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(54) **ANDROGRAPHIS PANICULATA EXTRACT**

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(57) **ABSTRACT**

An extract of *Andrographis paniculata* extract containing andrographolide, 14-deoxyandrographolide, 14-deoxy-11, 12-dehydroandrographolide, neoandrographolide, polysaccharides, and flavanoids. Also disclosed is a pharmaceutical composition containing such an extract and its use for treating inflammatory bowel disease.

ANDROGRAPHIS PANICULATA EXTRACT

BACKGROUND

[0001] Inflammatory bowel disease includes chronic gastrointestinal disorders characterized by infiltration of inflammatory cells into the mucosa of the digestive tract. Ulcerative colitis and Crohn's disease are two prevalent conditions among them.

[0002] Ulcerative colitis takes place in the large intestine (i.e., colon). The inner lining of the disordered intestine becomes inflamed and develops ulcers.

[0003] Crohn's disease most commonly affects the end of the small intestine (i.e., terminal ileum) and parts of the large intestine. It causes inflammation that extends much deeper into the layers of the intestinal wall than ulcerative colitis.

[0004] Both ulcerative colitis and Crohn's disease are attributed to dysregulation of pro-inflammatory cytokine, including TNFα and IL-1β. See, e.g., McClane S. J. et al., *Journal of Parenteral and Enteral Nutrition* 23, 1999. Therapeutic agents have been developed based on down-regulation of pro-inflammatory cytokine. For example, 5-aminosalicylic acid, an inhibitor of TNFα signaling events, has been used to treat ulcerative colitis. See, e.g., *Therapeutic Immunology* Ed. Austen, K F., Blackwell Publishing, 2001, 159-167. However, most inflammatory bowel disease therapeutics have limited efficacy or significant side effects.

[0005] There is still a need to develop more effective therapeutic agents for treating inflammatory bowel disease.

SUMMARY

[0006] This invention is based on a surprising finding that an extract of *Andrographis paniculata* effectively exerts a curative effect against inflammatory bowel disease. The extract contains andrographolide lactones, polysacchlorides, and flavanoids; constituting 10-22% (preferably 13-17%), 18-28% (preferably 20-25%), and 10-15% (preferably 12-14) of the dry weight of the extract, respectively. The andrographolide lactones include andrographolide, 14-deoxyandrographolide, 14-deoxy-11,12-dehydroandrographolide, and neoandrographolide, which constitute 2-20% (preferably 3-10%, more preferably 6-10%), 0.01-6% (preferably 0.01-2%, more preferably 0.01-1%), 1-6% (preferably 2-5%, more preferably 2-4%), and 1-5% (preferably 2-4%) of the dry weight of the extract, respectively.

[0007] Another aspect of this invention relates to a method of treating inflammatory bowel disease (including Crohn's disease and ulcerative colitis). The method includes administering to a subject in need of the treatment an effective amount of the above-described extract.

[0008] Also within the scope of this invention are a pharmaceutical composition containing the extract described above and a pharmaceutically acceptable carrier, the use of such a composition to treat inflammatory bowel disease, and the use of such a composition for the manufacture of a medicament for treating this disease.

[0009] Details of several embodiments of the invention are set forth in the description below. Other features, objects, and advantages of the invention will be apparent from the description, and also from the claims.

DETAILED DESCRIPTION

[0010] To prepare the extract of this invention, one can immerse the aerial part of *Andrographis paniculata* in 80-95% ethanol, collect the ethanol phase, and then remove the ethanol. An actual example is provided below. The extract thus obtained can be further purified by thin layer chromatography, flash column chromatography, high performance liquid chromatography, or any other suitable methods.

[0011] This invention includes methods of treating inflammatory bowel disease by administering to a subject in need thereof an effective amount of the extract of this invention. The term "an effective amount" refers to the amount of the extract which is required to confer one of the above-described therapeutic effects in the subject. Effective amounts may vary, as recognized by those skilled in the art, depending on route of administration, excipient usage, and the possibility of co-usage with other agents. Preferably, the effective amount is 1-100 mg/kg/day based on the dry weight of the extract. The term "treating" refers to administering the extract to a subject that has inflammatory bowel disease, or has a symptom of the disease, or has a predisposition toward the disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the disease, the symptoms of the disease, or the predisposition toward the disease.

[0012] To practice one of the above-described methods, one administers to a subject in need thereof orally, rectally, parenterally, by inhalation spray, or via an implanted reservoir a composition that is either the above-mentioned extract alone or a mixture of the extract and a pharmaceutically acceptable carrier. The term "parenteral" as used herein includes subcutaneous, intracutaneous, intravenous, intramuscular, intraarticular, intraarterial, intrasynovial, intrasternal, intrathecal, intralesional and intracranial injection or infusion techniques.

[0013] An oral composition can be any orally acceptable dosage form including, but not limited to, tablets, capsules, emulsions and aqueous suspensions, dispersions and solutions. Commonly used carriers for tablets include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added to tablets. Tablets may also be coated for delivery or cosmetic effects. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions or emulsions are administered orally, the active ingredient can be suspended or dissolved in an oily phase combined with emulsifying or suspending agents. If desired, certain sweetening, flavoring, or coloring agents can be added.

[0014] A rectal composition can be any rectally acceptable dosage form including, but not limited to, cream, gel, emulsion, suspension, suppository, and tablet. One preferred dosage form is a suppository having a shape and size designed for introduction into the rectal orifice of the human body. A suppository usually softens, melts, or dissolves at body temperature. Suppository excipients include, but are not limited to, theobroma oil (cocoa butter), glycerinated gelatin, hydrogenated vegetable ails, mixtures of polyethylene glycols of various molecular weights, and fatty acid esters of polyethylene glycol.

[0015] A sterile injectable composition (e.g., aqueous or oleaginous suspension) can be formulated according to techniques known in the art using suitable dispersing or wetting agents (such as, for example, Tween 80) and suspending agents. The sterile injectable preparation can also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are mannitol, water, Ringer's solution

and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium (e.g., synthetic mono- or di-glycerides). Fatty acids, such as oleic acid and its glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically-acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions can also contain a long-chain alcohol diluent or dispersant, or carboxymethyl cellulose or similar dispersing agents.

[0016] An inhalation composition can be prepared according to techniques well known in the art of pharmaceutical formulation and can be prepared as solutions in saline, employing benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, fluorocarbons, and/or other solubilizing or dispersing agents known in the art.

[0017] A topical composition can be formulated in form of oil, cream, lotion, ointment and the like. Suitable carriers for the composition include vegetable or mineral oils, white petrolatum (white soft paraffin), branched chain fats or oils, animal fats and high molecular weight alcohols (greater than C12). The preferred carriers are those in which the active ingredient is soluble. Emulsifiers, stabilizers, humectants and antioxidants may also be included as well as agents imparting color or fragrance, if desired. Additionally, transdermal penetration enhancers may be employed in these topical formulations. Examples of such enhancers can be found in U.S. Pat. Nos. 3,989,816 and 4,444,762. Creams are preferably formulated from a mixture of mineral oil, self-emulsifying beeswax and water in which mixture the active ingredient, dissolved in a small amount of an oil, such as almond oil, is admixed. An example of such a cream is one which includes about 40 parts water, about 20 parts beeswax, about 40 parts mineral oil and about 1 part almond oil. Ointments may be formulated by mixing a solution of the active ingredient in a vegetable oil, such as almond oil, with warm soft paraffin and allowing the mixture to cool. An example of such an ointment is one which includes about 30% almond and about 70% white soft paraffin by weight.

[0018] A carrier in a pharmaceutical composition must be "acceptable" in the sense of being compatible with the active ingredient of the formulation (and preferably, capable of stabilizing it) and not deleterious to the subject to be treated. For example, solubilizing agents, such as cyclodextrins (which form specific, more soluble complexes with one or more of active compounds of the extract), can be utilized as pharmaceutical excipients for delivery of the active compounds. Examples of other carriers include colloidal silicon dioxide, magnesium stearate, cellulose, sodium lauryl sulfate, and D&C Yellow #10.

[0019] A suitable in vitro assay can be used to preliminarily evaluate the efficacy of the above-described extract in inhibiting expression of TNFα or IL-1β. The extract can further be examined for its efficacy in treating inflammatory bowel disease by in vivo assays. For example, the extract can be administered to an animal (e.g., a mouse model) or human having inflammatory bowel disease and its therapeutic effects are then accessed. Based on the results, an appropriate dosage range and administration route can also be determined.

[0020] Without further elaboration, it is believed that the above description has adequately enabled the present invention. The following specific examples are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. All of the publications, including patents, cited herein are hereby incorporated by reference in their entirety.

EXAMPLE 1

Preparation of the *Andrographis paniculata* Extract

[0021] Dry powder of *Andrographis paniculata* leaves (350 kg) was immersed in 90% ethanol (2,100 kg). The mixture was refluxed at 75-80° C. for two hours. The ethanol phase was collected and the solid residue was subjected to extraction again. The ethanol solutions were combined, filtered, and concentrated to afford a wet mixture having a density of 1.00-1.10 g/ml.

[0022] A small amount of the mixture was dried and analyzed for its composition by high performance liquid chromatography and spectrophotometry. The result showed that the dry extract contained andrographolide lactones (14.8% of the dry weight of the extract), polysaccharides (24.6%) and flavanoids (12.8%). Among the andrographolide lactones, andrographolide was at 9.2% of the dry weight of the extract, 14-deoxyandrographolide at <0.1%, 14-deoxy-11,12-dehydroandrographolide 2.6%, and neoandrographolide 3.0%. Dextrin was added (0.03 kg) to the wet mixture, which was then spray-dried (inlet: 185-195° C.; outlet: 90-100° C.). The solid extract thus obtained was ground, sieved, and packaged to form tablets and capsules as described below.

[0023] Tablets were prepared as follows. Starch (10 g) and sugar (10 g) were mixed with purified water (80.0 g) to yield a paste. Separately, the extract (500.0 g), starch (140.0 g), microcrystalline cellulous (337.5 g), and the paste were mixed, wet granulized, and dried at 55° C. The dried granules (957.6 g) and magnesium stearate (2.4 g) were mixed for 5 minutes. The final mixture was compressed to form tablets (400 mg/tablet, eqv. to 200 mg the extract/tablet). The tablets were film-coated with a paste prepared by mixing hypromellose (7.5 g), propylene glycol (1.6 g), titanium dioxide (3.0 g), Food Drug & Cosmetic color lake (0.4 g), and purified water (87.5 g) to afford the desired *Andrographis paniculata* extract-containing tablets.

[0024] Capsules were prepared as follows. The extract (340.0 g), pre-dried starch (221.0 g), silicon dioxide (2.125 g), and microcrystalline cellulous (34.0 g) were mixed. The mixture was filled into #0 hard-shell capsules using a capsule filling board to form the desired *Andrographis paniculata* extract-containing capsules (351.25 mg the mixture/capsule, eqv. to 200 mg the extract/capsule).

EXAMPLE 2

Inhibition of TNFα and IL-1β Expression

[0025] Peripheral blood monocytes (PBMC) cells are isolated from fresh blood using Ficoll-Paque Plus (Amersham Bioscience), according to the protocol provided by the manufacturer. The cells are suspended in RPMI 1640 media containing 10% FBS at a concentration of 1×10^5 cells/ml and seeded in a 96-well plate. Each reaction is carried out in three wells.

[0026] 10 μl of the extract of *Andrographis paniculata* in DMSO is added into each well (final concentrations: 0.1, 0.3, 1, 3, 10, and 30 μg/ml). Dexamethason (final concentration: 10 μM) is used as positive control. 10 μl of the media is used as a negative control. The plate is incubated at 37° C. under 5% CO_2 for 15 minutes. After 10 μl aliquots of 100 μg/ml

lipopolysaccharide are added to all wells except for the negative controls, the plate is incubated at 37° C. under 5% CO_2 overnight.

[0027] The plate is spun at 1000 rpm for 15 minutes and the supernatants are collected. Concentrations of TNFα and IL-1β are measured using the TNFα ELISA (Enzyme Linked Immunosorbent Assay) Kit and IL1-β ELISA Kit (Jingmei Bioengineer Technology).

[0028] The inhibition ratio is calculated as follows:

$$\text{Inhibition Ratio}(\%) = \left(1 - \frac{C_{extract} - C_{Control}}{C_{LPS} - C_{Control}}\right) \times 100$$

[0029] where $C_{extract}$ is the concentration of TNFα or IL-1β in PBMC cells treated with the extract and LPS, C_{LPS} is the concentration of TNFα or IL-1β in PBMC cells treated with LPS and dexamethason, and $C_{Control}$ is the concentration of TNFα or IL-1β in PBMC cells without being treated with LPS or the extract.

EXAMPLE 3

Treatment of Inflammatory Bowel Disease in Mouse Model

[0030] Balb/c male mice (18-24 g, purchased from Chinese Academy of Science animal center) are anaesthetized with 1% pentobarbital sodium at 0.05 mg/10 g. 1.5 mg of 2,4,6-trinitrobenzenesulfonic acid in 50% ethanol is administered slowly to each mouse (except blank control mice) via a catheter to induce inflammatory bowel disease. Blank control mice only receive 0.1 ml of 50% ethanol. The mice are treated with the test sample 24 hours and 2 hours prior to the inflammatory bowel disease administration and daily for 5 days after the administration.

[0031] The body weight of each mouse is monitored every day before and after the 2,4,6-trinitrobenzenesulfonic acid administration. The mice are sacrificed 24 hours after the last administration of test samples. Colons are removed and weighed. Furthermore, the colon weight to body weight ratio is calculated and adhesion between colon and other organs is also monitored.

[0032] Samples of colon tissues located precisely 2 cm above the anal canal are obtained, fixed in 10% buffered phosphate, embedded in paraffin, sectioned, and stained with hematoxylin/eosin. The degree of inflammation on microscopic cross sections is graded from 0 to 4 (0: no signs of inflammation; 1: a very low level of inflammation; 2: a low level of leukocyte infiltration; 3: a high level of leukocyte infiltration, a high vascular density, and a thickened colon wall; and 4: transmural infiltrations, loss of goblet cells, a high vascular density, and a thickened colon wall).

EXAMPLE 4

Clinical Treatment of Ulcerative Colitis

[0033] To study the efficacy of the *Andrographis paniculata* extract in treating ulcerative colitis, a randomized, double-dummy, active controlled 8-week clinical trial was conducted at 5 locations in Shanghai, China in compliance with to the International Conference on Harmonisation-Good Clinical Practice (ICH-GCP) guidelines. 120 patients with colonoscopy-confirmed mildly to moderately active ulcerative colitis were assigned to two groups (60 patients/group). One group was treated with the *Andrographis paniculata* extract-containing tablets mentioned above (3 times daily, 2 tablets each time,), and the other was treated with 5-amino-2-hydroxybenzoic acid, i.e., Etiasa (3 times daily, 500-mg granule each time,). All other medications were excluded. The therapeutic effects were assessed biweekly using a scale similar to the partial Mayo Scoring System, and the clinical symptom score reduction (≧50% reduction in symptoms) was calculated. Scores were then retrospectively calculated using the standard partial Mayo scores (PMS), clinical response (improvement ≧2 points or final score of 0) and remission (≦1 PMS score at week 8). Colonoscopies at the beginning and at the end of treatment were rated with a modified Baron score, and biopsies taken during colonoscopy were graded histologically with a scale of 0-3.

[0034] Patients in the two groups had similar demographics. In each group, the mean duration of disease ranged from 3.5-3.7 years and the baseline mean PMS was 3.8. In the 53 intent-to-treat patients treated with the extract, the clinical symptom score reduction was 27% in the patients at week 2 and improved to 56% in the patients by week 8. The 55 intent-to-treat Etiasa treated patients showed similar reduction. The clinical response rate at week 8 was 58% in the patients treated with the extract and 58% in the patients treated with Etiasa. The remission rate at week 8 was 43% in the patients treated with the extract and 58% in the patients treated with Etiasa. The results of PMS at the baseline and week 8 in both groups are statistically significant ($p<0.0002$).

[0035] Endoscopically, at week 8, 28% of the patients treated with the extract and 24% of those treated with Etiasa were in complete remission (Baron score of 0); and 47% of the patients treated with the extract and 42% of those treated with Etiasa had scores reduced by at least two grades.

[0036] Histologically, 19 of the patients treated with the extract and 15 of the patients treated with Etiasa were evaluated. 10 of the 19 patients treated with the extract showed decrease of inflammation scores by 25-50% at week 8, and so did 6 of the 15 patients treated with Etiasa. In the extract-treated group, 12⁄15 entering with elevated C-reactive protein levels showed normal levels at week 8, compared to 4⁄6 in the Etiasa-treated group. The results in both groups are statistically significant ($p<0.0001$).

[0037] The results indicated that the extract was effective in treating ulcerative colitis. Surprisingly, its efficacy was comparable to or even better than Etiasa.

OTHER EMBODIMENTS

[0038] A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention. Accordingly, other embodiments are also within the scope of the following claims.

[0039] The details of one or more embodiments of the invention are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawings, and from the claims.

1. An extract of Andrographis paniculata, comprising andrographolide lactones, polysaceharides, and flavonoids; wherein the andrographolide lactones, the polysaceharides, and the flavonoids constitute 10-22%, 18-28%, and 10-15% of the dry weight of the extract, respectively; and the andrographolide lactones include andrographolide, 14-deoxyandrographolide, 14-deoxy- 11, 12-dehydrogen-an-

drographolide, and neoandrographolide, which constitute 2-20%, 0.01-6%, 1-6%, and 1-5% of the dry weight of the extract, respectively.

2. The extract of claim **1**, wherein the andrographolide lactones, the polysaccharides, and the flavonoids constitute 13-17%, 20-25%, and 12-14% of the dry weight of the extract, respectively.

3. The extract of claim **1**, wherein the andrographolide, the 14-deoxyandrographolide, the 14-deoxy-11, 12-dehydroandrographolide, and the neoandrographolide constitute 3-10%, 0.01-2%, 2-5%, and 2-4% of the dry weight of the extract, respectively.

4. The extract of claim **3**, wherein the andrographolide, the 14-deoxyandrographolide, the 14-deoxy-11,12-dehydrogen-andrographolide, and the neoandrographolide constitute 6-10%, 0.01-1%, 2-4%, and 2-4% of the dry weight of the extract, respectively.

5. The extract of claim **2**, wherein the andrographolide, the 14-deoxyandrographolide, the 14-deoxy-11, 12-dehydrogen-andrographolide, and the neoandrographolide constitute 3-10%, 0.01-2%, 2-5%, and 2-4% of the dry weight of the extract, respectively.

6. The extract of claim **5**, wherein the andrographolide, the 14-deoxyandrographolide, the 14-deoxy-11, 12-dehydrogen-andrographolide, and the neoandrographolide constitute 6-10%, 0.01-1%, 2-4%, and 2-4% of the dry weight of the extract, respectively.

7. The extract of claim **1**, wherein the polysaccharides, the flavonoids, the andrographolide, the 14-deoxyandrographolide, the 14-deoxy-11,12-dehydrogen-andrographolide, and the neoandrographolide constitute 24.6%, 12.8%, 9.2%, <0.1%, 2.6%, and 3.0% of the dry weight of the extract, respectively.

8. An extract prepared from Andrographis paniculata, wherein the extract is prepared by a process comprising:

mixing an Andrographis paniculata plant with 90-95% ethanol at 75-80° C.,

separating the supernatant from the mixture, and

removing ethanol from the supernatant to provide the extract.

9. A pharmaceutical composition comprising:

an extract of Andrographis paniculata containing andrographolide lactones, polysaccharides, and flavonoids; wherein the andrographolide lactones, the polysaccharides, and the flavanoids constitute 10-22%, 18-28%, and 10-15% of the dry weight of the extract, respectively; and the andrographolide lactones include andrographolide, 14-deoxyandrographolide, 14-deoxy-11 ,12-dehydrogen-andrographolide, and neoandrographolide, which constitute 2-20%, 0.01-6%, 1-6%, and 1-5% of the dry weight of the extract, respectively; and

a pharmaceutically acceptable carrier.

10. The pharmaceutical composition of claim **9**, wherein the andrographolide lactones, the polysaccharides, and the flavonoids constitute 13-17%, 20-25%, and 12-14% of the dry weight of the extract, respectively.

11. The pharmaceutical composition of claim **9**, wherein the andrographolide, the 14-deoxyandrographolide, the 14-deoxy- 11, 12-dehydrogen-andrographolide, and the neoandrographolide constitute 3-10%, 0.01-2%, 2-5%, and 2-4% of the dry weight of the extract, respectively.

12. The pharmaceutical composition of claim **9**, wherein the polysaccharides, the flavonoids, the andrographolide, the 14-deoxyandrographolide, the 14-deoxy- 11,12-dehydrogen-andrographolide, and the neoandrographolide constitute 24.6%, 12.8%, 9.2%, <0.1%, 2.6%, and 3.0% of the dry weight of the extract, respectively.

13-25. (canceled)

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