



Immunomodulatory Activity of Methanolic Extract of *Ruta graveolens*

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ABSTRACT

Many herbal remedies individually or in combination have been recommended in various medical treatises for the cure of different diseases. However, the use of plant remedies, known to possess natural antioxidant, antibacterial, antiviral, immunomodulatory and many other activities, has increased in the last decade in human and animal medicine, as it is perceived as a natural approach to treat disease. Traditionally, leaves of the herb *Ruta graveolens* are claimed to possess immunobiological activity and hence the reason behind evaluating the immunomodulatory potential of methanolic extract of *Ruta graveolens* (MERG). (MERG) supposed to contain 1-Phenyl-naphthalene lignans as the secondary metabolites having potent immunobiological activities. The immunomodulatory activity was evaluated by the assay of Immunoglobulin (Ig) production-stimulating activity. Oral administration of the extract significantly stimulated the Ig production at 100 mg/kg dose levels and it increases with increased concentration of the extract. The study demonstrates that *Ruta graveolens* can be used as a potent immunomodulator.

1. Introduction

The immune system is known to be involved in the etiology and pathophysiological mechanisms of several diseases. The function and efficiency of the immune system may be influenced by many exogenous and endogenous factors resulting in either immunosuppression or immunostimulation. Several agents have been shown to possess an activity to normalize or modulate pathophysiological processes and are called immunomodulatory agents. Along with the available drugs, a large number of herbal drugs are promoted in traditional Indian treatments, for their immunomodulating activity [1, 2].

Immunomodulatory agents of plants enhance the immune responsiveness of an organism against a pathogen by activating the immune system [3]. The modulation of immune response by various agents in order to alleviate the disease has been of interest since many years and the concept of Indian Rasayana in Ayurveda has similarity with it. Immunomodulators are being used as an adjuvant in conditions of immunodeficiency in cancer and in acquired immunodeficiency syndrome [4].

Ruta graveolens commonly known as 'Sudab' is strongly aromatic evergreen perennial herb. The methanolic extract of the herb shows antipyretic, diuretic, antitoxic, antitumoral, antifungal and antifertility, antiplatelet and cytotoxic activities. It is recommended herb for painful menstruation, stomach trouble, cramps in the bowels, nervousness, hysteria, spasms, convulsions, pain in the head, confusion, dizziness, colic and convulsions in children, sciatica, pain in the joints and gout [5-7]. It is also believed to resist poison. In homeopathic dentistry, ruta is used to relieve pain. Other homeopathic uses for ruta include treatment of plantar warts on the feet, blood and mucus in stools, pain in the rectum, rectal prolapsed and general weakness and depression [8]. The current wave of interest is indeed directed to the above mentioned aspects.

Lignans represent a characteristic and important group of biologically active polyphenolic metabolites, derived from two phenylpropanoid units [9]. Their rich and varied structural diversity and miscellaneous biological activities have always attracted considerable attention of phytochemists, occasionally including also botanists, pharmacologists, environmentalists and recently even experts for a superior and safe food production [10].

At present, the growing interest in bioactive lignans, is motivated by a potential use of these compounds as phytopharmaceuticals or nutraceuticals, i.e. biologically active food supplements [11-16], preventively efficient to protect health against the increasing number of chronic diseases, or health problems resulting from excessive environmental stress and working activity. It is known that the activity of lignans depends to a great extent on the type and position of substituents. The natural lignan lactones show a considerably high activity with a potency to be effective in assumed therapeutic exploitation. It has achieved the maximum activity revealing a specific course of immune action.

2. Experimental Methods

2.1 Plant Material

Aerial parts of *Ruta graveolens* L. (family Euphorbiaceae) were collected in the month of November from Shree Shail Medifarm, Nagpur, plant nursery, Maharashtra, India. A Voucher specimen of the plant has been deposited in the Herbarium, Department of Botany, Rashtra Sant Tukdoji Mararaj Nagpur University, Nagpur for further reference. Fresh leaves of *Ruta graveolens* were washed with distilled water so as to remove dust and other foreign particles. The leaves were then left on a clean surface to dry well. The leaves were air-dried under shade for 10-15 days. Then the dried material was grinded to fine powder using an electric grinder and stored in air tight bottles. The powdered material was used further, for, phytochemical screening and preparation of extracts.

2.2 Extraction Methodology

Powdered aerial parts (500 g) were packed in soxhlet apparatus. The drug was defatted with petroleum ether (60-80 °C) for about 30-35 complete cycles. Defatted material was extracted with two liters of petroleum ether by soxhlet apparatus and then the extracted material is successively extracted with methanol and water and finally maceration at room temperature, then these extracts were dried by rotary vacuum dryer.

2.3 Experimental Animals

Male wistar rats (150-200 g) were used and housed three animals per cage with paddy husk as bedding. Animals were housed at temperature of 25±2 °C and relative humidity of 30-60%. A 12:12 light and dark cycle was

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followed. Animals had free access to pelleted feed and purified water ad libitum. The animals were divided into three groups consisting of six animals each. The methanolic extract of *Ruta graveolens* was administered for 5 days at a dose of 100 mg/kg/day (Group I), 200 mg/kg/day (Group II), 400 mg/kg/day (Group III) for assessment of immunomodulatory effect. The animal experimental protocols were approved by the Institute of Animals Ethics Committee.

2.4 Assay of Ig Production-Stimulating Activity

Blood withdrawn from retro-orbital vein aseptically, centrifuged and serum was used for the determination of IgM levels. The Ig production-stimulating activity was examined by measuring the amount of secreted Igs by ELISA method [17]. A goat anti-human IgM antibody solution at 1 µg/mL (Cappel, Durham, NC, USA) in a 50 mM sodium carbonate-bicarbonate buffer (pH 9.6) was added to a 96-well microplate (Nunc, Roskilde, Denmark) at 100 µL/well and incubated for 2 h at 37 °C. After washing the wells three times with 0.05% Tween 20-PBS (PBS-T-Phosphate buffer saline -T), each well was blocked with PBS containing 1% (w/v) BSA for 2 h at 37 °C. Following the blocking reaction, each well was treated with 50 µL of the culture supernatant for 1 h at 37 °C. After washing with PBS-T each well was then treated for 1 h at 37 °C with 100 µL of the horseradish peroxidase (HRP)-conjugated anti-human IgM or IgG antibody (Biosource International, Camarillo, CA, USA) diluted at 1:1000 in PBS containing 1% BSA. After washing again 0.6mg/mL of 2, 2'-azino-bis(ethylbenzothiazoline-6-sulfonic acid) (ABTS) dissolved in a 0.03% H₂O₂-0.05 M citrate buffer (pH 4.0) was added to each well at 100 µL and the absorbance at 415 nm was measured after adding 100 µL of 1.5% oxalic acid to terminate the coloring reaction.

3. Results and Discussion

The oral administration of the plant extracts significantly stimulated the Ig production-stimulating activity. The result of Ig production-stimulating activity was shown in the Table 1 and Fig. 1 below. With a dose of 100, 200 and 400 mg/kg, the Ig production was 18.53 ± 4.23, 21.10 ± 3.30, and 31.57 ± 8.73 respectively in comparison to the corresponding value served by PBS (Phosphate Buffer Saline) and PHA (Phytohaemagglutinin) as the negative and positive control respectively. The IgM production was enhanced in a concentration dependent manner.

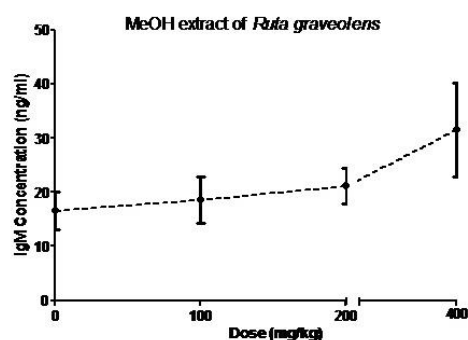


Fig. 1 Effect of plant extract on IgM production

Table 1 Effect of *Ruta graveolens* on IgM production

S. No.	Compound	100 mg/kg	200 mg/kg	400 mg/kg
1	MeOH extract of <i>Ruta graveolens</i>	18.53 ± 4.23	21.10 ± 3.30	31.57 ± 8.73
2	PHA	50.52 ± 3.23		
3	PBS	6.01 ± 1.85		

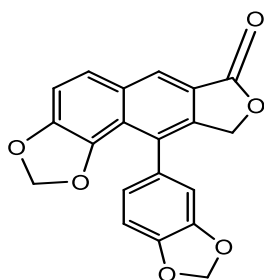


Fig. 2 Helioxanthin (Lignan) [*R. graveolens* extract (MeOH)]

Phytochemical screening of the methanolic extract of *Ruta graveolens* shows the presence of 1-Phenyl-naphthalene lignans [18]. Similar lignan derivatives were synthesized in the laboratory [19, 20] and analysed for immunomodulatory potential, showing sharp potency for immunostimulation [21, 22], here not discussed in the paper. The herbal extract when evaluated for immunomodulatory properties shows maximum production of immunoglobulins and thus can be concluded that its pharmacological activities were mainly due to the presence of 1-Phenyl-naphthalene lignan - Helioxanthin in *Ruta graveolens* (Fig. 2). *Ruta graveolens* has ability to modulate humoral immune responses by acting at various levels in immune mechanism such as Ig production.

The present study was carried out to determine the immunomodulatory activity of methanolic extracts of *Ruta graveolens* at various dose levels. Antibody molecules, a product of B-lymphocytes and plasma cells, are central to humoral immune responses; IgG and IgM are the major immunoglobulins which are involved in the complement activation, opsonization, neutralization of toxins etc., [23]. Methanolic extracts of *Ruta graveolens* showed significant ($p < 0.05$) increase in haemagglutination titer compared to vehicle control. The present investigation suggests that methanolic extract of *Ruta graveolens* stimulate the humoral immunity. All over findings suggest that methanolic extract of *Ruta graveolens* containing occurring 1-phenyl-naphthalene lignans positively modulates the immunity of the host.

4. Conclusion

Immunomodulatory agents of plant origin enhance the immune responsiveness of the organism against a pathogen by activating the immune system. However these agents should be subjected to systemic studies to substantiate the therapeutic claims made with regard to their clinical utility. Due to economic constraints, providing modern medical healthcare in developing countries such as India is still a far reaching goal. Therefore, it is prudent to look for options in herbal medicine as immunomodulatory as well. The methanolic extract of *Ruta graveolens* possesses significant immunomodulatory activity. We should work continuously towards establishing the scientific basis of use of such plants in immune disease. Such an ethno medical approach for disease is a practical, cost effective and logical for its treatment.

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