ijlpr 2021; doi 10.22376/ijpbs/lpr.2021.11.4.L120-126

International Journal of Life science and Pharma Research

Research Article

Biotechnology For Prospective Medical Science

Anti - Inflammatory Activity of Quercetin and Kaempferol Is Limited to Tlr4 Stimulation in Monocytes

Pothuganti Manoj Kumar[1*], Madhuri Padala[1], Sudharshan Reddy Nelli[2] and Surya Satyanarayana Singh[1]

[1]Department of Biochemistry, University College of Science, Osmania University, Hyderabad – 500 007, Telangana, India

[2]Department of Cancer and Translational Research, Dr. D. Y. Patil Biotechnology and Bioinformatics Institute, Dr. D. Y. Patil Vidyapeeth, Pune – 411 033, Maharastra, India

Abstract: Flavonoids are known to exert anti-inflammatory activity but their mechanism at receptor level and their comparative potencies are less studied. Objective of the study is to evaluate, rank order few flavonoids for their cytokine inhibitory activity in monocytes (THP-1) and explore the inhibitory signature among toll-like receptors (TLRs). We tested flavonoids for their potential to inhibit LPS induced cytokines IL-6, TNF alpha in human monocyte cell line THP-1 and mouse macrophage cell line using Enzyme linked immunosorbent assay (ELISA) assays. Quercetin and Kaempferol reduced LPS induced cytokine secretion in monocyte and macrophage cell lines. Quercetin and Kaempferol demonstrated dose dependent inhibition of cytokines IL-6 and TNF alpha. Quercetin demonstrated IC_{50} of 6.9μM whereas Kaempferol demonstrated IC_{50} of 14.3μM in cytokine reduction. In THP-1, NF-kB activity is measured using secreted embryonic alkaline phosphatase (SEAP) reporter with different toll-like receptor activation. TLR4 stimulated NF-kB activity was inhibited by Quercetin and Kaempferol in THP-1 cell line, while these flavonoids did not impact signaling of other TLR ligands Pam3CSK4 (TLR1/2) FLS (TLR2/6), Flagellin (TLR5) and R848 (TLR7/8). I. Quercetin and Kaempferol modulate NF-kB activity when stimulated with TLR4, but not with TLR1/2, TLR2/6, TLR5 and TLR7/8 in Monocytes. Understanding the inhibition profile of flavonoids against Toll receptors will help in design better inhibitor which can alleviate inflammatory diseases

Keywords: Chrysin, Hesperidin, Flavonoids, Quercetin, Catechin, Kaempferol, cytokines

***Corresponding Author**

Pothuganti Manoj Kumar , Department of Biochemistry, University College of Science, Osmania University, Hyderabad – 500 007, Telangana, India

Recieved On 22 April, 2021
Revised On 13 July, 2021
Accepted On 20 July, 2021
Published On 30 July, 2021

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Pothuganti Manoj Kumar, Madhuri Padala, Sudharshan Reddy Nelli, Surya Satyanarayana Singh , Anti - Inflammatory Activity of Quercetin and Kaempferol Is Limited To Tlr4 Stimulation in Monocytes.(2021).Int. J. Life Sci. Pharma Res.11(4), L120-126 http://dx.doi.org/10.22376/ijpbs/lpr.2021.11.4.L120-126

1. INTRODUCTION

Flavonoids belong to a group of polyphenols which are majorly sub classified into flavones, flavonols, flavanones, isoflavones and anthocyanidins. The classification is based on differences in structural and functional groups position at which the B ring is attached to the C ring. Flavonoids were known for their beneficial effects on health long before they were isolated as effective compounds. More than 9000 varieties of flavonoids have been identified, many of which are responsible for the attractive colors of flowers, fruit, and leaves [1]. Honey consists of bio active components like flavonoids, Phenolic acids, tannins and Coumarin. The flavonoid content of honey largely depends on the geographical conditions, season and flora of the region. Honey extracts were shown to have anti-inflammatory properties. Kaempferol, Quercetin, Hesperidin and chrysin were well studied and abundant honey flavonoids [2]. There are several studies reporting anti-inflammatory properties of honey flavonoids. Malaysian honey extracts showed anti-inflammatory activity by reducing nitric oxide production and showed protection from TNF Cytotoxicity [3]. Honey reduced paw size in carrageenan model and granuloma weight in cotton pellet model [4]. Flavonoids reduced the expression of iNOS and cox-2 genes in LPS activated N13 microglia cells [5]. Kaempferol demonstrated protective effect on LPS and ATP induced inflammatory response from cardiac fibroblasts [6]. Kaempferol ameliorated H9N2 swine influenza virus induced acute lung injury .Quercetin reported to inhibit LPS induced Nitric oxide and TNF alpha secretion in murine macrophages [7]. Quercetin also reported to reduce IL-1 stimulated IL-6 release from Mast cells [8]. Catechins show their beneficial effects by scavenging free radicals, chelating metal ions, also by producing phase II detoxification enzymes and antioxidant enzymes [9]. Hesperidin reduces inflammation and oxidative damage in pleural exudates, also protected IL-1B induced inflammation in osteoarthritis chondrocytes [10]. Hesperidin protects against intestinal inflammation by up regulating Treg cells [11]. Chrysin showed effectiveness in atopic dermatitis by suppressing inflammation of keratinocytes and also reduced endothelial inflammation [12-13]. The Innate immune system is equipped with pattern recognition receptors (PRR) as the first line of defense. PRR comprise of Pathogen-associated molecular patterns (PAMPs) and Damage associated molecular patterns (DAMPs). These responses are essential for the clearance of infection and generation of adaptive immunity. In mammals, classes of PRRs including Toll-like receptors (TLRs), Nod like receptors (NLRs), RIG-1-like receptors (RLRs), AIM2-like receptors (ALRs), C-type lectin receptors (CLRs), and intracellular DNA sensors. Among these, Toll like receptors are well characterized. In humans, 10 TLRs and in mice 12 TLRS were identified till date. Each TLR structurally contains an ectodomain with leucine-rich repeats (LRRs) for PAMPs recognition, a cytoplasmic Toll/IL-1 receptor (TIR) domain that initiates downstream signaling and transmembrane domain [14]. Our objective was to compare the anti inflammatory activity of flavonoids in a single assay system. We tested flavonoids for inhibition of LPS induced IL-6 and TNF alpha in monocytes and macrophages. Apart from TLR4 mediated effect , we screened flavonoids for inhibitory effect on other toll like receptors. All TLRs signal to either NF-kb or IRF3/7. We have tested flavonoids against toll-like receptors expressed in monocyte cell line for their inhibition of NF-kB activity. This study will help identifying the inhibition patterns of flavonoids against different toll like receptors. Identification of TLR inhibition profile of flavonoids will help in understanding structure activity relation of flavonoids and TLR receptors

2. MATERIALS AND METHODS

2.1 Materials

Catechin (# C1788), Chrysin (#C80105), Glycolic acid (#G8284) , Kaempferol (#K0133), Quercetin (# Q4951) and Coumarin (# C4261), Lipopolysaccharide (# L2630) were purchased from sigma Aldrich. Human IL-6 (# 88-7066-22) and Human TNF alpha (# 88-7346-22), ELISA kits were purchased from e-biosciences. Pam3CSK4 (# tlrl-pms), Rec flagellin (# rec-flast), FSl-1 (# tlrl-fsl), R848 (# tlrl-R848) , THP-1 blue NF-kb SEAP (#thp-nfkb) reporter cell line were purchased from Invivogen. XTT (# X6493) was purchased from Thermo Fischer scientific limited.

2.2 Cell lines and culture conditions

THP-1 and RAW264.7 cell lines were obtained from National center for cell sciences, Pune, INDIA. THP-1 cells were cultured in RPMI-1640 media (gibco) with 10% fetal bovine serum and 50 µM beta mercaptoethanol. The cells were maintained with a density of 0.2 to 0.8 million per ml with medium change or passage for 2-3 days. RAW 264.7 cells were cultured in Dulbecco's Modified Eagle's Medium with 10% fetal bovine serum. All the cultures were maintained in CO2 incubator with 5% CO_2 and 95% humidity

2.3 Cell viability assay

Cell viability was measured using XTT (2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide) dye. Actively metabolizing cells convert the water-soluble XTT to an orange colored formazan product[15]. PMS (N-methyl dibenzopyrazine methyl sulfate) is an activator which enhances reduction of XTT. THP-1 (10000 cells per well) and RAW 264.7 (3000 cells per well) were seeded in 96 well plate, cells were treated with Flavonoids at mentioned concentrations and incubated for 72 hours. XTT was prepared by dissolving 1mg/ml in incomplete media and heat for 3-5 minutes at 50^0C. PMS as activator added to make XTT working solution. XTT working solution added to cells and incubated until color develop. Absorbance measured at 492 nm in plate reader. Absorbance is proportional to cell viability (manufacturer's protocol was used).

2.4 Cytokine release assays

Cells were seeded in 96 well plates and treated with flavonoids for 1 hour followed by addition of LPS and incubated overnight at 37°C incubator with 5% CO_2. Supernatants collected and ELISA carried out using kit protocol for measurement of cytokines[16] (Kit manufacturer's protocol was used)

2.5 NF-kB reporter assay

THP-1 NF-kb cells were maintained in 100 µg/ml of Normocin. THP-1 NF-kB reporter (SEAP) cells line was seeded in 96 well plate at a density of 30000 cells per well. Flavonoids were added at mentioned concentrations and

incubated for 30 minutes, followed by addition of TLR ligands/stimulants. Cells were incubated overnight. 180 µl of Quanti blue reagent was added to 20 µl of cell culture supernatant. Incubated for 1-3 hours or until color turns from pink to blue. Absorbance measured at 620 nm. Absorbance is proportional to activity of NF-kB. Basal activity is wells where stimulant was not added. Cells where DMSO and stimulant was added considered as 100% activity. % Activity of flavonoid treatment was calculated using formula (absorbance value in Flavonoid treated well/ absorbance value in DMSO)*100. Percent activity was represented in bar graph with Mean and standard deviation

3. STATISTICAL ANALYSIS

Data was exported and analyzed using Microsoft Excel 2007 (Microsoft Corporation) for Cell viability, ELISA and reporter assays. Statistical significance analyses were performed using MedCalc for Windows, version 19.4 (MedCalc Software, Ostend, Belgium). IC_{50} values were calculated using non linear regression method with four parameter analysis in Graphpad Prism version 8.4.3.686 for Windows, GraphPad Software, La Jolla California USA.

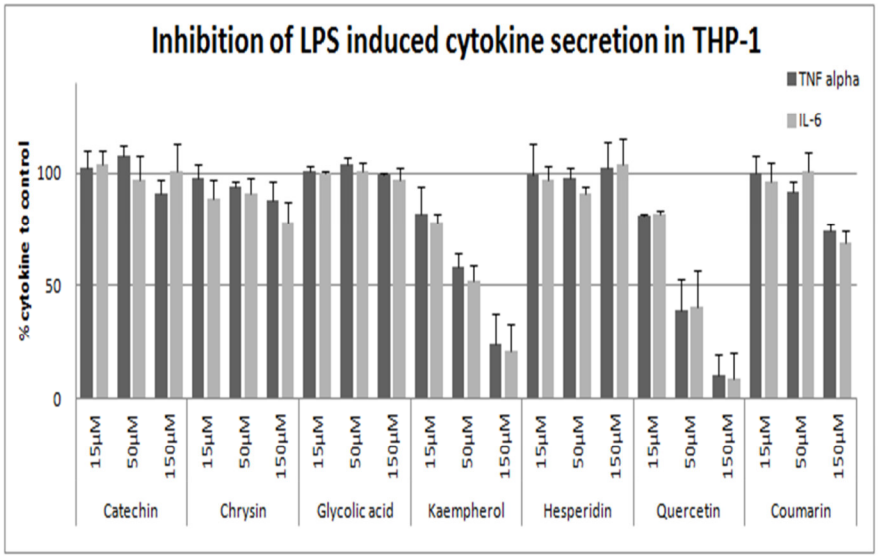
4. RESULTS AND DISCUSSION

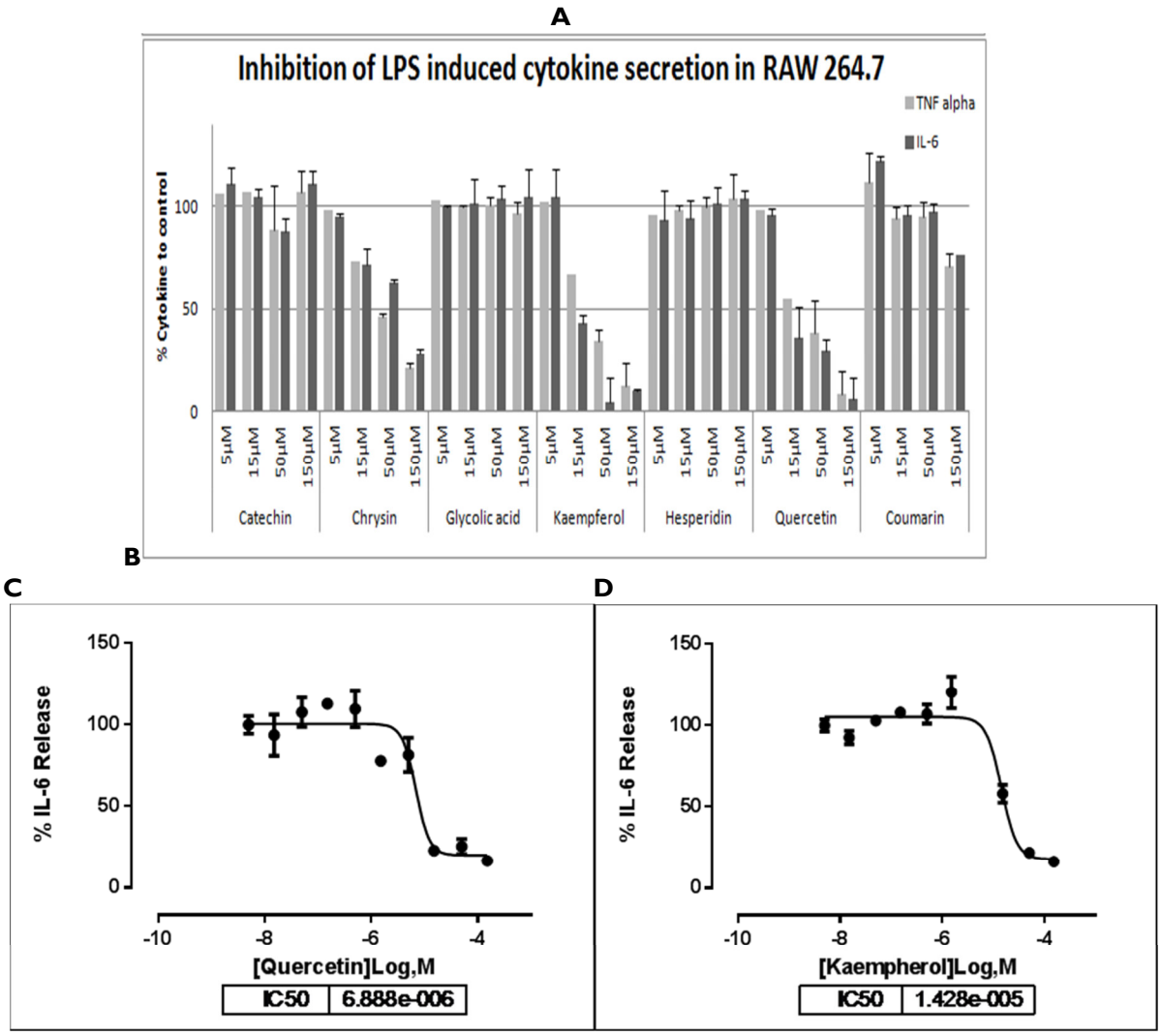
4.1 Impact of flavonoids on monocyte and macrophage cell viability

To understand the impact of flavonoids on cell viability, flavonoids were tested in THP-1 and RAW264.7 cell lines for their viability. Flavonoids were treated at concentrations ranging from 3µM to 300 µM were incubated with cells for 48 hours. Cell viability was measured using XTT viability assay. There is no significant change in viability observed when treated cells with flavonoids up to 300µM (data not shown). Quercetin and Kaempferol was known to inhibit cell proliferation of cancer cells at 96 hours [17-20], but in monocytes and macrophages we did not observe any cell proliferation inhibition at up to 48 hours. This suggest that the cytokine reduction observed with flavonoids is not because of cytotoxicity effect and indeed it is by signaling mechanism

4.2 Cytokine inhibition by flavonoids

Flavonoids Catechin, chrysin, Kaempferol, Hesperidin, Quercetin, Coumarin and Glycolic acid were tested in Raw264.7 and THP-1 for their cytokine inhibiting ability when stimulated with LPS. In literature Flavonoids or alcoholic extracts demonstrated anti inflammatory activity in several studies. But to our understanding this is first study comparing flavonoids for their ability to inhibit cytokine in single assay system. This will help understand and rank order their potency in inhibiting cytokines. This knowledge will help to design better inhibitors using computational rational design. In RAW 264.7 cell line Chrysin, Kaempferol and Quercetin demonstrated potent inhibition of IL-6 and TNF alpha while Catechin, Hesperidin, Coumarin and Glycolic acid did not show significant inhibition (figure 1A). In THP-1 compounds Quercetin, Kaempferol demonstrated potent inhibition of IL-6 and TNF alpha cytokines while Chrysin, Catechin, Hesperidin, Coumarin and Glycolic acid did not demonstrate significant inhibition of IL-6 and TNF alpha under LPS stimulation conditions (figure 1B). Cytokine inhibition pattern for all tested compounds were similar in THP-1 and RAW 264.7 cell lines with an exception that Chrysin. Chrysin showed reduction in RAW 264.7 cell line but not in THP-1 cell line Figure 1A and 1B. The difference in chrysin profiles between both these cell lines could be due to either because of complex signaling difference in human versus mouse cell line or monocyte and macrophage cell systems. Active flavonoids were tested in THP-1 for IC_{50} generation to understand their rank order potencies in inhibition of IL-6. Quercetin observed to be the most potent flavonoid followed by Kaempferol. IC_{50} for Quercetin, Kaempferol observed to 6.6 µM and 14 µM respectively for inhibition of IL-6 (figure 1C and 1D). Chrysin showed activity only in mouse macrophage cell line not in human monocyte THP-1 cell line.





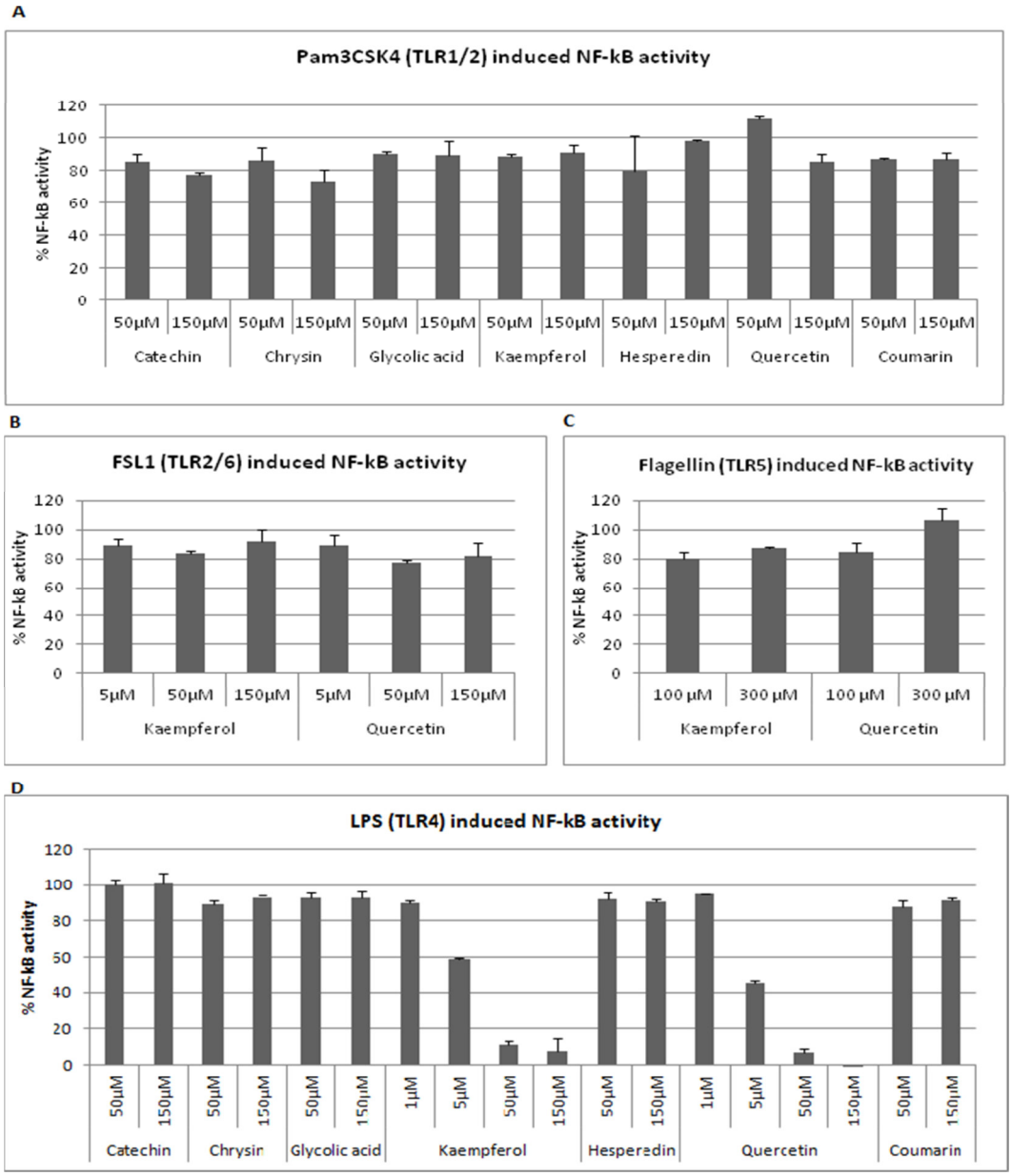
Values represent mean ± standard deviation (n=3) C. Dose response curve for Quercetin in THP-1 cell line for inhibition of IL-6. D. Dose response curve for Kaempferol in THP-1 cell line for inhibition of IL-6 Values represent mean ± standard deviation (n=3)

Fig 1: Effect of flavonoids on cytokine inhibition with LPS stimulation. A. TNF alpha and IL-6 inhibition with flavonoids in RAW 264.7 cell line. B. TNF alpha and IL-6 inhibition with flavonoids in THP-1 cell line.

4.3 Cytokine inhibition is correlated to NF-kB reporter inhibition by flavonoids

NF-kB reporter assay system is a system where plasmid contains NF-kB promoter element fol- lowed by alkaline phosphatase gene (SEAP). The increase in NF-kB activity leads to increase in alkaline phosphatase expression which is secreted in cell culture supernatant. The secreted SEAP activity can be measured using Quanti blue substrate. This system allows monitoring the NF-kB activity with different TLR stimulus. In humans 10 TLR (TLR1-10) receptors are identified and well characterized where as in mouse 12 toll like receptors (TLR1-9, 11-13) were identified till date. We explored whether flavonoids inhibit activity of other Toll like receptors. We have tested TLR1/2, TLR2/6, TLR4 and TLR5 using NF-kB reporter assay. Since TLR7/8 signals through both IRF3/7 and NF-Kb as well, so downstream cytokines used for evaluation of inhibition.TLR3 and TLR9 expression is reported to be limited in THP-1 cell line, The stimulation with TLR3 and TLR9 did not yield significant stimulation in reporter cell line, so TLR3 and TLR9 were not evaluated for inhibition by flavonoids. The inhibition data for all NF-Kb experiments was represented as percent activity for uniform representation of their potencies. Percent activity was calculated in comparison to stimulated control. Stimulation with ligand is considered as 100% activity. Percent activity in flavonoids was calculated using formula [value in treatment/ value in stimulated control] X 100.Staurosporine,a multi kinase inhibitor is used as a positive control which inhibits NF-kB signaling Pam3CSK4 (Pam3CysSerLys4) is a synthetic triacylated lipopeptide , activator of TLR1/2 ligand [21]. Cells were stimulated with 35ng/μl of Pam3CSK4, which showed 8 fold increase in absorbance values. Catechin, chrysin, Kaempferol, Hesperidin, Quercetin, Coumarin and Glycolic acid did not demonstrate significant inhibition of NF-kB reporter activity. Percent activity of NF-kb reporter values were represented in Figure 2A. FSL1 is a synthetic lipopeptide derived from Mycoplasma salivarium. FSL-1 contains a diacylated cysteine residue and is recognized by the TLR2/TLR6 [22]. Cells were stimulated with 2.5ng/μl of FSL1 which gave 2.5 fold increases in absorbance values. Quercetin and Kaempferol did not significantly inhibit FSL1 induced NF-kB activity. LPS induced NF-kb activity demonstrated good correlation with LPS induced cytokine assay in THP-1 cells. LPS activates NF-kB through TLR 4-myddosome pathway. Potent inhibition of NF-kB activity

observed with Quercetin, Kaempferol in a dose dependent manner under LPS stimulation. Chrysin did not show significant inhibition of NF-kB reporter activity. This observation is in line with cytokine inhibition in THP-1. All the other tested flavonoids did not show significant inhibition of NF-kB reporter activity with LPS stimulation. Extracellular flagellin is recognized by Toll-like receptor 5 [23]. We have used recombinant Flagellin in reporter experiments. Cells were stimulated with 400ng/μl of recombinant flagellin which showed 5 fold increase in NF- kB reporter activity. Quercetin and Kaempferol did not significantly inhibit TLR5 induced NF-kB activity. Single stranded viral RNA is natural ligand but we have used R848 which is a synthetic agonist for TLR7/8[24]. TLR7/8 signals through both myd88 pathway and TRAF pathway leads to increase in NF- kb mediated pro inflammatory cytokine genes and IRF3/7 induced Type-I interferon genes. R848 is synthetic agonist for TLR7/8, Quercetin and Kaempferol did not modulate R848 induced IFN alpha secretion (Figure 3A). Quercetin and Kaempferol were most potent among tested flavonoids in modulating the TLR4 induced cytokine inhibition or NF-kB activity. Similar rank ordering potency observed for Quercetin and Keampferol in inhibiting intra cellular calcium , pro inflammatory mediators release in mast cells[25] while these flavonoids did not impact TLR1/2, TLR2/6 and TLR5 induced NF-kB activity.



***Inhibition by flavonoids (values normalized to stimulated control). All values were represented as Mean*± Standard deviation (n=3).**

Fig 2: Inhibition of NF-kb activity with Flavonoids under different TLR stimulations in THP-1 cell line. A. Pam3CSK4 TLR1/2). B.FSL1 (TLR2/6), C.Flagellin (TLR5), D.LPS (TLR4) induced NF-kB activity,

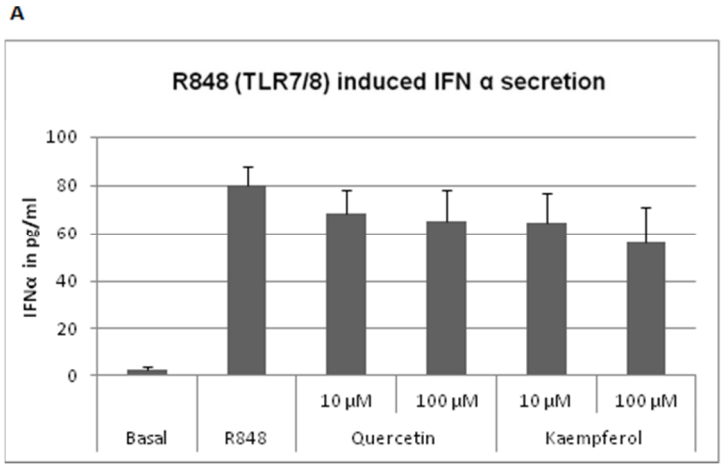


Fig. 3: Effect on cytokine secretion with Flavonoids. A.R848 induced IFN*α* in THP-1 cell line (not significant) .

Quercetin effectiveness anti inflammatory models DCB induced atopic dermatitis and other acute, chronic inflammation models [26,27]. This validates the cytokine inhibitory potential of Quercetin and there is a good translation from invitro to in vivo. Quercetin potency can be optimized using computer aided drug design methods to make potent, efficacious inhibitors to compensate unmet medical need for inflammatory diseases. Quercetin binding protein and binding mechanism need to be evaluated for better understanding which will be useful in design of better TLR inhibitors for treating inflammatory conditions

5. CONCLUSION

Flavonoids Quercetin and Kaempferol have shown cytokine inhibition in monocyte and macrophage cell line. These flavonoids also demonstrated LPS induced NF-kB activity inhibition, but not with the other TLRs like TLR1/2, TLR2/6, TLR5 and TLR7/8. Quercetin and Kaempferol disrupts the TLR4 signaling pathway alone and not the other TLR signaling in monocytes. Evaluation of binding mechanism and signaling target of Quercetin will help in designing more potent and efficacious compounds to meet unmet medical needs for inflammatory diseases.

6. AUTHORS CONTRIBUTION STATEMENT

Pothuganti Manoj Kumar and Surya Satyanarayana Singh conceptualized, designed the work. Pothuganti Manoj Kumar and Madhuri Padala conducted experiments and collected data. Pothuganti Manoj Kumar, Sudharshan Reddy Nelli and Surya Satyanarayana Singh analyzed, interpreted the results. Pothuganti Manoj Kumar drafted the article and Surya Satyanarayana Singh approved the final version to be published. All the authors read and approved the final version of the manuscript.

7. ACKNOWLEDGEMENT

We acknowledge the resource and facility support for the study was provided by the Department of Biochemistry, University college of Sciences, Osmania University. The generous support for carrying out the study at CFRD, Osmania University is acknowledged

8. CONFLICT OF INTEREST

Conflicts of interest declared none.

9. REFERENCES

1. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. J Nutr Sci. 2016;5:e47. doi: 10.1017/jns.2016.41, PMID 28620474.
2. G Vallianou NG. Honey and its anti-inflammatory, anti-bacterial and anti-oxidant properties. General Med. 2014;02(2):2-. doi: 10.4172/2327-5146.1000132.
3. Kassim M, Achoui M, Mustafa MR, Mohd MA, Yusoff KM. Ellagic acid, phenolic acids, and flavonoids in Malaysian honey extracts demonstrate in vitro anti-inflammatory activity. Nutr Res. 2010;30(9):650-9. doi: 10.1016/j.nutres.2010.08.008, PMID 20934607.
4. Owoyele BV, Adenekan OT, Soladoye AO. Effects of honey on inflammation and nitric oxide production in Wistar rats. Zhong Xi Yi Jie He Xue Bao. 2011;9(4):447-52. doi: 10.3736/jcim20110415, PMID 21486559.
5. Candiracci M, Piatti E, Dominguez-Barragán M, García-Antrás D, Morgado B, Ruano D, et al. Anti-inflammatory activity of a honey flavonoid extract on lipopolysaccharide-activated N13 microglial cells. J Agric Food Chem. 2012;60(50):12304-11. Avail- able from. doi: 10.1021/jf302468h, PMID 23176387.
6. Tang XL, Liu JX, Dong W, Li P, Li L, Hou JC, Zheng YQ, Lin CR, Ren JG. Protective effect of kaempferol on LPS plus ATP-induced inflammatory response in cardiac fibroblasts. Inflammation. 2015;38(1):94-101. doi: 10.1007/s10753-014-0011-2, PMID 25189464.
7. K RM, Ghosh B. K RM, Ghosh B. Quercetin inhibits LPS-induced nitric oxide and tumor necrosis factor-*α* product- tion in murine macrophages. Int J Immunopharmacol. 1999;21(7):435-43. doi: 10.1016/S0192-0561(99)00024-7.
8. Kandere-Grzybowska K, Kempuraj D, Cao J, Cetrulo CL, Theoharides TC. Regulation of IL- 1-induced selective IL-6 release from human mast cells and inhibition by quercetin. Br J Pharmacol. 2006;148(2):208-15. doi: 10.1038/sj.bjp.0706695. PMID 16532021.
9. Bernatoniene J, Kopustinskiene DM. The Role of Catechins in cellular Responses to Ox- idative Stress.

Molecules. 2018;23(4):965-. doi: 10.3390/molecules23040965, PMID 29677167.
10. Adefegha SA, Rosa Leal DBR, Olabiyi AA, Oboh G, Castilhos LG. Hesperidin attenuates inflamma- tion and oxidative damage in pleural exudates and liver of rat model of pleurisy. Redox Rep. 2017;22(6):563-71. doi: 10.1080/13510002.2017.1344013, PMID 28657497.
11. Fu Z, Chen Z, Xie Q, Lei H, Xiang S. Hesperidin protects against IL-1β-induced inflammation in human osteoarthritis chondrocytes. Exp Ther Med. 2018;16(4):3721-7. doi: 10.3892/etm.2018.6616, PMID 30233731.
12. Choi JK, Jang YH, Lee S, Lee SR, Choi YA, Jin M, Choi JH, Park JH, Park PH, Choi H, Kwon TK, Khang D, Kim SH. Chrysin attenuates atopic dermatitis by suppressing inflammation of keratinocytes. Food Chem Toxicol. 2017;110:142-50. doi: 10.1016/j.fct.2017.10.025, PMID 29050978.
13. Zhao S, Liang M, Wang Y, Hu J, Zhong Y, Li J, Huang K, Li Y. Chrysin suppresses vascular endothelial inflammation via inhibiting the NF-κB signaling pathway. J Cardiovasc Pharmacol Ther. 2019;24(3):278-87. doi: 10.1177/1074248418810809, PMID 30497287.
14. Kawasaki T, Kawai T. Toll-like receptor signaling pathways. Front Immunol. 2014;5:461. doi: 10.3389/fimmu.2014.00461, PMID 25309543.
15. Roehm, Neal W., et al. "An improved colorimetric assay for cell proliferation and viability utilizing the tetrazolium salt XTT." J. Immunol. Methods, vol. 142, no. 2, 13 Sept. 1991, pp. 257-65, doi:10.1016/0022-1759(91)90114-U.
16. The enzyme-linked immunosorbent assay (ELISA). Bulletin of the World Health Organization vol. 54,2 (1976): 129-39.
17. Da J, Xu M, Wang Y, Li W, Lu M, Wang Z. Kaempferol promotes apoptosis while inhibiting cell proliferation via androgen-dependent pathway and suppressing vasculogenic mimicry and invasion in prostate cancer. Anal Cell Pathol (Amst). 2019;2019:1907698. doi: 10.1155/2019/1907698, PMID 31871879.
18. Paliwal S, Sundaram J, Mitragotri S. Induction of cancer-specific cytotoxicity towards human prostate and skin cells using quercetin and ultrasound. Br J Cancer. 2005;92(3):499-502. doi: 10.1038/sj.bjc.6602364, PMID 15685239.
19. Samarghandian S, Afshari JT, Davoodi S. Chrysin reduces proliferation and induces apoptosis in the human prostate cancer cell line pc-3. Clinics (Sao Paulo). 2011;66(6):1073-9. doi: 10.1590/s1807-59322011000600026, PMID 21808878.
20. Zhu L, Xue L. Kaempferol suppresses proliferation and induces cell cycle arrest, apoptosis, and DNA damage in breast cancer cells. Oncol Res. 2019;27(6):629-34. doi: 10.3727/096504018X15228018559434, PMID 29739490.
21. Jin MS, Kim SE, Heo JY, Lee ME, Kim HM, Paik S-G, Lee H, Lee JO. Crystal structure of the TLR1-TLR2 heterodimer induced by binding of a tri-acylated lipopeptide. Cell. 2007;130(6):1071-82. doi: 10.1016/j.cell.2007.09.008, PMID 17889651.
22. Kurkjian CJ, Guo H, Montgomery ND, Cheng N, Yuan H, Merrill JR, Sempowski GD, Brickey WJ, Ting JP. The toll–like Receptor 2/6 agonist, FSL–1 lipopeptide, therapeutically mitigates acute radiation syndrome. Sci Rep. 2017;7(1):17355. doi: 10.1038/s41598-017-17729-9, PMID 29230065.
23. Lu J, Sun PD. The structure of the TLR5-flagellin complex: A new mode of pathogen detection, conserved receptor dimerization for signaling. Sci Signal. 2012;5(223):pe11. doi: 10.1126/scisignal.2002963, PMID 22720339.
24. Dockrell DH, Kinghorn GR. Imiquimod and resiquimod as novel immunomodulators. J Antimicrob Chemother. 2001;48(6):751-5. doi: 10.1093/jac/48.6.751, PMID 11733457.
25. Kempuraj D, Madhappan B, Christodoulou S, Boucher W, Cao J, Papadopoulou N, Cetrulo CL, Theoharides TC. Flavonols inhibit proinflammatory mediator release, intracellular calcium ion levels and protein kinase C theta phosphorylation in human mast cells. Br J Pharmacol. 2005;145(7):934-44. doi: 10.1038/sj.bjp.0706246, PMID 15912140.
26. Kaidama WM, Gacche R. Anti-inflammatory activity of quercetin in acute and chronic phases of inflammation in guinea pigs. Am J Phytomed Clin Ther. 2015;3:129-36.
27. Lee HN, Shin SA, Choo GS, Kim HJ, Park YS, Kim BS, Kim SK, Cho SD, Nam JS, Choi CS, Che JH, Park BK, Jung JY. Anti inflammatory effect of quercetin and galangin in LPS stimulated RAW264.7 macrophages and DNCB induced atopic dermatitis animal models. Int J Mol Med. 2018;41(2):888-98. doi: 10.3892/ijmm.2017.3296, PMID 29207037.