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Immunomodulatory activity of butanol fraction of *Gentiana olivieri* Griseb. on Balb/C mice

Satnam Singh[1], Yadav CPS[2], Malleshappa N Noolvi[1*]

[1]*Department of Pharmaceutical Sciences, ASBASJSM College of Pharmacy, Bela (Ropar)-140111, Punjab, India*
[2]*MJRP College of Health Care and Allied Sciences Mahatma Jyoti Rao Phoole University, Jaipur, Rajasthan, India*

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ABSTRACT

Objective: To explore the immunomodulatory properties of 80% ethanol extract and butanol fraction of *Gentiana olivieri* (*G. olivieri*) Griseb on Balb/C mice. **Methods:** The study was performed with basic models of immunomodulation such as the humoral antibody response (hemoglutination antibody titres), cell mediated immune response (delayed type hypersensitivity and *in vivo* carbon clearance or phagocytosis). Ethanol (80%) extract of flowering aerial parts of *G. olivieri* and its butanol fraction were administered p.o. (orally) to the mice. Levamisole, 2.5 mg/kg was used as standard drug. **Results:** There was a potentiation of immune response to sheep red blood cells by cellular and humoral mediated mechanisms comparable to levamisole (2.5 mg/kg) by both 80% ethanol extract and the butanol fraction at doses of 50-200 mg/kg in male Balb/C mice. Both significantly (P<0.01) potentiated the humoral immune response in cyclophosphamide (250 mg/kg) immunosupressed mice at 100 and 200 mg/kg of each extract and fraction as compared to control. The potentiation of delayed type hypersensitivity response was statistically significant (P<0.01) at 200 mg/kg of ethanol extract and 100, 200 mg/kg of butanol fraction as compared to control. The phagocytosis was significant at 200 mg/kg with butanol fraction of *G. olivieri*. **Conclusions:** The results reveal the immunostimulant effects of plant *G. olivieri* in mice by acting through cellular and humoral immunity in experimental models of immunity in mice. Butanol fraction is the most effective at a dose level of 200 mg/kg.

1. Introduction

The use of medicinal plant products as immunomodulators as possible therapeutic measure is becoming a new subject of scientific investigations[1]. Traditionally, the plant *Gentiana olivieri* (*G. olivieri*) Grieseb is used for treatment of a variety of disorders. The plant is reported to be sudorific in Ayurveda[2], widely used in east and south-east Anatolia as bitter tonic, stomachic and to combat some mental disorders in the different regions of Turkey. Macerated dried flowering herb in water is used to lower the blood pressure in type-2 diabetic patients, while infusion (2%-3%) is used as appetizer and as antipyretic[3]. The plant is known to possess a number of alkaloids, triterpenoid acids, fats, bitter secoiridoids glycosides, flavonoids (iso-orientin and its derivatives) and xanthones[2-5]. The presence of these phytoconstituents was confirmed by different qualitative tests performed on different extracts and fraction of *G. olivieri* (not shown in this study).

The different active phytoconstituents of plant such as polysacchrides, lectins, peptides, flavonoids have been reported to modulate the immune system in different experimental models[6]. Therefore, the chemical profile indicates herb *G. olivieri* may be a good source of immunomodulatory agents. Further the plant is known to possess hepatoprotective, antidiabetic, antimicrobial and anti-inflammatory bioactivities. However, till date no scientific evaluations are conducted for its immunomodulatory activity. Thus, this study was designed to evaluate the immunomodulatory activity of 80% ethanol extract and butanol fraction of aerial part of *G. olivieri* Griseb. in different experimental models of cellular and humoral immunity in mice.

*Corresponding author: Dr. Malleshappa N Noolvi, Professor and Head, Department of Pharmaceutical Chemistry, ASBASJSM College of Pharmacy, Bela (Ropar)-14011, Punjab, India.
Tel: + 91-9417563874
Fax: + 911881263655
E-mail: pharmacistsatnam@rediffmail.com, mnoolvi@yahoo.co.uk
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2. Materials and methods

2.1. Plant material

G. olivieri Griseb. (flowering aerial part) was procured from Himalya Herbal Store, Saharanpur, UP (India). The sample was identified on the basis of exomorphic characters, chemical reaction and review of literature by Dr. Singh HB, Taxonomist, NISCAIR, CSIR New Delhi. The voucher specimen of the sample (NISCAIR/RHMD/Consult/2009-10/1255/1259) was deposited in the NISCAIR, RHM Division, Dr. KS Krishna Marg (Near Pusa Gate), New Delhi (India).

2.2. Extraction and fractionation-phytochemicals

Aerial flowering herb (1 kg) was macerated with 80% ethanol (1:5 ratio) for 70 h. The solvent was filtered, marc was drained and the procedure was repeated thrice for the complete extraction of phytochemical. The combined extracts were reduced to one eighth of their original volume under rotavapour (Heidolph Hei Vap Advantage, MLIG3) at 50 ℃ and lyophilized to get a yield of 37 g 80% ethanol extract of *G. olivieri* Griseb. This lyophilized extract was thoroughly treated with butanol to get 9.8 g butanol fraction.

2.3. Animals

Male Balb/C mice (*Mus musculus*) 8-10 weeks old and weighing 18-22 g, in groups of six each were used for the study. The animals were housed under standard laboratory conditions with a temperature of (23±1) ℃, relative humidity of (55±10)%, 12/12 h light-dark cycles and fed with a standard pellet diet (Lipton India Ltd.) and water was given *ad libitum*. None of the animals were sacrificed throughout the study. Drugs for oral administration were freshly prepared as a homogenized suspension of 80% ethanol extract and butanol fraction of *G. olivieri* at doses of 50, 100, 200 mg/g each in gum acacia and administered orally, once daily for the duration of the experiment to Balb/C mice. Levamisole at the dose of 2.5 mg/kg (p.o.), was used as a standard immunostimulant drug. Cyclophosphamide and cyclosporine-A were used as the standard immunosuppressive agents at 250 and 5 mg/kg (p.o.).

2.4. Chemicals

Bovine albumin saline (BSA) was purchased from Himedia Mumbai. Ethylene diamine tetra acetic acid (EDTA), cyclophosphamide, cyclosporin-A and levamisole were purchased from Sigma Aldrich, New Delhi. All other reagents used were of analytical grade.

Fresh blood was collected from a healthy sheep from a local farmer. Sheep red blood cells (SRBCs) were washed thrice with normal saline adjusted to a concencentration of 0.1 mL containing 5×10^9 cells for immunisation and challenge.

2.5. Experimental protocols

All experimental protocols and the number of animals used for the experimental work were duly approved by the Institutional Animals Ethics Committee (IAEC); vide approval No. ASCB/IAEC/02/10/014, dated June 05,2010.

2.5.1. Humoral antibody response

The mice were divided into 10 groups, each consisting of 6 animals. Mice in group I (control) were given 0.1% bovine serum albumin (BSA saline) 0.3 mL/mouse for 7 days. Mice in group II (sensitized control) were given SRBCs on day 0. Mice in group III-VIII were given cyclophosphamide 250 mg/kg on day 0 and 80% ethanol extract and butanol fraction of *G. olivieri* at doses of 50, 100, 200 mg/kg bw (orally) for seven days. Mice in group IX and X were given levamisol 2.5 mg/kg and cyclophosphamide 250 mg/kg, respectively on day 0. The animals were immunized by injecting 200 μL of 5×10^9 SRBCs/mL intraperitonially (i.p.) on day 0. Blood samples were collected in microlitre tubes from individual animals of all the groups by retroorbital vein puncture on day 8. The blood samples were centrifuged and the serum was separated. Then, haemoglutination primary and secondary titres were performed[7,8].

2.5.2. Delayed type hypersensitivity

A new area of research is the discovery or/and development of immunomodulatory agents that are free from any toxic side effects and can be used for a long duration, resulting in continuous immuno-activation[9]. Animals were divided into ten groups of 6 each. Group I and II served as control and sensitized control, respectively as in humoral antibody response titre. Mice in group III-VIII were administered both extract and fraction of *G. olivieri* after SRBCs sensitization and once daily for seven days. Levamisole (2.5 mg/kg) and cyclosporine-A (5 mg/kg) were administered as standard immunostimulant (group IX) and T-cell suppressor (group X), respectively. The mice were then challenged by injecting the same amount of SRBCs intradermally into the right hind footpad, whereas left hind footpad served as control[10,11].

The footpad thickness was measured with sphaeromicrometer (pitch 0.01 mm) at 0, 24 and 48 h of SRBCs challenge.

2.5.3. In vivo carbon clearance test

The mice were divide into 8 groups. Each group consists of 6 animals. Group I (control) was given 1% sodium carboxy methyl cellulose in water (0.3 mL/mouse, orally) for 5 days, Mice in group II-VIII were given different concentrations of ethanol extract and butanol fraction of *G. olivieri* at doses of 50, 100, 200 mg/g, p.o., and standard drug (levimasole 2.5 mg/kg, p.o.) for 5 days. At the end of 5 days, after the gap of 48 h, the mice were injected, *via* the tail vein, with carbon ink suspension (10 μL/g bw). Blood samples were drawn (in EDTA solution 5 μL), from the retroorbital vein, at interval of 0 and 15 min. A 25 μL sample was mixed with 0.1% sodium

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