

Original Research Article

Anti-obesity potential of Porelis, a standardized extract of purple tea (*Camellia sinensis*), via modulation of obesity-related inflammation in RAW264.7 macrophages and anti-adipogenesis in 3T3-L1 cells

Kuluvar Gouthamchandra^{1*}, Heggar Venkataramana Sudeep¹, Bhoomika Ranganath¹, Amritharaj¹, Lingaraju Harakanahalli Basavegowda², TP Prasanna Kumara³, Shyam Prasad Kodimule^{2,3}

¹Department of Biomedical Research, ²Department of Phytochemistry, ³Department of Analytical Development Research Laboratory, R & D Centre for Excellence, Vidya Herbs Pvt. Ltd, #14A, Jigani I Phase, Bangalore-560105, Karnataka, India

*For correspondence: **Email:** bmrl@vidyaherbs.com, goutham06@gmail.com

Sent for review: 26 July 2024

Revised accepted: 2 January 2025

Abstract

Purpose: To assess the effect of Porelis, a standardized extract of purple tea (*Camellia sinensis*) on inflammation in RAW 264.7 cells, anti-adipogenesis and lipid accumulation in 3T3-L1 cells.

Methods: Viability of RAW 264.7 and 3T3L1 cells was determined by MTT assay. Lipopolysaccharide (LPS) was used to induce inflammation in RAW264.7 cells. Nitric oxide (NO) generation and levels of anti-inflammatory cytokines (TNF- α and IL-6) were determined using Griess reagent and ELISA, respectively. In addition, Oil-O red staining was carried out to assess the differentiation of 3T3L cells. Furthermore, Western blotting was used to quantify the protein expressions of PPAR- γ , C/EBP and SREBP-1 while RT-PCR was used to determine leptin and FAS mRNA expression.

Results: Porelis comprised 3 – 5 % of 1,2-di-O-galloyl-4,6-O-(S)-hexahydroxydiphenoyl- β -D-glucose (GHG). Its use significantly inhibited the generation of NO, IL-6 and TNF- α in LPS-stimulated RAW 264.7 cells ($p < 0.0001$). Interestingly, there was a significantly inhibited adipocyte differentiation, fat accumulation and protein expression of PPAR γ and CEBP α in a dose-dependent manner ($p < 0.0001$). The mRNA levels of leptin and FAS were significantly ($p < 0.0001$) upregulated in differentiated adipocytes compared to preadipocytes. Furthermore, treatment with Porelis significantly inhibited mRNA expression levels of leptin and FAS.

Conclusion: Porelis may exert anti-inflammatory activity by inhibiting inflammatory responses in RAW264.7 macrophages as well as anti-obesity activity by blocking fat accumulation via inhibition of adipogenesis in 3T3L adipocytes. This study shows that Porelis could be a promising dietary supplement for prevention of inflammation and obesity.

Keywords: Porelis, Purple tea, Cytokines, Adipogenesis, Oil-O red staining, Transcription factors

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Pharmaceutical Research is indexed by Scopus, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

The World Health Organization (WHO) states that obesity is one of the most prominent, but often ignored, public health issues. It raises the

risk of several diseases, such as cardiovascular disease, type 2 diabetes, non-alcoholic fatty liver disease (NAFLD), hypertension and certain types of cancers [1,2]. Additionally, obesity has been linked to a chronic inflammatory state that is

characterized by abnormal cytokine production and activation of inflammatory signaling pathways. Chronic inflammation is a major factor in the enhancement of metabolic diseases including insulin resistance and atherosclerosis that are connected to obesity [3-5]. Therefore, reducing the expression of cytokines and enzymes related to inflammation could be helpful in the reduction of diseases connected to inflammation.

Adipogenesis represents the biological process by which a fibroblast differentiates into a preadipocyte, and then into a mature adipocyte. By preventing adipogenesis and fat deposition, the amount of adipose tissue is reduced [6,7]. Over the past few decades, there has been a growing trend in the use of herbal extracts as a health supplement for anti-adipogenesis. Numerous phytochemicals have demonstrated antiadipogenic properties both *in vitro* and *in vivo* [8-10].

Purple tea is a newly developed tea presently grown in Kenya. It is the result of 25 years of studies on cloning conducted by the Kenya Tea Research Foundation. Purple tea extract has been shown in a recent study to modify the microbial composition of the gut and prevent microbial dysbiosis in the host. As a result, it may play a role in modifying susceptibility to obesity, hepatic disorders and insulin resistance that are associated with a high-fat diet [6]. On the other hand, little is known about the effects of purple tea on adipogenesis. The current study explored the effect of Porelis, a standardized extract of purple tea (*Camellia sinensis*) comprised of 3 – 5 % GHG (1,2-di-O-galloyl-4,6-O-(S)-hexahydroxydiphenoyl- β -D-glucose) on inflammation in RAW 264.7 cells, anti-adipogenesis and lipid accumulation in 3T3-L1 adipocytes.

EXPERIMENTAL

Sample preparation

Porelis, obtained from the Department of Phytochemistry, Vidya Herbs Pvt. Ltd, Bengaluru, Karnataka, India, was dissolved in water at the proper concentrations required for each experiment.

Cell culture

The RAW 264.7 murine macrophage and 3T3-L1 mouse adipocyte cells were purchased from NCCS (Pune, India). Cell lines were maintained separately in growth media (DMEM supplemented with 10 % FBS) and were

changed every two to three days, to keep the cells healthy. They were subsequently sub-cultured after they achieved 80 % confluence.

Determination of cell viability

Cell viability was assessed using the MTT test by following established procedures [12]. Briefly, murine macrophage RAW 264.7 and 3T3-L1 mouse adipocyte cells (5×10^3 / well) were seeded in separate 96-well plates and incubated overnight. The cells were then treated with different concentrations (50 – 500 $\mu\text{g}/\text{mL}$) of Porelis and incubated at 37 °C in 5 % CO₂ and 95 % air. The medium was replaced, after 24 h, with 100 μL aliquot of MTT reagent and allowed to remain at 37 °C for 4 h. After carefully decanting the supernatant, 100 μL of DMSO was added and the absorbance at 570 nm was read to determine the formazan concentration, which correlates to the number of living cells.

Determination of nitric oxide (NO) in RAW 264.7 macrophages

Production of nitric oxide (NO) in supernatants of cultured RAW264.7 macrophages was determined using the Griess reagent. Briefly, RAW macrophages were plated in 6-well plates at a density of 1×10^5 cells/well and incubated overnight. Subsequently, the cells were exposed to two different concentrations (100 and 200 $\mu\text{g}/\text{mL}$) of Porelis, followed by LPS (5 $\mu\text{g}/\text{mL}$; Sigma-Aldrich) stimulation for 24 h. After treatment, Griess reagent (100 μL) was mixed with 100 μL of cell culture supernatant in 96 well plates and the mixture was incubated for 10 minutes at room temperature. Thereafter, the absorbance was read at 550 nm using Tecan Infinite Multimode Plate Reader.

Evaluation of cytokine secretion level in macrophages

The levels of inflammatory cytokines, such as TNF- α and IL-6, were determined in the cell culture supernatant of RAW 264.7 macrophages using commercially available ELISA kit (Krishgen Biosystems, Mumbai, India) according to manufacturer's instructions.

Cell culture and adipocyte differentiation

To achieve standard adipocyte differentiation, the 3T3-L1 cells were grown to confluence in growth medium (DMEM + 10 % FBS). Two days post-confluence, preadipocytes were induced with differentiation media (DMEM supplemented + 10 % FBS + 1 μM dexamethasone (DEX) + 0.5 mM methyl isobutyl xanthine (MIX), + 1 $\mu\text{g}/\text{mL}$

insulin). After two days, the culture medium was switched to fresh maintenance medium (DMEM + 10 % FBS + 5 µg/mL insulin) and was subsequently changed to DMEM supplemented with 10 % FBS (following two days of incubation), which was further replaced every two days until the analysis was conducted on days 6 and 8. Two different concentrations of Porelis 100 and 200 µg/mL were added to the medium on Day 4 during 8-day differentiation.

Oil red O staining

Intracellular lipid accumulation was quantified with Oil Red O staining method [13]. Briefly, 3T3-L1 cells (0.2×10^6 /well) cultured in 6-well plates, were grown as described in adipocyte differentiation for 8 days. On day 8, the cells were washed with PBS, fixed with 10 % formaldehyde, and then stained for 1 h with a filtered solution of 60 % Oil Red O in 100 % aqueous 2-isopropanol. To quantify intracellular lipids, stained lipid droplets were dissolved in isopropanol (3 mL per well). The extracted dye was transferred into a 96-well plate and the absorbance was read with multimode microplate reader (Tecan Infinite) at 500 nm.

Western blotting

3T3-L1 adipocytes were collected and lysed with RIPA lysis buffer for 30 minutes. Bradford reagent was used to determine the protein concentrations. Equal amounts of protein from each sample were loaded and separated on a 10 % SDS-PAGE. Following their electrophoretic separation, proteins were transferred to a nitrocellulose membrane using a semi-dry transfer and blocked with 5 % skim milk for 1 h at room temperature. The membranes were incubated (4 °C, overnight) with primary antibodies – PPAR γ (sc-7273) and SREBP-1 (sc-365513) from Santa Cruz Biotechnology – followed by incubation with anti-rabbit or anti-mouse secondary antibodies (Santa Cruz Biotechnology) and protein bands were

visualized using an enhanced chemiluminescence system Image quant (GE).

Determination of mRNA expression levels

Total RNA was isolated from adipocytes using TRI-Reagent (Sigma–Aldrich) according to manufacturer's instructions. cDNA synthesis was performed with 1 µg of total RNA using cDNA synthesis kit (Bio-Rad) in accordance with the manufacturer's directions. Quantification of gene expression in Porelis-treated differentiated adipocytes was determined using a CFX96 Touch™ Real-Time PCR Detection system (Bio-Rad Laboratories). Primers used for amplification of cDNAs are shown in Table 1. The reaction mixtures were incubated for an initial denaturation at 94 °C for 10 min, followed by 40 PCR cycles: 40 s at 95 °C, 30 s at 59 °C and 30 s at 72 °C. The Relative quantification of gene expression was evaluated using the $2^{-\Delta\Delta Cq}$ method. All reactions were performed in triplicate.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 10.2.0 software. The treatment effect was determined using one-way ANOVA followed by Tukey's multiple comparison test. Data are presented as mean \pm standard error of the mean (SEM). Statistical significance was established at $**p < 0.001$ and $***p < 0.0001$.

RESULTS

Effect of Porelis on cell viability

To evaluate the effect of Porelis on the viability of RAW 264.7 macrophages, an MTT assay was performed and the results are shown in Figure 1 A. Porelis had no cytotoxic effect at a concentration of up to 300 µg/mL, while at higher concentrations of 400 and 500 µg/mL, cell viability of RAW 264.7 macrophages reduced to 91.69 and 82.72 %, respectively.

Table 1: Primers used for amplification of cDNAs

| Gene | Sequence |
|--------------------------------------------------|-------------------------------------------------------------------------------|
| Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) | Forward: 5' -ATGACATCAAGAAGGTGGTG-3' Reverse: 5' -CAT ACCAGGAAATGAGCTTG-3' |
| Leptin | Forward: 5'-TGAGTTTGTCCAAGATGGACC-3' Reverse: 5'-GCCATCCAGGCTCTCTGG-3' |
| FAS | Forward: 5'-AGACCCGAACTCCAAGTTATTC-3' Reverse: 5'-GCAGCTCCTTGATACTTCTCC-3' |

Therefore, the concentration range of 100 – 200 µg/mL was chosen for further experiments.

Effect of porelis on LPS-induced nitric oxide (NO) production

Nitric oxide (NO) is an established indicator of inflammation in LPS-stimulated macrophages. Figure 1 B shows that when RAW 264.7 macrophages were treated with LPS (5µg/mL), the amount of NO produced rose from 1.31 µmol/L to 59.44 µmol/L. Interestingly, Porelis pre-treatment exhibited a dose-dependent reduction in the elevated NO production. Nitric oxide production in the cell culture supernatant was significantly reduced to 29.56 µmol/L at the concentration of 100 µg/mL of Porelis.

Impact of Porelis on cytokine levels in LPS-induced RAW264.7 cells

When inflammation occurs, LPS-stimulated macrophages release a significant amount of pro-inflammatory cytokines that contribute to inflammation. As shown in Figures 1 C and D, LPS stimulation in RAW264.7 cells significantly increased levels of TNF-α and IL-6. Interestingly, pre-treatment of two different concentrations (100 and 200 µg/mL) of Porelis, dose-dependently inhibited the release of TNF-α and IL-6.

Effect of Porelis on cell viability of 3T3L-1 adipocytes

As shown in Figure 2 A, 3T3L-1 adipocytes were stimulated to differentiate via treatment with different concentrations of Porelis (100 – 500 µg/mL). The MTT assay revealed that Porelis up to 200 µg/mL did not cause any cytotoxic effect on cells, while at higher concentrations, the viability of cells reduced significantly. Therefore, the concentration range of 50 – 100 µg/mL was selected for further experiments.

Effect of Porelis on accumulation of lipid droplets in mature adipocytes

To investigate the effect of Porelis on adipocyte differentiation, accumulation of intracellular lipids during the 8-day adipocyte differentiation process was examined by microscopic examination followed by Oil Red O staining in differentiated 3T3-L1 adipocytes. As shown in Figures 2 B and C, treatment with 50 and 100 µg/mL of Porelis inhibited lipid content in 3T3-L1 adipocytes by 33 and 44 %, respectively compared to control.

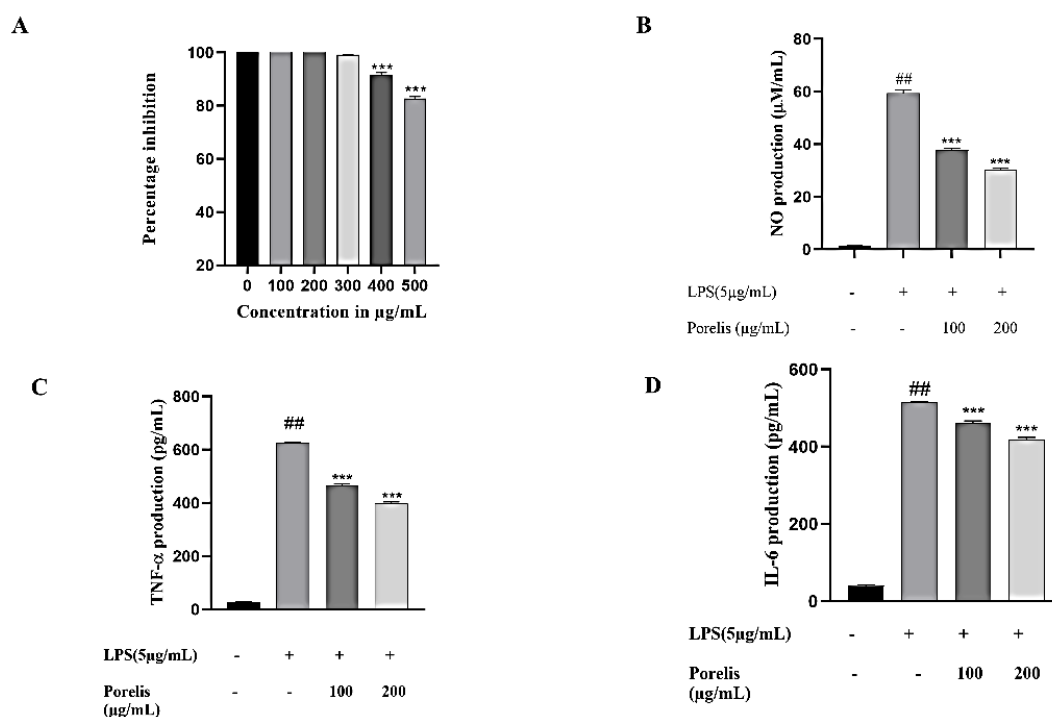


Figure 1: Effect of Porelis on cell viability of RAW 264.7 cells (A); Nitric oxide production level (B); and cytokine levels of TNF-α and IL-6 (C & D) in LPS-stimulated RAW 264.7 cells. ##*P* < 0.0001 vs untreated control; ****p* < 0.0001 vs LPS treated cells

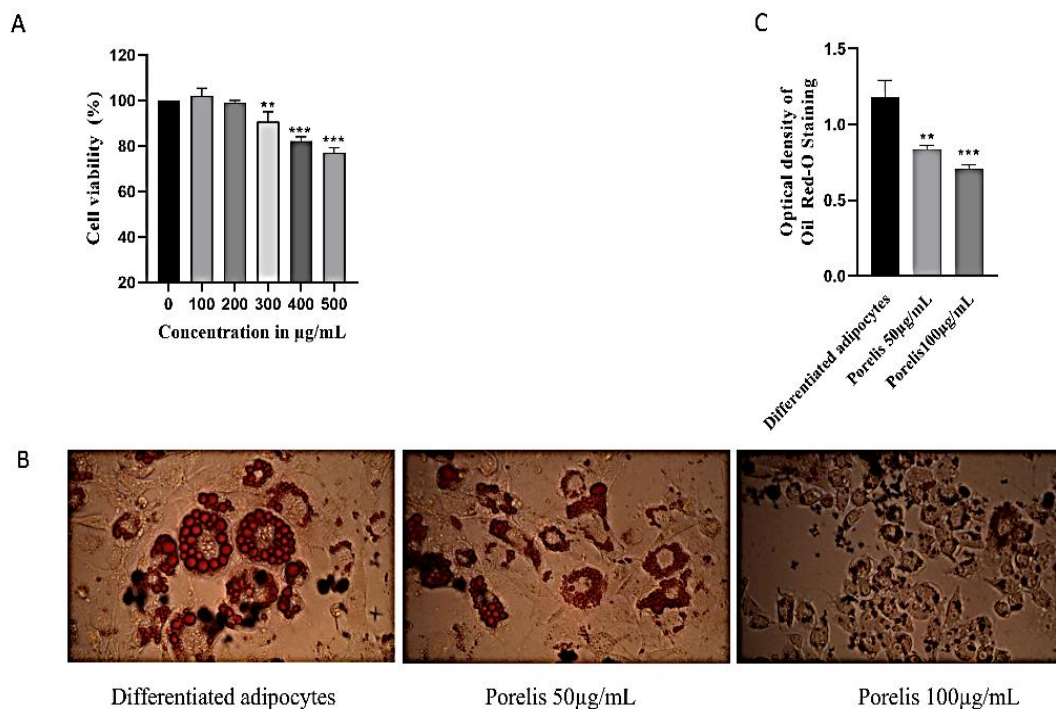


Figure 2: Effect of Porelis on the cell viability of differentiated 3T3L cells (A); Intracellular lipids were stained with Oil Red O staining (B); Absorbance was spectrophotometrically determined at 500 nm after Oil red O staining (C). ** $P < 0.01$ and *** $p < 0.001$ vs differentiated adipocytes

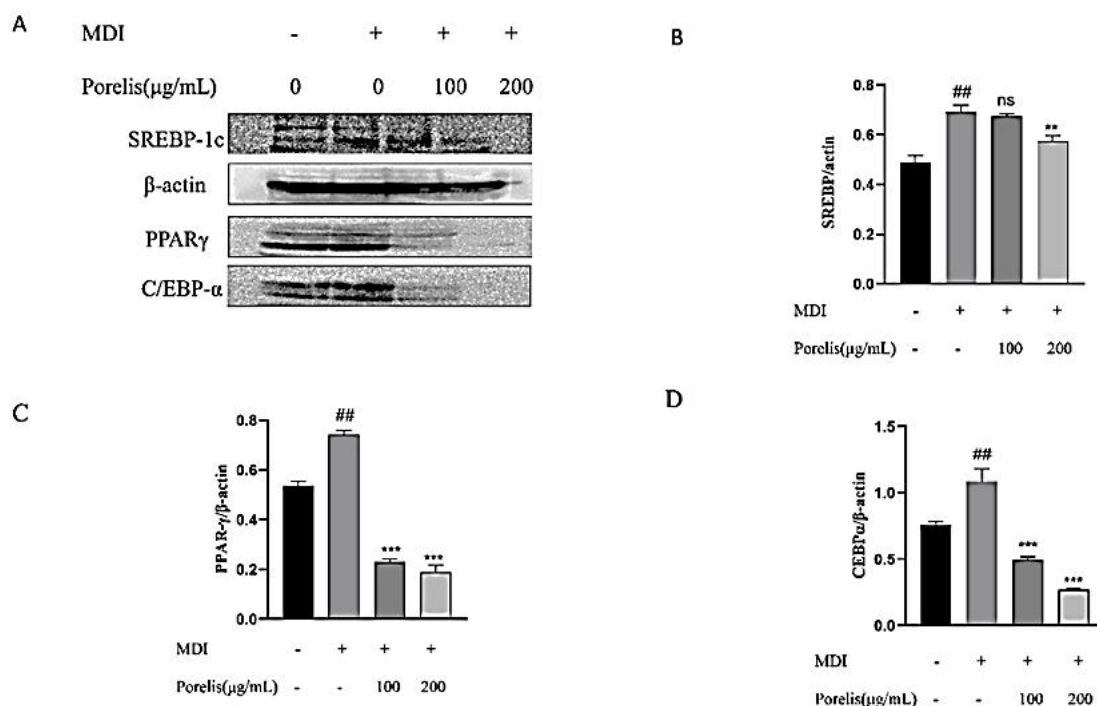


Figure 3: Effect of Porelis on protein expression levels of PPARγ, C/EBPα and SREBP (A). Densitometry analysis of SREBP (B), PPARγ (C) and C/EBPα (D). ## $P < 0.001$ vs preadipocyte cells. ## $P < 0.01$ and *** $p < 0.001$ vs differentiated adipocytes

Effect of Porelis on protein expression levels of adipogenesis-related factors

Differentiation of pre-adipocyte into mature adipocyte is tightly regulated by sequential activation of several transcriptional factors, including SREBP-1c, PPAR- γ and C/EBP- α .

Following treatment of differentiated adipocytes with Porelis (50 and 100 $\mu\text{g}/\text{mL}$), protein expression levels of SREBP-1c, PPAR- γ and C/EBP- α were significantly decreased (Figures 3 A – D) when compared with fully differentiated mature adipocytes.

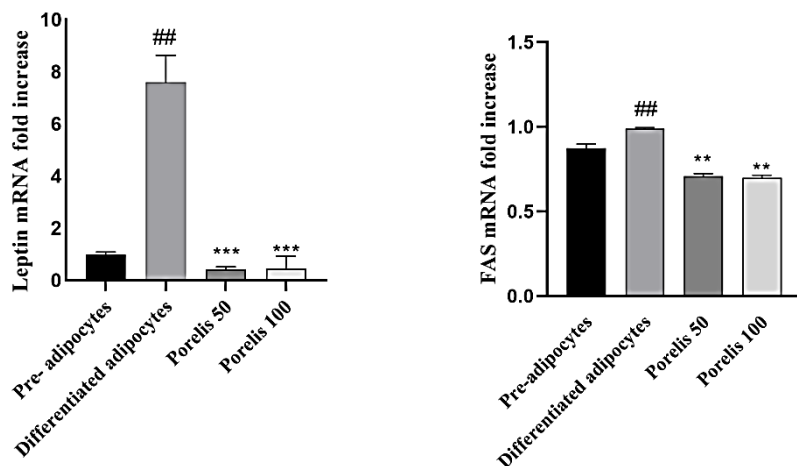


Figure 4: mRNA expression of Leptin, and adipogenic enzyme FAS has been analysed. ## $P < 0.001$ vs preadipocyte cells. ** $P < 0.001$ and *** $p < 0.0001$ vs differentiated adipocytes

Effect of Porelis on mRNA expression of mature adipocytes

As shown in Figures 4 A and B, differentiated adipocytes exhibited a significant increase in mRNA expression of leptin and FAS by 8 folds and 1.5 folds, respectively, compared to preadipocytes ($p < 0.001$). Interestingly, differentiated adipocytes' mRNA expression of Leptin and FAs was reduced significantly by treatment with 50 and 100 $\mu\text{g}/\text{mL}$ of Porelis ($p < 0.0001$).

DISCUSSION

Standardized plant extracts provide a comprehensive approach to health by utilizing traditional herbal knowledge to treat various health issues. They offer a sustainable, affordable and natural substitute for synthetic medications. Plant-based molecules have played a crucial role in developing numerous lifesaving drugs. Purple tea, derived from the *Camellia sinensis* plant, is rich in phytochemicals, particularly anthocyanins, which are responsible for its distinct purple colour. The biological activities of *Camellia sinensis* have not been thoroughly demonstrated, apart from a few numbers of bioactivities, such as antioxidant and anti-obesity and other biological properties [14,15]. The current study evaluated the potential pleiotropic effects of Porelis, a standardized purple tea extract.

Lipopolysaccharide (LPS) is an endotoxin and a component of Gram-negative bacteria. It is a potent stimulator of strong inflammatory response by activating pro-inflammatory mediators and cytokines such as NO, TNF- and IL-6, and through signal transduction via toll-like receptor 4. Nitric oxide (NO), TNF- α and IL-6 are mainly produced by macrophages and are involved in the acute phase of inflammation by recruitment of neutrophils and activation of arachidonic acid metabolism [16,17]. In order to verify the beneficial effect, LPS was used to induce inflammatory response in RAW264.7 macrophages and concurrently administered Porelis. It was found that Porelis was able to considerably reduce the levels of NO, IL-6 and TNF- α in cell culture supernatants. This protection against inflammatory response might be due to presence of polyphenolic compounds including GHG in Porelis. Results from this study are consistent with previous studies [18,19].

Adipogenesis has gained attention in recent years as a key player in the pathophysiology of obesity and its comorbidities. Preadipocytes undergo a complicated, multi-step process called adipogenesis to develop into mature, lipid-containing adipocytes. Transient high expression of CCAAT/enhancer-binding proteins (C/EBP), C/EBP δ and C/EBP β takes place in the early phases of development. Key transcription factors of adipogenesis, C/EBP β/δ and peroxisome

proliferator-activated receptor γ (PPAR γ), are stimulated during the intermediate stage of adipogenesis. In the final stage of differentiation, PPAR γ and C/EBP α work together to promote differentiation and induce a few adipocyte-specific genes, including perilipin, fatty acid synthase (FAS), adipocyte protein 2 (aP2) and lipoprotein lipase (LPL). One of the most widely utilized *in vitro* models for this study is the differentiation of the 3T3-L1 preadipocyte fibroblast clonal cell line into mature fat cells [20,21]. PPAR γ plays crucial role in adipogenesis and PPAR γ has been a critical target in developing anti-obesity drugs. Drugs that target peroxisome proliferator-activated receptor- γ have been shown to possess anti-inflammatory effects as well. The results of this study indicate that adipocytes treated with Porelis could lower lipid droplet accumulation and significantly inhibit protein expression of PPAR γ , C/EBP α and SREBP1 along with leptin and FAS mRNA expression compared to untreated adipocytes. These results are consistent with previously reported studies [22,23].

CONCLUSION

Through a putative anti-adipogenesis mechanism, Porelis, a standardized extract of *Camelina sinensis* inhibits lipid accumulation and preadipocyte differentiation in 3T3-L1 without affecting their viability. Porelis is a promising dietary supplement for inflammation and anti-obesity.

DECLARATIONS

Acknowledgement/Funding

The authors express their gratitude to the Department of Phytochemistry and Analytical Development Laboratory at Vidya Herbs Pvt Ltd in Bangalore, India, specifically to Mr. Chandrappa S, Dr Deepak and Dr Vedamuthy BM. We also thank the members of our lab for their insightful recommendations. Financial support was provided by Vidya Herbs Pvt Ltd, Bangalore. India.

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Kuluvar Gouthamchandra and Heggar Venkataramana Sudeep drafted the original manuscript. Bhoomika Ranganath Amritharaj, Lingaraju Harakanahalli Basavegowda, TP Prasanna Kumara and Shyam Prasad Kodimule produced the data and prepared Figures 1 – 4. Kuluvar Gouthamchandra and Shyam Prasad Kodimule designed and analyzed the experiment and results. All authors read and approved the final draft of the manuscript for publication.

Open Access

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

REFERENCES

1. Gregg EW, Shaw JE. Global health effects of overweight and obesity. *N Engl J Med* 2017; 377(1): 80-81 DOI: <https://doi.org/10.1056/NEJMe1706095>
2. Piché ME, Tchernof A, Després JP. Obesity phenotypes, diabetes, and cardiovascular diseases. *Circ Res* 2020; 126(11): 1477-1500. <https://doi.org/doi:10.1161/CIRCRESAHA.120.316101>
3. Ellulu MS, Patimah I, Khaza'ai H, Rahmat A, Abed Y. Obesity and inflammation: the linking mechanism and the complications. *Arch Med Sci* 2017; 13(4): 851-863. <https://10.5114/aoms.2016.58928>.
4. Varra FN, Varras M, Varra VK, Theodosios-Nobelos P. Molecular and pathophysiological relationship between obesity and chronic inflammation in the manifestation of metabolic dysfunctions and their inflammation mediating treatment options (Review). *Mol Med Rep* 2024; 29(6): 95. <https://10.3892/mmr.2024.13219>.
5. Stępień M, Stępień A, Wlazłel RN, Paradowski M, Banach M, Rysz J. Obesity indices and inflammatory markers in obese non-diabetic normo- and hypertensive patients: a

- comparative pilot study. *Lipids Health Dis* 2014; 8; 13: 29. <https://doi.org/10.1186/1476-511X-13-29>
6. Fernyhough M, Bucci L, Hausman G, Antonio J, Vierck J, Dodson M. Gaining a solid grip on adipogenesis. *Tissue Cell* 2005; 37: 335–338. <https://doi.org/10.1016/j.tice.2005.03.007>
 7. Rayalam S, Della-Fera M, Baile C. Phytochemicals, and regulation of the adipocyte life cycle. *J Nutr Biochem* 2008; 19, 717–726. <https://doi.org/10.1016/j.jnutbio.2007.12.007>
 8. Boonsong T, Pakwan S, Chawnawa W. Anti-adipogenesis and antioxidative defense status of a crude extract of 'Kalasin 2' peanut sprouts in 3T3-L1 mouse adipocytes. *Biochem Cell Biol* 2019; 97(6): 740–749. <https://doi.org/10.1139/bcb-2018-0391>
 9. Lim SH, Lee HS, Han HK, Choi CI. Saikosaponin A and D inhibit adipogenesis via the AMPK and MAPK Signaling Pathways in 3T3-L1 Adipocytes. *Int J Mol Sci* 2021; 22(21): 11409, <https://doi.org/10.3390/ijms222111409>
 10. Guo L, Park SY, Kang HM, Kang NJ, Hwang DY, Choi YW. Edible vital melon fruit extract inhibits adipogenesis and ameliorates high-fat diet-induced obesity. *Biomed Res Int* 2022; 22: 2369650. <https://doi.org/10.1155/2022/2369650>
 11. Lin YC, Lu HF, Chen JC, Huang HC, Chen YH, Su YS, Tung CY, Huang C. Purple-leaf tea (*Camellia sinensis* L.) ameliorates high-fat diet-induced obesity and metabolic disorder through the modulation of the gut microbiota in mice. *BMC Complement Med Ther* 2020; 20(1): 376. <https://doi.org/10.1186/s12906-020-03171-4>
 12. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983; 65(1-2): 55-63. [https://doi.org/10.1016/0022-1759\(83\)90303-4](https://doi.org/10.1016/0022-1759(83)90303-4)
 13. Ramírez-Zacarias JL, Castro-Muñozledo F, Kuri-Harcuch W. Quantitation of adipose conversion and triglycerides by staining intracytoplasmic lipids with Oil red O. *Histochem* 1992; 97(6): 493-497. <https://doi.org/10.1007/BF00316069>
 14. de Moura C, Kabbas Junior T, Pedreira FRO, Azevedo L, Furtado MM, Sant'Ana AS, Franchin M, Gonzaga VR, Cui Y, Wen M, et al. Purple tea (*Camellia sinensis* Var. Assamica) leaves as a potential functional ingredient: From extraction of phenolic compounds to cell-based antioxidant/biological activities. *Food Chem Toxicol* 2022; 159: 112668. <https://doi.org/10.1016/j.fct.2021.112668>
 15. Shimoda H, Hitoe S, Nakamura S, Matsuda H. Purple tea and its extract suppress diet-induced fat accumulation in mice and human subjects by inhibiting fat absorption and enhancing hepatic carnitine palmitoyl transferase expression. *Int J Biomed Sci* 2015; 11(2): 67-75.
 16. Wynn TA, Chawla A, Pollard JW. Macrophage biology in development, homeostasis and disease. *Nature* 2013; 496: 445–455. <https://doi.org/10.1038/nature12034>
 17. Krausgruber T, Blazek K, Smallie T, Alzabin S, Lockstone H, Sahgal N, Hussell T, Feldmann M, Udalova IA. IRF5 promotes inflammatory macrophage polarization and TH1-TH17 responses. *Nat Immunol* 2011; 12: 231–238. <https://doi.org/10.1038/ni.1990>
 18. Suriyaprom S, Srisai P, Intachaisri V, Kaewkod T, Pekkoh J, Desvaux M, Tragoolpua Y. Antioxidant and anti-inflammatory activity on LPS-stimulated RAW 264.7 macrophage cells of white mulberry (*Morus alba* L.) leaf extracts. *Molecules* 2023; 28(11): 4395. <https://doi.org/10.3390/molecules28114395>
 19. Nguyen TQC, Duy Binh T, Pham TLA, Nguyen YDH, Thi Xuan Trang D, Nguyen TT, Kanaori K, Kamei K. Anti-inflammatory effects of *Lasia spinosa* leaf extract in lipopolysaccharide-induced RAW 264.7 macrophages. *Int J Mol Sci* 2020; 21(10): 3439. 2020. <https://doi.org/10.3390/ijms21103439>
 20. Fajas L, Schoonjans K, Gelman L, Kim JB, Najib J, Martin G, Fruchart JC, Briggs M, Spiegelman BM, Auwerx J. Regulation of peroxisome proliferator-activated receptor gamma expression by adipocyte differentiation and determination factor 1/sterol regulatory element binding protein 1: implications for adipocyte differentiation and metabolism. *Mol Cell Biol* 1999; 19(8): 5495-503. <https://doi.org/10.1128/MCB.19.8.5495>
 21. Rosen ED, Walkey CJ, Puigserver P, Spiegelman BM. Transcriptional regulation of adipogenesis. *Genes Dev* 2000; 14: 1293–1307.
 22. Jakab J, Miškić B, Mikšić Š, Juranić B, Čosić V, Schwarz D, Včev A. Adipogenesis as a potential anti-obesity target: a review of pharmacological treatment and natural products. *Diabetes Metab Syndr Obes* 2021; 14: 67-83. <https://doi.org/10.2147/DMSO.S281186>
 23. Hengpratom T, Ngernsoungnern A, Ngernsoungnern P, Lowe GM, Eumkeb G. Antiadipogenesis of *Oroxylum indicum* (L.) Kurz Extract via PPARγ2 in 3T3-L1 adipocytes. *Evid Based Complement Alternat Med* 2020; 5: 2020. <https://doi.org/10.1155/2020/6720205>