Rzeszów University Press 2025
ISSN 2544-1361 (online)
doi: 10.15584/ejcem.2025.1.16

*http://www.ejcem.ur.edu.pl*
*European Journal of Clinical and Experimental Medicine*
*Eur J Clin Exp Med 2025; 23 (1): 100–109*

**ORIGINAL PAPER**

# In vitro and in vivo models of immunomodulatory activity of a hydroalcoholic fraction of *Turnera ulmifolia* Linn

Vuppula Sruthi, G. Baba Shankar Rao

Department of Pharmacognosy, Anurag University, Venkatapur, Hyderabad, Telangana, India

**ABSTRACT**

**Introduction and aim**. The present study explores the immunomodulatory effects of the hydroalcoholic extract of *Turnera ulmifolia* through in vitro and in vivo models.

**Material and methods**. The study examined cytotoxicity, cytokine production, and nitric oxide (NO) levels using RAW 264.7 murine macrophage cells, while *in vivo* assessments were performed using BALB/c mice.

**Results**. *In vitro, T. ulmifolia* extract significantly increased cytokine levels and NO production in unstimulated cells while effectively inhibiting overproduction in LPS-stimulated cells, suggesting immunomodulatory and anti-inflammatory activities. In *vivo* experiments demonstrated that *T. ulmifolia* extract enhanced the immune response by improving macrophage phagocytic activity, increasing delayed-type hypersensitivity, increasing serum hemolysin levels, and enhancing thymus and spleen indices. These results highlight the potential of the *T. ulmifolia* extract as an immunomodulatory agent, regulating cytokine secretion and enhancing immune responses without causing cytotoxicity.

**Conclusion**. The findings indicate promising therapeutic applications for *T. ulmifolia* extract in modulating immune function and inflammation.

**Keywords.** hydroalcoholic, immunomodulatory activity, in vitro, in vivo, *Turnera ulmifolia*

## Introduction

*Turnera ulmifolia* Linn., sometimes called ramgoat dashalong, is a flowering plant classified under the *Passifloraceae* family. *T. ulmifolia,* a plant widely spread across the Americas, including South and Central America and the Caribbean, is renowned for its therapeutic powers and decorative value. The plant often exhibits a prostrate growth habit, reaching heights of up to one meter, with conspicuous yellow flowers and leaves with toothed edges.[1] This species thrives in many environments, including open forests, places that have been disrupted, and beside highways, often seen in tropical and subtropical countries. The dashalong ramgoat plant has a long-standing history of being used in traditional herbal treatments in its indigenous habitat. Multiple components of the plant, including leaves, stems, and roots, are used to create decoctions, infusions, and poultices to treat a diverse array of diseases.[2,3] *T. ulmifolia* is highly regarded in traditional Caribbean medicine for its alleged ability to reduce inflammation, relieve pain, increase urine production, and relax muscles. It is often used to alleviate symptoms of diseases such as lung infections, gastrointestinal problems, menstrual cramps, and urinary tract infections. Moreover, it is widely believed that the plant has aphrodisiac characteristics and is sometimes used to increase desire and sexual performance.[4,5]

*T. ulmifolia* is planted for decorative reasons due to its beautiful leaves, vivid yellow blooms, and thera-

**Corresponding author:** G. Baba Shankar Rao, e-mail: babaphd2010@gmail.com

Received: 30.06.2024 / Revised: 13.09.2024 / Accepted: 19.09.2024 / Published: 30.03.2025

Sruthi V, Rao GBS. In vitro and in vivo models of immunomodulatory activity of a hydroalcoholic fraction of *Turnera ulmifolia* Linn. *Eur J Clin Exp Med.* 2025;23(1):100–109. doi: 10.15584/ejcem.2025.1.16.

peutic applications. Commonly cultivated in gardens, parks and landscapes, this plant is an ornamental addition that enhances the aesthetic appeal and visual appeal of outdoor areas. Furthermore, the plant offers nectar and a home for pollinators such as bees and butterflies, thus improving local biodiversity and ecological well-being.[6,7] Although *v T. ulmifolia* has been highly valued in traditional medicine for many years, there is a lack of scientific studies on its pharmacological qualities and possible therapeutic uses. Additional research is required to clarify the bioactive substances found in the plant, their modes of operation, and their effectiveness and safety characteristics. Furthermore, initiatives aimed at preserving the natural populations of *T. ulmifolia* and advocating for sustainable farming methods can guarantee its accessibility for future generations while benefiting local people who depend on its medicinal and economic advantages.[8-10] To explore the pharmacological potential of *T. ulmifolia,* we extracted the plant using a hydroalcoholic solvent, and the active fraction was subjected to *in vivo* and *in vitro* immunomodulatory activity.

*T. ulmifolia,* commonly known as ramgoat dashalong, has been widely used in traditional medicine for its various therapeutic benefits, such as anti-inflammatory, analgesic, and aphrodisiac properties. Despite its historical and widespread use, the pharmacological potential of *T. ulmifolia* has not been thoroughly explored in modern scientific research. There remains a significant gap in understanding its immunomodulatory effects, particularly its potential to regulate the immune system and combat inflammation without cytotoxic side effects. Given the growing interest in herbal medicines for immune modulation, this study aims to address the lack of rigorous scientific investigation of the plant's bioactive compounds. By focusing on both *in vitro* and *in vivo* models, this research seeks to uncover the immunomodulatory activities of *T. ulmifolia* extract, contributing valuable insights to both the fields of immunopharmacology and research on natural products.

## Aim

This study is unique in that it systematically evaluates the immunomodulatory effects of *T. ulmifolia* through a comprehensive approach that includes both *in vitro* and *in vivo* models. This research utilizes advanced methodologies to assess cytokine production, nitric oxide (NO) levels, and macrophage function, providing novel information on the plant's biphasic effect on immune responses. Additionally, this work distinguishes itself by demonstrating the ability to enhance immune responses in unstimulated cells while simultaneously inhibiting excessive immune reactions in stimulated conditions, suggesting dual immunomodulatory and anti-inflammatory effects. These findings significantly advance our understanding of the pharmacological properties of *T. ulmifolia,* positioning it as a potential therapeutic agent for immune-related conditions.

## Material and methods

### *Reagents*

Glowderma Labs Pvt. Ltd. (Mumbai, India) supplied levamisole hydrochloride. In contrast, Infobio Lifesciences (Delhi, India) supplied Mouse TNF-α, IL-1β, IL-6, and IFN-γ ELISA kits from Krishgen Biosystems, Mumbai, India (catalog numbers: TNF-α: KET7012, IL-1β: KET7013, IL-6: KET7014, IFN-γ: KET7015). The remaining chemicals utilized in the study were acquired from HiMedia Laboratories in Mumbai, India.

### *Preparation of T. ulmifolia extracts*

The protocol for the preparation of *T. ulmifolia* extracts involves the extraction of plant or biological samples using a mixture of ethanol and water in a 70:30 ratio. This extract is then subjected to column fractionation, which is based on the polarity of the solvent system. Various solvents are used sequentially and the sample is placed on top of a silica gel column (60-120 mesh size) to ensure even distribution. A cotton bolus is placed above and below the sample to maintain uniformity. Different solvents, including ethyl acetate, benzene-ethyl acetate mixtures, chloroform, and acetone, are used, each with specific run times until the complete separation of the components is achieved. The fractions obtained are subsequently analyzed using thin-layer chromatography (TLC) to determine the number and type of compounds present in each fraction, and further analysis performed on the most promising fraction for compound identification.

### *Cell culture*

Aaranya Biosciences Private Limited supplied the RAW 264.7 murine macrophage cell line. Cells were cultured in DMEM, supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. They were kept at 37°C in a humidified incubator with an atmosphere of 5% $CO_2$ and 95% air.

### *Cell viability assay*

The MTT assay, used to assess cell viability, was obtained from HiMedia Laboratories, Mumbai, India. RAW 264.7 cells were seeded at a density of $4\times10^5$ cells/mL into 96-well plates and allowed to incubate overnight. The following day, different concentrations of *T. ulmifolia* extract (ranging from 0–150 µg/mL, diluted in 0.1% DMSO) were added to the cells and incubated for 24 hours. After incubation, 50 µL of MTT reagent was added to each well, and the cells were further incubated for 4 hours. The MTT reagent was then removed and 100 µL of DMSO was added to each well to dissolve

the formazan crystals formed by viable cells. The optical density (OD) was measured at 570 nm using a microplate reader to determine cell viability.

***Cytokine assays***

RAW 264.7 cells were seeded in 96-well plates at a density of $4\times10^5$ cells/mL. The cells were then treated for 24 hours with 1 μg/mL of lipopolysaccharide (LPS) or with 10, 25, or 150 μg/mL of trifluoroacetic acid (diluted in 0.1% DMSO). After treatment, cytokine analysis was performed on the cell-free supernatants. Concentrations of TNF-α, IL-1β, IL-6, and IFN-γ in the supernatants were measured using ELISA kits, following the manufacturer's instructions.

***Nitrite measurement***

NO production of NO was determined by measuring the accumulation of nitrite in the cell culture medium using the Griess procedure. RAW 264.7 cells were seeded in 96-well plates and treated with *T. ulmifolia* extract at concentrations of 10, 25, 100, and 150 μg/mL (dissolved in 0.1% DMSO). Cells were either exposed to 1 μg/mL of LPS or left untreated for 24 hours. After incubation, cell supernatants were collected, and NO generation was evaluated using the Griess reagent. Equal volumes of the samples and Griess reagent were mixed and incubated at room temperature for 15 minutes. Absorbance was measured at 540 nm using a microplate reader. The nitrite concentration was calculated on a standard sodium nitrite dilution curve.

***Animals***

BALB/c mice weighing 18–22 g were purchased from the National Institute of Nutrition (NIN) in Hyderabad, India. The animals were housed in isolation cages with unrestricted access to food and water. Before the experiments, the mice were allowed at least one week to acclimate to the experimental environment. All experimental procedures involving animals were conducted strictly in accordance with the guidelines established by the Ethical Committee for the Experimental Use of Animals.

***In vivo immunomodulatory effect of T. ulmifolia extract***

*Carbon clearance test to measure macrophage phagocytic activity in mice*

Six groups of mice (n=6 per group) were randomly assigned as follows: NC (normal control) group, DC (disease control) group, TU-200 group (treated with 200 mg/kg of *T. ulmifolia* extract), TU-400 group (treated with 400 mg/kg of *T. ulmifolia* extract), and a standard group. Mice in the TU-200 and TU-400 groups were administered the extract orally (p.o.) using sodium carboxymethyl cellulose (CMC) as a vehicle for seven days. The standard group received levamisole (LEV) at a dose of 30 mg/kg intraperitoneally (i.p.). Mice in the NC and DC groups were given sodium CMC p.o. On days 2, 4, and 6 of the experiment, mice in all groups except the Standard group were administered cyclophosphamide (CTX) at a dose of 40 mg/kg ip to induce immunosuppression. One hour after the final dose, India ink (diluted to 10 μL/kg) was injected into the caudal vein of each mouse. Blood samples (20 μL) were collected from the tail vein at two minutes and ten minutes after injection. The collected blood samples were immediately mixed with 2 mLof 1 mg/mL sodium carbonate ($Na_2CO_3$) solution to lyse the red blood cells and stabilize the ink particles. The absorbance of the samples was measured at 650 nm using a microplate reader. The phagocytic index was calculated using the following formula:

$$\text{Phagocytic index}=(t_{10} - t_2)/(\log OD_2 - \log OD_{10})$$

Where:

$t_{10}$=10 minutes post-injection

$t_2$=2 minutes after injection

$OD_2$=absorbance at 2 minutes

$OD_{10}$=absorbance at 10 minutes

After the final blood collection, the mice were sacrificed and the spleen and liver were carefully removed and weighed. Organ weights were expressed as a ratio of body weight to the combined weight of the spleen and liver, providing additional information on the mice's immune function.

*Assessing mice for delayed-type hypersensitivity*

The footpad edema test assessed mice's delayed-type hypersensitivity (DTH) response. The experimental design was maintained, with the animals divided into appropriate groups. On the second day of drug administration, each mouse was sensitized via an intraperitoneal (i.p.) injection of 0.2 mL of 2% sheep red blood cell (SRBC) suspension. This sensitization initiated the immune response in preparation for the challenge. On the seventh day of drug administration, a challenge was performed by injecting 20 μL of a 20% SRBC suspension subcutaneously into the right posterior footpad of each mouse. This subcutaneous injection was performed to induce the DTH response in the immune-sensitized mice. After 24 hours, the thickness of the right (injected) and left (non-injected) rear foot pads was measured using a vernier caliper. The differential thickness of the footpads was recorded before and after the injection of SRBC to assess the degree of inflammation. The degree of DTH was determined by calculating the difference in thickness between the right (injected) and left (non-injected) footpads before and after the SRBC challenge. This differential measurement indicated the inflammatory response, with more significant swelling indicating a stronger DTH response. This method is designed to assess the cellular immune response in mice and can be used to evaluate the immunomodulatory effects of various substances. The test can be accurately replicated and

results reliably reproduced by following these detailed steps.

*Determination of serum hemolysin level in mice*

The serum hemolysin assay was performed to assess the humoral immune response in mice. The experimental design remained consistent and the mice were grouped as appropriate. On the third day of treatment, each mouse received 0.2 mL of a 2% sheep red blood cell (SRBC) suspension via intraperitoneal (i.p.) injection to stimulate the immune system and induce antibody production. Exactly 24 hours after SRBC injection, blood samples were taken from the brachial plexus of mice. The blood collected was immediately processed to obtain serum by centrifugation at 3000 rpm for 10 minutes. The serum was then diluted 100-fold in physiological saline for further analysis. A hemolysin reaction mixture was prepared by combining 1 mL of the diluted mouse serum, 0.5 mL of a 10% sheep red blood cell (SRBC) suspension (serving as the antigen), and 0.5 mL of a 10% complement solution. The mixture was then incubated to assess the hemolytic activity of the serum, which indicates the presence of hemolysin antibodies that interact with SRBC in the presence of the complement. The reaction mixture was incubated in a 37°C water bath for 30 minutes to allow the hemolysin (antibodies) in the serum to lyse the SRBC in the presence of complement. After 30 minutes of incubation, the reaction was stopped by placing the tubes in an ice bath. The reaction mixture was centrifuged at 3000 rpm for 10 minutes to separate the lysed cells from the supernatant. The supernatant absorbance was then measured at 540 nm using a microplate reader, indicating the hemolysin activity in the serum. The absorbance values reflect the level of hemolysin antibodies produced by the mice, with a higher absorbance indicating greater hemolytic activity and a stronger humoral immune response.

*Immune organ index determination*

After seven days of drug administration, the experimental design remained unchanged and the mice were weighed to record their body weights. Subsequently, mice were humanely euthanized via cervical dislocation. After euthanasia, the thymus and spleen were carefully removed from each mouse. Care was taken to remove any extraneous tissues attached to the organs. The thymus and spleen were then weighed separately using a precision balance. Organ weights were recorded in milligrams (mg). Organ weights were normalized to the body weight of each mouse to calculate thymus and spleen indices. These were expressed in milligrams of organ weight per gram of body weight (mg/g). This index provides insight into the effect of the drug on the immune organs of mice.

***Statistical analysis***

Statistical analysis was performed for the investigation of the immunomodulatory activity of the *T. ulmifolia* extract using standard methods to ensure the accuracy and validity of the findings. Data are expressed as the mean±standard deviation (SD) of independent experiments. The differences between groups were evaluated using one-way analysis of variance (ANOVA) followed by post hoc tests such as Tukey's multiple comparison test. Significance was considered when p-values were less than 0.05 ($p<0.05$), indicating a statistically significant difference. Furthermore, for comparisons involving multiple groups, values such as $p<0.01$ and $p<0.001$ were reported to indicate a higher significance level (SSPS, IBM, Armonk, NY, USA).

***Ethical approval***

The study was carried out according to ethical standards and ethical approval was obtained from Flair Labs, located at Plot No. B 510, Palsana, Surat-394315, Gujarat, India. The study was approved under the registration number 1250/PO/RcBi/S/23/CPCSEA.

## Results

***In vitro studies***

*Effect of lipopolysaccharide extract on cell viability*

*The extract of T. ulmifolia* did not induce significant cytotoxicity in the concentration range of 0–100 µg/mL in RAW 264.7 macrophage cells, whether in the presence or absence of LPS stimulation (Fig.1). This is evident from the minimal variation in cell viability values, all of which remained close to or above 0.8, with most reaching or exceeding 1.0. In particular, the highest concentration of *T. ulmifolia* extract (100 µg/mL) led to a slight reduction in cell viability without LPS. However, this was not statistically significant, suggesting that the extract was non-toxic at the tested doses. The ability to maintain or slightly enhance cell viability, particularly at 10 and 25 µg/mL, suggests that the extract of *T. ulmifolia* may have an immunostimulatory effect without inducing cellular damage. Furthermore, in the presence of LPS, which is known to activate macrophages and cause an inflammatory response, cell viability remained relatively stable. This indicates that the *T. ulmifolia* extract may modulate the immune response without exerting cytotoxic effects.

*Effect of T. ulmifolia extract on cytokine release in RAW 264.7 macrophages that are unstimulated or stimulated by LPS*

This work investigated how the extract of *T. ulmifolia* affected the cytokine release in RAW 264.7 macrophages. The findings demonstrated that relative to the control group, *T. ulmifolia* extract at doses of 10, 25, 100, and 150 µg/mL markedly improved the production

of TNF-α, IL-1β, IL-6, and IFN-γ ($p<0.05$ or $p<0.01$ or $p<0.001$) (Fig 2). Significantly, this induction showed immunomodulatory activity in innate immunity as it was carried out without any cytotoxic effects and produced a favorable modulation of RAW 264.7 macrophage function. To assess the anti-inflammatory effects of the *T. ulmifolia* extract in a laboratory environment, we carried out measurements on how much inflammatory cytokine was present in RAW 264.7 cell supernatants after exposure to LPS stimulation.

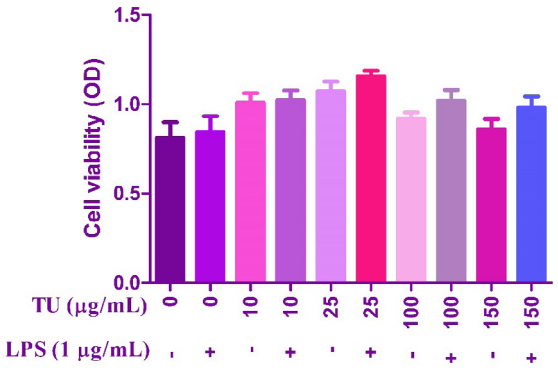


**Fig. 1.** Effect of *T. ulmifolia* extract on RAW 264.7 viability

LPS stimulation markedly ($p<0.01$) boosted cytokine production compared to the control group. Concentrations of 10, 25, 100, and 150 µg/mL of *T. ulmifolia* extract markedly decreased cytokine synthesis (Fig. 3). A dose-dependent pattern of this inhibition ($p<0.05$ or $p<0.01$) suggested anti-inflammatory action. The results imply that the extract of *T. ulmifolia* may affect RAW 264.7 macrophages' cytokine release, impacting the immune system and lowering inflammation.

***Effects of T. ulmifolia extract on NO production in RAW 264.7 macrophages with and without LPS stimulation***

Our investigation found that the extract of *T. ulmifolia* has a concentration range that could promote NO generation in unstimulated RAW 264.7 macrophages and be non-cytotoxic. Comparing the concentrations of the *T. ulmifolia* extract at 10, 25, 100, and 150 µg/mL to the control group, the former significantly increased NO generation ($p<0.05$) (Fig. 4). Proportional to dose, the extract of *T. ulmifolia* at doses of 25, 100, and 150 µg/mL markedly reduced NO overproduction of NO as against the LPS-stimulated group ($p<0.05$, $p<0.01$, and $p<0.01$) (Fig. 5). The results show that the extract of *T. ulmifolia* affects RAW 264.7 macrophages' release of the inflamma-

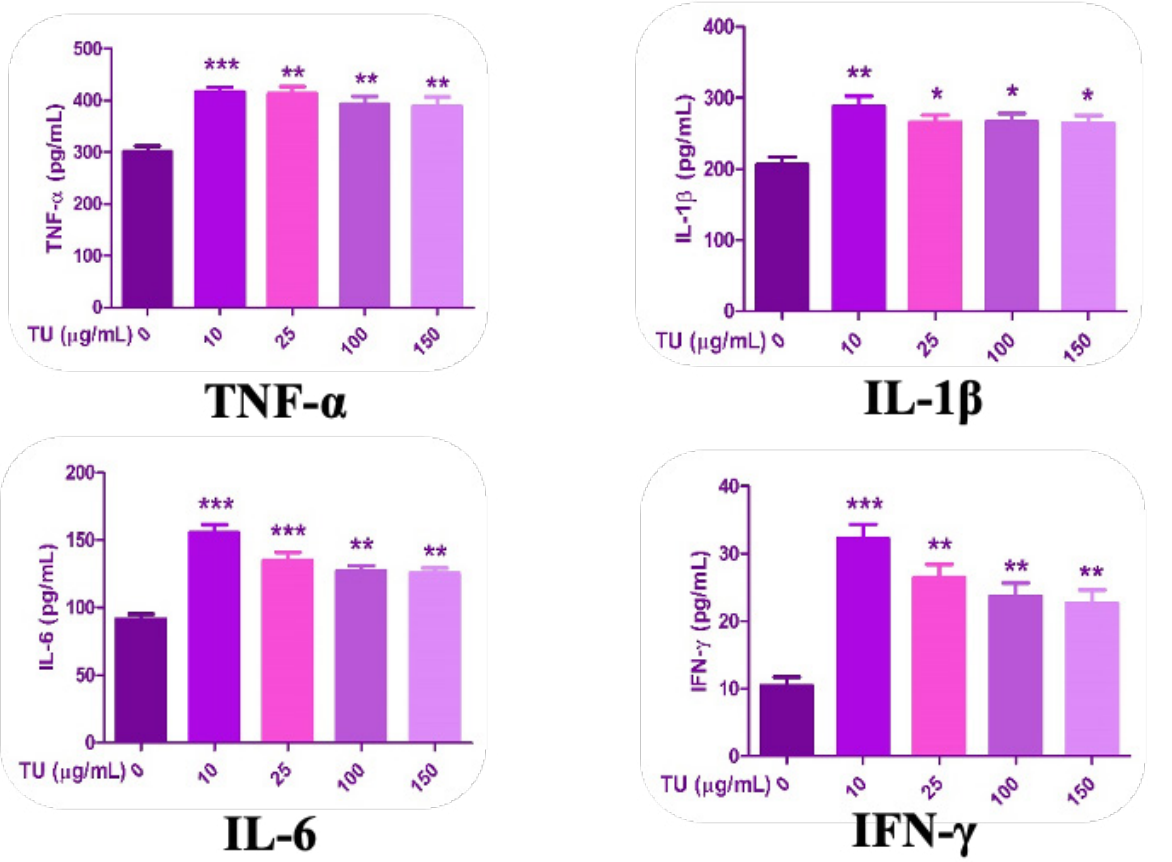


**Fig. 2.** Effect of the *T. ulmifolia* extract on cytokine release in unstimulated RAW 264.7 macrophages, *** – $p<0.001$, ** – $p<0.01$, * – $p<0.05$, with respect to the control

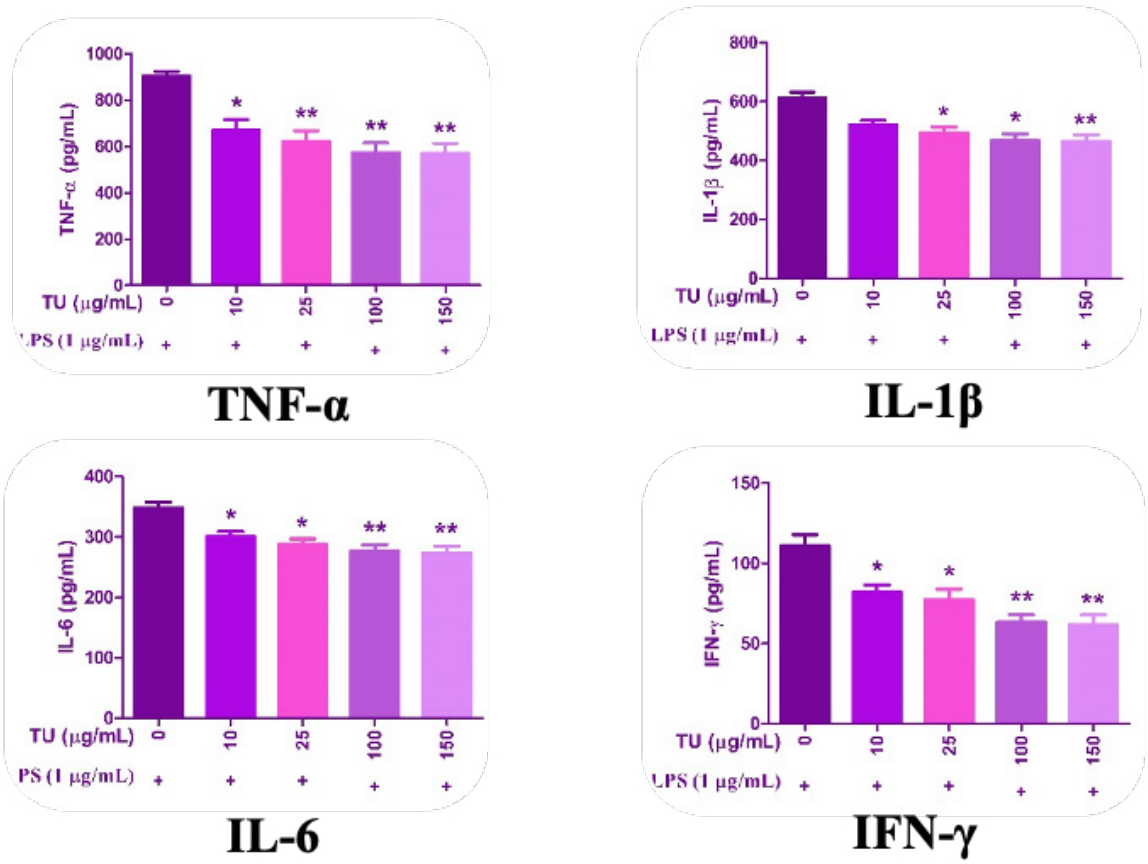


**Fig. 3.** Impact of the *T. ulmifolia* extract on the production of cytokines by RAW 264.7 macrophages stimulated by LPS, ** – p<0.01, * – p<0.05, with respect to the control

tory mediator NO by RAW 264.7 macrophages in both directions, therefore having immunomodulatory effects.

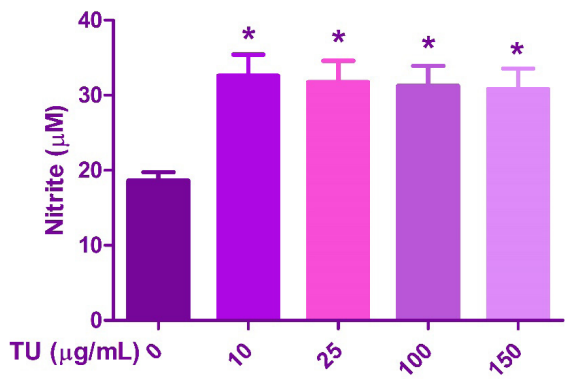


**Fig. 4.** Effects of *T. ulmifolia* extract on NO production in non-stimulated RAW 264.7 macrophages, * – p<0.05, with respect to control

### *Immunomodulatory effects of the T. ulmifolia extract in vivo*

*Effects of T. ulmifolia extract on mice's macrophage phagocytic activity*

The phagocytic index was significantly lower in the DC group (2.5±0.12) compared to the normal control group (NC) group (3.6±0.17), reflecting immune suppression in the DC group. However, treatment with *T. ulmifolia* extract restored phagocytic activity, with the TU-200 group showing a phagocytic index comparable to the NC group (3.6±0.17) and the TU-400 group showing a slight decrease (3.4±0.078). This suggests that *T. ulmifolia* extract enhances the innate immune response by promoting macrophage phagocytic function, particularly at the dose of 200 mg/kg (Fig. 6).

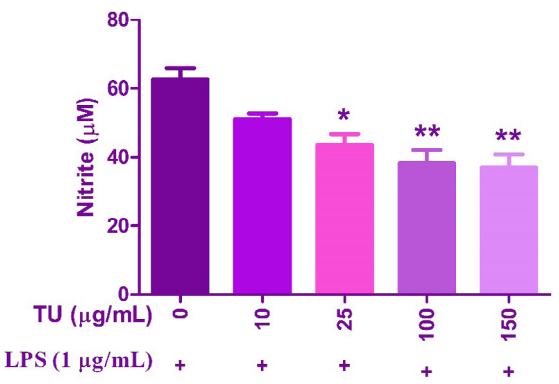


**Fig. 5. Effects of the** *T. ulmifolia* extract on NO production in RAW 264.7 macrophages stimulated by LPS, ** – p<0.01, * – p<0.05, with respect to the control

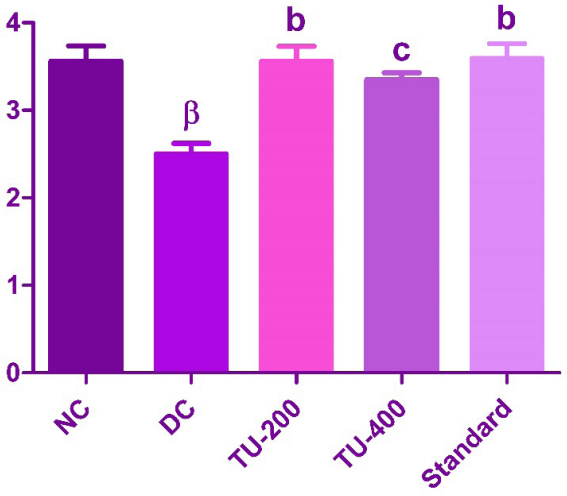


**Fig. 6.** Effects of *T. ulmifolia* extract on macrophage phagocytic function, $^{\beta}$ – p<0.01, when compared to the NC group, $^{b}$ – p<0.01, $^{c}$ – p<0.05, when compared to the DC group

*Impact of T. ulmifolia extract on mice with DTH*

Footpad thickness, a measure of the DTH response, was significantly reduced in the DC group (0.73±0.12 mm) compared to the NC group (1.5±0.07 mm), indicating an altered immune response. Both the TU-200 (1.3±0.10 mm) and TU-400 (1.2±0.092 mm) groups showed a significant increase in footpad thickness compared to the DC group, demonstrating that the extract of *T. ulmifolia* helps restore cell-mediated immunity in immunosuppressed mice. However, the response was slightly lower than in the NC group, indicating a partial but significant recovery of immune function (Fig. 7).

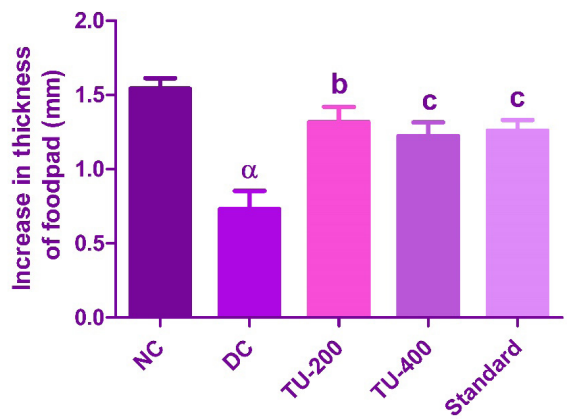


**Fig. 7.** Effects of *T. ulmifolia* extract on delayed-type hypersensitivity, $^{\alpha}$ – p<0.001, when compared to the NC group, $^{b}$ – p<0.01, $^{c}$ – p<0.05, when compared to the DC group

*Effects of T. ulmifolia extract on serum hemolysin level in mice*

Serum hemolysis levels were markedly lower in the DC group (0.16±0.024 OD value) compared to the NC group (0.31±0.035 OD value), showing reduced humoral immune function. Treatment with extracts of *T. ulmifolia* significantly increased serum hemolysis levels, with TU-200 (0.30±0.021 OD value) and TU-400 (0.28±0.021 OD value) approaching the levels of the NC group. This indicates that *T. ulmifolia* extract effectively enhances the humoral immune response, probably by increasing antibody production (Fig. 8) .

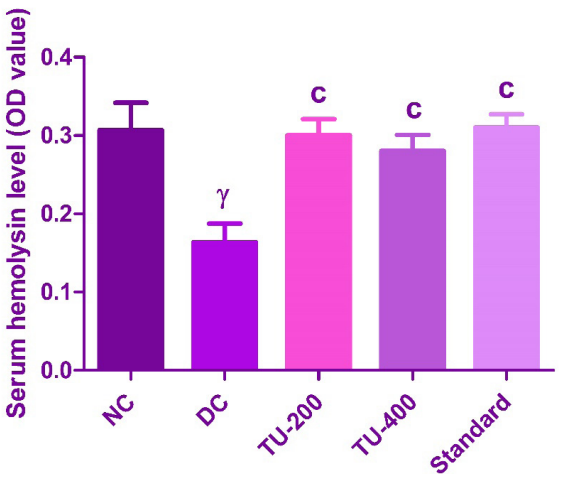


**Fig. 8.** Effects of *T. ulmifolia* extract on serum hemolysin level, $^{\gamma}$ – p<0.01 when compared to the NC group, $^{c}$ – p<0.05 when compared to the DC group

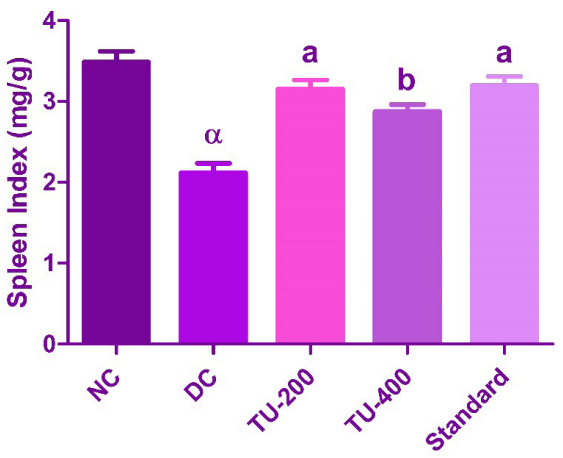


**Fig. 9.** Effects of *T. ulmifolia* extract on spleen index, $^{\alpha}$ – p<0.001, when compared to the NC group, $^{a}$ – p<0.001, $^{b}$ – p<0.01, when compared to the DC group

*Effects of T. ulmifolia extract on immune organ index in mice*

The thymus and spleen indices (Fig. 9 and Fig. 10 ), which serve as indicators of immune organ health, were

significantly lower in the DC group (1.2±0.091 mg/g for thymus, 2.1±0.12 mg/g for spleen) compared to the NC group (2.7±0.17 mg/g for thymus, 3.5±0.14 mg/g for spleen), reflecting immunosuppression. Both thymus and spleen indices increased significantly in the TU-200 and TU-400 groups, and the TU-200 group showing values closer to those of the NC group.

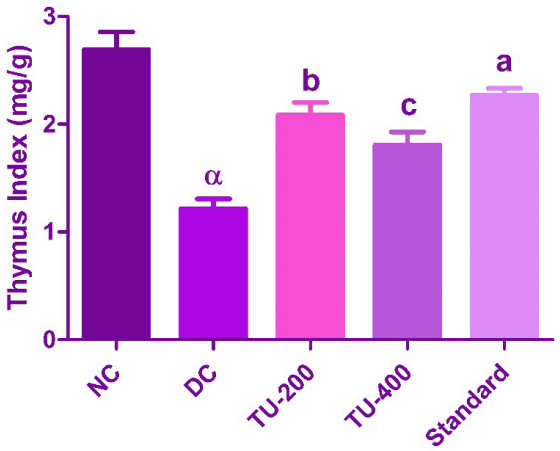


**Fig. 10.** Effects of *T. ulmifolia* extract on the thymus index, [α] – p<0.001 when compared to the NC group, [a] – p<0.001, [b] – p<0.01, [c] – p<0.05, when compared to the DC group

## Discussion

The RAW 264.7 cell line is a valuable tool for assessing *in vitro* the immunomodulatory effects of numerous compounds. Among the immunomodulatory agents secreted by these macrophages are cytokines such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and leukocyte adhesion molecules. Together, these chemicals promote the development of T and B lymphocytes, trigger the phagocytosis process in macrophages, and kill germs as part of secondary immune responses.[11] Cytokines play an essential role in modulating immune responses.[12] TNF-α is a tumor necrosis factor and a vital host regulatory molecule.[13] Macrophages release TNF-α and IL-1β, categorized as "early response cytokines", in reaction to inflammatory stimuli. These cytokines help raise endothelial cells' adhesion molecule levels, facilitating the migration and movement of phagocytes to tissue damage sites.[14] In contrast, IL-6 is essential for the host's immunological response, rapidly synthesizing proteins, and maintenance of homeostasis.[15,16]

Inflammatory cytokine overproduction can have detrimental effects, such as systemic exposure that may be fatal and collateral to normal cells. However, it is vital to remember that tissue healing and host survival from infections depend on the release of inflammatory cytokines.[17,18]

Although a volatile gas, NO is essential for several physiological functions, including inflammation, neurotransmission, and immunological responses.[19] It shows a dual biological role in affecting the activity of resident cells, tumor cells, and immune cells in many tissues and organs at suitable concentrations or, at low levels, acting as neurotransmitters.[20] On the other hand, excessive or uncontrollably released NO can cause damage to inflammatory tissue and host cell death. Therefore, controlling NO production may help reduce inflammatory and immunological disorders.[21]

In phagocytosis, specialized cells, called phagocytes, take in foreign substances and destroy them, including bacteria, tumor cells, inorganic fragments, and tissue waste. It is critical to the defense mechanisms. Phagocytes, which include neutrophils, monocytes, and macrophages, are among the first to the invasion of respond to a pathogenic organism.[22] When the epithelial barrier is penetrated, macrophages, ancient and evolutionary conserved cells in multicellular creatures, act as the first line of defense. A reflection of the phagocytic activity of the mononuclear phagocytic system, the macrophage phagocytic index is regarded as a diagnostic for identifying nonspecific immunity.[23] T-lymphocytes can develop into sensitized lymphocytes in response to antigen presentation, which can cause aberrant reactive inflammation to arise locally. This allergic inflammation exhibits necrosis and cell degradation and has a delayed start.[24] The presence of serum hemolysin antibodies can be used to estimate the degree of humoral immunity accurately. In reaction to different antigens, B-lymphocytes release this antibody.[25] This suggests that the *T. ulmifolia* extract helps restore immune organ health, particularly at the dose of 200 mg /kg, supporting the overall immune response.[26]

The results of this study reveal the significant immunomodulatory activity of the hydroalcoholic extract of *T. ulmifolia* in both *in vitro* and *in vivo* models. In vitro experiments using RAW 264.7 murine macrophage cells demonstrated that the extract could enhance the production of key cytokines (TNF-α, IL-1β, IL-6, and IFN-γ) and NO without causing cytotoxic effects. The increase in cytokine production indicates that *T. ulmifolia* extract has an immunostimulatory effect on macrophages, which are critical players in the innate immune response. However, it is essential to note that the extract also exhibited anti-inflammatory activity by inhibiting the overproduction of cytokines and NO in LPS-stimulated cells. This dual immunomodulatory and anti-inflammatory effect is significant because an excessive inflammatory response can lead to tissue damage and other adverse effects. At the same time, cytokines are necessary for effective immune function.[27] In *vivo* studies further support the immunomodulatory potential. The extract enhanced macrophage phagocytic activity, as observed in the carbon-clearance test in mice. This enhancement of the innate immune response was par-

ticularly evident at the 200 mg/kg dose, suggesting an optimal concentration for the efficacy of the extract. Additionally, the extract positively influenced cell-mediated immunity, as shown by the increased DTH response in immunosuppressed mice. The improvement in the DTH response points to the role in restoring cell-mediated immunity, an essential aspect of the immune system's ability to recognize and respond to foreign antigens.[28] The study also found that *T. ulmifolia* increased serum hemolysin levels, boosting the humoral immune response. The production of hemolysin antibodies by B-lymphocytes is a vital part of the body's defense mechanism against pathogens, and the ability to elevate these levels highlights its potential as an immunostimulant. Furthermore, the increase in thymus and spleen indices in treated mice suggests that the extract may positively impact the health and functionality of these critical immune organs.[29,30] Overall, the findings of both *in vitro* and *in vivo* models suggest that the extract of *T. ulmifolia* has significant immunomodulatory effects, enhancing immune responses and providing anti-inflammatory benefits. The biphasic effect of stimulating cytokine production in unstimulated cells and inhibiting excessive cytokine production in activated cells indicates that the extract can modulate immune responses in a balanced manner. These properties make *T. ulmifolia* extract a promising candidate for developing therapeutic agents targeting immune-related conditions, where fine-tuning of the immune system is crucial. More research is needed to isolate the active compounds responsible for these effects and to elucidate the underlying mechanisms of action.

## Conclusion

The findings of this study demonstrated that the *T. ulmifolia* extract has significant immunomodulatory effects, both *in vitro* and *in vivo*. *In vitro* studies revealed that the extract improved cytokine production and nitric oxide levels in unstimulated RAW 264.7 macrophage cells while inhibiting the overproduction of these immune mediators in LPS-stimulated cells, thus exhibiting immunostimulatory and anti-inflammatory properties. Furthermore, in vivo studies in BALB/c mice showed that the *T. ulmifolia* extract improved macrophage phagocytic function, increased DTH response, and elevated serum hemolysin levels, further indicating a strong immunostimulatory effect. The extract also improved the thymus and spleen indices, suggesting its ability to enhance overall immune responses. These results highlight the potential of *T. ulmifolia* extract as an effective immunomodulatory agent with promising applications in the management of immune-related disorders.

## Declarations

***Funding***

The authors received no external funding.

***Author contributions***

Conceptualization, V.S. and G.B.S.R.; Methodology, V.S.; Software, V.S.; Validation, V.S. and G.B.S.R.; Formal Analysis, V.S.; Investigation, V.S.; Resources, G.B.S.R.; Data Curation, V.S.; Writing – Original Draft Preparation, V.S.; Writing – Review & Editing, G.B.S.R.; Visualization, V.S.; Supervision, G.B.S.R.; Project Administration, G.B.S.R.; Funding Acquisition, G.B.S.R.

***Conflicts of interest***

The authors declare that they have no potential conflicts of interest regarding the research, authorship, and publication of this article.

***Data availability***

All data generated or analyzed during this study are included in the published article. Any additional data supporting the findings of this study are available from the corresponding author upon reasonable request.

***Ethics approval***

Ethical approval for the study was obtained from Flair Labs, located at PLOT NO B 510, PALSANA, Surat-394315, Gujarat, India, under the registration number 1250/PO/RcBi/S/23/CPCSEA.

## References

1. Pino JA. Essential oil of Turnera ulmifolia leaves from Cuba. *Nat Prod Commun.* 2010;5(11):1829-1830.
2. Brito NJ, López JA, do Nascimento MA, et al. Antioxidant activity and protective effect of Turnera ulmifolia Linn. var. elegans against carbon tetrachloride-induced oxidative damage in rats. *Food Chem Toxicol.* 2012;50(12):4340-4347. doi: 10.1016/j.fct.2012.08.003
3. Coutinho HD, Costa JG, Lima EO, Falcão-Silva VS, Siqueira Júnior JP. Herbal therapy associated with antibiotic therapy: potentiation of the antibiotic activity against methicillin – resistant Staphylococcus aureus by Turnera ulmifolia L. *BMC Complement Altern Med.* 2009;9(1):13. doi: 10.1186/1472-6882-9-13
4. Santos KKA, Matias EFF, Souza CES, et al. Anti-Candida activity of Mentha arvensis and Turnera ulmifolia. *J Med Food.* 2012;15(3):322-324. doi: 10.1089/jmf.2011.0128
5. Janine L, Ribeiro C, Pereira T, et al. Investigation ofTurnera ulmifoliaeffects in pregnant rats and offspring. *Pharm Biol.* 2009;47(11):1071-1077. doi: 10.3109/13880200902995533
6. Turnera ulmifolia (West Indian holly). *Plantwise Plus Knowle*. 2022; Species Pages. doi: 10.1079/pwkb.species.55353
7. Prabu D, Nappinnai M, Ponnudurai K, Thirugnanasambanthan A, Srinivasan S, Ramvikas M. Effects of turnera

ulmifolia (Linn.) leaves on blood glucose level in normal and alloxan-induced diabetic rats. *Iran J Pharmacol Ther*. 2009;8(2):77-81.

8. Kumar S, Taneja R, Sharma A. The GenusTurnera: A Review Update. *Pharm Biol*. 2005;43(5):383-391. doi: 10.1080/13880200590962926
9. Viel AM, Figueiredo CCM, Granero FO, et al. Anti-glycation, antioxidant, and cytotoxicity activities of crude extract of Turnera ulmifolia L. before and after microencapsulation process. *J Pharm Biomed Anal*. 2022;219:114975. doi: 10.1016/j.jpba.2022.114975
10. Chandrasekhar N, Vinay SP. Yellow-colored blooms of Argemone mexicana and Turnera ulmifolia mediated synthesis of silver nanoparticles and study of their antibacterial and antioxidant activity. *Appl Nanosci*. 2017;7(8):851-861. doi: 10.1007/s13204-017-0624-5
11. Guo Z, Xu HY, Xu L, Wang SS, Zhang XM. In Vivo And In Vitro Immunomodulatory And Anti-Inflammatory Effects Of Total Flavonoids Of Astragalus. *AJTCAMA*. 2016;13(4):60-73. doi: 10.21010/ajtcam.v13i4.10
12. Vilcek J, Lee TH. Tumor necrosis factor. New insights into the molecular mechanisms of its multiple actions. *JBC*. 1991;266(12):7313-7316.
13. Lejeune. Efficiency of recombinant human TNF in human cancer therapy. *Cancer Immun*. 2024;6.
14. Laskin DL, Laskin JD. Role of macrophages and inflammatory mediators in chemically induced toxicity. *J Toxicol*. 2001;160(1-3):111-118. doi: 10.1016/s0300--483x(00)00437-6
15. Liu YW, Shang HF, Wang CK, Hsu FL, Hou WC. Immunomodulatory activity of dioscorin, the storage protein of yam (Dioscorea alata cv. Tainong No. 1) tuber. *Food and Chemical Toxicology*. 2007;45(11):2312-2318. doi: 10.1016/j.fct.2007.06.009
16. Hu XD, Yang Y, Zhong XG, et al. Anti-inflammatory effects of Z23 on LPS-induced inflammatory responses in RAW264.7 macrophages. *J Ethnopharmacol*. 2008;120(3):447-451. doi: 10.1016/j.jep.2008.09.026
17. Glauser MP. The Inflammatory Cytokines. *Drugs*. 1996;52(2):9-17. doi: 10.2165/00003495-199600522-00004
18. Männel DN, Echtenacher B. TNF in the inflammatory response. *J Immun*. 2000;74:141-161.
19. Mayer B, Hemmens B. Biosynthesis and action of nitric oxide in mammalian cells. *Trends Biochem Sci*. 1997;22(12):477-481. doi: 10.1016/s0968-0004(97)01147-x
20. Palmer RMJ, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature*. 1988;333(6174):664-666. doi: 10.1038/333664a0
21. Zhang X, Li H, Feng H, et al. Valnemulin downregulates nitric oxide, prostaglandin E2, and cytokine production via inhibition of NF-κB and MAPK activity. *Int Immunopharmacol*. 2009;9(7-8):810-816. doi: 10.1016/j.intimp.2009.02.018
22. Birk RW, A. Gratchev, N. Hakiy, et al. Alternative Aktivierung antigenpräsentierender Zellen. *Der Hautarzt*. 2001;52(3):193-200. doi: 10.1007/s001050051289
23. Kang MC, Kim SY, Kim YT, et al. In vitro and in vivo antioxidant activities of polysaccharide purified from aloe vera (Aloe barbadensis) gel. *Carbohydr. Polym*. 2014;99:365-371. doi: 10.1016/j.carbpol.2013.07.091
24. Heriazon A, Yager JA, Sears W, Mallard BA. Induction of delayed-type hypersensitivity and interferon-gamma to Candida albicans and anti-hen-egg white lysozyme antibody as phenotypic markers of enhanced bovine immune response. *Vet Immunol Immunopathol*. 2009;129(1-2):93-100. doi: 10.1016/j.vetimm.2008.12.019
25. Tsai JP, Chen HW, Cheng ML, et al. Analysis of host versus tumor interaction in cancer patients: opposing role of transforming growth factor-β1 and interleukin-6 in the development of in situ tumor immunity. *Immunobiol*. 2005;210(9):661-671. doi: 10.1016/j.imbio.2005.07.004
26. Gao H, Li G, Huang J, et al. Protective effects of Zhuyeqing liquor on the immune function of normal and immunosuppressed mice in vivo. *BMC Complement Altern Med*. 2013;13(1):252. doi: 10.1186/1472-6882-13-252
27. Makare N, Bodhankar S, Rangari V. Immunomodulatory activity of alcoholic extract of Mangifera indica L. in mice. *J Ethnopharmacol*. 2001;78(2-3):133-137. doi: 10.1016/s0378-8741(01)00326-9
28. De Agostino Biella C, Salvador MJ, Dias DA, Dias-Baruffi M, Pereira-Crott LS. Evaluation of immunomodulatory and anti-inflammatory effects and phytochemical screening of Alternanthera tenella Colla (Amaranthaceae) aqueous extracts. *Mem Inst Oswaldo Cruz*. 2008;103(6):569-577. doi: 10.1590/s0074-02762008000600010
29. Liu C, Xi T, Lin Q, et al. Immunomodulatory activity of polysaccharides isolated from Strongylocentrotus nudus eggs. *Int. Immunopharmacol*. 2008;8(13-14):1835-1841. doi: 10.1016/j.intimp.2008.09.005
30. Costa JFO, David JPL, David JM, et al. Immunomodulatory activity of extracts from Cordia superba Cham. and Cordia rufescens A. DC. (Boraginaceae), plant species native from Brazilian Semi-arid. *Rev. bras. farmacogn*. 2008;18(1). doi: 10.1590/s0102-695x2008000100004