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(54) Title: COMPOSITION AND USES THEREOF

(57) Abstract: The invention relates to compositions comprising *Pinus pinaster* stem bark extract, papain, and *Aloe vera* extract, and methods of manufacturing same. There are also described methods of treating or preventing a variety of conditions, including treating or preventing elevated blood glucose, pre-diabetes, type 2 diabetes, autoimmune diseases, reducing or decreasing inflammation, treating or preventing diseases characterised by elevated levels of inflammation, and lowering blood cholesterol, said methods comprising administering an effective amount of a composition according to the invention to a subject in need thereof. Uses of the composition of the invention for the manufacture of a medicament for treating or preventing a variety of conditions are also described.

# Composition and uses thereof

## Technical Field

[0001] The present invention relates generally to the field of health supplements. More specifically, the present invention relates to a composition comprising *Pinus pinaster* stem bark extract, papain and *Aloe vera* extract and to its use in improving health, for example, regulating blood sugar levels and treating, delaying or preventing conditions associated with or caused by elevated blood sugar levels.

## Priority

[0002] The present application claims priority from Australian provisional application AU 2015901913, the entire contents of which are incorporated herein by cross-reference.

## Background

*Pre-Diabetes and Diabetes*

[0003] According to Diabetes Australia, nearly one in four adults over the age of 25 years has either diabetes or a condition known as 'pre-diabetes'. Pre-diabetes is a condition in which blood glucose levels are higher than normal, but not high enough to be diagnosed as type 2 diabetes mellitus (T2DM). Pre-diabetes is a condition in which blood glucose levels are higher than normal, although not high enough to be classified as diabetes. Pre-diabetes may have no signs or symptoms. People diagnosed with pre-diabetes have a higher risk of developing T2DM and cardiovascular (heart and circulation) disease. Pre-diabetes is diagnosed by a blood test that checks the blood glucose level. Any blood glucose test that shows higher than normal blood glucose levels needs to be followed up by a physician. This is likely to involve an oral glucose tolerance test (OGTT). The results of the OGTT will show whether the blood glucose levels are in the normal, pre-diabetes or diabetes range. If a person is diagnosed with pre-diabetes, they will have one or both of the following

- impaired fasting glucose (IFG) – this is when the fasting blood glucose level is higher [in the range of 6.1 mmol/L to 6.9 mmol/L] than normal, but still below the level for a diagnosis of diabetes.

- impaired glucose tolerance (IGT) – is when the blood glucose level two hours after an OGTT is higher [in the range of 7.8 to 11.0 mmol/L] than normal, but is still below the

level for a diagnosis of diabetes. The fasting blood glucose level may be in the normal range.

Sometimes the oral diabetes drug metformin (Glucophage) may be recommended by a doctor if an individual is at high risk of progressing to T2DM. This includes people whose body mass index is over 35, people who are younger than 60 years and women with a history of gestational diabetes.

*Type 2 Diabetes*

[0004] Islet dysfunction and peripheral insulin resistance are both present in T2DM and are both necessary for the development of hyperglycemia. In both type 1 diabetes mellitus (T1DM) and T2DM, large, prospective clinical studies have shown a strong relation between time-averaged mean values of glycemia, measured as glycated hemoglobin (HbA1c), and vascular diabetic complications. These studies are the basis for the American Diabetes Association's current recommended treatment goal that HbA1c should be <7%. The measurement of the HbA1c concentration is considered the gold standard for assessing long-term glycemia. However, it does not reveal any information on the extent or frequency of blood glucose excursions, but provides an overall mean value only. Postprandial hyperglycemia occurs frequently in patients diagnosed with T2DM receiving active treatment and can occur even when metabolic control is apparently good. Interventional studies indicate that reducing post-meal glucose excursions is as important as controlling fasting plasma glucose in persons with T2DM and impaired glucose tolerance. Evidence exists for a causal relation between post-meal glucose increases and microvascular and macrovascular outcomes. Therefore, treatments with different compounds that have specific effects on postprandial glucose regulation is accompanied by a significant improvement of many pathways supposed to be involved in diabetic complications, including endothelial dysfunction, inflammation, and nuclear factor-kappaB activation. The goal of therapy should be to achieve glycemic status as near to normal as safely possible in all 3 components of glycemic control, these including HbA1c, fasting glucose, and post-meal glucose peak. The overall adverse effect in failing to control these factors is to increase the risk of disease, in particular in the older age groups.

[0005] There is a need for new methods and compositions that provide one or more health benefits, such as improving overall health, improving organ function, treating and preventing elevated blood sugar related diseases such as T2DM and its related chronic diseases, treating and preventing conditions associated with or causative from elevated blood sugar such as pre-

diabetes, treating inflammation and its associated diseases, and/or lowering blood cholesterol.

## Summary of the Invention

[0006] The present inventor has identified a composition comprising *Pinus pinaster* stem bark extract, papain, and *Aloe vera* extract that exerts a number of health benefits, for example, the ability to improve organ function, reduce inflammation, and regulate blood sugar levels.

[0007] Accordingly, in a first aspect, the present invention provides a composition comprising *Pinus pinaster* stem bark extract, papain, and *Aloe vera* extract.

[0008] In a second aspect, the present invention provides a composition comprising a therapeutically effective amount of *Pinus pinaster* stem bark extract, papain, and *Aloe vera* extract.

[0009] In a third aspect, the present invention provides an aqueous composition comprising about 2.6 mg/mL *Pinus pinaster* stem bark extract, about 2.4 mg/mL papain, and about 1.75 mg/mL *Aloe vera* extract.

[0010] The following options may be used in conjunction with the first, second or third aspects above either individually or in any suitable combination.

[0011] The *Aloe vera* extract may be *Aloe vera* leaf extract.

[0012] The therapeutically effective amount of *Pinus pinaster* stem bark extract may correspond to a daily adult human dosage of from about 60 mg to about 1500 mg *Pinus pinaster* stem bark extract; the therapeutically effective amount of papain may correspond to a daily adult human dosage of from about 30 mg to about 1200 mg papain; and the therapeutically effective amount of *Aloe vera* extract may correspond to a daily adult human dosage of from about 15 mg to about 600 mg *Aloe vera* extract.

[0013] In one embodiment, the therapeutically effective amount of *Pinus pinaster* stem bark extract corresponds to a daily adult human dosage of about 260 mg; the therapeutically effective amount of papain corresponds to a daily adult human dosage of about 240 mg; and the therapeutically effective amount of *Aloe vera* extract corresponds to a daily adult human dosage of about 175 mg.

[0014] The composition may further comprise an acid. The acid may be acetic acid. The acid may be present in a concentration effective to adjust the pH of the composition to between about

3.2 and about 4.2.

[0015] The composition may further comprise honey.

[0016] The composition may further comprise one or more additives selected from the group consisting of: a preservative, a flavouring agent, a salt, and a pigment. The composition may comprise a salt, and the salt may be sodium chloride.

[0017] The composition may comprise an additional component selected from the group consisting of: an omega-3 fatty acid, a phytonutrient, a source of protein, an amino acid, an antioxidant, a vitamin, a mineral, a plant extract, and mixtures thereof.

[0018] The composition may be synergistic.

[0019] The composition may be adapted for oral administration. For example, the composition may be in a form selected from the group consisting of: a tablet, a capsule, a chewable tablet, a soft gel capsule, a sachet, a powder, granules, a liquid, a syrup, a liquid suspension, an emulsion, a solution, and combinations thereof. The composition may be an aqueous composition.

[0020] The composition may have a pH of about 3.7.

[0021] The *Pinus pinaster* stem bark extract may be present at a concentration of from about 2 mg/mL to about 10 mg/mL. The *Pinus pinaster* stem bark extract may be present at a concentration of about 2.6 mg/mL.

[0022] The papain may be present at a concentration of from about 1 mg/mL to about 5 mg/mL. The papain may be present at a concentration of about 2.4 mg/mL.

[0023] The *Aloe vera* extract may be present at a concentration of from about 0.5 mg/mL to about 4 mg/mL. The *Aloe vera* extract may be present at a concentration of about 1.75 mg/mL.

[0024] The composition may comprise glacial acetic acid at a concentration of from about 1 mg/mL to about 8 mg/mL. The glacial acetic acid may be present at a concentration of about 2 mg/mL.

[0025] The composition may comprise honey at a concentration of from about 50 mg/mL to about 200 mg/mL. The honey may be present at a concentration of about 100 mg/mL.

[0026] In an embodiment, each mL of the composition comprises *Pinus pinaster* (Maritime Pine) stem bark concentrated extract equivalent to 1.56 g dry *Pinus pinaster* stem bark

standardised to contain 1.82 mg procyanidins, 2.4 mg papain, 3 mg sodium chloride, and *Aloe vera* dried inner leaf juice equivalent to 350 mg fresh juice.

[0027] The composition may further comprise a source of omega-3 fatty acids selected from the group consisting of fish oil, krill, plant sources containing omega-3 fatty acids, flaxseed, walnut, algae, and combinations thereof. The omega-3 fatty acids may be selected from the group consisting of alpha-linolenic acid ("ALA"), docosahexaenoic acid ("DHA"), stearidonic acid ("SD") and combinations thereof. The omega-3 fatty acids may be provided in an amount of about 0.25 g to 5.0 g per day.

[0028] The composition may further comprise a phytonutrient selected from the group consisting of flavanoids, allied phenolic compounds, polyphenolic compounds, terpenoids, alkaloids, sulfur-containing compounds, and combinations thereof. The phytonutrient may be selected from the group consisting of carotenoids, plant sterols, quercetin, curcumin, limonin, and combinations thereof.

[0029] The composition may comprise a source of protein. The source of protein may provide the composition with at least 10 g of high quality protein. The source of protein may be selected from the group consisting of dairy based proteins, plant based proteins, animal based proteins, artificial proteins, and combinations thereof. The dairy based proteins may be selected from the group consisting of casein, micellar casein, caseinates, casein hydrolysate, whey, whey protein micelles, whey hydrolysates, whey concentrates, whey isolates, milk protein concentrate, milk protein isolate, and combinations thereof. The plant based proteins may be selected from the group consisting of soy protein, pea protein, canola protein, wheat and fractionated wheat proteins, corn proteins, Zein proteins, rice proteins, oat proteins, potato proteins, peanut proteins, green pea powder, green bean powder, spirulina, proteins derived from vegetables, beans, buckwheat, lentils, pulses, single cell proteins, and combinations thereof.

[0030] The composition may further comprise an amino acid selected from the group consisting of alanine, arginine, asparagine, aspartate, citrulline, cysteine, glutamate, glutamine, glycine, histidine, hydroxyproline, hydroxyserine, hydroxytyrosine, hydroxylysine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, taurine, threonine, tryptophan, tyrosine, valine, and combinations thereof. The amino acid may be a branched chain amino acid selected from the group consisting of isoleucine, leucine, valine, and combinations thereof.

[0031] The composition may further comprise an antioxidant selected from the group consisting

of astaxanthin, carotenoids, coenzyme Q10 ("CoQ10"), flavonoids, glutathione, Goji (Wolfberry), hesperidin, lactowolfberry, lignan, lutein, lycopene, polyphenols, selenium, vitamin A, vitamin C, vitamin E, Zeaxanthin, and combinations thereof.

[0032] The composition may further comprise a vitamin selected from the group consisting of vitamin A, Vitamin B1 (thiamine), Vitamin B2 (riboflavin), Vitamin B3 (niacin or niacinamide), Vitamin B5 (pantothenic acid), Vitamin B6 (pyridoxine, pyridoxal, or pyridoxamine, or pyridoxine hydrochloride), Vitamin B7 (biotin), Vitamin B9 (folic acid), and Vitamin B12 (various cobalamins; commonly cyanocobalamin in vitamin supplements), vitamin C, vitamin D, vitamin E, vitamin K, K1 and K2 (i.e., MK-4, MK-7), choline and combinations thereof.

[0033] The composition may further comprise a mineral selected from the group consisting of boron, calcium, chromium, copper, iodine, iron, magnesium, manganese, molybdenum, nickel, phosphorus, potassium, selenium, silicon, tin, vanadium, zinc, and combinations thereof.

[0034] The composition may further comprise a plant extract selected from the group consisting of the *Malvaceae* family, such as *Abelmoschus moschatus Medik,* or from the *Asteraceae* family, such as *Achiliea santolina* L., or *Achyrocline satureioides* (Lam.) DC, or *Artemisia dracunculus* L. (dragon herb) or *Cichorium intybus* L., or *Eclipta alba* (L) Hassk, or from the *Lamiaceae* family such as *Ajuga iva* L. *Schreberr* (Medit), or *Ocimum sanctum* Linn. (Tulasi), or *Origanum vulgare* L. or from the *Annonaceae* family such as *Annona squamosa* L., or from the *Gentianeacea* family such as *Anthocleista djalonensis* A. Chev (cabbage tree), or *Anthocleista Schweinfurthii,* or *Anthocleista vogelii* Planch, *Enicostemma littorale Blume* or *Gentiana olivieri* L., or from the *Oxalidaceae* family such as *Averrhoa bilimbi* L, or *Biophytum sensitivum* (L) DC, or from the *Leguminosae* family such as *Bauhinia candicans* Benth, or from the *Bixaceae* family such as *Bixa orellana* L., or from the *Nyctaginaceae* family such as *Boerhaavia diffusa* L., or from the *Brassicaceae* family such as *Brassica nigra* (L) Koch, or *Eruka sativa,* or *Lepidium sativum* L., or from the *Caesalpiniaceae* family such as *Capparis spinosa L.* or *Cassia auriculata* L. or *Tamarindus indica* L., or from the *Capparidaceae* family such as *Capparis spinosa* L., or from the *Apiaceae family* such as *Carum carvi* L., or from the family *Rutaceae* such as *Clausena anisata* (Wild) Benth., or from the family *Palmae* such as *Cocos nucifera* Linn. (Coconut palm), or from the *Cucurbitaceae* family such as *Cogniauxia podoleana,* or *Ibervillea sonorae* S., or from the family *Conimelinaceae* such as *Commelina communis* L., or from the family *Zingiberaceae* such as *Curcuma longa* L., or from the family *Poaceae* such as *Cynodon dactylon* Pers. (Bermuda grass), or from the family *Ginkgoaceae* such as *Ginkgo biloba* L., or from the

family *Papilionaceae* such as *Glycyrrhiza uralensis* Fish., or from the family *Asclepiadaceae,* such as *Gongronema latifolium* Benth., or *Gymnema montanum* Hook, or from the family *Sterculiaceae* such as *Helicteres isora* L., As., or from the *Rubiaceae* family such as *Hintonia standleyana,* or from the *Gramineae* family such as *Hordeum vulgare* L. (Barley), or from the *Convolvulaceae* family such as *Ipomoea aquatic* Forsk., or *Ipomea batata* Linn (Sweet potato), or from the *Loranthaceae* family such as *Loranthus micranthus* Linn, or from the *Moraceae* family such as *Morus indica*. L., or from the *Musaceae* family such as *Musa sapientum* Kuntz (Banana), or from the *Euphorbiaceae* family such as *Phyllanthus amarus* Schum. Thonn, or *Phyllanthus niruri* L., or *Phyllanthus sellowianus* Mull. Arg., or from the *Piperacea* family such as *Piper longum,* or from the *Myrtaceae* family such as *Psidium guajava* L., or *Syzygium alternifolium* (Wt) Walp, or from the *Lythraceae* family such as *Punica granatum* L. (pomegranate), or from the *Papilionaceae* family such as *Retama raetam (RR)* (Forssk) Webb., or from the *Adoxaceae* family such as *Sambucus nigra* L., or from the Apocynaceae family such as *Sanguis draxonis,* or from the *Anacardiaceae* family such as *Sclerocarya birea* (A. Rich), or from the *Scrophariaceae* family such as *Scoparia dulcis* L., or from the *Caryophyllaceae* family such as *Spergularia purpurea,* or from the *Chenopodiaceae* family such as *Suaeda fruticosa* (SF) Euras, or from the *Combretaceae* family such as *Terminalia chebula* Retz., or *Terminalia bellirica* (Gaertn), or from the *Menispermaceae* family such as *Tinospora cordifolia* Miers., or from the *Urticaceae* family such as *Urtica pilulifera* L., or from the *Astereaceae* family such as *Vernonia amygdalina* Del., or from the *Solanaceae* family such as *Withania soimifera* (L) Dunal, or from the *Zygophyllaceae* family such as *Zygophyllum gaetulum* Emb and Maire and combinations thereof.

[0035] In a fourth aspect, the present invention provides an aqueous composition comprising: 2.40 mg/mL papain; 2.60 mg/mL dry *Pinus pinaster* stem bark extract; 1.75 mg/mL dry *Aloe vera* inner leaf juice; 100.00 mg/mL honey; 2.00 mg/mL glacial acetic acid; 3.00 mg/mL sodium chloride; 400 µg/mL potassium sorbate; 400 µg/mL sodium benzoate; 10.00 mg/mL wildberry flavour UA71225; and 800 µg/mL anthocyanin extract.

[0036] In a fifth aspect, the present invention provides an aqueous composition comprising papain, *Pinus pinaster* stem bark extract, and *Aloe vera* inner leaf juice in a ratio of papain : dry *Pinus pinaster* stem bark extract : dry *Aloe vera* inner leaf juice of 2.40 : 2.60 : 1.75 by weight.

[0037] In one embodiment, there is provided an aqueous composition comprising papain, *Pinus pinaster* stem bark extract, *Aloe vera* inner leaf juice, and honey in a ratio of papain : dry *Pinus*

*pinaster* stem bark extract : dry *Aloe vera* inner leaf juice : honey of 2.40 : 2.60 : 1.75 : 100 by weight.

[0038] In another embodiment, there is provided an aqueous composition comprising papain, *Pinus pinaster* stem bark extract, *Aloe vera* inner leaf juice, and glacial acetic acid in a ratio of papain : dry *Pinus pinaster* stem bark extract : dry *Aloe vera* inner leaf juice : glacial acetic acid of 2.40 : 2.60 : 1.75 : 2.00 by weight.

[0039] In another embodiment, there is provided an aqueous composition comprising papain, *Pinus pinaster* stem bark extract, *Aloe vera* inner leaf juice, honey, and glacial acetic acid in a ratio of papain : dry *Pinus pinaster* stem bark extract : dry *Aloe vera* inner leaf juice : honey : glacial acetic acid of 2.40 : 2.60 : 1.75 : 100 : 2.00 by weight.

[0040] In yet another embodiment, there is provided an aqueous composition comprising papain, *Pinus pinaster* stem bark extract, *Aloe vera* inner leaf juice, honey, glacial acetic acid, and sodium chloride in a ratio of papain : dry *Pinus pinaster* stem bark extract : dry *Aloe vera* inner leaf juice : honey : glacial acetic acid : sodium chloride of 2.40 : 2.60 : 1.75 : 100 : 2.00 : 3.00 by weight.

[0041] In a further embodiment, there is provided an aqueous composition comprising papain, dry *Pinus pinaster* stem bark extract, dry *Aloe vera* inner leaf juice, honey, glacial acetic acid, sodium chloride, potassium sorbate, sodium benzoate, wildberry flavour UA71225 and anthocyanin extract in a ratio of papain : dry *Pinus pinaster* stem bark extract : dry *Aloe vera* inner leaf juice : honey : glacial acetic acid : sodium chloride : potassium sorbate : sodium benzoate : wildberry flavour UA71225 : anthocyanin extract of 2.40 : 2.60 : 1.75 : 100 : 2.00 : 3.00 : 0.40 : 0.40 : 10.00 : 0.80 by weight.

[0042] In a sixth aspect, the present invention provides a method of treating or preventing elevated blood glucose, comprising administering to a subject in need thereof an effective amount of a composition according to any one of the first to fifth aspects above.

[0043] In a seventh aspect, the present invention provides a method of preventing or delaying the onset of type 2 diabetes mellitus (T2DM), comprising administering to a subject in need thereof an effective amount of a composition according to any one of the first to fifth aspects above.

[0044] In an eighth aspect, the present invention provides a method of treating or preventing pre-diabetes, comprising administering to a subject in need thereof an effective amount of a

composition according to any one of the first to fifth aspects above.

[0045] In a ninth aspect, the present invention provides a method of reducing inflammation, comprising administering to a subject in need thereof an effective amount of a composition according to any one of the first to fifth aspects above.

[0046] In a tenth aspect, the present invention provides a method of decreasing cytokine-induced inflammation comprising administering to a subject in need thereof an effective amount of a composition according to any one of the first to fifth aspects above.

[0047] In an eleventh aspect, the present invention provides a method of decreasing an oxidative stress response comprising administering to a subject in need thereof an effective amount of a composition according to any one of the first to fifth aspects above.

[0048] In a twelfth aspect, the present invention provides a method of treating or preventing a disease characterised by elevated levels of cytokine-induced inflammation, comprising administering to a subject in need thereof an effective amount of a composition according to any one of the first to fifth aspects above.

[0049] In a thirteenth aspect, the present invention provides a method of treating or preventing a disease characterised by elevated levels of oxidative stress, comprising administering to a subject in need thereof an effective amount of a composition according to any one of the first to fifth aspects above.

[0050] In one embodiment of the twelfth or thirteenth aspects above, the disease is atherosclerosis, liver inflammation associated with type 2 diabetes and obesity, endometriosis, or osteoarthritis.

[0051] In a fourteenth aspect, the present invention provides a method of reducing one or more side effects of a diabetes medication selected from the group consisting of metformin, sulfonylureas, meglitinides, thiazolidinediones, DPP-4 inhibitors, GLP-1 receptor agonists, SGLT2 inhibitors, and insulin therapy, the method comprising administering to a subject in need thereof an effective amount of a composition according to any one of the first to fifth aspects above. The side effect may be abdominal or stomach discomfort, decreased appetite, or diarrhoea.

[0052] In a fifteenth aspect, the present invention provides a method of treating or preventing a dysfunction associated with excess blood glucose, comprising administering to a subject in need

thereof an effective amount of a composition according to any one of the first to fifth aspects above. The dysfunction may be selected from the group consisting of: increased thirst and hunger, frequent urination, fatigue/lethargy, blurry vision, sleepiness, and muscle weakness.

[0053] In one embodiment of the sixth to fifteenth aspects above, the composition is administered in combination with metformin or another blood glucose lowering medication.

[0054] In a sixteenth aspect, the present invention provides a method of improving organ function comprising administering to a subject in need thereof an effective amount of a composition according to any one of the first to fifth aspects above.

[0055] In a seventeenth aspect, the present invention provides a method of lowering blood cholesterol, comprising administering to a subject in need thereof an effective amount of a composition according to any one of the first to fifth aspects above. The blood cholesterol may be selected from the group consisting of: low density lipoproteins (LDL), high density lipoproteins (HDL), trigylcerides and total cholesterol. In one embodiment, the present invention provides a method of lowering low density lipoprotein (LDL) blood cholesterol levels, comprising administering to a subject in need thereof an effective amount of a composition according to any one of the first to fifth aspects above. In another embodiment, the present invention provides a method of increasing high density lipoprotein (HDL) blood cholesterol levels, comprising administering to a subject in need thereof an effective amount of a composition according to any one of the first to fifth aspects above. In yet another embodiment, the present invention provides a method of decreasing the ratio of Total Cholesterol to HDL, comprising administering to a subject in need thereof an effective amount of a composition according to any one of the first to fifth aspects above. In yet another embodiment, the present invention provides a method of decreasing blood triglyceride levels, comprising administering to a subject in need thereof an effective amount of a composition according to any one of the first to fifth aspects above.

[0056] The effective amount of the composition may correspond to: a daily adult human dosage of from about 60 mg to about 1500 mg *Pinus pinaster* stem bark extract; a daily adult human dosage of from about 30 mg to about 1200 mg papain; and a daily adult human dosage of from about 15 mg to about 600 mg *Aloe vera* extract. For example, the effective amount of the composition may correspond to: a daily adult human dosage of about 260 mg *Pinus pinaster* stem bark extract; a daily adult human dosage of about 240 mg papain; and a daily adult human dosage of about 175 mg *Aloe vera* extract. The daily adult human dosage of *Pinus pinaster* stem

bark extract, papain and *Aloe vera* extract may be provided to the subject in a single dose, or the daily adult human dosage of *Pinus pinaster* stem bark extract, papain and *Aloe vera* extract may be provided to the subject in multiple doses.

[0057] In one embodiment of the sixth to seventeenth aspects above, the subject is selected from the group consisting of the elderly, those with a medical condition of pre-diabetes or at risk of developing pre-diabetes, and combinations thereof.

[0058] In an eighteenth aspect, the present invention provides a method of improving immune function, comprising administering to a subject in need thereof an effective amount of a composition according to any one of the first to fifth aspects above. In one embodiment, improved immune function is characterised by an increased white blood cell count, e.g., increased levels of neutrophils.

[0059] In a nineteenth aspect, the present invention provides a method of treating an autoimmune disease, comprising administering to a subject in need thereof an effective amount of a composition according to any one of the first to fifth aspects above. The autoimmune disease may be selected from the group consisting of: lupus, arthritis, psoriasis, and type I diabetes. In one embodiment, the present invention provides a method of treating lupus, comprising administering to a subject in need thereof an effective amount of a composition according to any one of the first to fifth aspects above. The treatment of lupus may reduce the number of lesions, e.g., sub cutaneous lesions, in or on the subject. In another embodiment, the present invention provides a method of treating arthritis, comprising administering to a subject in need thereof an effective amount of a composition according to any one of the first to fifth aspects above. In one embodiment, the arthritis is rheumatoid arthritis. In a further embodiment, the present invention provides a method of treating psoriasis, comprising administering to a subject in need thereof an effective amount of a composition according to any one of the first to fifth aspects above. In yet a further embodiment, the present invention provides a method of treating type I diabetes, comprising administering to a subject in need thereof an effective amount of a composition according to any one of the first to fifth aspects above.

[0060] In a twentieth aspect, the present invention provides a method of treating emphysema, comprising administering to a subject in need thereof an effective amount of a composition according to any one of the first to fifth aspects above.

[0061] In a twenty first aspect, the present invention provides a method of increasing general

wellbeing, comprising administering to a subject in need thereof an effective amount of a composition according to any one of the first to fifth aspects above. In one embodiment, a measure of general wellbeing includes one or more of: increased energy levels, improved vitality, better sleep, improved breathing ability, improved levels of physical activity, improved mental functioning, and/or improved physical functioning.

[0062] In the method of any one of the sixth to twenty first aspects above, the composition may be administered to an adult human subject at a dosage of 30 to 100 mL per day, wherein each mL of the composition comprises: *Pinus pinaster* (Maritime Pine) stem bark concentrated extract equivalent to 1.56 g dry *Pinus pinaster* stem bark standardised to contain 1.82 mg procyanidins, 2.4 mg papain, 3 mg sodium chloride, and *Aloe vera* dried inner leaf juice equivalent to 350 mg fresh juice. In an embodiment, each mL of the composition comprises: 2.40 mg papain; 2.60 mg dry *Pinus pinaster* stem bark extract; 1.75 mg dry *Aloe vera* inner leaf juice; 100.00 mg honey; 2.00 mg glacial acetic acid; 3.00 mg sodium chloride; 400 µg potassium sorbate; 400 µg sodium benzoate; 10.00 mg wildberry flavour UA71225; and 800 µg anthocyanin extract, or equivalents thereof. The composition may be administered at a total daily dosage of about 30 to about 100 mL in two separate doses. For example, the composition may be administered in two separate doses of about 15 to about 50 mL, one dose after a morning meal and one dose after an evening meal.

[0063] In a twenty second aspect, the present invention provides use of a composition according to any one of the first to fifth aspects above for the manufacture of a medicament for: treating or preventing elevated blood glucose; or preventing or delaying the onset of type 2 diabetes mellitus (T2DM); or treating or preventing pre-diabetes; or reducing inflammation; or decreasing cytokine-induced inflammation; or decreasing an oxidative stress response; or treating or preventing a disease characterised by elevated levels of cytokine-induced inflammation; or treating or preventing a disease characterised by elevated levels of oxidative stress; or reducing one or more side effects of a diabetes medication selected from the group consisting of metformin, sulfonylureas, meglitinides, thiazolidinediones, DPP-4 inhibitors, GLP-1 receptor agonists, SGLT2 inhibitors, and insulin therapy; or treating or preventing a dysfunction associated with excess blood glucose; or improving organ function; or lowering blood cholesterol in a subject.

[0064] In a twenty third aspect, the present invention provides use of a composition according to any one of the first to fifth aspects above for the manufacture of a medicament for: improving

immune function; treating an autoimmune disease, e.g., lupus, arthritis, psoriasis, or type I diabetes; treating emphysema; or increasing general wellbeing, e.g., increasing energy levels, improving vitality, improving sleep, improving breathing ability, improving levels of physical activity, improving mental functioning, and/or improving physical functioning in a subject.

[0065] In one embodiment, the present invention provides use of a composition according to any one of the first to fifth aspects above for the manufacture of a medicament for treating or preventing elevated blood glucose in a subject.

[0066] In one embodiment, the present invention provides use of a composition according to any one of the first to fifth aspects above for the manufacture of a medicament for preventing or delaying the onset of type 2 diabetes mellitus (T2DM) in a subject.

[0067] In one embodiment, the present invention provides use of a composition according to any one of the first to fifth aspects above for the manufacture of a medicament for treating or preventing pre-diabetes in a subject.

[0068] In one embodiment, the present invention provides use of a composition according to any one of the first to fifth aspects above for the manufacture of a medicament for reducing inflammation in a subject.

[0069] In one embodiment, the present invention provides use of a composition according to any one of the first to fifth aspects above for the manufacture of a medicament for decreasing cytokine-induced inflammation in a subject.

[0070] In one embodiment, the present invention provides use of a composition according to any one of the first to fifth aspects above for the manufacture of a medicament for decreasing an oxidative stress response in a subject.

[0071] In one embodiment, the present invention provides use of a composition according to any one of the first to fifth aspects above for the manufacture of a medicament for treating or preventing a disease characterised by elevated levels of cytokine-induced inflammation in a subject. The disease may be atherosclerosis, liver inflammation associated with type 2 diabetes and obesity, endometriosis, or osteoarthritis.

[0072] In one embodiment, the present invention provides use of a composition according to any one of the first to fifth aspects above for the manufacture of a medicament for treating or preventing a disease characterised by elevated levels of oxidative stress in a subject. The disease

may be atherosclerosis, liver inflammation associated with type 2 diabetes and obesity, endometriosis, or osteoarthritis.

[0073] In one embodiment, the present invention provides use of a composition according to any one of the first to fifth aspects above for the manufacture of a medicament for reducing one or more side effects of a diabetes medication selected from the group consisting of metformin, sulfonylureas, meglitinides, thiazolidinediones, DPP-4 inhibitors, GLP-1 receptor agonists, SGLT2 inhibitors, and insulin therapy in a subject. The side effect may be abdominal or stomach discomfort, decreased appetite, or diarrhoea.

[0074] In one embodiment, the present invention provides use of a composition according to any one of the first to fifth aspects above for the manufacture of a medicament for treating or preventing a dysfunction associated with excess blood glucose in a subject. The dysfunction may be selected from the group consisting of: increased thirst and hunger, frequent urination, fatigue/lethargy, blurry vision, sleepiness, and muscle weakness.

[0075] In one embodiment, the present invention provides use of a composition according to any one of the first to fifth aspects above for the manufacture of a medicament for improving organ function in a subject.

[0076] In one embodiment, the present invention provides use of a composition according to any one of the first to fifth aspects above for the manufacture of a medicament for lowering blood cholesterol in a subject.

[0077] In one embodiment of the twenty second aspect above, the subject may be taking metformin or another blood glucose lowering medication, and/or the subject may be selected from the group consisting of the elderly, those with a medical condition of pre-diabetes or at risk of developing pre-diabetes, and combinations thereof.

[0078] In one embodiment, the present invention provides use of a composition according to any one of the first to fifth aspects above for the manufacture of a medicament for improving immune function in a subject. In another embodiment, the present invention provides use of a composition according to any one of the first to fifth aspect above for treating an autoimmune disease, e.g., lupus, arthritis, psoriasis, or type I diabetes in a subject. In a further embodiment, the present invention provides use of a composition according to any one of the first to fifth aspects above for the manufacture of a medicament for treating emphysema in a subject. In yet a further embodiment, the present invention provides use of a composition according to any one of

the first to fifth aspects above for the manufacture of a medicament for increasing general wellbeing, e.g., increasing energy levels, improving vitality, improving sleep, improving breathing ability, improving levels of physical activity, improving mental functioning, and/or improving physical functioning in a subject.

## Brief Description of the Drawings

[0079] Embodiments of the present invention will now be described, by way of example only with reference to the accompanying figures wherein:

[0080] **Figure 1** shows that a composition according to Example 1 decreases inflammatory markers (a) TNF-alpha-induced VCAM-1, (b) TNF-alpha-induced ICAM-1 and (c) TNF-alpha-induced ELAM in human coronary artery endothelial cells. HCAECs were exposed to the indicated amounts (μL/mL) of the composition of Example 1 for 3 hours prior to exposure to TNF-alpha.

[0081] **Figure 2** shows that a composition according to Example 1 decreases protein levels of (a) TNF-alpha-induced VCAM-1 and (b) TNF-alpha-induced ICAM-1 in human coronary artery endothelial cells. HCAECs were exposed to the indicated amounts (μL/mL) of the composition of Example 1 for 3 hours prior to exposure to TNF-alpha.

[0082] **Figure 3** shows that a composition according to Example 1 decreases activation of the key mediator of inflammation, NFkB. HCAECs were exposed to the indicated amounts (μL/mL) of the composition of Example 1 prior to exposure to TNF-alpha.

[0083] **Figure 4** shows that a composition according to Example 1 decreases cytokine-induced ROS levels in TNF-alpha-induced HCAECs. HCAECs were exposed to the indicated amounts (μL/mL) of the composition of Example 1 for 3 hours prior to exposure to TNF-alpha.

[0084] **Figure 5** shows that a composition according to Example 1 decreases (a) NOX-4 and increases (b) SOD-1 levels in TNF-alpha-induced HCAECs. HCAECs were exposed to the indicated amounts (μL/mL) of the composition of Example 1 prior to exposure to TNF-alpha.

[0085] **Figure 6** shows that a composition according to Example 1 ingested by athletes pre-training decreases CRP levels in their blood post-training (dark grey = runners, light grey = cyclists).

[0086] **Figure 7** shows that a composition according to Example 1 blocks VCAM-1 levels from

rising in response to the pro-inflammatory cytokine, TNF-α. HCAECs were exposed to concentrations equivalent to a 50 mL, 25 mL, 12.5 mL and 6.25 mL dose of the composition of Example 1 prior to exposure to TNF-alpha.

[0087] **Figure 8** shows that human coronary artery endothelial cells exposed to an inflammatory stimulus, TNF-α, after pre-treatment (24h) with plasma obtained from individuals 3 h after ingestion of a composition of Example 1 demonstrated a reduced level of inflammation response (measured by VCAM-1 levels) compared to cells pretreated with plasma from the same individuals pre-ingestion of the composition.

## Definitions

[0088] As used in this application, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the phrase 'a composition' also includes a plurality of compositions.

[0089] As used herein, the term "comprising" means "including." Variations of the word "comprising", such as "comprise" and "comprises," have correspondingly varied meanings. Thus, for example, a composition "comprising" *Pinus pinaster* stem bark extract, papain and *Aloe vera* extract may consist exclusively of *Pinus pinaster* stem bark extract, papain and *Aloe vera* extract, or it may include one or more additional components (e.g., acid, honey, preservatives, etc.).

[0090] It will be understood that use the term "about" herein in reference to a recited numerical value includes the recited numerical value and numerical values within plus or minus ten percent of the recited value.

[0091] As used herein, the term "subject" is understood to include an animal, especially a mammal, and more especially a human, that is receiving or intended to receive treatment or has or is at risk of having a medical condition that can benefit from the composition as described herein. For example, the animal may include, but is not limited to, mammals such as rodents, aquatic mammals, domestic animals such as dogs and cats, farm animals such as sheep, pigs, cows and horses, and humans. It is also contemplated that the term 'subject' applies to any animals that are capable of the effect exhibited or intended to be exhibited by the compositions as described herein.

[0092] It will be understood that use of the term "between" herein when referring to a range of

numerical values encompasses the numerical values at each endpoint of the range. For example, a pH of between about 3.2 and about 4.2 is inclusive of a pH of 3.2 and a pH of 4.2.

[0093] As used herein, the terms "aqueous" and "aqueous composition" will be understood as referring to a composition formulated in or with water, and in particular, purified water. In some embodiments, the aqueous compositions will be predominantly water, for example, up to 99% water by weight or by volume. In other embodiments, the aqueous compositions will comprise at least some water, for example, more than 1% water by weight or by volume.

## Detailed Description

[0094] The following detailed description conveys the present invention, including exemplary embodiments thereof, in sufficient detail to enable those of ordinary skill in the art to practice the present invention. Features or limitations of the various embodiments described do not necessarily limit other embodiments of the present invention or the present invention as a whole. Hence, the following detailed description does not limit the scope of the present invention, which is defined only by the claims.

[0095] The present disclosure relates generally to a composition comprising *Pinus pinaster* stem bark extract in combination with *Aloe vera* extract (alternatively known as *A. barbadensis* Mill., *Aloe indica* Royle, *Aloe perfoliata* L. var. *vera* and *A. vulgaris* Lam) and papain and methods and uses thereof for the therapeutic and/or prophylactic treatment, amelioration and/or regulation of disease states or pathological conditions that are associated with excess blood glucose (hyperglycemia), for improving organ function, and for reducing inflammation. The compositions and methods may, for example, be suitable for treating and/or preventing a dysfunction associated with excess blood glucose, which may include disturbances in everyday physiological functions such as increased thirst and hunger, frequent urination, fatigue/lethargy, blurry vision, sleepiness, and/or muscle weakness. Such associated conditions may be compounded by pharmaceutical administrations (e.g., metformin), which may cause or exacerbate abdominal or stomach discomfort, decreased appetite, diarrhoea, and a general feeling of discomfort.

### COMPOSITION

#### *Pinus pinaster stem bark extract*

[0096] The composition according to the present invention comprises *Pinus pinaster* stem bark

extract. This *Pinus pinaster* stem bark extract is made from the bark of a European coastal pine tree called the *Landes* or *maritime pine*, whose scientific name is *Pinus maritima* or *Pinus pinaster*. Without being bound by theory, the pine bark extract may have antioxidant properties that make it suitable for a wide range of healing and preventative purposes.

[0097] The *Pinus pinaster* stem bark extract may be derived from any suitable source, for example, extracted from the bark of the maritime pine tree using methods known in the art, or it may be obtained commercially, e.g., as the product Pycnogenol® supplied by Horphag Research USA Inc. or as Pinus pinaster L supplied by Pharmactive Biotech Products SL. Preferably, the *Pinus pinaster* stem bark extract is Pycnogenol® supplied by Horphag Research USA Inc. The *Pinus pinaster* stem bark extract may be provided in the composition according to the invention in solid form, e.g., as a dry powder, or a dry powder compacted into a tablet, or dry powder in a capsule, or in a liquid form, e.g., dry powder dissolved or suspended in a solution, suspension or emulsion. Where the composition comprising *Pinus pinaster* stem bark extract is provided as an aqueous composition, dry powdered *Pinus pinaster* stem bark extract may be dissolved in the water. In the context of this specification, the term 'extract' is to be given its broadest construction and is understood to refer to a plant and its components or preparation, or component of a plant or herb or preparation derived therefrom, which, when used in the composition according to the invention in an effective amount, promotes a health benefit in the subject such as regulation of high blood glucose levels.

[0098] The *Pinus pinaster* stem bark extract may be present in the composition in any suitable amount, e.g., at a therapeutically effective amount. A therapeutically effective amount of *Pinus pinaster* stem bark extract may correspond to a daily adult human dosage of from about 60 mg to about 1500 mg *Pinus pinaster* stem bark extract. In one embodiment, the *Pinus pinaster* stem bark extract is present in the composition in a therapeutically effective amount of corresponding to a daily adult human dosage of about 260 mg.

[0099] Where the composition is formulated as liquid, e.g., an aqueous liquid, *Pinus pinaster* stem bark extract may be present in the composition in a concentration ranging from about 2.0 mg/mL to about 10 mg/mL. For example, *Pinus pinaster* stem bark extract may be present in the composition in a concentration ranging from about 2.0 mg/mL to about 3.0 mg/mL, or from about 2.0 mg/mL to about 5.0 mg/mL, or from about 4.0 mg/mL to about 8.0 mg/mL, or from about 6.0 mg/mL to about 10.0 mg/mL, e.g., at a concentration of 2.0 mg/mL, 2.2 mg/mL, 2.4 mg/mL, 2.6 mg/mL, 2.8 mg/mL, 3.0 mg/mL, 3.2 mg/mL, 3.4 mg/mL, 3.6 mg/mL, 3.8 mg/mL,

4.0 mg/mL, 4.5 mg/mL, 5.0 mg/mL, 5.5 mg/mL, 6.0 mg/mL, 6.5 mg/mL, 7.0 mg/mL, 7.5 mg/mL, 8.0 mg/mL, 8.5 mg/mL, 9.0 mg/mL, 9.5 mg/mL, or 10 mg/mL, where the amount of *Pinus pinaster* stem bark extract is expressed as the amount of dry *Pinus pinaster* stem bark extract per mL of the composition. It is contemplated herein that the amount of dry *Pinus pinaster* stem bark extract per mL includes *Pinus pinaster* stem bark extract added to the liquid composition in dry form, but also covers addition of *Pinus pinaster* stem bark extract in other forms (e.g., in a liquid concentrate) in different amounts, provided that the overall concentration of *Pinus pinaster* stem bark extract after addition is equivalent to the concentration of dry *Pinus pinaster* stem bark extract per mL of the composition.

[0100] The amounts of "dry *Pinus pinaster* stem bark extract" referred to herein are calculated on the basis of the exemplified embodiments of the invention, for example, the composition in Table 1, in which the dry *Pinus pinaster* stem bark extract added to the composition comprises approximately 65-75% procyanidins. In one embodiment, the 2.60 mg dry *Pinus pinaster* stem bark extract per mL in Table 1, equivalent to 1.56 g dry *Pinus pinaster* stem bark, is standardised to contain about 1.82 mg procyanidins. It will be understood that alternative sources or forms of *Pinus pinaster* stem bark extract may be available, in which case the amount of *Pinus pinaster* stem bark extract used in the composition of the invention may be adjusted so as to provide an equivalent amount of procyanidins rather than a necessarily equivalent amount of *Pinus pinaster* stem bark extract.

[0101] In one embodiment, the *Pinus pinaster* stem bark extract is present in the aqueous composition in a concentration of 2.6 mg/mL.

***Papain***

[0102] The composition according to the present invention comprises papain. Papain is a cysteine protease enzyme extracted from the papaya plant (*Carica papaya*).

[0103] The papain may be derived from any suitable source, for example, the leaves, latex, roots, and/or fruits of the papaya plant using methods known in the art, or it may be obtained commercially, e.g., as the product Papaina (USP) as supplied by Biocon Limited, Bangalore, India. It may be added to the composition according to the invention in solid form, e.g., as a dry powder, or in any other suitable form, e.g., suspended or dissolved in an aqueous buffer solution, or as a concentrated liquid. Where the composition comprising papain is provided as an aqueous composition, pure, powdered, dry papain may be dissolved in the water.

[0104] The papain may be present in the composition in any suitable amount, e.g., at a therapeutically effective amount. A therapeutically effective amount of papain may correspond to a daily adult human dosage of from about 30 mg to about 1200 mg papain. In one embodiment, the papain is present in the composition in a therapeutically effective amount corresponding to a daily adult human dosage of about 240 mg.

[0105] Where the composition is formulated as liquid, e.g., an aqueous liquid, papain may be present in the composition in a concentration ranging from about 1.0 mg/mL to about 8 mg/mL. For example, papain may be present in the composition in a concentration ranging from about 1.0 mg/mL to about 3.0 mg/mL, or from about 2.0 mg/mL to about 5.0 mg/mL, or from about 4.0 mg/mL to about 6.0 mg/mL, or from about 5.0 mg/mL to about 8.0 mg/mL, e.g., at a concentration of 1.0 mg/mL, 1.2 mg/mL, 1.4 mg/mL, 1.6 mg/mL, 1.8 mg/mL, 2.0 mg/mL, 2.2 mg/mL, 2.4 mg/mL, 2.6 mg/mL, 2.8 mg/mL, 3.0 mg/mL, 3.2 mg/mL, 3.4 mg/mL, 3.6 mg/mL, 3.8 mg/mL, 4.0 mg/mL, 4.5 mg/mL, 5.0 mg/mL, 5.5 mg/mL, 6.0 mg/mL, 6.5 mg/mL, 7.0 mg/mL, 7.5 mg/mL, or 8.0 mg/mL, where the amount of papain is expressed as the amount of dry papain per mL of the composition. It is contemplated herein that the amount of dry papain per mL includes papain added to the liquid composition in dry form, but also covers addition of papain in other forms (e.g., in a liquid concentrate) in different amounts, provided that the overall concentration of papain after addition is equivalent to the concentration of dry papain per mL of the composition.

[0106] In one embodiment, the papain is present in the aqueous composition in a concentration of 2.4 mg/mL.

***Aloe vera***

[0107] The composition according to the present invention comprises *Aloe vera* extract. *Aloe vera* is alternatively known as *A. barbadensis* Mill., *Aloe indica* Royle, *Aloe perfoliata* L. var. *vera* and *A. vulgaris* Lam. The *Aloe vera* extract may be extracted from any suitable part of the *Aloe vera* plant, for example, the *Aloe vera* extract may be *Aloe vera* leaf extract, e.g., made by crushing the whole *Aloe vera* leaf, or may be *Aloe vera* gel extract, or may be *Aloe vera* latext extract. Preferably, the *Aloe vera* extract is dry *Aloe vera* inner leaf juice extract.

[0108] The *Aloe vera* extract may be derived from any suitable source, for example, the leaves, latex, or gel of the *Aloe vera* plant using methods known in the art or it may be obtained commercially, e.g., as the product Aloe barbadensis Miller, spray dried supplied by Capitol

Ingredients. It may be utilised in the composition according to the invention in solid form, e.g., as a dry inner leaf juice powder, or in any other suitable form, e.g., as a liquid, gel, or paste. Thus, as used herein, *Aloe vera* extract may refer to dry *Aloe vera* inner leaf juice extract. Where the composition comprising *Aloe vera* extract is provided as an aqueous composition, powdered dry *Aloe vera* inner leaf juice extract may be dissolved in the water.

[0109] The *Aloe vera* extract may be present in the composition in any suitable amount, e.g., in a therapeutically effective amount. A therapeutically effective amount of *Aloe vera* extract may correspond to a daily adult human dosage of from about 15 mg to about 600 mg *Aloe vera* extract. In one embodiment, the *Aloe vera* extract, e.g., *Aloe vera* inner leaf juice extract, is present in the composition in a therapeutically effective amount corresponding to a daily adult human dosage of about 175 mg.

[0110] Where the composition is formulated as liquid, e.g., an aqueous liquid, *Aloe vera* inner leaf juice extract may be present in the composition in a concentration ranging from about 0.5 mg/mL to about 4 mg/mL. For example, *Aloe vera* inner leaf juice extract may be present in the composition in a concentration ranging from about 0.5 mg/mL to about 2.0 mg/mL, or from about 1.5 mg/mL to about 3.5 mg/mL, or from about 2.0 mg/mL to about 4.0 mg/mL, e.g., at a concentration of 0.5 mg/mL, 0.7 mg/mL, 0.9 mg/mL, 1.0 mg/mL, 1.2 mg/mL, 1.4 mg/mL, 1.5 mg/mL, 1.6 mg/mL, 1.7 mg/mL, 1.8 mg/mL, 1.9 mg/mL, 2.0 mg/mL, 2.2 mg/mL, 2.4 mg/mL, 2.6 mg/mL, 2.8 mg/mL, 3.0 mg/mL, 3.2 mg/mL, 3.4 mg/mL, 3.6 mg/mL, 3.8 mg/mL, or 4.0 mg/mL, where the amount of *Aloe vera* inner leaf juice extract is expressed as the amount of spray dried *Aloe vera* inner leaf juice extract per mL of the composition. It is contemplated herein that the amount of spray dried *Aloe vera* inner leaf juice extract per mL includes spray dried *Aloe vera* inner leaf juice extract added to the liquid composition in dry form, but also covers addition of *Aloe vera* inner leaf juice extract in other forms (e.g., in a liquid or gel concentrate) in different amounts, provided that the overall concentration of *Aloe vera* inner leaf juice extract after addition is equivalent to the concentration of spray dried *Aloe vera* inner leaf juice extract per mL of the composition. In this regard, the amounts of "spray dried *Aloe vera* inner leaf juice extract" referred to herein are calculated on the basis of the exemplified embodiments of the invention, for example, the composition in Table 1, in which the spray dried *Aloe vera* inner leaf juice extract added to the composition comprises approximately 40-50% maltodextrin and wherein the ratio of fresh plant to spray dried material is about 180-210:1. It will be understood that alternative methods for the preparation of *Aloe vera* extract may be available, in which case the amount of *Aloe vera* extract used in the composition of the invention

may be adjusted to account for differences in the concentration of actual *Aloe vera* sourced material and or additives present.

[0111] In one embodiment, the *Aloe vera* inner leaf juice extract is present in the aqueous composition in a concentration of 1.75 mg/mL.

[0112] The composition of the present disclosure including *Pinus pinaster* stem bark extract, papain and *Aloe vera* extract may be administered in combination with other compounds to assist in the regulation of blood glucose levels. This may permit tailoring of the taste profile of the compositions to improve compliance and thus efficacy and/or to deliver additional or complementary health benefits.

***Honey***

[0113] Accordingly, the composition according to the present invention may comprise honey. Any suitable honey or mixture of honeys may be used.

[0114] The honey may be derived from any suitable source from any suitable floral species, e.g., it may be obtained commercially as British Pharmacopoeia (BP) grade honey, for example, as supplied by Capilano Honey Limited, Australia. The honey may be added to the composition according to the invention as a pure honey in the form of a concentrated, viscous mixture. It may be heated prior to addition to lower the viscosity and increase ease of dispersion or solubilisation in the composition.

[0115] The honey may be present in the composition in any suitable amount. For example, pure honey may be present in the composition at a daily adult human dosage of from about 1.5 g to about 30 g/day, e.g., at a daily human dosage of from about 1.5 g to about 5 g, or from about 5 g to about 10 g, or from about 7 g to about 12 g, or from about 10 g to about 20 g, or from about 15 g to about 30 g, or from about 20 g to about 25 g, or at a daily human dosage of about 1.5 g, 2 g, 5 g, 7.5 g, 10 g, 12.5 g, 15 g, 17.5 g, 20 g, 22.5 g, 25 g, 27.5 g, or 30 g. In one embodiment, the honey is present in the composition in an amount corresponding to a daily adult human dosage of about 10 g.

[0116] Where the composition is formulated as liquid, e.g., an aqueous liquid, pure honey may be present in a concentration ranging from about 50 mg/mL to about 200 mg/mL. For example, pure honey may be present in the composition in a concentration ranging from about 50 mg/mL to about 100 mg/mL, or from about 75 mg/mL to about 150 mg/mL, or from about 100 mg/mL

to about 200 mg/mL, e.g., at a concentration of 50 mg/mL, 60 mg/mL, 70 mg/mL, 80 mg/mL, 90 mg/mL, 100 mg/mL, 110 mg/mL, 120 mg/mL, 130 mg/mL, 140 mg/mL, 150 mg/mL, 160 mg/mL, 170 mg/mL, 180 mg/mL, 1900 mg/mL, or 200 mg/mL. In one embodiment, the honey is present in the aqueous composition in a concentration of 100 mg/mL.

***Acid***

[0117] The composition according to the present invention may comprise an acid. Any suitable acid or mixture of acids may be used, for example, the acid may be an organic or an inorganic acid, or a mixture of any one or more organic and/or inorganic acids. The acid may be of any suitable concentration, e.g., may be concentrated (e.g., substantially anhydrous) or dilute. In one embodiment, the acid is a food acid, e.g., acetic acid. In a preferred embodiment, the acid is acetic acid. In another embodiment, the acid is glacial acetic acid. The acid may be added to assist the composition with entering the blood stream of a subject, which may in turn enable the composition or one or more of its component ingredients to reach parts of the subject's body that are, e.g., inflamed. The acid may be added to the composition as vinegar.

[0118] The acid may be present in the composition in an amount sufficient to acidify the composition to a pH of between about 3.2 to about 4.2, for example, between about 3.2 and about 3.7, about 3.5 and about 4.0, about 3.7 and about 4.2, or about 4.0 and about 4.2, e.g., at a pH of about 3.2, about 3.3, about 3.4, about 3.5, about 3.6, about 3.7, about 3.8, about 3.9, about 4.0, about 4.1, or about 4.2.

[0119] Where the acid is glacial acetic acid, the acid may be present in the composition in any suitable amount. For example, the glacial acetic acid may be present in the composition at a daily adult human dosage of from about 30 mg to about 1200 mg/day, e.g., at a daily human dosage of from about 30 mg to about 100 mg, or from about 50 mg to about 250 mg, or from about 150 mg to about 250 mg, or from about 200 mg to about 500 mg, or from about 500 mg to about 750 mg, or from about 700 mg to about 1000 mg, or from about 900 mg to about 1200 mg, or at a daily human dosage of about 30 mg, 50 mg, 100 mg, 200 mg, 300 mg, 400 mg, 500 mg, 600 mg, 700 mg, 800 mg, 900 mg, 1000 mg, 1100 mg, or 1200 mg. In one embodiment, the glacial acetic acid is present in the composition in an amount corresponding to a daily adult human dosage of about 200 mg.

[0120] Where the composition is formulated as liquid, e.g., an aqueous liquid, the glacial acetic acid may be present in the composition in a concentration ranging from about 1 mg/mL to about

8 mg/mL. For example, the glacial acetic acid may be present in the composition in a concentration ranging from about 1.0 mg/mL to about 3.0 mg/mL, or from about 2.0 mg/mL to about 5.0 mg/mL, or from about 3.0 mg/mL to about 6.0 mg/mL, or from about 4.0 mg/mL to about 8.0 mg/mL, e.g., at a concentration of 1.0 mg/mL, 1.2 mg/mL, 1.4 mg/mL, 1.6 mg/mL, 1.8 mg/mL, 2.0 mg/mL, 2.2 mg/mL, 2.4 mg/mL, 2.6 mg/mL, 2.8 mg/mL, 3.0 mg/mL, 3.2 mg/mL, 3.4 mg/mL, 3.6 mg/mL, 3.8 mg/mL, 4.0 mg/mL, 4.5 mg/mL, 5.0 mg/mL, 5.5 mg/mL, 6.0 mg/mL, 6.5 mg/mL, 7.0 mg/mL, 7.5 mg/mL, or 8.0 mg/mL. In one embodiment, the glacial acetic acid is present in the aqueous composition in a concentration of 2 mg/mL.

[0121] The glacial acetic acid may be obtained commercially, e.g., as supplied by Bronson & Jacobs Pty Limited, Sydney, Australia.

***Synergism***

[0122] The composition according to the present invention may comprise a synergistic mixture of active ingredients. The synergism may refer to the effect exerted by the composition in regulating blood sugar levels in a subject, or may refer to the effect exerted by the composition in treating or preventing pre-diabetes, diabetes, or any associated dysfunctions. For example, the composition comprising *Pinus pinaster* stem bark extract, papain and *Aloe vera* extract may act synergistically to regulate blood sugar levels or treat or prevent pre-diabetes, diabetes or any associated dysfunctions in a subject. The composition comprising *Pinus pinaster* stem bark extract, papain, *Aloe vera* extract and honey may additionally or alternatively act synergistically to regulate blood sugar levels or treat or prevent pre-diabetes, diabetes or any associated dysfunctions in a subject. The composition comprising *Pinus pinaster* stem bark extract, papain, *Aloe vera* extract, and acid, e.g., acetic acid, may additionally or alternatively act synergistically to regulate blood sugar levels or treat or prevent pre-diabetes, diabetes or any associated dysfunctions in a subject. The composition comprising *Pinus pinaster* stem bark extract, papain, *Aloe vera* extract, honey and acid, e.g., acetic acid, may additionally or alternatively act synergistically to regulate blood sugar levels or treat or prevent pre-diabetes, diabetes or any associated dysfunctions in a subject.

***Additives***

[0123] The composition of the invention may additionally include any suitable additives. For example, the additives may include a preservative, a flavouring agent, a salt, a pigment, or a mixture of any two or more of these.

[0124] Suitable preservatives are known in the art, for example benzoic acid, sodium benzoate, sodium or potassium sorbate, sorbic acid, hydroxybenzoate, etc. Accordingly, the composition disclosed herein may comprise one or more preservatives. The preservatives may be added to inhibit growth of bacterial or other harmful organisms in the composition during manufacture and storage. Suitable preservatives for use in the composition according to the present invention include sodium benzoate and potassium sorbate. Suitable preservatives may be added to the composition as disclosed herein in any suitable amount. For example, where the composition is formulated as liquid, e.g., an aqueous liquid, the preservatives may be present in a concentration ranging from about 500 μg/mL to about 1000 μg/mL, or from about 500 μg/mL to about 750 μg/mL, or from about 650 μg/mL to about 800 μg/mL, or from about 800 μg/mL to about 1000 μg/mL, e.g., about 500 μg/mL, about 600 μg/mL, about 700 μg/mL, about 800 μg/mL, about 900 μg/mL, or about 1000 μg/mL, or otherwise as may be determined by one skilled in the art. In one embodiment, sodium benzoate is present in the aqueous composition in a concentration of 400 μg/mL and potassium sorbate is present in the aqueous composition in a concentration of 400 μg/mL.

[0125] Suitable flavouring agents are also known in the art and are commercially available, e.g., suitable flavouring agents for use in the composition according to the present invention may include SM10483- Natural Strawberry Flavour(TIF-20550), SM00401- Natural Orange Oil Sweet Aust 48-7685, SM10458 Orange Passionfruit Flavour (UA-71735), or Wildberry flavour UA71225 as supplied by Ungerer Australia Pty Limited. Accordingly, the composition disclosed herein may comprise one or more flavouring agents. The flavouring agents may improve the flavour and/or odour of the composition and result in increased compliance and therefore efficacy. Suitable flavouring agents may be added to the composition as disclosed herein in any suitable amount. For example, where the composition is formulated as liquid, e.g., an aqueous liquid, the flavouring agent may be present in a concentration ranging from about 5 mg/mL to 15 mg/mL, e.g., from about 5 mg/mL to about 7 mg/mL, from about 7 mg/mL to about 12 mg/mL, or from about 12 mg/mL to about 15 mg/mL, e.g., at about 5 mg/mL, about 6 mg/mL, about 7 mg/mL, about 8 mg/mL, about 9 mg/mL, about 10 mg/mL, about 11 mg/mL, about 12 mg/mL, about 13 mg/mL, about 14 mg/mL, about 15 mg/mL, or otherwise as may be determined by one skilled in the art. In one embodiment, the flavouring agent – preferably Wildberry flavour UA71225 – is present in the aqueous composition in a concentration of 10 mg/mL.

[0126] Suitable salts are known in the art, for example sodium and potassium chloride.

Accordingly, the composition disclosed herein may comprise one or more salts. One suitable salt for use in the composition according to the present invention is sodium chloride. The salt may be added in any suitable amount. For example, where the composition is formulated as liquid, e.g., an aqueous liquid, the salt, preferably sodium chloride, may be present in a concentration ranging from about 1.0 mg/mL to about 6.0 mg/mL, e.g., in a range of from about 1.0 mg/mL to about 3.0 mg/mL, or from about 2.0 mg/mL to about 5.0 mg/mL, or from about 4.0 mg/mL to about 6.0 mg/mL, e.g., at a concentration of 1.0 mg/mL, 1.5 mg/mL, 2.0 mg/mL, 2.5 mg/mL, 3.0 mg/mL, 3.5 mg/mL, 4.0 mg/mL, 4.5 mg/mL, 5.0 mg/mL, 5.5 mg/mL, or 6.0 mg/mL. In one embodiment, sodium chloride is present in the aqueous composition in a concentration of 3.00 mg/mL.

[0127] Suitable pigments are known in the art, for example carotenoids, chlorophylls and anthocyanins, etc. Accordingly, the composition disclosed herein may comprise one or more pigments. The pigments may be added to increase the visual appeal of the composition. Suitable pigments for use in the composition according to the present invention include anthocyanin extract. Suitable pigments may be added to the composition as disclosed herein in any suitable amount. For example, anthocyanin extract may be present in the composition in a concentration of between about 500 μg/mL and about 1000 μg/mL, or between about 500 μg/mL and about 750 μg/mL, or between about 650 μg/mL and about 800 μg/mL, or between about 800 μg/mL and about 1000 μg/mL, e.g., about 500 μg/mL, about 600 μg/mL, about 700 μg/mL, about 800 μg/mL, about 900 μg/mL, or about 1000 μg/mL, or otherwise as may be determined by one skilled in the art. In one embodiment, the pigment – preferably anthocyanin extract – is added to the aqueous composition at a concentration of 800 μg/mL.

[0128] The amounts of pigments, e.g., "anthocyanin extract" referred to herein are calculated on the basis of the exemplified embodiments of the invention, for example, the composition in Table 1, in which the anthocyanin extract added to the composition comprises approximately 25-35% maltodextrin. It will be understood that alternative sources or forms of anthocyanin extract may be available that contain more, less or no maltodextrin, in which case the amount of anthocyanin extract used in the composition of the invention may be adjusted so as to account for differences in the anthocyanin content of an anthocyanin extract used in the preparation of the inventive composition.

[0129] Other suitable additives, such as thickeners (e.g., maltodextrin), diluents, buffers, additional therapeutic agents, bioavailability enhancers, side-effect suppressing components,

binders, etc. may also be used in the composition disclosed herein. These additives preferably do not negatively affect the efficacy or therapeutic benefits of the composition.

***Additional components***

[0130] In addition to the above additives, the composition according to the present invention may comprise one or more additional components.

[0131] For example, the composition may comprise an antioxidant such as ascorbic acid (vitamin C). Common additional antioxidants are vitamins A, E, and the mineral selenium or the group of antioxidants found in pine bark extract known as oligomeric proanthocyanidins, (OPCs / PCOs), astaxanthin, carotenoids, coenzyme Q10 ("CoQ10"), flavonoids, glutathione, Goji (Wolfberry), hesperidin, lacto-Wolfberry, lignan, lutein, lycopene, polyphenols, selenium, vitamin A, vitamin C, vitamin E, zeaxanthin, or combinations thereof.

[0132] The composition may comprise a mineral including boron, calcium, chloride, chromium, copper, iodine, iron, magnesium, manganese, molybdenum, nickel, phosphorus potassium, selenium, silicon, sodium, tin, vanadium, zinc, or combinations thereof in any suitable form, e.g., in the form of a mineral or other bioavailable complex.

[0133] The composition may comprise a phytonutrient selected from the group consisting of flavanoids, allied phenolic compounds, polyphenolic compounds, terpenoids, alkaloids, sulphur-containing compounds, or combinations thereof. The phytonutrient may be selected from the group consisting of carotenoids, plant sterols, quercetin, curcumin, limonin, or combinations thereof.

[0134] The composition may comprise a herbal medicine known to have or suspected of having anti-diabetic effects. Such herbal medicine may include a medicinal plant, for example, from the *Malvaceae* family, such as *Abelmoschus moschatus Medik,* or from the *Asteraceae* family, such as *Achiliea santolina* L., or *Achyrocline satureioides* (Lam.) DC, or *Artemisia dracunculus* L. (dragon herb) or *Cichorium intybus* L., or *Eclipta alba* (L) Hassk, or from the *Lamiaceae* family such as *Ajuga iva* L. *Schreberr* (Medit), or *Ocimum sanctum* Linn. (Tulasi), or *Origanum vulgare* L. or from the *Annonaceae* family such as *Annona squamosa* L., or from the *Gentianeacea* family such as *Anthocleista djalonensis* A. Chev (cabbage tree), or *Anthocleista Schweinfurthii,* or *Anthocleista vogelii* Planch, *Enicostemma littorale Blume* or *Gentiana olivieri* L., or from the *Oxalidaceae* family such as *Averrhoa bilimbi* L, or *Biophytum sensitivum* (L) DC, or from the *Leguminosae* family such as *Bauhinia candicans* Benth, or from the *Bixaceae* family

such as *Bixa orellana* L., or from the *Nyctaginaceae* family such as *Boerhaavia diffusa* L., or from the *Brassicaceae* family such as *Brassica nigra* (L) Koch, or *Eruka sativa,* or *Lepidium sativum* L., or from the *Caesalpiniaceae* family such as *Capparis spinosa L.* or *Cassia auriculata* L. or *Tamarindus indica* L., or from the *Capparidaceae* family such as *Capparis spinosa* L., or from the *Apiaceae family* such as *Carum carvi* L., or from the family *Rutaceae* such as *Clausena anisata* (Wild) Benth., or from the family *Palmae* such as *Cocos nucifera* Linn. (Coconut palm), or from the *Cucurbitaceae* family such as *Cogniauxia podoleana,* or *Ibervillea sonorae* S., or from the family *Conimelinaceae* such as *Commelina communis* L., or from the family *Zingiberaceae* such as *Curcuma longa* L., or from the family *Poaceae* such as *Cynodon dactylon* Pers. (Bermuda grass), or from the family *Ginkgoaceae* such as *Ginkgo biloba* L., or from the family *Papilionaceae* such as *Glycyrrhiza uralensis* Fish., or from the family *Asclepiadaceae,* such as *Gongronema latifolium* Benth., or *Gymnema montanum* Hook, or from the family *Sterculiaceae* such as *Helicteres isora* L., As., or from the *Rubiaceae* family such as *Hintonia standleyana,* or from the *Gramineae* family such as *Hordeum vulgare* L. (Barley), or from the *Convolvulaceae* family such as *Ipomoea aquatic* Forsk., or *Ipomea batata* Linn (Sweet potato), or from the *Loranthaceae* family such as *Loranthus micranthus* Linn, or from the *Moraceae* family such as *Morus indica*. L., or from the *Musaceae* family such as *Musa sapientum* Kuntz (Banana), or from the *Euphorbiaceae* family such as *Phyllanthus amarus* Schum. Thonn, or *Phyllanthus niruri* L., or *Phyllanthus sellowianus* Mull. Arg., or from the *Piperacea* family such as *Piper longum,* or from the *Myrtaceae* family such as *Psidium guajava* L., or *Syzygium alternifolium* (Wt) Walp, or from the *Lythraceae* family such as *Punica granatum* L. (pomegranate), or from the *Papilionaceae* family such as *Retama raetam (RR)* (Forssk) Webb., or from the *Adoxaceae* family such as *Sambucus nigra* L., or from the Apocynaceae family such as *Sanguis draxonis,* or from the *Anacardiaceae* family such as *Sclerocarya birea* (A. Rich), or from the *Scrophariaceae* family such as *Scoparia dulcis* L., or from the *Caryophyllaceae* family such as *Spergularia purpurea,* or from the *Chenopodiaceae* family such as *Suaeda fruticosa* (SF) Euras, or from the *Combretaceae* family such as *Terminalia chebula* Retz., or *Terminalia bellirica* (Gaertn), or from the *Menispermaceae* family such as *Tinospora cordifolia* Miers., or from the *Urticaceae* family such as *Urtica pilulifera* L., or from the *Astereaceae* family such as *Vernonia amygdalina* Del., or from the *Solanaceae* family such as *Withania soimifera* (L) Dunal, or from the *Zygophyllaceae* family such as *Zygophyllum gaetulum* Emb and Maire.

[0135] The composition may comprise one or more vitamins and/or amino acids. The vitamins may be selected from, but not limited to: vitamins A, $B_1$, $B_2$, $B_3$, $B_5$, $B_6$, $B_9$, $B_{12}$, C, D, E, K, K1

and K2 (i.e. MK-4, MK-7), folic acid or biotin. The amino acids may be selected from, but are not limited to: alanine, arginine, aspartic acid, cystine, citrulline, glycine, glutamate, glutamine, histidine, hydroxyproline, hydroxyserine, hydroxytyrosine, hydroxylysine, isoleucine, lysine, methionine, phenylalanine, proline, serine, taurine, threonine, tryptophan tyrosine, valine, or ornithine.

[0136] The composition may comprise a source of omega-3 or omega-6 fatty acids, for example, fish oil, krill, plant sources of omega-3, flaxseed, canola oil, walnut, and algae. Examples of omega-3 fatty acids include, for example, omega-linolenic acid ("ALA"), docosahexaenoic acid ("DHA"), stearidonic acid (SDA), eicosapentaenoic acid ("EPA"), or combinations thereof. Examples of omega-6 fatty acids include linoleic acid ("LA"), arachidonic acid ("ARA"). The omega-3 fatty acids may be provided in an amount of about 0.25 g to 5.0 g per day, such as from about 1.0 to 3.0 g per day.

[0137] The composition may comprise a phenolic compound, including, for example, monophenols (such as, for example, apiole, carnosol, carvacrol, dillapiole, rosemarinol); flavonoids (polyphenols) including flavonols (such as, for example, quercetin, fingerol, kaempferol, myricetin, rutin, isorhamnetin), flavanones (such as, for example, fesperidin, naringenin, silybin, eriodictyol), flavones (such as, for example, apigenin, tangeritin, luteolin), flavan-3-ols (such as, for example, catechins, (+)-catechin, (+)-gallocatechin, (–)-epicatechin, (—)-epigallocatechin, (–)epigallocatechin gallate (EGCG), (–)-epicatechin 3-gallate, theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate, theaflavin-3,3'-digallate, thearubigins), anthocyanins (flavonals) and anthocyanidins (such as, for example, pelargonidin, peonidin, cyanidin, delphinidin, malvidin, petunidin), isoflavones (phytoestrogens) (such as, for example, daidzein (for mononetin), genistein (biochanin A), glycitein), dihydroflavonols, chalcones, coumestans (phytoestrogens), and Coumestrol; Phenolic acids (such as: Ellagic acid, Gallic acid, Tannic acid, Vanillin, curcumin); hydroxycinnamic acids (such as, for example, caffeic acid, chlorogenic acid, cinnamic acid, ferulic acid, coumarin); lignans (phytoestrogens), silymarin, secoisolariciresinol, pinoresinol and lariciresinol); tyrosol esters (such as, for example, tyrosol, hydroxytyrosol, oleocanthal, oleuropein); stilbenoids (such as, for example, resveratrol, pterostilbene, piceatannol) and punicalagins; terpenes (isoprenoids) including carotenoids (tetraterpenoids) including carotenes (such as, for example, beta-carotene, [3-carotene, gama-carotene, delta-carotene, lycopene, neurosporene, phytofluene, phytoene), and xanthophylls (such as, for example, canthaxanthin, cryptoxanthin, aeaxanthin, astaxanthin, lutein, rubixanthin); monoterpenes (such as, for example, limonene, perillyl alcohol); saponins; lipids

including: phytosterols (such as, for example, campesterol, beta sitosterol, gamma sitosterol, stigmasterol), tocopherols (vitamin E), and omega-3, 6, and 9 fatty acids (such as, for example, gamma-linolenic acid); triterpenoid (such as, for example, oleanolic acid, ursolic acid, betulinic acid, moronic acid); betalains, including betacyanins (such as: betanin, isobetanin, probetanin, neobetanin); and betaxanthins (non glycosidic versions) (such as, for example, indicaxanthin, and vulgaxanthin); organosulfides, including, for example, dithiolthiones (isothiocyanates) (such as, for example, sulphoraphane); and thiosulphonates (allium compounds) (such as, for example, allyl methyl trisulfide, and diallyl sulfide), indoles, glucosinolates, including, for example, indole-3-carbinol; sulforaphane; 3,3'-diindolylmethane; sinigrin; allicin; alliin; allyl isothiocyanate; piperine; syn-propanethial-S-oxide; protein inhibitors, which include, for example, protease inhibitors; other organic acids including oxalic acid, phytic acid (inositol hexaphosphate); tartaric acid; and anacardic acid; or combinations thereof.

[0138] The composition may comprise a protein. Non-limiting examples of proteins include dairy based proteins, plant based proteins, animal based proteins and artificial proteins. Dairy based proteins may be selected from the group consisting of casein, micellar casein, caseinates, casein hydrolysate, whey, whey hydrolysates, whey concentrates, whey isolates, milk protein concentrate, milk protein isolate, or combinations thereof. Plant based proteins may include, for example, soy protein (e.g., all forms including concentrate and isolate), pea protein (e.g., all forms including concentrate and isolate), canola protein (e. g., all forms including concentrate and isolate), wheat and fractionated wheat proteins, corn and it fractions including zein, rice, oat, potato, peanut, and any proteins derived from beans, buckwheat, lentils, pulses, single cell proteins, or combinations thereof. Animal based proteins may be selected from the group consisting of beef, poultry, fish, lamb, seafood, or combinations thereof.

[0139] The composition may comprise a source of phytochemicals, e.g., flavonoids and allied phenolic and polyphenolic compounds, terpenoids, e.g., carotenoids and plant sterols and alkaloids and sulfur containing compounds.

[0140] The composition may comprise a source of carbohydrates. Any suitable carbohydrate may be used, including, but not limited to, sucrose, lactose, glucose, fructose, corn syrup solids, maltodextrin, modified starch, amylose starch, tapioca starch, corn starch or combinations thereof.

[0141] The composition may comprise grains. The grains may include, for example, whole grains, which may be obtained from different sources. The different sources may include

semolina, cones, grits, flour and micronized grain (micronized flour), and may originate from a cereal or a pseudo-cereal. The grain may be a hydrolysed whole grain component, i.e., an enzymatically digested whole grain component or a whole grain component digested by using at least an alpha-amylase, which alpha-amylase shows no hydrolytic activity towards dietary fibers when in the active state. The hydrolsed whole grain component may be further digested by the use of a protease, which protease shows no hydrolytic activity towards dietary fibers when in the active state. The hydrolyzed whole grain component may be provided in the form of a liquid, a concentrate, a powder, a juice, a puree, or combinations thereof.

[0142] The composition may comprise a source of fat. The source of fat may include any suitable fat or fat mixture. For example, the fat source may include, but is not limited to, vegetable fat (such as olive oil, corn oil, sunflower oil, high-oleic sunflower, flax seed oil, rapeseed oil, canola oil, high oleic canola oil, hazelnut oil, soy oil, palm oil, coconut oil, blackcurrant seed oil, borage oil, lecithins, and the like), animal fats (such as milk fat), or combinations thereof. The source of fat may also be less refined versions of the fats listed above (e.g., olive oil for polyphenol content).

[0143] Where the composition is formulated as an oral supplement, it may contain *Pinus pinaster*, *Aloe vera* and papain in addition to an appropriate nutritional profile that contains 10 or more grams of high quality protein, which may be provided as whey protein micelle, lipids with the EPA and DHA, and carbohydrates for energy and palatability. Vitamins such as vitamin D and minerals and ingredients such as lactowolfberry may also be included.

[0144] The composition may comprise a probiotic strain.

[0145] The composition may comprise all three branched chain amino acids, leucine, isoleucine, and valine.

***Composition Form***

[0146] The composition according to the present invention may be formulated for administration in any suitable manner. For example, the composition may be formulated for oral administration, e.g., as a read-to-drink liquid mixture, solution, suspension, emulsion, syrup or in a liquid comestible such as a soft drink, juice, sports drink, milk drink, yogurt drink, soup, etc. The composition may be an aqueous composition. Alternatively, the composition may be formulated for oral administration in the form of a tablet, capsule, chewable tablet, or soft gel capsule.

[0147] In one embodiment, the composition disclosed herein is an aqueous composition comprising: 2.40 mg/mL papain; 2.60 mg/mL dry *Pinus pinaster* stem bark extract; 1.75 mg/mL dry *Aloe vera* inner leaf juice; 100.00 mg/mL honey; 2.00 mg/mL glacial acetic acid; 3.00 mg/mL sodium chloride; 400 μg/mL potassium sorbate; 400 μg/mL sodium benzoate; 10.00 mg/mL wildberry flavour UA71225; and 800 μg/mL anthocyanin extract.

[0148] In another embodiment, the composition disclosed herein is aqueous composition comprising papain, dry *Pinus pinaster* stem bark extract, dry *Aloe vera* inner leaf juice, honey, glacial acetic acid, sodium chloride, potassium sorbate, sodium benzoate, wildberry flavour UA71225 and anthocyanin extract in a ratio of papain : dry *Pinus pinaster* stem bark extract : dry *Aloe vera* inner leaf juice : honey : glacial acetic acid : sodium chloride : potassium sorbate : sodium benzoate : wildberry flavour UA71225 : anthocyanin extract of 2.40 : 2.60 : 1.75 : 100 : 2.00 : 3.00 : 0.40 : 0.40 : 10.00 : 0.80 by weight. As such, compositions comprising the same ingredients in the same relative proportions but in different total volumes are contemplated, e.g., concentrated or dilute aqueous compositions having the same relative proportions of ingredients.

[0149] The composition disclosed herein, in particular the aqueous compositions disclosed herein, may have a pH of between about 3.2 and about 4.2, e.g., a pH of about 3.2, about 3.5, about 3.7, about 3.9, or about 4.1, or may be adjusted so as to have a pH of between about 3.2 and about 4.2, e.g., a pH of about 3.2, about 3.5, about 3.7, about 3.9, or about 4.1. In one embodiment, the composition disclosed herein, in particular the aqueous compositions disclosed herein, has a pH of about 3.7.

[0150] A further alternative formulation contemplated in the invention is provision of the composition in dry form as a powder or granules, or as a dry or liquid concentrate, which can then be diluted with water or another liquid, such as milk or fruit juice, to yield a ready-to-drink composition. The powder or granules may be provided in a sachet, which is optionally soluble. Alternatively, the composition may be consumed as a powder in the absence of a drink or additional food product. The composition may be assembled and packaged as a powder for dissolution at the time of use.

[0151] Where the composition is provided in dry form, e.g., in the form of powder or granules, the composition may comprise a therapeutically effective amount of *Pinus pinaster* stem bark extract corresponding to a daily adult human dosage of from about 60 mg to about 1500 mg *Pinus pinaster* stem bark extract; a therapeutically effective amount of papain corresponding to a daily adult human dosage of from about 30 mg to about 1200 mg papain; and a therapeutically

effective amount of *Aloe vera* extract corresponding to a daily adult human dosage of from about 15 mg to about 600 mg *Aloe vera* extract. In one embodiment, a dry form of the composition disclosed herein comprises a therapeutically effective amount of *Pinus pinaster* stem bark extract corresponding to a daily adult human dosage of about 260 mg; a therapeutically effective amount of papain corresponding to a daily adult human dosage of about 240 mg; and a therapeutically effective amount of *Aloe vera* extract corresponding to a daily adult human dosage of about 175 mg.

[0152] The composition described herein may be added to a food or beverage such as a water-based beverage, a milk-based beverage, a yoghurt-based beverage, another dairy-based beverage, a milk-substitute based beverage such as soy milk or oat milk, or a juice-based beverage, water, a soft drinks, a carbonated drink, or a nutritional beverage, (including a concentrated stock solution of a beverage and a dry powder for preparation of such a beverage), or in a baked product such as a cracker, a bread, a muffin, a roll, a bagel, a biscuit, a cereal, a bar such as a muesli bar, a health food bar and the like, a dressing, a sauce, a custard, a yoghurt, a pudding, a pre-packaged frozen meal, a soup or a confectionery item.

[0153] The composition according to the present invention may be formulated for oral administration using any suitable method known in the art using suitable excipients, diluents and fillers. In general, oral compositions are prepared by uniformly and intimately bringing into association the components of the composition with a liquid carrier or finely divided solid carrier, or both and then, if necessary, shaping the product into the desired composition.

[0154] For example, when the composition is formulated as capsules, the components of the composition may be formulated with one or more pharmaceutically acceptable carriers such as starch, lactose, microcrystalline cellulose and/or silicon dioxide. Additional ingredients may include lubricants such as magnesium stearate and/or calcium stearate. The capsules may optionally be coated, for example, with a film coating or an enteric coating and/or may be formulated so as to provide slow or controlled release of the composition therein.

[0155] Tablets may be prepared by compression or moulding, optionally with one or more additional ingredients. Compressed tablets may be prepared by compressing in a suitable machine the components of the composition in a free-flowing form such as a powder or granules, optionally mixed with a binder, lubricant (for example magnesium stearate or calcium stearate), inert diluent or a surface active/dispersing agent. Moulded tablets may be made by moulding a mixture of the powdered composition moistened with an inert liquid diluent, in a suitable

machine. The tablets may optionally be coated, for example, with a film coating or an enteric coating and/or may be formulated so as to provide slow or controlled release of the composition therein.

[0156] If the compositions are formulated to be administered orally, the compositions may be a liquid oral supplement. Such liquid oral supplements may comprise additional beneficial ingredients, for example, as outlined in the section herein entitled 'Additional Components', which may allow for more efficient use of the administered composition for preservation of regulated blood glucose levels.

**METHODS OF TREATMENT AND USES**

[0157] The composition of the invention as described herein is suitable for treatment and prevention of a variety of conditions associated with or caused by elevated blood sugar. For example, the composition may be suitable for treating and/or preventing any dysfunction associated with excess blood glucose, which may include, for example, disturbances in everyday physiological functions such as increased thirst and hunger, frequent urination, fatigue/lethargy blurry vision, sleepiness, and muscle weakness. An associated dysfunction may result from, result in, be characterised by, or otherwise be associated with a high blood glucose level in a subject and may directly or indirectly result from the high blood glucose level. In some cases, the associated dysfunction may be temporally separated from when the high blood glucose level is recorded. As high blood glucose levels may lead to disturbances in overall physiological function and may induce or promote a chronic disease, reducing blood glucose levels to a normal range may be beneficial in reducing the risk of metabolic disorders, and thus treatment of high blood glucose levels may also treat chronic diseases or symptoms thereof. Accordingly, methods of treating chronic diseases associated with elevated blood glucose levels or symptoms thereof are also contemplated herein.

[0158] Accordingly, methods for stimulating regulation of high blood glucose levels in a subject in need of same are provided herein. Methods for alleviating or minimising the adverse effects of high blood glucose levels in a subject in need of same are provided. Methods for preserving a blood glucose level in the normal physiological range in a subject in need of same are provided. The methods include administering to the individual a composition as described in the section herein entitled 'COMPOSITION'. The compositions of the present disclosure may also include other active or inactive ingredients as discussed herein.

[0159] For example, the present invention provides a method of treating or preventing elevated blood glucose, comprising administering to a subject in need thereof an effective amount of a composition as described in the section herein entitled 'COMPOSITION'. There is also provided a method of treating or preventing pre-diabetes, comprising administering to a subject in need thereof an effective amount of a composition as described in the section herein entitled 'COMPOSITION'.

[0160] There is further provided a method of reducing inflammation, comprising administering to a subject in need thereof an effective amount of a composition as described in the section herein entitled 'COMPOSITION'. The inflammation may be, for example, in an athlete, and in particular, an athlete undergoing intense exercise/training or endurance exercise. There is still further provided a method of decreasing cytokine-induced inflammation, or of decreasing oxidative stress responses, comprising administering to a subject in need thereof an effective amount of a composition as described in the section herein entitled 'COMPOSITION'. There is also provided a method of treating or preventing a disease characterised by elevated levels of cytokine-induced inflammation, or characterised by elevated levels of oxidative stress, comprising administering to a subject in need thereof an effective amount of a composition as described in the section herein entitled 'COMPOSITION'. The disease may be atherosclerosis, liver inflammation associated with type 2 diabetes and obesity, endometriosis, or osteoarthritis.

[0161] There is also provided a method of reducing one or more side effects, in particular, adverse side effects, of a diabetes medication selected from the group consisting of metformin, sulfonylureas, meglitinides, thiazolidinediones, DPP-4 inhibitors, GLP-1 receptor agonists, SGLT2 inhibitors, and insulin therapy, comprising administering to a subject in need thereof an effective amount of a composition as described in the section herein entitled 'COMPOSITION'. Common adverse side effects may include abdominal or stomach discomfort, decreased appetite, or diarrhoea.

[0162] There is further provided a method of treating or preventing a dysfunction associated with excess blood glucose, comprising administering to a subject in need thereof an effective amount of a composition as described in the section herein entitled 'COMPOSITION'. The dysfunction may be increased thirst and hunger, frequent urination, fatigue/lethargy, blurry vision, sleepiness, or muscle weakness, or a combination thereof.

[0163] The composition of the present invention may be administered in accordance with the methods described herein alone, or in combination with metformin or one or more other blood

glucose lowering medication(s).

[0164] Still further, there is provided a method of improving organ function comprising administering to a subject in need thereof an effective amount of a composition as described in the section herein entitled 'COMPOSITION'. The term "improving organ function" means that a composition is administered to, or a method is used for, a subject for a period effective to improve function as determined by comparison with function in subjects not being administered the composition or using the method or determined by comparison with function in subjects prior to being administered the composition or using the method. Any suitable method of assessing organ function can be used to determine whether an improvement occurs. In one embodiment, organ function is assessed by the level(s) of one or more biomarkers in a tissue or fluid sample, for example systemic inflammation content of blood. Elevated levels in blood of human C-Reactive Protein (CRP), in particular the slightly to moderately elevated levels are an indicator of when there is inflammation in the body.

[0165] The composition of the invention as described herein is suitable for treating and regulating high cholesterol levels. In particular, the composition of the invention may be suitable for lowering blood cholesterol in subjects in need thereof. Accordingly, there is provided a method of lowering blood cholesterol comprising administering to a subject in need thereof an effective amount of a composition as described in the section herein entitled 'COMPOSITION'.

[0166] The blood cholesterol may be selected from the group consisting of: low density lipoproteins (LDL), high density lipoproteins (HDL), trigylcerides and total cholesterol, or a combination thereof. For example, the composition may be used to lower low density lipoprotein (LDL) or 'bad' blood cholesterol levels in a subject, may additionally or alternatively be used to increase high density lipoprotein (HDL) or 'good' blood cholesterol levels, or may be used to decrease blood triglyceride levels. The composition may be used to decrease the ratio of Total Cholesterol to HDL in the blood of a subject.

[0167] The term 'increasing' and related terms 'increase', 'increased' and the term 'decreasing' and related terms 'decrease', 'decreased' mean that a composition is administered to, or a method is used for, a subject for a period effective to change the level of the recited marker, e.g., HDL or LDL cholesterol, as determined by comparison with the levels of the recited marker in subjects not being administered the composition or in subjects not using the method, or determined by comparison with a level of the marker in a subject prior to the subject being

administered the composition or prior to using the method. Any suitable method of assessing levels of the recited marker, e.g., cholesterol, may be used to determine whether an increase or decrease occurs, and methods for measuring blood cholesterol are known in the art.

[0168] The composition of the invention as described herein is also suitable for improving immune function, and accordingly, a method of improving immune function in a subject in need thereof by administering an effective amount of a composition as described in the section herein entitled 'COMPOSITION' is also provided herein. Improved immune function may be measured using any suitable immune function indicator, for example, as an increased white blood cell count, where the white blood cells are, e.g., neutrophils, using methods known in the art.

[0169] The composition of the invention as described herein is further suitable for treating an autoimmune disease, and accordingly, methods of treating an autoimmune disease comprising administering to a subject in need thereof an effective amount of a composition as described in the section herein entitled 'COMPOSITION' are also provided herein. The autoimmune disease may be any autoimmune disease, for example, it may be selected from the group consisting of: lupus, arthritis, psoriasis, and type I diabetes. Where the autoimmune disease is lupus, the treating may include decreasing the number and/or severity of lesions, e.g., sub cutaneous lesions, in or on the subject. Where the autoimmune disease is arthritis, in particular rheumatoid arthritis, the treating may include decreasing swelling and pain in the subject. Where the autoimmune disease is psoriasis, the treating may include reducing the number and/or severity of skin plaques, decreasing swelling or inflammation, and/or decreasing pain. Where the autoimmune disease is type I diabetes, the treating may enable a reduction in the amount of insulin otherwise required for effective management of blood sugar levels.

[0170] The composition of the invention as described herein may also be used to treat emphysema, and accordingly, there is provided a method of treating emphysema comprising administering to a subject in need thereof an effective amount of a composition as described in the section herein entitled 'COMPOSITION'.

[0171] The composition of the invention as described herein may be used to increase general wellbeing in a subject, and accordingly, there is provided a method of increasing general wellbeing comprising administering to a subject in need thereof an effective amount of a composition as described in the section herein entitled 'COMPOSITION'. A measure of increased general wellbeing may include one or more of: increased energy levels, better sleep,

improved breathing ability, improved levels of physical activity, and/or improved physical functioning.

[0172] The composition as described herein may have potent antioxidant activity and anti-inflammatory actions and support a healthy immune system. For example, it may have excellent radical scavenging properties and may support the health and function of the immune system. These properties may cause or assist the observed therapeutic effects of the compositions described herein for a range of diseases and conditions.

[0173] Also contemplated herein are uses of a composition as described in the section herein entitled 'COMPOSITION' for the manufacture of a medicament for: treating or preventing elevated blood glucose; or preventing or delaying the onset of type 2 diabetes mellitus (T2DM); or treating or preventing pre-diabetes; or reducing inflammation; or decreasing cytokine-induced inflammation; or decreasing an oxidative stress response; or treating or preventing a disease characterised by elevated levels of cytokine-induced inflammation; or treating or preventing a disease characterised by elevated levels of oxidative stress; or reducing one or more side effects of a diabetes medication selected from the group consisting of metformin, sulfonylureas, meglitinides, thiazolidinediones, DPP-4 inhibitors, GLP-1 receptor agonists, SGLT2 inhibitors, and insulin therapy; or treating or preventing a dysfunction associated with excess blood glucose; or improving organ function; or lowering blood cholesterol; or improving immune function; or treating an autoimmune disease, e.g., lupus, arthritis, psoriasis, or type I diabetes; or treating emphysema; or increasing general wellbeing, e.g., increasing energy levels, improving vitality, improving sleep, improving breathing ability, improving levels of physical activity, improving mental functioning and/or improving physical functioning in a subject.

[0174] In the methods and uses described herein, the subject may be elderly, have a medically diagnosed or undiagnosed condition of pre-diabetes, or be at risk of developing pre-diabetes. The subject may be taking metformin or other blood glucose lowering medications at the time of taking the composition described herein, or may have been treated with metformin or other blood glucose lowering medications prior to taking the composition.

[0175] In the methods and uses described herein, the terms "treating", "treatment" and the like refer to any and all applications which remedy, or otherwise hinder, retard, or reverse the progression of, a disease or at least one symptom of a disease, including reducing the severity of a disease. Thus, treatment does not necessarily imply that a subject is treated until complete recovery from a disease. Similarly, the terms "preventing", "prevention" and the like refer to

any and all applications that prevent the establishment of a disease or otherwise delay the onset of a disease.

[0176] The composition described herein may further comprise one or more additives or additional components as described in the sections herein entitled 'Additives' and 'Additional Components' respectively such that the composition may provide other health benefits, for example, assisting with muscle protein synthesis.

***Dosages***

[0177] In general, the composition described herein may be administered in any suitable dose amount that is effective as a health supplement and/or as therapeutic agent to achieve the desired health outcome. Those skilled in the art will appreciate that single or multiple administrations of the composition disclosed herein can be carried out with dose levels and dosing regimens being determined as required depending on the circumstances and on the condition of the subject to be treated. The skilled addressee can readily determine suitable dosage regimes. A broad range of doses may be applicable. Dosage regimens may be adjusted to provide the optimum therapeutic response. Those skilled in the art will appreciate that the exact amounts and rates of administration of the composition disclosed herein will depend on a number of factors such as the particular composition being administered, the age, body weight, general health, sex and dietary requirements of the subject, as well as any drugs or agents used in combination or coincidental with the composition. For example, several divided doses may be administered hourly, daily, weekly, monthly or at other suitable time intervals or the dose may be proportionally reduced as indicated by the exigencies of the situation. Based on the teaching herein, those skilled in the art will, by routine trial and experimentation, be capable of determining suitable dosage regimes on a case-by-case basis.

[0178] Compositions and methods of the present disclosure may be employed as an adjunct to other therapies or treatments for chronic diseases such as pre-diabetes, diabetes and conditions associated therewith. Accordingly, compositions and methods disclosed herein may be co-administered with other agents that may facilitate a desired therapeutic outcome, for example, one more renal drugs, or may be sequentially administered with other agents, for example, other blood-sugar regulating or diabetes medications, such as insulin. By "co-administered" is meant simultaneous administration in the same formulation or in two different formulations via the same or different routes or sequential administration by the same or different routes. By "sequential" administration is meant a time difference of from seconds, minutes, hours or days

between the administration of the agents, compositions or treatments. Sequential administration may be in any order.

[0179] In one embodiment, the composition of the present disclosure may be provided to an adult human subject so as to deliver a total amount of *Pinus pinaster* stem bark extract (equivalent to dry *Pinus pinaster* stem bark extract) of from about 60 mg to about 1500 mg per subject per day, e.g., from about 60 mg/day to about 100 mg/day, from about 75 mg/day to about 125 mg/day, from about 100 mg/day to about 200 mg/day, from about 150 mg/day to about 250 mg/day, from about 200 mg/day to about 300 mg/day, or from about 250 mg/day to about 350 mg/day, or from about 300 mg/day to about 400 mg/day, or from about 400 mg/day to about 600 mg/day, or from about 500 mg/day to about 700 mg/day, or from about 600 mg/day to about 800 mg/day, or from about 700 mg/day to about 900 mg/day, or from about 800 mg/day to about 1000 mg/day, or from about 900 mg/day to about 1100 mg/day, or from about 1000 mg/day to about 1200 mg/day, or from about 1000 mg/day to about 1500 mg/day, such as about 60 mg/day, about 100 mg/day, about 150 mg/day, about 200 mg/day, about 220 mg/day, about 240 mg/day, about 260 mg/day, about 280 mg/day, about 300 mg/day, about 320 mg/day, about 340 mg/day, about 360 mg/day, about 380 mg/day, about 400 mg/day, about 500 mg/day, or about 600 mg/day, or about 800 mg/day, or about 1000 mg/day, or about 1200 mg/day or about 1500 mg/day. These total daily dosages may be provided in any suitable number of doses, e.g., in a single dose, or in multiple (i.e., two, three, four or more doses). The doses may comprise an equal amount of *Pinus pinaster* stem bark extract. For example, in one dosage regime, a subject may be administered two doses of a composition according to the invention per day, wherein each composition comprises from about 100 mg to about 200 mg of *Pinus pinaster* stem bark extract, e.g., from about 100 mg to about 150 mg, or from about 120 mg to about 175 mg, or from about 150 mg to about 200 mg of *Pinus pinaster* stem bark extract, for example, about 100 mg, 110 mg, 120 mg, 130 mg, 140 mg, 150 mg, 160 mg, 170 mg, 180 mg, 190 mg or 200 mg *Pinus pinaster* stem bark extract.

[0180] The composition of the present disclosure may be provided to an adult human subject so as to deliver a total amount of *Pinus pinaster* stem bark extract (equivalent to dry *Pinus pinaster* stem bark extract) of about 260 mg per subject per day, said amount being effective for treating or preventing elevated blood glucose, or preventing or delaying the onset of type 2 diabetes mellitus (T2DM), or treating or preventing pre-diabetes; or reducing inflammation, or decreasing cytokine-induced inflammation; or decreasing an oxidative stress response, or treating or preventing a disease characterised by elevated levels of cytokine-induced inflammation, or

treating or preventing a disease characterised by elevated levels of oxidative stress, or reducing one or more side effects of a diabetes medication selected from the group consisting of metformin, sulfonylureas, meglitinides, thiazolidinediones, DPP-4 inhibitors, GLP-1 receptor agonists, SGLT2 inhibitors, and insulin therapy, or treating or preventing a dysfunction associated with excess blood glucose, or improving organ function, or improving immune function, or treating an autoimmune disease, e.g., lupus, arthritis, psoriasis, or type I diabetes, or treating emphysema, or increasing general wellbeing, e.g., increasing energy levels, improving vitality, improving sleep, improving breathing ability, improving levels of physical activity, improving mental functioning, and/or improving physical functioning.

[0181] The amount of *Pinus pinaster* stem bark extract may be increased to more than 260 mg per day depending on the severity of the disease or condition to be treated. Once the disease or condition has been effectively ameliorated, the adult subject may decrease the dosage to about 100 mg per day for maintenance purposes.

[0182] In another embodiment, the composition of the present disclosure may be provided to an adult human subject so as to deliver a total amount of papain (equivalent to dry papain) of from about 30 mg to about 1200 mg per subject per day, e.g., from about 30 mg/day to about 80 mg/day, from about 70 mg/day to about 120 mg/day, from about 100 mg/day to about 200 mg/day, from about 150 mg/day to about 250 mg/day, from about 200 mg/day to about 300 mg/day, or from about 250 mg/day to about 350 mg/day, or from about 300 mg/day to about 400 mg/day, or from about 400 mg/day to about 600 mg/day, or from about 600 mg/day to about 800 mg/day, or from about 800 mg/day to about 1000 mg/day, or from about 900 mg/day to about 1200 mg/day, such as about 60 mg/day, about 100 mg/day, about 150 mg/day, about 200 mg/day, about 220 mg/day, about 240 mg/day, about 260 mg/day, about 280 mg/day, about 300 mg/day, about 320 mg/day, about 340 mg/day, about 360 mg/day, about 380 mg/day, about 400 mg/day, about 500 mg/day, about 600 mg/day, or about 800 mg/day, or about 1000 mg/day or about 1200 mg/day. These total daily dosages may be provided in any suitable number of doses, e.g., in a single dose, or in multiple (i.e., two, three, four or more doses). The doses may comprise an equal amount of papain.

[0183] In another embodiment, the composition of the present disclosure may be provided to an adult human subject so as to deliver a total amount of *Aloe vera* extract (equivalent to dry *Aloe vera* extract, particularly equivalent to spray dried *Aloe vera* inner leaf juice extract) of from about 15 mg to about 600 mg per subject per day, e.g., from about 15 mg/day to about 80

mg/day, from about 50 mg/day to about 100 mg/day, from about 75 mg/day to about 150 mg/day, from about 100 mg/day to about 200 mg/day, from about 150 mg/day to about 300 mg/day, or from about 250 mg/day to about 350 mg/day, or from about 300 mg/day to about 400 mg/day, or from about 400 mg/day to about 600 mg/day, such as about 15 mg/day, about 30 mg/day, about 60 mg/day, about 90 mg/day, about 120 mg/day, about 175 mg/day, about 200 mg/day, about 250 mg/day, about 300 mg/day, about 340 mg/day, about 380 mg/day, about 400 mg/day, about 500 mg/day, or about 600 mg/day. These total daily dosages may be provided in any suitable number of doses, e.g., in a single dose, or in multiple (i.e., two, three, four or more doses). The doses may comprise an equal amount of *Aloe vera* extract.

[0184] When the composition according to the present invention is formulated as an aqueous composition having from about 2 mg/mL to about 10 mg/mL *Pinus pinaster* stem bark extract, from about 0.5 mg/mL to about 4 mg/mL *Aloe vera* extract, and about 1 mg/mL to about 8 mg/mL papain, a suitable total dosage of the aqueous composition may be from about 30 mL to about 150 mL per adult human subject per day, e.g., from about 30 mL to about 50 mL, or from about 50 mL to about 100 mL, or from about 100 mL to about 150 mL per subject per day, e.g., about 30 mL, 40 mL, 50 mL, 60 mL, 70 mL, 80 mL, 90 mL, 100 mL, 110 mL, 120 mL, 130 mL, 140 mL, or 150 mL. In an embodiment, from about 30 to about 100 mL or from about 30 mL to about 50 mL of a composition according to the invention is given to an adult human subject per day to maintain general health and wellbeing. In an embodiment, from about 50 mL to about 150 mL of a composition according to the invention is given to an adult human subject per day to treat a condition described herein, for example, to treat elevated blood sugar, pre-diabetes and/or their associated conditions. In an embodiment, from 50 to 100 mL of a composition according to the invention is given to an adult human subject per day to treat a condition described herein, for example, to treat elevated blood sugar, pre-diabetes and/or their associated conditions. As noted above, the total dosage may be administered to an adult human subject in one dose, or in multiple (i.e., two, three, four or more) smaller doses. In an embodiment, where a daily amount is administered as two doses, the doses may be taken one after a morning meal and one after an evening meal. In an embodiment, the doses may be equal doses, e.g., two equal doses of about 50 mL.

[0185] Accordingly, when the composition according to the present invention is formulated as an aqueous composition having from about 2 mg/mL to about 10 mg/mL *Pinus pinaster* stem bark extract, from about 0.5 mg/mL to about 4 mg/mL *Aloe vera* extract, and about 1 mg/mL to about 8 mg/mL papain, a suitable dosage may be from about 1 mL/kg/day to about 15

mL/kg/day, or from about 1 mL/kg/day to about 5 mL/kg/day, from about 3 mL/kg/day to about 7 mL/kg/day, or from about 10 mL/kg/day to about 15 mL/kg/day, e.g., about 1 mL/kg/day, about 3 mL/kg/day, about 5 mL/kg/day, about 7 mL/kg/day, about 9 mL/kg/day, about 11 mL/kg/day, about 13 mL/kg/day, or about 15 mL/kg/day.

[0186] Paediatric dosages of a composition comprising *Pinus pinaster* stem bark extract, *Aloe vera* extract and papain may be in the range of about 15% to about 90% of the daily adult human dosages listed herein.

**MANUFACTURE**

[0187] The disclosure herein further provides a method of manufacturing an aqueous composition, said method comprising combining *Pinus pinaster* stem bark extract, papain, and *Aloe vera* extract in water. As outlined in the section herein entitled '*Pinus pinaster* stem bark extract', the *Pinus pinaster* stem bark extract may be added to the water as a solid or dry powder. As outlined in the section herein entitled 'Papain', the papain may be added to the water as a solid or dry powder. As outlined in the section herein entitled '*Aloe vera*', the *Aloe vera* extract may be added to the water as a solid or dry powder. In one embodiment, the *Aloe vera* extract is *Aloe vera* leaf extract, in particular, dry inner leaf juice. The *Pinus pinaster* stem bark extract, papain, and *Aloe vera* extract may be combined in water in a single step, and dissolved or suspended in the water by mixing. Any suitable amount of water may be used.

[0188] The method may further comprise adding an acid. The acid may be an acid as described in the above section entitled 'Acid'. In one embodiment, the acid is glacial acetic acid, and is partially diluted in water prior to adding to the *Pinus pinaster* stem bark extract, papain, and *Aloe vera* extract in water. The acid is preferably added to the composition after the *Pinus pinaster* stem bark extract, papain, and *Aloe vera* extract have been added.

[0189] The method may still further comprise adding honey. The honey may be as described in the section herein entitled 'Honey'. In one embodiment, the honey is pre-heated prior to adding it to the water and *Pinus pinaster* stem bark extract, papain, and *Aloe vera*. In embodiments where acid is added to the composition, the honey is preferably added to the composition after the acid has been added and mixed.

[0190] In one embodiment, the method of manufacturing an aqueous composition of the invention comprises:

(a) dissolving sodium chloride and preservatives (potassium sorbate and sodium benzoate) in

water to form a solution;

(b) adding papain, *Pinus pinaster* stem bark extract, dry *Aloe vera* juice and a pigment (anthocyanin extract) to the solution formed in step (a) with stirring to form a mixture;

(c) adding pre-diluted glacial acetic acid to the mixture formed in step (b); and

(d) adding a flavouring agent and pre-heated honey to the mixture formed in step (c).

[0191] It will be appreciated by persons of ordinary skill in the art that numerous variations and/or modifications can be made to the present invention as disclosed in the specific embodiments without departing from the spirit or scope of the present invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

## Examples

[0192] The present invention will now be described with reference to specific examples, which should not be construed as in any way limiting.

***Example 1: Composition***

[0193] A composition according to an embodiment of the present invention is given in Table 1.

**Table 1**

| **Component** | **Content (/mL)** | **Content (/100 mL)** |
|---|---|---|
| Sodium chloride | 3.00 mg | 0.3 g |
| Papain | 2.40 mg | 0.24 g |
| *Pinus pinaster* stem bark extract, dry (Equiv. *Pinus pinaster* stem bark dry – 1.56 g) | 2.60 mg | 0.26 g |
| *Aloe vera* inner leaf juice dry (Equiv. aloe vera leaf inner fresh – 350 mg) (Equiv. hydroxyanthacene derivatives calculated as barbaloin – 1.75 ng) (Equiv. maltodextrin – 875 µg) | 1.75 mg | 0.175 g |
| Potassium sorbate (Equiv. potassium – 0.104mg) | 400 µg | 40 mg |
| Sodium benzoate (Equiv. sodium – 0.064 mg) | 400 µg | 40 mg |
| Honey | 100.00 mg | 10 g |
| Acetic acid - glacial | 2.00 mg | 0.2 g |
| Wildberry flavour UA71225 | 10.00 mg | 1 g |
| Anthocyanin extract (Equiv. maltodextrin – 280 µg) | 800 µg | 80 mg |
| Water - purified | 912.00 mg | 91.2 g |

***Example 2: Sample synthesis***

[0194] An example synthesis of 100 mL of the composition given in Example 1 is as follows:

Sodium chloride (0.3 g), potassium sorbate (40 mg) and sodium benzoate (40 mg) were dissolved in water (11.90 g). To the resultant solution, papain (0.24 g; Papaina (USP), Biocon Limited, Bangalore, India), *Pinus pinaster* stem bark extract (0.26 g; Pycnogenol®, Horphag Research USA Inc. (procyanidins 65-75%)), *Aloe vera* juice dry (0.175 g; Aloe barbadensis Miller, spray dried supplied by Capitol Ingredients (fresh plant:product 180-210:1; 40-50% maltodextrin)) and anthocyanin extract (80 mg; Anthocyanin extract powder, Naturex (25-35% maltodextrin, 65-75% anthocyanin extract (E163))) were added slowly, with stirring. To this mixture, a pre-diluted solution of glacial acetic acid (0.2 g; Bronson & Jacobs Pty Limited, Sydney, Australia) in water (4.76 g) was added. To the resultant mixture, water (69.05 g), wildberry flavour (1 g; wildberry flavour UA71225, Ungerer Australia Pty Limited) and pre-heated honey (10 g; British Pharmacopoeia (BP) grade honey, Capilano Honey Limited, Australia) were added and the resultant mixture stirred until homogeneous. Further water (5.49 g) was then added and gentle stirring continued to give a brownish maroon suspension.

***Example 3: Laboratory Studies***

[0195] The results of laboratory studies exploring the effect of a composition according to the Example 1, denoted 'Example 1', on supressing the inflammatory and oxidative responses in cytokine–activated human coronary artery endothelial cells.

*Introduction*

[0196] Chronic inflammation and oxidative stress underlies the pathogenesis of a number of lifestyle diseases including the major heart disease, atherosclerosis, and type 2 diabetes. Atherosclerosis is the major cause of mortality and morbidity in Western societies, with death rates exceeding those caused from any cancer. Type 2 diabetes is in epidemic proportions because of lifestyle imbalance, and importantly to our medical care system, type 2 diabetes increases the risk of atherosclerosis 2-4 fold, and is the leading reason for kidney failure and subsequent dependency on dialysis. Atherosclerosis is the development of lipid-filled lesions in arterial walls. The lesions develop because LDL particles accumulate in the vessel wall. In the vessel wall, the LDL particles become oxidised and in their oxidised state, the LDL particles initiate an inflammatory reaction in the cells that comprised the vessel wall. The endothelial cells that line the lumen of the artery are especially activated and in their activated state, the cells

express cell adhesion molecules. These adhesion molecules bind leukocytes, including monocytes, and in the bound state, the monocytes are induced to migrate into the vessel wall. Once in the vessel wall, the monocytes differentiate into macrophages and the macrophages engulf the oxidised LDL particles. These oxidised LDL particles cause macrophage apoptosis and necrosis. The death of the macrophage causes the engulfed lipid to spill out of the cell and this coalesces with other destroyed macrophages to form the early fatty streak characteristic of an atherosclerotic lesion.

[0197] The important cell adhesion molecules that are expressed by dysfunctional endothelial cells are VCAM-1, ICAM-1 and ELAM. These are recognised markers of an inflammatory response in all cell types, but are especially important in the pathogenesis of any vascular disease. VCAM-1 and ICAM-1 are regulated by a key inflammatory mediator, nuclear factor kappa B (NFkB). If NFkB is activated then cells will increase their expression of VCAM-1 and ICAM-1.

[0198] NFkB becomes activated when cells are exposed to inflammatory cytokines and reactive oxygen species (or free radicals, ROS). Increased ROS accumulate in tissues and cells when the enzymes that produce ROS are expressed in higher levels, and are more active, than the enzymes and proteins that scavenge or metabolise the ROS. This imbalance leads to a state of cellular oxidative stress, which activates regulators such as NFkB and initiates an inflammatory response. If these responses continue unchecked the cell will enter apoptosis. A major enzyme involved in ROS production (superoxide) in endothelial cells is NADPH oxidase 4 (NOX4) while a major enzyme with antioxidant function is superoxide dismutase-1 (SOD-1).

[0199] In this study, it is reported that Example 1 was able to decrease the expression of cytokine-activated VCAM-1, ICAM-1 and ELAM in human coronary artery endothelial cells (HCAECs) via a mechanism that is associated with the decreased activation of NFkB. In keeping with the suppression of the inflammatory markers, it is reported that Example 1 decreased ROS levels in cells, with an accompanying decrease in NOX4 but increase in SOD-1, expression. The decrease in inflammatory and oxidative responses culminated in a decrease in monocyte adhesion to the endothelial cells.

*Methods*

[0200] Human coronary artery endothelial cells were cultured in standard laboratory conditions. They were pre-incubated with Example 1 (50, 25, 12.5, 6.25 μl) for 3 hours before they were

stimulated with the inflammatory cytokine, TNFalpha, for 1 (mRNA) or 3 (protein) hours. Cells were then assayed for cell adhesion molecule, NOX-4 and SOD-1 expression, NFkB activation, superoxide levels, and monocyte adhesion.

*Results*

[0201] Example 1 decreases TNFalpha-induced VCAM-1, ICAM-1 and ELAM expression (see **Figure 1**). HCAECs were exposed to increasing volumes of Example 1 for 3 hours. After 3 hours, HCAECs were activated with an inflammatory insult, TNFalpha for 1 hour. In HCAEC that were not exposed to Example 1, TNFalpha increased VCAM-1 expression by 15-fold, ICAM-1 by 10-fold, and ELAM by 100-fold. Example 1 supplementation was able to suppress TNFalpha induced mRNA levels for all the CAMs. The Example 1 dose of 25uL/mL is equivalent of consuming 100 mL of Example 1. The results suggest that Example 1 is having an anti-inflammatory effect in HCAECs.

[0202] The next experiment examined if CAM protein levels were also decreased by Example 1 in the presence of inflammatory stimuli. As above, HCAECs were exposed to Example 1 for 3 hours and then activated with TNFalpha for 3 hours. Example 1 was found to significantly decrease protein levels of both VCAM-1 and ICAM-1 (**Figure 2**, ELAM was not measured). Due to the half-life of membrane proteins, the effective suppression of Example 1 was not as great as for the mRNA, which has a much quicker turnover.

[0203] The expression of VCAM-1, ICAM-1 and HAM are regulated by NFkB. Therefore, it was next determined if Example 1 decreased the activation of NFkB. As shown in **Figure 3**, Example 1 decreases activation of NFkB.

[0204] NFkB is a redox sensitive protein. Increased ROS levels will therefore activate this regulatory factor. The next experiment explored whether Example 1 was able to decrease ROS accumulation in human coronary artery endothelial cells. To this end, HCAECs were exposed to Example 1 for 3 hours before being loaded with the oxidant-sensitive fluorescent probe 2'7'-dichlorofluorescin (DCFH). The cells were then activated with TNFalpha for 15 mins. DCFH can be rapidly oxidised to a highly fluorescent compound called dehydrodichlorofluorescin by various ROS and peroxynitrite and it was determined that after TNFalpha activation, fluorescence intensity increased dramatically. This effect was significantly abrogated in HCAECs that were pre-exposed to Example 1. **Figure 4** shows that Example 1 decreases cytokine-induced ROS levels in HCAECs.

[0205] Given the finding that Example 1 decreased ROS levels within TNFalpha-activated HCAECs, it was next investigated if Example 1 modulated the expression of genes encoding for the ROS generating enzyme, NOX4 and the ROS metabolising enzyme, SOD-1. Results show that Example 1 decreased NOX4 mRNA levels by a small but significant level. Example 1 was shown to restore SOD-1 mRNA levels back to baseline. In **Figure 5**, Example 1 is shown to decrease NOX-4 and increase SOD-1 levels in HCAECs.

*Conclusions*

[0206] Example 1 decreases cytokine-induced inflammatory and oxidative stress responses in human coronary artery endothelial cells. The mechanism involves suppressing the activation of the key mediator of inflammation, NFkB, by decreasing the generation of ROS and thereby lowering the level of oxidative stress in HCAECs in response to inflammatory stimuli. As the onset of type 2 diabetes is driven by inflammation and oxidative stress, increases in blood levels of glucose, triglycerides, cholesterol and other toxic substances associates with diabetes leads to the deposit of triglycerides/cholesterol in the liver, and fat tissue deposition leads to inflammation of the liver, inflammation may drive the onset of insulin resistance, and if left unchecked, type 2 diabetes. More generally, inflammation and oxidative stress underlies a number of chronic diseases including atherosclerosis, liver inflammation associated with type 2 diabetes and obesity, endometriosis, and osteoarthritis. The laboratory findings for Example 1 suggest that this supplement may decrease the symptoms and causes associated with these chronic diseases.

***Example 4 – Case Study 1***

[0207] Two overweight middle-aged subjects in their mid-to-late 50's diagnosed with elevated blood glucose levels were administered a liquid preparation of the composition according to Example 1. This first person, 58 years old, was overweight and diagnosed as type 2 diabetic with high blood glucose levels. After one month at a dose of 100 mL / day for 6 months, this subject presented with improved (lowered) blood glucose levels. The second person, in their mid 50's and also overweight with high blood glucose saw, after one month at a dose of 100 mL / day, a reduction in blood sugar to the normal range and improved mobility/physical functioning.

***Example 5 – Case Study 2: Patient 'AF'***

[0208] A pre-diabetic/borderline female diabetic was given a daily single dose (50 mL) of the

Example 1 composition over the course of two years starting from February 2012 whilst maintaining a consistent diet and exercise regime. The blood sugar results obtained (measured as fasting plasma glucose levels) are given in Table 2.

| Table 2 | |
|---|---|
| **Date** | **Test result (mmol/L)** |
| 12 September 2011 (before administration of composition) | 5.8 |
| 21 April 2012 | 5.5 |
| 26 October 2013 | 5.2 |

[0209] The patient took the single dose above each day between February 2012 and August 2014, but from August 2014 to November 2014, did not take any of the composition. The fasting plasma glucose level result after this time is shown in Table 3.

| Table 3 | |
|---|---|
| **Date** | **Test result (mmol/L)** |
| 17 November 2014 | 6.2 |

[0210] These results demonstrate a general decline in blood glucose levels when using the composition in Example 1, and an increase in blood glucose levels when not using it.

[0211] Patient AF also reported being overweight, suffering from severe arthritis in her legs, knees, wrists and thumbs, and suffering from inflammation, as well as having severe pain and swelling in her legs due to injuries sustained in a motorbike accident. Patient AF also reported recurring cellulitis in her left leg.

[0212] After taking about 50-80 mL per day of the composition of Example 1 in the morning for a period of approximately three years, Patient AF is reportedly able to walk, work full time and lead a relatively normal lifestyle. Patient AF has also reported that the cellulitis, pain and swelling are kept at bay by the composition of Example 1, with no cellulitis recurrence since taking this composition. Patient AF also reported that her blood pressure has been kept within a normal range as a result of taking the composition of Example 1.

***Example 6 – Case Study 3: Patient 'FC'***

[0213] A 34 year old overweight man diagnosed with stomach cancer was given a daily dose of 100 mL of the composition of Example 1 for a period of 2 months. Blood samples were collected approximately 4 months prior to the first dose of the composition and shortly after the last dose at 2 months. Fasting blood cholesterol results obtained from testing these two samples are given in Table 4.

**Table 4**

| Blood component | Concentration in sample (mg/dl) 4 months prior to taking Example 1 composition | Concentration in sample (mg/dl) after 2 months of taking Example 1 composition |
|---|---|---|
| Cholesterol | 275 | 173 |
| Triglycerides | 375 | 253 |
| HDL | 55 | 45 |
| LDL | 145 | 77 |

[0214] As shown in Table 4, cholesterol, triglycerides, HDL and LDL reading have all reduced significantly after taking the composition of Example 1. The patient also reported a reduction in the swelling in his ankles after taking the composition of Example 1.

[0215] Patient FC also reported generally feeling unwell, having gout attacks, high liver enzymes and low energy levels. After 3 months of taking the composition of Example 1, along with diet changes, Patient FC reported a reduction in his weight of about 3 kg in two months. Previous weight loss from diet changes alone only resulted in a loss of 4 kg over a period of approximately four months.

[0216] Patient FC also reported that dry redness in his eyes late at night was reduced after taking the composition of Example 1, along with tinges of blood in his nose disappearing and thick build-up of greenish mucus disappearing indicative of less dryness in the area.

[0217] Patient FC further reported that numbness in his arm has gone, which discussion with a doctor indicates could be a result of the reduction in his blood cholesterol. Uncontrollable dandruff and inflamed scalp conditions have also improved significantly since taking the composition of Example 1. Shedding and inflammation of skin around and inside of his ear lobe has also reportedly gone.

[0218] With small diet changes and after taking the composition of Example 1 for 3 months, Patient FC reported that his blood work shows balancing of lipids in the blood, substantially reduced liver enzymes, and reduction in blood sugar to normal levels. He also reported substantially improved general energy and focus/concentration, especially when pain in his joints goes away.

***Example 7 – Athlete study***

*Introduction*

[0219] Nitric oxide is a powerful vasodilator (dilates the blood vessels in the body) but it also has a number of other very beneficial roles on skeletal muscles. Nitric oxide is produced from arginine, an amino acid obtained from the diet from dairy products, beef, pork, poultry, gelatin, seafood, wheat germ, oatmeal, granola, peanuts and other nuts, seeds, chick peas and soybeans. Arginine is converted to nitric oxide by an enzyme called nitric oxide synthase. In blood vessels, the enzyme is called endothelial nitric oxide synthase, abbreviated to eNOS.

[0220] If a healthy diet is consumed, there will be ample arginine in the diet. What is in limited amounts in the body is the enzyme, eNOS. Therefore, only so much nitric oxide can be generated at any one time because the enzyme is in rate limiting amounts.

[0221] When the composition of Example 1 is consumed, it acts on the endothelial cells in the blood vessels to switch on the synthesis of eNOS. As there is more eNOS then it is possible to produce more nitric oxide. Moreover, the composition activates eNOS- it makes the enzyme work faster so nitric oxide is generated more rapidly. This is very important for the active athlete.

[0222] Nitric oxide has the ability to dilate blood vessels. Dilated blood vessels allow more efficient nutrient delivery to active muscles and other tissues. More efficient blood supply will also allow higher levels of oxygen to be transported at a quicker rate to tissues, including our active muscles. Dilated blood vessels also include the venous system which allows faster elimination of toxic waste products from the body.

[0223] Nitric oxide is important during exercise because it is able to boost the function of mitochondria in cells, including those in skeletal muscle cells. Mitochondria are the energy generating units in cells. The substrate that goes into mitochondria are simple sugars such as glucose and these are converted into the energy unit, ATP. Nitric oxide boosts the speed at which

glucose is converted to ATP and it facilitates the entry of glucose into the cell. The faster glucose can be taken up by cells, the faster it can be converted into ATP. NO is a natural stimulant of muscle growth and repair. This will benefit the active athlete increasing strength and endurance.

[0224] Inflammation is a natural consequence of endurance exercise. The production of pro-inflammatory cytokines such as IL-1β, TNF-α, IFN-γ, is increased during intense and prolonged exercise (MacKinnon, 1999). The inflammatory cytokines will cause damage within skeletal muscle (Malm, 2001). It has been demonstrated that if blood is sampled 30 min after an Ironman event, TNF-α levels rise 3.5-fold, IFN-γ levels rise by 16.5-fold and IL-1β levels increase by 7-fold and all of these cytokines stay high for the next 48 hours, damaging muscle fibres.

[0225] Pro-inflammatory cytokines increase the stress hormones, catecholamines and corticosteroids. This is best demonstrated by the dramatic rise in cortisol levels by endurance exercise. Acute phase inflammatory markers also rise during endurance exercise including ultramarathons and Ironman events. C Reactive Protein (CRP) is one of these acute phase inflammatory markers.

[0226] A cytokine involved in coordinating glucose homeostasis (eg glycogen resynthesis) and anti-inflammatory responses, IL-6, increases during endurance exercise. It has been measured 24 hours after an Ironman when levels are high and these remain high 5 days after the Ironman event.

[0227] The increase in inflammatory cytokines is, in part, due to the trauma caused to skeletal muscles during and after endurance exercise. The leading causes of trauma are eccentric muscle contractions, as in the long distance triathlon, (b) the impact of the extremities against the ground and (c) the number of repetitions of the same movement. The damage to the muscles may lead to a phenomenon of delayed onset muscle soreness (DOMS) that develops 24-48 hours post-endurance exercise that is accompanied by prolonged muscle strength loss, a reduced range of motion, and high levels of creatine kinase activity in the blood. Muscle swelling and DOMS is all consistent with muscle inflammation that is mediated by infiltrating neutrophils and macrophages that produce inflammatory cytokines but also prostaglandin E2 (PGE2), and this is a key mediator of pain 24-48 hours after an exercise session. Inflammatory activity within skeletal muscles can also be driven by local endothelial cells and smooth muscle cells. The cytokine inflammatory response persists for at least 5 days post-recovery as a low-grade systemic inflammatory response. For those that start training again, even at a level $1/5^{th}$ of the training prior to an Ironman race, the low-grade systemic inflammatory response can persist for

longer.

[0228] The initial increase in pro-inflammatory cytokines is followed by elevated levels of anti-inflammatory cytokines. These anti-inflammatory cytokines such as IL-10 will act to dampen the pro-inflammatory response and prevent the overshooting of inflammation. Well-trained athletes are able to balance the acute exercise-induced inflammation by increasing their levels of IL-10. Increased IL-10 levels are associated with better performance.

[0229] Subjects that drop out of Ironman races due to self-reported fatigue symptoms have much higher pre-race CRP and IL-6 levels than those athletes that completed the race. The levels in the athletes that dropped out of the race were 316 to 1442% higher than the average pre-race concentration for those athletes that completed the race. Overtraining/prolonged training may lead to a prolonged inflammatory condition and lead to underperformance syndrome.

*Results*

[0230] A laboratory study has shown that the composition of Example 1 can reduce plasma concentrations of the inflammatory marker, acute phase reactant, CRP (see **Figure 6** – dark grey denotes runners and light grey denotes cyclists). Athletes were asked to complete a long run (15-20km) or long ride (90km+) on two separate occasions, 3-weeks apart. Blood sampling was performed prior to exercise and within 15 minutes post-exercise. On one of the occasions, the composition of Example 1 was ingested, prior to the training run or ride. On the other occasion a placebo supplement was ingested. The athletes were blinded to the supplement that they ingested each time.

[0231] ELISA tests showed that CRP levels were markedly decreased in the blood of the athletes post-training after the composition of Example 1, whereas there was no effect of the placebo supplement (slight increase in CRP levels after exercise). A decrease in CRP reflects a decrease in systemic inflammation caused by endurance exercise training. In turn, this may reduce muscle damage due to inflammation.

[0232] It was next tested if the composition of Example 1 has direct anti-inflammatory actions in human cells. To test this human coronary artery endothelial cells, the cells that line the arteries that supply blood to the tissues of the body, were exposed to the composition of Example 1 at concentrations equivalent to a 50-, 25-, 12.5- and 6.25- mL dose of the composition of Example 1. It was shown that the composition of Example 1 was able to suppress inflammation caused by

the pro-inflammatory cytokine, TNFα, in human coronary artery endothelial cells as assessed by measuring an inflammatory marker in the cells called VCAM-1 (see **Figure 7**).

[0233] In the above experiment, the composition of Example 1 was added directly to the cells from the bottle. However, in the body the composition of Example 1 is digested and components are absorbed into the blood stream where they pass through the liver and undergo type II metabolism. Therefore, the original molecules in the composition of Example 1 may be modified and it is these modified components that will cause the cells in the body to respond.

[0234] To test if the modified components of the composition of Example 1 are effective within the blood stream as anti-inflammatory molecules, subjects were asked to consume 100 ml of the composition of Example 1. Blood sampling was completed immediately before composition consumption and 3 hours after consumption. From the blood collected, the plasma was isolated. The plasma was then used to treat human coronary artery endothelial cells. Cells were treated for 24 hours with the plasma and then the cells were exposed to an inflammatory stimulus, pro-inflammatory cytokine, TNF-α. We then measured the extent of inflammation by monitoring VCAM-1 levels. Results are given in **Figure 8**.

***Example 8 – Phase IIa Clinical Study***

[0235] A phase IIa placebo controlled randomised clinical study has commenced with approximately 150 participants diagnosed with pre-diabetes. The clinical study is supplementing participants with a composition according to Example 1 [with or without metformin] or placebo for 12 weeks to ameliorate blood glucose levels as evidenced by an improvement in blood glucose levels according to the following outcomes:

**Primary:**

— Impaired Fasting Glucose (IFG) [measured by fasting glucose levels]

**Secondary:**

— Impaired Glucose Tolerance (IGT) [measured by Oral Glucose Tolerance Test (OGTT)]
— hs-CRP
— HbA1c levels
— Safety (ELFTs, Urea, FBC)
— HDL, LDL, TGs, Lipid profile

— Quality of Life (SF-12v2 Questionnaire)

**Tertiary:**

— BP

— Waist: hip ratio,

— Weight

— Dietary habits (3-day diet recall)

— Physical activity (International Physical Activity Questionnaire – IPAQ)

[0236] Inclusion criteria:

[0237] Participants ≥ 18 years of age at time of entry on study; Male and Female, with Impaired fasting blood glucose

- Initial online / telephone assessment will involve the Australian Type 2 Diabetes Risk Assessment Tool (AUSDRISK) questionnaire. Potential participants must have a score above 6 to qualify for next stage of assessment of meeting inclusion criteria.
- Participants must demonstrate IFG – defined as fasting blood glucose 6.1 – 6.9 mmol/L
- BMI ≥ 25
- Waist measurement: men ≥ 102 cm; women ≥ 88 cm
- Cognitive ability to understand informed consent process and to give informed consent to the experimental treatment;
- Participants agree to undergo venepuncture on multiple occasions
- Participants agree to adhere to the study protocol, including not changing diet or exercise patterns over the 12 week study period.

[0238] Exclusion criteria:

[0239] Any clinically relevant abnormal findings, which in the opinion of the investigators/clinicians, may put the participant at risk of adverse events because of participation in the clinical trial including:

1) Physical examination; Clinical chemistry; Haematology; Urinalysis; Vital signs;
2) Diagnosis of Type II Diabetes Mellitus
3) Taking Anti-Obesity medications such as Orlistat

4) Taking oral blood glucose-lowering medications such as Sulfonylureas, Biguanides, Alpha-glucosidase inhibitors, Thiazolidinediones
5) Taking any dietary supplements such as herbal, multivitamin or mineral, probiotics, fish oils etc, prior to commencing study. However, if prescribed by their doctor they must NOT stop taking any form of dietary supplement. This will however make them ineligible to participate in the study.
6) Any allergies or past reactions to ingredients in treatment and placebo supplements, including *Pinus pinaster* or extracts thereof, papain (*Carica papaya*), aloe vera, honey, potassium sorbate and sodium benzoate
7. Alcohol abuse;
8. The use of any illicit drugs;
9. Pregnancy or nursing of an infant;
10. Commencing lifestyle interventions such as dietary changes and increasing exercise duration/intensity;
11. Any psychiatric disorders by history or examination that would prevent completion of the study or result in possible adverse events for the participant.

[0240] Once the eligibility criteria have been met, the participants will be randomized into two study arms namely, Group I and Group II. Group I to receive placebo and Group II to receive a composition of the invention. This study design was chosen as in an outpatient setting each participant can be considered to be a control subject in both arms of the study, thereby each participant acting also as their own control.

***Example 9 – Case Study 4: Patient 'SG' (Diabetes Type 1)***

SG is a 23 year old female of normal weight diagnosed with type I diabetes on 9 April 2015 after experiencing blurred vision, a huge thirst and fatigue. On 10 April 2015, SG's blood glucose level was measured at 23.8 mmol/L.

SG was administered 100 mL per day of the composition of Example 1 in the mornings from 16 April 2015 to 15 May 2015. Administration of the composition of Example 1 ceased after 15 May 2015 for a few days, during which time SG's blood sugar levels began rising. Then, the composition of Example 1 was administered at a daily dose of 50 mL, taken as a single dose each morning. Blood glucose charts for SG are shown in Tables 5(a)-(k).

These charts show that SG's injected insulin levels are very low – which may be due to the composition of Example 1 helping the body to use the insulin efficiently, hence using less. SG's specialist commented that SG had attained a balance of blood sugar levels expected after 6 months, not 6 weeks. SG's blood sugar levels dropped whilst taking the composition of Example 1.

SG is now on an insulin pump and is maintaining the 50 mL per day of the composition of Example 1 – which SG believes helps her feel normal, and is allowing her to dial in less insulin that is recommended for her carbohydrate intake.

**Table 5(a)**

WEEK BEGINNING: 6/4/15 (DATE)

| **Insulin Injections** | | | | | | **Monitoring Blood Glucose** | | | | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Type of Insulin | Units given | | | | Breakfast | | Lunch | | Dinner | | Before Supper or Bed | Overnight | Activity, illness, diet changes, time of hypos (noting blood glucose and treatment) |
| | | Breakfast | Lunch | Dinner | Before Bed | Before | After | Before | After | Before | After | | | |
| Mon | | | | | | | | | | | | | | Eat<br>Blood Glucose level |
| | | | | | | | | | | | | | | |
| Tues | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| Wed | | | | | | | | | | | | | | Insulin |
| | | | | | | | | | | | | | | |
| Thu | NR | 6 | 6 | 6 | | | | | 23.8<br>17.1 | | 19.8 | | | NR |
| | Lantus | | | 10 | 6 pm | | | | | | | | | |
| Fri | NR | 6 | 8 | 8 | | 12.8 | 15.8 | | 11.2 | | 18.9 | | | eat |
| | Lantus | | | 12 | | | | | | | | | | |
| Sat | | 8 | 8 | 8 | | 10.4 | 14.8 | | 14.4 | | 8.7 | | | 2 hour |
| | | | | 12 | | | | | | | | | | |
| Sun | | 8 | | | | 7.2 | 7.0 | 11.3 | 10.3 | 16.1 | 11.0 | | | Test |
| | | | | | | | | | | | | | | |

**Table 5(b)**

WEEK BEGINNING: 13/4/15 (DATE)

| Insulin Injections | | | | | | Monitoring Blood Glucose | | | | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Type of Insulin | Units given | | | | Breakfast | | Lunch | | Dinner | | Before Supper or Bed | Overnight | Activity, illness, diet changes, time of hypos (noting blood glucose and treatment) |
| | | Breakfast | Lunch | Dinner | Before Bed | Before | After | Before | After | Before | After | | | |
| Mon | NR | 8 | 8 | 8 | | 7.5 | 9.4 | 6.9 | (9.4) 4.4 | | 6.3 | | | |
| | L | | | 12 | | | | | | | | | | |
| Tues | NR | 8 | 6 | 8 | | 7.9 | 5.9 | | 8.5 | | 15.0 | | | |
| | L | | | 12 | | | | | | | | | | |
| Wed | NR | 8 | 8 | 8 | | 10.7 | 7.1 | 6.4 | 5.0 | 12.0 | 9.5 | 7.2 | | |
| | L | | | 12 | | | | | | | | | | |
| Thu | NR | 8 | 8 | 8 | | 6.5 | 6.7 | | 5.7 | 9.9 | 6.3 | 5.9 | | |
| | L | | | 12 | | | | | | | | | | |
| Fri | NR | 8 | 8 | 8 | | 8.0 | 9.3 | | 4.1 | | 9.5 | | | |
| | L | | | 12 | | | | | | | | | | |
| Sat | NR | 8 | 7 | 7 | | 6.5 | 3.4 | 5.3 | 4.4 | 9.4 | 2.7 5.2 | 9.2 | | |
| | L | | | 12 | | | | | | | | | | |
| Sun | NR | 7 | 6 | 7 | | 6.6 | 2.9 5.4 | 7.2 | 10.1 8.6 | | 5.9 | 8.2 | | Walking at 2:00 |
| | L | | | 12 | | | | | | | | | | |

## Table 5(c)

WEEK BEGINNING: 20/4/15 (DATE)

| Insulin Injections | | | | | | Monitoring Blood Glucose | | | | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Units given | | | | Breakfast | | Lunch | | Dinner | | | | Activity, illness, diet changes, time of hypos (noting blood glucose and treatment) |
| | Type of Insulin | Breakfast | Lunch | Dinner | Before Bed | Before | After | Before | After | Before | After | Before Supper or Bed | Overnight | |
| Mon | | 7 | 5 | 4 | | 7.2 | 2.7<br>6.0 | 6.2 | 6.8 | | 13.9 | | | |
| | | | | 12 | | | | | | | | | | |
| Tues | | 4 | 5 | 4 | | 5.6 | 5.0 | 5.0 | 11.8 | 8.9 | 6.9 | 7.7 | | |
| | | | | | | | | | | | | | | |
| Wed | | 4 | 5 | 4 | | 5.5 | 8.4 | 6.8 | 6.2 | | 10.2 | 10.8 | | |
| | | | | | | | | | | | | | | |
| Thu | | 4 | 5 | 4 | | 7.2 | 4.5 | 8.6 | 3.4 | | 9.3 | 10.2 | | |
| | | | | 12 | | | | | | | | | | |
| Fri | | 3 | 4 | 4 | | 6.7 | 8.4 | 8.9 | 6.3 | 9.3 | 11.2 | 9.2 | 6.5 | |
| | | | | | | | | | | | | | | |
| Sat | | 3 | 5 | 4 | | 6.4 | 11.5 | 11.0 | 12.3 | | 7.7 | | 6.4 | |
| | | | | 12 | | | | | | | | | | |
| Sun | | 4 | 4 | 4 | | 6.5 | 6.0 | 5.0 | 4.9 | 6.7 | 13.1 | | | |
| | | | | 12 | | | | | | | | | | |

## Table 5(d)

WEEK BEGINNING: 27/4/15 (DATE)

| Insulin Injections | | | | | | Monitoring Blood Glucose | | | | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Units given | | | | Breakfast | | Lunch | | Dinner | | | | Activity, illness, diet changes, time of hypos (noting blood glucose and treatment) |
| | Type of Insulin | Breakfast | Lunch | Dinner | Before Bed | Before | After | Before | After | Before | After | Before Supper or Bed | Overnight | |
| Mon | | 4 | 4 | 4 | | 5.9 | 7.5 | | 3.8<br>7.2 | | 5.6 | 12.0 | 9.2 | |
| | | | | 12 | | | | | | | | | | |
| Tues | | 4 | 4 | 4 | | 6.7 | 5.2 | | 6.2 | 10.1 | 13.8 | 10.8 | | |
| | | | | 12 | | | | | | | | | | |
| Wed | | 4 | 4 | 4 | | 7.1 | 5.3 | 7.6 | 7.4 | | 8.3 | | | |
| | | | | 12 | | | | | | | | | | |
| Thu | | 4 | 4 | 4 | | 5.9 | 8.0 | 6.0 | 9.9 | 9.3 | 8.0 | | | |
| | | | | 12 | | | | | | | | | | |
| Fri | | 4 | 4 | 4 | | 7.3 | 9.3 | 8.9 | 6.7 | 5.8 | 10.1 | | 8.0 | |
| | | | | 12 | | | | | | | | | | |
| Sat | | 4 | 4 | 5 | | 6.9 | 7.3 | | 10.0 | 9.4 | 9.00 | 10.3 | | |
| | | | | 12 | | | | | | | | | | |
| Sun | | 4 | 4 | 4 | | 5.3 | 7.0 | | 8.2 | | 7.3 | 10.0 | | |
| | | | | 12 | | | | | | | | | | |

## Table 5(e)

WEEK BEGINNING: 5/5/15 (DATE)

| Insulin Injections | | | | | | Monitoring Blood Glucose | | | | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Type of Insulin | Units given | | | | Breakfast | | Lunch | | Dinner | | Before Supper or Bed | Overnight | Activity, illness, diet changes, time of hypos (noting blood glucose and treatment) |
| | | Breakfast | Lunch | Dinner | Before Bed | Before | After | Before | After | Before | After | | | |
| Mon | | 4 | 4 | 5 | | 5.4 | 5.8 | | 6.0 | | 8.3 | 9.7 | | |
| | | | | 12 | | | | | | | | | | |
| Tues | | 4 | 4 | 4 | | 5.7 | 5.4 | | 4.6 | | 6.2 | 8.9 | | |
| | | | | 12 | | | | | | | | | | |
| Wed | | 4 | 4 | 4 | | 6.0 | 7.2 | | 5.7 | | 8.3 | 10.7 | | |
| | | | | 12 | | | | | | | | | | |
| Thu | | 4 | 5 | 4 | | 5.4 | 3.3 | 11.0 | 3.6 | | 4.6 | 7.0 | 7.1 | |
| | | | | 12 | | | | | 6.0 | | | | | |
| Fri | | 4 | 3 | 3 | | 6.2 | 6.2 | 4.4 | 7.6 | | 10.7 | | 6.1 | |
| | | | | 12 | | | | | | | | | | |
| Sat | | 3 | 3 | 4 | | 5.9 | 6.2 | | 5.5 | | 9.5 | 9.9 | 6.8 | |
| | | | | 12 | | | | | | | | | | |
| Sun | | 4 | 4 | 5 | | 6.3 | 8.9 | | 6.4 | | 8.9 | 6.9 | | |
| | | | | 12 | | | | | | | | | | |

**Table 5(f)**

WEEK BEGINNING: 11/5/15 (DATE)

| Insulin Injections | | | | | | Monitoring Blood Glucose | | | | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Type of Insulin | Units given | | | | Breakfast | | Lunch | | Dinner | | Before Supper or Bed | Overnight | Activity, illness, diet changes, time of hypos (noting blood glucose and treatment) |
| | | Breakfast | Lunch | Dinner | Before Bed | Before | After | Before | After | Before | After | | | |
| Mon | | 4 | 4 | 4 | | 4.8 | 6.8 | | 8.9 | | 7.4 | | | |
| | | | | 12 | | | | | | | | | | |
| Tues | | 3 | 4 | 4 | | 7.0 | 9.6 | | 8.0 | | 7.3 | | | |
| | | | | 12 | | | | | | | | | | |
| Wed | | 4 | 3 | 3 | | 6.3 | 10.3 | | 7.5 | | 11.0 | | | |
| | | | | 12 | | | | | | | | | | |
| Thu | | 4 | 4 | 4 | | 5.4 | 5.7 | | 7.4 | | 10.8 | | | |
| | | | | 12 | | | | | | | | | | |
| Fri | | 3 | 3 | 5 | | 5.1 | 6.4 | | 7.6 | | 9.7 | | | |
| | | | | 12 | | | | | | | | | | |
| Sat | | 3 | 4 | 3 | | 4.7 | 4.9 | | 6.8 | | 7.6 | | | |
| | | | | 12 | | | | | | | | | | |
| Sun | | 3 | 4 | 4 | | 5.3 | 6.5 | | 7.0 | | 9.0 | | | |
| | | | | 12 | | | | | | | | | | |

## Table 5(g)

WEEK BEGINNING: 18/5/15 (DATE)

| Insulin Injections | | | | | | Monitoring Blood Glucose | | | | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Type of Insulin | Units given | | | | Breakfast | | Lunch | | Dinner | | Before Supper or Bed | Overnight | Activity, illness, diet changes, time of hypos (noting blood glucose and treatment) |
| | | Breakfast | Lunch | Dinner | Before Bed | Before | After | Before | After | Before | After | | | |
| Mon | | 3 | 3 | 3 | | 5.7 | 5.5 | | 7.2 | | 7.0 | | | |
| | | | | 12 | | | | | | | | | | |
| Tues | | | | | | 5.4 | | | | | | | | |
| | | | | 12 | | | | | | | | | | |
| Wed | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| Thu | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| Fri | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| Sat | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| Sun | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |

## Table 5(h)

WEEK BEGINNING: 25/5/15 (DATE)

| | Insulin Injections | | | | | Monitoring Blood Glucose | | | | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Type of Insulin | Units given | | | | Breakfast | | Lunch | | Dinner | | Before Supper or Bed | Overnight | Activity, illness, diet changes, time of hypos (noting blood glucose and treatment) |
| | | Breakfast | Lunch | Dinner | Before Bed | Before | After | Before | After | Before | After | | | |
| Mon | | 3 | 3 | 4 | | 6.1 | 7.3 | | 9.0 | | 8.5 | | | |
| | | | | 12 | | | | | | | | | | |
| Tues | | 3 | 3 | 3 | | 5.9 | 10.6 | | 8.0 | | 12.3 | | | |
| | | | | 12 | | | | | | | | | | |
| Wed | | 3 | 3 | 3 | | 6.8 | 9.4 | | 7.7 | | 8.3 | | | |
| | | | | 12 | | | | | | | | | | |
| Thu | | 3 | 3 | 3 | | 5.3 | 7.9 | | 6.5 | | 8.0 | | 3.9<br>7.4 | |
| | | | | 12 | | | | | | | | | | |
| Fri | | 3 | 3 | 3 | | 10.0 | 4.1 | | 8.3 | | 6.7 | | | |
| | | | | 10 | | | | | | | | | | |
| Sat | | 2 | 3 | 3 | | 7.0 | 8.2 | | 7.0 | | 8.3 | | | |
| | | | | 10 | | | | | | | | | | |
| Sun | | 2 | 3 | 2 | | 5.8 | 9.5 | 7.4 | 3.0<br>7.3 | | 6.9 | | | |
| | | | | 10 | | | | | | | | | | |

**Table 5(i)**

WEEK BEGINNING: 1/6/15 (DATE)

| Insulin Injections | | | | | | Monitoring Blood Glucose | | | | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Type of Insulin | Units given | | | | Breakfast | | Lunch | | Dinner | | Before Supper or Bed | Overnight | Activity, illness, diet changes, time of hypos (noting blood glucose and treatment) |
| | | Breakfast | Lunch | Dinner | Before Bed | Before | After | Before | After | Before | After | | | |
| Mon | | 2 | 2 | 4 | | 7.3 | 6.3 | | 9.0 | | 7.3 | | | |
| | | | | 10 | | | | | | | | | | |
| Tues | | 2 | 3 | 3 | | 6.8 | 9.7 | | 9.8 | | 12.0 | | | |
| | | | | 10 | | | | | | | | | | |
| Wed | | 2 | 3 | 3 | | 6.4 | 10.3 | | 6.3 | | 5.6 | | | |
| | | | | 10 | | | | | | | | | | |
| Thu | | 2 | 2 | 3 | | 5.9 | 6.3 | 5.0 | 7.5 | | 11.3 | 10.0 | | |
| | | | | 10 | | | | | | | | | | |
| Fri | | 2 | 2 | 4 | | 7.5 | 8.0 | | 7.7 | | 6.2 | | | |
| | | | | 10 | | | | | | | | | | |
| Sat | | 2 | 3 | 4 | | 6.5 | 10.4 | | 7.0 | | 10.9 | | | |
| | | | | 10 | | | | | | | | | | |
| Sun | | 3 | 3 | 3 | | 7.4 | 7.0 | | 10.7 | | 10.6 | | | |
| | | | | 10 | | | | | | | | | | |

## Table 5(j)

WEEK BEGINNING: 8/6/15 (DATE)

| Insulin Injections | | | | | | Monitoring Blood Glucose | | | | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Type of Insulin | Units given | | | | Breakfast | | Lunch | | Dinner | | Before Supper or Bed | Overnight | Activity, illness, diet changes, time of hypos (noting blood glucose and treatment) |
| | | Breakfast | Lunch | Dinner | Before Bed | Before | After | Before | After | Before | After | | | |
| Mon | | 2 | 4 | 3 | | 6.7 | 8.3 | | 5.3 | | 11.7 | | | |
| | | | | 10 | | | | | | | | | | |
| Tues | | 3 | 3 | 3 | | 7.4 | 10.0 | | 9.5 | | 12.8 | 11.9 | | |
| | | | | 10 | | | | | | | | | | |
| Wed | | 3 | 3 | 3 | | 6.5 | 8.5 | | 11.8 | | 5.7 | | | |
| | | | | 10 | | | | | | | | | | |
| Thu | | 3 | 3 | 3 | | 6.5 | 6.8 | | 7.5 | | 11.4 | | | |
| | | | | 10 | | | | | | | | | | |
| Fri | | 3 | 3 | 3 | | 7.7 | 7.0 | | 7.2 | | 8.1 | | | |
| | | | | 10 | | | | | | | | | | |
| Sat | | 3 | 3 | 3 | | 6.1 | 7.8 | | 9.0 | | 8.4 | | | |
| | | | | 10 | | | | | | | | | | |
| Sun | | 3 | 3 | 3 | | 7.6 | 11.3 | | 11.1 | | 13.2 | | | |
| | | | | 10 | | | | | | | | | | |

**Table 5(k)**

WEEK BEGINNING: 15/6/15 (DATE)

| Insulin Injections | | | | | | Monitoring Blood Glucose | | | | | | | | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Type of Insulin | Units given | | | | Breakfast | | Lunch | | Dinner | | Before Supper or Bed | Overnight | Activity, illness, diet changes, time of hypos (noting blood glucose and treatment) |
| | | Breakfast | Lunch | Dinner | Before Bed | Before | After | Before | After | Before | After | | | |
| Mon | | 3 | 3 | 4 | | 6.6 | 9.9 | | 9.0 | | 13.9 | | | |
| | | | | 10 | | | | | | | | | | |
| Tues | | 3 | | | | 5.2 | 10.1 | | | | | | | |
| | | | | | | | | | | | | | | |
| Wed | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| Thu | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| Fri | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| Sat | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| Sun | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |

***Example 10 – Case Study 5: Patient 'A' (high cholesterol)***

Patient A is a 51 year old male who has had high cholesterol for approximately 20 years, and who is reasonably fit and is not overweight.

Patient A has been taking 100 mL of the composition of Example 1 each day for a period of 3 weeks. The results of a blood test on 14 January 2015 (prior to taking the composition of Example 1) and a blood test on 24 June 2015 (after taking the composition of Example 1 for 3 weeks) gave the results in Table 6:

| Table 6 | | | |
|---|---|---|---|
| *Blood component* | *Reading on 24/06/15* | *Previous reading on 14/01/15* | *Units* |
| HDL | 1.3 | 1.1 | mmol/L |
| LDL | 5.6 | 5.8 | mmol/L |
| Total Chol/HDL Ratio | 5.8 | 6.9 | |
| Neutrophils<br>ANC | 47%<br>2.3 | 41%<br>1.8 | |

The results in Table 6 show that the HDL (good cholesterol) levels in Patient A have improved whilst the LDL (bad cholesterol) levels have reduced, resulting in a significant change/reduction to Patient A's overall Total Cholesterol/HDL Ratio. Table 6 also shows an improvement in white blood cell count (neutrophils).

Patient A has also noticed that he is sleeping better and has more energy during the day whilst taking the composition of Example 1.

Patient A has not been taking Lipitor® or any other statins for over 18 months, as he has found the side effects of Lipitor® to be muscle aches and pain, tiredness, joint pain and a general feeling of not being well. His diet and exercise regime has not changed in the past 12 months.

***Example 11 – Case Study 6: Patient 'PL' (Diabetes Type 2)***

Patient PL is a 63 year old male diagnosed with Diabetes Type 2 in March 2012. As part of his treatment for Diabetes 2, PL has been taking metformin since diagnosed. He is of thin build and is not overweight.

PL commenced taking a daily dose of 80 to 100 mL of the composition of Example 1 on 1 May 2015 and ceased taking metformin at the same time. The results of PL's blood tests (measuring HBA1c) are given in Table 7.

| Table 7 | | | |
|---|---|---|---|
| **Date** | **Test result (HBA1c; %)** | **Test result (HBA1c (IFCC); mmol/mol)** | **Test result (eAG; mmol/L)** |
| 26 June 2012 | 6.3 H | - | 7.4 |
| 26 March 2013 | 6.5 H | 47 H | 7.8 |
| 1 July 2015 | 6.0 H | 42 H | 7.0 |

As shown in Table 7, PL's recent blood test (1 July 2015) indicates an HBA1c level of 6.0 after taking the composition of Example 1 for a period of 2 months, which is down from the previous test level of 6.5 (on 26 March 2013). eAG levels are also down after taking the composition of Example 1. PL has advised that blood glucose self-monitoring has seen his BGL reduce from a general level of approximately 8.0 mmol/L prior to taking the composition of Example 1 to approximately 6.0 mmol/L now (i.e., after taking the composition of Example 1).

***Example 12 – Case Study 7: Patient 'TG' (lupus)***

Patient TG is a male suffering from lupus for the past 18 years. Recently, he began taking the composition of Example 1 at a dosage of 100 mL per day in the morning. After approximately 2-3 weeks (having taken 1.5 L), the sub cutaneous lesions on TG's face and scalp for years have reportedly disappeared and have not returned.

TG also reports episodes of clarity that last for hours, better immune system function, and generally feeling better as a result of taking the composition of Example 1.

***Example 13 – Case Study 8: Patient 'MS' (emphysema)***

Patient MS is a male patient diagnosed with emphysema, who has been taking 100 mL of the composition of Example 1 daily for a period of a few years prior to December 2014 since having surgery to remove most of his right lung.

Patient MS suffered from major shortness of breath and found it difficult to climb stairs after removal of most of his right lung. However, after taking the composition of Example 1 for one month, he reported that he was breathing more easily and experiencing less difficulty climbing stairs. After three months of taking the composition of Example 1, Patient MS reported that his X-rays and MRI results showed no signs of emphysema. He reported that his doctor was amazed

at this result and this type of lung disease does not usually go away and instead usually gets worse over time.

Patient MS also reported that his overall health has been exceptional since taking the composition of Example 1, and that he has not had a cold, flu or other common ailment for years. He also notes that there has been no decrease in his lung capacity since taking the composition of Example 1.

***Example 14 – Case Study 9: Patient JW (high cholesterol and high blood pressure)***

Patient JW is a female patient suffering from heart problems, high cholesterol, high blood pressure, and breathing problems. She began taking 50 mL of the composition of Example 1 daily in the morning about 1 year prior to December 2014. In December 2014, she reported that her high cholesterol had reduced and that she had increased energy as a result of taking the composition of Example 1.

***Example 15 – Case Study 10: Patient 'S' (psoriasis)***

Patient S is a female patient suffering from psoriasis of the scalp. After taking the composition of Example 1 for a few days, Patient S reported that the psoriasis on her scalp improved.

***Example 16 – Case Study 11: Patient 'M' (psoriasis)***

Patient M is a female patient suffering from plaque psoriasis. After taking 30 mL of the composition of Example 1 daily each morning for around 7 weeks, Patient M reported reduced redness with her flaky skin and less plaque showing, particularly on her knees.

CLAIMS:

1. A composition comprising *Pinus pinaster* stem bark extract, papain, and *Aloe vera* extract.

2. A composition comprising a therapeutically effective amount of *Pinus pinaster* stem bark extract, papain, and *Aloe vera* extract.

3. The composition according to claim 1 or claim 2, wherein the *Aloe vera* extract is *Aloe vera* leaf extract.

4. The composition according to claim 2 or claim 3, wherein
the therapeutically effective amount of *Pinus pinaster* stem bark extract corresponds to a daily adult human dosage of from about 60 mg to about 1500 mg *Pinus pinaster* stem bark extract;
the therapeutically effective amount of papain corresponds to a daily adult human dosage of from about 30 mg to about 1200 mg papain; and
the therapeutically effective amount of *Aloe vera* extract corresponds to a daily adult human dosage of from about 15 mg to about 600 mg *Aloe vera* extract.

5. The composition according to any one of claims 2 to 4, wherein
the therapeutically effective amount of *Pinus pinaster* stem bark extract corresponds to a daily adult human dosage of about 260 mg;
the therapeutically effective amount of papain corresponds to a daily adult human dosage of about 240 mg; and
the therapeutically effective amount of *Aloe vera* extract corresponds to a daily adult human dosage of about 175 mg.

6. The composition according to any one of claims 1 to 5, wherein the composition further comprises an acid.

7. The composition according to claim 6, wherein the acid is acetic acid.

8. The composition according to claim 6 or claim 7, wherein the composition comprises acid in a concentration effective to adjust the pH of the composition to between about 3.2 and about 4.2.

9. The composition according to any one of claims 1 to 8, wherein the composition further comprises honey.

10. The composition according to any one of claims 1 to 9, wherein the composition comprises one or more additives selected from the group consisting of: a preservative, a flavouring agent, a salt, and a pigment.

11. The composition according to any one of claims 1 to 10, wherein the composition comprises a salt, and wherein the salt is sodium chloride.

12. The composition according to any one of claims 1 to 11, further comprising an additional component selected from the group consisting of: an omega-3 fatty acid, a phytonutrient, a source of protein, an amino acid, an antioxidant, a vitamin, a mineral, a plant extract, and mixtures thereof.

13. The composition according to any one of claims 1 to 12, wherein the composition is synergistic.

14. The composition according to any one of claims 1 to 13, wherein the composition is adapted for oral administration.

15. The composition according to claim 14, wherein the composition is in a form selected from the group consisting of: a tablet, a capsule, a chewable tablet, a soft gel capsule, a sachet, a powder, granules, a liquid, a syrup, a liquid suspension, an emulsion, a solution, and combinations thereof.

16. The composition according to any one of claims 1 to 14, wherein the composition is an aqueous composition.

17. The composition according to any one of claims 1 to 16, wherein the composition has a pH of about 3.7.

18. The composition according to claim 16 or claim 17, wherein the *Pinus pinaster* stem bark extract is present at a concentration of from about 2 mg/mL to about 10 mg/mL.

19. The composition according to any one of claims 16 to 18, wherein the *Pinus pinaster* stem bark extract is present at a concentration of about 2.6 mg/mL.

20. The composition according to any one of claims 16 to 19, wherein the papain is present at a concentration of from about 1 mg/mL to about 5 mg/mL.

21. The composition according to any one of claims 16 to 20, wherein the papain is present at a concentration of about 2.4 mg/mL.

22. The composition according to any one of claims 16 to 21, wherein the *Aloe vera* extract is present at a concentration of from about 0.5 mg/mL to about 4 mg/mL.

23. The composition according to any one of claims 16 to 22, wherein the *Aloe vera* extract is present at a concentration of about 1.75 mg/mL.

24. The composition according to any one of claims 16 to 23, wherein the composition comprises glacial acetic acid at a concentration of from about 1 mg/mL to about 8 mg/mL.

25. The composition according to claim 24, wherein the glacial acetic acid is present at a concentration of about 2 mg/mL.

26. The composition according to any one of claims 15 to 25, wherein the composition comprises honey at a concentration of from about 50 mg/mL to about 200 mg/mL.

27. The composition according to claim 26, wherein the honey is present at a concentration of about 100 mg/mL.

28. An aqueous composition comprising about 2.6 mg/mL *Pinus pinaster* stem bark extract, about 2.4 mg/mL papain, and about 1.75 mg/mL *Aloe vera* extract.

29. The composition according to claim 28, further comprising about 100 mg/mL honey.

30. The composition according to claim 28 or claim 29, further comprising about 2 mg/mL glacial acetic acid.

31. An aqueous composition comprising:
2.40 mg/mL papain;
2.60 mg/mL *Pinus pinaster* stem bark extract;
1.75 mg/mL *Aloe vera* inner leaf juice;
100.00 mg/mL honey;
2.00 mg/mL glacial acetic acid;
3.00 mg/mL sodium chloride;
400 μg/mL potassium sorbate;
400 μg/mL sodium benzoate;
10.00 mg/mL wildberry flavour UA71225; and
800 μg/mL anthocyanin extract.

32. An aqueous composition comprising papain, *Pinus pinaster* stem bark extract, *Aloe vera* inner leaf juice, honey, glacial acetic acid, sodium chloride, potassium sorbate, sodium benzoate, wildberry flavour UA71225 and anthocyanin extract
in a ratio of
papain : dry *Pinus pinaster* stem bark extract : dry *Aloe vera* inner leaf juice : honey : glacial acetic acid : sodium chloride : potassium sorbate : sodium benzoate : wildberry flavour UA71225 : anthocyanin extract
of 2.40 : 2.60 : 1.75 : 100 : 2.00 : 3.00 : 0.40 : 0.40 : 10.00 : 0.80 by weight.

33. An aqueous composition comprising papain, dry *Pinus pinaster* stem bark extract, dry *Aloe vera* inner leaf juice, honey, glacial acetic acid, and sodium chloride
in a ratio of
papain : dry *Pinus pinaster* stem bark extract : dry *Aloe vera* inner leaf juice : honey : glacial acetic acid : sodium chloride
of 2.40 : 2.60 : 1.75 : 100 : 2.00 : 3.00 by weight.

34. An aqueous composition comprising papain, *Pinus pinaster* stem bark extract, and *Aloe vera* inner leaf juice in a ratio of
papain : dry *Pinus pinaster* stem bark extract : dry *Aloe vera* inner leaf juice
of 2.40 : 2.60 : 1.75 by weight.

35. A method of treating or preventing elevated blood glucose, comprising administering to a subject in need thereof an effective amount of a composition according to any one of claims 1 to 34.

36. A method of preventing or delaying the onset of type 2 diabetes mellitus (T2DM), comprising administering to a subject in need thereof an effective amount of a composition according to any one of claims 1 to 34.

37. A method of treating or preventing pre-diabetes, comprising administering to a subject in need thereof an effective amount of a composition according to any one of claims 1 to 34.

38. A method of reducing inflammation, comprising administering to a subject in need thereof an effective amount of a composition according to any one of claims 1 to 34.

39. A method of decreasing cytokine-induced inflammation, comprising administering to a subject in need thereof an effective amount of a composition according to any one of claims 1 to 34.

40. A method of decreasing an oxidative stress response, comprising administering to a subject in need thereof an effective amount of a composition according to any one of claims 1 to 34.

41. A method of treating or preventing a disease characterised by elevated levels of cytokine-induced inflammation, comprising administering to a subject in need thereof an effective amount of a composition according to any one of claims 1 to 34.

42. A method of treating or preventing a disease characterised by elevated levels of oxidative stress, comprising administering to a subject in need thereof an effective amount of a composition according to any one of claims 1 to 34.

43. The method of claim 41 or claim 42, wherein the disease is atherosclerosis, liver inflammation associated with type 2 diabetes and obesity, endometriosis, or osteoarthritis.

44. A method of reducing one or more side effects of a diabetes medication selected from the group consisting of metformin, sulfonylureas, meglitinides, thiazolidinediones, DPP-4

inhibitors, GLP-1 receptor agonists, SGLT2 inhibitors, and insulin therapy, the method comprising administering to a subject in need thereof an effective amount of a composition according to any one of claims 1 to 34.

45. The method of claim 44, wherein the side effect is abdominal or stomach discomfort, decreased appetite, or diarrhoea.

46. A method of treating or preventing a dysfunction associated with excess blood glucose, comprising administering to a subject in need thereof an effective amount of a composition according to any one of claims 1 to 34.

47. The method of claim 46, wherein the dysfunction is selected from the group consisting of: increased thirst and hunger, frequent urination, fatigue/lethargy, blurry vision, sleepiness, and muscle weakness.

48. The method of any one of claims 35 to 47, wherein the composition is administered in combination with metformin or another blood glucose lowering medication.

49. A method of improving organ function comprising administering to a subject in need thereof an effective amount of a composition according to any one of claims 1 to 34.

50. A method of lowering blood cholesterol, comprising administering to a subject in need thereof an effective amount of a composition according to any one of claims 1 to 34.

51. The method of claim 50, wherein the blood cholesterol is selected from the group consisting of: low density lipoproteins (LDL), high density lipoproteins (HDL), trigylcerides and total cholesterol.

52. A method of treating an autoimmune disease, comprising administering to a subject in need thereof an effective amount of a composition according to any one of claims 1 to 34.

53. The method of claim 52, wherein the autoimmune disease is selected from the group consisting of: lupus, arthritis, psoriasis, and type I diabetes.

54. The method according to any one of claims 35 to 53, wherein the effective amount of the composition corresponds to:
a daily adult human dosage of from about 60 mg to about 1500 mg *Pinus pinaster* stem bark extract;
a daily adult human dosage of from about 30 mg to about 1200 mg papain; and
a daily adult human dosage of from about 15 mg to about 600 mg *Aloe vera* extract.

55. The method according to any one of claims 35 to 54, wherein the effective amount of the composition corresponds to:
a daily adult human dosage of about 260 mg *Pinus pinaster* stem bark extract;
a daily adult human dosage of about 240 mg papain; and
a daily adult human dosage of about 175 mg *Aloe vera* extract.

56. The method according to claim 54 or claim 55, wherein the daily adult human dosage of *Pinus pinaster* stem bark extract, papain and *Aloe vera* extract is provided to the subject in a single dose.

57. The method according to claim 54 or claim 55, wherein the daily adult human dosage of *Pinus pinaster* stem bark extract, papain and *Aloe vera* extract is provided to the subject in multiple doses.

58. Use of a composition according to any one of claims 1 to 34 for the manufacture of a medicament for:
treating or preventing elevated blood glucose; or
preventing or delaying the onset of type 2 diabetes mellitus (T2DM) ; or
treating or preventing pre-diabetes; or
reducing inflammation; or
decreasing cytokine-induced inflammation ; or
decreasing an oxidative stress response; or
treating or preventing a disease characterised by elevated levels of cytokine-induced inflammation; or
treating or preventing a disease characterised by elevated levels of oxidative stress; or

reducing one or more side effects of a diabetes medication selected from the group consisting of metformin, sulfonylureas, meglitinides, thiazolidinediones, DPP-4 inhibitors, GLP-1 receptor agonists, SGLT2 inhibitors, and insulin therapy; or
treating or preventing a dysfunction associated with excess blood glucose; or
improving organ function; or
lowering blood cholesterol;
in a subject.

59. The method of any one of claims 35 to 57 or the use of claim 58, wherein the subject is selected from the group consisting of the elderly, those with a medical condition of pre-diabetes or at risk of developing pre-diabetes, and combinations thereof.

60. The use of claim 58 or claim 59, wherein the subject is taking metformin or another blood glucose lowering medication.

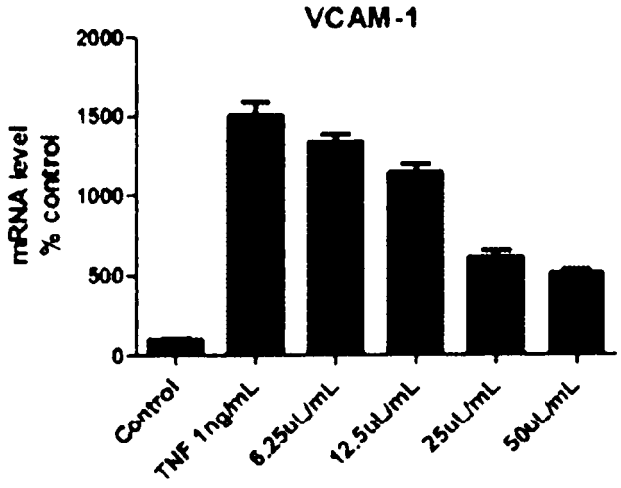
61. A method of manufacturing an aqueous composition, said method comprising:
combining *Pinus pinaster* stem bark extract, papain, and *Aloe vera* extract in water.

62. The method of claim 61, wherein the method further comprises:
adding an acid.

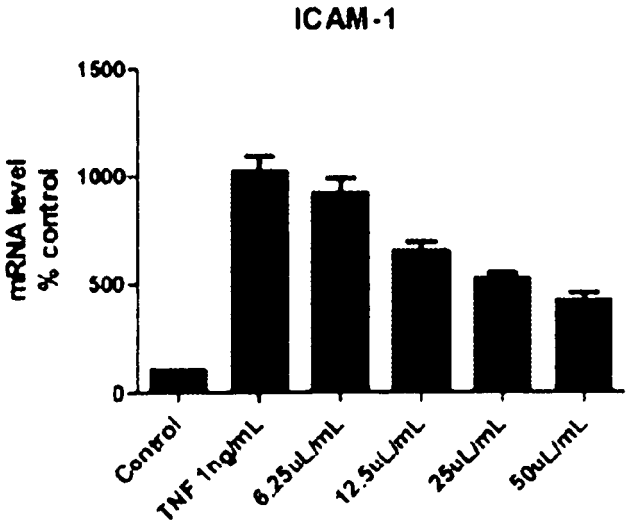
63. The method of claim 62, wherein the acid is glacial acetic acid.

64. The method of any one of claims 61 to 63, further comprising:
adding honey.

(a)


VCAM-1
mRNA level
% control
2000
1500
1000
500
0
Control
TNF 1ng/mL
6.25uL/mL
12.5uL/mL
25uL/mL
50uL/mL

(b)


ICAM-1
mRNA level
% control
1500
1000
500
0
Control
TNF 1ng/mL
6.25uL/mL
12.5uL/mL
25uL/mL
50uL/mL

(c)

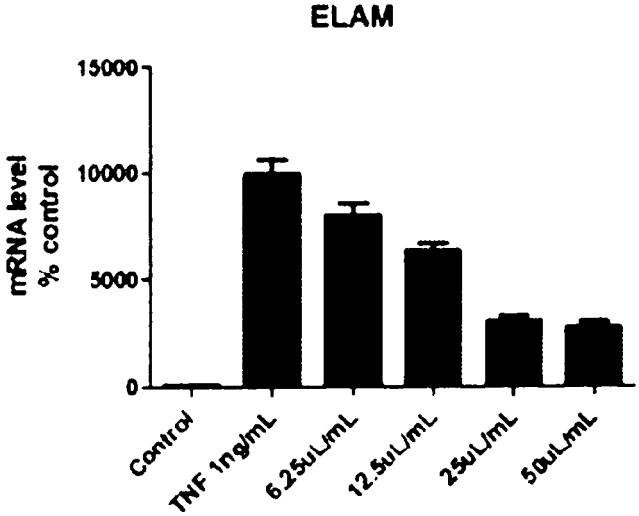

ELAM
mRNA level
% control
15000
10000
5000
0
Control
TNF 1ng/mL
6.25uL/mL
12.5uL/mL
25uL/mL
50uL/mL

FIG. 1

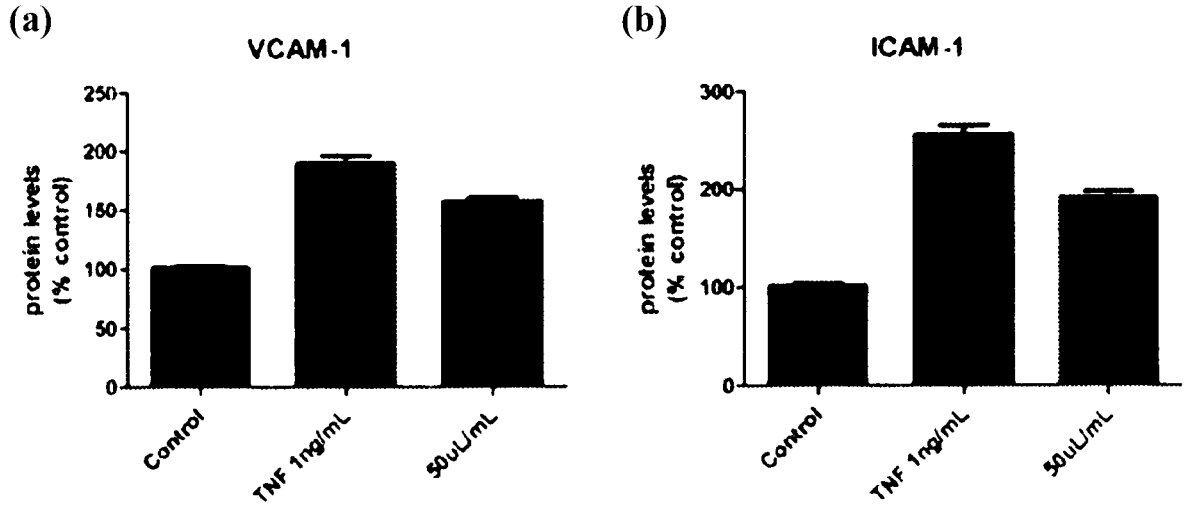

(a)
VCAM-1
protein levels (% control)
250
200
150
100
50
0
Control
TNF 1ng/mL
50uL/mL
(b)
ICAM-1
protein levels (% control)
300
200
100
0
Control
TNF 1ng/mL
50uL/mL

FIG. 2

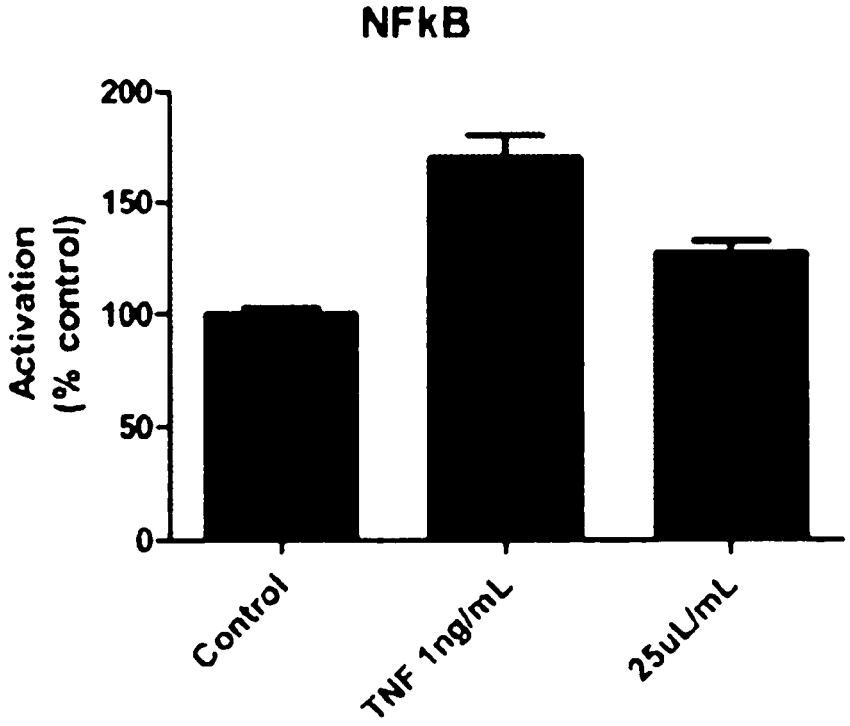

NFkB
Activation (% control)
200
150
100
50
0
Control
TNF 1ng/mL
25uL/mL

FIG. 3

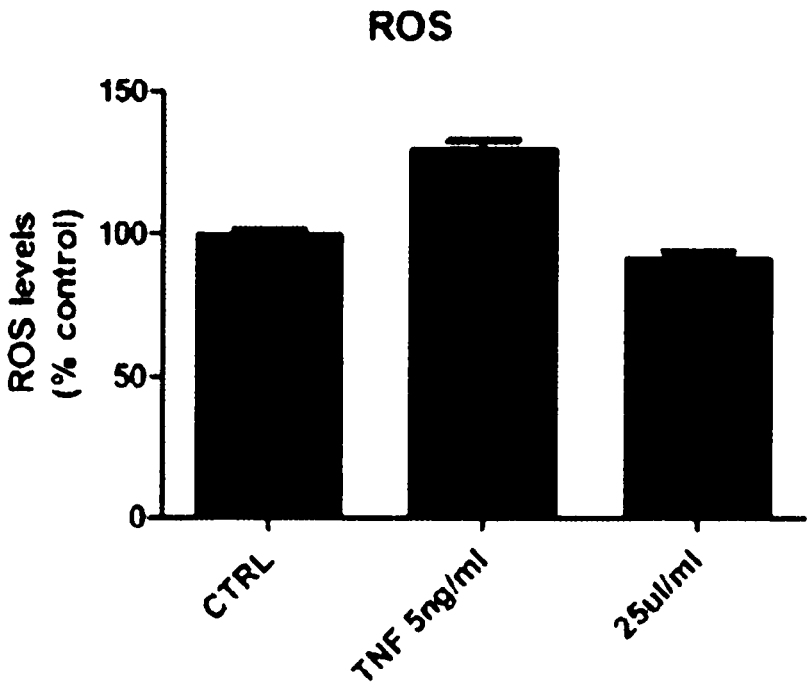

ROS
ROS levels (% control)
150
100
50
0
CTRL
TNF 5ng/ml
25ul/ml

FIG. 4

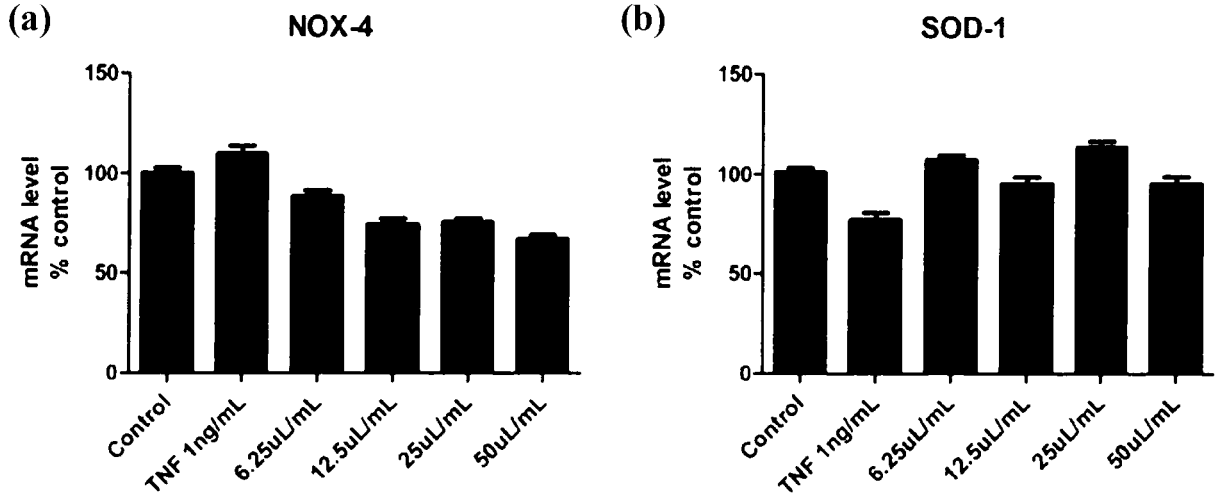

(a)
NOX-4
mRNA level % control
150
100
50
0
Control
TNF 1ng/mL
6.25uL/mL
12.5uL/mL
25uL/mL
50uL/mL
(b)
SOD-1
mRNA level % control
150
100
50
0
Control
TNF 1ng/mL
6.25uL/mL
12.5uL/mL
25uL/mL
50uL/mL

FIG. 5

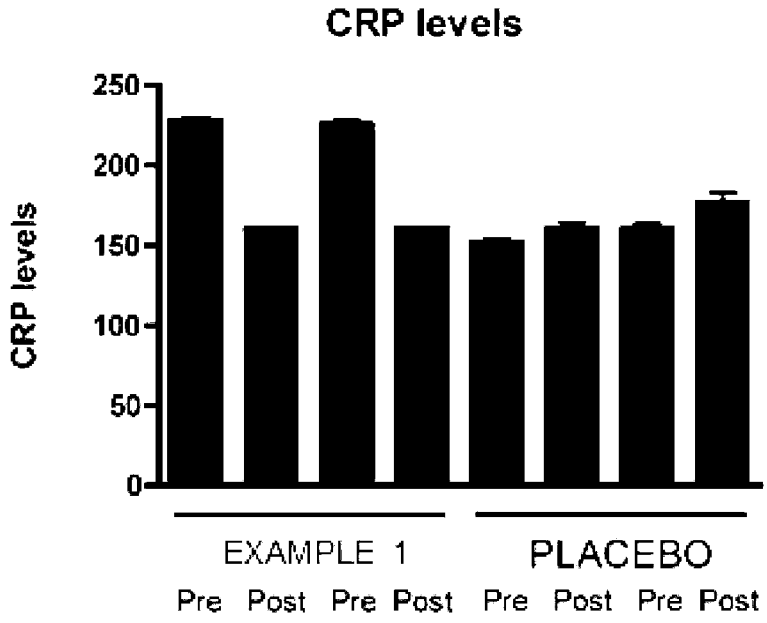

CRP levels
CRP levels
250
200
150
100
50
0
EXAMPLE 1
PLACEBO
Pre Post Pre Post Pre Post Pre Post

FIG. 6

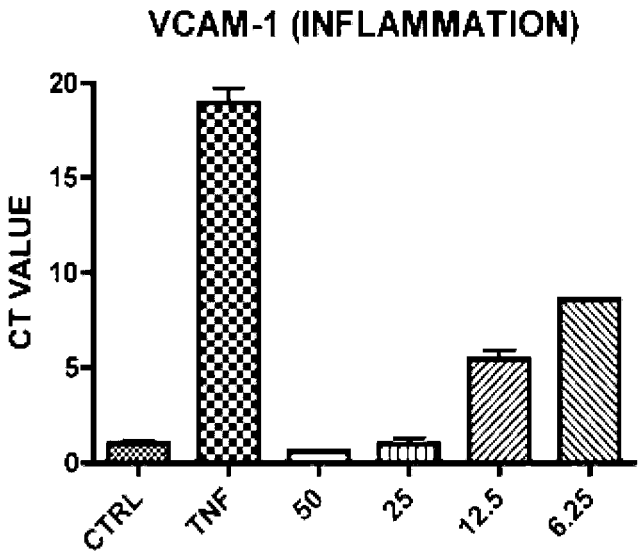

VCAM-1 (INFLAMMATION)
CT VALUE
20
15
10
5
0
CTRL
TNF
50
25
12.5
6.25

FIG. 7

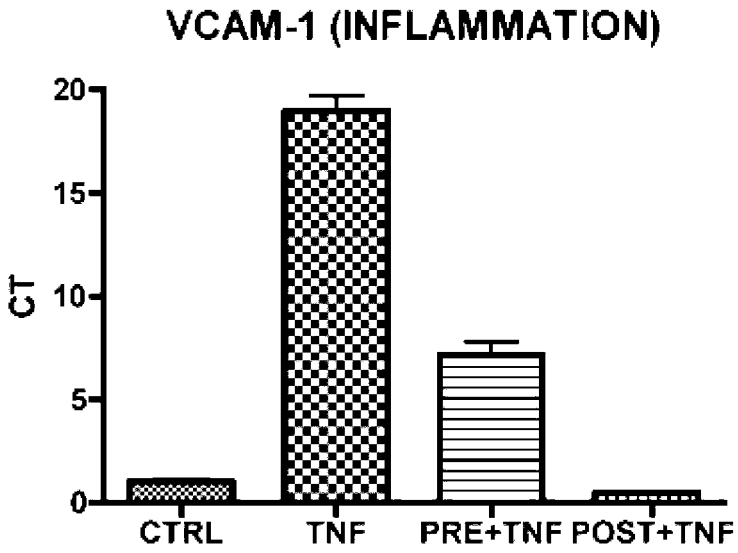



FIG. 8

**INTERNATIONAL SEARCH REPORT**

International application No.
**PCT/AU2015/000406**

A. CLASSIFICATION OF SUBJECT MATTER

***A61K 36/886 (2006.01) A61K 36/15 (2006.01) A61K 38/48 (2006.01)***

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Databases: MEDLINE, EPODOC, WPIAP, MINTEL GNPD, CAPLUS, BIOSIS. Keywords: Pinus pinaster, pinus maritime, cluster pine, maritime pine, pycnogenol, lands pine, papain, cysteine protease proteinase papaya, papaina, Aloe (vera or barbadensis or indica or perfoliata or vulgaris or chinesis or elongate or fava or lanzae or maculata or rubenscens or variegate), arborvitae, pine, bark, extract, honey, vinegar, acetic acid, blood sugar, diabetes, glucose, diabetes mellitus, T2DM, blood glucose, inflammation, atherosclerosis, osteoarthritis, autoimmune, cholesterol, oxidative stress, leaf extract as well as synomyns and similar terms.

Applicant and/or Inventor searches of the patent and non-patent literature was performed using Patentscope (http://www.wipo.int/patentscope/en/), and PubMed (http://www.ncbi.nlm.nih.gov/pubmed)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| | Documents are listed in the continuation of Box C | |

[X] Further documents are listed in the continuation of Box C [X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 12 August 2015 | 12 August 2015 |

| **Name and mailing address of the ISA/AU** | **Authorised officer** |
|---|---|
| AUSTRALIAN PATENT OFFICE<br>PO BOX 200, WODEN ACT 2606, AUSTRALIA<br>Email address: pct@ipaustralia.gov.au | Monica Graham<br>AUSTRALIAN PATENT OFFICE<br>(ISO 9001 Quality Certified Service)<br>Telephone No. 0262833179 |

| INTERNATIONAL SEARCH REPORT<br>C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT | | International application No.<br>**PCT/AU2015/000406** |
|---|---|---|
| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| X | MINTEL GNPD database record ID: 10066213. "Joint Saver." Company: Figuerola Laboratories, USA. Date Published: April 2000.<br>(see whole document) | 1-3, 6, 10, 12-16, 38, 39, 41, 43, 52, 53, 58 and 61 |
| X | MINTEL GNPD database record ID: 1916018. "Purifying Seaweed Clay Mask." Company: The Organic Pharmacy, UK. Date Published: November 2012.<br>(see whole document) | 1-3, 6, 10, 12 and 13 |
| X | US 2011/0129546 A1 (UMBERT MILL, I.) 02 June 2011<br>(Example 1 and claims) | 1-3, 6, 12, 13, 38, 39, 41, 52, 53 and 58 |
| X | https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=368098 Details the clinical trial from ANZCTR titled "An investigative study on the safety and efficacy of French Maritime Pine Bark Extract, Papain and Aloe Vera in pre-diabetic participants". Date submitted: 03 March 2015. Date registered: 13 March 2015. Sponsored by: Arborvitae Health and Wellbeing Pty Ltd. Retrieved online 28 July 2015.<br>(see whole document) | 1-5, 10-16, 18-23, 28, 34 and 61 |
| A | WO 2000/057726 A1 (THE NUTRASWEET COMPANY) 05 October 2000 | |
| A | WO 2008/079898 A1 (PHARMWEST, INC.) 03 July 2008 | |

| INTERNATIONAL SEARCH REPORT<br>Information on patent family members | International application No.<br>**PCT/AU2015/000406** |
|---|---|

This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

| Patent Document/s Cited in Search Report | | Patent Family Member/s | |
|---|---|---|---|
| **Publication Number** | **Publication Date** | **Publication Number** | **Publication Date** |
| US 2011/0129546 A1 | 02 June 2011 | US 2011129546 A1 | 02 Jun 2011 |
| | | CA 2728664 A1 | 23 Dec 2009 |
| | | CN 102123705 A | 13 Jul 2011 |
| | | EP 2311454 A2 | 20 Apr 2011 |
| | | EP 2311454 B1 | 18 Mar 2015 |
| | | ES 2316312 A1 | 01 Apr 2009 |
| | | ES 2316312 B1 | 08 Feb 2010 |
| | | JP 2011524884 A | 08 Sep 2011 |
| | | WO 2009153373 A2 | 23 Dec 2009 |
| WO 2000/057726 A1 | 05 October 2000 | WO 0057726 A1 | 05 Oct 2000 |
| | | AR 023208 A1 | 04 Sep 2002 |
| | | AU 4038800 A | 16 Oct 2000 |
| WO 2008/079898 A1 | 03 July 2008 | WO 2008079898 A1 | 03 Jul 2008 |
| | | US 2010055138 A1 | 04 Mar 2010 |

**End of Annex**

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

(19)中华人民共和国国家知识产权局

(12)发明专利申请

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(85)PCT国际申请进入国家阶段日
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PCT/AU2015/000406 2015.07.14

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(71)申请人 崖柏健康与福利私人有限公司
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(51)Int.Cl.
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*A61K 36/15*(2006.01)
*A61K 38/48*(2006.01)

权利要求书4页 说明书46页 附图5页

(54)发明名称

组合物和其用途

(57)摘要

本发明涉及包含海岸松茎皮提取物、木瓜蛋白酶和芦荟提取物的组合物及制造其的方法。还描述了治疗或预防多种状况的方法，包括治疗或预防升高的血糖、糖尿病前期、2型糖尿病、自身免疫性疾病，减轻或减少炎症，治疗或预防以升高水平的炎症为特征的疾病，和降低血液胆固醇，所述方法包括将有效量的根据本发明的组合物施用给需要其的受试者。还描述了本发明的组合物用于制造用于治疗或预防多种状况的药物的用途。

CN 107735099 A

1.组合物,包含海岸松茎皮提取物、木瓜蛋白酶和芦荟提取物。

2.组合物,包含治疗有效量的海岸松茎皮提取物、木瓜蛋白酶和芦荟提取物。

3.根据权利要求1或权利要求2所述的组合物,其中所述芦荟提取物是芦荟叶提取物。

4.根据权利要求2或权利要求3所述的组合物,其中

所述海岸松茎皮提取物的治疗有效量对应于约60mg至约1500mg海岸松茎皮提取物的每日成人剂量;

所述木瓜蛋白酶的治疗有效量对应于约30mg至约1200mg木瓜蛋白酶的每日成人剂量;和

所述芦荟提取物的治疗有效量对应于约15mg至约600mg芦荟提取物的每日成人剂量。

5.根据权利要求2至4中任一项所述的组合物,其中

所述海岸松茎皮提取物的治疗有效量对应于约260mg的每日成人剂量;

所述木瓜蛋白酶的治疗有效量对应于约240mg的每日成人剂量;和

所述芦荟提取物的治疗有效量对应于约175mg的每日成人剂量。

6.根据权利要求1至5中任一项所述的组合物,其中所述组合物还包含酸。

7.根据权利要求6所述的组合物,其中所述酸是乙酸。

8.根据权利要求6或权利要求7所述的组合物,其中所述组合物包含以有效调节所述组合物的pH至约3.2和约4.2之间的浓度的酸。

9.根据权利要求1至8中任一项所述的组合物,其中所述组合物还包含蜂蜜。

10.根据权利要求1至9中任一项所述的组合物,其中所述组合物包含一种或多种选自防腐剂、调味剂、盐和色素的添加剂。

11.根据权利要求1至10中任一项所述的组合物,其中所述组合物包含盐,并且其中所述盐为氯化钠。

12.根据权利要求1至11中任一项所述的组合物,进一步包含选自以下的另外的组分:ω-3脂肪酸、植物营养素、蛋白质源、氨基酸、抗氧化剂、维生素、矿物质、植物提取物及其混合物。

13.根据权利要求1-12中任一项所述的组合物,其中所述组合物是协同的。

14.根据权利要求1-13中任一项所述的组合物,其中所述组合物适于口服给药。

15.根据权利要求14所述的组合物,其中所述组合物处于选自以下的形式:片剂、胶囊剂、咀嚼片剂、软凝胶胶囊剂、囊剂、粉剂、颗粒剂、液体、糖浆剂、液体悬浮液、乳剂、溶液及其组合。

16.根据权利要求1至14中任一项所述的组合物,其中所述组合物是含水组合物。

17.根据权利要求1至16中任一项所述的组合物,其中所述组合物具有约3.7的pH。

18.根据权利要求16或权利要求17所述的组合物,其中所述海岸松茎皮提取物以约2mg/mL至约10mg/mL的浓度存在。

19.根据权利要求16至18中任一项所述的组合物,其中所述海岸松茎皮提取物以约2.6mg/mL的浓度存在。

20.根据权利要求16至19中任一项所述的组合物,其中所述木瓜蛋白酶以约1mg/mL至约5mg/mL的浓度存在。

21.根据权利要求16至20中任一项所述的组合物,其中所述木瓜蛋白酶以约2.4mg/mL

的浓度存在。

22.根据权利要求16至21中任一项所述的组合物,其中所述芦荟提取物以约0.5mg/mL至约4mg/mL的浓度存在。

23.根据权利要求16至22中任一项所述的组合物,其中所述芦荟提取物以约1.75mg/mL的浓度存在。

24.根据权利要求16至23中任一项所述的组合物,其中所述组合物包含浓度为约1mg/mL至约8mg/mL的冰醋酸。

25.根据权利要求24所述的组合物,其中所述冰醋酸以约2mg/mL的浓度存在。

26.根据权利要求15至25中任一项所述的组合物,其中所述组合物包含浓度为约50mg/mL至约200mg/mL的蜂蜜。

27.根据权利要求26所述的组合物,其中所述蜂蜜以约100mg/mL的浓度存在。

28.含水组合物,包含约2.6mg/mL海岸松茎皮提取物、约2.4mg/mL木瓜蛋白酶和约1.75mg/mL的芦荟提取物。

29.根据权利要求28所述的组合物,进一步包含约100mg/mL蜂蜜。

30.根据权利要求28或权利要求29所述的组合物,进一步包含约2mg/mL冰醋酸。

31.含水组合物,其包含:

2.40mg/mL木瓜蛋白酶;

2.60mg/mL海岸松茎皮提取物;

1.75mg/mL芦荟内叶汁;

100.00mg/mL蜂蜜;

2.00mg/mL冰醋酸;

3.00mg/mL氯化钠;

400μg/mL山梨酸钾;

400μg/mL苯甲酸钠;

10.00mg/mL野莓香料UA71225;和

800μg/mL花青素提取物。

32.含水组合物,包含以按重量计木瓜蛋白酶:干燥的海岸松茎皮提取物:干燥的芦荟内叶汁:蜂蜜:冰醋酸:氯化钠:山梨酸钾:苯甲酸钠:野莓香料UA71225:花青素提取物的比为2.40:2.60:1.75:100:2.00:3.00:0.40:0.40:10.00:0.80的木瓜蛋白酶、海岸松茎皮提取物、芦荟内叶汁、蜂蜜、冰醋酸、氯化钠、山梨酸钾、苯甲酸钠、野莓香料UA71225和花青素提取物。

33.含水组合物,包含以按重量计木瓜蛋白酶:干燥的海岸松茎皮提取物:干燥的芦荟内叶汁:蜂蜜:冰醋酸:氯化钠的比为2.40:2.60:1.75:100:2.00:3.00的木瓜蛋白酶、干燥的海岸松茎皮提取物、干燥的芦荟内叶汁、蜂蜜、冰醋酸和氯化钠。

34.含水组合物,包含以按重量计木瓜蛋白酶:干燥的海岸松茎皮提取物:干燥的芦荟内叶汁的比为2.40:2.60:1.75的木瓜蛋白酶、海岸松茎皮提取物和芦荟内叶汁。

35.治疗或预防升高的血糖的方法,包括向需要其的受试者施用有效量的根据权利要求1至34中任一项所述的组合物。

36.预防或延缓2型糖尿病(T2DM)发作的方法,包括向需要其的受试者施用有效量的根

据权利要求1至34中任一项的组合物。

37.治疗或预防糖尿病前期的方法，包括向需要其的受试者施用有效量的根据权利要求1至34中任一项所述的组合物。

38.减轻炎症的方法，包括向需要其的受试者施用有效量的根据权利要求1至34中任一项所述的组合物。

39.减少细胞因子诱导的炎症的方法，包括向需要其的受试者施用有效量的根据权利要求1至34中任一项所述的组合物。

40.减少氧化应激反应的方法，包括向需要其的受试者施用有效量的根据权利要求1至34中任一项所述的组合物。

41.治疗或预防以升高水平的细胞因子诱导的炎症为特征的疾病的方法，包括向需要其的受试者施用有效量的根据权利要求1至34中任一项所述的组合物。

42.治疗或预防以升高水平的氧化应激为特征的疾病的方法，包括向需要其的受试者施用有效量的根据权利要求1至34中任一项所述的组合物。

43.权利要求41或权利要求42所述的方法，其中所述疾病是动脉粥样硬化、与2型糖尿病和肥胖相关的肝脏炎症、子宫内膜异位症或骨关节炎。

44.减少选自以下的糖尿病药物的一种或多种副作用的方法：二甲双胍、磺脲类、氯茴苯酸类、噻唑啉二酮类、DPP-4抑制剂、GLP-1受体激动剂、SGLT2抑制剂、和胰岛素疗法，所述方法包括向需要其的受试者施用有效量的根据权利要求1至34中任一项的组合物。

45.权利要求44所述的方法，其中所述副作用是腹部或胃部不适、食欲减退、或腹泻。

46.治疗或预防与过量血糖相关的功能障碍的方法，包括向需要其的受试者施用有效量的根据权利要求1至34中任一项所述的组合物。

47.权利要求46所述的方法，其中所述功能障碍选自：增加的口渴和饥饿、尿频、疲劳/嗜睡、视力模糊、想睡和肌肉无力。

48.权利要求35至47中任一项所述的方法，其中所述组合物与二甲双胍或另一种降血糖药物组合施用。

49.改善器官功能的方法，包括向需要其的受试者施用有效量的根据权利要求1至34中任一项所述的组合物。

50.降低血液胆固醇的方法，包括向需要其的受试者施用有效量的根据权利要求1至34中任一项所述的组合物。

51.权利要求50所述的方法，其中所述血液胆固醇选自低密度脂蛋白(LDL)、高密度脂蛋白(HDL)、甘油三酯和总胆固醇。

52.治疗自身免疫性疾病的方法，包括向需要其的受试者施用有效量的根据权利要求1至34中任一项的组合物。

53.权利要求52所述的方法，其中所述自身免疫性疾病选自：狼疮、关节炎、银屑病和I型糖尿病。

54.根据权利要求35至53中任一项所述的方法，其中所述组合物的有效量对应于：

约60mg至约1500mg海岸松茎皮提取物的每日成人剂量；

约30mg至约1200mg木瓜蛋白酶的每日成人剂量；和

约15mg至约600mg芦荟提取物的每日成人剂量。

55.根据权利要求35至54中任一项所述的方法，其中所述组合物的有效量对应于：

约260mg海岸松茎皮提取物的每日成人剂量；

约240mg木瓜蛋白酶的每日成人剂量；和

约175mg芦荟提取物的每日成人剂量。

56.根据权利要求54或权利要求55所述的方法，其中将海岸松茎皮提取物、木瓜蛋白酶和芦荟提取物的所述每日成人剂量以单次剂量提供给所述受试者。

57.根据权利要求54或权利要求55所述的方法，其中将海岸松茎皮提取物、木瓜蛋白酶和芦荟提取物的所述每日成人剂量以多次剂量提供给所述受试者。

58.根据权利要求1至34中任一项所述的组合物用于制备药物的用途，所述药物用于在受试者中：

治疗或预防升高的血糖；或

预防或延缓2型糖尿病(T2DM)的发作；或

治疗或预防糖尿病前期；或

减轻炎症；或

减少细胞因子诱导的炎症；或

减少氧化应激反应；或

治疗或预防以升高水平的细胞因子诱导的炎症为特征的疾病；或

治疗或预防以升高水平的氧化应激为特征的疾病；或

降低选自以下的糖尿病药物的一种或多种副作用：二甲双胍、磺脲类、氯茴苯酸类、噻唑啉二酮类、DPP-4抑制剂、GLP-1受体激动剂、SGLT2抑制剂、和胰岛素疗法；或

治疗或预防与过量血糖相关的功能障碍；或

改善器官功能；或

降低血液胆固醇。

59.权利要求35至57中任一项所述的方法或权利要求58所述的用途，其中所述受试者选自老年人、具有糖尿病前期的医学状况或处于发展糖尿病前期风险的那些，和其组合。

60.权利要求58或权利要求59所述的用途，其中所述受试者服用二甲双胍或另一种降血糖药物。

61.制造含水组合物的方法，所述方法包括：将海岸松茎皮提取物、木瓜蛋白酶和芦荟提取物在水中组合。

62.权利要求61所述的方法，其中所述方法进一步包括：添加酸。

63.权利要求62所述的方法，其中所述酸是冰醋酸。

64.权利要求61至63中任一项所述的方法，进一步包括：添加蜂蜜。

# 组合物和其用途

## 技术领域

[0001] 本发明总体上涉及保健品(健康补充品,health supplement)领域。更具体地,本发明涉及包含海岸松(Pinus pinaster)茎皮(stem bark)提取物、木瓜蛋白酶和芦荟提取物的组合物,并涉及其在增进健康,例如调节血糖水平和治疗、延迟或预防与升高的血糖水平有关或由升高血糖水平引起的状况中的用途。

[0002] 优先权

[0003] 本申请要求澳大利亚临时申请AU2015901913的优先权,其全部内容通过交叉引用并入本文。

[0004] 背景

[0005] 糖尿病前期和糖尿病

[0006] 根据澳大利亚糖尿病协会(Diabetes Australia),25岁以上的成年人中近四分之一具有糖尿病或称为"糖尿病前期(pre-diabetes)"的状况。糖尿病前期是其中血糖水平高于正常但不足够高以诊断为2型糖尿病(T2DM)的状况。糖尿病前期是这样的状况,其中血糖水平高于正常,虽然不足够高以被归类为糖尿病。糖尿病前期可能没有体征或症状。被诊断为糖尿病前期的人具有较高的发展T2DM和心血管(心脏和循环)疾病的风险。糖尿病前期是通过检查血糖水平的血液测试来诊断的。任何显示高于正常血糖水平的血糖测试都需要医生进行跟踪。这可能涉及口服葡萄糖耐量试验(OGTT)。OGTT的结果将显示血糖水平是否在正常、糖尿病前期或糖尿病范围内。如果一个人被诊断为糖尿病前期,那么他们将有以下的一种或两种

[0007] • 空腹血糖受损(IFG)——这是当空腹血糖水平高于[在6.1mmol/L至6.9mmol/L的范围]正常,但仍低于糖尿病诊断的水平时。

[0008] • 葡萄糖耐量降低(葡萄糖耐量受损,impaired glucose tolerance)(IGT)——是当OGTT后2小时的血糖水平高于[在7.8至11.0mmol/L的范围]正常,但仍低于糖尿病诊断的水平时。空腹血糖水平可以在正常范围内。

[0009] 如果个体处于发展为T2DM的高风险,有时可由医生推荐口服糖尿病药物甲福明二甲双胍(Glucophage)。这包括体重指数超过35岁的人、小于60岁的人以及有妊娠糖尿病史的妇女。

[0010] 2型糖尿病

[0011] 胰岛功能障碍和外周胰岛素抵抗都存在于T2DM中,并且两者都是发生高血糖症所必需的。在1型糖尿病(T1DM)和T2DM中,大的前瞻性临床研究已显示糖血(血糖过多,glycemia)的时间平均的均值(以糖化血红蛋白(HbA1c)测量)与血管糖尿病并发症之间存在密切关系。这些研究是美国糖尿病协会目前推荐的治疗目标——HbA1c应<7%——的基础。HbA1c浓度的测量被认为是评估长期糖血的金标准。然而,它并没有透露任何关于血糖偏移(血糖波动,blood glucose excursion)的范围或频率的信息,而仅仅提供了总体平均值。餐后高血糖症在接受积极治疗的诊断为T2DM的患者中经常发生,并且即使在代谢控制明显好的情况下也可发生。介入研究表明,降低餐后葡萄糖偏移与控制空腹血糖在具有

T2DM和葡萄糖耐量降低的患者中同等重要。存在餐后葡萄糖升高与微血管和大血管结局之间因果关系的证据。因此，用对餐后葡萄糖调节具有特异作用的不同化合物的治疗伴随着假定在糖尿病性并发症——包括内皮功能障碍、炎症和核因子-κB活化——中涉及的许多途径的显著改善。治疗的目标应该是在血糖控制的所有三个组成部分——这些包括HbA1c、空腹血糖和餐后葡萄糖峰值——中尽可能安全地达到接近正常的血糖状态。未控制这些因素的整体不利影响将增加疾病的风险，尤其是在较高年龄组中。

[0012] 对提供一种或多种健康益处的新方法和组合物存在需要，所述健康益处如改善整体健康、改善器官功能、治疗和预防升高血糖相关的疾病如T2DM及其相关慢性疾病、治疗和预防与升高的血糖如糖尿病前期相关或由升高的血糖如糖尿病前期引起的状况、治疗炎症及其相关疾病、和/或降低血液胆固醇。

[0013] 发明概述

[0014] 本发明人已经鉴定了包含海岸松茎皮提取物、木瓜蛋白酶和芦荟提取物的组合物，其发挥许多健康益处，例如，改善器官功能、减轻炎症和调节血糖水平的能力。

[0015] 因此，第一方面，本发明提供包含海岸松茎皮提取物、木瓜蛋白酶和芦荟提取物的组合物。

[0016] 第二方面，本发明提供包含治疗有效量的海岸松茎皮提取物、木瓜蛋白酶和芦荟提取物的组合物。

[0017] 第三方面，本发明提供含有约2.6mg/mL海岸松茎皮提取物、约2.4mg/mL木瓜蛋白酶和约1.75mg/mL芦荟提取物的含水组合物。

[0018] 以下选项可单独或以任何合适的组合与上述第一、第二或第三方面结合使用。

[0019] 芦荟提取物可以是芦荟叶提取物。

[0020] 海岸松茎皮提取物的治疗有效量可对应于约60mg至约1500mg海岸松茎皮提取物的每日成人剂量；木瓜蛋白酶的治疗有效量可对应于约30mg至约1200mg木瓜蛋白酶的每日成人剂量；和芦荟提取物的治疗有效量可以对应于约15mg至约600mg芦荟提取物的每日成人剂量。

[0021] 在一个实施方式中，海岸松茎皮提取物的治疗有效量对应于约260mg的每日成人剂量；木瓜蛋白酶的治疗有效量对应于约240mg的每日成人剂量；和芦荟提取物的治疗有效量对应于约175mg的每日成人剂量。

[0022] 组合物可以进一步包含酸。酸可以是乙酸。酸可以以有效调节组合物的pH值于约3.2和约4.2之间的浓度存在。

[0023] 组合物可以进一步包含蜂蜜。

[0024] 组合物可进一步包含一种或多种选自防腐剂、调味剂、盐和色素的添加剂。组合物可以包含盐，并且盐可以是氯化钠。

[0025] 组合物可以包含另外的组分，所述另外的组分选自ω-3脂肪酸、植物营养素(phytonutrient)、蛋白质源、氨基酸、抗氧化剂、维生素、矿物质、植物提取物、和其混合物。

[0026] 组合物可以是协同的。

[0027] 组合物可适用于口服给药。例如，组合物可以是选自以下的形式：片剂、胶囊剂、可咀嚼片剂、软凝胶胶囊剂、囊剂、粉剂、颗粒剂、液体、糖浆剂、液体悬浮液、乳剂、溶液、和其组合。组合物可以是含水组合物。

[0028] 组合物可具有约3.7的pH。

[0029] 海岸松茎皮提取物可以以约2mg/mL至约10mg/mL的浓度存在。海岸松茎皮提取物可以以约2.6mg/mL的浓度存在。

[0030] 木瓜蛋白酶可以以约1mg/mL至约5mg/mL的浓度存在。木瓜蛋白酶可以以约2.4mg/mL的浓度存在。

[0031] 芦荟提取物可以以约0.5mg/mL至约4mg/mL的浓度存在。芦荟提取物可以以约1.75mg/mL的浓度存在。

[0032] 组合物可以包含浓度为约1mg/mL至约8mg/mL的冰醋酸。冰醋酸可以以约2mg/mL的浓度存在。

[0033] 组合物可以包含浓度为约50mg/mL至约200mg/mL的蜂蜜。蜂蜜可以以约100mg/mL的浓度存在。

[0034] 在一个实施方式中,每mL组合物包含:相当于1.56g干海岸松茎树皮——标准化为含有1.82mg原花青素——的海岸松茎皮浓缩提取物、2.4mg木瓜蛋白酶、3mg氯化钠和芦荟干燥的内叶汁——相当于350mg新鲜的汁。

[0035] 组合物可以进一步包含ω-3脂肪酸的来源,其选自鱼油、磷虾、含有ω-3脂肪酸的植物来源、亚麻籽、核桃、藻类、和其组合。ω-3脂肪酸可以选自α-亚麻酸(“ALA”)、二十二碳六烯酸(“DHA”)、十八碳四烯酸(“SD”)及其组合。ω-3脂肪酸可以以每天约0.25g至5.0g的量提供。

[0036] 组合物可进一步包含植物营养素,其选自黄酮类化合物(flavanoids)、同类的(allied)酚类化合物、多酚化合物、萜类化合物、生物碱类、含硫化合物及其组合。植物营养素可以选自类胡萝卜素、植物甾醇类、槲皮素、姜黄素、柠檬苦素和它们的组合。

[0037] 组合物可以包含蛋白质源。蛋白质源可以提供给组合物至少10g的高质量蛋白质。蛋白质源可以选自基于乳品的蛋白质、基于植物的蛋白质、基于动物的蛋白质、人造蛋白质、和其组合。基于乳品的蛋白质可选自酪蛋白、胶束酪蛋白、酪蛋白酸盐、酪蛋白水解产物、乳清、乳清蛋白胶束、乳清水解产物、乳清浓缩物、乳清分离物、乳蛋白浓缩物、乳蛋白分离物及其组合。基于植物的蛋白质可选自大豆蛋白,豌豆蛋白,低芥酸菜子蛋白(canola protein),小麦和分级小麦蛋白,玉米蛋白,玉米醇溶蛋白,稻米蛋白,燕麦蛋白,马铃薯蛋白,花生蛋白,青豌豆粉,绿豆粉,螺旋藻,源自蔬菜、豆(beans)、荞麦、扁豆、豆类(pulses)的蛋白质,单细胞蛋白,和其组合。

[0038] 组合物可进一步包含选自以下的氨基酸:丙氨酸、精氨酸、天冬酰胺、天冬氨酸、瓜氨酸、半胱氨酸、谷氨酸、谷氨酰胺、甘氨酸、组氨酸、羟脯氨酸、羟丝氨酸、羟酪氨酸、羟赖氨酸、异亮氨酸、亮氨酸、赖氨酸、甲硫氨酸、苯丙氨酸、脯氨酸、丝氨酸、牛磺酸、苏氨酸、色氨酸、酪氨酸、缬氨酸及其组合。氨基酸可以是选自异亮氨酸、亮氨酸、缬氨酸及其组合的支链氨基酸。

[0039] 组合物可进一步包含选自以下的抗氧化剂:虾青素、类胡萝卜素类、辅酶Q10(“CoQ10”)、黄酮类化合物、谷胱甘肽、枸杞、橙皮苷、乳枸杞(lactowolfberry)、木酚素、叶黄素、番茄红素、多酚、硒、维生素A、维生素C、维生素E、玉米黄质及其组合。

[0040] 组合物可进一步包含选自以下的维生素:维生素A、维生素B1(硫胺素)、维生素B2(核黄素)、维生素B3(烟酸或烟酰胺)、维生素B5(泛酸)、维生素B6(吡哆醇、吡哆醛、吡哆胺

或吡哆醇氢氯化物)、维生素B7(生物素)、维生素B9(叶酸)、和维生素B12(各种钴胺素;维生素补充剂中常见的氰钴胺素)、维生素C、维生素D、维生素E、维生素K、K1和K2(即MK-4、MK-7)、胆碱、及其组合。

[0041] 组合物可进一步包含选自硼、钙、铬、铜、碘、铁、镁、锰、钼、镍、磷、钾、硒、硅、锡、钒、锌及其组合的矿物质。

[0042] 组合物可进一步包含植物提取物,其选自锦葵科(Malvaceae),如黄秋葵(Abelmoschus moschatus)Medik,或选自菊(Asteraceae)科,如Achiliea santolina L.、或Achyrocline satureioides(Lam.)DC、或龙蒿(Artemisia dracunculus L.)(龙草)或菊苣(Cichorium intybus L.)、或旱莲草(Eclipta alba)(L)Hassk,或选自唇形科(Lamiaceae)如Ajuga iva L.Schreberr(Medit)或罗勒圣地Linn.(Ocimum sanctum Linn.)(Tulasi)/或牛至(Origanum vulgare L.)或选自番荔枝科如番荔枝,或选自Gentianeacea科如Anthocleista djalonensis A.Chev(卷心菜树)、或Anthocleista Schweinfurthii、或Anthocleista vogelii Planch、Enicostemma littorale Blume或Gentiana olivieri L.,或选自酢浆草科如三敛(Averrhoa bilimbi L)、或感应草属(L)DC,或选自豆科如Bauhinia candicans Benth,或选自红木科如红木,或选自紫茉莉科如印度黄细心(Boerhaavia diffusa L.),或选自十字花科如黑芥(L)Koch、Eruka sativa、或独行菜(Lepidium sativum L.),或选自云实科如刺山柑(Capparis spinosa L.)或耳叶番泻(Cassia auriculata L.)或酸豆(Tamarindus indica L.),或选自白花菜科如刺山柑,或选自伞形科如藏茴香(葛缕子,Carum carvi L.),或选自芸香科如Clausena anisata(野生)Benth,或选自棕榈科如椰子Linn.(椰子树),或选自葫芦科,如Cogniauxia podoleana或Ibervillea sonorae S.,或选自Conimelinaceae科如鸭跖草,或选自姜科如姜黄(Curcuma longa L.),或选自禾本科如狗牙根(Cynodon dactylon Pers.)(百慕大草),或选自银杏科如银杏,或选自蝶形花科,如甘草(Glycyrrhiza uralensis Fish.),或选自萝摩科如Gongronema latifolium Benth.、或匙羹藤(Gymnema montanum Hook),或选自梧桐科如火索麻(Helicteres isora L.)、As.,或选自茜草科如Hintonia standleyana,或禾本科如大麦,或选自旋花科如蕹菜(Ipomoea aquatic Forsk.)、或红薯(Ipomea batata Linn)(甘薯),或选自桑寄生科如Loranthus micranthus Linn,或选自桑科如桑籼(Morus indica.L.),或选自芭蕉科如Musa sapientum Kuntz(香蕉),或选自大戟科如苦味叶下珠(Phyllanthus amarus)Schum.Thonn或珠子草(Phyllanthus niruri)L.、或Phyllanthus sellowianus Mull.Arg.,或选自胡椒(Piperacea)科如荜拔(Piper longum),或选自桃金娘科如番石榴、或Syzygium alternifolium(Wt)Walp,或选自千屈菜科家族如雷塔马石榴,或选自蝶形花科(Papilionaceae)如鹰爪豆(Retama raetam)(RR)(Forssk)Webb.,或选自五福花科(Adoxaceae)如西泽接骨木,或选自夹竹桃科如血竭(Sanguis draxonis),或选自漆树科如Sclerocarya birea(A.Rich),或选自Scrophariaceae科如野甘草(Scoparia dulcis L.),或选自石竹科如紫花漆姑草(Spergularia purpurea),或选自藜科如碱蓬紫穗槐(Suaeda fruticosa)(SF)Euras,或选自使君子科如诃子(Terminalia chebula Retz.)、或毗黎勒(Terminalia bellirica)(Gaertn),或选自防己科(Menispermaceae),如心叶青牛胆(Tinospora cordifolia Miers.),或选自荨麻科(Urticaceae)如小荨麻(Urtica pilulifera L.),或选自菊科,如扁桃斑鸠菊(Vernonia amygdalina Del.),或选

自茄科(Solanaceae)如催眠睡茄(Withania soimifera(L)Dunal),或选自蒺藜科(Zygophyllaceae)如Zygophyllum gaetulum Emb和Maire及其组合。

[0043] 第四方面,本发明提供含水组合物,其包含:2.40mg/mL木瓜蛋白酶;2.60mg/mL干燥的海岸松茎皮提取物;1.75mg/mL干燥的芦荟内叶汁;蜂蜜100.00mg/mL;2.00mg/mL冰醋酸;3.00mg/mL氯化钠;400μg/mL山梨酸钾;400μg/mL苯甲酸钠;10.00mg/mL野莓风味物(wildberry flavour)UA71225;和800μg/mL花青素提取物。

[0044] 第五方面,本发明提供含水组合物,其包含按重量计木瓜蛋白酶:干燥的海岸松茎皮提取物:干燥的芦荟内叶汁的比为2.40:2.60:1.75的木瓜蛋白酶、海岸松茎皮提取物和芦荟内叶汁。

[0045] 在一个实施方式中,提供包含按重量计木瓜蛋白酶:干燥的海岸松茎皮提取物:干燥的芦荟内叶汁:蜂蜜的比为2.40:2.60:1.75:100的木瓜蛋白酶、海岸松茎皮提取物、芦荟内叶汁和蜂蜜的含水组合物。

[0046] 在另一实施方式中,提供包含按重量计木瓜蛋白酶:干燥的海岸松茎皮提取物:干燥的芦荟内叶汁:冰醋酸的比为2.40:2.60:1.75:2.00的木瓜蛋白酶、海岸松茎皮提取物、芦荟内叶汁和冰醋酸的含水组合物。

[0047] 在另一个实施方式中,提供包含按重量计木瓜蛋白酶:干燥的海岸松茎皮提取物:干燥的芦荟内叶汁:蜂蜜:冰醋酸的比为2.40:2.60:1.75:100:2.00的木瓜蛋白酶、海岸松茎皮提取物、芦荟内叶汁、蜂蜜和冰醋酸的含水组合物。

[0048] 在另一个实施方式中,提供包含按重量计木瓜蛋白酶:干燥的海岸松茎皮提取物:干燥的芦荟内叶汁:蜂蜜:冰醋酸:氯化钠的比为2.40:2.60:1.75:100:2.00:3.00的木瓜蛋白酶、海岸松茎皮提取物、芦荟内叶汁、蜂蜜、冰醋酸和氯化钠的含水组合物。

[0049] 在另一个实施方式中,提供包含以按重量计木瓜蛋白酶:干燥的海岸松茎皮提取物:干燥的芦荟内叶汁:蜂蜜:冰醋酸:氯化钠:山梨酸钾:苯甲酸钠:野莓香料UA71225:花青素提取物的比为2.40:2.60:1.75:100:2.00:3.00:0.40:0.40:10.00:0.80的木瓜蛋白酶、海岸松茎皮提取物、芦荟内叶汁、蜂蜜、冰醋酸、氯化钠、山梨酸钾、苯甲酸钠、野莓香料UA71225和花青素提取物的含水组合物。

[0050] 第六方面,本发明提供治疗或预防升高的血糖的方法,包括向需要其的受试者施用有效量的根据上述第一至第五方面中任一个的组合物。

[0051] 第七方面,本发明提供预防或延缓2型糖尿病(T2DM)发病的方法,包括向需要其的受试者施用有效量的根据上述第一至第五方面中任一个的组合物。

[0052] 第八方面,本发明提供治疗或预防糖尿病前期的方法,包括向需要其的受试者施用有效量的根据上述第一至第五方面中任一个的组合物。

[0053] 第九方面,本发明提供减轻炎症的方法,包括向需要其的受试者施用有效量的根据上述第一至第五方面中任一个的组合物。

[0054] 第十方面,本发明提供减少细胞因子诱导的炎症的方法,包括向需要其的受试者施用有效量的根据上述第一至第五方面中任一个的组合物。

[0055] 第十一方面,本发明提供减少氧化应激反应的方法,包括向需要其的受试者施用有效量的根据上述第一至第五方面中任一个的组合物。

[0056] 第十二方面,本发明提供治疗或预防以升高水平的细胞因子诱导的炎症为特征的

疾病的方法，包括向需要其的受试者施用有效量的根据上述第一至第五方面中任一个的组合物。

[0057] 第十三方面，本发明提供治疗或预防以升高水平的氧化应激为特征的疾病的方法，包括向需要其的受试者施用有效量的根据上述第一至第五方面中任一个的组合物。

[0058] 在上述第十二或第十三方面的一个实施方式中，疾病是动脉粥样硬化、与2型糖尿病和肥胖相关的肝脏炎症、子宫内膜异位症或骨关节炎。

[0059] 第十四方面，本发明提供减少选自以下的糖尿病药物的一种或多种副作用的方法：二甲双胍、磺脲类、氯茴苯酸类、噻唑啉二酮类、DPP-4抑制剂、GLP-1受体激动剂、SGLT2抑制剂、和胰岛素疗法，所述方法包括向需要其的受试者施用有效量的根据上述第一至第五方面中任一个的组合物。副作用可以是腹部或胃部不适、食欲减退、或腹泻。

[0060] 第十五方面，本发明提供治疗或预防与过量血糖相关的功能障碍的方法，包括向需要其的受试者施用有效量的根据上述第一至第五方面中任一个的组合物。功能障碍可以选自：增加的口渴和饥饿、尿频、疲劳/嗜睡、视力模糊、想睡和肌肉无力。

[0061] 在上述第六至第十五方面的一个实施方式中，组合物与二甲双胍或另一种降血糖药物组合施用。

[0062] 第十六方面，本发明提供改善器官功能的方法，包括向需要其的受试者施用有效量的根据上述第一至第五方面中任一个的组合物。

[0063] 第十七方面，本发明提供降低血液胆固醇的方法，包括向需要其的受试者施用有效量的根据上述第一至第五方面中任一个的组合物。血液胆固醇可以选自低密度脂蛋白(LDL)、高密度脂蛋白(HDL)、甘油三酯和总胆固醇。在一个实施方式中，本发明提供降低低密度脂蛋白(LDL)血液胆固醇水平的方法，包括向需要其的受试者施用有效量的根据上述第一至第五方面中任一个的组合物。在另一个实施方式中，本发明提供增加高密度脂蛋白(HDL)血液胆固醇水平的方法，包括向需要其的受试者施用有效量的根据上述第一至第五方面中任一个的组合物。在又一个实施方式中，本发明提供降低总胆固醇与HDL的比的方法，包括向需要其的受试者施用有效量的根据上述第一至第五方面中任一个的组合物。在又一个实施方式中，本发明提供降低血液甘油三酯水平的方法，包括向需要其的受试者施用有效量的根据上述第一至第五方面中任一个的组合物。

[0064] 组合物的有效量可以对应于：约60mg至约1500mg海岸松茎皮提取物的每日成人剂量；约30mg至约1200mg木瓜蛋白酶的每日成人剂量；和约15mg至约600mg芦荟提取物的每日成人剂量。例如，组合物的有效量可以对应于：约260mg海岸松茎皮提取物的每日成人剂量；约240mg木瓜蛋白酶的每日成人剂量；和约为175mg芦荟提取物的每日成人剂量。可以以单次剂量向受试者提供海岸松茎皮提取物、木瓜蛋白酶和芦荟提取物的每日成人剂量，或者可以以多个剂量向受试者提供海岸松茎皮提取物、木瓜蛋白酶和芦荟提取物的每日成人剂量。

[0065] 在上述第六至第十七方面的一个实施方式中，受试者选自老年人、具有糖尿病前期的医学状况或处于发展糖尿病前期风险的人、和其组合。

[0066] 第十八方面，本发明提供改善免疫功能的方法，包括向需要其的受试者施用有效量的根据上述第一至第五方面中任一个的组合物。在一个实施方式中，改善的免疫功能的特征在于增加的白细胞计数，例如，增加的嗜中性粒细胞水平。

[0067] 第十九方面，本发明提供治疗自身免疫性疾病的方法，包括向需要其的受试者施用有效量的根据上述第一至第五方面中任一个的组合物。自身免疫性疾病可以选自：狼疮、关节炎、银屑病和I型糖尿病。在一个实施方式中，本发明提供治疗狼疮的方法，包括向需要其的受试者施用有效量的根据上述第一至第五方面中任一个的组合物。狼疮的治疗可以减少受试者中或受试者上的损伤例如皮下损伤的数量。在另一个实施方式中，本发明提供治疗关节炎的方法，包括向需要其的受试者施用有效量的根据上述第一至第五方面中任一个的组合物。在一个实施方式中，关节炎是类风湿关节炎。在另外的实施方式中，本发明提供治疗银屑病的方法，包括向需要其的受试者施用有效量的根据上述第一至第五方面中任一个的组合物。在又一个实施方式中，本发明提供治疗I型糖尿病的方法，包括向需要其的受试者施用有效量的根据上述第一至第五方面中任一个的组合物。

[0068] 第二十方面，本发明提供治疗气肿的方法，包括向需要其的受试者施用有效量的根据上述第一至第五方面中任一个的组合物。

[0069] 第二十一方面，本发明提供增加总体幸福(健康，wellbeing)的方法，包括向需要其的受试者施用有效量的根据上述第一至第五方面中任一个的组合物。在一个实施方式中，总体幸福的测量包括以下一项或多项：增加的能量水平、改善的活力、更好的睡眠、改善的呼吸能力、改善的体力活动水平、改善的心理功能和/或改善的身体功能。

[0070] 在上述第六至第二十一方面中任一个的方法中，组合物可以以每天30至100mL的剂量施用于成年人受试者，其中每mL所述组合物包含：相当于1.56g干燥的海岸松茎皮——标准化为含有1.82mg原花青素——的海岸松茎皮浓缩提取物、2.4mg木瓜蛋白酶、3mg氯化钠和相当于350mg新鲜汁的芦荟干内叶汁。在一个实施方式中，每mL组合物包含：2.40mg木瓜蛋白酶；2.60mg干燥的海岸松茎皮提取物；1.75mg干燥的芦荟内叶汁；100.00mg蜂蜜；2.00mg冰醋酸；3.00mg氯化钠；400μg山梨酸钾；400μg苯甲酸钠；10.00mg野莓风味物UA71225；和800μg花青素提取物或其等价物。组合物可以以两个独立的剂量以约30至约100mL的总日剂量施用。例如，组合物可以以约15至约50mL的两个独立剂量施用，在早餐后一个剂量和在晚餐后一个剂量。

[0071] 第二十二方面，本发明提供根据上述第一至第五方面中任一个的组合物用于制备药物的应用，所述药物用于在受试者中：治疗或预防升高的血糖；或预防或延缓2型糖尿病(T2DM)的发病；或治疗或预防糖尿病前期；或减轻炎症；或减少细胞因子诱导的炎症；或减少氧化应激反应；或治疗或预防以升高水平的细胞因子诱导的炎症为特征的疾病；或治疗或预防以升高水平的氧化应激为特征的疾病；或降低选自二甲双胍、磺脲类、氯茴苯酸类、噻唑啉二酮类、DPP-4抑制剂、GLP-1受体激动剂、SGLT2抑制剂和胰岛素疗法的糖尿病药物的一种或多种副作用；或治疗或预防与过量血糖相关的功能障碍；或改善器官功能；或降低血液胆固醇。

[0072] 第二十三方面，本发明提供根据上述第一至第五方面中任一个的组合物用于制备药物的用途，所述药物用于在受试者中：改善免疫功能；治疗自身免疫性疾病，例如狼疮、关节炎、银屑病或I型糖尿病；治疗气肿；或增加总体幸福，例如增加能量水平、改善活力、改善睡眠、改善呼吸能力、改善体力活动水平、改善心理功能、和/或改善身体功能。

[0073] 在一个实施方式中，本发明提供根据上述第一至第五方面中任一个的组合物用于制备用于治疗或预防受试者中升高的血糖的药物的用途。

[0074] 在一个实施方式中，本发明提供根据上述第一至第五方面中任一个的组合物用于制备用于预防或延缓受试者中2型糖尿病(T2DM)发病的药物的用途。

[0075] 在一个实施方式中，本发明提供根据上述第一至第五方面中任一个的组合物用于制备用于治疗或预防受试者中的糖尿病前期的药物的用途。

[0076] 在一个实施方式中，本发明提供根据上述第一至第五方面中任一个的组合物用于制备用于减轻受试者中炎症的药物的用途。

[0077] 在一个实施方式中，本发明提供根据上述第一至第五方面中任一个的组合物用于制备用于减少受试者中细胞因子诱导的炎症的药物的用途。

[0078] 在一个实施方式中，本发明提供根据上述第一至第五方面中任一个的组合物用于制备降低受试者中氧化应激反应的药物的用途。

[0079] 在一个实施方式中，本发明提供根据上述第一至第五方面中任一个的组合物用于制备用于治疗或预防受试者中以升高水平的细胞因子诱导的炎症为特征的疾病的药物的用途。该疾病可以是动脉粥样硬化、与2型糖尿病和肥胖相关的肝脏炎症、子宫内膜异位症或骨关节炎。

[0080] 在一个实施方式中，本发明提供根据上述第一至第五方面中任一个的组合物用于制备用于治疗或预防受试者中以升高水平的氧化应激为特征的疾病的药物的用途。该疾病可以是动脉粥样硬化、与2型糖尿病和肥胖相关的肝脏炎症、子宫内膜异位症或骨关节炎。

[0081] 在一个实施方式中，本发明提供根据上述第一至第五方面中任一个的组合物在制备用于在受试者中降低糖尿病药物的一种或多种副作用的药物中的用途，所述糖尿病药物选自二甲双胍、磺脲类、氯茴苯酸类、噻唑啉二酮类、DPP-4抑制剂、GLP-1受体激动剂、SGLT2抑制剂和胰岛素疗法。副作用可以是腹部或胃部不适、食欲减退或腹泻。

[0082] 在一个实施方式中，本发明提供根据上述第一至第五方面中任一个的组合物用于制备用于治疗或预防受试者中与过量血糖相关的功能障碍的药物的用途。功能障碍可以选自：增加的口渴和饥饿、尿频、疲劳/嗜睡、视力模糊、想睡和肌肉无力。

[0083] 在一个实施方式中，本发明提供根据上述第一至第五方面中任一个的组合物用于制备用于改善受试者中器官功能的药物的用途。

[0084] 在一个实施方式中，本发明提供根据上述第一至第五方面中任一个的组合物用于制备用于降低受试者中血液胆固醇的药物的用途。

[0085] 在上述第二十二方面的一个实施方式中，受试者可以服用二甲双胍或其它降血糖药物，和/或受试者可以选自老年人、具有糖尿病前期的医学状况或者处于发展糖尿病前期的风险的那些、及其组合。

[0086] 在一个实施方式中，本发明提供根据上述第一至第五方面中任一个的组合物用于制备用于改善受试者中免疫功能的药物的用途。在另一个实施方式中，本发明提供根据上述第一至第五方面中任一个的组合物用于治疗受试者中的自身免疫性疾病，例如狼疮、关节炎、银屑病或I型糖尿病的用途。在另一个实施方式中，本发明提供根据上述第一至第五方面中任一个的组合物用于制造用于治疗受试者中气肿的药物的用途。在又一个实施方式中，本发明提供根据上述第一至第五方面中任一个的组合物用于制造用于在受试者中增加总体幸福，例如增加能量水平、改善活力、改善睡眠、改善呼吸能力、改善体力活动水平、改善心理功能、和/或改善的身体功能的药物的用途。

[0087] 附图简述

[0088] 现在将仅通过例子参考附图来描述本发明的实施方式，其中：

[0089] 图1显示根据实施例1的组合物减少人冠状动脉内皮细胞中炎症标志物(a)TNF-α诱导的VCAM-1、(b)TNF-α诱导的ICAM-1和(c)TNF-α诱导的ELAM。在暴露于TNF-α之前，将HCAEC暴露于指示量(μL/mL)的实施例1的组合物3小时。

[0090] 图2显示根据实施例1的组合物减少人冠状动脉内皮细胞中(a)TNF-α诱导的VCAM-1和(b)TNF-α诱导的ICAM-1的蛋白质水平。在暴露于TNF-α之前，将HCAEC暴露于指示量(μL/mL)的实施例1的组合物3小时。

[0091] 图3显示根据实施例1的组合物减少炎症关键介质NFkB的活化。在暴露于TNF-α之前，将HCAEC暴露于指示量(μL/mL)的实施例1的组合物。

[0092] 图4显示根据实施例1的组合物降低TNF-α诱导的HCAEC中的细胞因子诱导的ROS水平。在暴露于TNF-α之前，将HCAEC暴露于指示量(μL/mL)的实施例1的组合物3小时。

[0093] 图5显示根据实施例1的组合物降低TNF-α诱导的HCAEC中的(a)NOX-4并增加(b)SOD-1水平。在暴露于TNF-α之前，将HCAEC暴露于指示量(μL/mL)的实施例1的组合物。

[0094] 图6显示运动员训练前摄入的根据实施例1的组合物降低了训练后他们血液中的CRP水平(深灰色＝跑步者，浅灰色＝骑车者)。

[0095] 图7显示根据实施例1的组合物阻断VCAM-1水平响应于促炎细胞因子TNF-α而升高。在暴露于TNF-α之前，将HCAEC暴露于相当于实施例1的组合物的50mL、25mL、12.5mL和6.25mL剂量的浓度。

[0096] 图8显示用在摄取实施例1的组合物3小时后从个体获得的血浆预处理(24h)后，暴露于炎性刺激物TNF-α的人冠状动脉内皮细胞与用来自组合物预摄入的相同个体的血浆预处理的细胞相比显示降低水平的炎症反应(通过VCAM-1水平测量)。

[0097] 定义

[0098] 如在本申请中所使用的，除非上下文另外清楚地指出，否则单数形式“一(a)”、“一(an)”、“所述(the)”包括复数指代。例如，短语“一种组合物”也包括多种组合物。

[0099] 如本文所使用的，术语“包含(comprising)”意思是“包括(including)”。单词“包含”的变化，如“包含(comprise)”和“包含(comprises)”具有相应的不同含义。因此，例如，“包含”海岸松茎皮提取物、木瓜蛋白酶和芦荟提取物的组合物可以仅由海岸松茎皮提取物、木瓜蛋白酶和芦荟提取物组成，或者它可以包括一种或多种另外的组分(例如酸、蜜糖、防腐剂等)。

[0100] 应该理解，本文关于所陈述的数值使用术语“约”包括所陈述的数值和在所陈述的值的正负百分之十内的数值。

[0101] 如本文所用，术语“受试者”被理解为包括正在接受或打算接受治疗或者具有或处于具有可受益于本文所述的组合物的医学状况的风险的动物，尤其是哺乳动物，更特别地是人。例如，动物可以包括但不限于，哺乳动物如啮齿类、水生哺乳动物、家畜如狗和猫、农场动物如绵羊、猪、牛和马、以及人类。还考虑术语“受试者”适用于能够展现或打算由本文所述的组合物展现的作用的任何动物。

[0102] 应该理解，当提到数值范围时，本文术语“在...之间”的使用涵盖在范围的每个端点处的数值。例如，约3.2至约4.2之间的pH值包括3.2的pH值和4.2的pH值。

[0103] 如本文所用，术语“含水的”和“含水组合物”将被理解为是指在水——并且特别是纯净水——中或者与水——并且特别是纯净水——一起配制的组合物。在一些实施方式中，含水组合物将主要为水，例如按重量计或按体积计多至99％的水。在其它实施方式中，含水组合物将包含至少一些水，例如超过按重量计或按体积计1％的水。

[0104] 详细说明

[0105] 以下详细描述足够详细地传达了本发明，包括其示例性实施方式，以使本领域的普通技术人员能够实施本发明。所描述的各种实施方式的特征或限制不一定限制本发明的其它实施方式或整个本发明。因此，下面的详细描述不限制仅由权利要求限定的本发明的范围。

[0106] 本公开内容总体上涉及包含海岸松茎皮提取物与芦荟提取物(可选地称为巴巴多斯芦荟(A.barbadensis Mill.)，印度芦荟(Aloe indica Royle)，不夜城芦荟(Aloe perfoliata L.)var.vera和A.vulgaris Lam)和木瓜蛋白酶的组合的组合物以及方法和用途，用于治疗性和/或预防性治疗、改善和/或调节与过量血糖(高血糖症)相关的疾病状态或病理状况，用于改善器官功能，和用于减轻炎症。例如，组合物和方法可适于治疗和/或预防与过量血糖相关的功能障碍，其可包括日常生理功能紊乱，如增加的口渴和饥饿、尿频、疲劳/嗜睡、视力模糊、想睡、和/或肌肉无力。这样的相关状况可以通过药物施用(例如二甲双胍(metfonnin))混合，这可引起或加剧腹部或胃部不适、食欲减退、腹泻和总体不适感。

[0107] 组合物

[0108] 海岸松茎皮提取物

[0109] 根据本发明的组合物包含海岸松茎皮提取物。这种海岸松茎皮提取物由称为陆地或海松的欧洲海岸松树——学名海岸松(Pinus maritima或Pinus pinaster)——的树皮制成。不受理论束缚，松树皮提取物可具有抗氧化特性，使其适于广泛的愈合和预防目的。

[0110] 海岸松茎皮提取物可以来源自任何合适的来源，例如使用本领域已知的方法从海松树的树皮中提取，或者其可以商业获得，例如作为由Horphag Research USA Inc.提供的产品Pycnogenol®，或作为由Pharmactive Biotech Products SL提供的海岸松L。优选地，海岸松茎皮提取物是由Horphag Research USA Inc.提供的Pycnogenol®。海岸松茎皮提取物可以以固体形式，例如作为干粉、或压紧成片剂的干粉、或胶囊中的干粉，或以液体形式，例如，溶解或悬浮于溶液、悬浮液或乳剂中的干粉，提供于根据本发明的组合物中。在包含海岸松茎皮提取物的组合物作为含水组合物提供的情况下，可以将干粉的海岸松茎皮提取物溶解在水中。在本说明书的上下文中，术语“提取物”应被赋予其最广泛的解释，并被理解为指植物及其组分或制剂，或植物或草的组分或由其衍生的制剂，其当以有效量用于根据本发明的组合物时促进受试者的健康益处，如调节高血糖水平。

[0111] 海岸松茎皮提取物可以以任何合适的量，例如以治疗有效量存在于组合物中。海岸松茎皮提取物的治疗有效量可对应于约60mg至约1500mg海岸松茎皮提取物的每日成人剂量。在一个实施方式中，海岸松茎皮提取物以对应于约260mg的每日成年人剂量的治疗有效量存在于组合物中。

[0112] 在将组合物配制成液体，例如水性液体的情况下，海岸松茎皮提取物可以以约2.0mg/mL至约10mg/mL范围的浓度存在于组合物中。例如，海岸松茎皮提取物可以以约2.0mg/mL至约3.0mg/mL、或约2.0mg/mL至约5.0mg/mL、或约4.0mg ml至约8.0mg/mL、或约

6.0mg/mL至约10.0mg/mL范围的浓度，例如以2.0mg/mL、2.2mg/mL、2.4mg/mL、2.6mg/mL、2.8mg/mL、3.0mg/mL、3.2mg/mL、3.4mg/mL、3.6mg/mL、3.8mg/mL、4.0mg/mL、4.5mg/mL、5.0mg/mL、5.5mg/mL、6.0mg/mL、6.5mg/mL、7.0mg/mL、7.5mg/mL、8.0mg/mL、8.5mg/mL、9.0mg/mL、9.5mg/mL、或10mg/mL的浓度存在于组合物中，其中海岸松茎皮提取物的量表示为每mL组合物干海岸松茎皮提取物的量。本文考虑每mL干燥的海岸松茎树皮提取物的量包括以干燥形式加到液体组合物中的海岸松茎皮提取物，但也涵盖不同量的以其它形式(例如在液体浓缩物中)的海岸松茎树皮提取物的添加，条件是加入后的海岸松茎皮提取物的总浓度等于每mL组合物干海岸松茎皮提取物的浓度。

[0113] 本文所指的“干燥的海岸松茎皮提取物”的量是基于本发明的示例性实施方式，例如表1中的组合物计算的，其中加到组合物中的干燥的海岸松茎皮提取物包含大约65-75％的原花青素。在一个实施方式中，表1中每mL 2.60mg干燥的海岸松茎皮提取物——等于1.56g干燥的海岸松茎皮——被标准化为含有约1.82mg原花青素。应该理解的是，海岸松茎皮提取物的替代来源或形式可以是可用的，在这种情况下，本发明组合物中使用的海岸松茎皮提取物的量可以被调整以提供相当量的原青花素而不是必然相当量的海岸松茎皮提取物。

[0114] 在一个实施方式中，海岸松茎皮提取物以2.6mg/mL的浓度存在于含水组合物中。

[0115] 木瓜蛋白酶

[0116] 根据本发明的组合物包含木瓜蛋白酶。木瓜蛋白酶是从木瓜植物(番木瓜)中提取的半胱氨酸蛋白酶。

[0117] 木瓜蛋白酶可以使用本领域已知的方法从任何合适的来源获得，例如木瓜植物的叶、胶乳、根和/或果实，或者其可以商业获得，例如作为由Biocon Limited,Bangalore,India提供的产品Papaina(USP)。它可以以固体形式，例如作为干粉，或以任何其他合适的形式，例如悬浮或溶解在水性缓冲溶液中，或作为浓缩液体加到本发明的组合物中。在包含木瓜蛋白酶的组合物作为含水组合物提供的情况下，可以将纯粉状的干木瓜蛋白酶溶解在水中。

[0118] 木瓜蛋白酶可以以任何合适的量例如以治疗有效量存在于组合物中。木瓜蛋白酶的治疗有效量可对应于约30mg至约1200mg木瓜蛋白酶的每日成人剂量。在一个实施方式中，木瓜蛋白酶以对应于约240mg的每日成人剂量的治疗有效量存在于组合物中。

[0119] 在组合物被配制成液体，例如含水液体的情况下，木瓜蛋白酶可以以约1.0mg/mL至约8mg/mL范围的浓度存在于组合物中。例如，木瓜蛋白酶可以以约1.0mg/mL至约3.0mg/mL、或约2.0mg/mL至约5.0mg/mL、或约4.0mg/mL至约6.0mg/mL、或约5.0mg/mL至约8.0mg/mL范围的浓度，例如，以1.0mg/mL、1.2mg/mL、1.4mg/mL、1.6mg/mL、1.8mg/mL、2.0mg/mL、2.2mg/mL、2.4mg/mL、2.6mg/mL、2.8mg/mL、3.0mg/mL、3.2mg/mL、3.4mg/mL、3.6mg/mL、3.8mg/mL、4.0mg/mL、4.5mg/mL、5.0mg/mL、5.5mg/mL、6.0mg/mL、6.5mg/mL、7.0mg/mL、7.5mg/mL、或8.0mg/mL的浓度存在于组合物中，其中木瓜蛋白酶的量被表示为每mL组合物干木瓜蛋白酶的量。本文考虑每mL干木瓜蛋白酶的量包括以干的形式加到液体组合物中的木瓜蛋白酶，也包括以不同量的以其他形式(例如以液体浓缩物)加入木瓜蛋白酶，条件是加入后木瓜蛋白酶的总浓度相当于每mL组合物干木瓜蛋白酶的浓度。

[0120] 在一个实施方式中，木瓜蛋白酶以2.4mg/mL的浓度存在于含水组合物中。

[0121] 芦荟

[0122] 根据本发明的组合物包含芦荟提取物。芦荟也可选地称为巴巴多斯芦荟(A.barbadensis Mill.),印度芦荟(Aloe indica Royle),不夜城芦荟(Aloe perfoliata L.)var.vera和A.vulgaris Lam。芦荟提取物可以从芦荟植物的任何合适的部分提取,例如,芦荟提取物可以是芦荟叶提取物,例如通过粉碎整个芦荟叶制成,或者可以是芦荟凝胶提取物,或者可以是芦荟胶乳(latext)提取物。优选地,芦荟提取物是干的芦荟内叶汁提取物。

[0123] 芦荟提取物可以使用本领域已知的方法从任何合适的来源获得,例如芦荟植物的叶、胶乳或凝胶,或者其可以商业获得,例如作为由Capitol Ingredients提供的产品巴巴多斯芦荟——喷雾干燥的。它可以以固体形式,例如作为干的内叶汁粉末,或以任何其他合适的形式,例如作为液体、凝胶或糊用于根据本发明的组合物。因此,如本文所用,芦荟提取物可以指干的芦荟内叶汁提取物。在包含芦荟提取物的组合物作为含水组合物提供的情况下,粉状的干芦荟内叶汁提取物可以溶解在水中。

[0124] 芦荟提取物可以以任何合适的量例如以治疗有效量存在于组合物中。芦荟提取物的治疗有效量可对应于约15mg至约600mg芦荟提取物的每日成人剂量。在一个实施方式中,芦荟提取物例如芦荟内叶汁提取物以相当于约175mg的每日成人剂量的治疗有效量存在于组合物中。

[0125] 如果组合物被配制为液体,例如含水液体,则芦荟内叶汁提取物可以以约0.5mg/mL至约4mg/mL范围的浓度存在于组合物中。例如,芦荟内叶汁提取物可以以约0.5mg/mL至约2.0mg/mL、或约1.5mg/mL至约3.5mg/mL、或约2.0mg/mL至约4.0mg/mL范围的浓度,例如以0.5mg/mL、0.7mg/mL、0.9mg/mL、1.0mg/mL、1.2mg/mL、1.4mg/mL、1.5mg/mL、1.6mg/mL、1.7mg/mL、1.8mg/mL、1.9mg/mL、2.0mg/mL、2.2mg/mL、2.4mg/mL、2.6mg/mL、2.8mg/mL、3.0mg/mL、3.2mg/mL、3.4mg/mL、3.6mg/mL、3.8mg/mL或4.0mg/mL的浓度存在于组合物中,其中芦荟内叶汁提取物的量表示为每mL组合物的喷雾干燥的芦荟内叶汁提取物的量。本文考虑每mL喷雾干燥的芦荟内叶汁提取物的量包括以干燥形式加到液体组合物中的喷雾干燥的芦荟内叶汁提取物,也包括以不同量的以其它形式(例如以液体或凝胶浓缩物)加入芦荟内叶汁提取物,条件是加入后芦荟叶内汁提取物的总浓度相当于每mL组合物的喷雾干燥的芦荟内叶汁提取物的浓度。就此而言,本文所指的“喷雾干燥的芦荟内叶汁提取物”的量是基于本发明的示例性实施方式,例如表1中的组合物计算的,其中加到组合物中的喷雾干燥的芦荟内叶汁提取物包含大约40-50%的麦芽糊精,其中新鲜的植物与喷雾干燥的物质的比为约180-210:1。应该理解,用于制备芦荟提取物的备选方法可以是可用的,在这种情况下,本发明组合物中使用的芦荟提取物的量可以被调整以解释实际芦荟来源材料和或存在的添加剂的浓度差异。

[0126] 在一个实施方式中,芦荟内叶汁提取物以1.75mg/mL的浓度存在于含水组合物中。

[0127] 包括海岸松茎皮提取物、木瓜蛋白酶和芦荟提取物的本公开的组合物可以与其他化合物组合施用以协助调节血糖水平。这可以允许调整组合物的味道特征(taste profile)以改善依从性和因此的功效和/或以递送另外的或补充的健康益处。

[0128] 蜂蜜

[0129] 因此,根据本发明的组合物可以包含蜂蜜。可以使用任何合适的蜂蜜或蜂蜜混合

物。

[0130] 蜂蜜可以源自任何合适的来自任何合适的花卉物种的来源，例如，其可以商业获得如英国药典(BP)级蜂蜜，例如，由澳大利亚的Capilano Honey Limited提供。蜂蜜可以以浓缩的粘性混合物的形式作为纯蜂蜜加入根据本发明的组合物中。它可以在加入之前加热以降低粘度并增加组合物中分散或增溶的容易度。

[0131] 蜂蜜可以以任何合适的量存在于组合物中。例如，纯蜂蜜可以以约1.5g至约30g/天的每日成人剂量，例如以约1.5g至约5g、或约5g至约10g、或约7g至约12g、或约10g至约20g、或约15g至约30g、或约20g至约25g的每日成人剂量，或以约1.5g、2g、5g、7.5g、10g、12.5g、15g、17.5g、20g、22.5g、25g、27.5g或30g的每日成人剂量存在于组合物中。在一个实施方式中，蜂蜜以对应于约10g的每日成人剂量的量存在于组合物中。

[0132] 在组合物被配制成液体例如含水液体的情况下，纯蜂蜜可以以约50mg/mL至约200mg/mL范围的浓度存在。例如，纯蜂蜜可以以约50mg/mL至约100mg/mL、或约75mg/mL至约150mg/mL、或约100mg/mL至约200mg/mL范围的浓度，例如以50mg/mL、60mg/mL、70mg/mL、80mg/mL、90mg/mL、100mg/mL、110mg/mL、120mg/ml、130mg/mL、140mg/mL、150mg/mL、160mg/mL、170mg/mL、180mg/mL、1900mg/mL或200mg/mL的浓度存在于组合物中。在一个实施方式中，蜂蜜以100mg/mL的浓度存在于含水组合物中。

[0133] 酸

[0134] 根据本发明的组合物可以包含酸。可以使用任何合适的酸或酸的混合物，例如，酸可以是有机酸或无机酸，或者任何一种或多种有机酸和/或无机酸的混合物。酸可以具有任何合适的浓度，例如可以浓缩的(例如基本上无水)或稀释的。在一个实施方式中，酸是食物酸，例如乙酸。在优选的实施方式中，酸是乙酸。在另一个实施方式中，酸是冰醋酸。可以加入酸以帮助组合物进入受试者的血流，这又可以使组合物或其一种或多种组分成分到达例如发炎的受试者身体部位。酸可以作为醋加到组合物中。

[0135] 酸可以以足以将组合物酸化至约3.2和约4.2之间，例如在约3.2和约3.7、约3.5和约4.0、约3.7和约4.2、或约4.0和约4.2之间的pH，例如在约3.2、约3.3、约3.4、约3.5、约3.6、约3.7、约3.8、约3.9、约4.0、约4.1或约4.2的pH的量存在于组合物中。

[0136] 在酸是冰醋酸的情况下，酸可以以任何合适的量存在于组合物中。例如，冰醋酸可以以约30mg至约1200mg/天的每日成人剂量，例如以在约30mg至约100mg、或约50mg至约250mg、或约150mg至约250mg、或约200mg至约500mg、或约500mg至约750mg、或约700mg至约1000mg、或约900mg至约1200mg的每日人类剂量，或以约30mg、50mg、100mg、200mg、300mg、400mg、500mg、600mg、700mg、800mg、900mg、1000mg、1100mg或1200mg的每日人类剂量存在于组合物中。在一个实施方式中，冰醋酸以对用于约200mg的每日成人剂量的量存在于组合物中。

[0137] 在组合物被配制为液体例如含水液体的情况下，冰醋酸可以约1mg/mL至约8mg/mL范围的浓度存在于组合物中。例如，冰醋酸可以以约1.0mg/mL至约3.0mg/mL、或约2.0mg/mL至约5.0mg/mL、或约3.0mg/mL至约6.0mg/mL、或约4.0mg/mL至约8.0mg/mL范围的浓度，例如以1.0mg/mL、1.2mg/mL、1.4mg/mL、1.6mg/mL、1.8mg/mL、2.0mg/mL、2.2mg/mL、2.4mg/mL、2.6mg/mL、2.8mg/mL、3.0mg/mL、3.2mg/mL、3.4mg/mL、3.6mg/mL、3.8mg/ml、4.0mg/mL、4.5mg/mL、5.0mg/mL、5.5mg/mL、6.0mg/mL、6.5mg/mL、7.0mg/mL、7.5mg/mL或8.0mg/mL的浓

度存在于组合物中。在一个实施方式中，冰醋酸以2mg/mL的浓度存在于含水组合物中。

[0138] 冰醋酸可以商业获得，例如由澳大利亚悉尼的Bronson&Jacobs Pty Limited供应。

[0139] 协同作用(增效作用，synergism)

[0140] 根据本发明的组合物可以包含活性成分的协同混合物。协同作用可以指组合物在调节受试者中的血糖水平中所起的作用，或者可以指组合物在治疗或预防糖尿病前期、糖尿病或任何相关的功能障碍中所起的作用。例如，包含海岸松茎皮提取物、木瓜蛋白酶和芦荟提取物的组合物可协同地调节血糖水平或治疗或预防受试者中的糖尿病前期、糖尿病或任何相关的功能障碍。包含海岸松茎皮提取物、木瓜蛋白酶、芦荟提取物和蜂蜜的组合物可另外地或可选地协同地起作用以调节血糖水平或治疗或预防受试者中的糖尿病前期、糖尿病或任何相关的功能障碍。包含海岸松茎皮提取物、木瓜蛋白酶、芦荟提取物和酸例如乙酸的组合物可另外地或可选地协同地起作用以调节血糖水平或治疗或预防受试者中的糖尿病前期、糖尿病或任何相关的功能障碍。包含海岸松茎皮提取物、木瓜蛋白酶、芦荟提取物、蜂蜜和酸例如乙酸的组合物可另外地或可选地协同地起作用以调节血糖水平或治疗或预防受试者中的糖尿病前期、糖尿病或任何相关的功能障碍。

[0141] 添加剂

[0142] 本发明的组合物可以另外包括任何合适的添加剂。例如，添加剂可以包括防腐剂、调味剂、盐、色素或这些中任何两种或更多种的混合物。

[0143] 合适的防腐剂是本领域已知的，例如苯甲酸、苯甲酸钠、山梨酸钠或山梨酸钾、山梨酸、羟基苯甲酸盐等。因此，本文公开的组合物可以包含一种或多种防腐剂。可以添加防腐剂以在制造和储存过程中抑制组合物中的细菌或其他有害生物体的生长。用于根据本发明的组合物的合适的防腐剂包括苯甲酸钠和山梨酸钾。合适的防腐剂可以以任何合适的量加入到本文公开的组合物中。例如，在组合物被配制为液体例如含水液体的情况下，防腐剂可以以范围为约500μg/mL至约1000μg/mL、或约500μg/mL至约750μg mL、或约650μg/mL至约800μg/mL、或约800μg/mL至约1000μg/mL，例如约500μg/mL、约600μg/mL、约700μg/mL、约800μg/mL、约900μg/mL或约1000μg/mL、或者另外如本领域技术人员可以确定的浓度存在。在一个实施方式中，苯甲酸钠以400μg/mL的浓度存在于含水组合物中，山梨酸钾以400μg/mL的浓度存在于含水组合物中。

[0144] 合适的调味剂在本领域中也是已知的并且是可商业获得，例如，用于根据本发明的组合物的合适的调味剂可以包括SM10483-天然草莓香料(TIF-20550)、SM00401-天然橙油甜Aust 48-7685、SM10458橙西番莲果香料(UA-71735)、或野莓香料UA71225——由Ungerer Australia Pty Limited提供。因此，本文公开的组合物可以包含一种或多种调味剂。调味剂可改善组合物的风味和/或气味，并导致增加的依从性和因此有效性。合适的调味剂可以以任何合适的量加入到本文公开的组合物中。例如，在组合物被配制为液体例如含水液体的情况下，调味剂可以以范围为约5mg/mL至15mg/mL，例如约5mg/mL至约7mg/mL、约7mg/mL至约12mg/mL、或约12mg/mL至约15mg/mL，例如约5mg/mL、约6mg/mL、约7mg/mL、约8mg/mL、约9mg/mL、约10mg/mL、约11mg/mL、约12mg/mL、约13mg/mL、约14mg/mL、约15mg/mL或者另外如本领域技术人员可以确定的浓度存在。在一个实施方式中，调味剂——优选野莓香料UA71225——以10mg/mL的浓度存在于含水组合物中。

[0145] 合适的盐是本领域已知的，例如氯化钠和氯化钾。因此，本文公开的组合物可以包含一种或多种盐。用于根据本发明的组合物中的一种合适的盐是氯化钠。盐可以以任何合适的量加入。例如，在将组合物配制为液体例如含水液体的情况下，盐，优选氯化钠，可以以范围为约1.0mg/mL至约6.0mg/mL，例如以约1.0mg/mL至约3.0mg/mL、或约2.0mg/mL至约5.0mg/mL、或约4.0mg/mL至约6.0mg/mL范围的浓度，例如以1.0mg/mL、1.5mg/mL、2.0mg/mL、2.5mg/mL、3.0mg/mL、3.5mg/mL、4.0mg/mL、4.5mg/mL、5.0mg/mL、5.5mg/mL或6.0mg/mL的浓度存在。在一个实施方式中，氯化钠以3.00mg/mL的浓度存在于含水组合物中。

[0146] 合适的色素是本领域已知的，例如类胡萝卜素、叶绿素和花青素等。因此，本文公开的组合物可以包含一种或多种色素。可以添加色素以增加组合物的视觉吸引力。用于根据本发明的组合物中的合适的色素包括花青素提取物。合适的色素可以以任何合适的量加入到本文公开的组合物中。例如，花青素提取物可以以约500μg/mL和约1000μg/mL之间、或约500μg/mL和约750μg/mL之间、或约650μg/mL和约800μg/mL之间、或约800μg/mL和约1000μg/mL之间，例如约500μg/mL、约600μg/mL、约700μg/mL、约800μg/mL、约900μg/mL或约1000μg/mL、或者如本领域技术人员可以确定的浓度存在于组合物中。在一个实施方式中，色素——优选花青素提取物——以800μg/mL的浓度加入含水组合物中。

[0147] 色素，例如，本文所指“花青素提取物”的量是基于本发明的示例性实施方式，例如表1中的组合物计算的，其中加入到组合物中的花青素提取物包含约25-35％麦芽糊精。应当理解，可以获得含有更多、更少或不含麦芽糊精的替代来源或形式的花青素提取物，在这种情况下，可以调节本发明组合物中使用的花青素提取物的量，以解释(导致，account for)用于制备发明的组合物的花青素提取物的花青素含量的差异。

[0148] 在本文公开的组合物中也可以使用其它合适的添加剂，如增稠剂(例如麦芽糊精)、稀释剂、缓冲剂、另外的治疗剂、生物利用度增强剂、副作用抑制组分、粘合剂等。这些添加剂优选地没有不利地影响组合物的功效或治疗益处。

[0149] 另外的组分

[0150] 除了上述添加剂之外，根据本发明的组合物可以包含一种或多种另外的组分。

[0151] 例如，组合物可以包含抗氧化剂如抗坏血酸(维生素C)。常见的另外的抗氧化剂是维生素A、E和矿物硒或松树皮提取物中发现的称为低聚原花青素、(OPCs/PCOs)、虾青素、类胡萝卜素、辅酶Q10(“CoQ10”)、黄酮类化合物、谷胱甘肽、枸杞、橙皮苷、乳-枸杞(lacto-Wolfberry)、木脂素、叶黄素、番茄红素、多酚、硒、维生素A、维生素C、维生素E、玉米黄质的抗氧化剂组、或其组合。

[0152] 组合物可包含以任何合适形式，例如以矿物质或其他生物可利用的复合物的形式的矿物质——包括硼、钙、氯化物、铬、铜、碘、铁、镁、锰、钼、镍、磷钾、硒、硅、钠、锡、钒、锌或其组合。

[0153] 组合物可以包含选自如下的植物营养素：黄酮类化合物、同类的酚类化合物、多酚化合物、萜类、生物碱类、含硫化合物及其组合。植物营养素可以选自类胡萝卜素、植物甾醇、槲皮素、姜黄素、柠檬苦素和其组合。

[0154] 组合物可以包含已知具有或怀疑具有抗糖尿病作用的草药。这样的草药可以包括药用植物，例如来自锦葵科(Malvaceae)，如黄秋葵(Abelmoschus moschatus)Medik，或来自菊科(Asteraceae)，如Achiliea santolina L.、或Achyrocline satureioides(Lam.)

DC、或龙蒿(Artemisia dracunculus L.)(龙草)或菊苣(Cichorium intybus L.)、或旱莲草(Eclipta alba)(L)Hassk,或来自唇形科(Lamiaceae)如Ajuga iva L.Schreberr(Medit)或罗勒圣地Linn.(Ocimum sanctum Linn.)(Tulasi)/或牛至(Origanum vulgare L.)或来自番荔枝科如番荔枝,或来自Gentianeacea科如Anthocleista djalonensis A.Chev(卷心菜树)、或Anthocleista Schweinfurthii、或Anthocleista vogelii Planch、Enicostemma littorale Blume或Gentiana olivieri L.,或来自酢浆草科(Oxalidaceae)如三敛(Averrhoa bilimbi L)、或感应草(Biophytum sensitivum)(L)DC,或来自豆科如Bauhinia candicans Benth,或来自红木科如红木(Bixa orellana L.),或来自紫茉莉科如印度黄细心(Boerhaavia diffusa L.),或来自十字花科如黑芥(L)Koch、或Eruka sativa、或独行菜(Lepidium sativum L.),或来自云实科(Caesalpiniaceae)如刺山柑(Capparis spinosa L.)或耳叶番泻(Cassia auriculata L.)或酸豆(Tamarindus indica L.),或来自白花菜科家族如刺山柑,或来自伞形科如藏茴香(葛缕子,Carum carvi L),或来自芸香科如Clausena anisata(野生)Benth,或来自棕榈科如椰子Linn.(椰子树),或来自葫芦科,如Cogniauxia podoleana或Ibervillea sonorae S.,或来自Conimelinaceae科如鸭跖草,或来自姜科如姜黄(Curcuma longa L.),或来自禾本科如狗牙根(Cynodon dactylon Pers.)(百慕大草),或来自银杏科如银杏,或来自蝶形花科(Papilionaceae),如甘草(Glycyrrhiza uralensis Fish.),或来自萝摩科如Gongronema latifolium Benth.、或匙羹藤(Gymnema montanum Hook),或来自梧桐科如火索麻(Helicteres isora L.)、As.,或来自茜草科如Hintonia standleyana,或禾本科如大麦,或来自旋花科如蕹菜(Ipomoea aquatic Forsk.).、红薯(Ipomea batata Linn)(甘薯),或来自桑寄生科如Loranthus micranthus Linn),或来自桑科如Morus indica.L.,或来自芭蕉科家族如Musa sapientum Kuntz(香蕉),或来自大戟科如苦味叶下珠(Phyllanthus amarus)Schum.Thonn或珠子草(Phyllanthus niruri)L.、或Phyllanthus sellowianus Mull.Arg.,或来自胡椒科(Piperacea)如荜拔(Piper longum),或来自桃金娘科如番石榴、或Syzygium alternifolium(Wt)Walp,或来自千屈菜科如石榴,或来自蝶形花科(Papilionaceae)如鹰爪豆(Retama raetam)(RR)(Forssk)Webb.,或来自五福花科(Adoxaceae)如西泽接骨木,或来自夹竹桃科如血竭(Sanguis draxonis),或来自漆树科如Sclerocarya birea(A.Rich),或来自Scrophariaceae科如野甘草(Scoparia dulcis L.),或来自石竹科如紫花漆姑草(Spergularia purpurea),或来自藜科如碱蓬紫穗槐(Suaeda fruticosa)(SF)Euras,或来自使君子科如诃子(Terminalia chebula Retz.)、或毗黎勒(Terminalia bellirica)(Gaertn),或来自防己科(Menispermaceae),如心叶青牛胆(Tinospora cordifolia Miers.),或来自荨麻科(Urticaceae)家族如小荨麻(Urtica pilulifera L.),或来自菊科(Astereaceae),如扁桃斑鸠菊(Vernonia amygdalina Del.),或来自茄科(Solanaceae)如催眠睡茄(Withania soimifera(L)Dunal),或来自蒺藜科(Zygophyllaceae)如Zygophyllum gaetulum Emb和Maire。

[0155] 组合物可以包含一种或多种维生素和/或氨基酸。维生素可以选自但不限于:维生素A、$B_1$、$B_2$、$B_3$、$B_5$、$B_6$、$B_9$、$B_{12}$、C、D、E、K、K1和K2(即MK-4、MK-7)、叶酸或生物素。氨基酸可以选自但不限于:丙氨酸、精氨酸、天冬氨酸、胱氨酸、瓜氨酸、甘氨酸、谷氨酸、谷氨酰胺、组氨酸、羟脯氨酸、羟丝氨酸、羟酪氨酸、羟赖氨酸、异亮氨酸、赖氨酸、甲硫氨酸、苯丙氨酸、脯氨

酸、丝氨酸、牛磺酸、苏氨酸、色氨酸酪氨酸、缬氨酸或鸟氨酸。

[0156] 组合物可以包含ω-3或ω-6脂肪酸的来源，例如鱼油、磷虾、ω-3植物来源、亚麻籽、芥花籽油(canola oil)、核桃和藻类。ω-3脂肪酸的实例包括例如ω-亚麻酸(“ALA”)、二十二碳六烯酸(“DHA”)、十八碳四烯酸(SDA)、二十碳五烯酸(“EPA”)或其组合。ω-6脂肪酸的实例包括亚油酸(“LA”)、花生四烯酸(“ARA”)。ω-3脂肪酸可以以每天约0.25g至5.0g，如每天约1.0至3.0g的量提供。

[0157] 组合物可以包含酚类化合物，包括，例如，一元酚(如，例如，芹菜脑、鼠尾草酚、香芹酚、莳萝脑(dillapiole)、rosemarinol)；黄酮类化合物(flavonoids)(多酚)，包括黄酮醇(如，例如，槲皮素、fingerol、山奈酚、杨梅黄酮、芦丁、异鼠李素)、黄烷酮类(如，例如，fesperidin、柚皮素、水飞蓟宾、圣草酚)、黄酮(如例如，芹菜素、柑桔黄酮、木犀草素)、黄烷-3-醇(如，例如，儿茶素、(+)-儿茶素、(+)-表没食子儿茶素、(-)-表儿茶素、(-)-表没食子儿茶素、(-)表没食子儿茶素没食子酸酯(EGCG)、(-)-表儿茶素3-没食子酸酯、茶黄素、茶黄素-3-没食子酸酯、茶黄素-3’-没食子酸酯、茶黄素-3,3’-二没食子酸酯、茶红素)、花青素(黄酮(flavonals))和花青素(anthocyanidin)(如，例如，花葵素、芍药花青素、花青定、翠雀定、锦葵花素、矮牵牛素)、异黄酮(植物雌激素)(如，例如，黄豆苷元(用于mononetin)、染料木黄酮(鹰嘴豆芽素A)、黄豆黄素)、二氢黄酮醇、查耳酮、香豆素类(coumestans)(植物雌激素)和香豆雌酚；酚酸(如：鞣花酸、没食子酸、单宁酸、香兰素、姜黄素)；羟基肉桂酸(如，例如，咖啡酸、绿原酸、肉桂酸、阿魏酸、香豆素)；木脂素(植物雌激素)、水飞蓟素、开环异落叶松脂素(secoisolariciresinol)、松脂醇和落叶松脂素)；酪醇酯(如，例如，酪醇、羟基酪醇、橄榄刺激醛(oleocanthal)、橄榄苦苷)；芪类(stilbenoids)(如，例如，白藜芦醇、紫檀芪、四羟反式芪)和石榴(punicalagins)；萜类(类异戊二烯)，包括类胡萝卜素(四萜类)——包括胡萝卜素(如，例如，β-胡萝卜素、[3-胡萝卜素、γ-胡萝卜素、δ-胡萝卜素、番茄红素、链孢红素(neurosporene)、六氢番茄红素、八氢番茄红素)和黄叶素(如，例如，角黄素、隐黄质、玉米黄质(aeaxanthin)、虾青素、叶黄素、玉红黄质)；单萜类(如，例如，柠檬烯、紫苏子醇)；皂苷类；脂类，包括：植物甾醇类(如，例如，菜油甾醇、β谷甾醇、γ谷甾醇、豆甾醇)、生育酚(维生素E)和ω-3,6和9脂肪酸(如，例如，γ-亚麻酸)；三萜类化合物(如，例如，齐墩果酸、熊果酸、桦木酸、模绕酮酸(moronic acid))；甜菜红碱，包括β花青素(如：甜菜苷、异甜菜苷、probetanin、neobetanin)；和β-叶黄素(非糖苷形式)(如，例如，梨果仙人掌黄素和仙人掌黄素)；有机硫化物，包括，例如，二硫代硫酮(异硫氰酸酯类)(如，例如，萝卜硫素(sulphoraphane))；和硫代磺酸盐(葱属化合物)(如，例如，烯丙基甲基三硫化物和二烯丙基硫化物)、吲哚、硫代葡萄糖盐，包括，例如，吲哚-3-甲醇；萝卜硫素；3,3’-二吲哚基甲烷；黑芥子甙；大蒜素；蒜氨酸；异硫氰酸丙烯酯；胡椒碱；顺式-硫代丙醛(propanethial)-S-氧化物；蛋白质抑制剂，其包括例如蛋白酶抑制剂；其他有机酸，包括草酸、植酸(肌醇六磷酸)；酒石酸；和漆树酸；或其组合。

[0158] 组合物可以包含蛋白质。蛋白质的非限制性实例包括基于乳品的蛋白质、基于植物的蛋白质、基于动物的蛋白质和人造蛋白质。基于乳品的蛋白质可选自酪蛋白、胶束酪蛋白、酪蛋白酸盐、酪蛋白水解产物、乳清、乳清水解产物、乳清浓缩物、乳清分离物、乳蛋白浓缩物、乳蛋白分离物及其组合。基于植物的蛋白质可以包括，例如，大豆蛋白(例如，包括浓缩物和分离物的所有形式)、豌豆蛋白(例如，包括浓缩物和分离物的所有形式)、低芥酸菜

子蛋白(例如,包括浓缩物和分离物的所有形式)、小麦和分级小麦蛋白、玉米及其部分——包括玉米醇溶蛋白、稻米、燕麦、马铃薯、花生、以及来源于豆、荞麦、扁豆、豆类、单细胞蛋白质的任何蛋白质、或其组合。基于动物的蛋白质可以选自牛肉、家禽、鱼肉、羔羊肉、海鲜或其组合。

[0159] 组合物可以包含植物化学物质来源,例如,黄酮类化合物、同类的酚类化合物、多酚化合物、萜类化合物例如类胡萝卜素和植物甾醇和生物碱类和含硫化合物。

[0160] 组合物可以包含碳水化合物来源。可以使用任何合适的碳水化合物包括但不限于蔗糖、乳糖、葡萄糖、果糖、玉米糖浆固体、麦芽糊精、改性淀粉、直链淀粉、木薯淀粉、玉米淀粉或其组合。

[0161] 组合物可以包含谷物。谷物可以包括,例如,可以从不同来源获得的全谷物。不同的来源可以包括粗面粉、松果、粗磨粉、面粉和微粉化的谷物(微粉化的面粉),并且可以来源于谷类或假谷类。谷物可以是水解的全谷物组分,即酶促消化的全谷物组分或通过使用至少α-淀粉酶消化的全谷物组分,该α-淀粉酶在处于活性状态时对膳食纤维不显示水解活性。水解的全谷物组分可以通过使用蛋白酶进一步消化,该蛋白酶在对处于活性状态时对膳食纤维不显示水解活性。水解的全谷物组分可以以液体、浓缩物、粉末、汁、浓汤或其组合的形式提供。

[0162] 组合物可以包含脂肪来源。脂肪来源可以包括任何合适的脂肪或脂肪混合物。例如,脂肪来源可以包括但不限于植物脂肪(如橄榄油、玉米油、向日葵油、高油酸向日葵、亚麻子油、菜籽油、芥花籽油、高油酸芥花籽油、榛子油、大豆油、棕榈油、椰子油、黑醋栗籽油、琉璃苣油、卵磷脂等)、动物脂肪(如乳脂)、或其组合。脂肪来源也可以是上面列出的脂肪的较不精制的形式(例如,用于多酚含量的橄榄油)。

[0163] 在组合物被配制为口服补充剂的情况下,除了含有10克或更多克优质蛋白质的合适的营养概况之外,其可以含有海岸松、芦荟和木瓜蛋白酶,所述优质蛋白质可以作为乳清蛋白胶束、具有EPA和DHA的脂质、和用于能量和适口性的碳水化合物而被提供。维生素如维生素D和矿物质和成分如乳枸杞也可以包括在内。

[0164] 组合物可以包含益生菌菌株。

[0165] 组合物可以包含全部三种支链氨基酸:亮氨酸、异亮氨酸和缬氨酸。

[0166] 组合物形式

[0167] 根据本发明的组合物可以被配制用于以任何合适的方式的施用。例如,所述组合物可以被配制用于口服给药,例如作为即饮(read-to-drink)液体混合物、溶液、悬浮液、乳剂、糖浆或在诸如软饮料、汁、运动饮料、乳饮料、酸奶饮料、汤等的液体食物中。组合物可以是含水组合物。或者,组合物可以被配制用于以片剂、胶囊、咀嚼片或软凝胶胶囊形式口服给药。

[0168] 在一个实施方式中,本文公开的组合物是含水组合物,其包含:2.40mg/mL木瓜蛋白酶;2.60mg/mL干燥的海岸松茎皮提取物;1.75mg/mL干燥的芦荟内叶汁;100.00mg/mL蜂蜜;2.00mg/mL冰醋酸;3.00mg/mL氯化钠;400μg/mL山梨酸钾;400μg/mL苯甲酸钠;10.00mg/mL的野莓香料UA71225;和800μg/mL花青素提取物。

[0169] 在另一个实施方式中,本文公开的组合物是包含以按重量计木瓜蛋白酶:干燥的海岸松茎皮提取物:干燥的芦荟内叶汁:蜂蜜:冰醋酸:氯化钠:山梨酸钾:苯甲酸钠:野莓香

料UA71225:花青素提取物的比为2.40:2.60:1.75:100:2.00:3.00:0.40:0.40:10.00:0.80的木瓜蛋白酶、干燥的海岸松茎皮提取物、干燥的芦荟内叶汁、蜂蜜、冰醋酸、氯化钠、山梨酸钾、苯甲酸钠、野莓香料UA71225和花青素提取物的含水组合物。因此,考虑了包含以相同的相对比例但以不同的总体积的相同成分的组合物,例如具有相同的相对比例的成分的浓缩或稀释的含水组合物。

[0170] 本文公开的组合物,特别是本文公开的含水组合物可以具有约3.2至约4.2之间的pH,例如约3.2、约3.5、约3.7、约3.9或约4.1的pH,或者可被调节以便具有约3.2至约4.2之间的pH、例如约3.2、约3.5、约3.7、约3.9或约4.1的pH。在一个实施方式中,本文公开的组合物,特别是本文公开的含水组合物具有约3.7的pH。

[0171] 本发明考虑的另一种可选的制剂是作为粉末或颗粒,或者作为干的或液体浓缩物以干燥形式的组合物提供,其然后可以用水或其它液体如牛奶或果汁稀释,以产生即饮组合物。粉末或颗粒可以提供在任选可溶的小袋中。可选地,组合物可以在没有饮料或另外的食物产品的情况下作为粉末消耗。组合物可以组装和包装为在使用时用于溶解的粉末。

[0172] 在组合物以干燥形式例如以粉末或颗粒形式提供的情况下,组合物可包含治疗有效量的海岸松茎皮提取物——对应于约60mg至约1500mg的每日成年人剂量的海岸松茎皮提取物;治疗有效量的木瓜蛋白酶——对应于约30mg至约1200mg的每日成人剂量的木瓜蛋白酶;和治疗有效量的芦荟提取物——对应于约15mg至约600mg的每日成人剂量的芦荟提取物。在一个实施方式中,干燥形式的本文公开的组合物包含对应于约260mg的每日成年人剂量的治疗有效量的海岸松茎皮提取物;对应于约240mg的每日成人剂量的治疗有效量的木瓜蛋白酶;和对应于约175mg的每日成人剂量的治疗有效量的芦荟提取物。

[0173] 本文所述的组合物可以添加到食品或饮料,如基于水的饮料、基于乳的饮料、基于酸奶的饮料、基于乳品的饮料、基于乳代用品的饮料如豆乳或燕麦乳、或基于汁的饮料、水、软饮料、碳酸饮料、或营养饮料(包括饮料的浓缩储备溶液和用于制备这种饮料的干粉)、或在烘焙产品如薄脆饼干、面包、松饼、卷、百吉饼(bagel)、饼干、谷物、诸如燕麦坚果棒(muesli bar)、保健食品棒等的棒、敷料、酱、奶蛋糊、酸奶、布丁、预包装的冷冻餐、汤或糖果物品中。

[0174] 根据本发明的组合物可以使用合适的赋形剂、稀释剂和填充剂利用本领域已知的任何合适的方法配制用于口服给药。一般而言,口服组合物通过将组合物的组分与液体载体或细碎的固体载体或两者均匀且紧密地结合在一起,然后如果需要,将产品塑形为期望的组合物来制备。

[0175] 例如,当组合物配制成胶囊剂时,组合物的组分可以与一种或多种药学上可接受的载体如淀粉、乳糖、微晶纤维素和/或二氧化硅一起配制。另外的成分可以包括润滑剂如硬脂酸镁和/或硬脂酸钙。胶囊可以任选地例如用膜包衣或肠溶衣包被和/或可以被配制以提供其中组合物的缓慢或受控释放。

[0176] 片剂可以通过压制或模制,任选地与一种或多种另外的成分一起制备。压制片剂可以通过在合适的机器中压制以自由流动形式的组合物中的组分如粉末或颗粒,任选与粘合剂、润滑剂(例如硬脂酸镁或硬脂酸钙)、惰性稀释剂或表面活性剂/分散剂混合来制备。模制片剂可以通过在合适的机器中模制用惰性液体稀释剂润湿的粉状组合物的混合物来制备。片剂可以任选地例如用膜包衣或肠溶衣包被和/或可以被配制以提供其中组合物的

缓慢或受控释放。

[0177] 如果组合物被配制为口服给药，则组合物可以是液体口服补充剂。这样的液体口服补充剂可以包含另外的有益成分，例如，如在本文标题为“另外的组分”的部分中所概述的，其可以考虑到施用的组合物用于保存调节的血糖水平的更有效地应用。

[0178] 治疗方法和用途

[0179] 如本文所述的本发明的组合物适用于治疗和预防与升高的血糖相关或由升高的血糖引起的多种状况。例如，组合物可以适用于治疗和/或预防与过量血糖相关的任何功能障碍，所述功能障碍可以包括，例如，日常生理功能紊乱如增加的口渴和饥饿、尿频、疲劳/嗜睡、视力模糊、想睡、和肌肉无力。相关的功能障碍可以由受试者中的高血糖水平引起，导致受试者中的高血糖水平，以受试者中的高血糖水平为特征，或以其他方式与受试者中的高血糖水平相关，并且可直接或间接地由高血糖水平引起。在某些情况下，相关的功能障碍可以与当记录到高血糖水平时暂时分开。由于高血糖水平可以导致整体生理功能紊乱并且可以诱发或促进慢性疾病，所以将血糖水平降低至正常范围可有利于降低代谢紊乱的风险，并且因此高血糖水平的治疗还可以治疗其慢性疾病或症状。因此，本文还考虑了治疗与升高的血糖水平有关的慢性疾病或其症状的方法。

[0180] 因此，本文提供在需要其的受试者中刺激高血糖水平调节的方法。提供在需要其的受试者中减轻或最小化高血糖水平的不良作用的方法。提供在需要其的受试者中保持血糖水平在正常生理范围内的方法。方法包括向个体施用如本文标题为“组合物”的部分中所描述的组合物。本公开的组合物还可以包括如本文所讨论的其它活性或非活性成分。

[0181] 例如，本发明提供治疗或预防升高的血糖的方法，其包含向需要其的受试者施用有效量的如本文标题为“组合物”的部分中所述的组合物。还提供治疗或预防糖尿病前期的方法，包括向需要其的受试者施用有效量的如本文标题为“组合物”的部分中所述的组合物。

[0182] 进一步提供减轻炎症的方法，包括向需要其的受试者施用有效量的如本文标题为“组合物”的部分中所述的组合物。炎症可以，例如，在运动员中和特别是正在进行剧烈运动/训练或耐力运动的运动员。还提供减少细胞因子诱导的炎症或减少氧化应激反应的方法，包括向需要其的受试者施用有效量的如本文标题为“组合物”的部分中所述的组合物。还提供治疗或预防以升高水平的细胞因子诱导的炎症为特征，或以升高水平的氧化应激为特征的疾病的方法，包括向需要其的受试者施用有效量的如本文标题为“组合物”的部分中所述的组合物。疾病可以是动脉粥样硬化、与2型糖尿病和肥胖相关的肝脏炎症、子宫内膜异位症或骨关节炎。

[0183] 还提供减少选自二甲双胍、磺脲类、氯茴苯酸类、噻唑啉二酮类、DPP-4抑制剂、GLP-1受体激动剂、SGLT2抑制剂和胰岛素疗法的糖尿病药物的一种或多种副作用，特别是不良副作用的方法，包括向需要其的受试者施用有效量的如本文标题为“组合物”的部分中所述的组合物。常见的不良副作用可包括腹部或胃部不适、食欲减退或腹泻。

[0184] 进一步提供治疗或预防与过量血糖相关的功能障碍的方法，包括向需要其的受试者施用有效量的如本文标题为“组合物”的部分中所述的组合物。功能障碍可以是增加的口渴和饥饿、尿频、疲劳/嗜睡、视力模糊、想睡、或肌肉无力、或其组合。

[0185] 本发明的组合物可以根据本文所述的方法单独地或与二甲双胍或一种或多种其

他降血糖药物(一种或多种)组合施用。

[0186] 更进一步,提供改善器官功能的方法,包括向需要其的受试者施用有效量的如本文标题为“组合物”的部分中所述的组合物。术语“改善器官功能”是指将组合物施用给或方法用于受试者持续有效改善功能——如通过与未施用所述组合物或使用所述方法的受试者中的功能进行比较所确定,或通过与施用所述组合物或使用所述方法之前受试者中的功能进行比较所确定——的时期。评估器官功能的任何合适的方法都可以用来确定是否发生改善。在一个实施方式中,器官功能通过组织或流体样品中一种或多种生物标志物的水平(或多个)来评估,例如血液的全身性炎症含量。人体C-反应蛋白(CRP)的血液中的升高水平,特别是轻度至中度升高的水平是体内存在炎症时的指标。

[0187] 如本文所述的本发明的组合物适用于治疗和调节高胆固醇水平。特别地,本发明的组合物可适用于降低需要其的受试者中的血液胆固醇。因此,提供降低血液胆固醇的方法,包括向需要其的受试者施用有效量的如本文标题为“组合物”的部分中所述的组合物。

[0188] 血液胆固醇可以选自低密度脂蛋白(LDL)、高密度脂蛋白(HDL)、甘油三酯和总胆固醇、或其组合。例如,组合物可以用于降低受试者中的低密度脂蛋白(LDL)或“不良”血液胆固醇水平,可另外或可选地用于增加高密度脂蛋白(HDL)或“好的”血液胆固醇水平,或者可用于降低血液甘油三酯水平。组合物可用于降低受试者血液中总胆固醇与HDL的比。

[0189] 术语“增加(increasing)”和相关术语“增加(increase)”、“增加(increased)”以及术语“减少(decreasing)”和相关术语“减少(decrease)”、“减少((decreased)”是指组合物施用给或方法用于受试者持续有效改变所述标志物例如HDL或LDL胆固醇的水平——如通过与未施用所述组合物的受试者中或使用所述方法的受试者中的所述标志物的水平进行比较所确定,或通过与施用所述组合物或使用所述方法之前受试者中的所述标志物的水平进行比较所确定——的时期。可以使用任何合适的评估所述标记物例如胆固醇的水平的方法来确定是否发生增加或减少,并且用于测量血液胆固醇的方法是本领域已知的。

[0190] 如本文所述的本发明的组合物也适用于改善免疫功能,因此,本文也提供通过施用有效量的如本文标题为“组合物”的部分中所述的组合物来改善需要其的受试者中的免疫功能的方法。改善的免疫功能可以使用本领域已知的方法,使用任何合适的免疫功能指示物例如增加的白细胞计数来来测量,其中白细胞是例如嗜中性粒细胞。

[0191] 本文所述的本发明的组合物进一步适用于治疗自身免疫性疾病,因此,本文也提供治疗自身免疫疾病的方法,包括向需要其的受试者施用有效量的如本文标题为“组合物”的部分中所述的组合物。自身免疫性疾病可以是任何自身免疫疾病,例如,它可以选自:狼疮、关节炎、银屑病和I型糖尿病。在自身免疫性疾病是狼疮的情况下,治疗可以包括减少受试者中或受试者上的损伤例如皮下损伤的数量和/或严重性。在自身免疫性疾病是关节炎,特别是类风湿性关节炎的情况下,治疗可以包括减少受试者中的肿胀和疼痛。在自身免疫性疾病是银屑病的情况下,治疗可以包括减少皮肤斑块的数量和/或严重性,减少肿胀或炎症,和/或减少疼痛。在自身免疫疾病是I型糖尿病的情况下,治疗可以使得血糖水平有效控制另外所需的胰岛素的量减少。

[0192] 如本文所述的本发明的组合物也可以用于治疗气肿,并且因此提供治疗气肿的方法,包括向需要其的受试者施用有效量的如本文标题为“组合物”的部分中所述的组合物。

[0193] 如本文所述的组合物可用于增加受试者中的总体幸福,并且因此提供增加总体幸

福的方法，包括向需要其的受试者施用有效量的如本文标题为“组合物”的部分中所述的组合物。增加的总体幸福的测量可以包括以下中的一个或多个：增加的能量水平、更好的睡眠、改善的呼吸能力、改善的体力活动水平和/或改善的身体功能。

[0194] 如本文所述的组合物可具有有效的抗氧化剂活性和抗炎作用并支持健康的免疫系统。例如，它可以具有优异的自由基清除性质并且可以支持免疫系统的健康和功能。这些性质可以引起或有助于本文所述的组合物针对一系列疾病和状况的观察到的治疗效果。

[0195] 本文还考虑如本文标题为“组合物”的部分中所述的组合物用于制备药物的用途，所述药物在受试者中用于：治疗或预防升高的血糖；或预防或延缓2型糖尿病(T2DM)的发病；或治疗或预防糖尿病前期；或减轻炎症；或减少细胞因子诱导的炎症；或减少氧化应激反应；或治疗或预防以升高水平的细胞因子诱导的炎症为特征的疾病；或治疗或预防以升高水平的氧化应激为特征的疾病；或降低选自二甲双胍、磺脲类、氯茴苯酸类、噻唑啉二酮类、DPP-4抑制剂、GLP-1受体激动剂、SGLT2抑制剂和胰岛素疗法的糖尿病药物的一种或多种副作用；或治疗或预防与过量血糖相关的功能障碍；或改善器官功能；或降低血液胆固醇；或改善免疫功能；或治疗自身免疫性疾病，例如狼疮、关节炎、银屑病或I型糖尿病；或治疗气肿；或增加总体幸福，例如增加能量水平、改善活力、改善睡眠、改善呼吸能力、改善体力活动水平、改善心理功能、和/或改善身体功能。

[0196] 在本文所述的方法和用途中，受试者可以是老年人，具有医学诊断或未确诊的糖尿病前期状况，或处于发展糖尿病前期的风险。受试者在服用本文所述的组合物时可以服用二甲双胍或其他降血糖药物，或者在服用组合物之前可以已经用二甲双胍或其它降血糖药物治疗。

[0197] 在本文所述的方法和用途中，术语“治疗(treating)”、“治疗(treatment)”等是指治疗(补救，remedy)或以其它方式阻碍、延缓或逆转疾病或疾病的至少一种症状的进展——包括减轻疾病的严重性——的任何和所有应用。因此，治疗未必意味着受试者被治疗直到从疾病中完全恢复。类似地，术语“预防(preventing)”、“预防(prevention)”等是指防止疾病的建立或以其它方式延迟疾病发作的任何和所有应用。

[0198] 本文所述的组合物可以进一步包含一种或多种如本文标题为“添加剂”和“另外的组分”的部分中分别陈述的添加剂或另外的组分，使得组合物可以提供其他健康益处，例如帮助肌肉蛋白质合成。

[0199] 剂量

[0200] 一般而言，本文所述的组合物可以以作为健康补充剂和/或作为治疗剂有效实现期望健康结果的任何合适的剂量施用。本领域技术人员将会理解，本文公开的组合物的单次或多次施用可以用根据需要取决于情况和待治疗的受试者的状况而确定的剂量水平和给药方案来进行。熟练的收件人可以容易地确定合适的剂量方案。广泛的剂量可以适用。给药方案可以调整以提供最佳的治疗反应。本领域技术人员将会理解，本文公开的组合物的施用的确切的量和速率将取决于许多因素，如正被施用的特定组合物、受试者的年龄、体重、总体幸福、性别和饮食要求、以及与组合物组合或同时使用的任何药物或剂。例如，可以每小时、每天、每周、每月或以其他合适的时间间隔施用几个分剂量，或者剂量可以如情况的紧急所示，按比例减少。基于本文的教导，本领域技术人员将通过常规试验和实验能够逐个确定合适的给药方案。

[0201] 本公开的组合物和方法可以作为用于慢性疾病如糖尿病前期、糖尿病和与其相关的状况的其它疗法或治疗的辅助。因此，本文公开的组合物和方法可与可促进期望治疗结果的其它剂，例如，一种或多种肾脏药物，共同施用，或者可与其它剂，例如，其它血糖调节或糖尿病药物，如胰岛素，连续施用。通过“共同施用”是指通过相同或不同途径在相同制剂或两种不同制剂中同时施用，或者通过相同或不同途径连续施用。通过“连续”施用是指施用剂、组合物或治疗之间的秒、分、小时或天的时间差。连续施用可以以任何顺序。

[0202] 在一个实施方式中，可以将本公开的组合物提供给成年人受试者以递送以下总量的海岸松茎皮提取物(相当于干燥的海岸松茎皮提取物)，每个受试者每天约60mg至约1500mg，例如约60mg/天至约100mg/天、约75mg/天至约125mg/天、约100mg/天至约200mg/天、约150mg/天至约250mg/天、约200mg/天至约300mg/天、或约250mg/天至约350mg/天、或约300mg/天至约400mg/天、或约400mg/天至约600mg/天、或约500mg/天至约700mg/天、或约600mg/天至约800mg/天、或约700mg/天至约900mg/天、或约800mg/天至约1000mg/天、或约900mg/天至约1100mg/天、或约1000mg/天至约1200mg/天、或约1000mg/天至约1500mg/天，如约60mg/天、约100mg/天、约150mg/天、约200mg/天、约220mg/天、约240mg/天、约260mg/天、约280mg/天、约300mg/天、约320mg/天、约340mg/天、约360mg/天、约380mg/天、约400mg/天、约500mg/天、或约600mg/天、或约800mg/天、或约1000mg/天、或约1200mg/天或约1500mg/天。这些总的每日剂量可以以任何合适数量的剂量，例如以单次剂量或以多个剂量(即，二、三、四或更多个剂量)提供。剂量可以包括等量的海岸松茎皮提取物。例如，在一个剂量方案中，受试者可以每天被施用两个剂量的根据本发明的组合物，其中每种组合物包含约100mg至约200mg的海岸松茎皮提取物，例如约100mg约150mg、或约120mg至约175mg、或约150mg至约200mg的海岸松茎皮提取物，例如约100mg、110mg、120mg、130mg、140mg、150mg、160mg、170mg、180mg、190mg或200mg海岸松茎皮提取物。

[0203] 本公开的组合物可以提供给成年人受试者，以便递送每个受试者每天约260mg总量的海岸松皮提取物(相当于干燥的海岸松茎皮提取物)，所述量有效用于治疗或预防升高的血糖，或预防或延迟2型糖尿病(T2DM)的发作，或治疗或预防糖尿病前期；或减轻炎症，或减少细胞因子诱导的炎症；或减少氧化应激反应，或治疗或预防以升高水平的细胞因子诱导的炎症为特征的疾病，或治疗或预防以升高水平的氧化应激为特征的疾病，或降低选自二甲双胍、磺脲类、氯茴苯酸类、噻唑啉二酮类、DPP-4抑制剂、GLP-1受体激动剂、SGLT2抑制剂和胰岛素疗法的糖尿病药物的一种或多种副作用，或治疗或预防与过量血糖相关的功能障碍，或改善器官功能，或改善免疫功能，或治疗自身免疫性疾病，例如狼疮、关节炎、银屑病或I型糖尿病，或治疗气肿；或增加总体幸福，例如增加能量水平、改善活力、改善睡眠、改善呼吸能力、改善体力活动水平、改善心理功能、和/或改善身体功能。

[0204] 取决于待治疗的疾病或状况的严重性，海岸松茎皮提取物的量可以增加至每天超过260mg。一旦疾病或状况已得到有效改善，则为了维持目的，成人受试者可以将剂量减少至每天约100mg。

[0205] 在另一个实施方式中，可以将本公开的组合物提供给成人受试者，以便递送以下总量的木瓜蛋白酶(相当于干木瓜蛋白酶)，每个受试者每天约30mg至约1200mg，例如约30mg/天至约80mg/天、约70mg/天至约120mg/天、约100mg/天至约200mg/天、约150mg/天至约250mg/天、约200mg/天至约300mg/天、或约250mg/天至约350mg/天、或约300mg/天至约

400mg/天、或约400mg/天至约600mg/天、或约600mg/天至约800mg/天、或约800mg/天至约1000mg/天、或约900mg/天至约1200mg/天，如约60mg/天、约100mg/天、约150mg/天、约200mg/天、约220mg/天、约240mg/天、约260mg/天、约280mg/天、约300mg/天、约320mg/天、约340mg/天、约360mg/天、约380mg/天、约400mg/天、约500mg/天、约600mg/天、或约800mg/天、或约1000mg/天或约1200mg/天。这些总的每日剂量可以以任何合适数量的剂量，例如以单次剂量或以多个剂量(即，二、三、四或更多个剂量)提供。剂量可以包含等量的木瓜蛋白酶。

[0206] 在另一个实施方式中，可以将本公开的组合物提供给成人受试者，以便将以下总量的芦荟提取物(相当于干燥的芦荟提取物，特别相当于喷雾干燥的芦荟叶内汁液提取物)，每个受试者每天约15mg至约600mg，例如约15mg/天至约80mg/天、约50mg/天至约100mg/天、约75mg/天至约150mg/天、约100mg/天至约200mg/天、约150mg/天至约300mg/天、或约250mg/天至约350mg/天、或约300mg/天至约400mg/天、或约400mg/天至约600mg/天，如约15mg/天、约30mg/天、约60mg/天、约90mg/天、约120mg/天、约175mg/天、约200mg/天、约250mg/天、约300mg/天、约340mg/天、约380mg/天、约400mg/天、约500mg/天、或约600mg/天。这些总的每日剂量可以以任何合适数量的剂量，例如以单次剂量或以多个剂量(即，二、三、四或更多个剂量)提供。剂量可以包含等量的芦荟提取物。

[0207] 当将根据本发明的组合物被配制为具有约2mg/mL至约10mg/mL海岸松茎皮提取物、约0.5mg/mL至约4mg/mL芦荟提取物和约1mg/mL至约8mg/mL木瓜蛋白酶的含水组合物时，含水组合物的合适的总剂量可以是每天每个成年人受试者约30mL至约150mL，例如每天每个受试者约30mL至约50mL、约50mL至约100mL、或约100mL至约150mL，例如约30mL、40mL、50mL、60mL、70mL、80mL、90mL、100mL、110mL、120mL、130mL、140mL或150mL。在一个实施方式中，每天向成年人受试者给予约30至约100mL或约30mL至约50mL的根据本发明的组合物以维持总体健康和幸福。在一个实施方式中，每天给予成人受试者约50mL至约150mL的根据本发明的组合物以治疗本文所述的状况，例如治疗升高的血糖、糖尿病前期和/或它们的相关状况。在一个实施方式中，每天给予成人受试者50至100mL的根据本发明的组合物以治疗本文所述的状况，例如治疗升高的血糖、糖尿病前期和/或它们的相关状况。如上所述，总剂量可以以一个剂量或以多个(即，二、三、四或更多个)较小剂量施用给成年人受试者。在一个实施方式中，在每天的量以两个剂量施用的情况下，剂量可以在早餐后服用一次和晚餐后服用一次。在一个实施方式中，剂量可以是相等的剂量，例如约50mL的两个相等的剂量。

[0208] 因此，当根据本发明的组合物被配制为具有约2mg/mL至约10mg/mL海岸松茎皮提取物、约0.5mg/mL至约4mg/mL芦荟提取物和约1mg/mL至约8mg/mL木瓜蛋白酶的含水组合物时，合适的剂量可以为约1mL/kg/天至约15mL/kg/天、或约1mL/kg/天至约5mL/kg/天、约3mL/kg/天至约7mL/kg/天、或约10mL/kg/天至约15mL/kg/天，例如约1mL/kg/天、约3mL/kg/天、约5mL/kg/天、约7mL/kg/天、约9mL/kg/天、约11mL/kg/天、约13mL/kg/或约15mL/kg/天。

[0209] 包含海岸松茎皮提取物、芦荟提取物和木瓜蛋白酶的组合物的儿科剂量可以在本文列出的每日成人剂量的约15％至约90％的范围内。

[0210] 制造

[0211] 本文的公开内容进一步提供制造含水组合物的方法，所述方法包括在水中组合海岸松茎皮提取物、木瓜蛋白酶和芦荟提取物。如本文标题为“海岸松茎皮提取物”的部分中所概述的，海岸松茎皮提取物可作为固体或干粉添加到水中。如本文标题为“木瓜蛋白酶”

的部分所概述的，木瓜蛋白酶可作为固体或干粉添加到水中。如本文标题为“芦荟”的部分所概述的，芦荟提取物可作为固体或干粉添加到水中。在一个实施方式中，芦荟提取物是芦荟叶提取物，特别是干的内叶汁。海岸松茎皮提取物、木瓜蛋白酶和芦荟提取物可以在水中一步组合，并通过混合而溶解或悬浮在水中。可以使用任何合适量的水。

[0212] 方法可以进一步包括添加酸。酸可以是如上面标题为“酸”的部分中所述的酸。在一个实施方式中，酸是冰醋酸，并且在加到水中的海岸松茎皮提取物、木瓜蛋白酶和芦荟提取物之前在水中部分稀释。优选在加入了海岸松茎皮提取物、木瓜蛋白酶和芦荟提取物之后，将酸加入到组合物中。

[0213] 方法还可以进一步包括添加蜂蜜。蜂蜜可以是如本文标题为“蜂蜜”的部分中所述。在一个实施方式中，在将蜂蜜加入水和海岸松茎皮提取物、木瓜蛋白酶和芦荟之前将蜂蜜预先加热。在向组合物添加酸的实施方式中，优选在酸已被添加和混合之后，将蜂蜜加入到组合物中。

[0214] 在一个实施方式中，制造本发明的含水组合物的方法包括：

[0215] (a)将氯化钠和防腐剂(山梨酸钾和苯甲酸钠)溶于水中以形成溶液；

[0216] (b)在搅拌下将木瓜蛋白酶、海岸松茎皮提取物、干燥的芦荟汁和色素(花青素提取物)加入到步骤(a)中形成的溶液中以形成混合物；

[0217] (c)将预稀释的冰醋酸加入到步骤(b)中形成的混合物中；和

[0218] (d)将调味剂和预热的蜂蜜加入到步骤(c)中形成的混合物中。

[0219] 本领域的普通技术人员将会理解，在不背离广义描述的本发明的精神或范围的情况下，可以对如在具体实施方式中所公开的本发明进行许多变化和/或修改。因此，本实施方式在所有方面都被认为是说明性的而不是限制性的。

## 实施例

[0220] 现在将参照具体实施例来描述本发明，具体实施例不应被解释为以任何方式进行限制。

[0221] 实施例1：组合物

[0222] 表1中给出了根据本发明的实施方式的组合物。

[0223]

| 表 1 | | |
|---|---|---|
| **组分** | **含量 (/mL)** | **含量 (/100 mL)** |
| 氯化钠 | 3.00 mg | 0.3 g |
| 木瓜蛋白酶 | 2.40 mg | 0.24 g |
| 海岸松茎皮提取物，干燥的(相当于海岸松茎皮干燥的– 1.56 g) | 2.60 mg | 0.26 g |
| 芦荟内叶汁，干燥的(相当于芦荟叶内部新鲜的 – 350 mg) (相当于计算为芦荟苷的羟基蒽衍生物– 1.75 ng) (相当于麦芽糊精– 875 μg) | 1.75 mg | 0.175 g |
| 山梨酸钾 (相当于钾 – 0.104mg) | 400 μg | 40 mg |
| 苯甲酸钠 (相当于钠 – 0.064 mg) | 400 μg | 40 mg |
| 蜂蜜 | 100.00 mg | 10 g |
| 醋酸–冰的 | 2.00 mg | 0.2 g |
| 野梅香料 UA71225 | 10.00 mg | 1 g |
| 花青素提取物(相当于麦芽糊精– 280 μg) | 800 μg | 80 mg |
| 水–纯的 | 912.00 mg | 91.2 g |

[0224] 实施例2:样品合成

[0225] 实施例1中给出的100mL组合物的样品合成如下：

[0226] 将氯化钠(0.3g)、山梨酸钾(40mg)和苯甲酸钠(40mg)溶于水(11.90g)中。在搅拌下向得到的溶液缓慢加入木瓜蛋白酶(0.24g;Papaina(USP),Biocon Limited,班加罗尔,印度)、海岸松茎皮提取物(0.26g;Pycnogenol®,Horphag Research USA Inc.(原花青素65-75％))、芦荟汁干燥的(0.175g;Aloe barbadensis Miller,喷雾干燥的,由Capitol Ingredients提供(新鲜植物:产品180-210:1;40-50％麦芽糊精))和花青素提取物(80mg;花青素提取物粉、Naturex(25-35％麦芽糊精、65-75％花青素提取物(E163)))。向该混合物加入在水(4.76g)中的冰醋酸(0.2g;Bronson&Jacobs Pty Limited,悉尼,澳大利亚)的预稀释溶液。向得到的混合物加入水(69.05g)、野莓香料(1g;野莓香料UA71225,Ungerer Australia Pty Limited)和预热的蜂蜜(10g;英国药典(BP)级蜂蜜,Capilano Honey Limited,澳大利亚),并搅拌所得混合物直至均匀。然后加入另外的水(5.49g)并继续温和搅拌,得到褐色栗色悬浮液。

[0227] 实施例3:实验室研究

[0228] 探索根据实施例1的组合物(称为“实施例1”)对抑制细胞因子激活的人冠状动脉内皮细胞中的炎性和氧化反应的效果的实验室研究的结果。

[0229] 引言

[0230] 慢性炎症和氧化应激是包括主要的心脏疾病、动脉粥样硬化和2型糖尿病在内的

许多生活方式疾病的发病机制的基础。动脉粥样硬化是西方社会死亡率和发病率的主要原因，死亡率超过任何癌症引起的死亡率。2型糖尿病由于生活方式的不平衡而处于流行的比例，对我们的医疗保健系统来说重要的是，2型糖尿病使动脉粥样硬化的风险增加2-4倍，并且是导致肾脏衰竭和随后依赖透析的首要原因。动脉粥样硬化是动脉壁中充满脂质的损害的发展。由于LDL颗粒在血管壁中积累，所以病变发展。在血管壁中，LDL颗粒被氧化并处于其氧化状态，LDL颗粒在组成血管壁的细胞中引发炎症反应。衬于动脉管腔里面的内皮细胞尤其被激活，并且在其激活状态下，细胞表达细胞粘附分子。这些粘附分子结合包括单核细胞在内的白细胞，并且在结合状态下，诱导单核细胞迁移到血管壁中。一旦在血管壁中，单核细胞就分化成巨噬细胞，并且巨噬细胞吞入氧化的LDL颗粒。这些氧化的LDL颗粒引起巨噬细胞凋亡和坏死。巨噬细胞的死亡导致被吞入的脂质散出细胞，并且这与其它破坏的巨噬细胞合并，形成动脉粥样硬化损害特征性的早期脂肪纹。

[0231] 由功能失调的内皮细胞表达的重要细胞粘附分子是VCAM-1、ICAM-1和ELAM。这些是所有细胞类型中炎症反应的公认标志物，但在任何血管疾病的发病机制中都是特别重要的。VCAM-1和ICAM-1受关键炎症介质核因子κB(NFkB)的调节。如果NFkB被激活，那么细胞将增加它们的VCAM-1和ICAM-1的表达。

[0232] 当细胞暴露于炎性细胞因子和活性氧种类(或自由基，ROS)时，NFkB被激活。当产生ROS的酶以高水平表达并且比清除或代谢ROS的酶和蛋白质更活跃时，增加的ROS在组织和细胞中积累。这种不平衡导致细胞氧化应激状态，其激活诸如NFkB的调节剂并引发炎症反应。如果这些反应继续未加抑制，则细胞将进入凋亡。参与内皮细胞中ROS产生(超氧化物)的主要酶是NADPH氧化酶4(NOX4)，而具有抗氧化功能的主要酶是超氧化物歧化酶-1(SOD-1)。

[0233] 在该研究中，据报道，实施例1能够通过与NFkB活化减少有关的机制来减少人冠状动脉内皮细胞(HCAECs)中细胞因子活化的VCAM-1、ICAM-1和ELAM的表达。与炎症标志物的抑制一起，据报道，实施例1减少了细胞中的ROS水平，NOX4表达伴随地减少而SOD-1表达增加。炎症和氧化反应的减少最终导致单核细胞与内皮细胞粘附减少。

[0234] 方法

[0235] 在标准实验室条件下培养人冠状动脉内皮细胞。在用炎性细胞因子TNFα刺激1(mRNA)或3(蛋白质)小时之前，将它们与实施例1(50、25、12.5、6.25μl)预温育3小时。然后测定细胞的细胞粘附分子、NOX-4和SOD-1表达、NFkB活化、超氧化物水平和单核细胞粘附。

[0236] 结果

[0237] 实施例1减少TNFα诱导的VCAM-1、ICAM-1和ELAM表达(见图1)。将HCAEC暴露于体积增加的实施例1持续3小时。3小时后，将HCAEC用炎症损伤TNFα激活1小时。在未暴露于实施例1的HCAEC中，TNFα使VCAM-1表达增加15倍，ICAM-1增加10倍，ELAM增加100倍。实施例1补充能够抑制所有CAM的TNFα诱导的mRNA水平。25uL/mL的实施例1的剂量相当于消耗100mL的实施例1。结果提示实施例1在HCAEC中具有抗炎作用。

[0238] 下一个实验检查了在存在炎性刺激下CAM蛋白水平是否也被实施例1降低。如上所述，将HCAEC暴露于实施例1持续3小时，然后用TNFα激活3小时。发现实施例1显著减少VCAM-1和ICAM-1的蛋白质水平(图2，未测量ELAM)。由于膜蛋白的半衰期，实施例1的有效抑制作用不如对mRNA的大，其周转更快。

[0239] VCAM-1、ICAM-1和HAM的表达由NFκB调节。因此，接下来确定实施例1是否减少NFkB的活化。如图3所示，实施例1减少了NFkB的活化。

[0240] NFkB是一种氧化还原敏感蛋白。增加的ROS水平将因此激活这种调节因子。下一个实验探索了实施例1是否能够减少人冠状动脉内皮细胞中的ROS积累。为此，HCAEC在装载氧化剂敏感的荧光探针2'7'-二氯荧光素(DCFH)之前暴露于实施例1持续3小时。然后将细胞用TNFα活化15min。DCFH可被各种ROS和过氧亚硝酸盐迅速氧化成称为脱氢二氯荧光素的高度荧光化合物，并且确定在TNFα活化后，荧光强度显著增加。这种效果在预先暴露于实施例1的HCAEC中被显著地消除。图4显示实施例1降低HCAEC中细胞因子诱导的ROS水平。

[0241] 考虑到实施例1降低TNFα活化的HCAEC内的ROS水平的发现，接下来研究实施例1是否调节编码产生ROS的酶、NOX4和ROS代谢酶，SOD-1的基因的表达。结果显示，实施例1使NOX4mRNA水平降低了很小但显著的水平。显示实施例1使SOD-1mRNA水平恢复至基线。在图5中，显示实施例1减少HCAEC中的NOX-4和增加SOD-1水平。

[0242] 结论

[0243] 实施例1减少人冠状动脉内皮细胞中细胞因子诱导的炎性和氧化应激反应。该机制涉及通过减少ROS的产生并由此降低响应于炎性刺激的HCAEC中的氧化应激水平来抑制炎症的关键介质NFkB的活化。由于2型糖尿病的发作是由炎症和氧化应激驱动的，葡萄糖、甘油三酯、胆固醇和其他与糖尿病相关的有毒物质的血液水平升高导致肝脏中甘油三酯/胆固醇的沉积，脂肪组织沉积导致肝脏炎症，炎症可驱动胰岛素抵抗的发生，如果未经检查，导致2型糖尿病。更一般地，炎症和氧化应激是许多慢性疾病的基础，所述慢性疾病包括动脉粥样硬化、与2型糖尿病和肥胖相关的肝脏炎症、子宫内膜异位和骨关节炎。实施例1的实验室结果提示，该补充剂可以减少与这些慢性疾病相关的症状和原因。

[0244] 实施例4-病例研究1

[0245] 被诊断为升高的血糖水平的50岁中后期的两名超重中年受试者被施用根据实施例1的组合物的液体制剂。第一个人，58岁，超重，被诊断为具有高血糖水平的2型糖尿病患者。在持续6个月的100mL/天的剂量下一个月后，该受试者表现出改善的(降低的)血糖水平。第二人，55岁左右，也超重，高血糖，在100mL/天的剂量下一个月后，看到血糖降低到正常范围，和改善的运动性/身体功能。

[0246] 实施例5-病例研究2:患者'AF'

[0247] 在2012年2月开始的两年期间内，给予糖尿病前期/边界女性糖尿病患者每日单次剂量(50mL)的实施例1组合物，同时保持一致的饮食和锻炼方案。表2给出了获得的血糖结果(以空腹血糖水平测量)。

[0248]

**表 2**

| **日期** | **测试结果 (mmol/L)** |
|---|---|
| 2011 年 9 月 12 日<br>(在组合物施用之前) | 5.8 |
| 2012 年 4 月 21 日 | 5.5 |
| 2013 年 10 月 26 日 | 5.2 |

[0249] 患者于2012年2月至2014年8月之间每天服用上述单次剂量，但从2014年8月至2014年11月，未服用任何组合物。这个时间之后的空腹血糖水平在表3中示出。

[0250]

| 表 3 | |
|---|---|
| **日期** | **测试结果 (mmol/L)** |
| 2014 年 11 月 17 日 | 6.2 |

[0251] 这些结果表明当使用实施例1中的组合物时血糖水平普遍下降，而不使用时血糖水平升高。

[0252] 患者AF也报告超重，她的腿、膝盖、腕和拇指患有严重的关节炎，并且她患有炎症，以及由于在摩托车事故中遭受的损伤而具有腿部重度疼痛和肿胀。患者AF也报告她的左腿反复蜂窝织炎。

[0253] 在早晨服用实施例1的组合物约50-80mL每天，持续大约三年的时间后，据报告，患者AF能够行走，全职工作并且导致相对正常的生活方式。患者AF还报告了蜂窝织炎、疼痛和肿胀由实施例1的组合物控制(kept at bay)，自从服用该组合物后没有蜂窝织炎复发。患者AF还报告，由于服用实施例1的组合物，她的血压已保持在正常范围内。

[0254] 实施例6-病例研究3：患者‘FC’

[0255] 对诊断有胃癌的34岁超重男性给予每日剂量为100mL的实施例1的组合物持续2个月的时期。在第一剂量的组合物之前大约4个月和在2个月时最后一次剂量之后不久收集血样。表4给出了测试这两个样品获得的空腹血液胆固醇结果。

[0256]

| 表 4 |
|---|

[0257]

| 血液组分 | 服用实施例 1 组合物之前 4 个月样品中的浓度(mg/dl) | 服用实施例 1 组合物 2 个月之后样品中的浓度(mg/dl) |
|---|---|---|
| 胆固醇 | 275 | 173 |
| 甘油三酯 | 375 | 253 |
| HDL | 55 | 45 |
| LDL | 145 | 77 |

[0258] 如表4所示，在服用实施例1的组合物之后，胆固醇、甘油三酯、HDL和LDL读数全部显著降低。在服用实施例1的组合物之后，患者还报告了他的脚踝肿胀减轻。

[0259] 患者FC也报告普遍感觉不适，具有痛风发作、高的肝脏酶和低的能量水平。在服用实施例1的组合物3个月之后，随着饮食改变，患者FC报告在两个月内其体重减轻约3kg。以前因单独的饮食改变而导致的体重减轻仅在大约四个月的时间内导致4kg的减轻。

[0260] 患者FC还报告，在服用实施例1的组合物之后，深夜他的眼睛中的干发红减少，以

及他的鼻子中的血色消失，并且呈绿色的粘液的厚积聚物消失，指示该区域中更少的干燥。

[0261] 患者FC进一步报告，他的手臂麻木已经消失，与医生的讨论显示可能是他的血液中胆固醇降低的结果。自从服用实施例1的组合物，不可控制的头皮屑和发炎的头皮状况也显著改善。据报道，他的耳垂周围和内侧的皮肤脱落和炎症也消失了。

[0262] 具有少量饮食改变，并且在服用实施例1的组合物3个月之后，患者FC报告他的血液工作显示血液中的脂质平衡、大幅度降低的肝脏酶、以及血糖降低至正常水平。他还报告，整体能量和集中/专注度大幅度改善，特别是当他的关节疼痛消失的时候。

[0263] 实施例7-运动员研究

[0264] 介绍

[0265] 一氧化氮是一种强有力的血管扩张剂(扩张体内的血管)，但它对骨骼肌也有许多其他非常有益的作用。一氧化氮由精氨酸产生，精氨酸是从乳制品、牛肉、猪肉、家禽、明胶、海鲜、麦胚、燕麦、格兰诺拉麦片、花生和其他坚果、种子、鹰嘴豆和大豆的饮食中获得的氨基酸。精氨酸通过称为一氧化氮合酶的酶转化为一氧化氮。在血管中，该酶被称为内皮一氧化氮合酶，缩写为eNOS。

[0266] 如果饮食健康，则饮食中会有充足的精氨酸。酶，eNOS，在体内的量被限制。因此，任何时候都只能产生这么多的一氧化氮，因为该酶处于的限制速度的量。

[0267] 当实施例1的组合物被消耗时，其作用于血管中的内皮细胞以开启eNOS的合成。因为有更多的eNOS，所以可以产生更多的一氧化氮。而且，所述组合物激活eNOS——它使得酶工作得更快，所以一氧化氮生成得更快。这对活动的运动员来说非常重要。

[0268] 一氧化氮具有扩张血管的能力。扩张的血管允许更有效的营养输送到活动的肌肉和其他组织。更有效的血液供应也将允许更高水平的氧以更快的速度运输到组织，包括我们活动的肌肉。扩张的血管还包括静脉系统，其允许更快地从身体清楚有毒废物产物。

[0269] 一氧化氮在运动过程中是重要的，因为它能够提高细胞中线粒体，包括骨骼肌细胞中的线粒体的功能。线粒体是细胞中的能量产生单位。进入线粒体的底物是简单的糖如葡萄糖，并且这些被转化成能量单位ATP。一氧化氮提高了葡萄糖转化为ATP的速度，并且它促进葡萄糖进入细胞。葡萄糖可以被细胞摄取得越快，它就可以越快地转换成ATP。NO是肌肉生长和修复的天然兴奋剂。这将有利于活动的运动员增加力量和耐力。

[0270] 炎症是耐力锻炼的自然结果。促炎细胞因子如IL-1β、TNF-α、IFN-γ的产生在强烈和延长时间的运动期间增加(MacKinnon，1999)。炎性细胞因子会造成骨骼肌内的损伤(Malm，2001)。已经证明，如果在铁人比赛后30分钟取血样，则TNF-α水平升高3.5倍，IFN-γ水平升高16.5倍，IL-1β水平升高7倍，并且所有这些细胞因子在接下来的48小时持续高，损害肌肉纤维。

[0271] 促炎细胞因子增加应激激素，儿茶酚胺和皮质类固醇。这由耐力运动引起的皮质醇水平急剧上升而最好地证明。在包括超马拉松和铁人比赛在内的耐力运动期间，急性期炎症标志物也上升。C反应蛋白(CRP)是这些急性期炎症标志物之一。

[0272] 在耐力运动期间，在协调葡萄糖稳态(例如糖原再合成)和抗炎反应中涉及的细胞因子IL-6增加。其在铁人比赛之后的24小时——那时水平是高的——已被测量，并且在铁人比赛之后5天这些仍然高。

[0273] 炎性细胞因子的增加部分是由于在耐力运动期间和之后对骨骼肌造成的创伤。创

伤的主要原因是偏心(eccentric)肌肉收缩,如在长距离马拉松中,(b)四肢对地面的冲击和(c)相同运动的重复次数。对肌肉的损害可导致耐力运动后24-48小时发生的延迟性肌肉酸痛(DOMS)现象,其伴随着持续长时间的肌肉力量丧失、减少的运动范围和血液中高水平的肌酸激酶活性。肌肉肿胀和DOMS与由产生炎性细胞因子以及前列腺素E2(PGE2)——这是运动时间之后24-48小时疼痛的关键介质——的浸润中性粒细胞和巨噬细胞介导的肌肉炎症是一致的。骨骼肌内的炎症活动也可以由局部内皮细胞和平滑肌细胞驱动。细胞因子炎症应答在恢复后作为低度全身性炎症应答持续至少5天。对于那些再次开始训练的人来说,即使在铁人竞赛之前的训练水平的第1/5,低度全身性炎症应答也可以持续更长时间。

[0274] 促炎细胞因子的初始增加随后是抗炎细胞因子水平的升高。这些抗炎细胞因子如IL-10将起到抑制(缓冲,dampen)促炎反应的作用并防止炎症的过度反应(超调,overshooting)。训练有素的运动员能够通过提高他们的IL-10水平来平衡急性运动诱发的炎症。增加的IL-10水平与更好的表现相关联。

[0275] 由于自我报告的疲劳症状而退出铁人竞赛的受试者比完成竞赛的那些运动员具有更高的多的赛前CRP和IL-6水平。退出竞赛的运动员中的水平比完成竞赛的那些运动员的平均赛前浓度高316%至1442%。过度训练/持续长时间的训练可导致持续长时间的炎症状况并导致表现欠佳表现综合症(underperformance syndrome)。

[0276] 结果

[0277] 实验室研究已经显示,实施例1的组合物可以降低炎症标志物,急性期反应物CRP的血浆浓度(参见图6-深灰色表示跑步者,浅灰色表示骑车者)。要求运动员在两个不同的场合,相隔3周,完成长距离跑(15-20km)或长距离骑行(90km+)。运动前和运动后15分钟内进行血液采样。在一种场合下,在训练跑或骑行之前摄入实施例1的组合物。在另一种场合下,摄入安慰剂补充物。运动员不知道每次他们摄入的补充剂。

[0278] ELISA试验显示,在实施例1的组合物之后,训练后在运动员的血液中CRP水平显著减少,而安慰剂补充物没有效果(运动后CRP水平轻微增加)。CRP减少反映耐力运动训练引起的全身炎症减少。反过来,这可以减少由于炎症的肌肉损伤。

[0279] 接下来测试实施例1的组合物是否在人类细胞中具有直接的抗炎作用。为了测试该人体冠状动脉内皮细胞,将衬于将血液供给身体组织的动脉里面的细胞暴露于以相当于50-、25-、12.5-、和6.25-mL剂量的实施例1的组合物的浓度的实施例1的组合物。显示实施例1的组合物能够抑制人冠状动脉内皮细胞中由促炎细胞因子TNFα引起的炎症,如通过测量称为VCAM-1的细胞中的炎症标志物所评估的(参见图7)。

[0280] 在上述实验中,将实施例1的组合物直接加入到来自瓶子的细胞中。然而,在身体中,实施例1的组合物被消化,并且组分被吸收到血流中,在那里它们通过肝脏并经历II型代谢。因此,实施例1的组合物中的原始分子可以被修饰,并且这些修饰的组分会引起体内的细胞响应。

[0281] 为了测试实施例1的组合物的修饰组分在血流内作为抗炎分子是否有效,要求受试者吃完100mL实施例1的组合物。在组合物消耗之前和消耗之后3小时立即完成血液采样。从收集的血液中分离血浆。然后将血浆用于处理人冠状动脉内皮细胞。用血浆处理细胞24小时,然后将细胞暴露于炎性刺激物,促炎细胞因子TNF-α。然后我们通过监测VCAM-1水平来测量炎症的程度。结果在图8中给出。

[0282] 实施例8-IIa期临床研究

[0283] IIa期安慰剂控制的随机临床研究已经用大约150位被诊断为糖尿病前期的参与者开始。临床研究正在给参与者补充根据实施例1的组合物[有或没有二甲双胍]或安慰剂持续12周以改善血糖水平,如根据以下结果通过血糖水平的改善所证明的:

[0284] 第一:

[0285] -空腹血糖受损(IFG)[通过空腹血糖水平测量]

[0286] 第二:

[0287] -葡萄糖耐量降低(IGT)[通过口服葡萄糖耐量试验(OGTT)测量]

[0288] -hs-CRP

[0289] -HbA1c水平

[0290] -安全性(ELFT、尿素、FBC)

[0291] -HDL,LDL,TGs,脂质特征

[0292] -生活质量(SF-12v2调查表)

[0293] 第三:

[0294] -BP

[0295] -腰:臀比,

[0296] -重量

[0297] -饮食习惯(3天饮食回忆)

[0298] -体力活动(国际体力活动调查表-IPAQ)

[0299] 纳入标准:

[0300] 参加者在进入研究时≥18岁;男性和女性,具有空腹血糖受损

[0301] • 最初的在线/电话评估将包括澳大利亚2型糖尿病风险评估工具(AUSDRISK)调查表。潜在的参与者必须具有高于6的分数以在符合纳入标准的下一个评估阶段取得资格。

[0302] • 参与者必须显示IFG——定义为空腹血糖6.1-6.9mmol/L

[0303] • BMI≥25

[0304] • 腰围测量:男子≥102cm;女性≥88cm

[0305] • 了解知情同意过程并给予实验治疗知情同意的认知能力;

[0306] • 参与者同意在多个场合经历静脉穿刺

[0307] • 参与者同意遵守研究协议,包括在12周研究期间里不改变饮食或运动模式。

[0308] 排除标准:

[0309] 任何临床相关的异常发现,这在研究人员/临床医师的意见中可因参与临床试验而使参与者面临不良事件的风险,包括:

[0310] 1)体检;临床化学;血液学;尿液分析;生命体征

[0311] 2)II型糖尿病的诊断

[0312] 3)服用诸如奥利司他的抗肥胖药物

[0313] 4)口服降血糖药物如磺脲类、双胍类、α-葡萄糖苷酶抑制剂、噻唑啉二酮类

[0314] 5)在开始研究之前服用任何膳食补充剂,如草药、多种维生素或矿物质、益生菌、鱼油等。然而,如果由他们的医生开处方,则他们不得停止服用任何形式的膳食补充剂。然而,这将使他们没有资格参加研究。

[0315] 6)对治疗中的成分和安慰剂补充剂——包括海岸松或其提取物、木瓜蛋白酶(番木瓜)、芦荟、蜂蜜、山梨酸钾和苯甲酸钠——的任何过敏或过去的反应

[0316] 7.酒精滥用;

[0317] 8.使用任何非法药物;

[0318] 9.怀孕或哺乳婴儿;

[0319] 10.开始生活方式干预如饮食变化和增加运动持续时间/强度;

[0320] 11.由历史或检查引起的任何精神障碍,其将阻止完成研究或导致参与者可能的不良事件。

[0321] 一旦已经符合资格标准,则参与者将被随机分为两个研究组,即I组和II组。I组接受安慰剂,II组接受本发明的组合物。该研究设计被选为在门诊中,每个参与者可以被认为是在两个研究组中的对照受试者,从而每个参与者也充当他们自己的对照。

[0322] 实施例9-病例研究4:患者'SG'(1型糖尿病)

[0323] SG是在经历模糊的视力、巨大的口渴和疲劳之后于2015年4月9日诊断为I型糖尿病的23岁体重正常的女性。于2015年4月10日,SG的血糖水平测量为23.8mmol/L。

[0324] SG于2015年4月16日至2015年5月15日早晨施用每天100mL实施例1的组合物。实施例1的组合物的施用在2015年5月15日之后停止几天,在此期间SG的血糖水平开始升高。然后,实施例1的组合物以50mL的每日剂量施用,每天早晨作为单次剂量服用。表5(a)-(k)中示出了SG的血糖图。

[0325] 这些图显示SG的注射的胰岛素水平非常低——这可能是由于实施例1的组合物帮助身体有效使用胰岛素,因此使用更少。SG的专家评论,SG已经在6个月而不是6周之后达到了预期的血糖水平的平衡。服用实施例1的组合物时,SG的血糖水平下降。

[0326] SG现在在胰岛素泵上,并且保持每天50mL的实施例1的组合物——SG相信其帮助她感觉正常,并且正允许她拨入更少的胰岛素,其被推荐用于她的碳水化合物摄入。

[0327] 表5(a)

[0328] 周开始6/4/15(日期)

[0329]

| 胰岛素注射剂 | | | | | | 监测血糖 | | | | | | | | 附注 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 胰岛素类型 | 给予的单位 | | | | 早餐 | | 午餐 | | 晚餐 | | 晚餐前或睡前 | 一晚上 | 活性、疾病、饮食变化、疑病的时间（记录血糖和处理） |
| | | 早餐 | 午餐 | 晚餐 | 睡前 | 之前 | 之后 | 之前 | 之后 | 之前 | 之后 | | | |
| 周一 | | | | | | | | | | | | | | 吃<br>血糖水平 |
| | | | | | | | | | | | | | | |
| 周二 | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| 周三 | | | | | | | | | | | | | | 胰岛素 |
| | | | | | | | | | | | | | | |
| 周四 | N/R | 6 | 6 | 6 | | | | | 23.8<br>17.1 | | 19.8 | | | N/R |
| | Lantus | | | 10 | 6 pm | | | | | | | | | |
| 周五 | N/R | 6 | 8 | 8 | | 12.8 | 15.8 | | 11.2 | | 18.9 | | | 吃 |
| | Lantus | | | 12 | | | | | | | | | | |
| 周六 | | 8 | 8 | 8 | | 10.4 | 14.8 | | 14.4 | | 8.7 | | | 2小时 |
| | | | | 12 | | | | | | | | | | |
| 周日 | | 8 | | | | 7.2 | 7.0 | 11.3 | 10.3 | 16.1 | 11.0 | | | 测试 |
| | | | | | | | | | | | | | | |

[0330] 表5(b)

[0331] 周开始:13/4/15(日期)

[0332]

| | 胰岛素注射剂 | | | | | 监测血糖 | | | | | | | | 附注 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 给予的单位 | | | | 早餐 | | 午餐 | | 晚餐 | | 晚餐前或睡前 | 一晚上 | 活性、疾病、饮食变化、疑病的时间（记录血糖和处理） |
| | 胰岛素类型 | 早餐 | 午餐 | 晚餐 | 睡前 | 之前 | 之后 | 之前 | 之后 | 之前 | 之后 | | | |
| 周一 | N/R | 8 | 8 | 8 | | 7.5 | 9.4 | 6.9 | (9.4) 4.4 | | 6.3 | | | |
| | L | | | 12 | | | | | | | | | | |
| 周二 | N/R | 8 | 6 | 8 | | 7.9 | 5.9 | | 8.5 | | 15.0 | | | |
| | L | | | 12 | | | | | | | | | | |
| 周三 | N/R | 8 | 8 | 8 | | 10.7 | 7.1 | 6.4 | 5.0 | 12.0 | 9.5 | 7.2 | | |
| | L | | | 12 | | | | | | | | | | |
| 周四 | N/R | 8 | 8 | 8 | | 6.5 | 6.7 | | 5.7 | 9.9 | 6.3 | 5.9 | | |
| | L | | | 12 | | | | | | | | | | |
| 周五 | N/R | 8 | 8 | 8 | | 8.0 | 9.3 | | 4.1 | | 9.5 | | | |
| | L | | | 12 | | | | | | | | | | |
| 周六 | N/R | 8 | 7 | 7 | | 6.5 | 3.4 | 5.3 | 4.4 | 9.4 | 2.7 /5.2 | 9.2 | | |
| | L | | | 12 | | | | | | | | | | |
| 周日 | N/R | 7 | 6 | 7 | | 6.6 | 2.9 /5.4 | 7.2 | 10.1 /8.6 | | 5.9 | 8.2 | | 在 2:00 步行 |
| | L | | | 12 | | | | | | | | | | |

[0333] 表5(c)

[0334] 周开始:20/4/15(日期)

[0335]

| | 胰岛素注射剂 | | | | | 监测血糖 | | | | | | | | 附注 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 胰岛素类型 | 给予的单位 | | | | 早餐 | | 午餐 | | 晚餐 | | 晚餐前或睡前 | 一晚上 | 活性、疾病、饮食变化、疑病的时间(记录血糖和处理) |
| | | 早餐 | 午餐 | 晚餐 | 睡前 | 之前 | 之后 | 之前 | 之后 | 之前 | 之后 | | | |
| 周一 | | 7 | 5 | 4 | | 7.2 | 2.7 /6.0 | 6.2 | 6.8 | | 13.9 | | | |
| | | | | 12 | | | | | | | | | | |
| 周二 | | 4 | 5 | 4 | | 5.6 | 5.0 | 5.0 | 11.8 | 8.9 | 6.9 | 7.7 | | |
| | | | | | | | | | | | | | | |
| 周三 | | 4 | 5 | 4 | | 5.5 | 8.4 | 6.8 | 6.2 | | 10.2 | 10.8 | | |
| | | | | | | | | | | | | | | |
| 周四 | | 4 | 5 | 4 | | 7.2 | 4.5 | 8.6 | 3.4 | | 9.3 | 10.2 | | |
| | | | | 12 | | | | | | | | | | |
| 周五 | | 3 | 4 | 4 | | 6.7 | 8.4 | 8.9 | 6.3 | 9.3 | 11.2 | 9.2 | 6.5 | |
| | | | | | | | | | | | | | | |
| 周六 | | 3 | 5 | 4 | | 6.4 | 11.5 | 11.0 | 12.3 | | 7.7 | | 6.4 | |
| | | | | 12 | | | | | | | | | | |
| 周日 | | 4 | 4 | 4 | | 6.5 | 6.0 | 5.0 | 4.9 | 6.7 | 13.1 | | | |
| | | | | 12 | | | | | | | | | | |

[0336] 表

[0337] 5(d)

[0338] 周开始:27/4/15(日期)

[0339]

| | 胰岛素注射剂 | | | | | 监测血糖 | | | | | | | | 附注 活性、疾病、饮食变化、疑病的时间（记录血糖和处理） |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 胰岛素类型 | 给予的单位 | | | | 早餐 | | 午餐 | | 晚餐 | | 晚餐之前或睡前 | 一晚上 | |
| | | 早餐 | 午餐 | 晚餐 | 睡前 | 之前 | 之后 | 之前 | 之后 | 之前 | 之后 | | | |
| 周一 | | 4 | 4 | 4 | | 5.9 | 7.5 | | 3.8 /7.2 | | 5.6 | 12.0 | 9.2 | |
| | | | | 12 | | | | | | | | | | |
| 周二 | | 4 | 4 | 4 | | 6.7 | 5.2 | | 6.2 | 10.1 | 13.8 | 10.8 | | |
| | | | | 12 | | | | | | | | | | |
| 周三 | | 4 | 4 | 4 | | 7.1 | 5.3 | 7.6 | 7.4 | | 8.3 | | | |
| | | | | 12 | | | | | | | | | | |
| 周四 | | 4 | 4 | 4 | | 5.9 | 8.0 | 6.0 | 9.9 | 9.3 | 8.0 | | | |
| | | | | 12 | | | | | | | | | | |
| 周五 | | 4 | 4 | 4 | | 7.3 | 9.3 | 8.9 | 6.7 | 5.8 | 10.1 | | 8.0 | |
| | | | | 12 | | | | | | | | | | |
| 周六 | | 4 | 4 | 5 | | 6.9 | 7.3 | | 10.0 | 9.4 | 9.00 | 10.3 | | |
| | | | | 12 | | | | | | | | | | |
| 周日 | | 4 | 4 | 4 | | 5.3 | 7.0 | | 8.2 | | 7.3 | 10.0 | | |
| | | | | 12 | | | | | | | | | | |

[0340] 表

[0341] 5(e)

[0342] 周开始:5/5/15(日期)

[0343]

| | 胰岛素注射剂 | | | | | 监测血糖 | | | | | | | | 附注<br>活性、疾病、饮食变化、疑病的时间（记录血糖和处理） |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 胰岛素类型 | 给予的单位 | | | | 早餐 | | 午餐 | | 晚餐 | | 晚餐之前或睡前 | 一晚上 | |
| | | 早餐 | 午餐 | 晚餐 | 睡前 | 之前 | 之后 | 之前 | 之后 | 之前 | 之后 | | | |
| 周一 | | 4 | 4 | 5 | | 5.4 | 5.8 | | 6.0 | | 8.3 | 9.7 | | |
| | | | | 12 | | | | | | | | | | |
| 周二 | | 4 | 4 | 4 | | 5.7 | 5.4 | | 4.6 | | 6.2 | 8.9 | | |
| | | | | 12 | | | | | | | | | | |
| 周三 | | 4 | 4 | 4 | | 6.0 | 7.2 | | 5.7 | | 8.3 | 10.7 | | |
| | | | | 12 | | | | | | | | | | |
| 周四 | | 4 | 5 | 4 | | 5.4 | 3.3<br>/11.1 | 11.0 | 3.6<br>/6.0 | | 4.6 | 7.0 | 7.1 | |
| | | | | 12 | | | | | | | | | | |
| 周五 | | 4 | 3 | 3 | | 6.2 | 6.2 | 4.4 | 7.6 | | 10.7 | | 6.1 | |
| | | | | 12 | | | | | | | | | | |
| 周六 | | 3 | 3 | 4 | | 5.9 | 6.2 | | 5.5 | | 9.5 | 9.9 | 6.8 | |
| | | | | 12 | | | | | | | | | | |
| 周日 | | 4 | 4 | 5 | | 6.3 | 8.9 | | 6.4 | | 8.9 | 6.9 | | |
| | | | | 12 | | | | | | | | | | |

[0344] 表

[0345] 5(f)

[0346] 周开始:11/5/15(日期)

[0347]

| | 胰岛素注射剂 | | | | | 监测血糖 | | | | | | | | 附注 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 胰岛素类型 | 给予的单位 | | | | 早餐 | | 午餐 | | 晚餐 | | 晚餐前或睡前 | 一晚上 | 活性、疾病、饮食变化、疑病的时间（记录血糖和处理） |
| | | 早餐 | 午餐 | 晚餐 | 睡前 | 之前 | 之后 | 之前 | 之后 | 之前 | 之后 | | | |
| 周一 | | 4 | 4 | 4 | | 4.8 | 6.8 | | 8.9 | | 7.4 | | | |
| | | | | 12 | | | | | | | | | | |
| 周二 | | 3 | 4 | 4 | | 7.0 | 9.6 | | 8.0 | | 7.3 | | | |
| | | | | 12 | | | | | | | | | | |
| 周三 | | 4 | 3 | 3 | | 6.3 | 10.3 | | 7.5 | | 11.0 | | | |
| | | | | 12 | | | | | | | | | | |
| 周四 | | 4 | 4 | 4 | | 5.4 | 5.7 | | 7.4 | | 10.8 | | | |
| | | | | 12 | | | | | | | | | | |
| 周五 | | 3 | 3 | 5 | | 5.1 | 6.4 | | 7.6 | | 9.7 | | | |
| | | | | 12 | | | | | | | | | | |
| 周六 | | 3 | 4 | 3 | | 4.7 | 4.9 | | 6.8 | | 7.6 | | | |
| | | | | 12 | | | | | | | | | | |
| 周日 | | 3 | 4 | 4 | | 5.3 | 6.5 | | 7.0 | | 9.0 | | | |
| | | | | 12 | | | | | | | | | | |

[0348] 表

[0349] 5(g)

[0350] 周开始:18/5/15(日期)

[0351]

| | 胰岛素注射剂 | | | | | 监测血糖 | | | | | | | | 附注<br>活性、疾病、饮食变化、疑病的时间（记录血糖和处理） |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 胰岛素类型 | 给予的单位 | | | | 早餐 | | 午餐 | | 晚餐 | | 晚餐之前或睡前 | 一晚上 | |
| | | 早餐 | 午餐 | 晚餐 | 睡前 | 之前 | 之后 | 之前 | 之后 | 之前 | 之后 | | | |
| 周一 | | 3 | 3 | 3 | | 5.7 | 5.5 | | 7.2 | | 7.0 | | | |
| | | | | 12 | | | | | | | | | | |
| 周二 | | | | | | 5.4 | | | | | | | | |
| | | | | 12 | | | | | | | | | | |
| 周三 | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| 周四 | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| 周五 | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| 周六 | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| 周日 | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |

[0352] 表

[0353] 5(h)

[0354] 周开始:25/5/15(日期)

[0355]

| 胰岛素注射剂 | | | | | | 监测血糖 | | | | | | | | 附注 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 给予的单位 | | | | 早餐 | | 午餐 | | 晚餐 | | 晚餐前或睡前 | 一晚上 | 活性、疾病、饮食变化、疑病的时间（记录血糖和处理） |
| | 胰岛素类型 | 早餐 | 午餐 | 晚餐 | 睡前 | 之前 | 之后 | 之前 | 之后 | 之前 | 之后 | | | |
| 周一 | | 3 | 3 | 4 | | 6.1 | 7.3 | | 9.0 | | 8.5 | | | |
| | | | | 12 | | | | | | | | | | |
| 周二 | | 3 | 3 | 3 | | 5.9 | 10.6 | | 8.0 | | 12.3 | | | |
| | | | | 12 | | | | | | | | | | |
| 周三 | | 3 | 3 | 3 | | 6.8 | 9.4 | | 7.7 | | 8.3 | | | |
| | | | | 12 | | | | | | | | | | |
| 周四 | | 3 | 3 | 3 | | 5.3 | 7.9 | | 6.5 | | 8.0 | | 3.9 /7.4 | |
| | | | | 12 | | | | | | | | | | |
| 周五 | | 3 | 3 | 3 | | 10.0 | 4.1 | | 8.3 | | 6.7 | | | |
| | | | | 10 | | | | | | | | | | |
| 周六 | | 2 | 3 | 3 | | 7.0 | 8.2 | | 7.0 | | 8.3 | | | |
| | | | | 10 | | | | | | | | | | |
| 周日 | | 2 | 3 | 2 | | 5.8 | 9.5 | 7.4 | 3.0 /7.3 | | 6.9 | | | |
| | | | | 10 | | | | | | | | | | |

[0356] 表

[0357] 5(i)

[0358] 周开始:1/6/15(日期)

[0359]

| | 胰岛素注射剂 | | | | | 监测血糖 | | | | | | | | 附注<br>活性、疾病、饮食变化、疑病的时间（记录血糖和处理） |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 胰岛素类型 | 给予的单位 | | | | 早餐 | | 午餐 | | 晚餐 | | 晚餐之前或睡前 | 一晚上 | |
| | | 早餐 | 午餐 | 晚餐 | 睡前 | 之前 | 之后 | 之前 | 之后 | 之前 | 之后 | | | |
| 周一 | | 2 | 2 | 4 | | 7.3 | 6.3 | | 9.0 | | 7.3 | | | |
| | | | | 10 | | | | | | | | | | |
| 周二 | | 2 | 3 | 3 | | 6.8 | 9.7 | | 9.8 | | 12.0 | | | |
| | | | | 10 | | | | | | | | | | |
| 周三 | | 2 | 3 | 3 | | 6.4 | 10.3 | | 6.3 | | 5.6 | | | |
| | | | | 10 | | | | | | | | | | |
| 周四 | | 2 | 2 | 3 | | 5.9 | 6.3 | 5.0 | 7.5 | | 11.3 | 10.0 | | |
| | | | | 10 | | | | | | | | | | |
| 周五 | | 2 | 2 | 4 | | 7.5 | 8.0 | | 7.7 | | 6.2 | | | |
| | | | | 10 | | | | | | | | | | |
| 周六 | | 2 | 3 | 4 | | 6.5 | 10.4 | | 7.0 | | 10.9 | | | |
| | | | | 10 | | | | | | | | | | |
| 周日 | | 3 | 3 | 3 | | 7.4 | 7.0 | | 10.7 | | 10.6 | | | |
| | | | | 10 | | | | | | | | | | |

[0360] 表

[0361] 5(j)

[0362] 周开始:8/6/15(日期)

[0363]

| | 胰岛素注射剂 | | | | | 监测血糖 | | | | | | | | 附注<br>活性、疾病、饮食变化、疑病的时间（记录血糖和处理） |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 胰岛素类型 | 给予的单位 | | | | 早餐 | | 午餐 | | 晚餐 | | 晚餐之前或睡前 | 一晚上 | |
| | | 早餐 | 午餐 | 晚餐 | 睡前 | 之前 | 之后 | 之前 | 之后 | 之前 | 之后 | | | |
| 周一 | | 2 | 4 | 3 | | 6.7 | 8.3 | | 5.3 | | 11.7 | | | |
| | | | | 10 | | | | | | | | | | |
| 周二 | | 3 | 3 | 3 | | 7.4 | 10.0 | | 9.5 | | 12.8 | 11.9 | | |
| | | | | 10 | | | | | | | | | | |
| 周三 | | 3 | 3 | 3 | | 6.5 | 8.5 | | 11.8 | | 5.7 | | | |
| | | | | 10 | | | | | | | | | | |
| 周四 | | 3 | 3 | 3 | | 6.5 | 6.8 | | 7.5 | | 11.4 | | | |
| | | | | 10 | | | | | | | | | | |
| 周五 | | 3 | 3 | 3 | | 7.7 | 7.0 | | 7.2 | | 8.1 | | | |
| | | | | 10 | | | | | | | | | | |
| 周六 | | 3 | 3 | 3 | | 6.1 | 7.8 | | 9.0 | | 8.4 | | | |
| | | | | 10 | | | | | | | | | | |
| 周日 | | 3 | 3 | 3 | | 7.6 | 11.3 | | 11.1 | | 13.2 | | | |
| | | | | 10 | | | | | | | | | | |

[0364] 表

[0365] 5(k)

[0366] 周开始:15/6/15(日期)

[0367]

| 胰岛素注射剂 | | | | | | 监测血糖 | | | | | | | | 附注 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 胰岛素类型 | 给予的单位 | | | | 早餐 | | 午餐 | | 晚餐 | | 晚餐前或睡前 | 一晚上 | 活性、疾病、饮食变化、疑病的时间（记录血糖和处理） |
| | | 早餐 | 午餐 | 晚餐 | 睡前 | 之前 | 之后 | 之前 | 之后 | 之前 | 之后 | | | |
| 周一 | | 3 | 3 | 4 | | 6.6 | 9.9 | | 9.0 | | 13.9 | | | |
| | | | | 10 | | | | | | | | | | |
| 周二 | | 3 | | | | 5.2 | 10.1 | | | | | | | |
| | | | | | | | | | | | | | | |
| 周三 | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| 周四 | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| 周五 | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| 周六 | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |
| 周日 | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | |

[0368] 实施例10-病例研究5:患者‘A’(高胆固醇)

[0369] 患者A是一名51岁的男性,他具有高胆固醇持续大约20年,并且他身体健康(reasonably fit),不超重。

[0370] 患者A每天服用100mL实施例1的组合物持续3周的时间。在2015年1月14日的血液测试(在服用实施例1的组合物之前)和在2015年6月24日的血液测试(在服用实施例1的组合物3周之后)的结果在表6中给出结果:

[0371]

| 表 6 | | | |
|---|---|---|---|
| *血液组分* | *在 24/06/15 的读数* | *在 14/01/15 的先前的读数* | *单位* |
| HDL | 1.3 | 1.1 | mmol/L |
| LDL | 5.6 | 5.8 | mmol/L |
| 总胆固醇/HDL 比 | 5.8 | 6.9 | |
| 中性粒细胞<br>ANC | 47%<br>2.3 | 41%<br>1.8 | |

[0372] 表6中的结果显示患者A中的HDL(良好的胆固醇)水平已经改善,而LDL(不良胆固醇)水平已经降低,导致患者A全部的总胆固醇/HDL比显著改变/降低。表6还显示白细胞计数(中性粒细胞)的改善。

[0373] 患者A还注意到,在服用实施例1的组合物的同时,他白天睡得更好,并且具有更多的能量。

[0374] 患者A在超过18个月里没有服用Lipitor®或任何其它他汀类药物,因为他发现Lipitor®的副作用是肌肉疼痛、疲倦、关节痛以及总体感觉不好。他的饮食和运动方案在过去的12个月里没有改变。

[0375] 实施例11-病例研究6:患者'PL'(2型糖尿病)

[0376] 患者PL是一名63岁的男性,于2012年3月被诊断患有2型糖尿病。作为针对糖尿病2的他的治疗的一部分,PL自诊断以来一直服用二甲双胍。他具有瘦的体格,不超重。

[0377] PL于2015年5月1日开始服用每日剂量80至100mL的实施例1的组合物,并且同时停止服用二甲双胍。表7给出了PL的血液测试(测量HBA1c)的结果。

[0378]

| 表 7 | | | |
|---|---|---|---|
| **日期** | **测试结果 (HBA1c; %)** | **测试结果 (HBA1c (IFCC); mmol/mol)** | **测试结果 (eAG; mmol/L)** |
| 2012 年 6 月 26 日 | 6.3 H | - | 7.4 |
| 2013 年 3 月 26 日 | 6.5 H | 47 H | 7.8 |
| 2015 年 7 月 1 日 | 6.0 H | 42 H | 7.0 |

[0379] 如表7所示,PL的近期血液测试(2015年7月1日)指示,在服用实施例1的组合物2个月时间之后6.0的HBA1c水平,从先前的测试水平6.5(于2013年3月26日)下降。服用实施例1

的组合物之后，eAG水平也下降。PL已经建议，血糖自我监测已经看到，他的BGL从服用实施例1的组合物之前大约8.0mmol/L的一般水平降低到现在大约6.0mmol/L(即，在服用实施例1的组合物之后)。

[0380] 实施例12-病例研究7：患者‘TG’(狼疮)

[0381] 患者TG是过去18年来患有狼疮的男性。最近，他开始在早晨以每天100mL的剂量服用实施例1的组合物。在大约2-3周之后(已经服用1.5L)，TG的脸和头皮多年来的皮下损伤据报告已经消失，并且没有复发。

[0382] TG还报告由于服用实施例1的组合物引起的持续数个小时的清楚、更好的免疫系统功能和总体感觉更好的事件。

[0383] 实施例13-病例研究8：患者‘MS’(肺气肿)

[0384] 患者MS是被诊断患有肺气肿的男性患者，由于手术切除了其大部分右肺，他在2014年12月之前每天服用100mL实施例1的组合物持续几年时间。

[0385] 患者MS患有严重呼吸短促，发现他的右肺大部分切除后难以爬楼梯。然而，在服用实施例1的组合物持续一个月之后，他报告他更容易呼吸，爬楼梯的困难较小。在服用实施例1的组合物三个月之后，患者MS报告其X射线和MR1结果显示没有肺气肿迹象。他报告说，他的医生对此结果感到惊讶，这种肺病通常不会消失，反而通常会随着时间而变得更坏。

[0386] 患者MS也报告说，自从服用实施例1的组合物，他的整体健康已经是异常的(优异的，exceptional)，并且他多年来没有患过感冒、流感或其他常见病。他还指出，自从服用实施例1的组合物，他的肺容量没有下降。

[0387] 实施例14-病例研究9：患者JW(高胆固醇和高血压)

[0388] 患者JW是一名患有心脏问题、高胆固醇、高血压和呼吸问题的女性患者。在2014年12月之前的大约1年，她每天早上开始服用50mL实施例1的组合物。在2014年12月，她报告说，由于服用实施例1的组合物，她的高胆固醇已经降低，并且她具有增加的能量。

[0389] 实施例15-病例研究10：患者‘S’(银屑病)

[0390] 患者S是患有头皮银屑病的女性患者。在服用实施例1的组合物几天后，患者S报告她的头皮上的银屑病得到改善。

[0391] 实施例16-病例研究11：患者‘M’(银屑病)

[0392] 患者M是患有斑块状银屑病的女性患者。每天每个早晨服用30mL实施例1的组合物约7周之后，患者M报告她的薄片状的皮肤发红减少且更少的斑块表现，特别是在她的膝盖上。

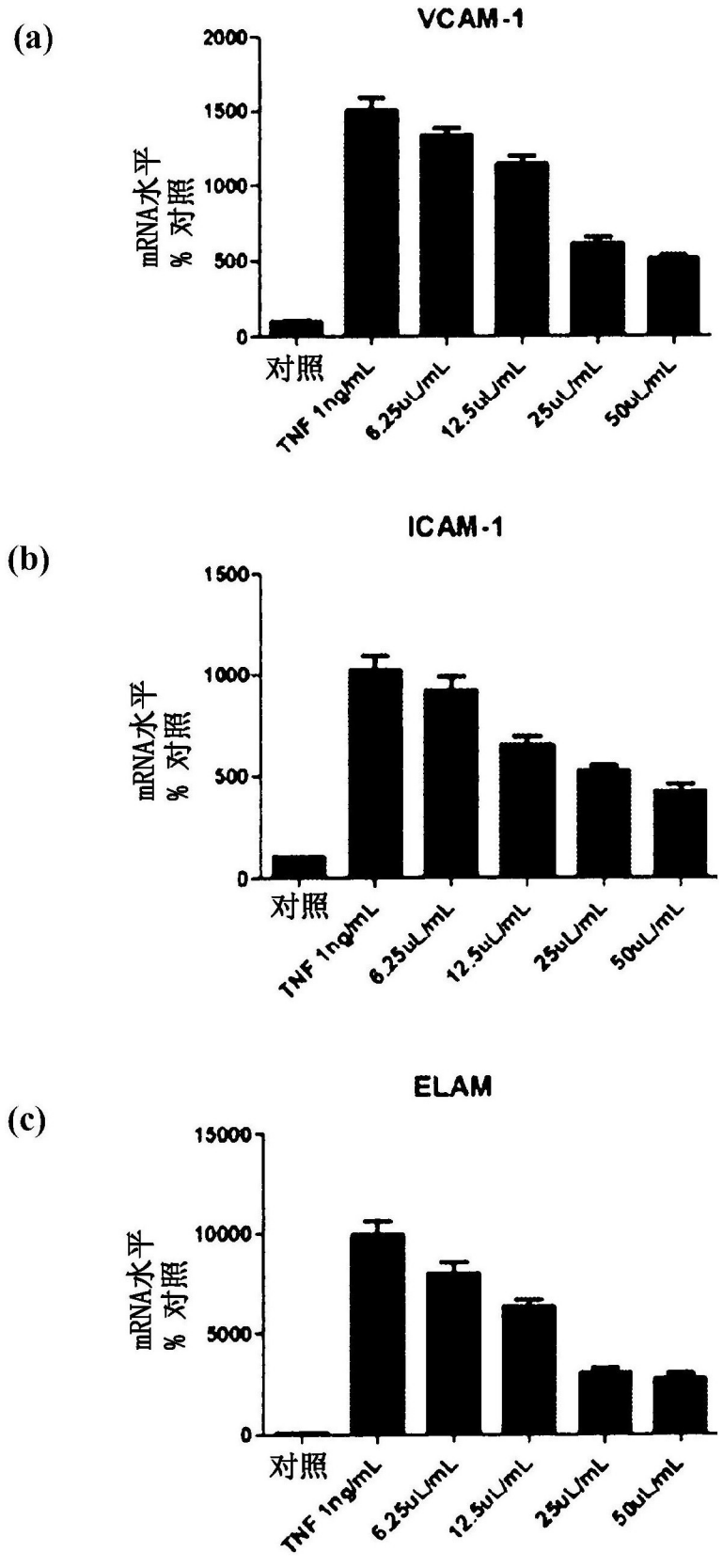
(a)
VCAM-1
mRNA水平
% 对照
2000
1500
1000
500
0
对照
TNF 1ng/mL
6.25uL/mL
12.5uL/mL
25uL/mL
50uL/mL
(b)
ICAM-1
mRNA水平
% 对照
1500
1000
500
0
对照
TNF 1ng/mL
6.25uL/mL
12.5uL/mL
25uL/mL
50uL/mL
(c)
ELAM
mRNA水平
% 对照
15000
10000
5000
0
对照
TNF 1ng/mL
6.25uL/mL
12.5uL/mL
25uL/mL
50uL/mL

图1

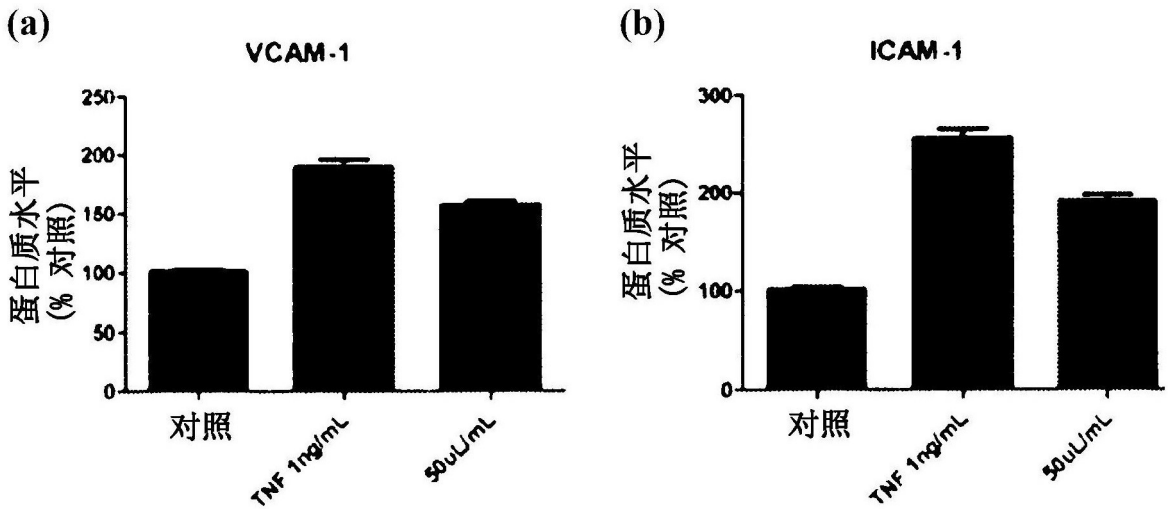
(a)
VCAM-1
蛋白质水平
(% 对照)
250
200
150
100
50
0
对照
TNF 1ng/mL
50uL/mL
(b)
ICAM-1
蛋白质水平
(% 对照)
300
200
100
0
对照
TNF 1ng/mL
50uL/mL

图2

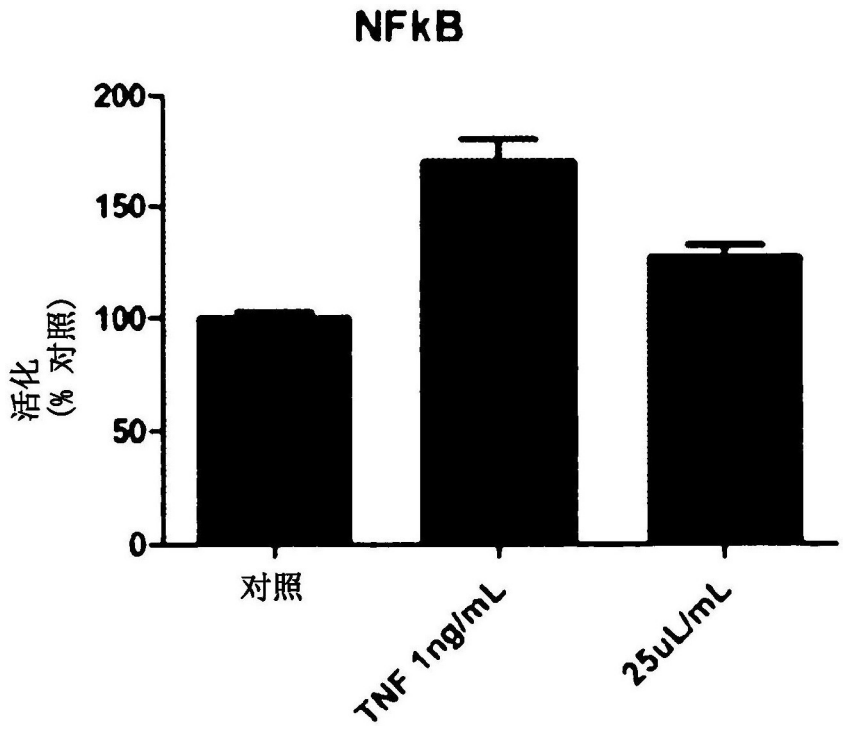
NFkB
活化
(% 对照)
200
150
100
50
0
对照
TNF 1ng/mL
25uL/mL

图3

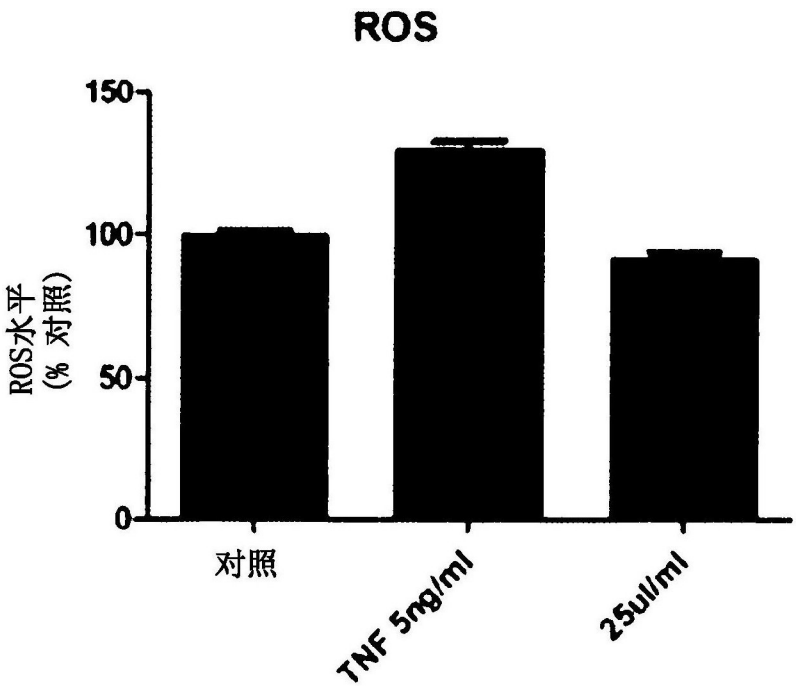
ROS
150
100
50
0
ROS水平
(% 对照)
对照
TNF 5ng/ml
25ul/ml

图4

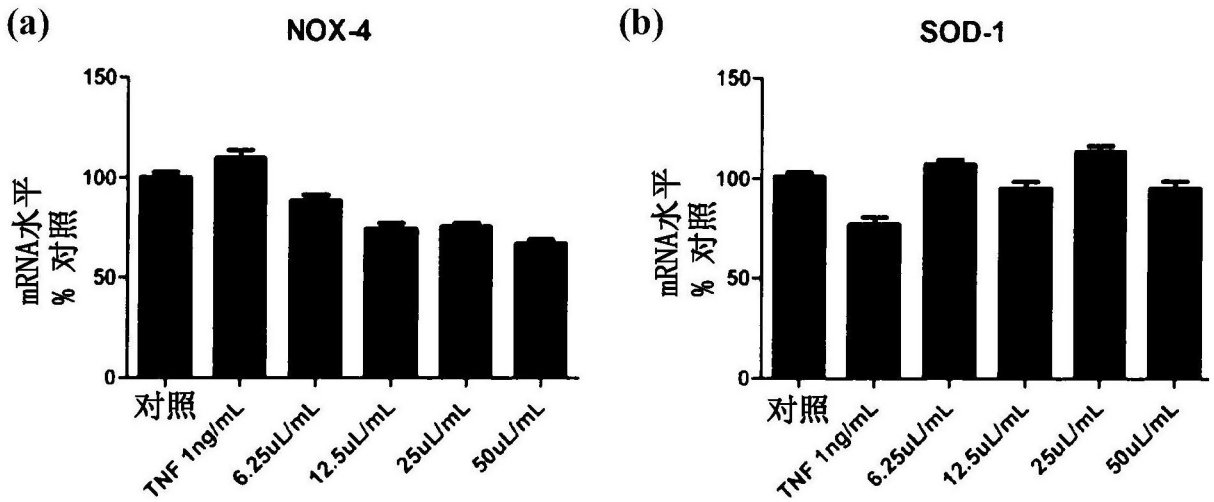
(a)
NOX-4
150
100
50
0
mRNA水平
% 对照
对照
TNF 1ng/mL
6.25uL/mL
12.5uL/mL
25uL/mL
50uL/mL
(b)
SOD-1
150
100
50
0
mRNA水平
% 对照
对照
TNF 1ng/mL
6.25uL/mL
12.5uL/mL
25uL/mL
50uL/mL

图5

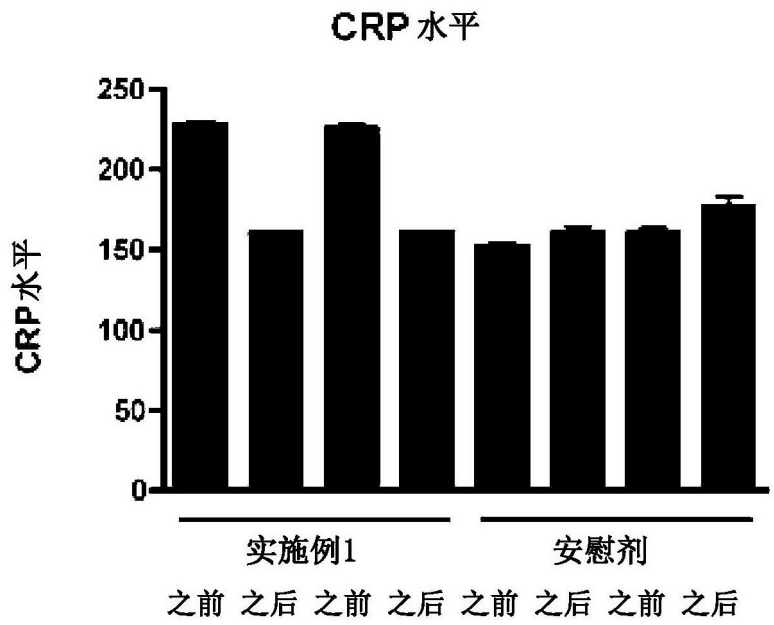
CRP水平
CRP水平
250
200
150
100
50
0
实施例1
安慰剂
之前 之后 之前 之后 之前 之后 之前 之后

图6

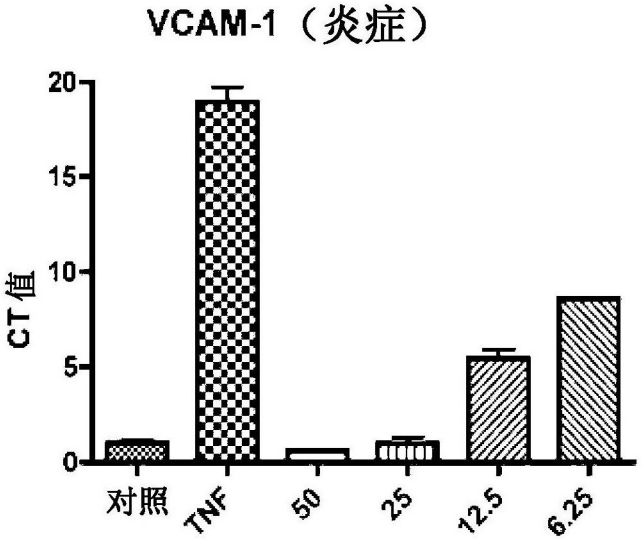
VCAM-1（炎症）
CT值
20
15
10
5
0
对照
TNF
50
25
12.5
6.25

图7

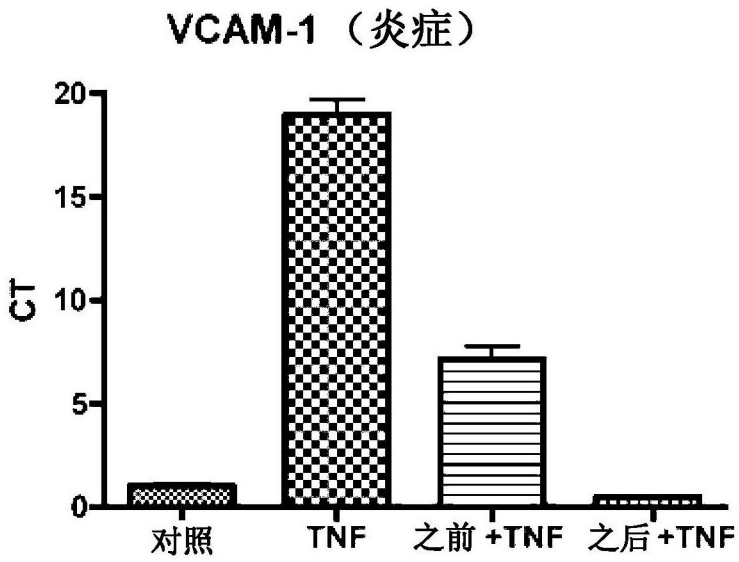


图8