Available online at www.sciencedirect.com
ScienceDirect
Phytomedicine 14 (2007) 792–798
Phytomedicine
www.elsevier.de/phymed

Antidyslipidemic activity of polyprenol from *Coccinia grandis* in high-fat diet-fed hamster model ☆

Geetu Singh[a], Prasoon Gupta[a], Preeti Rawat[a], Anju Puri[b], Gitika Bhatia[b], Rakesh Maurya[a,*]

[a]*Medicinal and Process Chemistry Division, Central Drug Research Institute, Chattar Manzil Palace, Lucknow 226 001, India*
[b]*Biochemistry Division, Central Drug Research Institute, Chattar Manzil Palace, Lucknow 226 001, India*

Received 21 December 2006; accepted 2 April 2007

Abstract

Ethanol extract of *Coccinia grandis* (L.) Voigt showed significant triglyceride (TG) and cholesterol-lowering effects in dyslipidemic hamster model. Ethanolic extract was fractionated into chloroform, *n*-butanol and water-soluble fractions and were evaluated. Activity was proved to be concentrated in chloroform-soluble fraction. Chloroform-soluble fraction containing active component was subjected to repeated column chromatography, furnished a polyprenol characterized as C_{60}-polyprenol (**1**) isolated for the first time from this plant. It significantly decreased serum TG by 42%, total cholesterol (TC) 25% and glycerol (Gly) 12%, accompanied HDL-C/TC ratio 26% in high-fat diet (HFD)-fed dyslipidemic hamsters at the dose of 50 mg/kg body weight. Results are comparable to standard drug fenofibrate at the dose of 108 mg/kg. Based on these investigations, it was concluded that the compound polyprenol (**1**) isolated from leaves of *C. grandis* possess marked antidyslipidemic activity.

Keywords: *Coccinia grandis*; Polyprenols; Antidyslipidemia; Hamster model

Introduction

Increased levels of cholesterol or lipid in blood plasma is a state called hyperlipidemia, which can lead to serious problems such as coronary heart disease and other cardiovascular diseases (Simons, 2002). Consequently, there is an increase in interest in finding a naturally occurring, plant-based compound, which could reduce the increased levels of lipid in plasma.

Coccinia grandis (L.) Voigt (syn. *Coccinia indica* Wight & Arn., *Coccinia cordifolia* (L.) Cogn., *Cephalandra indica* Naud.) belongs to family Cucurbitaceae, commonly known as Kundru in Hindi and Ivy Gourd in English, is a vegetable grown wildly throughout India. It is a perennial climbing herb with tuberous roots, fruiting throughout the year. The leaves and roots of the plant showed antidiabetic activity comparable to tolbutamide (Brahmachari and Augusti, 1963). The medicinal use of the plant can be traced to ancient period where the juice of the roots and leaves were used in the treatment of diabetes mellitus, bronchitis, skin diseases, tongue-sores and ear ache (Satyavati et al., 1987). Ethanolic extract of fruit of *Coccinia indica* has also been reported to show hepatoprotective effects (Rao et al., 2003). Previous phytochemical examination of this plant indicated the presence of triterpenoides (Vaishnav et al., 2001; Bhakuni et al., 1962), carotenoides (Barua and Goswami, 1979), flavonoids

☆CDRI communication no.: 6626.

*Corresponding author. Tel.: +91 522 2212411 18x4440; fax: +91 522 2223405/2223938/2229504.
E-mail address: mauryarakesh@rediffmail.com (R. Maurya).

0944-7113/$ - see front matter © 2007 Elsevier GmbH. All rights reserved.
doi:10.1016/j.phymed.2007.06.008

(Vaishnav and Gupta, 1996), alkaloids (Qudrat-i-Khuda et al., 1965) and fatty acids (Siddiqui et al., 1973).

Since no previous attempts have been made to examine the antidyslipidemic effects, of *C. grandis*, we focused on finding the active component of the plant, which can regulate blood lipid profile in dyslipidemia levels. The present communication deals with a bioassay-guided isolation of the dyslipidemic principles from *C. grandis*.

Materials and methods

Plant material

C. grandis leaves were collected from Lucknow (Uttar Pradesh, India) in the month of October 2003 and were identified and authenticated by the Department of Botany Division of Central Drug Research Institute. Voucher specimen (No. 4536) is kept in the herbarium of the institute.

Preparation of extract

Powdered leaves of *C. grandis* (7.0 kg) were placed in a glass percolator with ethanol (25.0 l) and are allowed to stand at room temperature for about 16 h (overnight). The percolate was collected. This process of extraction was repeated for four times. The combined extract was filtered, concentrated under vacuum using rotavapor at 40 °C, weight of extract obtained was 480.0 g.

Fractionation of ethanolic extract

Ethanolic extract of *C. grandis* (430.0 g) was macerated 10 times with chloroform (1.0 l each). The chloroform-soluble fraction was concentrated under reduced pressure yielding brown viscous oil 160.0 g. The chloroform-insoluble residue obtained was then suspended in distilled water (500 ml) and then extracted in a separatory funnel with *n*-BuOH saturated with water (300 ml × 8). The *n*-BuOH and water-soluble fractions were evaporated to dryness under vacuum using rotavapor at 45 °C, yielded residues 66.0 and 204.0 g, respectively.

Analytical material and methods

IR spectra (neat) were recorded on a Perkin-Elmer RX-1 spectrophotometer. NMR spectra were run on an AVANCE DPX 200 and Bruker DRX 300 spectrometer using C_6D_6 and $CDCl_3$ as solvent and TMS as internal standard. ESMS was recorded in the instrument mass spectrometer make/model-micromass (UK)/QUATRO II. Column chromatography was performed using flash silica gel (230–400 mesh). Preparative and analytical high-pressure liquid chromatography (HPLC) was performed on the Shimadzu model LC-8A instrument.

Isolation and identification of polyprenol (1)

A chloroform-soluble fraction (140.0 g) was subjected to column chromatography over silica gel (2.8 kg, 230–400 meshes) with benzene as eluent, and gave a polyprenol fraction (16.5 g). This was rechromatographed over silica gel (400.0 g, 230–400 meshes) eluting with a mixture of hexane–ethyl acetate (99:1) and gave compound **1**. Further purification was carried out using preparative HPLC on a Shimadzu Shimpack CLC-ODS, C-18 column (20.0 mm i.d. × 250 mm) using refractive index detector (RID-10A) with acetone–water (96:4) to give polyprenol (**1**, 500 mg) as light yellow oil. Compound **1** was identified by means of ES-MS, ^{1}H NMR and ^{13}C NMR as C_{60}-polyprenol (**1**) and comparisons with the reported spectral data (Song et al., 1996). Compound **1** is reported for the first time from this plant.

Fingerprinting/standardization of active fraction

Chloroform fraction (active) was standardized using polyprenol **1**, by HPLC. The chloroform extract (50 mg/ml) was loaded (in 20 μl volume) on the RP-18 reverse-phase column, Shimpack ODS, C-18 column (4.6 mm i.d. × 25 cm length) with mobile phase acetone:water (96:4) at 0.5 ml/min flow rate using the RID-10A detector. Similar procedure was followed for polyprenol **1**.

Animals

Golden Syrian hamster (*Mesocricetus auratus*), male 12-week old, 110–120 g body weight were used. A group of eight animals (four/cage) were kept in controlled conditions, temperature 25–26 °C, relative humidity 60–80% and 12/12 h light/dark cycle (light from 8.00 a.m. to 8.00 p.m.). The animals with identification marks were acclimatized for 7 days before experiments. Experimental protocols were approved by our institutional ethical committee, which follows guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), which complies with international norms of INSA.

High-fat diet (HFD)

The HFD was prepared by mixing the normal pellet diet with groundnut oil, cholesterol (Sigma), deoxycholesterol (Sigma), and fructose (Sigma) in a ratio of 610 g:300 ml:5 g:5 g:100 g, respectively, to a final weight of 1.0 kg. This homogenous soft cake was molded in the shape of pellets of about 3 g each. Animals of all the groups were fed with HFD (10 g/animal) for 4 days