

PHARMACEUTICAL USE OF GRAPTOPETALUM AND RELATED PLANTS

FIELD OF THE INVENTION

5 This invention relates to the therapeutic and prophylactic effects of Graptopetalum and related plants, particularly for liver-related diseases and medical conditions.

REFERENCES

U.S. Patent No. 5,023,252.

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15 Giladi, H., et al. (2003). Small interfering RNA inhibits hepatitis B virus replication in mice. *Mol Ther.* 8(5):769-776.

Pines, M. (1997). Halofuginone, a specific inhibitor of collagen type I synthesis, prevents dimethylnitrosamine-induced liver cirrhosis. *J Hepatol.* 27(2):391-398.

20 Saller, R., et al. (2001). The use of silymarin in the treatment of liver diseases. *Drugs.* 61(14):2035-2063.

25 All of the publications, patents and patent applications cited above or elsewhere in this application are herein incorporated by reference in their entirety to the same extent as if the disclosure of each individual publication, patent application or patent was specifically and individually indicated to be incorporated by reference in its entirety.

BACKGROUND OF THE INVENTION

The liver is a multi-functional organ. Its basic functions can be divided into three categories: (1) the vascular functions for storage and filtration of blood, (2) the metabolic functions which are involved with most of the metabolic systems of the

body, and (3) the secretory and excretory functions responsible for bile formation. Major activities of the liver include detoxification and elimination of both endogenous and exogenous toxins, deamination of amino acids and formation of urea, regulation of blood sugar through the formation of glycogen, production of plasma proteins, production and secretion of bile, and phagocytosis of particulate matter from the splanchnic (intestinal) circulation.

As a result of liver's many different roles, when the liver is damaged, its various functions are disturbed simultaneously in different combinations, depending on the nature and location of the damage. Liver damage from any source may result in liver regeneration, necrosis (cell death), degeneration, inflammation, fibrosis, or combinations of these processes. Although the liver has great functional reserves, with progressive injury, disruption of liver function can have life-threatening consequences. Cirrhosis, which is a common end-stage liver disease, is one of the top ten causes of death in the Western world.

Despite the significance and potential severity of liver disease, therapeutic approaches are limited. Many types of liver disease are the result of viruses (e.g., hepatitis A, B, C, D, and E), and effective anti-viral therapies are rare and often cause potentially severe side effects. Other liver diseases are the results of previous toxic exposure (such as alcoholic cirrhosis and exposure to toxic plants, or environmental pollutants). In still other cases, liver diseases are the result of poorly understood interplay of various factors, including genetic factors, environmental conditions, and immune system activity (autoimmune hepatitis). No matter what the causes are, therapeutic approaches to liver diseases are complicated by two factors. Since the liver is responsible for detoxification, any use of drugs may increase the burden upon the liver and lead to deterioration of the condition. Furthermore, liver fibrosis, which occurs in many liver diseases and conditions, is generally an irreversible process. Consequently, the usable portion of the liver decreases with the progress of fibrosis, resulting in increasing complications of other organs that rely on liver functions.

Therefore, a pharmaceutical agent for the liver should ideally prevent fibrosis from occurring or progressing. In addition, it is also desirable if the agent does not pose a metabolic burden on the liver, as well as has few or no side effects.

SUMMARY OF THE INVENTION

The present invention provides, *inter alia*, compositions that comprise Graptopetalum. The inventors discovered that this common plant can protect animals from a toxic substance that causes inflammation and fibrosis of the liver, weight loss, spleen swelling, and ultimately death. With the administration of Graptopetalum, inflammation and fibrosis were significantly inhibited; body weight, liver weight, and spleen weight of the animals were nearly normal; and the survival rate was greatly improved. In particular, Graptopetalum inhibits proliferation of activated hepatic stellate cells, which play a pivotal role in liver fibrosis. Consistent with this observation, Graptopetalum inhibits the accumulation of collagen and prevents fibrosis to occur or progress. In addition, the composition, even at a high dosage, displayed no detectable side effects. Echeveria had similar effects.

Accordingly, one aspect of the present invention provides a pharmaceutical composition comprising Graptopetalum or Echeveria. The Graptopetalum may be any Graptopetalum species or in any form of preparation. Preferably, the Graptopetalum species is *Graptopetalum paraguayense*. Similarly, the Echeveria may be any Echeveria species or in any form of preparation. Preferably, the Echeveria species is *Echeveria peacockii*. The Graptopetalum or Echeveria is preferably an extract, particularly an extract of one or more aqueous solvents, nonpolar solvents, alcohol solvents, or mixtures thereof.

The pharmaceutical composition may further comprise a pharmaceutically acceptable excipient or carrier. The pharmaceutical composition may also comprise at least one other agent, particularly an agent that has anti-inflammatory, anti-steatosis, anti-viral, or anti-fibrosis, activities. For example, the agent may be selected from the group consisting of arbutin, lemon extract, cucumber extract, mercaptosuccinic acid, mercaptodextran, kojic acid, derivatives of kojic acid, vitamin C, hydroquinone, glutathione, cysteine, mulberry extract, licorice extract, and bearberry extract.

The pharmaceutical composition may be formulated for any suitable route of administration, preferably oral or topical administration.

Another aspect of the present invention provides a method of preventing or treating a liver disease or medical condition in a subject, comprising administering an

effective amount of the pharmaceutical composition described herein to the subject, wherein the liver disease or medical condition is selected from the group consisting of liver inflammation, liver steatosis, liver fibrosis, liver cirrhosis, and hepatitis B.

When used for liver fibrosis or cirrhosis, it is preferable that the composition is
5 capable of reducing the expression of smooth muscle α -actin, particularly in hepatic stellate cells.

Another aspect of the present invention provides a method for inhibiting hepatic stellate cell proliferation, comprising contacting hepatic stellate cells with the pharmaceutical composition comprising Graptopetalum or Echeveria. Yet another
10 aspect of the present invention provides a method for selectively killing hepatic stellate cells, comprising contacting hepatic stellate cells with the pharmaceutical composition comprising Graptopetalum or Echeveria. The hepatic stellate cells are preferably located in a mammal, and most preferably in a human.

Also provided is a method for inhibiting hepatitis B virus surface antigen
15 expression in a mammal, comprising administering to the mammal the pharmaceutical composition. The mammal is preferably a human.

In addition to Graptopetalum and Echeveria, other succulent plants in the family Crassulaceae can also be used in the same manner as described herein. This family includes the genera Adromischus, Aeonium, Aichryson, Bryophyllum,
20 Chiastophyllum, Cotyledon, Crassula, Cremanthodium, Diamorpha, Dudleya, Echeveria, Graptopetalum, Greenovia, Hylotelephium, Hypagophytum, Jovibarba, Kalanchoe, Kitchingia, Lenophyllum, Meterostachys, Monanthes, Orostachys, Pachyphytum, Perrierosedum, Pistorinia, Prometheum, Pseudosedum, Rhodiola, Rosularia, Sedella, Sedum, Sempervivum, Sinocrassula, Tacitus, Telmissa, Thompsonella, Tylecodon,
25 Umbilicus, and Villadia. Graptopetalum and Echeveria are the preferred genera for the practice of the present invention.

Other aspects of the present invention provides methods for treating or preventing fibrosis or inflammation of other tissues and organs, such as the lung, kidney, and bladder, by using Graptopetalum or its related plants.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the number of surviving rats that received vehicle control (Control), DMN and Graptopetalum (DMN-GP), DMN and silymarin (DMN-Silymarin), or DMN alone (DMN).

5 **Figure 2** shows the anti-proliferative effect of crude Graptopetalum extracts on cultured rat hepatic stellate cells. Rat hepatic stellate cells were incubated in 250 or 500 µg/ml of Graptopetalum crude extract, and the number of surviving cells was counted everyday. The survival rate shown is the percent survival rate as compared to the control cells which did not receive Graptopetalum.

10 **Figure 3** shows the anti-proliferative effect of various Graptopetalum extracts on cultured rat hepatic stellate cells. Rat hepatic stellate cells were incubated in the indicated Graptopetalum extract, and the number of surviving cells was counted everyday. The survival rate shown is the percent survival rate as compared to the control cells which did not receive Graptopetalum.

15 **Figure 4** shows the expression levels of HbsAg in Hep3B or HepA2 cells incubated in the indicated concentrations of Graptopetalum extract.

20 **Figure 5** shows the antiproliferative effect of various Graptopetalum extracts on cultured mice lung fibroblasts. Primary cultured lung fibroblasts were incubated in the indicated Graptopetalum extract, and the number of surviving cells was counted after incubation for five days. The survival rate shown is the percent survival rate as compared to the control cells, which did not receive Graptopetalum extract.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to compositions comprising Graptopetalum and/or related plants, and uses thereof. Graptopetalum can protect animals from a
25 toxic substance that causes inflammation and fibrosis of the liver, weight loss, spleen swelling, and ultimately death. In particular, Graptopetalum inhibits proliferation of activated hepatic stellate cells, which play a pivotal role in liver fibrosis. The anti-fibrosis activities of Graptopetalum are not limited to the liver. Graptopetalum also inhibits proliferation of lung fibroblasts, and protects animals from a toxic substance
30 that causes inflammation and fibrosis in the lung. Therefore, Graptopetalum is a

prophylactic and therapeutic agent, particularly for tissue fibrosis. Plants related to *Graptopetalum* can be used in the same manner.

Prior to describing the invention in further detail, the terms used in this application are defined as follows unless otherwise indicated.

5 Definitions

“*Graptopetalum*”, as used herein, refers to any plant in the genus of *Graptopetalum*, or part or parts thereof. The plant parts may include, without being limited to, leaves, flowers, stalks, roots, fruits, and seeds. This term also encompasses modified forms of the plant or part(s), such as powders, extracts, dried extracts,
10 homogenates, granules, or precipitates. Combinations of more than one species of *Graptopetalum*, or parts thereof, are also contemplated. The *Graptopetalum* is preferably *G. paraguayense*.

“*Echeveria*”, as used herein, refers to any plant in the genus of *Echeveria*, or part or parts thereof. The plant parts may include, without being limited to, leaves,
15 flowers, stalks, roots, fruits, and seeds. This term also encompasses modified forms of the plant or part(s), such as powders, extracts, dried extracts, homogenates, granules, or precipitates. Combinations of more than one species of *Echeveria*, or parts thereof, are also contemplated. The *Echeveria* is preferably *Echeveria peacockii*.

20 An “extract” is a solution obtained by soaking or mixing a substance to be extracted with a solvent. Typically, an extract is prepared by drying *Graptopetalum* and subsequently cutting or grinding the dried material. The extraction process may then be performed with the help of an appropriate choice of solvent, typically water, ethanol, ethanol/water mixture, methanol, propanol, iso-propanol, butanol, iso-
25 butanol, acetone, hexane, petroleum ether, or other organic solvents by means of, e.g., maceration, percolation, repercolation, counter-current extraction, turbo-extraction, or carbon-dioxide hypercritical (temperature/pressure) extraction. The extract may then be further evaporated and thus concentrated to yield a soft extract (*extractum spissum*) and/or eventually a dried extract, *extracum siccum*, by means of, e.g., spray drying,
30 vacuum drying, fluid-bed drying, or freeze-drying. Preferably, the extract is prepared without boiling *Graptopetalum* or its related plant. It is also preferable that no oil is used to extract the plants.

“Preventing” a disease or medical condition means preventing the symptoms of the disease or medical condition from manifestation prior to onset of the disease or medical condition.

5 “Treating” a disease or medical condition means the reduction or complete removal of the symptoms of the disease or medical condition after onset of the disease or medical condition.

A “liver disease or medical condition” is a disease or medical condition involved with damages of the liver, or any part thereof. Liver diseases and medical conditions include, without being limited to, liver fibrosis, liver cirrhosis, chronic
10 hepatitis (such as A, B, C, D, E, or other forms), non-alcoholic fatty liver, alcoholic fatty liver, and liver tumors (particularly hepatic carcinoma).

An “effective amount” is an amount of an agent sufficient to achieve the intended purpose. For example, an effective amount of Graptopetalum to treat liver cirrhosis is an amount sufficient to reduce or remove the symptoms of liver cirrhosis,
15 as compared to the symptoms manifest in the absence of Graptopetalum. The effective amount of a given agent will vary with factors such as the nature of the agent, the route of administration, the size and species of the animal to receive the agent, and the purpose of the administration. The effective amount in each individual case may be determined empirically by a skilled artisan according to the disclosure
20 herein and established methods in the art.

The term “unit dosage forms” refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of Graptopetalum calculated to produce the desired effect, in association with a suitable pharmaceutical excipient.

25 Effects of Graptopetalum on fibrosis and inflammation

Graptopetalum is a succulent plant with leaves arranged in the shape of rosettes. The genus Graptopetalum contains 12 species, including *G. paraguayense* (Ghost Plant), *G. amethystinum* (Lavender Pebbles), *G. bellum* (also known as *Tacitus bellus*), and *G. macdougallii*.

30 In order to examine the effects of Graptopetalum on damaged liver, we utilized an animal model of liver injuries. Thus, rats were injected with a toxic

chemical, dimethylnitrosamine (DMN), which caused inflammation and fibrosis of the liver. Liver functions were lower in these animals, as indicated by a variety of biochemical parameters. Body weight and liver weight both declined, while spleen weight increased, and the majority of these animals died within weeks. In contrast, the rats that received Graptopetalum in addition to DMN had nearly normal body and liver weights, and their spleens did not swell (Example 1). The majority of these animals survived, and their liver function indicators, including the glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), prothrombin time (PT), platelet, bilirubin and albumin levels, were relatively normal (Example 2). Therefore, Graptopetalum dramatically reduced the damaging effects of DMN on the liver. A known therapeutic agent for the liver, silymarin (Saller et al., 2001), was examined in parallel as a positive control. Silymarin was also capable of increasing animal survival, body and liver weight, as well as liver functions. Graptopetalum, however, was even more effective than silymarin.

DMN is known to cause liver inflammation and fibrosis. Indeed, after DMN treatment for three weeks, the liver showed massive necrosis and infiltration of inflammatory cells upon a histochemical analysis (Example 3). The rats that also received Graptopetalum, on the other hand, had little or no necrosis or infiltration, indicating that Graptopetalum can be used to treat or prevent inflammation.

The effects of Graptopetalum on liver fibrosis were also examined. After DMN treatment, a fine, uniform granulation was observed on the surface of the liver. Microscopic analysis revealed cirrhotic-like structural patterns in the liver: fibrous connective tissue components in Glisson's sheath, pseudolobule formation, formation of fibrotic septa, and thickened reticulin fibers joining the central areas. Animals treated with both DMN and silymarin still exhibited intense centrilobular and periportal deposition of fibrous connective tissues. In contrast, the livers of DMN-treated rats that received Graptopetalum showed only mild bridging fibrosis, diminished fibrosis in both the periportal and centrilobular areas, and reduced deformation of the liver acinus (Example 4).

In a fibrotic liver, extracellular matrix accumulates. The increased levels of extracellular matrix are crucial for further progression of fibrosis, which ultimately leads to liver cirrhosis. Since collagen is a main component in the extracellular

matrix, we determined the collagen content of animals treated with DMN alone or the combination of DMN and Graptopetalum. Although there were very low levels of collagen deposition in the livers of control rats, DMN-treated rats displayed bundles of collagen surrounding the lobules and large fibrous septa, indicating the onset of severe fibrosis. Quantitative analysis of fibrosis revealed that the Graptopetalum and silymarin treatments significantly reduced the DMN-induced hepatic collagen content, by 80% and 25%, respectively (Example 5).

Specific liver cells, the hepatic stellate cells (HSC), are responsible for the increase in extracellular matrix in the fibrotic liver. In normal livers, HSCs, which are lipocytes in nature, are the primary storage depot for retinoids. These cells can be identified by prominent intracellular droplets that contain vitamin A. Upon activation, HSCs undergo cell proliferation and increased fibrogenesis to result in fibrosis (see, e.g., Friedman, 1993). Since activated HSCs, as opposed to resting HSCs, produce large amounts of smooth muscle α -actin (α -SMA) mRNA and protein, α -SMA is marker of activated HSC and liver fibrosis. As expected, intense, specific staining for α -SMA was observed in the pericentral area of the DMN-treated livers. This DMN-induced expression of α -SMA was suppressed dramatically by oral administration of Graptopetalum or silymarin, while Graptopetalum was more effective than silymarin (Example 6). Taken together, these results thus indicate that Graptopetalum can reduce or prevent liver fibrosis, perhaps through its effects on HSCs.

We incubated cultured HSCs with Graptopetalum to determine the possible mechanism of Graptopetalum action on HSCs. The results show that Graptopetalum inhibited HSC proliferation in a time- and dosage-dependent manner. After a few days of incubation, the HSC changed in morphology, detached from the culture container, and died (Example 7). In contrast, hepatocytes, which were incubated in the same fashion, were not significantly affected. Thus, Graptopetalum is selectively cytotoxic to HSC.

Accordingly, the present invention provides a method of treating or preventing liver fibrosis or cirrhosis in a subject by administering an effective amount of Graptopetalum to the subject. The amount of Graptopetalum is preferably sufficient to reduce collagen formation, to inhibit HSC proliferation, and/or to inhibit α -SMA

mRNA or protein expression, in the liver of the subject. The subject may begin to receive Graptopetalum before any sign of liver fibrosis is manifest; alternatively, the subject may receive Graptopetalum after liver fibrosis, or even cirrhosis, has begun. Almost all chronic liver conditions eventually cause liver cirrhosis. Therefore,

5 Graptopetalum can be used to ameliorate the symptoms of a variety of liver diseases or conditions, including chronic hepatitis (such as A, B, C, D, E, or other forms), non-alcoholic fatty liver, alcoholic fatty liver, and liver tumors (particularly hepatic carcinoma).

Another aspect of the present invention provides a method of inhibiting HSC
10 proliferation by using Graptopetalum. HSC proliferation may be measured by counting HSC numbers, determining biochemical markers of HSC (such as α -SMA mRNA or protein expression), determining collagen levels, or any other methods established in the art.

Another aspect of the present invention provides a method for preventing or
15 treating inflammation in the liver of a subject, comprising administering an effective amount of Graptopetalum to the subject. It is contemplated that Graptopetalum can be used alone or in conjunction with other anti-inflammatory agents. These other anti-inflammatory agents may include, without being limited to, glucocorticoids, aspirin, ibuprofen, cyclopentenone prostaglandins, sesquiterpene lactones,
20 sulfasalazine, chlorpheniramine maleate, clemastine, and deoxyspergualin. When Graptopetalum is administered in combination with at least one other anti-inflammatory agent, Graptopetalum and the other agent may be mixed in one composition for administration to the subject, or they can be administered separately.

We further discovered that Graptopetalum can inhibit the expression of
25 hepatitis B virus surface antigen (Example 8). Since interference with the expression of the hepatitis B virus surface antigen has been shown to inhibit hepatitis B virus replication (see, e.g., Giladi et al., 2003), Graptopetalum can be used to inhibit replication of the virus as well. The present invention thus provides a method for treating or preventing hepatitis B virus infection in a subject, comprising
30 administering to the subject an effective amount of Graptopetalum. Graptopetalum can be used alone or in conjunction with other anti-viral agents. These other anti-viral agents may include, without being limited to, interferon, lamivudine (3TC), adefovir,

ribavirin, specific antibodies for the virus of interest, and combinations thereof.

When Graptopetalum is administered in combination with at least one other anti-viral agent, Graptopetalum and the other agent may be mixed in one composition for administration to the subject, or they can be administered separately.

5 It is contemplated that Graptopetalum can also be used to treat or prevent fibrosis of other tissues or organs, such as the lung, kidney, bladder, or the like. Thus, administration of Graptopetalum, such as by the methods described herein, can reduce or prevent abnormal accumulation of extracellular matrix material in a tissue. Similarly, Graptopetalum can be used to treat or prevent inflammation in tissues or
10 organs other than the liver as well. Examples 10 and 11 demonstrate the inhibitory effects of Graptopetalum on lung fibroblast proliferation and lung fibrosis/inflammation.

 The subject that receives Graptopetalum according to the present invention is preferably a mammal, more preferably a mammal selected from the group consisting
15 of human, non-human primate, feline, canine, murine, rodent, equine, porcine, bovine and ovine, and most preferably a human. Preferably, the subject either suffers from a liver disease or medical condition, or is at risk for a liver disease or medical condition. A subject is at risk for a liver disease or medical condition if, for example, the subject consumes an above-normal level of alcohol frequently, has a family history of chronic
20 liver diseases or conditions, expects to need blood transfusions, or is easily exposed to any hepatitis virus or hazardous material.

 Graptopetalum does not have detectable side effects. As shown in Example 7, even long term exposure to high doses of Graptopetalum did not impose any significant damage to experimental animals. Therefore, it is of particular interest for a
25 subject to use Graptopetalum as a health/nutrition food on a regular basis. Thus, Graptopetalum can be mixed in food or feed, made into a drink, taken in the form of tablets, capsules, pills, powders, or the like. For the ease of regular consumption, packets comprising Graptopetalum powders can be prepared that can be easily made into a drink. Cookies, crackers, cakes, chips, or other snacks that contain
30 Graptopetalum are also contemplated. In general, the recommended daily dosage of Graptopetalum is the equivalent of from 0.1 grams to 30 grams of the Graptopetalum whole plant (wet weight) per kilogram body weight. The dosage is preferably 0.3 to

20, more preferably 0.5 to 15, and yet more preferably 1 to 10 (grams per kilogram body weight).

Although the preferred route of administration is oral, Graptopetalum can be administered in any other manner. For example, Graptopetalum can be administered
5 subcutaneously, intravascularly, intravenously, intraperitoneally, topically, nasally, or pulmonarily.

We further discovered that Echeveria, a plant related to Graptopetalum, had effects similar to those described herein. Echeveria and Graptopetalum are both genera in the family of Crassulaceae. This is a family of succulent, tender and cold-
10 hardy plants, including the genera Adromischus, Aeonium, Aichryson, Bryophyllum, Chiasmophyllum, Cotyledon, Crassula, Cremanthodium, Diamorpha, Dudleya, Echeveria, Graptopetalum, Greenovia, Hylotelephium, Hypagophytum, Jovibarba, Kalanchoe, Kitchingia, Lenophyllum, Meterostachys, Monanthes, Orostachys, Pachyphytum, Perrierosedum, Pistorinia, Prometheum, Pseudosedum, Rhodiola, Rosularia, Sedella,
15 Sedum, Sempervivum, Sinocrassula, Tacitus, Telmissa, Thompsonella, Tylecodon, Umbilicus, and Villadia. It is contemplated that other succulent plants from the Crassulaceae family can be used in lieu of, or in combination of, Graptopetalum and/or Echeveria. For example, the prophylactic or therapeutic composition of this invention may comprise Graptopetalum and optionally one or more plants from the
20 Crassulaceae family, preferably *Echeveria*, more preferably *Echeveria peacockii*.

Compositions

This invention also includes pharmaceutical compositions which contain, as the active ingredient, Graptopetalum, as well as pharmaceutically acceptable carriers or excipients. As discussed above, other succulent plants from the Crassulaceae
25 family can be used in lieu of, or in combination of, Graptopetalum. Therefore, although the preparation and use of compositions comprising Graptopetalum are discussed below, it is contemplated that other succulent plants from the Crassulaceae family are useful in the same manner.

In making the compositions of this invention, the Graptopetalum is usually
30 mixed with an excipient, diluted by an excipient, or enclosed within such a carrier which can be in the form of a capsule, sachet, paper, or other container. When the pharmaceutically acceptable excipient serves as a diluent, it can be a solid, semi-solid,

or liquid material, which acts as a vehicle, carrier, or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments containing, for example, up to 10% by weight of the active compound, soft and hard gelatin capsules, suppositories, sterile injectable solutions, and sterile packaged powders.

Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, sterile water, syrup, and methyl cellulose. The formulations can additionally include lubricating agents such as talc, magnesium stearate, and mineral oil; wetting agents; emulsifying and suspending agents; preserving agents such as methyl- and propylhydroxy-benzoates; sweetening agents; and flavoring agents. The compositions of the invention can be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures known in the art.

For preparing solid compositions such as tablets, the principal active ingredient is mixed with a pharmaceutical excipient to form a solid preformulation composition containing a homogeneous mixture of a compound of the present invention. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills, and capsules.

The tablets or pills of the present invention may be coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer which serves to resist disintegration in the stomach and permit the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, acetyl alcohol, and cellulose acetate.

The liquid forms in which the compositions of the present invention may be incorporated for administration orally or by injection include aqueous solutions, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as corn oil, cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

Compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. The liquid or solid compositions may contain suitable pharmaceutically acceptable excipients as described herein. Preferably the compositions are administered by the oral or nasal respiratory route for local or systemic effect. Compositions in preferably pharmaceutically acceptable solvents may be nebulized by use of inert gases. Nebulized solutions may be inhaled directly from the nebulizing device, or the nebulizing device may be attached to a face mask tent, or intermittent positive pressure breathing machine. Solution, suspension, or powder compositions may be administered, preferably orally or nasally, from devices which deliver the formulation in an appropriate manner.

Another preferred formulation employed in the methods of the present invention employs transdermal delivery devices ("patches"). Such transdermal patches may be used to provide continuous or discontinuous infusion of Graptopetalum in controlled amounts. The construction and use of transdermal patches for the delivery of pharmaceutical agents is well known in the art. See, for example, U.S. Pat. No. 5,023,252, herein incorporated by reference. Such patches may be constructed for continuous, pulsatile, or on-demand delivery of pharmaceutical agents.

Other suitable formulations for use in the present invention can be found in Remington's Pharmaceutical Sciences.

The pharmaceutical composition may be provided in convenient kits wherein the necessary materials are packaged into suitable containers. The compositions can be administered in a single dose, or multiple doses (i.e., more than one dose). The multiple doses can be administered concurrently, or consecutively (e.g., over a period of days or weeks). The compositions are preferably formulated in a unit dosage form;

each dosage containing the equivalent amount of from about 1 to 500 grams of the whole *Graptopetalum* plant (wet weight).

It is further contemplated that the compositions of the present invention may be administered in conjunction with a known anti-inflammatory agent, anti-steatosis agent, anti-viral, or anti-fibrosis agent. In addition, other substances commonly used
 5 to protect the liver, such as silymarin, can also be used in conjunction with the *Graptopetalum* composition. All of these agents or substances can be mixed in the same composition as *Graptopetalum* or administered separately.

The following examples are offered to illustrate this invention and are not to
 10 be construed in any way as limiting the scope of the present invention.

EXAMPLES

In the examples below, the following abbreviations have the following meanings. Abbreviations not defined have their generally accepted meanings.

	°C	=	degree Celsius
15	hr	=	hour
	min	=	minute
	sec	=	second
	μM	=	micromolar
	mM	=	millimolar
20	M	=	molar
	L or l	=	liter
	ml	=	milliliter
	μl	=	microliter
	G or g	=	gram
25	mg	=	milligram
	μg	=	microgram
	DMEM	=	Dulbecco's modified Eagle's medium
	EGF	=	epidermal growth factor
	FBS	=	fetal bovine serum
30	MEM	=	modified Eagle's medium
	PBS	=	phosphate buffered saline
	PDGF	=	platelet derived growth factor
	PEO	=	polyethylene oxide

Materials and Methods

Reagents

DMN and silymarin were obtained from Sigma Company (St. Louis, MO). Anti- α -SMA and anti-type I collagen antibodies were purchased from Calbiochem-Merck (Cambridge, MA). Fast Green was purchased from Curr, BDH Chemical (Canada), and hematoxylin/eosin staining reagents were obtained from Sigma Company (St. Louis, MO). Kits of GOT, GPT, bilirubin, and albumin were obtained from Wako (Tokyo, Japan). The organic solvents were purchased from Sigma Chemical Co. (St. Louis, MO). Whatman No. 1 filter paper was bought from Fisher Scientific (Fair Lawn, NY).

Preparation of Graptopetalum

Graptopetalum was purchased from a local herb farm in Taiwan. Graptopetalum was washed with distilled water and air dried overnight, then freeze dried at -50°C with a freeze dryer. Graptopetalum was then ground to powder (100 mesh), lyophilized, and stored under sealing condition at 4°C until use. Unless indicated otherwise, Graptopetalum extract was prepared by dissolving Graptopetalum powder in water and gradually adding ethanol to a final concentration of 80%. After centrifugation at $1400\times g$ for 20 min, the supernatant was filtered through a $0.22\text{-}\mu\text{m}$ filter and evaporated to dryness in a rotary evaporator. The recovery of Graptopetalum extract after ethanol treatment was 65% (w/w). The dried extract was dissolved in water to a concentration of 50 mg/ml as a stock solution. The stock solution was then diluted to the appropriate working solution.

Animals

Male Sprague-Dawley rats, weighing 300 to 350 g, were purchased from the National Laboratory Animal Breeding and Research Center, National Science Council, Taiwan. All experiments were performed in compliance with The National Laboratory Animal Breeding and Research Center's guidelines.

Preparation of rats as an animal model for liver injury and fibrosis

Liver injury and fibrosis model rats were prepared by administration of DMN as previous reported (Pines et al., 1997) with minor modification. Briefly, DMN was diluted with phosphate-buffered saline (PBS) and injected intraperitoneally (i.p.) into

rats on the first three days of each week, at seven mg/kg per day, for three weeks. The control group received injection of PBS alone.

Administration of Graptopetalum or silymarin

Seventy-two rats were divided randomly into four experimental groups (n= 18
5 in each group) as follows:

- (1) PBS-water (injection of PBS i.p. and oral administration of water);
- (2) DMN-water (injection of DMN i.p. and oral administration of water);
- (3) DMN-Graptopetalum (injection of DMN i.p. and oral administration of Graptopetalum one week after the first DMN injection); and
- 10 (4) DMN-Silymarin (injection of DMN i.p. and oral administration of silymarin one week after the first DMN injection).

Graptopetalum powder or silymarin was dissolved in water and given orally once per day at a dosage of 1 g/kg body weight (Graptopetalum powder) or 0.4 g/kg body weight (silymarin) for five weeks starting from seven days after the first
15 injection of DMN. Control animals received the same volume of distilled water alone. After the six-week treatment, the rats were weighed and sacrificed, blood samples were collected for determination of biochemical indicators (including GOT, GPT, bilirubin, albumin, prothrombin time, and platelet number), and livers and spleens were excised, weighed, and fixed in formaldehyde for histochemistry.

20 *Histopathological examination of the liver*

Liver specimens were fixed with phosphate-buffered formaldehyde, embedded in paraffin, and stained with hematoxylin-eosin. Differential staining of collagenous and noncollagenous proteins was performed with 0.1% Sirius red and 0.1% fast green as a counter staining in saturated picric acid. By this procedure collagen is stained
25 red. The extent of fibrosis and architectural alteration was scored with a semiquantitative method by an independent pathologist in a blind fashion using liver preparation from each group as described previously (Desmet et al., 1994). Liver tissue sections were photographed using a microscope (Olympus IX70, Japan).

EXAMPLE 1

Graptopetalum prevents body and liver weight loss induced by DMN

To determine the effects of Graptopetalum on liver injury and fibrosis, an animal model was established using DMN, a known toxic substance to the liver.

- 5 Animals which received DMN, as described in Materials and Methods, were then given Graptopetalum and the effects determined. The effects of Graptopetalum were also compared to those of silymarin, which has known hepatoprotective activities. Normal animals, which did not receive DMN, were used as controls.

- 10 The effects of oral administration of Graptopetalum on body and liver weights of rats with and without i.p. injection of DMN are shown in Table 1. Treatment with DMN caused a significant decrease in rat body and liver weights (DMN-water group) compared with control rats (PBS-water group). Orally fed Graptopetalum markedly prevented this DMN-mediated rat body and liver weight loss (DMN-Graptopetalum group); Graptopetalum also reduced DMN-mediated rat spleen weight increase. No
15 changes in body, liver, or spleen weight were observed in normal control rats. These results indicate that Graptopetalum significantly reduced the detrimental impacts of DMN.

Table 1

Body, Liver, Spleen Weight and Survival Number after 6 Weeks of Treatment				
	Survival Number	Body Weight (g)	Liver Weight (g)	Spleen Weight (g)
Control	18	556.5 ± 11.3 (n=18)	24.0 ± 1.4 (n=18)	1.0 ± 0.0 (n=18)
DMN	5	439.5 ± 10.6 (n=5)	14.0 ± 2.8 (n=5)	2.0 ± 0.2 (n=5)
DMN-GP	12	528.5 ± 7.8 (n=12)	23.0 ± 1.2 (n=12)	1.0 ± 0.2 (n=12)
DMN-Silymarin	10	472.0 ± 14.1 (n=10)	19.5 ± 1.4 (n=10)	1.5 ± 0.2 (n=10)

n, number of survived rats in the group

- 20 In addition, after 6 weeks, 13 out of 18 rats died in the DMN-treated group, 8 of 18 rats died in the DMN-silymarin-treated group, and only 6 of 18 rats died in the DMN-treated group orally fed with Graptopetalum (Figure 1). All control rats (18 of each group) not treated with DMN, Graptopetalum, or silymarin survived. Therefore,

Graptopetalum effectively protected the animals from the toxicity of DMN, and it was more effective than silymarin.

EXAMPLE 2

Effects of Graptopetalum on serum bilirubin, albumin, platelets, and prothrombin time

5

To further investigate the effects of Graptopetalum on liver functions, indicators of liver functions were measured. As shown in Table 2, co-administration of Graptopetalum prevented increases in serum GOT and GPT levels after DMN treatment. Moreover, serum bilirubin levels and prothrombin time increased in DMN-treated rats in comparison to normal controls. However, oral administration of Graptopetalum daily significantly prevented the bilirubin increase and albumin decrease in DMN-treated rats. In addition, treatment with DMN resulted in marked reduction of serum platelets and prolongation of prothrombin time. In contrast, improvement of prothrombin time was significant, and numbers of platelet were consistently higher in the DMN-Graptopetalum-treated groups.

15

Table 2

The Level of GOT, GPT, PT, PLT, Bilirubin and Albumin of Serum after 6 Weeks of Treatment						
	GOT (U/L)	GPT (U/L)	PT (sec)	PLT (10 ³ /ul)	Bilirubin (μmole/L)	Albumin (g%)
Control (n=18)	83.0 ± 15.5	51.0 ± 11.3	12.1 ± 0.4	925.0 ± 49.4	0.13 ± 0.01	4.9 ± 0.3
DMN (n=5)	651.5 ± 41.7	273.5 ± 13.4	19.8 ± 1.1	171.0 ± 85.5	1.27 ± 0.04	3.2 ± 0.2
DMN-GP (n=12)	149.5 ± 2.1	71.0 ± 11.3	13.9 ± 1.4	818.0 ± 66.5	0.14 ± 0.02	4.9 ± 0.2
DMN-Silymarin (n=10)	459.4 ± 37.6	217.5 ± 34.6	17.6 ± 1.2	334.3 ± 41.5	1.00 ± 0.04	3.5 ± 0.4

GOT, Glutamic Oxaloacetic Transaminase

GPT, Glutamic Pyruvic Transaminase

PT, Prothrombin Time

PLT, Platelet

n, number of survived rats in the group

Accordingly, Graptopetalum significantly enhanced liver functions.

EXAMPLE 3

Effects of Graptopetalum on inflammatory and necrosis responses following DMN administration

Since DMN-induced liver injury is considered to be involved in inflammatory responses, we next addressed the question whether Graptopetalum prevents necrosis or inflammatory responses in the liver following DMN intoxication. The results show that massive necrosis in the pericentral and mid-zonal area, with infiltration of inflammatory cells, was observed in the liver three weeks following DMN treatment. Co-administration of Graptopetalum, however, prevented the DMN-induced necrosis and inflammation. Thus, Graptopetalum can drastically prevent necrosis and inflammatory responses in the liver.

EXAMPLE 4

Prevention of hepatic fibrogenesis by Graptopetalum in DMN-treated rats

Intraperitoneal administration of DMN resulted in a uniform fine granulation of the surface of the liver. Microscopic analysis revealed cirrhotic-like structural patterns in the liver of animals that had received DMN for six weeks: fibrous connective tissue components in Glisson's sheath, pseudolobule formation, formation of fibrotic septa, and thickened reticulin fibers joining the central areas. Animals treated with both DMN and silymarin still exhibited intense centrilobular and periportal deposition of fibrous connective tissues. In contrast, the livers of DMN-treated rats that received Graptopetalum for five weeks (DMN-Graptopetalum group) showed only mild bridging fibrosis, diminished fibrosis in both the periportal and centrilobular areas, and reduced deformation of the liver acinus. Thus, Graptopetalum can be used to reduce fibrogenesis of liver, which is a key development in liver cirrhosis.

EXAMPLE 5

Graptopetalum reduced hepatic collagen and hydroxyproline content

In a fibrotic liver, extracellular matrix accumulates. The increased levels of extracellular matrix are crucial for further progression of fibrosis, which ultimately leads to liver cirrhosis. Since collagen is a main component in the extracellular matrix, we determined the collagen content of animals treated with DMN alone or the combination of DMN and Graptopetalum.

The content of collagen in liver was detected by Fast Green staining. The DMN-treated rats displayed a gradual and marked increase in collagen fiber content in the liver and bundles of collagen surrounding the lobules, resulting in large fibrous septa, indicating the onset of severe fibrosis. In the DMN-silymarin-treated rats, liver collagen content was also markedly higher than that in healthy controls. However, the thickening of these collagen bundles, which stained red in the staining method used, was reduced markedly in DMN-Graptopetalum-treated rats. There were very low levels of collagen deposition in the livers of control rats. Quantitative analysis of fibrosis by image analysis techniques reveal that the Graptopetalum and silymarin treatments significantly reduced the DMN-induced hepatic collagen content by 80% and 25%, respectively.

Hepatic fibrosis was also quantified by the measurement of hepatic hydroxyproline. It was found that the hydroxyproline content of the DMN-treated group (820 ± 31 $\mu\text{g/g}$) was significantly higher than that of the DMN-Graptopetalum-treated group (558 ± 53 $\mu\text{g/g}$), DMN-silymarin-treated group (749 ± 72 $\mu\text{g/g}$), and control group (498 ± 22 $\mu\text{g/g}$).

Consistent with the previous two examples, these results demonstrate that Graptopetalum markedly prevented hepatic fibrosis.

EXAMPLE 6

Graptopetalum suppressed α -SMA expression in DMN-treated rats

Next, the expression of α -SMA, a marker of activated HSCs, was measured. In a fibrotic liver, extracellular matrix accumulates. The increased levels of extracellular matrix are crucial for further progression of fibrosis, which ultimately leads to liver cirrhosis. Specific liver cells, the hepatic stellate cells (HSC), are responsible for the increase in extracellular matrix. Since activated HSCs, as opposed to resting HSCs, produce large amounts of smooth muscle α -actin (α -SMA) mRNA and protein, α -SMA is an indicator of activated HSC and liver fibrosis.

α -SMA was measured by immunohistochemical staining in the liver six weeks after the first DMN administration. As expected, intense, specific staining for α -SMA was observed in the pericentral area of the DMN-treated livers. This DMN-induced expression of α -SMA was suppressed dramatically by oral administration of

Graptopetalum or silymarin, while Graptopetalum was more effective than silymarin. Livers from rats that received vehicle controls showed negative staining for α -SMA, as expected. Again, these findings indicate that Graptopetalum prevented fibrogenic responses in the liver.

EXAMPLE 7

The anti-proliferative effect of Graptopetalum extracts on cultured rat hepatic stellate cells

Various Graptopetalum extracts were prepared to determine the activity of Graptopetalum on hepatic stellate cell. The extracts were prepared as follows:

Crude aqueous extract

Whole plants of Graptopetalum were lyophilized and powdered. Five grams of the powdered material were extracted twice with 250 mL of water at 25°C for 2 hours on a rotary shaker at 100 rpm. The extract was pink in color after lyophilization.

Water-ethanol extract

The crude aqueous extract described above was stirred with activated charcoal (0.036 g/mL extracts) for 30 min. The mixture was then filtered with Whatman No. 1 filter paper, washed twice with distilled water, and extracted with 0.6 L 50% EtOH. After filtration and vacuum evaporation, 300 mg of dried ethanol extract was obtained as a dark brown paste. The main constituents were glycosides, oligomeric gallotannins, and flavonoids. Terpenoids and saponin were also detected. The dark brown paste was soluble in water and partially soluble in methanol. Precipitates from methanol were analyzed, and they contained polysaccharides and glycoproteins.

Ethanol extract

The activated charcoal described above, after being extracted with 50% ethanol, was then extracted with 0.6 L 95% ethanol. After filtration and vacuum evaporation, 200 mg of dried ethanol extract was obtained. The main constituents of this dark brown paste were flavonoids and terpenoids.

Acetone extract

The activated charcoal described in the last paragraph, after being extracted with 95% ethanol, was extracted with 0.6 L acetonitrile, followed by 0.6 L 70% acetone. The extracts were combined, filtered and vacuum evaporated to yield 50 mg of dried acetone extract. The main constituents were oligomeric gallotannins, flavonoids, saponin, and triterpenoids. This dark brown paste was soluble in water, ethanol, and methanol.

Chloroform extract

The activated charcoal, after being extracted with acetone as described in the last paragraph, was extracted with 0.6 L chloroform. After filtration and vacuum evaporation, dried acetone extract was obtained as a dark brown paste. The main constituents were flavonoids, saponin, and triterpenoids. The dark brown paste was insoluble in water and partially soluble in acetone. The acetone precipitate was white in color.

Crude methanolic extract

Whole plants of *Graptopetalum* were lyophilized and powdered. Five grams of the powdered material were extracted twice with 250 mL of methanol at 25°C for two hours on a rotary shaker at 100 rpm. The extract was filtered and vacuum evaporated. The resulting product was partially water soluble. The water-soluble portion contained 200 milligrams of reddish flavonoids, and the water-insoluble portion contained 400 milligrams of flavonoids and triterpens.

The effects of *Graptopetalum* fractions on the proliferation of HSC was examined. The number of cells increased from $1.2 \pm 0.3 \times 10^5$ (n=9) to $3.6 \pm 0.5 \times 10^5$ (n=9) cells per well during the six-day period of culture. *Graptopetalum* extract inhibited the cell growth of HSC in a dose- and time-dependent manner. The addition of high dose *Graptopetalum* crude extract (500 µg/ml) to cultured activated rat HSCs and hepatocytes resulted in striking morphologic alterations in HSCs after five days as judged by light microscopy. Thus, HSCs changed from a flattened fibroblastic phenotype with distinct cell-cell interfaces to a substratum-detached, rounded morphology, leaving a striking extracellular matrix net structure on the bottom of the culture flasks. In contrast, the crude extract of *Graptopetalum* had almost no effect on

hepatocytes at the same dose. Therefore, Graptopetalum was preferentially toxic to HSCs and ultimately resulted in cell death (Figure 2). Without wishing to be limited to a theory, this selective cytotoxic effect may be the basis of the anti-fibrotic activities of Graptopetalum.

5 The anti-proliferative effects of various Graptopetalum extracts, including the water extract, water-ethanol extract, ethanol extract, methanol extract, acetone extract, and chloroform extract as described above, were determined. As shown in Figure 3, all the tested extracts of Graptopetalum exhibited cytotoxic effects on hepatic stellate cells, indicating that all these extracts have anti-fibrotic activities.

10 The safety of Graptopetalum was demonstrated by further experiments. Daily oral administration of a high dose of Graptopetalum (5 g/kg body weight) for 90 days did not cause any apparent adverse side effects in rats, and there was no evidence of mortality or hepatic damage upon an examination of histological sections of the liver. Moreover, these Graptopetalum-treated rats did not exhibit any significant change in
15 body weight, liver weight, or serum GOT, GPT, bilirubin, and albumin levels in comparison with the control group or a low-dose Graptopetalum-treatment group (1 g/kg) (data not shown). Therefore, Graptopetalum is safe to normal cells even at a high dosage, although it effectively kills activated hepatic stellate cells.

EXAMPLE 8

20 Inhibition of HbsAg expression

 The effects of Graptopetalum on the hepatitis B virus was examined using human hepatoma Hep3B and HepA2 cells, which constitutively express the surface antigen of hepatitis B virus (HbsAg). After incubating Hep3B and HepA2 cells with Graptopetalum extracts, the expression levels of HbsAg were examined with an
25 ELISA assay. As depicted in Figure 4, treatment with 250 µg/ml Graptopetalum significantly reduced the secretion of HbsAg from Hep3B and HepA2 cells, by 75% and 40%, respectively. Therefore, Graptopetalum can be used to inhibit hepatitis B virus replication.

EXAMPLE 9

The effects of Graptopetalum on clinical patients

Fourteen patients were diagnosed with hepatic steatosis (10 men, 4 women; mean age 49 years). The diagnosis was confirmed by ultrasonography examination showing hepatic steatosis in all patients. The patients received an oral dose of 60-200 mg/kilogram body weight per day of Graptopetalum (dry powder) for six months and reexamined with ultrasonography. The results indicate that almost all patients completely recovered from hepatic steatosis. Thus, Graptopetalum is very effective in patients with fatty liver.

In addition, Graptopetalum significantly improved the biochemical markers of patients with light or moderate liver fibrosis. For example, in one study, Graptopetalum significantly reduced the levels of serum GOT, GPT, and bilirubin in 10 of 16 patients (11 men and 5 women; mean age 52 years) while it elevated the level of serum albumin. Based on their ultrasonography images, it was also found that Graptopetalum significantly reduced liver fibrosis in several patients. Moreover, Graptopetalum improved the quality of life for 4 patients with terminal stage of liver cirrhosis (3 men and 1 woman, mean age 51 years).

EXAMPLE 10

Effects of Graptopetalum on lung fibrosis

Isolation of lung fibroblasts: Minced pieces of lung tissue was digested for 1 h with collagenase (0.5 mg/ml) and DNase (0.05 mg/ml) in Hanks' balanced salt solution which contained 0.5 mM EDTA. Cells were separated from the digested tissue by filtration through polyamide nylon mesh, collected by centrifugation, and washed once in medium. After resuspension in DMEM containing 10% FCS, cells were plated on culture plates. After three passages, cultures contained approximately 100% lung fibroblasts. Primary cultured lung fibroblasts were treated with various Graptopetalum extracts for five days. After the incubation, cell number was directly counted by the Trypan blue dye exclusion method.

The results show that all tested Graptopetalum extracts inhibited cell growth of lung fibroblast in a dose-dependent manner (Figure 5). The anti-proliferative effect

on lung fibroblasts thus can serve as the basis of the anti-fibrotic activities of Graptopetalum in the lung.

EXAMPLE 11

The effects of Graptopetalum on the lung

5 An animal model of bleomycin-induced pulmonary fibrosis was used to study the effect of Graptopetalum on the lung. Male C57BL/6 mice (6-8 weeks old) were anesthetized with ketamine and given intratracheal injections of either 0.15 U/kg of bleomycin (Blenoxane; Sigma-Aldrich, St. Louis, Missouri, USA) or sterile saline. The mice were sacrificed for analysis 1, 3, 7, 14, 21 days after exposure to bleomycin.
10 In separate experiments, mice treated with bleomycin were given daily oral administration of 1 g/kg body weight of Graptopetalum powder or water until sacrifice. Animal use for these studies was approved by the Institutional Animal Care and Use Committees of the Taichung Veterans General Hospital, Taiwan.

 Five animals from each treatment group were randomly chosen for histologic
15 evaluation of their lungs at the end of the experiment, as follows. After sacrifice, the lung tissue was fixed by a buffered 10% formalin solution for 48 h and embedded in paraffin. The tissues were then sectioned at 3 μ m, stained with hematoxylin and eosin, and examined for inflammatory cell infiltration and pulmonary fibrosis. The criteria for scoring pulmonary fibrosis were as follows: Grade 0 = normal lung;
20 Grade 1 = minimal fibrous thickening of alveolar or bronchiolar walls; Grade 2 = increased fibrosis with definite damage to the lung structure and the formation of fibrous bands or small fibrous masses; and Grade 3 = severe distortion of the lung structure and large fibrous areas.

 All mice survived until the time of killing, but the mice treated with only
25 bleomycin suffered significant weight loss and were in a worse condition than the mice treated with Graptopetalum. In the histological studies, lungs from the bleomycin/water group showed diffuse and marked infiltration of inflammatory cells, as well as increased alveolar wall thickness with typical fibrotic changes. In contrast, lungs from the bleomycin/Graptopetalum group showed fewer fibrotic lesions and
30 local infiltrations of inflammatory cells. Lungs from the Graptopetalum group, which did not receive bleomycin, did not show any identifiable lesions, demonstrating that Graptopetalum itself did not cause any change to the lung. The pulmonary fibrosis

scores for the bleomycin/Graptopetalum group (1.4 ± 0.5) were significantly lower than that for the bleomycin/water group (2.6 ± 0.4). The scores for the normal control group (saline/water) and the Graptopetalum group (saline/Graptopetalum) were approximately 0 and 0, respectively.

5 These results are consistent with those in Example 10, which indicate that Graptopetalum inhibits the proliferation of lung fibroblasts. Accordingly, Graptopetalum is effective against pulmonary fibrosis and inflammation.

 A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without
10 departing from the spirit and scope of the invention.

We claim:

1. A pharmaceutical composition comprising Graptopetalum or Echeveria.
2. The pharmaceutical composition as claimed in claim 1 wherein the
5 Graptopetalum is *Graptopetalum paraguayense*.
3. The pharmaceutical composition as claimed in claim 1 or 2 wherein the Graptopetalum or Echeveria is an extract.
4. The pharmaceutical composition as claimed in claim 3 wherein the
10 extract is an extract of one or more aqueous solvents, nonpolar solvents, alcohol solvents, or mixtures thereof.
5. The pharmaceutical composition as claimed in any one of claims 1-4 further comprising a pharmaceutically acceptable excipient or carrier.
6. The pharmaceutical composition as claimed in any one of claims 1-5 suitable for oral or topical administration.
- 15 7. A method of preventing or treating a liver disease or medical condition in a subject, comprising administering an effective amount of the pharmaceutical composition of any one of claims 1-6 to the subject, wherein the liver disease or medical condition is selected from the group consisting of liver inflammation, liver steatosis, liver fibrosis, liver cirrhosis, and hepatitis B.
- 20 8. The method as claimed in claim 7 wherein the liver disease or medical condition is liver inflammation.
9. The method as claimed in claim 7 wherein the liver disease or medical condition is liver fibrosis.
10. The method as claimed in claim 9 wherein the expression of smooth
25 muscle α -actin is reduced.
11. The method as claimed in claim 10 wherein the expression of smooth muscle α -actin is reduced in hepatic stellate cells.
12. The method as claimed in claim 7 wherein the liver disease or medical condition is liver cirrhosis.

13. The method as claimed in claim 7 wherein the liver disease or medical condition is hepatitis B.

14. The method as claimed in claim 7 wherein the liver disease or medical condition is liver steatosis

5 15. A method for inhibiting hepatic stellate cell proliferation, comprising contacting hepatic stellate cells with the pharmaceutical composition of any one of claims 1-6.

16. The method as claimed in claim 15 wherein the hepatic stellate cells are located in a mammal.

10 17. The method as claimed in claim 16 wherein the mammal is human.

18. A method for selectively killing hepatic stellate cells, comprising contacting hepatic stellate cells with the pharmaceutical composition of any one of claims 1-6.

15 19. The method as claimed in claim 18 wherein the hepatic stellate cells are located in a mammal.

20. The method as claimed in claim 19 wherein the mammal is human.

21. A method for inhibiting hepatitis B virus surface antigen expression in a mammal, comprising administering to the mammal the pharmaceutical composition of any one of claims 1-6.

20 22. The method as claimed in claim 21 wherein hepatitis B virus replication is inhibited in the mammal.

23. The method as claimed in claim 22 wherein the mammal is human.

24. A method for preventing or treating fibrosis or inflammation in a mammal, comprising administering the composition of any one of claims 1-6 to the
25 mammal.

25. The method as claimed in claim 24 wherein the fibrosis or inflammation occurs in an organ selected from the group consisting of lung, kidney, and bladder.

26. The method as claimed in claim 24 or 25 wherein the composition is
30 administered orally.

27. The method as claimed in claim 24 or 25 wherein the composition is administered by injections.

28. The method as claimed in any one of claims 24-27 further comprising administering to the mammal an additional therapeutic or prophylactic agent for
5 inflammation or fibrosis.

29. Use of Graptopetalum or Echeveria in the manufacture of a medicament for therapeutic use.

30. The use as claimed in claim 29 wherein the Graptopetalum is *Graptopetalum paraguayense*.

10 31. The use as claimed in claim 30 wherein the Graptopetalum or Echeveria is an extract.

32. Use of Graptopetalum or Echeveria as a medicament for the treatment of liver disease.

15 33. The use as claimed in claim 32 wherein the Graptopetalum is *Graptopetalum paraguayense*.

34. The use as claimed in claim 32 wherein the Graptopetalum or Echeveria is an extract.

35. The use as claimed in claim 32 wherein the liver disease is liver inflammation, liver steatosis, liver fibrosis, liver cirrhosis, or hepatitis B.

20 36. The use as claimed in claim 32 wherein the medicament reduces the expression of smooth muscle α -actin.

37. The use as claimed in claim 36 wherein the medicament reduces the expression of smooth muscle α -actin in hepatic stellate cells.

25 38. The use as claimed in claim 32 wherein the medicament inhibits hepatic stellate cell proliferation.

39. The use as claimed in claim 32 wherein the medicament inhibits hepatitis B virus surface antigen expression in a mammal.

40. The use as claimed in claim 21 wherein hepatitis B virus replication is inhibited in the mammal.

41. The use as claimed in claim 32 wherein the medicament prevents or treats fibrosis or inflammation in a mammal.

42. The use as claimed in claim 41 wherein the fibrosis or inflammation occurs in an organ selected from the group consisting of lung, kidney, and bladder.

5 43. The use as claimed in claim 40 or 41 wherein the medicament is formulated for oral administration.

44. The use as claimed in claim 40 or 41 wherein the medicament is formulated for administration by injection.