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(54) Title: LIQUID COMPOSITION FOR TOPICAL APPLICATION

Abrégé/Abstract:
The invention concerns a composition, in particular a non-solid pharmaceutical composition for local application comprising, as active principle, at least glycerol or a concentrated solution of glycerol, saccharose, sorbitol or mannitol, the active principle concentration of said composition being osmotically active towards plasma.
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(54) Title: NON-SOLID COMPOSITION FOR LOCAL APPLICATION
(54) Titre: COMPOSITION NON SOLIDE POUR APPLICATION LOCALE

(57) Abstract: The invention concerns a composition, in particular a non-solid pharmaceutical composition for local application comprising, as active principle, at least glycerol or a concentrated solution of glycerol, saccharose, sorbitol or mannitol, the active principle concentration of said composition being osmotically active towards plasma.

(57) Abrégé: Composition notamment pharmaceutique non solide pour application locale comprenant, à titre de principe actif, au moins du glycérol ou une solution concentrée de glycérol, de saccharose, de sorbitol ou de mannitol, la concentration en principe actif de ladite composition étant osmotiquement active vis-à-vis du plasma.
LIQUID COMPOSITION FOR TOPICAL APPLICATION

This invention relates to a new liquid or viscous composition notably pharmaceutical containing a hypertonic solution or glycerin, and their use for the treatment of oral ulcers and the superficial injuries.

The development of lesions in the form of ulcers in the buccal cavity, and occasionally on other parts of the body is a very common phenomenon. Most individuals are susceptible to develop oral ulcers and small topical injuries. Although ulcers do not constitute a fully fledged illness, they cause considerable pain and discomfort.

From physiopathological point of view, an ulcer can be considered as a localised breach of the superficial zones of the skin or mucosa. This injury exposes the underlying and deeper parts of the ulcer to more severe traumatisms, which is manifested by rupture of localised blood vessels and degradation of deeper layers of the tissue. These minor injuries are exposed to micro-organisms, particularly the streptococci and staphylococci responsible for secondary infections, which leads to secondary lesions in the form of oral ulcers.

The development of such lesions is often associated with traumatic injuries and itches but the formation of oral ulcers on the mucosa may also be related to other factors, which are not yet fully understood. In addition to the traumatic lesions, the development of blisters and oral ulcers can also be due to certain elements in the food, which alter mucosal surface. The
deficiency of certain vitamins, such as the vitamin A is also responsible for mucous membrane fragility, which breaks easily following small injuries.

Clinically, the ulcers and superficial injuries are small lesions of the mucosa or epidermis (a few millimetres to a few centimetres), purplish or yellowish in colour, that let open the underlying tissue layers and the blood vessels. These lesions constitute an ideal site for bacterial proliferation. The presence of pyogenic bacteria is a common phenomenon. The body defence mechanisms and the tissue healing processes are immediately activated after the appearance of tissue injury and start the healing process. The immunity system fights against bacterial growth finally to prepare the damaged zone for regeneration.

Although the healing process is relatively rapid for skin lesions, it may take minimum seven to ten days to completely heal an oral ulcer. This prolonged healing process is related to the fact that oral ulcers are constantly in contact with food, which contains non-pathogenic micro-organisms. Thus, the lesion is constantly exposed to bacteria, which are ready to multiply in a favourable environment. The constant movements of mouth, for example while speaking, equally increases healing time and delays injury repair.

All currently available treatments are directed to stop or reduce bacterial growth in the lesion but have no effect on the tissue regeneration process necessary for a rapid healing. Most of the available treatments for ulcers contain antibiotics or antiseptic agents. Often these treatments are for topical application.
In case of severe infection, antibiotics are used orally. The major disadvantage of these treatments is that they act only on the secondary bacterial infection but have no effect on the tissue regeneration. Very often, people have the tendency to scrape affected zone, which provokes an inflammation and can aggravate the extension of lesion. Another major disadvantage of currently available treatments is that they do not reduce the healing period, people continue suffering from pain and increase in the size of the lesion.

Therefore, an ideal treatment for oral ulcers must possess the following three major qualities:

- Eliminate micro-organisms present inside the lesion, finally to prepare a favourable ground for cellular growth,
- Accelerate tissue regeneration to stimulate healing and to minimise recovery period,
- Should be non-toxic and should be free of side-effects.

Till today, no product with these three properties of removing bacteria from the lesion, stimulating healing and being non-toxic, was discovered.

The glycerin or concentrated solutions, for example the concentrated sugar solutions were often used as preservatives, for example in jams, or as excipient but no pharmacological properties, particularly for the treatment of ulcers were assigned to these products. Surprisingly, we discovered that the bacteria can be easily removed from the ulcer in a very short period of time by the application of a concentrated osmotically active solution compared to
the plasma, and that the healing period can be considerably reduced by adding a substance capable to stimulate cell proliferation.

The present invention therefore concerns a non-solid and preferably a liquid composition for topical application containing glycerol or a concentrated solution of glycerol, sucrose, sorbitol or mannitol as active product, the concentration of such a non-solid composition being osmotically active compared to plasma particularly the blood plasma.

In the preferred form of the preparation, this non-solid composition is a pharmaceutical preparation.

According to current invention, the term non-solid is applied to the liquid as well as gluey (viscous) preparations.

Our observations show that pure glycerol or a concentrated solution of sucrose, sorbitol, mannitol or glycerin (glycerol) applied on an open superficial injury induce accelerated flow of plasma from the injury and stimulate lesion healing.

The increased outward plasma flow is a result of osmotic process between the inner and the outer parts of the wound. According to the law of diffusion, the glycerol or any hypertonic solution tries to penetrate into the tissue. However, due to the large size of molecules in these solutions, their penetration into the tissue is not possible. On the contrary, the highly permeable hypotonic plasma around the damaged capillaries of the injury drains out to balance the osmotic equilibrium. The topical application of a hypertonic solution on an injured tissue therefore produces exudation of a
large amount of plasma from the wound. During this process, the micro-
organisms present at the level of the lesion are eliminated along with the
flow of plasma which immediately reduces bacterial load inside the wound.
Therefore the concentrated solutions allow to drain superficial injuries and
ulcers.

This plasma exudation equally brings many immunity factors
(immunoglobins, complement system, leukocytes) participating in microbial
elimination, which prepares a favourable ground for ulcer healing.
Furthermore, the glycerol, the concentrated solutions of sucrose, sorbitol,
mannitol or glycerine (glycerol) are very less toxic or at all non-toxic for
health and can be used orally without any side-effect.

The preferential compositions according to the present invention
concerns use of pure glycerol as active principle. Non-solid compositions of
sucrose or mannitol can also be preferred.

Under optimal conditions of preparation according to this invention,
the concentration of active principle in the non-solid composition should
allow to obtain a solution having osmotic concentration superior to plasma:
iminimum 300 milliosmoles (mOsm), preferably superior to 500 mOsm,
notably superior to 800 mOsm and specifically superior to1 mOsm. This
cosmetic capacity is assigned through the incorporation of active principle in
the solution at a concentration of minimum 30%, preferably minimum 60%,
particularly 90% and specifically minimum 95%, the remaining osmotic
capacity can be obtained by the addition of other osmotically active
ingredients.
Under preferential conditions of preparation, the concentration of active principle in the non-solid composition is such that the volume of diluant (solvent) is less than 70%, preferably less than 40%, notably less than 20%, preferably less than 10%.

The association of these osmotically active products with antibiotics or antiseptic, either natural or synthetic, helps to enhance antibacterial properties. The association of these osmotically active substances with another ingredient capable to stimulate cell proliferation equally helps to accelerate the speed of healing.

For these reasons, the current invention also concerns a non-solid composition as explained above in which an osmotically active substance is associated with at least one antiseptic or a product capable to stimulate cell growth. Such an association represents an excellent remedy for the treatment of ulcers, superficial injuries, and burns, for postoperative care and to accelerate healing with minimum scar tissue formation.

Non-solid compositions according to present invention can be mixed with different substances capable to stimulate cell proliferation particularly with plant extracts used traditionally or not for dermatological ailments (Mimosa tenuiflora, Quercus, Aesculus hippocastanum, Geranium robertianum, Cupressus sempervirens, Vitis vinifera, Ribes nigrum, Centella asiatica, Matricaria Chamomilla and particularly the Alchemilla vulgaris) or with any other substance with «growth factor» type activity (example : escine, tannins, procynadolic, oligomers, mimosides) or with a bacteriostatic or bacteriocidal antibiotics (examples papaïne, geranine).
These compositions particularly pharmaceuticals, can be liquid or viscous and can be presented in pharmaceutical forms commonly employed in human medicine, for example elongated tubes containing solutions or sprays manufactured employing traditional methods.

The active principles can be incorporated in any commonly used excipient such as the aqueous or non-aqueous excipients, different humidifying agents the preservatives and the thickening agents.

This invention also concerns the use of glycerol or the concentrated solutions of glycerol sucrose, sorbitol or mannitol in osmotically active concentrations compared to the plasma for a method of treatment for human or animal body, i.e. as a drug.

The drugs according to the present invention can be used for the preventive or curative treatment of ulcers. They can also be used for the treatment of ulcers on the mucosa or skin epidermis other than blisters.

The usual dose varies according to the person treated and according to the type of injury, for example, 2 to 6 topical oral applications of 2 drops of the composition given in the example number 3 on each ulcer per day for a period of 3 days.

The current invention also includes the method of preparation of the compositions given above, characterised by the mixing of an osmotically active solution with an pharmaceutically acceptable excipient.
This invention is principally related to the use of glycerol or a concentrated solution of glycerol, sucrose, sorbitol or mannitol, in osmotically active concentrations compared to plasma, to produce a drug directed to treat small lesions on the mucosa or epidermis, notably the ulcers.

The preferential conditions of preparation of such non-solid and preferably liquid compositions are given below which are also applied to other formulations given in this patent.

The following examples illustrate the patent request.

10-ml tubes with a 4cm long canula were prepared by formulating the following composition:

Example 1

Water 60-ml
Sorbitol 40g
Shake to obtain a clear solution

Example 2

Water 50-ml
Glycerol 50-ml

Example 3

10-ml tubes with a 4cm long canula were prepared by weighing the following composition:

Water 45%
Xanthan gum 0.5%
Methyl parahydroxy benzoate 0.15%
Hydroalcoholic extract of Lady’s Mantle* 5.0%
Blackcurrant perfume 0.43%
Glycerol qsp 100%

*Obtained from Biosphère, France: 150 g dried leaves mixed with 500-ml water and 500-ml ethanol.

Example 4
Glycerin 97-ml

Dried extract of Alchemilla vulgaris: 3g
Mix.

Example 5
Glycerol 90%
Blackcurrant extract 9%
Extract of Azadirachta indica 1%
Mix.

Example 6
Glycerin 96.5%
Extract of Alchemilla vulgaris 3.0%
Extract of Azadirachta indica 0.5%

Example 7
Different capacity tubes were prepared according to the following formula:
Extract of horse chestnut 8.1%
Cypress extract 5.0%
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geranium robertianum extract</td>
<td>4.0%</td>
</tr>
<tr>
<td>Escin</td>
<td>0.3%</td>
</tr>
<tr>
<td>Papain</td>
<td>0.1%</td>
</tr>
<tr>
<td>Carbomer</td>
<td>0.5%</td>
</tr>
<tr>
<td>Alcohol</td>
<td>4.0%</td>
</tr>
<tr>
<td>Phenonip</td>
<td>0.5%</td>
</tr>
<tr>
<td>PEG-7 Glyceryl cocoate</td>
<td>3.0%</td>
</tr>
<tr>
<td>Glycerol</td>
<td>30%</td>
</tr>
<tr>
<td>Water</td>
<td>qsp 100%</td>
</tr>
</tbody>
</table>

Example 8

Different capacity tubes were prepared according to the following formula:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extract of Alchemilla vulgaris</td>
<td>9.8%</td>
</tr>
<tr>
<td>Vitis vinifera</td>
<td>2.0%</td>
</tr>
<tr>
<td>Mimosa tenuiflora</td>
<td>5.0%</td>
</tr>
<tr>
<td>Carbomer</td>
<td>0.4%</td>
</tr>
<tr>
<td>PEG-7 Glyceryl cocoate</td>
<td>2.0%</td>
</tr>
<tr>
<td>Phenonip</td>
<td>0.5%</td>
</tr>
<tr>
<td>Triethanolamine</td>
<td>0.2%</td>
</tr>
<tr>
<td>Fragrance</td>
<td>0.2%</td>
</tr>
<tr>
<td>Glycerol</td>
<td>10-40%</td>
</tr>
<tr>
<td>Water</td>
<td>qsp 100%</td>
</tr>
</tbody>
</table>

Example 9

Different capacity tubes were prepared according to the following formula:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercus extract</td>
<td>0.5%</td>
</tr>
<tr>
<td>Escine</td>
<td>0.1%</td>
</tr>
<tr>
<td>Azadirachta indica</td>
<td>1.1%</td>
</tr>
</tbody>
</table>
Methyl parahydroxybenzoate 0.15%
Xanthan Gum 0.5%
Blackcurrant extract 0.43%
Glycerol 50%
Water qsp 100%

PHARMACOLOGICAL STUDIES

30 rats (IOPS, IFFA-CREDO 200+/− 20g) were shaved (3x3 cm) on the right side of the back. A wound of 0.4x0.4 cm was created with the help of a scissors and knife. 30 minutes after wounding, clotted blood was removed and 0.2-ml of glycerine containing 3% Alchemilla vulgaris extract was applied on the wounds of 10 rats. Other 10 rats received 0.2-ml distilled water.

The complete recovery time and the healing index were calculated every day over 10 days. The recovery time was reduced by 48% in glycerine – 3% Alchemilla vulgaris treated group with a healing index of 2.1 in the treated group compared to 3.3 in the control group.

With glycerin alone, the wound healing time was reduced by 26% with a healing index of 2.7. These results show that glycerin alone helps wound healing but the association of glycerin with a product capable to stimulate cellular mitotic activity markedly enhances the speed of healing.

The effect of different plant extracts on the rate of epithelial cell proliferation was determined *in-vitro*. Bovine kidney cells (MDBK) were
cultured in 96 well tissue culture micro-plated (10^5 cells / ml ; 100μl/ well). Different concentrations of plant extracts were added to the culture medium on day0 (n= 16 / dilution). Cells were incubated for 72 hours (37°C- 5% CO_2) and total number of cells was determined after trypsinization by MTT staining.

Only 5 out of 26 plant extracts tested showed activity to stimulate cell proliferation in the following order: Alchemilla vulgaris, Mimosa tenuiflora, Quercus, Aesculus hippocastanum, Geranium robertianum, Cupressus sempervirens, Vitis vinifera, Ribes nigrum.

**CLINICAL STUDY**

10-ml tubes were prepared, containing either a solution of 97% glycerin with 3% hydroglycerinated extract of Alchemilla vulgaris (3%dried plant extract w/w) as given in the example 4, or a preparation containing 97% ethyl alcohol (96%) and 3% hydroalcoholic extract of Alchemilla vulgaris (3% dried extract w/w).

18 subjects having problems of oral ulcers were included in a pilot clinical trial. 8 control subjects tested product containing hydroalcoholic extract while 10 other participants received the product with hydroglycerinated extract. 2 drops of the product were applied 3 times a day after meals up to complete ulcer healing. The time required for complete healing was determined in the two groups.
The mean healing period was 2.7 days in the group treated with hydroglycerinated extract compared to 6.3 days in the controls.

The use of osmetically active substances or glycerin, alone or in association with other ingredients capable to stimulate cellular mitotic activity, stimulate cell proliferation, superficial wound healing and notably oral ulcer recovery.
CLAIMS

1. A non-solid formulation for topical application for the treatment of oral ulcers and superficial skin injuries comprising:
   - glycerol, sucrose, sorbitose or mannitol as active principle, and
   - an extract of Alchemilla vulgaris as stimulating cell proliferation agent,
the formulation being osmotically active compared to blood plasma.

2. The non-solid formulation according to Claim 1, wherein said active principle is glycerol.

3. The non-solid formulation according to Claim 1, wherein said active principle is sorbitol or mannitol.

4. The non-solid formulation according to any one of Claims 1 to 3, wherein the formulation has an osmotic strength greater than 300 milliosmoles (mOsm).

5. The non-solid formulation according to Claim 4, wherein the formulation has an osmotic strength greater than 500 milliosmoles (mOsm).

6. The non-solid formulation according to any one of Claims 1 to 5, wherein the active principle concentration is such that the quantity of diluant (solvent) is less than 20 % by volume.

7. The non-solid formulation according to any one of Claims 1 to 6, wherein the active principle is associated with an antiseptic or an antibiotic product.

8. The non-solid formulation according to any one of Claims 1 to 7, wherein the formulation is a pharmaceutical composition or is in the form of an oral hygiene formulation.
9. A non-solid formulation for the preparation of a composition for the treatment of oral ulcers and superficial skin injuries, said formulation comprising:
- glycerol, as active principle, and
- a stimulating cell proliferation agent,
the formulation being osmotically active compared to blood plasma.

10. The formulation according to Claim 9, wherein the stimulating cell proliferation agent is an extract of Alchemilla vulgaris.

11. The use of a non-solid formulation for the treatment of oral ulcers and superficial skin injuries, wherein said non-solid formulation comprises:
- glycerol, as active principle, and
- a stimulating cell proliferation agent,
the formulation being osmotically active compared to blood plasma.

12. The use according to Claim 11, wherein the stimulating cell proliferation agent is an extract of Alchemilla vulgaris.

13. The use of a non-solid formulation for the preparation of a medicament for the treatment of oral ulcers and superficial skin injuries, wherein said non-solid formulation comprising:
- glycerol, as active principle, and
- a stimulating cell proliferation agent,
the formulation being osmotically active compared to blood plasma.

14. The use according to Claim 13, wherein the stimulating cell proliferation agent is an extract of Alchemilla vulgaris.