

ORIGINAL PAPER

Homeopathic remedies with antineoplastic properties have immunomodulatory effects in experimental animals



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Background: Our previous work suggests that *Thuja occidentalis*, *Carcinosinum* and *Ruta graveolens* have antineoplastic properties. The mechanism of this action has not previously been studied. We studied the hypothesis that the mechanism of action is through the immune modulation.

Methods: We evaluated the effects of *Thuja occidentalis*, *Carcinosinum* and *Ruta graveolens* 1M, 200c and 30c on the immune system of Balb/c mice. The homeopathic preparations were administered orally for ten consecutive days. Haematological parameters (Total White Blood Cell (WBC) Count, Differential Count and Haemoglobin content), haematopoietic parameters (bone marrow cellularity and α -esterase positive cells) and immune parameters for antibody response and lymphoid cell proliferation were assessed using standard methods. Results were analysed by statistical comparison with the control.

Results: We observed significant enhancement of haematological parameters including total WBC count, haematopoietic parameters such as bone marrow cellularity and the number of α -esterase positive cells, other parameters of immune response such as circulating antibody titre and the number of plaque forming cells (PFC), particularly with higher dilutions of *Thuja* and *Ruta*. Enhanced proliferation of B and T lymphoid cells was also observed. No toxic effects were observed.

Conclusions: The results suggest immunomodulatory activity of homeopathic preparations in high dilution. This may be a mechanism through which homeopathic preparations act. *Homeopathy* (2015) 104, 211–219.

Keywords: *Thuja*; *Carcinosinum*; *Ruta*; Immunomodulatory activity

Introduction

Ultra diluted homeopathic medicines may strengthen the physiological system of the organism to effectively combat a pathological condition, possibly targeting the immune sys-

tem for their action. Few scientific studies have been conducted to validate their efficacy, reproducibility and mechanism. A few experimental and clinical studies were reported which can be considered as promising cases. For instance, a report regarding the complex homeopathic medication named Canova, revealed that its *in vitro* treatment on bone marrow cells could stimulate the differentiation of mono nuclear cells and activation of progenitor cells and stromal cells.¹ Preclinical animal model studies on ultra dilutions of thymic hormones and cytokines proved their immunostimulatory roles under immunosuppressed condition.² A different study reported the specific modulation of IgG and IgM mediated antibody responses in

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experimental animals by a homeopathic preparation of keyhole limpet haemocyanin (KLH) antigen.³ Similarly various animal experimental studies regarding the capability of many homeopathic remedies to modulate the immune effectors were also documented.^{4–7} However, drugs possessing immunomodulatory effects will be demanded during every pathological condition including malignancy and various immunosuppressed conditions like in the course of chemotherapy or radiotherapy. Recently, a few immune therapies are being widely used but they are also capable to create anaphylactic reactions, adverse and even fatal side effects.⁸ Here comes the importance of natural and non-toxic immunomodulators which can boost up the immune system thereby rendering a powerful defense mechanism to the body. Several plant products were reported to act as immunomodulators by regulating the expression of various cytokines required for differentiation, maturation and activation of various cells of immune system.^{9–12}

All the homeopathic medicines selected for the present study are used by homeopathic practitioners to treat benign tumours, ulcer and some malignant conditions.¹³ Detailed studies were reported which could prove the immunopharmacological properties of *Thuja occidentalis*, whose aqueous-alcoholic extract or their fractions or extract containing herbal preparations, can induce antibody response, cytokine induction, T cell response and HIV virucidal properties in pre-clinical experimental conditions.^{14–17} Clinical data also showed the efficacy of the extract containing preparations against respiratory tract infections and common cold.^{18,19} Even though the fresh plant extract has intoxicating properties, the preparations that contain diluted amounts of the same was proved to be clinically safe. The toxic property has been explained owing to the presence of rich quantities of a monoterpene called thujone. But this compound was reported to be safe and non-toxic up to a daily dose of 75 mg in humans.^{20,21} *Ruta graveolens*, is a traditionally used medicinal plant especially in alternative medicines. Its potentized preparation at 200c was reported to inhibit tumour development in N-nitrosodiethylamine (NDEA) induced hepatocarcinoma models in rats, DLA induced solid tumour model, EAC induced ascites tumour model and 3-methylcolanthrene induced sarcoma model in mice.^{22–24} There are other reports also which proved the clastogenic potential of *Ruta graveolens* and its homeopathic preparation on bone marrow cells in mice.²⁵ However a study on normal monkey kidney cell line, *in vitro*, showed that it is non-toxic to normal cells.²⁶ *Carcinosinum*, the potentized preparations from carcinomas and has been used against malignant conditions, was also subjected to various scientific studies. It was reported that *Carcinosinum* 200c could induce apoptosis in DLA cells *in vitro*, mediated through the upregulation of p53 gene.²⁴ Another study checked the effect of intermittent use of *Carcinosinum* 200c along with *Natrum sulphuricum* 30c against azo dye induced hepatocarcinoma which resulted in synergistic therapeutic effects.²⁷ It has also been reported that potentized preparations of *Thuja occidentalis*

and *Carcinosinum* could exert preferential cytotoxic effects against breast cancer cell lines by altered expression of cell cycle regulatory proteins as well as induction of the apoptotic cascade.²⁸ However, due to methodological constraints, further experimental studies are recommended to confirm these results.²⁹

The immunostimulatory effects of potentized preparations are not well studied or yet been proved experimentally. The present study evaluated the effect of homeopathic preparations of *Thuja*, *Carcinosinum* and *Ruta* at their different potencies on the immune system in normal conditions through verification of various haematological factors and other significant parameters which can assess the response status of immune system.

Materials and methods

Homeopathic medicines and method of administration

1M, 200c and 30c potencies of the homeopathic drugs namely *Thuja* (*Thuja occidentalis*), *Carcinosinum* and *Ruta* (*Ruta graveolens*) in potentized alcohol, were purchased from Willmar Schwabe, Germany. 90%, 30c potentized alcohol was provided specially for our research purpose by Similia Homoeo Laboratories, Aluva, Kerala, India. This was used as the vehicle control. 1 mL of each homeopathic preparation was diluted to 10 mL with autoclaved distilled water and 100 μ L of it is administered to each animal. All the medicines as well as the vehicle control were administered orally for ten consecutive days.

Chemicals

RPMI-1640 medium and Concanavalin A (Con A) was purchased from Sigma Aldrich, (St Louis, MO). Foetal Calf Serum was purchased from Biological Industries, Israel. Pararosaniline and α -naphthyl acetate were obtained from Loba Chemie, Mumbai. Harris' haematoxylin was purchased from Nice Chemicals, Cochin, Kerala. Radioactive (³H) thymidine was purchased from the Board of Radiation and Isotope Technology, Mumbai, India. All other chemicals used were of analytical reagent grade.

Animals

Inbred Balb/c mice (male, 4–6 weeks old) were maintained at Amala Cancer Research Centre breeding section, in well ventilated cages under controlled environmental conditions, and fed with normal mice chow (Sai Feed, India) and water *ad libitum*. All animal experiments were performed with prior permission from Institute Animal Ethical Committee (IAEC) and strictly adhering to the rules and regulations of the Committee for the purpose of Control and Supervision of Experiment on Animals (CPSCEA), constituted by the Animal Welfare Division, Government of India.

Experiment design

In all the experiments, drug administration, observations and the analysis of results were conducted blind.

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