SHST
Horticulture and Forestry Society of Transylvania

Okoko T and Robert FO (2022)
**Notulae Scientia Biologicae**
Volume 14, Issue 3, Article number 11294
DOI:10.15835/nsb14311294
**Research Article**

NSB
Notulae Scientia Biologicae

# Inhibitory potential of rutin on lipopolysaccharide-induced toxicity and inflammatory response of raw U937 cells and macrophages

Tebekeme OKOKO*, Faith O. ROBERT

*Niger Delta University, Department of Biochemistry, Nigeria; tebebuddy@yahoo.com (*corresponding author); fthrobert@yahoo.co.uk*

## Abstract

Rutin is an important flavonoid found in plants with enormous pharmacological activities in various experimental models while lipopolysaccharide is an amphipathic glycolipid with potent inflammatory activity. The protective effect of rutin on lipopolysaccharide-mediated cytotoxity and inflammatory effect on U937 cells and macrophages was investigated. U937 cells were incubated with or without rutin (50 - 200 μM) and later exposed to lipopolysaccharide (5 μg/mL). Cell viability and the production of reactive oxygen species were later analyzed. In the other experiment, the cells were differentially-induced to macrophages and incubated with or without rutin before lipopolysaccharide exposure. The secretion of cytokines and expression of some transcription factors and enzymes were analyzed. It revealed that incubating cells with lipopolysaccharide alone caused significant cell death and production of reactive oxygen species which were reduced when cells were pre-incubated with rutin. Exposure of macrophages to lipopolysaccharide also resulted in significant secretion of both TNF-α and IL-6 which was reduced by rutin. Endotoxin also enhanced the expression of the transcription factors (NF-κB and iNOS) while reduced the expression of the antioxidant enzymes superoxide dismutase and catalase. The lipopolysaccharide-induced alterations in transcription were significantly reduced when macrophages were pre-incubated with rutin. Implications of the findings are discussed.

***Keywords:*** cytotoxicity; cytokines; lipopolysaccharide; macrophages; rutin; transcription factors

## Introduction

The consumption of plants/plant products for medicinal purposes has been a routine practice for decades if not for centuries. It is believed that the medicinal potentials of these plants stem from important phytochemicals which are often products of secondary metabolism. Polyphenols, especially flavonoids have attracted serious attention because of their immense bioactivities in both *in vivo* and *in vitro* studies (Terao, 2009; Pieta, 2010). Rutin (also known as vitamin P or rutoside) is an important flavonoid found in plants such as tea leaves, apples etc with enormous pharmacological properties (Al-Dhabi *et al.*, 2015; Ganeshpurkar and Saluja, 2017). Known pharmacological activities of rutin in experimental models include anti-neurodegradative, anti-hepatotoxic, anti-inflammatory, hypouricemic, antioxidant etc. (Al-Dhabi *et al.*, 2015; Iriti *et al.*, 2017; Enogieru *et al.*, 2018).

Lipopolysaccharide (also known as endotoxin) is located at the outer leaflet of the outer membrane of Gram-negative bacteria (Wang and Quin, 2010). Lipopolysaccharide is an amphipathic glycolipid which gives

*Received: 07 Jun 2022. Received in revised form: 26 Jul 2022. Accepted: 04 Aug 2022. Published online: 23 Sep 2022.*
From Volume 13, Issue 1, 2021, Notulae Scientia Biologicae journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

Gram-negative bacteria a strong permeability barrier and enables the organisms thrive in harsh environmental conditions (Snyder and Mclntosh, 2000; Qiao *et al*., 2014; Rios *et al*., 2016). Lipopolysaccharide is a virulence factor that binds to a specific target (especially cells of the innate immune system) and the resulting complex activates signal transduction pathways, transcription factors and induces gene expression (Guha *et al*., 2001). The events cause cell activation and production of endogenous mediators including pro-inflammatory cytokines, adhesion molecules, acute proteins, vasoactive amines, nitric oxide, prostaglandins which could be chemotactic (Zhang and Ghosh, 2000; Guha *et al*., 2001; Triantafilou *et al*., 2002). The current work investigates the protective effect of rutin against lipopolysaccharide-mediated cytotoxicity, production of reactive oxygen species and activation of the inflammatory response.

## Materials and Methods

*Cell culture and toxicity*

The cell line U937 was grown in complete RPMI medium as reported by Okoko and Oruambo (2009). Briefly, cells were grown in a $CO_2$ (5%) incubator at 37 °C with RPMI supplemented with heat inoculated fetal calf serum, *L*-glutamine (0.02M) and penicillin-streptomycin. Cell numbers were maintained at 5 x $10^4$ cells/mL before exposure to either rutin or lipopolysaccharide. For toxicity study, cells were incubated with or without rutin (50 - 200 µM) for 24 h before exposure to lipopolysaccharide (5 µg/mL) at 37 °C for 1 h. Cell viability was analysed via the MTT reduction assay according to Zhou et al. (2006) as modified (Okoko and Ndoni, 2021). The production of reactive oxygen species (ROS) was also assessed according to the method of Koga and Meydani (2001) as modified (Okoko, 2020).

*Cytokine production*

Cell line U937 was subjected to PMA (phorbol-12, myristate-13.acetate)-induced differentiation as described (Okoko and Oruambo, 2009). Media were removed and replaced with or without rutin and incubated for 24 h at 37 °C. Cells (now differentiated to macrophages) were subsequently incubated with or without lipopolysaccharide (5 µg/mL) and production of TNF-α and IL-6 analysed via cytokine capture ELISA as described (Okoko and Oruambo, 2009).

*Quantitative RT-PCR*

The expression of the transcription factors inducible nitric oxide synthase (iNOS), nuclear factor kappa B (NF-κB), and the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) was performed as reported by Okoko and Ndoni (2021). Briefly, total RNA was extracted from cells followed by cDNA synthesis. Sequences specific to iNOS, NF-κB, SOD and CAT were amplified with primer pairs listed in Table 1. Real-Time PCR data were analyzed and presented as fold change in expression to the GAPDH housekeeping gene of same sample.

*Data analysis*

Values are expressed as mean ± SEM from six replicates. Comparisons were done by subjecting raw data to analysis of variance followed by Duncan's multiple range tests. Significance was set at $p < 0.05$.

**Table 1.** Primers pairs for RT-PCR

| mRNA | Primer sequence (5′-3′) |
|---|---|
| iNOS | FP: GTGCCACCTCCAGTCCAG<br>RP:GCTGCCCCAGTTTTTGATCC |
| NF-κB | FP:GCCTTGCATCTAGCCACAGAG<br>RP:GATGTCAGCACCAGCCTTCAG |
| SOD | FP:GACTGAAGGCCTGCATGGATTC<br>RP: CACATCGGCCACACCATCTTTG |
| CAT | FP:CTTCGACCCAAGCAACATGC<br>RP:GATAATTGGGTCCCAGGCGATG |
| GAPDH | FP:GTCGGAGTCAACGGATTTGGTC<br>RP:CTTCCCGTTCTCAGCCTTGAC |

## Results

*Cell toxicity and production of ROS*

Cell viability was determined via the MTT reduction assay and presented in Figure 1. It revealed that LPS caused significant cytotoxicity when compared to controls. Pre-incubating cells with rutin prior to LPS exposure enhanced cell viability closer to control value. Viable cells following pre-incubation with 100 µM rutin was significantly higher than treatment with 50 µM rutin ($p < 0.05$). However, there was no significant difference in viable cells between treatment with 100 µM and 200 µM rutin ($p > 0.05$). As shown in Figure 2, incubating the cells with LPS alone resulted in significant production of ROS when compared with control ($p < 0.05$). However, pre-incubating cells with rutin (at different concentrations) reduced production of ROS which was concentration-dependent ($p < 0.05$).

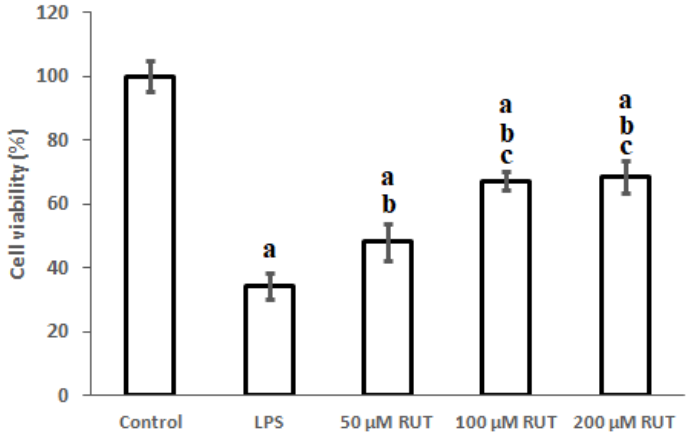


**Figure 1.** Effect of rutin on lipopolysaccharide-induced cell death in U937 cells

LPS, cells treated with 5 µg/mL lipopolysaccharide only; 50µM RUT, cells supplemented with 50 µM RUT before exposure to lipopolysaccharide (5 µg/mL); 100 µM RUT, cells supplemented with 100 µM RUT before exposure to lipopolysaccharide (5 µg/mL); 200 µM RUT, cells supplemented with 200 µM RUT before exposure to lipopolysaccharide (1 µg/mL). Each bar represents mean ± S.E.M of six replicates expressed as % viability in comparison to control. [a]Significantly different from control; [b]significantly different from LPS and [c]significantly different from 50 µM RUT. $p < 0.05$

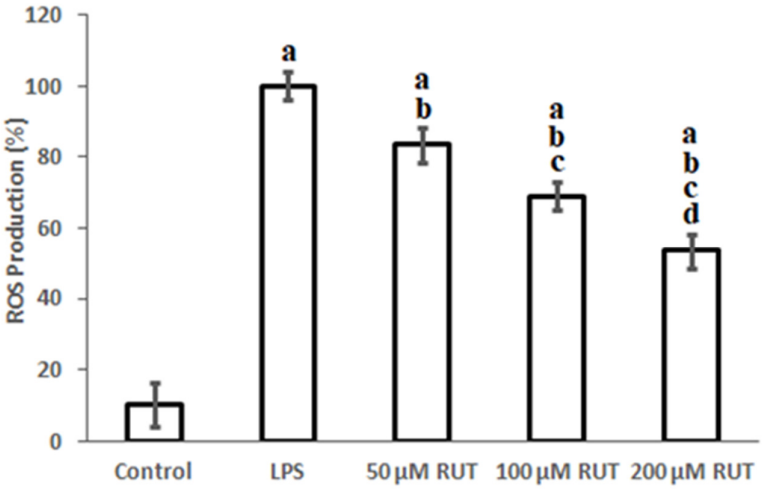


**Figure 2.** Effect of rutin on lipopolysaccharide-induced production of ROS in U937 cells
LPS, cells treated with 5 µg/mL lipopolysaccharide only; 50 µM RUT, cells supplemented with 50 µM RUT before exposure to lipopolysaccharide (5 µg/mL); 100 µM RUT, cells supplemented with 100 µM RUT before exposure to lipopolysaccharide (5 µg/mL); 200 µM RUT, cells supplemented with 200 µM RUT before exposure to lipopolysaccharide (1 µg/mL). Each bar represents mean ± S.E.M of six replicates expressed as % viability in comparison to control. [a]Significantly different from control; [b]significantly different from LPS and [c]significantly different from 50 µM RUT; [d]significantly difference from 100 µM RUT. $p < 0.05$

*Excretion of TNF-α and IL-6*

The effect of rutin on LPS-induced production of pro-inflammatory cytokines in U937-derived macrophages is presented in Figure 3. Lipopolysaccharide caused significant production of TNF-α and IL-6 which was reduced following pre-incubation with rutin (at different concentrations). There was no significant difference in the reduction of cytokine production following pre-treatment of macrophages with 50 µM and 100 µM rutin. However, pre-treatment of cells with 200 µM rutin significantly reduced cytokine production when compared with pre-incubation with other rutin concentrations ($p < 0.05$).

*Quantitative RT-PCR*

The effect of rutin on the expression of transcription factors and antioxidant enzymes is shown in Figure 4. Lipopolysaccharide significantly stimulated the expression of the transcription factors iNOS and NF-κB in U937-derived macrophages when compared to cells not treated with LPS ($p < 0.05$). Rutin significantly reduced the LPS-mediated inductions which was not concentration-dependent. Incubating the macrophages with LPS alone caused significant reduction in the expression of SOD and CAT. However, pre-incubating the macrophages with rutin prior to LPS exposure significantly enhanced the expression of both SOD and CAT closer to control values. However, the variations among pre-treatments of cells with different concentrations of rutin were not significant ($p < 0.05$).

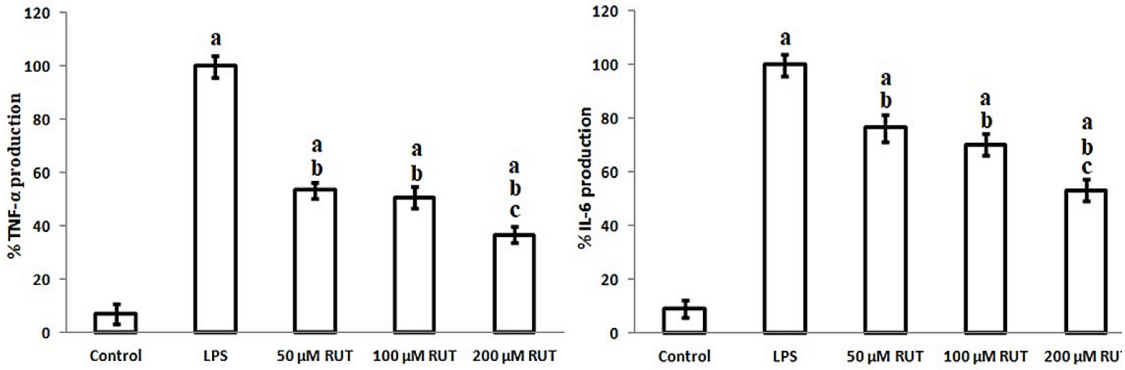


**Figure 3**. Production of TNF-α (left) and IL-6 (right) in U937-derived macrophages
LPS, cells treated with 5 µg/mL lipopolysaccharide only; 50 µM RUT, cells supplemented with 50 µM RUT before exposure to lipopolysaccharide (5 µg/mL); 100 µM RUT, cells supplemented with 100 µM RUT before exposure to lipopolysaccharide (5 µg/mL); 200 µM RUT, cells supplemented with 200 µM RUT before exposure to lipopolysaccharide (1 µg/mL). Each bar represents mean ± S.E.M of six replicates expressed as % viability in comparison to control. [a]Significantly different from control; [b]significantly different from LPS and [c]significantly different from 50 µM RUT; significantly difference from 100 µM RUT. $p < 0.05$

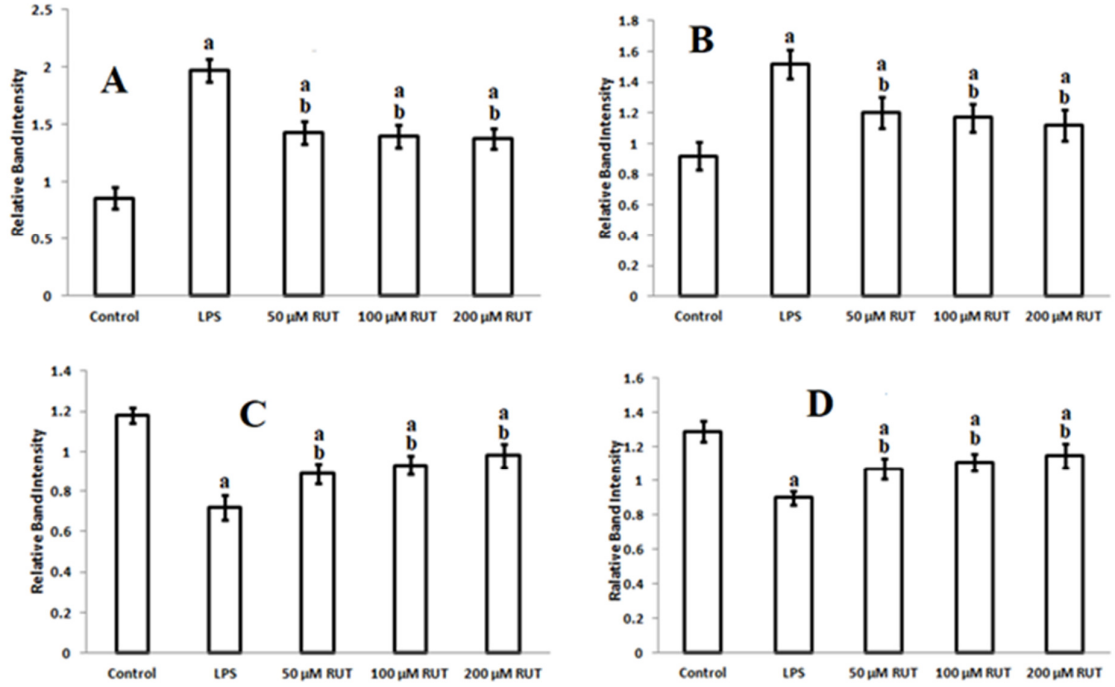


**Figure 4.** Expression of (A) iNOS; (B) NF-κB; (C) SOD; and (D) CAT in U937-derived macrophages
LPS, cells treated with 5 µg/mL lipopolysaccharide only; 50 µM RUT, cells supplemented with 50 µM RUT before exposure to lipopolysaccharide (5 µg/mL); 100 µM RUT, cells supplemented with 100 µM RUT before exposure to lipopolysaccharide (5 µg/mL); 200 µM RUT, cells supplemented with 200 µM RUT before exposure to lipopolysaccharide (1 µg/mL). Each bar represents mean ± S.E.M of six replicates expressed as % viability in comparison to control. [a]Significantly different from control; [b]significantly different from LPS. $p < 0.05$

## Discussion

Lipopolysaccharidde (LPS) is a major virulence factor of bacteria due to its powerful role in host pathogen relationship thus used as a model to induce infection and inflammation (Maldonado *et al*., 2016; Zhang *et al*., 2018). Though inflammation is a process employed by immune cells to fight infection, persistence is cytotoxic thus it is essential that a balance exists between immune activation and deactivation (to maintain immune homeostasis) (Gerber and Mosser, 2001). The dysregulation of the inflammatory response has been implicated in several pathological conditions such as septic shock, rheumatoid arthritis, diabetes, cancer, organ failure and even death and other chronic inflammatory diseases (Nicolls *et al*., 2007; Chen *et al*., 2018; Muller *et al*., 2019; Nakamura *et al*., 2020).

In this experiment, LPS induced significant toxicity and production of reactive oxygen species (ROS) in raw U937 cells which was reduced by rutin. It has been reported that LPS elicits either apoptotic or necrotic cell death which in some cases, is a consequence of the production of ROS (Meßmer *et al*., 1999; Simon and Fernández, 2009; Ozal *et al*., 2018). Flavonoids (including rutin) are well known antioxidants which inhibit the production of reactive oxygen species either directly or indirectly. They protect cells by inducing the expression of phase II detoxification proteins such as glutathione, γ-glutamylcystein ligase, glutathione S-transferase and NAD(P)H:quinine oxidoreductase in different cell systems (Maher and Hanneken, 2005; Angeloni *et al*., 2007). Though the cell has robust mechanisms to maintain oxidation/ antioxidation balance, excessive production of ROS could trigger a shift in this homeostasis and rutin could be an important flavonoid that protects cells from the effect of these highly reactive species.

The inflammatory process (most times triggered by non-self-signals) is the mechanism the immune system employs to remove pathogens, toxic compounds etc to initiate the healing process (Chen *et al*., 2018). The process activates various signaling pathways that modulate the production of cytokines, vasoactive amines, nitric oxide, etc (Dinarello, 1997; Zhang and An, 2007; Chen *et al*., 2018). Lipopolysaccharide induces the secretion of the pro-inflammatory cytokines TNF-α and IL-6 from U937-derived macrophages in this current experiment which could be attributable to many regulatory factors. This has been reported as a major mechanism of lung inflammation from exposure to some gram-negative bacteria (Park *et al*., 2017; Shi *et al*., 2018; Liu *et al*., 2018). However, in this current work, pre-incubation of the macrophages with rutin significantly reduced cytokine production which could be concentration-dependent.

The data also revealed the expression of iNOS and NF-κB was upregulated by LPS. The production of nitric oxide (NO) is catalyzed by iNOS thus up-regulation of its expression increases NO levels. Nitric oxide is an inorganic signal molecule that activates a dysregulated immune response (Kim *et al*., 2017). The signal molecule also reacts with superoxide radical to produce the highly reactive peroxynitrite which reacts freely with important macromolecules to cause tissue damage (Radi, 2018; Ahmad *et al*., 2019). This reveals that improper upregulation of iNOS production could alter redox balance (Somasundaram *et al*., 2019). The RT-PCR data revealed that rutin reduced LPS-mediated expression of iNOS and NF-κB. Nuclear factor kappa B (NF-κB) is a transcriptional factor that induces the production of pro-inflammatory cytokines and iNOS thus it is regarded as one of the most important factors during the inflammatory process and a potential drug target (Ferraz *et al*., 2020). Flavonoids inhibit the production of inflammatory mediators such as ROS, nitric oxide, iNOS, cytokines and the expression of NF-κB (Leyva *et al*., 2016; Salaritabar *et al*., 2017; Ferraz *et al*., 2020).

It has been reported that rutin decreases the activation of NF-κB expression in human embryonic cell line which correlates with the reduced production of IL-6, TNF-α and NO (Choy *et al*., 2019).

In order to further investigate the antioxidant potential of rutin, its effect on the elaboration of the enzymes catalase and superoxide dismutase was analyzed. These are important enzymes that convert highly reactive oxidants to water thus help maintain oxidation/antioxidation balance. The results revealed that pre-treatment of transformed U937 cells (i.e. U937-derived macrophages) with rutin prior to LPS exposure

significantly promoted the activities of the enzymes at the transcription level. The mechanism via which rutin reversed the LPS-mediated alterations is a subject of further investigation.

## Conclusions

Lipopolysaccharide at the concentration of 5 µg/mL caused significant cytotoxicity which could be ascribed to the production of ROS. The glycolipid also enhanced the production of pro-inflammatory cytokines which could be mediated via inhibition of key regulatory and antioxidant genes. But the supplementation of rutin significantly reversed the LPS-induced alteration close to control levels which could be concentration-dependent. In summary, rutin significantly reduced LPS-mediated cytotoxicity and production of ROS via the alteration of the inflammatory response.

## Authors' Contributions

Both authors read and approved the final manuscript.

**Ethical approval** (for researches involving animals or humans)

Not applicable

## Acknowledgements

Authors are thankful to the authorities of the Niger Delta University for support.

## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

## References

Ahmad R, Hussain A, Ahsan H (2019) Peroxynitrite: cellular pathology and implications in autoimmunity. Journal of Immunoassay and Immunochemistry 40:123-138. *https://doi.org/10.1080/15321819.2019.1583109*

Al-Dhabi NA, Arasu MV, Park CH, Park, S.U. (2015). An up-to-date review of rutin and its biological and pharmacological activities. EXCLI Journal 14:59. *http://dx.doi.org/10.17179/excli2014-663*

Angeloni C, Spencer JPE, Leoncini E, Biagi PL, Hrelia S. (2007). Role of quercetin and its *in vivo* metabolites in protecting H9c2 cells against oxidative stress. Biochimie 89:73-82. *https://doi.org/10.1016/j.biochi.2006.09.006*

Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X, Zhao L. (2018). Inflammatory responses and inflammation-associate diseases in organs. Oncotarget 9(6):7204. *https://dx.doi/10.18632/oncotarget.23208*

Choy KW, Murugan D, Leong XF, Abas R, Alias A, Mustafa MR. (2019). Flavonoids as natural anti-inflammatory agents targeting nuclear factor-kappa B (NFκB) signaling in cardiovascular diseases: a mini review. Frontiers in Pharmacology 10:1295. *https://doi.org/10.3389/fphar.2019.01295*

Dinarello C A. (1997) Role of pro- and anti-inflammatory cytokines during inflammation: experimental and clinical findings. Journal of Biological Regulators and Homeostatic Agents 11:91-103. *https://europepmc.org/article/med/9498158*

Enogieru AB, Haylett W, Hiss DC, Bardien S, Ekpo OE. (2018). Rutin as a potent antioxidant: implications for neurodegenerative disorders. Oxidative Medicine and Cellular Longevity 6241017. *https://doi.org/10.1155/2018/6241017*

Ferraz CR, Carvalho TT, Manchope MF, Artero NA, Rasquel-Oliveira FS, Fattori V, Casagrande R, Verri WA. (2020). Therapeutic potential of flavonoids in pain and inflammation: mechanisms of action, pre-clinical and clinical data, and pharmaceutical development. Molecules 25(3):762. *https://doi.org/10.3390/molecules25030762*

Ganeshpurkar A, Saluja AK. (2017). The pharmacological potential of rutin. Saudi Pharmaceutical Journal 25:149-164. *https://doi.org/10.1016/j.jsps.2016.04.025*

Gerber JS, Mosser DM. (2001). Reversing lipopolysaccharide toxicity by ligating the macrophage Fcγ receptors. The Journal of Immunology 166:6861-6868. *https://doi.org/10.4049/jimmunol.166.11.6861*

Guha M, O'Connell MA, Pawlinski R, Hollis A, McGovern P, Yan SF, Stern D, Mackman N. (2001). Lipopolysaccharide activation of the MEK-ERK1/2 pathway in human monocytic cells mediates tissue factor and tumor necrosis factor α expression by inducing Elk-1 phosphorylation and Egr-1 expression. Blood, The Journal of the American Society of Hematology 98:1429-1439. *https://doi.org/10.1182/blood.V98.5.1429*

Iriti M, Kubina R, Cochis A, Sorrentino R, Varoni EM, Kabała-Dzik A, Azzimonti B, Dziedzic A, Rimondini L, Wojtyczka RD. (2017). Rutin, a quercetin glycoside, restores chemosensitivity in human breast cancer cells. Phytotherapy Research 31:1529-1538. *https://doi.org/10.1002/ptr.5878*

Kim SH, Park SY, Park YL, Myung DS, Rew JS, Joo YE. (2017). Chlorogenic acid suppresses lipopolysaccharide-induced nitric oxide and interleukin-1β expression by inhibiting JAK2/STAT3 activation in RAW264.7 cells. Molecular Medicine Reports 16:9224-9232. *https://doi.org/10.3892/mmr.2017.7686*

Koga T, Meydani M. (2001). Effect of plasma metabolites of (+)-catechin and quercetin on monocyte adhesion to human aortic endothelial cells. American Journal of Clinical Nutrition 73:941-948. *https://doi.org/10.1093/ajcn/73.5.941*

Leyva-López N, Gutierrez-Grijalva EP, Ambriz-Perez DL, Heredia JB. (2016). Flavonoids as cytokine modulators: a possible therapy for inflammation-related diseases. International Journal of Molecular Sciences 17:921. *https://doi.org/10.3390/ijms17060921*

Liu X, Yin S, Chen Y, Wu Y, Zheng W, Dong H, Bai Y, Qin Y, Li J, Feng S, Zhao P. (2018). LPS-induced proinflammatory cytokine expression in human airway epithelial cells and macrophages via NF-κB, STAT3 or AP-1 activation. Molecular Medicine Reports 17:5484-5491. *https://doi.org/10.3892/mmr.2018.8542*

Maher P, Hanneken A. (2005). Flavonoids protect retinal ganglion cells from oxidative stress–induced death. Investigative Ophthalmology and Visual Science 46:4796-4803. *https://doi.org/10.1167/iovs.05-0397*

Maldonado RF, Sa-Correia I, Valvano MA. (2016). Lipopolysaccharide modification in Gram-negative bacteria during chronic infection. FEMS Microbiology Reviews 40:480-493. *https://doi.org/10.1093/femsre/fuw007*

Meßmer UK, Briner VA, Pfeilschifter J. (1999). Tumor necrosis factor-α and lipopolysaccharide induce apoptotic cell death in bovine glomerular endothelial cells. Kidney International 55:2322-2337. *https://doi.org/10.1046/j.1523-1755.1999.00473.x*

Muller L, Benedetto SD, Pawelec G. (2019) The immune system and its dysregulation with aging. In: Harris, J. and Korolchuk V (Eds). Biochemistry and Cell Biology of ageing: Path II Clinical Science. Subcelluar Biochemistry, pp 21-43. *https://doi.org/10.1007/978-981-13-3681-2_2*

Nakamura K, Smyth MJ, Martinet L. (2020) Cancer immunoediting and immune dysregulation in multiple myeloma. Blood 136:2731-2740. *https://doi.org/10.1182/blood.2020006540*

Nicolls MR, Haskins K, Flores SC. (2007). Oxidant stress, immune dysregulation and vascular function in type I diabetes. Antioxidants and Redox Signaling 97:879-889. *https://doi.org/10.1089/ars.2007.1631*

Okoko T. (2020) Glycine and selenium (separately and in combination) reduced bromate-mediated oxidative stress and inflammatory response in U937 cells. Asian Journal of Biochemistry, Genetics and Molecular Biology 6:8-16. *https://doi.org/10.9734/ajbgmb/2020/v6i130142*

Okoko T, Ndoni SA (2021) Protective effect of kolaviron on bromate-induced toxicity on raw U937 cells and macrophages. Malaysian Journal of Biochemistry and Molecular Biology 2021:169-174.

Okoko T, Oruambo IF (2009). Inhibitory activity of quercetin and its metabolite on lipopolysaccharide-induced activation of macrophage U937 cells. Food and Chemical Toxicology 47:809-812. *https://doi.org/10.1016/j.fct.2009.01.013*

Ozal SA, Turkekul K, Gurlu V, Guclu H, Erdogan S. (2018). Esculetin protects human retinal pigment epithelial cells from lipopolysaccharide-induced Inflammation and cell death. Current Eye Research 43:1169-1176. *https://doi.org/10.1080/02713683.2018.1481517*

Park JY, Chung TW, Jeong YJ, Kwak CH, Ha SH, Kwon KM, ... Magae J (2017). Ascofuranone inhibits lipopolysaccharide–induced inflammatory response via NF-kappaB and AP-1, p-ERK, TNF-α, IL-6 and IL-1β in RAW 264.7 macrophages. PLoS One 12:e0171322. *https://doi.org/10.1371/journal.pone.0171322*

Pietta PG (2000). Flavonoids as antioxidants. Journal of Natural Products 63:1035-1042. *https://doi.org/10.1021/np9904509*

Qiao S, Luo Q, Zhao Y, Zhang XC, Huang Y. (2014). Structural basis for lipopolysaccharide insertion in the bacterial outer membrane. Nature 511:108-111. *https://doi.org/10.1038/nature13484*

Radi R. (2018) Oxygen radicals, nitric oxide and peroxynitrite: Redox pathways in molecular medicine. Proceedings of the National Academy of Sciences 115:5839-5848. *https://doi.org/10.1073/pnas.1804932115*

Rios ECS, de Lima TM, Moretti AIS, Soriano FG. (2016). The role of nitric oxide in the epigenetic regulation of THP-1 induced by lipopolysaccharide. Life Science 147:110-116. *https://doi.org/10.1016/j.lfs.2016.01.041*

Salaritabar A, Darvishi B, Hadjiakhoondi F, Manayi A, Sureda A, Nabavi SF, ... Bishayee A. (2017). Therapeutic potential of flavonoids in inflammatory bowel disease: A comprehensive review. World Journal of Gastroenterology 23:5097. *https://doi.org/10.3748/wjg.v23.i28.5097*

Snyder DS, Mclntosh TJ (2000) The lipopolysaccharide barrier: correlation of antibiotic susceptibility with antibiotic permeability and fluorescent probe binding kinetics. Biochemistry 39:11777-11787. *https://doi.org/10.1021/bi000810n*

Shi L, Dong N, Ji D, Huang X, Ying Z, Wang X, Chen C. (2018). Lipopolysaccharide-induced CCN1 production enhances interleukin-6 secretion in bronchial epithelial cells. Cell Biology and Toxicology 34:39-49. *https://doi.org/10.1007/s10565-017-9401-1*

Simon F, Fernández R. (2009). Early lipopolysaccharide-induced reactive oxygen species production evokes necrotic cell death in human umbilical vein endothelial cells. Journal of Hypertension 27:1202-1216. *https://doi.org/10.1097/HJH.0b013e328329e31c*

Somasundaram V, Basudhar D, Bharadwaj G, No JH, Ridnour LA, Cheng RY, ... Wink DA (2019). Molecular mechanisms of nitric oxide in cancer progression, signal transduction, and metabolism. Antioxidants and Redox Signaling 30:1124-1143. *https://doi.org/10.1089/ars.2018.7527*

Terao J. (2009). Dietary flavonoids as antioxidants. In: Food factors for health promotion. Vol. 61. Karger Publishers, pp 87-94. *https://doi.org/10.1159/000212741*

Triantafilou M, Miyake K, Golenbock DT, Triantafilou K (2002). Mediators of innate immune recognition of bacteria concentrate in lipid rafts and facilitate lipopolysaccharide-induced cell activation. Journal of Cell Science 115:2603-2611. *https://doi.org/10.1242/jcs.115.12.2603*

Wang X, Quinn PJ (2010). Lipopolysaccharide: biosynthetic pathway and structure modification. Progress in Lipid Research 49:97-107. *https://doi.org/10.1016/j.plipres.2009.06.002*

Zhang JM, An J (2007) Cytokines, inflammation and pain. International Anaesthesiology Clinics 45(2):27. *https://doi.org/10.1097/AIA.0b013e318034194e*

Zhang G, Ghosh S (2000). Molecular mechanisms of NF-κB activation induced by bacterial lipopolysaccharide through Toll-like receptors. Journal of Endotoxin Research 6:453-457.

Zhang WY, Wang H, Qi S, Wang X, Li X, Zhou K, Zhang Y, Gao MQ (2018). CYP1A1 relieves lipopolysaccharide-induced inflammatory responses in bovine mammary epithelial cells. Mediators of Inflammation 4093285. *https://doi.org/10.1155/2018/4093285*

Zhou Q, Xie H, Zhang L, Stewart JK, Gu X-X, Ryan JJ (2006). cis-Terpenones as an effective chemoprotective agent against aflatoxin-B1-induced cytotoxicity and TCDD-induced P450 1A/B activity in HepG2 cells. Chemical Research in Toxicology 19:1415-1419. *https://doi.org/10.1021/tx0601307*

**Notes:**

- Material disclaimer: The authors are fully responsible for their work and they hold sole responsibility for the articles published in the journal.
- Maps and affiliations: The publisher stay neutral with regard to jurisdictional claims in published maps and institutional affiliations.
- Responsibilities: The editors, editorial board and publisher do not assume any responsibility for the article's contents and for the authors' views expressed in their contributions. The statements and opinions published represent the views of the authors or persons to whom they are credited. Publication of research information does not constitute a recommendation or endorsement of products involved.