TAMARIND SEED POLYSACCHARIDE FOR USE IN THE TREATMENT OF INFLAMMATORY DISEASES

Technical field of the invention

The present invention relates to the use of tamarind seed polysaccharide as an anti-inflammatory and to anti-inflammatory compositions which contain it as active ingredient.

Background of the invention

Tamarind seed polysaccharide (TSP) is a natural polysaccharide polymer obtained from the seeds of *Tamarindus indica,* an evergreen plant which can reach a height of 15 metres and produces fruit in the form of pods. It is very common in India, Africa and throughout the Far East, where it is mainly grown as a food. The fruit contains large seeds with a high percentage of polysaccharides, which have the function of accumulating and preserving vital energy-giving substances. Tamarind seeds, which were originally considered as waste products, have subsequently found different applications after grinding to obtain a farinaceous product (known as tamarind gum or tamarind nut powder). The most important of these applications is in the textile industry and the paper industry, where tamarind gum is used as a sizing and gluing agent, and in the food industry where, like other polysaccharides, it is used as a thickener, gelling agent, stabiliser and binder in various products. Raw tamarind gum is a commercially available product containing from 65% to 73% by weight of polysaccharide, from 15% to 23% by weight of protein material, from 3% to 8% by weight of fats and oils, and from 2% to 4% by weight of ashes, together with smaller amounts of raw fibre, tannins and other impurities.

More recently, TSP has also been used in the pharmaceutical field as active ingredient in tear substitutes (WO2009/044423), as a carrier for slow-release ophthalmic medicaments for topical administration

(WO97/28787) and, more generally, as an excipient due to its mucoadhesive characteristics (WO2006/131262).

Summary of the invention

The present invention refers to the use of tamarind seed polysaccharide in treatment of inflammatory diseases.

The invention also relates to an anti-inflammatory pharmaceutical and/or dermocosmetic composition comprising tamarind seed polysaccharide as active ingredient mixed with one or more acceptable excipients.

Furthermore, the invention concerns a method of treating a patient having an inflammatory disease comprising administering a therapeutically effective amount of Tamarind seed polysaccharide.

Detailed description of the invention

We have now found that tamarind seed polysaccharide (hereinafter called TSP) has anti-inflammatory properties, especially when administered topically.

Object of the present invention is therefore the use of TSP as an anti-inflammatory and, in particular, the use of TSP as an anti-inflammatory for topical administration.

A further object of the present invention is an anti-inflammatory pharmaceutical and/or dermocosmetic composition comprising TSP mixed with one or more acceptable excipients and, more particularly, an anti-inflammatory pharmaceutical and/or dermocosmetic composition for topical administration comprising TSP mixed with one or more acceptable excipients.

TSP can be used, as an anti-inflammatory according to the present invention, alone or in combination with other active ingredients.

A further object of the present invention is therefore the use of TSP as an anti-inflammatory in combination with one or more active ingredients and, more particularly, the use of TSP as an anti-inflammatory for topical

administration in combination with one or more active ingredients.

Anti-inflammatory pharmaceutical and/or dermocosmetic compositions and, more particularly, anti-inflammatory pharmaceutical and/or dermocosmetic compositions for topical administration, comprising TSP in combination with one or more active ingredients mixed with one or more acceptable excipients, also form the object of the present invention.

According to the invention, "TSP" means a polysaccharide-enriched fraction obtained from tamarind gum commercially available, for example from Dainippon Sumitomo Pharma Ltd. under the Glyloid® trademark or from Indena SpA under the Xilogel® trademark.

The active ingredients which can be used in combination with TSP are, for example, antimicrobial agents, anti-inflammatory agents, analgesic agents, wound-healing agents.

The preferred antimicrobial agents are antibiotics such as clindamycin, erythromycin, benzylpenicillin, tetracycline, chloramphenicol, vancomycin and linezolid.

Anti-inflammatory agents include steroidal anti-inflammatory drugs, such as cortisone, and non-steroidal anti-inflammatory drugs, such as acetylsalicylic acid and ibuprofen, which have analgesic activity.

Wound-healing agents may be of natural or synthetic origin.

Particularly preferred is the combination of TSP with other extracts of plant origin, especially extracts of plant origin which possess antimicrobial and/or anti-inflammatory and/or analgesic and/or wound-healing properties.

The use of TSP in combination with other extracts of plant origin can have a synergic effect on anti-inflammatory activity.

Preferred examples of extracts of plant origin which may be used in combination with TSP are extracts of *Helichrysum italicum, Ferula* spp., *Aesculus hippocastanum* and *Zanthoxylum bungeanum.*

The extracts of *Helichrysum italicum, Ferula* spp. and *Aesculus hippocastanum* are known individually for their anti-inflammatory action.

The active ingredients in *Helichrysum italicum* extract are contained in the aerial parts of the plant, which contains non-flavonoid prenylated polyphenols. The extracts of *Helichrysum italicum* are known and can be prepared by conventional methods.

The active ingredients of *Ferula* spp. extract are contained in the whole plant, which contains ferutinin. The extracts of *Ferula* spp. are known and can be prepared by conventional methods.

The active ingredients of *Aesculus hippocastanum* extract are contained in the seed of the plant and in the bark, which contains proanthocyanidin A2 (PA2).

The extracts of *Aesculus hippocastanum* are known and can be prepared by known methods.

The active ingredients in *Zanthoxylum bungeanum* extract are contained in the pericarp, which contains alkamides. The extracts of *Zanthoxylum bungeanum* are known and can be prepared by conventional methods.

For use as an anti-inflammatory according to the present invention, TSP is formulated in suitable pharmaceutical and/or dermocosmetic compositions, preferably in topical pharmaceutical and/or dermocosmetic compositions. The topical pharmaceutical and/or dermocosmetic compositions according to the invention comprise TSP mixed with one or more suitable excipients and may be, for example, in the form of cream, ointment, gel, gum, toothpaste, mouthwash or shampoo.

TSP may generally be used in quantities of between 0.1% and 5% by weight, preferably between 0.1% and 2% by weight, and even more preferably between 0.2% and 1% by weight.

The other active ingredients optionally present in combination with TSP

are used in suitable effective quantities. In the case of other extracts of plant origin used in combination with TSP, their quantity may generally be between 0.1% and 5% by weight, preferably between 0.1% and 2% by weight, and even more preferably between 0.2% and 1% by weight.

Examples of suitable excipients that may be used in the compositions according to the invention are solvents, diluents, gliding agents, preservatives, gums, sweeteners, coating agents, binders, disintegrating agents, lubricants, suspending agents, dispersing agents, colorants, flavouring agents, non-stick agents, surfactants, plasticisers, emulsifiers, chelating agents and emollients.

The solvent preferably used is water, but alcohols or other organic solvents may also be used, possibly mixed with water.

The choice of excipients is part of the normal knowledge of one skilled in the art, and will mainly depend on the pharmaceutical and/or dermocosmetic form chosen.

For example, a cream can be prepared by incorporating TSP in a topical carrier consisting of liquid paraffin, dispersed in an aqueous medium by means of lubricants. An ointment can be prepared by mixing TSP with a topical carrier such as mineral oil or wax. A gel can be prepared by mixing TSP with a topical carrier containing a gelling agent.

The pharmaceutical and/or dermocosmetic composition according to the invention may also be a, woven or non-woven, material coated and/or impregnated with a mixture of TSP with a suitable carrier or a matrix in which TSP is dispersed so that it comes into contact with the skin for transdermal administration. Specific examples are sticking plasters, gauze, towelettes, etc.

The choice of type of pharmaceutical and/or dermocosmetic form will depend mainly on the area to be treated and is part of the normal knowledge of one skilled in the art. For example, a gum or mouthwash may be more suitable to treat the oral cavity, whereas a cream, ointment, lotion or towelettes may be

suitable for the skin of the face.

The treatment with TSP according to the present invention is effective in stimulating the anti-inflammatory response, especially when administered topically to the skin and to the mucosa.

The compositions according to the invention are therefore useful to treat any disorder of the skin or mucosa associated with an inflammatory state.

The term "skin" is used according to the present invention in its conventional meaning, namely an external organ including the epithelial tissue. The term "mucosa" is also used with its usual meaning, which relates to all the mucosal barriers in the body, such as the gastrointestinal, pulmonary, sublingual, buccal, rectal, vaginal, nasal, urethral and ocular barriers.

The compositions to which this invention relates are preferably applied by topical administration directly to the inflamed area of the skin or mucosa and/or to the surrounding area.

Numerous disorders of the skin and mucosa associated with inflammatory states are known. For example, one disorder of the skin or mucosa which can be effectively treated or prevented by applying a composition according to the invention is dermatitis.

Dermatitis is a surface inflammation of the skin characterised by the formation of blisters, erythema, oedema, exudative lesions, desquamative lesions, scab-forming lesions and intense itching. Dermatitis can be the contact, atopic or seborrhoeic type. The main treatment consists of removing the trigger, generally an irritant substance or an allergen, but that is not always possible.

A particular form of dermatitis is psoriasis, a chronic hyperproliferative inflammatory skin disease that affects approximately 1-2% of the population. Every year about 150,000 new cases of psoriasis are recorded, and approximately 400 deaths are caused by this disease. The impact of psoriasis

on the patients' life is not limited to its effect on their physical appearance, but also affects their physical capacity and longevity. The most common type of psoriasis is chronic plaque psoriasis, generally a chronic condition with periods of remission and flares, characterised by erythematous desquamative plaques most frequently located on the scalp or on the extensor surfaces of the elbows and knees.

The treatment of dermatitis is generally based on corticosteroids, which present considerable side effects such as reduced immune response which leads to secondary bacterial infections, especially those caused by fungi or Candida. Moreover, this treatment requires frequent periods of suspension, and cannot be used during the acute exudative stage of the disease.

Alternatively, psoriasis can be treated by topical administration of dithranol, vitamin D3 analogues or tazarotene. These active ingredients present various side effects, such as irritation, toxicity and carcinogenicity. Phototherapy with UVA or UVB radiation can also be used to treat psoriasis, especially in patients who fail to respond to topical treatment. However, phototherapy is not devoid of side effects, such as the risk of erythema, blistering and premature aging of the skin in the case of UVB radiation, or nausea, erythema, headache, skin pain, actinic keratosis, premature aging of the skin, irregular pigmentation and squamous-cell carcinoma in the case of UVA radiation.

The compositions according to the invention allow all types of dermatitis to be treated effectively.

Even inflammations of the mucosa, especially the oral, rectal and vaginal mucosa, can be treated with the compositions according to the invention.

In particular, TSP can be used for topical treatment of inflammations of the oral mucosa such as mucositis and stomatitis.

The terms mucositis and stomatitis are often used interchangeably, although the two disorders can present some differences.

Mucositis is a toxic inflammatory reaction which affects the gastrointestinal tract and can be caused by exposure to chemotherapy agents or ionising radiation. Mucositis is generally manifested as an erythematous lesion similar to a burn or as a random ulcerative lesion from focal to diffuse.

Stomatitis is an inflammatory reaction that affects the oral mucosa, with or without ulceration, which can be caused or intensified by pharmacological treatments, especially chemotherapy, or by radiotherapy.

The degree of stomatitis can range from mild to severe and the patient with severe stomatitis may be unable to eat or drink or take medicinal products by mouth.

Many women suffer from mouth ulcers at certain stages of the menstrual cycle and at the same time present the same type of ulcers in the genital tract, especially the vulva and vagina. They are sometimes very severe and can cause urine retention and require strong analgesics and sedatives.

The most serious form is known as Behçet's Syndrome.

According to the invention, the more general term of mucositis will also be used to indicate stomatitis.

Erythematous mucositis may appear as early as three days after exposure to chemotherapy or radiotherapy, but more commonly appears after 5-7 days. The progress to ulcerative mucositis takes place within 7 days of the start of chemotherapy and can sometimes become so severe as to require discontinuance of the pharmacological treatment. Mucositis can involve the mouth and the oropharyngeal tract as well as the gastrointestinal tract from mouth to anus. In the present context, unless otherwise specified, reference is made to mucositis which relates to the more easily accessible regions such as the mouth, pharynx, oesophagus and rectum.

As a high percentage (30-40%) of patients who receive chemotherapy develop mucositis of varying degrees of severity, there is a particular need for an effective, convenient treatment. No effective treatment is currently available and attempts to solve the problem involve the use of analgesics, antiseptics and oral hygiene measures or attenuation of the symptoms.

Moreover, the problem is not limited to cancer patients, because mucositis frequently also occurs in patients with HIV, especially when associated with Kaposi's sarcoma, in patients suffering from non-Hodgkin's lymphoma, in debilitated elderly patients and in patients receiving treatments with BRMs (Biological Response Modifiers) such as interleukin-2, interferons, lymphocytes activated by lymphokines and the like.

The following examples are provided to further illustrate the present invention.

Examples

Example 1 - Cream for topical use

TSP (tamarind seed polysaccharide)	0.400% (w/w)
Water	75.150% (w/w)
Montanov 202	5.000% (w/w)
Lanol 99	10.000% (w/w)
Sepilift DPHP	1.000% (w/w)
Monoi Butter	2.000% (w/w)
Sepifeel One	1.000% (w/w)
Aquaxyl	3.000% (w/w)
Panthenol	1.200% (w/w)
Tocopheryl acetate	0.600% (w/w)
Sepicide HB	0.300% (w/w)
Perfume	0.250% (w/w)
Sodium hydroxide (30% sol.)	0.100% (w/w)
Total	100.000% (w/w)

Example 2 - Oral paste

TSP (tamarind seed polysaccharide)	0.300% (w/w)
Zanthoxylum bungeanum extract	0.300% (w/w)
Sorbitol	50.000% (w/w)
Distilled water	35.040% (w/w)
Hydrated silica	8.000% (w/w)
Carboxymethylcellulose	5.000% (w/w)
Potassium sorbate	0.300% (w/w)
Sodium benzoate	0.300% (w/w)
PEG-40 hydrogenated castor oil	0.160% (w/w)
Sodium saccharine	0.100% (w/w)
Flavouring	0.500% (w/w)
Total	100.000% (w/w)

Example 3 - Vaginal cream

TSP (tamarind seed polysaccharide)	0.200% (w/w)
Ferula spp. extract	0.200% (w/w)
Distilled water	73.300% (w/w)
Liquid paraffin	15.000% (w/w)
Cetearyl alcohol - PEG-20 stearate	9.000% (w/w)
Dimethicone	1.000% (w/w)
Phenoxyethanol	0.500% (w/w)
Imidazolidinyl urea	0.300% (w/w)
Propylparaben	0.150% (w/w)
Methylparaben	0.150% (w/w)
Sodium EDTA	0.100% (w/w)
Lactic acid	0.100% (w/w)
Total	100.000% (w/w)

Example 4 - Myeloperoxidase expression in skin PMNs

Tamarind Seed Polysaccharide (either alone and in combination with standardized botanical extracts) was tested for its ability to reduce inflammation cellular response through the measurement of the inhibition of myeloperoxidase (MPO) activity in polymorphonuclear neutrophils (PMNs).

Inflammation, in particular during the acute phase, causes the migration of PMNs towards the inflammation site by chemotaxis. It is also known that the degree of MPO activity is directly proportional to the number of PMNs present in the tissue and to the inflammatory response. Thus, a reduction of the number of PMNs corresponds to a reduction in the activity of myeloperoxidase and it can be then concluded that the cellular response to inflammation is reduced accordingly.

Human skin PMNs were cultivated in bacteriological Petri dishes with 100 mm diameter. Cell culture medium was RPMI-1640 supplemented with Penicillin (100 U/ml), Streptomycin (100 mcg/ml), L-glutamin (2 mM) and 10% heat-inactivated and filtered FCS (Foetal Calf Serum). PMNs were then stimulated with the addition of LPS (lipopolysaccharides) to the cultivation media.

Different standardized botanical extracts (at 0.2% concentration in the medium), either alone or in combination with Tamarind Seed Polysaccharide (at 0.2% concentration in the medium), were added to the Petri dishes. Dexamethasone (known to be a strong inhibitor of inflammatory responses and of MPO activity) was used at 0.1% as a positive reference compound. The details are reported in Table 1.

Table 1

Products	**Concentration**
Basal value (after stimulation with LPS)	-
Positive reference (Dexamethasone)	0.1%
Tamarind Seed Polysaccharide (TSP)	0.2%
Helichrysum italicum extract (HIE)	0.2%
HIE + TSP	0.2% + 0.2%
Ferula hermonis extract (FHE)	0.2%
FHE + TSP	0.2% + 0.2%
Zanthoxylum alatum (ZA)	0.2%
ZA + TSP	0.2% + 0.2%

After 6 hours of incubation, the overall inhibition of myeloperoxidase (MPO) activity was quantified in the PMNs cell supernatants using an enzyme immunoassay kit (ELISA). Results are reported in Table 2.

Table 2

Tested products	**MPO Inhibition of Expression (Δ% vs. basal)**
Basal value (after stimulation with LPS)	-
Positive reference (Dexamethasone)	-30%*
Tamarind Seed Polysaccharide (TSP)	-40%*
Helichrysum italicum extract (HIE)	-20%*
HIE + TSP	-95%**
Ferula hermonis extract (FHE)	-15%*
FHE + TSP	-93%**
Zanthoxylum alatum extract (ZAE)	-30%*
ZAE + TSP	-88%**

$p < 0.01$ vs. control

***$p<0.01$ vs. positive reference (Dexamethasone)*

CLAIMS

1. An anti-inflammatory pharmaceutical and/or dermocosmetic composition comprising tamarind seed polysaccharide in combination with an extract of *Ferula hermonis* as active ingredients, mixed with one or more acceptable excipients.

2. A composition according to claim 1, for topical administration.

3. A composition according to claim 1 or 2, in the form of cream, ointment, gel, lotion, gum, toothpaste, mouthwash or shampoo.

4. A composition according to claims 1-3, comprising at least a further active ingredient selected from the group consisting of antimicrobial agents, anti-inflammatory agents, analgesic agents and wound-healing agents.

5. A composition according to claims 1-4, wherein the tamarind seed polysaccharide quantity ranges from 0.1% to 5% by weight.

6. A composition of claims 1-5 for use as a medicament for the treatment of inflammation diseases.

7. Use of an anti-inflammatory pharmaceutical composition comprising tamarind seed polysaccharide in combination with an extract of *Ferula hermonis* as active ingredients, mixed with one or more acceptable excipients in the manufacture of a medicament for the treatment of inflammation diseases.

8. The use according to claim 7, wherein the composition further comprises at least a further active ingredient selected from the group consisting of antimicrobial agents, anti-inflammatory agents, analgesic agents and wound-healing agents.

9. The use according to claim 7 or 8, wherein the tamarind seed

polysaccharide quantity ranges from 0.1% to 5% by weight.