

ORIGINAL ARTICLE

Ghee Hiang's Sesame Oil Mitigates Inflammation: Insights from an In Vitro Macrophage Model

Ubashini Vijakumaran¹, Nooreliana Zulaiha Zulkifli¹, Chean Ring Leong², Woei Yenn Tong¹, Muhammad Fauzi Daud^{1*}

¹ Institute of Medical Science Technology, Universiti Kuala Lumpur (UniKL), A1-1, Jalan TKS 1, Taman Kajang Sentral, Selangor, 43000 Kajang, Selangor, Malaysia

² Malaysian Institute of Chemical and Bioengineering Technology, Universiti Kuala Lumpur, 78000, Alor Gajah, Melaka, Malaysia

ABSTRACT

Introduction: Sesame oil, derived from *Sesamum indicum* seeds, is enriched with bioactive compounds proven for their antioxidant and anti-inflammatory properties. However, limited studies have explored the immunomodulatory potential of traditionally extracted sesame oils, such as Ghee Hiang's sesame oil, in macrophage-driven inflammation models. **Materials and Methods:** RAW264.7 macrophage cells were stimulated with lipopolysaccharides (LPS) to induce inflammation and treated with several different concentrations of sesame oil extract. Cytotoxicity was assessed via the MTT assay, while cytokine levels of TNF- α and IL-10 were quantified using ELISA. **Results:** The results showed that Ghee Hiang's sesame oil was non-toxic at concentrations up to 200 $\mu\text{g/ml}$ and exhibited significant immunomodulatory effects, reducing TNF- α levels by up to 65% and increasing IL-10 levels by 132% in a dose-dependent manner. This indicates a shift towards an anti-inflammatory macrophage phenotype. **Conclusion:** These findings suggest that Ghee Hiang's sesame oil has potent anti-inflammatory properties, potentially serving as a natural therapeutic candidate for inflammation control. Its ability to downregulate pro-inflammatory cytokines and enhance anti-inflammatory responses positions it as a promising candidate for future studies involving chronic inflammatory diseases and nutraceutical development.

Malaysian Journal of Medicine and Health Sciences (2026) 22(SUPP3): 31-35. doi:10.47836/mjmhs.22.s3.5

Keywords: Sesame oil, ghee hiang, anti-inflammatory, immunomodulation, macrophages

Corresponding Author:

Muhammad Fauzi Daud, PhD

Email: mfauzid@unikl.edu.my

Tel: +603-8739 5894

INTRODUCTION

Sesame oil, derived from the seeds of *Sesamum indicum*, is widely recognized for its nutritional and medicinal value, owing to its rich composition of lipids, proteins, and diverse bioactive compounds including tocopherols, sesamin, sesamol, and other lignans (1, 2). Globally, sesame is cultivated on approximately 14 million hectares with an annual production of about 6.8 million tonnes (1). Traditionally used in Asian medicine and cuisine, sesame oil is now gaining scientific attention for its pharmacological activities. Previous studies have reported that sesame oil exhibits antioxidant, antimicrobial, antihypertensive, and cardioprotective effects, with many of these benefits attributed to its polyunsaturated fatty acids (PUFAs) and lignan content (3-6). In particular, Ghee Hiang's sesame oil, a traditionally extracted Malaysian sesame oil product,

was shown by Yenn et al. (2023) to possess significant antibacterial and antioxidant activities, highlighting its potential as a bioactive-rich therapeutic oil (7).

Building upon these findings, it is plausible that Ghee Hiang's sesame oil may also exert immunomodulatory effects, as similar bioactive compounds particularly sesamin and sesamol have been shown to regulate immune responses (8). These compounds are known to inhibit key inflammatory pathways such as nuclear factor-kappa B (NF- κB) and cyclooxygenase-2 (COX-2) (9), leading to reduced secretion of pro-inflammatory cytokines like tumor necrosis factor-alpha (TNF- α), interleukin-1 beta (IL-1 β), and interleukin-6 (IL-6), while promoting the production of anti-inflammatory cytokines such as interleukin-10 (IL-10) (8, 10). A meta-analysis of randomised controlled trials reported that sesame consumption reduced serum level interleukin-6 (IL-6) (WMD - 0.90; 95% CI (-1.71, - 0.09) but did not alter level of CRP and TNF- α significantly (11).

Moreover, in vivo studies in mice have demonstrated that aqueous extracts of sesame oil suppress inflammation-

associated genes involved in atherosclerosis and upregulate those involved in reverse cholesterol transport and anti-inflammatory responses (12). Sesamol, in particular, has been shown to alleviate oxidative stress and airway inflammation in asthmatic mice by increasing glutathione levels and reducing malondialdehyde expression (13). Despite these insights, the immunomodulatory potential of Ghee Hiang's sesame oil has not been previously explored. Given that the method of oil extraction may influence its bioactive compound profile and potency, there is a scientific basis to investigate whether traditionally processed sesame oil like Ghee Hiang's can modulate immune cell responses *in vitro*.

Therefore, the present study aims to evaluate the anti-inflammatory and immunomodulatory effects of Ghee Hiang's sesame oil using RAW264.7 macrophage cells stimulated with lipopolysaccharide (LPS) to induce inflammation. This research focuses on its cytotoxicity profile and its ability to suppress TNF- α and enhance IL-10 production, indicators of immune modulation, thereby expanding the pharmacological profile of Ghee Hiang's sesame oil.

MATERIALS AND METHODS

Study Design

The study was designed as an *in vitro* analysis to assess the immunomodulatory, anti-inflammatory, and cytotoxicity effects of Ghee Hiang's sesame oil. RAW264.7 macrophage cells were used to evaluate the oil's effects on immune modulation, inflammation, and cell viability. Cells were exposed to varying doses of sesame oil and then stimulated with lipopolysaccharides (LPS) to trigger an inflammatory response. MTT assay was used to determine cell viability, and cytokine levels were measured to assess the inflammatory activity.

Materials and Chemicals

The materials used included Ghee Hiang's sesame oil, LPS (Sigma-Aldrich), and reagents for cytokine and cytotoxicity assays. RAW264.7 macrophage cells were maintained in DMEM media completed with 10% fetal bovine serum (FBS) and were sourced from iCell Bioscience. Cytokine levels were measured using ELISA kits for TNF- α and IL-10 (Elabsience). MTT assay (BBI/Sangon Biotech) used to detect cell viability.

Extraction of Sesame Oil

To extract the sesame oil sample, liquid partitioning was performed. The process involved using a separation funnel, where two distinct layers were formed. The upper layer, light brown in color, represented the organic phase, while the bottom layer was dark brown. The upper organic layer was collected as the methanolic extract of the sesame oil. After the drying process, the extract had a brown appearance, and an extraction yield of 1.74% was obtained.

Cytotoxicity Testing

To evaluate the cytotoxicity of Ghee Hiang's sesame oil, RAW264.7 cells were cultured in 24-well plates, and different concentrations of sesame oil (200–1000 $\mu\text{g/mL}$) were added to the cells and incubated for 48 hours. At the end of the treatment, MTT solution was added at a concentration (5 mg/mL) were added to the treated cells and incubated for 40 minutes at 37°C. The formazan crystal was dissolved using DMSO. The intensity of formazan was detected at 570nm through a microplate reader. Cell viability was quantified as a percentage relative to untreated control cells, and the half-maximal inhibitory concentration (IC_{50}) was calculated to evaluate the cytotoxic potential of the sesame oil extract. Based on the cytotoxicity results, concentrations of 100 $\mu\text{g/mL}$ and 200 $\mu\text{g/mL}$ were selected for subsequent assays, as these doses were below the cytotoxic threshold and ensured macrophage viability. Similar non-toxic concentration ranges have been reported for Ghee Hiang sesame oil extract showing strong antioxidant and antibacterial activities (7).

Pro-inflammatory Testing

RAW264.7 cells were treated with LPS for 24 hours to induce pre inflammation condition. Afterwards, the cells were treated with Ghee Hiang's sesame oil extract at two different concentrations, 100 $\mu\text{g/ml}$ and 200 $\mu\text{g/ml}$, for 24 hours. The levels of pro-inflammatory TNF- α cytokine were measured using ELISA.

Anti-inflammatory Testing

In a separate experiment, RAW264.7 cells were first stimulated with LPS for 24 hours to induce inflammation. Afterwards, the cells were treated with Ghee Hiang's sesame oil extract at concentrations of 100 $\mu\text{g/ml}$ and 200 $\mu\text{g/ml}$ for 24 hours. The levels of anti-inflammatory IL-10 cytokine were measured using ELISA.

Data Collection and Statistical Analysis

Data are presented as mean \pm standard deviation (SD) from three independent experimental replicates. Cytokine concentrations were measured using ELISA and expressed as percentages relative to the untreated control group. Likewise, cell viability in the cytotoxicity assay was calculated as a percentage of the control. Statistical analysis was conducted using one-way ANOVA followed by Tukey's post hoc test, with $p < 0.05$ considered statistically significant.

RESULTS

Cytotoxicity Effects

The cytotoxicity of Ghee Hiang's sesame oil on RAW264.7 cells was assessed using the MTT assay. Cell viability was unaffected at lower concentrations (up to approximately 250 $\mu\text{g/ml}$), with over 90% cell viability observed (Figure 1). However, at higher concentrations (300 $\mu\text{g/ml}$ and above), cell viability decreased, with the IC_{50} concentration being approximately 357.6 $\mu\text{g/ml}$.

This indicates that Ghee Hiang's sesame oil is non-toxic at lower concentrations (below 300 µg/ml), which were used in the assays to study the pro-inflammatory and anti-inflammatory functions.

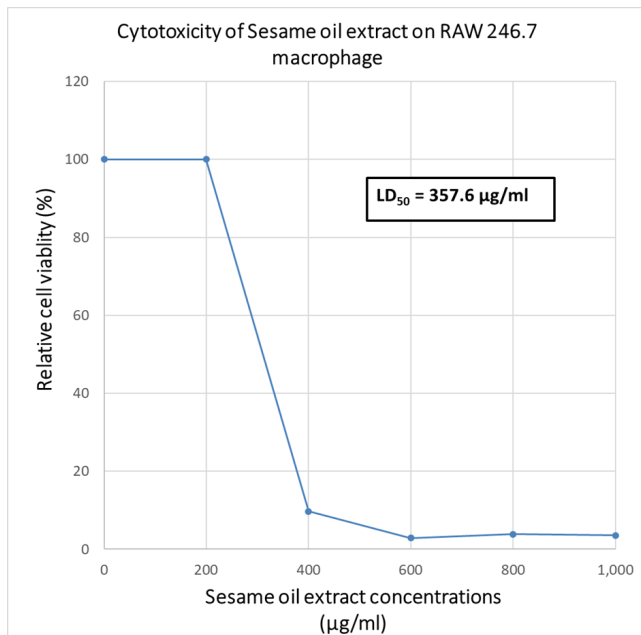


Fig. 1: Cytotoxicity of Sesame oil extract on RAW246.7 macrophages. (n = 3, three independent experiments)

Pro-inflammatory effects

Ghee Hiang's sesame oil extract reduced the production of pro-inflammatory cytokine (TNF-alpha) compared to the control group in a dose-dependent manner. At 100 µg/ml concentration, TNF-α levels were reduced by 55% and reduced by 65% at the highest non-toxic concentration (200 µg/ml) as shown in Figure 2.

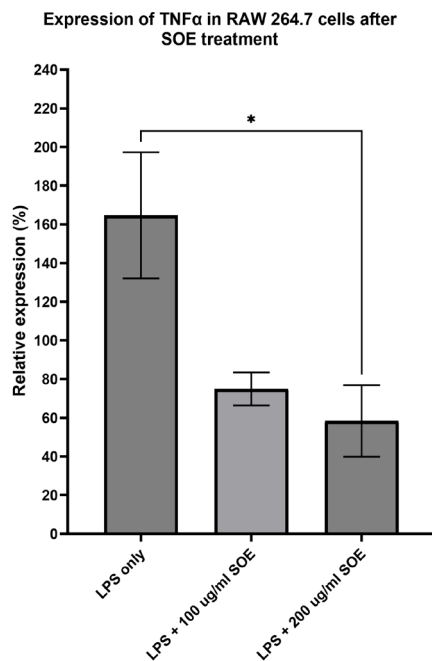


Fig. 2: Expression of TNFα in RAW 264.7 cells after SOE treatment. *p<0.01, (n = 3, three independent experiments)

Anti-inflammatory Effects

Ghee Hiang's sesame oil extract increased anti-inflammatory cytokine production (IL-10) compared to the control group in a dose-dependent manner. At 100 µg/ml concentration, IL-10 levels were elevated by 29%, and increased by 132% at the highest non-toxic concentration (200 µg/ml) as shown in Figure 3.

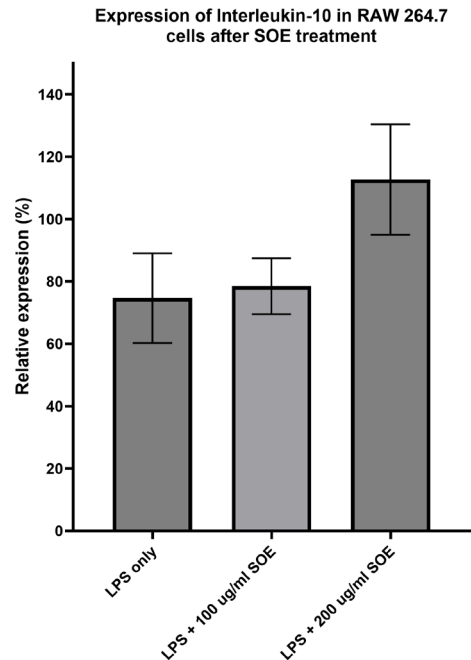


Fig. 3: Expression of Interleukin-10 in RAW 264.7 cells after SOE treatment. (n = 3, three independent experiments)

DISCUSSION

The cytotoxicity analysis revealed that Ghee Hiang's sesame oil is well-tolerated by RAW264.7 macrophage cells at concentrations up to 200 µg/ml, making it suitable for further testing in immune and inflammation-related assays. The IC₅₀ value (approximately 357.6 µg/ml) suggests that the oil extract begins to exert cytotoxic effects at higher concentrations. Meanwhile, a study was done on two different types of sesame from Indonesia exhibited IC₅₀ DPPH 8.88–44.21 µg/mL and IC₅₀ ABTS 24.91–141.19 µg/mL (14).

With regard to immunomodulation, a reduction in the pro-inflammatory cytokine TNF-α indicates that Ghee Hiang's sesame oil was observed to reduce inflammatory activity. This suggests that the oil extract can shift macrophages from a pro-inflammatory state towards a more balanced or anti-inflammatory phenotype, which could be particularly beneficial in managing conditions of chronic inflammation. The findings confirm that Ghee Hiang's sesame oil possesses potent anti-inflammatory properties, likely due to its rich content of bioactive compounds like sesamin. By significantly reducing levels of pro-inflammatory cytokines in an LPS-induced inflammation model, the oil extract demonstrates potential as a natural anti-inflammatory agent, with

implications for its use in mitigating inflammation-related conditions.

The bioactive compounds in sesame oil, particularly sesamin, are believed to contribute to these immunomodulatory and anti-inflammatory effects. Previous studies have shown that these compounds inhibit key inflammatory pathways, including NF- κ B and MAPK signaling, thereby reducing the production of pro-inflammatory mediators and enhancing the expression of anti-inflammatory cytokines such as IL-10 (8, 9). Furthermore, Hsu et al. demonstrated that sesamol attenuates endotoxin-induced oxidative stress and multiple organ injury in rats by reducing nitric oxide production, suppressing inducible nitric oxide synthase expression, and enhancing antioxidant enzyme activities, thereby protecting against inflammation-associated tissue damage (15). Sesamol also alleviates high-fat-diet-induced hepatic insulin resistance in mice by enhancing adiponectin secretion from adipose tissue and activating hepatic AMPK signaling, thereby improving glycogen synthesis and reducing gluconeogenesis (16).

The combination of cytotoxicity testing and cytokine modulation findings provides a strong foundation for exploring the therapeutic potential of Ghee Hiang's sesame oil. Its ability to reduce inflammation while remaining non-toxic at lower concentrations suggests it could be useful in managing inflammatory conditions. Further *in vivo* and clinical studies will be essential to validate these findings and to investigate its potential for systemic applications.

This study was limited to *in vitro* assays using RAW264.7 macrophage cells. While the cytotoxicity and cytokine modulation findings are promising, additional *in vivo* studies and clinical trials are needed to confirm the safety and efficacy of Ghee Hiang's sesame oil for therapeutic use. Future studies should also investigate the molecular mechanisms involved and assess long-term effects on immune regulation and inflammation.

Although this study demonstrated the immunomodulatory effects of Ghee Hiang's sesame oil extract, the chemical constituents were not directly analyzed. Previous studies have reported that traditionally extracted sesame oils contain bioactive lignans such as sesamin, sesamol, and sesamolol, which are responsible for their antioxidant and anti-inflammatory properties. These compounds have been shown to modulate inflammatory pathways, including NF- κ B and MAPK signaling, leading to reduced production of pro-inflammatory cytokines and enhanced antioxidant capacity (8, 17). A recent report also confirmed the antibacterial and antioxidant activities of Ghee Hiang's sesame oil extract, further supporting its high bioactive compound content (7). Future studies incorporating GCMS or HPLC profiling will be essential to identify and quantify these constituents and to correlate their abundance with the observed biological

effects.

CONCLUSION

This study demonstrated that Ghee Hiang's sesame oil extract exhibits immunomodulatory and anti-inflammatory properties in LPS-stimulated RAW264.7 macrophage cells. The extract was well tolerated up to 200 μ g/mL and significantly reduced the production of the pro-inflammatory cytokine TNF- α while maintaining cell viability. These findings suggest that Ghee Hiang's sesame oil shows potential as a natural anti-inflammatory agent warranting further *in vivo* validation to confirm its efficacy and elucidate its underlying mechanisms.

ACKNOWLEDGMENTS

We would like to thank Ghee Hiang Manufacturing Co. Sdn. Bhd for the financial support provided to the project. We also thank Dr. Nurshamimi Nor Rashid and the Drug Design and Development Research Group, University of Malaya for generously providing the RAW264.7 macrophage cell line used in this study.

REFERENCES

1. Langyan S, Yadava P, Sharma S, Gupta NC, Bansal R, Yadav R, et al. Food and nutraceutical functions of sesame oil: An underutilized crop for nutritional and health benefits. *Food Chemistry*. 2022;389:132990.
2. Ma X, Wang Z, Zheng C, Liu C. A comprehensive review of bioactive compounds and processing technology of sesame seed. *Oil Crop Science*. 2022;7(2):88-94.
3. Orsavova J, Misurcova L, Ambrozova JV, Vicha R, Mlcek J. Fatty Acids Composition of Vegetable Oils and Its Contribution to Dietary Energy Intake and Dependence of Cardiovascular Mortality on Dietary Intake of Fatty Acids. *Int J Mol Sci*. 2015;16(6):12871-90.
4. Sheikuduman MS. Anti-microbial activity of sesame oil. *International Journal of Research in Phytochemistry and Pharmacology*. 2011;1.
5. Hsu E, Parthasarathy S. Anti-inflammatory and Antioxidant Effects of Sesame Oil on Atherosclerosis: A Descriptive Literature Review. *Cureus*. 2017;9(7):e1438.
6. Karatzi K, Stamatelopoulos K, Lykka M, Mantzouratou P, Skalidi S, Manios E, et al. Acute and Long-Term Hemodynamic Effects of Sesame Oil Consumption in Hypertensive Men. *The Journal of Clinical Hypertension*. 2012;14(9):630-6.
7. Yenn T, Hashim N, Lim L, Leong CR, Wen Nee T. Antibacterial and Antioxidant Activities of Ghee Hiang Sesame Oil Extract. *Malaysian Journal of Medicine and Health Sciences*. 2023;19:75-81.
8. Majdalawieh A, Yousef S, Abu-Yousef I, Nasrallah G. Immunomodulatory and anti-inflammatory effects of sesamin: mechanisms of action and future

directions. *Critical Reviews in Food Science and Nutrition*. 2021;62:1-32.

9. Wu XL, Liou CJ, Li ZY, Lai XY, Fang LW, Huang WC. Sesamol suppresses the inflammatory response by inhibiting NF- κ B/MAPK activation and upregulating AMP kinase signaling in RAW 264.7 macrophages. *Inflamm Res*. 2015;64(8):577-88.
10. Mostashari P, Mousavi Khaneghah A. Sesame Seeds: A Nutrient-Rich Superfood. *Foods*. 2024;13(8):1153.
11. Rafiee S, Faryabi R, Yargholi A, Zareian MA, Hawkins J, Shivappa N, et al. Effects of Sesame Consumption on Inflammatory Biomarkers in Humans: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Evid Based Complement Alternat Med*. 2021;2021:6622981.
12. Narasimhulu CA, Selvarajan K, Burge KY, Litvinov D, Sengupta B, Parthasarathy S. Water-Soluble Components of Sesame Oil Reduce Inflammation and Atherosclerosis. *J Med Food*. 2016;19(7):629-37.
13. Liou C-J, Chen Y-L, Yu M-C, Yeh K-W, Shen S-C, Huang W-C. Sesamol Alleviates Airway Hyperresponsiveness and Oxidative Stress in Asthmatic Mice. *Antioxidants*. 2020;9(4):295.
14. Ruslan K, Happyniar S, Fidrianny I. Antioxidant potential of two varieties of *Sesamum indicum* L. collected from Indonesia. *Journal of Taibah University Medical Sciences*. 2018;13(3):211-8.
15. Hsu DZ, Li YH, Chu PY, Chien SP, Chuang YC, Liu MY. Attenuation of endotoxin-induced oxidative stress and multiple organ injury by 3,4-Methylenedioxyphenol in rats. *Shock*. 2006;25(3):300-5.
16. Zheng W, Yang J, Zhang Q, Cheng M, Shaukat H, Qin H. Sesamol Alleviates High-Fat Diet-Induced Hepatic Insulin Resistance in C57BL/6 J Mice Through AMPK Activation Mediated by Adipose Adiponectin. *Plant Foods Hum Nutr*. 2023;78(4):720-7.
17. Oboulbiga EB, Douamba Z, Compaoré-Séréme D, Semporé JN, Dabo R, Semde Z, et al. Physicochemical, potential nutritional, antioxidant and health properties of sesame seed oil: a review. *Front Nutr*. 2023;10:1127926.