Saudi Pharmaceutical Journal 32 (2024) 101956

HOSTED BY

Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Zerumbone reduces TLR2 stimulation-induced M1 macrophage polarization pattern via upregulation of Nrf-2 expression in murine macrophages

Marwa Qadri [a,b,*], Zenat Khired [c], Reem Alaqi [b], Sandy Elsayed [d], Abdulaziz Alarifi [e,f], Rayan Ahmed [a], Hussain Alhamami [g], Amani Khardali [h,i], Walaa Hakami [a]

[a] Department of Pharmacology and Toxicology, College of Pharmacy, Jazan University, 45142, Saudi Arabia
[b] Inflammation Pharmacology and Drug Discovery Unit, Health Science Research Center (HSRC), Jazan University, 45142, Saudi Arabia
[c] Surgical Department, Faculty of Medicine, Jazan University, 45142, Saudi Arabia
[d] Department of Pharmacology and Toxicology, Faculty of Pharmacy, October University for Modern Sciences and Arts (MSA), Giza 12451, Egypt
[e] Department of Basic Sciences, College of Science and Health Professions, King Saud bin Abdulaziz University for Health Sciences, Riyadh, Saudi Arabia
[f] King Abdullah International Medical Research Center, Riyadh, Saudi Arabia
[g] Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia
[h] Department of Clinical Pharmacy, College of Pharmacy, Jazan University, Jizan 45142, Jazan, Saudi Arabia
[i] Pharmacy Practice Research Unit, College of Pharmacy, Jazan University, Jizan 45142, Jazan, Saudi Arabia

ARTICLE INFO

Keywords:
Hyperuricemia
Gout
sUA
TLR2
Macrophages
Polarization
Zerumbone
Anti-inflammatory
Antioxidant

ABSTRACT

Hyperuricemia contributes significantly to gout arthritis pathogenesis, which promotes urate crystal deposition in the joints and activates joint-resident macrophages and circulating monocytes to initiate a state of inflammatory arthritis. In the joint, macrophages have an immune defense role where the presence of urate crystals results in the inflammatory mediators secretion, inflammatory cells recruitment to the joint, and shift macrophage population toward M1 pro-inflammatory phenotypes. Current treatment modalities of gout arthritis have side effects that limit their use in the elderly. A novel treatment that targets macrophage polarization to re-establish homeostasis may initiate a drug discovery program of novel disease-modifying agents for gout. Zerumbone (Zer) is a sesquiterpenoid bioactive compound found in the rhizome of Zingiberaceae family and possesses anti-inflammatory, antioxidant, and anti-proliferative activity. Our study hypothesized that soluble uric acid (sUA) and Pam3CSK4 (TLR2 agonist) reduce the anti-inflammatory function of murine M2 bone marrow-derived macrophages and change the expression of M2 genetic markers toward M1 phenotypes. We observed that priming of M2 macrophages with sUA and Pam3CSK4 significantly decreased M2 specific markers expression, e.g., Arg-1, Ym-1, and Fizz-1, enhanced mRNA expression of IL-1β, TNF-α, CXCL2, and iNOS and increased oxidative stress in M2 macrophages, as exhibited by a reduction in Nrf2 expression. We also aimed to study the impact of Zer on reducing the pro-inflammatory effect of sUA in TLR2-stimulated M2 macrophages. We noticed that Zer treatment significantly reduced L-1β and TNF-α production following Pam3CSK4 + sUA treatment on M2 macrophages. Furthermore, Zer reduced the caspase-1 activity without altering cytosolic NLRP3 content in challenged M2 BMDMs. We also observed that Zer significantly enhanced M2-associated marker's expression, e.g., Arg-1, Ym-1, and Fizz-1, and augmented Nrf-2 and other antioxidant proteins, including HMOX1 and srxn1expression following Pam3CSK4 + sUA treatment. We draw the conclusion that Zer is a potentially effective anti-inflammatory treatment for gout arthritis linked to hyperuricemia.

1. Introduction

Gout is a common inflammatory joint disorder characterized by joint swelling, redness, pain, and interspersing periods of chronic inflammation and articular damage (Wen et al., 2022; Ragab, 2017; Duan et al., 2022). The primary risk factor for gout arthritis pathogenesis is

* Corresponding author at: Department of Pharmacology and Toxicology, College of Pharmacy, Jazan University, Inflammation Pharmacology and Drug Discovery Unit, Health Science Research Center (HSRC), Jazan University, Al Maarefah Rd, Jazan, Saudi Arabia 45142.
E-mail address: mqadri@jazanu.edu.sa (M. Qadri).

https://doi.org/10.1016/j.jsps.2024.101956
Received 10 December 2023; Accepted 9 January 2024
Available online 11 January 2024

hyperuricemia. When the serum uric acid level reaches saturation, it may deposit as insoluble urate needle-shaped crystals, causing chronic inflammation and tissue damage (Chen-Xu et al., 2019). Joint-resident macrophages phagocytose urate crystals via toll-like receptors (TLRs) 2 and 4 (Liu-Bryan et al., 2005; Qadri et al., 2018). TLRs has the ability to identify a collection of heterogeneous damage-associated molecular patterns (DAMPs) such as uric acid (Chen et al., 2007; Liew et al., 2005). The interaction of TLRs with their ligands promotes nuclear translocation of nuclear factor κB (NF-κB) and enhances the transcription of pro-inflammatory cytokines and chemokines. Furthermore, the engulfment of urate crystals by macrophage results in the downstream NLRP3 inflammasome assembly and interleukin1β (IL-1β) release via activation of caspase-1.

Synovial-resident macrophages exist on a polarization spectrum with two distinct populations: M1, pro-inflammatory, and M2, anti-inflammatory macrophages at opposite poles (Kemble and Croft, 2021; Kennedy et al., 2011; Saeki and Imai, 2020; Qadri et al., 2021). M1 macrophages activate their bactericidal response by upregulating inducible nitric oxide synthase (iNOS) and enhancing pro-inflammatory cytokine secretion, e.g., IL-1β, tumor necrosis factor- α (TNF-α) (Kelley et al., 2019; Kushiyama et al., 2012). Conversely, M2 macrophages typically express arginase-1 (Arg-1) to convert arginine to L-ornithine and, therefore, deprive iNOS from its substrate (Liu-Bryan et al., 2005; Kelley et al., 2019; Denes et al., 2012). Additionally, M2 macrophages have the capacity to promote the release of anti-inflammatory cytokines (Italiani and Boraschi, 2014; Rath et al., 2014).

Priming macrophages via TLRs by systemic factors such as fatty acids, uric acid, or lipopolysaccharide (LPS) enhances the nuclear translocation of NF-κB, IL-1β release and upregulates the expression of NLRP3 inflammasome components (Lu et al., 2008; Zheng et al., 2015; Joosten et al., 2010; Braga et al., 2017). Recent reports have shown that uric acid enhanced the M1-like phenotype activity of human macrophages (Martínez-Reyes et al., 2020). In addition, monosodium urate crystal (MSU) engulfment by M2 macrophages enhanced the release of the IL-1β (Garcia-Melchor et al., 2015). Currently, available first-line therapies for gouty arthritis are associated with severe toxicity that limits their use. Therefore, a definitive treatment for hyperuricemia-induced gout remains an unmet clinical need.

Zerumbone (Zer) is a phytochemical compound that is isolated from the rhizomes of ginger and possess a diverse biological property, including anti-inflammatory, antioxidant, and antiproliferative activity (Yeh et al., 2022; Singh et al., 2019; Girisa et al., 2019; Haque et al., 2018; Moreira da Silva et al., 2018; Abdelwahab et al., 2010; Sulaiman et al., 2010). Previous findings have shown that Zer ameliorated the inflammatory response in experimental inflammation model in a dose-dependent manner (Sulaiman et al., 2010). Previous research has confirmed that Zer inhibited the mRNA level of iNOS, IL-1β release, and NLRP3 activity in TLR4 agonist-induced stimulation of murine and human macrophages (Haque et al., 2018; Su et al., 2021). Recent reports have highlighted the antioxidant effect of Zer. These reports have further confirmed that the anti-inflammatory properties of Zer are attributed to the nuclear translocation of Nrf2 and the resulting activation of the antioxidant response element (ARE) in experimental animals (Leung et al., 2017; Yang et al., 2018). The aim of this study is to investigate the role of Zer in modulating the TLR2 agonist-induced proinflammatory M1-like phenotype activation and M2-specific gene alteration toward the M1-specific profile following sUA treatment. We also evaluated whether the regulatory role of Zer was mediated through upregulation of transcription factor Nrf2 and subsequent reduction of oxidative stress in murine bone marrow-derived macrophages.

2. Materials and methods

2.1. Experimental animals and generation of bone marrow-derived macrophages (BMDMs) from bone marrow progenitors

Murine bone marrow isolation procedure was conducted as previously described (Zhang et al., 2008; Qadri et al., 2018), utilizing C57BL/6 mice aged between 10 and 14-week-old were obtained from the animal house at the Health Science Research Center (HSRC) in Jazan, Saudi Arabia. Obtaining of murine bone marrow was approved by the Standing Scientific Committee at Jazan University. C57BL/6 mice were kept in a polycarbonate cage supplemented with rice husk bedding purchased from the local market. During the trial, the mice were housed at HSRC, meeting the international standard laboratory environments, including a temperature of 25 ± 2 °C and humidity of 45–55 %. The mice had unlimited access to food and water and had not been diagnosed with any disease before the experiments. During the experiment, the mice were euthanized with CO_2, followed by rapid cervical dislocation. The excess muscle around the femur and tibia was removed using sharp scissors or razor blades soaked in ethanol. Bone marrow cells were flushed from the femurs and tibia after dissection using a sterile DMEM/F12 medium filled in a syringe (Thermo Fisher Scientific) + 10 ng/mL murine M−CSF (R&D Systems) as described previously (Zhang et al., 2008; Qadri et al., 2018). The isolated cells were undergone centrifugation. After centrifugation, the supernatant was removed, and the cells were plated in sterile flasks in 15 mL in DMEM/F12 medium + 10 ng/mL M−CSF (Zhang et al., 2008) at 37 °C in 5 % CO2 incubator. 3 mL of PBS without Ca & Mg (VWR) was used to wash the attached cells on days 3 & 5. On day 7, the differentiated attached BMDMs were washed and incubated with 3 mL enzyme-free cell detaching solution (VWR) at 37 °C for 5 min. The cells were then scraped with a sterile scrapper, and 10 mL of DMEM/F12 medium + 10 ng/mL M−CSF was added. After centrifugation, BMDMs were resuspended 10 mL of DMEM/F12 medium + 10 ng/mL M−CSF.

2.2. Polarization of BMDMs into M2 macrophages

On day 7, a total of 700,000 BMDMs were plated overnight in 6-well plates at 37 °C. On the next day, M2 macrophages were induced by interleukin-4 (IL-4) (20 ng/mL; R&D Systems) for 24 h, as we previously described (Qadri et al., 2021). M2 macrophage markers expression was examined using RT-qPCR. Using TRIzol reagent (Solarbio Life Science, China), RNA was extracted from the cells, and the amount of extracted RNA was determined using a NanoDrop spectrophotometer. We utilized ABscript II cDNA First-Strand Synthesis Kit (AB clonal technologies, China) to synthesize the first strand of the cDNA. RT-qPCR was performed using 2X universal SYBR green fast qPCR mix (AB clonal technologies, China). Genes of interest included iNOS and CD86; M1 markers, Arg-1, Yum-1, Fizz-1, CD206, and CD163 are M2 markers, and β-actin was used to normalize the expression of the gene of interest. The primers were designed and obtained from Macrogen, Korea (Table 1). The relative expression of iNOS, CD86, Arg-1, Yum-1, Fizz-1, CD206, and CD163 was determined using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).

2.3. Priming and activation of M2 BMDMs

M2 macrophages were washed with sterile PBS without Ca & Mg and primed with soluble uric acid (sUA) (50 mg/dL; Sigma-Aldrich) for 24 h (Crişan et al., 2017). On the next day, a toll-like receptor 2 ligand (Pam3CSK4; 10 ng/mL; Invivogen) (Feng et al., 2019) ± 10 μM Zerumbone (Zer; SolarBio life science) was incubated with cells in serum free DMEM/F12 for 24 h. M2 Macrophages were treated with Zer for 3 h before adding Pam3CSK4. The rationale for selecting Zer concentration is based on the previous observation that 10 μM Zer significantly abolished IL-1β release in LPS-challenged murine macrophage (Su et al.,

Table 1
PCR primers sequences.

Primer Name	Forward (5′ to 3′)	Reverse (5′ to 3′)
Murine β-Actin	AAGGCCAACCGTGAAAAGAT	GTGGTACGACCAGAGGCATAC
Murine iNOS	ACATCGACCCGTCCACAGTAT	CAGAGGGGTAGGCTTGTCTC
Murine CD86	TGGGCGCAGAGAAACTTGAT	AAGCCCGTGTCCTTGATCTG
Murine Arg-1	CTCCAAGCCAAAGTCCTTAGAG	GGAGCTGTCATTAGGGACATCA
Murine Fizz-1	CCTGCTGGGATGACTGCTACT	AGATCCACAGGCAAAGCCAC
Murine Ym-1	AGAAGGGAGTTTCAAACCTGGT	GTCTTGCTCATGTGTGTAAGTCA
Murine CD206	TTGGACGGATAGATGGAGGG	CCAGGCAGTTGAGGAGGTTC
Murine CD163	GGCTAGACGAAGTCATCTGCAC	CTTCGTTGGTCAGCCTCAGAGA
Murine TLR2	CGCCCTTTAAGCTGTGTCTC	CGATGGAATCGATGATGTTG
Murine TLR4	CCTGATGACATTCCTTCT	AGCCACCAGATTCTCTAA
Murine IL-1β	GAAATGCCACCTTTTGACAGTG	TGGATGCTCTCATCAGGACAG
Murine TNF-α	CAGGCGGTGCCTATGTCTC	CGATCACCCCGAAGTTCAGTAG
Murine CXCL2	AGTGAACTGCGCTGTCAATG	TTAGCCTTGCCTTTGTTCAG
Murine Nrf2	CTGAACTCCTGGACGGGACTA	CGGTGGGTCTCCGTAAATGG
Murine HMOX-1	AGGTACACATCCAAGCCGAGAA	CTCTGGACACTGACCCTTCTG
Murine srnx1	CCCAGGGTGGCGACTACTA	GTGGACCTCACGAGCTTGG

2021).

2.4. Impact of Zer on M1 and M2 macrophages markers' expressions following sUA andTLR2 stimulation

M2 macrophages in 6-well plates were stimulated with 50 mg/dL sUA and treated with Pam3CSK4 ± 10 μM Zer for 24 h as described above. Isolation and quantification of RNA, synthesis of first strand cDNA, and RT-qPCR were conducted as above. Genes of interest included iNOS; M1 marker, Arg-1, Yum-1, and Fizz-1 are M2 markers (Table 1).

2.5. Impact of Zer treatment on TLR2, TLR4, Nrf2, HMOX-1, srxn-1 expression and secreted cytokines and chemokines expression and production by challenged M2 macrophages

M2 macrophages were primed with 50 mg/dL sUA and treated with Pam3CSK4 ± 10 μM Zer as described above. Isolation and quantification of RNA, synthesis of first strand cDNA, and RT-qPCR were conducted as above, using designed primers TLR2, TLR4, Nrf2, HMOX-1, srxn-1, L-1β, TNF-α, and CXCL2 (Table 1). The relative expression was determined as above. To quantify the protein level of secreted cytokines, cells were stimulated with sUA, as previously mentioned, followed by a 24 h Pam3CSK4 ± 10 μM Zer incubation. On the following day, secreted IL-1β and TNF-α levels were determined using ready to use ELISA kits (R&D Systems).

2.6. Impact of Zer treatment on downstream NLRP3 inflammasome activity and caspase-1 activity in M2 macrophages following sUA andTLR2 stimulation

As mentioned above, M2 BMDMs were stimulated with sUA, then pretreated with 10 μM Zer for 3 h before adding Pam3CSK4 for another 3 h. After 6 h, the total protein content per well was measured using the Pierce BCA protein assay kit (Fisher Scientific). Cytosolic NLRP3 protein and caspase-1 activity were measured using commercially available kits. We used 5 μg protein to determine cytosolic NLRP3 (MyBioSource) content and caspase-1 activity (Solarbio Life Science; colorimetric assay). Protein content in each experimental group was quantified to the total protein content in each treatment group.

2.7. Statistical analysis

Multiple group comparisons of parametric data were conducted using one-way ANOVA followed by Tukey's post-hoc test. For statistical significance, a p-value of less than 0.05 was considered. We conducted three independent experiments per group, and the data are displayed as mean ± S.D.

3. Results

3.1. Polarization of BMDMs into M2 macrophages

We examined the expression of M2 macrophage markers in BMDMs following IL-4 addition. IL-4 treatment resulted in a higher expression of M2 macrophage genetic markers in BMDMs (Fig. 1). These markers include CD206 (C-type mannose receptor), arginase-1 (Arg-1), chitinase-like protein-1 (Ym-1), resistin-like molecule α (Fizz-1), and CD163 (hemoglobin-haptoglobin scavenger receptor). Moreover, the M1 phenotypic markers, including a cluster of differentiation 86 (CD86) and inducible nitric oxide synthase (iNOS), were lower in BMDMs following IL-4 stimulation (Fig. 1).

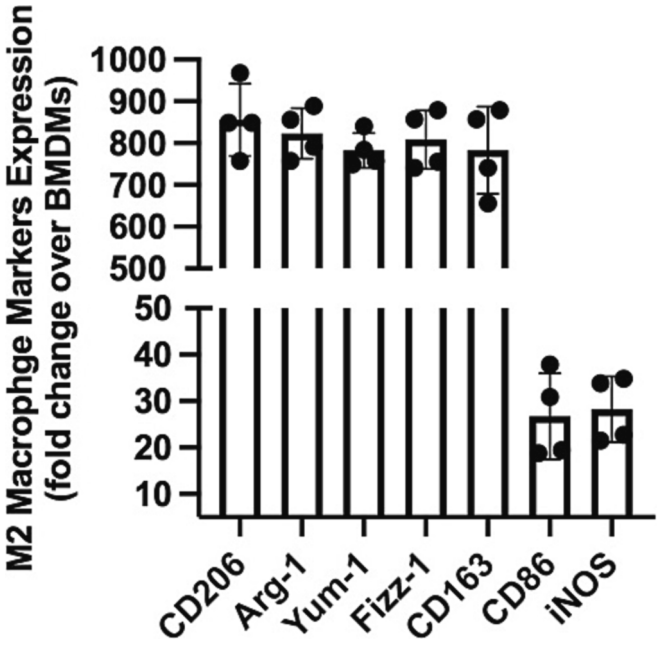

Fig. 1. Characterization of M2 macrophage markers expression in bone marrow-derived macrophages (BMDMs) following the addition of IL-4. The mRNA levels of M2 markers, including CD206 (C-type mannose receptor), arginase-1 (Arg-1), chitinase-like protein-1 (Ym-1), resistin-like molecule α (Fizz-1), and CD163 (hemoglobin-haptoglobin scavenger receptor) were higher in BMDMs. The M1 markers, including cluster of differentiation 86 (CD86) and inducible nitric oxide synthase (iNOS), were lower in BMDMs following IL-4 induction.

3.2. Impact of Zer treatment on M1 and M2 macrophages markers' expressions following sUA and TLR2 stimulation

To examine the phenotypical markers of M1 and M2 in the context of inflammatory conditions, we focused on Arg-1, Yum-1, and Fizz-1 as classical markers of M2 macrophages and iNOS as M1 marker. The isolated BMDMs were polarized into M2 using IL-4, as we previously described. The polarized M2 macrophages were primed with sUA and treated with TLR2 agonist (Pam3CSK4). We observed that sUA + Pam3CSK4 pretreatment significantly increased gene expression of iNOS, an M1 phenotypic marker, in M2 BMDMs (***p = 0.0001; Fig. 2A) and resulted in a significant reduction in M2 markers, including Arg-1 (****p < 0.0001; Fig. 2B), Ym-1 (****p < 0.0001; Fig. 2C) and Fizz-1 (****p < 0.0001; Fig. 2D) compared to either treatment alone. To investigate the efficacy of Zer treatment on reducing M1-like phenotype activation, M2 BMDMs were primed with sUA and treated with Pam3CSK4 ± Zer. We noticed that Zer treatment significantly abolished iNOS mRNA expression in M2 macrophages (**p = 0.0019; Fig. 2A) following sUA + Pam3CSK4 addition. Interestingly, Zer treatment enhanced the gene expression M2 markers Arg-1 (****p < 0.0001; Fig. 2B), Ym-1 (****p < 0.0001; Fig. 2C) and Fizz-1 (****p < 0.0001; Fig. 2D) compared to sUA + Pam3CSK4 group.

3.3. Impact of Zer treatment on TLR2 and TLR4 gene expression following sUA andTLR2 stimulation

To examine the efficacy of Zer on the expression of phagocytic receptors TLR2 and TLR4, the M2 BMDMs were stimulated as described above. We observed that sUA + Pam3CSK4 pretreatment resulted in a significant enhancement of TLR2 (****p < 0.0001, **p = 0.0039; Fig. 3A) and TLR4 expression (***p = 0.0003, *p = 0.0472; Fig. 3B) in polarized M2 macrophages compared to either treatment alone, whereas Zer treatment expectedly decreased the mRNA level of TLR2 (***p = 0.0004; Fig. 3A) and TLR4 (*p = 0.0157; Fig. 3B) in M2 polarized BMDMs that were treated with sUA + Pam3CSK4.

3.4. Impact of Zer treatment on the mRNA expression of secreted cytokine and chemokine by M2 macrophages following sUA andTLR2 stimulation

To study the efficacy of Zer on the expression of cytokines and chemokines by M2 macrophages, M2 BMDMs were primed with sUA and treated Pam3CSK4 ± Zer. Our study showed an increased mRNA expression of the M1 proinflammatory cytokines and chemokines, including IL-1β (****p < 0.0001, *p = 0.0268; Fig. 4A), TNF-α (****p < 0.0001, ***p = 0.0004; Fig. 4B) and chemokine ligand-2 (CXCL2) (****p < 0.0001, **p = 0.0032; Fig. 4C) mRNA level in stimulated M2 BMDMs compared to either treatment alone. However, Zer treatment significantly reduced IL-1β (****p < 0.0001; Fig. 4A), TNF-α (****p < 0.0001; Fig. 4B) and CXCL2 (***p = 0.0004; Fig. 4C) mRNA expression after sUA + Pam3CSK4 in M2 BMDMs.

3.5. Impact of Zer treatment on secreted cytokines production by M2 macrophages following sUA andTLR2 stimulation

We also examined the impact of Zer on the secretion of the M1 proinflammatory cytokines and chemokines. Similar to the enhanced expression of M1 cytokine profile, we observed that sUA + Pam3CSK4 pretreatment increased the protein level of IL-1β (****p < 0.0001; Fig. 5A) and TNF-α (****p < 0.0001; Fig. 5B) compared to either treatment alone. Pam3CSK4 alone enhanced the production of IL-1β (**p = 0.0068; Fig. 5A) and TNF-α (*p = 0.0320; Fig. 5B) protein levels compared to control. However, Zer treatment significantly reversed sUA + Pam3CSK4 stimulation effect on IL-1β (***p = 0.0003; Fig. 5A) and TNF-α (****p < 0.0001; Fig. 5B) protein level in M2 BMDMs.

3.6. Impact of Zer treatment on downstream activation of NLRP3 inflammasome following sUA andTLR2 stimulation

We also studied the efficacy of Zer on the activation of NLRP3 inflammasome in M2 BMDMs following sUA + Pam3CSK4. We observed that cytosolic NLRP3 protein levels in M2 BMDMs were significantly enhanced following sUA + Pam3CSK4 addition compared to sUA alone (*p = 0.0230; Fig. 6). Zer treatment did not alter cytosolic NLRP3 protein levels in sUA + Pam3CSK4 treated M2 BMDMs. ns; not significant.

3.7. Impact of Zer treatment on caspase-1 activity following sUA andTLR2 stimulation

We studied the impact of Zer on the downstream activation of caspase-1 in M2 BMDMs following sUA + Pam3CSK4 stimulation. We observed that Pam3CSK4 alone enhanced caspase-1 activity in M2 BMDMs compared to the control (***p = 0.0006; Fig. 7). Similarly, sUA

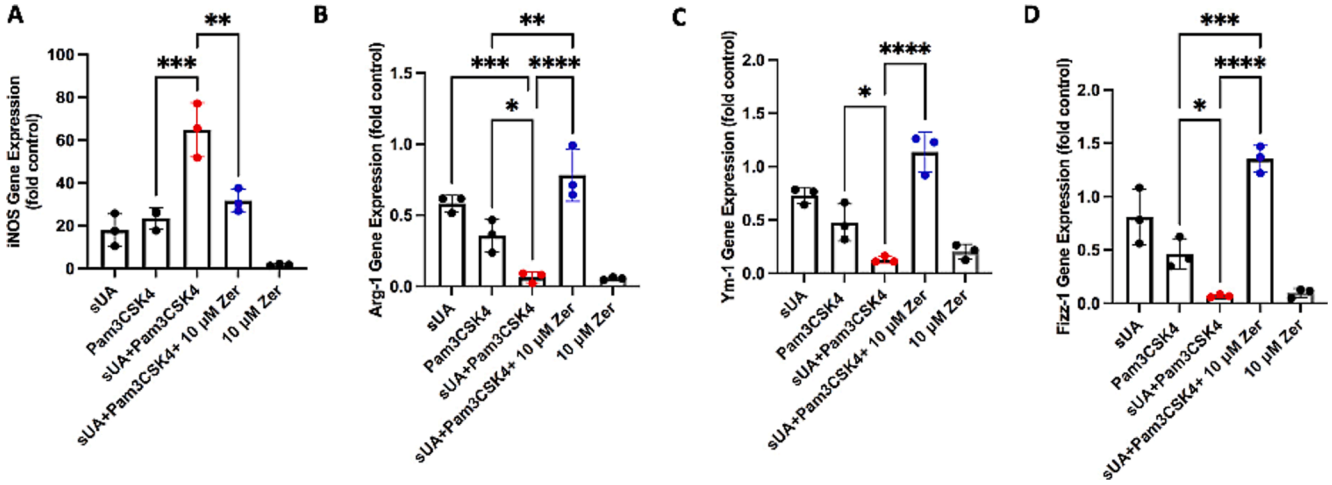

Fig. 2. Impact of zerumbone (Zer) on the mRNA level of inducible nitric oxide synthase (iNOS), arginase-1 (Arg-1), chitinase-like protein 3 (Ym-1), and resistin-like molecule α (Fizz-1) in M2 bone marrow-derived macrophages (BMDMs) following soluble uric acid (sUA) and Pam3CSK4; toll-like receptor (TLR2) agonist pretreatment. **(A)** sUA + Pam3CSK4 pretreatment increased iNOS expression in M2 BMDMs compared to either treatment alone (***p = 0.0001). Zer reduced iNOS expression in M2 macrophages (**p = 0.0019) compared to sUA + Pam3CSK4 group. **(B)** sUA + Pam3CSK4 reduced M2 markers expression Arg-1 (*p = 0.0007), **(C)** Ym-1 (*p = 0.0011) and **(D)** Fizz-1 (*p = 0.0008) compared to sUA or Pam3CSK3 alone. Zer enhanced the gene expression of Arg-1 (****p < 0.0001), **(C)** Ym-1 (****p < 0.0001), and **(D)** Fizz-1 (****p < 0.0001) compared to sUA + Pam3CSK4 group.

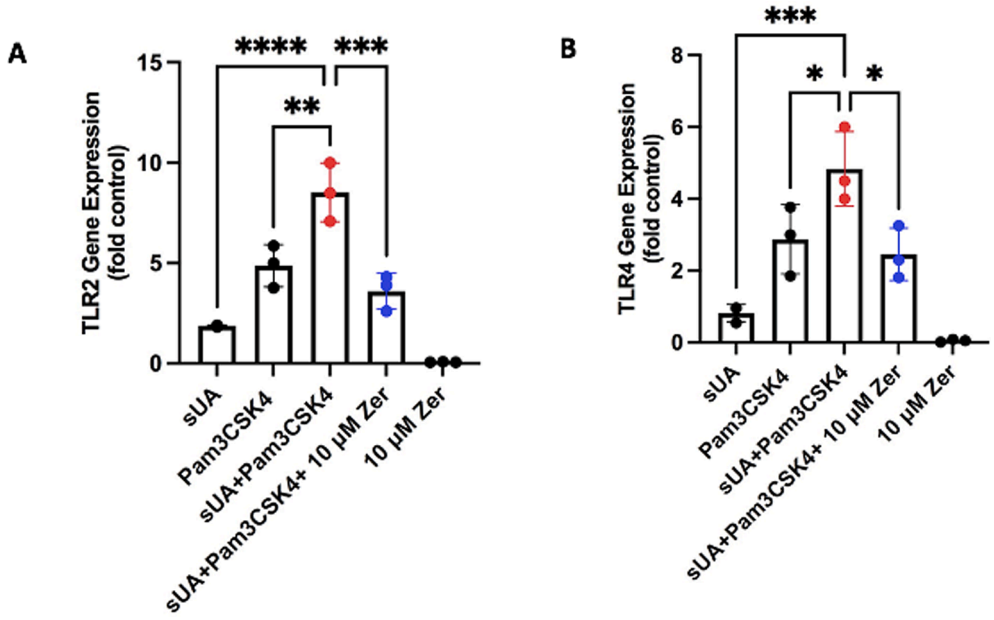

Fig. 3. Impact of zerumbone (Zer) treatment on TLR2 and TLR4 expression in challenged M2 macrophages. **(A)** sUA + Pam3CSK4 increased of TLR2 (****p < 0.0001, **p = 0.0039) and **(B)** TLR4 (***p = 0.0003, *p = 0.0472) expression compared to either treatment alone. Zer abolished TLR2 (***p = 0.0004) and TLR4 (*p = 0.0157) mRNA level in M2 polarized BMDMs following sUA + Pam3CSK4 treatment.

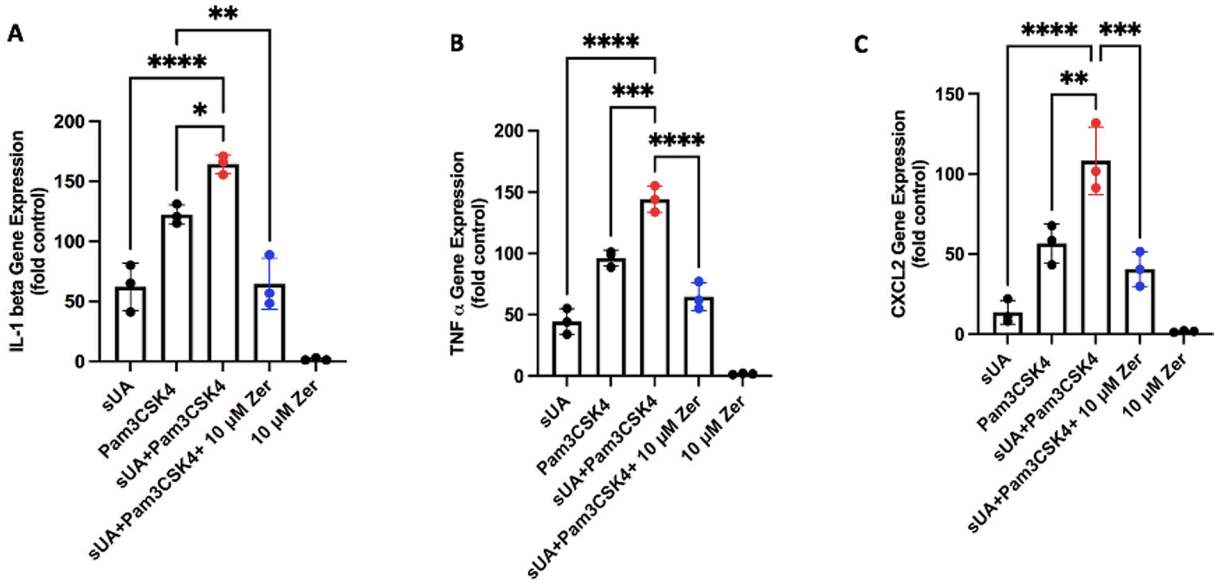

Fig. 4. Effect of zerumbone (Zer) treatment on secreted cytokines and chemokines expression by M2 challenged BMDMs. sUA + Pam3CSK4 pretreatment increased (**A**) IL-1β (****p < 0.0001, *p = 0.0268), (**B**) TNF-α (****p < 0.0001, ***p = 0.0004) and (**C**) CXCL2 (****p < 0.0001, **p = 0.0032) mRNA expression in stimulated M2 BMDMs in comparison to either treatment alone. Zer treatment reduced (**A**) IL-1β (****p < 0.0001), (**B**) TNF-α (****p < 0.0001) and (**C**) CXCL2 (***p = 0.0004) mRNA expression after sUA + Pam3CSK4 in M2 BMDMs.

+ Pam3CSK4 pretreatment increased caspase-1 activity (****p < 0.0001, **p = 0.0029; Fig. 7) compared to either treatment alone. Zer treatment significantly reduced caspase-1 activity in M2 polarized BMDMs that were previously treated with sUA + Pam3CSK4 (***p = 0.0002; Fig. 7). sUA and Zer did not alter the basal activity of caspase-1 in stimulated M2 BMDMs. ns, not significant; Fig. 7.

3.8. Impact of Zer treatment on Nrf2, HMOX-1, and srxn-1 expression following sUA andTLR2 stimulation

We investigated nuclear factor erythroid 2-related factor (Nrf2) expression in M2 BMDMs following sUA + Pam3CSK4 treatment, and we observed that sUA + Pam3CSK4 pretreatment significantly reduced Nrf2 expression in M2 BMDMs (**p = 0.0018, ***p = 0.0008; Fig. 8) compared to either treatment alone. Nrf2 expression enhancement with sUA and Pam3CSK4 did not show a significance level in M2 macrophages compared to the control. However, Nrf2 expression was increased three-fold over sUA + Pam3CSK4 group following 10 µM Zer treatment in M2 BMDMs (****p < 0.0001; Fig. 8A). 10 µM Zer did not alter the basal expression of Nrf2. ns, not significant; Fig. 8A. Moreover, we examined the heme oxygenase-1 (HMOX-1) mRNA expression in M2 macrophages, we noticed that sUA + Pam3CSK4 pretreatment

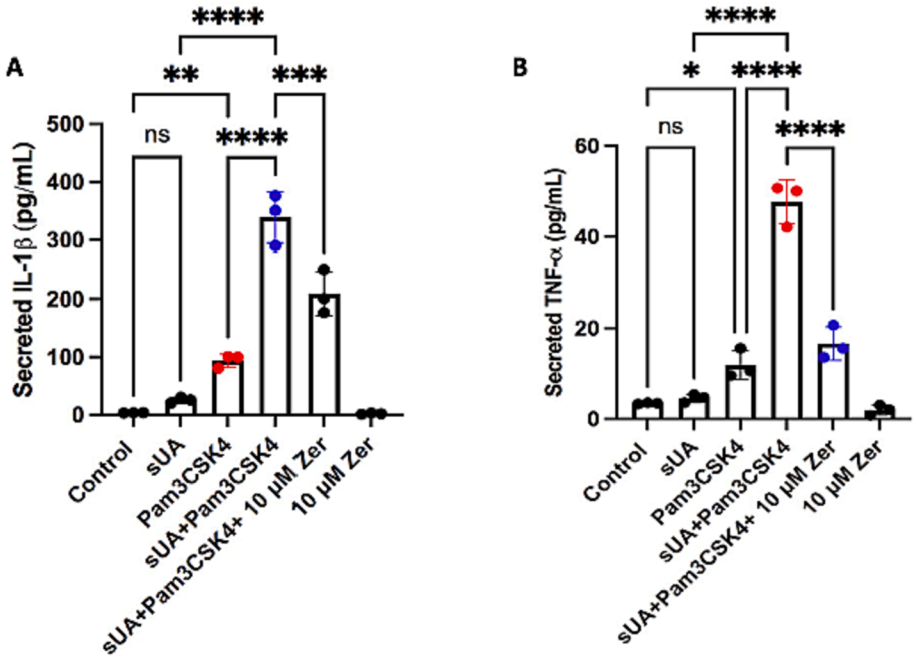

Fig. 5. Efficacy of zerumbone (Zer) on IL-1β and TNF-α secretion after sUA + Pam3CSK4 stimulation in M2 BMDMs. Pam3CSK4 alone enhanced the production of (**A**) IL-1β (**p = 0.0068) and (**B**) TNF-α (*p = 0.0320) protein levels compared to control. Similarly, sUA + Pam3CSK4 pretreatment increased the protein level of (**A**) IL-1β (****p < 0.000) and (**B**) TNF-α (****p < 0.0001) in comparison to either treatment alone. Zer treatment reduced (**A**) IL-1β (***p = 0.0003) and (**B**) TNF-α (****p < 0.0001) protein level in M2 BMDMs following sUA + Pam3CSK4 stimulation.

significantly reduced HMOX-1 expression in M2 BMDMs (***p = 0.0002, *p = 0.0340; Fig. 8B) compared to either treatment alone. HMOX-1 expression was increased three-fold and half over sUA + Pam3CSK4 group following 10 μM Zer treatment in M2 BMDMs (****p < 0.0001; Fig. 8B). 10 μM Zer did not alter the basal expression of HMOX-1. ns, not significant; Fig. 8B. The mRNA expression of sulfiredoxin-1 (srxn-1) was studied in M2 BMDMs following sUA + Pam3CSK4 stimulation. We observed that sUA + Pam3CSK4 pretreatment significantly reduced srxn-1 expression in M2 BMDMs (***p = 0.0007, *p = 0.0088; Fig. 8C) compared to either treatment alone. However, srxn-1 expression was increased three-fold and half over sUA + Pam3CSK4 group following 10 μM Zer treatment in M2 BMDMs (****p < 0.0001; Fig. 8C). 10 μM Zer did not alter the basal expression of srxn-1. ns, not significant; Fig. 8C.

(A) sUA + Pam3CSK4 pretreatment reduced Nrf2 expression in M2 BMDMs (**p = 0.0018, ***p = 0.0008) compared to either treatment alone. sUA and Pam3CSK4 alone did not alter the Nrf2 expression in M2 macrophages compared to the control. Nrf2 expression increased following 10 μM Zer treatment in M2 BMDMs compared to sUA + Pam3CSK4 group (****p < 0.0001). 10 μM Zer did not alter the basal expression of Nrf2. ns, not significant.

(B) sUA + Pam3CSK4 pretreatment reduced HMOX-1 expression in M2 BMDMs (***p = 0.0002, *p = 0.0340) compared to either treatment alone. HMOX-1 expression increased following 10 μM Zer treatment in M2 BMDMs compared to sUA + Pam3CSK4 group (****p < 0.0001). 10 μM Zer did not alter the basal expression of HMOX-1. ns, not significant.

(C) sUA + Pam3CSK4 pretreatment reduced srxn-1 expression in M2 BMDMs (***p = 0.0007, *p = 0.0088) compared to either treatment alone. srxn-1 expression increased following 10 μM Zer treatment in M2 BMDMs compared to sUA + Pam3CSK4 group (****p < 0.0001). 10 μM Zer did not alter the basal expression of srxn-1. ns, not significant.

4. Discussion

Gout is a relatively common inflammatory joint disease due to the precipitation of monosodium urate (MSU) crystal in synovial joints, resulting in joint inflammation and chronic tissue damage (Wen et al., 2022; Ragab, 2017; Duan et al., 2022). Hyperuricemia is considered the primary pathogenic factor in the etiopathogenesis of gout arthritis, where the precipitation of MSU crystals in the lower limbs joints and cartilage causes synovitis and cartilage degeneration through stimulation of the innate immune receptor (Ragab, 2017; Chen-Xu et al., 2019). Uric acid acts as a danger signal, resulting in a low grade of inflammation in gout and other metabolic disorders (Wu et al., 2020; Kushiyama et al., 2012). In response to innate immune danger e.g., uric acid in the joint microenvironment, synovial macrophages can be activated by urate crystals to enhance the secretion of IL-1β, recruit inflammatory monocytes, and shift the macrophages population into a pro-inflammatory phenotype which in turn drives the acute inflammatory response (Liu et al., 2022; Zhao et al., 2022). Evidence of synovial macrophage activation and disease severity in gout arthritis was reported recently. Priming macrophages via toll-like receptors (TLRs) by endogenous ligands triggers signaling cascades that activate NF-κB translocation, pro interleukin-1 beta (pro-IL-1β) production, and NLRP3 inflammasome expression (Lu et al., 2008; Zheng et al., 2015; Joosten et al., 2010; Braga et al., 2017). In addition to innate activation of resident macrophages by TLRs, macrophages can be polarized into two distinct phenotypes as a response to danger signals in the joint microenvironment, M1 and M2 macrophages, or "proinflammatory or classically" and "anti-inflammatory or alternatively" activated macrophages (Italiani and Boraschi, 2014; Garcia-Melchor et al., 2015; Ponomarev et al., 2013). In the synovial joints, signaling cascades of persistent TLRs activation may induce a change of macrophage phenotype from M2 into M1, which would be one of the possible causes of painful episodes of acute gouty attack.

Available evidence supports that uric acid plays a role in altering the polarization patterns of macrophages. We have shown previously that activation of human monocytes with sUA and TLR4 agonist enhanced

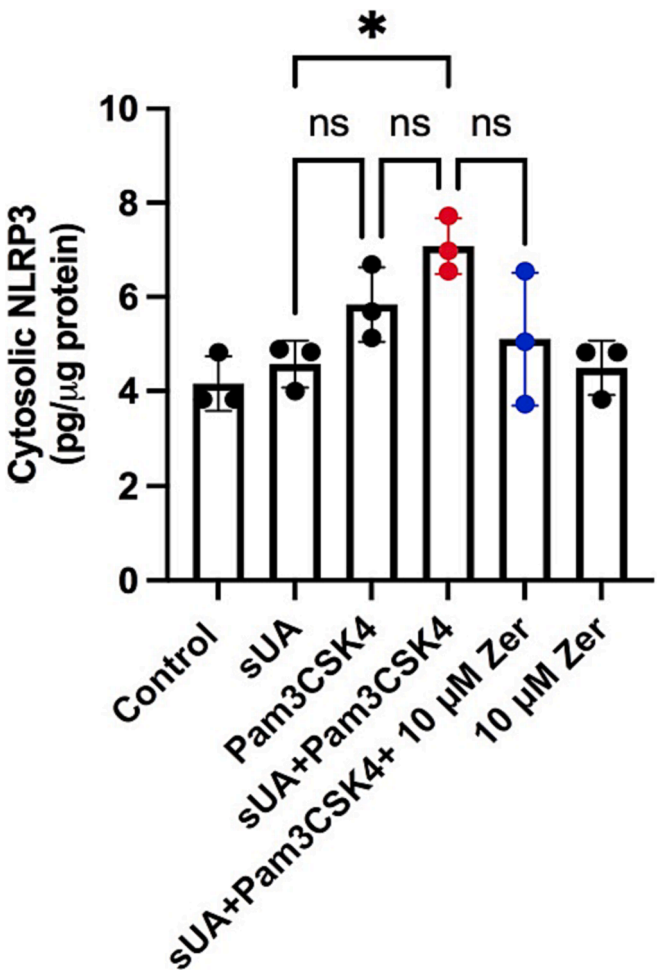

Fig. 6. Impact of zerumbone (Zer) on cytosolic NLRP3 inflammasome level after sUA + Pam3CSK4 stimulation in M2 BMDMs. Cytosolic NLRP3 protein levels in M2 BMDMs increased following sUA + Pam3CKS4 addition compared to sUA alone (*p = 0.0230). Zer treatment did not alter cytosolic NLRP3 protein levels in sUA + Pam3CSK4 treated M2 BMDMs. ns; not significant.

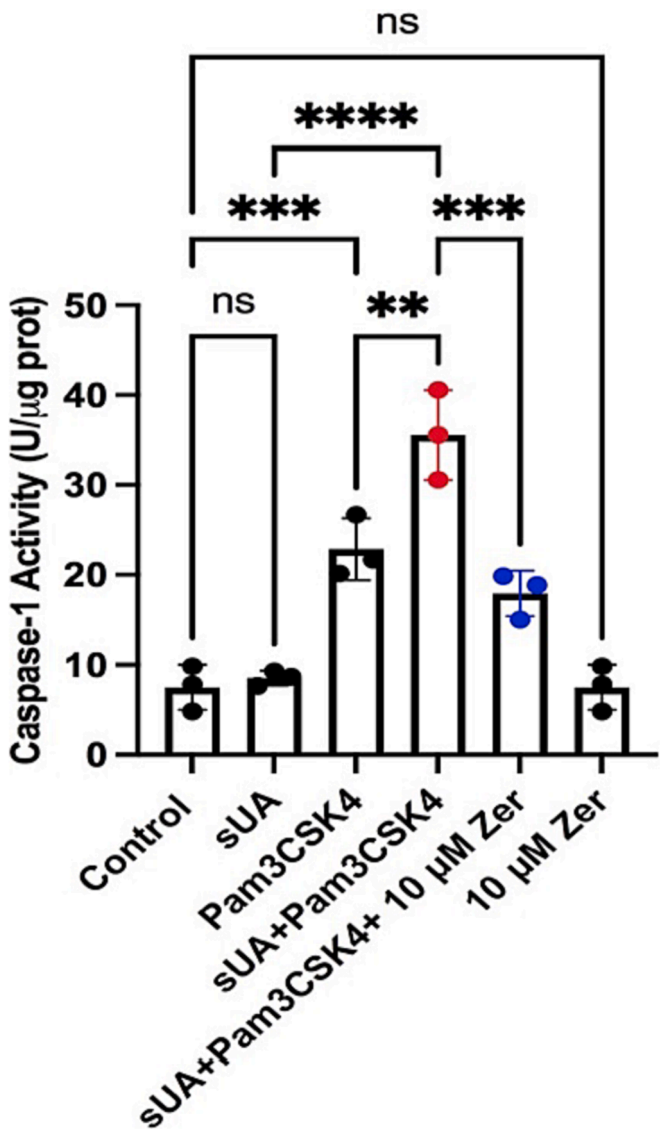

Fig. 7. Impact of zerumbone (Zer) on caspase-1 activity in challenged M2 bone marrow-derived macrophages (BMDMs). Pam3CSK4 alone enhanced caspase-1 activity in M2 BMDMs compared to the control (***p = 0.0006). Similarly, sUA + Pam3CSK4 pretreatment increased caspase-1 activity (****p < 0.0001, **p = 0.0029) compared to either treatment alone. Zer treatment reduced caspase-1 activity in M2 polarized BMDMs following sUA + Pam3CSK4 (***p = 0.0002). sUA did not change the basal activity of caspase-1 in stimulated M2 BMDMs. ns, not significant.

the monocyte's ability to internalize the urate crystal and downstream stimulation of NLRP3 and IL-1β production (Qadri et al., 2021). A study by Martínez-Reyes et al. (2020), has shown that uric acid enhanced the production of TNF-α in human macrophages and skewed the polarization of macrophages into a pro-inflammatory state (Martínez-Reyes et al., 2020). In addition to our previous experience, this study revealed that priming of M2 bone-marrow-derived macrophages with sUA and TLR2 agonist; Pam3CSK4 altered the expression profile of M2 macrophages toward M1-like phenotype. We also observed that sUA + Pam3CSK4 significantly increased gene expression of iNOS in M2 murine macrophages. M2 macrophage has an anti-inflammatory activity to antagonize the pro-inflammatory activity of M1 macrophages. Arg1, Fizz1, and Ym1 are among the distinct markers that are expressed by M2 macrophages (Jablonski et al., 2015). In this study, we observed that sUA and TLR2 ligand significantly reduced M2-specific genetic markers, Arg-1, Fizz-1, and Ym1. This finding proves that M2 macrophage activity is impaired in the context of acute inflammation.

Zerumbone (Zer) is a substance known as sesquiterpenoid that is found in the rhizomes of ginger (family: Zingiberaceae) and possesses numerous pharmacological properties, including anti-inflammatory, antioxidant, anti-immunomodulatory, anti-microbial, anti-cancer, and neuroprotective properties (Yeh et al., 2022; Singh et al., 2019; Girisa et al., 2019; Haque et al., 2018; Moreira da Silva et al., 2018; Abdelwahab et al., 2010; Sulaiman et al., 2010). Previous studies have reported on Zer's safety profile. Rahman et al. (2014), reported that the oral administration of Zer-loaded nanostructured lipid carrier showed no signs of toxicity in the murine model (Rahman et al., 2014). Recently, the role of Zer in regulating macrophage homeostasis was investigated. It was discovered that Zer reduced iNOS expression and enhanced IL-10 production and Arg-1 activity (Yeh et al., 2022). Similarly, in our study we noticed that Zer treatment reversed the effect of TLR2 engagement on iNOS expression in M2 BMDMS macrophages. Surprisingly, we found that Zer did not only augment the expression of Arg-1 in M2 macrophages but also resulted in a marked increase in the expression of Fizz-1 and Ym1 in challenged M2 BMDMs, suggesting the significant role of Zer in aiding in the resolution of inflammation and skews macrophages polarization toward M2 anti-inflammatory phenotypes.

Toll-like receptors (TLRs) play an integral role in responding to external pathogens and internal danger signals (Chen et al., 2007; Liew et al., 2005). Biglycan, fibronectin, fragments of low molecular weight

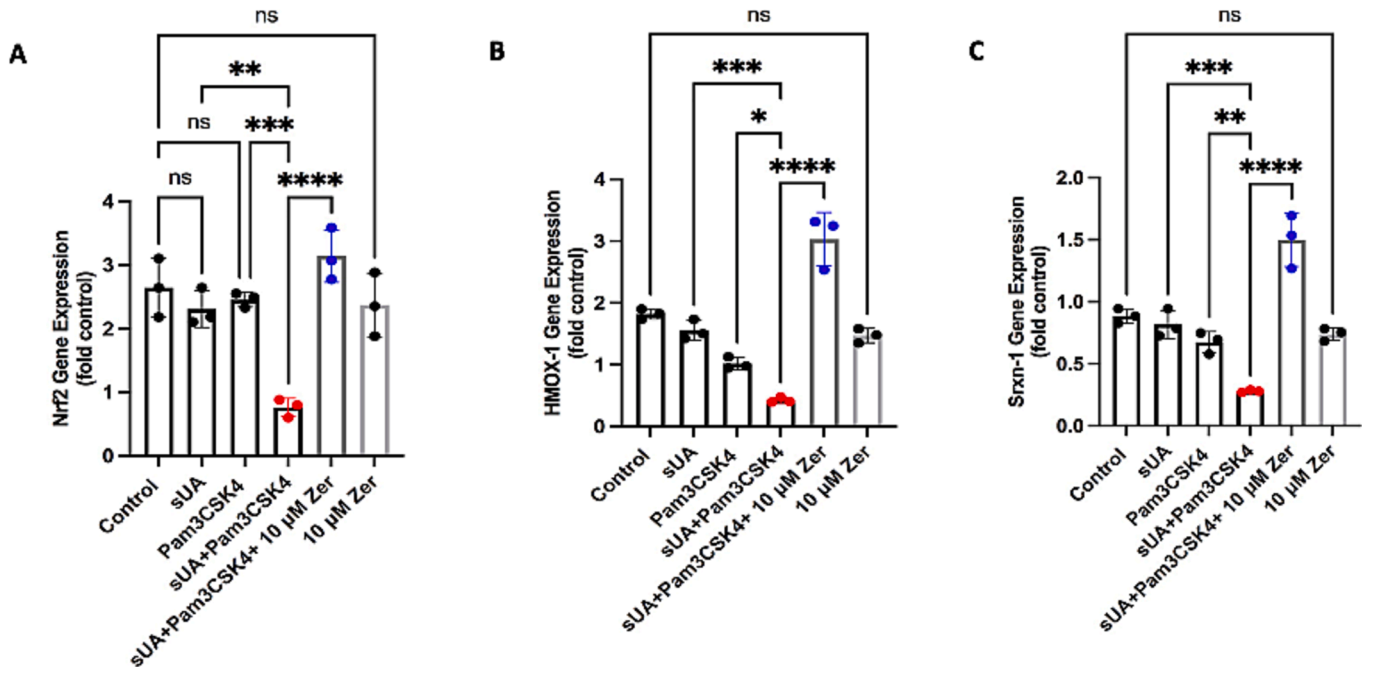

Fig. 8. Effect of zerumbone (Zer) treatment on nuclear factor erythroid 2-related factor (Nrf2), heme oxygenase-1 (HMOX-1), and sulfiredoxin-1 (srxn-1) gene expression in stimulated M2 bone marrow-derived macrophages (BMDMs).

hyaluronan, and endogenous danger like MSU crystals are DAMPs that are known to stimulate TLRs in the joint in degenerative joint diseases e.g., gout and osteoarthritis (Piccinini and Midwood, 2010; Huang, 2013; Lees et al., 2015; Okamura et al., 2001; Schaefer et al., 2005). Activation of TLRs by DAMPs stimulates signaling pathways, leading to the downstream activation of NLRP3 inflammasome and IL-1β (Chen et al., 2007; Liew et al., 2005). We previously reported that TLR2 and TLR4 are crucial for urate crystal internalization, NLRP3 inflammasome activation, and IL-1β production in human monocytes and murine macrophages (Qadri et al., 2018). Previous reports have shown that stimulation of TLR4 signaling pathways changed the function of monocyte/macrophage subset toward M1-like phenotype (Orr et al., 2012; Wang et al., 2014). Finding a pharmacological agent that modulates the upstream signaling receptor's expression is crucial for urate crystal-mediated inflammation. Haque et al., 2018, found that Zer diminished LPS-mediated activation of IL-1β in human macrophages (Haque et al., 2018). Interestingly, we noticed that Zer treatment abolished the mRNA level TLR2 and TLR4 in M2 polarized BMDMs that were previously challenged with sUA + Pam3CSK4. Furthermore, the anti-inflammatory efficacy of Zer was also noticed as Zer treatment attenuated the magnitude of IL-1β, TNF-α and CXCL2 expression and IL-1β, and TNF-α secretion in M2 BMDMs.

Numerous chronic inflammatory diseases are largely influenced by the activation of the NLRP3 inflammasome, and a number of studies reported an excessive activity of the inflammasome, resulting in destructive inflammation in patients (Davis et al., 2011). NLRP3 inflammasome activation function to the process of pro-IL-1β to mature IL-1β through caspase-1 activation (Franchi et al., 2009). One study reported that Zer diminished the NLRP3 stimulation following nigericin; an inflammasome activator in murine macrophages (Su et al., 2021). In contrary, our experimental results demonstrated that Zer did not alter the cytosolic NLRP3 protein level in sUA + Pam3CSK4 in treated M2 BMDMs. Surprisingly, Zer failed to change the cytosolic content of NLRP3 but resulted in a significant attenuation in caspase-1 activity in challenged M2 BMDMs, suggesting that the effect of Zer on reducing caspase-1activity following sUA + Pam3CK4 is NLRP3 independent effect as the processing of caspase-1 is dependent on the nature of the stimulus (He et al., 2020).

Nuclear factor erythroid 2-related factor (Nrf2) contributes significantly to cellular defense against oxidative insult via binding to the antioxidant response element (ARE). This binding enhances the expression of genes coding for antioxidant proteins, including HMOX1 and srxn1 (He et al., 2020; Loboda et al., 2016; Wu et al., 2020). Previous findings demonstrated that Zer inhibited reactive oxygen species (ROS) generation via upregulation of Nrf2 expression (Wu et al., 2020; Yang et al., 2018; Leung et al., 2017; Yang et al., 2018). Interestingly, our finding showed that Zer significantly alleviated the negative effects of sUA and Pam3CSK4 on Nrf2 expression in M2 BMDMs. Moreover, Zer treatment enhanced the expression of HMOX-1 and srxn1 in M2 macrophages, which might be significant in reducing proinflammatory M1-like function and contributing to the anti-inflammatory effect of Zer. Since our study was conducted only in vitro, more research should be undertaken to determine Zer's effectiveness in an in vivo experimental setting in order to comprehend the effect of Zer in systemic conditions.

5. Conclusion

sUA and Pam3CSK4 combined to skew M2 macrophage polarization pattern into M1-like function exhibited by increased M1 genetic marker expression, pro-inflammatory cytokines expression and secretion, and enhanced oxidative stress in M2 macrophages. The anti-inflammatory effect of Zer in macrophages is facilitated by upregulation of Nrf2 and subsequent reduction of oxidative stress in M2 BMDMs, as exhibited by its ability to restore the expression of Arg-1, Fizz-1 and Ym1, markers of M2 macrophage polarization following sUA and Pam3CSK4 treatment.

Acknowledgments & Dedication

The authors extend their appreciation to the Deputyship for Research & Innovation, Ministry of Education in Saudi Arabia, for funding this research work through the project number ISP23-121

Institutional review board statement

The design trials were done with consent Standing Committee for Scientific Research - Jazan University (Reference no. REC-45/03/770).

CRediT authorship contribution statement

Marwa Qadri: Conceptualization, Formal analysis, Funding acquisition, Methodology, Supervision, Writing – original draft, Writing – review & editing. **Zenat Khired:** Conceptualization, Formal analysis, Writing – review & editing. **Reem Alaqi:** Methodology, Writing – review & editing. **Sandy Elsayed:** Writing – review & editing. **Abdulaziz Alarifi:** Writing – review & editing. **Rayan Ahmed:** Writing – review & editing. **Hussain Alhamami:** Writing – review & editing. **Amani Khardali:** Writing – review & editing. **Walaa Hakami:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abdelwahab, S.I., Abdul, A.B., Devi, N., Taha, M.M.E., Al-Zubairi, A.S., Mohan, S., Mariod, A.A., 2010. Regression of cervical intraepithelial neoplasia by zerumbone in female Balb/c mice prenatally exposed to diethylstilboestrol: involvement of mitochondria-regulated apoptosis. Exp. Toxicol. Pathol. 62 (5), 461–469.

Braga, T.T., Forni, M.F., Correa-Costa, M., Ramos, R.N., Barbuto, J.A., Branco, P., Castoldi, A., Hiyane, M.I., Davanso, M.R., Latz, E., 2017. Soluble uric acid activates the NLRP3 inflammasome. Sci. Rep. 7 (1), 39884.

Chen, K., Huang, J., Gong, W., Iribarren, P., Dunlop, N.M., Wang, J.M., 2007. Toll-like receptors in inflammation, infection and cancer. Int. Immunopharmacol. 7 (10), 1271–1285.

Chen-Xu, M., Yokose, C., Rai, S.K., Pillinger, M.H., Choi, H.K., 2019. Contemporary prevalence of gout and hyperuricemia in the United States and decadal trends: the National Health and Nutrition Examination Survey, 2007–2016. Arthritis Rheumatol. 71 (6), 991–999.

Crişan, T.O., Cleophas, M.C., Novakovic, B., Erler, K., van de Veerdonk, F.L., Stunnenberg, H.G., Netea, M.G., Dinarello, C.A., Joosten, L.A., 2017. Uric acid priming in human monocytes is driven by the AKT–PRAS40 autophagy pathway. Proc. Natl. Acad. Sci. 114 (21), 5485–5490.

Davis, B.K., Wen, H., Ting, J.-P.-Y., 2011. The inflammasome NLRs in immunity, inflammation, and associated diseases. Annu. Rev. Immunol. 29, 707–735.

Denes, A., Lopez-Castejon, G., Brough, D., 2012. Caspase-1: is IL-1 just the tip of the ICEberg? Cell Death Dis. 3 (7), e338–e.

Duan, L., Zhong, J., Yang, Y., Zhu, X., 2022. Advances in pathogenesis and therapies of gout. Front. Immunol. 13, 890204.

Feng, Y., Mu, R., Wang, Z., Xing, P., Zhang, J., Dong, L., Wang, C., 2019. A toll-like receptor agonist mimicking microbial signal to generate tumor-suppressive macrophages. Nat. Commun. 10 (1), 2272.

Franchi, L., Eigenbrod, T., Muñoz-Planillo, R.l., Núñez, G., 2009. The inflammasome: a caspase-1-activation platform that regulates immune responses and disease pathogenesis. Nat. Immunol. 10, 241–247.

Garcia-Melchor, E., Guma, M., Yague, J., Juan, M., Harper, J., 2015. THU0023 soluble uric acid exerts a suppressive effect on the response of macrophages to monosodium urate crystals. Ann. Rheum. Dis. 74, 201.

Girisa, S., Shabnam, B., Monisha, J., Fan, L., Halim, C.E., Arfuso, F., Ahn, K.S., Sethi, G., Kunnumakkara, A.B., 2019. Potential of zerumbone as an anti-cancer agent. Molecules 24 (4), 734.

Haque, M.A., Jantan, I., Harikrishnan, H., 2018. Zerumbone suppresses the activation of inflammatory mediators in LPS-stimulated U937 macrophages through MyD88-dependent NF-κB/MAPK/PI3K-Akt signaling pathways. Int. Immunopharmacol. 55, 312–322.

He, F., Ru, X., Wen, T., 2020. NRF2, a transcription factor for stress response and beyond. Int. J. Mol. Sci. 21 (13), 4777.

Huang, Q.Q., Pope, R.M., 2013. The role of glycoprotein 96 in the persistent inflammation of rheumatoid arthritis. Arch. Biochem. Biophys. 530 (1), 1–6.

Italiani, P., Boraschi, D., 2014. From monocytes to M1/M2 macrophages: phenotypical vs. functional differentiation. Front. Immunol. 5, 514.

Jablonski, K.A., Amici, S.A., Webb, L.M., Ruiz-Rosado, J.d.D., Popovich, P.G., Partida-Sanchez, S., Guerau-de-Arellano, M., 2015. Novel markers to delineate murine M1 and M2 macrophages. PLoS One 10 (12), e0145342.

Joosten, L.A., Netea, M.G., Mylona, E., Koenders, M.I., Malireddi, R.S., Oosting, M., Stienstra, R., van de Veerdonk, F.L., Stalenhoef, A.F., Giamarellos-Bourboulis, E.J., 2010. Fatty acids engagement with TLR2 drive IL-1β production via ASC-caspase-1 pathway by urate crystals in gouty arthritis. Arthritis Rheum. 62 (11), 3237.

Kelley, N., Jeltema, D., Duan, Y., He, Y., 2019. The NLRP3 inflammasome: an overview of mechanisms of activation and regulation. Int. J. Mol. Sci. 20 (13), 3328.

Kemble, S., Croft, A.P., 2021. Critical role of synovial tissue–resident macrophage and fibroblast subsets in the persistence of joint inflammation. Front. Immunol. 12, 715894.

Kennedy, A., Fearon, U., Veale, D.J., Godson, C., 2011. Macrophages in synovial inflammation. Front. Immunol. 2, 52.

Kushiyama, A., Okubo, H., Sakoda, H., Kikuchi, T., Fujishiro, M., Sato, H., Kushiyama, S., Iwashita, M., Nishimura, F., Fukushima, T., 2012. Xanthine oxidoreductase is involved in macrophage foam cell formation and atherosclerosis development. Arterioscler. Thromb. Vasc. Biol. 32 (2), 291–298.

Lees, S., Golub, S.B., Last, K., Zeng, W., Jackson, D.C., Sutton, P., Fosang, A.J., 2015. Bioactivity in an aggrecan 32-mer fragment is mediated via Toll-like receptor 2. Arthritis Rheumatol. 67 (5), 1240–1249.

Leung, W.-S., Yang, M.-L., Lee, S.-S., Kuo, C.-W., Ho, Y.-C., Huang-Liu, R., Lin, H.-W., Kuan, Y.-H., 2017. Protective effect of zerumbone reduces lipopolysaccharide-induced acute lung injury via antioxidative enzymes and Nrf2/HO-1 pathway. Int. Immunopharmacol. 46, 194–200.

Liew, F.Y., Xu, D., Brint, E.K., O'Neill, L.A., 2005. Negative regulation of toll-like receptor-mediated immune responses. Nat. Rev. Immunol. 5 (6), 446–458.

Liu, L., Zhu, L., Liu, M., Zhao, L., Yu, Y., Xue, Y., Shan, L., 2022. Recent insights into the role of macrophages in acute gout. Front. Immunol. 13, 955806.

Liu-Bryan, R., Scott, P., Sydlaske, A., Rose, D.M., Terkeltaub, R., 2005. Innate immunity conferred by toll-like receptors 2 and 4 and myeloid differentiation factor 88 expression is pivotal to monosodium urate monohydrate crystal–induced inflammation. Arthritis Rheum. 52 (9), 2936–2946.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. Methods 25 (4), 402–408.

Loboda, A., Damulewicz, M., Pyza, E., Jozkowicz, A., Dulak, J., 2016. Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism. Cell. Mol. Life Sci. 73, 3221–3247.

Lu, Y.-C., Yeh, W.-C., Ohashi, P.S., 2008. LPS/TLR4 signal transduction pathway. Cytokine 42 (2), 145–151.

Martínez-Reyes, C.P., Manjarrez-Reyna, A.N., Méndez-García, L.A., Aguayo-Guerrero, J. A., Aguirre-Sierra, B., Villalobos-Molina, R., López-Vidal, Y., Bobadilla, K., Escobedo, G., 2020. Uric acid has direct proinflammatory effects on human macrophages by increasing proinflammatory mediators and bacterial phagocytosis probably via URAT1. Biomolecules 10 (4), 576.

Moreira da Silva, T., Pinheiro, C.D., Puccinelli Orlandi, P., Pinheiro, C.C., Soares Pontes, G., 2018. Zerumbone from Zingiber zerumbet (L.) smith: a potential prophylactic and therapeutic agent against the cariogenic bacterium Streptococcus mutans. BMC Complement. Altern. Med. 18 (1), 1–9.

Okamura, Y., Watari, M., Jerud, E.S., Young, D.W., Ishizaka, S.T., Rose, J., Chow, J.C., Strauss, J.F., 2001. The extra domain A of fibronectin activates toll-like receptor 4. J. Biol. Chem. 276 (13), 10229–10233.

Orr, J.S., Puglisi, M.J., Ellacott, K.L., Lumeng, C.N., Wasserman, D.H., Hasty, A.H., 2012. Toll-like receptor 4 deficiency promotes the alternative activation of adipose tissue macrophages. Diabetes 61 (11), 2718–2727.

Piccinini, A., Midwood, K., 2010. DAMPening inflammation by modulating TLR signalling. Mediators Inflamm. 2010.

Ponomarev, E.D., Veremeyko, T., Weiner, H.L., 2013. MicroRNAs are universal regulators of differentiation, activation, and polarization of microglia and macrophages in normal and diseased CNS. Glia 61 (1), 91–103.

Qadri, M., Almadani, S., Jay, G.D., Elsaid, K.A., 2018. Role of CD44 in regulating TLR2 activation of human macrophages and downstream expression of proinflammatory cytokines. J. Immunol. 200 (2), 758–767.

Qadri, M., Jay, G.D., Zhang, L.X., Wong, W., Reginato, A.M., Sun, C., Schmidt, T.A., Elsaid, K.A., 2018. Recombinant human proteoglycan-4 reduces phagocytosis of urate crystals and downstream nuclear factor kappa B and inflammasome activation and production of cytokines and chemokines in human and murine macrophages. Arthritis Res. Ther. 20 (1), 1–16.

Qadri, M., ElSayed, S., Elsaid, K.A., 2021. Fingolimod Phosphate (FTY720-P) Activates Protein Phosphatase 2A in Human Monocytes and Inhibits Monosodium Urate Crystal-Induced Interleukin-1β Production. J. Pharmacol. Exp. Ther. 376 (2), 222–230.

Qadri, M., Jay, G.D., Zhang, L.X., Schmidt, T.A., Totonchy, J., Elsaid, K.A., 2021. Proteoglycan-4 is an essential regulator of synovial macrophage polarization and inflammatory macrophage joint infiltration. Arthritis Res. Ther. 23, 1–16.

Rahman, H.S., Rasedee, A., Othman, H.H., Chartrand, M.S., Namvar, F., Yeap, S.K., Abdul Samad, N., Andas, R.J., Muhammad Nadzri, N., Anasamy, T., 2014. Acute toxicity study of zerumbone-loaded nanostructured lipid carrier on BALB/c mice model. BioMed Res. Int.

Rath, M., Müller, I., Kropf, P., Closs, E.I., Munder, M., 2014. Metabolism via arginase or nitric oxide synthase: two competing arginine pathways in macrophages. Front. Immunol. 5, 532.

Saeki, N., Imai, Y., 2020. Reprogramming of synovial macrophage metabolism by synovial fibroblasts under inflammatory conditions. Cell Commun. Signal. 18 (1), 1–14.

Schaefer, L., Babelova, A., Kiss, E., Hausser, H.-J., Baliova, M., Krzyzankova, M., Marsche, G., Young, M.F., Mihalik, D., Götte, M.J.T., 2005. "The matrix component biglycan is proinflammatory and signals through Toll-like receptors 4 and 2 in macrophages. J. Clin. Investigation 115 (8), 2223–2233.

Singh, Y.P., Girisa, S., Banik, K., Ghosh, S., Swathi, P., Deka, M., Padmavathi, G., Kotoky, J., Sethi, G., Fan, L., 2019. Potential application of zerumbone in the prevention and therapy of chronic human diseases. J. Funct. Foods 53, 248–258.

Su, C.-C., Wang, S.-C., Chen, I.-C., Chiu, F.-Y., Liu, P.-L., Huang, C.-H., Huang, K.-H., Fang, S.-H., Cheng, W.-C., Huang, S.-P., 2021. Zerumbone suppresses the LPS-induced inflammatory response and represses activation of the NLRP3 inflammasome in macrophages. Front. Pharmacol. 12, 652860.

Sulaiman, M., Perimal, E., Akhtar, M., Mohamad, A., Khalid, M., Tasrip, N., Mokhtar, F., Zakaria, Z., Lajis, N., Israf, D., 2010. Anti-inflammatory effect of zerumbone on acute and chronic inflammation models in mice. Fitoterapia 81 (7), 855–858.

Wang, N., Liang, H., Zen, K., 2014. Molecular mechanisms that influence the macrophage M1–M2 polarization balance. Front. Immunol. 5, 614.
Wen, P., Luo, P., Zhang, B., Zhang, Y., 2022. Mapping knowledge structure and global research trends in gout: a bibliometric analysis from 2001 to 2021. Front. Public Health 10, 924676.
Wu, Z., Lu, Z., Ou, J., Su, X., Liu, J., 2020. Inflammatory response and oxidative stress attenuated by sulfiredoxin-1 in neuron-like cells depends on nuclear factor erythroid-2-related factor 2. Mol. Med. Rep. 22 (6), 4734–4742.
Yang, H.-L., Lee, C.-L., Korivi, M., Liao, J.-W., Rajendran, P., Wu, J.-J., Hseu, Y.-C., 2018. Zerumbone protects human skin keratinocytes against UVA-irradiated damages through Nrf2 induction. Biochem. Pharmacol. 148, 130–146.
Yeh, W.-L., Huang, B.-R., Chen, G.-W., Charoensaensuk, V., Tsai, C.-F., Yang, L.-Y., Lu, D.-Y., Chen, M.-K., Lin, C., 2022. Role of zerumbone, a phytochemical sesquiterpenoid from Zingiber zerumbet Smith, in maintaining macrophage polarization and redox homeostasis. Nutrients 14 (24), 5402.
Zhang, X., Goncalves, R., Mosser, D.M., 2008. The isolation and characterization of murine macrophages. Curr. Protocols Immunol. 83 (1), 14.11–14.14.
Zhao, L., Ye, W., Zhu, Y., Chen, F., Wang, Q., Lv, X., Hua, Y., Du, Z., Zhu, X., Yu, Y., 2022. Distinct macrophage polarization in acute and chronic gout. Lab. Invest. 102 (10), 1054–1063.
Zheng, S.-C., Zhu, X.-X., Xue, Y., Zhang, L.-H., Zou, H.-J., Qiu, J.-H., Liu, Q., 2015. Role of the NLRP3 inflammasome in the transient release of IL-1β induced by monosodium urate crystals in human fibroblast-like synoviocytes. J. Inflamm. 12 (1), 1–9.