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In vitro: The Modulating Effect of Myricetin on the Atherosclerosis Related Processes in THP1 Macrophages

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Authors' contributions

This work was carried out in collaboration among all authors. Author RFA validated the study, performed the statistical analysis, wrote the original, draft of the manuscript. Author EAH conceptualization, methodology, and supervision of the study. Authors SJA and SYS investigated the analyses of the study, and author KG managed the review and editing of manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To assess the anti-atherosclerotic effects of Myricetin (pharmaceutical) in human THP-1 macrophages following IFN-γ or MCP-1 stimulation.
Study Design: The protective effects of myricetin against atherosclerosis was evaluated using the

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humanTHP-1 macrophages and studying the following parameters namely, cell viability, cell proliferation, cell migration, inflammation related gene expression and cholesterol efflux *in vitro.*

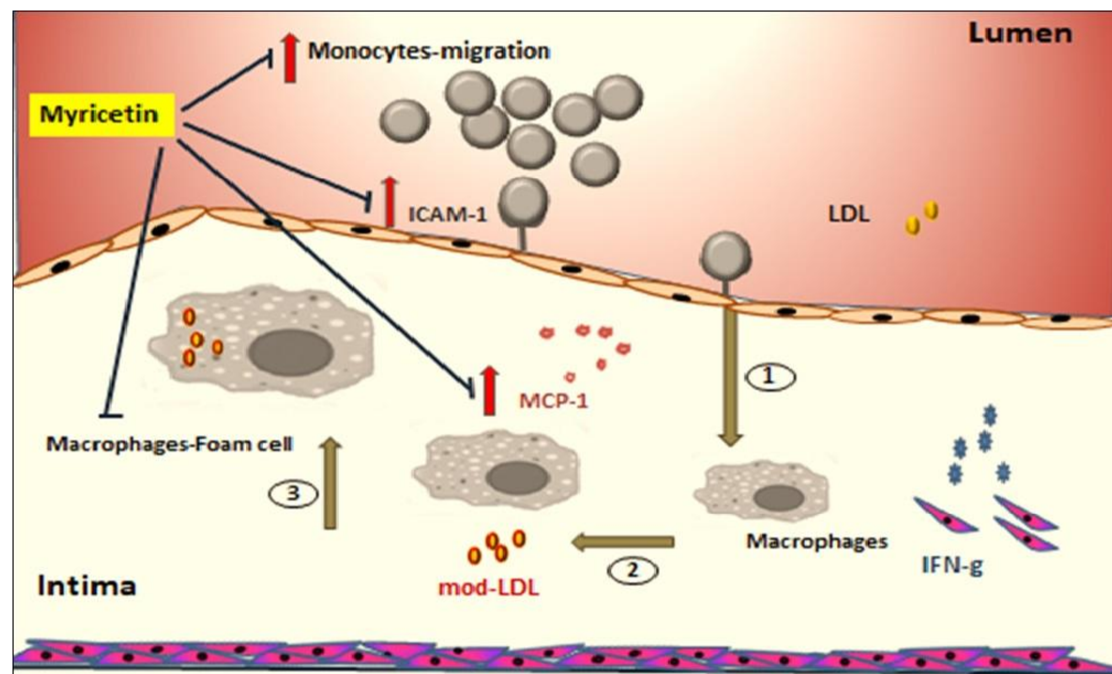
Place and Duration of Study: THP-1 cell line: Department of biochemistry (faculty of science), Cell Culture Unit, Experimental Biochemistry Unit (King Fahad Medical Research Centre), King Abdul Aziz University, between September 2019 and September 2020.

Methodology: The THP-1 cell lines were differentiated into macrophages by incubation with PMA (160 nM) for 24 hours. The viability percentage was determined using Pierce LDH cytotoxicity assay kit, the percentage change in macrophages proliferation was evaluated by crystal violet dye, the RNA was extracted then the cDNA was synthesized and the quantitative real time polymerase chain reaction (qRT-PCR) was done for inflammation-related genes, ICAM-1 and MCP-1. The percentage of monocyte migration and cholesterol efflux were also calculated.

Results: Cytotoxicity assays demonstrated no significant toxicity with myricetin at 25μM and 50 μM concentrations on THP-1 macrophages. The quantitative real-time RT-PCR (qRT-PCR) demonstrated a significant increase in interferon gamma (IFN-γ) mediated expression of both intercellular adhesion molecule (ICAM-1) and monocyte chemo-attractant protein-1 (MCP-1) by 2.1 and 7.1 fold respectively, compared to the control. Treatment with myricetin (25 μM and 50 μM) significantly inhibited the IFN-γ induced overexpression of ICAM-1 by 42.86% & 71.34% and MCP-1 by 53.52% & 87.32% respectively. Myricetin (25 μM) significantly reduced the migration of monocytes by 33.66% compared to MCP-1. The cholesterol efflux from THP-1 macrophages treated with myricetin was significantly increased by 47% and 57% in the absence and presence of IFN-γ, respectively compared to the control.

Conclusion: Myricetin has anti-inflammatory effects and supports cholesterol efflux, which can help in prevention of atherosclerosis. Furthermore, myricetin did not exhibit any cytotoxic effects and therefore is a safe phytochemical which can complement conventional therapeutics.

Graphical Abstract



Keywords: Cell migration; cholesterol efflux; gene expression; macrophages; myricetin.

1. INTRODUCTION

The cardiovascular disease appears to be the world-leading cause of morbidity and death. The World Health Organization, reports that 17.9 million people die of CVD every year that accounts for approximately 31% of the world's deaths. By 2030, the mortality due to CVD is predicted to increase to 23.6 million globally [1]. In recent years there is an increase in the

incidence of CVD in the Kingdom of Saudi Arabia [2]. This is largely attributed to the increased risk of dyslipidemia resulting from dietary and sedentary lifestyle [3]. Atherosclerosis is the major reason for all cardiovascular diseases such as stroke, heart attacks and, congestive heart failure. It is defined as a chronic inflammatory condition marked by the development of arterial sclerosis and the release of inflammatory substances secreted by macrophages. Macrophages via its secretions orchestrate the immune response in atherosclerosis [4].

The onset of the atherosclerosis disease pathology develops after dysfunction of the blood vessels and possible elevated plasma low-density lipoprotein (LDL) concentration. Cytokines play a significant role in all phases of atherosclerosis, from initial monocytes recruitment to advanced plaque formation and rupture. Macrophages and T-cells secrete one of the key cytokines namely, interferon gamma (IFN-γ), that has pleiotropic functions. It is expressed at high levels in atherosclerotic injuries together with another inflammation related cytokine namely, tumor necrosis factor (TNF)-α [5]. These cytokines are attracted to the area of lipid aggregation in response to the chemokine monocyte chemo-attractant protein-1 (MCP-1) [6]. The interaction between immune cells and the lipids deposits in the intima of the arteries later leads to generation of abnormal plaques [7]. IFN-γ additionally promotes the expression of adhesion particles like intracellular adhesion molecule-1 (ICAM-1) [8]. The monocytes become tethered to ICAM-1 and penetrate the intima of the blood vessel in response to the inflammatory stimuli from the lining of the vessel wall.

Currently lipid lowering agents are widely used in the management of atherosclerosis, however not all patients respond to the therapy. Moreover, the associated side-effects with various anti-lipidemic agents had prompted research into natural plant-based products which have anti-inflammatory/anti-atherosclerotic properties [9]. Recently, several studies have identified that nutraceuticals, phytochemicals and/or its derivatives can promote human health. The foods which are rich in flavonoids have many health advantages, namely antioxidants and anti-inflammatory characteristic [10]. Myricetin (Myr) is hexahydroxy flavone (Fig. 1) and is structurally and functionally similar to fisetin, luteolin, and quercetin [11]. Myricetin it is extracted from most vegetables including hot peppers, onions and spinach [12], berries and tea [13]. Interestingly, there is a strong correlation between increased intake of fruits and vegetables and the prevention of CVDs [14].

Based on this study, we assessed the anti-inflammatory and anti-atherosclerotic effects of Myr in human THP-1 macrophages following IFN-γ stimulation or MCP-1 stimulation by studying the following parameters, namely cell viability, cell proliferation, real-time gene expression, monocyte migration and cholesterol efflux *in-vitro.*

2. MATERIALS AND METHODS

2.1 Cell Culture and Chemical Reagents

The THP-1 human monocyte cell line (TIB202) was a kind gift from the Molecular Biomedicine Unit at the King Faisal specialist Hospital & Research Centre (KFSH&RC, Riyadh, KSA). The THP-1 cell line was cultured using RPMI-1640 medium (A1049101) supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS, A3840002), 2 mmol/l L-glutamine 200 mM (25030081) and 1 % antibiotic solution (15140122, Penicillin-streptomycin) which were obtained from (Invitrogen, Waltham, Massachusetts, USA) and incubated at 37°C in a humidified incubator containing 5% CO_2. Differentiation of THP-1 monocytes into macrophages was achieved by incubation with 160 nM phorbol-12-myristate-13-acetate (PMA, J63916, Alfa Aesar, Melbourne, Victoria, Australia) [15].

Myricetin was purchased from Sigma-Aldrich Company (70050, Gillingham, UK). Ten milligrams of Myr dissolved in 3 ml dimethyl sulfoxide (DMSO, D12345, Invitrogen, Waltham, Massachusetts, USA) as main stock (10 mM) and the required working concentrations were freshly prepared from 1mM sub-stock of Myr.

2.2 Cell Viability Assay

Preliminary evaluations of THP-1 macrophages viability and proliferation were performed with various concentrations of myricetin (10, 25, 50, 100, 200) µM for 24 hours (data not included) to select the optimal concentrations for subsequent experiments. Two concentrations of Myr namely, 25 µM and 50 µM were selected to evaluate viability of THP-1 macrophages with or without IFN-γ (250 U/ml) [15] (13265, Sigma-Aldrich,

Gillingham, UK). The lactate dehydrogenase (LDH) enzyme which is released following cell membrane injury and hence reflects the cell viability was determined using Pierce LDH cytotoxicity assay kit (8895, Thermo Fisher, Waltham, Massachusetts, USA). Briefly, THP-1 monocytes were seeded at a density of 4.11 x 10^5 cells/cm^2 in 96-well plate with total volume 100 µl of complete media and differentiated into macrophages by incubation with PMA (160 nM) for 24 hours. To induce foam cell genesis, IFN-γ (250 U/ml) was added for three hours to one set of the samples and vehicle in the other. The differentiated cells were eventually treated with Myr (25 µM and 50 µM) and incubated in the 5% CO_2 incubator for another 24 hours. The cell supernatant 100 µl was pipetted into a new 96-well plate and 100 µl of assay buffer was added and incubated at 37 ^{0}C for 30 minutes. Then 100 µl of stop solution was added and the absorbance at 490 nm with a background wavelength of 680 nm was determined using the Bio-Tek micro plate reader (Winooski, VT, USA). Results of LDH release were computed as % change of viability compared to vehicle control. The adherent macrophages were used parallelly for the proliferation assay described below.

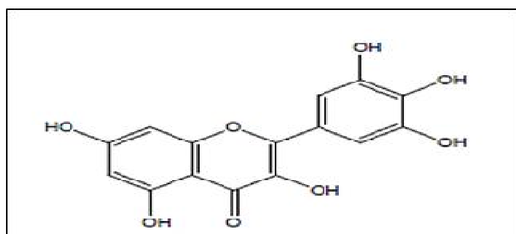


Fig. 1. Chemical structure of myricetin

2.3 Cell Proliferation Assay

The crystal violet dye binds to the deoxyribonucleic acid in viable cells, and hence used as a proliferation assay. The adherent macrophages that remained from the above experimental plates following utilization of the cell supernatant for the LDH assay were used for the cell proliferation assay. Briefly, 100 µl of crystal violet (0.2% (w/v) in 10% ethanol) was added to each well and incubated at room temperature for 5 minutes. The dye was then removed and washed thrice with 100 µl phosphate buffered saline (PBS, 10010015, Invitrogen, Waltham, Massachusetts, USA. Then 100 µl of monosodium phosphate buffer (0.1M NaH_2PO_4 in 50% (w/v) ethanol) was added to each well and the plate was agitated for 5 minutes on a shaker at room temperature (RT). The absorbance at 570 nm was determined using the Bio-Tek microplate reader. The results were represented as cell proliferation percentage compared to the vehicle control.

2.4 RNA Extraction, Reverse Transcription, Quantitative PCR

THP-1 monocytes were seeded in a six-well plat at a density of (1 x 10^6) cells/well in 3 ml total volume of culture medium and differentiated into macrophages as previously described. The cells were treated with IFN-γ (250 U/ml) for 3 hours to induce the target genes. Then myricetin (25 µM and 50 µM) were added and the macrophages were incubated in a 5% CO_2 incubator for 24 hours, before RNA extraction using RNeasy mini kit (74104, Qiagen, Hilden, Germany) according to the manufacturer protocol. The RNA quality and quantity were determined using (NanoDrop, Technologies, Wilmington, DW, USA). The cDNA was then synthesized with random hexamers using ImProm-II reverse transcription system kit (A3800, Promega, Madison, WI, USA). The following genes related to inflammation namely, ICAM-1 and MCP-1 were analyzed. GAPDH was used as the internal control. The primers were obtained from earlier published work [15] and the quantitative real time polymerase chain reaction (qRT-PCR) was done using with SYBRGreen master mix (204054, Qiagen, Hilden, Germany) via StepOnePlus real-time PCR system (Applied Biosystems, Beverly, MA, USA) [16]. Relative quantitation was done using comparative Ct ($2^{\wedge-(\Delta\Delta CT)}$) method.

2.5 Migration Assay

Cell migration was performed by seeding the THP-1 monocytes ($5x10^5$cells/ml) in cell culture inserts (8 µm pore size). Serum free media (0.5 ml) was added in the upper chamber of Falcon 12-well companion plates (SPL Insert hanging, 35224, SPL life sciences, Pocheon, Korea) then treated with Myr 25 µM or vehicle (DMSO). The lower chambers of the treatment arms contained the chemokine, MCP-1 (20 ng/ml; SRP3109, Sigma-Aldrich, Gillingham, UK) or Myr 25 µM in 1 ml complete medium and the plate was incubated in a humidified 5% CO_2 incubator for three hours. After incubation, monocytes numbers that migrated to the lower half of the insert and those from the undersurface of the membrane were collected and counted using a hemocytometer. The migration of monocytes in the presence or absence of myricetin was

expressed as percentage increase or decrease compared to control. The MCP-1 percentage was considered attributably as 100 %

2.6 Cholesterol Efflux

Cholesterol efflux assay was performed as published earlier [17] using the Abcam Cholesterol efflux kit (ab196985, Cambridge, UK,) according to the manufacturer's instructions. The assay was used to evaluate the potential of Myr to efflux cholesterol from THP-1 macrophages in presence/absence of IFN-γ (250 U/ml) stimuli. Briefly, the THP-1 monocytes were seeded in a 96-well black plate with clear flat bottom at a density of (4.11×10^5) cells/cm^2 in 100 µl of complete culture medium and incubated for 1 hour to allow the cells to settle down. They were then differentiated into macrophages by overnight incubation with PMA 160 nM in 5% CO_2 incubator. To induce foam cell genesis, IFN-γ (250 U/ml) was added for three hours to one set of the samples and vehicle in the other. Subsequently, the incubated macrophages were washed with serum medium. Then 100 µl of freshly prepared mix of (labeling reagent + equilibration buffer) were added to each well and incubated in 37°C humidified incubator for 16 hours. After overnight incubation, the labeling mix was removed, and macrophages were washed with 200 µl serum free medium. The medium was then removed and Myr 25 µM in 100 µl culture medium was added to each well in both sets of samples and incubated for another 24 hours. The cell supernatant was transferred into a new 96 wells plate and, therefore the adherent monolayer of macrophages was solubilized with 100 µl of cell lysis buffer upon a shaker at room temperature for 30 minutes. The fluorescence (Ex/Em) = 485/515 nm was measured for both plates. The percentage of cholesterol efflux was calculated by dividing (the fluorescence intensity of the medium only) by the (total of fluorescence intensity of medium + cell lysate) then multiplying by 100. The final values were presented as percentage change of vehicle control which attributably presented as equal as 100%.

2.7 Statistics

Statistical analysis was performed using IBM-SPSS version 25. One-way ANOVA with Tukey post hoc analysis was performed for comparison of more than two groups and t-test independent samples was used when two groups were compared. The results were expressed as mean ± standard error of the mean (SEM) from three independent experiments and a *P*-value ≤ 0.05 was considered as statistically significant.

3. RESULTS AND DISCUSSION

3.1 Myricetin has no Significant Cytotoxic Effect on THP-1 Macrophages Viability

The THP-1 macrophages viability was determined by the LDH cytotoxicity assay after 24 hours of incubation with Myr (25 µM and 50 µM). The percentage of viability were (100, 95.09, 95.93, 98.13, 101.45, 101.52) % respectively. The results were showed a mild decrease by 4.91 % and 4.07 % respectively compared to the control. Moreover, the viability of macrophages after stimulated by IFN-γ (250 U/ml) alone showed a mild decrease by 1.87 %. In contrast, the combinations of Myr (25 µM and 50 µM) and IFN-γ (250 U/ml) showed mild increase by 1.45 % and 1.52 % compared to the control. These changes in values observed with myricetin or combinations of myricetin and IFN-γ were not statistically significant (Fig. 2).

3.2 Myricetin Exhibited No Significant Cytotoxic Change on THP-1 Macrophages Proliferation

The crystal violet assay showed negligible changes and the results were (100, 99.78, 92.11, 105.78, 94.89, 94.22) % of THP-1 proliferation. The results were exhibited mild decrease in THP-1 macrophages proliferation with Myr (25 µM and 50 µM) by 0.22 % and 7.89 % respectively compared to vehicle. Moreover, the combination of Myr (25 µM and 50 µM) and IFN-γ (250 U/ml) showed a decrease in proliferation by 5.11 % and 5.78% respectively, compared to the control. Stimulation with IFN-γ (250 U/ml) alone showed a mild increase by 5.78% compared to the control. However, these differences in the proliferation were not statistically significant (Fig. 3).

3.3 Myricetin Decreased MCP-1, ICAM-1 Expression in THP-1 Macrophages Induced by IFN-γ

The inflammation related genes ICAM-1 and MCP-1 demonstrated significant increases in their expression compared to the control, following induction with IFN-γ. The increases in ICAM-1 and MCP-1 were 2.1 fold and 7.1 fold respectively, compared to the control (Fig. 4A,4B) and these increases were highly

significant (*P*=0.005, *P*=0.000). In contrast, the combinations of Myr and IFN- γ showed decreases in the expression of ICAM-1 and MCP-1 compared IFN-γ stimulated group. The decreases observed with combination of Myr (25 µM and 50 µM) and IFN-γ were 1.2, 0.6 fold change in ICAM-1 and 3.3, 0.9 fold change in MCP-1 respectively. These decreases were 42.86 % & 71.34 % with ICAM-1 and 53.52 % & 87.32 % with MCP-1 respectively. These decreases in comparison to IFN-γ were statistically significant with ICAM-1 (*P*=0.035, *P*=0.002) and also with MCP-1 (*P*=0.000) (Fig. 4A,4B)

3.4 Myricetin Inhibited THP-1 Monocytes Migration

THP-1 monocytes demonstrated an increase in migration with both MCP-1 and Myr (25 µM) compared to the vehicle control. The percentages of THP-1 monocytes migration were (100, 66.34) % and these percentages increase by 60.00% and 26.34% respectively. The increase observed with MCP-1 alone was statistically significant (*P*=0.000) compared to the control (Fig. 5). Compared to MCP-1, which is known to have chemotactic potential, Myr showed a decrease by 33.66% and this decrease in value was statistically significant (*P*= 0.000).

3.5 Myricetin Increased Cholesterol Efflux in THP-1 Macrophages

THP-1 macrophages exhibited an increase in the cholesterol efflux percentage in all the treated macrophages when compared to vehicle. The cholesterol efflux results were 100, 138.26, 147.39, 156.52) % respectively. The increase in cholesterol efflux with IFN-γ by 38.26 % was statistically not significant compared to the control. In contrast, the increases in cholesterol efflux following Myr 25 µM and combination of Myr (25 µM) + IFN-γ (250 U/ml) by 47.39 % and 56.52 % respectively, were statistically significant compared to the control (*P*=0.049 and *P*=0.028) (Fig. 6).

4. DISCUSSION

Myricetin is a common flavonoid that has various biological activities as anti-diabetic anti-oxidant, anti-cancer, and anti-inflammatory advantages [13]. In this work, the viability and proliferation of treated THP-1 macrophages were similar to untreated cells which indicated that myricetin had no cytotoxicity. Our present findings were similar to earlier studies in which RAW264.7 macrophages were incubated overnight with various concentrations of Myr up to 200 µM. The EZ-Cytox cell viability assay kit was used and the cytotoxicity of RAW264.7 macrophages was not shown [18]. Houa et al. had also reported that RAW264.7 macrophages exposed to LPS demonstrated no cytotoxicity following MTT assay when pretreated with 25 µM of myricetin [19]. The protective effects of macrophages may be due to their inherent biological properties and may also be attributed to the rich presence of hydroxyl groups in myricetin chemical structure [20]. There are many related advantages in the use of myricetin as anti-atherogenic therapy. It has antidyslipidemic, antioxidant, anti-inflammatory activities which may help in the prevention of hyperlipidemia and related cardiovascular diseases [21,22]

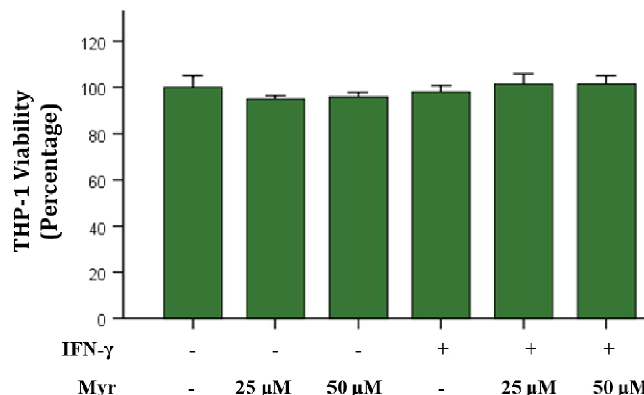


Fig. 2. The effect of myricetin on THP-1 macrophages viability: THP-1 macrophages were treated for 24 h with myricetin (25 µM and 50 µM) with/without IFN γ (250 U/mL) for LDH assay. Results from three independent experiments are presented as mean ± SEM

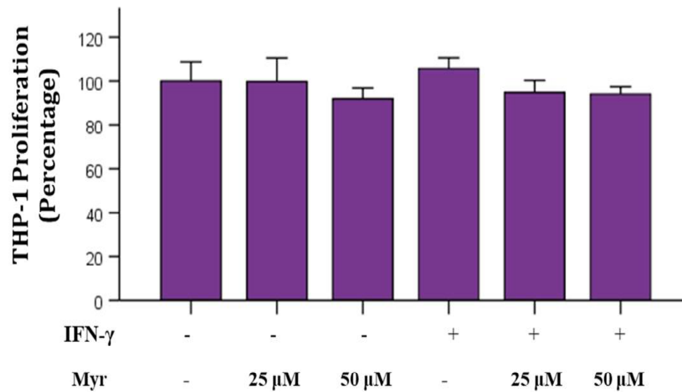


Fig. 3. The effect of myricetin on THP-1 macrophages proliferation: THP-1 macrophages were treated for 24 h with myricetin (25 µM and 50 µM) with/without IFN γ (250 U/mL) for Crystal violet test. Results from three independent experiments are presented as mean ± SEM

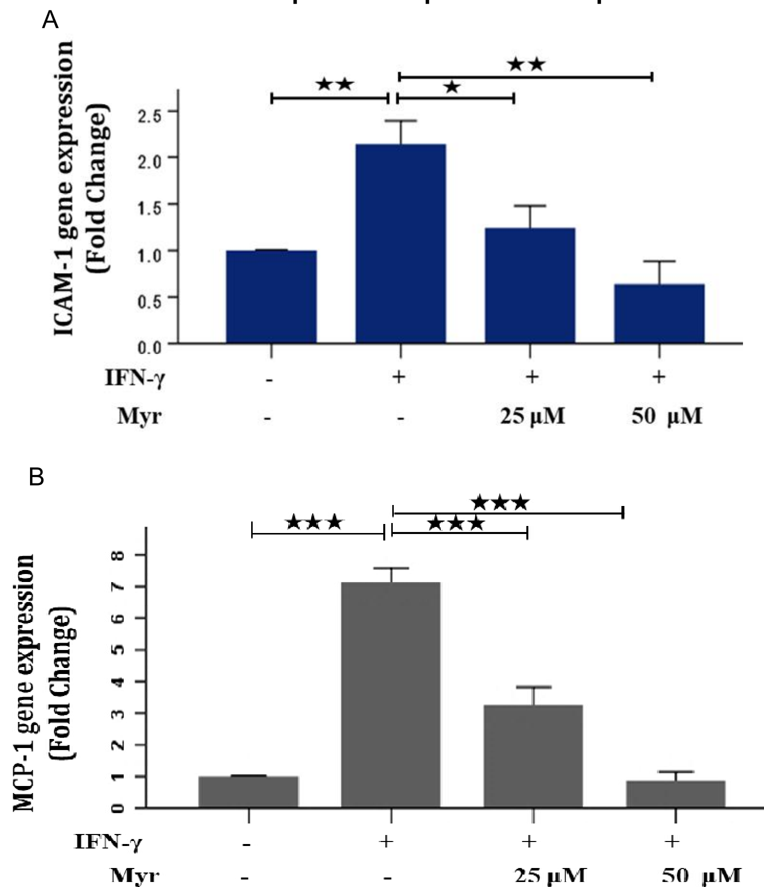


Fig. 4(a) Gene expression analysis of ICAM-1: The gene expression was determined by using real-time RT-PCR. Data analysis and relative quantitation were done using the comparative Ct method (ΔΔ Ct). Results are computed from three independent experiments and compared to vehicle. (b) Gene expression analysis of MCP-1: The gene expression was determined by using real-time RT-PCR. Data analysis and relative quantitation were done using the comparative Ct method (ΔΔ Ct). Results are computed from three independent experiments and compared to vehicle. * indicates statistical significance (*P* ≤ 0.05); ** indicates statistical significance (*P* ≤ 0.01), * indicates statistical significance (*P* ≤ 0.001).**

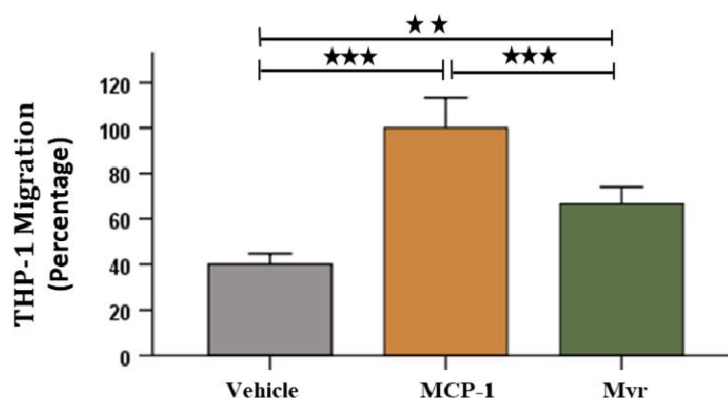


Fig. 5. The effect of myricetin on THP-1 monocytes migration: THP-1 monocytes were evaluated for their migration properties using inserts. The serum-starved THP-macrophages were plated in the upper chamber and treated with either vehicle (DMSO) or myricetin (25 µM) for three hours. The migrated cells were then counted using a hemocytometer. The results from three independent experiments are expressed as mean ± SEM. ** indicates statistical significance (*P* ≤ 0.01); * indicates statistical significance (*P* ≤ 0.001).**

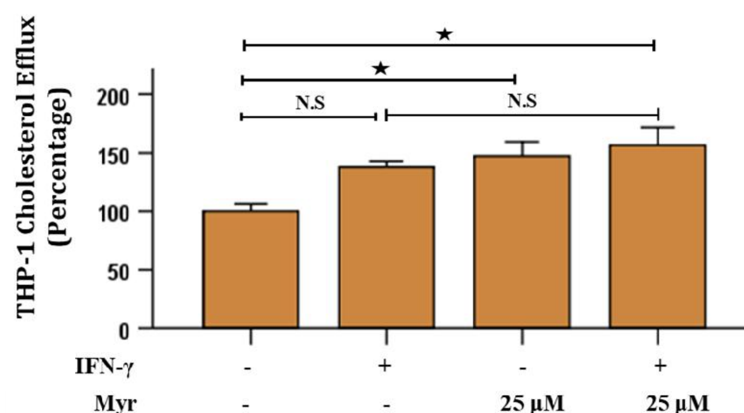


Fig. 6. The Effect of myricetin on cholesterol efflux of THP-1 macrophage: The percentage of cholesterol efflux was significantly increased by 47% and 57% when incubated 24 h with myricetin (25 µM) in the absence/and presence of IFN- γ (250 U/mL). The results from three independent experiments are expressed as Mean ± SEM. * indicates statistical significance (*P* ≤ 0.05).

Macrophages play a considerable function in atherosclerosis initiation and progression [23]. IFN-γ plays a crucial function in the inflammatory process and is modulated by activated macrophages. Upon inflammation, the activated endothelium expresses ICAM-1 on their surfaces which in turn increase the expression of MCP-1. In the present study, both ICAM-1 and MCP-1 gene expressions were increased after IFN-γ stimulation. Increase in MCP-1 expression following IFN-γ stimulation is understood to be mediated through an ERK-dependent pathway [24]. In Wei-Siong et al. [25] study, an ICAM-1 mRNA expression was up-regulated by IFN-γ activation through nuclear factor kappa B (NF- kB) and interferon regulatory factor-1 (IRF-1) expression.

The expression of ICAM and MCP-1 genes showed a decrease with myricetin 25 µM, 50 µM (Fig. 3A, 3B). Inflammation caused by IFN-γ was reported to be suppressed due to the regulatory influence of flavonoids on inflammatory cytokines and chemokines [26]. The inhibition of inflammation related genes as observed in the

present study were in line with an earlier report, where the exposure to myricetin before LPS induction decreased the inflammatory activity in RAW264.7 macrophages [19]. This anti-inflammatory effect may due myricetin capacity to suppress pro-inflammatory mediators' production by inhibiting NF-κB and STAT-1 signals pathways.

Min and others have earlier demonstrated that the secretion and gene expression of many pro-inflammatory molecules are attenuated after being treated with quercetin in PMACI stimulated HMC-1 cells [27]. Monocytes mainly rely on MCP-1 chemokines which induce inflamed arteries in the trafficking of low-density lipoprotein [28]. Monocyte chemoattractant protein-1 (MCP-1) regulates the migration and infiltration of monocytes/macrophages significantly. For cardiovascular diseases, it has been demonstrated that MPC-1 deficiency results in a substantial reduction in arterial lipid deposition [29]. Our study identified that myricetin, inhibited the monocytes recruited towards MCP-1. Therefore, myricetin might help to thwart the chemokine stimulation and migration of monocytes/macrophages towards the inflamed/damaged vessel wall and thus prevent plaque formation. Myricetin possesses both antioxidant and anti-inflammatory functions, and so is a promising drug for CVD prevention and or protection as an alternative therapy [30].

Arterial injury following high LDL cholesterol is the initial stage in the pathogenesis of atherosclerosis, where the LDL diffuses into the intima of the blood vessel and becomes modified and acts as a nidus for plague generation. Secretion and activation of the cytokines and chemokines recruits macrophages, which then engulf modified LDL. We observed that a percentage increase in the cholesterol efflux from THP-1 macrophages stimulated with IFN-γ was non-significant. This is clearly indicating the pro-atherogenic nature of IFN-γ that may promote the expression of lipid uptake related genes [8]. Also, it can reduce LXR expression in THP-1 macrophage-derived foam cells via the JAK/STAT-1 signaling pathway and thereupon reduce expression of ABCA1 and cholesterol efflux [31]. Furthermore, IFN-γ led to increase in the inflammation associated genes ICAM and MCP-1.

IFN-γ was also reported to inhibit the scavenger receptor A, in murine and human macrophages which then is associated with an increase in the levels of esterified cholesterol [32,33]. The cholesterol efflux was significantly increased in THP-1 macrophages treated with myricetin with and without IFN-γ stimulation. Our findings were in line with an earlier study where myricetin and other flavonoids prevented plasma LDL from oxidation and reduced macrophage intake of ox-LDL in U937 differentiated cells [34]. Meng and others [35] also observed that myricetin blocks the buildup of cholesterol in foam cells by reduced CD36 expression. Study by Plourde and others explain that preventing foam cell development can be achieved by the inhibition of the oxLDL binding to SRs through nano-lipoblockers. This blocker work across its anion groups with SR-A collagenous domain [36] and this may clarify the way of myricetin binding throughout the hydroxyl groups. These findings provide a potential mechanism for myricetin which exhibits anti-atherosclerotic, anti-inflammatory and anti-hyperlipidemic effects on THP-1 macrophages

5. CONCLUSION

The present study has identified that myricetin regulates the atherosclerosis key process. It inhibited the gene expression of the inflammatory markers namely MCP-1 and ICAM-1 in THP-1 macrophages activated by interferon gamma which is a well characterized pro-inflammatory cytokine in atherosclerosis. Also, it increased cholesterol efflux which in turn inhibited the foam cell formation during disease onset. Our results exhibited that myricetin did not exhibit any cytotoxicity in THP-1 macrophages and hence is safe. Therefore, it may be useful as a prophylaxis in the prevention and/or treatment of CVD along with conventional therapeutics. Future research should further develop and confirm these initial findings by applying bioinformatics studies and some human *in vivo* study for validating the anti-atherosclerosis effect of myricetin.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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