# Role of *Curcuma longa* Extracts in Enhancing Immune Response (IFN-γ, IL-12) Against Experimental Toxoplasmosis in Mice

Eman Habeeb Asleel
[1]Department of Biology, College of Science, University of Kirkuk, Kirkuk, Iraq

*Corresponding author's email: scbd24010@uokirkuk.edu.iq

## Abstract

Background : Toxoplasmosis is an opportunistic parasitic disease of public and veterinary health importance caused by the obligate intracellular parasite *Toxoplasma gondii*. This experimental study will assessed the effect of turmeric plant extracts (*Curcuma longa*) on increasing cellular immunity levels characterized by higher levels of cytokines IFN-γ and IL-12 in mouse models experimentally infected with *T. gondii* . The study was conducted on sixty Swiss albino mice divided into four equal groups (15 mice per group): a healthy control group, an untreated infected group, and two infected groups treated with two different doses of ethanolic turmeric extract (200 and 400 mg/kg). Two weeks after treatment, the levels of IFN-γ and IL-12 in the serum were measured using the ELISA technique, in addition to evaluating the parasitic load in the brain tissues. The results revealed a significant increase in IFN-γ and IL-12 levels in the two treated groups compared to the untreated infected group ($P<0.05$), along with a noticeable decrease in the cerebral parasitic load. The higher dose (400 mg/kg) was more effective with statistical significance. The herbal plant, Curcuma (the botanical genus name of the plant), and curcumin, as its active phenolic compound with actual therapeutic effects

**Keywords:** *Curcuma longa, Curcuma , Toxoplasma gondii, IFN-γ, IL-12, immune response, experimental mice.*

## Introduction

One-third of the world's population is thought to have latent cystic stage toxoplasmosis, making it one of the most common parasite diseases in the world (Bradyzoites) of the parasite *Toxoplasma gondii* in their tissues without showing clear clinical symptoms in most cases [1]. This organism is classified within the phylum Apicomplexa, and it is an obligatory intracellular parasite. The only definitive hosts able to complete the full sexual life cycle are the domestic cat and the Felidae family, whereas a variety of mammals and birds (including humans) host the asexual stages [2]. This infection assumes exceptional clinical importance in immunocompromised groups such as, those with human immunodeficiency virus (HIV) infection, cancer patients undergoing chemotherapy, organ transplant recipients as well as the fetus if primary infection occurs during pregnancy. Those initial forms may later change into a rapidly replicating phase (Tachyzoites), producing acute encephalitis (Toxoplasmic encephalitis) and severe visceral lesions that may be fatal if not promptly treated.[3]. What complicates the therapeutic landscape is that the currently available drug protocols—particularly the combination of pyrimethamine and sulfadiazine—are limited in their effectiveness against tissue cysts, relatively high in cost, and accompanied by side effects that may necessitate discontinuation of treatment [4].

In light of these challenges, there is an increasing need to explore natural therapeutic alternatives with higher

ISSN 2072-3857

immunological efficacy and safety. The turmeric plant (*Curcuma longa* L.) has garnered With growing scientific interest as a treasure trove of phytochemical compounds with multifaceted biological activity. This plant belongs to the Zingiberaceae family and has accompanied traditional medicine in South Asia and the Arabian Peninsula for thousands of years. Its distinctive yellow pigment, *Curcuma* , has become the subject of thousands of contemporary academic studies [5].

*Curcuma* [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] is the main phenolic compound that constitutes about 75-80% of the total *Curcuma* oids in the rhizome of C. longa, along with two accompanying compounds, dimethoxy *Curcuma* and bis-demethoxy *Curcuma* . These compounds are characterized by their antioxidant, anti-inflammatory, and antimicrobial effects, making them interesting therapeutic candidates [6]. And regarding their immune effects specifically, studies have proven that *Curcuma* intervenes in the regulation of vital cellular signaling pathways, including the Janus Kinase – Signal Transducer and Activator of Transcription (JAK-STAT ) and NF-κB pathways , and modifies the activity of macrophages, dendritic cells, and CD4+/CD8+ T cells in a manner that may enhance resistance against intracellular infectious agents [7].

The effective immune response against *T. gondii* primarily revolves around cell-mediated immunity, particularly the IL-12/IFN-γ axis. Interleukin-12 (IL-12), T helper 1 (Th1) cells and natural killer (NK) cells are stimulated to release interferon-gamma (IFN-γ) by antigen-presenting cells including macrophages and dendritic cells, which in turn activates macrophages to destroy the parasites thru mechanisms that include the production of nitric oxide (NO) and the regulation of tryptophan availability via the enzyme IDO (Indoleamine 2,3-dioxygenase) [8] [9]. Research has shown that *Curcuma* is capable of enhancing this vital immune axis, opening promising avenues for its use as an adjuvant or therapeutic alternative in the management of toxoplasmosis.

Although there have been individual studies addressing aspects of this relationship, the understanding of the precise molecular mechanisms thru which turmeric enhances the immune response against *T. gondii* remains limited, especially concerning the optimal dosage, the most suitable treatment duration, and the interplay between different cytokines. This study aims to fill these gaps by evaluating the effects of two different doses of the ethanolic extract of C. longa on the levels of IFN-γ and IL-12 in a Swiss albino mouse model experimentally infected with *T. gondi*i, while observing the implications on the cerebral parasitic load, which may pave the way for developing integrative therapeutic protocols that harness the potential of this ancient medicinal plant.

This study aims to achieve the following objectives: to evaluate the effect of the ethanolic extract of *Curcuma longa* on IFN-γ cytokine levels in the serum of experimentally infected mice with *Toxoplasma gondii*. And determining the levels of interleukin IL-12 and their changes in response to treatment with C. longa extracts at two different doses, and comparing the efficacy of the two doses of the extract (200 and 400 mg/kg) in enhancing the immune response

## Material and Methods

### 2-1 Experimental Animals

Twenty male Swiss albino mice, weighing 25–30 grams and aged 6–8 weeks, were procured from the authorized animal farm

ISSN 2072-3857

for this investigation. The animals were kept in a controlled setting with a temperature of 22±2°C and a 12-hour light/dark cycle. They were also given unlimited access to sterilized water and feed. Every procedure was carried out in compliance with the ARRIVE criteria, which are ethical guidelines for animal research.

**2-2 Study Groups**

Four groups of five mice each were randomly selected from among the mice:

• Group 1 (Healthy control): Healthy mice that were neither infected nor treated.

• Group 2 (infected untreated): Infected with *T. gondii* and given saline solution.

• The third group (treatment - low dose): Infected and treated with C. longa extract (200 mg/kg/day).

• Group four (treatment - high dose): Infected and treated with C. longa extract (400 mg/kg/day).

**3.2 Preparation of the plant extract**

The dried roots of C. longa were purchased from a local market with approval from a botanical identifying lab. Powdered samples were soaked in 70% ethanol at 1:10 (w/v) for 72 hours with occasional stirring. The extract was filtered, then dried at low pressure (40 1C) in a rotary evaporator to yield a crude powder. The powder was dissolved in 0.5% DMSO solution (for daily use) and was delivered through a gastric tube of Gavage[8].

**4.2 Experimental Infection**

The mice from groups two, three and four were intravenously infected with 10^3 tachyzoites of RH strain of *T. gondi*i, suspended in 0.2 ml of saline. Therapy commenced 24 h post-infection and was continued for the next 14 days without interruption.

**5.2 Sampling and laboratory analyses**

On the fifteenth day, under mild anesthesia, blood samples were collected from the retro-orbital plexus of each mouse. Serum was separated by centrifugation at 3000 rpm for 10 min and stored at −80°C until analysis. The levels of IFN-γ and IL-12 were measured using sandwich ELISA kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

**6.2 Statistical Analysis**

Tukey's post-hoc test and one-way ANOVA were used in the analysis of the data using SPSS version 26. At $P<0.05$, differences were deemed statistically significant. The mean ± standard deviation (Mean ±SD) was used to express the results

**Results and Discussion**

**1.3 IFN-γ Level in Mouse Serum**

The two extract-treated groups' IFN-γ levels were significantly higher than those of the untreated infected group, according to the data. The group treated with 200 mg/kg had an average of 118.4±12.7 pg/mL, whereas the untreated group had an average of 62.8±8.3 pg/mL. The group treated with 400 mg/kg had the highest value (194.6±18.3 pg/mL). Every group's differences were statistically significant ($P<0.01$) (Table 1).

**2.3 IL-12 Level in Mouse Serum**

The level of IL-12 followed the same trend, as it significantly increased in both treatment groups. The untreated infected group only showed a slight increase (51.7±7.1 pg/mL) compared to the control group (38.4±5.2 pg/mL), whereas the group treated with 400 mg/kg reached (152.8±15.6 pg/mL), reflecting a clear stimulation of the Th1 immune axis ($P<0.001$).

ISSN 2072-3857

### 3.3 Cerebral parasitic load

The parasitic load significantly decreased in both treatment groups; it dropped from (28.6±4.9×10^3/g tissue) in the untreated group to (15.3±3.2×10^3/g tissue) at the low dose, and (8.7±2.1×10^3/g tissue) at the high dose, representing a relative decrease of 46.5% and 69.6%, respectively ($P<0.01$).

The results of this study provide strong experimental evidence that the ethanolic extract of *Curcuma longa* enhances the cellular immune response against *Toxoplasma gondii* in a dose-dependent manner, as evidenced by the gradual increase in IFN-γ and IL-12 levels in the treated groups compared to the untreated infected group. This trend is consistent with what was observed in the study by [10] which observed a similar increase in Th1 immune-stimulating cytokines in mouse models treated with *Curcuma* .

The proposed molecular mechanism behind this multifaceted effect is that studies have shown *Curcuma* inhibits the SOCS1 protein (Suppressor of Cytokine Signaling 1), which obstructs the JAK-STAT4 pathway responsible for IL-12 production in macrophages and dendritic cells [11] .Moreover, *Curcuma* activates the Nrf2/HO-1 pathway, which enhances the oxidative efficiency of macrophages and increases their ability to kill intracellular parasites. It is important to note that IFN-γ in turn activates the enzyme iNOS (Inducible nitric oxide synthase), which secretes nitric oxide (NO) as one of the main cellular weapons against intracellular parasites; this partially explains the significant reduction in cerebral parasitic load [12]. What is striking in the current study's results is the clear direct relationship between the dose (200 vs. 400 mg/kg) and cytokine levels on one hand, and the reduction in parasitic load on the other. This relationship contributes to supporting the conclusion that *Curcuma* does not exert its effect thru a single mechanism but rather thru an interconnected network of molecular signals working together, which often explains the phenomenon of the enhanced effect at higher doses within the safe dosage range. The study by [13] . Animal safety for a dose of up to 8000 mg/kg without acute toxic effects, providing a wide therapeutic margin.

These results cannot be interpreted in isolation from the scientific literature related to the IL-12/IFN-γ axis in the resistance to *T. gondi*i. [14] documented that the parasite primarily induces IL-12 thru pattern recognition receptors (PRRs) such as TLR11 and TLR12, which recognize the profilin protein in the parasite. *Curcuma* is likely to enhance the expression of these receptors on the surface of macrophages or improve their sensitivity, thereby doubling the immune system's ability to respond quickly to any parasitic challenge. This hypothesis is consistent with what [14] of enhanced dendritic cell activity and increased MHC-II expression after *Curcuma* treatment.

From a therapeutic application perspective, researchers raise a fundamental question about the possibility of translating these experimental results into actual clinical therapeutic protocols. The limited bioavailability of *Curcuma* , which has long been considered a barrier to its medical applications—since its oral absorption is less than 1% can be overcome with nanoformulations or those enhanced with piperine or liposomal forms, which have shown an improvement in bioavailability by up to twenty times [15] [16].

ISSN 2072-3857

**Table (1): IFN-γ and IL-12 Levels and Parasite Burden in the Study Groups (Mean ± Standard Deviation)**

| Parasite Burden (×10³/g Tissue) | IL-12 (pg/mL) | IFN-γ (pg/mL) | Group |
|---|---|---|---|
| 0 | 38.4 ± 5.2 | 45.2 ± 6.1 | Healthy Control |
| 28.6 ± 4.9 | 51.7 ± 7.1 | 62.8 ± 8.3 | Infected Untreated |
| 15.3 ± 3.2 | 96.3 ± 10.2 | 118.4 ± 12.7 | Infected + 200 mg/kg |
| 8.7 ± 2.1 | 152.8 ± 15.6 | 194.6 ± 18.3 | Infected + 400 mg/kg |

## Conclusion

In light of the obtained results, the following can be concluded: The ethanolic extract of *Curcuma longa* significantly dose-dependently enhances the levels of IFN-γ and IL-12 in mice experimentally infected with *T. gondi*i. The treatment with C. longa extract results in a significant reduction in the cerebral parasitic load, indicating a dual effect: an immune-stimulating and a direct antiparasitic effect. The 400 mg/kg dose is more effective than the 200 mg/kg dose in all measured variables.

**Acknowledgment:** The author would like to express sincere gratitude to the Department of Biology, College of Science, University of Kirkuk, for providing the facilities and support necessary to conduct this research. Special thanks are extended to all laboratory staff for their technical assistance and cooperation throughout the study. The author also appreciates the valuable guidance and encouragement provided by colleagues and faculty members during the completion of this work.
**Funding:** This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

## References

**[1] Aggarwal, B. B., Sundaram, C., Malani, N., & Ichikawa, H. (2007). Curcumin: The Indian solid gold.** *Advances in Experimental Medicine and Biology, 595*, 1–75. https://doi.org/10.1007/978-0-387-46401-5_1

**[2] Alday, P. H., & Doggett, J. S. (2017).** Existing drugs as broad-spectrum and target-based treatments for *Toxoplasma gondii*. *Infection and Drug Resistance, 10*, 177–197. https://doi.org/10.2147/IDR.S106397

**[3] Anand, P., Kunnumakkara, A. B., Newman, R. A., & Aggarwal, B. B. (2007).** Bioavailability of curcumin: Problems and promises**.** *Molecular Pharmaceutics, 4*(6), 807–818. https://doi.org/10.1021/mp700113r

**[4] Blanco, B., Balana-Fouce, R., & Reguera, R. M. (2023).** *Toxoplasma gondii*: Advances in immune evasion and

therapeutic approaches. *Frontiers in Cellular and Infection Microbiology, 13*, 1135723. https://doi.org/10.3389/fcimb.2023.1135723

**[5] Dunay, I. R., Gajurel, K., Dhakal, R., Liesenfeld, O., & Montoya, J. G. (2018).** Treatment of toxoplasmosis: Historical perspective, animal models, and current clinical practice. Clinical Microbiology Reviews, 31(4), e00057-17. https://doi.org/10.1128/CMR.00057-17

**[6] Dubey, J. P. (2010).** *Toxoplasmosis of animals and humans* (2nd ed.). CRC Press.

[7] Hunter, C. A., & Sibley, L. D. (2012). Modulation of innate immunity by *Toxoplasma gondii* virulence effectors. *Nature Reviews Microbiology, 10*(11), 766–778. https://doi.org/10.1038/nrmicro2858

**[8] Jurenka, J. S. (2009).** Anti-inflammatory properties of curcumin, a major constituent of *Curcuma longa*: A review of preclinical and clinical research**.** *Alternative Medicine Review*, *14*(2), 141–153

**[9] Mahmoudvand, H., Tavakoli, R., Shakibaie, M., Minaei, S., Ezatkhah, F., Jahanbakhsh, S., & Sharifi, I. (2019)**. *In vitro and in vivo activities of curcumin on Toxoplasma gondii infection: A systematic review*. Journal of Parasitic Diseases, 43(3), 369–379. https://doi.org/10.1007/s12639-019-01107-9

**[10] Daryani, A., Sharif, M., Sarvi, S., Aarabi, M., Mizani, A., Ahmadpour, E., Rahimi, M. T., & Gholami, S. (2017)**. *The potential use of curcumin for treatment of toxoplasmosis: A review*. Frontiers in Microbiology, 8, 1208. https://doi.org/10.3389/fmicb.2017.01208

**[11] Montoya, J. G., & Liesenfeld, O. (2004).** Toxoplasmosis. *The Lancet*, *363*(9425), 1965–1976. https://doi.org/10.1016/S0140-6736(04)16412-X

**[12] Prasad, S.,&Aggarwal, B. B. (2011).** Turmeric, the golden spice: From traditional medicine to modern medicine. In I. F. F. Benzie & S. Wachtel-Galor (Eds.), *Herbal medicine: Biomolecular and clinical aspects* (2nd ed.). CRC Press.

**[13] Robert-Gangneux, F., & Dardé, M. L. (2012).** Epidemiology of and diagnostic strategies for toxoplasmosis. *Clinical Microbiology Reviews*, *25*(2), 264–296. https://doi.org/10.1128/CMR.05013-11

**[14] Shakibaei, M., Kraehe, P., Popper, B., Shakibaei, M. M., & Buhrmann, C. (2019)**. Curcumin potentiates antitumor activity of oxaliplatin by interfering with the NF-κB signaling pathway**.** *Molecular Cancer*, *18*(1), 116.

**[15] Pappas, G., Roussos, N., & Falagas, M. E. (2009).** *Toxoplasmosis snapshots: Global status of Toxoplasma gondii seroprevalence and implications for pregnancy and congenital toxoplasmosis*. International Journal for Parasitology, 39(12), 1385–1394. https://doi.org/10.1016/j.ijpara.2009.04.003

**[16] Yarovinsky, F. (2014).** Innate immunity to *Toxoplasma gondii* infection. *Nature Reviews Immunology*, *14*(2), 109–121. https://doi.org/10.1038/nri3598

ISSN 2072-3857