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(54) Title: GALACTOMANNAN POLYSACCHARIDE COMPOSITION FOR THE TREATMENT OF NONALCOHOLIC STEATOHEPATITIS AND NONALCOHOLIC FATTY LIVER DISEASE

(57) Abstract: Aspects of the invention provides provide methods for treatment of NASH and associated liver fibrosis. In particular, aspects of the invention relate to the therapeutic formulation comprising a galactomannan polysaccharide compound for the treatment of NASH and associated liver fibrosis.

GALACTOMANNAN POLYSACCHARIDE COMPOSITION FOR THE TREATMENT OF NONALCOHOLIC STEATOHEPATITIS AND NONALCOHOLIC FATTY LIVER DISEASE

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. provisional Application Serial No. 61/535,655, filed September 16, 2011, and U.S. provisional Application Serial No. 61/656,288, filed June 6, 2012, the entire disclosure of each of which is incorporated herein by reference in their entireties.

BACKGROUND OF THE INVENTION

[0002] Nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) are common liver disorders in the United States. Histopathologically, these disorders resemble alcoholic liver disease, but they occur in people who drink little or no alcohol. The pathological changes in the liver include, but are not limited to, fat accumulation in hepatocytes, evidence of hepatocellular degeneration, infiltrates of inflammatory cells, deposition of excess fibrous tissue, hepatocellular nodule formation, cirrhosis, and hepatocellular carcinoma. To date, no specific therapies for these disorders exist. Therefore, there is a need to provide methods for treatment of nonalcoholic steatohepatitis with or without associated liver fibrosis.

SUMMARY OF THE INVENTION

[0003] Aspects of the invention relate to methods of treating a subject having a fatty liver, nonalcoholic fatty liver disease (NALFD), nonalcoholic steatohepatitis (NASH), nonalcoholic steatohepatitis with liver fibrosis, nonalcoholic steatohepatitis with cirrhosis, or nonalcoholic steatohepatitis with cirrhosis and hepatocellular carcinoma, using a therapeutic composition comprising a galactose-containing polysaccharide

compound in an acceptable pharmaceutical carrier for parenteral or enteral administration. In some aspects, the invention relate to compositions having a galactomannan polysaccharide for the treatment of fatty liver, NALFD, NASH, NASH with liver fibrosis, NASH with cirrhosis, or NASH with cirrhosis and hepatocellular carcinoma. Other aspects of the invention relate to the use of a galactomannan polysaccharide in the manufacture of a pharmaceutical composition for the treatment of fatty liver, NALFD, NASH, NASH with liver fibrosis, NASH with cirrhosis, or NASH with cirrhosis and hepatocellular carcinoma. In some embodiments, an admixture having a galactomannan polysaccharide and a therapeutic agent can be used for the treatment or in the manufacture of a pharmaceutical composition for treatment of fatty liver, NALFD, NASH, NASH with liver fibrosis, NASH with cirrhosis, or NASH with cirrhosis and hepatocellular carcinoma.

[0004] In some embodiments, the method comprises the steps of obtaining a composition for parenteral or enteral administration comprising a galactomannan polysaccharide compound in an acceptable pharmaceutical carrier; administering to a subject an effective dose of the composition for parenteral administration, the subject having one of a fatty liver, NALFD, NASH, NASH with liver fibrosis, NASH with cirrhosis, or NASH with cirrhosis and hepatocellular carcinoma.

[0005] In some embodiments, the effective dose of the composition, when administered in a subject in need thereof, can result in reduction of at least one point in severity of NALFD or NASH grading scoring systems, reduction of the level of serum markers of NASH activity, reduction of NASH disease activity or reduction in the medical consequences of NASH.

[0006] In some embodiments, the efficacy of the composition for parenteral administration can be determined by administering the composition to animal models of NASH, including but not limited, to mice rendered diabetic and fed a high fat diet, resulting in at least 5% reduction in hepatocellular fat accumulation, at least 5% reduction in liver infiltration of inflammatory cells, or at least a 5% reduction in liver collagen content as determined by morphometric quantification.

[0007] In some embodiments, the effective dose of the composition, when administered in a subject in need thereof, can result in the reduction in the accumulation of fat in the liver (steatosis) as determined from liver histological sections by assessment of micro-vesicular and macro-vasicular fat particles in hepatocytes. In some embodiments, the accumulation of fat in the liver is reduced by at least 10% as assessed in percentage of hepatocytes with fat and graded as per NAFLD grading system or by image analysis.

[0008] In some embodiments, the effective dose of the composition, when administered in a subject in need thereof, can result in the reduction of hepatocyte ballooning as determined from liver histological section by assessment of swelling of hepatocytes indicating toxicity and inability to regulate cellular volume. In some embodiments, the hepatocyte ballooning is reduced by at least 10% as assessed in percentage of swollen hepatocytes and graded as per NAFLD grading system.

[0009] In some embodiments, the effective dose of the composition, when administered in a subject in need thereof, can result in the reduction in the infiltration of inflammatory cells in liver histological specimens as assessed by the number of neutrophils and lymphocytes in portal, central and lobular areas of the liver specimens. In some embodiments, the infiltration of inflammatory cells in liver histological specimens is reduced by at least 10% less as assessed in percentage of inflammatory cells graded using the NAFLD grading system.

[00010] In some embodiments, the effective dose of the composition, when administered in a subject in need thereof, can result in the reduction in the level of serum markers of NASH activity. In some embodiments, the serum markers of NASH activity can include, but are not limited to, serum levels of transaminases, serum levels of coenzyme Q reduced or oxidized, or a combination of other serum markers of NASH activity known in the art.

[00011] In some embodiments, the effective dose of the composition, when administered in a subject in need thereof, can result in the reduction of liver fibrosis or cirrhosis based on evidence comprising a reduction of the level of the biochemical

markers of fibrosis, non invasive testing of liver fibrosis or cirrhosis or liver histologic grading of fibrosis or cirrhosis.

[00012] In some embodiments, the effective dose of the composition, when administered in a subject in need thereof, can result in the reduction of at least one point in severity of NAFLD or NASH grading scoring systems, including but not limited to, NAFLD activity score (NAS), proposed by the NASH Clinical Research Network (established in 2002 by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)), a widely used scoring system.

[00013] In some embodiments, the effective dose of the composition, when administered in a subject in need thereof, can result in the reduction in the medical consequences of NASH with liver fibrosis or cirrhosis comprising portal hypertension, reduced hepatic protein synthesis, hyperbilirubinemia, or encephalopathy.

[00014] In some embodiments, the effective dose of the composition, when administered in a subject in need thereof, can result in the reduction in disease activity based on at least a 10% reduction in liver tissue or serum galectin-3.

[00015] In some embodiments, the effective dose of the composition, when administered in a subject in need thereof, can result in the reduction of accumulation of collagen in the liver as determined by quantitative analysis of Sirius Red staining of liver histological sections. In some embodiments, the reduction of accumulation of collagen in the liver is reduced by at least 5% less as assessed in percentage of liver tissue staining positive for Sirius red indicating collagen.

[00016] In some embodiments, the galactomannan polysaccharide consists essentially of galactose and mannose residues and resulting from a sufficiently controlled depolymerization of galactomannan so as to result in a galactomannan polysaccharide composition with a defined average molecular weight.

[00017] In some embodiments, the galactomannan polysaccharide compound consists essentially of galactose and mannose residues and resulting from a sufficiently controlled depolymerization of galactomannan so as to result in a homogenous galactomannan polysaccharide has an average weight of 4,000 to 60,000 D as assayed by GPC-MALLS.

[00018] In some embodiments, the galactomannan polysaccharide compound has a ratio of mannose to galactose molecules in a range of 1:1 to 1:4.

[00019] In some embodiments, the galactomannan polysaccharide compound has a ratio of mannose to galactose molecules of 1.7:1.

[00020] In some embodiments, the compound is a galactomannan polysaccharide used in combination with a therapeutically effective amount of cysteamine or a pharmaceutically acceptable salt thereof, or cystamine or a pharmaceutically acceptable salt thereof.

[00021] In some embodiments, the compound is a galactomannan polysaccharide used in combination with a therapeutically effective amount of various anti-oxidant compounds including but not limited to parenteral or oral administration of compositions comprising glycyrrhizin, schisandra, ascorbic acid, L-glutathione, silymarin, lipoic acid, and d-alpha-tocopherol.

[00022] In some embodiments, the compound is a galactomannan polysaccharide used in combination with a therapeutically effective amount of various anti-oxidant compounds including but not limited to parenteral or oral administration of compositions comprising a water soluble Vitamin E preparation, mixed carotenoids, or selenium.

[00023] In some embodiments, the compound is a galactomannan polysaccharide used in combination with a therapeutically effective amount of parenteral or oral administration of lecithin or vitamin B complex.

[00024] In some embodiments, the compound is a galactomannan polysaccharide used in combination with a therapeutically effective amount of bile salt preparations including but not limited to ursodeoxycholic acid, chenodeoxycholic acid of other naturally occurring or synthetic bile acids or bile acid salts.

[00025] In some embodiments, the compound is a galactomannan polysaccharide used in combination with a therapeutically effective amount of antagonists and/or inverse agonists of the Cannabinoid-1 (CB1) receptor.

[00026] In some embodiments, the compound is a galactomannan polysaccharide used in combination with a therapeutically effective amount of a PPAR (peroxisome proliferator-activated receptor) activity regulator.

[00027] In some embodiments, the compound is a galactomannan polysaccharide used in combination with a therapeutically effective amount of a benzothiazepine or benzothiepine compound having a thioamide bond and a quaternary ammonium substituent.

[00028] In some embodiments, the compound is a galactomannan polysaccharide used in combination with a therapeutically effective amount of an RNA antisense construct to inhibit protein tyrosine phosphatase PTPRU.

[00029] In some embodiments, the compound is a galactomannan polysaccharide used in combination with a therapeutically effective amount of a heteroatom-linked substituted piperidine and derivatives thereof useful as histamine H.sub.3 antagonists.

[00030] In some embodiments, the compound is a galactomannan polysaccharide used in combination with a therapeutically effective amount of a axacyclopentane derivative that inhibits stearoyl-coenzyme alpha delta-9 desaturase.

[00031] In some embodiments, the compound is a galactomannan polysaccharide used in combination with a therapeutically effective amount of a acylamide compound having secretagogue or inducer activity of adiponectin.

[00032] In some embodiments, the compound is a galactomannan polysaccharide used in combination with a therapeutically effective amount of quaternary ammonium compounds.

[00033] In some embodiments, the compound is a galactomannan polysaccharide used in combination with a therapeutically effective amount of a isoflavone compound.

[00034] In some embodiments, the compound is a galactomannan polysaccharide composition used in combination with a therapeutically effective amount of a macrolide antibiotic.

[00035] In some embodiments, the compound is a galactomannan polysaccharide used in combination with a therapeutically effective amount of Glatiramer acetate (also known as Copolymer 1, Cop-1, or Copaxone - as marketed by Teva Pharmaceuticals), an immunomodulator drug currently used to treat multiple sclerosis.

[00036] In some embodiments, the compound is galactomannan polysaccharide used in combination with a therapeutically effective amount of a stain, for example but not limited to HMG-CoA reductase inhibitors such as atorvastatin and simvastatin.

[00037] In some embodiments, the compound is a galactomannan polysaccharide used in combination with a therapeutically effective amount of an n-acetyl cysteine.

[00038] In some embodiments, the compound is a galactomannan polysaccharide used in combination with a therapeutically effective amount of another galectin inhibitor that may inhibit single galectin proteins or a set of galectin proteins including but not limited small organic inhibitors of galectin, monoclonal antibodies, RNA inhibitors, small binding peptides, or protein inhibitors or any combinations of the foregoing.

[00039] In some embodiments, the compound is a used in combination with a therapeutically effective amount of a monoclonal antibody to inhibit lysyl oxidase (or other proteins that cross link collagens) or monoclonal antibody to connective tissue growth factor.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[00040] The present invention will be further explained with reference to the attached drawings, wherein like structures are referred to by like numerals throughout the several views. The drawings shown are not necessarily to scale, with emphasis instead generally being placed upon illustrating the principles of the present invention.

[00041] FIGURE 1 is a schematical representation of the Experimental Design of Therapy in STAM Mouse Model of Steatohepatitis

[00042] FIGURE 2A is a graph showing the changes in body weight of STAM mice in treatment groups at weeks 6-9. FIGURE 2B is a graph showing the changes in body weight of STAM mice in treatment groups at weeks 9-12.

[00043] FIGURE 3A is a graph showing the whole blood glucose in STAM mice between the treatment groups at weeks 6-9. FIGURE 3B is a graph showing the whole blood glucose in STAM mice between the treatment groups at weeks 9-12.

[00044] FIGURE 4 shows the histology of the normal and the NASH mouse model stained with hematoxylin and eosin (H&E) and with Sirius Red.

[00045] FIGURE 5A shows the histology comparison of STAM mice between the treatment groups. FIGURE 5B is a graph showing the NAFLD activity score in STAM mice in the treatment groups. FIGURE 5C is a graph showing the steatosis grade score in STAM mice in the treatment groups.

[00046] FIGURE 6 shows the liver histology with Sirius red staining of experimental groups at weeks 6-9 and 9-12.

[00047] FIGURE 7A is a graph showing the comparison of Sirius red-positive area in liver histology between experimental groups at weeks 6-9. FIGURE 7B is a graph showing the comparison of Sirius red-positive area in liver histology between experimental groups at weeks 9-12. FIGURE 7C is a graph showing the comparison of Sirius red-positive area in liver histology between experimental groups for all animals

[00048] FIGURE 8A shows the immunohistochemical staining of alpha-Smooth Muscle Actin (SMA) in liver tissue of experimental groups. FIGURE 8B is a graph showing the digital morphometry of alpha-Smooth Muscle Actin (SMA) in liver tissue of experimental groups.

[00049] FIGURE 9A shows the immunohistochemical staining of galectin-3 in liver tissue of experimental groups. FIGURE 9B is a graph showing the digital morphometry of galectin-3 in liver tissue of experimental groups.

DETAILED DESCRIPTION OF THE INVENTION

[00050] Detailed embodiments of the present invention are disclosed herein; however, it is to be understood that the disclosed embodiments are merely illustrative of the invention that may be embodied in various forms. In addition, each of the examples given in connection with the various embodiments of the invention is intended to be illustrative, and not restrictive. Further, the figures are not necessarily to scale, some features may be exaggerated to show details of particular components. In addition, any measurements, specifications and the like shown in the figures are intended to be illustrative, and not restrictive. Therefore, specific structural and functional details

disclosed herein are not to be interpreted as limiting, but merely as a representative basis for teaching one skilled in the art to variously employ the present invention.

[00051] Unless otherwise specified, all percentages expressed herein are weight/weight.

[00052] The major feature in Nonalcoholic Fatty Liver Disease (NAFLD) is fat accumulation in hepatocytes with minimal inflammation. These patients are usually identified on the basis of a liver biopsy performed because of mildly elevated liver transaminase levels in the serum or the suspicion of fatty liver on non-invasive testing such as computerized tomography or ultrasound.

[00053] A subset of individuals with NAFLD are found to have Nonalcoholic Steatohepatitis (NASH) which is fatty liver with the addition of the development of infiltration of inflammatory cells (including but not limited to neutrophils or lymphocytes) within the lobule, central vein and portal areas and evidence of damage to hepatocytes including but not limited to ballooning degeneration. This inflammatory state of NASH may result in the deposition of fibrous tissue, including but not limited to collagen, which can lead to cirrhosis, nodule formation, and eventually hepatocellular carcinoma.

[00054] The disease progress is insidious since most people with NASH feel well and are not aware that they have a liver problem. Despite the lack of symptoms, NASH can be severe and can lead to the deposition of fibrotic material in the liver which can result in severe scarring and/or cirrhosis and, in some cases, hepatocellular carcinoma. Therefore, there is a need for clinical tests that could identify NASH early and follow its progression.

[00055] NAFLD and NASH are common disorders. It is reported by the U.S. National Institutes of Health that 10-20 percent of Americans have NAFLD and 3-5 percent have NASH. Both are becoming more common because of the greater numbers of people with obesity and diabetes, including children and adolescents. The fact that NASH can progress to cirrhosis makes this a major health problem.

[00056] Although NASH has become more common, its underlying cause is still not clear. It most often occurs in middle-aged persons who overweight or obese, many of whom have metabolic syndrome, insulin resistance, or overt diabetes. However,

NASH is not simply obesity that affects the liver. NASH can affect children and adolescents.

[00057] The proximal cause of liver injury in NASH is not known. Multiple theories have been proposed, with some experimental data to suggest their involvement. Some of these include, but are not limited to, hepatocyte resistance to the action of insulin, production of inflammatory cytokines by fat cells and other inflammatory cells that damage the liver and recruit additional inflammatory cells and oxidative stress in hepatocytes with production of reactive oxygen radicals that damage liver cells and induce inflammation.

[00058] Currently, no specific therapies for NASH exist and only general health recommendations are currently provided to patients. These include weight reduction, eating a balanced and healthy diet, increasing physical activity, and avoidance of alcohol and unnecessary medications. Weight loss can improve serum liver tests in some patients with NASH and may improve evidence of histological liver damage, but it does not reverse severe liver disease and not all patients with NASH are overweight.

[00059] A variety of experimental approaches have been evaluated, or are under evaluation in patients with NASH including the use of antioxidants, such as vitamin E, selenium, betaine, and anti-diabetic agents including metformin, rosiglitazone, and pioglitazone. All clinical results to date have been disappointing.

[00060] The galectin-3 protein has recently been implicated in the pathogenesis of NASH. Galectins (also known as galaptins or S-lectin) are a family of lectins which bind beta-galactoside. Galectin as general name was proposed in 1994 for a family of animal lectins (Barondes, S.H., et al.: Galectins: a family of animal b-galactoside-binding lectins. Cell 76, 597-598, 1994), The family is defined by having at least one characteristic carbohydrate recognition domain (CRD) with an affinity for beta-galactosides and sharing certain sequence elements. Within the same peptide chain, some galectins have a CRD with only a few additional amino acids, whereas others have two CRDs joined by a link peptide, and one (galectin-3) has one CRD joined to a different type of domain. The galectin carbohydrate recognition domain (CRD) is a beta-sandwich of about 135 amino acids. The two sheets are slightly bent with 6 strands

forming the concave side and 5 strands forming the convex side. The concave side forms a groove in which carbohydrate is bound (Leffler H, Carlsson S, Hedlund M, Qian Y, Poirier F (2004). "Introduction to galectins". Glycoconj. J. 19 (7-9): 433–40).

[00061] A wide variety of biological phenomena have been shown to be related to galectins, e.g., development, differentiation, morphogenesis, tumor metastasis, apoptosis, RNA splicing, etc. However, relatively little is known about the mechanism by which galectins exert these functions, particularly in terms of carbohydrate recognition.

[00062] Generally, the carbohydrate domain binds to galactose residues associated with glycoproteins. At least fifteen mammalian galectin proteins have been identified which have one or two carbohydrate domain in tandem.

[00063] Each galectin protein has a galactose binding domain and other domains that allow homo- or hetero-dimerization to other galectin proteins. Galectin proteins are expressed in a broad range of cells and tissues at low levels under physiological conditions and are found in the nucleus, cytoplasm, and are secreted into the extracellular space by a non-traditional secretory pathway.

[00064] The galactose binding domain of galectins binds to galactose containing glycoproteins located on the cell surface or on extracellular matrix proteins. The dimerization domains on galectins promote interaction of galectin proteins, thereby creating interaction between membrane or matrix glycoproteins. These interactions promote cell-cell, cell-matrix, and matrix-matrix interactions and association of membrane receptors that can cause activation, inactivation, or modulation of cell receptor activity leading to modulation of intracellular signaling and subsequent events.

[00065] Certain galectin molecules are markedly up-regulated and secreted in high amounts from cells in pathological situations. Multiple inflammatory cells, including but not limited to macrophages and lymphocytes, in tissue inflammation states and repair (fibrosis, scarring) express galectins, particularly galectin-1 and galectin-3.

[00066] Mice that lack the galectin-3 gene have been used to explore the function of galectin-3 in a number of disease states that include inflammation and fibrogenesis as key components. These galectin-3 knockout mice have been shown to be resistant

to liver fibrogenesis due to toxin administration, lung fibrogenesis, and kidney fibrogenesis.

[00067] Galectin-3 knockout mice have also been used to explore the importance of galectin-3 in NASH. In these experiments, mice were fed a high fat diet to induce the development of NAFLD and NASH. Normal mice readily developed fatty liver, inflammatory infiltrates in the liver and liver fibrosis. In stark contrast, the galectin-3 knockout mice did not develop as much fatty liver, and had minimal inflammatory infiltrate and fibrosis. These data suggest that galectin-3 might be an important target for therapy of NASH.

[00068] Inhibition of galectin-3 is one potential mechanism underlying the efficacy of galactomannan oligosaccharide compositions in this invention.

[00069] The term “effective dose” means the amount of galactomannan oligosaccharide or other agent in combination with the galactomannan oligosaccharide composition that, when administered as a parental dose or in an oral formulation to an animal or human with NAFLD, NASH, or NASH with fibrosis or cirrhosis, is capable of improving NAS score by at least one point or reducing percent collagen area by at least 5%. In some embodiments, a therapeutically effective dose can be evaluated by a reduction of at least 10% in the level of galectin-3 in liver tissue or serum. In some embodiments, a therapeutically effective dose can be evaluated by a change in the level of galectin-3 in serum.

[00070] The term "pharmaceutically acceptable carrier" refers to any and all solvents, dispersion media, e.g., human albumin or cross-linked gelatin polypeptides, coatings, antibacterial and antifungal compounds, isotonic, e.g., sodium chloride or sodium glutamate, and absorption delaying compounds, and the like that are physiologically compatible. The use of such media and compounds for pharmaceutically active substances is well known in the art. Preferably, the carrier is suitable for oral, intravenous, intramuscular, subcutaneous, parenteral, spinal or epidural administration (e.g., by injection or infusion). Depending on the route of administration, the active compound can be coated in a material to protect the compound from the action of acids and other natural conditions that can inactivate the compound.

[00071] The term “efficacy” means demonstrating an improvement in the liver histology findings associated with NASH or NASH with fibrosis or cirrhosis as determined by the NAS score or percent collagen.

[00072] In some embodiments, the method comprises the steps of obtaining a composition for parenteral or enteral administration comprising a galactomannan polysaccharide compound in an acceptable pharmaceutical carrier. In some embodiments, the compound is a galactomannan oligosaccharide consisting essentially of galactose and mannose residues and resulting from a sufficiently controlled depolymerization of galactomannan so as to result in a galactomannan polysaccharide composition with a defined average molecular weight.

[00073] As used herein, the term “"depolymerization" refers to partial or complete hydrolysis of the polysaccharide backbone occurring for example when the polysaccharide is treated chemically and resulting in fragments of reduced size when compared with the original polysaccharide.

[00074] Galactomannan can be obtained from a variety of natural sources such as plants and microbial sources. The polysaccharide can also be synthetically made. Galactomannan can be derived from carob gum (Ceratonia siliqua), guar gum (Cyamopsis tetragonoloba), and honey locust (Gleditsia triacanthos), are examples of commercial available galactomannans. The polysaccharides include, but are not limited to, galactomannans available from a number of plant and microbial sources. For example, the galactomannan can be a derivative of Guar gum from seeds of Cyamopsis tetragonoloba. Yet in other embodiments, the galactomannan can be a derivative of Gleditsia triacanthos, medicago falcate, Trigonella Foenum-graecum and microbial like Ceratonia siliqua Xanthomonas campestris, yeast and mold galactomannan, Arabinogalactan (from Larix occidentalis), Rhamnogalacturonan (from potato), Carrageenan (from Eucheuma Seaweed), and the Locust Bean Gum (from Ceratonia siliqua.

[00075] As used herein, the term “backbone" means the major chain of a polysaccharide, or the chain originating from the major chain of a starting polysaccharide, having saccharide moieties sequentially linked by either .alpha or .beta

glycosidic bonds. A backbone may comprise additional monosaccharide moieties connected thereto at various positions along the sequential chain.

[00076] In some embodiments, the galactomannan polysaccharide composition consists essentially of galactose and mannose residues and resulting from a sufficiently controlled depolymerization of galactomannan so as to result in a homogenous galactomannan polysaccharide. In some embodiments, the galactomannan polysaccharide has an average weight of 4,000 to 60,000 D, as assayed by GPC-MALLS (galactomannan).

[00077] In some embodiments, the galactomannan polysaccharide composition has a ratio of mannose to galactose molecules in a range of 1:1 to 1:4.

[00078] In some embodiments, the galactomannan polysaccharide composition has a ratio of mannose to galactose molecules of 1.7.

[00079] In some embodiments, the galactomannan polysaccharide composition is produced as described in US 7,893,252 incorporated expressly by reference in its entirety for all purposes. The process is designed to generate a highly pure soluble and homogeneous oligomer with an average molecular weight in the range of about 48,000 daltons, and mannose to galactose ratio in the range of about 1.7:1. The process incorporates four major phases: controlled depolymerization to produce the desired galactomannan oligomer and three purification steps, removal of insoluble impurities, removal of water soluble impurities, removal of organic soluble impurities, and finally freeze drying to generate a pure and stable form of galactomannan powder. In some embodiments, the product is in the form of a highly soluble oligomer of Galactomannan (GM).

[00080] Galactomannan can be packaged and delivered as a sterile concentrated solution in a single use vial, while bulk galactomannan can be produced and stored as powder. The process described herein is for both bulk drug and final drug product. The galactomannan drug product can be combined and administered together with a therapeutically effective amount of a therapeutic agent to form the active ingredients of a pharmaceutical preparation. In some embodiments, the drug product can contain

normal saline for infusion (about 0.9 M sodium chloride in water) and has a pH of about 6.5.

[00081] In some embodiments, the compound is a galactomannan polysaccharide composition can be used in combination with a therapeutically effective amount of a therapeutic agent. In some embodiment, the galactomannan polysaccharide can be used in admixture. "Admixture" means more than one component mixed together to form a combination. For purposes of the present invention, "admixture" means the mixture of two or more compounds at any time prior or subsequent to, or concomitant with, administration.

[00082] In one embodiment, the therapeutic agent can be cysteamine or a pharmaceutically acceptable salt thereof, or cystamine or a pharmaceutically acceptable salt thereof. [7,994,226, incorporated expressly by reference for all purposes.]

[00083] In some embodiments, the compound is a galactomannan polysaccharide composition can be used in combination with a therapeutically effective amount of various anti-oxidant compounds including but not limited to parenteral or oral administration of compositions comprising glycyrrhizin, schisandra, ascorbic acid, L-glutathione, silymarin, lipoic acid, and d-alpha-tocopherol. [7,078,064, incorporated expressly by reference for all purposes.]

[00084] In some embodiments, the compound is a galactomannan polysaccharide composition can be used in combination with a therapeutically effective amount of various anti-oxidant compounds including but not limited to parenteral or oral administration of compositions comprising a water soluble Vitamin E preparation, mixed carotenoids, or selenium [6,596,762, incorporated expressly by reference for all purposes.]

[00085] In some embodiments, the compound is a galactomannan polysaccharide composition can be used in combination with a therapeutically effective amount of parenteral or oral administration of lecithin or vitamin B complex [7,018,652; 6,180,139, incorporated expressly by reference for all purposes.]

[00086] In some embodiments, the compound is a galactomannan polysaccharide composition can be used in combination with a therapeutically effective amount of bile

salt preparations including but not limited to ursodeoxycholic acid, chenodeoxycholic acid of other naturally occurring or synthetic bile acids or bile acid salts. [6,297,229, incorporated expressly by reference for all purposes.]

[00087] In some embodiments, the compound is a galactomannan polysaccharide composition can be used in combination with a therapeutically effective amount of antagonists and/or inverse agonists of the Cannabinoid-1 (CB1) receptor. [7,999,107; 7,906,652, incorporated expressly by reference for all purposes.]

[00088] In some embodiments, the compound is a galactomannan polysaccharide composition can be used in combination with a therapeutically effective amount of a PPAR (peroxisome proliferator-activated receptor) activity regulators. [7,994,353, incorporated expressly by reference for all purposes.]

[00089] In some embodiments, the compound is a galactomannan polysaccharide composition can be used in combination with a therapeutically effective amount of a benzothiazepine or benzothiepine compound represented by the following formula having a thioamide bond and a quaternary ammonium substituent. [7,973,030, incorporated expressly by reference for all purposes.]

[00090] In some embodiments, the compound is a galactomannan polysaccharide composition can be used in combination with a therapeutically effective amount of an RNA antisense construct to inhibit protein tyrosine phosphatase PTPRU. [7,897,583, incorporated expressly by reference for all purposes.]

[00091] In some embodiments, the compound is a galactomannan polysaccharide composition can be used in combination with a therapeutically effective amount of a heteroatom-linked substituted piperidine and derivatives thereof useful as histamine H.sub.3 antagonists. [7,846,946, incorporated expressly by reference for all purposes.]

[00092] In some embodiments, the compound is a galactomannan polysaccharide composition can be used in combination with a therapeutically effective amount of a axacyclopentane derivative that inhibits stearoyl-coenzyme alpha delta-9 desaturase. [7,754,745, incorporated expressly by reference for all purposes.]

[00093] In some embodiments, the compound is a galactomannan polysaccharide composition can be used in combination with a therapeutically effective amount of a

acylamide compound having secretagogue or inducer activity of adiponectin. [7,732,637, incorporated expressly by reference for all purposes.]

[00094] In some embodiments, the compound is a galactomannan polysaccharide composition can be used in combination with a therapeutically effective amount of quaternary ammonium compounds. [7,312,208, incorporated expressly by reference for all purposes.]

[00095] In some embodiments, the compound is a galactomannan polysaccharide composition can be used in combination with a therapeutically effective amount of a isoflavone compound. [6,592,910, incorporated expressly by reference for all purposes.]

[00096] In some embodiments, the compound is a galactomannan polysaccharide composition can be used in combination with a therapeutically effective amount of a macrolide antibiotic. [5,760,010, incorporated expressly by reference for all purposes.]

[00097] In some embodiments, the compound is a galactomannan polysaccharide composition can be used in combination with a therapeutically effective amount of Glatiramer acetate (also known as Copolymer 1, Cop-1, or Copaxone - as marketed by Teva Pharmaceuticals), an immunomodulator drug currently used to treat multiple sclerosis.

[00098] In some embodiments, the compound is a galactomannan polysaccharide used in combination with a therapeutically effective amount of pentraxin proteins, including but not limited to recombinant pentraxin-2.

[00099] In some embodiments, the compound is a galactomannan polysaccharide composition can be used in combination with a therapeutically effective amount of a stain, for example but not limited to HMG-CoA reductase inhibitors such as atorvastatin and simvastatin.

[000100] In some embodiments, the compound is a galactomannan polysaccharide composition can be used in combination with a therapeutically effective amount of an n-acetyl cysteine.

[000101] In some embodiments, the compound is a galactomannan polysaccharide composition can be used in combination with a therapeutically effective amount of another galectin inhibitor that may inhibit single galectin proteins or a set of galectin

proteins, including but not limited to, small organic inhibitors of galectin, monoclonal antibodies, RNA inhibitors, small binding peptides, protein inhibitors or combinations thereof.

[000102] In some embodiments, the compound is a galactomannan polysaccharide composition can be used in combination with a therapeutically effective amount of a monoclonal antibody to inhibit lysyl oxidase (or other proteins that cross link collagens) or monoclonal antibody to connective tissue growth factor.

[000103] In some aspects of the invention, the compound comprises molecules which have a first portion, which is typically a carbohydrate, and which is capable of binding to galectins, joined to a second portion which inactivates or otherwise moderates the activity of a protein. This second portion need not be a carbohydrate and can comprise a material which cross links or otherwise denatures the segment of protein comprising an active portion of the galectin protein, or an active portion of another protein which interacts with the galectin. Such materials include active species such as sulfur or other chalcogen elements alone or in combination such as thiols, sulfhydryls and the like. Other active species may comprise cyano groups, thiocyanates, alkylating agents, aldehydes and the like.

[000104] In some embodiments, a NASH therapeutic formulation with suitable or increased efficacy in the treatment of NASH or NALD. In some embodiments, the NASH therapeutic formulation includes and effective dose of the galactomannan polysaccharide compound or composition. In some embodiments, the NASH therapeutic formulation can be administered alone or co-administered with an effective dose of a therapeutic agent in a mixture or regimen. The formulation my further include an additional NASH therapeutic agent or excipients in which the formulation is in a powder form or in a liquid form.

[000105] In another embodiment, an effective dose of the galactomannan polysaccharide composition can be administered in a formulation for oral administration. The formulation may include methods of physical alterations of the compound or additions of various agents that enhance the oral absorption of the galactose-containing polysaccharide.

[000106] In one embodiment, the galactomannan polysaccharide composition and other compounds described, are proposed as therapy alone or in combination with other compounds listed above, for human NASH as a method of ameliorating or reversing hepatocyte fat accumulation, intra-portal and intra-lobular inflammatory infiltrate, and fibrosis, including but not limited to collagen deposition in the peri-sinusoidal space, cirrhosis, and for preventing progression to hepatocellular carcinoma. Moreover, it is proposed that these improvements in liver disease pathology will have a resultant positive effect on the health of the individuals by reducing complications of liver fibrosis and cirrhosis, including the development of hepatocellular carcinoma.

[000107] In another embodiment, an effective dose of the galactomannan polysaccharide composition can be administered via a variety of routes including, parenteral via an intravenous infusion given as repeated bolus infusions or constant infusion, intradermal injection, subcutaneously given as repeated bolus injection or constant infusion, or oral administration.

[000108] An effective parental dose (given intravenously, intraperitoneally, or subcutaneously) of galactose containing polysaccharide to an experimental animal is within the range of 10 mg/kg up to 180 mg/kg body weight, or 10 mg/kg, or 30 mg/kg, or 60 mg/kg, or 120 mg/kg, or 180 mg/kg body weight.

[000109] An effective parenteral dose (given intravenously, intraperitoneally, or subcutaneously) of the galactomannan oligosaccharide composition to an experimental animal can be administered three times weekly, twice weekly, once weekly, once every two weeks, once monthly, or as a constant infusion.

[000110] An effective parental dose (given intravenously or subcutaneously) of galactomannan oligosaccharide composition to a human subject is within the range of 0.5 mg/kg up to 25 mg/kg body weight, or 1 mg/kg, or 2 mg/kg, or 5 mg/kg, or 7.5 mg/kg, or 10 mg/kg body weight, or 15 mg/kg body weight.

[000111] An effective parenteral dose (given intravenously or subcutaneously) of galactose containing polysaccharide to a human subject can be administered three times weekly, twice weekly, once weekly, once every two weeks, once monthly, or as a constant infusion.

[000112] In another embodiment, a therapeutically effective dose can be evaluated by a change of at least 10% in the level of the serum biomarkers of NASH. In some embodiments, the serum biomarkers of NASH can include but not limited to hyaluronic acid and other breakdown products of collagens, cytokeratin-18 and other cytoskeletal cellular proteins, tissue inhibitor of metalloprotease I and II and other liver derived collagen and matrix proteases. These compounds and biomarkers may be measured in the serum or in the liver tissue using immunoassays and the levels can be correlated with severity of disease and treatment.

[000113] In another embodiment, a therapeutically effective dose can be evaluated by a change of at least 10% in the level of the serum biomarkers of NASH including but not limited to reactive oxygen products of lipid or protein origin, coenzyme Q reduced or oxidized forms, and lipid molecules or conjugates. These biomarkers can be measured by various means including immunoassays and electrophoresis and their levels can be correlated with severity of disease and treatment.

[000114] In another embodiment, a therapeutically effective dose can be evaluated by a change of at least 10% in the level of the serum biomarkers of NASH including but not limited to cytokines that include but are not limited to TNF-alpha, TGF-beta or IL-8, osteopontin, or a metabolic profile of serum components that is indicative of NASH presence or severity (these include serum and urine markers). A profile of one or more of these cytokines, as measured by immunoassay or proteomic assessment by LC mass spec, may provide an assessment of activity of the disease and a marker to follow in therapy of the disease.

[000115] In another embodiment, a therapeutically effective dose can be evaluated by a change of at least 10% in the pathophysiologic spectrum of NASH which includes histopathological findings on liver biopsy. Histopathological findings on liver biopsy can include but are not limited to evidence of intra-hepatocellular fat, hepatocellular toxicity including but not limited to hyaline bodies, inflammatory cell infiltrates (including but not limited to lymphocytes and various subsets of lymphocytes and neutrophils), changes in bile duct cells, changes in endothelial cells, number of Kupffer cell macrophages, collagen deposition (including but not limited to peri-sinusoidal, portal and central

collagen deposition and portal to central bridging collagen deposition, hepatocellular nodules that distort the normal architecture, hepatocellular atypia consistent with malignant transformation, and various scales and methods that combine various sets of observations for grading the severity of NASH. Such histological assessments are the sine-qua-non of NASH diagnosis and therefore integrally related to assessment of therapy.

[000116] In another embodiment, a therapeutically effective dose can be evaluated by a change of at least 10% in the clinical manifestations of NASH including but not limited to clinical testing of stage and severity of the disease, clinical signs and symptoms of disease, and medical complications. Clinical testing of stage and severity of NASH include but are not limited to hematologic testing (including but not limited to red blood cell count and morphology, white blood cell count and differential and morphology, platelet count and morphology), serum or plasma lipids including but not limited to triglycerides, cholesterol, fatty acids, lipoprotein species and lipid peroxidation species, serum or plasma enzymes (including but not limited to aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (AP), gamma glutamyltranspeptidase (GGTP), lactate dehydrogenase (LDH) and isoforms, serum or plasma albumin and other proteins indicative of liver synthetic capacity, serum or plasma levels of bilirubin or other compounds indicative of the ability of the liver to clear metabolic byproducts, serum or plasma electrolytes (including but not limited to sodium, potassium, chloride, calcium, phosphorous), coagulation profile including but not limited to prothrombin time (PT), partial thromoplastin time (PTT), specific coagulation factor levels, bleeding time and platelet function. Clinical testing also includes but is not limited to non-invasive and invasive testing that assesses the architecture, structural integrity or function of the liver including but not limited to computerized tomography (CT scan), ultrasound (US), ultrasonic elastography (including but not limited to FibroScan) or other measurements of the elasticity of liver tissue, magnetic resonance scanning or spectroscopy, percutaneous or skinny needle or transjugular liver biopsy and histological assessment (including but not limited to staining for different components using affinity dyes or immunohistochemistry), measurement of hepatic portal-venous

wedge pressure gradient, or other non-invasive or invasive tests that may be developed for assessing severity of NASH in the liver tissue.

[000117] In another embodiment, a therapeutically effective dose can be evaluated by a change of at least 10% in clinical signs and symptoms of disease include fatigue, muscle weight loss, spider angiomata, abdominal pain, abdominal swelling, ascites, gastrointestinal bleeding, other bleeding complications, easy bruising and ecchymoses, peripheral edema, hepatomegaly, nodular firm liver, somnolence, sleep disturbance, and coma. Medical complications of NASH are related to cirrhosis and include ascites, peripheral edema, esophageal and other gastrointestinal tract varices, gastrointestinal bleeding, other bleeding complications, emaciation and muscle wasting, hepatorenal syndrome, and hepatic encephalopathy. An additional complication of NASH related cirrhosis is the development of complications sufficiently severe to warrant placement on liver transplantation list or receiving a liver transplantation.

[000118] In another embodiment, a therapeutically effective dose has an effect on NASH liver disease and/or fibrosis in the absence of any effect on whole blood glucose in patients with diabetes or serum lipids in patients with elevated serum lipids.

EXAMPLE 1: METHOD OF MANUFACTURING GALACTOMANNAN COMPOUND

[000119] The following is merely an illustrative example of the production of a therapeutic polysaccharide that is not meant to limit the invention. In this case, the galactomannan oligosaccharide composition produced has been labeled GM-CT-01 in this application.

[000120] A purification and manufacturing process for a galactomannan compound is described. High grade Guar gum was dissolved in warm water at 1% at 45°C. for 2 hr. The pH is reduced to 2.2 with 1 M HCl and solution was heated to 95°C for 2 hours. Then pH is adjusted to 5.8 with 1 M NaOH. The solution was then cooled to 20°C and filtered with glass filter. Next CuSO4/Na--K tartrate was added and the precipitate was collected on 200 mesh filter, wash with water solution and then washed in 5% HCl in 96% EtOH. It was then washed with 75% EtOH and twice with 96% EtOH, and finally

vacuum freeze-dried as white solids. Galactomannan, from a readily available source (e.g., Guar gum), was selected for process optimization and manufacturing. The soluble galactomannan oligomer was tested as described in Example 2.

[000121] The manufacturing process described above produces a product in the form of a highly soluble oligomer of Galactomannan (GM) from certified premium Guar Gum powder (from seeds of Cyamopsis tetragonoloba). The process is designed to generate a highly pure soluble and homogeneous oligomer with an average molecular weight in the range of about 48,000 daltons (D), and mannose to galactose ratio in the range of about 1:7. The process incorporated four major phases: controlled depolymerization to produce the desired galactomannan oligomer, and three purification steps, removal of insoluble impurities, removal of water soluble impurities, removal of organic soluble impurities, and finally freeze drying to generate a pure and stable form of galactomannan powder.

EXAMPLE 2: METHOD OF TREATMENT OF A MOUSE MODEL OF STEATOHEPATITIS

[000122] The experimental model used in this example is the mouse in which diabetes was induced and a high fat diet was administered, a model that has been called STAM mice. As shown in FIGURE 1, diabetes is induced immediately following birth with a single injection of streptozotocin and then four weeks later the mice are started on a high fat diet. This is a proven model in which the mice consistently develop NASH with hepatocyte fat accumulation, evidence of hepatocyte toxicity, portal and lobular inflammatory infiltrates, peri-sinusoidal fibrosis, advanced fibrosis with nodule formation, cirrhosis, and ultimately hepatocellular carcinoma in a certain percentage of animals.

[000123] The progression of disease appearance is fatty liver (FL) by five weeks of age, steatohepatitis (NASH) by 7 weeks of age, fibrosis (Fib) by 9 weeks of age, nodule formation (N) by 13 weeks of age, and some animals developing hepatocellular carcinoma (HC) by 16 weeks of age (FIGURE 1).

[000124] GM-CT-01, produced as described in Example 1, was given in a dose of 120 mg/kg twice weekly intravenously for four (4) weeks at the each of the starting times indicated. Early treatment was given for weeks 6 through 9 and late therapy was given for weeks 9 through 12.

[000125] The changes in body weight of animals sacrificed after the early and late treatment periods are shown in FIGURE 2A-2B. Three was no difference in body weight for animals treated with vehicle (normal saline alone) as compared to animals treated with GM-CT-01. This indicates at a gross level that there was little or no toxic effect of the treatments on the animals and any changes detected are unlikely due to the overall health of the animals.

[000126] The comparison of whole blood glucose between treatment groups is shown in FIGURE 3. FIGURES 3A-3B shows that the blood glucose levels were markedly elevated in both the vehicle control and GM-CT-01 groups with no statistical differences at either the early or late treatment schedule. The normal blood glucose in mice is approximately 100 mg/dL and the average in the STAM animals was between 700 and 800 mg/dL, hence demonstrating that all animals had overt diabetes.

[000127] FIGURE 4 shows the histology of the liver in normal and NASH mice. Liver sections stained with hematoxylin and eosin (H&E) show a marked difference between the normal liver and NASH liver, with the NASH liver showing large fat filled hepatocytes, ballooning degeneration of hepatocytes and an infiltrate of inflammatory cells. Liver sections stained with Sirius red highlight the presence of type I collagen. FIGURE 4 shows very little collagen in the normal liver, but increased collagen localized around central veins and in the peri-sinusoidal space in the NASH liver. This result demonstrates that the pathology of NASH was achieved in this mouse model.

[000128] The NAFLD activity score is used to evaluate the severity of liver disease on histological sections of liver and gives points for three aspects of NASH pathology including, steatosis (0 (<5%), 1 (5-33%), 2 (33-66%), or 3 (>66%)), hepatocyte ballooning (0 (none), 1, (few), or 3 (many)), and lobular inflammation (0 (no foci), 1 (<2 foci/200x field), 2 (2-4 foci/200x field), or 3 (>4 foci/200x field)). The total number of points is the NAFLD activity score.

[000129] FIGURE 5A shows the histology and FIGURE 5B shows the comparison of NFLD activity score on liver histology in STAM mice between the vehicle and GM-CT-01 treatment groups in the early treatment group. The score for the vehicle treated mice was an average of 5, confirming the presence of NASH. While there was not a statistically significant reduction in the NAFLD activity score in the groups of STAM mice treated with GM-CT-01, there was a statistically significant reduction in steatosis when compared to vehicle animals. These data show that GM-CT-01 reduces the pathology in the liver associated with NASH.

[000130] FIGURE 6 shows the comparison of Sirius red-positive area in liver biopsies of STAM mice between the vehicle and GM-CT-01 treatment group. Sirius red is a histological stain that has a specific affinity for collagen fibers, staining them red, and is therefore a quantitative tool for assessing the degree of fibrosis in liver biopsies. In both the early and late treatment groups there is a reduction in Sirius red staining seen in the GM-CT-01 treated animals versus vehicle control.

[000131] The area of Sirius red staining on liver histopathological sections from each of the two treatment groups was assessed using computer assisted morphometric analysis. FIGURE 7A-B shows that collagen percent area was reduced in the early and late treatment groups, and this difference became statistically significant when both groups were combined (FIGURE 7C). These results demonstrate that treatment with GM-CT-01 reduces liver fibrosis in mice with NASH.

[000132] The data further show that the efficacy of GM-CT-01 has an effect on NASH liver disease and fibrosis in the absence of any effect on whole blood glucose. The blood glucose was not reduced in the treatment groups versus the control groups indicating that the liver disease can be treated without effective treatment of diabetes.

[000133] The primary cell that lays down collagen in the liver is the activated stellate cell. The inflammatory infiltrate in NASH activates quiescent stellate cells which, in their activated state, secrete collagen that forms the fibrous tissue associated with the disease. FIGURE 8A shows immunohistochemical staining for the protein alpha-Smooth Muscle Actin, which is a marker for activated stellate cells. Treatment with GM-CT-01 caused a reduction in alpha-Smooth Muscle Actin which indicates that

activated stellate cells are markedly reduced (FIGURE 8B). This is one of mechanism for the reduced collagen deposition with treatment.

[000134] FIGURE 9A shows immunohistochemical staining of galectin-3 protein in liver tissue of vehicle and GM-CT-01 treated experimental groups. There is high level expression of galectin-3 predominantly in macrophages in the vehicle treated animal. Treatment with GM-CT-01 results in a marked reduction of galectin-3 in the liver associated with improved pathology of the disease (FIGURE 9B).

[000135] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications of changes in light thereof are to be included within the spirit and purview of this application and scope of the appended claims. All publication, patents and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

CLAIMS

1. A method comprising the steps of:

obtaining a composition for parenteral or enteral administration comprising a galactomannan polysaccharide in an acceptable pharmaceutical carrier;

administering to a subject in need thereof an effective dose of the composition, wherein administration results in at least one of the following:

reduction of at least one point in severity of nonalcoholic fatty liver disease or nonalcoholic steatohepatitis grading scoring systems, reduction of the level of serum markers of nonalcoholic steatohepatitis activity, reduction of nonalcoholic steatohepatitis disease activity or reduction in the medical consequences of nonalcoholic steatohepatitis,

wherein the subject has at least one of the following: fatty liver, nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, nonalcoholic steatohepatitis with liver fibrosis, nonalcoholic steatohepatitis with cirrhosis, or nonalcoholic steatohepatitis with cirrhosis and hepatocellular carcinoma.

2. The method of claim 1 wherein administration results in reduction of the accumulation of fat in the liver by at least 10%.

3. The method of claim 1 wherein administration results in reduction of the accumulation of fat in the liver as assessed in percentage of hepatocytes with fat in the liver.

4. The method of claim 3 wherein the reduction in the accumulation of fat in the liver is assessed by ultrasound or magnetic resonance imaging protocols.

5. The method of claim 1 wherein administration results in reduction of the hepatocyte ballooning by at least 10%.

6. The method of claim 5 wherein the reduction of hepatocyte ballooning is assessed in percentage of swollen hepatocytes.

7. The method of claim 1 wherein administration results in reduction of infiltration of neutrophils and lymphocytes by at least 10% in portal, central and lobular areas of a liver specimen.

8. The method of claim 1 wherein administration results in reduction of accumulation of collagen in the liver by at least 5%.

9. The method of claim 8 wherein the reduction of the accumulation of collagen in the liver is determined non-invasively by tissue stiffness/elasticity measurement using ultrasound or magnetic resonance elastography.

10. The method of claim 1 wherein the serum markers of nonalcoholic steatohepatitis activity are selected from the group consisting of transaminases, coenzyme Q reduced or oxidized, or a combination thereof.

11. The method of claim 1 wherein the reduction of the medical consequences of NASH with liver fibrosis or cirrhosis comprises reduction of portal hypertension, reduction in hepatic protein synthetic capability, hyperbilirubinemia, or encephalopathy.

12. The method of claim 1 wherein administration results in reduction by at least 10% of galectin-3 in liver tissue or serum.

13. The method of claim 1 wherein in the step of administering the galactomannan polysaccharide is co-administered with an effective amount of a therapeutic agent.

14. The method of claim 13 wherein the therapeutic agent is one of cysteamine or a pharmaceutically acceptable salt thereof, cystamine or a pharmaceutically acceptable salt thereof, an anti-oxidant compound, lecithin, vitamin B complex, a bile salt preparations, an antagonists of Cannabinoid-1 (CB1) receptor, an inverse agonists of Cannabinoid-1 (CB1) receptor, a peroxisome proliferator-activated receptor) activity regulators, a benzothiazepine or benzothiepine compound, an RNA antisense construct to inhibit protein tyrosine phosphatase PTPRU, a heteroatom-linked substituted piperidine and derivatives thereof, an axacyclopentane derivative, acylamide compound having secretagogue or inducer activity of adiponectin, a quaternary ammonium compound, Glatiramer acetate, pentraxin proteins, a HMG-CoA reductase inhibitor, n-acetyl cysteine, isoflavone compound, a macrolide antibiotic, a galectin inhibitor, an antibody, or any combination of the foregoing.

15. The method of claim 14 wherein the anti-oxidant compound comprises a water soluble Vitamin E preparation, mixed carotenoids, selenium or combinations thereof.

16. The method of claim 14 wherein the bile salt preparation comprises ursodeoxycholic acid, chenodeoxycholic acid of naturally occurring bile acids or bile acid salts, chenodeoxycholic acid of synthetic bile acids or bile acid salts or combinations thereof.

17. The method of claim 14 wherein the prentaxin protein is a recombinant pentraxin-2.

18. The method of claim 14 wherein the HMG-CoA reductase inhibitors comprises atorvastatin, simvastatin or combinations thereof.

19. The method of claim 14 wherein the galectin inhibitor comprises small organic inhibitors of galectin, monoclonal antibodies, RNA inhibitors, small binding peptides, protein inhibitors or combinations thereof.

20. The method of claim 14 wherein the antibody is an antibody against lysyl oxidase or an antibody against connective tissue growth factor.

21. The method of claim 1 wherein in the step of obtaining the galactomannan polysaccharide has a mannose to galactose ratio ranging from 1:1 to 1:4.

22. The method of claim 1 wherein in the step of obtaining the galactomannan polysaccharide has a mannose to galactose ratio of 1.7:1.

23. The method of claim 1 wherein in the step of obtaining the galactomannan polysaccharide has a molecular weight of about 48,000 D.

24. The method of claim 1 wherein in the step of obtaining the galactomannan polysaccharide has an average molecular weight ranging from 4,000 D to 60,000D.

25. A composition having a galactomannan polysaccharide for the treatment of fatty liver, nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, nonalcoholic steatohepatitis with liver fibrosis, nonalcoholic steatohepatitis with cirrhosis, or nonalcoholic steatohepatitis with cirrhosis and hepatocellular carcinoma.

26. The composition of claim 25 wherein the galactomannan polysaccharide has a ratio of mannose to galactose of 1.7:1.

27. The composition of claim 25 wherein the galactomannan polysaccharide has an average molecular weight ranging from 4,000 to 60,000 D.

28. Use of a galactomannan polysaccharide in the manufacture of a pharmaceutical composition for the treatment of fatty liver, nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, nonalcoholic steatohepatitis with liver fibrosis, nonalcoholic steatohepatitis with cirrhosis, or nonalcoholic steatohepatitis with cirrhosis and hepatocellular carcinoma.

29. The use of claim 28 wherein the galactomannan polysaccharide has a ratio of mannose to galactose of 1.7:1.

30. The use of claim 28 wherein the galactomannan polysaccharide has an average molecular weight ranging from 4,000 to 60,000 D.

31. An admixture having a galactomannan polysaccharide and a therapeutic agent for the treatment of fatty liver, nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, nonalcoholic steatohepatitis with liver fibrosis, nonalcoholic steatohepatitis with cirrhosis, or nonalcoholic steatohepatitis with cirrhosis and hepatocellular carcinoma.

32. Use of an admixture having a galactomannan polysaccharide and a therapeutic agent in a pharmaceutically acceptable carrier in the manufacture of a pharmaceutical composition for the treatment of fatty liver, nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, nonalcoholic steatohepatitis with liver fibrosis, nonalcoholic steatohepatitis with cirrhosis, or nonalcoholic steatohepatitis with cirrhosis and hepatocellular carcinoma.

33. The admixture of claim 31 wherein the therapeutic agent is one of cysteamine or a pharmaceutically acceptable salt thereof, cystamine or a pharmaceutically acceptable salt thereof, an anti-oxidant compound, lecithin, vitamin B complex, a bile salt preparations, an antagonists of Cannabinoid-1 (CB1) receptor, an inverse agonists of Cannabinoid-1 (CB1) receptor, a peroxisome proliferator-activated

receptor) activity regulators, a benzothiazepine or benzothiepine compound, an RNA antisense construct to inhibit protein tyrosine phosphatase PTPRU, a heteroatom-linked substituted piperidine and derivatives thereof, an axacyclopentane derivative, acylamide compound having secretagogue or inducer activity of adiponectin, a quaternary ammonium compound, Glatiramer acetate, pentraxin proteins, a HMG-CoA reductase inhibitor, n-acetyl cysteine, isoflavone compound, a macrolide antibiotic, a galectin inhibitor, an antibody, or any combination of the foregoing.

34. The admixture of claim 31 wherein the galactomannan polysaccharide has a ratio of mannose to galactose of 1.7:1.

35. The admixture of claim 31 wherein the galactomannan polysaccharide has an average molecular weight ranging from 4,000 to 60,000 D.

FIGURE 1

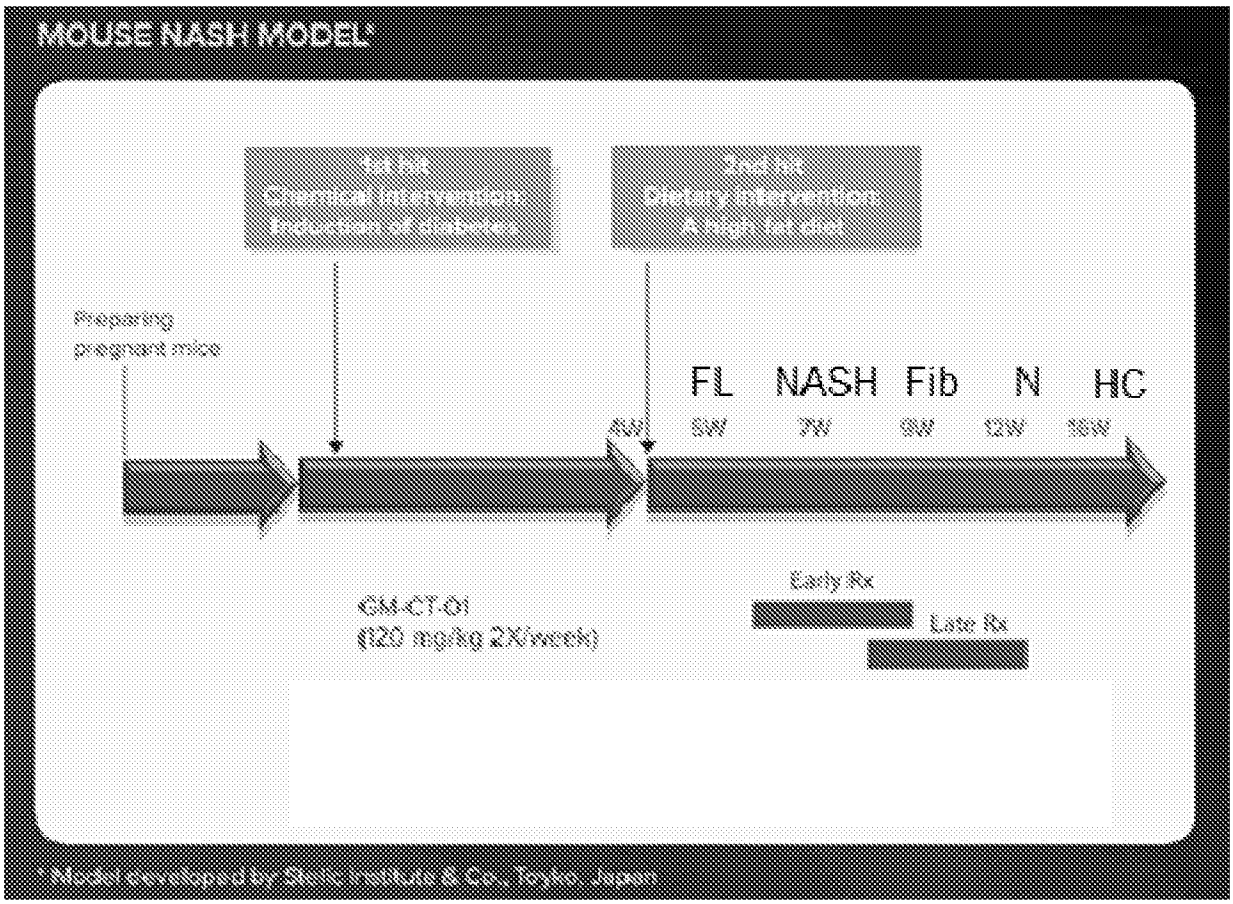
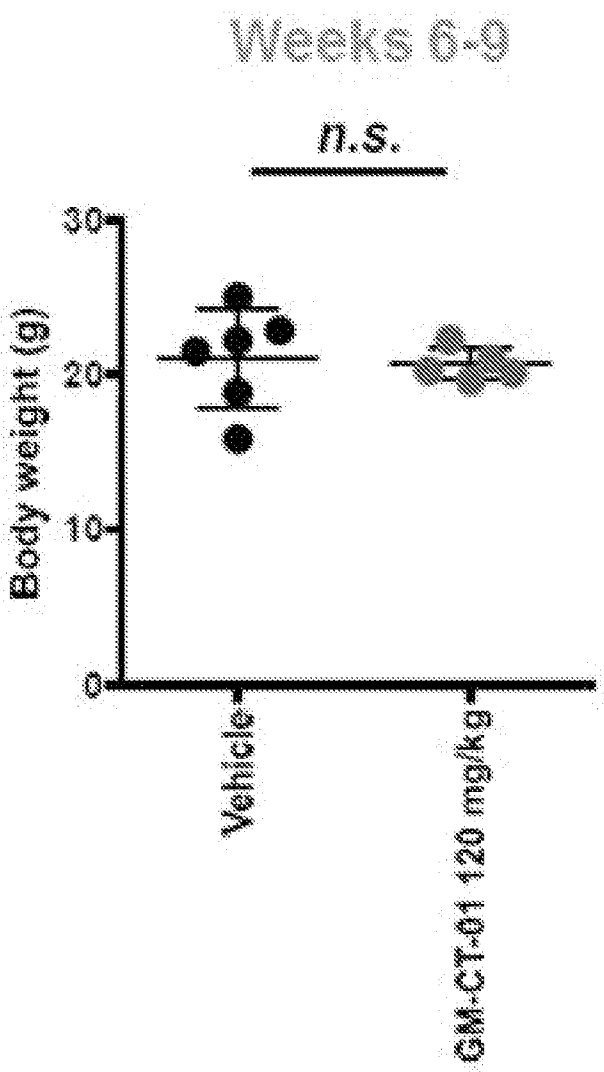

MOUSE NASH MODEL*
Preparing pregnant mice
FL
NASH
Fib
N
HC
5W
7W
9W
12W
16W
4W
Early Rx
Late Rx
CM-CT-01
(120 mg/kg 2X/week)

FIGURE 2

A.


Weeks 6-9
n.s.
Body weight (g)
30
20
10
0
Vehicle
GM-CT-01 120 mg/kg

B.

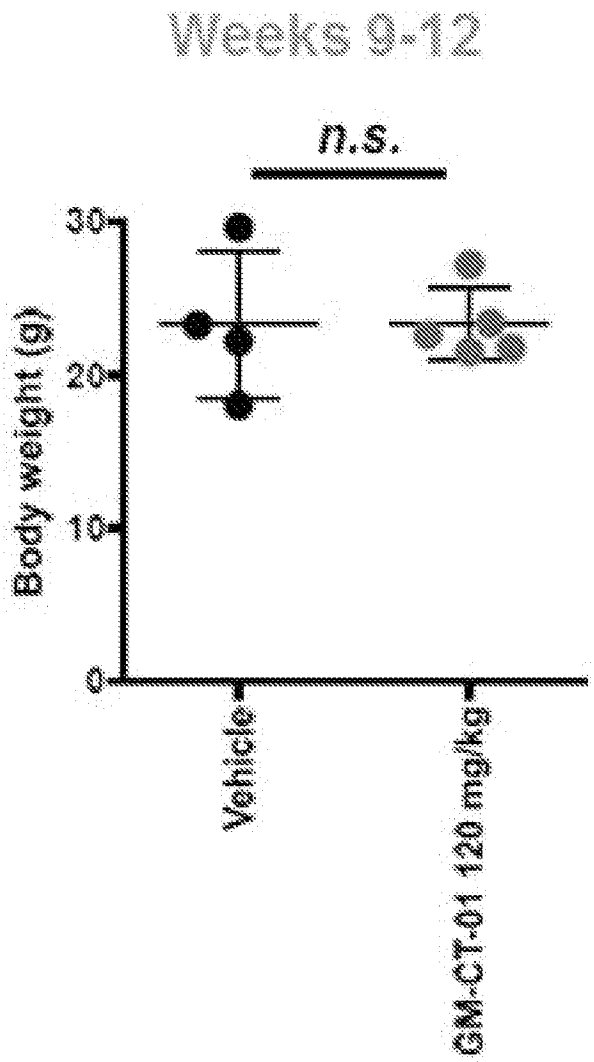
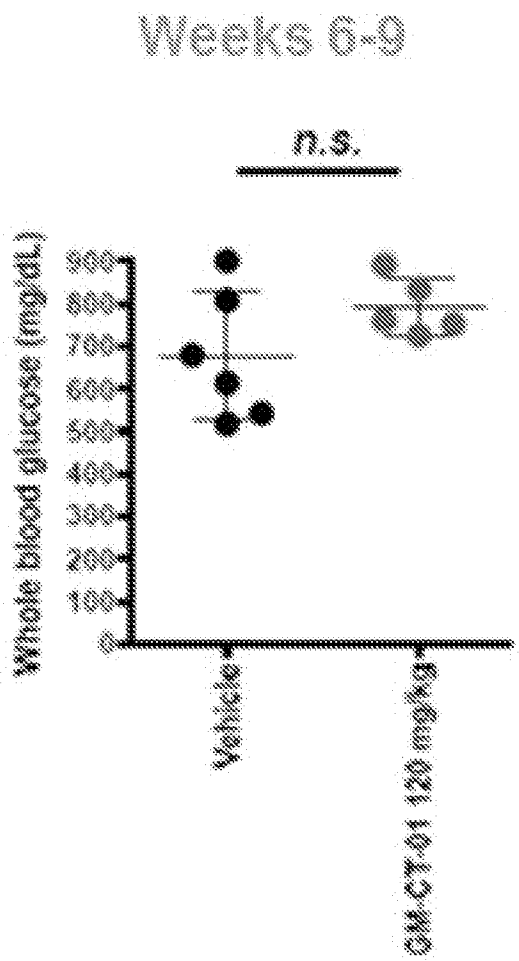

Weeks 9-12
n.s.
Body weight (g)
30
20
10
0
Vehicle
GM-CT-01 120 mg/kg

FIGURE 3

A.


Weeks 6-9
n.s.
Whole blood glucose (mg/dL)
900
800
700
600
500
400
300
200
100
0
Vehicle
GM-CT-01 120 mg/kg

B.

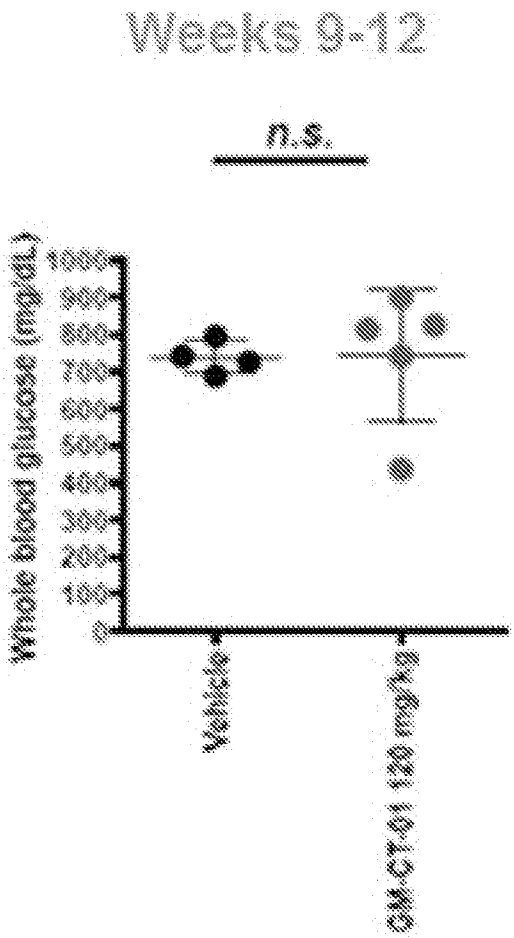

Weeks 9-12
n.s.
Whole blood glucose (mg/dL)
1000
900
800
700
600
500
400
300
200
100
0
Vehicle
GM-CT-01 120 mg/kg

FIGURE 4

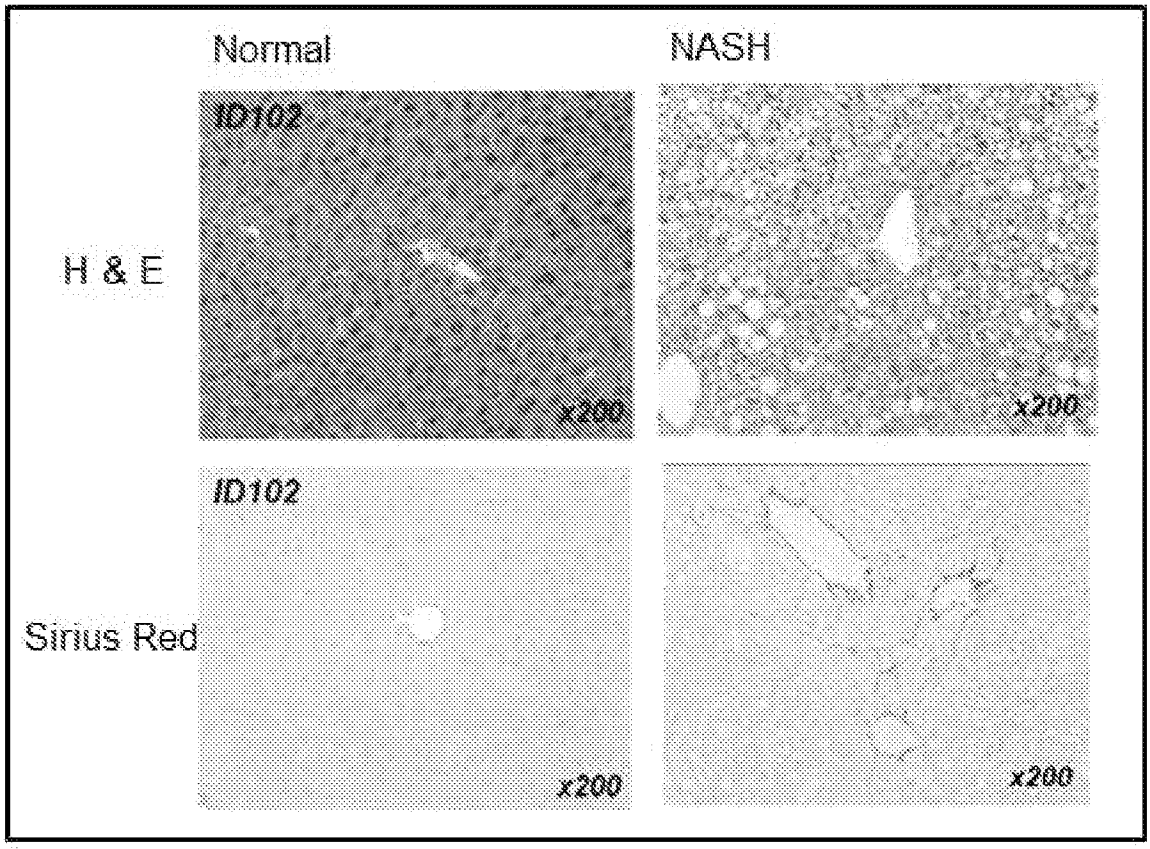
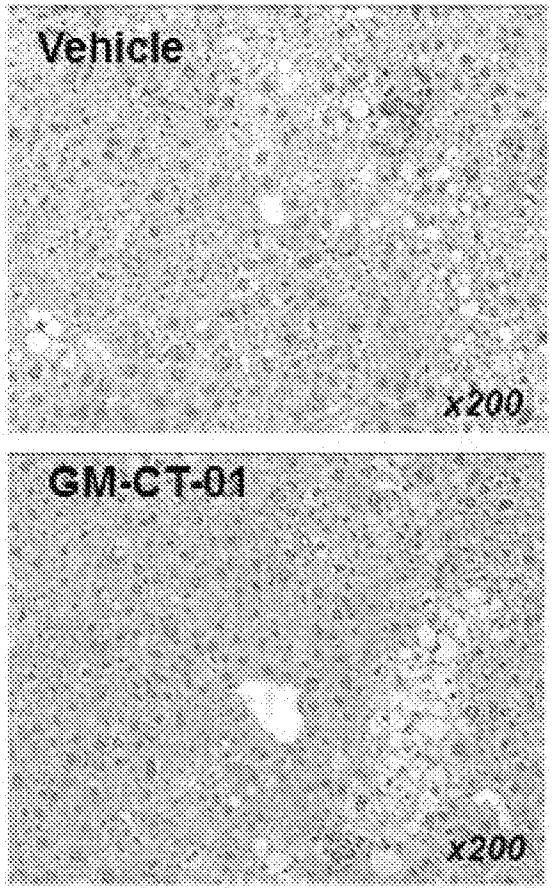
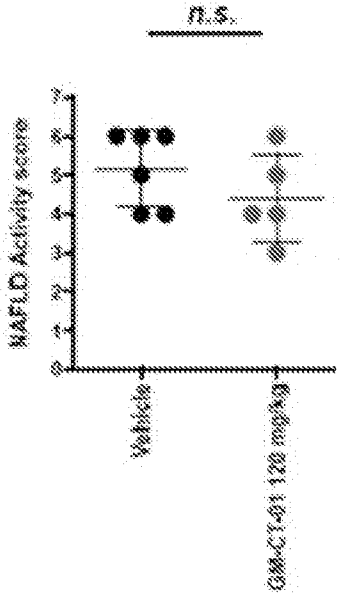


FIGURE 5

A.


Vehicle
x200
GM-CT-01
x200

B.


n.s.
NAFLD Activity score
Vehicle
GM-CT-01 120 mg/kg

C.

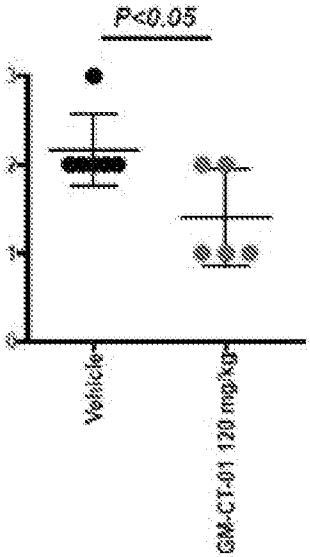

P<0.05
Steatosis grade (score)
Vehicle
GM-CT-01 120 mg/kg

FIGURE 6

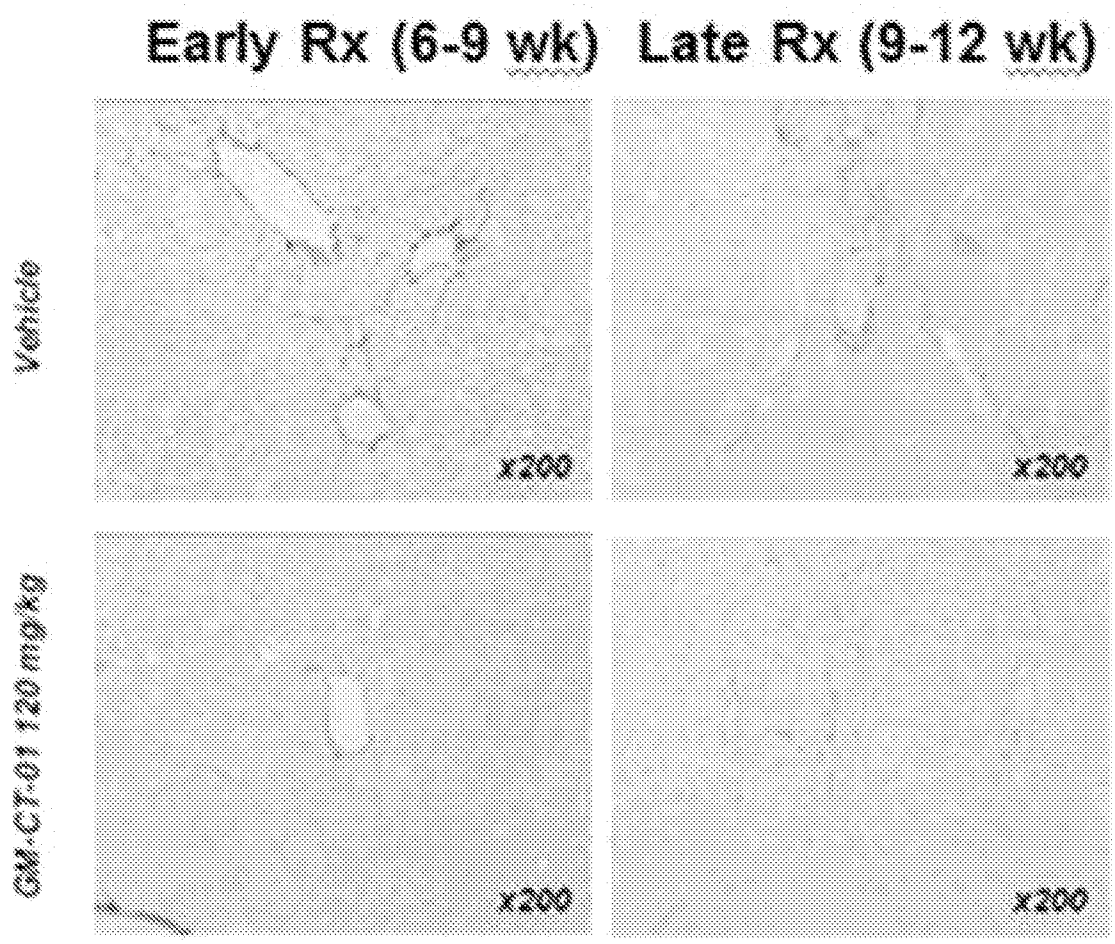

Early Rx (6-9 wk)
Late Rx (9-12 wk)
Vehicle
CM-CT-01 120 mg/kg
x200
x200
x200
x200

FIGURE 7

A. B. C.

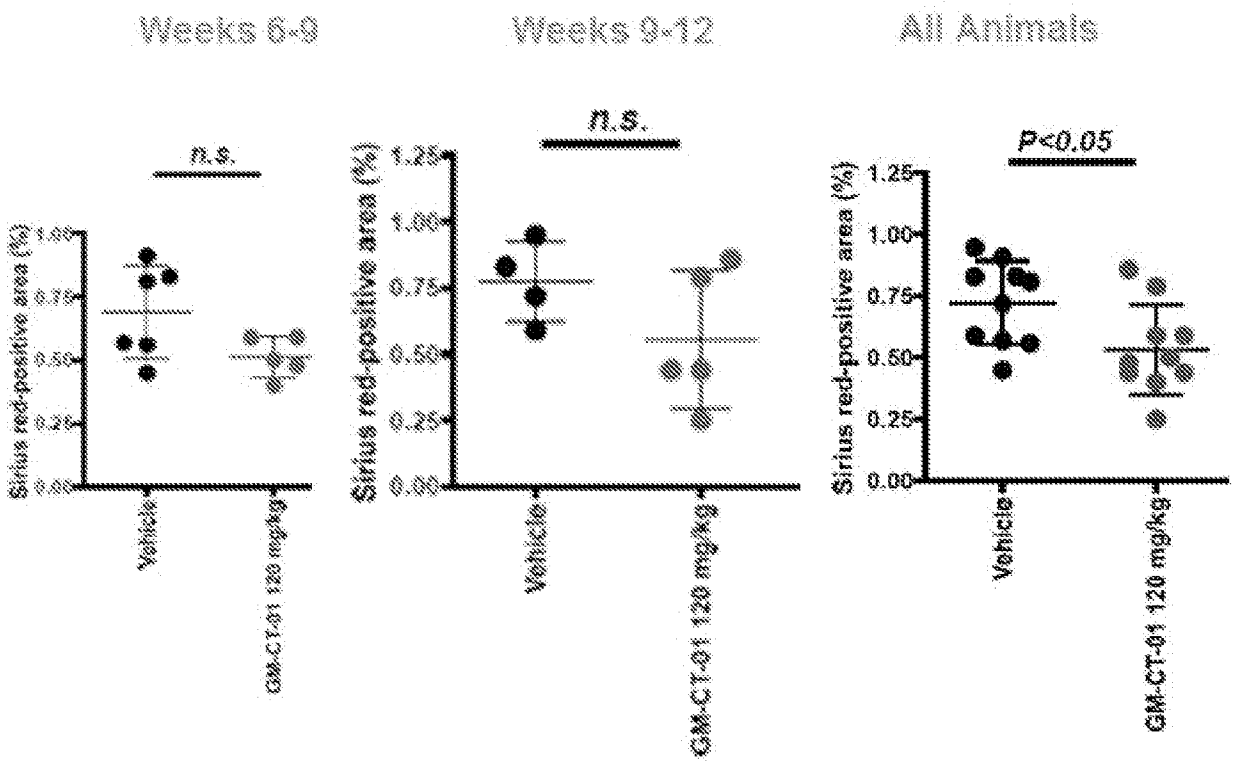
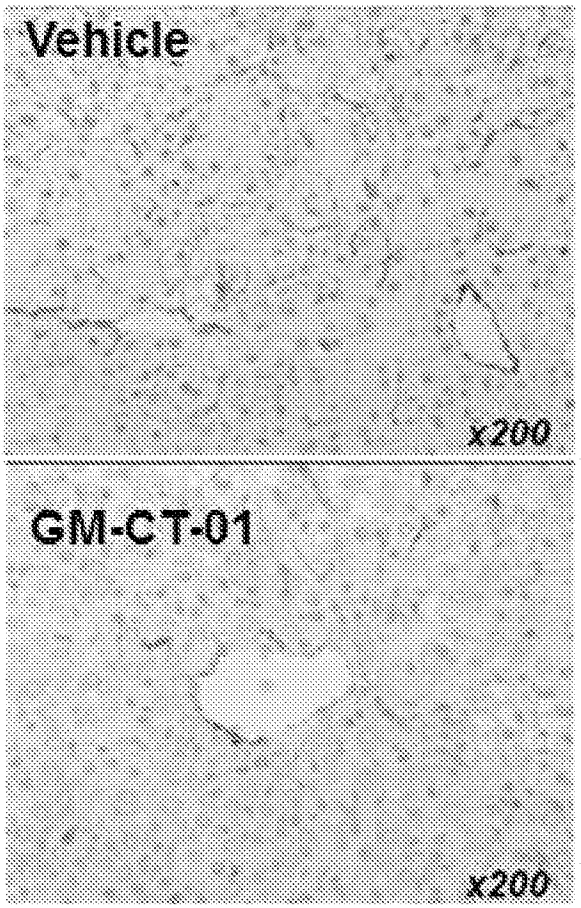

Weeks 6-9
Weeks 9-12
All Animals
n.s.
n.s.
P<0.05
Sirius red-positive area (%)
1.25
1.00
0.75
0.50
0.25
0.00
Vehicle
GM-CT-01 120 mg/kg

Figure 8

A.


Vehicle
x200
GM-CT-01
x200

B.

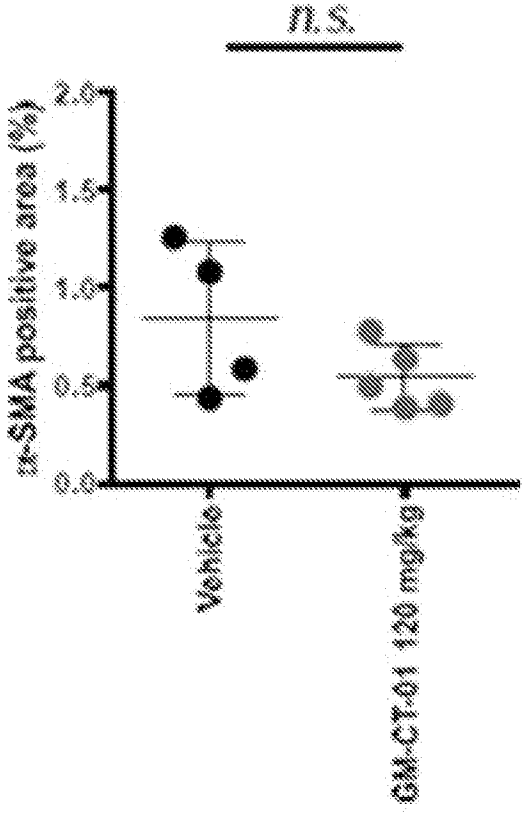
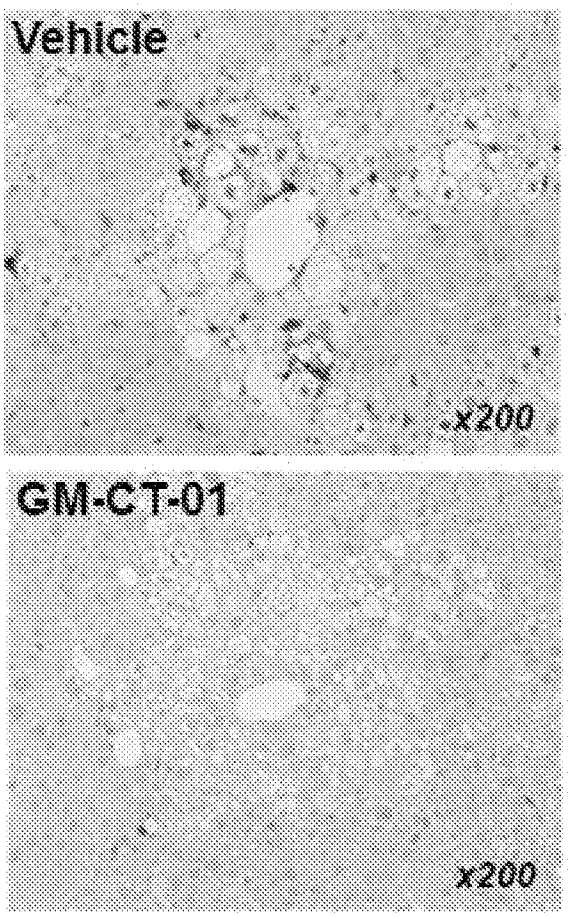
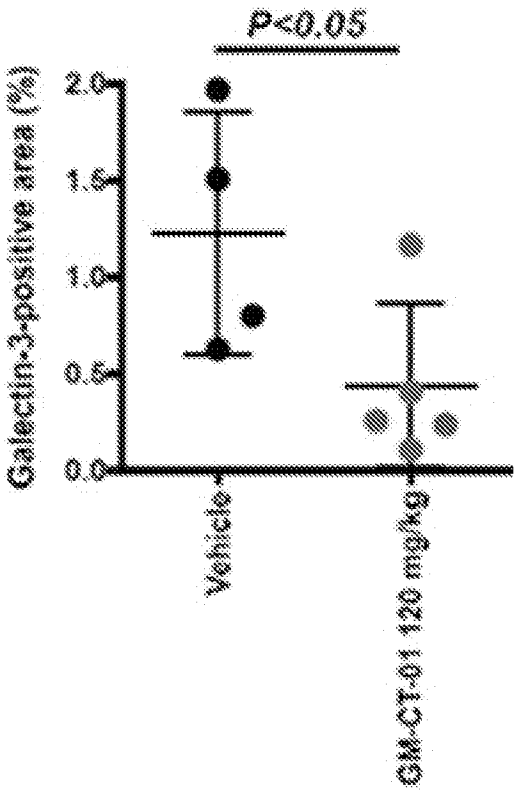

n.s.
α-SMA positive area (%)
2.0
1.5
1.0
0.5
0.0
Vehicle
GM-CT-01 120 mg/kg

Figure 9

A.


Vehicle
x200
GM-CT-01
x200

B.


P<0.05
Galectin-3-positive area (%)
2.0
1.5
1.0
0.5
0.0
Vehicle
GM-CT-01 120 mg/kg

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/55348

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A01N 43/04 (2012.01)
USPC - 514/54
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC: 514/54

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 514/54, 55; 536/2, 123
(keyword limited; terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase; PubWEST (PGPB,USPT,USOC,EPAB,JPAB); Google; PubMed
Search terms: galactomannan, nonalcoholic, steatohepatitis, liver, hepatic, fibrosis, fatty, ultrasound, MRI, galectin-3, pentraxin-2, PRM-151, collagen, elasticity, ballooning

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2008/0107622 A1 (PLATT et al.) 8 May 2008 (08.05.2008) para [0006]-[0008], [0047]-[0048], [0061], [0064], [0069], [0106]-[0107]	1-35
Y	US 2011/0003757 A1 (KUROSAKI et al.) 6 January 2011 (06.01.2011) para [0002], [0030], [0051], [0070]	1-35
Y	WO 2010/058295 A2 (CALES et al.) 27 May 2010 (27.05.2010) abstract; pg 16, ln 9-17	4, 10-11
Y	GHEORGHE et al. Real-time sonoelastography - a new application in the field of liver disease. J. Gastrointestin. Liver Dis. December 2008 (12.2008), Vol. 17, No. 4, pages 469-474; abstract; pg 469, para 1	9
Y	HENDERSON et al. Galectin-3 regulates myofibroblast activation and hepatic fibrosis. Proc. Natl. Acad. Sci. USA. 28 March 2006 (28.03.2006), Vol. 103, No. 13, pages 5060-5065; abstract; pg 5060, para 4	12, 19
Y	US 2008/0194575 A1 (BERAZA et al.) 14 August 2008 (14.08.2008) para [0070]	15-16
Y	US 2011/0183948 A1 (LEVINE et al.) 28 July 2011 (28.07.2011) para [0212]	17

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 November 2012 (01.11.2012)	26 DEC 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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