

Immunological Impact of *Ricinus communis* Leaves Extract on Isolated Human Peripheral Blood Mononuclear Cells

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Abstract

Background: Plants are the source of many important drugs. *Ricinus communis* is utilized as a traditional folkloric remedy for the treatment of a wide extend of diseases around the world. Large number of phytochemicals such as alkaloids, terpenes, and phenolic compounds that have an established anticancer, antimicrobial, and immunological impact presence in many plant families. **Objective:** The aim of the present study was to investigate the immunological impact of *R. communis* leaves extract on human immune response using isolated human peripheral blood mononuclear cells (PBMNC). **Materials and Methods:** Cells were isolated using the gradient centrifugation method and treated with *R. communis* leaves extract at different concentrations, and then, the cytotoxic effect was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, and the immunologic effect was estimated by measuring the cytokine levels via the enzyme-linked immunosorbent assay technique. **Results:** Plant extract showed a low cytotoxic effect on PBMNC at therapeutic doses and high doses (1000 µg/mL) used. Plant extract caused a significant reduction in the levels of interleukin (IL)-4 and IL-17 at higher doses and increased IFN-γ at both doses used. **Conclusion:** *R. communis* leaves extract appears to be safe for medical uses and has immunomodulatory effects.

Keywords: Cytokines, cytotoxicity, immunomodulation, PBMNC, *Ricinus communis*

INTRODUCTION

Plants are the richest and most abundant source of organic natural compounds used for therapeutic purposes.^[1] Traditional remedies include several crude medications that can treat a variety of illnesses and disorders.^[2] Phytochemicals such as terpenes, alkaloids, and phenolic compounds have been discovered in *Ricinus communis* L., which is used traditionally for the management of a wide range of disorders worldwide.^[3] These compounds are useful for pharmacological action as anticancer, antimicrobial, insecticide, antioxidant, antidiabetic, analgesic, and anticonvulsant activity.^[4]

Plants containing essential oils, such as *R. communis*, have gained popularity and scientific interest. These chemicals have a diverse range of pharmacological effects. One of its benefits is that they attack bacteria without increasing antibiotic resistance, which is a problem created by the long-term usage of synthetic antibiotics.^[5] The chemical

constituents of *R. communis* leaves showed the existence of alkaloids, ricinine, flavones, mono- and sesquiterpenoid, and many other chemicals.^[6] *R. communis* has been reported to have anti-inflammatory potential mostly related to the presence of flavonoids.^[3]

Lymphocytes have a similar appearance (involving T and B cells and natural killers) but differ in function.^[7] One of the lymphocyte's functions is the secretion of cytokines; tiny proteins secreted by cells that play a specific role in the interactions and communications among cells. As well as in identifying the nature and duration of immune responses.^[8,9]

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Submission: 22-Nov-2022 **Accepted:** 21-Dec-2022 **Published:** 27-Apr-2023

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How to cite this article: Immunological impact of *Ricinus communis* leaves extract on isolated human peripheral blood mononuclear cells. Med J Babylon 2023;20:120-3.

Access this article online

Quick Response Code:



Website:
www.medjbabylon.org

DOI:
10.4103/MJBL.MJBL_287_22

MATERIALS AND METHODS

Separation of peripheral blood mononuclear cells

Density gradient techniques were used to separate human peripheral blood mononuclear cells (PBMNC).^[10] In brief, 5 mL of venous blood was collected from healthy 20–30-year-old volunteers, left to cool to ambient temperature for 30 min, and then slowly and carefully added to the surface of 5 mL of lymphoprep (1.077 g/mL) (Capricorn Scientific GmbH, Germany) without mixing. Separation of the PBMNC was carried out by centrifuging the tube contents at 400 g for 30 min. PBMN layers were transferred to a new tube, rinsed twice with phosphate buffer saline, and centrifuged for 10 min at 400 rcf. Pellets were then cultivated in a T25 flask for 24 h at 37°C and 5% CO₂ in a T25 flask.^[11]

Preparation of *R. communis* leaves extract

Fresh *R. communis* leaves were obtained from several locations within the Babylon governorate. The plant leaves were carefully washed with tap water and dried for 2 weeks in the dark at room temperature. After drying, leaves were grounded to a fine powder and soaked in water (1:20 weight to volume) for 3 days before extraction. Soxhlet was used to achieve the aqueous extraction, which was dried for 48 h at 40°C.

MTT assay

Isolated PBMNs were cultured immediately in 96 tissue culture plates at a cell density of 10×10^3 cells/well. After that, 200 µL of complete growth medium containing the plant extract at serial concentrations of 1000, 500, 250, 125, 62.5, and 31.25 µg/mL was added to each well and incubated for 24 h at 37°C and 5% CO₂. For each concentration, five duplicates were used. The cells' vitality was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test by removing the media from the wells, washing the cells with phosphate-buffered solution, and then, adding the MTT dye. The conversion of dye's yellow color into purple color after incubation for 2–4 h is proportional to the viable fraction of the cells.

Cytokines assessment

Cells were seeded in 48-well plates, which is divided into three groups to assess the immunomodulatory effects of *R. communis* aqueous extract: the first group was the untreated cells (control group), and the second and third groups were treated with 500 and 125 µg/mL *R. communis* aqueous extract, respectively. The supernatant of each well was obtained after 48 h of incubation for immunoassay using the Enzyme-Linked Immunosorbent Assay technique to determine the levels of interleukin (IL)-4, IL-10, and IL-17, and interferon-gamma (IFN-γ) using cytokines kits (Elabscience Biotechnology, Texas, USA).

Statistical analysis

Sigmaplot (v12.5) software (CA, United States) was used to analyze the data. One-way analysis of variance test is used to determine the presence of significant differences among the means of the groups. Statistical significance was defined when *P*-values < 0.05.

Ethical approval

The study was carried out under ethical principles that are consentient with the Declaration of Helsinki. The volunteers agreed to participate in the study and provided informed consents. The study protocol and the subject information and the consent form were reviewed and approved by a local ethics committee according to document number 116, dated October 24, 2022.

RESULTS

Cytotoxicity of *R. communis* extract on isolated PBMNCs

Plant extract showed relatively low cytotoxicity on the cells [Figure 1]. Only at the concentrations of 500 and 1000 µg/mL, a significant (*P* ≤ 0.05) decrease in the viability was shown compared with the control group.

Phytochemical analysis of *R. communis* leaves extract revealed the existence of high fractions of flavonoids.^[12] Flavonoids are common effective plant compounds.^[13]

The cytotoxicity of the plant at higher doses may be related to flavonoids' activity. Flavonoids' inhibitory effects affect cell viability via the stimulation of apoptosis. Flavonoids can inhibit the growth of many types of cell lines, mostly by arresting the cell cycle and induction of apoptotic pathways.^[14] In malignant cells, flavonoids interact with and arrest the cell cycle in G2/M phases, as well as suppress the production of cyclin B. The main cause of G2/M phase arrest is assumed to be the suppression of cyclin B protein, which is regulated by cyclin B gene. Inhibiting the release of the vascular permeability factor vascular endothelial growth factors, which is involved in angiogenesis and vasculogenesis, is thought to be the cause of angiogenesis inhibition *in vitro*.^[15]

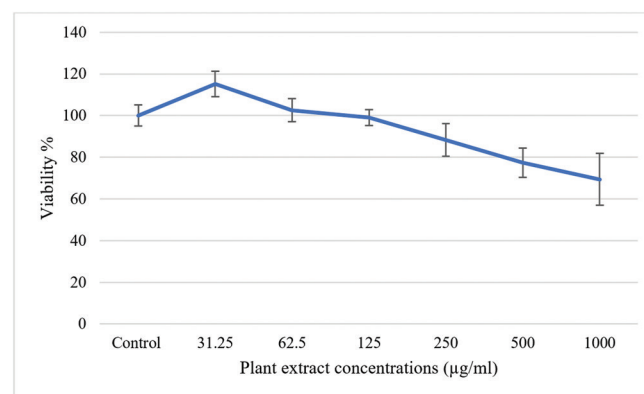


Figure 1: Cytotoxicity assay of aqueous leaves extracts of *Ricinus communis* on the isolated PBMNC

Influence of *R. communis* extract on cytokines levels

Extract of *R. communis* caused a significant decrease ($P \leq 0.05$) in IL-4 levels at concentrations of 125 and 500 $\mu\text{g/mL}$, whereas IL-10 showed no significant variation after the treatment with the plant extract [Figure 2].

Because IL-4 regulates lymphocyte responses, the aqueous leaves extract may be effective in suppressing allergies and other immunological illnesses by lowering the amounts of these cytokines and limiting the differentiation of Nave cluster of differentiation T cells into Th2 cells.^[16]

Low concentrations of phytochemicals such as quercetin and lutein stimulated the expression of the anti-inflammatory cytokines such as IL-4 and IL-10, but not at higher doses.^[17]

IL-10 offers the main negative regulatory feedback on proinflammatory cytokine release.^[18] In the regular immune response, when the naive T cells encounter an antigen or a mitogen, they will be activated and differentiated into two types of effector cells called Th1 and Th2. Th2 cells produce mainly IL-4 and IL-10.^[19] In turn, increased IL-10 signaling can obstruct proinflammatory cytokine release by directly targeting immune effectors. It may indirectly modify immune response by preventing the maturation of macrophage and dendritic cells; this will, in turn, limit the host's ability for antigen presentation and chemokine secretion.^[20]

There was a significant ($P \leq 0.001$) suppression of the IL-17 only at (500 $\mu\text{g/mL}$) of leaf extract in comparison

with the control group, as illustrated in [Figure 2]. IL-17 acts as an inflammation mediator. It causes granulocyte colony-stimulating factor and chemokine release. IL-17 is crucial during infection to kill extracellular bacteria and fungi by producing antimicrobial peptides such as defensin. This cytokine is also involved in the etiology of autoimmune disorders and allergies. Inhibition of IL-17 release may, thus, be beneficial in the treatment of chronic autoimmune diseases including rheumatoid arthritis and multiple sclerosis.^[21]

Anthocyanins are a group of water-soluble flavonoids that influence the expression of IL-17. Anthocyanin reduced Th17 cells *in vitro* and *in vivo* and prevented the expression of proinflammatory cytokines (tumor necrosis factor-, IL-6, IL-17, and IL-21).^[22] IFN- γ produced from PBMN showed a significant ($P \leq 0.001$) increase when treating lymphocytes with the plant extract [Figure 2].

Boroujerdnia *et al.*'s^[23] study revealed that the activated T cells in the normal immune system can differentiate into Th1 cells, which further can secrete many proinflammatory cytokines mainly IFN- γ .

IFN- γ increases the effectiveness of the immune system against microbial and parasitic infections by improving its competence to deliver antimicrobial and antiparasitic effector functions. Undoubtedly, such an important immune mediator is under stringent regulatory control, increasing the production of IFN- γ within the regulated range can boost immunity and help to fight infections,

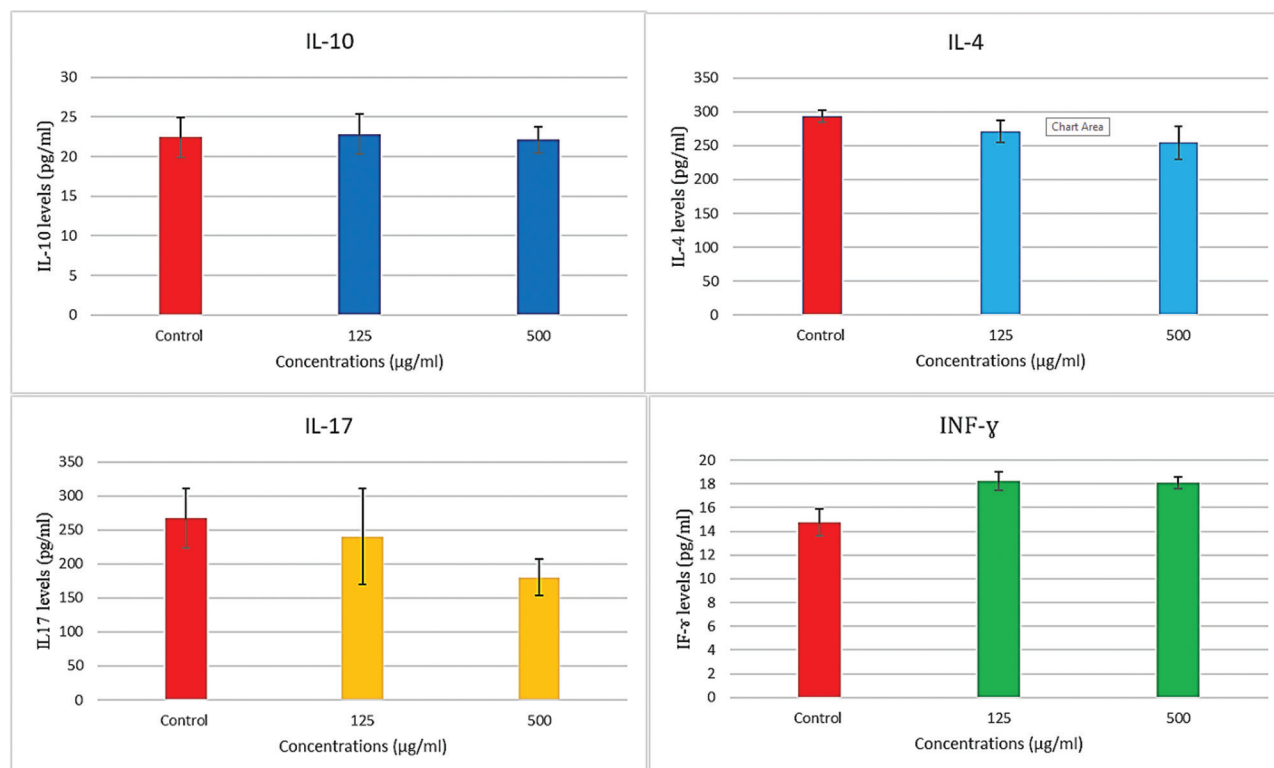


Figure 2: Effect of *Ricinus communis* on IL-4, IL-10, IL-17 and IFN- γ cytokines production

whereas overproduction of IFN- γ may cause tissue damage and necrosis.^[24] Quercetin can also boost lymphocytes' intracellular and released IFN- γ levels, as well as IFN synthesis and release. These two cytokines are thought to have a key function in the control of human immunological responses.^[25]

CONCLUSION

Our data reveal that *R. communis* aqueous leaves extract had a cytotoxic effect on isolated PBMNs only at higher concentrations. The aqueous leaves extract significantly modulate the immune response in term of cytokine secretion in a dose-dependent manner.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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