International Journal of Herbal Medicine
Available online at www.florajournal.com

E-ISSN: 2321-2187
P-ISSN: 2394-0514
www.florajournal.com
IJHM 2024; 12(2): 01-04
Received: 02-12-2023
Accepted: 07-01-2024

Naveen Chandra
Sri Dharmasthala Manjunatheshwara Centre, Centre for Research in Ayurveda and Allied Sciences, (AYUSH Centre for Excellence) Laxminarayana Nagar, PO Kuthpady-Udupi, Karnataka, India

Vishwanatha U
Sri Dharmasthala Manjunatheshwara Centre, Centre for Research in Ayurveda and Allied Sciences, (AYUSH Centre for Excellence) Laxminarayana Nagar, PO Kuthpady-Udupi, Karnataka, India

Kiran Khatau
Lodaat Pharma, 1415 West 22nd Street-Tower Floor, Oak Brook, Illinois 60523, USA

Dr. Sanjay Tamoli
Target Institute of Medical Education & Research, 402/A, A, B, C, Jaswanti Allied Business Centre, Off Link Road, Malad West, Mumbai, Maharashtra, India

Corresponding Author:
Dr. Sanjay Tamoli
Target Institute of Medical Education & Research, 402/A, A, B, C, Jaswanti Allied Business Centre, Off Link Road, Malad West, Mumbai, Maharashtra, India

Evaluation of immunity enhancing potential of *Ocimum sanctum* (TulsiOdaat™) through an *in-vitro* cytotoxicity and phagocytic activity assessment on mouse macrophage RAW 264.7 cell line

Naveen Chandra, Vishwanatha U, Kiran Khatau and Dr. Sanjay Tamoli

DOI: https://doi.org/10.22271/flora.2024.v12.i2a.923

Abstract
Tulsi (*Ocimum sanctum*), commonly known as Holy Basil, has traditionally been used a remedy of common infections of the respiratory tract, and as an immunity booster. The herb has potential anti-microbial, anti-bacterial, and anti-viral effects. The present study evaluated the cytotoxic and phagocytic activity of a standardized extract of *Ocimum sanctum* (TulsiOdaat™) in mouse macrophages RAW 264.7 cell lines. Phagocytic activity was evaluated by observing the amount of engulfed zymosans (absorbance at 405 nm) isolated from yeasts. The study demonstrated that TulsiOdaat™ significantly enhanced the phagocytic activity of macrophages compared to the vehicle treated/control group at a non-toxic concentration of 3 µg/mL. The observations made in the present study confirm that TulsiOdaat™ stimulates macrophages and enhances their phagocytic activity and therefore, may have potential enhancing effects on innate immunity. The results corroborate observations of previous studies on *Ocimum sanctum* species, which may augment the immune response against foreign antigens or disease-causing pathogens.

Keywords: Cytotoxic, Phagocytic, Zymosans, Macrophages, Ocimum sanctum, Tulsi, immunity, TulsiOdaat™

1. Introduction
The COVID-19 pandemic has emphasised the importance of immunity. Co-morbid conditions associated with weak immunity have been considered factors for increased mortality and increased risk of serious complications from COVID-19 [1]. In addition to COVID-19, other common viral and bacterial infections may have significant complications for individuals with lower or compromised immunity. Advisories from international government agencies have suggested medicinal and non-medicinal interventions for boosting immunity that may prevent the liklihood of infection as well as reduce its severity.

Herbs in various forms have been traditionally recommended as well as scientifically studied for their immune enhancing and immunomodulatory effects [2]. Phytoconstituents present in these herbs, including alkaloids, flavanoids, polyphenols, etc., have been attributed to possessing these potential benefits. *Ocimum sanctum* (OS), popularly known as Holy Basil, is one of the oldest and most well-known herbs in traditional systems of medicine [3]. Traditionally, OS has been utilized for its specific action on the respiratory system in the prevention and treatment of infections and inflammation related conditions.

Bioactive compounds of OS have been investigated for their various medicinal properties and their effects at the molecular level [4]. Eugenol (1-hydroxy-2-methoxy-4-allylbenzene), the active constituent present in *Ocimum sanctum*, has been found to be largely responsible for the therapeutic potentials of OS. Laboratory studies have shown that OS protects against toxic chemical-induced injury by increasing levels of anti-oxidants such as glutathione and enhancing the activity of anti-oxidant enzymes, such as SuperOxide Dismutase (SOD), catalase and other toxic agents [5-6].

The objective of the present study is to investigate the dose dependent phagocytic activity and cytotoxic activity of standardized *Ocimum sanctum* extract (TulsiOdaat™) in mouse macrophages RAW 264.7 cell line. This study aims to explore immunomodulatory (phagocytic) and cytotoxicity effects of TulsiOdaat™ developed by LODAAT Pharma. *Ocimum sanctum* extract - TulsiOdaat™ is *Ocimum sanctum* extract standardised to contain - 2.5% Ursolic Acid.

2. Materials and Methods

The Mouse Monocyte Macrophage (RAW 264.7) cell line was procured from the National Centre for Cell Service, Pune and Sub cultured at Sri Dharmasthala Manjunatheshwara Centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udupi as per standard procedure. The cell line was used for cytotoxicity and phagocytic activity studies in mouse macrophage cell line (RAW264.7) using coded test products.

2.1 Cell culture and MTT Assay- Cytotoxicity of test products against Mouse Macrophage cell line RAW264.7

Cytotoxicity of the test product, TulsiOdaat™, against Mouse Macrophage cell line (RAW 264.7) was carried out by MTT assay with modification (Mosmann, T. 1983). Mouse Monocyte Macrophage (RAW 264.7) cell line was procured from NCCS Pune and sub cultured using Dulbecco's Modified Eagle Medium (DMEM) with fetal bovine serum (FBS). Between 70-80% confluent RAW 264.7 cell line was taken and the medium from the culture flask was removed. The cells were washed twice with sterile phosphate buffer saline (PBS) without disturbing the cells. The wash solution from the culture flask was then removed. The cells were detached from the flask by scraping the surface of flask using a sterile cell scraper. After the cells were detached from the flask, between 1-2 ml of fresh medium (DMEM medium with 10% fetal bovine serum) was added to the flasks and the cell suspension was transferred to 15 ml sterile centrifuge tube. Cells were centrifuged at 800 rpm for 5 minutes. After centrifugation, the pellet was washed twice with PBS and re-suspended with growth medium (DMEM medium with 10% FBS). 100 µl of tryphan blue (0.04%) was pipetted to a vial and an equal volume of cell suspension was added to the same vial. Both were mixed carefully, loaded to haemocytometer, and counted under an inverted microscope. After counting the cells, 50, 000 cells/well in 100 µl of medium was added to a 96-well plate and the plate was incubated with a CO_2 incubator for 24 hours. After 24 hours, the old medium from the 96-well plate was discarded, and cells were washed once with PBS using a multichannel pipette. Various concentrations of the coded test product, TulsiOdaat™, was dissolved in serum free DMEM medium and added to different wells respectively in the 96-well plate and incubated for 24 hours. Control cells were supplemented with routine growth medium. After completion of incubation time, 20 µL of MTT dye (5 mg/mL in PBS) was added to all wells and the plate was covered with aluminium foil and incubated in a CO_2 incubator at 37 °C for 4 hours. After 4 hours, 100 µL of 0.4 NHCl and isopropanol (1:24) was added to all the wells and mixed to dissolve the crystals. Using a multi plate reader, the absorbance was recorded at 570 nm and 640 nm reference range. The percentage of viable cells was calculated using the formula - % of viable cells = [(Test sample-blank) / (Control-blank)] x 100

2.2 Phagocytic activity of Mouse Macrophage cell line (RAW 264.7)

The phagocytic activity of RAW 264.7 cells was determined using a CytoSelect™ 96-well phagocytosis assay kit (Zymosan Colorimetric format, Cell Biolabs Inc., San Diego, CA, USA) as per the manufacturer's instructions. Cells were seeded in a 96-well plate at 1 x 105 cells/well and allowed to attach to the plate for 24 hours. The cells were then treated with TulsiOdaat™ (3 µg/mL & 6 µg/mL) or with 0.25% Dimethyl sulfoxide (DMSO) as a control group. The second group of cells was treated with 50 µg/ml of Lipopolysaccharide (LPS) as the positive control group. Subsequently, non-opsonized zymosan was added and the amount of engulfed zymosan (Absorbance) was measured at 405nm after 2 h incubation at 37 °C by microplate reader (TECAN, Infinite M NANO· Austria).

2.3 Plant material and Extracts

The present study was conducted on TulsiOdaat™, a proprietary extract of *Ocimum sanctum* developed by LODAAT Pharma. TulsiOdaat™ contains standardised extract of *Ocimum sanctum* containing 2.5% Ursolic Acid

2.4 Statistical analysis

Data are presented as mean ± standard deviation (SD). Statistical analysis was done by two-tailed Student t-test using graphpad software, Statistical significance between the treatment groups and control was assessed. Significant differences were considered at $p<0.05$. Data has been presented as graphs and tables.

3. Results

3.1 Effect of extract of *Ocimum sanctum* (TulsiOdaat™) on cell viability

Mouse macrophages RAW 264.7 cell line showed increasing cytotoxicity with increasing doses/ concentration of TulsiOdaat™. Cell viability was >60% at concentration of TulsiOdaat™ up to 80 µg/mL. Further, cell viability remained between 25-50% at doses between 80- 1000 µg/mL of TulsiOdaat™. The cell viability dropped below 25% at doses above 1000 µg/mL. (Figure1).

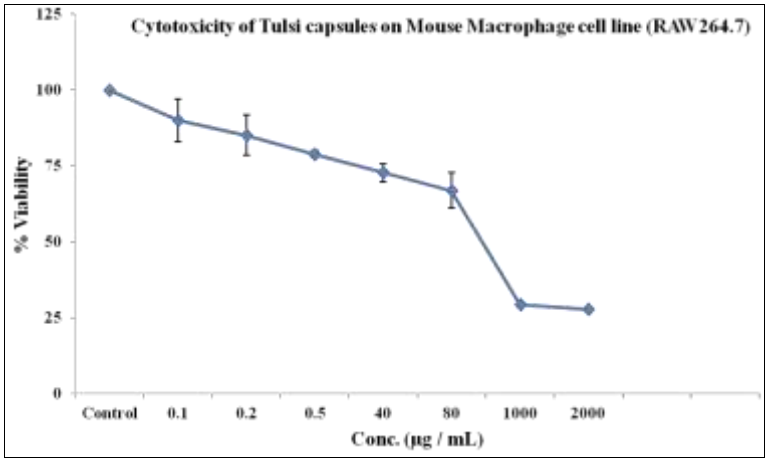

Fig 1: Cytotoxicity of TulsiOdaat™ against (RAW264.7) cell line.

3.2 Phagocytic activity of extract of *Ocimum sanctum* (TulsiOdaat™)

Phagocytosis is a critical immunological function of macrophages as a defense to protect the host from foreign antigens/pathogens. Phagocytic activity was evaluated by observing the amount of engulfed zymosans (absorbance at 405 nm) isolated from yeasts. The study demonstrated that the standardized extract of *Ocimum sanctum* (TulsiOdaat™) significantly enhanced the phagocytic activity of macrophages compared to vehicle treated/control group at non-toxic concentration of 3 µg/mL (Figure 2, Table 1) in mouse macrophage RAW 264.7 cell line. The study demonstrated that the absorbance with TulsiOdaat™ at 3 µg/mL concentration (mean OD value 0.431±0.0261) was significantly higher (p<0.0034) than absorbance observed with the control group (mean OD value 0.393±0.0020). However, increasing the dosage of TulsiOdaat™ did not result in an observable increase in absorbance as the absorbance at 6 µg/mL concentration (mean OD value 0.467±0.0005) was closer to the absorbance at dosage of 3 µg/mL.

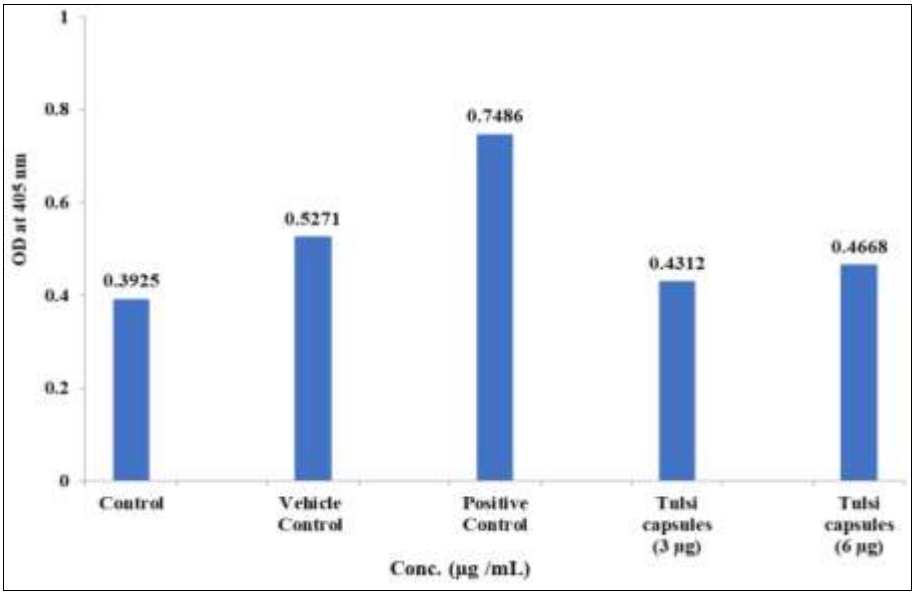

Fig 2: Phagocytic activity of mouse macrophage cell line (RAW 264.7) of TulsiOdaat™ The values are presented as Mean OD values ± SD. Control (Zymosan free group); Vehicle control (without LPS); Positive Control (with LPS)

Table 1: OD mean & SD values of Phagocytic activity of TulsiOdaat™

Experimental Groups	Mean OD Values	SD	p value between the groups
Control	0.393	0.0020	*p*-0.1765 (vs. control) *p*-0.0350 (vs. vehicle control) *p*-0.0034 (vs. positive control)
Vehicle Control	0.527	0.0002	
Positive Control	0.749	0.0003	
TulsiOdaat™ (3 µg)	0.431	0.0261	
TulsiOdaat™ (6 µg)	0.467	0.0005	*p*-0.0004 (vs. control) *p*-0.0001 (vs. vehicle control) *p*-0.0001 (vs. positive control)

4. Discussions

In this study, we evaluated the cytotoxic and phagocytic activity of a proprietary, standardized extract of *Ocimum sanctum* (OS), commercially known as TulsiOdaat™. After screening for cytotoxicity in the mouse macrophage RAW 264.7 cell line, the non-cytotoxic doses/concentration of TulsiOdaat™, (3 µg/mL and 6 µg/mL), were tested for the phagocytic activity. The experiment compared the activity of TulsiOdaat™ with a positive control (LPS) and the untreated/control group. TulsiOdaat™, at a dose of 3 µg/mL, demonstrated a similar phagocytic activity to positive control (LPS) and higher activity than the untreated/control group. Increasing the dose to 6 µg/mL did not show any enhanced activity and was found to be similar to the dose of 3 µg/mL.

Previous scientific research has revealed that *Ocimum sanctum* has anti-bacterial, anti-viral and anti-fungal activities that includes activity against many pathogens responsible for human infections. *Ocimum sanctum* has also been shown to boost defenses against infective threats by enhancing immune responses in non-stressed and stressed animals and healthy humans. There is experimental evidence that *Ocimum sanctum* may help in the treatment of various human bacterial infections including urinary tract infections, skin and wound infections, typhoid fever, cholera, tuberculosis, gonorrhea, acne, herpes simplex, leishmaniasis, various pneumonias and fungal infections, as well as mosquito-borne diseases such as dengue, malaria, and filariasis [7-13].

The results of our *in vitro* study corroborate the observations of previous studies on *Ocimum sanctum* and confirms that this proprietary, standardized extract of *Ocimum sanctum*, commercially known as TulsiOdaat™, activates macrophages and enhances their phagocytic activity, which could help in augmenting the immune response against foreign antigens or disease-causing pathogens.

5. Conclusions

The observations made in the present study confirm that TulsiOdaat™ stimulates macrophages and enhances their phagocytic activity, and therefore, may have potential enhancing effects on innate immunity. Clinical studies are underway on TulsiOdaat™ to further validate the effect as an immunity booster compound. Further studies are needed to evaluate *Ocimum sanctum* and TulsiOdaat™.

6. Acknowledgement
The Authors acknowledge, Sri Dharmasthala Manjunatheshwara Centre for Research in Ayurveda and Allied Sciences (AYUSH Centre for Excellence and Recognized SIROs by DSIR) Laxminarayana Nagar, P.O. Kuthpady – 574 118, Udupi [Karnataka] for providing the facility to carry out the experimental studies.

7. Disclosure
TulsiOdaat™ - *Ocimum sanctum* extract was supplied by LODAAT Pharma.

8. Conflict of Interest: None.

9. References
1. Lauren Callender, *et al*. The Impact of Pre-existing Comorbidities and Therapeutic Interventions on COVID-19, Front. Immunol. 2020 Aug, 11.
2. Kanika Khanna, Sukhmeen Kaur Kohli, Herbal immune-boosters: Substantial warriors of pandemic COVID-19 battle, Phytomedicine 2021 May;85:153361
3. Marc Maurice Cohen, Tulsi - *Ocimum sanctum*: A herb for all reasons, Journal of Ayurveda & Integrative Medicine. 2014 Oct-Dec;5(4):251-259
4. Vasudevan P, Kashyap S, Sharma S. Bioactive botanicals from basil (*Ocimum* sp.) J Sci. Ind. Res. (C). 1999;58:332-8.
5. Shivananjappa M, Joshi M. Aqueous extract of *Tulsi* (*Ocimum sanctum*) enhances endogenous antioxidant defenses of human hepatoma cell line (HepG2) J Herbs Spices Med Plants. 2012;18:331-48.
6. Manikandan P, Murugan RS, Abbas H, Abraham SK, Nagini S. *Ocimum sanctum* Linn. (Holy Basil) ethanolic leaf extract protects against 7, 12-dimethylbenz (a) anthracene-induced genotoxicity, oxidative stress, and imbalance in xenobiotic-metabolizing enzymes. J Med Food. 2007;10:495-502.
7. Mediratta PK, Sharma KK, Singh S. Evaluation of immunomodulatory potential of *Ocimum sanctum* seed oil and its possible mechanism of action. J Ethnopharmacol. 2002;80:15-20.
8. Hemalatha R, Babu KN, Karthik M, Ramesh R, Kumar BD, Kumar PU. Immunomodulatory activity and Th1/Th2 cytokine response of *Ocimum sanctum* in myelo-suppressed swiss albino mice. Trends Med Res. 2011;6:23-31.
9. Tripathi AK, Rajora VS, Gupta DK, Shukla SK. Immunomodulatory activity of *Ocimum sanctum* and its influence on cyclophosphamide induced immunosupression. Indian J Anim. Sci. 2008;78:33-6.
10. Goel A, Singh DK, Kumar S, Bhatia AK. Immunomodulating property of *Ocimum sanctum* by regulating the IL-2 production and its mRNA expression using rat's splenocytes. Asian Pac. J Trop. Med. 2010;3:8-12.
11. Pavaraj M, Balasubramanian V, Baskaran S, Ramasamy P. Development of immunity by extract of medicinal plant *Ocimum sanctum* on common carp Cyprinus carpio (L.) Res. J Immunol. 2011;4:12-8.
12. Chitra G, Krishnaveni N. Immunostimulatory effect of *Ocimum sanctum* leaf extract on the Indian major carp, *Catla catla*. Plant Archives. 2011;11:213-4.
13. Mondal S, Varma S, Bamola VD, Naik SN, Mirdha BR, Padhi MM, *et al*. Double-blinded randomized controlled trial for immunomodulatory effects of Tulsi (*Ocimum sanctum*.) leaf extract on healthy volunteers. J Ethnopharmacol. 2011;136:452-6