (6) allodynia. Patients were examined at onset and at monthly intervals using a neurologic disability scale, nerve conduction studies, computer-assisted sensory examination for vibration and thermal cooling and warming, quantitative sudomotor axon reflex test and quantitative flare response. There was no statistical evidence of efficacy of capsaicin cream over placebo for any of the pain indices. At early time points (1-4 weeks), there were a small number of indices that favoured the placebo. The percent of limbs that improved on the investigator's global scale were 51.3 vs. 53.8 at 4 weeks, 56.4 vs. 64.1 at 8 weeks and 59 vs. 66.7 at 12 weeks for capsaicin vs. placebo; no statistically significant difference was found. All the safety indices showed no difference between sides.

Mathias 1995: In an open-labelled prospective pilot study 23 patients with chronic neck pain received topically capsaicin cream (0.025%) 4 times daily for 5 weeks. The primary outcome variables 'visual analogue pain scale' and 'pain relief scale' improved statistically significantly.

McCarthy & McCarty, 1992: Topical capsaicin 0.075% was evaluated for the treatment of the painful joints of rheumatoid arthritis (RA) and osteoarthritis (OA) in a 4 week double blind, placebo controlled randomised trial. Twenty-one patients were selected, all of whom had either RA (n=7) or OA (n=14) with painful involvement of the hands. Assessments of pain (VAS), functional capacity, morning stiffness, grip strength, joint swelling and tenderness (dolorimeter) were performed before randomisation. Treatment was applied to each painful hand joint 4 times daily with reassessment at 1, 2 and 4 weeks after entry. One subject did not complete the study. Capsaicin reduced tenderness (p less than 0.02) and pain (p less than 0.02) associated with OA, but not RA as compared with placebo. A local burning sensation was the only adverse effect noted. These findings suggested – in the opinion of the authors -that topical capsaicin was a safe and potentially useful drug for the treatment of painful OA of the hands.

McCleane 2000a: The aim of this study was to assess if the pain of OA is reduced by topical capsaicin and to determine whether addition of glyceryl trinitrate has an effect on analgesic efficacy and tolerability of capsaicin. A randomised, double-blind, placebo controlled study was carried out on 200 adult patients attending a Pain Clinic with osteoarthritis pain. Patients applied one of four creams topically over the affected joint over a 6 week period. Creams contained either placebo (vehicle), 0.025% capsaicin, 1.33% glyceryl trinitrate or 0.025% capsaicin + 1.33% glyceryl trinitrate. Analgesic efficacy, tolerability of cream and analgesic consumption were assessed. One hundred sixty-seven out of 200 patients completed the study. Baseline visual analogue scores (0-10 scale) for pain was 6.40. There was a significant reduction in pain scores in the glyceryl trinitrate group (mean decrease 0.59, $p<0.05$, 95% confidence limits 0.04-1.14), 0.025% capsaicin group (mean decrease 0.5, $p<0.05$, 95% confidence limits 0.05-1.05) and the glyceryl trinitrate capsaicin group (mean decrease 1.1, $p<0.05$, 95% confidence limits 0.22-1.98). Baseline discomfort of application scores were similar for all but the capsaicin groups (they were significantly higher (by 2.1 units, $p<0.001$)). The odds ratio in favour of continuing treatment was 2.1 (95% confidence limits 1-4.4) for glyceryl trinitrate and 2.4 (95% confidence limits 1.2-5.1) for capsaicin and 5.0 (95% confidence limits 3.8-6.4) for capsaicin glyceryl trinitrate combination.

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of
Capsicum frutescens L., fructus
EMA/HMPC/674138/2013   Page 50/67

The study showed that topical capsaicin and glyceryl trinitrate have an analgesic effect in painful osteoarthritis. When used together this effect is increased with the combination being more tolerable than capsaicin alone.

McCleane 2000b: The author assessed the analgesic effect of topical administration of 3.3% doxepin hydrochloride, 0.025% capsaicin and a combination in patients with chronic neuropathic pain. A randomised, double-blind, placebo controlled study was carried out on 200 adult patients for 4 weeks. Overall pain was significantly reduced by doxepin, capsaicin and the combination to similar extent. The analgesia had a more rapid onset with the combination.

Paice *et al.* 2000: Distal symmetrical peripheral neuropathy (DSPN) is a particularly distressing pain syndrome associated with HIV disease. This multicentre controlled, randomised, double-masked clinical trial studied patients with HIV-associated DSPN and compared measures of pain intensity, pain relief, sensory perception, quality of life, mood, and function for patients who received topical capsaicin (0.075%) to the corresponding measures for patients who received the vehicle only. Twenty-six subjects were enrolled in the study. At the end of 1 week, subjects receiving capsaicin tended to report higher current pain scores than did subjects receiving the vehicle (Mann-Whitney test; p=0.042). The dropout rate was higher for the capsaicin group (67%) than for the vehicle group (18%) (chi 2 test of association; p=0.014). There were no other statistically significant differences between the capsaicin and vehicle groups with respect to current pain, worst pain, pain relief, sensory perception, quality of life, mood, or function at study entry or at any time during the 4-week trial. These results suggest capsaicin is ineffective in relieving pain associated with HIV-associated DSPN.

Peikert *et al.* 1991: In order to evaluate the efficacy, time-course of action and predictors of response to topical capsaicin, 39 patients with chronic post-herpetic neuralgia (PHN), median duration 24 months, were treated with 0.025% capsaicin cream for 8 weeks. During therapy the patients rated their pain on a VAS and a verbal outcome scale. A follow-up investigation was performed 10-12 months after study onset on the patients who had improved. Nineteen patients (48.7%) substantially improved after the 8-week trial; 5 (12.8%) discontinued therapy due to side-effects such as intolerable capsaicin-induced burning sensations (4) or mastitis (1); 15 (38.5%) reported no benefit. The decrease in VAS ratings was significant after 2 weeks of continuous application. Of the responders 72.2% were still improved at the follow-up; only one-third of them had continued application irregularly. Treatment effect was not dependent on patients' age, duration or localisation of PHN (trigeminal involvement was excluded), sensory disturbance or pain character. Treatment response was not correlated with the incidence, time-course or severity of capsaicin-induced burning.

Scheffler *et al.* 1991: An 8-week, double-blind, vehicle-controlled study was conducted to determine the effectiveness of topical capsaicin 0.075% cream in relieving pain associated with diabetic neuropathy. Fifty-four patients were selected who experienced moderate to very severe pain, which interfered with sleep or activities on a daily basis, and who were unresponsive or intolerant to conventional therapy. The results after 8 weeks showed a statistically significant difference in favour of the capsaicin-treated patients, with 90% of these patients improved. According to the authors, the results of this study indicated that topical capsaicin 0.075% cream is safe and effective in managing painful diabetic neuropathy.

Tandan *et al.* 1992b: The authors conducted an 8-week controlled study with topical 0.075% capsaicin in subjects with chronic severe PDN who were unresponsive or intolerant to conventional therapy. In 22 randomly assigned subjects, either capsaicin or vehicle cream was applied to painful areas 4 times/day. Pain measurements were recorded at baseline and at 2-weeks intervals for 8 weeks.

Capsaicin treatment was more beneficial than vehicle treatment in the overall clinical improvement of pain status, as measured by physician's global evaluation (p=0.038) and by a categorical pain severity scale (p=0.057). Decrease in mean pain intensity by a VAS was 16% in capsaicin-treated and 4.1% in vehicle-treated subjects. Mean pain relief on VAS was 44.6 and 23.2%, respectively. In a follow-up open-label study, approximately 50% of subjects reported improved pain control or were cured, and 25% each were unchanged or worse. A burning sensation at the application site was noted by some subjects but both its magnitude and duration decreased with time.

Watson & Evans, 1992: This paper describes a randomised parallel trial of topical 0.075% capsaicin versus vehicle (placebo) in the post-mastectomy pain syndrome (PMPS). The study was double-blind in design; however, this was compromised by the burning sensation induced by capsaicin.
The authors could not demonstrate a significant difference in the VAS for steady pain although a trend was present. A significant difference was found, however, in the VAS for jabbing pain, in category pain severity scales, and in overall pain relief scales in favour of capsaicin. Five of 13 patients on capsaicin were categorised as good-to-excellent responses with 8 (62%) having 50% or greater improvement. Only 1 of 10 cases had a good response to vehicle with 3 rated as 50% or better.

Watson *et al.* 1993: A double-blind, vehicle-controlled study of 143 patients with chronic postherpetic neuralgia (PHN) was performed to evaluate the degree of efficacy of topically applied capsaicin 0.075% cream. In addition, the safety and efficacy of long-term application of topical capsaicin in PHN was assessed by following patients in an open-label study for up to 2 years. In the double-blind phase, 143 patients with PHN of 6 months' duration or longer were enrolled. Since epidemiologic studies of patients who received no treatment have shown that only 10% to 25% of those with PHN after 1 month will still have pain at 1 year, two separate efficacy analyses were performed: one with all evaluable patients (n=131) and the other with 93 patients whose PHN lasted for longer than 12 months prior to study start up. All efficacy variables, including the physician's global evaluation of reduction in PHN pain, changes in pain severity on the categorical scale, VAS for pain severity, VAS for pain relief, and functional capacity scale, showed significant improvement at nearly all time points throughout the study for both patient groups, based on duration of PHN pain. In contrast, the group receiving vehicle cream remained essentially unchanged. Data from the long-term, open-label phase (up to 2 years, n=77), which immediately followed the 6-week blinded phase, showed that the clinical benefit in patients treated for a short (6-week) period with topical capsaicin could be maintained or amplified in most patients (86%) during prolonged therapy. There were no serious adverse effects observed or reported throughout the trial; in fact, the only side effect associated with capsaicin treatment was the burning or stinging at local sites of application (in 9% of patients) during exposures of up to 2 years (long-term phase).

Winocur *et al.* 2000 (only abstract available): The authors aimed to determine the effectiveness of topical capsaicin cream application on localised pain in the temporomandibular joint (TMJ) area.
A randomised, double-blind, placebo-controlled study was conducted on 30 patients suffering from unilateral pain in the TMJ area. Patients were randomly divided into experimental and placebo groups; they were instructed to apply 0.025% capsaicin cream or its vehicle to the painful TMJ area 4 times daily for 4 weeks. Subjective parameters of present pain, most severe pain, effect of pain on daily activities, and pain relief were assessed each week on a VAS. Muscle and joint sensitivity to palpation on the painful and contralateral joints and maximal mouth opening (assisted/passive and non-assisted/active) were examined weekly by the same experienced examiner. Capsaicin cream produced no statistically significant influence on measured variables when compared to placebo. Both experimental and placebo groups showed statistically significant improvement in most variables during the experiment.

Migraine, headache

Cianchetti 2010: The authors attempted to verify whether topical periarterial capsaicin could ameliorate pain in absence of and during a migraine attack. On 23 migraineurs showing pain at pressure on scalp arteries, topical capsaicin 0.1% or Vaseline jelly was administered on painful arteries in absence of migraine attack. In those having pain reduction > 50%, the same comparison was made during a migraine attack. Topical capsaicin caused > 50% reduction of arterial pain in absence of attack in 17/23 patients, as opposed to two with Vaseline. During attacks of mild to moderate intensity, > 50% improvement was obtained in 11/17 with capsaicin and in one with Vaseline.

Marks *et al.* 1993: The authors attempted to determine whether capsaicin is effective in aborting cluster headache (CH) attacks. Patients in acute cluster were randomised to receive either capsaicin or placebo in the ipsilateral nostril for 7 days. Patients recorded the severity of each headache for 15 days. Headaches on days 8-15 of the study were significantly less severe in the capsaicin group vs. the placebo group. There was also a significant decrease in headache severity in the capsaicin group on days 8-15 compared to days 1-7, but not in the placebo group. Episodic CH patients appeared to benefit more than chronic CH patients.

Pruritus

Ellis *et al.* 1993: Safety and efficacy of topical capsaicin were evaluated in patients with pruritic psoriasis. Patients applied capsaicin 0.025% cream (n=98) or vehicle (n=99) four times a day for 6 weeks in this double-blind study. Efficacy was based on a physician's global evaluation and a combined psoriasis severity score including scaling, thickness, erythema, and pruritus. Capsaicin-treated patients demonstrated significantly greater improvement in global evaluation (p=0.024 after 4 weeks and p=0.03 after 6 weeks) and in pruritus relief (p=0.002 and p=0.06, respectively), as well as a significantly greater reduction in combined psoriasis severity scores (p=0.03 and p=0.036, respectively). The most frequently reported side effect in both treatment groups was a transient burning sensation at application sites.

Lysy *et al.* 2003: This clinical trial was performed using capsicin in patients with pruritus ani. Firstly, a pilot open study was carried out on five patients to establish which of two doses was the most acceptable by comparing effectiveness and side effects. Secondly, a double blind, placebo controlled, crossover study of topical capsaicin was performed. This study involved two four week treatment phases separated by a one week washout phase. Forty-four patients were randomised to receive locally either active capsaicin (0.006%) or placebo (menthol 1%) ointment over a four week period (22 patients per group). After four weeks of treatment and a one week washout period, the placebo group began to receive capsaicin while the treated group received placebo (menthol 1%) for another four weeks. At the end of the controlled study, responders from both groups continued with capsaicin treatment in an open labelled manner. Thirty-one of 44 patients experienced relief during capsaicin treatment periods and did not respond to menthol; all patients not responding to capsaicin also failed on menthol (p<0.0001). In 13 patients, treatment with capsaicin was unsuccessful: 8 patients did not respond to capsaicin treatment, one responded equally to capsaicin and placebo, and four others dropped out because of side effects. During the follow up period (mean 10.9 (SD 5.8) months), 29 "responders" needed a mean application of capsaicin every day (1.6 (SD 1.2); range 0.5-7 days) to remain symptom free (or nearly symptom free).

Makhlough *et al.* 2010: The study aimed to evaluate the therapeutic effect of capsaicin on pruritus, compared with placebo, in patients on haemodialysis (HD). This randomised double-blinded cross-over clinical trial was performed on 34 patients on HD with uremic pruritus. The patients were divided into 2 groups, one group received capsaicin ointment (0.03%) and the other, placebo, for 4 weeks.

Treatment was stopped for 2 weeks as washout period and continued as a cross-over technique. Pruritus scores were analysed and compared. Thirty-four patients on long-term HD, 14 men and 20 women with a mean age of 57.0±18.6 years were studied. The mean of pruritus score before capsaicin treatment was 15.9±6.3, which was reduced to 6.4±3.9, 4.7±3.1, 3.2±2.9, and 2.5±2.5 on weeks 1 to 4, respectively (p<0.001). In the placebo group, pruritus score before treatment was 15±6 on average, and it was 11.7±5.8, 9.4±5.9, 7.9±5.5, and 7.2±5.5, respectively, on weeks 1 to 4 (p<0.001). There was no significant difference in pruritus scores before the treatment between the two groups, but after each week, the difference was significant (p<0.001). Repeated measurement test showed that decreasing in pruritus severity in the capsaicin group was more than that in the placebo group during treatment period (p<0.001).

Ständer *et al.* 2001: The aim of this concentration- and regimen-ranging study was to evaluate the efficacy, safety, and practicability of capsaicin in the topical treatment of prurigo nodularis in a large series of patients. A total of 33 patients with prurigo nodularis of various origins were selected to receive capsaicin (0.025% to 0.3%) 4 to 6 times daily for 2 weeks up to 10 months. The consecutive follow-up period was up to 6 months. In 7 patients, skin biopsy specimens were taken before, during, and after therapy and investigated histologically, immunohistochemically, and ultra-structurally. All 33 patients could be evaluated for efficacy. After cessation of the symptoms of neurogenic inflammation, such as burning sensations or erythema, all of them experienced a complete elimination of pruritus within 12 days. In addition, capsaicin largely contributed to the gradual healing of the skin lesions. After discontinuation of the therapy, pruritus returned in 16 of 33 patients within 2 months. At the ultrastructural level, no degenerative changes of cutaneous nerves could be found during or after capsaicin therapy. Depletion of substance P was demonstrated by confocal laser scanning microscopy thus confirming the specific effect of capsaicin *in vivo*.

Szeimies *et al.* 1994: The authors reported a case of successful treatment of recalcitrant pruritus after infusion therapy with hydroxyethyl starch. The previous attempts to treat the pruritus with antihistaminics, other antipruritic agents, UVB irradiation and neuroleptic drugs were ineffective. Topical capsaicin (0.05%) twice daily relieved the symptoms without side-effects.

Tarng *et al.* 1996: This study aimed to assess the efficacy and safety of capsaicin 0.025% cream in the treatment of haemodialysis (HD)-related pruritus and to further explore the underlying path mechanism. Nineteen HD patients with idiopathic, moderate (n=5) to severe (n=14) pruritus were examined in a double-blind, placebo-controlled, crossover study and 17 of them completed the study. Topical agent of capsaicin or placebo base cream was applied to localised areas of pruritus 4 times a day. The severity of pruritus and treatment-related side effects (cutaneous burning/stinging sensations, dryness, or erythema) were evaluated weekly. The results showed (1) that 14 of 17 patients reported marked relief and 5 of these 14 patients had complete remission of pruritus during capsaicin treatment (Wilcoxon signed-ranks test, 2p<0.001); (2) capsaicin was significantly more effective than placebo (Mann-Whitney rank sum test, 2p<0.001) and a prolonged antipruritic effect was observed 8 weeks post treatment; (3) no serious side effects were noted during the study and (4) there were no significant changes in serum concentrations of albumin, calcium, phosphorus, alkaline phosphatase, or intact parathyroid hormone during the treatment with either capsaicin or placebo.

Weisshaar *et al.* 2000: The authors investigated the antipruritic effect of topical capsaicin on serotonin-induced reactions in 10 healthy volunteers in comparison to untreated skin (UPS) and placebo substance (vehicle)-treated skin (VS). On the first day, serotonin iontophoresis was performed in untreated skin. One week later, the treatments started, using either capsaicin 0.05% liniment or a placebo liniment (vehicle) 3 times daily over a 5-day period. On day 6, serotonin was applied by iontophoresis within the pretreated skin.

After another 1-week break, the treatments were performed vice versa on the corresponding infrascapular region. Weal and flare areas were planimetrically evaluated. Itch sensations were documented by the volunteer on a scale over a 24 min follow-up period. The examination also comprised alloknesis, which stands for induction of perifocal sensations by usually non-itching stimuli. In capsaicin-treated skin, serotonin-induced wheals were significantly larger post-application compared to non-pretreated skin. Wheals were significantly larger in VS than in UPS. Comparison of serotonin-induced flares in the different study arms did not reveal any significant differences. Itch sensations were not significantly reduced by topical capsaicin application. The areas of alloknesis were smaller in capsaicin-treated skin compared to VS and UPS, but did not reach significant value. In conclusion, topical capsaicin application was not effective in serotonin-induced itching in healthy volunteers. According to the authors, serotonin is most unlikely to play a role in the mechanism of action of capsaicin.

Weisshaar *et al.* 2003: Eleven pruritic patients on hemodialysis (HD) and ten controls were treated with capsaicin 0.05% liniment on the upper back three times daily for 5 days. Study parameters to be investigated were wheal and flare reactions, itch and alloknesis (perifocal itch sensation induced by usually non-itching stimuli) after serotonin and histamine iontophoresis in treated and untreated skin. There were no significant differences in any parameter before and after HD. In both groups, itching was not significantly reduced by capsaicin compared to untreated skin. Itching, however, was significantly lower in capsaicin-pretreated patients when compared to controls. In the opinion of the authors, topical capsaicin showed some antipruritic potency in HD patients in this experimental model and may therefore be considered as a co-medication in HD patients.

Other indications

Harada & Okajima, 2007: The cutaneous application of 0.01% capsaicin to faces of 17 healthy female volunteers for seven days increased the cheek skin elasticity.

De Stefano *et al.* 1997 (only abstract available): In a preliminary study 0.025% topically capsaicin cream, daily administered showed after a cold-induced vasospasm an improvement of the temperature recovery of the acral cutaneous blood flow. According to the authors, the findings suggested a role for topical capsaicin in the therapy of Raynaud's phenomenon.

Führer *et al.* 2011: A total of 116 outpatients with upper GI symptoms participated in this double-blind, placebo-controlled trial of which 73 patients received a final diagnosis of functional dyspepsia. Patients were administered a capsule containing 0.75 mg capsaicin or placebo. A graded questionnaire evaluated the severity of nine upper GI symptoms before and after capsule ingestion and an aggregate symptom score was calculated. A final score of >9 was considered as a positive test. In functional dyspepsia, median perception scores were 10.8 (interquartile range: 4.5-18.8) after ingestion of capsaicin and 0.5 (0-2.5) after placebo (p<0.001). Thirty-seven functional dyspepsia patients (54%) had a positive test after capsaicin ingestion, whereas only four (11%) patients with upper GI symptoms but without functional dyspepsia were capsaicin positive [median perception score: 1.5 (0-5.0)]. After placebo, symptom scores were low and not significantly different among patient groups (p>0.05). Clinical characteristics, age, and gender distribution was similar in capsaicin positive and capsaicin negative functional dyspepsia patients (p>0.05). The value of patient blinding was good.

Muhiddin *et al.* 1994: The authors presented a case report of the effective use of a capsaicin cream (0.025%) in chronic, previously not sufficiently curable erythromyalgia in a 68 years old lady.

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of
Capsicum frutescens L., fructus
EMA/HMPC/674138/2013   Page 55/67

Clinical trials with high concentration dermal patches

Backonja *et al.* 2008: NGX-4010, a high-concentration (8%) capsaicin dermal patch, was developed to treat patients with neuropathic pain. The authors reported the results of a randomised, double blind, 12-week study of the efficacy and safety of one application of NGX-4010 in patients with postherpetic neuralgia (PHN). In this multicentre, double-blind, parallel-group trial, 402 patients were randomly assigned to one 60 min application of NGX-4010 (640 µg/cm^2 [8% capsaicin]) or a low-concentration capsaicin control patch (3.2 µg/cm^2 [0.04% capsaicin]). Patients were aged 18-90 years, had had PHN for at least 6 months, and had an average baseline numeric pain rating scale (NPRS) score of 3 to 9. The primary efficacy endpoint was percentage change in NPRS score from baseline to weeks 2-8. Analysis was by intention to treat. Patients who were randomly assigned to NGX-4010 (n=206) had a significantly greater reduction in pain during weeks two to eight than did patients who had the control patch (n=196). The mean changes in NPRS score were -29.6%vs -19.9% (difference -9.7%, 95% CI -15.47 to -3.95; p=0.001). 87 (42%) patients who received NGX-4010 and 63 (32%) controls had a 30% or greater reduction in mean NPRS score (odds ratio [OR] 1.56, 95% CI 1.03 to 2.37; p=0.03). The patients who had NGX-4010 had significant improvements in pain during weeks two to 12 (mean change in NPRS score -29.9% vs. -20.4%, difference -9.5, -15.39 to -3.61; p=0.002). Transient blood pressure changes associated with changes in pain level were recorded on the day of treatment, and short-lasting erythema and pain at the site of application were common, self-limited, and generally mild to moderate in the NGX-4010 group and less frequent and severe in the controls. Interpretation by the authors: one 60 min application of NGX-4010 provided rapid and sustained pain relief in patients with PHN. No adverse events were associated with treatment except for local reactions at the site of application and those related to treatment-associated pain.

Backonja *et al.* 2010: The authors tried to assess the efficacy, tolerability, and safety of NGX-4010, a high-concentration capsaicin dermal patch (capsaicin 640 µg/cm^2, 8%) in patients with PHN. Patients were randomised to receive NGX-4010 or a low-concentration capsaicin control patch (3.2 µg/cm^2 [0.04% capsaicin]) in a 4-week, double-blind study. This was followed by an open-label extension phase (up to 48 weeks total) where patients could receive up to three additional treatments no sooner than 12 weeks after initial treatment. The primary efficacy variable was mean change from baseline in mean morning and evening NPRS scores. During days 8-28 after the double-blind treatment, NGX-4010 patients had a mean change in NPRS scores from baseline of -32.7% compared with -4.4% for control patients (p=0.003). Mean NPRS scores decreased from baseline during week 1 in both treatment groups, remained relatively stable through week 12 in NXG-4010 patients, but returned to near baseline during weeks 2-4 in controls. Mean change in NPRS scores from baseline during weeks 2-12 was -33.8% for NGX-4010 and +4.9% for control recipients. A similar decrease in NPRS scores from baseline was maintained with subsequent NGX-4010 treatments, regardless of the number of treatments received. Transient increases in application site pain were adequately managed with analgesics. No increases in application site reactions or adverse events were observed with repeated treatments. No patients discontinued the study due to an adverse event.

Irving *et al.* 2011: The authors tried to confirm the efficacy, tolerability, and safety of NGX-4010, an 8% capsaicin dermal patch (capsaicin 640 µg/cm^2), in patients with PHN. A total of 418 patients were randomised to receive a single 60 min application of NGX-4010 or a 0.04% capsaicin control patch (3.2 µg/cm^2) in a multicentre, double-blind, confirmatory, phase 3 study. Patients were 18-90 years old with a diagnosis of PHN, pain for at least 6 months, and an average baseline NPRS score of 3-9. Outcome measures: the primary efficacy end point was the percentage change in NPRS score from baseline to weeks 2-8. NGX-4010 recipients had a significantly greater mean reduction from baseline in pain during weeks 2-8 compared with the control group (32% vs. 24.4%; p=0.011).

A ≥ 30% reduction in mean NPRS scores was achieved in 46% of NGX-4010 recipients compared with 34% of controls (p=0.02). Pain was significantly lower in NGX-4010 recipients than controls by week 2, and greater pain reduction was maintained throughout the remaining 12-week study period. Most treatment-emergent adverse events were application site specific (notably erythema and pain), transient, and generally mild to moderate in severity. The authors concluded that in patients with PHN, a single 60 min application of NGX-4010 produced significant reduction in pain that was maintained over a 12-week period.

Irving *et al.* 2012: The objective was the analyses of integrated data from 4 controlled PHN studies evaluated the effect of NGX-4010, a capsaicin 8% patch, administered alone or together with systemic neuropathic pain medications. Patients recorded their "average pain for the past 24 hours" daily for 12 weeks using an 11-point NPRS. Efficacy assessment included the percentage NPRS score reduction from baseline during weeks 2 to 8 and 2 to 12, the proportion of patients responding during weeks 2 to 8 and 2 to 12 and the Patient Global Impression of Change (PGIC) at weeks 8 and 12. During the studies, 302 NGX-4010 and 250 control (capsaicin, 0.04% wt/wt) patients were using at least one systemic neuropathic pain medication; 295 NGX-4010 and 280 control patients were not. During weeks 2 to 8, NGX-4010 patients reported greater reductions in NPRS scores compared with control both in patients using systemic neuropathic pain medications (26.1% vs. 18.1%, p=0.0011) and in patients not using these medications (36.5% vs. 26.2%, p=0.0002). Patients not using systemic neuropathic pain medications reported a greater reduction in pain compared with patients using these medications in both, NGX-4010 and control groups, resulting in comparable treatment differences between NGX-4010 and control regardless of systemic neuropathic pain medication use. Similar results were seen during weeks 2 to 12, for the responder and PGIC analyses. Transient, capsaicin-related application site reactions were the most common adverse events and not affected by systemic neuropathic pain medication use. The authors concluded that a single 60 min NGX-4010 treatment reduced PHN for up to 12 weeks regardless of concomitant systemic neuropathic pain medication use.

Simpson *et al.* 2008: The authors reported a placebo-controlled study of a high-concentration capsaicin dermal patch (NGX-4010) for the treatment of painful HIV-distal sensory polyneuropathy (HIV-DSP). This double-blind multicentre study randomised 307 patients with painful HIV-DSP to receive NGX-4010 or control, a low-concentration capsaicin patch. After application of a topical anaesthetic, NGX-4010 or control was applied once for 30, 60, or 90 min to painful areas on the feet. The primary efficacy endpoint was percent change in NPRS from baseline in mean "average pain for past 24 hours" scores from weeks 2 to 12. A single NGX-4010 application resulted in a mean pain reduction of 22.8% during weeks 2 to 12 as compared to a 10.7% reduction for controls (p=0.0026). Following a transient treatment-related pain increase, pain was reduced; significant improvement was apparent by week 2 and continued throughout the controlled 12-week observation period. Mean pain reductions in the NGX-4010 30-, 60 and 90 min groups were 27.7%, 15.9%, and 24.7% (p=0.0007, 0.287, and 0.0046 vs. control). One third of NGX-4010-treated patients reported >or=30% pain decrease from baseline compared to 18% of controls (p=0.0092). Self-limited, mild-to-moderate local skin reactions were commonly observed. The authors concluded that a single NGX-4010 application was safe and provided at least 12 weeks of pain reduction in patients with HIV-associated distal sensory polyneuropathy.

Webster *et al.* 2010a: This multicentre, double-blind, controlled study randomised 299 postherpetic neuralgia (PHN) patients to receive either NGX-4010, a high-concentration capsaicin (8%) patch, or a low-concentration capsaicin (0.04%) control patch for 30, 60, or 90 minutes. The mean percent reductions in NPRS score from baseline to weeks 2 through 8 were significantly greater in the total NGX-4010 group (26.5%, p=0.0286) and the 90 min NGX-4010 group (27.8%, p=0.0438) compared

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of
Capsicum frutescens L., fructus
EMA/HMPC/674138/2013   Page 57/67

to the pooled control group (17.3%). After review of the data suggested a difference between genders in reporting of pain scores and a higher proportion of males (61%) in the 60 min NGX-4010 group, post hoc gender-stratified analyses were performed and showed that the 60 min NGX-4010 group also had a significantly larger mean percent reduction in average pain scores (28%, p=0.0331). Pain reduction in the 30 min NGX-4010 group, although similar in magnitude to the other doses, was not significantly different from control in either of these analyses. Similar results were observed during weeks 2 through 12. Most treatment-emergent adverse events were application-site specific, transient and mostly mild to moderate in severity.

Webster *et al.* 2010b: The authors evaluated the safety and efficacy of a single 60 min application of NGX-4010, an 8% capsaicin patch, in patients with PHN. This multicentre, double-blind, controlled study randomised 155 patients 2:1 to receive either NGX-4010 or a 0.04% capsaicin control patch. Patients were at least 18 years old with PHN for at least 3 months, and an average Numeric Pain Rating Scale (NPRS) score of 3 to 9. The primary efficacy endpoint was the percentage change in NPRS score from baseline to weeks 2-8. The mean percent reduction in "average pain for the past 24 hours" NPRS scores from baseline to weeks 2-8 was greater in the NGX-4010 group (36.5%) compared with control (29.9%) although the difference was not significant (p=0.296). PGIC analysis demonstrated that more NGX-4010 recipients considered themselves improved (much, or very much) compared with control at weeks 8 and 12, but the differences did not reach statistical significance. Post hoc analyses of patients with PHN for at least 6 months showed significantly greater reductions in "average pain for the past 24 hours" NPRS scores from baseline to weeks 2-8 in NGX-4010 patients compared to controls (37.6% vs. 23.4%; p=0.0291). PGIC analysis in this subgroup demonstrated that significantly more NGX-4010 recipients considered themselves much or very much improved compared with control at week 12 (40% vs. 20%; p=0.0403). The authors concluded that although treatment appeared to be safe and well tolerated, a single 60 min application of NGX-4010 failed to show efficacy in this study which included patients with PHN for less than 6 months. Large reductions in pain observed among control patients with pain for less than 6 months may have been due to spontaneous resolution of PHN, may have confounded the results of the pre-specified analyses.

Webster *et al.* 2011: The authors tried to assess efficacy, safety, and tolerability of NGX-4010, capsaicin 8% patch, in patients with peripheral neuropathic pain. This open-label, uncontrolled, 12-week study enrolled 25 patients with postherpetic neuralgia (PHN), one with HIV-distal sensory polyneuropathy, and 91 with painful diabetic neuropathy (PDN). Patients received pre-treatment with one of three 4% lidocaine topical anaesthetics followed by a single 60 or 90 min NGX-4010 application. The primary efficacy variable was the percentage change in Numeric Pain Rating Scale scores from baseline to Weeks 2-12. Adverse events (AEs), laboratory parameters, vital signs, neurosensory examinations, dermal assessments, treatment-related pain scores, and medication use for treatment-related pain were collected. PDN and PHN patients achieved a 31% and 28% mean pain decrease from baseline during Weeks 2-12, respectively, and 47% and 44%, respectively, were responders (≥30% pain decrease). Mild or moderate treatment-site-related burning and pain were the most common AEs and there was no evidence of impaired neurosensory function.

Review articles

Derry & Moore, 2012:

Objectives: To review the evidence from controlled trials on the efficacy and tolerability of topically applied low-concentration (< 1%) capsaicin in chronic neuropathic pain in adults. Search strategy: Cochrane CENTRAL, MEDLINE, EMBASE and Oxford Pain Relief Database, searched to July 2012.

Main results: Six studies (389 participants in total) compared regular application of low dose (0.075%) capsaicin cream with placebo cream. The study medication was in all cases synthetic capsaicin.

Authors' conclusions: There were insufficient data to draw any conclusions about the efficacy of low-concentration capsaicin cream in the treatment of neuropathic pain.

Rapporteur's comment: the authors compared results from studies with patients suffering on neuropathic pain of very heterogeneous origin (Cancer, postherpetic neuralgia, post-mastectomy pain syndrome). The outcome of the review is of minor relevance for the HMPC monograph.

Derry *et al.* 2013: Objectives: To review the evidence from controlled trials on the efficacy and tolerability of topically applied high-concentration (8%) capsaicin in chronic neuropathic pain in adults. Main results: Six studies were included, involving 2073 participants. Four studies involved 1272 participants with postherpetic neuralgia. All efficacy outcomes were significantly better than control. At both 8 and 12 weeks there was a significant benefit for high-concentration over low-concentration topical capsaicin for participants reporting themselves to be much or very much better, with point estimates of the numbers needed to treat (NNTs) of 8.8 (95% confidence interval (CI) 5.3 to 26) and 7.0 (95% CI 4.6 to 15) respectively. More participants had average 2 to 8-week and 2 to 12-week pain intensity reductions over baseline of at least 30% and at least 50% with active treatment than control, with NNT values between 10 and 12. Two studies involved 801 participants with painful HIV neuropathy. In a single study the NNT at 12 weeks for participants to be much or very much better was 5.8 (95% CI 3.8 to 12). Over both studies more participants had average 2 to 12-week pain intensity reductions over baseline of at least 30% with active treatment than control, with an NNT of 11.

Authors' conclusions: High-concentration topical capsaicin used to treat postherpetic neuralgia and HIV-neuropathy generates more participants with high levels of pain relief than does control treatment using a much lower concentration of capsaicin.

Gagnier *et al.* 2007: Objectives: To determine the effectiveness of herbal medicine compared with placebo, no intervention, or "standard/accepted/conventional treatments" for nonspecific low back pain.

Results: Ten trials were included in this review. Two high-quality trials utilising *Harpagophytum procumbens* (Devil's claw) found strong evidence for short-term improvements in pain and rescue medication for daily doses standardised to 50 mg or 100 mg harpagoside with another high-quality trial demonstrating relative equivalence to 12.5 mg per day of rofecoxib. Two moderate-quality trials utilising *Salix alba* (White willow bark) found moderate evidence for short-term improvements in pain and rescue medication for daily doses standardised to 120 mg or 240 mg salicin with an additional trial demonstrating relative equivalence to 12.5 mg per day of rofecoxib. Three low-quality trials using *Capsicum frutescens* (Cayenne) using various topical preparations (including the above cited trials by Keitel *et al.* 2001, Frerick *et al.* 2003 and a trial by Ginsberg & Famaey, 1987 [reference not available]) found moderate evidence for favourable results against placebo and one trial found equivalence to a homeopathic ointment.

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of *Capsicum frutescens* L., fructus
EMA/HMPC/674138/2013

Page 59/67

Authors' conclusions: *Harpagophytum procumbens, Salix alba*, and *Capsicum frutescens* seem to reduce pain more than placebo. Additional trials testing these herbal medicines against standard treatments will clarify their equivalence in terms of efficacy.

Gagnier 2010: Objective: The objective of this paper was to review and summarise the evidence surrounding natural health products for chronic non-specific low back pain. Results: The author included two systematic reviews and 2 additional randomised controlled trials published subsequently to these reviews. The authors found strong evidence for 50 mg harpagoside per dose of an aqueous extract of *Harpagophytum procumbens* per day reduces pain more than placebo. The authors found moderate evidence for 100 mg harpagoside per dose of an aqueous extract of *Harpagophytum procumbens* compared to placebo, for an extract of willow bark yielding 120 mg salicin per day compared with placebo, for 240 mg of salicin per day in reducing pain to a greater extent than placebo, for 240 mg of salicin per day as equivalent to 120 mg salicin, for no differences in pain and function between a 60 mg daily harpagoside dose of an aqueous extract of *Harpagophytum procumbens* and 12.5 mg rofecoxib per day, for no difference in pain and overall improvement between Spiroflor SRL homeopathic gel and Cremor Capsici Compositus FNA, the capsici oleoresin gel, for intramuscular B12 when compared with placebo. The authors found limited evidence for topical *Capsicum frutescens* in the form of cream or a Capsicum plaster for reducing pain more than placebo, for lavender oil in the treatment of chronic NSLBP, or vitamin C, zinc, and manganese in addition to prolotherapy.

Assessor's comments to Gagnier 2007 and 2010: The only clinical trial considered by the authors which is relevant for the monograph is the trial by Keitel et al. 2001. The study of Frerick 2003 was only considered in the 2007 review, where the conclusions were more promising for Capsicum compared to 2010. Both clinical trials were in the opinion of the authors of high methodological quality.

Gooding *et al.* 2010: Systematic review of topical capsaicin in the treatment of pruritus: 6 randomised controlled trials were included. The authors concluded that there is no convincing evidence for the use of capsaicin to treat pruritus in any medical condition.

Hautkappe *et al.* 1998: To determine the effectiveness of capsaicin for painful cutaneous disorders and neural dysfunction, the authors analysed data from 33 reports (MEDLINE search of 1966-96) on the efficacy of capsaicin. Outcome measures consisted of the response rate and degree of pain relief. Results from placebo-controlled trials were pooled when possible; effect of treatment was estimated by the method of DerSimonian and Laird. Pain relief for post-mastectomy syndrome and cluster headache was greater with capsaicin than with placebo; also, psoriasis and pruritus responded better to capsaicin. Uncontrolled studies and case reports have indicated that pain or dysfunction was less at the end of capsaicin therapy for neck pain, loin pain/haematuria syndrome, oral mucositis, rhinopathy, reflex sympathetic dystrophy syndrome, detrusor hyperreflexia, and cutaneous pain due to tumour of the skin.

Authors' conclusions: Capsaicin is effective for psoriasis, pruritus, and cluster headache; it is often helpful for the itching and pain of post-mastectomy pain syndrome, oral mucositis, cutaneous allergy, loin pain/haematuria syndrome, neck pain, amputation stump pain, and skin tumour; and it may be beneficial for neural dysfunction (detrusor hyperreflexia, reflex sympathetic dystrophy, and rhinopathy). A universal problem for many of the studies analysed was the absence of a "burning placebo" such as camphor.

Mason 2004: The author reviewed the data from clinical trials using topical capsaicin in different types of pain.

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of
Capsicum frutescens L., fructus
EMA/HMPC/674138/2013   Page 60/67

Authors' conclusions: compared with placebo, treatment of neuropathic pain with 0.075% capsaicin for 8 weeks would benefit one additional patient for every 6 treated; compared with placebo, treatment of musculoskeletal pain with 0.025% capsaicin for 4 weeks would benefit one additional patient for every 8 treated.

McCormack 2010: Review of the clinical data regarding the use of a dermal patch containing 8% capsaicin in patients with peripheral neuropathic pain. The 30 min application of the patch was significantly better than control for the reduction from baseline in mean Numeric Pain Rating Scale scores during weeks 2-12. The efficacy was maintained for up to 1 year.

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

Herbal preparations mentioned in the monograph

No data available. As no data on efficacy and safety for children and adolescents are available the use is not recommended in this age group.

<u>Other herbal preparations</u>

Kim *et al.* 2006: The authors investigated the postoperative analgesic efficacy of capsicum plaster (containing Capsicum powder and a tincture, total capsaicin content 0.046%) at Zusanli (ST-36) points after paediatric hernia repair. This double-blind, sham-controlled study was designed in 108 children, aged 4 month to 9 years, undergoing unilateral hernia repair, and was randomly assigned to three treatment regimens: group Zusanli (Z) = capsicum plaster at Zusanli acupoints and placebo tape on the shoulder as a non-acupoint, group Sham (S) = capsicum plaster on the shoulders and placebo tape at Zusanli acupoints, and group control (C) = placebo tape at Zusanli acupoints and on the shoulder. The postoperative pain scores and analgesic requirements during 24 hours postoperatively were assessed. Total meperidine consumption was significantly lower in group Z (0.87 ± 0.35 mg/kg) compared with group C (1.27 ± 0.41 mg/kg) and S (1.22 ± 0.45 mg/kg) ($p < 0.001$). The pain scores on both the objective pain scale and the Children Hospital of Ontario Pain Scale, were significantly lower in group Z compared with the other groups at 6 and 24 hours postoperatively, but not at the 10 min and 1 hour postoperative time periods.

4.4. Overall conclusions on clinical pharmacology and efficacy

Data from clinical trials with medicated plasters as well as with semi-solid dosage forms indicate some efficacy of herbal preparations containing a defined amount of capsaicinoids in indications related to muscular pain. In considerably higher concentrations pure synthetic capsaicin is used as cutaneous patch also for the treatment of neuropathic pain.
Following the general regulatory discussions on capsaicin containing medicinal products there is a common understanding that applications for new medicinal products cannot be based on a bibliographic or generic approach alone. The clinical efficacy of locally applied capsaicin (semi-solid dosage forms, medicated plasters) depends in a high degree on the excipients used. Therefore a bridging of clinical data from a certain medicinal product to another one without supporting data may not be acceptable from a regulatory point of view. Data on pharmaceutical as well as clinical equivalence might be necessary in order to show the relevance of the literature data for the product concerned.
Capsaicinoids are considered to be responsible for the clinical efficacy. Therefore finished products have to be standardised to a certain content of capsaicinoids. As for standardised herbal preparations the extraction solvent and the DER are of less importance, well-established use is also proposed for herbal preparations not tested in clinical trials.

5. Clinical Safety/Pharmacovigilance

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

Data relevant for the monograph

No interactions studies have been performed.

Other data

Lee & Ryan, 2003: The authors performed a double-blind, randomised, controlled, pilot study comparing the effect of magnesium-aluminium-hydroxide-simeticone suspension (MgAl) with that of saline in the treatment of dermal capsaicin exposures. Ten volunteers were sprayed with a commercial defensive spray containing 10% capsaicin on the flexor surface of both forearms. A dressing embedded with MgAl suspension was randomly applied to one arm and a saline-embedded dressing was applied to the other arm. Pain was assessed on a 10-cm visual analog scaleVAS at 0, 10, 20, 30, 60, 90, and 120 minutes. Mean pain scores were significantly lower in the MgAl group as compared with the saline group at 10, 20, and 30 minutes. Differences in pain scores were not statistically significant at times 60, 90, and 120 minutes.

Rapporteur's comment: although this clinical trial does not contribute to the assessment of the safety and efficacy of capsicum-containing herbal medicines the results indicate a useful method if capsicum-containing herbal medicines are overdosed.

Published SmPCs

In approved SmPCs of medicated plasters and semisolid dosage forms containing herbal preparations of Capsicum the following interactions are mentioned:

> The plaster/ointment is not intended to be applied at the same time as other topical products [e.g. other rubefacients (which increase the perfusion and cause a reddening of the skin) or pain relieving gels] at the same application site.

> Interactions with other products applied at the same application site may even occur up to 12 hours after the plaster has been removed.

> These statements cannot be substantiated by published data. However, as this is mentioned in the product information for many medicinal products containing capsicum preparations or capsaicin this wording is also taken for the monograph.

Data on dietary use of chili pepper

Archer & Jones, 2002: There are four cookeries in the United States that are noted for their high pepper content: Mexican-American, Cajun, white Creole, and black Creole. Each is largely confined to a single ethnic-cultural group which is concentrated in some counties. By use of county population and mortality data, significantly higher rates for stomach and liver cancer were found in counties inhabited by these four ethnic-cultural groups than in matched control counties. This involved both sexes. The cancer increase was dependent on the concentration of these groups in a county. These results strengthen and extend an earlier case-control study which found odds ratios above 5 for the stomach cancer association with capsaicin pepper. It is further evidence that capsaicin is a human carcinogen.

Mathew *et al.* 2000: A prospective case-control study was conducted in Trivandrum, India, to evaluate the dietary risk factors for stomach cancer. One hundred ninety- four patients with stomach cancer

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of
Capsicum frutescens L., fructus
EMA/HMPC/674138/2013
Page 62/67

registered at the Regional Cancer Centre (RCC), Trivandrum, Kerala, India, during the period 1988-1991 were considered as cases. A minimum of one control (n=305), matched for age (±5 years), sex, religion and residential area was selected from the visitors to RCC during the same period. Interviews were carried out using a predetermined structured food frequency questionnaire. The information collected also included socio-demographic/economic background, tobacco chewing, tobacco smoking and alcohol habits. Data were analysed using a multiple logistic regression model. Odds ratios for all dietary variables were estimated. Increased risks were observed with higher consumption of rice (OR 3.9; 95% CI 1.6-10). Risk was high for those consuming spicy food (OR 2.3; 95% CI 1.1- 5), high consumption of chilli (OR 7.4; 95% CI 4 - 13.5) and consumption of high-temperature food (OR 7; 95% CI 3.7-12.9). On multivariate analysis, high consumption of rice, high consumption of chilli and consumption of high- temperature food were found to be independent risk factors.

Milke *et al.* 2006: Although the ingestion of chilli has been associated with gastroesophageal reflux (GER) symptoms, there are no studies that have explored the effect of a chronic ingestion of different kinds of chilli with a variable content of capsaicin as a cause of GER. The effect of chilli on oesophageal 24 hours pH monitoring was studied in 12 healthy subjects without GER symptoms before and after of ingestion one of two kinds of chilli. Patients were randomised to ingest 3 g daily of cascabel chilli (*Capsicum annum* coraciforme containing 880 ppm of capsaicin) or ancho chilli (*Capsicum annum* grossum containing 488 ppm of capsaicin). After chilli ingestion, the Johnson De Meester Index (JDI) increased significantly [basal: 7 (1-14), after chilli: 13 (2-69), p=0.0047]. When considering both kinds of chilli separately, the JDI varied, although non significantly with the ancho chilli [basal: 3 (1-8), after chilli: 10 (2-69), p=0.11], and significantly with the cascabel chilli [basal: 10 (5-14), after chilli: 18 (2-44), p=0.028]. The results suggest that the chronic ingestion of chilli induces GER, and that the magnitude of the induced reflux seems to be related to the kind of chilli.

Myers *et al.* 1987 (only abstract available): The authors assessed the effects of red and black pepper on the gastric mucosa using double-blind intragastric administration of test meals containing red pepper (0.1-1.5 g) or black pepper (1.5 g) to healthy human volunteers; aspirin (655 mg) and distilled water were used as positive and negative controls, respectively. Serial gastric washes were performed after test meal administration and gastric contents were analysed for DNA, pepsin, blood, sodium, potassium, parietal cell secretion, and non-parietal cell secretion. Both red pepper and black pepper caused significant increases in parietal secretion, pepsin secretion, and potassium loss. Gastric cell exfoliation (as reflected in DNA loss into gastric contents) was increased after red or black pepper administration; the increase after red pepper administration was dose dependent. Mucosal micro-bleeding was seen after spice administration and one subject had grossly visible gastric bleeding after both red pepper and black pepper administration. There were no significant differences from control between the test meals, in non-parietal volume, fractional recovery of the gastric secretions, or sodium secretion. Finally, no spice was significantly different from aspirin in any parameter studied; indeed, aspirin was comparable to the higher doses of pepper. The long-term result of daily pepper ingestion is unknown. Whether spices are detrimental, beneficial (e.g., inducing an adaptive cytoprotective response), or have no significant long-term effect on the gastric mucosa is unknown and deserves further study.

Nakadaira *et al.* 2009: The authors investigated whether or not an association between chili pepper consumption and gallbladder cancer (GBC) in the presence of gallstones was present in Hungary, where mortality from GBC is high and chili peppers are frequently consumed. In a case-control study, the authors compared 41 female GBC patients with gallstones (GS) and 30 gender and GS-matched hospital controls. Trained staff interviewed all subjects to determine socioeconomic status, family

history, past history and life style habits (smoking, alcohol intake, dietary habits and elimination habits).

Because mean ages differed significantly between the case and control groups, age-adjusted odds ratios (ORs) were calculated. A shorter education period (< 10 years/≥16 years) was indicated to be a risk factor (age-adjusted OR (95% CI): 3.2 (1.2-8.7)). In addition, the intake of Hungarian hot pepper (yes/no) was found to be significantly higher in the GBC cases than in controls (age-adjusted OR (95% CI): 8.4 (2.3-30.4)). There were no differences between the case and control groups for other variables. Multivariate logistic regression analysis retained only Hungarian hot pepper consumption as a significant independent risk factor for GBC. It's age-adjusted OR was 16.2 (95%CI: 2.1-126.2), while there were no differences associated with low education, frequent consumption of fresh fruit and vegetables, low socioeconomic status or smoking. Hungarian hot pepper consumption was identified as a risk factor for GBC by multivariate logistic regression analysis.

Solanke 1973 (only abstract available): The effect of red pepper suspension on gastric acid secretion was studied in patients with proven duodenal ulcer and those admitted to hospital with diagnoses other than duodenal ulceration. Two forms of the red pepper suspension were used-one form was the ordinary red pepper suspension (A), and the other was the pH adjusted suspension (B). The patients were divided into Groups (A) and (B). Each group had both duodenal and non-duodenal patients. Distilled water was used as a control in each patient. The instillation of the suspension through a nasogastric tube eliminated the burning effect of the red pepper suspension and the admixture of saliva with the test suspension. There was a significant increase in gastric acid secretion after treatment with red pepper in both groups of patients, more especially in duodenal ulcer patients. The results of this study lend support to the idea that the consumption of red pepper leads to increased gastric acid production which may aggravate the symptoms of duodenal ulceration. The amount of red pepper used in this study was minute when compared with the amount added to meals in some countries.

5.2. Patient exposure

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

5.3. Adverse events and serious adverse events and deaths

Adverse events reported from clinical trials

Chrubasik *et al.* 2010: Three patients out of 140 in the treatment group suffered from ADRs with causal relationship to the study medication in a way that they left the study: unpleasant local heat sensation (2 patients), pruritus (1 patient). In general heat sensation was recognised by approximately 80% of the patients in the treatment group, pruritus was reported by about 15%. Skin irritation was observed in 2 patients after 3 weeks (1.4%).

Frerick *et al.* 2003: Out of 160 patients in treatment group 12 patients terminated prematurely the study due to excessively severe heat sensations after application of the medicated plaster. Local erythema under the plaster was recorded in 68% of the patients in the treatment group after 3 weeks.

Keitel *et al.* 2001: Out of 74 patients in treatment group 15 reported local sensation of warmth and itching. More severe reactions were recorded in five cases: inflammatory contact eczema, urticaria, minute haemorrhagic spots, vesiculation or dermatitis.

Case reports

Meneghini & Angelini, 1979: Case report: a man (37 years old) developed an allergic contact dermatitis after several days of administration of an anti-rheumatic cream. Patch testing revealed *Capsicum* (no more details) as the only relevant constituent of the cream.

Patanè *et al.* 2009: Case report of arterial hypertensive crisis and acute myocardial infarction in a 59-year-old Italian man with high levels of thyroid stimulating hormone and with an abundant ingestion of chili peppers (no further information available) which occurred on the day before.

Patanè *et al.* 2010: Case report of an arterial hypertensive crisis in a 19-year-old Italian man with an abundant ingestion of peppers and of chili peppers (no further information available) the preceding day.

Raccagni *et al.* 1995: Case report of an erythema-multiforme-like contact dermatitis due to topical application of a home-made Capsicum tincture (no further details).

Sayin *et al.* 2012: Case report of a myocardial infarction in a 41-year-old man who took Capsicum pills (no further details available) for weight loss.

Bley *et al.* 2012 (review): The authors are of the opinion that the postulated link between capsaicin consumption and stomach or gall bladder cancer deserves a very critical re-evaluation, because the respective epidemiological studies were small and capsaicin-containing food may be contaminated by known carcinogens. Moreover studies with high-purity capsaicin revealed a low genotoxic and carcinogenic potential.

Published SmPCs

In approved SmPC of medicated plasters and semisolid dosage forms the following adverse events are mentioned:

> The active ingredient causes increased local blood circulation with marked reddening of the skin and a sensation of warmth. This reaction is part of the normal pharmacological action of the herbal preparation.

> Skin hypersensitivity and allergic reactions (e.g. urticaria, blisters or vesiculation at the application site) may occur. The frequency is not known. The treatment is to be stopped in such cases immediately.

> If, in individual cases, burning sensation or stinging or itching are experienced as excessive, treatment should be discontinued.

> For medicated plasters also the frequency 'rare (~1 /10,000 to <1/1,000) is mentioned for these side effects. This statement cannot be substantiated by published data. However, as this is mentioned in the product information for many medicinal products containing capsicum preparations or capsaicin this wording is also taken for the monograph.

As the clinical trials were performed over 3 weeks and there is some evidence that the rate of adverse events increases with the duration of the treatment a break of at least 2 weeks is advised after 3 weeks of continuous use of *Capsicum* preparations.

5.4. Laboratory findings

No data available.

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of
Capsicum frutescens L., fructus
EMA/HMPC/674138/2013
Page 65/67

5.5. Safety in special populations and situations

<u>Herbal preparations:</u> No data available.

The use in children and adolescents is not considered as safety and efficacy in this age group is not supported by published data from clinical trials.

Although no data from clinical trials with pregnant women are available the approved summaries of product characteristics for medicated plasters indicate that after careful risk-benefit assessment a use of medicated plasters containing *Capsicum* preparations could be acceptable. The same approach is acceptable for semi-solid dosage forms.

5.6. Overall conclusions on clinical safety

Capsaicinoids are known to be very aggressive compounds, particularly when applied to mucous membranes. From preclinical tests it is also evident that prolonged contact of sensitive neurons to capsaicin may lead to possibly irreversible damage of the neuronal membranes. Therefore restrictions should apply for the medicinal use of herbal preparations containing capsaicins.

In order to limit the risk for irreversible neuronal damage the duration of continuous use should be restricted to a maximum of 3 weeks. The administration is contraindicated on broken skin, wounds and eczemas. Contact with mucous membranes should be avoided. As capsaicinoids act via a thermosensitive receptor the application of additional sources of heat should be avoided.

The use in children and adolescents is not considered in the monograph as safety and efficacy in this age group is not supported by published data from clinical trials.

Medicated plasters or semi-solid dosage form should only be used during pregnancy and lactation after a careful risk-benefit assessment as safety during pregnancy and lactation has not been fully established.

The reported side effects are related to hypersensitivity reactions. In such cases the treatment should be discontinued.

In case of cutaneous application for short term no interactions have to be anticipated. So far no interactions were reported.

Therefore it can be concluded that the medicinal use of herbal preparations from *Capsicum* fruits in herbal medicinal products is safe as long the precautionary measures set out in the monograph are applied.

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of
Capsicum frutescens L., fructus
EMA/HMPC/674138/2013
Page 66/67

6. Overall conclusions

Based on the data documented in the assessment report, a European Union herbal monograph is established on the well establish use of several preparations of *Capsicum anuum* L. var *minimum* (Miller) Heiser and small fruited varieties of *Capsicum frutescens* L., fructus. The use of *Capsicum* preparations (included in the monograph) fulfil the requirements 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 efficacy is plausible on for the following indication: Herbal medicinal product for the relief of muscle pain such as low back pain.

Capsaicinoids are considered to be responsible for the clinical efficacy. Therefore finished products have to be standardised to a certain content of capsaicinoids. This can be achieved either by using defined amounts of standardised extracts or by adjusting the content during the manufacturing of the finished product using variable amounts of an extract and variable amounts of excipients for standardisation. As for standardised herbal preparations the extraction solvent and the DER are of less importance, well-established use is also proposed for herbal preparations not tested in clinical trials.

Proposed ATC code: M02AB.

Following the general regulatory discussions on capsaicin containing medicinal products for cutaneous use there is a common understanding that applications for new medicinal products cannot be based on a bibliographic or generic approach alone. The clinical efficacy of locally applied capsaicin (semi-solid dosage forms, medicated plasters) depends in a high degree on the excipients used. Therefore a bridging of clinical data from a certain medicinal product to another one without supporting data may not be acceptable from a regulatory point of view. Data on pharmaceutical as well as clinical equivalence might be necessary in order to show the relevance of the literature data for the product concerned.

Contact with the responsible national agencies is advisable prior to an application (National scientific advice).

From preclinical tests it is evident that prolonged contact of sensitive neurons to capsaicin may lead to possibly irreversible damage of the neuronal membranes. Therefore the use should be restricted to a maximum of 3 weeks of continuous application.

Annex

List of references

Assessment report on *Capsicum annuum* L. var. *minimum* (Miller) Heiser and small fruited varieties of
Capsicum frutescens L., fructus
EMA/HMPC/674138/2013   Page 67/67