Modulation of cytokine production from cultured mononuclear cells of leukemia patients by Hypericum triquetrifolium Turra methanolic extract

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ORIGINAL ARTICLE

ABSTRACT
The effect of Hypericum triquetrifolium Turra (Family: Hypericaceae) methanolic extract on an in vitro cytokine release (IL-2, IL-4, IL-10, IL-17A and IFN-γ) from cultured mononuclear cells was determined. The cells were obtained from acute lymphoblastic (ALL) and acute myelogenous leukaemic (AML) Iraqi patients. Two concentrations (1.146 and 1.719 µg/ml) of the extract were tested. They were correspondent to 20 and 30%, respectively of the plant IC50 (5.73 µg/ml). Assessments of cytokine levels in supernatants of cultured cells revealed that ALL, AML or control cells responded differently to the plant extract in their production of IL-2, IL-10, IL-17A and IFN-γ, but leukaemic cells were better than control cells in their response, while there was no effect on IL-4 production. The results suggested that H. triquetrifolium methanolic extract exerted immunomodulatory effects on cultured cells.

1. Introduction

The immune response modulation, as a possible therapeutic strategy by using medicinal plants or their secondary metabolites, has become a fruitful subject for scientific investigations. They are traditionally used in the treatment of various ailments and considered to be superior over the conventionally employed medicines, which are known to have unfavorable side effects (Chouhan, Islamuddin, Sahal, & Afrin, 2014). One of these plants is Hypericum triquetrifolium Turra (Family: Hypericaceae). It is a promising medicinal plant from Eastern Europe and the Mediterranean area, and traditionally used for its sedative, anti-helminthic, anti-inflammatory and anti-septic effects (Saad, Azaizeh, & Said, 2005). In addition, several studies have reported the potential use of its essential oil and crude extracts as therapeutic substances, mainly in the treatment of burns and gastroenteritis, and as anti-nociceptive and anti-oxidant drug. This species also has healing and diuretic properties and is used to treat kidney, urinary bladder, liver and migraine complications (Conforti, Loizzo, Statti, & Menichini, 2007). In addition, several biological potentials have described for different extracts of H. triquetrifolium or its natural products; for instance, anti-oxidant, anti-microbial, immunological, anti-mutagenic and anti-tumor potentials (Rouis et al., 2013; Saad et al., 2011).

Acute lymphoblastic leukemia (ALL) and acute myelogenous leukemia (AML) are a clonal malignancies, in which hematopoietic differentiation is profoundly blocked, and as a consequence, an overproduction of immature blasts is overwhelmed. During the genesis of these aberrant cells, some properties are established that enable leukemia cells to survive and proliferate, and counteract growth signals mediated by immunological mediators, such as cytokines (van Etten, 2007). Recent investigations also suggest that cytokines play an important role in progression of leukemia, and their modulation may have a therapeutic potential (Chiarini et al., 2015).

One of the approaches that are employed to determine immunological effects of medicinal plants or their products is assessment of cytokine production in vitro and in vivo (Saxena et al., 2016). Cytokines are low molecular weight glycoproteins produced by a number of cell types, predominantly leukocytes that regulate immunity, inflammation and hematopoiesis (Oppenheim, 2013). They are produced from various cells during the effector phases of immune responses and regulate a number of physiological and pathological functions including innate immunity, acquired immunity and inflammatory responses (Levine, 2013). However, none of the Hypericum species have been investigated in leukemic cells to determine their effects on cytokine
production by these cells, but PubMed searching revealed the existence of 16 studies that tested the potential of different species of the genus *Hypericum* on cytokine production from cells of different origins, and encouraging results were presented (Canning et al., 2010; Hu et al., 2006; Novelli et al., 2014; Saad et al., 2011). Accordingly, a study was designed to determine the effect of *H. triquetrifolium* methanolic extract on an *in vitro* cytokine release (IL-2, IFN-γ, IL-4, IL-10 and IL-17A) from cultured mononuclear cells obtained from ALL and AML Iraqi patients.

2. Materials and methods

2.1. Reagents

Aluminum chloride (BDH, UK), fetal calf serum, histopaque, penicillin, phytohemagglutinin, RPMI-1640 medium, rutin, streptomycin (Sigma, St. Louis, MO), heparin (Leo Pharmaceutical, Denmark), methanol (Fluka, Switzerland) and trypan blue (Pharmacia Fine chemical, Sweden) were purchased from their respective companies.

2.2. Plant collection and extraction

Dr. Saman Abdulrahman Ahmad (Department of Field Crops, College of Agriculture Science, University of Sulaymaniyah, Iraq) supplied the leaves of *H. triquetrifolium* as a powdered dried material. The plant was collected from the mountain regions in Tasloga (Sulaymaniyah); a city located 330 km north-east the capital Baghdad. The leaf powder was subjected to extraction with methanol (Fu et al., 2010). Briefly, 50 grams of the plant leaf powder were extracted with 80% methanol (250 ml) at 65 °C for 3 hours using the soxhlet apparatus. The extract solution was concentrated to dryness under a reduced pressure in a rotary evaporator to yield a dried crude extract, which was frozen at −20 °C until use to prepare the required concentrations. Two concentrations (1.146 and 1.719 μg/ml) of the extract were tested. They were correspondent to 20 and 30%, respectively of the IC50 (5.73 μg/ml) of *H. triquetrifolium* methanolic extract that was tested in four cancer cell lines and one normal primary cell culture (Conforti et al., 2007).

2.3. Determination of flavonoids

Total flavonoids content was spectrophotchemically determined in the methanolic extract of *H. triquetrifolium* as a rutin (flavonoid standard) equivalent by the aluminium chloride colorimetric method (Sakanaka, Tachibana, & Okada, 2005).

2.4. Leukemia patients and controls

The ethical committee at the Iraqi Ministry of Health approved the study. The leukemia patients (ALL and AML; each of 8 cases) were referred to the Baghdad Teaching Hospital for diagnosis and treatment. The diagnosis was based on a clinical examination and laboratory evaluations, which were carried out by the consultant medical staff at the hospital. The patients were Iraqi Arabs, and their age range was 25–40 years. They were firstly diagnosed, and none of them was under therapy. A further 7 apparently healthy controls were also investigated. They were university staff and students who had no history or signs of leukemia, and matched patients for ethnicity and age.

2.5. Isolation of mononuclear cells

Peripheral blood (8–10 ml) was obtained under aseptic conditions from each participant by a venipuncture, and distributed into two aliquots. The first (4 ml) was dispensed in a plain tube and used for the collection of serum, while the second aliquot was dispensed in a test tube containing heparin (10 IU/ml) and used for the isolation of mononuclear cells, which were enriched by a density-gradient centrifugation, using histopaque as a separating medium. After enrichment, the isolated cells were suspended in RPMI-1640 medium, cell viability was assessed by a dye exclusion test (trypan blue) and the count was adjusted to 2–3 x 10^6 cells/ml (Ad’hiah, 1990).

2.6. Experimental design

The experiments were designed to assess *in vitro* production of cytokines from cultured mononuclear cells obtained from ALL and AML patients and controls. To achieve such aim, six cultures were set-up for each participant. In culture I, cells were not treated with any material (negative control), while in culture II, cells were stimulated with phytohemagglutinin (PHA) only. For cultures III and IV, cells were treated with any material (negative control), while in culture II, cells were stimulated with phytohemagglutinin (PHA) only. For cultures III and IV, cells were treated with 1.146 and 1.791 μg/ml of plant extract, respectively and stimulated with PHA. In case of cultures V and VI, the cells were only treated with the two concentrations of plant extract, respectively.

2.7. Culture establishment

Following the experimental design presented in section 2.6, counted cells (1 ml) were transferred to a test tube, to which 0.1 ml of PHA and/or a plant extract was added. The tube was incubated at 37 °C for 48 hours (5% CO2 and 80% relative humidity). After incubation, the tube was centrifuged (2000 rpm
for 5 minutes and the supernatant was collected, distributed into aliquots, and frozen at –20 °C until assessment of cytokines.

2.8. Assessment of cytokines

Sera and cell culture supernatants of leukemia patients (ALL and AML) and controls were assessed for the level of five cytokines (IL-2, IL-4, IL-10, IL-17A and IFN-γ) by means of an ELISA method using commercially available kits (PeproTech; UK).

2.9. Statistical analysis

The level of cytokines was given as mean ± standard deviation (SD), and differences between means were assessed by analysis of variance (ANOVA) followed by Duncan test, using the statistical package SPSS version 13.0. The difference was considered significant when the probability was equal or less than 0.05, 0.01 or 0.001.

3. Results

3.1. Total flavonoids

The extract was found to contain 115.73 μg/ml flavonoids. Such finding is in a good agreement with a previous study (Çirak, Radušienė, Janulis, Ivanauskas, & Çamaş, 2011), in which it was demonstrated that *H. triquetrifolium* grown in Turkey is a rich source of flavonoids, such as rutin, hyperoside, apigenin-7-O-glucoside, kaempferol, quercitrin, quercetin and amentoflavone.

3.2. IL-2

Serum level of IL-2 showed a significant increase in AML patients (28.2 ± 3.9 pg/ml) compared to ALL patients (23.7 ± 1.9 pg/ml) or controls (10.2 ± 3.2 pg/ml), and the difference was also significant between ALL patients and controls. In culture supernatant, the cells of patients and controls responded differently to the type of treatment. The PHA was significantly able to increase IL-2 level in supernatant of ALL and AML cells (10.4 ± 3.8 and 10.3 ± 3.3 pg/ml, respectively), compared to the corresponding means in untreated cultures (7.4 ± 1.7 and 5.7 ± 2.8 pg/ml, respectively) or PHA-treated control cells (5.9 ± 3.4 pg/ml). Combining PHA and *H. triquetrifolium* methanolic extract contributed to a significant increase in IL-2 level especially at the second concentration of the extract (1.791 μg/ml) in ALL patients (36.4 ± 1.8 pg/ml) compared to controls (11.5 ± 1.5 pg/ml) or AML patients (25.3 ± 1.9 pg/ml). The extract alone and at the second concentration was also effective in inducing the cultured cells of ALL and AML patients to produce a significantly higher level of IL-2 (30.8 ± 1.1 and 21.1 ± 2.6 pg/ml, respectively) compared to controls (21.1 ± 2.6 pg/ml) (Table 1).

### Table 1. Effect of *H. triquetrifolium* methanolic extract on IL-2 production from cultured mononuclear cells of ALL and AML patients.

| Culture number | Plant extract concentration (μg/ml) | ALL (No. = 8) | Controls (No. = 7) | AML (No. = 8) | p Value* |
|----------------|-------------------------------------|---------------|-------------------|---------------|----------|
| I (Untreated) |                                     | 7.4 ± 1.7a    | 2.8 ± 1.5a        | 5.7 ± 2.8a    | 0.05     |
| II (PHA only) |                                     | 10.4 ± 3.8a   | 5.9 ± 3.4a        | 10.3 ± 3.3a   | 0.05     |
| III (PHA + Extract) |                               | 23.1 ± 1.6a   | 12.8 ± 3.9a       | 11.7 ± 1.3a   | 0.01     |
| IV (PHA + Extract) |                               | 36.4 ± 1.8a   | 11.5 ± 1.5a       | 25.3 ± 1.9a   | 0.001    |
| V (Extract only) |                                 | 27.2 ± 1.9a   | 12.4 ± 3.1a       | 16.2 ± 1.8c   | 0.001    |
| VI (Extract only) |                                 | 30.8 ± 1.1b   | 14.4 ± 1.5a       | 21.1 ± 2.6a   | 0.001    |
| Serum          |                                     | 23.7 ± 1.9a   | 10.2 ± 3.2c       | 28.2 ± 3.9a   | 0.001    |

ALL: acute lymphoblastic leukemia; AML: acute myelogenous leukemia; PHA: phytohemagglutinin; SD: standard deviation; Different superscript letters represent a significant difference (p ≤ 0.05) between means of columns (cultures I–VI and serum).

*Probability of difference between means of ALL, controls and AML.

3.3. IL-4

Serum level of IL-4 showed a significant decrease in ALL and AML patients (1.5 ± 0.7 and 3.7 ± 2.5 pg/ml, respectively) compared to controls (6.2 ± 1.6 pg/ml). With respect to cultures, the level of IL-4 showed no significant difference between ALL, AML and controls, and the same observation was made when the comparison was made between the different types of treatments for each group of subjects, in which the level of IL-4 was approximated in the six types of cultures that were set in ALL and AML patients or controls (Table 2).

3.4. IL-10

Sera of ALL and AML patients shared a significant increased serum level of IL-10 (38.3 ± 10.4 and 32.9 ± 10.1 pg/ml, respectively) compared to controls (28.1 ± 4.3 pg/ml), while the response of their cultured cells was subjected to the type of treatment. The cells of ALL, control and AML subjects responded similarly to PHA treatment (18.4 ± 8.4, 17.8 ± 5.1 and 17.2 ± 5.2 pg/ml, respectively), while combining PHA and *H. triquetrifolium* methanolic extract revealed some differences in a dose-dependent manner. The highest IL-10 level was observed in supernatants of control (38.9 ± 5.8 pg/ml) and ALL.
Table 2. Effect of *H. triquetrifolium* methanolic extract on IL-4 production from cultured mononuclear cells of ALL and AML patients.

| Culture number | Plant extract concentration (µg/ml) | ALL (No. = 8) | Controls (No. = 7) | AML (No. = 8) | p Value |
|----------------|-------------------------------------|---------------|-------------------|--------------|---------|
| I (Untreated)  | –                                   | 3.3 ± 1.5³     | 3.5 ± 1.7³        | 3.1 ± 1.6³   | NS      |
| II (PHA only)  | –                                   | 4.7 ± 1.3³     | 5.6 ± 1.4³        | 6.5 ± 3.3³   | NS      |
| III (PHA + Extract) | 1.146                     | 3.9 ± 1.6³     | 3.6 ± 1.3³        | 4.5 ± 1.4³   | NS      |
| IV (PHA + Extract) | 1.791                     | 3.6 ± 0.7³     | 4.5 ± 1.5³        | 4.4 ± 1.3³   | NS      |
| V (Extract only) | 1.146                     | 4.2 ± 0.9³     | 3.6 ± 2.3³        | 4.7 ± 1.3³   | NS      |
| VI (Extract only) | 1.791                     | 3.7 ± 1.9³     | 4.9 ± 1.5³        | 4.4 ± 0.8³   | NS      |
| Serum          |                                  | 1.5 ± 0.4³     | 6.2 ± 2.6³        | 3.7 ± 2.5³   | 0.001   |

ALL: acute lymphoblastic leukemia; AML: acute myelogenous leukemia; NS: not significant; PHA: phytohemagglutinin; SD: standard deviation; Different superscript letters represent a significant difference (p ≤ 0.05) between means of columns (cultures I–VI and serum).

*Probability of difference between means of ALL, controls and AML.

(34.1 ± 5.7 pg/ml) cells at the concentration 1.791 µg/ml, while the supernatant of AML cells showed a less level (23.6 ± 8.6 pg/ml) and the difference was significant. Treating cultured cells with the plant extract induced a similar increased production of IL-10, but it did not reach the synergistic effects of PHA and the plant extract (Table 3).

### 3.5. IL-17A

The sera of ALL and AML patients showed a significant increased level of IL-17A as compared to controls (22.6 ± 6.5 and 27.4 ± 5.3, respectively vs. 3.2 ± 1.4 pg/ml). The cultured cells of AML and controls were better than ALL cells in their production of IL-17A after the different treatments (PHA only, PHA + plant extract or plant extract only). The highest production was observed in PHA + plant extract cultures (culture IV) of AML and control subjects (41.1 ± 9.8 and 41.4 ± 6.2 pg/ml, respectively), which were significantly higher than the corresponding culture of ALL patients (23.7 ± 6.8 pg/ml) (Table 4).

### 3.6. IFN-γ

The sera of ALL patients showed a significant increased level of IFN-γ (70.1 ± 5.5 pg/ml) compared to control (17.6 ± 6.5 pg/ml) or AML (28.1 ± 4.6 pg/ml) sera, but AML patients also manifested a significant increased level compared to controls. The best response of cultured cells in the production of IFN-γ was observed in supernatants of cultures treated with PHA + plant extract at the concentration 1.791µg/ml in ALL and AML patients, and the difference was significant compared to the corresponding supernatants of controls (109.8 ± 18.5 and 86.1 ± 7.1, respectively vs. 33.6 ± 8.4 pg/ml) (Table 5).

4. Discussion

The presented results strongly suggest that *H. triquetrifolium* methanolic extract impacted the cells of ALL and AML patients, as well as controls to produce important cytokines that have a role in enhancing cell-mediated immune response (IL-2 and IFN-γ), regulating immune response (IL-10) and mediating anti-inflammatory response (IL-17A); all of which may have a role in controlling the malignant transformation of cells or their progression and produced by important T cells; T helper (Th)1, Treg and Th17 (Levine, 2013; Oppenheim, 2013). In this regard, various medicinal plant-derived factors have been reported to regulate the production of cytokines; for instance, flavonoids (Cuevas, Saavedra, Salazar, & Abdalla, 2013). In fact, the chemical analysis of methanolic extract revealed that *H. triquetrifolium* is a rich source of flavonoids, which were observed at a concentration of 115.73 µg/ml. It has also been augmented that flavonoids that were extracted from different plant sources (e.g. grape seeds, green tea and strawberry) exerted significant immunomodulatory effects by modulating Th1 and Th2-derived...
cytokines and other immune cells (Leyva-López, Gutierrez-Grijalva, Ambriz-Perez, & Basilio Heredia, 2016; Nair et al., 2002; Peluso, Miglio, Morabito, Ioannone, & Serafini, 2015).

One of these cytokines is IL-2, which showed an increased level in culture supernatants of plant extract-treated cells. CD4 + T cells are a major source of IL-2, and the increased level of IL-2 might be due to the richness of *H. triquetrifolium* extract in flavonoids that might have enhanced these cells to produce IL-2 (Kunishi, Tai, & Yamamoto, 2001; Pae & Wu, 2013). It has been demonstrated further that flavonoids can have a wide range of immunomodulatory effects by affecting CD4 + T cells to increase their capacity in IL-2 production both *in vitro* and *in vivo* (Maghraby et al., 2010).

IL-10 was a further target for *H. triquetrifolium* methanolic extract, which exerted stimulating effects on cells to produce IL-10. Again, flavonoids (quercetin) have been demonstrated to have a positive effect on cells to secret IL-10 (Wang et al., 2014). In this context, it has been discussed that flavonoids have immunomodulatory and anti-inflammatory properties and the stimulated activities of numerous cell types (lymphocytes and macrophages) can be influenced by particular flavonoids to produce certain cytokines (Peluso et al., 2015).

The modulation of immune response to combat diseases has long been a topic of interest. Macrophages are the first line of defences in the innate immunity against microbial infection. They engulf and kill microorganisms and present antigens that elicit adaptive immune responses (Farhadi, Mohammadi-Motlagh, Seyfi, & Mostafaie, 2014). Macrophages secrete cytokines, such as interleukins, TNF-α and IFNs, as well as inflammatory mediators, such as nitric oxide (Herbst, Schaible, & Schneider, 2011). The effects of *H. triquetrifolium* methanolic extract on inducing macrophages to release immunomodulatory cytokines, such as IFN-γ, were possibly determined. The results showed that the extract could modulate immunity by inducing macrophages to intracellular expression of IFN-γ. IFN-γ also stimulates macrophages and is involved in the development of Th1 cells. The cellular effects of IFN-γ include up-regulation of pathogen recognition, antigen processing and presentation, inhibition of viruses, inhibition of cellular proliferation, and modifying apoptosis and immunomodulation (Belguendouz et al., 2011; Lyu & Park, 2005). These findings are important and *H. triquetrifolium* can be considered as a very suitable candidate for modulating macrophage function. *In vitro* and *in vivo* studies demonstrated the regulatory effect of phenolic compounds (e.g. the flavones apigenin and luteolin) on macrophage modulation to secret inflammatory mediators (Soromou et al., 2012; Verbeek, Ploomp, Van Tol, & Van Noort, 2004). Accordingly, *H. triquetrifolium* might have exerted an immunostimulatory effect on cultured cells by enhancing the

### Table 4. Effect of *H. triquetrifolium* methanolic extract on IL-17A production from cultured mononuclear cells of ALL and AML patients.

| Culture number | Plant extract concentration (µg/ml) | ALL (No. = 8) | Controls (No. = 7) | AML (No. = 8) | p value* |<  
|----------------|------------------------------------|---------------|--------------------|---------------|----------|---
| I (Untreated)  | –                                  | 1.5 ± 0.4C    | 2.3 ± 1.3C         | 2.7 ± 1.5C    | NS       |---
| II (PHA only)  | 24.9 ± 7.6B                      | 26.2 ± 7.9B   | 36.9 ± 6.9B        | 0.01         | ---      |---
| III (PHA + Extract) | 21.6 ± 5.1A     | 29.5 ± 7.3A   | 34.7 ± 5.5A        | 0.001        | ---      |---
| IV (PHA + Extract) | 1.791 | 41.4 ± 6.2A   | 41.1 ± 9.5B        | 0.001        | ---      |---
| V (Extract only) | 1.146 | 24.9 ± 4.9B   | 24.1 ± 3.8B        | 0.001        | ---      | ---
| VI (Extract only) | 1.791 | 26.6 ± 3.6A   | 26.2 ± 3.4A        | 0.001        | ---      |---
| Serum          | 22.6 ± 6.5B                      | 32.1 ± 1.4C   | 27.4 ± 5.3B        | 0.001        | ---      |---

ALL: acute lymphoblastic leukemia; AML: acute myelogenous leukemia; NS: not significant; PHA: phytohemagglutinin; SD: standard deviation; Different superscript letters represent a significant difference (p ≤ 0.05) between means of columns (cultures I–VI and serum).

*Probability of difference between means of ALL, controls and AML.

### Table 5. Effect of *H. triquetrifolium* methanolic extract on IFN-γ production from cultured mononuclear cells of ALL and AML patients.

| Culture number | Plant extract concentration (µg/ml) | ALL (No. = 8) | Controls (No. = 7) | AML (No. = 8) | p value* |<  
|----------------|------------------------------------|---------------|--------------------|---------------|----------|---
| I (Untreated)  | –                                  | 8.4 ± 1.8B    | 3.3 ± 1.3C         | 6.7 ± 3.8B    | 0.01     |---
| II (PHA only)  | 40.4 ± 8.9E                       | 13.1 ± 2.5C   | 12.5 ± 3.8B        | 0.001        | ---      |---
| III (PHA + Extract) | 70.9 ± 14.1E  | 32.1 ± 8.5A   | 66.6 ± 13.6A       | 0.001        | ---      |---
| IV (PHA + Extract) | 1.791 | 33.6 ± 8.4A   | 86.1 ± 7.1A        | 0.001        | ---      |---
| V (Extract only) | 1.146 | 34.2 ± 12.6A  | 61.9 ± 8.1B        | 0.001        | ---      |---
| VI (Extract only) | 1.791 | 33.1 ± 11.1A  | 61.6 ± 6.5B        | 0.001        | ---      |---
| Serum          | 70.1 ± 5.5E                       | 17.6 ± 6.5A   | 28.1 ± 4.6B        | 0.001        | ---      |---

ALL: acute lymphoblastic leukemia; AML: acute myelogenous leukemia; PHA: phytohemagglutinin; SD: standard deviation; Different superscript letters represent a significant difference (p ≤ 0.05) between means of columns (cultures I–VI and serum).

*Probability of difference between means of ALL, controls and AML.
expression of IFN-γ, which is involved in lymphocyte activation, and may indirectly affect the activation of NK cell type by inducing macrophages to secret cytokines, which consequently stimulate cell-mediated immunity (Abood, Fahmi, Abdulla, & Ismail, 2014).

The results also showed that the plant extract modulated IL-17A production in a concentration-dependent manner. It might be reasonable to hypothesize that flavonoids of the plant extract may regulate RORγt (the first transcription factor selectively expressed in Th17 cells), which is regulated by STAT3 (signal transducer and activator of transcription 3). The latter acts as an important mediator in multiple biological processes induced by different cytokines (Ivanov, Zhou, Littman, & Ivanov, 2007; Wei, Laurence, Elias, & O’Shea, 2007).

It was also observed that ALL, AML or control cultured mononuclear cells responded differently to the plant extract in their production of IL-2, IL-10, IL-17A and IFN-γ, but leukemic cells were better than control cells in their response, and this may suggest that these cells were pre-committed to interior immunological effects and the plant extract came to enhance such effects. Some herbal medicines are suggested to alter the activity of immune function through a dynamic regulation of informational molecules such as cytokines. This may offer an explanation for the effects of herbs on function of immune system and other tissue, especially in leukemia, and H. triquetrifolium might be one of these herbal medicines that regulate RORγt (the first transcription factor selectively expressed in Th17 cells), which is regulated by STAT3 (signal transducer and activator of transcription 3). The latter acts as an important mediator in multiple biological processes induced by different cytokines (Ivanov, Zhou, Littman, & Ivanov, 2007; Wei, Laurence, Elias, & O’Shea, 2007).

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5. Conclusions

The in vitro immunomodulatory potential of H. triquetrifolium methanol extract is suggested, and the profiles of IL-2, IL-10, IL-17A and IFN-γ in the supernatant of cultured ALL, AML and control cells are in favor of such suggestion. However, further investigations based on in vivo evidence are certainly required.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Abood, W. N., Fahmi, I., Abdulla, M. A., & Ismail, S. (2014). Immunomodulatory effect of an isolated fraction from Tinospora crispa on intracellular expression of INF-γ, IL-6 and IL-8. BMC Complementary and Alternative Medicine, 14, 205. doi:10.1186/1472-6882-14-205

Ad’hiah, A. H. (1990). Immunogenetic studies in selected human diseases. Ph.D. Thesis, Department of Human Genetics, University of Newcastle upon Tyne, England.

Belguendouz, H., Messaoudene, D., Lahmar, K., Ahmedli, L, Medjebier, O., Hartani, D., ..., Touil-Boukoffa, C. (2011). Interferon-γ and nitric oxide production during Behc¸et uveitis: immunomodulatory effect of interleukin-10. Journal of Interferon & Cytokine Research, 31, 643–651. doi:10.1089/jir.2010.0148

Canning, S., Waterman, M., Orsi, N., Ayres, J., Simpson, N., & Dye, L. (2010). The Efficacy of Hypericum perforatum (St John’s Wort) for the treatment of premenstrual syndrome: a randomized, double-blind, placebo-controlled trial. CNS Drugs, 24, 207–225. doi:10.2165/11530120-000000000-00000

Chiarini, F., Lonetti, A., Evangelisti, C., Buontempo, F., Orsini, E., Evangelisti, C., ..., Martelli, A. M. (2015). Advances in understanding the acute lymphoblastic leukemia bone marrow microenvironment: from biology to therapeutic targeting. Biochimica et Biophysica Acta, 1863, 449–463. doi:10.1016/j.bbamcr.2015.08.015

Chouhan, G., Islamuddin, M., Sahal, D., & Afrin, F. (2014). Exploring the role of medicinal plant-based immunomodulators for effective therapy of leishmaniasis. Frontiers in Immunology, 5, 193. doi:10.3389/fimmu.2014.00193

Çırak, C., Radušienė, J., Janulis, V., Ivanauskas, L., & Čamaš, N. (2011). Phenolic constituents of Hypericum triquetrifolium Turra (Guttiferae) growing in Turkey: variation among populations and plant parts. Turkish Journal of Biology, 35, 449–456. doi:10.3906/biy-1002-36

Conforti, F., Loizzo, M. R., Statti, A. G., & Menichini, F. (2007). Cytotoxic activity of antioxidant constituents from Hypericum triquetrifolium Turra. Natural Products Research, 21, 42–46. doi:10.1080/14786410500356243

Cuevas, A., Saavedra, N., Salazar, L. A., & Abdalla, D. S. P. (2013). Modulation of immune function by polyphenols: possible contribution of epigenetic factors. Nutrients, 5, 2314–2332. doi:10.3390/nu5072314
Farhadi, L, Mohammadi-Motlagh, H. -R, Seyfi, P, & Mostafae, A. (2014). Low concentrations of flavonoid - rich fraction of shallot extract induce delayed - type hypersensitivity and TH1 cytokine IFNγ expression in BALB/c mice. *International Journal of Molecular and Cellular Medicine*, 3, 16–25.

Fu, W., Chen, J., Cai, Y., Lei, Y., Chen, L., Pei, L., …, Ruan, J. (2010). Antioxidant, free radical scavenging, anti-inflammatory and hepatoprotective potential of the extract from *Parathelypteris nipponica* (Franch. et Sav.) Ching. *Journal of Ethnopharmacology*, 130, 521–528. doi:10.1016/j.jep.2010.05.039

Herbst, S., Schaible, U. E., & Schneider, B. E. (2011). Interferon gamma activated macrophages kill mycobacteria by nitric oxide induced apoptosis. *PLoS One*, 6, e19105. doi:10.1371/journal.pone.0019105

Ivanov, I. I., Zhou, L., & Littman, D. R. (2007). Transcriptional modulation of Th17 cell differentiation. *Science*, 318, 169–173. doi:10.1126/science.1147861

Kunishiro, K., Tai, A., & Yamamoto, I. (2001). Effects of rooisbos tea extract on antigen-specific antibody production and cytokine generation in vitro and in vivo. *Bioscience, Biotechnology, and Biochemistry*, 65, 2137–2145. doi:10.1271/bbb.65.2137

Lee, J. M., Hah, J. O., & Kim, H. S. (2012). The effect of red ginseng extract on inflammatory cytokines after chemotherapy in children. *Journal of Ginseng Research*, 36, 383–390. doi:10.5134/jgr.2012.36.4.383

Levine, S. J. (2013). Cytokines. In *Encyclopedia of biological chemistry* (2nd ed., pp. 613–675). USA: Elsevier Inc. doi:10.1016/B978-0-12-390. doi:10.5142/jgr.2012.36.4.383

Leyva-López, N., Gutierrez-Grijalva, E. P., Ambriz-Perez, D. L., & Basilio Heredia, J. (2016). Flavonoids as cytokine modulators: a possible therapy for inflammation-related diseases. *International Journal of Molecular Sciences*, 17, pii: E921. doi:10.3390/ijms17060921

Lyu, S. Y., & Park, W. -B. (2005). Production of cytokine and NO by RAW 264.7 macrophages and PBMC in vitro incubation with flavonoids. *Archives of Pharmacal Research*, 28, 573–581. doi:10.1007/ BF02977761

Maghraby, A. S., Shalaby, N., Abd-Alla, H. I., Ahmed, S. A., Khaled, H. M., & Bahgat, M. M. (2010). Immunostimulatory effects of extract of *Pulicaria crisp* before and after cultivation with flavonoids. *Pharmazie*, 65, 75–79. doi:10.1007/S12006-010-0024-9

Nair, N., Mahajan, S., Chawda, R., Kandaswami, C., Shanahan, T. C., & Schwartz, S. A., 2002. Grape seed extract activates Th1 cells in vitro. *Clinical and Vaccine Immunology*, 9, 470–476. doi:10.1128/CDLI.9.2, 470-476.2002

Novelli, M., Befify, P., Menegazzi, M., De Tata, V., Martino, L., Sgarbossa, A., …, Masiello, P. (2014). St. John’s wort extract and hyperforin protect rat and human pancreatic islets against cytokine toxicity. *Acta Diabetologica*, 51, 113–121. doi:10.1007/s00592-013-0518-2

Oppenheim, J. J. (2013). Cytokines, their receptors and signals. In *The autoimmune diseases* (5th ed., pp. 229–241). USA: Elsevier Inc. doi:10.1016/B978-0-12-384929-8.00083-6

Pae, M., & Wu, D. (2013). Immunomodulating effects of epigallocatechin-3-gallate from green tea: mechanisms and applications. *Food & Function*, 4, 1287–1303. doi:10.1039/ c3fo60076a

Peluso, I., Miglio, C., Morabito, G., Ioannone, F., & Serafini, M. (2015). Flavonoids and immune function in human: A systematic review. *Critical Reviews in Food Science & Nutrition*, 55, 383–395. doi:10.1080/10408398.2012.656770

Xiuying, P., Liang, J., Shang, R., Zhou, L., Wang, X., & Li, Y. (2012). Therapeutic efficacy of *Hypericum perforatum* L. extract for mice infected with an influenza A virus. *Canadian Journal of Physiology and Pharmacology*, 90, 123–130. doi:10.1139/y11-111

Rouis, Z., Abid, N., Koudja, S., Yangui, T., Elaissi, A., Cioni, P.L., …, Aouni, M. (2013). Evaluation of the cytotoxic effect and antibacterial, antifungal, and antiviral activities of *Hypericum triquetrifolium* Turra essential oils from Tunisia. *BMC Complementary and Alternative Medicine*, 13, 24. doi:10.1186/1472-6882-13-24

Saad, B., Abouatta, B. S., Basha, W., Hmade, A., Kmail, A., Khasib, S., & Said, O. (2011). *Hypericum triquetrifolium* – derived factors downregulate the production levels of LPS-induced nitric oxide and tumor necrosis factor-α in THP-1 cells. *Evidence-based Complementary and Alternative Medicine*, 2011. doi:10.1093/ecam/nen056

Saad, B., Azaizeh, H., & Said, O. (2005). Tradition and perspectives of Arab herbal medicine: A review. *Evidence-based Complementary and Alternative Medicine*, 2, 475–479. doi:10.1093/ecam/neh133

Sakanaka, S., Tachibana, Y., & Okada, Y. (2005). Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakino-ha-cha). *Food Chemistry*, 89, 569–575. doi:10.1016/j.foodchem.2004.03.013

Saxena, A., Yadav, D., Maurya, A. K., Kumar, A., Mohanty, S., Gupta, M. M., …, Bawankule, D. U. (2016). Diarylheptanoids from *Alnus nepalensis* attenuates LPS-induced inflammation in macrophages and endotoxic shock in mice. *International Immunopharmacology*, 30, 129–136. doi:10.1016/j.intimp.2015.12.002

Sorumou, L. W., Zhang, Z., Li, R., Chen, N., Guo, W., Huo, M., …, Deng, X. (2012). Regulation of inflammatory cytokines in lipopolysaccharide-stimulated RAW 264.7 murine macrophage by 7-O-methyl-naringenin. *Molecules*, 17, 3574–3585. doi:10.3390/molecules 17033574

Van Etten, R. A. (2007). Aberrant cytokine signaling in leukemia. *Oncogene*, 26, 6738–6749. doi:10.1038/sj.onc.1210758

Verbeek, R., Plomp, A. C., Van Tol, E. A. F., & Van Noort, J. M. (2004). The flavones luteolin and apigenin inhibit *in vitro* antigen-specific proliferation and interferon-gamma production by murine and human autoimmune T cells. *Biochemical Pharmacology*, 68, 621–629. doi:10.1016/j.bcp.2004.05.012

Wang, L., Chen, J., Wang, B., Wu, D., Li, H., Hu, H., …, Chai, Y. (2014). Protective effect of quercetin on lipopolysaccharide-induced acute lung injury in mice by inhibiting inflammatory cell influx. *Experimental Biology and Medicine (Maywood)*, 239, 1653–1662. doi:10.1177/ 1535370214537743

Wei, L., Laurence, A., Elias, K. M., & O’Shea, J. J. (2007). IL-21 is produced by Th17 cells and drives IL-17 production in a STAT3-dependent manner. *Journal of Biological Chemistry*, 282, 34605–34610. doi:10.1074/jbc.M705100200