To Carry Out Antiarthritic Activity of Quercetin and Curcumin Combination in Experimental Rat Model

Ms. Komal Mallikarjun Dikole, Dr.Hemant V. Kambale, Ms. Kore Monali Dattatray,

Ms.Manasi Siddheshwar Zade, Ms. Sushma subhash Chougule.

Loknete shri dadapatil pharate college of pharmacy , mandavgan pharata, pune, Maharashtra india.

Corresponding author

Ms. Komal Mallikarjun Dikole

ABSTRACT

Rheumatoid arthritis (RA) is a chronic inflammatory disorder characterized by progressive joint degeneration, oxidative stress, and elevated pro-inflammatory mediators. The present study aimed to evaluate the antiarthritic potential of a combined formulation of **quercetin and curcumin** in an experimental rat model. Arthritis was induced in Wistar rats using Complete Freund's Adjuvant (CFA), and animals were divided into control, arthritic control, standard (diclofenac), and treatment groups receiving quercetin, curcumin, or their combination. The therapeutic regimen was administered orally for 28 days. Antiarthritic activity was assessed through clinical scoring, paw edema measurement, body-weight analysis, and biochemical markers including rheumatoid factor (RF), C-reactive protein (CRP), TNF-α, IL-1β, and oxidative stress parameters. Histopathological evaluation of joint tissue was also performed.

Results demonstrated that the combination of quercetin and curcumin produced a **significant reduction in paw swelling**, restored body weight, and markedly improved inflammatory and oxidative stress biomarkers compared to individual treatments. The combination therapy also showed near-normal joint architecture with reduced synovial hyperplasia and cartilage erosion. These findings suggest a **synergistic interaction** between quercetin and curcumin, likely due to their complementary antioxidant and anti-inflammatory mechanisms. The study concludes that the quercetin–curcumin combination exhibits potent antiarthritic activity and may serve as a promising phytotherapeutic strategy for RA management.

KEY WORDS: Antiarthritic, Quercetin and Curcumin.

INTRODUCTION

Inflammation is a normal, essential, and protective response to any noxious stimulus that may threaten the host and may vary from a localized reaction to a complex response involving the whole organism Inflammation is nature's double-edged sword. Inflammation characterized by pain and swelling, is triggered as a healing response in the body when it is injured or attacked by negative bacteria and viruses. Once the body recovers, the inflammation goes away. Obviously, dark side of inflammation, inflammation that doesn't heal, that doesn't go away, is one of the most prevalent health problems today. Dangers of Conventional Treatment for Inflammation

Inflammation is the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. Inflammation is not a synonym for infection. Even in cases where inflammation is caused by infection it is incorrect to use the terms as synonyms: infection is caused by an exogenous pathogen, while inflammation is the response of the organism to the pathogen.

1.Conditions of inflammation

Many of the world's major diseases like infection, cancer, autoimmunity and allergy, critically involve the inflammation. Continued progress in understanding basic mechanisms of inflammation is essential for developing new abilities to treat and prevent diseases that affect millions worldwide.

2.Causes of Inflammation

Various agents may kill or damage cells as: Physical (heat or cold, Trauma, radiation), chemical (simple chemical poisons e.g.: acid, organic poisons e.g.: paraquat), Infection (bacteria, virus),Immunological (antigen – antibody, cell mediated)

3.Pathology of Inflammation

The classical signs of inflammation are: Redness (Rubor), Swelling (Tumor), Heat (color), Pain (Dolor), Loss of Function.

Rheumatoid arthritis (RA)

Rheumatoid arthritis (RA) is a chronic, inflammatory, autoimmune disease, the pathology of which is primarily and symmetrically localized in diarthrodial joints. The pathogenesis of RA is characterized by an inflamed synovium (lining the joint cavity), degradation of articular cartilage and erosion of sub-chondral bone. The systemic ramifications of the disease, with their attendant morbidity and mortality,

include cardiopathy, nephropathy, vasculopathy and pulmonary and cutaneous disorders (Firestein., 2001). The prevalence of RA in India is quite similar to that reported from the developed countries. Projected to the whole population, this would give a total of about seven million patients in India. It is higher than that reported from China, Indonesia,Philippines and rural Africa. (Malaviya et al., 1993). Although the cause of RA is unknown, the presentation of anarthritogenic self antigen to a genetically susceptible individual is believed to trigger the activation of auto-immunological pathways that lead to RA. Intense investigation of the cause of RA has uncovered many of the integral biochemical, cellular and molecular pathological components and pathways of this disease, leading to the discovery, development and marketing of new and novel therapeutics that target several seminal components of RA (Firestein, 2001). Thus, arthritis is an autoimmune disease in which inflammation is a predominant feature. Inflammation is the reactive state of hyperemia and exudation from blood vessels with consequent redness, heat, swelling and pain which a tissue manifests in response to physical or chemical injury or bacterial invasion.

Treatment

The primary objective is to improve or maintain functional status there by improving quality of life. Treatment of rheumatoid arthritis is a multifaceted approach that includes pharmacologic and nonpharmacologic therapies. Recent emphasis has been placed on aggressive treatment early in the disease course. The ultimate goal is to achieve complete disease remission, although this goal is seldom achieved. Additional goals of treatment include controlling disease activity and joint pain, maintaining the ability to function in daily activities or work, improving the quality of life, and slowing destructive joint changes. (Dipiro et al 2005)

Introduction To Cytokine Based Therapies

Cytokines frequently have multiple biologic functions with overlapping effects. Often they have proinflammatory as well as anti-inflammatory activity,although in most cases they can be classified predominantly as proinflammatory or anti-inflammatory cytokines. The therapeutic effects of cytokine targeted therapies also depend on the type of product, dose, dosing schedule, route of administration, mechanism of action, and patient population studied. Interleukin-1P (IL-lb) and TNF-a are the key proinflammatory cytokines implicated in the pathogenesis of RA

LITERATURE REVIEW

Rheumatoid arthritis (RA) is a chronic autoimmune disease marked by synovial inflammation, cartilage and bone destruction, oxidative stress, and elevated pro-inflammatory cytokines. Conventional therapies, though often effective, may present significant side effects, motivating research into safer, plant-derived alternatives. Among bioactive phytochemicals, Quercetin and Curcumin have emerged as promising candidates due to their anti-inflammatory, antioxidant, and immunomodulatory properties.

Anti-Arthritic & Anti-Inflammatory Properties of Curcumin

Curcumin, the principal curcuminoid from turmeric, has been widely studied for its role in inflammatory disorders including RA. A systematic review of animal studies (and some human data) found that curcumin consistently improved clinical and inflammatory parameters in RA models, via mechanisms including inhibition of signaling pathways such as mitogen-activated protein kinases (MAPKs), activator protein-1 (AP-1), and nuclear factor-kappa B (NF-κB).

In experimental models, curcumin has demonstrated the ability to reduce joint swelling, lower pro-inflammatory cytokines (e.g., TNF-α, IL-1β, IL-6), suppress pannus formation, reduce synovial hyperplasia, and protect cartilage and bone from degradation.For instance, in a collagen-induced arthritis (CIA) rat model, curcumin attenuated arthritis severity and improved histopathology of the joints.

However, a significant limitation of curcumin is its poor bioavailability — due to low absorption, rapid metabolism, and instability under physiological conditions. To address this, various formulations — such as milk-based suspensions, nano-formulations, or other delivery vehicles — have been explored. For example, a milk-based curcumin formulation improved both pharmacokinetic and pharmacodynamic parameters in arthritic rats, yielding better suppression of inflammation compared to standard curcumin suspension.

Anti-Arthritic & Anti-Inflammatory Properties of Quercetin

Quercetin, a flavonol found abundantly in many fruits and vegetables, exerts anti-oxidative and anti-inflammatory effects. In chronic adjuvant-induced arthritis in rodents, oral or subcutaneous administration of quercetin decreased arthritic scores and delayed disease progression in a dose-dependent manner.

On a cellular level, quercetin has been shown to inhibit macrophage-derived inflammatory cytokines and nitric oxide (NO), reduce neutrophil activation, suppress synoviocyte proliferation and angiogenesis, and inhibit key processes that contribute to the pathogenesis of arthritis. More recently, quercetin was found to inhibit the enzyme Adenosine Deaminase (ADA) in joint tissues of RA-induced rats; this was associated with lower levels of inflammatory cytokines, reduced rheumatoid factor (RF), C-reactive protein (CRP), and improved histopathology of joints. Moreover, formulations aimed at improving quercetin’s bioavailability — for example, nanoparticles — have shown enhanced anti-arthritic efficacy in animal models.

Further, in models of bone erosion associated with RA, quercetin has also shown capacity to modulate bone remodeling by influencing osteoblast/osteoclast activity, hinting at protective effects against bone destruction.

MATERIAL AND METHODS

Materials:

Animals:

Female Sprague Dawley rats weighing 180-200 gm

Instrument used:

Plethysmometer

Centrifuge

UV Spectrophotometer

Plantar test apparatus

Animal weighing electronic balance

Chemical weighing balance

Tissue Homogenizer

METHODS:

Ex-vivo parameters:

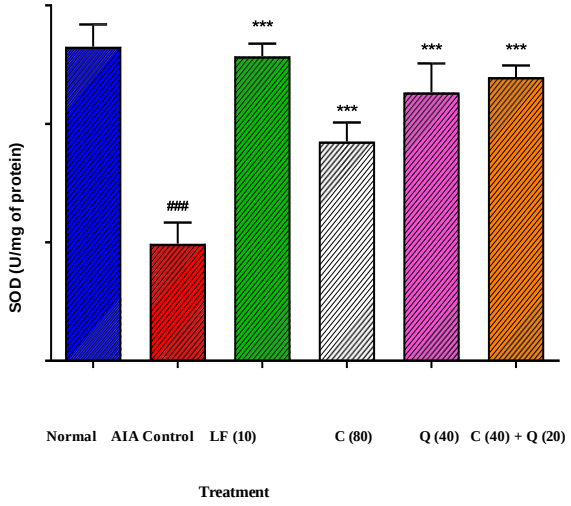
Tissue Parameters

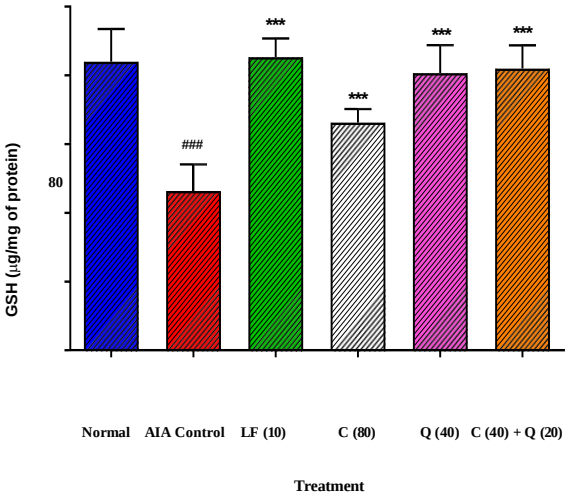
1) Determination of Lipid Peroxidation (MDA content)
2) Determination of Superoxide Dismutase (SOD)
3) Determination of Reduced glutathione (GSH)
4) Determination of NO:
5) Determination of tissue protein

RESULTS

1.Effect of curcumin, quercetin and their combination on FCA-induced alteration in hepatic SOD and GSH level:

Parameter	Hepatic SOD (U /mg of protein) and GSH µg/mg of protein) levels - Mean ± SEM					
	Normal	AIA Control	Leflu-nomide (10 mg/kg)	Curcumin (80 mg/kg)	Quercetin (40 mg/kg)	Curcumin (80 mg/kg) + Quercetin (40 mg/kg)
SOD (U /mg of protein)	5.31 ± 0.15	1.98 ± 0.15###	5.14 ± 0.09***	3.70 ± 0.13***	4.53 ± 0.20***	4.79 ± 0.08***
GSH (µg/mg of protein)	83.99 ± 3.88	46.36 ± 3.15###	85.32 ± 2.21***	66.28 ± 1.61***	80.66 ± 3.33***	81.97 ± 2.76***





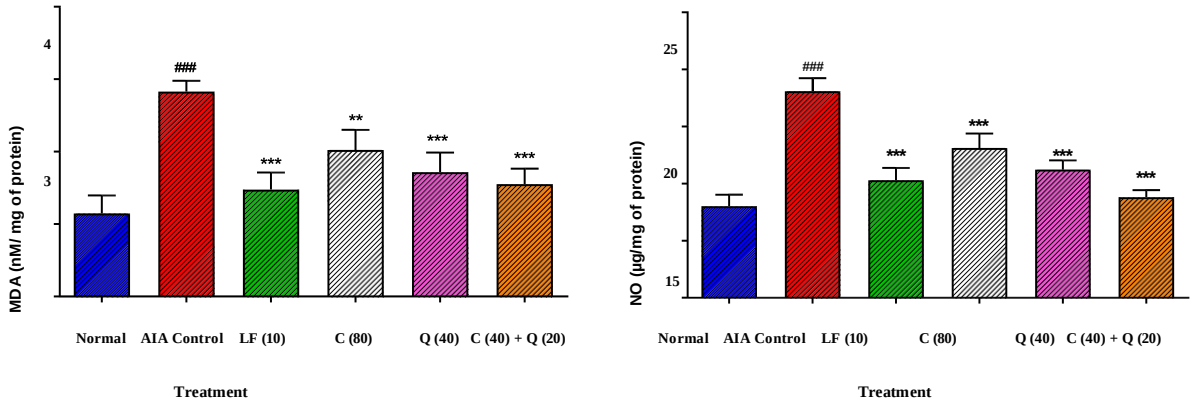
1.Effect of curcumin, quercetin and their combination on FCA-induced alteration in hepatic SOD and GSH level

Data were analyzed by One-way ANOVA followed by Dunnett's test. $^{###}P < 0.001$ as compared with normal group and $***P < 0.001$ as compared with AIA control group.

The hepatic SOD and GSH level in the AIA control rats was significantly decreased ($P < 0.001$) as compared to normal rats. The SOD and GSH level in the hepatic tissue of leflunomide (10 mg/kg), curcumin (80 mg/kg) alone and curcumin (40 mg/kg) in combination with quercetin (20 mg/kg) treated rats was significantly increased ($P < 0.001$) as compared to AIA control rats. The 28 days treatment of quercetin (40 mg/kg) significantly attenuated ($P < 0.001$) this FCA-induced decreased level of SOD and GSH as compared to AIA control rats.

2.Effect of curcumin, quercetin and their combination on FCA-induced alteration in hepatic MDA and NO level:

Parameter	Hepatic MDA (nM/mg of protein), nitric oxide (µg/ml) - Mean ± SEM					
	Normal	AIA Control	Leflu-nomide (10 mg/kg)	Curcumin (80 mg/kg)	Quercetin (40 mg/kg)	Curcumin (80 mg/kg) + Quercetin (40 mg/kg)
MDA (nM/mg of protein)	1.15 ± 0.10	2.83 ± 0.06$^{###}$	1.48 ± 0.10***	2.02 ± 0.11**	1.72 ± 0.11***	1.55 ± 0.09***
Nitric oxide (µg/ml)	8.02 ± 0.41	18.09 ± 0.46$^{###}$	10.30 ± 0.44***	13.12 ± 0.52***	11.23 ± 0.33***	8.80 ± 0.25***



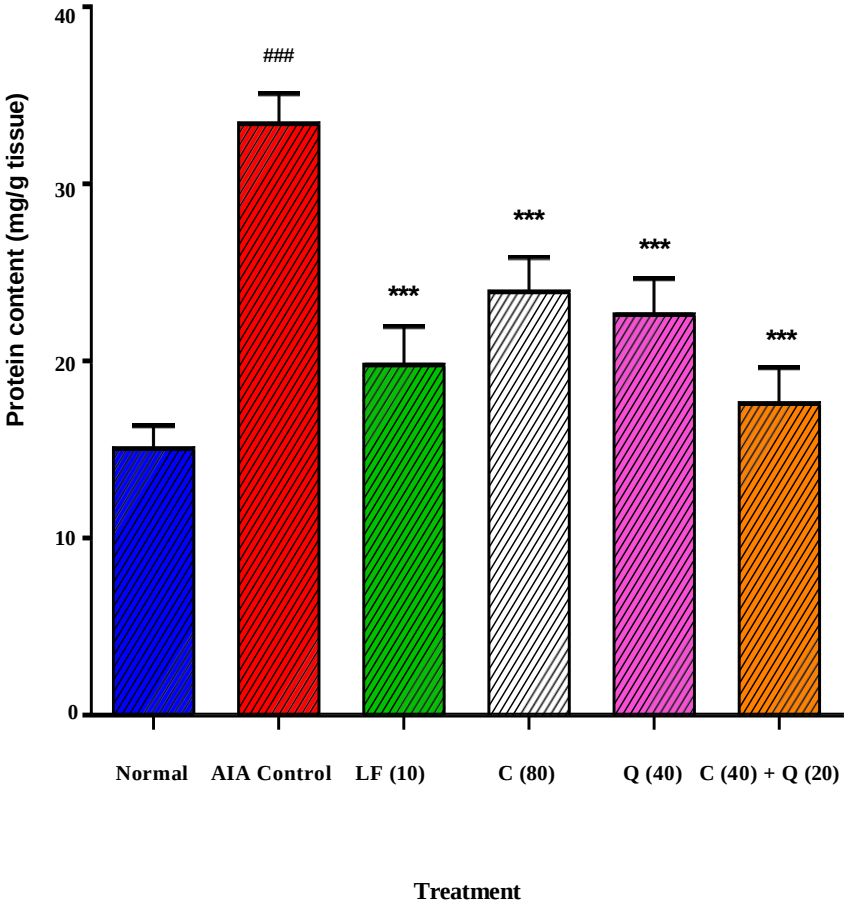
2. Effect of curcumin, quercetin and their combination on FCA-induced alteration in hepatic MDA and NO level

Data were analyzed by One-way ANOVA followed by Dunnett's test. $^{###}P < 0.001$ as compared with normal group and $^{**}P < 0.01$, $^{***}P < 0.001$ as compared with AIA control group.

There was significant increase in hepatic MDA and NO levels in AIA control rats as compared to normal rats. When compared to AIA control rats, the MDA and NO level in hepatic tissue of leflunomide (10 mg/kg), curcumin (80 mg/kg) alone and curcumin (40 mg/kg) in combination with quercetin (20 mg/kg) was significantly deceased ($P < 0.001$). The quercetin (40 mg/kg) alone treatment also produce significant decrease ($P < 0.001$) in MDA and NO level compared to AIA control rats. The curcumin (40 mg/kg) alone treatment also produce significant decrease ($P < 0.01$ and $P < 0.001$) in MDA and NO level compared to AIA control rats.

3.Effect of curcumin, quercetin and their combination on FCA-induced alteration in hepatic total protein level:

Hepatic total protein (mg/gm) - Mean ± SEM					
Normal	**AIA Control**	**Leflunomide (10 mg/kg)**	**Curcumin (80 mg/kg)**	**Quercetin (40 mg/kg)**	**Curcumin (80 mg/kg) + Quercetin (40 mg/kg)**
15.19 ± 0.46	33.53 ± 0.63$^{###}$	19.92 ± 0.83***	24.04 ± 0.74***	22.77 ± 0.77***	17.75 ± 0.76***



3.Effect of curcumin, quercetin and their combination on FCA-induced alteration in hepatic total protein level

Data were analyzed by One-way ANOVA followed by Dunnett's test. $^{###}P < 0.001$ as compared with normal group and $***P < 0.001$ as compared with AIA control group.

There was a significant increase ($P < 0.001$) in hepatic total protein level in AIA control group when compared to normal group. Administration of leflunomide (10 mg/kg) for 28 days significantly ($P < 0.001$) decrease total protein level in hepatic tissue compared to AIA control rats. Treatment with

curcumin (40 mg/kg) in combination with quercetin (20 mg/kg) also significantly ($P < 0.001$) decreased the hepatic total protein level compared to AIA control rats. Rats treated with curcumin (80 mg/kg) and quercetin (40 mg/kg) alone treated significantly decreased the hepatic total protein level ($P < 0.001$) as compared to AIA control rats.

REFERENCES :-

1. Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. Lancet. 2016 Oct 22;388(10055):2023-2038.
2. Ajay Sunil Sabale et.al , Anjali Sunil Sabale, Journal of Drug Delivery and Therapeutics,2024,To Carry Out Anti-Arthritic Activity of Combination of Quercetin and Curcumin Using FCA (Freund's Complete Adjuvant) Induced Arthritis in Laboratory Rats.
3. haleagrahara et al. 2009; mahmoud et al. 2013,Quercetin mitigates rheumatoid arthritis by inhibiting adenosine ndeaminase in rats.
4. Shen P, Lin W, Deng X, et al. Potential Implications of Quercetin in Autoimmune Diseases. Front Immunol. 2021;12:689044. doi:10.3389/fimmu.2021.
5. Lois Zoppi, b.a. Lois et.al 2022, news medical life sciences, Turmeric for Osteoarthritis.
6. Guardia T, Rotelli AE, Juarez AO, Pelzer LE. Anti-inflammatory properties of plant flavonoids. Effects of rutin, quercetin and hesperidin on adjuvant arthritis in rat. *Farmaco*. 2001;56(9):683–687.
7. Rotelli AE, Guardia T, Juarez AO, de la Rocha NE, Pelzer LE. Comparative study of flavonoids in experimental models of inflammation. *Pharmacol Res*. 2003;48(6):601–606.
8. Mengshi tang, yan zeng,weijun peng et.al 2022, taylor & francis,Pharmacological Aspects of Natural Quercetin in Rheumatoid Arthritis.
9. Nair R, Kalariya T, Chandra S Antibacterial activity of some selected Indian medicinal Flora Turk j Biol 2005 29;41-47
10. Narendhirakannan R.T., Subramanian S., Kandaswamy M., Anti-inflammatory and lysosomal stability actions of Cleome gynandra L. studied in adjuvant induced arthritic rats, Food and Chemical Toxicology 45 (2007) 1001–1012
11. Newbould BB. Chemotherapy of Arthritis Induced in Rats by Mycobacterial Adjuvant. British journal of pharmacology and chemotherapy 1963;21:127-36.
12. Obi FO, Ovat OD, Oriafo OSJ. Time-dependent prevention of carbontetra chloride-induced acute liver damage in the rat by Hibiscus rosa-sinensis petal anthocynidin extract administered in aqueous fifty percent ethanol. Biokemistri 2001;11:95–104.
13. Panayi GS. T-cell-dependent pathways in rheumatoid arthritis. Current opinion in rheumatology 1997;9(3):236-40.
14. Crunkhon, P., Meacock, S.C.R., 1971. Mediators of the inflammation induced in the rat paw by carrageenan. British Journal of Pharmacology 42, 392–402.
15. D'Amour F, Smith D (1941) A method for determining loss of pain sensation. J Pharmacol Exp

Ther 72~74-79.

16. Adhirajan N, Ravi kumar T, Shanmugasundram N, Babu M. in vivo and in vitro evaluation of hair growth potential of Hibiscus rosa-sinensis Linn. J Ethanopharmacol 2003;88:235–9.
17. Akarasereenont, P., Mitchell, J.A., Bakhle, C., Thiemermann, C., Vane,J.R.. Comparison of the induction of cyclooxygenase and nitric oxide synthase by endotoxin in endothelial cells and macrophages. European Journal of Pharmacology 1995;273, 121–128.
18. Amador-Patarroyo, M.J., Rodriguez-Rodriguez, A., Montoya-Ortiz, G., 2012. How does age at onset influence the outcome of autoimmune diseases? Autoimmune Dis 2012, 251730.
19. American Pharmaceutical Association 46, 515–519.
20. Aydin, S., Demir, T., Ozturk, Y., Baser, K.H.C., 1999. Analgesic activity of Nepeta italica L. Phytotherapy Research 13, 20–23
21. Badgujar LB, Ghosh P, Gaur V, Bodhankar SL, Effect of petroleum ether extract of Sphaeranthus indicus Linn. Of complete Freunds Adjuvant induced arthritis in laboratory rats. Pharmacologyonline 2009;2: 281-291
22. Basu K. Bharatiya Ousadhabalir Sankhipta Bhaisajya Tattwa. Calcutta: Hahnemann Publishing Company, 1946.
23. Bendele A: Animal models of rheumatoid arthritis. J Musculoskelet Neuronal Interact 2001, 1:377-3.

I