Turkish Online Journal of Qualitative Inquiry (TOJQI)
Volume 13, Issue 1, January 2022: 2146-2154

Anti-Rheumatoid Arthritic and Inflammatory property of Quisqualis indica Linn in wistar rat

[1]Mohammad Shoaib, [2]Diksha Singh, [3]Rajeev Shukla

[1,2,3] Institute of Pharmacy, Saraswati Higher Education and Technical, Varanasi.

Abstract

In India, *Quisqualis indica* major has a long history of medicinal usage. There aren't many reports on *Quisqualis indica's* anti-arthritic properties, though. Using Complete Freund's Adjuvant (CFA)-induced arthritis generated in female Wistar rats, the anti-arthritic effects of an n-methanolic extract of *Quisqualis indica* (MQI) were examined. As a positive control, diclofenac was utilized. MQI activity was determined by measuring the amount of paw edema, and neutrophils. Different phytoconstituents of *Quisqualis indica* and antioxidant activity were also examined. Diclofenac and the MQI extract both reduced paw oedema by 18 %, 14%, and 15 % at dosages of 150 and 300 mg/kg BW, respectively. MQI decreased the prevalence of arthritis and the arthritic index as well. The number of neutrophils significantly decreased in MQI-treated animals compared to untreated rats.

Keywords: *Quisqualis indica*, Rheumatoid arthritis, inflammatory joints, arthritis comorbidities.

1. Introduction

Rheumatoid arthritis (RA) is an immunological, chronic inflammatory joint disease that deteriorates joints and results in pain, stiffness, loss of function, and systemic comorbidities **[1]**. Chronic synovitis, which is a feature of RA, is brought on by ongoing immune cell infiltration into joints. A complicated network that fosters the generation of pro-inflammatory cytokines is formed by effector T cells, B cells, and other innate immune cells. These substances harm bone and cartilage by activating synoviocytes that resemble fibroblasts throughout the body **[2]**.

Macrophages that function by generating pro-inflammatory cytokines (TNF-, IL-1, and IL-6) and small-molecule inflammatory mediators, as well as innate immune cells like neutrophils and mast cells also contribute to synovitis **[3]**. The two categories of cytokines are pro- and anti-inflammatory. An essential therapeutic goal for the treatment of RA is the balance between these groups **[4]**.

Quisqualis inidica Linn. Shows the number of pharmacological or medicinal activities such as antipyretic activity, anti-staphylococcal activities, anti-inflammatory activities, etc., due to the presence of the various phytoconstituents in the plants. The species contains a wide range of bioactive substances, such as fatty acids, polysaccharides, vitamins, iridoid glycosides, alkaloids, terpenoids, phenolic compounds (derivatives of caffeine), and flavonoids **[5]**.

These substances are present in almost every component of the plant, including the seeds, leaves, flowers, and roots. Aucubine, a glycoside that is said to be a potent antitoxin, was discovered in the

chemical analysis of leaves **[6]**. *Qusqualis indica* 0.07% of oleanolic acid and 0.22% of ursolic acid, two significant terpenoids, are also present in leaves **[7]**.

Prostaglandin production that is catalyzed by cyclooxygenase-2 is selectively inhibited by ursolic acid. This inhibition may be the cause of the plant's anti-inflammatory properties. Major Dichloromethane extracts prevent leukocyte migration in mice with peritonitis brought on by thioglycolate. Additionally, mouse ear oedema brought on by croton oil exhibits anti-inflammatory action in response to ethanol extracts **[8]**.

A typical RA model that predicts the clinical efficacy of specific treatments for RA in humans is adjuvant arthritis in rats. In this investigation, female Wistar rats that had been given Complete Freund's Adjuvant to develop arthritis were tested for the activity of a *Qusqualis indica* major extract fraction **[9]**.

2. Material and methods

2.1. Drying & Pulverization of the plant material

The leaflets were cleansed to eliminate particulate matter and permitted to air dry in the sun for full dryness after collecting and authentication. The leaf extract was then ground in a mixer without any moisture **[10]**.

2.2. Plant extracts preparation

The fine powdered was packed securely in soxhlet apparatus and recovered with solvent methanol for 72 hrs. with intermittent shaking at 60°C. Evaporation condensed the extraction to a fraction of its initial volume, Phytochemical research was conducted on the methanolic extract of *Quisqualis indica (QI)* that resulted **[11]**.

2.3. Animals

This research employed Wistar rats weighing 150 g to -200 g. The animals came from the animals housed at the college name. The animals were randomly assigned to patients treated and housed in polypropylene enclosures with paddy husks as bedding when they arrived. The animals were kept at a temp of 24°C with a relative humidity of 30 - 70 percent. The A12:12 light: day cycles were used. All animals were given free access to the water and were fed commercial pelleted chow. All experimental techniques and methods adopted in this work were approved by the college name Animal Ethics Committe (688/02/C-CPCSEA), proposal number (NCP/IAEC/NO: 20121-2022), and followed the IACE criteria **[12]**.

2.4. Experimental approach

After a week of acclimatization in the animal house, the rats were sorted into five groups of five at random. Rats in groups III, IV, and V were given CFA and treated as shown in Table 1 whereas rats in group I served as the normal control group. Group II animals received CFA and functioned as the RA control group **[12]**.

Table 1: Experimental design

Group	Treatment
Group I	Negative Control
Group II	Positive control
Group III	CFA + Na diclofenac 4 mg/kg
Group IV	CFA + M*QI* 1500 mg/kg BW
Group V	CFA + M*QI* 300 mg/kg BW

From day 16 to day 46, extracts and Na diclofenac were taken orally every day.

MQI: Methanolic extract of *Quisqualis indica*.

RA: Rheumatoid arthritis.

CFA: Complete Freund's adjuvant.

2.5. Dosage choices

Since there has been reported safety up to a level of 6200 mg/kg, acute toxicity tests were not carried out. Based on a recent investigation employing thioglycollate-induced leukocyte migration in mice, the two dosages of 150 and 300 mg/kg BW were chosen **[13]**.

2.6. Evaluation of anti-RA

To create a baseline, the paw volume of each animal was measured on day 0. As already mentioned, arthritis was brought on. Injecting 0.15 mL of CFA into the right hind paw's plantar region was done quickly. On day 0, an equivalent quantity of saline was administered to the left paw. Using a plethysmometer, the volume of oedema was assessed every three days till day 47. The arthritis index was evaluated as described earlier every three days for three weeks to confirm the presence of RA. Depending on alterations in the redness and swelling of the toes, footpads, and ankles, the diagnostic severity of arthritis was graded, with a maximal score of 2 per paw. As the arthritic index, the mean of the cumulative value for all paws was used (AI). When the AI was more than 1, rats were regarded as having arthritis. MQI was administered orally to treated animals from days 16 to 46 at dosages of 150 or 300 mg/kg BW. Rats were given CMC or diclofenac sodium 4 mg/kg BW in the positive and negative control groups, accordingly **[14]**.

2.7. Homological variables

The retro-orbital puncture was used to get the blood samples. Using a Sysmex KX-21 haematology analyzer, total leukocytes and differential leukocyte counts were calculated at days 0 and 28 **[15]**.

3. Results:

3.1. Phytochemical screening:

It was determined the phytochemistry of *Quisqualis indica*. Table 2 provides a summary of the findings. Results for glycosides, alkaloids, flavonoids, tannin, and phenols in *Quisqualis indica* hexane extracts were favorable. For steroids, glycosides, terpenoids, saponins, alkaloids, flavonoids, tannins, phenols, and carbohydrates, *Quisqualis indica's* methanolic extract tested positive. Saponin, phenol, and alkaloids were detected in Petroleum ether extracts, and glycosides, alkaloids, flavonoids, tannins, phenol, and carbohydrates were detected in *Quisqualis indica* hexane chloroform extracts.

Table 2: Phytochemical screening of *Quisqualis indica* extracts.

Test	Hexane	Methanol	P. ether	Chloroform
Steroids	-	+	-	-
Glycosides	+	+	-	+
Terpenoids	-	+	-	-
Saponins	-	+	+	-
Alkaloids	+	+	+	+
Flavonoids	+	+	-	+
Tannins	+	+	-	+
Carbohydrate	-	+	-	+
Phenols	+	+	+	+

Note: ++: high content, +: moderate, - : Negative,

3.2. DPPH Radical Scavenging

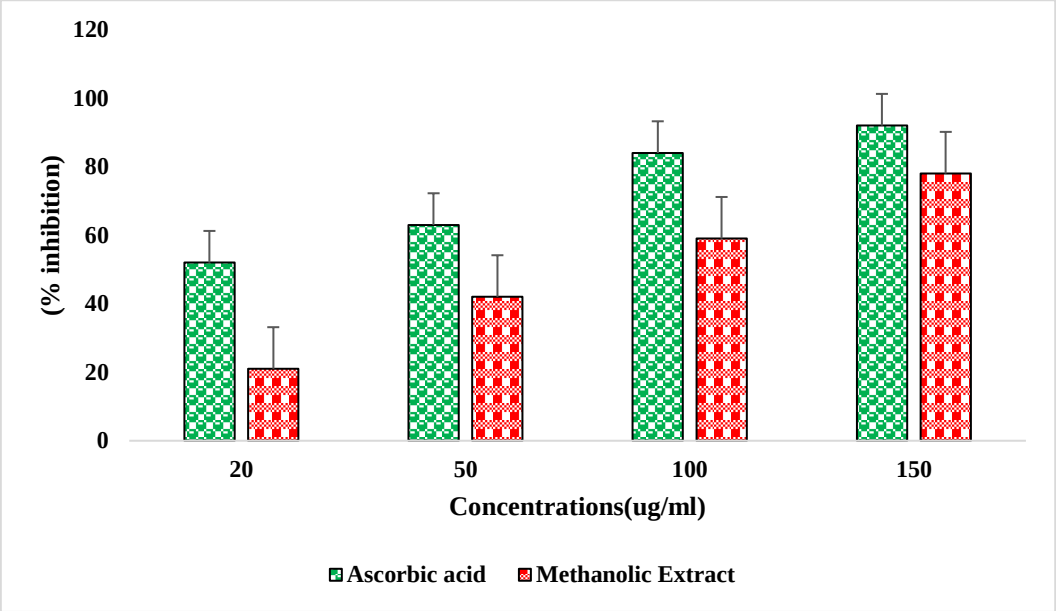

Figure 1: Antioxidant analysis of the methanolic extract and ascorbic acid.

The DPPH radical is a stable free radical that may accept an electron to transform into a stable molecule, making it a common substrate for testing antioxidant efficacy. The decrease in DPPH radical absorbency at 516 nm caused by antioxidants was used to quantify its reduction **[16].** The DPPH radical scavenging activity of the pericarp of *Quisqualis indica's* methanol extract is depicted in Figure 1 as a dose-response curve. The methanol extract's scavenging activity was 78 at a concentration of 150 g/ml. The investigation revealed that the extracts have the proton-donating ability and could act as free

radical inhibitors or scavengers, possibly acting as primary antioxidants, even if their DPPH radical scavenging powers were less than those of ascorbic acid (92%) at 150 g/ml.

3.3. Effect of prolonged treatment with MQI on paw volume

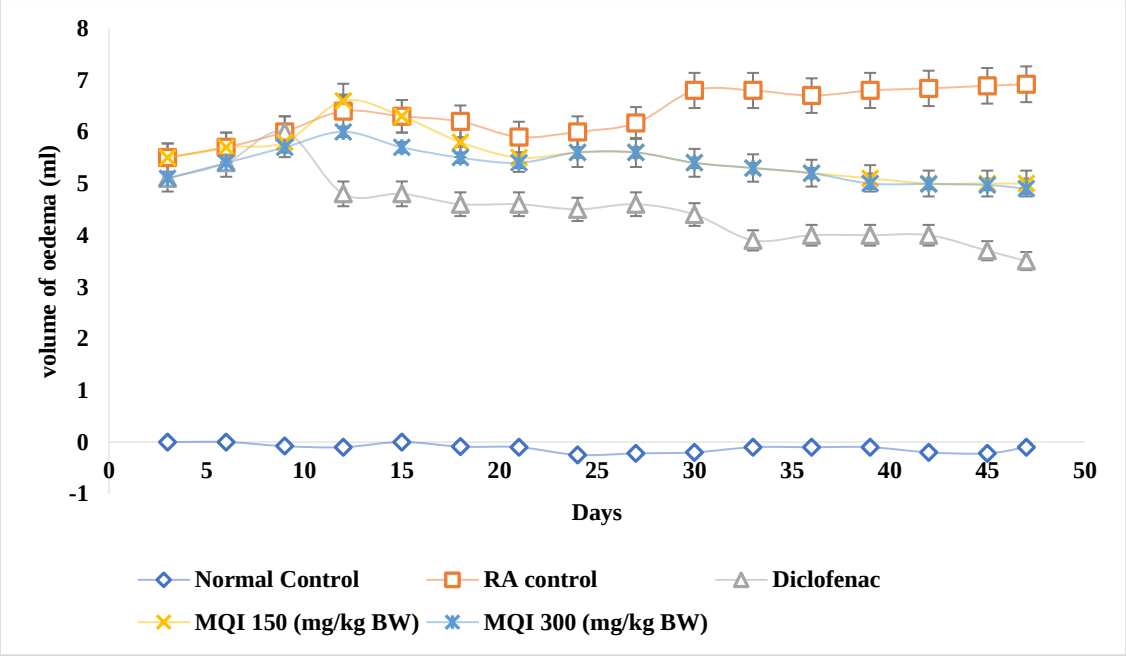

Figure 2: Assessing paw volume, the impact of IPM on CFA-induced arthritis was investigated.

The arthritic rats' hind paw swelling increased over time until day 47 after CFA injection (Fig. 2). When compared to RA controls, rats given 150 and 300 mg/kg BW of MQI, sodium diclofenac, or both showed significantly less paw oedema (<P 0.05).

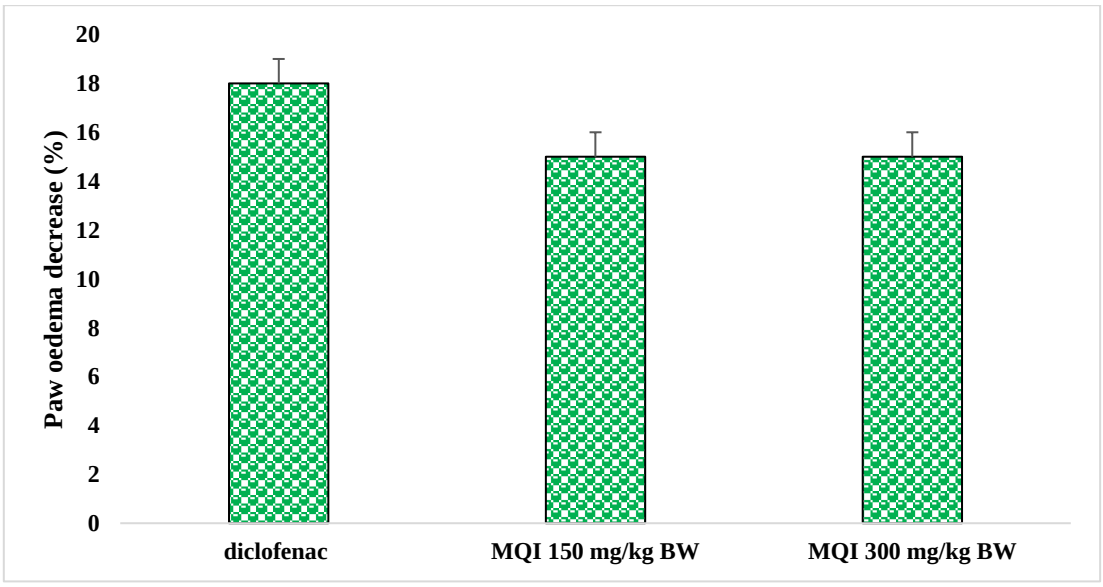

Figure 3. The reduction of paw oedema after treatment with diclofenac and the MQI extracts. n = 5, means, standard errors, #P< 0.05 with diclofenac. Diclofenac and MQI extract at dosages of 150 and 300 mg/kg BW prevent paw oedema.

MQI extracts at doses of 150 and 300 mg/kg BW and diclofenac inhibit paw oedema by 14 %, 15 %, and 18 %, respectively. From day 16 to day 47, rats treated with MQI and diclofenac exhibited considerably less severe arthritis (AI >1) than RA-control rats (Fig. 3).

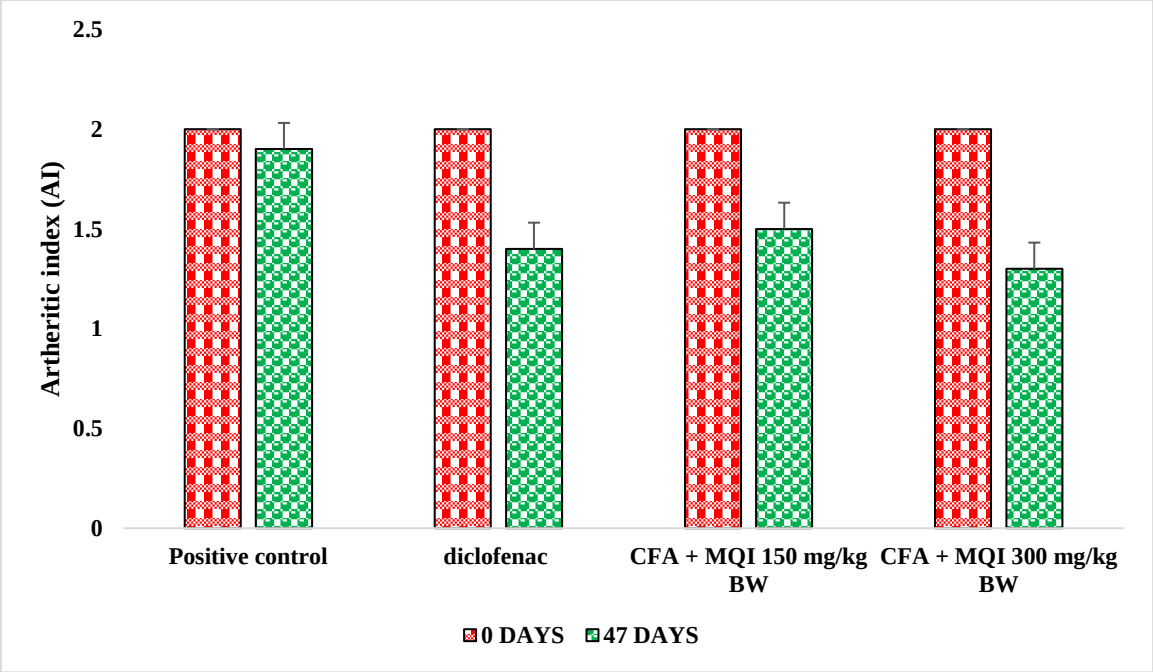

Figure 4. Arthritic Index (AI) comparison between day 16 and day 47 in treated animals. Results are presented as means SE, n = 5, P< 0.05 with RA Control, and #P <0.05 with diclofenac. QMI is extracted; RA stands for rheumatoid arthritis.

3.4. Effect of prolonged treatment with MQI on neutrophils

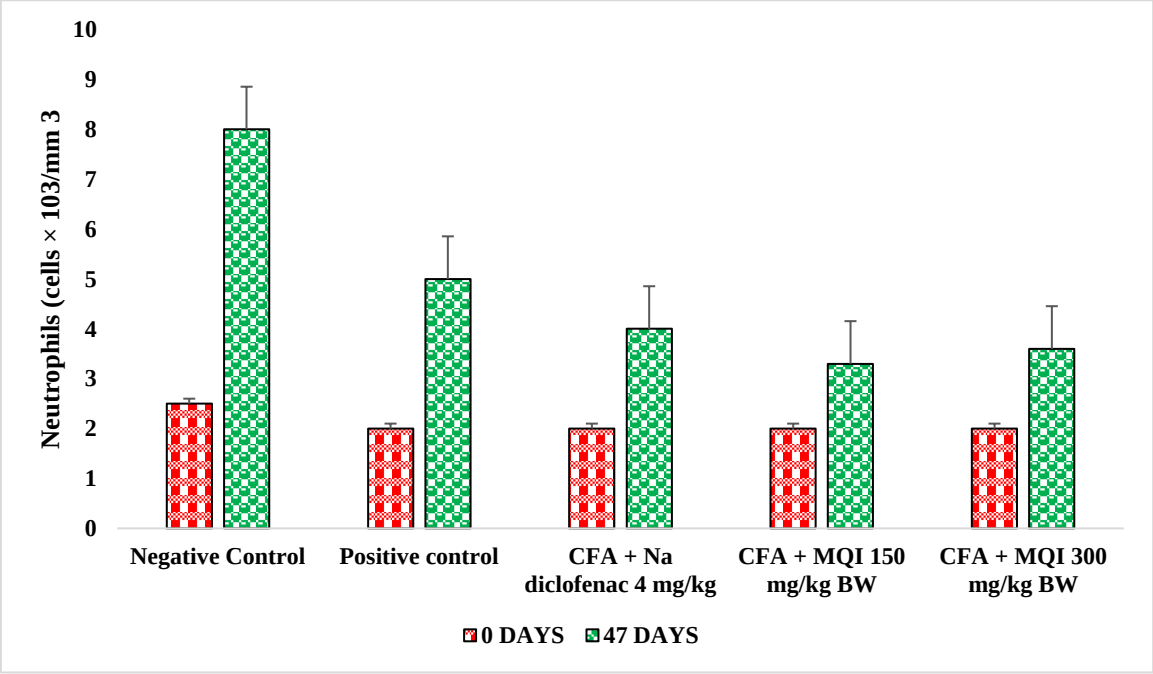

Figure 5: Arthritis's impact on neutrophil levels.

Results are presented as means SE, n = 5, P< 0.05 with RA Control, and #P <0.05 with diclofenac. MQI is extracted; RA stands for rheumatoid arthritis. Treatments with MQI and diclofenac resulted in a considerable drop in neutrophil numbers (Fig. 5).

4. Discussion

New exploration is currently being driven by a growing curiosity about the medicinal potential of medicinal plants. These initiatives highlight the preventative or therapeutic effectiveness, low toxicity, and lower number of adverse effects for numerous plant extracts when compared to traditional manufactured medications. *Quisqualis indica* has been developed into pharmaceutical forms and has been utilized for medical purposes, particularly anti-inflammation, for a long time in India **[17]**.

There are, however, few reports of *Quisqualis indica's* anti-inflammatory and anti-arthritic properties. On rats with arthritis brought on by CFA, we examined the effects of *Quisqualis indica* . Dichloromethane and extract of *Quisqualis indica* leaves were partitioned with methanolic fraction, which was then used to remove chlorophyll. This procedure was designed to increase the extract's secondary metabolite content.

An recognized in vivo model for RA pathogenesis research and the development of potential treatment targets is CFA-induced arthritis. Both clinical and serological characteristics of this model, such as the participation of paw oedema, arthrodynia, reduced body weight, and cartilage degradation, are comparable to those of human RA. CFA causes inflammation in the digits, soles, and ankles of the tested animals, which is evident by redness and swelling. Histamine, kinins, and prostaglandins are released into the circulation by CFA in joints **[18, 19]**. These substances increase the permeability of blood vessels and blood flow to the inflammatory area. Oedema, heat, erythema, and pain are brought on by this impact. Vascular permeability is increased, which aids granulocyte migration to inflammatory areas.

Paw edema, the arthritis index, arthritic incidence, and hematological alterations were all employed in this study to gauge the development of the illness. When arthritis was monitored by paw volume (Figs. 1 and 2), arthritic incidence and arthritic index, treatment with MQI significantly slowed its progression.

Compared to diclofenac, the MQI had a lower level of activity, but it nevertheless decreased the amount of oedema in the arthritic rats. The MQI at doses of 150 and 300 mg/kg BW suppressed paw oedema by 15.70% and 15.94%, respectively. Diclofenac inhibited paw oedema by 19.71%.

Every four days up until day 47, the severity of the arthritis in each group of CFA-induced rats was measured using the arthritis index. Rats were tested for RA using arthritis incidence, and the severity of the disease was assessed using the arthritis index scale.

Rats with RA exhibited alterations in the form of their foot soles as well as redness and swelling of their toes. By combining diclofenac with MQI, RA incidence was 80% lower. MQI additionally decreased AI. The AI of MQI was 1.5 and 1.3, respectively, for doses of 150 and 300 mg/kg BW, substantially different from diclofenac, which was 1.4. As a result, MQI fractions are a source of anti-inflammatory substances.

In rats with arthritis, MQI treatments decreased neutrophil numbers but had no effect on WBC or lymphocyte levels. These results were substantially different from both diclofenac-treated rats and RA control animals ($P < 0.05$).

The majority of leukocytes in inflammatory joints are neutrophils, and studies in both human and murine models of RA have shown the significance of these cells for the development and progression of the disease. Reactive oxygen species, a hazardous byproduct released by neutrophils, are thought to be partially to blame for the tissue damage that results from this process.

The MQI fraction that can reduce neutrophil migration may attenuate inflammatory processes in line with our earlier findings and the study by Reina et al., **[17]**. The two most significant compounds in *Quisqualis indica*, baicalein and aucubin, are known for their antioxidant properties and their capacity to scavenge free radicals. Neutrophils are important for articular inflammation and modulation of neutrophil functions, and they represent a target for pharmacological action in arthritis. Due to its capacity to prevent the release of pro-inflammatory cytokines by human mast cells, baicalein is also regarded as an anti-inflammatory substance.

Our results are in line with earlier studies that found *Quisqualis indica* to have anti-inflammatory and anti-arthritic properties in various pre-clinical settings.

5. Conclusion

By reducing inflammatory cells methanolic extract reduces the onset of arthritis in a RA rat model. The results of this study confirm the necessity for more MQI analysis, which should include in-depth mechanistic investigations and confirmed anti-arthritic effects in various animal models.

6. References

1. Gautam, S., Kumar, U., & Dada, R. (2020). Yoga and its impact on chronic inflammatory autoimmune arthritis. *Frontiers in Bioscience-Elite*, *13*(1), 77-116.
2. Ganesan, R., & Rasool, M. (2017). Fibroblast-like synoviocytes-dependent effector molecules as a critical mediator for rheumatoid arthritis: current status and future directions. *International reviews of immunology*, *36*(1), 20-30.
3. Chen, Z., Bozec, A., Ramming, A., & Schett, G. (2019). Anti-inflammatory and immune-regulatory cytokines in rheumatoid arthritis. *Nature Reviews Rheumatology*, *15*(1), 9-17.
4. Lubberts, E., & van den Berg, W. B. (2013). Cytokines in the pathogenesis of rheumatoid arthritis and collagen-induced arthritis. *Madame Curie Bioscience Database [Internet]*.
5. Sahu, J., Patel, P. K., & Dubey, B. (2012). Quisqualis indica Linn: A review of its medicinal properties. *Int. J. Pharm. Phytopharmacol. Res*, *1*(5), 313-321.
6. Mahajan, C. P., & Aher, A. N. (2017). A review on ethnobotanical, phytochemical and pharmacological activities of Quisqualis indica Linn. *Research Journal of Pharmacognosy and Phytochemistry*, *9*(1), 47-52.
7. Akram, M. A., Iqbal, N., Aqeel, M., Khalid, N., Alamri, S., Hashem, M., ... & Noman, A. Welcome to. *US Department of Agriculture*, *12*, 19-63.
8. Triastuti, A., Pradana, D. A., Saputra, D. E., Lianika, N., Wicaksono, H. R., Anisari, T. D., & Widyarini, S. (2022). Anti-rheumatoid activity of a hexane-insoluble fraction from Plantago major

in female Wistar rats induced by Complete Freund's Adjuvant. *Journal of Traditional and Complementary Medicine*, *12*(3), 219-224.

9. PATWARDHAN, B. EVIDENCE BASED AYURVEDA AND HERBAL MEDICINE.
10. Fernandez-Turiel, J. L., Georgakopoulos, A., Gimeno, D., Papastergios, G., & Kolovos, N. (2004). Ash deposition in a pulverized coal-fired power plant after high-calcium lignite combustion. *Energy & Fuels*, *18*(5), 1512-1518.
11. McCloud, T. G. (2010). High throughput extraction of plant, marine and fungal specimens for preservation of biologically active molecules. *Molecules*, *15*(7), 4526-4563.
12. Prakash, S., Chandra, G., & Verma, A. (2022). ASSESSMENT OF ANALGESIC ACTIVITY OF TRIDAX PROCUMBENS IN EXPERIMENTAL ANIMAL. *International Journal of Scientific Research*, *11*(5).
13. Laborde, E., Macsata, R. W., Meng, F., Peterson, B. T., Robinson, L., Schow, S. R., ... & Bernard, C. C. (2011). Discovery, Optimization, and Pharmacological Characterization of Novel Heteroaroylphenylureas Antagonists of C− C Chemokine Ligand 2 Function. *Journal of medicinal chemistry*, *54*(6), 1667-1681.
14. Farooq, S., Shaheen, G., Asif, H. M., Aslam, M. R., Zahid, R., Rajpoot, S. R., ... & Zafar, F. (2022). Preliminary Phytochemical Analysis: In-Vitro Comparative Evaluation of Anti-arthritic and Anti-inflammatory Potential of Some Traditionally Used Medicinal Plants. *Dose-Response*, *20*(1), 15593258211069720.
15. Srivastava, S., Singh, P., Jha, K. K., Mishra, G., Srivastava, S., & Khosa, R. L. (2012). Evaluation of anti-arthritic potential of the methanolic extract of the aerial parts of Costus speciosus. *Journal of Ayurveda and integrative medicine*, *3*(4), 204.
16. Hsu, C. F., Zhang, L., Peng, H., Travas-Sejdic, J., & Kilmartin, P. A. (2008). Scavenging of DPPH free radicals by polypyrrole powders of varying levels of overoxidation and/or reduction. *Synthetic Metals*, *158*(21-24), 946-952.
17. Xiong, Y., & Long, C. (2020). An ethnoveterinary study on medicinal plants used by the Buyi people in Southwest Guizhou, China. *Journal of ethnobiology and ethnomedicine*, *16*(1), 1-20.
18. Triastuti, A., Pradana, D. A., Saputra, D. E., Lianika, N., Wicaksono, H. R., Anisari, T. D., & Widyarini, S. (2022). Anti-rheumatoid activity of a hexane-insoluble fraction from Plantago major in female Wistar rats induced by Complete Freund's Adjuvant. *Journal of Traditional and Complementary Medicine*, *12*(3), 219-224.
19. Reina, E., Al-Shibani, N., Allam, E., Gregson, K. S., Kowolik, M., & Windsor, L. J. (2013). The effects of Plantago major on the activation of the neutrophil respiratory burst. *Journal of Traditional and Complementary Medicine*, *3*(4), 268-272.
20. Poorvashree, J., & Suneela, D. (2017). Novel drug delivery of dual acting prodrugs of hydroxychloroquine with aryl acetic acid NSAIDs: Design, kinetics and pharmacological study. *Drug Delivery and Translational Research*, *7*(5), 709-730.