Investigation of the Anti-Inflammatory and Immunomodulatory Effects of Mullein Species Extracts on Rheumatoid Arthritis

Stj. Dr. Mehmet Timur Başarıcı

Prof. Dr. Abdürrahim Koçyiğit

Bezmialem Vakıf University

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Introduction

- Rheumatoid Arthritis(RA)
  - long-term autoimmune disorder
  - Inflammation of joints as well as extra-articular involvement
  - Global prevalance: %0,46
  - F:M ratio: 2/1
  - More prevalent in developed countries

Pathophysiology/Etiopathology

The exact etiopathology is unknown

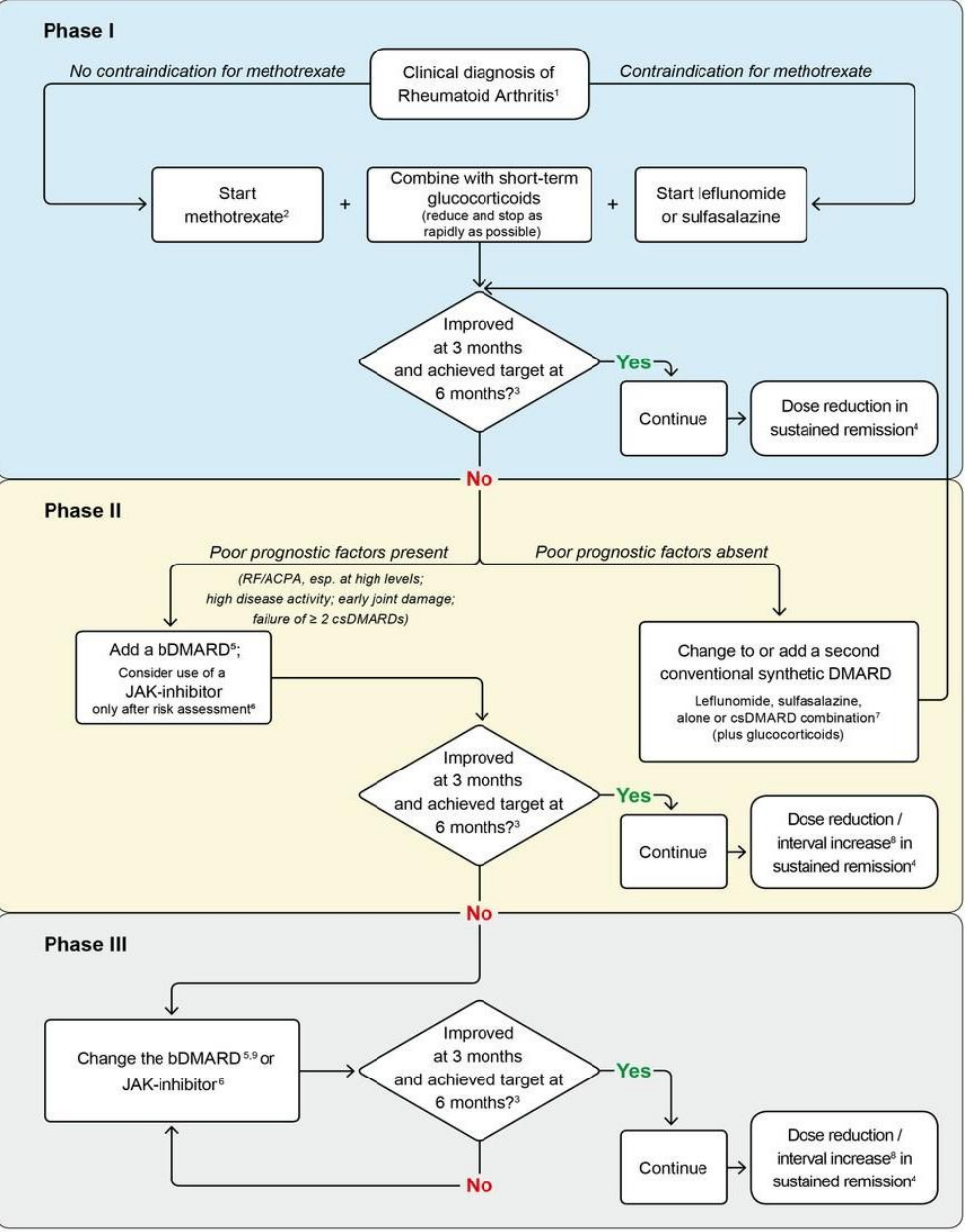
Immunomodulatory defect

TNF-α, IL-1 and IL-6 are dominant cytokines

Possible environmental factors(Bronchial stress, heat shock proteins, etc.)

HLA-DRB1 mutation

Mutations in NF-κB pathway



2010 ACR-EULAR classification criteria can support early diagnosis.
"Methotrexate should be part of the first treatment strategy". While combination therapy of csDMARDs is not preferred by the Task Force, starting with methotrexate does not exclude its use in combination with other csDMARDs although more adverse events without added benefit are to be expected, especially if MTX is combined with glucocorticoids.
The treatment target is clinical remission according to ACR-EULAR definitions or, if remission is unlikely to be achievable, at least low disease activity; the target should be reached after 6 months, but therapy should be adapted or changed if insufficient improvement (less than 50% of disease activity) is seen after 3 months.
Sustained remission: ≥ 6 months ACR/EULAR index based or Boolean remission.
Consider contraindications and risks. TNF-inhibitors (adalimumab, certolizumab, etanercept, golimumab, infliximab, including EMA/FDA approved bsDMARDs), abatacept, IL-6R inhibitors, or rituximab (under certain conditions); in patients who cannot use csDMARDs as comedication IL6-inhibitors and tsDMARDs have some advantages.
6. The following risk factors for cardiovascular events and malignancies must be considered when intending to prescribe a JAK-inhibitor: Age over 65 years, history of current or past smoking, other cardiovascular risk factors (such as diabetes, obesity, hypertension), other risk factors for malignancy (current or previous history of malignancy other than successfully treated NMSC), risk factors for thromboembolic events (history of MI or heart failure, cancer, inherited blood clotting disorders or a history of blood clots, as well as patients taking combined hormonal contraceptives or hormone replacement therapy, undergoing major surgery or immobile)
7. The most frequently used combination comprises methotrexate, sulfasalazine and hydroxychloroquine.
8. Dose reduction or interval increase can be safely done with all bDMARDs and tsDMARDs with little risk of flares; stopping is associated with high flare rates; most but not all patients can recapture their good state upon re-institution of the same bDMARD/tsDMARD, but before all this glucocorticoids must have been discontinued.
9. From a different or the same class.

Credit: *Annals of the Rheumatic Diseases* (2022). DOI: 10.1136/ard-2022-223356

- ACR(American College of Rheumatology) guidelines suggest usage the DMARDs(Disease-Modifying Antirheumatic Drugs)
- DMARDs:
  - Side effect profile
  - Cost

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- Verbascum, known as mullein, is a genus of over 450 species of flowering plants, with over 400 species in the Anatolia region.
- Verbascum has been used for:
  - Asthma
  - Eczema
  - Analgesic

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Aim of this study

To investigate the possible immunomodulatory anti-inflammatory effect of *Verbascum spp.*

To contribute to existing treatment algorithm by supporting the existing treatment options, as well as increasing efficacy of DMARDs

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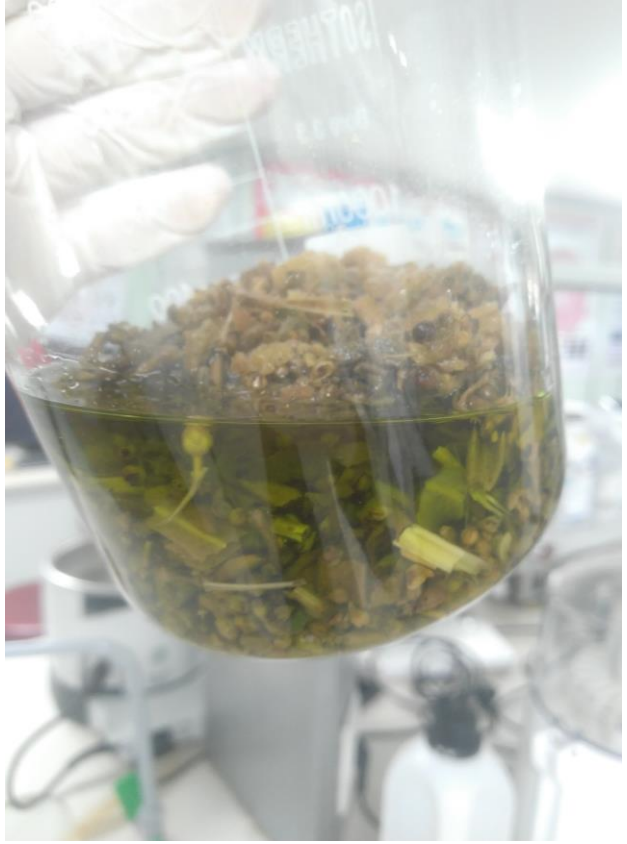
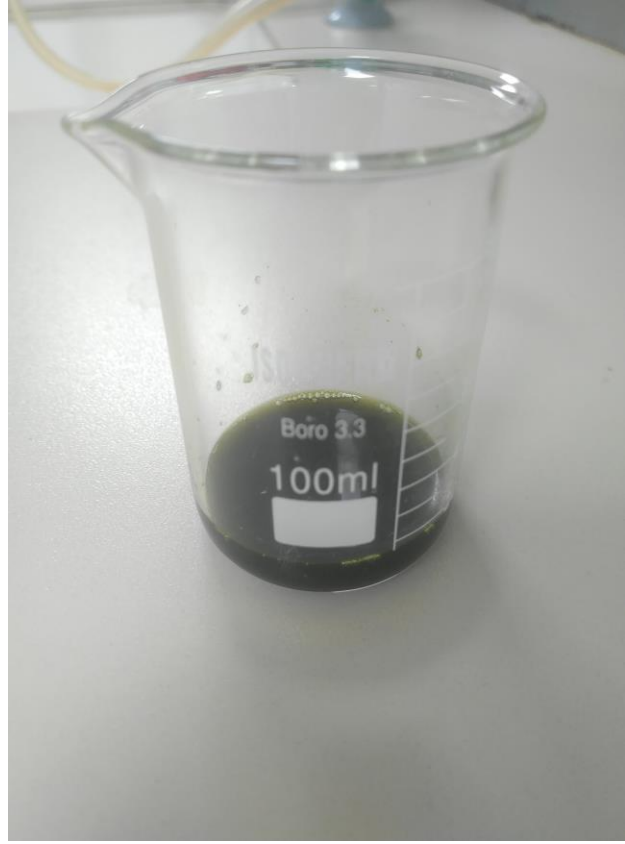
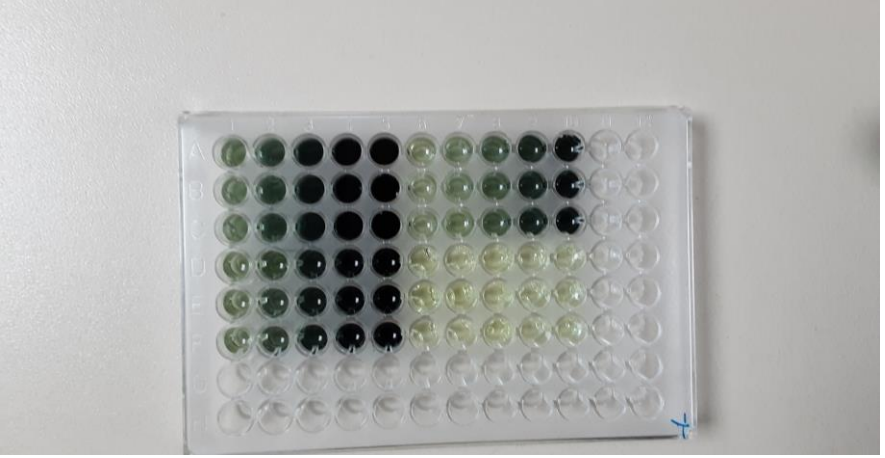
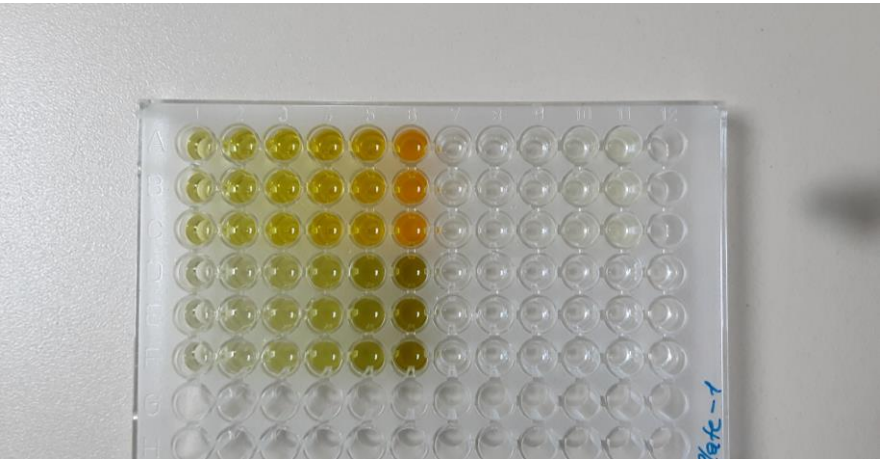


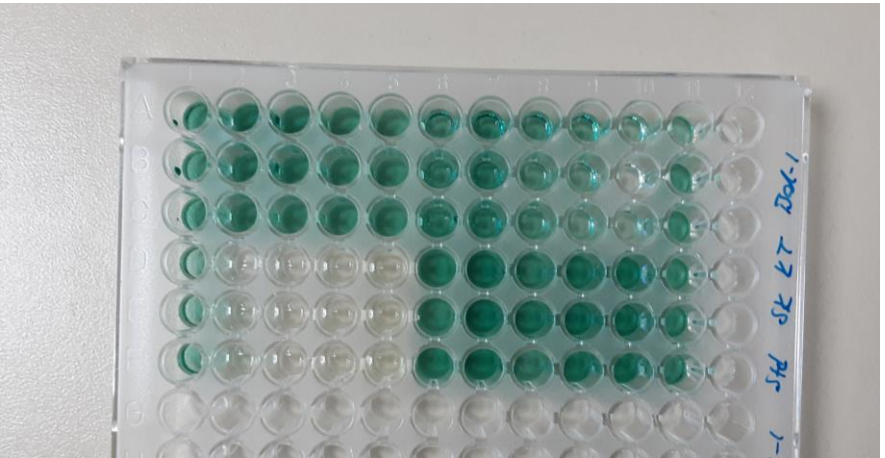


Material and Method

- 2 types of *Verbascum spp.* were collected and dried in the sun for 10 days
- Plants were extracted by using Slatnars method







• *Verbascum spp*. were further analyzed for its total phenolic, flavonoid content, and antioxidant capacity.

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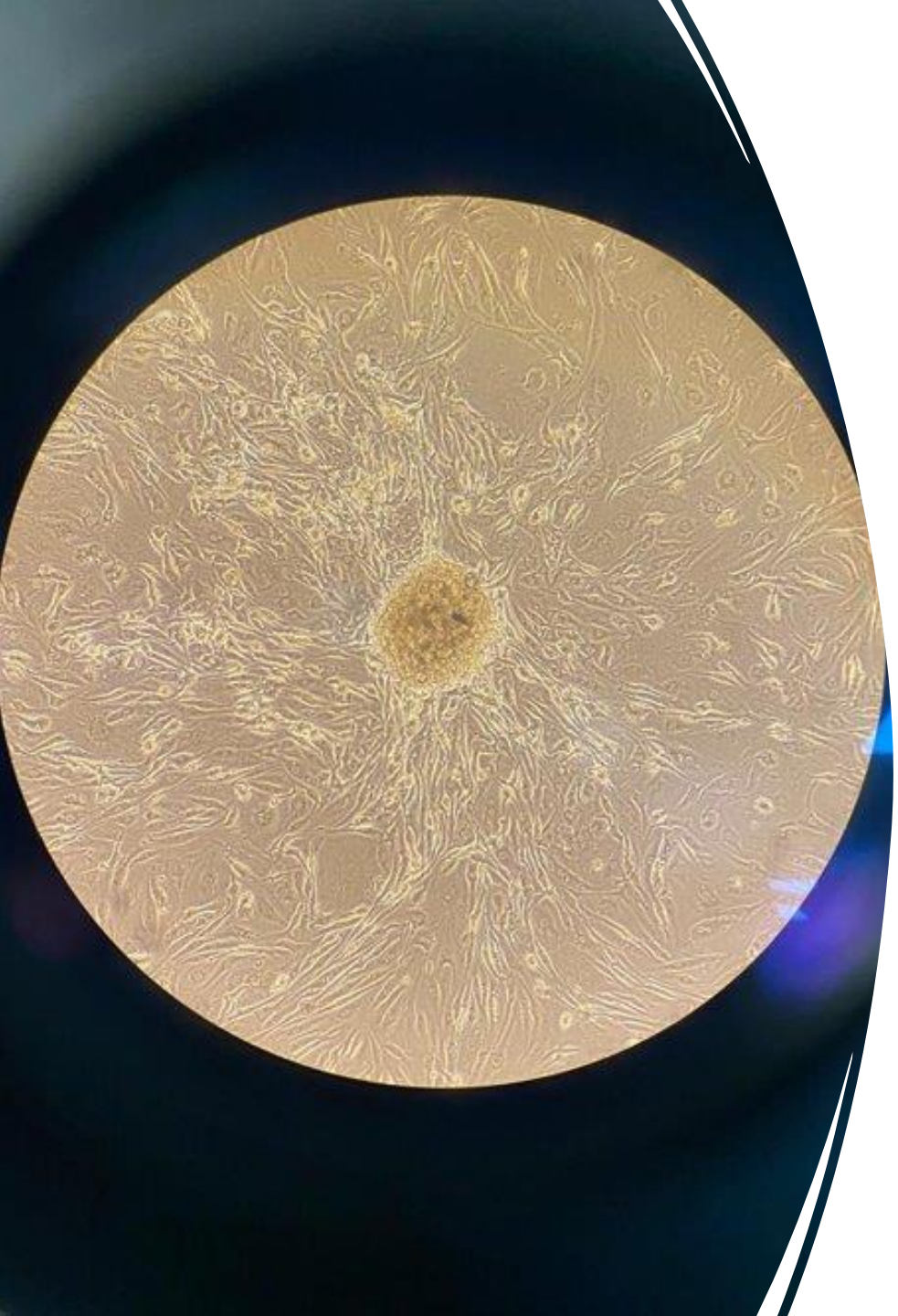

Preparing the cell culture

- Fibroblast-like synoviocytes(FLS) are targeted
  - Significant role in the pathogenesis of RA
- 2 synovium samples collected from RA patients
- MTT essay is used to determine an appropriate dosage

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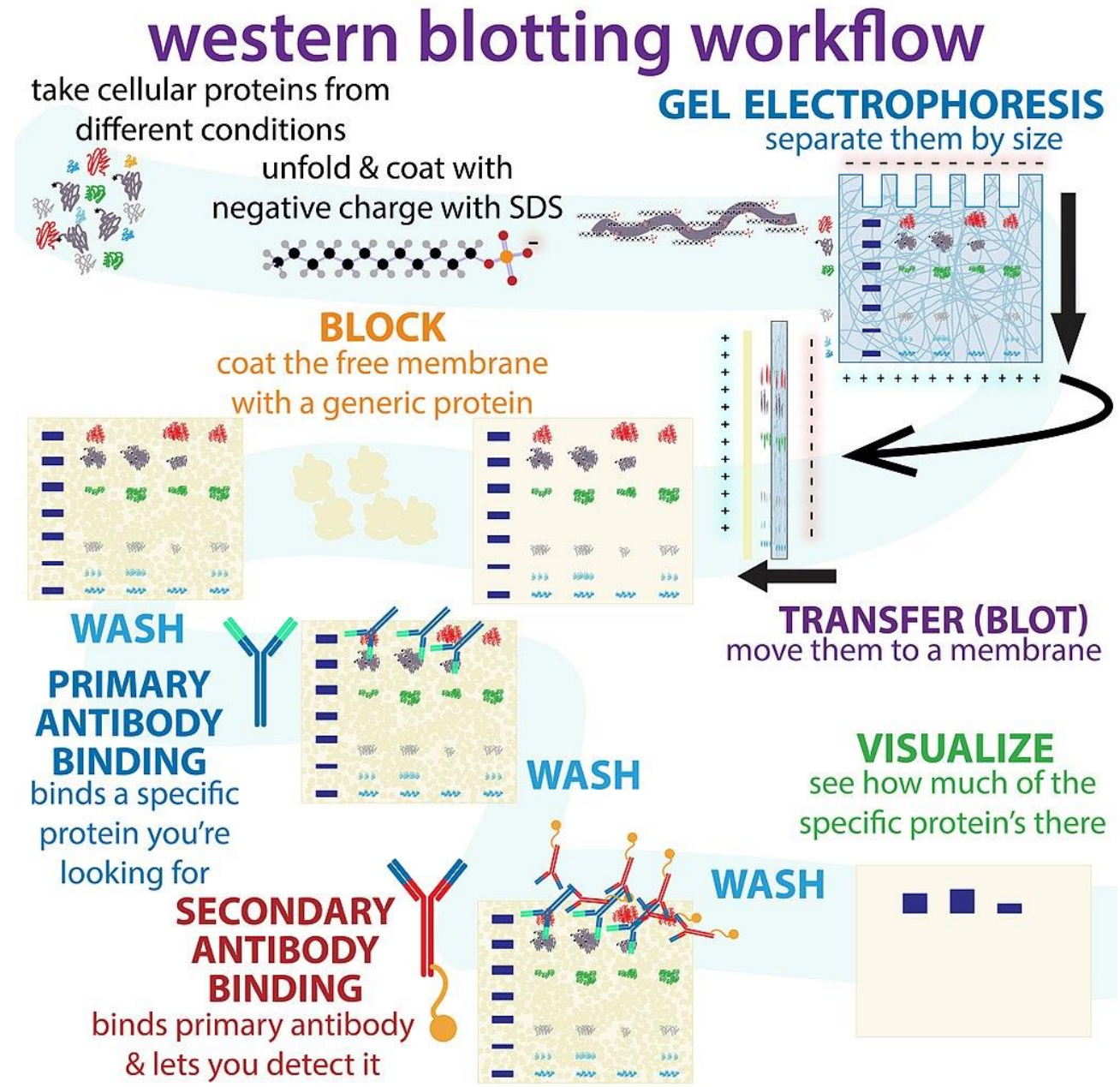

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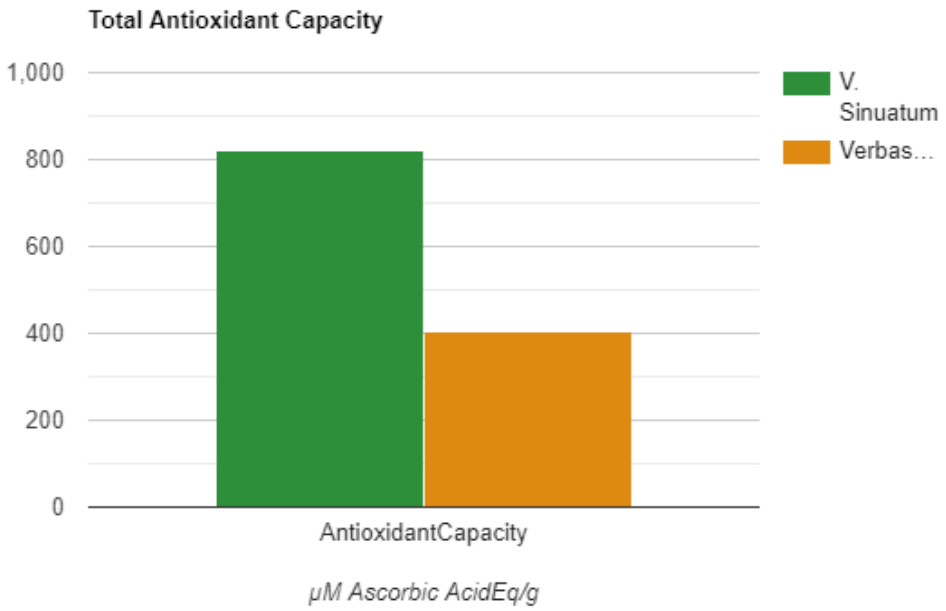
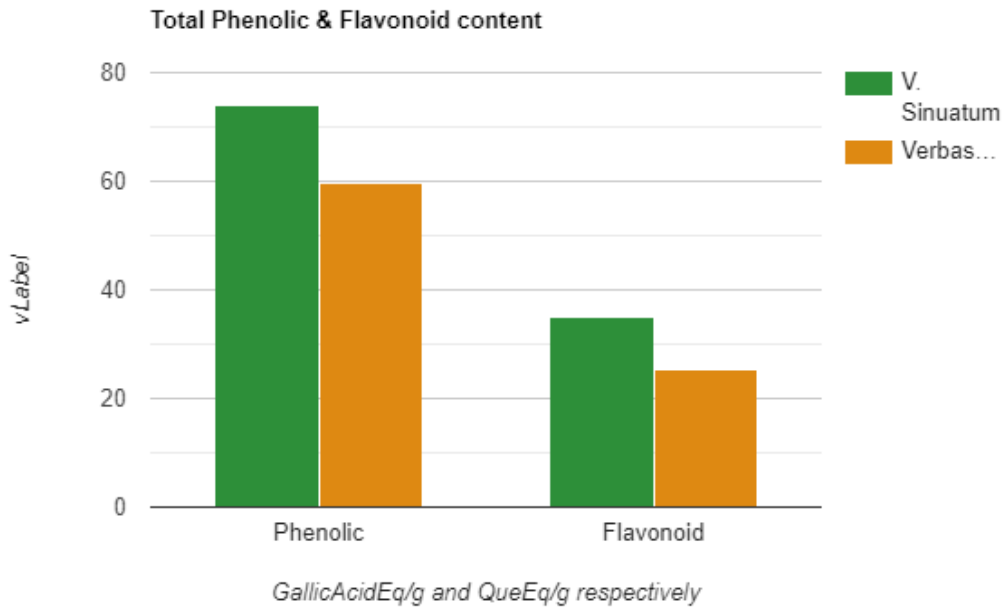
- Cells incubated with LPS to induce inflammation, then incubated with 50 and 100 ug/ml V*erbascum spp.* extracts.
- NF-κB protein levels were analyzed via western blotting

Results

- It has been found out that *V. Siniatum* and *Verbascum st.* extract yield:
- 74.14 and59,77mg GallicAcidEq/g phenolic content
- 34.96 and 25,26 mgQueEq/g flavonoid content
- Have 822,23 and 403,33µM ascorbic acid Eq/g antioxidant capacity, respectively

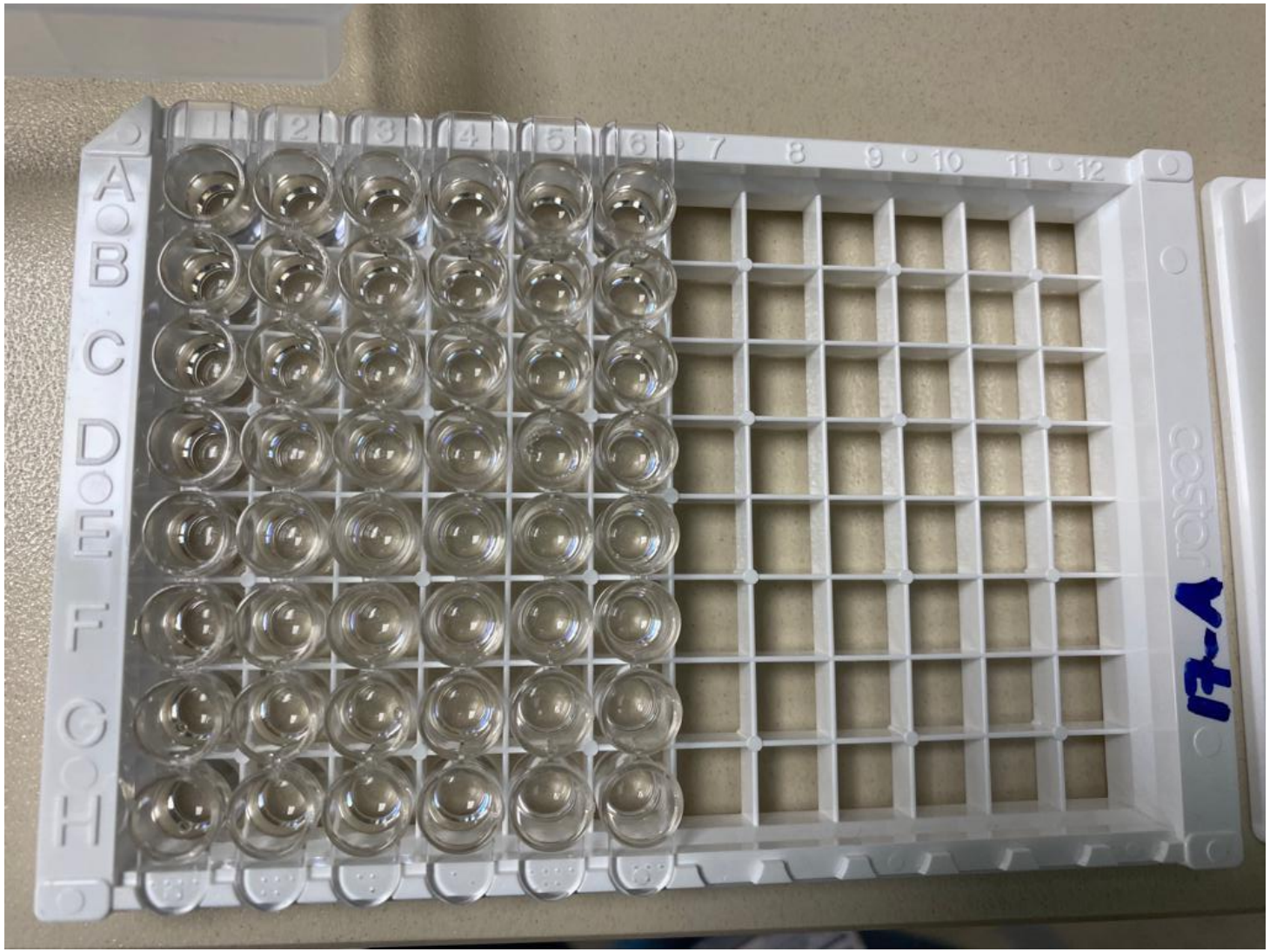




- MTT essays indicate 125 µg/ml range is safe to use, 50 µg/ml and 100 µg/ml concentrations are used
- FLS incubated in selected doses and resulting NF-κB are compared via Western blotting

- *V.Sinuatum* extract has the highest anti-inflammatory effect.

- TNF-α, IL-1β, IL-10 and IL-17A interleukins were inspected with ELİSA, but significant results could not be obtained due to defective kit



Conclusion

- Both Verbascum genus has anti-inflammatory effect compared to the control group, with *V. Sinuatum* having more potential than *Verbascum st*..

Weakness and strength

Weakness

- Lack of primary cell conformation
- Lack of further interleukin inspection due to financial issues

Strength

- This study is the first study to inspect the immunomodulatory effect of *Verbascum spp.* on a FLS model

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Thank you for your attention