Journal of Ethnopharmacology 125 (2009) 252–256

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Immunomodulatory activities of the ethanolic extract of *Caesalpinia bonducella* seeds

Shruti Shukla[a,*], Archana Mehta[a], Jinu John[a], Pradeep Mehta[a], Suresh Prasad Vyas[b], Savita Shukla[c]

[a] *Faculty of Life Sciences, Dr. H.S. Gour Vishwavidyalaya, Sagar 470003, Madhya Pradesh, India*
[b] *Department of Pharmaceutical Sciences, Dr. H.S. Gour Vishwavidyalaya, Sagar 470003, Madhya Pradesh, India*
[c] *P.M.B. Gujrati Science College, Devi Ahilya University, Indore 452-001, India*

ARTICLE INFO

Article history:
Received 6 April 2008
Received in revised form 16 May 2009
Accepted 7 July 2009
Available online 14 July 2009

Keywords:
Caesalpinia bonducella seeds
Immunostimulation
Neutrophil adhesion
HA titre
DTH response
Phagocytic activity

ABSTRACT

Caesalpinia bonducella FLEMING (Caesalpiniaceae) is a plant well known for its medicinal value in Indian Ayurveda. However, to prove its efficiency for the clinical utilization, more experimental data will be beneficial.

Aims of the study: The present study involved the investigation of immunomodulatory activities of ethanolic extract of *Caesalpinia bonducella* seeds.

Materials and methods: Neutrophil adhesion test, haemagglutinating antibody (HA) titre, delayed-type hypersensitivity (DTH) response, phagocytic activity and cyclophosphamide-induced myelosuppression were determined by *in vivo* experiments.

Results: The evaluation of immunomodulatory potential by oral administration of ethanolic seed extract of *Caesalpinia bonducella* (200–500 mg/kg) evoked a significant increase in percent neutrophil adhesion to nylon fibers as well as a dose-dependent increase in antibody titre values, and potentiated the delayed-type hypersensitivity reaction induced by sheep red blood cells. Also it prevented myelosuppression in cyclophosphamide drug treated rats and good response towards phagocytosis in carbon clearance assay.

Conclusions: The results obtained in this study indicate that *Caesalpinia bonducella* possesses potential immunomodulatory activity and has therapeutic potential for the prevention of autoimmune diseases.

1. Introduction

Modulation of immune responses to alleviate the diseases has been of interest for many years and the concept of 'Rasayana' is based on related principles (Patwardhan et al., 1990). Rasayana, listed as a class in the texts of traditional Indian medicine literature, consists of a number of plants reputed to promote physical and mental health, improve defence mechanisms of the body and enhance longevity. Besides, a number of medicinal plants as Rasayanas have been claimed to possess immunomodulatory activities. Some of the Rasayana drugs as immunomodulatory agents such as *Withania somnifera*, *Tinospora cordifolia*, *Asparagus racemosus* and *Mangifera indica* (Dahanukar and Thatte, 1997; Dhuley, 1997; Davis and Kuttan, 2000; Makare et al., 2001) are well known for their traditional uses. Furthermore, medicinal plants used for immunomodulation can provide potential alternatives to conventional chemotherapies for a variety of diseases, especially when the host defense mechanism has to be activated under the conditions of impaired immune response. The use of plant products in the indigenous system of medicines as immunomodulators, indeed, can modulate the body's immune system, as a variety of plant derivatives such as polysaccharides, lectins, peptides, flavonoids and tannins have been reported to modulate the immune system in various *in vivo* models (Shivaprasad et al., 2006).

Caesalpinia bonducella F., commonly known as Nata Karanja, a prickly shrub found throughout the hotter parts of India, Myanmar and Sri Lanka, has grey, hard, globular shaped seeds with a smooth shining surface. Seeds consist of a thick, brittle shell with a yellowish white bitter fatty kernel (Nadkarni, 1954). *Caesalpinia bonducella* is reported to have multiple therapeutic properties like antipyretic, antidiuretic, anthelmintic and antibacterial (Neogi and Nayak, 1958), anti-anaphylactic and antidiarrheal (Iyengar and Pendse, 1965), antiviral (Dhar et al., 1968), antiasthmatic (Gayaraja et al., 1978), anti-amoebic and anti-estrogenic (Raghunathan and Mitra, 1982). Further, it has also been revealed that *Caesalpinia bonducella* has been traditionally used for the treatment of tumor, inflammation and liver disorders (Kritkar and Basu, 1984). Besides, the aqueous solution of the outer shell of the seeds of *Caesalpinia bonducella* has also been used traditionally by the tribal people

* Corresponding author at: Department of Botany, Dr. H.S. Gour University, Sagar 470003, Madhya Pradesh, India. Tel.: +91 9893 460620; fax: +91 7582 223236.
E-mail address: s.microb@yahoo.com (S. Shukla).

0378-8741/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2009.07.002

of Andaman and Nicober Islands for the relief of the symptoms of diabetes mellitus. Blood sugar lowering activity of *Caesalpinia bonducella* has been primarily evaluated with significant results in rabbit (Rao et al., 1994) and rat models (Biswas et al., 1997; Sharma et al., 1997).

However, there is no scientific report available in the literature on the immunomodulatory activity of *Caesalpinia bonducella* seed extract. Therefore, the present study was undertaken to assess the immunomodulating activities of the ethanolic extract derived from the seeds of *Caesalpinia bonducella* in relation with its folklore medicinal properties.

2. Materials and methods

2.1. Plant material

The seeds of *Caesalpinia bonducella* were collected in March 2006 from Sagar District, Madhya Pradesh, India. Further taxonomic identification was conducted by Professor Pradeep Mehta at the Department of Botany, Dr. H.S. Gour University, Sagar, MP, India. A voucher specimen has been deposited in the herbarium at the Laboratory of Ecology under the voucher specimen number (Bot/H/2692).

2.2. Preparation of the extract

The air-dried seeds of *Caesalpinia bonducella* (50 g) were extracted with 500 ml of ethanol by using soxhlet apparatus. The crude extract was filtered, and evaporated under reduced pressure to give a viscous dark mass with a percentage yield of 3.0% (w/w).

2.3. Drugs

Accurately weighed quantities of the ethanol extract were suspended in 1% sodium carboxy methylcellulose (SCMC) to prepare suitable forms of the dosages. Cyclophosphamide was used as a standard immunosuppressant.

2.4. Preliminary phytochemical screening

To identify the essential constituents of the ethanolic extract of *Caesalpinia bonducella* seeds such as alkaloids, terpenes and steroids, saponins, flavonoids, polysaccharides and tannins, a preliminary phytochemical screening was carried out using various test methods of Draggendorff's and Mayer's test, Liebermann–Burchard test, foam formation test, lead acetate test, Molisch's and Fehling's test and ferric chloride test, respectively (Trease and Evans, 1983).

2.5. Experimental animals

Animal use protocol was approved by the Dr. Hari Singh Gour University, Sagar, MP, India (*Animal Eths Comm/IE/98/Reg No 379/01/ab/CPCSEA*) and was in accordance with International Standard on the care and use of experimental animals (CCAC, 1993). Swiss albino rats of either sex weighing between 100 and 125 g were used for the experiment of this study. Animals were housed under standard conditions of temperature (25 °C), 12 h/12 h light/dark cycles and fed with standard pellet diet and tap water.

2.6. Antigen

Fresh blood was collected from sheep sacrificed in the local slaughter house. Sheep red blood cells (SRBCs) were washed three times in large volumes of pyrogen free 0.9% normal saline and adjusted to a concentration of 0.5×10^9 cells/ml for immunization and challenge.

2.7. Toxicity assay

Caesalpinia bonducella dried ethanolic seed extract was dissolved in water and administered orally to different groups of rats in dosages ranging from 100 to 1000 mg/kg for the LD_{50} study using the modified method (Ghosh, 1971). There was no lethality in any of the groups after 7 days of treatment.

2.8. Neutrophil adhesion test

Wilkinson (1978) method was employed for neutrophil adhesion test. Rats of group I, were served as control and received 10 ml/kg normal saline, whereas groups II, III, IV and V were pre-treated with different concentrations of ethanolic extract of *Caesalpinia bonducella* seeds (200–500 mg/kg, oral). On day 14 of drug treatment, blood samples were collected by puncturing retro-orbital plexus into heparinized vials and analyzed for total leukocyte cell (TLC) and differential leukocyte cell (DLC) counts. After initial counts, blood samples were incubated with nylon fibers for 15 min at 37 °C. The incubated blood samples were again analyzed for TLC and DLC, respectively to give neutrophil index of blood samples. The percent neutrophil adhesion was calculated by the following formula:

$$\text{Neutrophil adhesion } (\%) = \frac{NI_u - NI_t}{NI_u} \times 100$$

where NI_u is the neutrophil index of untreated blood samples and NI_t is the neutrophil index of treated blood samples.

2.9. Haemagglutinating antibody (HA) titre

Puri et al. (1993) described the method for haemagglutinating antibody titre. The animals were immunized by injecting 0.1 ml of SRBCs suspension containing 0.5×10^9 cells intraperitoneally on day 0. Blood samples were collected in micro-centrifuge tubes from individual animal by retro-orbital puncture on day 7. The blood samples were centrifuged and serum was obtained. Antibody levels were determined by the hemagglutination technique. Equal volumes of individual serum samples of each group were pooled. Twofold serial dilutions of pooled serum samples made in 25 μl volume of normal saline in microtitration plates was added to 25 μl of 1% suspension of SRBCs in saline. After mixing, the plates were incubated at 37 °C for 1 h and examined for haemagglutination under microscope. The reciprocal of the highest dilution of the test serum agglutination was taken as the antibody titre.

2.10. Delayed-type hypersensitivity (DTH) response

The rats were challenged by injection of 0.5×10^9 cells SRBCs in right hind foot pad. Foot thickness was measured after +24 and +48 h of this challenge. The differences obtained for pre- and postchallenge foot thicknesses were taken for the measurement of DTH and were expressed in mm. The extract was administered orally on day 0 and continued till day 7 of challenge (Shivaprasad et al., 2006).

2.11. Phagocytic response

The method was described by Cheng et al. (2005). The animals were treated from day 0 to day 7 with different concentrations of the extract. On day 7, all the animals of the entire groups received the treatment of an intravenous injection of (0.3 ml per 30 g) Indian ink