ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
February 2022; 21(1):35-43.
Doi: 10.18502/ijaai.v21i1.8611

A Significant Increase in the Gene Expression of *GATA-3* Following the Treatment of Osteoarthritis Patients with Crocin

Javad Poursamimi[1,2], Zhaleh Shariati-Sarabi[3,4], Jalil Tavakkol-Afshari[5,6], and Mojgan Mohammadi[5,6]

[1] Department of Immunology, Faculty of Medicine, Zabol University of Medical Sciences, Zabol, Iran
[2] Allergy Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[3] Rheumatic Diseases Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[4] Department of Internal Medicine, Imam Reza Hospital, Mashhad University of Medical Sciences, Mashhad, Iran
[5] Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[6] Department of Immunology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Received: 24 August 2021; Received in revised form: 19 September 2021; Accepted: 1 October 2021

ABSTRACT

Osteoarthritis (OA) is known to be the most prevalent form of joint disease. We conducted this clinical trial to investigate the effects of Krocina™, a natural product containing crocin, on the gene expression of unique transcription factors of various T cell subsets in patients with OA.

We collected 40 peripheral blood samples of OA patients receiving Krocina™ and equal number of those who took a placebo (IRCT2015021910507N2, NCT03375814). RNA extraction was performed from the cultured peripheral blood mononuclear cells of the OA patients who received Krocina™ and placebo and SYBR Green Real-time PCR technique was applied to assess the relative gene expression of *T-bet*, *GATA3*, *ROR-γt*, and *FOXP3* as the unique transcription factors of various T cell subsets.

The relative gene expression of *T-bet* and *ROR-γt* insignificantly decreased in the Krocina™ receiving group as compared to the placebo group. In addition, the relative gene expressions of *GATA-3* and *FOXP3* after the treatment with Krocina™ showed a significant and insignificant increase, respectively. Moreover, an insignificant decrease was observed in the gene expression of *GATA-3* and *FOXP3* in the placebo group. A significant and insignificant decrease in the gene expression of *T-bet* and *ROR- γt* was detected in the OA patients who received a placebo. *GATA-3* is known as a unique transcription factor for the differentiation of T-cells to the Th2 subset.

The significant increase in the gene expression of *GATA-3* in the patients with OA treated with crocin may suggest the beneficial effect of crocin on shifting towards the Th2 subset and enhancing an anti-inflammatory condition.

Keywords: Crocin; Crocus; Gene expression; Osteoarthritis; Transcription factors

Corresponding Author: Mojgan Mohammadi, MD, PhD; Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran, Tel: (+98 51) 3711 2611/, (+98 913) 2428 291, E-mail: Mohammadimzh@mums.ac.ir

INTRODUCTION

Osteoarthritis is the most common form of joint disease which includes a local chronic inflammatory

response along with degeneration of articular cartilage.[1]

Overweight, gender, age, inappropriate physical condition, race, joint surgery, and malignancy are considered major OA risk factors.[2] Based on the joint radiographic images, histological and laboratory tests are exploited for the diagnosis of OA.[2]

Th1 and Th2 cells cytokine secretion imbalance is believed to be the most frequent factor in the pathogenesis of OA.[3,4] The crocin effects on the differentiation and percentage of Th1 cells may be indirect. For instance, in 2011, Bani et al showed that oral administration of alcoholic extract of Crocus sativus to mice drives a shift from Th1 toward Th2 response.[5] and consequently decreases levels of inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-18, interferon-γ (IFN-γ), and IL-1β.[6] Moreover, crocin is capable of suppressing the phosphorylated nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor-α, p-IκB kinaseα/β, and transcription factor-p65 expression in arthritis mice.[7] Park et al, in 2015, showed that crocin can decrease signal transducer and activator of transcription-1 (STAT-1) expression in a transformed aneuploid immortal keratinocyte cell line.[8]

T-bet and GATA binding protein-3 transcription factors have a vital role in the differentiation of CD4+ T cells into Th1 and Th2 effector cells.[9] T-bet is encoded by a conserved family of genes named T-box. It is a unique transcription factor for Th1 cell, which controls the expression of the Th1 cytokines, such as IFN-γ.[10] The cooperation between several factors, such as STAT-1, is required for the differentiation of naïve CD4+/CD3+ T cells to Th1 cells.[8]

GATA3 is also encoded by a gene with the same name. Naïve CD4+ T cells are differentiated to Th2 cells via the expression of the *GATA3* gene.[11] It plays a crucial role in the production of some cytokines such as IL-5 from the Th2 subset.[12]

Th17 is another T-cell subset involved in inflammatory status. Several cytokines, such as IL-1β, IL-6, transforming growth factor-β (TGF-β), and IL-23, participate in the differentiation and survival of Th17 cells.[13] Moreover, other transcription factors like Retinoic acid-related orphan receptor-γt (ROR-γt), Interferon regulatory factor-4 (IRF-4), basic leucine zipper transcription factor (BATF), and signal transducer and activator of transcription-3 (STAT-3) have a major influence on the differentiation of CD4+ naïve T cells to Th17 cells.[14–16]

Forkhead box P3 (FOXP3) is a pivotal regulatory transcription factor for the differentiation and development of CD4+naïve T cells to regulatory T lymphocytes. Any defects or loss of function due to mutations in the *FOXP3* gene, cause exacerbation of inflammatory disorders.[17] In the present clinical trial, we evaluated the effects of krocina™ on the gene expression of *T-bet*, *GATA-3*, *ROR-γt*, and *FOXP3* in patients with OA.

MATERIALS AND METHODS

Patient Selection

This case-control study was performed on RNA samples that were extracted from peripheral blood mononuclear cells (PBMCs) of patients with OA and banked in minus 80 degrees centigrade for further requirements. Further details regarding patients' selection have been shown in our recently published article.[18]

In brief, 40 patients suffering from OA were enrolled in our study and randomly divided into two groups, receiving Krocina™ and placebo. The clinical trial continued for four months. RNA was extracted from the cultured PBMCs of OA patients. Our clinical trial started in July 2016 and continued to June 2018 following the approval of the Ethics Committee of Mashhad University of Medical Sciences, Mashhad, Iran, with a reference number of IR.mums.fm.REC.13940279. For all the patients, sodium diclofenac was prescribed at a dosage of 50 mg daily as a painkiller.

Blood Sampling and Cell Culture

The blood samples were transferred into tubes containing EDTA. Peripheral blood mononuclear cells (PBMCs) were separated with Ficoll and centrifugation and then cultured in complete tissue medium (CTM) with 5 μg/mL Phytohemagglutinin and kept at 37°C in a humid incubator with 5% CO2.

Molecular Assessment Using the Real-time PCR Technique

RNA extraction from PBMCs was performed based on RNA Extraction kit instruction (www.Yektatajhiz.comCat.Number:YT9065).

Subsequently, RNA was employed to synthesize cDNA; utilizing a specific cDNA synthesis kit (Cat.Number: YT4500).

Initially, the primers were designed in house employing Beacon Designer software and blasted on the NCBI website (Primer-BLAST) to check the specificity. The sequence of the primers was presented in Table 1.

RT-PCR for relative genes expression rates of *T-bet*, *GATA-3*, *RoR-γt*, and *GAPDH* (as Internal Control) was performed according to manufacture protocol (Takara.Co and Afragen Biotech. Co). Briefly, 5 µL of SYBR Green Master Mix (Takara.Co), 0.4 µL (10 pmol/µL) of forwarding primer, 0.4 µL (10 pmol/µL) of reverse primer (Afragen Biotech. Co), 4 µL cDNA and 0.2 µL Sterilized water was added to a tube.

Table 1. Primer sequences designed for SYBR Green real-time PCR.

Gene name	Accession number	Sequence
T-bet-F	NM_013351.1	5′-ATTGCCGTGACTGCCTACCAGA-3′
T-bet-R		5′-GGAATTGACAGTTGGGTCCAGG-3′
GATA3-F	NM_001002295.1	5′-ACCACAACCACACTCTGGAGGA-3′
GATA3-R		5′-TCGGTTTCTGGTCTGGATGCCT-3′
ROR-γt-F	NM_005060.4	5′-CCCTGACAGAGATAGAGCACC-3′
ROR-γt-R		5′-TTCCCACATCTCCCACATGG-3′
FOXP3-F	NM_014009.3	5′-GGCACAATGTCTCCTCCAGAGA-3′
FOXP3-R		5′-CAGATGAAGCCTTGGTCAGTGC-3′
GAPDH-F	NM_001289746.1	5′-CACTAGGCGCTCACTGTTCTC-3′
GAPDH-R		5′-CCAATACGACCAAATCCGTTGAC-3′

F: forward primer; R: reverse primer; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

RT-PCR cycle was as follows: Hold 10 minutes at 95°C, followed by 40 cycles of denaturation at 95°C for 10 s, 30 seconds at 60 C for primers annealing and extention step 20 second in 72°C.

RT-PCR for relative gene expression rate of *FOXP3* was performed according to manufacture protocol (Takara.Co and Afragen Biotech. Co). 5 µL of SYBR Green Master Mix (Takara.Co), 0.25 µL (10 pmol/µL) of forwarding primer, 0.25 (10 pmol/µL) of reverse primer (Afragen Biotech. Co), 2 µL cDNA and 2.5 µL Sterilized water were added to a tube.

RT-PCR cycle was: Hold 2 minute at 95°C, 15 seconds at 95°C for denaturation, 15 seconds at 60°C for primers annealing, and extension step 20 seconds at 72°C.

Expression levels of mRNAs were calculated employing the $2^{-\Delta\Delta CT}$ formula.

Data Analysis

IBM SPSS Statistics 21 was employed for the data analysis. The mean differences and 95% confidence intervals were compared before and after the intervention. The parametric data were further analyzed using Paired Sample t-test while the nonparametric data were assessed via the Wilcoxon test. *p*-value less than 0.05 was considered to be statistically significant.

RESULTS

Demographic Data for Patients with Knee OA

It is noteworthy that five patients were excluded during the trial based on our inclusion and exclusion criteria [18]. Thirty-five patients with OA in both genders (women 66.7% and men 33.3%) aged 40-75 years were divided into the intervention group with 18 subjects (mean±SEM 58.7±1.51) and the placebo receiving group with 17 subjects (mean±SEM 57.64±1.72). They completed a four-month follow-up period. The patients in both groups had non-significant differences regarding age and gender.

The disease duration mean±SEM was 4.23±0.54 (Four years and 23 days) in the intervention and 4.26±0.57 (Four years and 26 days) in the placebo groups. The participants were randomized into two

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

groups, the intervention comprising 18 patients (11 women and 7 men), and the placebo with 17 patients (15 women and 2 men).

The Effect of Krocina™ and Placebo on the Gene Expression of *T-bet* Transcription Factor

Relative gene expression of *T-bet* in the intervention group showed an insignificant decrease ($p>0.05$). A significant decrease was observed in the gene expression of *T-bet* after the intervention ($p<0.05$) in the placebo group (Figure 1A).

The Effect of Krocina™ and Placebo on the Gene Expression of *GATA-3* Transcription Factor

Following the intervention, the relative gene expression of *GATA-3* in the intervention group showed a significant increase ($p<0.05$). Nevertheless, in the placebo group, an insignificant decrease of gene expression of *GATA-3* was observed after the intervention (Figure 1B).

The Effect of Krocina™ and Placebo on the Gene Expression of *ROR-γt* Transcription Factor

Relative gene expression of *ROR-γt* showed an insignificant decrease in the intervention and placebo groups after four months Figure 1C.

Hence, selecting a control group of patients with OA who do not take any conventional and interventional therapies might be a good choice for future studies to have a more reliable interpretation in this regard.

The Effect of Krocina™ and Placebo on the Gene Expression of *FOXP3* Transcription Factor

An insignificant increase was observed in relative gene expression of F*OXP3* in the intervention group after four months ($p>0.05$). However, in the placebo group, an insignificant decrease of gene expression of *FOXP3* was detected following the intervention Figure 1D.

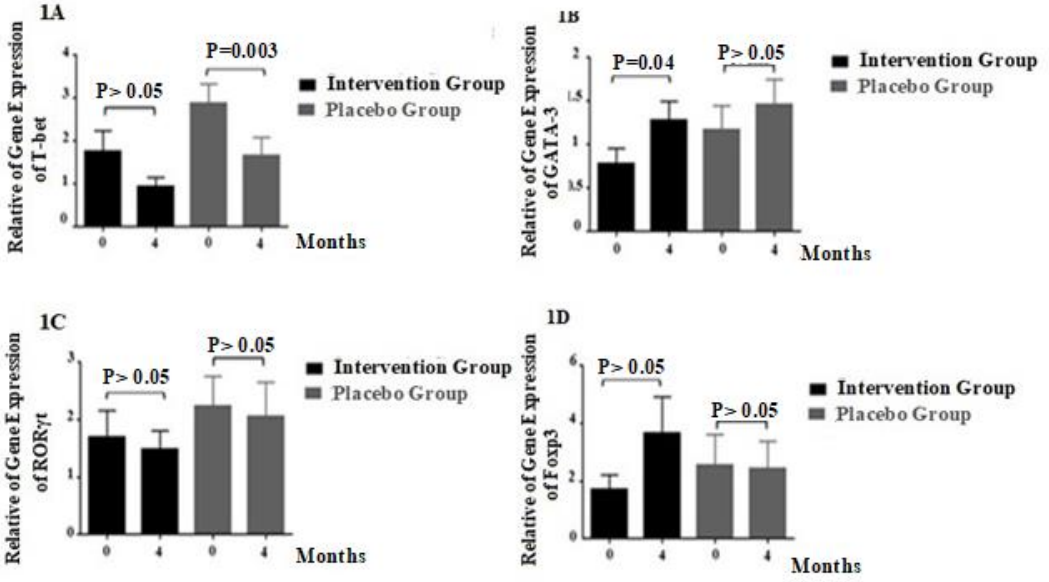

Figure 1. Relative gene expression of *T-bet* (1A), *GATA3* (1B), *ROR-γt* (1C), and *FOXP3* (1D) before and four months after the intervention.

.

DISCUSSION

OA is identified as an illness characterized by progressive destructive changes in joints and limitation in performing daily work.[1]

In the present study, we evaluated the effects of Krocina™ on the gene expression of various T-cell subsets transcription factors in patients with OA.

Our results revealed an insignificant decrease in the gene expression of *T-bet* in the intervention group at

the end of the four-month intervention whereas the same comparison in the placebo group showed a significant decrease.

The differentiation of the naive Th0 to Th1 lymphocytes requires a set of intracellular and extracellular factors to collaborate. For example, several transcription factors, such as the STAT-1, STAT-4, and T-bet, lead to the differentiation of naive CD3+CD4+ Th0 cells to IFN-γ secreting CD3+CD4+ T cells in antigen-dependent mechanisms.[19] It has been shown that the increase in the level of IL-12 in the microenvironment containing dendritic cell (DC) and macrophage (MQ) can activate the signal transduction pathway related to the STAT-4 transcription factor which is required for converting the naive Th0 cells to IFN-γ secreting CD3+ CD4+ Th1 cells. Additionally, IFN-γ leads to an increase in the expression of the T-bet in Th1 cells by STAT-1 pathway that mediates the proliferation of Th1 cells.[20]

Previous studies have evaluated the effects of saffron on the ratio of specific cytokines secreted from Th1/Th2 lymphocytes. However, we could not find any studies to evaluate the effect of saffron or its active ingredients on the gene expression of *T-bet*.

The majority of studies have focused on the effects of saffron and its active ingredients on pro-inflammatory cytokines and transcription factors like IFN- γ and NF-κB, respectively.[21]

The significant decrease in the gene expression of *T-bet* after four months in the placebo group might be attributed to the consumption of nonsteroidal anti-inflammatory drugs, such as sodium diclofenac by OA patients or placebo effects. In 2005, Wampold et al, reported the positive effects of placebo in medical treatment and psychotherapy. They reported that placebo consumption in patients with chronic diseases decreased the pain and anxiety affecting biochemical-neural pathways.[22]

Our findings herein shed light on a significant increase in the gene expression of *GATA-3* following four months in the intervention group as compared with time point zero.

GATA-3 is a unique transcription factor for the differentiation of Th0 lymphocytes to Th2 cells. GATA-3 can bind to the promoter of the Th2 cytokines, including IL-4, IL-5, and IL-13, which eventually prevents the proliferation and differentiation towards the Th1 subset.[11]

Recently, it has been reported that T-bet can regulate the function of GATA-3 and naive CD3+ CD4+T cells need competition between GATA-3 and T-bet for the differentiation towards Th1 and Th2 cells. It seems that the increase in the IL-4 level could help GATA-3 to win in this competition.[19,23] Moreover, the activation of the IL-4-STAT-6 pathway in Th2 cells can promote transcription of the *GATA-3* gene.[20] On the other hand, studies have depicted a competition between GATA-3 and FOXP3 transcription factors. Naive T cells could be differentiated into the particular Th2 or induced Treg cell subsets by specific transcription factors. Additionally, the presence of IL-4 in the tissue microenvironment can enhance the gene expression of *GATA-3* in competition with *FOXP3*. Researchers have reported that GATA-3 can be conjugated to the promoter of FOXP3 and inhibit its gene expression.[14] According to these data, the anti-inflammatory effect of crocin on improving OA clinical symptoms which were demonstrated in our study might in part correlate to its influence on the increase in *GATA-3* gene expression which is beneficial for the suppression of inflammation.

ROR-γt is defined as a unique transcription factor for the differentiation of IL-17 secreting Th17 cells.[24]

Based on our findings, the gene expression of *ROR-γt* after four months in the intervention and placebo groups insignificantly decreased.

For the differentiation of CD4+ CD25- naive T cells to IL-17 secreting Th17 cells, the concomitant presence of TGF-β and IL-6 in the microenvironment is necessary.[25] It has been reported that IL-6 can suppress the differentiation of CD4+CD25- naive T cells to Treg cells by linkage to *FOXP3* gene promoter. Researches in cell line have shown that the second promoter of FOXP3 could be conjugated to the promoter of ROR-γt and inhibit the conjugation of ROR-γt to IL-17A expressing gene.[25] Moreover, Mathur et al, in 2007, reported that other transcription factors, STAT3 and STAT4 for instance, had a crucial role in the differentiation and development of Th17 cells. They stated that naive CD4+ Th cells in cell culture medium containing TGF-β1 and IL-6 could actively express ROR-γt transcription factor via STAT3 pathway.[26]

Previous studies have also illustrated that the differentiation of naive CD4+ T cells to Th1, Th2, Th3, and Th17 cells and even Treg cells was dependent on Nuclear Factor of Activated T cells transcription factor and its subsets, including NFATc1 ،NFATc2 ،and NFATc3.[27–31] Activated NFAT has been observed to

conjugate to AP1 transcription factor and increases IL-2 expression synergistically. On the other hand, if it is conjugated to FOXP3 factor, IL-2 expression would be inhibited.[32–35] It was reported that the NFAT factor can regulate STAT4 and RUNX1, thereby activating lymphocytes via the IL-12 pathway. The concomitant collaboration of NFAT and RUNX1 is required for FOXP3 expression.[36,37] In return, activated FOXP3 can inhibit ROR-γt expression and the differentiation of CD4+naive T cells to Th17 cells by the inhibition of NFAT/AP1 factors.[38] Zhang et al, in 2011, showed that there were no significant differences in the percentage of peripheral Th17 cells and serum level of IL-17 in OA patients compared with healthy people.[38] Additionally, certain studies have implied a synergistic effect between ROR-γt and STAT3 in the proliferation, differentiation, and development of Th17 lymphocytes.[26] Since crocin was capable of inhibiting phosphorylation and activation of STAT3, it would inhibit ROR-γt expression in Th17 cells by the IL-6-STAT3 pathway.[39] Due to the influence of crocin on FOXP3, in a competition between ROR-γt and FOXP3, it would be augmented in order to develop iTreg cells. In addition, high expression of FOXP3 could prevent ROR-γt expression due to negative feedback.[25] Accordingly, a decreasing trend in the expression of ROR-γt in our study, even an insignificant one, following the treatment of OA patients with Krocina™ might be due to the anti-inflammatory effect of crocin. However, the same decreasing trend of ROR-γt in the placebo group might be related to the consumption of NSAIDs or the placebo effects.

Our findings indicated an insignificant increase and decrease in the expression level of FOXP3 after four months in the intervention and placebo groups, respectively. CD25+FOXP3+ Treg cells play a vital role in the regulation of immune responses.[40] Foxp3 is a unique transcription factor of Treg cells.[41,42] In an animal model of OA, a decrease in the number of peripheral blood Treg cells was reported, which was associated with the severity of the disease and clinical symptoms.[43] Several studies have shown that the gene expression of *FOXP3* is influenced by a combination of several factors, including GATA3,[14] tumor necrosis factor-alpha-induced protein 8-like 2,[44] STAT3,[45] miR-155,[46] and NFAT/RUNX1.[36,37]

In 2009, Raghvan et al, reported the presence of a few FOXP3 Treg cells in synovial OA patients. They also showed that the consumption of NSAIDs increased the expression of the *FOXP3* gene and the number of synovial FOXP3+ Treg cells; accordingly, it could ameliorate the condition of OA patients.[47]

Several studies have also reported that the high expression of FOXP3 happens due to the inhibiting effect of crocin on the NF-κB expression and consequently, the suppression of TNF-α and IFN-γ production.[8,48] Moreover, other studies implied that the consumption of NSAIDs in OA patients is correlated with the high expression of FOXP3.[43]

Based on these data, we could suggest that the treatment of OA patients with Krocina ™ influenced the decreasing trend of *FOXP3* gene expression at the end of the intervention even if the effect was not significant.

In conclusion, our study revealed that relative gene expression of *GATA-3* in patients with OA who were administered with Krocina™ 15 mg/daily, significantly increased compared to that in the placebo group, which might be attributed to the anti-inflammatory effects of crocin via the up-regulation of *GATA-3* as the unique transcription factor for the differentiation and development of Th2 cell subsets. Moreover, the significant decrease in the relative gene expression of *T-bet* in the placebo group was probably because of the effect of sodium diclofenac as a NSAID or placebo effect which needs further investigation including a control group of patients with OA. The increasing trend of *FOXP3* gene expression, even if it was not significant in the patients with OA who received Krocina™, might have been significantly provided that the dose of a drug or the follow-up period of the study increased in the future works. In sum, concerning the patients with OA who received Krocina™, the highest effects of crocin on the unique transcription factors of T-cell subsets belonged to the gene expression of *GATA-3* since the significant increase in this transcription factor might lead to the inhibition of inflammation and amelioration of OA.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

ACKNOWLEDGEMENTS

These data were extracted from the thesis of Javad Poursamimi, Ph.D candidate in clinical immunology at Mashhad University of Medical Sciences, Mashhad,

Iran. We are grateful to all of the participants in this clinical trial. This work was financially supported by the Vice-Chancellor for Research, Mashhad University of Medical Sciences, Mashhad, Iran. (No. 931577) and has been registered at www.IRCT.ir (IRCT2015021910507N2) and www.clinicaltrials.gov (NCT03375814).

REFERENCES

1. Ding Q, Zhong H, Qi Y, Cheng Y, Li W, Yan S, Wang X.. Anti-arthritic effects of crocin in interleukin-1β-treated articular chondrocytes and cartilage in a rabbit osteoarthritic model. Inflammation Research. 2013;62(1):17–25. https://doi.org/10.1007/s00011-012-0546-3.
2. Hochberg MC. Opportunities for the prevention of osteoarthritis. In Seminars in Arthritis and Rheumatism. 2010;39(5):321–2. https://doi.org/10.1016/j.semarthrit.2010.02.001.
3. Singh Y, Khan SA, Owais M, Abbas M, Parvez A, Kamal A. Th1/Th2 profile in patients suffering with osteoarthritis and rheumatoid arthritis: An analytical observational study. CHRISMED J Heal Res. 2014;1(4):258. https://doi.org/10.4103/2348-3334.142998.
4. Niu X, He D, Zhang X, Yue T, Li N, Zhang JZ, Dong C, Chen G. IL-21 regulates Th17 cells in rheumatoid arthritis. Hum Immunol. 2010;71(4):334–41.
5. Bani S, Pandey A, Agnihotri VK, Pathania V, Singh B. Selective Th2 upregulation by Crocus sativus: a neutraceutical spice. Evidence-Based Complementary and Alternative Medicine. 2011:1-9.
6. Adali F, Gonul Y, Aldemir M, Hazman O, Ahsen A, Bozkurt MF, Sen OG, Keles I, Keles H. Investigation of the effect of crocin pretreatment on renal injury induced by infrarenal aortic occlusion. J Surg Res. 2016;203(1):145-53. https://doi.org/10.1016/j.jss.2016.03.022.
7. Li L, Zhang H, Jin S, Liu C. Effects of crocin on inflammatory activities in human fibroblast-like synoviocytes and collagen-induced arthritis in mice. Immunol Res. 2018;66(3):406–13 https://doi.org/10.1007/s12026-018-8999-2.
8. Park JH, Lee KY, Park B, Yoon J. Suppression of T h2 chemokines by crocin via blocking of ERK-MAPK/NF-κ B/STAT 1 signalling pathways in TNF-α/IFN-γ stimulated human epidermal keratinocytes. Exp Dermatol. 2015;24(8):634-6.https://doi.org/10.1111/exd.12726.
9. Kanhere A, Hertweck A, Bhatia U, Gökmen MR, Perucha E, Jackson I, Lord GM, Jenner RG. T-bet and GATA3 orchestrate Th1 and Th2 differentiation through lineage-specific targeting of distal regulatory elements. Nat Commun. 2012;3(1):1-2. https://doi.org/10.1038/ncomms2260.
10. Faedo A, Ficara F, Ghiani M, Aiuti A, Rubenstein JL, Bulfone A. Developmental expression of the T-box transcription factor T-bet/Tbx21 during mouse embryogenesis. Mech Dev. 2002;116(1–2):157–60. https://doi.org/10.1016/S0925-4773(02)00114-4.
11. Zheng WP, Flavell RA.The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4 T cells. Cell. 1997;89(4):587–96. https://doi.org/10.1016/S0092-8674(00)80240-8.
12. Zhang DH, Cohn L, Ray P, Bottomly K, Ray A. Transcription factor GATA-3 is differentially expressed in murine Th1 and Th2 cells and controls Th2-specific expression of the interleukin-5 gene. J Biol Chem. 1997;272(34):21597-603. https://doi.org/10.1074/jbc.272.34.21597.
13. Nalbant A, Eskier D. Genes associated with T helper 17 cell differentiation and function. Front Biosci. 2016;8(3):427–35. https://doi.org/10.2741/e777.
14. Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, Cua DJ, Littman DR. The orphan nuclear receptor RORγt directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell. 2006;126(6):1121-33. https://doi.org/10.1016/j.cell.2006.07.035.
15. Mathur AN, Chang HC, Zisoulis DG, Stritesky GL, Yu Q, O'Malley JT, Kapur R, Levy DE, Kansas GS, Kaplan MH. Stat3 and Stat4 direct development of IL-17-secreting Th cells. J Immunol. 2007;178(8):4901–7. https://doi.org/10.4049/jimmunol.178.8.4901.
16. Yang XO, Pappu BP, Nurieva R, Akimzhanov A, Kang HS, Chung Y, Ma L, Shah B, Panopoulos AD, Schluns KS, Watowich SS. T helper 17 lineage differentiation is programmed by orphan nuclear receptors RORα and RORγ. Immunity. 2008;28(1):29–39. https://doi.org/10.1016/j.immuni.2007.11.016.
17. Rudensky AY. Regulatory T cells and Foxp3. Immunol Rev. 2011;241(1):260-8. https://doi.org/10.1111/j.1600-065X.2011.01018.x.
18. Poursamimi J, Shariati-Sarabi Z, Tavakkol-Afshari J, Mohajeri SA, Ghoryani M, Mohammadi M. Immunoregulatory effects of Krocina™, a herbal medicine made of crocin, on osteoarthritis patients: A

successful clinical trial in Iran. Iran J Allergy, Asthma Immunol. 2020;19(3):253–63. https://doi.org/10.18502/ijaai.v19i3.3453.

19. Szabo SJ, Dighe AS, Gubler U, Murphy KM. Regulation of the interleukin (IL)-12R β2 subunit expression in developing T helper 1 (Th1) and Th2 cells. J Exp Med. 1997;185(5):817-24. https://doi.org/10.1084/jem.185.5.817.
20. Glimcher LH, Murphy KM. Lineage commitment in the immune system: the T helper lymphocyte grows up. Genes Dev. 2000;14(14):1693–711. https://doi.org/https://10.1101/gad.14.14.1693.
21. Zhou C, Bai W, Chen Q, Xu Z, Zhu X, Wen A, Yang X. Protective effect of crocetin against burn-induced intestinal injury. J Surg Res. 2015;198(1):99–107. https://doi.org/10.1016/j.jss.2015.05.052.
22. Wampold BE, Minami T, Tierney SC, Baskin TW, Bhati KS. The placebo is powerful: estimating placebo effects in medicine and psychotherapy from randomized clinical trials. J Clin Psychol. 2005;61(7):835–54. https://doi.org/10.1002/jclp.20129.
23. Hwang ES, Szabo SJ, Schwartzberg PL, Glimcher LH. T helper cell fate specified by kinase-mediated interaction of T-bet with GATA-3. Science. 2005;307(5708):430-3. https://doi.org/10.1126/science.1103336.
24. Stockinger B, Veldhoen M, Martin B. Th17 T cells: linking innate and adaptive immunity. InSeminars Immunol. 2007;9(6):353-361. https://doi.org/10.1016/j.smim.2007.10.008.
25. Ichiyama K, Yoshida H, Wakabayashi Y, Chinen T, Saeki K, Nakaya M, Takaesu G, Hori S, Yoshimura A, Kobayashi T. Foxp3 Inhibits RORγt-mediated IL-17A mRNA Transcription through Direct Interaction with RORγt. J Biol Chem. 2008;283(25):17003–8. https://doi.org/10.1074/jbc.M801286200.
26. McCaffrey PG, Luo C, Kerppola TK, Jain J, Badalian TM, Ho AM, Burgeon E, Lane WS, Lambert JN, Curran T, Verdine GL.Isolation of the cyclosporin-sensitive T cell transcription factor NFATp. Science. 1993; 262(5134):750–4. https://doi.org/10.1126/science.8235597.
27. Macian F. NFAT proteins: key regulators of T-cell development and function. Nat Rev Immunol. 2005;5(6):472–84. https://doi.org/10.1038/nri1632.
28. Northrop JP, Ho SN, Chen L, Thomas DJ, Timmerman LA, Nolan GP, Admon A, Crabtree GR. NF-AT components define a family of transcription factors targeted in T-cell activation. Nature. 1994;369(6480):497-502. https://doi.org/10.1038/369497a0.
29. López-Rodríguez C, Aramburu J, Rakeman AS, Rao A. NFAT5, a constitutively nuclear NFAT protein that does not cooperate with Fos and Jun. Proc Natl Acad Sci. 1999;96(13):7214–9.
30. Hoey T, Sun YL, Williamson K, Xu X. Isolation of two new members of the NF-AT gene family and functional characterization of the NF-AT proteins. Immunity. 1995;2(5):461–72. https://doi.org/10.1016/1074-7613(95)90027-6.
31. Azzi JR, Sayegh MH, Mallat SG. Calcineurin inhibitors: 40 years later, can't live without. J Immunol. 191(12):5785–91. https://doi.org/10.4049/jimmunol.1390055.
32. Bettelli E, Dastrange M, Oukka M. Foxp3 interacts with nuclear factor of activated T cells and NF-κB to repress cytokine gene expression and effector functions of T helper cells. Proc Natl Acad Sci. 2005;102(14):5138–43. https://doi.org/10.1073/pnas.0501675102.
33. Wu Y, Borde M, Heissmeyer V, Feuerer M, Lapan AD, Stroud JC, Bates DL, Guo L, Han A, Ziegler SF, Mathis D. FOXP3 controls regulatory T cell function through cooperation with NFAT. Cell. 2006;126(2):375-87. https://doi.org/10.1016/j.cell.2006.05.042.
34. Macian F, López-Rodríguez C, Rao A. Partners in transcription: NFAT and AP-1. Oncogene. 2001;20(19):2476-89.
35. Hermann-Kleiter N, Baier G. NFAT pulls the strings during CD4+ T helper cell effector functions. Blood, J Am Soc Hematol. 2010;115(15):2989–97. https://doi.org/10.1182/blood-2009-10-233585.
36. Hu H, Djuretic I, Sundrud MS, Rao A.Transcriptional partners in regulatory T cells: Foxp3, Runx and NFAT. Trends Immunol. 2007;28(8):329–32. https://doi.org/10.1016/j.it.2007.06.006.
37. Kim B, Park B. Saffron carotenoids inhibit STAT3 activation and promote apoptotic progression in IL-6-stimulated liver cancer cells. Oncol Rep. 2018;39(4):1883–91. https://doi.org/10.3892/or. 2018. 6232.
38. Zhang L, Li JM, Liu XG, Ma DX, Hu NW, Li YG, Li W, Hu Y, Yu S, Qu X, Yang MX. Elevated Th22 cells correlated with Th17 cells in patients with rheumatoid arthritis. J Clin Immunol. 2011;31(4):606-14. https://doi.org/10.1007/s10875-011-9540-8.
39. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science. 2003;299(5609):1057–61. https://doi.org/10.1126/science.1079490.

40. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+ CD25+ regulatory T cells. Nat Immunol. 2003;4(4):330-6. https://doi.org/10.1038/ni904.
41. Khattri R, Cox T, Yasayko SA, Ramsdell F. An essential role for Scurfin in CD4+ CD25+ T regulatory cells. Nat Immunol. 2003;4(4):337-42. https://doi.org/doi:10.1038/ni909.
42. Mantel PY, Kuipers H, Boyman O, Rhyner C, Ouaked N, Rückert B, Karagiannidis C, Lambrecht BN, Hendriks RW, Crameri R, Akdis CA. GATA3-driven TH2 responses inhibit TGF-β1–induced FOXP3 expression and the formation of regulatory T cells. PLoS Biol. 2007;5(12):e329. https://doi.org/10.1371/journal.pbio.0050329.
43. Guo SY, Ding YJ, Li L, Zhang T, Zhang ZZ, Zhang ES. Correlation of CD. Genet Mol Res. 2015;14(3):7290–6. https://doi.org/10.4238/2015.July.3.4.
44. Sun H, Gong S, Carmody RJ, Hilliard A, Li L, Sun J, Kong L, Xu L, Hilliard B, Hu S, Shen H. TIPE2, a negative regulator of innate and adaptive immunity that maintains immune homeostasis. Cell. 2008;133(3):415-26. https://doi.org/10.1016/j.cell.2008.03.026.
45. Yang XO, Panopoulos AD, Nurieva R, Chang SH, Wang D, Watowich SS, Dong C. STAT3 regulates cytokine-mediated generation of inflammatory helper T cells. J Biol Chem. 2007;282(13):9358-63. https://doi.org/10.1074/jbc.C600321200.
46. Lu LF, Thai TH, Calado DP, Chaudhry A, Kubo M, Tanaka K, Loeb GB, Lee H, Yoshimura A, Rajewsky K, Rudensky AY. Foxp3-dependent microRNA155 confers competitive fitness to regulatory T cells by targeting SOCS1 protein. Immunity. Immunity. 2009;30(1):80–91. https://doi.org/10.1016/j.immuni.2008.11.010.
47. Raghavan S, Cao D, Widhe M, Roth K, Herrath J, Engström M, Roncador G, Banham AH, Trollmo C, Catrina AI, Malmström V. FOXP3 expression in blood, synovial fluid and synovial tissue during inflammatory arthritis and intra-articular corticosteroid treatment. Ann Rheum Dis. 2009;68(12):1908-15. https://doi.org/10.1136/ard.2008.100768.
48. Li S, Liu X, Lei J, Yang J, Tian P, Gao Y. Crocin protects podocytes against oxidative stress and inflammation induced by high glucose through inhibition of NF-κB. Cell Physiol Biochem. 2017;42(4):1481–92. https://doi.org/10.1159/000479212.