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ORIGINAL ARTICLE

Immunomodulatory activity of methanolic fruit extract of *Aegle marmelos* in experimental animals

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KEYWORDS

Aegle marmelos;
Immunomodulation;
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Abstract *Aim:* The aim of the present study was to investigate the immunomodulatory action of methanolic extract of *Aegle marmelos* fruit (FEAM) in experimental model of immunity.
Methods: Cellular immunity was carried out by neutrophil adhesion test and carbon clearance assay, whereas, humoral immunity was analyzed by mice lethality test and indirect haemagglutination assay. FEAM dose was selected by Stair case method (up and down) and administered at 100 and 500 mg/kg orally. The *Ocimum sanctum* (OSE, 100 mg/kg, *p.o*) was used as standard.
Results: FEAM at 100 and 500 mg/kg produced significant increases in adhesion of neutrophils and an increase in phagocytic index in carbon clearance assay. Both high and low doses of FEAM significantly prevented the mortality induced by bovine *Pasteurella multocida* in mice. Treatment of animals with FEAM and OSE significantly increased the circulating antibody titre in indirect haemagglunation test. Among the different doses, low one was more effective in cellular immunity models than the high. However, all the doses exhibited similar protection in humoral immunity procedures.
Conclusion: From the above findings, it is concluded that FEAM possesses potential for augmenting immune activity by cellular and humoral mediated mechanisms more at low dose (100 mg/kg) than high dose (500 mg/kg).

1. Introduction

A global reliance on alternative system of medicine for chronic and acute ailments resulted in an intense area of research and discovery of a number of herbs with potential to curb diseases. Among them, ample number of herbs has been exploited for modulation of immune system from Ayurvedic formulation either alone or in combinations.

Traditionally, various parts of the plant, *Aegle marmelos* Corr. (Rutaceae), used for the treatment of a variety of disorders (Nadkarni, 1986). The plant is reported to have multiple

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therapeutic properties such as anti-inflammatory, antipyretic and analgesic (Arul et al., 2005; Shankarananth et al., 2007), anti diabetic (Kamalakkannan et al., 2003; Arumugam et al., 2008; Kesari et al., 2006), anti diarrheal (Shoba and Thomas, 2001), anti hyperlipidemic (Vijaya et al., 2009), antifungal (Rana et al., 1997), antimicrobial, antibacterial and anti parasitic (Ulahannan et al., 2008), anti cancer (Gangadevi and Muthumary, 2008), anti malaria (Elango et al., 2009), hepatoproctective (Singh and Rao, 2008) and cardioprotective (Vimal and Devaki, 2004) potentials.

Environmental pollutants and dietary habits cause disturbances in immune activities and diet containing micronutrients and antioxidants are known to prevent these alterations (Bafna and Mishra, 2010). The use of herbs as immunomodulators in the indigenous system of medicines, indeed, can modulate the body's defence mechanism. The following active constituents of plant derivatives such as polysaccharides, lectins, peptides, flavonoids and tannins have been reported to modulate the immune system in different experimental *models* (Shivaprasad et al., 2006). The fruit of *Aegle marmelos* is reported to contain many functional and bioactive compounds such as carotenoids, phenolics, alkaloids, coumarins, flavonoids, terpenoids, and other antioxidants. In addition, it also has many vitamins and minerals including vitamin C, vitamin A, thiamine, riboflavin, niacin, calcium, and phosphorus (Das and Das, 1995). Therefore, the chemical profile indicates *Aegle marmelos* as good sources of immunomodulatory agents. Further, the fruit of the plant has been used for many disorders such as chronic diarrhoea & dysentery and act as a tonic for the heart and brain. It is widely used as indigenous traditional medicine for variety of stress disorders including immunodeficiencies (Das and Das, 1995). However, till date no scientific evaluations are conducted for confirming its role as immunostimulant. Thus, this study was designed to study the immunomodulatory activity of extract of *Aegle marmelos* fruit extract in different experimental models of cellular and humoral immunity in animals.

2. Materials and methods

2.1. Experimental animals

Laboratory bred Wistar albino rats (180–200 g) and albino mice (20–25 g) of either sex were housed at 25° ± 5 °C in a well-ventilated animal house under 12/12 h light/dark cycle. The mice were procured from Drug Testing Laboratory, Bangalore. The animals had free access to standard food pellets (Amrut Laboratory Animal feed, Maharashtra, India) containing (% w/w) protein 22.10, oil 4.13, fibre 3.15, ash 5.15, sand (silica) 1.12, and water *ad libitum*. Bedding material was removed and replaced with fresh paddy husk as often as necessary to keep the animals clean and dry. The animals were maintained under standard conditions in an animal house approved by Committee for the purpose of control and supervision on experiments on animals (CPCSEA). The experimental protocol was approved by Institutional ethical committee (KCP/IAEC-24/2008-09). The animals were subjected for quarantine (10 days) prior to experimentation

2.2. Procurement of plant material and extraction

Aegle marmelos fruits were purchased from S.K.R. Market Bangalore (India). The plant was identified and authenticated by Regional Research Institute (Bangalore, India) (RRCBI-Mus/02) The fruits were given to Phytotech Extracts Pvt. Ltd. (Bangalore, India) to get methanol fruit extract of *Aegle marmelos* (FEAM). The ethanolic extract of *Ocimum sanctum* leaves (Natural remedies, Bangalore, India) was used as standard immunomodulatory agent (Mediratta et al., 2002).

2.3. Chemicals and their sources

Leishmann's stain and gluteraldehyde were purchased from Merck (Mumbai, India). Indian ink from HIMEDIA (Mumbai, India). WBC diluting fluid and EDTA from Nice Chemicals (Cochin, India). *Pasteurella multocida* of bovine origin and its vaccine were (Institute of Animal Health and Veterinary Biologicals, Bangalore, India). Nylon fibers (Local market, Bangalore, India).

2.4. Antigen preparation

Fresh sheep blood was collected from the local slaughterhouse. 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 (Thomas et al., 2007).

2.5. Preliminary phytochemical screening of extract

Preliminary phytochemical analysis was carried out to check and identify the active constituents of the methanolic extract of *Aegle marmelos* fruit such as alkaloids, carbohydrates, flavonoids, terpenes and steroids, saponins and tannins by using test methods of Draggendorff's and Mayer's test, Molisch's and Fehling's test, lead acetate and magnesium ribbon test, Liebermann–Burchard test, foam formation test, ferric chloride test and gelatin test, respectively (Trease and Evans, 1983).

2.6. Acute toxicity studies (Ghosh, 1984)

The acute toxicity study was carried out to select the dose, by using up and down or stair case method. Two mice were selected with a dose of 50 mg/kg orally and examined for a period of 24 h for mortality. The subsequent doses are then increased by 1.5 factors to attain maximum non-lethal and minimum lethal dose. The extract was found to be safe at the dose of 5 g/kg *p.o.* According to office of pollution prevention and toxics (OPPT) guidelines (http://www.epa.gov/oppts/home/guideline.htm) (Kubavat and Asdaq, 2009), 1/10th and 1/50th of the maximum safe dose (5 g/kg) corresponding to 500 mg/kg and 100 mg/kg were selected as high and low doses, respectively.

2.6.1. Experimental protocol

The drug solutions were prepared in distilled water for oral administration. Immunomodulatory activity was checked both at cellular and humoral levels. Cellular immunity was evaluated by neutrophil adhesion test and carbon clearance assay, whereas, humoral immunity was analyzed by mice lethality test and indirect haemagglutination assay. All the experimental models had four common groups consisting of six animals each. Group I, was served as control and received (vehicle 1 ml/100 g, *p.o*), group II, received the ethanolic extract of