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Histological and Immunohistochemical study of Anti-Inflammatory effect of *Inula viscosa* (L). Aiton Leaf Extract in Formaldehyde-induced arthritis in Mice

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INTRODUCTION & AIM

Rheumatoid arthritis (RA) is an autoimmune disorder, caused by a complex interaction between genetic and environmental factors **[1];** characterized by synovial membrane inflammation (synovitis) which affects articular joints, leading to tissue destruction through cartilage and bone damage **[2]**. Despite the significant progress of current practices (early commencement of disease modifying anti-rheumatic drugs (DMARDs) and targeted therapy: tumor necrosis factor (TNF) inhibitors, interleukin-6 receptor (IL-6R) inhibitors, checkpoint modifiers (Abatacept), and B cell depletion (Rituximab)) in RA patient life management and remission, a notable percentage of non-response to treatment in many patients is observed **[3]**. Thus, our study aims to evaluate the potential anti-arthritic effect of *Inula viscosa* (I. viscosa), which is an endemism Mediterranean traditional plant, rich in phytochemicals responsible for various pharmacological activities especially anti-inflammatory effect **[4]**.

To validate its traditional use, leaves crude extract is used on an experimental model of arthritis, exploring *in vivo* histological an immunohistochemical effects.

METHOD

- Methanol crude extract of *I. viscosa* leaves (IVME) was obtained by maceration; for our study; After a 10 day Formaldehyde induced edema experimental protocol, mice were sacrificed (**Fig.1**).
- For histological analysis, right paws were removed and fixed in 10% formalin for 12 h then decalcified in 20% Nitric acid solution for 9 ±3 h, transverse sections were prepared, embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E) for the evaluation of bone and cartilage erosions.
- Immune cells infiltration was evaluated by immunohistochemical analyses of CD3+ (T cells), CD20+ (B cells) and CD68+ (monocytes) markers.

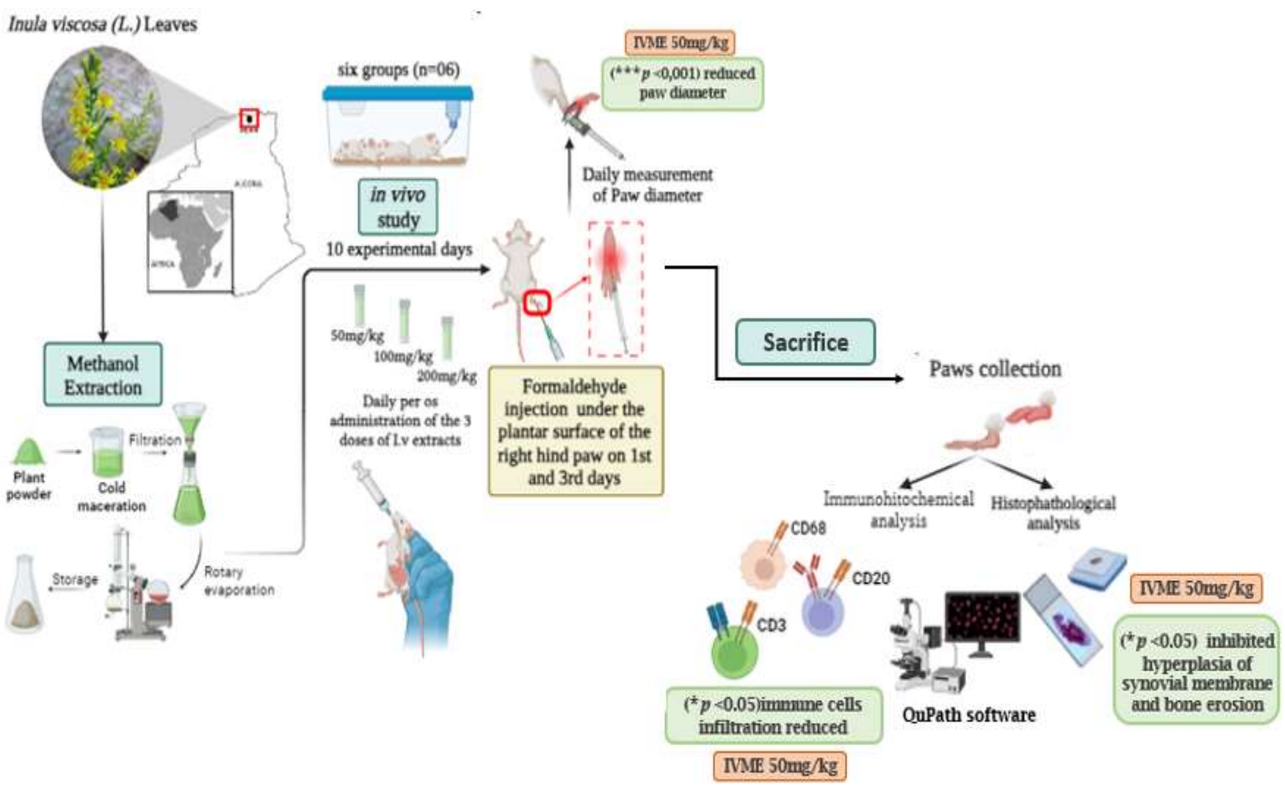

Fig. 1. Experimental protocol of histological and immunohistochemical study of IVME Anti-Inflammatory effect on NMRI albino mice. Approved by the local ethics committee (PBVE, University of Bejaia) (Ref.No.CE-LBVE-2024-114)

RESULTS & DISCUSSION

I/ Histological results

Bone erosion and pannus significantly (***p < 0.001) decreased in IVME 50mg/kg treated group, compared to disease group (Fig. 2D). Bone damage was still observed in IVME 100 mg/kg group (Fig. 2E), but significantly (**p < 0.01) less than disease group. IVME 200 mg/kg and Diclofenac treated groups (Fig. 2C and F), showed a significant (**p < 0.01) focal synovial hyperplasia with mild inflammatory infiltrates, and (***p < 0.001) small focal bone lesions at the surface of cortical bone compared to disease group (Fig 3 A & B).

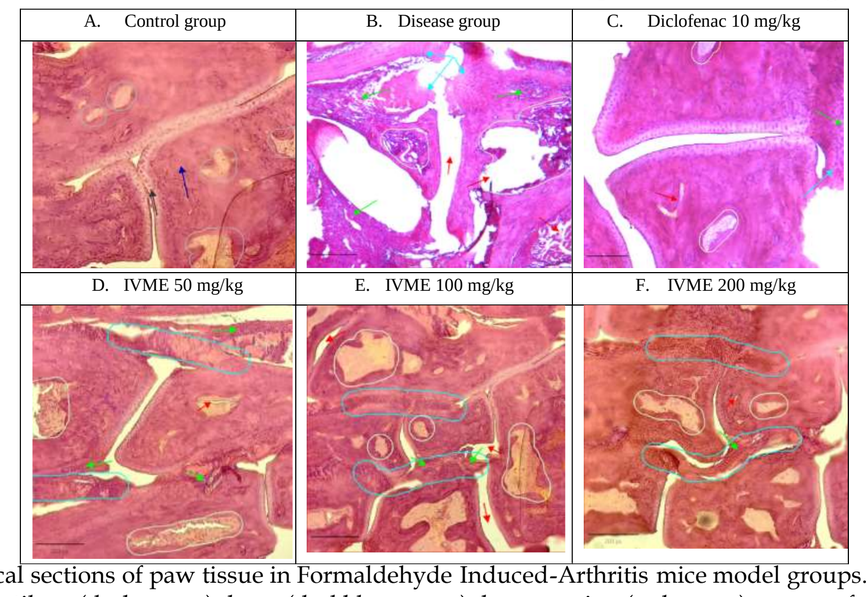

Fig .2. Histological sections of paw tissue in Formaldehyde Induced-Arthritis mice model groups. bone marrow (gray circle), cartilage (dark arrow), bone (darkblue arrow), bone erosion (red arrow), pannus formation and synovial hyperplasia (blue "cyan" circle), inflammatory infiltrate (green arrow).

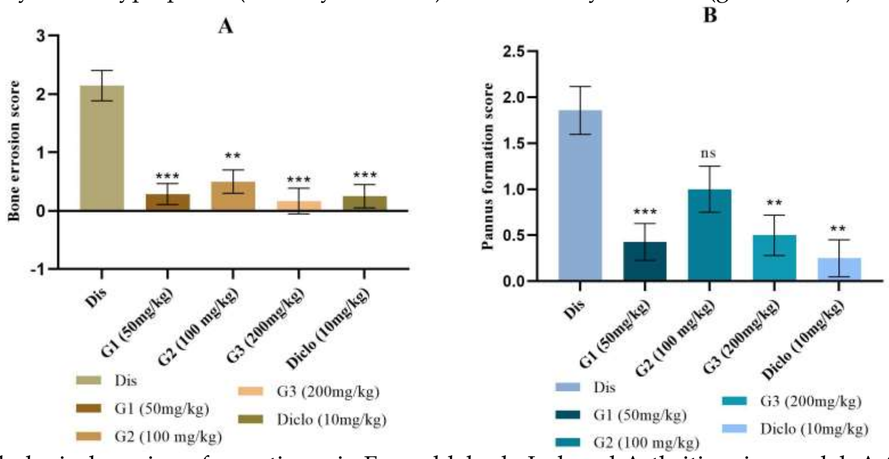

Fig .3. Histopathological scoring of paw tissue in Formaldehyde Induced-Arthritis mice model. A (Bone erosion) and B (Synovial inflammation). Statistical analysis by one-way ANOVA followed by Tukey's multiple comparisons.

2/ Immunohistochemical results

Quantitative staining scores of CD3+, CD20+, and CD68+, show low infiltration of adaptative immune-cells whereas it is predominated by CD68+ monocytes in disease group; revealing a "pauci-immune" synovitis pathotype; the results suggest that IVME significantly (*p value<0.05) reduced inflammation and the degree of cellular infiltration via an anti-inflammatory activity (**Fig. 4**).

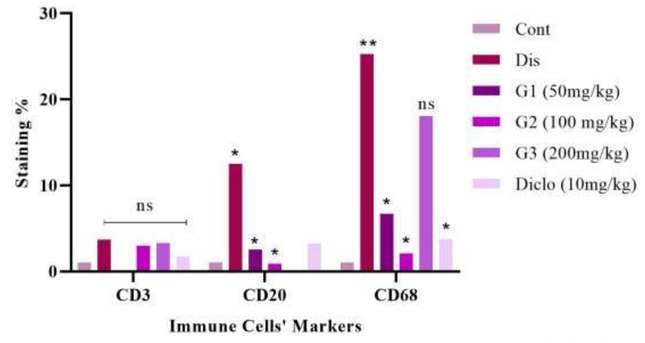

Fig. 4. Immunohistochemical semi-quantitative scoring of paw tissue in Formaldehyde Induced-Arthritis mice model, for expression of CD3, CD20 and CD68. All values are presented as mean w/ upper and lower limits. Statistical analysis by Ratio paired t-test.

CONCLUSION

In this present study, we demonstrated that IVME (50 mg/kg) treatment reduced inflammation of the joint, immune cells infiltration and exhibited bone protection activity, indicating the effective anti-inflammatory and anti-arthritic effect of *I. viscosa* leaves.

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