

# Immunomodulatory effects of homoeopathic medicines: A review of pre-clinical studies

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## Abstract

Immunomodulation is a kind of regulatory modification in the immune system so as to bring the desired response. In this review the immunomodulatory effects of homoeopathic medicines – *Rhus toxicodendron*, *Mercurius solubilis*, *Echinacea*, *Aconitum*, *Lachesis* and *Apis* – and homoeopathic combination remedy Canova<sup>®</sup> are discussed. The review was conducted using PubMed and references from the relevant articles. The keywords comprising the names of above homoeopathic medicines along with terms ‘immune function’, ‘cytokines’, etc., were used for search. The review shows that homoeopathic medicines produced modulation of immune function at multiple levels such as modulation of expression of genes, stimulation of macrophage and polymorph nuclear cells, changes in expression of surface receptors and induction of cytokines. Extensive studies are required to explore the immunomodulatory effects of vast number of homoeopathic medicines. Studies based on human-derived immune cells specifically need more attention.

**Keywords:** Cytokines, Homoeopathic medicines, Immunomodulation, Macrophage, Pre-clinical studies

## INTRODUCTION

Human immune system has evolved through eons of struggle between the host and pathogens.<sup>[1]</sup> It broadly comprises two components: the innate immunity and the acquired immunity. The innate immunity is non-specific immunity and exists from birth. It operates in a generalised fashion against multiple types of pathogens and deploys mechanical agents such as intact skin and mucosa as well as immune effectors cells such as phagocyte cells and natural killer cells. These cells have limited capabilities for identifying a vast diversity of pathogens which surround us. The acquired immunity is phylogenetically recently evolved and is more sophisticated than innate immunity. It is endowed with a large repertoire of lymphocytes, each with a specific type of receptor on its surface. Repeated exposure to a particular pathogen causes replication of selective T- and B-lymphocyte populations, thus enabling a stronger immune response on each subsequent encounter with the same antigen. The magnitude of diversity of T- and B-lymphocyte receptors can be appreciated by the fact that there are  $>10^{11}$  types of immunoglobulin receptors on B-lymphocytes and  $>10^{16}$  types of  $\alpha/\beta$  T-cell receptors on T-lymphocytes. This enormous diversity of receptors enables a

specific immune response to every possible antigen on the earth. The B-lymphocytes are responsible for humoral immunity which is mediated via immunoglobulin. The B-lymphocytes get activated when their immunoglobulin receptor binds to an antigen. On activation, they transform themselves into plasma cells, which synthesise antigen-specific antibodies. Some of the activated B-lymphocytes do not become plasma cells instead they turn into memory cells which continue to produce small amounts of antibodies long after the infection has been overcome. On re-encounter with the antigen, the memory cells quickly divide to produce new clones of appropriate plasma cells. Unlike B-cells, the receptors on T-lymphocytes cannot bind with antigens in their native form. The antigen is phagocytosed and fragmented by Antigen-Presenting Cells (APCs), for example – dendritic cells. The APCs have MHC molecules as their surface receptors. The most antigenic fragment (epitope) of an antigen is clubbed with MHC

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molecule and is presented by the APC to the T-lymphocyte. Besides this interaction of T-lymphocyte with epitope–MHC complex, there is an interaction of CD28 molecules on surface of T-lymphocyte with B7 molecules on APC or certain cytokines such as interleukin-1 (IL-1) produced by APC. These two types of interactions finally lead to activation of T-lymphocyte. Among various populations of lymphocytes, the CD4 T-lymphocytes play a crucial role in humoral as well as cell-mediated immunity. CD4 T-lymphocytes help B-lymphocytes to differentiate into antibody forming plasma cells. They also mediate delayed-type hypersensitivity (DTH) which gives rise to the characteristic granulomatous inflammation. They induce CD8 T-lymphocytes to differentiate into cytotoxic T-lymphocytes. Based on the patterns of their cytokine secretion, CD4 T-lymphocytes can be subdivided into Th1 cells, which produce IL-2, interferon- $\gamma$  (IFN- $\gamma$ ) and tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), and Th2 cells, which produce IL-4, IL-5, IL-6, IL-10 and IL-13.

### CYTOKINES ARE MEDIATORS OF IMMUNE RESPONSE

The mechanism of immune response is a complex process. It operates through a series of steps starting from phagocytosis of antigen by APCs, presentation of epitope of antigen by APCs to T-lymphocyte and ultimately stimulation of lymphocytes and initiation of humoral or cell-mediated immune response. Besides lymphocytes, granulocytes (neutrophils, eosinophils, basophils and mast cells) also participate in the immune response. These cells release several products such as cytokines, lymphokines, various enzymes, free oxygen radicals and reactive nitrogen species during the process of inflammation. For effective execution of immune response, a synergy exists between the cells of immune system and also between the immune system and the neuroendocrine system.<sup>[2]</sup> Various cytokines play a key role in maintaining this synergy and orchestrating the illness response. Cytokines are a diverse family of polypeptide hormones and growth factors that regulate many cellular processes. Although they differ in detail, all cytokines are four-helix bundles. Cytokines are produced by a variety of immune cells to communicate and orchestrate an immune response. Any imbalance in the cytokine network may lead to initiation and perpetuation of autoimmune diseases, tumor growth and various other diseases. In modern medicine, a novel approach of immunomodulatory therapy is being adopted in the treatment of both communicable and non-communicable diseases. Immunomodulatory therapies involving single and/or multiple Toll-like receptors, their targets and signaling pathways may be of value in both infective and non-infective pathobiologies.<sup>[3]</sup> Immunomodulation can also be implemented through cytokines by the administering anti-inflammatory cytokines such as IFN- $\beta$ , growth factors such as transforming growth factor  $\beta$ , IL-4 and IL-10 or by neutralising pro-inflammatory cytokines such as IL-2, IFN- $\gamma$ , IL-12, TNF- $\alpha$  and IL-1.<sup>[4]</sup> Currently, immunomodulatory cytokine therapy is instituted in a variety of diseases such

as cancer,<sup>[5]</sup> retinal disorders,<sup>[6]</sup> rheumatoid arthritis,<sup>[7]</sup> inflammatory bowel disease<sup>[8]</sup> and multiple sclerosis.<sup>[9]</sup>

### HOMOEOPATHY AND IMMUNOMODULATION

Homoeopathy is a system of therapeutics based on the key principle of ‘Like cures like’. When homoeopathic medicines are prescribed in ultra-diluted form on the basis of symptom similarity, they stimulate body’s own defense mechanisms to fight against the pathogen. Homoeopathy experiments<sup>[10-12]</sup> on basophils and mast cells showed that homoeopathic medicines in ultra-diluted form can influence degranulation of mast cells and basophils. Although these experiments were performed to explore the action of ultra-diluted substances, they raised the possibility that homoeopathic medicines act via their action on immune system.

Below is the review of some homoeopathy pre-clinical studies performed to explore the immunomodulatory action of homoeopathic medicines – *Rhus toxicodendron* (*rhustox*), *Mercurius solubilis* (*Merc sol*), *Echinacea*, *Aconitum*, *Lachesis* and *Apis* – and homoeopathic combination remedy Canova®. Canova® is a Brazilian medical formulation based on homoeopathic technique. It is composed of *Aconitum napellus*, *Thuja occidentalis*, *Bryonia alba*, *Arsenicum album* and *Lachesis muta* and <1% ethanol in distilled water. The medicine is also called by the name of *Metodo canova* (MC, *Canova* method). The following review was conducted using PubMed and references from the relevant articles. The keywords comprising the names of above homoeopathic medicines along with terms ‘immune function’, ‘cytokines’, etc. were used for search.

Patil *et al.*<sup>[13]</sup> evaluated the immunomodulatory activity of *Rhus tox*, in various dilutions, through *in vivo* and *in vitro* studies. *In vivo* experiments the effects of *Rhus tox* on humoral immune response and DTH were evaluated in C57BL/6 mouse model. Following the antigenic challenge by administration of sheep red blood cells (SRBCs), the haemagglutination assay was performed to assess rise in antibody titer. SRBC suspension was also injected in mice right hind paw, and changes in paw volume measured after 24 h by a plethysmometer were recorded as the intensity of DTH. The *in vitro* experiment assessed the effects of *Rhus tox* on chemotaxis of human polymorph nuclear (PMN) cells through ‘under agarose chemotaxis assay’ method and phagocytosis of *Candida albicans* by PMN cells by phagocytosis assay. The results of *in vivo* experiments showed a greater rise in antibody titer as compared to control group which was statistically significant. *Rhus tox* mother tincture, 6CH and 30CH dilutions showed significant stimulation of DTH response as compared to control group. In *In vitro* experiments, *Rhus tox* mother tincture and 6CH dilution stimulated chemotaxis of PMN cells, and *Rhus tox* mother tincture stimulated phagocytic activity of PMN cells.

de Oliveira *et al.*<sup>[14]</sup> conducted *in vivo* and *in vitro* experiments to investigate the effects of *Merc sol*, in various dilutions, on mice peritoneal macrophages. The various macrophage

parameters tested were change in morphology of macrophages and induction of cytokines TNF- $\alpha$ , IFN- $\gamma$ , IL-2, IL-4 and IL-5, nitric oxide (NO), superoxide (O<sub>2</sub><sup>-</sup>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). Mice received *Merc sol* in drinking water for 7 days. After 7 days, macrophages were harvested from mice peritoneal cavities and cultured in Dulbecco's Modified Eagle's medium. Two hours after plating, the cells were further treated with *Merc sol* or control solution. For morphological analysis and cytokine estimation, cells were treated with *Merc sol* 6CH, 12CH, 30CH and 200CH, and for reactive species analyses, cells were treated with *Merc sol* 6CH, 12CH and 200CH. Results of the experiments showed *Merc sol*-treated macrophages with typical morphology of activated state, such as wider spreading, numerous cellular projections and a large and euchromatic nucleus. The spreading ability was significantly higher in *Merc sol*-treated group which confirmed that 6CH, 12CH, 30CH and 200CH *Merc sol* induced macrophage activation. The cytokine quantification experiments showed that *Merc sol* induced IFN- $\gamma$  production at lower dilutions (6CH and 12CH), while higher dilutions (200CH) stimulated IL-4 production. NO production by macrophages was evaluated in the presence or absence of lipopolysaccharide (LPS) and IFN- $\gamma$ . *Merc sol* induced NO production at 6CH, 12CH and 200CH; however, in cells treated with LPS and IFN- $\gamma$ , it led to decrease in NO production. The O<sub>2</sub><sup>-</sup> formation was decreased whereas H<sub>2</sub>O<sub>2</sub> production was unaltered by *Merc sol* at any of the tested dilutions.

Erhard *et al.*<sup>[15]</sup> evaluated the effect of *Echinacea*, *Aconitum*, *Lachesis* and *Apis* extracts and their combinations on phagocytosis of human granulocytes. *Echinacea angustifolia* showed a stimulation of the phagocytic activity at all concentrations tested. *Aconitum*, *Lachesis* and *Apis* were not capable of influencing phagocytosis when used alone.

Da Rocha Piemonte *et al.*<sup>[16]</sup> investigated role of MC (*Canova* method) in enhancing immunological system responses acting through macrophages pathways. MC has the same composition as that of *Canova*<sup>®</sup>. Mice macrophages treated with MC show 86% of macrophage activation as compared to 15% control group. The number of activated macrophages increased proportionally with MC concentration. Macrophages cultured with two doses of MC showed that TNF- $\alpha$  production decreased when compared with control group.

de Oliveira *et al.*<sup>[17]</sup> evaluated effects of *Canova* on cytokine production and gene expression on mice macrophages. They found decrease in IL-4 and IL-2 *in vivo* production by cells from the *Canova* group. The gene expression profile was also modified in the *Canova* group. Statistical analysis of microarray data revealed 147 genes differentially expressed after mice treatment.

Abud *et al.*<sup>[18]</sup> evaluated the effects of *Canova* treatment on mouse bone marrow cells differentiation, proliferation and survival. The bone marrow cells were cultured and subjected to adherent cell experiments, cytokine quantification and

morphology assay. Flow cytometric immunophenotyping was performed for the detection of surface markers CD11b, CD11c, Ly-6G, CD45R, CD3 and TER119. The results showed increase in the number of adherent cells in *Canova* group. Almost all cells from *Canova* group were in activated state as defined by their morphological alterations. The cell clusters (cell niches) over the adherent cells were much more pronounced in the *Canova* group. The result of immunophenotyping showed that the number of cells was very similar to zero-hour group. However, after 72 h of culture, some surface markers floated equally between the groups. For example, CD11c and CD3 expressions increased after 72 h and returned to the 48 h level after 96 h of culture. CD45R expression decreased after 72 h and returned to 48 h values after 96 h. TER-119 expression increased after 72 h, and it was maintained after 96 h of culture. The expression of CD11b and Ly-6G did not change during the experiment.

## CONCLUSION

The results obtained from pre-clinical studies show that homoeopathic drugs, in various potencies, can influence mice bone marrow cells, macrophages, lymphocytes and PMN cells. The medicines produced modulation of immune function at multiple levels. These include modulation of expression of genes that code for receptors and other proteins in mice macrophages, stimulation of macrophages as evidenced by change in their morphology, expression of receptors on macrophage surface, chemotaxis of PMN cells and production of cytokines and reactive nitrogen and oxygen species from these immune cells. The homoeopathic medicines differed among themselves in respect to the type of cytokines they induced in mice macrophages. This evidence suggests that homoeopathic treatment has the potential for individual-specific immunomodulation. Variation in homoeopathic medicine action was also observed between different powers of homoeopathic dilutions used which corroborate the difference in their action observed on clinical plane. So far, the numbers of homoeopathic medicines studied for their immunomodulatory function are few as compared to the vast repertoire of homoeopathic medicines available to the profession. Majority of these studies utilised the mouse model. As such, the results of these studies may not be directly applicable on humans. In future, more pre-clinical studies are needed to explore the action of homoeopathic medicines at cellular level and their possible role in individual-specific immunomodulation. Pre-clinical studies based on human-derived immune cells merit particular attention.

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## Conflicts of interest

None declared.

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### होम्योपैथिक औषधियों के इम्यूनों मॉड्यूलेट्री प्रभाव: पूर्व नैदानिक अध्ययन की समीक्षा

इम्यूनों-मॉड्यूलेशन वांछित प्रयोग लाने के लिए प्रतिरक्षा प्रणाली में एक प्रकार का नियामक संशोधन है। इस समीक्षा में होम्योपैथिक औषधियों रस टोक्सीकोडेंड्रोन, मर्कुरियस सोल्यूबिलिस, एचीनीशिया, एकोनिटम, लेकेसिस व एपिस एवं होम्योपैथिक संयोजन औषधि कनोवा के इम्यूनों-मॉड्यूलेट्री प्रभाव की चर्चा की गई है। प्रासंगिक लेखों का संदर्भ लेते हुए और पबमेड की सहायता से इस लेख की समीक्षा की गई। उपरोक्त होम्योपैथिक औषधियों के नाम वाले सूचक शब्दों के साथ-साथ 'प्रतिरक्षा क्रिया', 'साइटोकिन्स' आदि का उपयोग अन्वेषण हेतु किया गया। यह समीक्षा दर्शाती है कि होम्योपैथिक औषधियाँ कई स्तर पर प्रतिरक्षा प्रणाली में उतार-चढ़ाव लाती हैं जैसे जीन अभिव्यक्ति में उतार-चढ़ाव, मैक्रोफेज और पॉलीमॉर्फ नाभिकीय कोशिकाओं की उत्तेजना, सतही-प्रापक की अभिव्यक्ति व साइटोकिन्स के प्रेरण में बदलाव। होम्योपैथिक औषधियों की एक बड़ी संख्या के इम्यूनों मॉड्यूलेट्री प्रभाव जानने के लिए विस्तृत अध्ययनों की आवश्यकता है। मानव-उत्प्रेरित प्रतिरक्षा कोशिकाओं पर आधारित अध्ययनों पर विशेष रूप से ध्यान देने की आवश्यकता है।

### Effets immunomodulateurs des médicaments homéopathiques : un examen d'études pré-cliniques

L'immunomodulation est une sorte de modification régulatrice dans le système immunitaire qui permet d'obtenir la réponse souhaitée. Dans cet examen, les effets immunomodulateurs des médicaments homéopathiques que sont *Rhus toxicodendron*, *Mercurius solubilis*, *Echinacea*, *Aconitum*, *Lachesis* et *Apis* ainsi que de *Canova*<sup>®</sup>, un remède qui est une combinaison de médicaments homéopathiques, sont abordés. L'examen a été conduit au moyen de PubMed et des mentions d'articles pertinents. Des mots-clés comprenant les noms des médicaments homéopathiques cités ci-dessus ainsi que les termes « fonction immunitaire », « cytokines », etc., ont été utilisés pour la recherche. L'examen montre que les médicaments homéopathiques ont provoqué une modulation de la fonction immunitaire à de multiples niveaux tels qu'une modulation de l'expression des gènes, une stimulation du macrophage et des cellules polymorphes nucléées, des modifications dans l'expression des récepteurs de surface et une induction de cytokines. Des études approfondies sont nécessaires pour explorer les effets immunomodulateurs d'un grand nombre de médicaments homéopathiques. Les études fondées sur les cellules immunitaires d'origine humaine demandent en particulier plus d'attention.

### **Efectos inmunomoduladores de los medicamentos homeopáticos: revisión de los estudios preclínicos** Resumen

La inmunomodulación es un tipo de modificación reguladora en el sistema inmunológico para dar lugar a la respuesta deseada. En esta revisión se discuten los efectos inmunomoduladores de los medicamentos *Rhustoxicodendron*, *MercuriusSolubilis*, *Echinacea*, *Aconitum*, *LachesisApis*, así como los del producto combinado Canova®. La revisión se realizó utilizando PubMed y las referencias de los artículos pertinentes. Las palabras clave que comprenden los nombres de los medicamentos homeopáticos anteriores junto con los términos "función inmune", "citoquinas", etc., se utilizaron para la búsqueda. La revisión muestra que los medicamentos homeopáticos provocaron la modulación de la función inmunitaria a múltiples niveles como la modulación de la expresión de genes, la estimulación de los macrófagos y las células polimorfonucleares, cambios en la expresión de los receptores superficiales y la inducción de citocinas. Se precisan estudios extensos para explorar los efectos inmunomoduladores de un gran número de medicamentos homeopáticos. En concreto, hay que prestar más atención a los estudios basados en las células inmunes derivadas del ser humano.

### **Immunomodulatorische Effekte von homöopathischen Arzneimitteln: Eine Rezension von vorklinische Studien**

Immunmodulation ist eine Art regulatorische Modifikation im Immunsystem, um die gewünschte Antwort zu bringen. In dieser Übersicht wurden die immunomodulatorischen Wirkungen homöopathischer Arzneimittel - *Rhus toxicodendron*, *Mercurius solubilis*, *Echinacea*, *Aconitum*, *Lachesis und Apis* - und des homöopathischen Kombinationsarzneimittels Canova® besprochen. Die Überprüfung wurde mit PubMed und Referenzen aus den relevanten Artikeln durchgeführt. Die Schlüsselwörter, die die Namen der obigen homöopathischen Arzneimittel zusammen mit den Begriffen "Immunfunktion", "Zytokine" usw. umfassen, wurden für die Suche verwendet. Die Daten zeigen, dass homöopathische Arzneimittel eine Modulation der Immunfunktion auf mehreren Ebenen, wie Modulation der Expression von Genen, Stimulation von Makrophagen- und polymorphen Zellkernen, Veränderungen der Expression von Oberflächenrezeptoren und Induktion von Zytokinen, bewirken. Umfangreiche Studien sind erforderlich, um die immunomodulatorischen Effekte einer großen Anzahl von homöopathischen Arzneimitteln zu untersuchen. Studien, die auf humanen Immunzellen basieren, benötigen speziell mehr Aufmerksamkeit

