Nutr Res Pract. 2025 Feb;19(1):143-153
https://doi.org/10.4162/nrp.2025.19.1.143
pISSN 1976-1457·eISSN 2005-6168

Nutrition Research and Practice

Short Communication

Check for updates

Anti-inflammatory effects of rutin in lipopolysaccharide-stimulated canine macrophage cells

Hyunsoo Jang [1*], **Su-Cheol Han** [2*], **Jisu Lee** [1], **Ha-Young Shin** [3], **Jeong Ho Hwang** [2,3§], **and Jung-Heun Ha** [1,4§]

[1]Department of Food Science and Nutrition, Dankook University, Cheonan 31116, Korea
[2]Companion Animal New Drug Development Center, Korea Institute of Toxicology, Jeongeup 56212, Korea
[3]Center for Large Animals Convergence Research, Korea Institute of Toxicology, Jeongeup 56212, Korea
[4]Research Center for Industrialization of Natural Neutralization, Dankook University, Yongin 16890, Korea

OPEN ACCESS

Received: Sep 9, 2024
Revised: Sep 24, 2024
Accepted: Oct 10, 2024
Published online: Oct 28, 2024

[§]Corresponding Authors:
Jeong Ho Hwang
Animal Model Research Group, Companion Animal New Drug Development Center, Institute of Toxicology, 30 Baekhak 1-gil, Jeongeup 56212, Korea.
Tel. +82-63-570-8528
Fax. +82-63-570-8897
Email. jeongho.hwang@kitox.re.kr

Jung-Heun Ha
Department of Food Science and Nutrition, Dankook University, 119 Dandae-ro, Cheonan 31116; Research Center for Industrialization of Natural Neutralization, Dankook University, 152 Jukjeon-ro, Yongin 16890, Korea.
Tel. +82-41-550-3479
Fax. +82-41-559-7955
Email. ha@dankook.ac.kr

*Hyunsoo Jang and Su-Cheol Han contributed equally to this work.

ABSTRACT

BACKGROUND/OBJECTIVES: Inflammatory responses are key pathological factors in various canine diseases, making the control of inflammatory responses vital for canine health. This study examined the anti-inflammatory effects of rutin on DH82 cells, a type of canine macrophage, against lipopolysaccharide (LPS)-induced inflammatory responses.
MATERIALS/METHODS: The inflammatory *in vitro* experimental model was established by stimulating canine macrophage DH82 cells with LPS. To evaluate the inflammation-preventative effects of rutin, analyses were conducted using enzyme-linked immunosorbent assay, western blot, and real-time quantitative reverse transcription polymerase chain reaction.
RESULTS: Rutin inhibited the LPS-induced increase in the protein and gene levels of pro-inflammatory cytokines (interleukin [IL]-1β, IL-6, tumor necrosis factor-α), while anti-inflammatory cytokines (IL-10, transforming growth factor-β1) levels remained unchanged. Furthermore, rutin suppressed the LPS-induced activation of phosphorylated extracellular signal-regulated kinase, Jun N-terminal kinase, inhibitor of nuclear factor kappa B, and nuclear factor kappa B (NF-κB) in DH82 cells.
CONCLUSION: Rutin exerts anti-inflammatory effects by inhibiting the mitogen-activated protein kinase-NF-κB signaling pathway and reducing the production of pro-inflammatory cytokines in DH82 cells.

Keywords: Dogs; macrophages; inflammation; rutin

INTRODUCTION

The pet industry has experienced significant growth and development due to evolving societal attitudes towards pets, increased pet ownership, and a greater willingness among pet owners to invest in their companions [1]. A growing focus on pet health and wellness is driving the expansion of pet healthcare, including services and products, such as veterinary care, nutritional supplements, and items designed to enhance pets' mental health and overall well-being [2]. In addition, there is increasing demand for preventative healthcare options for pets [3]. Unlike humans, pets have limited ways to communicate their health problems, which can result in substantial medical expenses when diseases emerge [4]. Considering the nature of pets, the optimal approach to sustaining pet health is linked to food intake [5].

ORCID iDs

Hyunsoo Jang
https://orcid.org/0009-0009-7586-5678
Su-Cheol Han
https://orcid.org/0000-0003-2006-9788
Jisu Lee
https://orcid.org/0000-0002-3327-1945
Ha-Young Shin
https://orcid.org/0000-0002-0901-4826
Jeong Ho Hwang
https://orcid.org/0000-0002-4763-8373
Jung-Heun Ha
https://orcid.org/0000-0001-5282-531X

Funding

This work was supported by the National Research Council of Science & Technology (NST) grant by the Korea government (MSIT) (grant number: CRC-21023-400).

Conflict of Interest

The authors declare no potential conflicts of interests.

Author Contributions

Conceptualization: Hwang JH, Ha JH; Data curation: Jang H, Han SC, Hwang JH, Ha JH; Formal analysis: Jang H, Han SC, Hwang JH, Ha JH; Funding acquisition: Hwang JH, Ha JH; Methodology: Jang H, Han SC, Lee J, Ha JH; Project administration: Hwang JH, Ha JH; Resources: Hwang JH, Ha JH; Software: Jang H, Han SC, Lee J, Shin HY, Hwang JH, Ha JH; Supervision: Hwang JH, Ha JH; Validation: Jang H, Han SC, Lee J, Shin HY, Hwang JH, Ha JH; Visualization: Jang H, Han SC; Writing - original draft: Jang H, Han SC, Hwang JH, Ha JH; Writing - review & editing: Jang H, Han SC, Lee J, Shin HY, Hwang JH, Ha JH.

Among pets, dogs are susceptible to various inflammation-related diseases. These conditions involve inflammatory reactions to injury, infection, or disease. The inflammatory reactions can be acute, protecting against harmful stimuli and initiating healing, or chronic, potentially causing various health complications [6]. Common conditions associated with heightened inflammatory responses include allergies, arthritis, inflammatory bowel syndrome, and dermatitis. Dogs may suffer from allergic reactions to food or environmental allergens [7]. Arthritis is often observed in older dogs, causing pain, stiffness, and reduced mobility due to deteriorating joint cartilage [8]. Inflammatory bowel syndrome manifests as increased inflammatory responses in the gastrointestinal tract, causing symptoms such as vomiting, diarrhea, and weight loss [9]. Furthermore, dermatitis can be triggered by contact with fleas or other irritants [10]. Elevated inflammatory responses are a common factor in these conditions.

Inflammation is a complex biological response to pathogens and cell damage. Macrophages mediate an inflammatory process, responding to infectious agents and cellular pathological responses by activating inflammatory signaling pathways, including nuclear factor kappa B (NF-κB) and mitogen activated protein kinase (MAPK) [11,12]. Lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria such as *Escherichia coli*, induces inflammation. LPS is commonly used to develop pathological experimental models for studying the anti-inflammatory effects of drugs and natural substances [13]. Exposing macrophages to LPS causes NF-κB activation and subsequent nuclear translocation, resulting in the release of pro-inflammatory mediators such as interleukin (IL)-1β, IL-6, tumor necrosis factor-alpha (TNF-α), and transforming growth factor beta 1 (TGF-β1) [14]. In addition, MAPK activation regulates the activities of inflammation-related transcription factors, affecting cellular proliferation, cell-cycle arrest, senescence, and apoptosis [15]. Therefore, investigating the anti-inflammatory potential of dietary components in dogs should evaluate the response of canine macrophages, such as DH82 cells, to inflammatory stimuli.

Rutin (3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoglucoside) is a strong candidate for preventing inflammatory responses in canine macrophages, specifically DH82 cells. Rutin is a flavonoid structured as a glycoside conjugated to quercetin (3,3′,4′,5,6-pentahydroxyflavone) widely found in buckwheat, vegetables, and fruits [16]. Research on rutin's biological and pharmacological effects shows rutin has anti-diabetic, anti-inflammatory, anti-tumor, antimicrobial, and antioxidant properties [17-21]. Studies involving tumors, viruses, and cell death show that rutin has anti-inflammatory effects, as evaluated through cell morphology and nitric oxide (NO), TNF-α, IL-1β, and IL-6 levels [22,23]. Moreover, rutin inhibits the induction of TNF-α by LPS and NF-κB activation, which is vital for treating vascular inflammatory diseases [24]. Rutin also reduces TNF-α and IL-1β activity and MAPK phosphorylation in mouse kidney cells, alleviating carbon tetrachloride (CCl_4)-induced inflammation [25]. Considering these health benefits, rutin has the potential for various industrial applications. However, its potential to protect against inflammatory responses in canine models has not been extensively studied.

This study explored the potential anti-inflammatory effects of rutin on canine macrophage DH82 cells in response to LPS treatment. In detail, we examined how rutin affects the post-translational activation of the MAPK–NF-κB pathway, inflammatory gene and protein expressions, and its underlying molecular mechanisms. Thus, this study aimed to assess rutin as a functional ingredient for preventing canine inflammatory responses.

Nutrition Research and Practice

MATERIALS AND METHODS

Canine DH82 cell culture

Canine DH82 cells were cultured in minimum essential medium supplemented with 15% fetal bovine serum and 1% penicillin/streptomycin and maintained in a humidified incubator with 5% CO_2 at 37°C (Vision Scientific Co., Ltd., Daejeon, Korea). The cells were seeded at a density of 7.0×10^5 cells/mL in 6-well plates (2 mL culture medium per well) and cultured until they reached 80–90% confluence. The cells were then treated with varying concentrations of rutin (10, 20, and 40 μM) for 24 h, and subsequently the cells were challenged with LPS (0.1 μg/mL).

Cell viability assays

Cell viability was assayed using an MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide). DH82 cells were seeded in a 96-well plate at a concentration of 2.4×10^4 cells/100 μL. The cells were treated with varying concentrations of rutin (0, 5, 10, 20, 40, 80, and 100 μM) to assess cytotoxicity of rutin. Following treatment, MTT solution (1 mg/mL) was added to each well, and the plate was incubated at 37°C for 1 h. Subsequently, optical density of the plate was measured at 540 nm using a microplate reader.

Cytokine enzyme-linked immunosorbent assays (ELISA)

The levels of secreted TNF-α and IL-10 were measured using commercially available ELISA kits (R&D Systems, Inc., Minneapolis, MN, USA). DH82 cells were seeded in 12-well plates at a density of 3.5×10^5 cells/mL and incubated for 24 h at 37°C. The cells were then treated with various concentrations of rutin (10, 20, 40 μM) for 24 h before inducing an inflammatory response using LPS (0.1 μg/mL) for 6 h. Following incubation, the culture supernatant was collected and centrifuged at 1,000 × g at 4°C for 10 min to remove debris before measuring the cytokine levels.

Western blot analysis

Total protein extraction and subsequent western blot analysis were performed as described in a previous in-house publication [26]. The primary and secondary antibodies used in the western blot analyses are detailed in **Supplementary Table 1**.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA extraction, cDNA synthesis, and real-time PCR was also executed as described previously with relative mRNA expressions were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [27]. The PCR primers used are detailed in **Supplementary Table 2**.

Statistical analysis

The data are presented as means with SDs. Statistical significance was determined using one-way analysis of variance, followed by Tukey's *post hoc* analysis for multiple comparisons using the SPSS program (Statistical Package for Social Science; IBM Corp., Armonk, NY, USA). Statistical significance was defined as $P < 0.05$ [28].

https://doi.org/10.4162/nrp.2025.19.1.143

Nutrition Research and Practice

RESULTS

Cytotoxic effects of rutin in DH82 cells

We executed an MTT cell viability assay to evaluate rutin's cytotoxic effects on DH82 cells at 0, 5, 10, 20, 40, 80, and 100 μM concentrations. Concentrations of rutin ≤ 80 μM showed no significant change in cell viability compared to the control. However, a significant decrease in cell viability was observed at 100 μM rutin (**Supplementary Fig. 1**). Subsequently, rutin was treated as 0, 10, 20, and 40 μM to investigate anti-inflammatory effects.

Rutin prevents LPS-induced TNF-α secretion in DH82 cells

M1 macrophages are activated by factors such as interferon (IFN)-γ, and their functions include producing pro-inflammatory cytokines, engulfing microbes, and triggering inflammatory immune responses. M1 macrophages generate NO or reactive oxygen intermediates to defend against bacteria and viruses. In contrast, M2 macrophages are activated by cytokines such as IL-4, IL-10, or IL-13, producing polyamines for cell growth or proline for collagen synthesis, making them pivotal in wound healing and tissue restoration. Rutin's anti-inflammatory effects on modulating LPS-inducible pro- and anti-inflammatory responses were assessed by measuring TNF-α and IL-10 levels in the culture media using ELISA. Rutin pre-treatment significantly prevented LPS-induced TNF-α production (**Fig. 1A**). However, no significant changes were found in the anti-inflammatory cytokine IL-10 secretions (**Fig. 1B**). Consequently, rutin pre-treatment significantly lowered the TNF-α/IL-10 ratio in our *in vitro* setting (**Fig. 1C**).

Rutin prevents LPS-induced activation of the MAPK–NF-κB pathway

Inflammatory responses are modulated both transcriptionally and post-translationally. Post-translational regulation of inflammatory reactions occurs through the activation of the MAPK–NF-κB pathway [2,29]. The MAPK–NF-κB pathway is activated by a wide range of external stimuli linked to inflammation, immune reactions, cell growth, differentiation, and survival [30]. An important step in activating the MAPK–NF-κB pathway is the phosphorylation-dependent activation of the inhibitor of nuclear factor kappa B (IκB) kinase complex. Phosphorylation of IκB is followed by ubiquitination and proteasome-mediated degradation of inhibitory IκB proteins, allowing NF-κB to enter the nucleus and initiate the transcription of target genes [31]. Since rutin reduced the production of LPS-induced pro-inflammatory cytokines, we hypothesized that rutin treatment might inhibit LPS-induced post-translational modifications in the MAPK–NF-κB axis. Indeed, LPS treatment significantly increased the

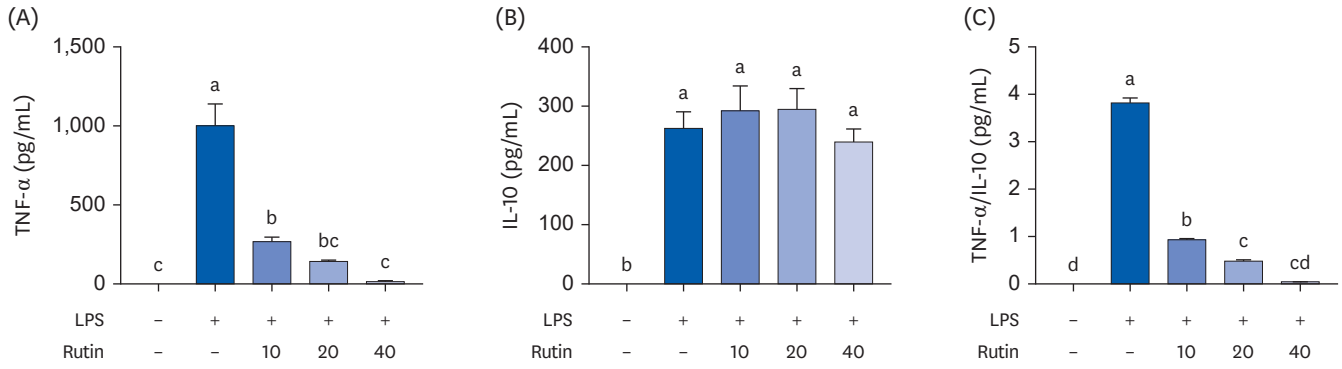

Fig. 1. The effect of rutin on LPS-induced pro/anti-inflammatory cytokines in DH82 cells. After 24 h of rutin treatment (0–40 μM), DH82 cells are exposed to LPS (0.1 μg/mL) for 6 h. (A) TNF-α levels, (B) IL-10 levels, (C) TNF-α/IL-10 ratio. Values are expressed as the mean ± SD.
LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-α; IL-10, interleukin 10.
[a,b,c,d]Means with different letters indicate significant differences ($P < 0.05$) as determined one-way analysis of variance, followed by Tukey's *post hoc* test.

https://doi.org/10.4162/nrp.2025.19.1.143

Nutrition Research and Practice

phosphorylation of the MAPK proteins, such as Jun N-terminal kinase (JNK) (**Fig. 2A and B**) and extracellular signal-regulated kinase (ERK) (**Fig. 2A and C**); however, rutin (40 μM) significantly inhibited this phosphorylation (**Fig. 2A-C**). Our findings suggested that rutin might prevent the post-translational modification in downstream targets of MAPK, including IκB and NF-κB. LPS exposure significantly increased the phosphorylation of IκB and NF-κB; however, rutin (40 μM) inhibited the phosphorylation of IκB (**Fig. 2A and D**) and NF-κB in canine macrophage cells (**Fig. 2A and E**). Thus, rutin treatment of DH82 cells inhibited the LPS-induced post-translational phosphorylation of proteins at multiple steps in the MAPK–NF-κB pathway.

Rutin prevents LPS-induced pro-inflammatory cytokine gene expression

Since rutin exposure prevented LPS-induced MAPK–NF-κB activation in DH82 cells, we hypothesized that rutin treatment would inhibit pro-inflammatory cytokine gene expression in DH82 cells since activating NF-κB triggers pro-inflammatory gene transcription. Exposure of DH82 cells to LPS (0.1 μg/mL) markedly increased pro-inflammatory cytokine mRNA levels, including *Il-6* (**Fig. 3A**), *Il-1β* (**Fig. 3B**), and *Tnf-α* (**Fig. 3C**). Treatment with rutin (10–40 μM) significantly reduced the mRNA levels of these pro-inflammatory genes (**Fig. 3A-C**). However, no significant differences were found in *Tgf-β1* levels (**Fig. 3D**). The anti-inflammatory cytokine *Il-10* showed no significant LPS induction following rutin pre-treatment (**Fig. 3E**), consistent with the protein secretion results (**Fig. 1B**).

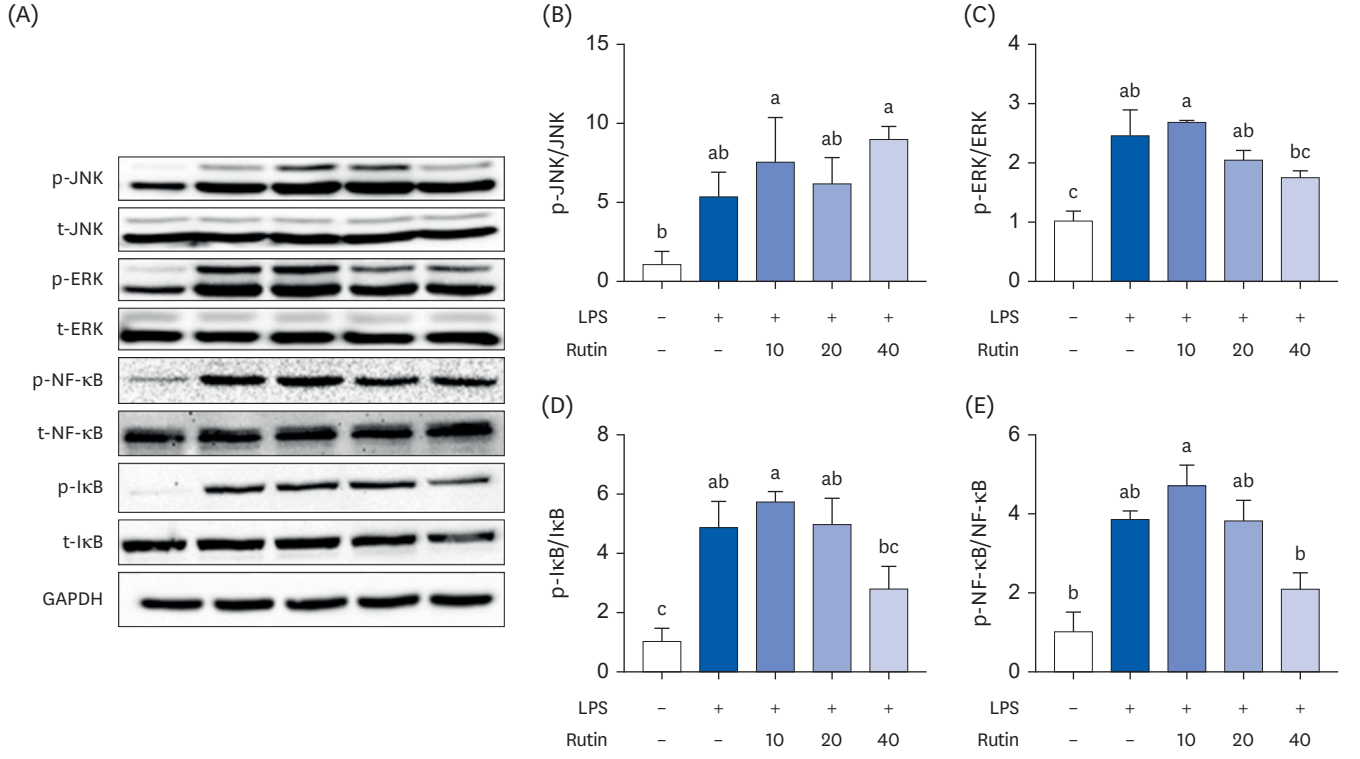

Fig. 2. The effect of rutin on LPS-induced proinflammatory gene expression, MAPK, and NF-κB activation in DH82 cells. After 24 h of rutin treatment (0–40 μM), DH82 cells are exposed to LPS (0.1 μg/mL) for 5 or 10 min. (A) Representative western blot images, (B) phosphorylated IκB levels, (C) phosphorylated NF-κB levels, (D) phosphorylated ERK levels, (E) phosphorylated JNK levels. Protein levels are adjusted relative to the GAPDH reference standard. Values are expressed as the mean ± SD.

LPS, lipopolysaccharide; JNK, Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor kappa B; IκB, inhibitor of nuclear factor kappa B; ERK, extracellular signal-regulated kinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; p, phosphorylation; t, total.

[a,b,c]Means with different letters indicate significant differences ($P < 0.05$) as determined one-way analysis of variance, followed by Tukey's *post hoc* test.

https://doi.org/10.4162/nrp.2025.19.1.143

Nutrition Research and Practice

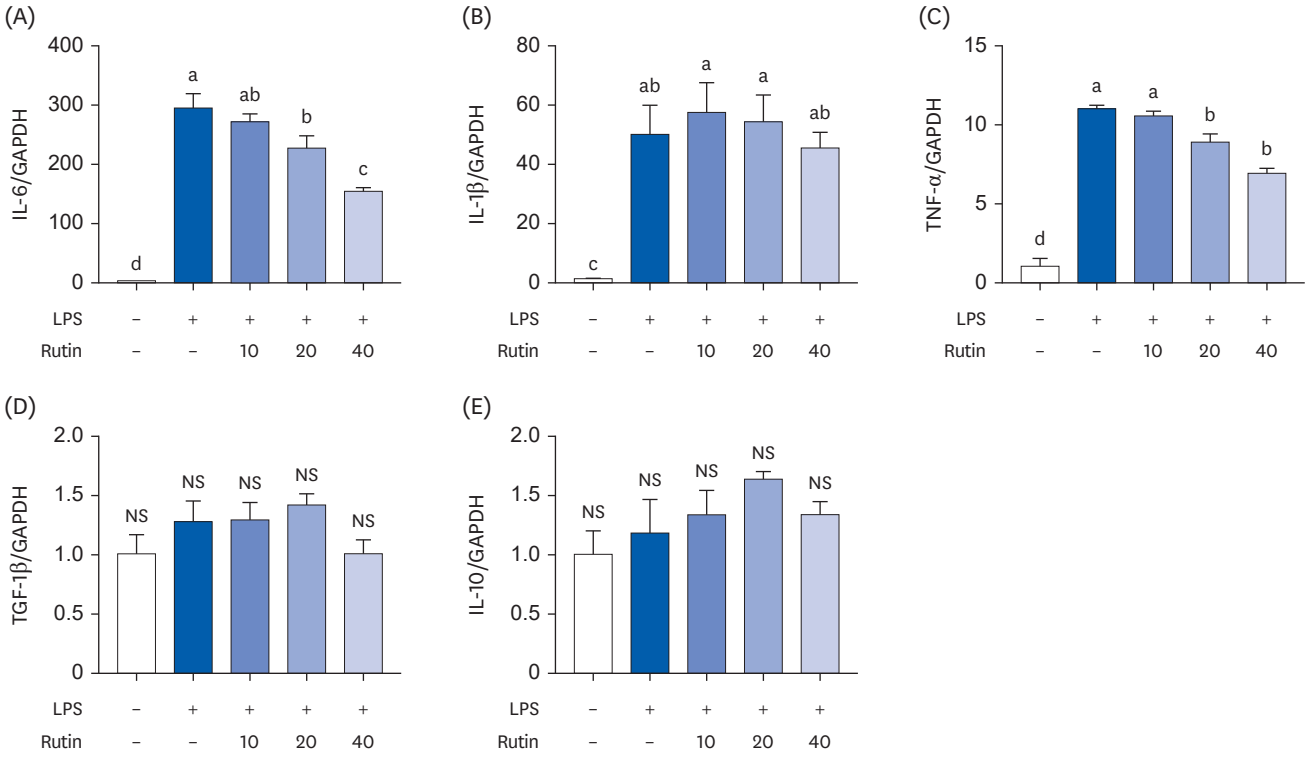

Fig. 3. The effect of rutin on LPS-induced mRNA inflammation-related expression in DH82 cells. After 24 h of rutin treatment (0–40 μM), DH82 cells are exposed to LPS (0.1 μg/mL) for 3 h. (A) IL-6 levels, (B) IL-1β levels, (C) TNF-α levels, (D) TGF-β1 levels, (E) IL-10 levels. The mRNA levels are adjusted relative to the GAPDH reference standard. Values are expressed as the mean ± SD.
LPS, lipopolysaccharide; IL, interleukin; TNF, tumor necrosis factor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NS, not statistically significant.
[a,b,c]Means with different letters indicate significant differences ($P < 0.05$) as determined one-way ANOVA, followed by Tukey's *post hoc* test.

DISCUSSION

In this study, we found that rutin prevented LPS-induced expression of inflammatory cytokines by modulating the MAPK–NF-κB pathway. Our results suggested that prophylactic treatment with rutin may help prevent potential inflammatory diseases in dogs. Pathological progression in dogs is closely linked to increase inflammation, which can occur locally (e.g., skin, intestines, and joints) or be widespread throughout the body [32]. Diagnosing inflammatory diseases in dogs can be difficult, emphasizing the importance of prevention by addressing toxic substances [33]. In this study, we developed a potential canine inflammatory disease model using DH82 cells (macrophages and monocytes) derived from a canine histiocytic sarcoma lineage [34]. DH82 cells have also been used to study abnormal inflammatory states, including leishmania, monocytic ehrlichiosis, and allergies [35-37]. This study focused on the mechanisms underlying inflammation-induced inhibition by rutin in LPS-induced conditions. Our findings indicate that rutin inhibits the activation of the MAPK–NF-κB pathway, reducing the expression of inflammatory cytokines.

M1 macrophage cell polarization is an activated state of pro-inflammatory immune cells; excessive activation contributes to inflammatory disease pathology. M1 macrophages secrete pro-inflammatory cytokines, including TNF-α and IL-6, promoting inflammatory responses [38]. LPS treatment in DH82 cells upregulates the levels of pro-inflammatory cytokines (e.g., TNF-α, IL-6, cyclooxygenase 2 [COX-2], and inducible nitric oxide synthase [iNOS])

https://doi.org/10.4162/nrp.2025.19.1.143

[39] at both the protein and mRNA levels. Activating the NF-κB signaling pathway induces the production of pro-inflammatory cytokines and chemokines. MAPK signaling works in conjunction with NF-κB as a downstream target. Consequently, the MAPK–NF-κB pathway is crucial to control overall inflammatory responses [40]. Our investigation showed that exposing DH82 cells to LPS induces MAPK phosphorylation, elevating the phosphorylation of IκB and NF-κB. The activation of inflammatory cytokine expression and the MAPK–NF-κB signaling pathway confirms that we established an inflammatory disease model in LPS-treated DH82 cells.

Rutin is a glycoside comprising the flavonol quercetin and the disaccharide rutinose [41]; it alleviates inflammation, oxidative stress, and diabetes (**Tables 1** and **2**) [42-51]. Rutin can alleviate inflammation locally and systemically. In a colitis model induced by dextran sodium sulfate, rutin effectively suppressed intestinal inflammation and oxidative stress, improving colonic permeability by increasing tight junction proteins [42]. In LPS-induced acute kidney injury, rutin mitigates the increased serum creatinine and blood urea nitrogen levels associated with pro-inflammatory cytokine regulation by preventing NF-κB activation and enhancing antioxidant enzyme activity [43]. Rutin also ameliorates pathologies in CCl_4-induced hepatic injury by reducing fibrosis and enhancing antioxidative capacities through upregulating nuclear factor erythroid 2–related factor 2 and heme oxygenase 1 expression [44]. In addition, rutin alleviates inflammation in a cyclophosphamide-induced liver injury model by enhancing antioxidant enzyme activity and regulating the expression of pro-inflammatory cytokines, NF-κB, iNOS, and COX-2 [45].

Table 1. Inflammation mitigating effects of rutin *in vivo*

Strain	Inducer	Treatment	Biological markers	Ref.
C57BL/6J mouse	DSS	20, 40 mg/kg for 8 days	Inhibition of colon length reduction; Proinflammatory cytokines; (IL-6, IL-8, TNF-α) ↓; ZO-1, MUC 2 ↓; NF-κB activation ↓	[42]
C57BL/6J mouse	LPS	50, 200 mg/kg for 24 h	Proinflammatory cytokines; (TNF-α, IL-6) ↓; NF-κB, TLR4, COX-2 ↓; MDA, caspase 3 ↓; GSH, SOD, catalase ↑	[43]
BALB/c mouse	CCl_4	10, 50, 150 mg/kg for 5 days	NF-κB, COX-2, TNF-α, iNOS ↓; Nrf2, HO-1 ↑; Transaminase ↓	[44]
Wistar rats	Cyclophosphamide	50, 100 mg/kg for 20 days	Proinflammatory cytokines; (TNF-α, IL-6) ↓; p38-MAPK, NF-κB, iNOS, COX-2 ↓; AST, ALT, LDH ↓; GSH, GR, GPX ↑	[45]
Sprague-Dawley rats	Fructose	50, 100 mg/kg for 4 weeks	NLRP3, ASC, caspase-1 ↓; Proinflammatory cytokines; (TNF-α, IL-1β, IL-6) ↓; Renal TG, TC, VLDL ↓; PPARα, CPT1, OCTN2 ↓	[49]

DSS, dextran sulfate sodium; IL, interleukin; TNF-α, tumor necrosis factor-alpha; ↑, increased; ↓, decreased; ZO-1, zonula occludens-1; MUC 2, mucin 2; NF-κB, nuclear factor kappa B; LPS, lipopolysaccharide; TLR, Toll-like receptor; COX, cyclooxygenase; MDA, 3,4-methylenedioxyamphetamine; GSH, glutathione; SOD, superoxide dismutase; iNOS, inducible nitric oxide synthase; Nrf2, nuclear factor erythroid 2-related factor 2; HO-1, heme oxygenase 1; MAPK, mitogen-activated protein kinase; AST, aspartate aminotransferase; ALT, alanine transaminase; LDH, lactate dehydrogenase; GR, glutathione reductase; GPX, glutathione peroxidase; NLRP3, nucleotide-binding domain, leucine-rich–containing family, pyrin domain–containing-3; ASC, apoptosis-associated speck-like protein; TG, triglyceride; TC, total cholesterol; VLDL, very low density lipoprotein; PPARα, peroxisome proliferator-activated receptor alpha; CPT1, carnitine palmitoyltransferase 1; OCTN2, organic cation/carnitine transporter 2.

Table 2. Inflammation mitigating effects of rutin *in vitro*

Strain	Inducer	Treatment	Biological markers	Ref.
RAW264.7	LPS	1.25, 2.5, 5, 10 uM	Proinflammatory cytokines; (TNF-α, IL-6, IL-1β) ↓; iNOS, COX-2 ↓; p38-MAPK, NF-κB ↓	[50]
RAW264.7	LPS	20, 50, 100 uM	iNOS, TLR4, MyD88, TRAF6 ↓; Activation NF-κB signaling pathway; (p65, IkB ↓)	[46]
RAW264.7	Palmitic acid	200 uM	TNF-α, IFN-γ, IL-1β, IL-6, MCP1 ↓; Gpr94, XBP-1, Edem1, Herp, CHOP ↓; ROS generation ↓	[51]
HepG2	Oleic acid	20, 40 uM	Hepatic triglyceride ↓; MDA, PPARα, SREBP-1, COX-2, LC3-B ↓; SOD activity ↑	[47]
hPDLSCs	TNF-α	10 umol/L	Osteogenic differentiation; (ALP, RUNX2) ↑; mTOR ↑	[48]

LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-alpha; IL, interleukin; ↑, increased; ↓, decreased; iNOS, inducible nitric oxide synthase; COX, cyclooxygenase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor kappa B; TLR, Toll-like receptor; MyD88, myeloid differentiation primary response 88; TRAF6, tumor necrosis factor receptor-associated factor 6; IκB, inhibitor of nuclear factor kappa B; IFN-γ, interferon-gamma; MCP1, monocyte chemoattractant protein-1; Gpr94, glucose-regulated protein 94; XBP-1, X-Box binding protein 1. Edem1, ER degradation-enhancing alpha-mannosidase-like protein 1; Herp, homocysteine-responsive stress protein; CHOP, CCAAT-enhancer-binding protein homologous protein; ROS, reactive oxygen species; MDA, 3,4-methylenedioxyamphetamine; PPARα, peroxisome proliferator-activated receptor alpha; SREBP-1, sterol regulatory element binding protein 1; SOD, superoxide dismutase; ALP, alkaline phosphatase; RUNX2, runt-related transcription factor 2; mTOR, mammalian target of rapamycin.

https://doi.org/10.4162/nrp.2025.19.1.143

Nutrition Research and Practice

Rutin has demonstrated anti-inflammatory activity in both *in vivo* and *in vitro* models. Rutin prevents inflammatory responses by regulating pro-inflammatory cytokines, iNOS, and COX-2 via the Toll-like receptor 4–myeloid differentiation primary response 88–TNF receptor associated factor 6–NF-κB pathway in LPS-induced mouse macrophages [46]. Furthermore, rutin attenuates palmitic acid-induced inflammation and oxidative stress in macrophages while enhancing antioxidant enzyme activity in liver cancer cells exposed to oleic acid [47]. In human periodontal ligament stem cells, rutin inhibits the mammalian target of rapamycin signaling pathway, protecting against TNF-α–induced abnormal osteogenic differentiation [48]. The imbalance of pro- and anti-inflammatory cytokines is a key feature of inflammatory diseases [52]; rutin maintains anti-inflammatory cytokine levels while suppressing pro-inflammatory cytokine expression. Rutin may suppress pro-inflammatory cytokine production by inhibiting NF-κB activation.

Our experimental settings may have significant limitations when considering the absorption and distribution of rutin in the gut. Rutin absorption through the gastrointestinal tract requires acid hydrolysis catalyzed by α-rhamnosidase and β-glucosidase enzymes of intestinal microorganisms [53]. Rutin, after undergoing acid hydrolysis, is converted into 3,4-dihydroxyphenylacetic acid, 3,4-dihydroxytoluene, m-hydroxyphenylacetic acid, 3-methoxy-4-hydroxyphenylacetic acid (homovanillic acid), and 3,5,7,3′,5′-pentahydroxyflavonol (quercetin). The hydrolysis process is necessary for the formation of these metabolites, which subsequently leads to reduced intestinal absorption and bioavailability of rutin compared to quercetin. [49]. Therefore, rutin may be metabolized into other compounds when administered to canine [54]. Addressing these issues of absorption and distribution requires further research to apply our findings practically, utilizing various potential metabolites of rutin.

SUPPLEMENTARY MATERIALS

Supplementary Table 1
Antibodies used for western blot analysis

Supplementary Table 2
Primer sequences for quantitative reverse transcription polymerase chain reaction

Supplementary Fig. 1
The effect of rutin on DH82 cell viability. After 24 h of rutin treatment (0–100 μM), the cell viability is measured using the MTT assay. Values are expressed as the mean ± SD. The ratios relative to the control are represented as percentages.

REFERENCES

1. Philpotts I, Dillon J, Rooney N. Improving the welfare of companion dogs-is owner education the solution? Animals (Basel) 2019;9:662. PUBMED | CROSSREF
2. Cargnello M, Roux PP. Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases. Microbiol Mol Biol Rev 2011;75:50-83. PUBMED | CROSSREF
3. Evason M, McGrath M, Stull J. Companion animal preventive care at a veterinary teaching hospital - knowledge, attitudes, and practices of clients. Can Vet J 2021;62:484-90. PUBMED
4. Rah H, Choi SH. Are Veterinary costs and socioeconomic status risk factors for companion animal relinquishment in the Republic of Korea? Animals (Basel) 2023;13:3406. PUBMED | CROSSREF

https://doi.org/10.4162/nrp.2025.19.1.143

5. Buff PR, Carter RA, Bauer JE, Kersey JH. Natural pet food: a review of natural diets and their impact on canine and feline physiology. J Anim Sci 2014;92:3781-91. PUBMED | CROSSREF
6. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X, Zhao L. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget 2017;9:7204-18. PUBMED | CROSSREF
7. Michálʼová A, Takáčová M, Karasová M, Kunay L, Grelová S, Fialkovičová M. Comparative study of classical and alternative therapy in dogs with allergies. Animals (Basel) 2022;12:1832. PUBMED | CROSSREF
8. Bunzendahl L, Moussavi A, Bleyer M, Dehnert J, Boretius S, Neumann S. Histological findings and T2 relaxation time in canine menisci of elderly dogs-an *ex vivo* study in stifle joints. Vet Sci 2023;10:182. PUBMED | CROSSREF
9. Díaz-Regañón D, García-Sancho M, Villaescusa A, Sainz Á, Agulla B, Reyes-Prieto M, Rodríguez-Bertos A, Rodríguez-Franco F. Characterization of the fecal and mucosa-associated microbiota in dogs with chronic inflammatory enteropathy. Animals (Basel) 2023;13:326. PUBMED | CROSSREF
10. Sousa CA, Halliwell RE. The ACVD task force on canine atopic dermatitis (XI): the relationship between arthropod hypersensitivity and atopic dermatitis in the dog. Vet Immunol Immunopathol 2001;81:233-7. PUBMED | CROSSREF
11. Bulua AC, Simon A, Maddipati R, Pelletier M, Park H, Kim KY, Sack MN, Kastner DL, Siegel RM. Mitochondrial reactive oxygen species promote production of proinflammatory cytokines and are elevated in TNFR1-associated periodic syndrome (TRAPS). J Exp Med 2011;208:519-33. PUBMED | CROSSREF
12. Viola A, Munari F, Sánchez-Rodríguez R, Scolaro T, Castegna A. The metabolic signature of macrophage responses. Front Immunol 2019;10:1462. PUBMED | CROSSREF
13. Skrzypczak-Wiercioch A, Sałat K. Lipopolysaccharide-induced model of neuroinflammation: mechanisms of action, research application and future directions for its use. Molecules 2022;27:5481. PUBMED | CROSSREF
14. Wei F, Zhu H, Li N, Yu C, Song Z, Wang S, Sun Y, Zheng L, Wang G, Huang Y, et al. Stevioside activates AMPK to suppress inflammation in macrophages and protects mice from LPS-induced lethal shock. Molecules 2021;26:858. PUBMED | CROSSREF
15. Plotnikov A, Zehorai E, Procaccia S, Seger R. The MAPK cascades: signaling components, nuclear roles and mechanisms of nuclear translocation. Biochim Biophys Acta 2011;1813:1619-33. PUBMED | CROSSREF
16. Ganeshpurkar A, Saluja AK. The pharmacological potential of rutin. Saudi Pharm J 2017;25:149-64. PUBMED | CROSSREF
17. Yang J, Guo J, Yuan J. *In vitro* antioxidant properties of rutin. Lebensm Wiss Technol 2008;41:1060-6. CROSSREF
18. Ivanov M, Novović K, Malešević M, Dinić M, Stojković D, Jovčić B, Soković M. Polyphenols as inhibitors of antibiotic resistant bacteria-mechanisms underlying rutin interference with bacterial virulence. Pharmaceuticals (Basel) 2022;15:385. PUBMED | CROSSREF
19. Satari A, Ghasemi S, Habtemariam S, Asgharian S, Lorigooini Z. Rutin: a flavonoid as an effective sensitizer for anticancer therapy; insights into multifaceted mechanisms and applicability for combination therapy. Evid Based Complement Alternat Med 2021;2021:9913179. PUBMED | CROSSREF
20. Yoo H, Ku SK, Baek YD, Bae JS. Anti-inflammatory effects of rutin on HMGB1-induced inflammatory responses *in vitro* and *in vivo*. Inflamm Res 2014;63:197-206. PUBMED | CROSSREF
21. Ghorbani A. Mechanisms of antidiabetic effects of flavonoid rutin. Biomed Pharmacother 2017;96:305-12. PUBMED | CROSSREF
22. Suebsaard P, Charerntantanakul W. Rutin, α-tocopherol, and l-ascorbic acid up-regulate type I interferon-regulated gene and type I and II interferon expressions and reduce inflammatory cytokine expressions in monocyte-derived macrophages infected with highly pathogenic porcine reproductive and respiratory syndrome virus. Vet Immunol Immunopathol 2021;235:110231. PUBMED | CROSSREF
23. Farha AK, Gan RY, Li HB, Wu DT, Atanasov AG, Gul K, Zhang JR, Yang QQ, Corke H. The anticancer potential of the dietary polyphenol rutin: current status, challenges, and perspectives. Crit Rev Food Sci Nutr 2022;62:832-59. PUBMED | CROSSREF
24. Fusco R, Siracusa R, Gugliandolo E, Peritore AF, D'Amico R, Cordaro M, Crupi R, Impellizzeri D, Gomiero C, Cuzzocrea S, et al. Micro composite palmitoylethanolamide/rutin reduces vascular injury through modulation of the Nrf2/HO-1 and NF-kB pathways. Curr Med Chem 2021;28:6287-302. PUBMED | CROSSREF
25. Elsawy H, Badr GM, Sedky A, Abdallah BM, Alzahrani AM, Abdel-Moneim AM. Rutin ameliorates carbon tetrachloride (CCl_4)-induced hepatorenal toxicity and hypogonadism in male rats. PeerJ 2019;7:e7011. PUBMED | CROSSREF
26. Lee J, Lee JK, Lee JJ, Park S, Jung S, Lee HJ, Ha JH. Partial replacement of high-fat diet with beef tallow attenuates dyslipidemia and endoplasmic reticulum stress in *db*/*db* mice. J Med Food 2022;25:660-74. PUBMED | CROSSREF

27. Kim J, Eun S, Jung H, Kim J, Kim J. *Boswellia serrata* extracts ameliorates symptom of irregularities in articular cartilage through inhibition of matrix metalloproteinases activation and apoptosis in monosodium-iodoacetate-induced osteoarthritic rat models. Prev Nutr Food Sci 2023;28:285-92. PUBMED | CROSSREF

28. Amelly LJ, Lee J, Jang H, Kang D, Kim I, Ha JH. Physicochemical properties of Yanggaeng with added tempeh powder. Prev Nutr Food Sci 2023;28:514-9. PUBMED | CROSSREF

29. Huang B, Yang XD, Lamb A, Chen LF. Posttranslational modifications of NF-κB: another layer of regulation for NF-κB signaling pathway. Cell Signal 2010;22:1282-90. PUBMED | CROSSREF

30. Guo Q, Jin Y, Chen X, Ye X, Shen X, Lin M, Zeng C, Zhou T, Zhang J. NF-κB in biology and targeted therapy: new insights and translational implications. Signal Transduct Target Ther 2024;9:53. PUBMED | CROSSREF

31. Hinz M, Scheidereit C. The IκB kinase complex in NF-κB regulation and beyond. EMBO Rep 2014;15:46-61. PUBMED | CROSSREF

32. Alfaro S, Acuña V, Ceriani R, Cavieres MF, Weinstein-Oppenheimer CR, Campos-Estrada C. Involvement of inflammation and its resolution in disease and therapeutics. Int J Mol Sci 2022;23:10719. PUBMED | CROSSREF

33. Kim SC, Kim S. Development of a dog health score using an artificial intelligence disease prediction algorithm based on multifaceted data. Animals (Basel) 2024;14:256. PUBMED | CROSSREF

34. Wellman ML, Krakowka S, Jacobs RM, Kociba GJ. A macrophage-monocyte cell line from a dog with malignant histiocytosis. In Vitro Cell Dev Biol 1988;24:223-9. PUBMED | CROSSREF

35. Herrmann I, Gotovina J, Fazekas-Singer J, Fischer MB, Hufnagl K, Bianchini R, Jensen-Jarolim E. Canine macrophages can like human macrophages be in vitro activated toward the M2a subtype relevant in allergy. Dev Comp Immunol 2018;82:118-27. PUBMED | CROSSREF

36. Ferrolho J, Simpson J, Hawes P, Zweygarth E, Bell-Sakyi L. Growth of *Ehrlichia canis*, the causative agent of canine monocytic ehrlichiosis, in vector and non-vector ixodid tick cell lines. Ticks Tick Borne Dis 2016;7:631-7. PUBMED | CROSSREF

37. Nadaes NR, Silva da Costa L, Santana RC, LaRocque-de-Freitas IF, Vivarini ÁC, Soares DC, Wardini AB, Gazos Lopes U, Saraiva EM, Freire-de-Lima CG, et al. DH82 canine and RAW264.7 murine macrophage cell lines display distinct activation profiles upon interaction with *Leishmania infantum* and *Leishmania amazonensis*. Front Cell Infect Microbiol 2020;10:247. PUBMED | CROSSREF

38. Chen S, Saeed AF, Liu Q, Jiang Q, Xu H, Xiao GG, Rao L, Duo Y. Macrophages in immunoregulation and therapeutics. Signal Transduct Target Ther 2023;8:207. PUBMED | CROSSREF

39. Jeong GH, Lee H, Woo SY, Lee HK, Chung BY, Bai HW. Novel aminopyridazine derivative of minaprine modified by radiolysis presents potent anti-inflammatory effects in LPS-stimulated RAW 264.7 and DH82 macrophage cells. Sci Rep 2023;13:10887. PUBMED | CROSSREF

40. Zhao H, Wu L, Yan G, Chen Y, Zhou M, Wu Y, Li Y. Inflammation and tumor progression: signaling pathways and targeted intervention. Signal Transduct Target Ther 2021;6:263. PUBMED | CROSSREF

41. Choi SS, Park HR, Lee KA. A comparative study of rutin and rutin glycoside: antioxidant activity, anti-inflammatory effect, effect on platelet aggregation and blood coagulation. Antioxidants 2021;10:1696. PUBMED | CROSSREF

42. Liu Y, Huang W, Ji S, Wang J, Luo J, Lu B. Sophora japonica flowers and their main phytochemical, rutin, regulate chemically induced murine colitis in association with targeting the NF-κB signaling pathway and gut microbiota. Food Chem 2022;393:133395. PUBMED | CROSSREF

43. Khajevand-Khazaei MR, Mohseni-Moghaddam P, Hosseini M, Gholami L, Baluchnejadmojarad T, Roghani M. Rutin, a quercetin glycoside, alleviates acute endotoxemic kidney injury in C57BL/6 mice via suppression of inflammation and up-regulation of antioxidants and SIRT1. Eur J Pharmacol 2018;833:307-13. PUBMED | CROSSREF

44. Domitrović R, Jakovac H, Vasiljev Marchesi V, Vladimir-Knežević S, Cvijanović O, Tadić Z, Romić Z, Rahelić D. Differential hepatoprotective mechanisms of rutin and quercetin in CCl_4-intoxicated BALB/cN mice. Acta Pharmacol Sin 2012;33:1260-70. PUBMED | CROSSREF

45. Nafees S, Rashid S, Ali N, Hasan SK, Sultana S. Rutin ameliorates cyclophosphamide induced oxidative stress and inflammation in Wistar rats: role of NFκB/MAPK pathway. Chem Biol Interact 2015;231:98-107. PUBMED | CROSSREF

46. Tian C, Liu X, Chang Y, Wang R, Yang M, Liu M. Rutin prevents inflammation induced by lipopolysaccharide in RAW 264.7 cells via conquering the TLR4-MyD88-TRAF6-NF-κB signalling pathway. J Pharm Pharmacol 2021;73:110-7. PUBMED | CROSSREF

47. Liu Q, Pan R, Ding L, Zhang F, Hu L, Ding B, Zhu L, Xia Y, Dou X. Rutin exhibits hepatoprotective effects in a mouse model of non-alcoholic fatty liver disease by reducing hepatic lipid levels and mitigating lipid-induced oxidative injuries. Int Immunopharmacol 2017;49:132-41. PUBMED | CROSSREF

48. Zhao B, Zhang W, Xiong Y, Zhang Y, Jia L, Xu X. Rutin protects human periodontal ligament stem cells from TNF-α induced damage to osteogenic differentiation through suppressing mTOR signaling pathway in inflammatory environment. Arch Oral Biol 2020;109:104584. PUBMED | CROSSREF

49. Manach C, Morand C, Demigné C, Texier O, Régérat F, Rémésy C. Bioavailability of rutin and quercetin in rats. FEBS Lett 1997;409:12-6. PUBMED | CROSSREF

50. Su KY, Yu CY, Chen YP, Hua KF, Chen YL. 3,4-Dihydroxytoluene, a metabolite of rutin, inhibits inflammatory responses in lipopolysaccharide-activated macrophages by reducing the activation of NF-κB signaling. BMC Complement Altern Med 2014;14:21. PUBMED | CROSSREF

51. Gao M, Ma Y, Liu D. Rutin suppresses palmitic acids-triggered inflammation in macrophages and blocks high fat diet-induced obesity and fatty liver in mice. Pharm Res 2013;30:2940-50. PUBMED | CROSSREF

52. Cicchese JM, Evans S, Hult C, Joslyn LR, Wessler T, Millar JA, Marino S, Cilfone NA, Mattila JT, Linderman JJ, et al. Dynamic balance of pro- and anti-inflammatory signals controls disease and limits pathology. Immunol Rev 2018;285:147-67. PUBMED | CROSSREF

53. Riva A, Kolimár D, Spittler A, Wisgrill L, Herbold CW, Abrankó L, Berry D. Conversion of rutin, a prevalent dietary flavonol, by the human gut microbiota. Front Microbiol 2020;11:585428. PUBMED | CROSSREF

54. Reinboth M, Wolffram S, Abraham G, Ungemach FR, Cermak R. Oral bioavailability of quercetin from different quercetin glycosides in dogs. Br J Nutr 2010;104:198-203. PUBMED | CROSSREF