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(54) **ORALLY INGESTIBLE PREPARATION OF MISTLETOE LECTINS AND METHOD**

ORAL EINNEHMBARE ZUBEREITUNG VON MISTELLECTINEN UND VERFAHREN

PREPARATION A BASE DE LECTINES DE GUI INGERABLE PAR VOIE ORALE ET METHODE ASSOCIEE

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- **EIFLER R ET AL: "Improved procedures for isolation of mistletoe lectins and their subunits: Lectin pattern of the european mistletoe" LECTINS: BIOLOGY,BIOCHEMISTRY,CLINICAL BIOCHEMISTRY, vol. 9, 1993, pages 144-151, XP002902377 Published by Wiley Eastern ltd (India)**

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Description

TECHNICAL FIELD

[0001] The present invention relates to medicinally useful preparations derived from mistletoe, methods for making such preparations and treatment methods employing such preparations. More specifically, the invention relates to an orally ingestible preparation of mistletoe lectins useful in the treatment of cancer and other diseases.

BACKGROUND ART

Lectins and immunomodulation

[0002] The cytotoxic cells of the immune system, cytolytic T cells (CTL), natural killer (NK) cells and macrophages, can seek out and ultimately lyse tumor cells either spontaneously or more often after appropriate activation. Spontaneous cytotoxic activity against tumor cells is mainly a result of NK cells. Various cytokines, alone or in combination, have been shown to augment antitumor activity : IL-2, IL-7, IL-12 and IFN-γ induce cytotoxic activity in NK and T-cells while IFN-γ and TNFα are potent activators of macrophages and monocytes. Most of the studies that have demonstrated these effects have been confined to *in vitro* systems although recently the antitumor effect of some of these cytokines has also been demonstrated *in vivo* in animals and also in humans. Lymphocytes cultured in the presence of high amounts of IL-2 are refered to as lymfokine-activated killer (LAK) cells. LAK cells are characterised by their ability to kill NK-resistant tumor cells without major histocompatibility complex (MHC) restriction. Although both NK and T cells are responsible for LAK activity, the former are responsible for mediating most of the activity. Macrophages and monocytes are known to accumulate around tumors. Following the TNFα and IFN-γ stimulated activation of these cells it is predicted that a local release of cytokines would occur from these activated cells directly into the tumor. This in turn would be expected to induce apoptosis and ultimately cause death of the tumor cells.

[0003] Besides cytokines, a variety of natural or synthetically produced protein mixtures have been reported to exert immunomodulating properties. The commercially available mistletoe extracts belong to this category of agents. Biochemical analysis has shown that the immunomodulating capacity is due to the presence of mistletoe lectins (ML-I, ML-II and ML-III) in the extracts.

Mistletoe extracts and use in cancer therapy.

[0004] Mistletoe extracts have been used in cancer therapy for more than 80 years, particularly in clinics in Austria, Switzerland and Germany. Use of these extracts has been heavily criticized by practitioners of traditional "school medicine" due to the lack of knowledge concerning the actual nature of active anti-cancer components in mistletoe. Recent work has now clearly shown that purified lectins (ML-I, ML-II, ML-III) present in mistletoe extracts possess both immunomodulatory and cytotoxic properties. The arguments which have been raised by advocates of "school medicine" are thus to a large extent no longer valid.

[0005] Treatment methods employing known mistletoe extracts have several disadvantages, however. In the first place, commercially available extracts vary greatly in terms of their composition, thus making both study and treatment regimes unreliable. Furthermore, it is generally believed that mistletoe is toxic. The most common method of clinically treating cancer patients with mistletoe extracts, therefore, has been through subcutaneous injection. Recent work, however, has shown that serum glycoproteins effectively bind to and thus will minimise the effects of mistletoe lectins injected into cancer patients. This suggests that the subcutaneous route is probably not very effective. An alternate delivery method is therefore desirable.

Dietary lectins and tumor growth

[0006] As has been shown in a series of publications since 1994 (see references), the growth of intraperitoneal or subcutaneous non-Hodgkin lymphoma tumors in NMRI mice can be reduced by feeding the animals a diet containing the lectin present in raw kidney bean (*Phaseolus vulgaris*), phytohaemagglutinin (PHA) or mistletoe lectin (ML-I). Other observations have shown that the development of a subcutaneous plasmacytoma tumor (MPC-11) in Balb/c mice can be greatly reduced by feeding a diet that included PHA. The lectins, being resistant to the proteolytic effect of gut enzymes, retain their biological activity in the alimentary canal. When added to the diet of experimental animals they bind to the surface of the gut mucosa and induce a fully reversible, dose-dependent hyperplastic growth of the small intestine. Simultaneously with the stimulated gut growth PHA induces an extensive absorption of amino acids and other nutrients from the intestinal lumen. Prior to the onset of hyperplastic growth, an extensive accumulation of extracellular polyamines occurs in the intestinal mucosa. Polyamines are molecules which play major roles in a series of important mechanisms controlling cell proliferation and, importantly, are involved in tumor growth.

[0007] In experiments to study the importance of the timing of feeding mice the PHA-containing diet with respect to when the tumor cells were injected, diets were changed on specific days. It was shown that the number of tumor cells was significantly lower when the lactalbumin-based (La) diet was replaced by one supplemented with PHA on the same day as tumor cells were injected. Recent experiments have shown that when PHA was added to the diet of mice bearing established NHL tumors then further growth was greatly retarded. Preliminary data have shown that PHA fed to rats causes a rapid in-

crease in TNFα production. Accelerated cellular turnover within the transplanted NHL tumor as a response to oral intake of ML-1 was seen as increased numbers of apoptotic cells with an increased area of serpiginous irregular dead cells, and the non-viable cells occupied a two fold increased area in the mice fed the lectin. Apoptoses were more numerous in the tumors of mice fed ML-I and these were identified by nick end-labelling around areas of non-viable tumor cells, at the advancing edge of the tumor and within intense lymphoid aggregates. Morphological studies of tumor sections showed a greatly reduced incidence of tumor vascularisation indicating that ML-1 induces the production of anti-angiogenic factors. Recent experiments performed with purified ML-III have shown that feeding of the lectin to mice bearing established NHL tumors was extremely effective in reducing further tumor growth.

[0008] While it has thus been shown in animal models that the purified mistletoe lectins are well tolerated when administered orally, the purified mistletoe lectins are extremely expensive and time consuming to produce, making them less than ideal candidates as a treatment method. There is a need, therefore, for a preparation of mistletoe lectins and related treatment method that can be administered orally and that is inexpensive to produce.

[0009] Although it is generally believed that mistletoe is toxic, a recent study concerning the outcome of 1754 exposures has shown that accidental ingestion of the plant is not associated with profound toxicity. There are, however, components present in mistletoe extracts that do induce nausea. These nausea-inducing compounds, including alkaloids and viscotoxins, have been mistakenly regarded as essential to the medicinal effectiveness of mistletoe extracts. (See, for example, US patents 5,637,563 and 5,547,674). As a result, no effort has been previously undertaken to produce a mistletoe preparation that can be satisfactorily administered orally to humans.

[0010] WO 97/11967 A discloses an antitumoral and immunostimulating effect of oral applied ML preparations. The document also mentions adverse reactions of parenteral applied ML preparations as well as that there are no documented antitumoral and immunostimulating effects of oral applied ML preparations.

[0011] A procedure for producing purified mistletoe lectins is described by R. Eifler, K. Pfüller, W. Göckeritz and U. Pfüller in "Lectins : Biology, Biochemistry and Clinical Biochemistry" vol. 9 (1993) pp 141-151, which is incorporated by reference herein.

[0012] The applicant has recognized that the complex procedure used by Eifler et al to isolate and purify the three individual mistletoe lectins (ML-I, II and III) is not suitable for producing a therapeutic, orally ingestible lectin preparation, however. The rationale being:

(i) The applicant has shown that ML-1, when added to the diets of mice on the same day as tumor cell injection, reduces the mass of non-Hodgkin lymphoma tumors related to controls. A clear dose-response was observed. At the highest amount of ML-1 ingested a total ablation of tumors was seen in 25% of animals. Furthermore, the applicant has recently shown that when purified ML-III was fed in the diet to mice 5 days after subcutaneous injection of Krebs II cells (non-Hodgkin lymphoma), then the growth of the established tumor was arrested. Since the three lectins have different biological specificities (e.g. sugar binding) then they may well act better in concert than if supplied separately. Purifying the individual lectins is therefore counterproductive from a medicinal point of view.

(ii) the complete purification procedure for the three lectins is considerably time consuming, and

(iii) the lectin preparation according to the invention showed surprisingly, and contrary to the accepted belief in the art, that it could be taken orally. Thus a small contamination by other proteins would not represent any major problem since proteins other than the lectins would be subject to breakdown by digestive enzymes.

[0013] The applicant has therefore modified and improved upon the procedure of Eifler et al. in order to arrive at a mistletoe lectin preparation that contains the lectins ML-I, ML-II and ML-III, possibly together with insignificant amounts of impurities, but which specifically excludes the nausea-inducing compounds otherwise present.

DISCLOSURE OF THE INVENTION

Objects and advantages of the invention

[0014] The obvious advantage of providing mistletoe lectins by the oral route is that large amounts of the lectins, through their binding to the gut mucosa followed by endocytosis, are in due course presented to lymphocytes of Peyers patches and thereby able to induce a major cytokine response. As has been observed, a major reduction in the weight of the spleen occurs following feeding PHA to mice. This can be attributed to a major release of lymphocytes into the blood circulation as a result of cytokine release from lymphocytes of Peyers patches. It is highly unlikely that such a response would be evoked by the small amounts of lectins that are able to reach the lymphatic tissue when mistletoe extracts are injected subcutaneously. Furthermore, seen from the patients point of view, it is obvious that taking the preparation of mistletoe lectins by the oral route is far more acceptable, and convenient, than by injection.

[0015] Based on observations from an animal model system, unpublished work and published data, the following immunomodulating effects are proposed following the oral intake of the mistletoe lectin preparation according to the invention:

1. ML's bind strongly to the gut mucosa.
2. ML's are effectively endocytosed through the mu-

cosa of the small intestine.
3. Binding of endocytosed ML's to lymphocytes of Peyers patches.
4. Stimulation of cytokine release and activation of NK cells etc.
5. Production and release of anti-angiogenic factors.
6. Cytotoxic effects on tumor cells.
7. Reduced tumor vascularisation.
8. Induction of apoptosis leading to tumor cell death.

[0016] In addition to the immunomodulating effects from oral intake, the mistletoe lectin preparation also provides a direct cytotoxic effect on tumors with which it comes into direct contact. The individual mistletoe lectins of the preparation are comprised of two chains, A and B. The B chain binds to receptors on the surface of tumor cells, causing endocytosis of the A chain (internalization of the A chain into the tumor cell). The A chain exhibits N-glycosidase activity which results in specific degradation of ribosomal 28S RNA, further resulting in inhibition of protein synthesis leading to tumor cell death.

Summary of the invention

Preparation of mistletoe lectins and process

[0017] According to the present invention, the nausea-inducing compounds are removed from a mistletoe extract by cationic exchange column chromatography, thus producing a preparation consisting essentially of lectins ML-I, ML-II and ML-III. The preparation may contain insignificant amounts of other, non-nausea inducing components without reducing the biological/medicinal effectiveness of the preparation.

Secondary medical indications and method of use

[0018] According to the present invention, the mistletoe lectin preparation may be administered as a raw chemical composition, or it can be used to produce a nutritional supplement and/or a pharmaceutical preparation that may be administered in therapeutically effective doses for the treatment of cancers as well as autoimmune diseases, such as arthritis, rheumatic diseases, asthma and emphysema, and subjects suffering from general fatigue. Such doses may be in a form suitable for oral, rectal, nasal, topical, vaginal or aerosol administration, or in a form suitable for inhalation or bladder infusion.

Use in conjunction with Arginine

[0019] As discussed in the examples, the applicant has discovered that the effectiveness of the mistletoe lectin preparation is enhanced when the preparation is administered together with a regimen of oral arginine. L-arginine can generally be described as a NO donor. It has been observed that arginine increases endothelium function in tumors, whereby the vessel walls in the tumor tighten up and are not as easily permeable to glucose and proteins. This in turn decreases the nutritional situation for the tumor, which supplements a similar mechanism and effect of the mistletoe lectin preparation. Arginine has also been observed to have a stimulating effect on the immune system, again complimenting a similar effect of the mistletoe lectin preparation.

Brief description of the drawings

[0020]

Fig. 1 is a schematic representation of the method of, producing the mistletoe lectin preparation according to the invention
Fig. 2 is a gel electrophoresis of the mistletoe lectin preparation.
Fig 3. is a gel electrophoresis of blood samples from a patient and a control.

Detailed description of the invention

Preparation and method.

[0021] Referring to Fig. 1, the following method is used to produce the mistletoe lectin preparation:

Source of mistletoe : *Visci Alba Herba* 4mm from Norsk Medisinaldepot (or Viscum album generally).

1. Mistletoe pulverized using a food processor.
2. Material mixed with 0.2M acetic acid (five times amount on a w/w basis). Stirred overnight at 4°C.
3. Rough plant remains removed by filtration through a coarse cloth followed by filtration through a 240 mm paper filter to remove turbidity.
4. Cationic exchange chromatography performed using approximately 5g SP-Sephadex cation exchanger/1.0 litre of crude mistletoe extract.
5. Column washed with 0.15M acetate buffer (pH 4.0) until absorption at 280 nm <0.05. This step removes components which do not have the capability of binding to the cationic exchanger.
6. ML-I, II and III lectins eluted collectively using a buffer containing 0.1M Tris-HCl, pH 8.0, and 0.5M NaCl.
7. Fractions with absorption at 280 nm >1.0 pooled and dialysed overnight (4°C) against 10 volumes of PBS (minus NaCl). The dialysis membrane has a cutoff size of MW 10,000 in order to remove molecules of low MW.
8. The preparation is aliquoted in 1 ml portions and frozen at -20°C for storage purposes.

[0022] The procedure according to the invention differs from that of Eifler et al in several ways:

a) the period of stirring with acetic acid was extended

in order to achieve maximal extraction of lectins.
b) filtration through a filter paper following the intial use of coarse cloth was found important to produce a turbid-free extract.
c) the paper filtered acetic acid extract was pumped on to the column rather than being pre-mixed with the cationic exchanger. This was found to be more convenient, especially when handling large volumes.
d) in order to achieve a concentrated, medicinally effective preparation of mistletoe lectins, only those fractions where absorbance at 280nm was high (>1.0) were chosen to collect and pool.
e) the pooled fractions were dialysed against salt-free buffer in order to remove low.MW components and to reduce the NaCl concentration since the preparation was to be taken orally.

Characterization of the mistletoe lectin preparation.

[0023]

1) The protein content of the final dialysed product, measured according to the method of Bradford, was 250μg/ml.

2) The absorbance of 1 ml of the final dialyzed product at 280 nm was >3.

3) Proteins present in the preparation were monitored by performing SDS polyacrylamide gel electrophoresis, as shown in Fig. 2. The major bands coincided with proteins within the range 29-35 kDa as expected for the subunits of the mistletoe lectins.

4) Biological activity confirmed using a cytotoxic assay where the ability of ML's to prevent growth of freshly seeded CHO cells was tested.

5) The stability of the preparation according to the invention was checked over a five-week period. The preparation proved to be stable at 4 degrees C, room temperature, minus 20 degrees C and minus 80 degrees C. It can be frozen and thawed rapidly at least five times without affecting its biological activity. When heated at 70 degrees C or above for a minimum of five minutes, however, then 100% biological activity is lost. This illustrates that the traditional manner of using boiling water to make mistletoe tea renders the mistletoe lectins biologically useless.

Method of use

[0024] According to one aspect of the invention, therapeutically effective doses of the mistletoe lectin preparation are administered orally to individuals suffering from cancer or other diseases, such as autoimmune diseases in order to induce an immunomodulating response. In a preferred embodiment, the preparation is administered in conjunction with a daily regimen of oral arginine.

[0025] According to another aspect of the invention, the mistletoe lectin preparation is administered in such a way as to provide direct contact between the preparation and tumor cells, in order to induce a direct cytotoxic response.

Determining dosage: Biological response to the preparation in human volunteers: Antibodies to ML's.

[0026] It has been shown that when purified ML-1 was presented orally to rats then antibodies could at later times be detected in the blood (Lavelle et al. Immunology 2000, 99, 30-37), incorporated by reference herein.

[0027] This has now been adapted, according to the present invention, to test human individuals in order to determine whether or not a biological response to mistletoe lectins has been elicited following the oral intake of the enriched mistletoe preparation. Immunoblotting is performed using blood serum as a potential source of antibodies. As can be seen in Fig. 3 the serum from subject A is negative while that of subject B is positive. Subject B had taken the preparation orally for several months while subject A (B's husband) had not. This procedure is thus used to monitor subjects, such that the appropriate dosage of the preparation could be determined i.e. the provision orally of sufficient lectin to promote an antibody response.

[0028] The dosage originally administered was 1 ml taken 3 times per week, on a Monday-Wednesday-Friday regime, for a period of up to six weeks. The dosages are taken on alternating days in order to permit de-saturation of the receptors of the small intestine. It has been observed in the case of tumors of the large intestine, where direct contact between the preparation and the tumor itself is also desirable, that the dosage may need to be increased in order to first saturate the receptors in the small intestine, so that the lectins are able to reach the tumor site.

[0029] The following doses have now shown to provide the best clinical effect:

The preparation according to the invention, provided in 1ml ampules, is mixed (for ease of consumption) in 2 dl water or another cold beverage, and taken three times per week as follows: Mondays 1 ml; Wednesdays 2 ml and Fridays 1 ml. Arginine is taken every day in the mornings and evenings. The appropriate dosage of arginine is dependent on body weight. In the preferred embodiment, a dosage of between 0.001 and 0.01 g of arginine per kg of body weight is used. For ease of consumption, the arginine is mixed with 2 dl water or other beverage.

Examples from human volunteers.

[0030] To date over 150 patients with different types

of cancer have been enrolled in a single-patient survey. No selection criteria have been applied. Patients include those with cancer in the digestive system, nervous system, different forms of gynecological cancer and skin cancer in terminal stages.

[0031] The patients have either been treated by staff of oncological departments without any offer of further treatment, or have been in terminal stages of hospital treatment. All patients have been in a poor state of health. It was desirable to investigate whether or not oral lectins could be offered as a positive form of treatment for this patient group.

[0032] Periodic blood samples have been taken and different forms of examination have been carried out (CT, X-ray, ultrasound)..Therapeutic consultation with the patients providing necessary information has been central in the treatment.

[0033] Summary of experience hitherto with this form of treatment:

A. For about 10% of the patients, decreased tumor size (both primary and secondary tumors) has been observed. Confirmed by CT, X-ray, ultrasound.

B. About 25% of the patients have shown stagnation of tumor growth with a reduction of secondary phenomena such as fluid in the peritoneal cavity, swelling around the tumor etc.

C. Another 30% have shown an arrest of tumor growth with stabilization of their condition.

D. In a further 30% of patients a much slower progression of tumor growth has been experienced than that expected without the treatment.

E. Approximately 5% of the patients have not shown any response.

[0034] In initial trials in human volunteers, several individuals have experienced clear signs of β-endorphin production. This is reflected in that 100% of the patients have reported a feeling of pleasant "warmness" and in general note a better general condition (sense of "ease"). In addition improved digestive function has been reported and a major decrease in pain level has been experienced.

[0035] There has been a clear indication that patients taking the preparation have had a higher level of tolerance during chemotherapy and/or radiotherapy than those not receiving the preparation.

[0036] Hitherto there have been no reports of any form of side-effects.

[0037] The preparation has in addition been used in the treatment of a limited number of patients suffering from auto-immune disease such as rheumatic disorders. The treatment has proved to have a marked effect by reducing joint swelling and decreasing the pain level, with a general improvement in the quality-of-life. The general effect on patients with rheumatic disorders is similar to that experienced with cancer patients who are under treatment.

Individual cases

[0038] The following are several indicative examples of specific observations:

A. One (bedridden) individual suffering from terminal prostate cancer with metastasis to the basal spine was given by his doctor in November 1998 a survival time of a matter of weeks. Within 2 months of oral intake of the preparation according to the invention his health had improved so drastically that he was able to attend his son's 50th birthday celebrations.

B. A 23 year female with intense abdominal pain, was diagnosed as suffering from stomach cancer. She was scheduled for surgery in order to remove her stomach. Began treatment with the mistletoe lectin preparation. Within 5 days she noted considerably less pain. Ten days later (4 days prior to operation) she was subjected to gastroscopy. The stomach tumor (the size of a plum) was considerably reduced in size and the operation postponed. Two months later a new gastroscopy showed no signs of the tumor and she was declared symptom free. This was almost certainly due to the direct cytoxic effect of the preparation on the tumor cells.

C. 55 year old male, operated in 1972 with a Bilroth II gastrectomy due to ulcer. 2000 detected adenocarcinoma of ventriculus with metastases to the peritoneum. Inoperable. Treated with palliative chemotherapy. No response to treatment, bad prognosis. Treatment with preparation according to the invention, later combined with arginine, stabilized health status. After one year of treatment, CT of abdomen showed virtual absence of ascites, no metastases and no original tumor. Clinically this patient is free of detectable cancer.

D. 49 year old female with ovarian cancer with metastasis to the lymph system and peritoneum. Inoperable. Clinically adenocarcinoma. Started treatment with preparation immediately. Three weeks later at staging procedure, in which a biopsy was to be performed, no tumor or metastasis was detectable. As a result, no chemotherapy or radiation treatment was deemed necessary. Clinical improvement to near normal health. This patient has been treated with the preparation and arginine alone.

E. 55 year old female diagnosed with breast cancer in 1998. Treated with operation, radiation and hormone therapy, yet still developed metastasis to lung and skeleton. After six weeks treatment with the preparation and arginine, x-ray and ultrasound scans showed decreased tumor and metastasis size. Clinically more energy and no pain. Discontinued use of painkillers.

F. 34 year old female. Cancer of the ovary six years prior, developed metastasis to the pelvis. Began treatment with the mistletoe preparation. At staging procedure three weeks later, the tumor was significantly reduced in size. Began treatment with radiation, chemotherapy and hypothermia, concomitant with treatment with the preparation. Results: no detectable tumor or metastasis.

Mechanisms of action

[0039] The observed patient data are consistent with the following properties of orally presented mistletoe lectins (ML), which are "negative" for tumor growth:

I) Stimulation of intestinal hyperplasia which induces a competition for nutritional factors between tumor and intestine.

II) Stimulation of lymphocyte infiltration into the tumor.

III) Local release of cytokines from macrophages and lymphocytes (e.g. tumor necrosis factor alpha) into the tumor. These are detrimental to the tumor and reduce growth.

IV) NKC (natural killer cells) activated and these "attack" the tumor cells.

V) Apoptosis ("natural cell death") occurs in the tumor cells as a result of II), III) and IV).

VI) Anti-angiogenic response, which results in a marked reduction in the number of blood capillaries in the tumor. Together with I) nutrient supply to the tumor reduced.

VII) If the tumor is "exposed" (e.g. stomach, colon) then ML may exert its cytotoxic effects i.e. uptake of ML by the tumor cells results in cell death through inhibition of protein synthesis.

Pharmaceutical preparation

[0040] While it is possible for the mistletoe lectin preparation according to the invention to be utilized for therapy as a raw chemical composition, it may be advantageous to present the mistletoe lectins in the form of a pharmaceutical preparation.

[0041] A further aspect of the invention is therefore a pharmaceutical preparation comprising the mistletoe lectin preparation, or a pharmaceutically acceptable salt or derivative thereof, together with one or more pharmaceutically acceptable carriers and possibly other therapeutic or prophylactic ingredients. Said carriers must be acceptable, in that they are compatible with the other ingredients in the preparation and pose no risk to the patient.

[0042] Pharmaceutical preparations include those that are adapted for oral, rectal, nasal, topical, or vaginal administration, as well as inhalation and bladder infusion.

[0043] The mistletoe lectin preparation, together with conventional additives, carriers or diluting agents, can be used to prepare pharmaceutical compositions, including individual doses thereof, in the form of tablets, filled capsules, or fluids such as solutions, mixtures, emulsions, elixirs or capsules filled with such, all for oral intake, as well as in the form of suppositories for rectal administration. Such pharmaceutical compositions and individual doses thereof can comprise conventional ingredients or principles, and such dosage-forms can contain any effective concentration of the active ingredients in accordance with the intended daily dosage range. Preparations that contain approximately 0.25 mg of the mistletoe lectin preparation per individual dosage unit are representative of an appropriate concentration.

[0044] The pharmaceutical preparation according to the invention can be administered in a wide range of dosage-forms. Carriers used to produce a pharmaceutical preparation containing the mistletoe lectin preparation can include both solid and liquid substances. Solid dosage-forms may include powders, tablets, pills, capsules, suppositories, or dispersible granules. A solid carrier can be one or more substances that function as a diluting agent, flavor additive, solvent, lubricant, suspension agent, binder, preservative, tablet-disintegrating substance or encapsulating material.

[0045] In powdered form, the carrier is a finely pulverized solid including lactose, hydroxypropylmethylcellulose and PVP, mixed with an appropriate amount of finely pulverized mistletoe lectin preparation.

[0046] Appropriate carriers for powder and tablet forms include magnesium carbonate, magnesium stearate, talc, sugar, lactose, pectin, dextrin, stiffeners, gelatins, tragacanth, methylcellulose, and sodium carboxymethylcellulose. The term "preparation" is meant to include dosage-forms where the active ingredients are enclosed in an encapsulating material whether or nor associated with a carrier, including capsules or lozenges.

[0047] Suppositories are produced by melting a low-melting point wax and distributing the mistletoe lectin preparation therein. The melted, homogeneous mixture is then poured into forms and allowed to cool.

[0048] Preparations appropriate for vaginal administration can be presented as presses, tampons, creams, gels, pastes, foams or sprays that include, in addition to the active ingredient, suitable carriers known in the art.

[0049] Preparations in liquid form include solutions, suspensions, and emulsions, for example aqueous or propylene glycol solutions, together with coloring agents, flavor additives, stabilizing agents or diluting agents as appropriate. Also included are preparations in solid form that are meant to be converted to liquid form shortly prior to consumption. These forms may include, in addition to the active ingredients, artificial colors, flavors, stabilizers, buffers, natural or artificial sweeteners, dispersing

agents, thickeners, dissolving agents and the like.

[0050] For topical administration to the epidermis, the mistletoe lectin preparation can be presented in the form of salves, creams, gels, skin washes or transdermal plasters. Salves and creams can be formulated with an aqueous or oil base, with the addition of suitable thickeners and/or gels. Skin washes can be prepared with an aqueous or oil base and may contain one or more emulsifying agents, stabilizers, dispersing agents, thickeners or fragrances.

[0051] Preparations suitable for topical administration in the mouth include lozenges that comprise active ingredients in an inert, flavored base, such as sucrose and arabica gum, as well as mouth washes containing the active ingredients in a liquid carrier.

[0052] Solutions or mixtures may be administered directly to the nasal cavity using conventional means, such as drops or sprays. The preparation may be produced in individual or multi-dose forms. Multi-dose forms would include a dropper, pipette or atomizer that delivers a predetermined volume of the preparation.

[0053] Administration to the respiratory tract may be achieved by the use of an aerosol preparation in which the active ingredients are placed in a pressurized container together with a suitable delivery agent, such as CFC, trichlorofluormethane, dichlorofluormethane, carbon dioxide or other suitable gas. The dosage may be controlled by an appropriate valve-system.

[0054] The pharmaceutical preparation is preferably provided in individual dosage units that contain a suitable amount of the active ingredients. The individual doses may be provided in a package, or possibly as a kit that includes a measuring device.

[0055] **All publications and articles identified below are specifically incorporated by reference into the preceding specification **

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[0056]

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Claims

1. A. composition of matter, consisting essentially of mistletoe lectin I (ML-I), mistletoe lectin II (ML-II), and mistletoe lectin III (ML-III), **characterized in that** said composition is free of nausea-inducing compounds and orally ingestible by mammals.

2. A composition of matter according to claim 1, **characterized in that** said the nausea inducing compounds are selected from the group consisting of alkaloids and viscotoxins.

3. A composition of matter according to any of claims 1, 2 **characterized in that** said mammals are human beings.

4. A composition of matter according to any of claims 1 to 3, **characterized in that** the protein content of said composition is ≥ 250 μg/ml.

5. A composition of matter according to any of claims 1 to 4, **characterized in that** the molecular weights (MW) of the individual ML subunits are 29-35 kDa.

6. A composition of matter according to any of claims 1 to 5, **characterized in that** the absorbance of 1 ml of said composition is greater than 3.0 at 280 nm.

7. A method to produce an orally ingestible composition of matter consisting essentially of mistletoe lectin I (ML-I), mistletoe lectin II (ML-II), and mistletoe lectin III (ML-III) free of nausea inducing compounds, **characterized in** comprising the following steps:

   i) pulverizing a start material consisting of suitable pieces of Viscum album,
   ii) mixing the pulverized material with five times the amount of 0.2 M acetic acid on w/w basis to form a solution and stirring said solution 12 hours at temperatures between 1 and 8°C,
   iii) filtering the solution through a course cloth, followed by a paper filter to remove iii) filtering the solution through a course cloth, followed by a paper filter to remove turbidity,
   iv) performing cationic exchange chromatography of the crude extract from (iii) on approximately 5 g SP-Sephadex cationic exchanger/1.0 litre of crude mistletoe extract,
   v) washing the column with 0.15 M acetate buffer (pH 4.0) until absorption at 280 nm < 0.5,
   vi) eluting ML-I, ML-II and ML-III collectively using a buffer containing 0.1 M Tris-HCl (pH 8.0) and 0.5 M NaCl,
   vii) pooling fractions with absorbance > 1.0 at 280 nm and dialyzing overnight (about 4°C) against 10 volumes of PBS (minus NaCl) using a membrane having a cutoff size of MW 10,000,
   viii) aliquoting the final extract in suitable portions.

8. Method according to claim 7, **characterized in that** the aliquots in step viii) are 1 ml.

9. Pharmaceutical preparation, **characterized in** comprising the composition of matter according to any of claims 1 to 6 together with pharmaceutically ac-

ceptable adjuvants, diluents, fillers, vehicles and preserving agents.

10. Pharmaceutical preparation according to claim 9, **characterized in** comprising a dosage-form suitable for oral administration.

11. Pharmaceutical preparation according to claim 9, **characterized in** comprising a dosage-form suitable for rectal administration.

12. Pharmaceutical preparation according to claim 9, **characterized in** comprising a dosage-form suitable for nasal administration.

13. Pharmaceutical preparation according to claim 9, **characterized in** comprising a dosage-form suitable for vaginal administration.

14. Pharmaceutical preparation according to claim 9, **characterized in** comprising a dosage-form suitable for topical administration.

15. Pharmaceutical preparation according to claim 9, **characterized in** comprising a dosage-form suitable for administration by inhalation.

16. Use of the composition of matter according to any of claims 1 to 6 to produce a pharmaceutical preparation for treating cancer and autoimmune diseases, such as arthritis, rheumatic diseases, asthma and emphysema, and subjects suffering from general fatigue in mammals.

17. Use according to claim 16 wherein the mammal is a human.

18. Use of the composition of matter according to any of claims 1 to 6 to produce a pharmaceutical preparation for treating a patient in need thereof, **characterized in** administering the composition of matter according to any of claims 1 to 6 to a patient in suitable doses, with or without a supplemental substance administered in suitable doses for augmenting the effect of the preparation.

19. Use of the composition of matter according to claims 1-6, **characterized in** administering the composition of matter orally.

20. Use of the composition of matter according to claims 1-6, **characterized in** administering the composition of matter rectally.

21. Use of the composition of matter according to claims 1-6, **characterized in** administering the composition of matter nasally.

22. Use of the composition of matter according to claims 1-6, **characterized in** administering the composition of matter vaginally.

23. Use of the composition of matter according to claims 1-6, **characterized in** administering the composition of matter topically.

24. Use of the composition of matter according to claims 1-6, **characterized in** administering the composition of matter by inhalation.

25. Use of the composition of matter according to any of claims 18 to 22, **characterized in that** the patient is suffering from cancer.

26. Use of the composition of matter according to any of claims 18 to 22, **characterized in that** the patient is suffering from auto immune disease or general fatigue.

27. Use of the composition of matter according to claims 26, **characterized in that** the auto immune diseases are selected from the group consisting of arthritis, rheumatic diseases, asthma and emphysema.

28. Use of the composition of matter according to any of claims 18 to 25, **characterized in that** said suitable doses are administered on non-consecutive days.

29. Use of the composition of matter according to claim 28, **characterized in that** said suitable doses are 1 ml three times a week.

30. Use of the composition of matter according to claim 28, **characterized in that** said suitable doses are 1 ml two days per week, and 2 ml on a day which falls between the days on which 1 ml is administered.

31. Use of the composition of matter according to any of claims 18 to 30 **characterized in that** the substance for augmenting the effect of the preparation is arginine.

32. Use of the composition of matter according to claim 31, **characterized in that** the dose for arginine is between 0.001 and 0.01 g of arginine per kg of body weight every morning and evening.

33. Kit, **characterized in that** it comprises an airtight package containing the composition of matter according to any of claims 1 to 6, arginine, and devices for metering dosages.

Patentansprüche

1. Stoffzusammensetzung, bestehend im wesentlichen aus Mistel-Lecithin 1 (ML-I), Mistel-Lecithin II (ML-II), und Mistel-Lecithin III (ML-III), **dadurch gekennzeichnet dass** die Zusammensetzung frei von Nausea-verursachenden Verbindungen und von Säugetieren oral einnehmbar ist.

2. Stoffzusammensetzung gemäß Anspruch 1, **dadurch gekennzeichnet, dass** die Nausea-verursachenden Verbindungen ausgewählt sind aus der Gruppe, bestehend aus Alkaloiden und Viscotoxinen.

3. Stoffzusammensetzung gemäß einem der Ansprüche 1, 2, **dadurch gekennzeichnet, dass** die Säugetiere Menschen sind.

4. Stoffzusammensetzung gemäß einem der Ansprüche 1 bis 3, **dadurch gekennzeichnet, dass** der Proteingehalt der Zusammensetzung > 250 μg/ml ist.

5. Stoffzusammensetzung gemäß einem der Ansprüche 1 bis 4, **dadurch gekennzeichnet, dass** die Molekulargewichte (MW) der individuellen ML-Untereinheiten 29-35 kDa betragen.

6. Stoffzusammensetzung gemäß einem der Ansprüche 1 bis 5, **dadurch gekennzeichnet, dass** die Absorption von 1 ml der Zusammensetzung bei 280 nm größer als 3,0 ist.

7. Verfahren zur Herstellung einer oral einnehmbaren Stoffzusammensetzung, die im Wesentlichen aus Mistellecitin I (ML-I), Mistellectiin II (ML-II), und Mistellectin III (ML-III) besteht und frei von Nauseaverursachenden Verbindungen ist, **dadurch gekennzeichnet, dass** es die folgenden Schritte umfasst:

   i) Pulverisieren eines Ausgangsmaterials, welches aus geeigneten Teilen von Viscum album besteht,
   ii) Mischen des pulverisierten Materials mit der fünffachen Menge an 0,2 M Essigsäure auf Gewichtsbasis, um eine Lösung zu bilden und 12 Stunden Rühren der Lösung bei Temperaturen zwischen 1 und 8°C,
   iii) Filtrieren der Lösung durch eine Gewebeschicht, gefolgt von einem Papierfilter, um Trübungen zu entfernen,
   iv) Durchführen von Kationenaustauschchromatographie des Rohextrakts aus (iii) auf ungefähr 5 g SP-Sephadex Kationenaustauscher/1,0 Liter Mistelrohextrakt,
   v) Waschen der Säule mit 0,15 M Acetatpuffer (pH 4,0), bis die Absorption bei 280 nm < 0,5 beträgt,
   vi) gleichzeitiges Eluieren von ML-I, ML-II und ML-III unter Verwendung eines Puffers, der 0,1 M Tris-HCl (pH 8,0) und 0,5 M NaCl enthält,
   vii) Zusammenführen der Fraktionen mit einer Aborption > 1,0 bei 280 nm und dialysieren über Nacht (ungefähr 4°C) gegen 10 Volumina von PBS (ohne NaCl) unter Verwendung einer Membran mit einem Rückhaltevermögen von MW 10.000,
   viii) Aufteilen des endgültigen Extrakts in geeignete Portionen.

8. Verfahren nach Anspruch 7, **dadurch gekennzeichnet, dass** die Teilmengen in Schritt viii) 1 ml betragen.

9. Pharmazeutisches Präparat, **dadurch gekennzeichnet, dass** es die Stoffzusammensetzung gemäß einem der Ansprüche 1 bis 6 zusammen mit pharmazeutisch akzeptablen Hilfsstoffen, Verdünnern, Füllstoffen, Vehikeln und Konservierungsmitteln enthält.

10. Pharmazeutisches Präparat nach Anspruch 9, **dadurch gekennzeichnet, dass** es eine Dosierungsform umfasst, die zur oralen Verabreichung geeignet ist.

11. Pharmazeutisches Präparat nach Anspruch 9, **dadurch gekennzeichnet, dass** es eine Dosierungsform umfasst, die zur rektalen Verabreichung geeignet ist.

12. Pharmazeutisches Präparat nach Anspruch 9, **dadurch gekennzeichnet, dass** es eine Dosierungsform umfasst, die zur nasalen Verabreichung geeignet ist.

13. Pharmazeutisches Präparat nach Anspruch 9, **dadurch gekennzeichnet, dass** es eine Dosierungsform umfasst, die zur vaginalen Verabreichung geeignet ist.

14. Pharmazeutisches Präparat nach Anspruch 9, **dadurch gekennzeichnet, dass** es eine Dosierungsform umfasst, die zur topischen Verabreichung geeignet ist.

15. Pharmazeutisches Präparat nach Anspruch 9, **dadurch gekennzeichnet, dass** es eine Dosierungsform umfasst, die zur Verabreichung durch Inhalation geeignet ist.

16. Verwendung der Stoffzusammensetzung gemäß einem der Ansprüche 1 bis 6 zur Herstellung eines pharmazeutischen Präparats zur Behandlung von

Krebs und Autoimmunerkrankungen, wie Arthritis, rheumatischen Erkrankungen, Asthma und Emphysemen, und von Personen, die an allgemeiner Erschöpfung leiden, in Säugetieren.

17. Verwendung nach Anspruch 16, wobei das Säugetier ein Mensch ist.

18. Verwendung der Stoffzusammensetzung gemäß einem der Ansprüche 1 bis 6 zur Herstellung eines pharmazeutischen Präparats zur Behandlung eines Patienten, der diese benötigt, **dadurch gekennzeichnet, dass** das Verabreichen der Stoffzusammensetzung gemäß einem der Ansprüche 1 bis 6 an eine Patienten in geeigneten Dosen erfolgt, mit oder ohne eine zusätzliche Substanz, die in geeigneten Dosen verabreicht wird, um die Wirkung des Präparats zu unterstützen.

19. Verwendung der Stoffzusammensetzung nach Ansprüchen 1 bis 6, **dadurch gekennzeichnet, dass** die Verabreichung der Stoffzusammensetzung oral erfolgt.

20. Verwendung der Stoffzusammensetzung nach Ansprüchen 1 bis 6, **dadurch gekennzeichnet, dass** die Verabreichung der Stoffzusammensetzung rektal erfolgt.

21. Verwendung der Stoffzusammensetzung nach Ansprüchen 1 bis 6, **dadurch gekennzeichnet, dass** die Verabreichung der Stoffzusammensetzung nasal erfolgt.

22. Verwendung der Stoffzusammensetzung nach Ansprüchen 1 bis 6, **dadurch gekennzeichnet, dass** die Verabreichung der Stoffzusammensetzung vaginal erfolgt.

23. Verwendung der Stoffzusammensetzung nach Ansprüchen 1 bis 6, **dadurch gekennzeichnet, dass** die Verabreichung der Stoffzusammensetzung topisch erfolgt.

24. Verwendung der Stoffzusammensetzung nach Ansprüchen 1 bis 6, **dadurch gekennzeichnet, dass** die Verabreichung der Stoffzusammensetzung durch Inhalation erfolgt.

25. Verwendung der Stoffzusammensetzung nach einem der Ansprüche 18 bis 22, **dadurch gekennzeichnet, dass** der Patient an Krebs leidet.

26. Verwendung der Stoffzusammensetzung nach einem der Ansprüche 18 bis 22, **dadurch gekennzeichnet, dass** der Patient an einer Autoimmunerkrankung oder allgemeiner Erschöpfung leidet.

27. Verwendung der Stoffzusammensetzung nach Anspruch 26, **dadurch gekennzeichnet, dass** die Autoimmunerkrankungen ausgewählt sind aus der Gruppe, bestehend aus Arthritis, rheumatischen Erkrankungen, Asthma und Emphysemen.

28. Verwendung der Stoffzusammensetzung nach einem der Ansprüche 18 bis 25, **dadurch gekennzeichnet, dass** die geeigneten Dosen an nicht aufeinander folgenden Tagen verabreicht werden.

29. Verwendung der Stoffzusammensetzung nach Anspruch 28, **dadurch gekennzeichnet, dass** die geeigneten Dosen 1 ml dreimal pro Woche betragen.

30. Verwendung der Stoffzusammensetzung nach Anspruch 28, **dadurch gekennzeichnet, dass** die geeigneten Dosen 1 ml an zwei Tagen pro Woche und 2 ml an einem Tag betragen, der zwischen den Tagen liegt, an welchen 1 ml verabreicht wird.

31. Verwendung der Stoffzusammensetzung nach einem der Ansprüche 18 bis 30, **dadurch gekennzeichnet, dass** die Substanz zum Unterstützen der Wirkung des Präparats Arginin ist.

32. Verwendung der Stoffzusammensetzung nach Anspruch 31, **dadurch gekennzeichnet, dass** die Dosis Arginin zwischen 0,001 und 0,01 g Arginin pro kg Körpergewicht jeden Morgen und Abend beträgt.

33. Kit, **dadurch gekennzeichnet, dass** es eine luftdichte Verpackung umfasst, welche die Stoffzusammensetzung nach einem der Ansprüche 1 bis 6, Arginin und Vorrichtungen zum Abmessen von Dosierungen enthält.

Revendications

1. Composition de matière essentiellement composée de lectine I de gui (ML-I), de lectine II de gui (ML-II) et de lectine III de gui (ML-III), **caractérisée en ce que** ladite composition est exempte de composés induisant une nausée et est ingérable par voie orale par les mammifères.

2. Composition de matière selon la revendication 1, **caractérisée en ce que** lesdits composés induisant une nausée sont choisis dans le groupe constitué par les alcaloïdes et les viscotoxines.

3. Composition de matière selon l'une quelconque des revendications 1 et 2, **caractérisée en ce que** lesdits mammifères sont des êtres humains.

4. Composition de matière selon l'une quelconque des revendications 1 à 3, **caractérisée en ce que** la te-

neur en protéines de ladite composition est supérieure ou égale à 250 μg/ml.

5. Composition de matière selon l'une quelconque des revendications 1 à 4, **caractérisée en ce que** les poids moléculaires (PM) des sous-unités individuelles de ML sont de 29 à 35 kDa.

6. Composition de matière selon l'une quelconque des revendications 1 à 5, **caractérisée en ce que** l'absorbance de 1 ml de ladite composition est supérieure à 3,0 à 280 nm.

7. Méthode de production d'une composition de matière ingérable par voie orale essentiellement composée de lectine I de gui (ML-I), de lectine II de gui (ML-II) et de lectine III de gui (ML-III) et exempte de composés induisant une nausée, **caractérisée en ce qu'**elle comprend les étapes suivantes :

i) pulvériser un produit de départ constitué de morceaux appropriés de Viscul album,
ii) mélanger la matière pulvérisée avec cinq fois la quantité d'acide acétique 0,2 M sur une base en poids pour former une solution et agiter ladite solution 12 heures à des températures entre 1 et 8°C,
iii) filtrer la solution à travers un drap grossier puis un filtre en papier pour éliminer la turbidité,
iv) réaliser une chromatographie d'échange cationique de l'extrait brut obtenu dans l'étape (iii) sur approximativement 5 g d'échangeur cationique SP-Séphadex/1,0 litre d'extrait brut de gui,
v) laver la colonne avec un tampon acétate 0,15 M (pH 4,0) jusqu'à ce que l'absorption à 280 nm soit inférieure à 0,5,
vi) éluer la ML-I, la ML-II et la ML-III collectivement en utilisant un tampon contenant du Tris-HCl 0,1 M (pH 8,0) et du NaCl 0,5 M,
vii) rassembler les fractions ayant une absorbance supérieure à 1,0 à 280 nm et dialyser pendant une nuit (environ 4°C) contre 10 volumes de PBS (moins NaCl) en utilisant une membrane possédant un seuil de coupure de PM de 10 000,
viii) aliquoter l'extrait final en portions appropriées.

8. Méthode selon la revendication 7, **caractérisée en ce que** les aliquotes de l'étape viii) sont de 1 ml.

9. Préparation pharmaceutique, **caractérisée en ce qu'**elle comprend la composition de matière selon l'une quelconque des revendications 1 à 6, avec des adjuvants, des diluants, des charges, des véhicules et des conservateurs pharmaceutiquement acceptables.

10. Préparation pharmaceutique selon la revendication 9, **caractérisée en ce qu'**elle comprend une forme pharmaceutique appropriée pour une administration par voie orale.

11. Préparation pharmaceutique selon la revendication 9, **caractérisée en ce qu'**elle comprend une forme pharmaceutique appropriée pour une administration par voie rectale.

12. Préparation pharmaceutique selon la revendication 9, **caractérisée en ce qu'**elle comprend une forme pharmaceutique appropriée pour une administration par voie nasale.

13. Préparation pharmaceutique selon la revendication 9, **caractérisée en ce qu'**elle comprend une forme pharmaceutique appropriée pour une administration par voie vaginale.

14. Préparation pharmaceutique selon la revendication 9, **caractérisée en ce qu'**elle comprend une forme pharmaceutique appropriée pour une administration par voie topique.

15. Préparation pharmaceutique selon la revendication 9, **caractérisée en ce qu'**elle comprend une forme pharmaceutique appropriée pour une administration par inhalation.

16. Utilisation de la composition de matière selon l'une quelconque des revendications 1 à 6 pour produire une préparation pharmaceutique destinée au traitement du cancer et des maladies auto-immunes, comme l'arthrite, les maladies rhumatismales, l'asthme et l'emphysème, et des sujets souffrant d'une fatigue générale chez les mammifères.

17. Utilisation selon la revendication 16, dans laquelle le mammifère est un humain.

18. Utilisation d'une composition de matière selon l'une quelconque des revendications 1 à 6 pour produire une préparation pharmaceutique destinée à traiter un patient en ayant besoin, **caractérisée en ce que** la composition de matière selon l'une quelconque des revendications 1 à 6 est administrée à un patient sous la forme de doses appropriées, avec ou sans une substance supplémentaire administrée sous la forme de doses appropriées pour augmenter l'effet de la préparation.

19. Utilisation de la composition de matière selon les revendications 1 à 6, **caractérisée en ce que** la composition de matière est administrée par voie orale.

20. Utilisation de la composition de matière selon les

revendications 1 à 6, **caractérisée en ce que** la composition de matière est administrée par voie rectale.

21. Utilisation de la composition de matière selon les revendications 1 à 6, **caractérisée en ce que** la composition de matière est administrée par voie nasale.

22. Utilisation de la composition de matière selon les revendications 1 à 6, **caractérisée en ce que** la composition de matière est administrée par voie vaginale.

23. Utilisation de la composition de matière selon les revendications 1 à 6, **caractérisée en ce que** la composition de matière est administrée par voie topique.

24. Utilisation de la composition de matière selon les revendications 1 à 6, **caractérisée en ce que** la composition de matière est administrée par inhalation.

25. Utilisation de la composition de matière selon l'une quelconque des revendications 18 à 22, **caractérisée en ce que** le patient souffre d'un cancer.

26. Utilisation de la composition de matière selon l'une quelconque des revendications 18 à 22, **caractérisée en ce que** le patient souffre d'une maladie auto-immune ou d'une fatigue générale.

27. Utilisation de la composition de matière selon la revendication 26, **caractérisée en ce que** les maladies auto-immunes sont choisies dans le groupe constitué par l'arthrite, les maladies rhumatismales, l'asthme et l'emphysème.

28. Utilisation de la composition de matière selon l'une quelconque des revendications 18 à 25, **caractérisée en ce que** lesdites doses appropriées sont administrées sur des jours non-consécutifs.

29. Utilisation de la composition de matière selon la revendication 28, **caractérisée en ce que** lesdites doses appropriées sont de 1 ml trois fois par semaine.

30. Utilisation de la composition de matière selon la revendication 28, **caractérisée en ce que** lesdites doses appropriées sont de 1 ml deux jours par semaine, et de 2 ml le jour qui tombe entre les jours où 1 ml est administré.

31. Utilisation de la composition de matière selon l'une quelconque des revendications 18 à 30, **caractérisée en ce que** la substance destinée à augmenter l'effet de la préparation est l'arginine.

32. Utilisation de la composition de matière selon la revendication 31, **caractérisée en ce que** la dose d'arginine se situe entre 0,001 et 0,01 g d'arginine par kg de poids corporel chaque matin et chaque soir.

33. Kit, **caractérisé en ce qu'**il comprend un emballage hermétique contenant la composition de matière selon l'une quelconque des revendications 1 à 6, l'arginine et des dispositifs permettant de mesurer les doses.

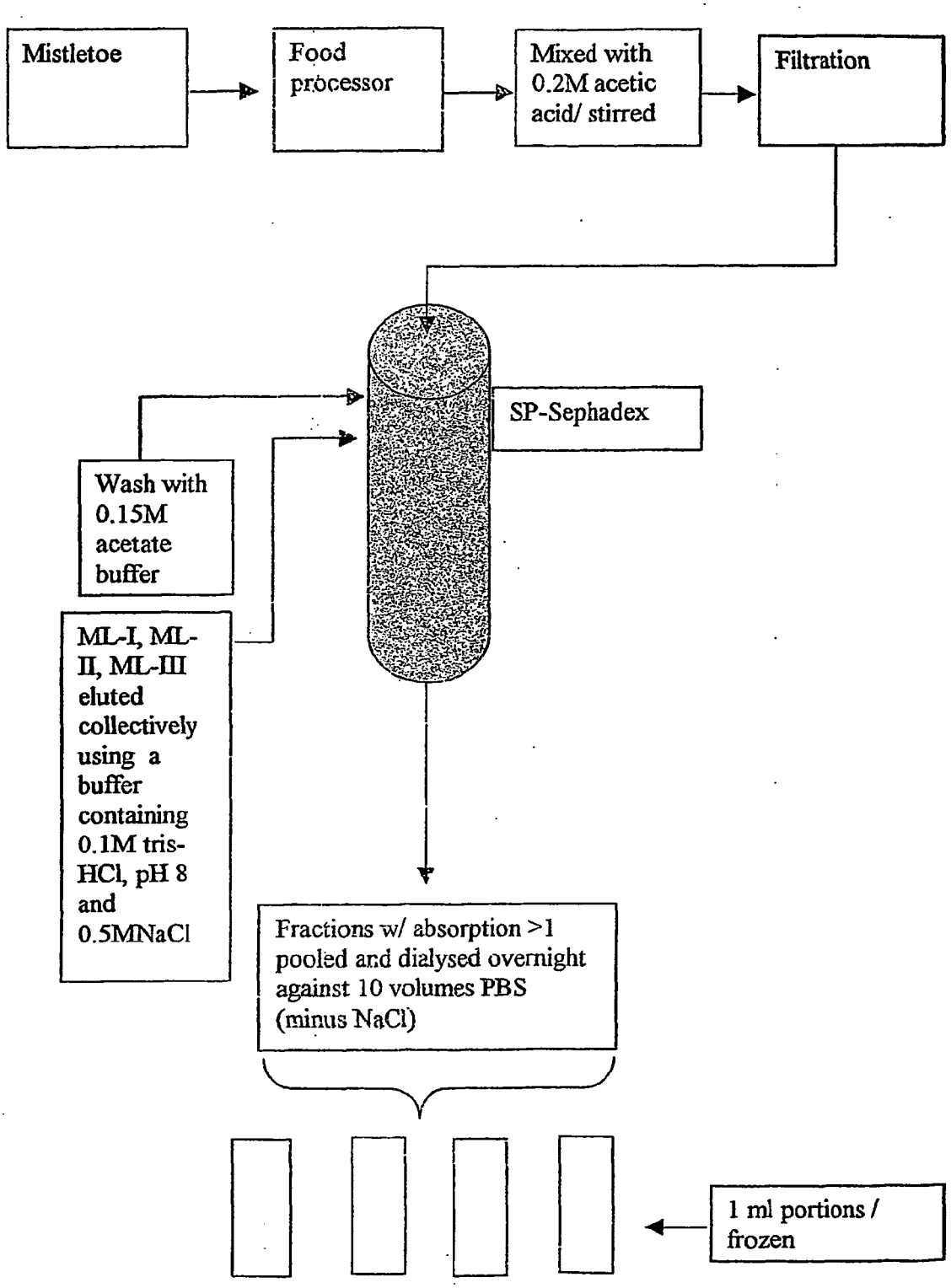
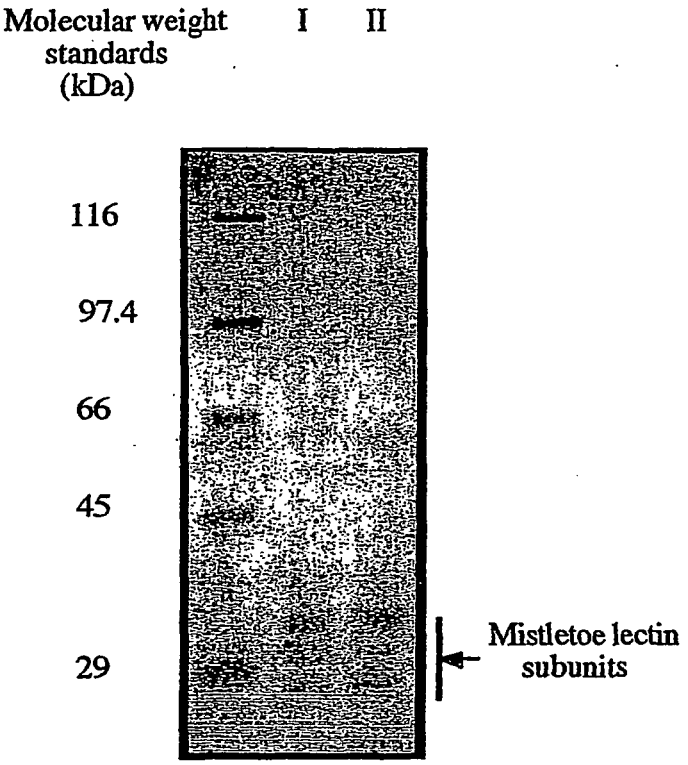

Mistletoe
Food processor
Mixed with 0.2M acetic acid/ stirred
Filtration
SP-Sephadex
Wash with 0.15M acetate buffer
ML-I, ML-II, ML-III eluted collectively using a buffer containing 0.1M tris-HCl, pH 8 and 0.5MNaCl
Fractions w/ absorption >1 pooled and dialysed overnight against 10 volumes PBS (minus NaCl)
1 ml portions / frozen

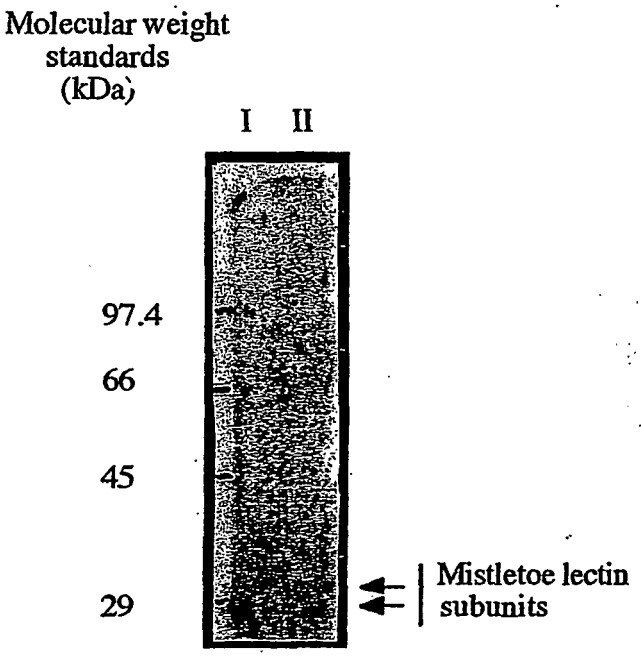
Figure 1

FIGURE 2.



I and II : two separate preparations of mistletoe lectins showing presence of individual protein subunits.

FIGURE 3.



I = serum from a person (control) who had not taken PALM.

II = serum from a person who had ingested PALM for > 6 months, showing positive reaction for presence of antibodies against mistletoe lectin subunits.

REFERENCES CITED IN THE DESCRIPTION

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