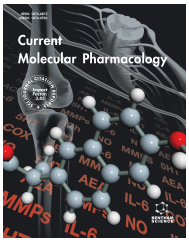
BENTHAM SCIENCE

CrossMark

Current Molecular Pharmacology

Content list available at: https://benthamscience.com/journal/cmp



RESEARCH ARTICLE

Curcumin and Berberine Arrest Maturation and Activation of Dendritic Cells Derived from Lupus Erythematosus Patients

Amin Reza Nikpoor[1], Mahmoud Mahmoudi[2,3], Abbas Shapouri-Moghaddam[2], Zahra Rezaieyazdi[4], Samaneh Mollazadeh[5], Nafiseh Tabasi[2], Atena Mansouri[6], Reyhane Modarres Moghadam[2], Amir Abbas Momtazi[7,8], Soran Kay Fi Najmaldin[9], Ramiar Kamal Kheder[10] and Seyed-Alireza Esmaeili[2,3,*]

[1]*Molecular Medicine Research Center, Hormozgan Health Institute, Hormozgan University of Medical Sciences, Bandar Abbas, Iran*
[2]*Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*
[3]*Immunology Department, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran*
[4]*Rheumatic Diseases Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*
[5]*Natural Products and Medicinal Plants Research Center, North Khorasan University of Medical Sciences, Bojnurd, Iran*
[6]*Cellular and Molecular Research Center, Birjand University of Medical Sciences, Birjand, Iran*
[7]*Department of Advanced Technologies in Medicine, School of Medicine, Neyshabur University of Medical Sciences, Neyshabur, Iran*
[8]*Healthy Ageing Research Centre, Neyshabur University of Medical Sciences, Neyshabur, Iran*
[9]*Department of Medical Analysis, Faculty of Applied Science, Tishk International University, Erbil, Iraq*
[10]*Medical Laboratory Science Department, College of Science, University of Raparin, Rania , Sulaymaniyah, Iraq*

Abstract:

Background:

Systemic lupus erythematosus (SLE) is a complex autoimmune disease recognized by elevated activity of autoimmune cells, loss of tolerance, and decreased regulatory T cells producing inhibitory cytokines. Despite many efforts, the definitive treatment for lupus has not been fully understood. Curcumin (CUR) and berberine (BBR) have significant immunomodulatory roles and anti-inflammatory properties that have been demonstrated in various studies. This study aimed to investigate the anti-inflammatory properties of CUR and BBR on human monocyte-derived dendritic cells (DCs) with an special focus on the maturation and activation of DCs.

Methods:

Human monocytes were isolated from the heparinized blood of SLE patients and healthy individuals, which were then exposed to cytokines (IL-4 and GM-CSF) for five days to produce immature DCs. Then, the obtained DCs were characterized by FITC-uptake assay and then cultured in the presence of CUR, BBR, or lipopolysaccharide (LPS) for 48 h. Finally, the maturation of DCs was analyzed by the level of maturation using flow cytometry or real-time PCR methods.

Results:

The results showed promising anti-inflammatory effects of CUR and BBR in comparison with LPS, supported by a significant reduction of not only co-stimulatory and antigen-presenting factors such as CD80, CD86, CD83, CD1a, CD14, and HLA-DR but also inflammatory cytokines such as IL-12.

Conclusion:

CUR and BBR could arrest DC maturation and develop a tolerogenic DC phenotype that subsequently promoted the expression of inhibitory cytokines and reduced the secretion of proinflammatory markers.

Keywords: Curcumin, Berberine, Dendritic cells, Cytokines, SLE, PCR methods.

Article History	Received: May 17, 2023	Revised: November 04, 2023	Accepted: December 11, 2023

1. INTRODUCTION

Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterized by autoantibody production, histiocytic infiltration, and organ dysfunction ranging from mild manifestations to seriously life-threatening complications [1, 2]. Beyond autoantibodies, SLE is determined by immune complex deposition, deregulated cytokine production, and recruitment of self-reacting T cells [3]. Pathogenically, the abnormal immune responses in SLE might be started by the activation of innate immunity accompanied by the recruitment of dendritic cells (DCs) to produce interferon α (IFNα), contributing to immune homeostasis [4]. DCs, powerful antigen-presenting cells (APCs), could establish a link between innate and adaptive immunity. In addition, these cells could present antigens, for example, self-antigens, to the T cells followed by triggering immune responses, either immune silence or immune activation, which uncovers the etiopathogenetic role of DCs in SLE supported by several studies [4, 5]. In this regard, immature and tolerogenic DCs affect immunomodulation by upregulation of inhibitory cytokines like IL-10, enhancement of transcription factors such as Indoleamine 2,3-dioxygenase (IDO), decrease of inflammatory cytokines like IL-12, and induction of Treg cells. DCs can also develop immunomodulation by changing the expression of their surface antigens, such as HLA-DR, CD80, CD86, CD1a, and CD83 [6 - 8]. As a result, DCs could be introduced as the pharmacological target of interest to develop novel therapies by elevating their tolerogenicity and reducing their immunogenicity. Current SLE treatment strategies, such as traditional immunosuppressants, are generally associated with not only multiple side effects, such as susceptibility to opportunistic pathogens and infections but also tolerability points worsening the disease condition [9], which propel the investigators to find more applicable alternative approaches [10 - 12].

Curcumin (CUR), a well-introduced natural compound, is a lipophilic polyphenol substance isolated from Turmeric, a curry spice originating from India [13]. Of note, this compound has been found to have various pharmacological activities, including anti-inflammatory and immunomodulatory effects. The noteworthy insight into the immunomodulatory features of CUR arises from its effects on DCs and the consequent reduction of the presentation of surface markers and secretion of IL-12 alongside the maintenance of immature DC phenotypes [14 - 17]. Based on this, CUR can induce maturation-arrested DCs, resulting in improved function of regulatory T cells [18]. Additionally, berberine (BBR) is another important natural compound recognized as an isoquinoline alkaloid. This substance could be extracted from cypress needles and *Coptis chinensis* [19]. BBR exerts surprising immunoregulatory effects mediated by decreased expression of inflammatory cytokines, including IFN-γ, IL-2, and IL-17 [20].

In an attempt to investigate the therapeutical potential of CUR and BBR on SLE, the current study was designed to examine the anti-inflammatory and immunomodulatory effects of CUR and BBR in the SLE setting regarding their influence on the induction of tolerogenic DCs, which could open a practical window to manage overactive immunity in SLE.

* Address correspondence to this author at the Immunology Research Center, Mashhad University of Medical, Sciences, Mashhad, Iran; Tel: +985138002379; +989191047300; E-mails: Imunoman2009@gmail.com, esmaeiliar@mums.ac.ir

2. MATERIALS AND METHODS

2.1. Isolation of Peripheral Blood Mononuclear Cells (PBMCs)

Blood samples (20 mL) were obtained from SLE patients and healthy controls, 3 volunteers in each group, and collected in a heparin tube. The SLE patients who had positive antinuclear antibody (ANA) and anti-double-stranded DNA were enrolled in the study before the administration of glucocorticoids and immunosuppressive drugs. All patients with SLE fulfilled at least four points of the revised classification criteria of the American College of Rheumatology (revised criteria of 1997). All SLE patients were newly diagnosed. All protocols used in this study were approved by the Ethics Committee of Mashhad University of Medical Sciences (Mashhad, Iran). SLE patients were recruited consecutively from the Rheumatology Centre. All participants signed a written informed consent form prior to participation in the study. To isolate PBMCs, blood samples were subjected to the Ficoll Hypaque (Cedarlane, Toronto, Canada) density gradient centrifugation. Then, the plastic adhesion method was applied to purify monocytes from PBMCs. Briefly, PBMCs were seeded in 24-well plates (1×10^6 cells/mL) and resuspended in the fresh RPMI-1640 medium (BioSera, London, UK) supplemented with 10% fetal bovine serum (FBS; GIBCO, UK), 100 I.U./mL Penicillin, 100 ug/ml streptomycin, and L-glutamine (Sigma-Aldrich, USA). Next, the cells were incubated in 5% CO_2 at 37°C for 2 h, removing non-adherent cells (lymphocytes and platelets) and thoroughly washing with PBS.

The expression levels of monocyte markers, CD14 and CD45, were examined by flow cytometry to evaluate the purity of isolated monocytes. In this regard, single cells were incubated with CD45 FITC/CD14R-PE, mouse IgG1 isotype (IQProducts, Groningen, The Netherlands) in phosphate-buffered saline (PBS) at 4°C for 1 h. In the final step, the labeled cells were evaluated by fluorescence-activated cell sorting (FACS) Calibur flow cytometer, and the results were analyzed by the Flowjo 7.6.2 software (Tree Star, Ashland, OR) [8].

2.2. Production of Immature DCs

Recombinant human cytokines, IL-4 and GM-CSF (RD, Minneapolis, MN, USA), were used to produce monocyte-derived immature DCs (IDC). For this purpose, isolated monocytes were cultured in a 24-well plate at the density of 2×10^5 cells/well and treated with complete RPMI-1640 medium containing IL-4 (10 ng/ml) and GM-CSF (10 ng/ml). Fifth day posttreatment, the IDCs were detached from the plastic surface and analyzed for expression of antigen-presenting molecules HLA-DR and CD1a, the co-stimulatory molecules CD86 and CD80, as well as CD14 as a monocyte

marker using flow cytometry. These cells were also evaluated for IL-12, IL-10, and IDO expression by real-time PCR method. After characterization, the obtained IDCs were used for subsequent tests [8].

2.3. Treatment of IDCs with CUR and BBR

To investigate the effects of CUR and BBR alone on the maturation of IDCs, the cells were seeded at the density of 2×10^5 cells/well in the presence of IL-4 (10 ng/ml) and GM-CSF (10 ng/ml) in four different conditions as follows; CUR (20 μM), BBR (20 μM), LPS as positive control (100 ng/ml; Sigma-Aldrich, USA). For the curcumin and berberine groups, the cells were first activated by LPS incubation and then matured into DCs for further experiments with curcumin and berberine over a period of 48 hours. Of note, DC maturation was assessed for both cells derived from healthy subjects and SLE individuals.

2.4. FITC-dextran Uptake Assay

FITC-dextran uptake assay was applied to check the phagocytosis potential and antigen uptake of IDCs. IDCs (2×10^5) were incubated with FITC dextran (1mg/mL, mol. wt. 40,000; Sigma) for 2 h at 37°C. Then, cells were washed twice with PBS to remove extra FITC dextran dye. Finally, the amount of FITC uptake was measured by flow cytometry and analyzed by Flowjo software [8].

2.5. Analysis of Surface Markers

Surface markers of IDCs and MDCs were examined on the fifth and seventh days posttreatment. In brief, the cells were collected at the number of 1×10^5/100 μl, and then isotype tubes were incubated with 10 μl of FITC-PE isotype. While test tubes were treated with 10 μl of CD80 FITC, CD83 FITC (Bio-Rad, Endeavour House, UK), CD14 PE, anti-HLA-DR-PE, CD1a PE, CD86 FITC (IQProducts, Groningen, The Netherlands) for one hour at 4°C. Then, the expression of various markers was read by flow cytometry and analyzed by Flowjo software.

2.6. RNA Isolation and Real-time PCR

Total RNA was extracted from both IDCs and CUR/BER matured DCs by the Tripure Isolation Reagent kit (Roche, Germany) according to the company's protocol. Then, RNA samples were subjected to reverse transcription using PrimeScript™ RT reagent Kit (Takara, Japan). Relative real-time PCR of the desired markers, including inflammatory molecules (IDO), anti-inflammatory cytokine (IL-10), and proinflammatory factors (IL-12) (Table **1**), was done in Rotor-gene Q (Qiagen, Germany) using SYBR Green (Takara, Japan) as indicator dye. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. Finally, the $2^{-\Delta Ct}$ method was used to evaluate the mRNA expression changes in genes of interest.

2.7. Statistical Analysis

All tests were accomplished in triplicate, and data were expressed as the mean±standard deviation (SD). The statistical analysis was performed using SSPS 11.5 (IBM company, Armonk, NY) and Prism 6 (GraphPad Software, San Diego, CA). ANOVA and complementary Tukey and Bonferroni tests analyzed significant associations between means of groups. $P \leq 0.05$ was considered statically significant.

3. RESULTS

3.1. Isolation and characterization of monocytes

Monocytes were isolated from PBMCs *via* adhesion features resulting in 21.5% purity as evidenced by the relatively high expression (86.4%) of monocyte markers (CD45 and CD14) (Fig. **1A**, **B**).

3.2. Production and Characterization of IDC

The culture of isolated monocytes in the presence of GM-CSF and IL-4 cytokines led to the production of floating IDCs 5 days posttreatment. Morphologically, the resulting IDCs had a denticulate round shape rather than a spherical phenotype of monocytes (data not shown). To ensure the ability of IDCs to uptake antigens, IDCs were incubated with FITC-dextran, and the results of flow cytometry analysis confirmed that more than 95.9% of the IDCs could uptake dextran, indicative of the potential of these cells to uptake CUR and BBR (Fig. **2A**). To further verify the production of IDCs, the expression of different surface markers was analyzed by flow cytometry. The results were in favor of monocyte differentiation to IDCs as it was demonstrated by high expression of HLA-DR (66%) and CD86 (64.6%), low level of CD80 (15.6%), moderate expression of CD1a (33%), low secretion of CD83 (15.3%) as a DC maturation marker, and very low level of CD14 (7.6%) as monocyte marker (Table **2**). In addition, the identity of IDCs was more verified by a significant increase of *IDO* expression compared to IL-12 and IL-10 ($P \leq 0.0001$). In contrast to *IL-10*, the expression of *IL-12* was slightly increased (Fig. **2B**).

Table 1. The primer sequences of real-time PCR.

Primers	Sense Primer	Anti-sense Primer
Human IDO	5′- TGTGGCAGCAACTATTATAAGATG-3′	5′- GGTACTCTTTACTGATTGTCCAG-3′
Human IL-12	5′- CGCAGCCTCCTCCTTGTG-3′	5′- GCAACTCTCATTCTTGGTTAATTCC-3′
Human 1L-10	5′- GGACTTTAAGGGTTACCTGG-3′	5′- GTCTGGGTCTTGGTTCTC-3′
Human GAPDH	5′-TGAAGGGGTCATTGATGGCA -3′	5′-AAGGTCGGAGTCAACGGATTT -3′

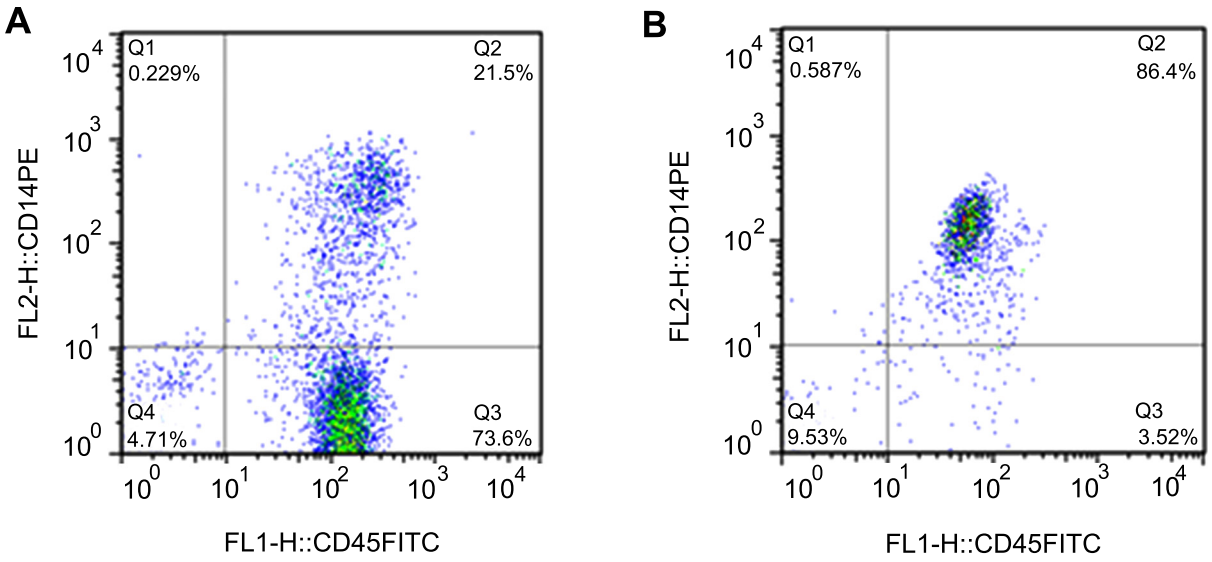


Fig. (1). Characterization of isolated monocytes. Flow cytometry analysis showed that the purity of monocytes in the PBMC population was 21.5% (**A**), and the frequency of the CD45 $^{+}$ and CD14 $^{+}$ subsets "monocytes" was 86.4% after sorting (**B**).

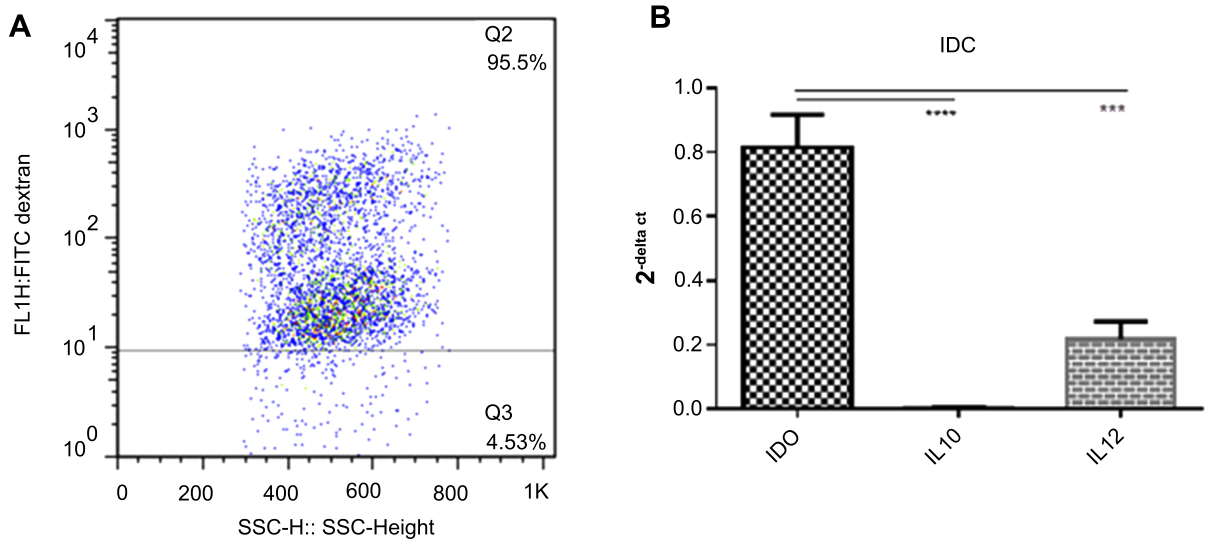


Fig. (2). Characterization of monocyte-derived immature DCs. (**A**) the results of the flow cytometry analysis showed that more than 95% of the immature DCs were able to uptake dextran-FITC. (**B**) Expression analysis of cytokines in DCs demonstrated significantly increased expression of *IDO* compared to *IL-10* and *IL-12*. Data are shown as mean ± SD. ***, **** indicate, respective P-values of ≤0.001 and ≤0.0001 between groups.

Table 2. Expression of cell surface markers in the immature dendritic cell (IDCs) subsets.

CD Marker	Expression (%)
HLA-DR	66 ± 2
CD86	64.6 ± 3
CD14	7.6 ±1.1
CD83	15.3 ± 2
CD1a	33 ± 6
CD80	15.6 ± 3.7

3.3. The Effects of CUR and BBR on IDC Maturation

To determine the effects of CUR and BBR on the development of MDCs, the IDCs derived from healthy and patient subjects were separately treated with these compounds alongside the above-mentioned cytokines (IL-4 plus GM-CSF). The results of flow cytometry analysis showed that the expression of CD14 marker, a monocyte factor, was notably decreased in cells matured with CUR and BBR compared to LPS control ($P \leq 0.0001$); however, this difference was not significant between healthy and SLE groups (Fig. **3A - F**). Likewise, CD86 expression, a co-stimulatory factor, was substantially decreased in IDC-derived from healthy and SLE subjects matured with CUR and BBR in comparison with LPS (healthy CUR and BBr *vs*. healthy LPs [$p = 0.0001$], SLE-CUR *vs*. SLE-LPS [$p = 0.0005$], SLE-BBR *vs*. SLE-LPS [$p \leq 0.005$]). HLA-DR, an antigen-presenting molecule, showed that its expression was reduced during IDC maturation in the presence of CUR and BBR in both healthy and lupus samples compared with the LPS group ($p = 0.0001$). Although the expression of the CD80 marker, a co-stimulatory factor, was significantly lessened in healthy-derived IDCS incubated with CUR and BBR in comparison to the LPS group ($p \leq 0.005$), its level just showed a significant reduction in CUR-treated cells compared to LPS group ($p \leq 0.005$). Analyzing the results of CD83 expression, a DC maturation marker, demonstrated that CUR and BBR treatment could not only noticeably reduce the amount of CD83 secretion in the healthy group (respectively, $p=0.0001$ and $p \leq 0.05$), but also this difference was noticeable between CUR and BBR treatment ($p \leq 0.005$). In contrast to BBR, CD83 was notably reduced in SLE-derived cells in the presence of CUR ($p \leq 0.005$). Likewise, CUR - and BBR-treated DCs significantly downregulated the expression of CD1a compared to LPS in both healthy and SLE-derived cells (healthy CUR and BBR *vs* healthy LPS [respectively, $p = 0.0001$ and $p \leq 0.05$], SLE-CUR and-BBR *vs* SLE-LPS [respectively, $p \leq 0.005$and $p \leq 0.05$]).

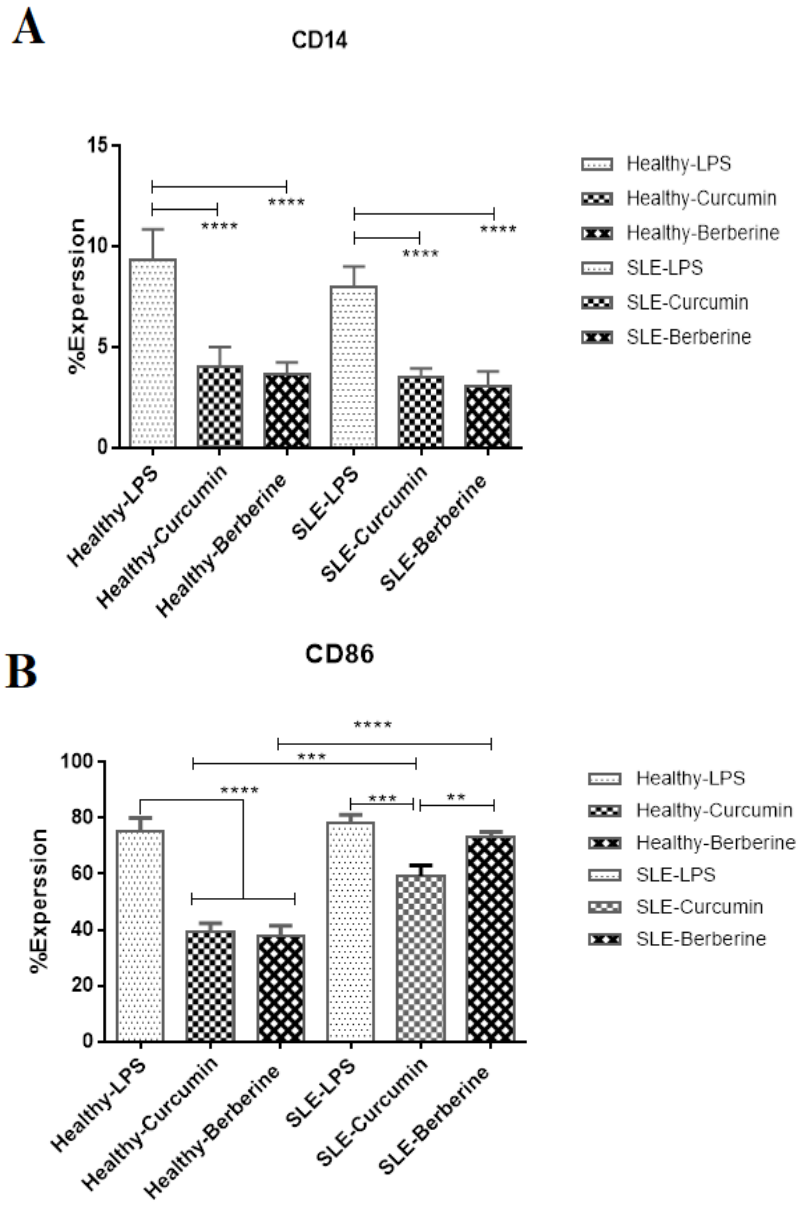


Fig. 3 contd.....

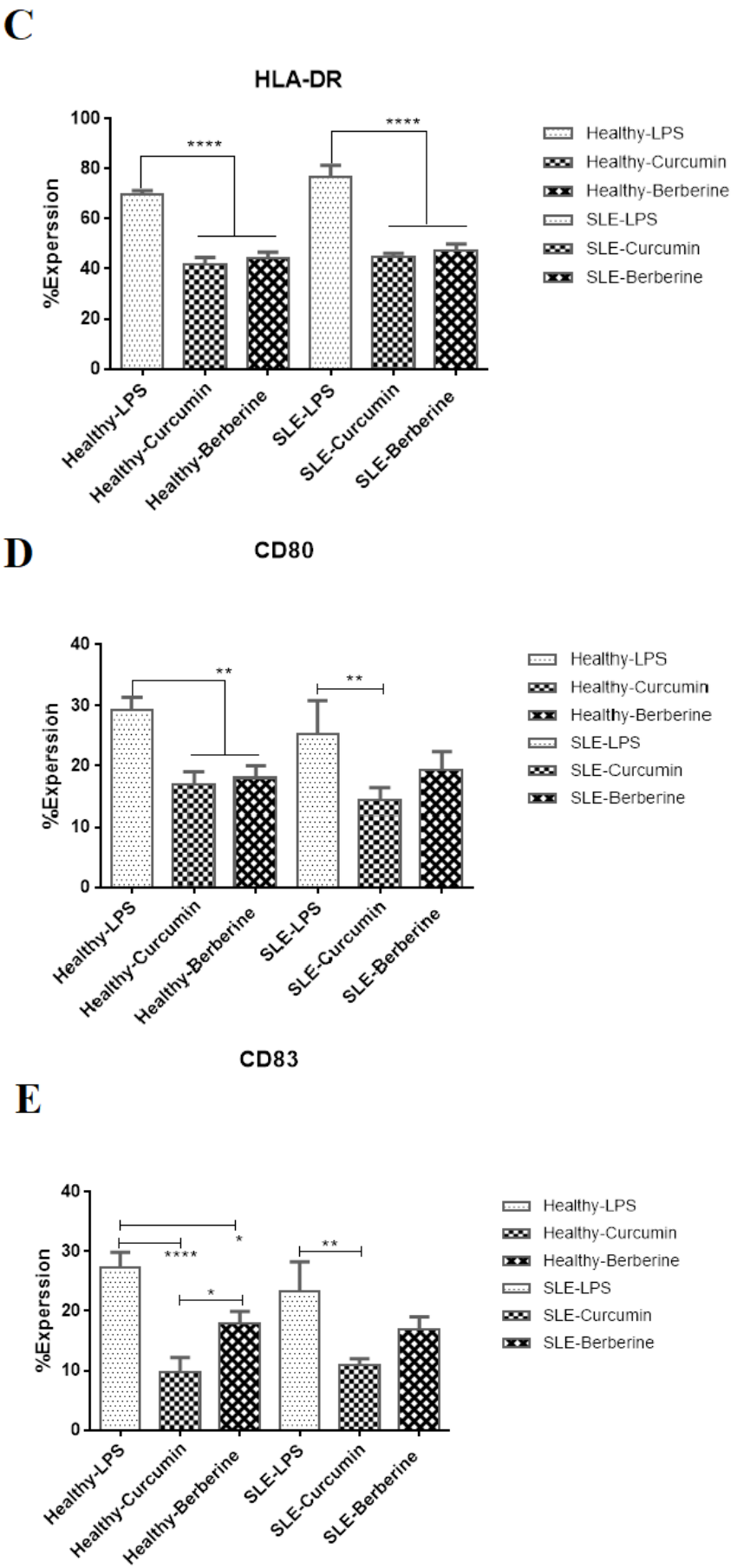

C
HLA-DR
%Experssion
100
80
60
40
20
0
****
****
Healthy-LPS
Healthy-Curcumin
Healthy-Berberine
SLE-LPS
SLE-Curcumin
SLE-Berberine
D
CD80
%Experssion
40
30
20
10
0
**
**
Healthy-LPS
Healthy-Curcumin
Healthy-Berberine
SLE-LPS
SLE-Curcumin
SLE-Berberine
CD83
E
%Experssion
40
30
20
10
0
****
*
*
**
Healthy-LPS
Healthy-Curcumin
Healthy-Berberine
SLE-LPS
SLE-Curcumin
SLE-Berberine

Fig. 3 contd.....

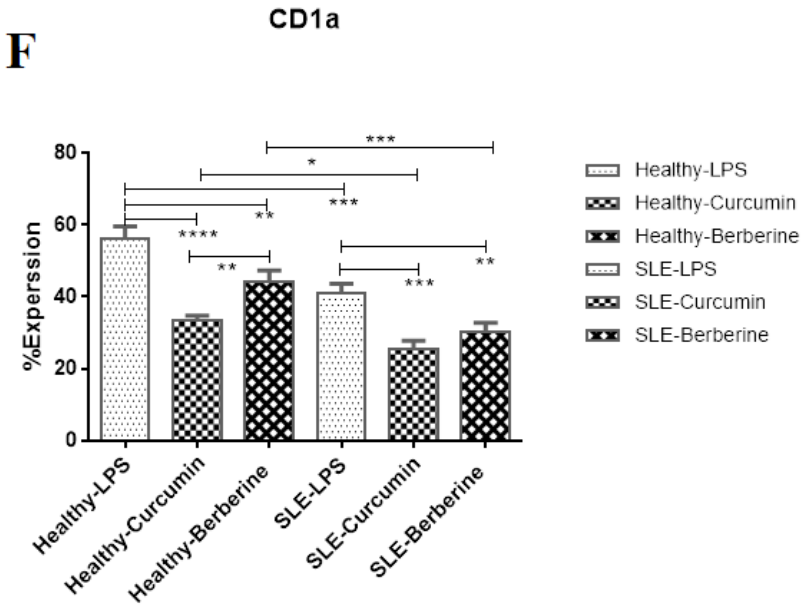


Fig. (3). CUR and BBR attenuated the expression of co-stimulatory, antigen-presenting, and maturation markers in human DCs derived from monocytes. Flow cytometry was used for measuring the levels of (**A**) CD14, (**B**) CD86, (**C**) HLA-DR, (**D**) CD80, (**E**) CD83, and (**F**) CD1a. *, **, ***, **** indicate respectively P-values of ≤ 0.05, ≤ 0.01, ≤0.001, and ≤0.0001 between groups**.**

To study the effects of CUR and BBR on the maturation process of IDCs in more detail, the mRNA levels of IL-12, IL-10, and IDO were evaluated by RT-qPCR. In LPS-treated DCs, the level of IL-12 was more than IDO and IL-10 (P-values ≤0.001). In curcumin/berberine-treated DCs, elevated levels of IDO and IL-10 were demonstrated in comparison with IL-12 but were not significant, respectively (P = 0.5699, P = 0.0983). (Fig. **4A - C**) (Healthy people). The comparison of IDO, IL-12, and IL-10 levels between LPS, Curcumin, and Berberine-treated DCs showed that the level of IDO and IL10 in both CUR/BER was increased compared to LPS. A significant increase was observed in the BER group, respectively (P-values ≤0.0155, P-values ≤0.0022) In spite of IL-12 that showed a significant reduction (P-values ≤0.0239)(Fig. **4A - C**)(SLE patient).

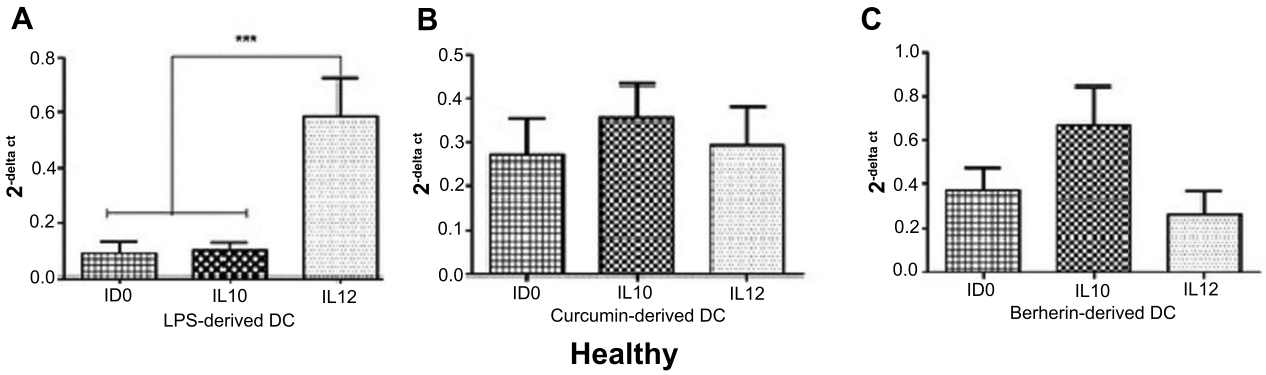


Fig. 4 contd.....

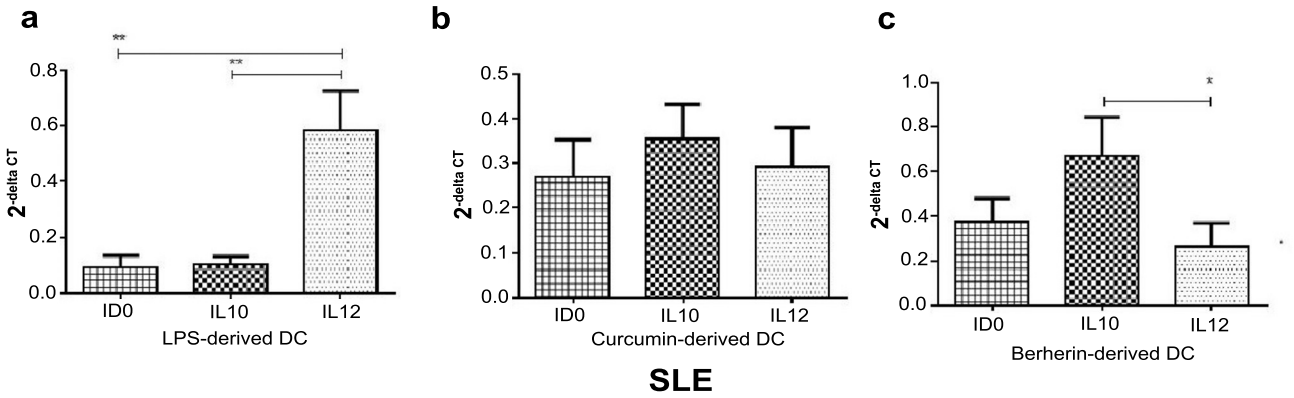


Fig. (4). CUR and BBR, in contrast to LPS, enhanced regulatory cytokine expression in treated DCS. In comparison with LPS, the real-time PCR displayed upregulation of IDO and IL-10 in DC treated with CUR and BBR. The increase in expression is evident, although the level of significance was not observed in all groups. Data are shown as mean ± SD, *** indicates P-values of ≤0.001, ** P-value ≤0.0022, * P-values ≤0.0155 between groups. ABS: healthy people, abc: SLE patient.

4. DISCUSSION

Accumulating evidence have demonstrated the effectiveness of CUR in alleviating the effects of several diseases, including SLE, by affecting various types of immune cells. Amongst them, it can refer to modulation of Th1, Th17, DCs, and Treg cells [16]. As well, BBR exhibits astonishing immunoregulatory effects and therapeutic function in SLE [21]. Based on this, the current study aimed to produce tolerogenic and non-inflammatory DCs using CUR and BBR. The results showed that IDC could be derived from co-culture of monocytes with IL-4 and GM-CSF, 5 days posttreatment which was approved by loss of monocyte adhesion and floating cells. Likewise, median expression of CD1a and high expression of CD86 and HLA-DR, alongside with the low amount of CD14 were other reasons confirming dendritic differentiation. It should bring to light that the expression of co-stimulators on the DCs surface has great type of importance in the activation of other immune cells.

In a similar study, Sim *et al*. generated DC under comparable conditions, as evidenced by low CD14 expression, high secretion of CD86 and HLA-DR, and a below-average production of CD83 consistent with our findings [22]. Our study's analysis of gene expression revealed that IDC could express high levels of IDO and low amounts of IL-12 and IL-10. In this regard, several studies have demonstrated the role of IDO as a negative regulator of the immune response [23]. According to a study by X.An *et al., the* expression of IDO in IDC led to accelerated apoptosis in Th2 cells and reduced allergic inflammation [24]. Since the uptake of CUR or BBR is necessary for the maturation of IDCS, our findings pointed out that all IDCs had the ability to uptake dextran-FITC. Similarly, Zhu *et al.* found that monocyte-derived DCs used for psoriasis treatment were able to uptake dextran-FITC dye as a measurable standard dye [25]. As well, Tarte *et al.* exhibited the ability of cytokine-produced IDCs to uptaking antigen and tumor apoptotic bodies [26]. In the current research, the results of treating monocyte-derived IDC with CUR or BBR alongside IL-4 plus GM-CSF for 48 h resulted in the production of tolerogenic and non-inflammatory DCs. In healthy subjects, LPS-matured DCs increased co-stimulator expression, which seems rational because MDCs are more suppliers and activators than IDCs. Moreover, the maturation process was confirmed *via* the notable expression of CD83, known as the DC maturation marker. Besides, the commitment of monocytes to mature DC was approved by high levels of CD1a and low expression of CD14, which is similar to the results of the Slobodin *et al.* study [27]. Moreover, enhanced expression of *IL-12* and decreased levels of *IDO* and *IL-10* in LPS-matured DCs of lupus case subjects were in agreement with the characteristics and phenotypes of these inflammatory cells. Since immuno-stimulants, LPS activates inflammatory pathways through TLR4, leading to the production of inflammatory mediators, inflammatory cell formation increases the amount of inflammatory cytokine, IL-12, and reduces the expression of inhibitory cytokines, IL-10 and IDO. Accordingly, Rigby *et al.* showed that LPS-stimulated DCs express high amounts of IL-12, while the levels of IL-10 were decreased [28]. It has been demonstrated that CUR could evoke immunomodulatory effects *via* suppressing proinflammatory cytokines like IL-12 in macrophages, splenic lymphocytes, DCs, and monocytes activated by LPS [29]. To some extent, the positive effects of CUR on human health could be explained by its capacity to elevate IL-10 expression [30]. As a result, CUR could block the response of DCs to immunostimulatory agents *via* inhibition of cytokines, maturation markers, and chemokine expression [20]. On the other hand, the therapeutic potential of BBR on autoimmune problems could be derived by the blockade of Th1/Th17 cells' inflammatory responses *via* IL-10 upregulation and IL-12 down-regulation [31]. In this regard, Hu *et al.* reported that BBR could selectively stimulate apoptosis in IL-12-producing DCs and shorten the DC lifespan [32]. Although our findings revealed that BBR had better effects on enhanced expression of *IDO* and *IL-10* than that of CUR, the exact reasons behind these differences need to be uncovered. The cells of lupus erythematosus have a specific phenotype that differs from that

of healthy individuals due to their genetic predisposition to SLE as well as frequent inflammation in the patient's body. The results of LPS-matured DCs isolated from SLE patients showed a reduction of CD1a and CD83 expression along with a little enhancement of co-stimulators (CD86 and HLA-DR) compared to healthy LPS-produced MDCs, which the different phenotypes of DC precursors could explain. In accordance with our results, Migita *et al.* demonstrated no special differences in TLR4 expression of lupus case-derived $CD14^+$ monocyte cells in spite of reduced TLR2 compared to healthy subjects-derived cells [33]. Molecularly, TLR4 is not only the main target of LPS but also substantial for the complete use of inflammatory responses. So, the reduction of TLR2 probably lessened the effects of LPS on DC maturation, which provides a plausible explanation for the difference between LPS-produced MDC in healthy and lupus cases. In more detail, Mozafarian *et al.* research displayed that monocyte-derived DCs from healthy subjects could increase the amounts of PDL1 and CTLA-4, 24 h-post stimulation in contrast to cells derived from lupus patients [34].

Mature DCs express co-stimulatory factors (CD86, CD83), antigen-presenting molecules (HLA-DR), and inflammatory cytokines (IL-12, IL-10) on their surface. So, impairment of the antigen-presenting apparatus of DCs hampered the T cells' imitated response [14]. In this context, our findings demonstrated that CUR and BBR-matured DCs downregulated CD86, CD80, CD83, CD1a, and HLA-DR. These findings were along with the Kim *et al.* study [15], which showed CUR inhibits the immunostimulatory activity of murine bone marrow-derived cells [14]. In the other part of our investigation, it was shown that administration of CUR and BBR to monocyte-derived DCS from SLE cases resulted in significant expression of CD83 and CD1a compared to cells from healthy individuals, which may be due to the original cell source and the influence of CUR and BBR on the cellular characteristics. In particular, lupus cells become more genetically accessible due to the ongoing inflammation in lupus patients. Although the exact mechanism by which CUR affects immune cells is not fully understood, there is evidence that CUR and BBR have inhibitory effects on the immune response. 12)). Consistent with our data, other studies highlight the potent function of CUR in suppressing CD86, CD80, and MHC-II on the DC surface, thereby attenuating immune stimulation [15]. Mechanically, CUR affects dendritic cells through the JAK/STAT signaling pathway and the transcription factors AP1 and NF-κB [35]. Additionally, DC subsets showed similar sensitivity to proapoptotic effects of BBR in a way that the ratio of mature to immature DCS selectively reduced following BBR treatment, an indicative role of BBR on DC departure, which is accompanied with production of reactive oxygen species (ROS), mitochondrial depolarization, and caspase 3 activation [32]. Since mature DCs play crucial roles in pathogenic inflammation and immune responses in rheumatoid arthritis (RA), BBR-induced apoptosis in mature DCs provides a major mechanism of immunomodulation that can account, at least in part, for the immunosuppressive and antiarthritic impacts observed in animal models of RA [32].

CONCLUSION

The results of this research may facilitate improved management of unwanted inflammation associated with SLE, given the importance of natural compounds as immuno-modulators. On the basis of our observations, our hypothesis is that CUR and BBR may have a disruptive effect on the inflammatory process by promoting the production of tolerogenic DCs. In addition, a more detailed understanding of the immunosuppressive mechanism behind the action of CUR and BBR is necessary for their clinical application as new anti-inflammatory agents. In addition, further studies will be conducted to optimize the bioavailability and physicochemical properties of CUR and BBR. This will help to establish them as effective adjuvant modulators.

AUTHOR CONTRIBUTIONS STATEMENT

Amin Reza Nikpoor, Nafiseh Tabasi, Atena Mansouri, Reyhane Modarres Moghadam, Ramiar Kamal Kheder, Amir Abbas Momtazi, and Abbas Shapouri Moghaddam participated in data collection, performing project and manuscript writing. Samaneh Mollazadeh and Tola Abdulsattar Faraj participated as the grammatical editors. Zahra Rezaieyazdi provided and confirmed SLE patients. Seyed-Alireza Esmaeili and Mahmoud Mahmoudi designed and drafted the article. All authors have fully read and approved the final manuscript.

LIST OF ABBREVIATIONS

SLE	=	Systemic Lupus Erythematosus
CUR	=	Curcumin
BRR	=	Berberine
DC	=	Dendritic Cell
CD	=	CD Marker
APC	=	Antigen Presenting Cells
TLR	=	Toll-like Receptors
LPS	=	Lipopolysaccharide

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All SLE patients were newly diagnosed. All protocols used in this study were approved by the Ethics Committee of Mashhad University of Medical Sciences (Mashhad, Iran). SLE patients were recruited consecutively from the Rheumatology Centre (approval no: IR.MUMS.MEDICAL.REC.1398.817 / 2019 July 23).

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All procedures performed in studies involving human participants were in accordance with the ethical standards of institutional and/or research committees and with the 1975 Declaration of Helsinki, as revised in 2013.

CONSENT FOR PUBLICATION

All participants signed a written informed consent form prior to participation in the study.

AVAILABILITY OF DATA AND MATERIALS

The data and supportive information are available within the article.

FUNDING

The funding body played no role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

CONFLICT OF INTEREST

The authors declare no competing financial and non-financial interests.

ACKNOWLEDGEMENTS

The authors appreciate the cooperation of Mashhad University of Medical Sciences and Hormozgan University of Medical Sciences.

REFERENCES

[1] Doria, A.; Iaccarino, L.; Ghirardello, A.; Zampieri, S.; Arienti, S.; Sarzi-Puttini, P.; Atzeni, F.; Piccoli, A.; Todesco, S. Long-term prognosis and causes of death in systemic lupus erythematosus. *Am. J. Med.,* **2006**, *119*(8), 700-706. [http://dx.doi.org/10.1016/j.amjmed.2005.11.034] [PMID: 16887417]

[2] Radmanesh, F.; Mahmoudi, M.; Yazdanpanah, E.; Keyvani, V.; Kia, N.; Nikpoor, A.R.; Zafari, P.; Esmaeili, S.A. The immunomodulatory effects of mesenchymal stromal cell□based therapy in human and animal models of systemic lupus erythematosus. *IUBMB Life,* **2020**, *72*(11), 2366-2381. [http://dx.doi.org/10.1002/iub.2387] [PMID: 33006813]

[3] Ohl, K.; Tenbrock, K. Inflammatory cytokines in systemic lupus erythematosus. *J. Biomed. Biotechnol.,* **2011**, *2011*, 1-14. [http://dx.doi.org/10.1155/2011/432595] [PMID: 22028588]

[4] Herrada, A.A.; Escobedo, N.; Iruretagoyena, M.; Valenzuela, R.A.; Burgos, P.I.; Cuitino, L.; Llanos, C. Innate immune cells' contribution to systemic lupus erythematosus. *Front. Immunol.,* **2019**, *10*, 772. [http://dx.doi.org/10.3389/fimmu.2019.00772] [PMID: 31037070]

[5] Esmaeili, S-A.; Taheri, R.A.; Mahmoudi, M.; Momtazi-Borojeni, A.A.; Morshedi, M.; Bahramifar, A.; Fasihi-Ramandi, M. Inhibitory effects of tolerogenic probiotics on migratory potential of lupus patient-derived DCs. *Iran. J. Basic Med. Sci.,* **2021**, *24*(11), 1509-1514. [PMID: 35317107]

[6] Klarquist, J.; Zhou, Z.; Shen, N.; Janssen, E.M. Dendritic cells in systemic lupus erythematosus: From pathogenic players to therapeutic tools. *Mediators Inflamm.,* **2016**, *2016*, 1-12. [http://dx.doi.org/10.1155/2016/5045248] [PMID: 27122656]

[7] Esmaeili, S.A.; Mahmoudi, M.; Momtazi, A.A.; Sahebkar, A.; Doulabi, H.; Rastin, M. Tolerogenic probiotics: Potential immunoregulators in Systemic Lupus Erythematosus. *J. Cell. Physiol.,* **2017**, *232*(8), 1994-2007. [http://dx.doi.org/10.1002/jcp.25748] [PMID: 27996081]

[8] Esmaeili, S.A.; Mahmoudi, M.; Rezaieyazdi, Z.; Sahebari, M.; Tabasi, N.; Sahebkar, A.; Rastin, M. Generation of tolerogenic dendritic cells using *Lactobacillus rhamnosus* and *Lactobacillus delbrueckii* as tolerogenic probiotics. *J. Cell. Biochem.,* **2018**, *119*(9), 7865-7872. [http://dx.doi.org/10.1002/jcb.27203] [PMID: 29943843]

[9] Kaewraemruaen, C.; Ritprajak, P.; Hirankarn, N. Dendritic cells as key players in systemic lupus erythematosus. *Asian Pac. J. Allergy Immunol.,* **2020**, *38*(4), 225-232. [PMID: 31837212]

[10] Mardani, F.; Mahmoudi, M.; Esmaeili, S.A.; Khorasani, S.; Tabasi, N.; Rastin, M. *In vivo* study: Th1–Th17 reduction in pristane□induced systemic lupus erythematosus mice after treatment with tolerogenic *Lactobacillus* probiotics. *J. Cell. Physiol.,* **2019**, *234*(1), 642-649. [http://dx.doi.org/10.1002/jcp.26819] [PMID: 30078223]

[11] Khorasani, S.; Mahmoudi, M.; Kalantari, M.R.; Lavi Arab, F.; Esmaeili, S.A.; Mardani, F.; Tabasi, N.; Rastin, M. Amelioration of regulatory T cells by *Lactobacillus delbrueckii* and *Lactobacillus rhamnosus* in pristane□induced lupus mice model. *J. Cell. Physiol.,* **2019**, *234*(6), 9778-9786. [http://dx.doi.org/10.1002/jcp.27663] [PMID: 30370554]

[12] Yazdanpanah, E.; Mahmoudi, M.; Sahebari, M.; Rezaieyazdi, Z.; Esmaeili, S.A.; Tabasi, N.; Jaberi, S.; Sahebkar, A.; Rastin, M. Vitamin D3 alters the expression of toll□like receptors in peripheral blood mononuclear cells of patients with systemic lupus erythematosus. *J. Cell. Biochem.,* **2017**, *118*(12), 4831-4835. [http://dx.doi.org/10.1002/jcb.26155] [PMID: 28544067]

[13] Kotha, R.R.; Luthria, D.L. Curcumin: biological, pharmaceutical, nutraceutical, and analytical aspects. *Molecules,* **2019**, *24*(16), 2930. [http://dx.doi.org/10.3390/molecules24162930] [PMID: 31412624]

[14] Shirley, S.A.; Montpetit, A.J.; Lockey, R.F.; Mohapatra, S.S. Curcumin prevents human dendritic cell response to immune stimulants. *Biochem. Biophys. Res. Commun.,* **2008**, *374*(3), 431-436. [http://dx.doi.org/10.1016/j.bbrc.2008.07.051] [PMID: 18639521]

[15] Kim, G.Y.; Kim, K.H.; Lee, S.H.; Yoon, M.S.; Lee, H.J.; Moon, D.O.; Lee, C.M.; Ahn, S.C.; Park, Y.C.; Park, Y.M. Curcumin inhibits immunostimulatory function of dendritic cells: MAPKs and translocation of NF-κ B as potential targets. *J. Immunol.,* **2005**, *174*(12), 8116-8124. [http://dx.doi.org/10.4049/jimmunol.174.12.8116] [PMID: 15944320]

[16] Momtazi-Borojeni, A.A.; Haftcheshmeh, S.M.; Esmaeili, S.A.; Johnston, T.P.; Abdollahi, E.; Sahebkar, A. Curcumin: A natural modulator of immune cells in systemic lupus erythematosus. *Autoimmun. Rev.,* **2018**, *17*(2), 125-135. [http://dx.doi.org/10.1016/j.autrev.2017.11.016] [PMID: 29180127]

[17] Chehreh, R.; Sayehmiri, K.; Shohani, M.; Vahidnia, S.; Tavan, H. Comparing the effect of herbal drugs and non-steroidal anti-inflammatory drugs on primary dysmenorrhea in iran: A systematic review and meta-analysis. *J. Chem. Health Risks,* **2021**, *11*(1), 41-53.

[18] Rogers, N.M.; Kireta, S.; Coates, P.T.H. Curcumin induces maturation-arrested dendritic cells that expand regulatory T cells *in vitro* and *in vivo*. *Clin. Exp. Immunol.,* **2010**, *162*(3), 460-473. [http://dx.doi.org/10.1111/j.1365-2249.2010.04232.x] [PMID: 21070208]

[19] Habtemariam, S. Berberine and inflammatory bowel disease: A concise review. *Pharmacol. Res.,* **2016**, *113*(Pt A), 592-599. [http://dx.doi.org/10.1016/j.phrs.2016.09.041] [PMID: 27697643]

[20] Rahimi, K.; Hassanzadeh, K.; Khanbabaei, H.; Haftcheshmeh, S.M.; Ahmadi, A.; Izadpanah, E.; Mohammadi, A.; Sahebkar, A. Curcumin: A dietary phytochemical for targeting the phenotype and function of dendritic cells. *Curr. Med. Chem.,* **2021**, *28*(8), 1549-1564. [http://dx.doi.org/10.2174/1875533XMTA2fNjQ33] [PMID: 32410550]

[21] Bao, Y.; Wu, Y.; Chen, T.; Jin, Y.; Sha, X.; Ge, X.; Meng, Y.; Guo, G.; Xia, Y.; Yang, J.; Sun, C.; Dong, C.; Ji, J.; Xue, Z.; Gu, Z. Berberine modulates lupus syndrome *via* the regulation of gut microbiota in MRL/Lpr mice *Research Square,* **2021**.

[22] Sim, W.J.; Malinarich, F.; Fairhurst, A-M.; Connolly, J.E. Generation of immature, mature and tolerogenic dendritic cells with differing metabolic phenotypes. *J. Vis. Exp.,* **2016**, (112), e54128-e54128. [PMID: 27404554]

[23] Furset, G.; Fløisand, Y.; Sioud, M. Impaired expression of indoleamine 2, 3□dioxygenase in monocyte□derived dendritic cells in response to Toll□like receptor□7/8 ligands. *Immunology,* **2008**, *123*(2), 263-271. [http://dx.doi.org/10.1111/j.1365-2567.2007.02695.x] [PMID: 17725606]

[24] An, X.J.; Bai, C.X.; Xia, J.B.; Dang, T.; Qian, P.; Qian, G.S.; Liao, W. Immature dendritic cells expressing indoleamine 2,3-dioxygenase suppress ovalbumin-induced allergic airway inflammation in mice. *J. Investig. Allergol. Clin. Immunol.,* **2011**, *21*(3), 185-192. [PMID: 21548446]

[25] Zhu, K-J.; Cheng, H.; Mao, X.H.; Lao, L.M.; Cen, J.P.; Ye, J. Increased endocytic activity in monocyte-derived dendritic cells in patients with psoriasis vulgaris. *Indian J. Med. Res.,* **2006**, *123*(1), 43-50. [PMID: 16567867]

[26] Tarte, K.; Fiol, G.; Rossi, J-F.; Klein, B. Extensive characterization of dendritic cells generated in serum-free conditions: Regulation of soluble antigen uptake, apoptotic tumor cell phagocytosis, chemotaxis and T cell activation during maturation *in vitro*. *Leukemia,* **2000**, *14*(12), 2182-2192. [http://dx.doi.org/10.1038/sj.leu.2401925] [PMID: 11187909]

[27] Slobodin, G.; Kessel, A.; Kofman, N.; Toubi, E.; Rosner, I.; Odeh, M. Phenotype of resting and activated monocyte-derived dendritic cells grown from peripheral blood of patients with ankylosing spondylitis. *Inflammation,* **2012**, *35*(2), 772-775. [http://dx.doi.org/10.1007/s10753-011-9373-x] [PMID: 21833763]

[28] Rigby, R.J.; Knight, S.C.; Kamm, M.A.; Stagg, A.J. Production of interleukin (IL)-10 and IL-12 by murine colonic dendritic cells in response to microbial stimuli. *Clin. Exp. Immunol.,* **2004**, *139*(2),

245-256.
[http://dx.doi.org/10.1111/j.1365-2249.2004.02674.x] [PMID: 15654823]

[29] Yuandani, ; Jantan, I.; Rohani, A.S.; Sumantri, I.B. Immunomodulatory effects and mechanisms of curcuma species and their bioactive compounds: A review. *Front. Pharmacol.,* **2021**, *12*, 643119.
[http://dx.doi.org/10.3389/fphar.2021.643119] [PMID: 33995049]

[30] Mollazadeh, H.; Cicero, A.F.G.; Blesso, C.N.; Pirro, M.; Majeed, M.; Sahebkar, A. Immune modulation by curcumin: The role of interleukin-10. *Crit. Rev. Food Sci. Nutr.,* **2019**, *59*(1), 89-101.
[http://dx.doi.org/10.1080/10408398.2017.1358139] [PMID: 28799796]

[31] Ehteshamfar, S.M.; Akhbari, M.; Afshari, J.T.; Seyedi, M.; Nikfar, B.; Shapouri-Moghaddam, A.; Ghanbarzadeh, E.; Momtazi-Borojeni, A.A. Anti□inflammatory and immune□modulatory impacts of berberine on activation of autoreactive T cells in autoimmune inflammation. *J. Cell. Mol. Med.,* **2020**, *24*(23), 13573-13588.
[http://dx.doi.org/10.1111/jcmm.16049] [PMID: 33135395]

[32] Hu, Z.; Jiao, Q.; Ding, J.; Liu, F.; Liu, R.; Shan, L.; Zeng, H.; Zhang, J.; Zhang, W. Berberine induces dendritic cell apoptosis and has therapeutic potential for rheumatoid arthritis. *Arthritis Rheum.,* **2011**, *63*(4), 949-959.
[http://dx.doi.org/10.1002/art.30202] [PMID: 21162100]

[33] Migita, K.; Miyashita, T.; Maeda, Y.; Nakamura, M.; Yatsuhashi, H.; Kimura, H.; Ishibashi, H.; Eguchi, K. Toll-like receptor expression in lupus peripheral blood mononuclear cells. *J. Rheumatol.,* **2007**, *34*(3), 493-500.
[PMID: 17295441]

[34] Mozaffarian, N.; Wiedeman, A.E.; Stevens, A.M. Active systemic lupus erythematosus is associated with failure of antigen-presenting cells to express programmed death ligand-1. *Rheumatology,* **2008**, *47*(9), 1335-1341.
[http://dx.doi.org/10.1093/rheumatology/ken256] [PMID: 18650228]

[35] Zhao, H.M.; Xu, R.; Huang, X.Y.; Cheng, S.M.; Huang, M.F.; Yue, H.Y.; Wang, X.; Zou, Y.; Lu, A.P.; Liu, D.Y. Curcumin suppressed activation of dendritic cells *via* JAK/STAT/SOCS signal in mice with experimental colitis. *Front. Pharmacol.,* **2016**, *7*, 455.
[http://dx.doi.org/10.3389/fphar.2016.00455] [PMID: 27932984]