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Stimulatory effects of *Cuminum cyminum* and flavonoid glycoside on Cyclosporine-A and restraint stress induced immune-suppression in Swiss albino mice

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ABSTRACT

Many herbs and spices are known to modulate the immune system and have been shown to restore the immunity in immuno-compromised individuals. Spices generally used to increase the taste and flavor of food also has the history of usage as an ayurvedic medicine. Therefore to explore the health modulating effects of *Cuminum cyminum* and to identify the active compound, immunomodulatory properties were evaluated using flowcytometry and ELISA in normal and immune-suppressed animals. *C. cyminum* and compound **1** stimulated the T cells and Th1 cytokines expression in normal animals. Swiss albino mice subjected to Cyclosporine-A induced immune-suppression were dosed orally with *C. cyminum* (25, 50, 100 and 200 mg/kg) on consecutive days. The results showed that administration significantly increased T cells (CD4 and CD8) count and Th1 predominant immune response in a dose dependent manner thereby suggesting immunomodulatory activity through modulation of T lymphocytes expression. In restraint stress induced immune-suppressed animals, compound **1** countered the depleted T lymphocytes, decreased the elevated corticosterone levels and size of adrenal glands and increased the weight of thymus and spleen. Based on the data we may conclude that *C. cyminum* is a potent immunomodulator and may develop as a lead to recover the immunity of immuno-compromised individuals.

1. Introduction

Immune system holds the key to optimum health where one minor disruption may open the door for many health risks. It has been proved that dysfunctional immune system is the root cause of many diseases and is also involved in the etiology and pathophysiological mechanisms [1]. Ayurveda, an ancient form of Indian medicine, gives emphasis on promotion of health, a concept of strengthening host defenses against different diseases [2] therefore, today there is an increasing demand of plant based agents which restore the immune system to function properly and when possible, gives an edge over the disease to heal itself and mounting evidence from epidemiological studies, animal research, clinical trials and research in nutritional biochemistry suggests that spices may be beneficial in many health related complications. The therapeutic potential of several plant species and the necessity for scientific validation of the use of plants in popular medicine have prompted increased interest in the field and a large number of plant species and their components have been shown to be potential immunomodulators acting as anti-stress and anti-cancer agents [3].

Varieties of spices have been used traditionally to prevent and treat diseases and are known to keep the immune system in a highly prepared state for any threat it may encounter. At present their pharmacologic activities, particularly stimulation of immune functions has been the focus of alternative medicine. Among the spices, *Cuminum cyminum* native of east India and east Mediterranean have gained its place as a spice in Indian, African, Chinese, Cuban and Mexican cuisines and is mainly used to increase the taste and flavor of food [4]. Many activities have been described for *C. cyminum* including their effect on immune system and to the best of our knowledge no study has been carried out till date to explore the immunomodulatory activity of this spice in immune-suppressed animals and the active compound responsible for the activity. Therefore the objective of this study was to evaluate the effect of this spice in both normal and immune-suppressed conditions, to identify the active compound responsible for the activity for which no detailed study has been reported so far and to delineate the mode of action. Our results suggest that, *C. cyminum* is a potent T cells response modifier and activity is

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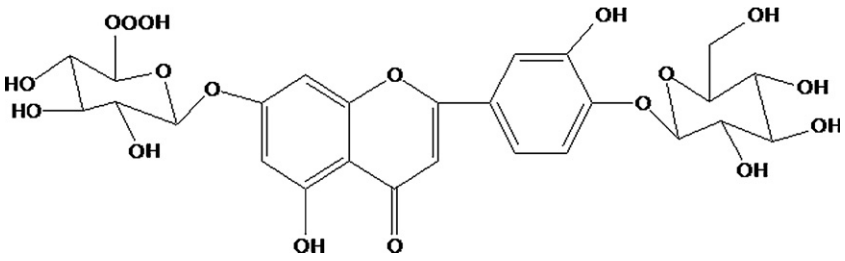

Fig. 1. Chemical structure of 7-(1-O-β-D-galacturonide)-4′-(1-O-β-glucopyranosyl)-3′4′,5,7-tetrahydroxyflavone (compound **1**).

mainly due to the flavonoid glycoside, 3′,5-dihydroxyflavone 7-O-β-D-galacturonide-4′-O-β-D-glucopyranoside (compound **1**).

2. Materials and methods

2.1. Plant material

Seeds of *C. cyminum* were supplied by plant survey division of Indian Institute of Integrative Medicine (IIIM) Jammu Tawi (India). A voucher specimen of the material has been deposited in the herbarium of IIIM Jammu.

2.1.1. Extraction and isolation

The seeds of *C. cyminum* (500 g) were dried and powdered. Powdered seeds were treated with petroleum ether (1 L) by percolation at room temperature for 16 h to remove oils and non-polar compounds. The defatted material (470 g) was then extracted with 50% aqueous alcohol (1 L) and kept overnight at room temperature. The marc was extracted three times more under similar conditions using 1 L solvent each time. The pooled extract was concentrated on rotavapour ($60 \pm 2\,^{\circ}C$) to remove alcohol and the remaining aqueous portion was lyophilized to yield an amorphous powder (80.5 g). The powder was investigated for immunomodulatory activity and has shown a very promising activity. In order to locate the activity, the aqueous-alcoholic extract was partitioned between n-Butanol and water. Removal of solvent from each phase gave the n-butanol (12.3 g) and aqueous (68.2 g) fractions. Evaluation of both the fractions showed that aqueous fraction is rich in chemical constituents.

2.1.2. Isolation of bioactive compound *1*

The aqueous fraction (50 g) was subjected to silica gel (60–120 mesh) chromatography. The column was eluted with ethyl acetate and then with methanol, gradually increasing the percentage of water. Fractions eluted in 5% water in methanol showed identical TLC pattern (run in n-butanol:acetic acid:water: 4:1:5) with one major spot having Rf value of 0.28 visualized by freshly prepared Borinate-PEG spray reagent (2-aminoethyl diphenylborinate, 1% in methanol: polyethylene glycol-4000, 5% ethanol, 1:1, v/v). All the fractions were pooled, dried and yellow powdered residue (8.5 g) was collected. From the residue compound **1** was isolated by following the method reported earlier by our group [5]. Compound **1** was identified as 7-(1-O-β-D-galacturonide)-4′-(1-O-β-D-glucopyranosyl)-3′,4′,5,7-tetrahydroxyflavone by comparison with an authentic sample (Fig. 1). The extract and compound **1** were analyzed by analytical high performance liquid chromatography (HPLC) conducted on Shimadzu HPLC equipment comprising an LC-10ATVP pump, SPD-MOA AVP photodiode-array (PDA) detector, and a Rheodyne 7010 SIL-10 ADVP injector with 50 μL sample loop, using a RP-18 column (5 μm, 250 mm × 4.0 mm) at 271 nm (Fig. 2 (a) and (b)). The mobile phase consisted of 1.5% acetic acid in water:acetonitrile (83:17) at a flow rate of 1.0 ml min^{-1}.

2.2. Animals

Swiss albino mice weighing 20–24 g, 10–12 weeks old were used for the study after obtaining the clearance from Institutional Animals Ethics Committee. All the animals were maintained at $22 \pm 2\,^{\circ}C$ with 12 h light/dark cycle and free access to pellet food (Lipton India Ltd) and water ad libitum. According to ethical regulations on animal research, all animals used in experimental work received humane care. Test material prepared as a homogenized suspension and was administered orally by gavage for the duration of the experiment.

2.3. Chemicals

FACS lysing solution, FACS permeabilizing solution, Golgi plug, FITC (Fluorescein isothiocynate) labeled anti-CD4 monoclonal antibodies, PE (Pycoerytherin) labeled anti-CD3, CD19, CD8, IFN-γ, IL-4, IL-2 and IL-12 monoclonal antibodies were purchased from B.D. Biosciences, Corticosterone estimation kit (Neogen corporation, USA), Cyclosporine-A (Sigma–Aldrich). All other reagents used were of analytical grade.

2.4. Antigenic stimulus

Sheep red blood cells (SRBC) suspension in Alsever solution was obtained from animals housed at IIIM, Jammu and was processed as described elsewhere [6].

2.5. Experimental design

Studies were carried out in 2 different sets:

Experiment 1: In normal animals.
Experiment 2: In immune-suppressed animals.

2.5.1. Experiment 1

2.5.1.1. Immunomodulatory activity in normal animals. The animals were divided into 10 groups each of eight animals and were injected with SRBC (200 μl, i.p.) on day 0 and 6 for immunization. From day 0 (2 h post-SRBC injection) to 6, *C. cyminum* at varying doses of 25, 50, 100 and 200 mg/kg and compound **1** at 1, 2 and 4 mg/kg were administered orally once daily in respective groups. Levamisole (2.5 mg/kg p.o.) was taken as standard drug as it is one of the known immune-stimulant which restores the suppressed immune functions of B and T cells [7]. After experimental period of 7 days, blood was collected by retro-orbital puncture in EDTA coated tubes for the estimation of CD3, CD19, IFN-gamma and IL-4 using flowcytometry.

2.5.2. Experiment 2

2.5.2.1. Studies in Cyclosporine-A induced immune-suppressed animals. Following group configurations were maintained for the study: normal control (NC), Immune-suppressed control (ISC), *C. cyminum* (25, 50, 100 and 200 mg/kg) and Levamisole (2.5 mg/kg).