Screening assessment of trimethoxy flavonoid and -(-)-epigallocatechin-3-gallate against formalin-induced arthritis in Swiss albino rats and binding properties on NF-κB-MMP9 proteins

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Abstract

Background: The isolated trimethoxy flavonoid 4a,5,8,8a-tetrahydro-5-hydroxy-3,7,8-trimethoxy-2-(3,4-dimethoxyphenyl) chromen-4-one (TMF) from methanolic stem extract of T chrysantha (METC) and -(-)-epigallocatechin-3-gallate (EGCG) can be used to suppress acute inflammation and arthritis as an ethical medicine in Ayurveda. The nuclear factor kappa beta (NF-κB) signaling is involved in the expression of inflammatory mediators such as TNF-α and IL-1β. A successive investigation of NF-κB–MMP9 signaling during the production of inflammatory mediators needs to be developed. The docking studies of compounds TMF and EGCG were carried out using Autodock 4.0 and Discovery studio Biovia 2017 software to find out the interaction between ligand and the target proteins. The antiarthritic potential of TMF, EGCG, and indomethacin was evaluated against formalin-induced arthritis in Swiss albino rats. Arthritis was assessed by checking the mean increase in paw diameter for 6 days via digital vernier caliper. The blood cell counter and diagnostic kits measured the different blood parameters and Rheumatoid factor (RF, IU/mL). The interleukin-1β (IL-1β) and tumor necrosis factor (TNFα) in serum were determined by ELISA, and the pERK, MMP9, and NF-κB expressions in the inflamed tissue were determined by Western blotting, respectively. The mRNA expression for inflammatory marker enzymes such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) was determined by qRT-PCR.

Results: Based on grid score, interactions, and IC₅₀ values in molecular docking studies, the TMF and EGCG can be effectively combined with proteins NF-kB and MMP9. The TMF-HD and EGCG-HD better suppressed the acute inflammation and arthritis with marked low-density pERK, MMP9, NF-κB, iNOS, COX-2 levels. The endogenous antioxidant levels were increased in TMF and EGCG treated rats.

Conclusion: The TMF and EGCG effectively unraveled acute inflammation and arthritis by suppressing NF-κB mediated MMP9 and cytokines.

Keywords: Acute inflammation, Arthritis, TMF, EGCG, NF-κB, MMP9, COX-2

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Background

The Ayurvedic system of medicine was reviewed by traditional drug practitioners in different countries and observed a large population depends upon...
phytochemicals for the prevention of different diseases [1]. Researchers also reviewed the latest incumbent of phytochemicals in the treatment of acute and chronic inflammations and musculoskeletal disorders [2, 3].

Arthritis can be categorized into inflammatory immunoarthritis (IIA), inflammatory non-immuno arthritis (INIA), and non-inflammatory non-immuno arthritis (NNIA). The IIA is under the category of a group of inflammatory disorders affected by the body’s defense mechanism (immune system) which affects the physiology of different organs. There are different contributing factors during the growth and development of IIA such as heredity, history of joint trauma, obesity, weight gain, endocrine disorders, cancerous growth, crystal deposition, and blood coagulation in the affected area [4]. The body’s immune system starts attacking its tissue instead of virus or bacteria. The three most common forms of IIA are rheumatoid arthritis (RA), ankylosing spondylitis (AS), psoriatic arthritis (PsA) [5]. Osteoarthritis is under the category of NNIIA only involved in the destruction of bone and joint cartilage. The cytokines, proteinases, oxygen derivatives, and interleukins (ILs) are inflammatory mediators found in the blood plasma and synovial fluid during IIA, which have been linked to inflammation and cartilage destruction. These mediators are synthesized by immune cells and released into an inflamed joint [6–8]. Post-Traumatic arthritis, gouty arthritis, septic arthritis, and Lyme arthritis are under the category of INIA.

The long-term medications used to relieve arthritis were not enough and cause abnormal liver and kidney function and also reduces the quality of life. Therefore, many scientists are in search of good curative anti-inflammatory drugs with few or no side effects [9, 10].

Our previous research revealed that the anti-tumor potential of METC was due to the suppression of sEGFR mediated ERK and STAT3 proteins [11]. Ospina et al. [12] reported the anti-inflammatory activity of methanolic extract of T chrysantha leaves but didn’t establish a mechanism of anti-inflammatory action. Garzon-Castano et al. [13] evaluated the antioxidant activity of the inner bark extract of T chrysantha. Scientists reported the linkage between EGFR and STAT3 with the NF-kB signaling pathway in both inflammation and cancerous growth. Expressions of EGF, MMP-2, MMP-9, STAT3, and VEGF were positively correlated with inflammation, tumor size, invasiveness, lymphatic and venous invasion, and metastasis of various carcinomas [14, 15]. In recent years, there has been a vast interest in the health benefits of polyphenols in the prevention of cancer, diabetes, weight reduction, and obesity. The nature synthesized different polyphenols such as caffeic acid (CA); gallic acid (GA); catechin (C); epicatechin (EC); gallocatechin (GC); catechin gallate (CG); gallocatechin gallate (GCG); epicatechin gallate (ECG); epigallocatechin (EGC); and -(-)-epigallocatechin-3-gallate [EGCG]. Among all the polyphenols, EGCG showed the most potent antiproliferative effects [16]. Most of the health beneficial effects of green tea therapy have been attributed to EGCG and related antioxidant activity [17, 18]. Consumption of EGCG (270 mg) in combination with caffeine (150 mg) has been shown to increase fat oxidation [19]. EGCG is one among 3 catechins (polyphenols) abundantly found in green tea, has been shown to inhibit the growth of many cancer cell lines and to suppress the phosphorylation of epidermal growth factor receptor (EGFR) [20].

Phytoconstituents present in the contributed plant T chrysantha are 2-Hydroxynaphthalene-1,4-dione, β-lapachone, 2-((dimethylamino)methyl)-3-methoxynaphthalene-1,4-dione, 4a,5,8,8a-tetrahydro-5-hydroxy-3,7,8-trimethoxy-2-(3,4-dimethoxyphenyl) [11, 21].

![Chemical structure of EGCG](image)

Osteoarthritis is due to the demethylation of certain CpG sites in the MMP9 promoter disturbing the synthesis of the MMP9 gene in cartilage tissues [22]. Over the last 20 years, the rheumatologist given attention to disease-modifying anti-rheumatic drugs (DMARDs) such as Amjevita, Cyltezo, Erelzi (TNF inhibitor), Rixathon (anti-CD20 antibody), Praia (RANKL antibody), Olumiant (JAK inhibitor) over non-steroidal anti-inflammatory drugs (NSAIDs), and corticosteroids for clinical remission of the disease as these can substantially decrease and/or delay joint deformity [23]. Despite the novel regimens, complete long-term disease remission was not successful for many patients.

The objective of this study was to discover an alternative remedy for IIA and NIJA because the frequency of consumption of conventional drugs such as NSAIDs, corticosteroids, and DMARDs by elderly patients leads to potentially adverse effects.

**Methods**

**Raw materials and chemicals**

Carrageenan (S.D. Fine Chemicals Limited, Bombay), Indomethacin (IPCA, Bombay), EGCG (Maysar herbal, Faridabad, Haryana), Formalin (Sisco research Lab),
TMF (isolated from METC), and all other chemicals obtained from Genaxy Scientific, Hyderabad, India.

**Docking analysis**
The matrix metalloproteinase (MMP) enzymes are actively involved in the pathogenesis of inflammation and disease processes such as arthritis and cancer. The inflammation is mostly attributed to NFkB signaling [24]. Citing the above literature findings docking studies of compounds TMF and EGCG was carried out using Autodock 4.0 and Discovery studio Biovia 2017 software to find out the interaction between ligand and the target protein. The crystal structure of transcription factor NFkB P50 homodimer bound to a KB site (1BFT), and MMP9 (6ESM) were derived from the protein data bank [25]. The molecular docking studies of TMF and EGCG on NFkB and MMP9 proteins were emphasized in Figs. 1, 2, 3, and 4 and Table 1.

**Source of animals with an ethical statement**
The adult male Wistar albino (WS) rats (175–225 g) were procured from Shri Venkateshwara Enterprises, Hyderabad and acclimatized to laboratory conditions for one week before investigation. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of the CMR College of pharmacy (Regd. No. CPCSEA/1657/IAEC/CMRCP/PhD/-19/86), India, dated 16/11/2019.

**Euthanasia and anesthesia**
Twenty-four hours after the last dose, all the animals were anesthetized by diethyl ether and sacrificed by cervical dislocation. The liver tissue was removed for estimation of antioxidant parameters, and the paw tissue was removed for estimation of associated inflammatory mediators. Before sacrifice the blood was collected from each rats by retro-orbital puncture for estimation of different blood parameters associated with arthritis.

![Fig. 1](image-url)  
**Fig. 1** Autodock of 1BFT (NF-κB p50) with TMF  
a The three-dimensional structural representation of predicted NF-κB p50.  
b The three-dimensional structure of TMF.  
c The molecular representations of Docked complexes.  
d 2D Interactions of ligand and Protein.  
e Interaction of ligand with NF-κB p50 at Methionine 284 via alkyl bond.  
f Interaction of ligand with NF-κB p50 at Iso-leucine 196, Proline 283, and Glutamic acid 285
Dose selection and formulation
The doses were selected by following the data from the literature and our previous research. EGCG 100 µg/mL (EGCG-LD), EGCG 200 µg/mL (EGCG-HD) and TMF 10 µg/mL (TMF-LD), TMF 15 µg/mL (TMF-HD) [21]. The powders were solubilized separately by pyrogen-free water with DMSO as a solubilizing agent. The LD50 dose value of EGCG was 2000 mg/kg, and the safe dose was 200 mg/kg [26].

Acute and chronic inflammatory models
Acute inflammatory model: carrageenan-induced footpad reaction in WS rats
The carrageenan (10 mg/mL; injection volume 0.1 mL) induced footpad reactions were performed in WS rats. Six test groups (TG) were selected by simple randomization technique, TGII, TGIII, TGIIV, TGV, and TGVI were administered with EGCG-LD, TMF-LD, EGCG-HD, TMF-HD, and Indomethacin (2 mg/kg, p.o), respectively [27]. TGI kept as carrageenan control. Acute edema was induced in the right hind paw of all WS rats by injecting 0.1 mL of the carrageenan solution. Treatment continued for up to 24 h. The right paw of each WS rat of all TGs served as normal control (noninflamed paw; 0.9%, 0.1 mL saline-injected) for comparison. The paw volume was measured by a plethysmometer at 0, 30, 60, and 120 min after carrageenan injection [28].

Chronic inflammatory model
In this model, formalin (2%v/v, 0.1 mL; SC) was injected recurrently at the right hind paw of the rats on the first and third days of the experiment [29]. Then the same methodology was followed in the acute inflammatory model with standard treatment of indomethacin (2 mg/kg, p.o) up to 6th days. Arthritis was evaluated by checking the mean increase in paw diameter for 6 days via a digital vernier caliper [30]. The difference in paw thickness and percentage of anti-arthritic effect were calculated for all groups on the 1st and 6th day of the experiment.
% Inhibition = 100 (1 − Vt/Vc)

Vc, joint diameter in control; Vt, joint diameter in treatment groups.

Hematology in a formalin-induced arthritis model The parameters like RBC, WBC, platelet, ESR, and Hemoglobin were measured by a blood cell counter (ERBA diagnostic Limited, India). Rheumatoid factor (RF, IU/mL) was determined by using the diagnostic kit (Laila Impex, Vijayawada). The total cholesterol level was measured by the available kit from SPAN diagnostic, India.

Estimation of ERK, MMP9, NF-κB expression by Western blotting Inflamed paw tissues were kept in isotonic KCl-0.01M phosphate buffer, centrifuged at 100,000×g for 60 min to remove tissue debris. 25% of tissue homogenate was suspended in 6.0 mL of 0.05 M phosphate buffer, pH 7.6, and EDTA. The amount of protein in each sample was measured using the Bradford assay employing bovine serum albumin (BSA) as a standard. Equal amounts of protein (2 mg) were then boiled for 10 min with an appropriate volume of sample buffer (350 nM Tris–HCl, pH 6.8, 1 M Urea, 1% 2-mercaptoethanol, 9.3% DTT, 13% sodium dodecyl sulfate (SDS), 0.06% bromophenol blue, and 30% glycerol). Samples were then resolved on a 12% SDS–polyacrylamide gel and separated at 150v for 4 h. The gel was then transferred overnight to the polyvinyl difluoride (PVDF) membrane at 48 °C. The membranes were blocked for 1 h at room temperature in 5% BSA in Tris-buffered saline with Tween-20 (TBST; 10 mM Tris–HCl, pH 7.9, 150 mM NaCl, and 0.05% Tween-20). The following primary antibodies were used: anti-NF-κB, anti-MMP-9, monoclonal anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody (Santa Cruz Biotech, Inc.), anti-ERK, and pERK antibody (Cell Signaling Technology, Inc.). The bands of all proteinomics were detected using the available software (LI-COR Biosciences). Both primary and secondary antibodies were diluted in blocking solution and washed with Tris-buffered saline containing 0.2% Tween-20.

Estimation of (IL)-1β and TNFα from serum by ELISA and iNOS, and COX-2 in the inflamed tissue by qRT-PCR The ELISA reader was used to measure the levels of (IL)-1β and TNFα in the serum using the standard detection procedure following the determination of OD value at 450 nm. The qRT-PCR assay was performed using the Power SYBR® Green master mix

**Fig. 3** Autodock of 1BFT (NF-kB p50) with EGCG
(Applied Biosystems 7300) with cycling conditions as follows: 95 °C for 15 s and 60 °C for 1 min for 40 cycles. The data analysis was performed using the $2^{-\Delta\Delta CT}$ method for relative quantification, and all sample values were normalized to the GAPDH mRNA expression value [21]. For iNOS and COX-2 detection, total RNA (2 μg) was reverse transcribed into cDNA with AMV reverse transcriptase (Promega, Madison, WI, USA). The extracted RNA was mixed with primer solution (20 μl) before analysis. GAPDH is used as a housekeeping gene.

### Quantitative densitometric analysis

The quantitative densitometric analysis of pERK, pSTAT3, MMP9, and

### Table 1 Docking analysis of TMF and EGCG

| Targeted proteins | Trimethoxy flavone | Binding affinities of phytocompound with target proteins (Kcal/mol)-AutoDock | Interactions of docked complexes | Inhibitory constant (IC₅₀) | (-)-Epigallocatechin gallate | Binding affinities of phytocompound with target proteins (Kcal/mol)-AutoDock | Interactions of docked complexes | Inhibitory constant (IC₅₀) |
|------------------|--------------------|--------------------------------------------------------------------------------|---------------------------------|---------------------------|-----------------------------|--------------------------------------------------------------------------------|---------------------------------|---------------------------|
| 1BFT             | — 5.69             | Arg-201, Asp-210, Asn-200, Glu-211                                             | 67.34 μM                        | — 7.82                    | Ser-203, Arg-201, Glu-211, Asn-200, Asp-210, Phe-213 and Ile-212             |
| 6ESM             | — 6.18             | Leu-188, Ala-189, Pro-246, Tyr-248, Met-247 and Gly-186                        | 29.56 μM                        | — 9.77                    | Lys-184, Asp-185, Glu-208, Leu-209, Gly-217, Tyr-218, Lys-214, Gly-213, Phe-250 and Tyr-248 |

**Fig. 4** Autodock of 6ESM (MMP9) with EGCG
NF-κB was performed using the quantity one software (Bio-Rad) at the Indian Institute of Chemical Biology, India. Band intensity was obtained for all proteins of each sample from three independent biological experiments [31].

The reduced glutathione (GSH), MPO, SOD, CAT activity MPO, a marker of neutrophil migration was estimated by measuring H$_2$O$_2$-dependent oxidation of O-dianisidine [32]. The clear supernatant of liver tissue homogenate was used for the assay of antioxidant enzymes SOD [33–35].

### Results

#### Docking analysis

The molecular docking studies demonstrated that TMF and EGCG can be effectively combined with proteins NF-κB and MMP9 (Figs. 1, 2, 3, 4). Grid Score, interactions, and IC$_{50}$ values are mentioned in Table 1. Negative values indicated that there is a combination, and positive values indicate no binding. Therefore, the smaller the score value, the stronger the binding force. Furthermore, we used a Discovery studio Biovia 2017 to study the ligand interactions of TMF and EGCG on NF-kB and MMP9 proteins. Both molecules are hydrophilic compounds that quickly penetrate the cell membrane. Based on the docked result, the TMF and EGCG are likely to be a potent agonist of NFkB and MMP9 proteins. The results showed that TMF and EGCG may have hydrogen bond interactions with Leu-188, Ala-189, Met-247, and Tyr-248.

#### Anti-inflammatory and anti-arthritic activity

The results of acute and chronic anti-inflammatory activity of EGCG and TMF in WS rats were summarised in Tables 1 and 2, respectively. The TMF-HD exhibited 55% inhibition of paw edema and 36% paw diameter, EGCG-HD has shown 45% inhibition of paw edema and 28% paw diameter which was comparable with standard drug indomethacin (Tables 2, 3; Fig. 5).

#### Effect of drugs on hematological parameters in a chronic inflammatory model

There was no anemic condition observed in the formalin-induced group. The WBC, Platelet, ESR, total cholesterol, and RF value also increased in the control group. Such types of changes were not observed in the TMF, EGCG, and indomethacin-treated groups (Table 4).

### Table 2 Carrageenan induced acute inflammatory model in WS rats

| Group | Treatment and dose | Paw volume (mL) | Inhibition (%) |
|-------|-------------------|----------------|---------------|
| I     | Carrageenan       | 0.8            | 1.9±0.02*     |
| II    | EGCG-LD           | 0.9            | 1.1±0.02**    | 43            |
| III   | TMF-LD            | 1              | 1.25±0.01**   | 35            |
| IV    | EGCG-HD           | 0.9            | 1.06±0.01*    | 45            |
| V     | TMF-HD            | 0.85           | 0.87±0.02*    | 55            |
| VI    | Indomethacin      | 0.8            | 0.85±0.03**   | 56            |

Values are mean S.E.M.; $n=6$; Treatment group *$p<0.001$; **$p<0.01$ vs. carrageenan control; *$p<0.05$ vs. saline control. The paw volume was measured in every 1 h and upto 6 h. The paw volume for normal control paw was 0.95±0.02.

### Table 3 Formalin induced arthritis model in WS rats

| Groups | Treatment and dose | Paw diameter in cm | Percentage inhibition (%) |
|--------|-------------------|--------------------|---------------------------|
| I      | Formalin control  | 3.46±0.01          | 3.86±0.01                 |
| II     | EGCG-LD           | 2.98±0.01**        | 2.95±0.02*                | 24            |
| III    | TMF-LD            | 2.95±0.01**        | 2.91±0.02*                | 25            |
| IV     | EGCG-HD           | 2.85±0.02*         | 2.80±0.02**               | 28            |
| V      | TMF-HD            | 2.65±0.01**        | 2.48±0.02*                | 36            |
| VI     | Indomethacin      | 2.40±0.01**        | 2.37±0.02*                | 39            |

Values are mean S.E.M.; $n=6$; **$p<0.001$ vs Normal control group; *$p<0.05$ vs. DSS control. The paw diameter of normal control paw was 2.35±0.05.

Report of estimated (IL)-1β and TNFα by ELISA, and iNOS, and COX-2 by qRT-PCR

The inflammatory cytokines TNF-α and IL-1β levels, and iNOS-mRNA and COX-2-mRNA expressions were significantly lowered in the TMF, EGCG, and indomethacin treated groups, as compared to the formalin-induced group. The decreased level was more obvious to confirm the anti-inflammatory potential of TMF and EGCG (Fig. 6 A–D).

Report of estimated p-ERK, MMP9, NF-κB proteins by Western blotting

In the TMF, EGCG, and indomethacin treated groups, the inflammatory marker proteins such as p-ERK, MMP9, NF-κB expressions were significantly reduced as compared to formalin control groups (Fig. 7).

Report of GSH, MPO, SOD, and CAT activity

The GSH and CAT levels were significantly decreased, and the MPO and SOD activity was significantly
increased in the formalin control group. In the TMF, EGCG, and indomethacin treated groups, the GSH, MPO, SOD, and CAT activity significantly restored to the normal level (Fig. 8 A–D).

**Statistical analysis**
All results (mean ± SEM) were analyzed for statistical significance by one-way analysis of variance (ANOVA) followed by Dunnett's test using GraphPad InStat version 3.05 (GraphPad Software, USA).

**Discussion**
IIA is an autoimmune inflammatory disorder where circulated immune cells T cells, B cells, and macrophages migrate and reside in the inflammatory loci [36]. Rheumatoid arthritis (RA), spondyloarthritis (SA) or ankylosing spondylitis (AS), psoriatic arthritis, and arthritis associated with inflammatory bowel disease (IBD) are the most common type of chronic IIA characterized by the presence of rheumatoid factor (RF), inflammatory cytokines such as tumor necrosis factor (TNF) and

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**Table 4** Hematological parameters

| Parameters | Normal control | Formalin control | EGCG-LD | TMF-LD | EGCG-HD | TMF-HD | Indomethacin |
|------------|----------------|------------------|---------|--------|---------|--------|--------------|
| Hb (g/dl)  | 14.50 ± 2.13   | 12.45 ± 3.06*    | 12.58 ± 2.52 | 12.52 ± 1.85* | 12.86 ± 0.54* | 12.88 ± 1.34 | 12.78 ± 3.64* |
| RBC (10⁶/mm³) | 8.50 ± 1.35   | 7.20 ± 1.83   | 7.38 ± 1.12*** | 7.18 ± 0.52   | 7.25 ± 0.72   | 7.55 ± 1.48   | 8.37 ± 0.52** |
| WBC (10³/mm³) | 5.45 ± 2.80   | 12.35 ± 1.49  | 7.32 ± 1.72*   | 6.82 ± 0.37*** | 6.23 ± 1.24** | 5.64 ± 0.68*  | 5.46 ± 2.45*** |
| Platelets (lakhs/mL) | 2.70 ± 1.65   | 5.35 ± 1.23*   | 3.37 ± 1.17**  | 3.78 ± 1.58*   | 3.64 ± 1.45*  | 2.72 ± 0.45*** | 3.13 ± 2.43** |
| ESR (60 min) | 3.45 ± 1.26   | 10.14 ± 1.35*  | 4.68 ± 1.37*   | 4.45 ± 1.75*   | 4.72 ± 1.43*  | 4.20 ± 1.57   | 4.55 ± 3.77*** |
| RF (IU/mL)  | 6.45 ± 0.94   | 19.73 ± 2.14   | 8.62 ± 0.32*   | 7.53 ± 0.25*   | 8.14 ± 0.73*  | 7.0 ± 0.76*   | 7.05 ± 2.76** |
| TC (mg/dl)  | 105.27 ± 2.17 | 134.27 ± 0.52* | 105.34 ± 3.51** | 117.59 ± 2.87*** | 106.54 ± 1.48** | 105.15 ± 1.80* | 120.20 ± 3.40 |

Values are mean S.E.M.; n = 6, *p < 0.001 vs normal control group; **p < 0.001, ***p < 0.01, ****p < 0.05 and vs. DSS Control
Fig. 6 Estimation of cytokines and inflammatory mediators: A Level of (IL)-1β, B TNFα expressions by ELISA, C iNOS mRNA, D COX-2 mRNA expressions by RT-PCR

Fig. 7 Immunoblot images of NF-κB, ERK, and MMP9 signaling with their quantification
(IL)-1β in the blood [37]. These factors are synthesized and released by macrophages, B-cells, and activated T-cells. The activated T-cell activates macrophages. The activated macrophages release inflammatory marker enzymes COX-2 and iNOS those act as a catalyst for the synthesis of PGE₂ and NOS or inflammatory reactions [38]. The IIA affects 0.6% population in Western countries with major determinants as gastrointestinal, cardiovascular disorders, and atherosclerosis [39]. To achieve clinical remission, the IIA should be routinely monitored along with adjustment of the treatment regimen.

In recent years, scientists actively involved in the discovery, evaluation, and development of MMP inhibitors. The pathogenesis of chronic inflammation and arthritis is due to MMP9 production by macrophages in the tissue [40]. The overexpression of MMP9 and the production of cytokines are under the control of the transcription factor NFkB production and activation [41, 42]. MMP9 inhibitors are categorized into specific and nonspecific MMP9 inhibitors. Food and Drug Administration approved doxycycline (Periostat®) as only one nonspecific MMP inhibitor as it is attenuated myocardial fibrosis by suppressing MMP-2 and MMP-9. SB-3CT is classified under the specific MMP-9 inhibitor which treated embolic focal cerebral ischemia. Inhibition of MMP-9 in a model of postoperative ileus reduced inflammation and improved motility [43, 44].

The matrix metalloproteinase (MMP) enzymes are actively involved in the pathogenesis of inflammation and disease processes such as arthritis and cancer [45]. During pathological inflammatory processes, MMPs modulate cytokine and chemokine activity and the generation of chemokine gradients at the cell surface. MMP-2, MMP-3, and MMP-9 can both up-and down-regulated IL-1β activity at sites of acute or chronic inflammation [46]. MMP7 (matrilysin) modulates the activities of the tumor necrosis factor (TNF) family, TNF-alpha, and FasL at the targeted cell surface during the production of bioactive cytokines [47].

The most abundant form of NF-kB protein dimer is usually known as P50-P65 dimer or NFkB1/RelA which is responsible for an inflammatory response [48]. This is called a nuclear transcription factor because the N terminus of dimer binds with DNA to have a function. Usually, NFkB resides in the cytoplasm in the resting stage after a combination of its Rel homology domain (RHD) with IK-Bα protein. In the resting stage, the NFkB can’t translocate to the nucleus and

![Fig. 8 MPO of inflamed tissue and SOD, GSH, and CAT levels in the hepatic tissue of rats in the chronic inflammatory model](image-url)
TMF and EGCG significantly normalize the levels of suppression of NF-κB signaling. The TNF-α and IL-1β inhibited by TMF, EGCG, and indomethacin through area developing inflammation. The COX-2 enzyme was the mouse causes infiltration of macrophages, T-cell, administration of formalin at the hind paw region of or MMP9 derived cytokines, and NF-κB [59, 60]. The inflammation of the rectum and anal regions is mostly attributed to NF-kB signaling [52].

The pro-inflammatory cytokines, oxidative stress, and TNF are responsible for the activation of the extracellular signal-regulated kinase (ERK). The activated ERK or p-ERK regulates different cellular processes such as proliferation, differentiation, inflammation, stress response, apoptosis [53]. The free radical scavengers or endogenous antioxidant molecules such as GSH, SOD, CAT are decreasing the ERK and IκBα phosphorylation [54]. That results in the activation of NF-κB signaling during therapy induced by flavonoid reach anti-inflammatory drugs. Scientists reported that EGCG having good antioxidant nature and scavenged ROS and RNS free radicals because of the phenol ring in its molecular structure [55]. Proteinomics such as MMP2, MMP9, Bax, Bcl-2, and cell cycle regulators such as EGFR, androgen receptor, Activator proteins 1(AP1) were found to be affected by EGCG [56].

Carrageenin-induced hind paw edema is the standard experimental model in the search for new acute anti-inflammatory drugs and is believed to be biphasic. The primary inflammatory reaction is due to the synthesis and release of histamine, leukotrienes, and interleukins, whereas the late phase is due to the release of prostaglandins (PGs) [57, 58]. The PGs (PGG2 and PGH2) are synthesized from a fatty acid-derived substance arachidonic acid (AA) by the COX-2 enzyme in response to immunological and chemical stimuli. Formalin induced arthritis in the mouse model is among the most acceptable autoimmune IA model for discovery of new anti-rheumatic drugs those can target both microphage or MMP9 derived cytokines, and NF-κB [59, 60]. The administration of formalin at the hind paw region of the mouse causes infiltration of macrophages, T-cell, B-cell, and neutrophils to the synovial lining or joint area developing inflammation. The COX-2 enzyme was inhibited by TMF, EGCG, and indomethacin through suppression of NF-κB signaling. The TNF-α and IL-1β stimulate cholesterol production in arthritic rats. The TMF and EGCG significantly normalize the levels of Hb, RBC, WBC, platelet count, ESR, cholesterol, and RF in a dose-dependent manner as compared with arthritic control and indomethacin treated groups.

Conclusions

Indians, mostly the peoples of South India depend upon flavonoid supplements to get relief from arthritis. Reciting our previous research evidence and traditional use we selected TMF and EGCG for research that has both antioxidant and anti-inflammatory actions. Studies in acute, chronic inflammatory models, and docking analysis demonstrated that TMF and EGCG having better acute and chronic anti-inflammatory potential. The possible anti-inflammatory mechanism of TMF and EGCG will be the suppression of NF-κB signaling, MMP9 gene expression, inflammatory cytokines, and inflammatory marker enzymes.

Abbreviations

IIA: Inflammatory Immunoarthritis; INIA: Inflammatory Non-Immuno Arthritis; NIA: Non-Inflammatory Arthritis; ERK: Extracellular-Signal-Regulated Kinase; MMP9: Matrix Metalloproteinase9; NF-κB: Nuclear Factor kappa Beta; EGCG: (-)-Epigallocatechin-3-Gallate; METC: Methanolic stem Extract of T. cheeyanthula; TMF: Trimethoxy flavonoid; TNF: Tomor Necrosis Factor; DMARDs: Disease-modifying anti-rheumatic drugs; iNOS: Nitric oxide synthase; COX-2: Cyclooxygenase-2; qRT-PCR: Quantitative Real-Time Polymerase Chain Reaction; GAPDH: Monoclonal anti-Glyceraldehyde 3-Phosphate Dehydrogenase; RF: Rheumatoid Factor; MPO: Myeloperoxidase; SOD: Superoxide Dismutase; CAT: Catalase; GSH: Reduced Glutathione; RANK: Receptor Activator of Nuclear Factor kappa Beta; DMSO: Dimethyl sulfoxide; IκBα: Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha.

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Authors’ contributions

SPP and UPP carried out the design, literature review, in vivo and in vitro analysis and wrote the manuscript. DSNBKP, SPM collected plant from Guntur, Andhrapradesh and helped in isolation and animal experiment. SPP and MR coordinated the research works. I ensured that all authors have read and approved the manuscript.

Availability of data and materials

The available data sets will be provided from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of the CMR College of pharmacy (Regd. No. CPCSEA/1657/IAEC/CMRCP/PhD/19/86), India, dated 16/11/2019.

Consent for publication
Not applicable.
Competing interests
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