

USE OF TOCOTRIENOLS IN THE TREATMENT OR PREVENTION OF ULCERATIVE COLITIS

FIELD OF THE INVENTION

[0001] The present invention relates to the field of molecular biology and biochemistry,
5 in particular the field of biochemistry and molecular biology relating to ulcerative colitis.

BACKGROUND OF THE INVENTION

[0002] Inflammatory bowel disease (IBD) is a common chronic gastrointestinal disorder
whose incidence in some countries occurs in more than 100,000 cases per year. IBD is an
umbrella term that covers a range of diseases. The two major forms of IBD include ulcerative
10 colitis (UC) and Crohn's Disease (CD). The similarity between these two diseases is that they
are both chronic wasting conditions characterized clinically by diarrhea and body weight loss
for which there is still no cure. Despite this common effect the characteristics of each disease
have distinct clinical, histopathological, pathophysiology, endoscopic, radiological features.
This has led to the generally accepted notion that CD and UC are distinct, if not discrete,
15 entities (Podolsky DK. 2002, Inflammatory bowel disease, N, Engl J Med, Vol 347, 417-
429).

[0003] In terms of area of the gastrointestinal tract affected CD can occur anywhere in the
gastrointestinal tract, but primarily in the ileum, whereas UC is restricted to the colon and
rectum. Histopathologically, CD is characterized by any or all of: skip lesions; fissuring
20 ulceration; transmural inflammation, and; granulomas whereas UC affects the mucosa and
upper submucosa, and does not have granulomas (other than to foreign bodies or ruptured
crypts). The differences in the immunological disturbances are significant. In CD the antigen
presenting cells and macrophages produce mainly interleukin-12 (IL-12) and IL-18 resulting
in the Th1-type polarization and production of pro-inflammatory cytokines including tumor
25 necrosis factor- α (TNF- α), interferon- γ (IFN- γ), and IL-2. Subsequently these cytokines
stimulate the antigen presenting cells to secrete other cytokines including IL-1, IL-6, IL-8,
IL-12, IL18, leading to a self sustaining cycle. In contrast UC exhibit a Th2 responses
characterized by increased secretion of IL-4, IL-5 and IL-13. Alternatively, a subgroup of UC
exhibits a Th17 responses characterized by increased secretion of IL-1 β , IL-6, IL-21 and IL-
30 23.

[0004] For animal studies of CD and UC chemically induced murine models have been
developed that resemble several important aspects of human CD and UC. Trinitrobenzene

sulfonic acid (TNBS) induced CD model uses hapten intrarectal administration of TNBS that results in pathology resembling CD in the distribution and location of lesions and allows the study of the early events in the initiation of CD. The pathology is driven by a potential initial IL-12 and TNF- α response, which preclude a counter regulatory IL-10 and TGF- β response.

5 Alternatively, the dextran sodium sulphate (DSS) induced UC model employs DSS to induce colitis. In contract to TNBS-induced CD, the pathology is not dependant on T cells and is therefore a more useful model to study UC. These models have become essential tools to investigate several important aspects of human CD (TNBS induced) and UC (DSS induced).

[0005] The initial diagnosis is very important because the two diseases react quite
10 differently to different treatments both in the mouse models and in patients (Triantafillidis et al. 2011, Current and emerging drugs for the treatment of inflammatory bowel disease. Drug Des Devel Ther.5: 185-210). Mesalazine also known as 5-aminosalicylic acid (5-ASA) a peroxisome proliferator-activated receptor- γ (PPAR- γ) agonists is an effective drug in the treatment of mild to moderately active distal UC however, the available data do not support a
15 role for mesalazine in the treatment of active CD (Triantafillidis et al. 2011)., Methotrexate can be used in patients with steroid dependant CD but it was not found efficacious in UC patients (Triantafillidis et al. 2011). Studies suggest that antibiotics are useful in the treatment of patients with CD but the role of antibiotics in UC is still open to further studies. While there are some treatments that have shown efficacy in both CD and UC such as
20 corticosteroids and Tacrolimus (Triantafillidis et al. 2011) these are more the exception than the rule and are prescribed to be used very differently depending whether it is to treat CD or UC. The severity of CD has led to a greater focus on the study and development of treatment for CD. Even the most effective drugs used to treat UC are only successful in about two thirds of patients, while often exhibiting potentially serious side effects. Although many
25 patients respond to existing therapies, available options for therapy remain inadequate for some patients with UC and alternative treatments are required.

[0006] UC is sometimes described as a chronic disease and sometimes as a recurrent disease. This is because the symptoms can be persistent or where some of the known treatments cause a remission, relapses are common. For most people, UC has a frustrating
30 pattern of flares and remissions. Thus one of the key aims in managing UC is to maintain a healthy colon by treatment as well as prevention of relapse.

SUMMARY OF THE INVENTION

[0007] A first aspect the present invention refers to a method for treating ulcerative colitis and/or preventing ulcerative colitis from occurring or reoccurring comprising administering

to a patient a composition comprising at least one tocotrienol, wherein the composition comprises the at least one tocotrienol in an amount that is pharmaceutically effective for the treatment and/or prevention of ulcerative colitis.

[0008] Other aspects of the invention would be apparent to a person skilled in the art with reference to the following drawings and discription of various non-limiting embodiments.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings. The drawings are not necessarily drawn to scale, emphasis instead generally being placed upon illustrating the principles of various embodiments. In the following description, various embodiments of the invention are described with reference to the following drawings in which:

[0010] **Figure 1.** Depicts the disease activity index of mice that were pre-treated for 1 week with either **A** 300mg/kg and **B** 150mg/kg doses of each treatment group prior to inducing UC with DSS, the treatment continued during and after the DSS treatment. * denotes statistical difference of groups versus DSS control using ANOVA Tukey/Kramer. P values < 0.05 were considered statistically significant.

[0011] **Figure 2.** Depicts the weight of mice that were pre-treated for 1 week with either 300mg/kg and 150mg/kg doses of each treatment group prior to inducing UC with DSS, the treatment continued during and after the DSS treatment. * denotes statistical difference of groups versus DSS control (P values < 0.05) on Day 17.

[0012] **Figure 3.** Depicts the histopathological score of mice that were pre-treated for 1 week with either 300mg/kg and 150mg/kg doses of each treatment group prior to inducing UC with DSS, the treatment continued during and after the DSS treatment. * denotes statistical difference of groups versus DSS control (P values < 0.05).

[0013] **Figure 4.** Depicts the length of the colon of mice that were pre-treated for 1 week with either 300mg/kg and 150mg/kg doses of each treatment group prior to inducing UC with DSS, the treatment continued during and after the DSS treatment, * denotes statistically significant from DSS control.

[0014] **Figure 5.** Depicts the weight of the spleen of mice that were pre-treated for 1 week with either 300mg/kg and 150mg/kg doses of each treatment group prior to inducing UC with DSS, the treatment continued during and after the DSS treatment, * denotes statistically significant from DSS control.

[0015] **Figure 6.** Depicts the weight of the colon after removing the fecal mater of mice that were pre-treated for 1 week with either 300mg/kg and 150mg/kg doses of each treatment group prior to inducing UC with DSS, the treatment continued during and after the DSS treatment, * denotes statistically significant from DSS control.

5 [0016] **Figure 7. (A)** A micrograph of colon cross sections of control mice that were not treated with DSS to induce UC. **(B)** A micrograph of colon cross sections of control mice that were not given a pretreatment prior to inducing UC with DSS or continuing treatment during and after the DSS treatment.

10 [0017] **Figure 8.** A micrograph of colon cross sections of mice that were pretreated for one week with 300mg/kg of tocotrienol rich fraction (DVL95) prior to inducing UC with DSS and continuing treatment with 300mg/kg DVL95 during and after the DSS treatment.

[0018] **Figure 9.** A micrograph of colon cross sections of mice that were pretreated for one week with 300mg/kg of Alpha tocopherol (ATP) prior to inducing UC with DSS and continuing treatment with 300mg/kg of ATP during and after the DSS treatment.

15 [0019] **Figure 10.** A micrograph of colon cross sections of mice that were pretreated for one week with 150mg/kg doses of tocotrienol rich fraction (DVL95) prior to inducing UC with DSS and continuing treatment with 150mg/kg doses of DVL95 during and after the DSS treatment.

20 [0020] **Figure 11.** A micrograph of colon cross sections of mice that were pretreated for one week with 150mg/kg of Alpha tocopherol (ATP) prior to inducing UC with DSS and continuing treatment with 150mg/kg of ATP during and after the DSS treatment.

[0021] **Figure 12.** Depicts the disease activity index of mice that were given DSS to induce UC and then treated with either 300mg/kg or 150mg/kg doses of each treatment group after the DSS treatment. * denotes statistical difference of groups versus DSS control using ANOVA P values < 0.05 were considered statistically significant.

[0022] **Figure 13.** Depicts the weight of mice that were given DSS to induce UC and then treated with either 300mg/kg or 150mg/kg doses of each treatment group after the DSS treatment * denotes statistical difference of groups versus DSS control (P values < 0.05) on Day 12.

30 [0023] **Figure 14.** Depicts the histopathological score of mice that were given DSS to induce UC and then treated with either 300mg/kg or 150mg/kg doses of each treatment group after the DSS treatment * Denotes statistical difference compared to DSS control.

[0024] **Figure 15.** Depicts the length of the colon of mice that were given DSS to induce UC and then treated with either 300mg/kg or 150mg/kg doses of each treatment group after the DSS treatment * Denotes statistical difference to DSS control.

[0025] **Figure 16.** Depicts the weight of the spleen of mice that were given DSS to induce UC and then treated with either 300mg/kg or 150mg/kg doses of each treatment group after the DSS treatment * Denotes statistical difference to DSS control.

[0026] **Figure 17.** Depicts the weight of the colon after removing the fecal mater of mice that were given DSS to induce UC and then treated with either 300mg/kg or 150mg/kg doses of each treatment group after the DSS treatment* Denotes statistical difference to DSS control.

DETAILED DESCRIPTION OF THE INVENTION

[0027] Managing ulcerative colitis requires maintaining a colon in a non-diseased state of relapse that requires both treatment of the active disease and prevention of a relapse. It has been demonstrated that a composition comprising at least one tocotrienol such as γ -tocotrienol (γ T3), or tocotrienol rich fraction (TRF) for example, are able to both prevent and treat the severe effects of UC. These findings are supported by *in vivo* data as can be observed from the experimental results referred to herein. The general principal of this aspect of the present invention is illustrated in the Figures based on examples in which a composition comprising at least one tocotrienol for example, γ -tocotrienol (γ T3), or tocotrienol rich fraction (TRF) demonstrated significant suppression of UC compared to the controls and other treatments such as 5-ASA.

[0028] Accordingly, a first aspect the present invention refers a method treating ulcerative colitis and/or preventing ulcerative colitis from occurring or reoccurring comprising administering to a patient a composition comprising at least one tocotrienol, wherein the composition comprises the at least one tocotrienol in an amount that is pharmaceutically effective for the treatment and/or prevention of ulcerative colitis.

[0029] The term “treat” or “treating” as used herein is intended to refer to providing a pharmaceutically effective amount of a composition comprising at least one tocotrienol, sufficient to reduce the development of a weakened and/or unhealthy state; and/or providing a subject or patient with a sufficient amount of the composition or medicament thereof so as to alleviate or eliminate a disease state/disorder and/or the symptoms of a disease state/disorder, and a weakened and/or unhealthy state. Examples of treatment include reducing the disease activity index, stopping or slowing the loss of body weight or reducing a histopathological score to below 3.

[0030] With “preventing ulcerative colitis from occurring or reoccurring it is referred to the act of preventing or hindering ulcerative colitis from occurring or reoccurring. In the present case, administering a composition referred to herein has the effect that the ulcerative colitis condition cannot develop in a patient or an animal body. Prevention is to be differentiated from “treatment” in which a composition referred to herein would be used for treating ulcerative colitis which already exist in the patient or animal body at the time of the administration or in other words for the treatment of a patient or animal body already suffering from a ulcerative colitis. Examples of preventing ulcerative colitis from occurring or reoccurring include maintaining a low disease activity index, a normal body weight or a histopathological score below 3 and avoiding a relapse to a UC state by maintaining a remission state.

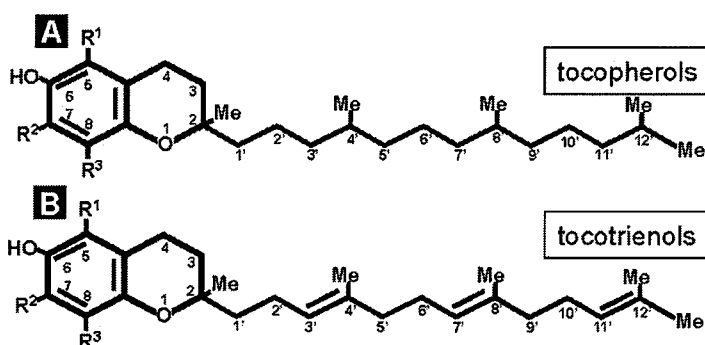
[0031] In general, “ulcerative colitis” (UC) is considered to refer to any one of the following characteristics: the UC occurs in the colon and/or has inflammation of the mucosa and/or the upper submucosa, and does not have granulomas (other than to foreign bodies or ruptured crypts). The antigen presenting cells and macrophages exhibit a Th2 responses characterized by increased secretion of IL-4, IL-5 and IL-13. Alternatively, a subgroup of UC exhibits a Th17 responses characterized by increased secretion of IL-1 β , IL-6, IL-21 and IL-23.

[0032] An amount that is pharmaceutically effective for the treatment and/or prevention of UC may includes at least 3mg/kg, more preferably at least 10mg/kg and most preferably at least 12mg/kg and may be as high as between 20mg/kg to 30mg/kg. The composition may comprises at least 30 weight % preferably at least 50 weight % of the at least one tocotrienol. In preferred embodiments the at least one tocotrienol is selected from the group consisting of α (alpha)-tocotrienol, β (beta)-tocotrienol, γ (gamma)-tocotrienol, δ (delta)-tocotrienol and mixtures of the aforementioned tocotrienols, preferably γ (gamma)-tocotrienol, δ (delta)-tocotrienol and mixtures thereof. In various embodiments the composition may further comprise at least one tocopherol.

[0033] As described herein, for the treatment of UC or prevention of UC from occurring or reoccurring, a composition comprising at least one tocotrienol is used. Tocotrienols (T3) are found mainly in palm oil but can also be found in other sources including but not limited to in annatto, rice bran etc. Together with tocopherols (T), they provide a significant source of anti-oxidant activity to all living cells. This common anti-oxidant attribute reflects the similarity in chemical structures of the tocotrienols and the tocopherols, which differ only in their structural side chain (contains farnesyl for tocotrienol or saturated phytyl side chain for

tocopherol). The common hydrogen atom from the hydroxyl group on the chromanol ring acts to scavenge the chain-propagating peroxy free radicals. Depending on the locations of methyl groups on their chromanol ring, tocopherols and tocotrienols can be distinguished into four isomeric forms: alpha (α), beta (β), gamma (γ), and delta (δ).

[0034] Different tocopherol and tocotrienol isoforms exist (see Formula I and II). Tocopherols consist of a chromanol ring and a 15-carbon tail derived from homogentisate (HGA) and phytyl diphosphate, respectively. On the other hand, tocotrienols differ structurally from tocopherols by the presence of three *trans* double bonds in the hydrocarbon tail. Formula I and Formula II and the description following it provide an overview about the known isoforms of tocopherols (T) and tocotrienols (T3).



Formula I

Formula II

[0035] Formula I (A): $R^1 = R^2 = R^3 = \text{Me (CH}_3\text{)}$, known as α (alpha)-tocopherol, is designated α -tocopherol or 5,7,8-trimethyltolcol; $R^1 = R^3 = \text{Me}$; $R^2 = \text{H}$, known as, β (beta)-tocopherol, is designated, β -tocopherol or 5,8-dimethyltolcol; $R^1 = \text{H}$; $R^2 = R^3 = \text{Me}$, known as γ (gamma)-tocopherol, is designated γ -tocopherol or 7,8-dimethyltolcol; $R^1 = R^2 = \text{H}$; $R^3 = \text{Me}$, known as δ (delta)-tocopherol, is designated δ -tocopherol or 8-methyltolcol. Formula II (B): $R^1 = R^2 = R^3 = \text{H}$, 2-methyl-2-(4,8,12-trimethyltrideca-3,7,11-trienyl)chroman-6-ol, is designated tocotrienol; $R^1 = R^2 = R^3 = \text{Me}$, formerly known as ζ_1 or ζ_2 -tocopherol, is designated 5,7,8-trimethyltocotrienol or α (alpha)-tocotrienol. The name tocochroman-3 has also been used; $R^1 = R^3 = \text{Me}$; $R^2 = \text{H}$, formerly known as ϵ -tocopherol, is designated 5,8-dimethyltocotrienol or β (beta)-tocotrienol; $R^1 = \text{H}$; $R^2 = R^3 = \text{Me}$, formerly known as γ -tocopherol, is designated 7,8-dimethyltocotrienol or (gamma) γ -tocotrienol. The name plastochroman-3 has also been used; $R^1 = R^2 = \text{H}$; $R^3 = \text{Me}$ is designated 8-methyltocotrienol or δ (delta)-tocotrienol.

[0036] The at least one tocotrienol used in the composition referred to herein can comprise or consist of at least one of tocotrienol, or a mixture of tocopherol, and tocotrienol. In some embodiments, the at least one tocotrienol used herein can be a mixture of at least one

at least one tocotrienol or a tocotrienol-rich fraction (TRF). A tocotrienol-rich fraction typically refers to a mixture of different isomers of tocotrienol and tocopherols, for example, α -tocopherol, α -tocotrienol, β -tocotrienol, γ -tocotrienol, and δ -tocotrienol. The tocotrienol-rich fraction can further include other components such as plant phytosterols, carotenoids and squalene to name only a few. Tocotrienol-rich fraction can for example be obtained from palm oil, rice bran, annatto, or grape seed. Typically a TRF comprises less than 30%, preferably less than 10% tocopherols of the total weight of the fraction. As such when the composition comprises a TRF the composition comprises less than 30%, preferably less than 10% tocopherol of the total weight of the composition.

[0037] In some embodiments, the at least one tocotrienol used in the composition referred to herein can comprise, consist essentially of or consist of γ -tocotrienol, δ -tocotrienol, tocotrienol-rich fraction (TRF), or mixtures thereof.

[0038] In various embodiments the composition contains the at least one tocotrienol as the pharmaceutical active ingredient, optionally as the sole pharmaceutical active ingredient.

[0039] In some embodiments, the composition may further comprise a probiotic. A probiotic is a live organisms, usually bacteria that is used as a treatment. In the case of UC the administration of a probiotic will affect the luminal flora of the colon. The luminal flora is suspected to play a role in UC etiology. The probiotic may include *Bifidobacterium longum*; *Streptococcus faecium*; *S. thermophilus*; *B. breve*, *B. infantis*, *Lactobacillus acidophilus*, *L. plantarum*, *L. casei*, *L. bulgaricus*, *Escherichia coli* Nissle 1917, *Saccharomyces boulardii* or mixtures thereof.

[0040] In some embodiments, the composition may further comprise a peroxisome proliferator-activated receptor- γ (PPAR- γ) agonist. The PPAR- γ agonist may include sulfasalazine, or 5-aminosalicylic acid (5-ASA). In a preferred embodiment the PPAR- γ agonist is 5-aminosalicylic acid (5-ASA).

[0041] In some embodiments, the composition may further comprise an immunosuppressive drug. The immunosuppressive drug may include Tacrolimus, cyclosporine, azathioprine or 6-Mercaptopurine. Preferably the immunosuppressive drug is administered together with the at least one tocotrienol to a patient suffering from refractory UC. Preferably cyclosporine is administered together with the at least one tocotrienol to a patient suffering from a severe flare-up of UC.

[0042] In some embodiments, the composition may further comprise a TNF alpha antagonist. The TNF alpha antagonist may include a TNF alpha antibody, preferably a TNF alpha monoclonal antibody.

5 [0043] In one embodiment, the at least one tocotrienol component can be comprised in an enriched formulation. "Enriched" means that at least one tocotrienol is comprised in an amount which is higher than in the normal mixture comprising all other tocotrienols. For example, tocotrienol isolated from, e.g., palm oil, comprises γ -tocotrienol and δ -tocotrienol in an amount of less than 10 wt.% based on the total weight of the oil. Thus, with respect to the
10 embodiments of the present invention, an "enriched" formulation means any formulation comprising a specific tocotrienol, for example, γ -tocotrienol or σ -tocotrienol or a mixture of γ -tocotrienol and δ -tocotrienol, in an amount of more than 0.1 % of the respective tocotrienol based on the total weight of the formulation (or composition).

[0044] In another embodiment, the enriched formulation can comprise a specific tocotrienol component in an amount of about 0.1 wt.%, 0.5 wt.%, 1 wt.%, 2 wt.%, 3 wt.%, 4
15 wt.%, 5 wt.%, 10 wt.%, 15 wt.%, 20 wt.%, 25 wt.%, 30 wt.%, 35 wt.%, 40 wt.%, 45 wt.%, 50 wt.%, 55 wt.%, 60 wt.%, 65 wt.%, 70 wt.%, 75 wt.%, 80 wt.%, 85 wt.%, 90 wt.%, 92 wt.%, 94 wt.%, 96 wt.%, 97 wt.% or 98 wt.% total content based on the total weight of the enriched formulation.

[0045] The amount of composition referred to herein can be administered to the patient at
20 any appropriate concentration as long as the composition provides the intended desired effect and does not cause an adverse effect to the patient. In some embodiments, the amount of composition administered to the patient can be between about 1 mg and about 1500 mg; between about 1 mg and about 1200 mg; about 1 mg and about 1000 mg; about 1 mg and about 800 mg; about 1 mg and about 500 mg; about 10 mg and about 1500 mg; about 25 mg
25 and about 1500 mg; about 30 mg and about 1500 mg; about 30 mg and about 1000 mg; about 40 mg and about 1000 mg; about 10 mg and about 800 mg; or between about 10 mg and about 500 mg. Preferably the amount is adjusted for the size of the patient such that an amount of at least 3mg/kg is administered, more preferably at least 10mg/kg and most preferably at least 12mg/kg and may be as high as between 20mg/kg to 30mg/kg. In one
30 embodiment, the patient is an animal. The animal can for example be a mammal such as, but are not limited to a human, pig, horse, mouse, rat, cow, dog or cat. Where the animal is a human preferably the dose is between 730 to 1,460mg for a 60kg human which is equivalent to between about 10mg/kg to about 25mg/kg.

[0046] In one embodiment, the composition can be administered as a softgel, a hard capsule, tablet, gel, sustained-release formulation, lotion, an enema, ointment, gel, spray, thin liquid, cream, injectable formulation, nanoparticle form or emulsion of nanoparticle or in encapsulated form.

5 [0047] In one embodiment, the composition can be administered in a water soluble form. Thus, when desired, the compositions referred to herein can be water solubilized by the addition of specific compounds. A water solubilized form of a composition referred to herein can be obtained, for example, by formulating it into a solid dispersion. Other methods of formulating water-dispersible or water-soluble tocotrienol forms are disclosed for example in
10 US Patent No. 5,869,704.

[0048] The term "solid dispersion" defines a system in a solid state (as opposed to a liquid or gaseous state) comprising at least two components, wherein one component is dispersed throughout the other component or components. For example, the components of the composition can be dispersed in a matrix comprised of a pharmaceutically acceptable
15 water-soluble polymer(s) and a pharmaceutically acceptable surfactant(s).

[0049] The term "solid dispersion" encompasses systems having small particles of one phase dispersed in another phase. These particles are typically of less than 400 μm in size, for example less than 100 μm , 10 μm , or 1 μm in size. When said dispersion of the components is such that the system is chemically and physically uniform or homogenous throughout or
20 consists of one phase (as defined in thermodynamics), such a solid dispersion will be called a "solid solution" or a "glassy solution." A glassy solution is a homogeneous, glassy system in which a solute is dissolved in a glassy solvent.

[0050] Such solid dispersions can be administered via different routes. For example, orally administered solid dosage forms include but are not limited to capsules, dragées,
25 granules, pills, powders, and tablets. Excipients commonly used to formulate such dosage forms include encapsulating materials or formulation additives such as absorption accelerators, antioxidants, binders, buffers, coating agents, colouring agents, diluents, disintegrating agents, emulsifiers, extenders, fillers, flavouring agents, humectants, lubricants, preservatives, propellants, releasing agents, sterilizing agents, sweeteners,
30 solubilizers, and mixtures thereof.

[0051] Excipients for orally administered compounds in solid dosage forms can include, but are not limited to agar, alginic acid, aluminium hydroxide, benzyl benzoate, 1,3-butylene glycol, castor oil, cellulose, cellulose acetate, cocoa butter, corn starch, corn oil, cottonseed oil, ethanol, ethyl acetate, ethyl carbonate, ethyl cellulose, ethyl laureate, ethyl oleate,

gelatine, germ oil, glucose, glycerol, groundnut oil, isopropanol, isotonic saline, lactose, magnesium hydroxide, magnesium stearate, malt, olive oil, peanut oil, potassium phosphate salts, potato starch, propylene glycol, talc, tragacanth, water, safflower oil, sesame oil, sesamin, sesamol, sodium carboxymethyl cellulose, sodium lauryl sulfate, sodium phosphate salts, soybean oil, sucrose, tetrahydro fur fury 1 alcohol, and mixtures thereof.

[0052] In one embodiment, a dosage form can comprise a solid solution or solid dispersion of at least one tocotrienol and at least one additional component. The matrix can comprise at least one pharmaceutically acceptable water-soluble polymer and at least one pharmaceutically acceptable surfactant. Suitable pharmaceutically acceptable water-soluble polymers include, but are not limited to, water-soluble polymers having a glass transition temperature (T_g) of at least 50°C, or at least 60 °C, or from about 80 °C to about 180 °C.

[0053] Water-soluble polymers having a T_g as defined above allow for the preparation of solid solutions or solid dispersions that are mechanically stable and, within ordinary temperature ranges, sufficiently temperature stable so that the solid solutions or solid dispersions can be used as dosage forms without further processing or be compacted to tablets with only a small amount of tableting aids.

[0054] The water-soluble polymer comprised in a dosage form referred to herein is a polymer that can have an apparent viscosity, when dissolved at 20°C in an aqueous solution at 2 % (w/v), of 1 to 5000 mPa s, or of 1 to 700 mPa s, or of 5 mPa s to 100 mPa s.

[0055] Water-soluble polymers suitable for use in a dosage form referred to herein can include, but are not limited to homopolymers and copolymers of N-vinyl lactams, especially homopolymers and copolymers of N-vinyl pyrrolidone, e.g. polyvinylpyrrolidone (PVP), copolymers of N-vinyl pyrrolidone and vinyl acetate or vinyl propionate; cellulose esters and cellulose ethers, in particular methylcellulose and ethylcellulose, hydroxyalkylcelluloses, in particular hydroxypropylcellulose, hydroxyalkylalkylcelluloses, in particular hydroxypropylmethylcellulose, cellulose phthalates or succinates, in particular cellulose acetate phthalate and hydroxypropylmethylcellulose phthalate, hydroxypropylmethylcellulose succinate or hydroxypropylmethylcellulose acetate succinate; high molecular polyalkylene oxides such as polyethylene oxide and polypropylene oxide and copolymers of ethylene oxide and propylene oxide; polyacrylates and polymethacrylates such as methacrylic acid/ethyl acrylate copolymers, methacrylic acid/methyl methacrylate copolymers, butyl methacrylate/2-dimethylaminoethyl methacrylate copolymers, poly(hydroxyalkyl acrylates), poly(hydroxyalkyl methacrylates); polyacrylamides, vinyl acetate polymers such as copolymers of vinyl acetate and crotonic acid, partially hydrolyzed polyvinyl acetate (also

referred to as partially saponified "polyvinyl alcohol"), polyvinyl alcohol; oligo- and polysaccharides such as carrageenans, galactomannans and xanthan gum, or mixtures of one or more thereof.

[0056] The term "pharmaceutically acceptable surfactant" as used herein refers to a pharmaceutically acceptable non-ionic surfactant. A dosage form referred to herein comprises at least one surfactant having a hydrophilic lipophilic balance (HLB) value of from 12 to 18, or from 13 to 17, or from 14 to 16. The HLB system attributes numeric values to surfactants, with lipophilic substances receiving lower HLB values and hydrophilic substances receiving higher HLB values.

[0057] In one embodiment, a dosage form referred to herein comprises one or more pharmaceutically acceptable surfactants selected from polyoxy ethylene castor oil derivatives, e.g. polyoxyethyleneglycerol triricinoleate or polyoxyl 35 castor oil (Cremophor[®] EL) or polyoxyethyleneglycerol oxystearate such as polyethylenglycol 40 hydrogenated castor oil (Cremophor[®] RH 40, also known as polyoxyl 40 hydrogenated castor oil or macrogolglycerol hydroxystearate) or polyethylenglycol 60 hydrogenated castor oil (Cremophor[®] RH 60); or a mono fatty acid ester of polyoxy ethylene (20) sorbitan, e.g. polyoxyethylene (20) sorbitan monooleate (Tween[®] 80), polyoxyethylene (20) sorbitan monostearate (Tween[®] 60), polyoxyethylene (20) sorbitan monopalmitate (Tween[®] 40), or polyoxyethylene (20) sorbitan monolaurate (Tween[®] 20). Other surfactants including those with HLB values of greater than 18 or less than 12 may also be used, e.g., block copolymers of ethylene oxide and propylene oxide, also known as polyoxyethylene polyoxypropylene block copolymers or polyoxyethylene polypropyleneglycol, such as Poloxamer[®] 124, Poloxamer[®] 188, Poloxamer[®] 237, Poloxamer[®] 388, or Poloxamer[®] 407.

[0058] Where two or more surfactants are used, the surfactant(s) having an HLB value of from 12 to 18 preferably accounts for at least 50 % by weight, more preferably at least 60 % by weight, of the total amount of surfactants used.

[0059] A dosage form referred to herein can also include additional excipients or additives such as flow regulators, lubricants, bulking agents (fillers) and disintegrants. Such additional excipients may comprise, without limitation, from 0 % to 15 % by weight of the total dosage form.

[0060] Dosage forms referred to herein can be provided as dosage forms consisting of several layers, for example laminated or multilayer tablets. They can be in open or closed form. "Closed dosage forms" are those in which one layer is completely surrounded by at least one other layer. Multilayer forms have the advantage that two active ingredients which

are incompatible with one another can be processed, or that the release characteristics of the active ingredient(s) can be controlled. For example, it is possible to provide an initial dose by including an active ingredient in one of the outer layers, and a maintenance dose by including the active ingredient in the inner layer(s). Multilayer tablets types may be produced by
5 compressing two or more layers of granules.

[0061] Furthermore, a film coat on the tablet can contribute to the ease with which a tablet can be swallowed. A film coat also improves taste and provides an elegant appearance. If desired, the film-coat may be an enteric coat. The film-coat usually includes a polymeric film-forming material such as hydroxypropyl methylcellulose, hydroxypropylcellulose, and
10 acrylate or methacrylate copolymers. Besides a film-forming polymer, the film-coat may further comprise a plasticizer, e.g. polyethylene glycol, a surfactant, e.g. a Tween[®] type, and optionally a pigment, e.g. titanium dioxide or iron oxides. The film-coating may also comprise talc as anti-adhesive. The film coat usually accounts for less than 5 % by weight of the dosage form.

[0062] Other specific forms of formulating the compositions referred to herein, include, but are not limited to native oil liquids of tocotrienols, such as palm oil, which can be used for the manufacture of a soft gel, a water soluble emulsion liquid form, which can be used for the manufacture of soft drinks, a cold water dispersible powder, which can be used for the manufacture of soft capsules and tablets, or beadlets, which can be used for the manufacture
20 of hard capsules.

[0063] For the manufacture of the compositions referred to herein in form of water soluble emulsion liquid, tocotrienol liquids are used as starting material to which one adds glycerine and blends of emulsifiers. Afterwards the mixture is homogenized into an emulsion.

[0064] Examples for emulsifiers which can be used for the formulation of water soluble
25 emulsion liquid include, but are not limited to glycerine fatty acid esters, acetic acid esters of monoglycerides, lactic acid esters of monoglycerides, citric acid esters of monoglycerides, succinic acid esters of monoglycerides, diacetyl tartaric acid esters of monoglycerides, polyglycerol esters of fatty acids, polyglycerol polyricinoleate, sorbitan esters of fatty acids, propylene glycol esters of fatty acids, starch derivatives, surfactants, sucrose esters of fatty
30 acids, calcium stearoyl di lactate, lecithin, or enzyme digested lecithin / enzyme treated lecithin.

[0065] Cold water dispersible powders of the compositions referred to herein can be manufactured by providing tocotrienol oil liquids as starting material. Emulsifiers, such as

modified corn starch, maltodextrin, cyclodextrins or corn starch, are added to the tocotrienol oil. The mixture can afterwards be spray dried into a dry powder.

[0066] Beadlets comprising compositions referred to herein can be obtained by providing tocotrienol oil liquids as starting material. Afterwards, gelatine, corn starch, sucrose and ascorbyl palmitate are added in one embodiment to the tocotrienol oil. The mixture is spray dried into dry beadlets.

[0067] Enema formulations which allow rectal introduction and delivery of the above compositions into the animal body can be manufactured by providing tocotrienol oil liquids as starting material. In another embodiment the compositions are formulated in a water-in-oil formulation.

[0068] The composition referred to herein can be administered into the patient via any suitable means as long as the intended therapeutic or disease control is achieved. In some embodiments, the composition can for example be administered into the patient via oral, or rectal, or intestinal or topical administration.

[0069] The pharmaceutical composition further includes a pharmaceutically acceptable carrier or excipient. The "carrier" or "excipient" can include any pharmaceutically acceptable carrier as long as the carrier is compatible with other ingredients of the formulation and not injurious to the patient. Accordingly, pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen. The pharmaceutically acceptable carrier or excipient can be any of cellulose, hydroxymethylcellulose, cellulose acetate phthalate (CAP) gellan gum, polyalcohol, polyvinyl alcohol, hyaluronic acid, polyacrylic acid, carbopol polymer, poloxamer, poly(oxyethylene) and poly(oxypropylene) and block copolymers thereof, polyethylene oxide, polycarbophil, chitosan, cyclodextrin, liposome, nanoparticle, microparticle including microsphere and nanosphere, niosome, pharmacosome, collagen shield, ocular film or combinations thereof.

[0070] In another aspect of the present invention, there is provided a pharmaceutical composition comprising at least one tocotrienol for the treatment and/or prevention of ulcerative colitis.

[0071] In another aspect of the present invention, there is provided use of a composition comprising at least one tocotrienol in an amount that is pharmaceutically effective for the treatment and/or prevention of ulcerative colitis.

[0072] In another aspect of the present invention, there is provided a method of manufacturing a composition comprising at least one tocotrienol in an amount that is pharmaceutically effective and suitable for use in the treatment and/or prevention of ulcerative colitis.

[0073] The invention illustratively described herein may suitably be practiced in the absence of any element or elements, limitation or limitations, not specifically disclosed herein. Thus, for example, the terms "comprising", "including", "containing", etc. shall be read expansively and without limitation. Additionally, the terms and expressions employed herein have been used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the inventions embodied therein herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

[0074] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[0075] Other embodiments are within the following claims and non-limiting examples. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

EXAMPLES

Example 1: Prevention

[0076] To determine the efficacy of the composition in the prevention of UC mice were given a treatment for 7 days and then induced to develop UC by administering DSS at 5% in

the mice' drinking water for 5 days while continuing the treatment during and after the DSS administration. The regimen is outlined in table 1.

Acclimatization/ Regular drinking water with Treatment daily	5% DSS in drinking water with Treatment daily	Regular drinking water with Treatment daily	Study termination
Day 1 ----- Day 7	Day 8 ----- Day 12	Day 13 ----- Day 19	
Body weight monitored daily and disease progression (clinical signs) observed after DSS treatment (2- 3 times per week)			Sample collection for histopath & blood analysis

Table 1: the daily treatment regimen

5

[0077] The treatments included Water alone without induction of UC with DSS; 5-ASA; a tocotrienol rich fraction (DVL-95); γ -tocotrienol (GD-T3); and alpha-tocopherol (ATP). A summary of the treatments and regimens are outlined in table 2.

Treatment Group	Animal Dose Level (mg/kg/day)	Route	Dose Regimen	Number of mice (Male)
Water alone (Non-treated)	0	Water	Days 1 - 19	8
DSS + Vehicle	5% DSS	Water	Days 1 - 19	8 - 12
DSS + DVL-95	300 mg/kg	Water	Days 1 - 19	4
	150 mg/kg			4
DSS + Gamma-Delta T3	300 mg/kg	Water	Days 1 - 19	4
	150 mg/kg			4
DSS + Alpha TP	300 mg/kg	Water	Days 1 - 19	4
	150 mg/kg			4
DSS + 5-ASA	ASA = 75 mg/kg	Oral Gavage	Day 8 - 19	4 - 8

10 **Table 2:** summary of the treatments and regimens

[0078] The Disease Activity Index (DAI) as described by Murano M et al, 2000; Makoto S et al, 2003; and Kim HS et al, 1992 was recorded 2 – 3 times weekly. Essentially the DAI is the combined score of weight loss, stool consistency and bleeding as outlined in table 3

15 divided by 3.

Body Weight loss	Weight Loss (%)	
	0	(-)
	1	1 – 5%
	2	5 – 10%
	3	10 – 15%
	4	> 15%
Fecal pellets	Stool consistency score	
	0	Normal fecal pellet
	2	Loose fecal pellet
	4	Frank diarrhea
Occult/Gross bleeding	Coloscreen occult blood card test	
	0	Negative
	2	Positive
	4	Gross bleeding

Table 3 : scores for calculating the disease activity index of UC.

[0079] Measurements of the disease activity index (DAI) were conducted as described above. In Figure 1 both 300mg/kg and 150mg/kg doses of DVL-95 and GD-T3 were effective in bringing down the disease activity index (DAI) to almost 0, similar to that of 5-ASA (the standard of care UC drug), but not for the ATP treated groups, which failed to lower DAI score. Data analyzed on Day 17 showed statistically significant difference of 5-ASA, DVL95 and GDT3 compared to DSS control. ATP group, up to 300mg/kg, on the other hand, were not statistically different from DSS.

[0080] Figure 2 demonstrates that the weight of the treated mice with both 300mg/kg and 150mg/kg doses of DVL-95, GD-T3 and 5-ASA were progressively increasing to almost healthy levels. However the ATP treatment did not rescue the weight of mice as they still developed weight loss indicative of UC flare-up.

[0081] Histopathology scoring of colons was conducted by a certified veterinary pathologist. The Inflammatory Index Scoring based on tissue sections stained with H & E was conducted as known in the art and described in CS Yang et al, 2009 and Yang GY et al, 2002. Essentially the scores of four individual inflammatory parameters in the Inflammatory Index Scoring are added to obtain the histopathological score. The grades of the Inflammatory Index Scoring are summarized in table 4

Feature graded	Grade	Description
Inflammation severity	0	Normal mucosa
	1	Mild inflammation
	2	Moderate inflammation
	3	Severe inflammation
Ulceration	0	Absent
	1	Present
Inflammation area	0	0%
	1	1 – 25%
	2	26 – 50%
	3	51 – 75%
	4	76 – 100%
Hyperplasia and Dysplasia	0	Normal
	1	Mild Hyperplasia
	2	Low-grade Dysplasia
	3	High-grade Dysplasia

Table 4: The grades of the Inflammatory Index Scoring

[0082] The Histopathological score was measured as described above. The most effective results were observed in the DVL-95 treatment groups whereby an absence of inflammation indices was observed in mice treated with 150mg/kg of the tocotrienol rich fraction and minimal inflammation indices was observed in mice treated with 300mg/kg. The control group (DSS) and ATP had the highest inflammation score (Figure 3).

[0083] Macroscopic measurements were taken of the length of the colon and the weight of the spleen as is known in the art. UC induced mice exhibited shortened colons. Shortening of the colon was prevented in mice pretreated with DVL-95, GD-T3 and 5-ASA but not those pretreated with ATP (Figure 4). DSS treated groups resulted in shortening of the colons. On the contrary, most of the DVL/GDT3 treated groups were able to improve this effect ($P < 0.05$), except the ATP group.

[0084] DSS treated groups of UC induced mice exhibited colitis-associated splenomegaly (enlargement of spleen), as determined by spleen weight. 5-ASA/DVL/GDT3

treated groups had reduced spleen enlargement compared to DSS groups and ATP groups ($P < 0.05$). Splenomegaly was prevented in mice pretreated with DVL-95, GD-T3 and 5-ASA but not those pretreated with ATP (Figure 5).

[0085] DSS treated groups of UC induced mice exhibited increased colonic weight compared to the 5-ASA/DVL/GDT3 treated groups (Figure 6).

[0086] The DSS induced UC was able to result in UC histology including mucosal inflammation and loss of crypts (see Figure 7 the comparison to the control samples of figure 7a and the DSS induced samples 7b). ATP treatment resulted in some ulceration (Figures 9 and 11) whereas the DVL-95 treatment resulted in sections similar to the control (Figures 8 and 10).

[0087] Administration of the TRF of DVL-95 was demonstrated to maintain the remission state during induction of UC and GD-T3 and 5-ASA minimized the UC flare-up after DSS induction of UC.

Example 2: Treatment

[0088] To determine the efficacy of the composition in the treatment of UC, mice induced to develop UC by administering DSS at 5% in the mices' drinking water for 5 days after which they were given treatment after the DSS administration. The regimen is outlined in table 5.

5% DSS in drinking water	Regular drinking water with Treatment daily	Study termination
Day 1 ----- Day 5	Day 6 ----- Day 12	Sample collection for histopath & blood analysis
Body weight monitored & disease progression (clinical signs) observed after DSS treatment (2- 3 times per week)		

Table 5: the daily treatment regimen.

[0089] The treatments included Water alone without induction of UC with DSS; water, 5-ASA; prednisone; and a tocotrienol rich reaction (DVL-95). A summary of the treatments and regimens are outlined in table 6.

Treatment Group	Animal Dose Level (mg/kg/day)	Route	Dose Regimen	Number of mice (Male)
DSS + Vehicle	5% DSS	Water	DSS (Day 1 – 5) Vehicle (Day 6 – 12)	6 - 12

DSS + 5-ASA	5% DSS 75mg/kg 5-ASA	Oral Gavage	DSS (Day 1 - 5) 5-ASA (Day 6 – 12)	4
DSS + Prednisolone	5% DSS 5mg/kg Prednisolone	Oral Gavage	DSS (Day 1 - 5) Pred (Day 6 – 12)	7
DSS + DVL-95	5% DSS 300mg/kg DVL95	Water	DSS (Day 1 - 5) DVL95 (Day 6 – 12)	7
DSS + DVL-95	5% DSS 150mg/kg DVL95	Water	DSS (Day 1 - 5) DVL95 (Day 6 – 12)	6
DSS + ATP	5% DSS 300mg/kg ATP	Water	DSS (Day 1 - 5) ATP (Day 6 – 12)	4 - 8
Non-treated control	Water	Water	-	8

Table 6: summary of the treatments and regimens

[0090] Measurements of the disease activity index (DAI) were conducted as described above. The results demonstrated that DVL-95 was the most effective treatment compared to the currently used treatments in bringing down the disease activity index (Figure 12). Data analyzed on Day 12 showed statistical difference of Prednisolone & DVL 95 (300mg/kg and 150mg/kg) compared to DSS control group.

[0091] Similarly, the weight of the treated mice with 300mg/kg dose of DVL-95, had the greatest rescue in mice weight compared with the standard of care 5-ASA and prednisolone (Figure 13). Data analyzed on Day 12 showed statistical difference of DVL-95 (300mg/kg) compared to DSS control group.

[0092] The Histopathological score was measured as described above. The Histopathological score was more effectively reduced in mice treated with 300mg/kg dose of DVL-95 than in any of the known treatments. (Figure 14). From the data, 5-ASA and 300mg/kg DVL 95 treated groups were able to reduce the inflammation scoring ($P < 0.05$).

[0093] Macroscopic measurements of the colon and the weight of the spleen demonstrated similar effective treatment. UC induced mice exhibited shortened colons. Shortening of the colon was recovered in mice treated with 300mg/kg doses of DVL-95, prednisolone and 5-ASA (Figure 15). 5-ASA, Pred and DVL-95 (300mg/kg & 150mg/kg) showed restoration of colon length to almost normal length, compared to ATP group.

[0094] UC induced mice exhibited splenomegaly (enlargement of the spleen), as determined by spleen weight. Splenomegaly was reduced in mice treated with DVL-95, prednisolone and 5-ASA (Figure 16). Most of the treatment groups showed statistically reduced spleen weight whereas ATP group was not able to ($P < 0.05$).

[0095] Most of the treatments showed improved colon weight ($P < 0.05$) to normal weight whereby ATP group was not able to. 300mg/kg doses of DVL-95, prednisolone and 5-

ASA showed a reduction in colon weight with treatment almost recovering the colon to the normal proximal weight (Figure 17).

[0096] Administration of the TRF of DVL-95 was demonstrated to be the most effective overall treatment of UC after DSS induction of UC.

5 [0097] Various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the inventions embodied herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

CLAIMS

What is claimed is:

1. A method of treating ulcerative colitis and/or preventing ulcerative colitis from occurring or reoccurring comprising administering to a patient a composition comprising at least one tocotrienol, wherein the composition comprises the at least one tocotrienol in an amount that is pharmaceutically effective for the treatment and/or prevention of ulcerative colitis.
2. The method of claim 1, wherein the composition comprises at least 30 wt.-%, preferably at least 50 wt.-%, of the at least one tocotrienol.
3. The method of claim 1 or 2, wherein the at least one tocotrienol is selected from the group consisting of α (alpha)-tocotrienol, β (beta)-tocotrienol, (gamma) γ -tocotrienol, δ (delta)-tocotrienol and mixtures of the aforementioned tocotrienols, preferably (gamma) γ -tocotrienol, δ (delta)-tocotrienol and mixtures thereof.
4. The method of any one of claims 1-3, wherein the composition further comprises at least one tocopherol.
5. The method of claims 4, wherein the tocopherol is selected from the group consisting of α -tocopherol, β (beta)-tocopherol, γ (gamma)-tocopherol, δ (delta)-tocopherol and mixtures of the aforementioned tocopherols.
6. The method of any one of claims 1 to 5, wherein the composition comprises less than 30 wt.-%, preferably less than 10 wt.-%, tocopherols.
7. The method of any one of claims 1 to 6, wherein the at least one tocotrienols comprises, consists essentially of or consists of (gamma) γ -tocotrienol, δ (delta)-tocotrienol, tocotrienol-rich fraction (TRF), or mixtures thereof.
8. The method of any one of claims 1 to 7, wherein the composition contains the at least one tocotrienol as the pharmaceutical active, optionally as the sole pharmaceutical active.

9. The method of any one of claims 1 to 8, wherein the composition comprising at least one tocotrienol further comprises a probiotic.

10. The method of any one of claims 1 to 8, wherein the composition comprising at least one tocotrienol further comprises a peroxisome proliferator-activated receptor- γ (PPAR- γ) agonist.

11. The method of claim 10 wherein the PPAR- γ agonist is 5-aminosalicylic acid (5-ASA) or sulfasalazine.

12. The method of any one of claims 1 to 8, wherein the composition comprising at least one tocotrienol further comprises an immunosuppressive drug.

13. The method of claim 12 wherein the immunosuppressive drug is Tacrolimus, cyclosporine, azathioprine or 6-Mercaptopurine.

14. The method of any one of claims 1 to 8, wherein the composition comprising at least one tocotrienol further comprises a TNF alpha antagonist.

15. The method of any one of claims 1 to 14, wherein the composition is administered in an amount of at least 3mg/kg, between about 1 mg to about 1500 mg or between about 500 mg and 1000 mg.

16. The method of any one of claims 1 to 15, wherein the patient is an animal.

17. The method of claim 16, wherein said animal is a mammal.

18. The method of claim 17, wherein the mammal is selected from the group consisting of human, pig, horse, mouse, rat, cow, dog and cat.

19. The method of any one of claims 1 to 18, wherein the composition is administered as a softgel, a hard capsule, a tablet, a gel, a liquid, an enema, a sustained-release formulation, an ointment, a cream, an injectable formulation, in nanoparticle form or in encapsulated form.

20. The method of any one of claims 1 to 18, wherein the composition is administered in a water soluble form.

5 21. The method of any one of claims 1 to 20, wherein the composition is administered orally or topically.

22. The method of any one of claims 1 to 21, wherein the composition further comprises a pharmaceutically acceptable excipient.

10 23. The method of any one of claims 1 to 22, wherein the at least one tocotrienol is comprised in an enriched formulation, wherein the enriched formulation comprises more than 0.1 wt.% of a specific tocotrienol component based on the total weight of the enriched formulation.

15 24. The method of claim 23, wherein the enriched formulation comprises an amount of the specific tocotrienol component which is selected from the group consisting of 0.1 wt.%, 0.5 wt.%, 1 wt.%, 2 wt.%, 3 wt.%, 4 wt.%, 5 wt.%, 10 wt.%, 15 wt.%, 20 wt.%, 25 wt.%, 30 wt.%, 35 wt.%, 40 wt.%, 45 wt.%, 50 wt.%, 55 wt.%, 60 wt.%, 65 wt.%,
20 70 wt.%, 75 wt.%, 80 wt.%, 85 wt.%, 90 wt.%, 92 wt.%, 94 wt.%, 96 wt.%, 97 wt.% and 98 wt.% total based on the total weight of the enriched formulation.