ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 17, Issue 12, 2024

Online - 2455-3891
Print - 0974-2441

Research Article

EXTRACELLULAR MODULATORY AND ANTI-INFLAMMATORY EFFECTS OF A COMBIATION OF CURCUMIN, PIPERINE, AND VIRGIN COCONUT OIL IN ANIMAL MODEL

ADITYA ASISH[1], SONY RAJAN[2], SVENIYA P JOSE[2], SHEETHAL S[2], RATHEESH M[2], SANDYA S*[1]

[1]Department of Inorganic and Physical Chemistry, Indian Institute of Science, Bengaluru, Karnataka, India.
[2]Department of Biochemistry, St. Thomas College, Palai, Kerala, India.
*Corresponding author: Sandya S; Email: sandya@iisc.ac.in

Received: 19 September 2024, Revised and Accepted: 28 October 2024

ABSTRACT

Objective: The current study investigated the potential of a novel herbal combination of curcumin, piperine, and virgin coconut oil to exhibit anti-inflammatory and anti-rheumatic properties against carrageenan-induced inflammation.

Method: A minimal dose of the drug formulation having a maximal anti-inflammatory effect was found (Dose III). Following the completion of the experiment, the animals were humanely euthanized, and paw tissue and serum samples were collected for further analysis. The expression levels of mRNA for Tumor Necrosis Factor-alpha (TNF-α), interleukin (IL)-6, PGE-2, and Thiobarbituric acid reactive substance (TBARS), as activities of cyclooxygenase -2, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and TBARS were measured and levels of matrix metalloproteinases (MMP)9 and MMP2 were measured using zymography and western-blotting.

Results: Anti-oxidant assays were performed in serum and synovial tissue, observed that the production of anti-oxidant enzymes such as SOD, GPx, CAT, and GSH was enhanced, and suppression of the accumulation of lipid peroxidation products in the group treated with the potent dose of the drug combination. A decrease in the mRNA expression of IL-6, IL-1β, and TNF α was noted in the treatment group, these are the upstream events of the inflammatory cascade. Radiological and histopathological analysis confirms that the drug formulation acted on MMPs to reduce its baneful consequence.

Conclusion: The drug formulation's anti-inflammatory and anti-rheumatic properties, combined with its multi-targeted mechanism, suggest its potential as a therapeutic agent for various inflammatory diseases.

Keywords: Inflammation, Cytokines, Matrix metalloproteinases, Curcumin, Piperine.

© 2024 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/) DOI: http://dx.doi.org/10.22159/ajpcr.2024v17i12.52641. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease that falls under the category of chronic inflammatory diseases. It mainly affects the diarthrodial joints causing cartilage and bone destruction, swelling of joints, autoantibodies production, hyperplasia of synovium, and infiltration of inflammatory cells. This can occur at any age but most commonly affects elderly people and among them, women are the main victims. The exact cause is unknown, researchers suggested that it may be due to genetic, hormonal, environmental, or a combination of all these factors. However, no diagnostic criteria are present for the complete prevention of this ailment. As this disease has injurious effects on organ systems such as cardiovascular, and pulmonary, the mortality rate also increases due to RA. After clinical assessment and laboratory tests, the treatment begins in the affected population, by using commercially available drugs, physical therapy, nutritional therapy, and surgery. The three main drug therapies for RA are non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and disease-modifying anti-rheumatic drugs (DMARDS-both synthetic and biological) [1]. They could relieve pain and inhibit inflammatory actions, but they have no effect on the improvement of disease and have many undesired side effects and some patients did not respond to treatment showing the heterogeneity of the disease.

Pathophysiology of RA involves the transmigration of immune cells and increased production of cyclooxygenase (COX) and matrix metalloproteinases (MMPs) in synovial fluid. The infiltrated immune cells such as leukocytes, macrophages, and mast cells produce several cytokines that are involved in every phase of the disease and cause an imbalance in pro-inflammatory and anti-inflammatory cytokines. These cytokines through different signaling pathways activate synoviocytes and chondrocytes to release MMPs resulting in tissue degradation, enhancing their production and causing local and systematic inflammation and their interaction with T and B cells resulting in autoimmunity by their proliferation and differentiation. Tumor Necrosis Factor-alpha (TNF-α) and interleukin (IL)-6 are the major proinflammatory cytokines. These are pleiotropic and function synergistically in developing chronic inflammation through a cascade of reactions. Many studies showed that in the isoforms of IL-1, the crucial one is IL-1β as it is produced only upon inflammatory signals. It also causes tissue degradation along with TNF-α and acts as secondary mediators in arthritis [2]. The main activated signaling pathway in inflammatory conditions is NF κB, which triggers the production of the above proinflammatory cytokines [3].

MMPs are a group of enzymes that degrade the extracellular matrix in a regulated way. Gelatinase (MMP2 and MMP9) is the main subgroup contributing to tissue degradation in RA. Their production is regulated at the cellular level and molecular level and NF κB is the main regulatory pathway. The overproduction of TNF-α and IL-6 cytokines leads to the excess production of MMPs. Therefore, regulation of cytokines can reverse the progression of RA. Even though many cytokine inhibitors were developed as drugs, the use of any one of the inhibitors would not be efficient in preventing RA.

Curcumin is the most prominent polyphenol extracted from Curcuma longa (commonly called turmeric), one of the spices from Asian countries and one of the most important ingredients in food. It is also

used in many cosmetic products and ayurvedic products for topical and internal treatment. Many studies have shown that curcumin has many biological and pharmacological effects such as anti-inflammatory, anti-oxidative, antitumor. [4]. It also possesses a beneficial influence on multiple chronic diseases. The anti-inflammatory and anti-oxidant property of curcumin is mainly used against RA. Curcumin can inhibit the production of proinflammatory cytokines such as TNF α, IL-6, and COX and also reduce oxidative stress with the help of antioxidant enzymes [5]. However, there are two main concerns regarding curcumin, low bioavailability and low aqueous solubility. Many studies have been conducted to evaluate the efficacy of curcumin against RA.

Black pepper (Piper nigrum) has been used as a spice since ancient times all around the globe. The principal alkaloid present in pepper is piperine. It was also used as a traditional medicine. It also has anti-inflammatory properties, protection from oxidative damage, etc. [6]. Literature states that alkaloid, piperine can boost the bioavailability of drugs by preventing glucuronidation in the liver and small intestine [7,8]. Curcumin in combination with piperine can increase the bioavailability of curcumin by increased absorption and reduced metabolism [9].

Curcumin is a lipophilic agent, which is very less soluble in water [10]. Due to its hydrophobic nature curcumin is easily eliminated from the body, so its lipid affinity can be used to enhance its persistence in the human body. Fresh coconut kernels are used to extract virgin coconut oil (VCO) and have wide nutraceutical and biopharmaceutical effects including antioxidant, anti-diabetic [11], anti-inflammatory [12], and hypolipidemic effects [13]. VCO in combination with turmeric was used as a food item and as a medicine in Ayurveda, was called golden milk. This combination was used for the treatment of digestive problems and skin-related diseases. Studies show that the bioavailability of curcumin can be boosted by the combination of VCO [14]. Hence, curcumin can be incorporated with pepper and VCO easily thus enhancing the therapeutic properties.

To identify a permanent cure for this disease, researchers were studying phytochemicals that may possess anti-inflammatory and anti-rheumatic properties. Natural medicines have a high demand over synthetic drugs. Many natural products have been shown to possess pharmaceutical properties. In this scenario, we intended to study the effect of a combination of curcumin, piperine, and VCO against carrageenan (Carr)-induced inflammation to identify the potent dose and investigate its anti-arthritic effect.

METHODS

Chemicals and solvents

Carr and Freund's complete adjuvant was sourced from Sigma Chemical (St. Louis, MO, USA). Unless otherwise specified, all chemicals and biochemicals used were of the highest grade available.

Preparation of different doses of formulation

Curcumin and powdered pepper were weighed according to Table 1. The combination is mixed with 1.5mL VCO. The mixture was then mixed thoroughly and centrifuged at 1500 RPM for 10 min in a cold centrifuge. 1.5 mL/Kg. Four different doses were given to the experimental animals. The mixture is stored at room temperature.

Animals

Male Wistar rats of age 4–5 weeks old, weighing 90–120g, were used for the *in vivo* study at Central for Animal Facility (CAF), IISC Bangalore. The animals were kept in isolator cages by maintaining a room temperature of 22±23°C with 50–70% relative humidity and a photoperiod of 12:12 h of light and dark cycle. The animals were provided with a standard pellet diet and had unrestricted access to fresh water. They received human treatment and care, by the ethical guidelines set by the institution's animal welfare committee. Experiment was conducted as per the guidelines of the Animal Ethics Committee CPCSEA (Registration No: CAF/Ethics/695/2019) according to the Institutional Animal Ethical Committee, CAF, Indian Institute of Science, Bangalore, India.

Experimental design

Carr induction and experimental design for acute inflammation

Anti-inflammatory activity was evaluated by the method of Carr induced edema in the sub-plantar region of the right hind paw of the rats [15]. Edema was induced by an injection of 0.1 mL of 1% freshly prepared suspension of Carr in saline. Male Wistar rats were grouped into five groups, with six rats in each, and were given a dose of the test sample (Carr Control, Carr+ Dose I, Carr+ Dose II, Carr+ Dose III, Carr+ Dose IV, respectively). One hour later, 0.1 mL suspension of 1% Carr (an edematogenic agent) in a 0.9% NaCl solution was injected into the sub-plantar tissue of the right hind paw. The paw volume was measured by a plethysmometer. The measures were taken at baseline (0 h, Vo) before the administration of the edematogenic agent, and then at 1,2,3,4, and 5 h post-injection (Vt). Edema was assessed by calculating the difference between Vt and Vo. Inhibition percentage was then determined using the following formula:

$$\%\ \text{inhibition} = \frac{(\text{Vt} - \text{Vo})\text{control} - (\text{Vt} - \text{Vo})\text{treated}}{(\text{Vt} - \text{Vo})\text{control}} \text{x}100$$

Adjuvant induction and experimental design for chronic inflammation

Experimental arthritis was induced at day 0 using 0.1mL of Complete Freund's Adjuvant, Complete Freund's Adjuvant (CFA) (Sigma Aldrich) containing heat-killed *Mycobacterium tuberculosis* by injecting to the right hind paw of animals of each group except the normal group. The animals were grouped into four categories, with six animals per group:
Group I – Normal Control (NC)
Group II – Arthritic Control (AA)
Group III – Standard Drug Indomethacin (dose 3 mg/kg/day)
Group IV – Drug combination (DC) (30 mg curcumin, 40 mg pepper, 1.5 mL VCO)

Body weight and paw edema were measured before CFA induction. After 1 week, the oral treatment started and continued up to day 21. The intensity of paw inflammation was evaluated by measuring the width of the paw joint using a plethysmometer on days 7, 14, 18, and 21. Body weight was also measured on the same days as the other parameters. At the end of the 21st day, rats were sacrificed and paw tissue and blood were collected for various biochemical analyses.

Preparation of tissue homogenate and blood sampling

After washing with 0.9% normal saline, the paw tissue was homogenized in phosphate-buffered saline (PBS) in 1:1 weight/volume at 4°C, followed by centrifugation for 10 min at 15,000× g in 4°C. Serum was extracted from collected blood samples through centrifugation. This serum and plasma were used for analyzing blood parameters such as hemoglobin, total white blood cell count, red blood cell, platelet count, and erythrocyte sedimentation rate.

Analysis of antioxidant activities

The catalase (CAT) enzyme was assayed by Aebi's method in 1984 [16]. The antioxidant activity of superoxide dismutase (SOD) was measured by following the method of Kakkar *et al*., 1984, and one unit of enzyme activity was defined as that amount of enzyme causing 50 % inhibition of NBT reduction per mg protein [17]. The evaluation of glutathione peroxidase (GPx) activity was done by the method of Paglia and Valentine, 1967. The activity of GPx was expressed in units of nanomoles of NADPH oxidized to NADP+ per min per mg of protein, using a molar extinction coefficient of 6.22×106 ($cm^{-1}M^{-1}$) for NADPH [18].

The assessment of Thiobarbituric acid reactive substance (TBARS) was done spectrophotometrically using the dual-heat treatment assay [19]. Reduced glutathione (GSH) is assayed using Kumar *et al*., 2020 method, levels were calculated using a molar extinction coefficient of the chromophore, that is, 1.36×104 M^{-1} cm^{-1} and results were expressed as μM per mg protein [20].

Assay of protein

Protein concentration was determined using the assay protocol established by Lowry *et al*. (1951) [21].

Radiological analysis (X-ray) and histopathological analysis

Hind limbs were removed and an X-ray was done at a local diagnostic center. The entire paw tissues were dissected and sectioned, then fixed in 10% formalin solution at room temperature. Ultra-thin sections (5 μm) were obtained after fixing the tissues in paraffin and staining using hematoxylin-eosin (H&E). Morphological changes within the tissue were examined and photographed under an optical microscope of 100× magnification (Olympus-Magnus trinocular microscope, Noida, India). Two independent observers, blind to the experimental protocol, examined the severity of inflammation.

Enzyme-linked immunosorbent assay (ELISA)

The efflux of proinflammatory cytokines such as IL-6, IL-1beta, and TNF-alpha and the inflammatory mediator PTGS2 in serum and tissue homogenate were determined using Sandwich ELISA kits (Elabscience, USA). The concentration of these cytokines was expressed in pg/mL.

Zymography and Western blotting

Gelatin zymography was performed according to the Itoh *et al*. 1997 method [22]. Simply put, tissue homogenate and serum were run on a non-reducing SDS-PAGE using a 7.5% gel containing 0.1% gelatin. After electrophoresis for 2 h, the gel was washed with 2.5% Triton X-100 by gentle shaking at room temperature and incubated overnight in 50 mM Tris-HCl (pH 7.5) containing 10 mM CaCl2 at 37°C. Following that, the gels were subjected to Coomassie brilliant blue staining.

Western blots were carried out according to a published method (Wang *et al*., 2005). In short, the protein concentration in tissue supernatant and serum was determined by Lowry's method. The samples were subjected to electrophoresis on a 10% SDS-PAGE gel. Following electrophoresis, the proteins were promptly transferred to a nitrocellulose membrane, which was then blocked overnight at 4°C. The membrane after washing with 0.05% Tween 20-PBS solution was probed with MMP-2 and MMP-9 antibodies (1:1000). For detection, horseradish peroxidase-conjugated secondary antibodies were employed (1:2000), followed by visualization using enhanced chemiluminescence substrate.

Reverse transcription-polymerase chain reaction (RT-PCR)

Gene activity levels of TNF-α, IL-6, IL-1β, and PTGS-2 mRNA were measured by semi-quantitative RT-PCR. Total RNA was isolated from frozen paw tissue using an RNA isolation mini kit (Thermo Fischer, USA) according to the manufacturer's instructions. Glyceraldehyde 3–phosphate dehydrogenase was kept as an internal control for RNA loading. The isolated RNA was quantified and an equal volume was reverse transcribed separately in two steps with the help of instructions given in the RT-PCR kit using primers shown in Table 1. PCR products were subjected to separation by electrophoresis on 1% agarose gel containing ethidium bromide, visualized under a UV–trans-illuminator.

Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences software (version 11.0 for Windows), with values presented as the mean and standard deviation±standard error of the mean. One-way ANOVA was employed for statistical evaluation, followed by Duncan's test to determine significant differences between groups at the significance level of p≤0.05.

RESULTS

Percentage of paw edema inhibition on the acute model

As shown in Table 2, the **Dose III** showed 87.5% of the paw edema inhibition. From this result, Dose III showed a potent inhibition of Carr-induced paw edema.

Effect of hematological parameters

Haematological parameters such as Hb, WBC, RBC, ESR and platelet count has been analysed and the results are shown in Table 3. Our results indicated that the parameters were towards the normal range in treated group compared to normal control group. Concentration of plasma C-reactive protein (CRP) was analysed (Fig. 1). Results shown that CRP concentration has significantly decreased in treated group compared to arthritic group (AA).

Anti-oxidant assay – activity of SOD

SOD was assayed for the identification of superoxide anion in an experimental model. A significant decrease in SOD was seen in an arthritic group compared to normal. There seem to be sufficient anti-oxidant properties in treated groups with standard drugs and combinations (Fig. 2).

Estimation of lipid peroxidation level

After the experiment (day 21), the NC group exhibited low levels of TBARS, which were deemed normal. Conversely, in the AA group, a significant elevation in TBARS production was observed in paw tissue. The TBARS of treated groups showed the same pattern as that of the NC groups indicating the inhibition of lipid peroxidase activity (Fig. 3).

Estimation of GSH

The concentration of GSH was evaluated to estimate endogenous defense against the generation of free radicals. There was a marked decrease in GSH content in the paw tissue of AA models compared to the NC. Arthritic rats treated with a DC showed a significant increase, like that of NC groups (Fig. 4).

Estimation of GPx

Accumulation of H_2O_2 can reduce the production of GPx enzyme. GPx is a scavenger of lipid peroxide. Their concentration is so diminished in the AA group, whereas their protective role is there in groups treated with standard drug and DC (Fig. 5).

CAT assay

CAT activity in paw tissue was significantly decreased in the arthritic model, and the condition is reversed by the administration of a DC and

Table 1: Primers used for reverse transcription-polymerase chain reaction

GENE	SENSE (5'-3')	ANTI-SENSE (5'-3')
TNF-α	ACTCCCAGAAAAGCAAGCAA	TGGAAGACTCCTCCCAGGTA
IL-6	CTTCCAGGCCAGTTGCCTT	AGTTTGGTGTCGCGGCGGAGCAC
IL-1β	GTGGCAGCTACCTATGTCTT	GAGAGGTGCTGATGTACCAG
PTGS-2	GGAGGTGGTGATAGCCGGTAT	TGGGTAATCCATAGAGCCCAG

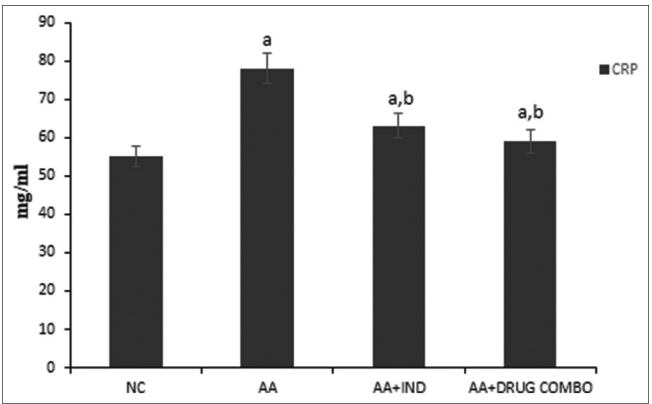

Fig. 1: Concentration of plasma C-reactive protein level. Values expressed as the average of six rats per group±standard deviation. Statistical analysis revealed significant differences: a – p <0.05 compared to the control group. b – p<0.05 compared to AA rats

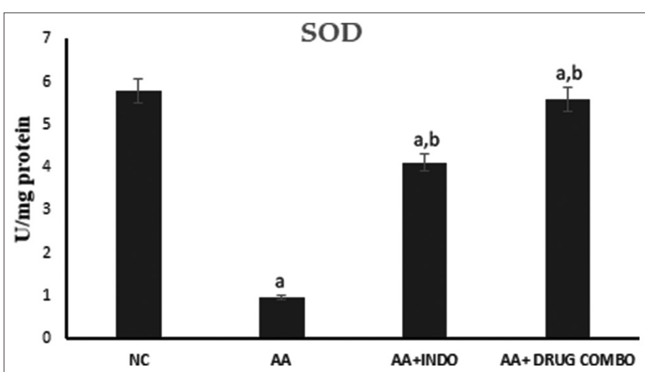

Fig. 2: Values expressed as the average of six rats per group±standard deviation. Statistical analysis revealed significant differences: a – p <0.05 compared to the control group. b – p<0.05 compared to AA rats. Superoxide dismutase Unit(U): enzyme concentration that inhibits 50% of chromogen production in 1 min

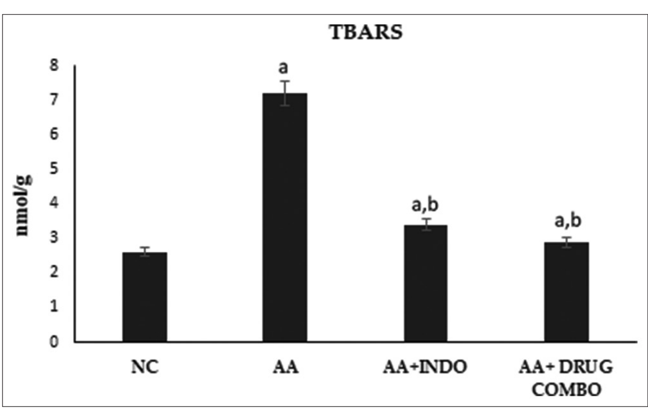

Fig. 3: The values are expressed as mean±standard deviation of with six rats in each group. a – Statistical difference found with NC group at p<0.05. b – Statistical difference with AA-induced rats at p<0.05

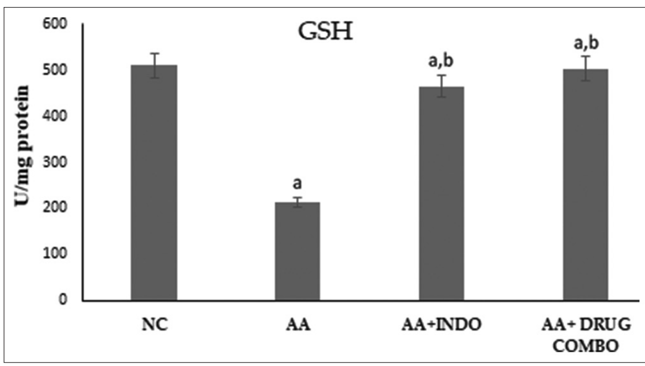

Fig. 4: Concentration of GSH level. Values expressed as the average of six rats per group±standard deviation. Statistical analysis revealed significant differences: a – p<0.05 compared to the control group. b – p<0.05 compared to AA rats

a standard drug (Fig. 6).

Analysis of radiological image

Radiographic assessments (X-RAYS) were conducted at the conclusion of the treatment period to evaluate the extent of recovery from the arthritic damage in the ankle joint of AA-induced arthritic rats treated with our novel DC and the standard drug indomethacin.

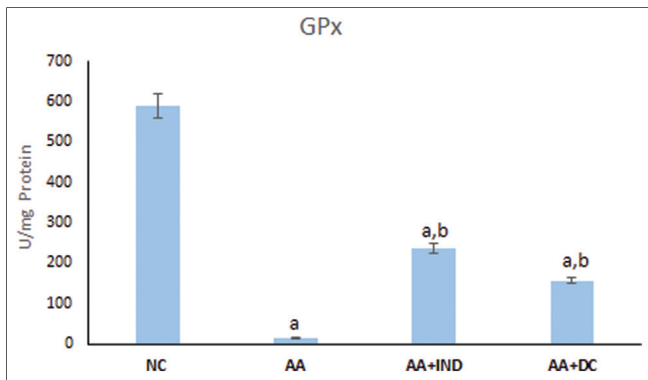

Fig. 5: Concentration of GPx level. Values expressed as the average of six rats per group±standard deviation. Statistical analysis revealed significant differences: a – p<0.05 compared to the control group. b – p<0.05 compared to AA rats

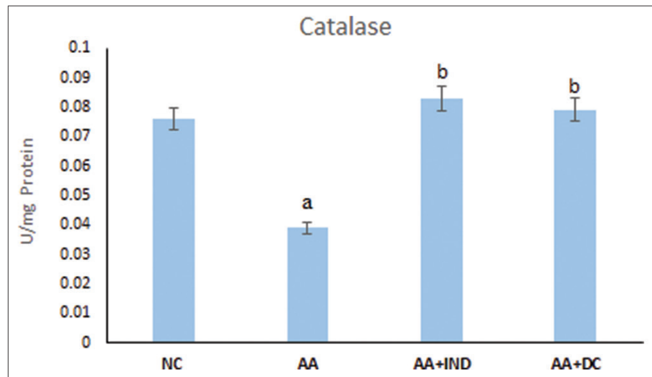

Fig. 6: Catalase activity. Values expressed as the average of six rats per group± standard deviation. Statistical analysis revealed significant differences: a – p<0.05 compared to the control group. b – p<0.05 compared to AA rats

Table 2: Paw edema Inhibition on acute model

Groups	Paw Measurement		
	0th h	3rd h	Percentage of edema inhibition
Control	3	3.8	
Dose I	2.8	3.2	50
Dose II	2.8	3.6	0
Dose III	2.8	2.9	87.5
Dose IV	2.7	2.8	87.5

AA-induced arthritic rats exhibited severe tissue swelling and pronounced bone destruction, whereas treated groups showed less swelling in joints and bone destruction. This clearly shows that treated groups have the prevention capacity for inflammation and bone damage (Fig. 7).

Analysis of histopathological images

Adjuvant-induced arthritic rats exhibited pronounced cellular infiltration and severe edema, whereas the indomethacin-treated group displayed a moderate degree of cellular infiltration and edema formation. The DC-given group also showed suppressed inflammatory cell infiltration, proliferated collagen, and edema (Fig. 8).

Effect of DC on inflammatory mediators

In both tissue and serum samples, the levels of inflammatory markers were high in the arthritic model but after the treatment, the levels are decreasing (Figs. 9 and 10).

Table 3: Inhibitory effect of drug combination on the concentration of various blood parameters

Description	Hemoglobin (%)	Total white blood cell count	Red blood cell	Erythrocyte sedimentation rate	Platelet count
NC	14.6 g	10890 cells/cu.mm	7.74 millions/cu.mm	20 mm/h	9.77 lakhs/cu.mm
AA	14.8 g	11410 cells/cu.mm	7.77 millions/cu.mm	22 mm/h	5.85 lakhs/cu.mm
AA+INDO	13.2 g	8560 cells/cu.mm	6.39 millions/cu.mm	17 mm/h	5.79 lakhs/cu.mm
AA+DC	14.1 g	10310 cells/cu.mm	5.20 millions/cu.mm	14 mm/h	4.56 lakhs/cu.mm

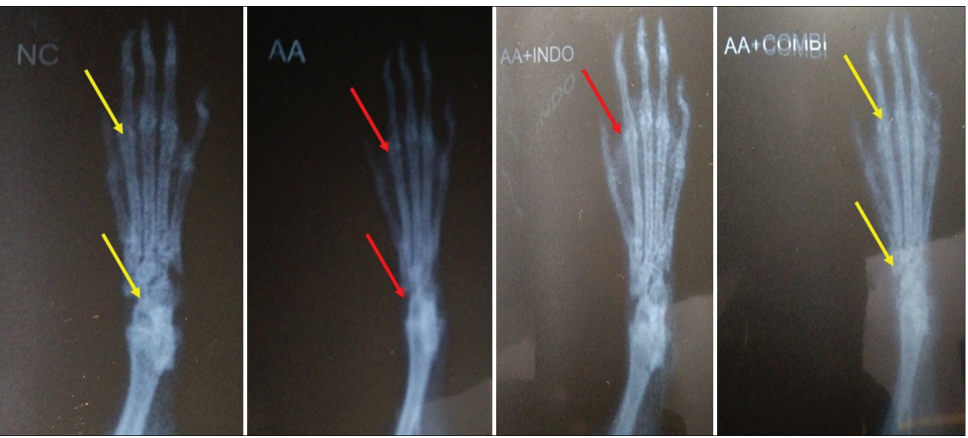

Fig. 7: Radiological imaging of Rat ankle joint

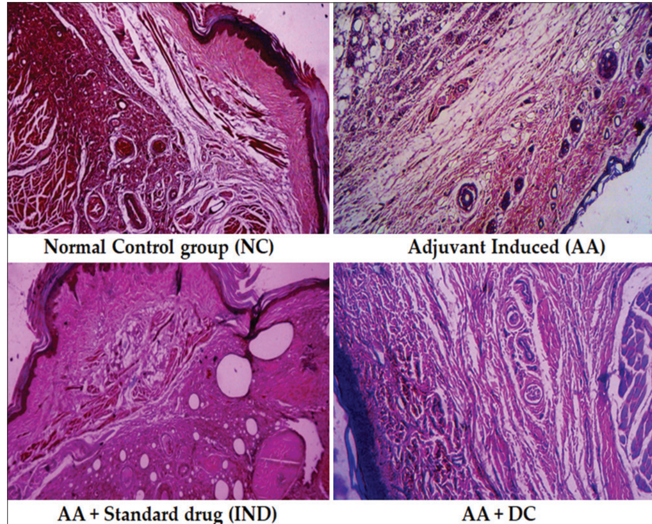

Fig. 8: Histopathology of paw tissue

Effect of DC on MMP-2 and MMP-9 level (by gelatin zymography and western blotting

The expression of the MMP-2 and MMP-9 genes was significantly increased in AA but the condition reversed on treatment (Fig. 11).

Effect of DC on PTGS-2, IL-6, IL-1beta and TNF-alpha

The gene level expression of PTGS-2, IL-6, IL-1beta, and TNF-alpha was upregulated in the AA group, and on the treatment they were downregulated (Fig. 12).

DISCUSSION

RA is a chronic autoimmune disease causing irreversible joint damage which ultimately leads to joint disability. Many synthetic drugs are available to reduce the symptoms, but till now no medicine claims the complete cure for this RA. The most commonly used drugs are NSAIDs, DMARDs, and corticosteroids, all can reduce pain and inflammation. However, most of them cause severe side effects. Hence, many researchers diverted to find a pharmacological product from nature. Many constituents extracted from medicinal plants possess

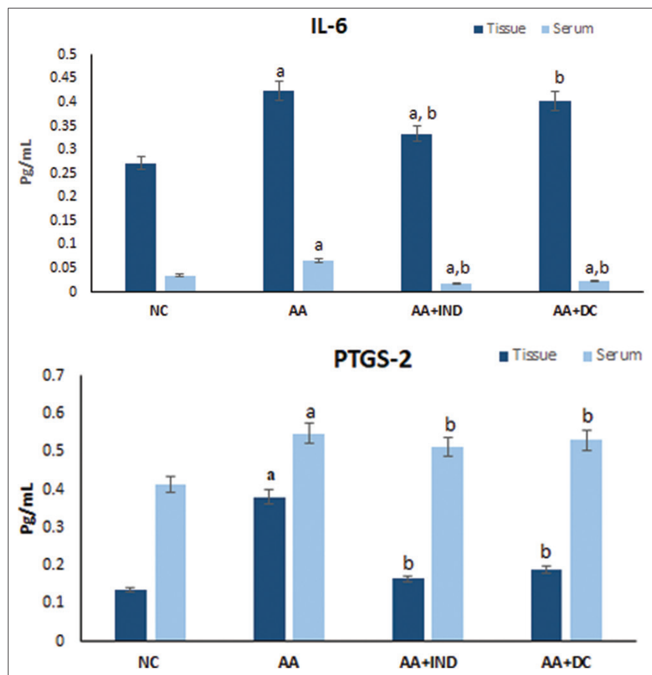

Fig. 9: Effect of drug combination on inflammatory markers IL-6 and PTGS-2 in tissue homogenate and serum. Values expressed as the average of six rats per group±standard deviation. Statistical analysis revealed significant differences: a – p<0.05 compared to the control group. b – p<0.05 compared to AA rats

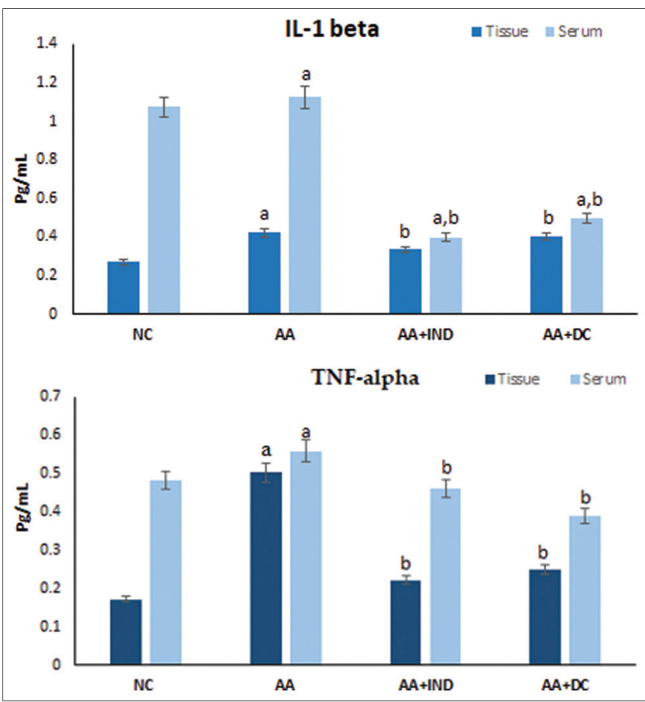

Fig. 10: Effect of drug combination on inflammatory markers interleukin-1beta and Tumor Necrosis Factor-alpha in tissue homogenate and serum. Values expressed as the average of six rats per group±standard deviation. Statistical analysis revealed significant differences: a – p<0.05 compared to the control group. b – p<0.05 compared to AA rats

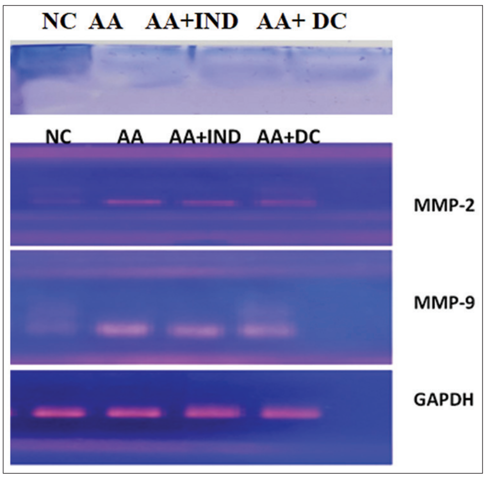

Fig. 11: Inhibitory effect of drug combination on level of MMP-2 and MMP-9. A: By gelatin zymography B: Western blotting. Glyceraldehyde 3-phosphate dehydrogenase was used as the control. MMP: Matrix metalloproteinases

pharmacological effects. For the treatment of RA, effects such as anti-inflammatory and anti-oxidant are important, by considering these researchers are studying some of the natural products possessing these characteristics. The *in vivo* model helps to study most of the human disease as it shows most of the clinical features of the disease.

In the present study, the phytochemicals called curcumin and piperine are mixed using VCO as the drug formulation, as each independently possesses the required properties against RA. In the acute inflammatory study, Carr was induced, and the minimal dose of the drug formulation had a maximal anti-inflammatory effect. In the chronic inflammation model, arthritis was induced with CFA, which causes pain, inflammation,

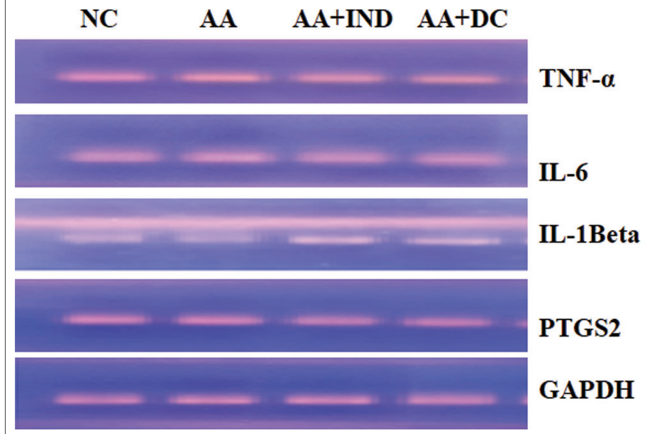

Fig. 12: Inhibitory effect of drug combination on gene expression of PTGS-2, IL-6, IL-1beta, and TNF-alpha by RT-PCR. Glyceraldehyde 3-phosphate dehydrogenase was used as the control. IL: interleukin, TNF: Tumor Necrosis Factor-alpha, RT-PCR: Reverse transcription-polymerase chain reaction

bone destruction, etc. The anti-inflammatory effect of the potent dose was compared with the standard drug, indomethacin. After the study period, paw tissues and serum were obtained and anti-oxidant assays were performed and found re-establishment of anti-oxidant enzymes such as CAT, GPx, SOD, and GSH, and can suppress the accumulation of lipid peroxidation products. The anti-inflammatory effect of the DC was further confirmed by radiographical imaging and histopathological analysis. A significant decrease in proinflammatory cytokines such as IL-6, IL-1β, and TNF α was noted in the group treated with drug formulation, indicating a potential anti-inflammatory effect. The disastrous effects of MMPs were overthrown by drug formulation. Our results suggested that the combinational drug formulation possesses anti-inflammatory and anti-oxidant properties and hence can be considered as a novel remedy for RA.

CONCLUSION

Two main crises of RA are inflammatory reactions and tissue degradation and both arise due to the imbalance in cytokines and metalloproteinases, respectively. The present study paved the way for the rising demand for a natural therapy for RA. Our study revealed that the combination of curcumin, piperine, and VCO, enhances the production of anti-oxidant enzymes such as SOD, GPx, CAT, and GSH. The regaining of body weight and the lowering of inflammation in treated groups reveals the possibility of RA disease being prevented by the phytochemical from nature itself. Radiographic and histopathological analyses were evident for the inhibitory activity of drug formulation against tissue damage. Although further research is required to fully establish the efficacy of this specific combination as an anti-arthritic agent, the current findings suggest promising potential. Hence, this drug formulation can be considered a novel therapeutic agent against RA.

FUNDING SOURCE

The funding for this project was provided by DST/SERB-CORE Research Grant 2018 (Sanction No. CRG/2018/002619).

CONFLICTS OF INTEREST

Authors declare no conflicts of interest.

AUTHOR'S CONTRIBUTIONS

Experimental conception and design, guidance, supervision, and review work for the research were done by Sandya Sukumaran

Analysis and interpretation of the data done by Sandya Sukumaran and Ratheesh Mohanan.

Statistical expertise: Svenia P. Jose and Sheethal S.

Collection and assembly of data, drafting of the article done by Aditya Asish

Experimental work, development, and optimization of the formulations, interpretation of result, and writing of this manuscript were done by Aditya Asish and Sony Rajan

All authors read and approve the final manuscript.

REFERENCES

1. Kuncha M, Naidu VG, Sahu BD, Gadepalli SG, Sistla R. Curcumin potentiates the anti-arthritic effect of prednisolone in Freund's complete adjuvant-induced arthritic rats. J Pharm Pharmacol. 2014 Jan;66(1):133-44. doi: 10.1111/jphp.12156, PMID 24138213
2. Lubberts E, van den Berg WB. Cytokines in the Pathogenesis of Rheumatoid Arthritis and Collagen-Induced Arthritis. Madame Curie Bioscience Database. TX: Austin. Landes Bioscience; 2000-2013.
3. Li J, Zhang H, Han Y, Hu Y, Geng Z, Su J. Targeted and responsive biomaterials in osteoarthritis. Theranostics. 2023 Jan 16;13(3):931-54. doi: 10.7150/thno.78639, PMID 36793867
4. Makuch S, Więcek K, Woźniak M. The immunomodulatory and anti-inflammatory effect of curcumin on immune cell populations, cytokines, and *in vivo* models of rheumatoid arthritis. Pharmaceuticals (Basel). 2021 Apr 1;14(4):309. doi: 10.3390/ph14040309, PMID 33915757
5. Guest PC. Studies on Biomarkers and New Targets in Aging Research in Iran: Focus on Turmeric and Curcumin. Berlin: Springer International Publishing; 2021.
6. Bang JS, Oh DH, Choi HM, Sur BJ, Lim SJ, Kim JY, *et al*. Anti-inflammatory and antiarthritic effects of piperine in human interleukin 1beta-stimulated fibroblast-like synoviocytes and in rat arthritis models. Arthritis Res Ther. 2009;11(2):R49. doi: 10.1186/ar2662, PMID 19327174
7. Atal CK, Dubey RK, Singh J. Biochemical basis of enhanced drug bioavailability by piperine: Evidence that piperine is a potent inhibitor of drug metabolism. J Pharmacol Exp Ther. 1985 Jan;232(1):258-62. PMID 3917507
8. Singh J, Dubey RK, Atal CK. Piperine-mediated inhibition of glucuronidation activity in isolated epithelial cells of the guinea-pig small intestine: evidence that piperine lowers the endogeneous UDP-glucuronic acid content. J Pharmacol Exp Ther. 1986 Feb;236(2):488-93. PMID 3080587
9. Shoba G, Joy D, Joseph T, Majeed M, Rajendran R, Srinivas PS. Influence of piperine on the pharmacokinetics of curcumin in animals and human volunteers. Planta Med. 1998 May;64(4):353-6. doi: 10.1055/s-2006-957450, PMID 9619120
10. Shehzad A, Rehman G, Lee YS. Curcumin in inflammatory diseases. BioFactors. 2013 Jan-Feb;39(1):69-77. doi: 10.1002/biof.1066, PMID 23281076
11. Narayanankutty A, Mukesh RK, Ayoob SK, Ramavarma SK, Suseela IM, Manalil JJ, *et al*. Virgin coconut oil maintains redox status and improves glycemic conditions in high fructose fed rats. J Food Sci Technol. 2016 Jan;53(1):895-901. doi: 10.1007/s13197-015-2040-8, PMID 26788013
12. Nair SS, Manalil JJ, Ramavarma SK, Suseela IM, Thekkepatt A, Raghavamenon AC. Virgin coconut oil supplementation ameliorates cyclophosphamide-induced systemic toxicity in mice. Hum Exp Toxicol. 2016 Feb;35(2):205-12. doi: 10.1177/0960327115578867, PMID 25805601
13. Narayanankutty A, Palliyil DM, Kuruvilla K, Raghavamenon AC. Virgin coconut oil reverses hepatic steatosis by restoring redox homeostasis and lipid metabolism in male Wistar rats. J Sci Food Agric. 2018 Mar;98(5):1757-64. doi: 10.1002/jsfa.8650, PMID 28862329
14. Narayanankutty A, Nair A, Illam SP, Upaganlawar A, RaghavamenonAC. Curcumin enriched VCO protects against 7,12-dimethyl benz[a] anthracene-induced skin papilloma in mice. Nutr Cancer. 2021;73(5):809-16. doi: 10.1080/01635581.2020.1778745, PMID 32530305
15. Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema in hind paw of the rat as an assay for antiiflammatory drugs. Proc Soc Exp Biol Med. 1962 Dec;111:544-7. doi: 10.3181/00379727-111-27849, PMID 14001233
16. Aebi H. Catalase *in vitro*. Methods Enzymol. 1984;105:121-6. doi: 10.1016/s0076-6879(84)05016-3, PMID 6727660
17. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys. 1984 Apr;21(2):130-2. PMID 6490072
18. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 1967 Jul;70(1):158-69. PMID 6066618
19. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol. 1990;186:421-31. doi: 10.1016/0076-6879(90)86135-i, PMID 2233309
20. Kumar A, Dhaliwal N, Dhaliwal J, Dharavath RN, Chopra K. Astaxanthin attenuates oxidative stress and inflammatory responses in complete Freund-adjuvant-induced arthritis in rats. Pharmacol Rep. 2020 Feb;72(1):104-14. doi: 10.1007/s43440-019-00022-z, PMID 32016833
21. Li J, Zhang H, Han Y, Hu Y, Geng Z, Su J. Targeted and responsive biomaterials in osteoarthritis. Theranostics. 2023 Jan 16;13(3):931-54. doi: 10.7150/thno.78639, PMID 36793867
22. Itoh K, Chiba T, Takahashi S, Ishii T, Igarashi K, Katoh Y, *et al*. An Nrf2/small Maf heterodimer mediates the induction of phase II detoxifying enzyme genes through antioxidant response elements. Biochem Biophys Res Commun. 1997 Jul 18;236(2):313-22. doi: 10.1006/bbrc.1997.6943, PMID 9240432