Journal of Traditional and Complementary Medicine 6 (2016) 160–167

Contents lists available at ScienceDirect

Journal of Traditional and Complementary Medicine

journal homepage: http://www.elsevier.com/locate/jtcme

Original article

Extract of a polyherbal formulation ameliorates experimental nonalcoholic steatohepatitis

Mohammed Azeemuddin [a], Mohamed Rafiq [a, *], Suryakanth Dattatraya Anturlikar [a], Lakkavalli Mohan Sharath Kumar [b], Pralhad Sadashiv Patki [a], Uddagiri Venkanna Babu [b], Ramakrishnan Shyam [c]

[a] Department of Pharmacology, R&D Center, The Himalaya Drug Company, Bangalore, India
[b] Department of Phytochemistry, R&D Center, The Himalaya Drug Company, Bangalore, India
[c] Chief Scientific Officer, R&D Center, The Himalaya Drug Company, Bangalore, India

ARTICLE INFO

Article history:
Received 17 September 2014
Received in revised form
24 November 2014
Accepted 4 December 2014
Available online 22 January 2015

Keywords:
antifibrotic
high-fat diet
insulin resistance
Liv.52
nonalcoholic steatohepatitis

ABSTRACT

The objective of the present study is to evaluate the effect of the extract of a well-known hepatospecific polyherbal formulation, Liv.52, in an experimental model of high-fat diet (HFD)-induced nonalcoholic steatohepatitis (NASH) in rats. Feeding a HFD for 15 weeks resulted in significant impairment of the lipid profile, elevation of hepatic enzyme markers, and insulin resistance in rats. The histological examination of the liver furthermore indicated fibrotic changes and fat deposition in hepatic tissues. The treatment with Liv.52 extract [125 mg/kg body weight per os (b.wt. p.o.)], which was administered from week 9 onward, reversed the HFD-induced changes to a statistically significant extent, compared to the untreated positive control animals. The effect observed with Liv.52 extract was comparable to that of pioglitazone (4 mg/kg b.wt.), a standard drug that is useful in the management of NASH. The treatment with Liv.52 extract significantly reduced steatosis, collagen deposition, and necrosis in hepatic tissues, which indicates its antifibrotic and antinecrotic properties. The results obtained in the present set of experiments indicate that Liv.52 extract effectively reverses metabolic and histological changes associated with HFD-induced NASH.

1. Introduction

The liver, which is the key organ of metabolism and excretion, is constantly endowed with the task of detoxifying xenobiotics, environmental pollutants, and chemotherapeutic agents. Disorders associated with this organ are numerous and varied. Nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH) are the most common liver diseases in the world. The mechanism involved in the pathogenesis of NAFLD/NASH has not been thoroughly investigated. Some studies show that insulin resistance has a key role in their pathogenesis. The exact mechanisms that mediate the transition from steatosis to NASH remain unknown, although oxidative stress and cytokine-mediated injuries may have a key role in NASH pathogenesis.[1,2] Under oxidative stress conditions, reactive oxygen species (ROS) lead to membrane lipid peroxidation, inflammatory responses, stimulation of stellate cells, and finally fibrosis.[3]

Modern medicine has not yet found a promising curative agent. Hence, the current use of peroxisome proliferator-activated receptor gamma (PPAR-γ), corticosteroids, and immunosuppressive agents only produce symptomatic relief. Furthermore, their usage is associated with the risks of relapse and adverse effects.[4–6]

The indigenous system of medicine in India has a long tradition of treating liver disorders with plant drugs. Liv.52 is one such polyherbal proprietary formulations. It is approved by the Government of India's Drug Regulatory Authority, the department of Ayurveda, Yoga, Naturopathy, Unani, Siddha and Homoeopathy (AYUSH) of the Ministry of Health and Family Welfare (New Delhi, India). The usefulness of Liv.52 in liver disorders of various etiologies is confirmed by a substantial number of clinical trials conducted across the globe. It is also beneficial in treating viral hepatitis, drug-induced hepatic damage, and alcoholic liver

* Corresponding author. Department of Pharmacology, R&D Center, The Himalaya Drug Company, Makali, Bangalore-562 162, Karnataka, India.
E-mail address: dr.rafiq@himalayawellness.com (M. Rafiq).
Peer review under responsibility of The Center for Food and Biomolecules, National Taiwan University.

http://dx.doi.org/10.1016/j.jtcme.2014.12.002

disorders.[7–10] The present study aimed to evaluate the effect of Liv.52 extract in an experimental model of high-fat diet (HFD)-induced nonalcoholic steatohepatitis (NASH) in rats. In the present study, pioglitazone, a standard reference drug that further validates the experimental model/procedure, was used for comparative study. The HFD model is a very appropriate model to induce NASH, obesity, and insulin resistance. "Overnutrition" with carbohydrates or fats or both may lead to obesity-related NAFLD. Insulin resistance and obese diabetes do not solely initiate steatohepatitis, but they do contribute to the progression of the entire spectrum of pathology of steatohepatitis, at least partly, via the upregulation of genes involved in lipogenesis, inflammation, and fibrogenesis. Agonists of PPAR-γ reportedly prevent the progression of NASH in a dietary model of NASH associated with obese diabetes.[11]

2. Composition and preparation of Liv.52 extract

The Liv.52 extract constitutes a mixture of roots of *Capparis spinosa* (續隨子根 xù suí zǐ), seeds of *Cichorium intybus* (菊苣 jú jù), whole plant of *Solanum nigrum* (龍葵 lóng kuí), *Terminalia arjuna* (bark), seed of *Cassia occidentalis* (望江南 wàng jiāng nán), *Achillea millefolium* (洋蓍草 yáng shī cǎo; aerial plant), and *Tamarix gallica* (whole plant). These crude herbal materials were subjected to pulverization to obtain their coarse powders, and then blended. They were then soaked in pure water and boiled in a steam-jacketed stainless steel reactor until the extraction was complete. The liquid extract thus obtained was filtered through a muslin cloth and concentrated in the reactors to attain 30% total solids. The soft extract was then subjected to spray drying to obtain the dry extract powder. It was tested for its quality and consistency at each manufacturing step per the accepted principles of good manufacturing practice (GMP) and good laboratory practice (GLP). Its botanical identification, quality parameters, and Ayurvedic criteria complied with the international guidelines and pharmacopoeial standards.[12,13]

3. High-performance liquid chromatography and liquid chromatography–mass spectrometry/mass spectroscopy fingerprinting of Liv.52 extract

3.1. Sample preparation and liquid chromatographic conditions

The concentration of the Liv.52 extract (DD-100) was 10 mg/mL in methanol. Liquid chromatography was performed by the Shimadzu LC-20AD series pump (Shimadzu Corporation, Kyoto, Japan) and DUG-20A3 series Shimadzu degasser (Shimadzu Corporation, Kyoto, Japan). Chromatographic separation was performed on the Luna C18 column (250 mm × 4.6 mm, 5 μm; Phenomenex, Torrance, CA, USA). For the separation, the mobile phase gradient consisted of water (J.T. Baker brand; Avantor Performance Materials, Inc., Center Valley, PA, USA) with 10mM ammonium acetate and 0.1% formic acid in pump A and acetonitrile (J.T. Baker brand; Avantor Performance Materials, Inc.) in pump B. The linear gradient program was set as follows: 0–27 minutes of 20% of acetonitrile to 80% acetonitrile (linear); 27–30 minutes of 80% acetonitrile to 20% acetonitrile (linear); followed by 30 minutes of 20% acetonitrile (isocratic) equilibration period for 2 minutes, delivered at a flow rate of 0.6 mL/min and a run time of approximately 32 minutes. Peak elution was monitored at 245 nm and 360 nm through the Shimadzu SPD-20A UV/VIS detector (Shimadzu Corporation). The injection volume of 20 μL was injected through the SIL-HTC Shimadzu Autosampler (Shimadzu Corporation). The ambient temperature was achieved through a CTO-10 AS VP column oven (Shimadzu Corporation) at 25 °C.

3.2. Mass spectrometric conditions

The API 2000 mass spectrometer (Applied Biosystems/MDS SCIEX, Ontario, Canada) was coupled with an electron spray ionization source and a chromatographic system. Batch acquisition and data processing were controlled by Analyst 1.5 version software.

Optimization of the mass spectroscopy (MS) parameters was performed with 2 mg/mL of test solutions that were prepared separately and diluted in methanol (J.T. Baker brand; Avantor Performance Materials, Inc.). The intensity response was checked in the positive ionization mode and negative ionization mode. The intense response was good in the positive mode. Interface conditions such as the declustering potential (40 V), ion spray voltage (3500 V), nebulizing gas [GS1 (55 psi) and GS2 (65 psi)], curtain gas (25 psi), focusing potential (400 V), entrance potential (10 V), and source temperature (420 °C) were optimized by multiple runs through liquid chromatography. Acquisition was performed by setting the mass of the analytes with the appropriate scan range. (Fig. 1).

4. Materials and methods

4.1. Drugs and chemicals

Liv.52 extract was procured from the Phytochemistry Department of The Himalaya Drug Company (Bangalore, India) and pioglitazone was procured from Sun Pharmaceuticals Industries Ltd. (Mumbai, India). Cholic acid and cholesterol were procured from HiMedia Laboratories Ltd. (Mumbai, India). All other chemicals used in the study were of reagent/analytical grade from reputed suppliers. Biochemical parameters such as glucose, cholesterol, triglycerides (TGs), serum glutamic oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), and alkaline phosphatase (ALP) were estimated in a biochemistry autoanalyzer (EM-360; Erba Diagnostics Mannheim GmbH, Mannheim, Germany) using ready-made assay kits (Erba kits; Erba Diagnostics Mannheim GmbH). Insulin was estimated using a radioimmunoassay kit (RIAK-1) supplied by Bhabha Atomic Research Centre/Board of Radiation and Isotope Technology (BARC/BRIT; Mumbai, India).

4.2. Experimental animals

Inbred male Wistar rats (260–280 g) were housed under standard conditions of temperature (22 ± 3 °C) at a relative humidity of 55 ± 5% and 12-hour light/dark cycle prior to and during the study. The normal group animals were fed a standard pellet diet (Provimi Animal Nutrition India Pvt. Ltd., Bangalore, Karnataka, India), and water *ad libitum*. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of The Himalaya Drug Company (Bangalore, India). The animals received humane care as prescribed by the guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India (New Delhi, India).

4.3. Composition of HFD

The HFD consisted of 87.5% normal laboratory rodent feed, 10% animal fat, 0.5% cholic acid, and 2% cholesterol.[14]

4.4. HFD-induced nonalcoholic steatohepatitis

Male Wistar rats (n = 36) that weighed 260–280 g were randomly divided into four groups consisting of nine animals each.