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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

Siva Prasad Panda[1]* , Uttam Prasad Panigrahy[2], Sarada Prasanna Mallick[3], DSNBK Prasanth[4] and Mitta Raghavendra[5]

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 IC_{50} 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

Panda *et al. Futur J Pharm Sci*   (2021) 7:207

Page 2 of 13

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 cartilages. 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].
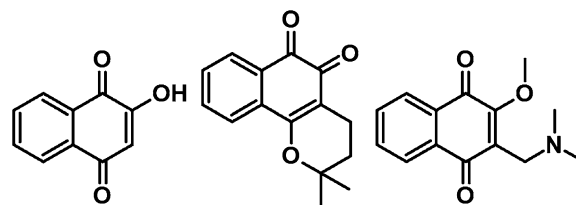
Osteoarthritis is due to the demethylation of certain CpG sites in the MMP9 promoter disturbing the synthesis of the MMP9 gene in cartilage tissues [22]. Over the last 20 years, the rheumatologist given attention to disease-modifying anti-rheumatic drugs (DMARDs) such as Amjevita, Cyltezo, Erelzi (TNF 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 NIIA 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),

Panda *et al. Futur J Pharm Sci*   (2021) 7:207

Page 3 of 13

TMF (isolated from METC), and all other chemicals obtained from Genaxy Scientific, Hyderabad, India.
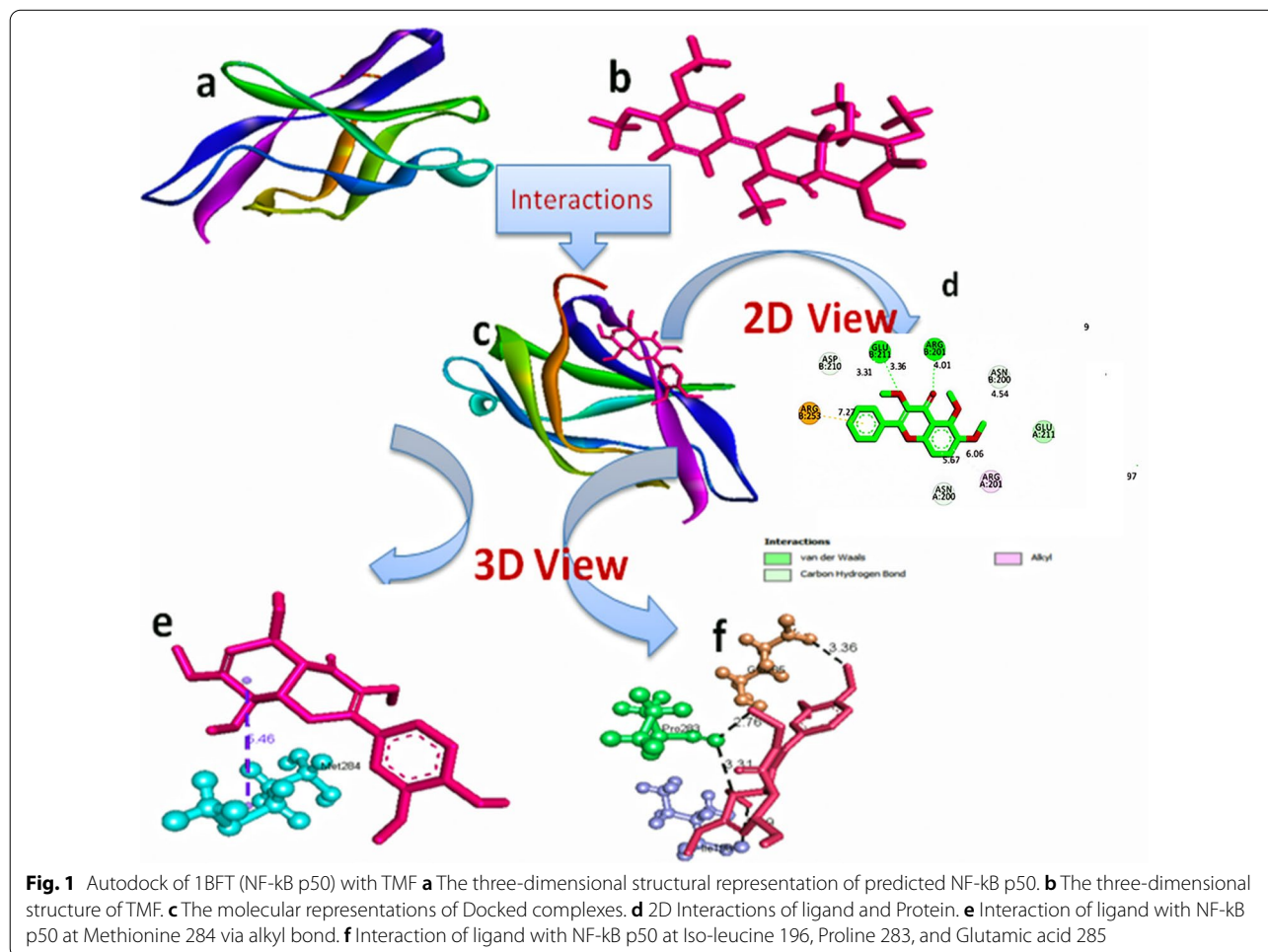
Docking analysis

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.
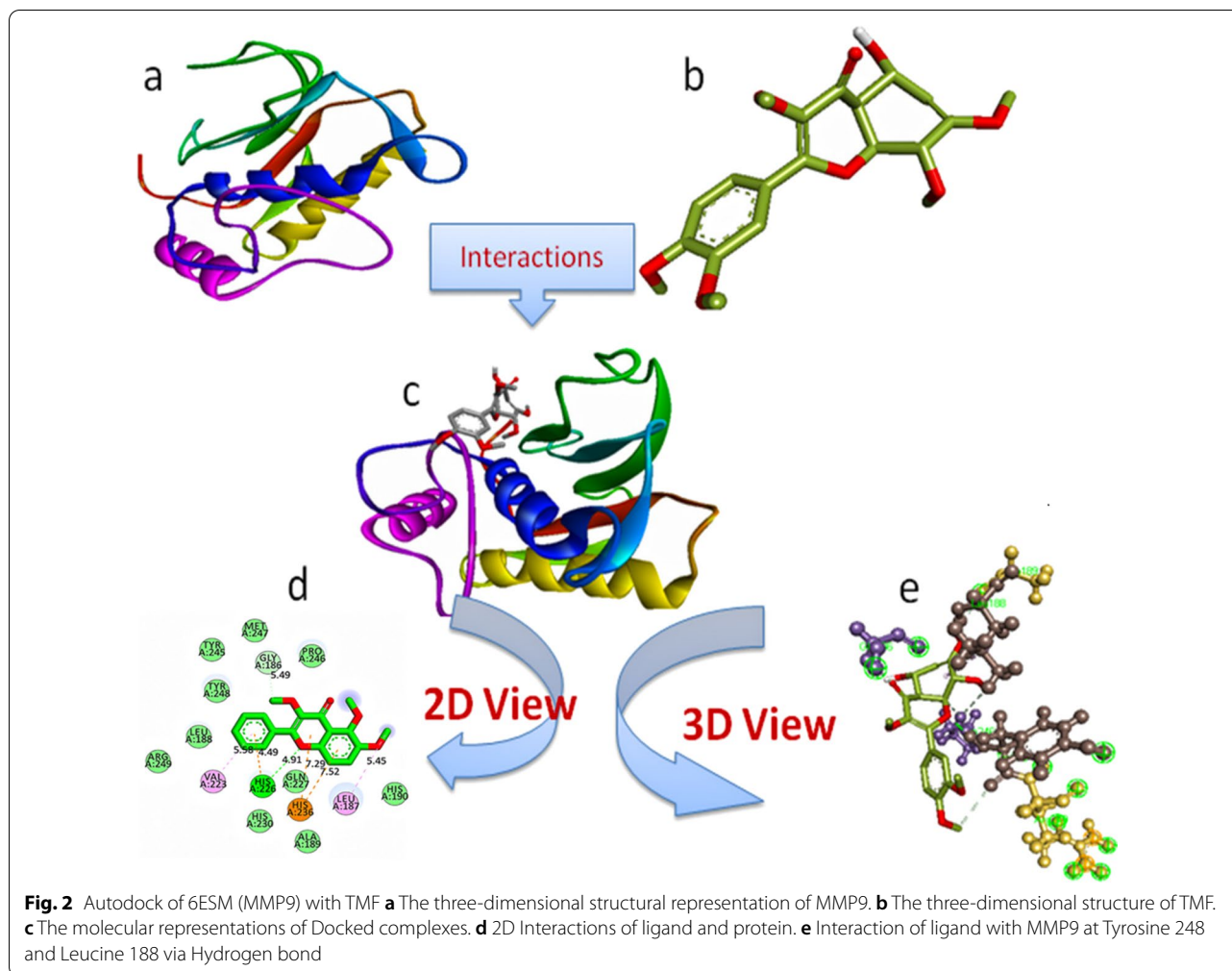
Source of animals with an ethical statement

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 Autodock of 1BFT (NF-kB p50) with TMF **a** The three-dimensional structural representation of predicted NF-kB 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-kB p50 at Methionine 284 via alkyl bond. **f** Interaction of ligand with NF-kB p50 at Iso-leucine 196, Proline 283, and Glutamic acid 285

Panda *et al. Futur J Pharm Sci*   (2021) 7:207

Page 4 of 13

Fig. 2 Autodock of 6ESM (MMP9) with TMF **a** The three-dimensional structural representation of MMP9. **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 MMP9 at Tyrosine 248 and Leucine 188 via Hydrogen bond

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 LD_{50} dose value of EGCG was 2000 mg/kg, and the safe dose was 200 mg/kg [26].
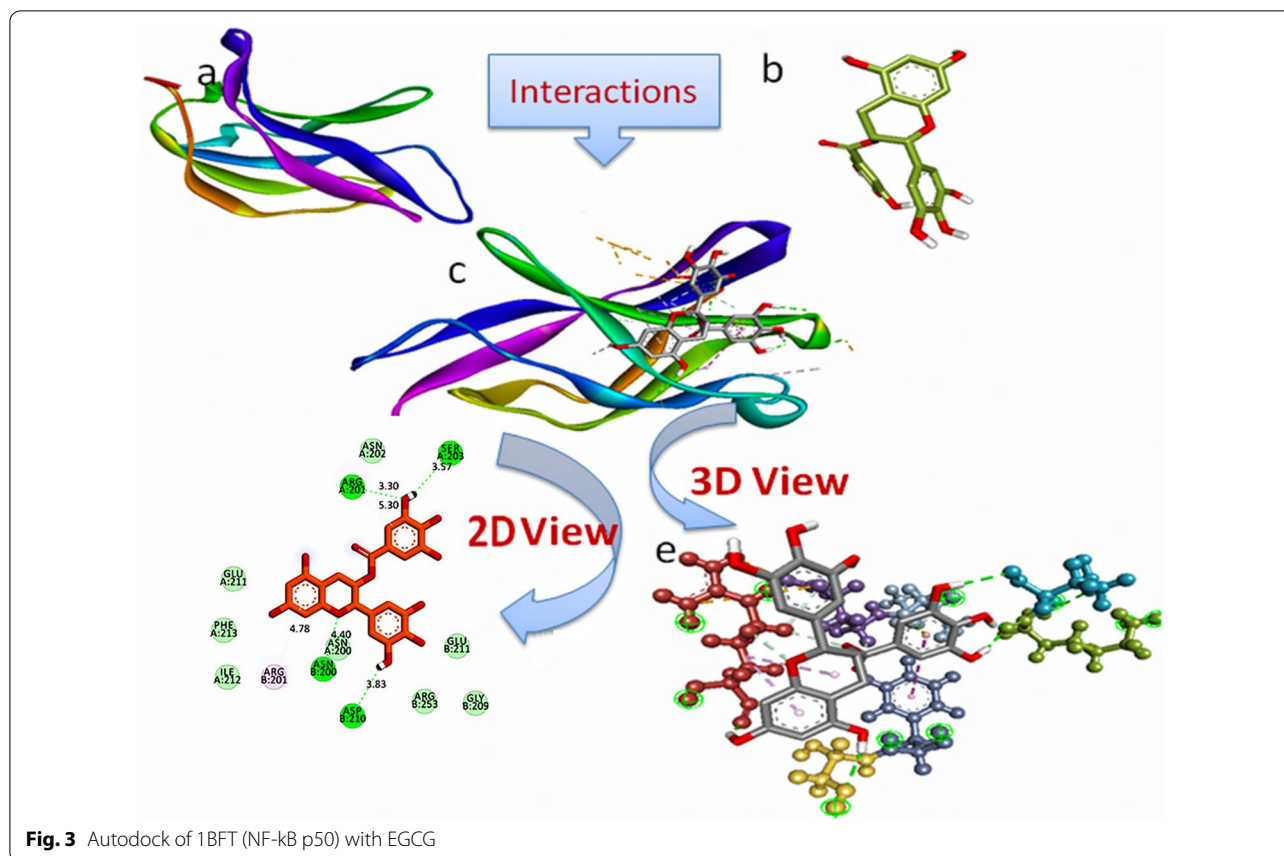
Acute and chronic inflammatory models

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, TGIV, 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.

Panda *et al. Futur J Pharm Sci* (2021) 7:207

Page 5 of 13

Fig. 3 Autodock of 1BFT (NF-kB p50) with EGCG

% Inhibition $= 100\,(1 - \mathrm{Vt/Vc}))$

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 Implex, 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 $100{,}000 \times g$ 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
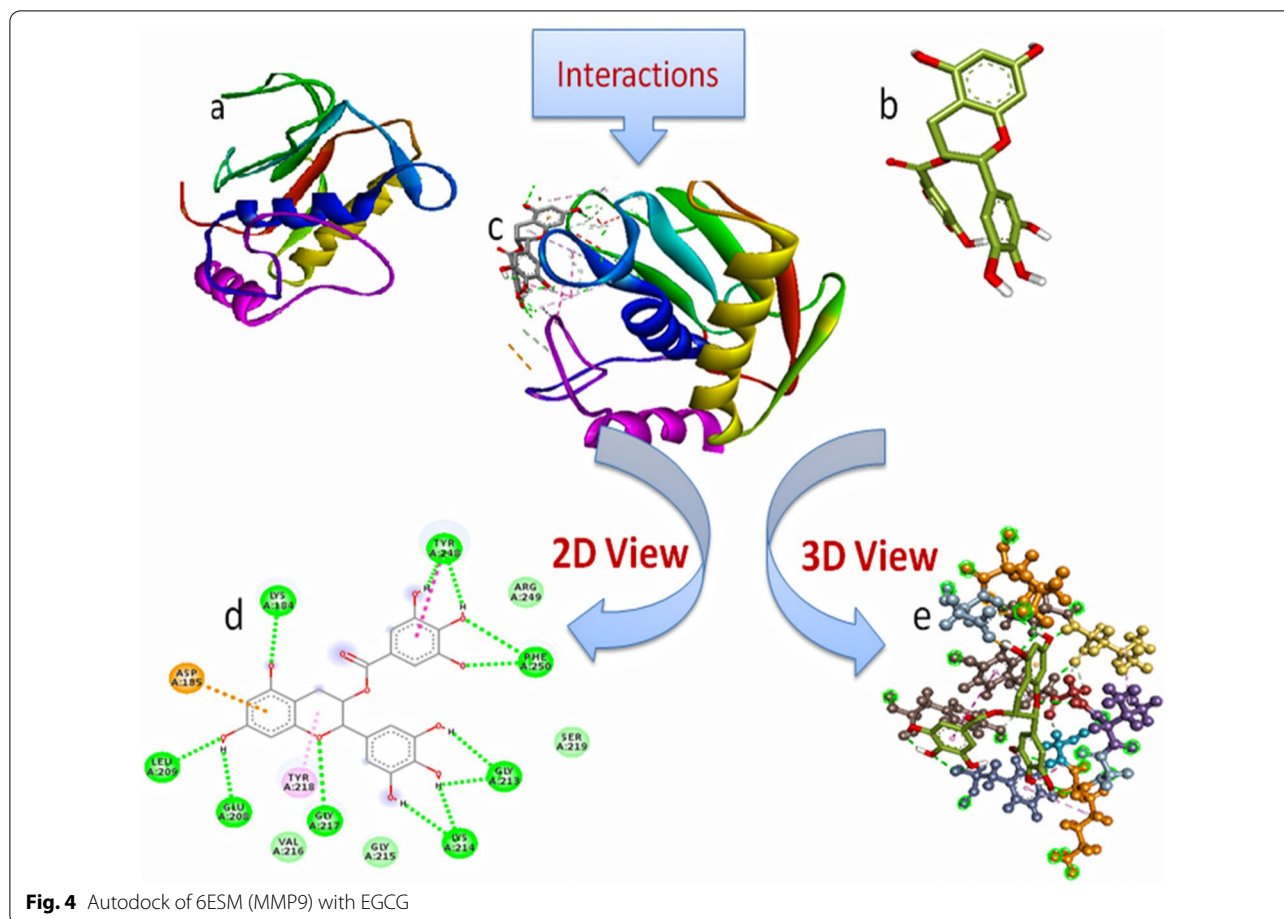
Panda *et al. Futur J Pharm Sci*   (2021) 7:207

Page 6 of 13

Fig. 4 Autodock of 6ESM (MMP9) with EGCG

Table 1 Docking analysis of TMF and EGCG

Targeted proteins	Trimethoxy flavone			(-)-Epigallocatechin gallate		
	Binding affinities of phytocompound with target proteins (Kcal/mol)-AutoDock	Interactions of docked complexes	Inhibitory constant (IC$_{50}$)	Binding affinities of phytocompound with target proteins (Kcal/mol)-AutoDock	Interactions of docked complexes	Inhibitory constant (IC$_{50}$)
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	1.85 μM
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	69.14 nM

(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

Panda *et al. Futur J Pharm Sci*   (2021) 7:207

Page 7 of 13

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_2O_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-kB 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 penetrates 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) (0 h)	Paw volume (mL) (6 h)	Inhibition (%)
I	Carrageenan	0.8	$1.9 \pm 0.02^{\#\#}$	
II	EGCG-LD	0.9	$1.1 \pm 0.02^{**}$	43
III	TMF-LD	1	$1.25 \pm 0.01^{**}$	35
IV	EGCG-HD	0.9	$1.06 \pm 0.01^{*}$	45
V	TMF-HD	0.85	$0.87 \pm 0.02^{*}$	55
VI	Indomethacin	0.8	$0.85 \pm 0.03^{**}$	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 (%)
		2nd day	6th day	
I	Formalin control	3.46 ± 0.01	3.86 ± 0.01	
II	EGCG-LD	$2.98 \pm 0.01^{**}$	$2.95 \pm 0.02^{*}$	24
III	TMF-LD	$2.95 \pm 0.01^{**}$	$2.91 \pm 0.02^{*}$	25
IV	EGCG-HD	$2.85 \pm 0.02^{*}$	$2.80 \pm 0.02^{**}$	28
V	TMF-HD	$2.65 \pm 0.01^{**}$	$2.48 \pm 0.02^{*}$	36
VII	Indomethacin	$2.40 \pm 0.01^{**}$	$2.37 \pm 0.02^{*}$	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. 6A–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
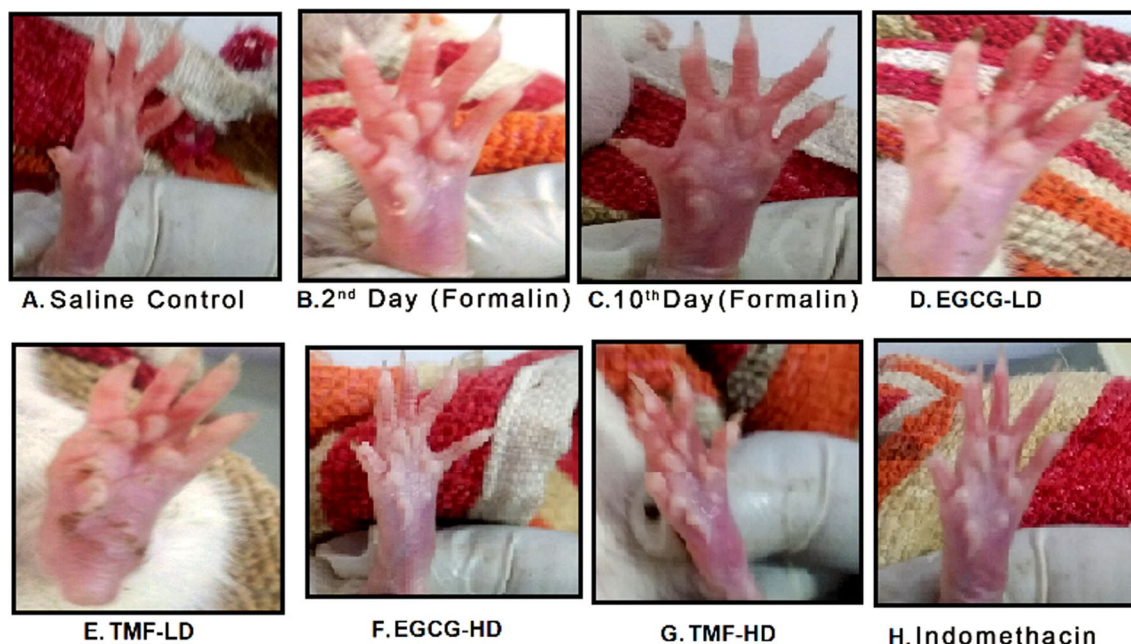
Panda *et al. Futur J Pharm Sci* (2021) 7:207

Page 8 of 13

Fig. 5 Footpad image of SW rats: **A** Normal saline control; **B**, **C** Development of arthritis after formalin injection; **D**–**H**. Arthritic status at the footpad region after treatment with EGCG-LD, TMF-LD, EGCG-HD, TMF-HD, and Indomethacin, respectively. Inflammatory arthritis in the footpad is effectively reduced after 6 days of treatment with drugs

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	$12.45 \pm 3.06^{\#}$	12.58 ± 2.52	$12.52 \pm 1.85^{*}$	$12.86 \pm 0.54^{*}$	12.88 ± 1.34	$12.78 \pm 3.64^{*}$
RBC (10^6/mm^3)	8.50 ± 1.35	7.20 ± 1.83	$7.38 \pm 1.12^{***}$	7.18 ± 0.52	7.25 ± 0.72	7.55 ± 1.48	$8.37 \pm 0.52^{**}$
WBC (10^3/mm^3)	5.45 ± 2.80	12.35 ± 1.49	$7.32 \pm 1.72^{*}$	$6.82 \pm 0.37^{**}$	$6.23 \pm 1.24^{**}$	$5.64 \pm 0.68^{*}$	$5.46 \pm 2.45^{***}$
Platelets (lakhs/mL)	2.70 ± 1.65	$5.35 \pm 1.23^{\#}$	$3.37 \pm 1.17^{**}$	$3.78 \pm 1.58^{*}$	$3.64 \pm 1.45^{*}$	$2.72 \pm 0.45^{***}$	$3.13 \pm 2.43^{**}$
ESR (60 min)	3.45 ± 1.26	$10.14 \pm 1.35^{\#}$	$4.68 \pm 1.37^{*}$	$4.45 \pm 1.75^{*}$	$4.72 \pm 1.43^{*}$	4.20 ± 1.57	$4.55 \pm 3.77^{***}$
RF (IU/mL)	6.45 ± 0.94	19.73 ± 2.14	$8.62 \pm 0.32^{*}$	$7.53 \pm 0.25^{*}$	$8.14 \pm 0.73^{*}$	$7.0 \pm 0.76^{*}$	$7.05 \pm 2.76^{**}$
TC (mg/dl)	105.27 ± 2.17	$134.27 \pm 0.52^{\#}$	$105.34 \pm 3.51^{**}$	$117.59 \pm 2.87^{**}$	$106.54 \pm 1.48^{**}$	$105.15 \pm 1.80^{*}$	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

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. 8A–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
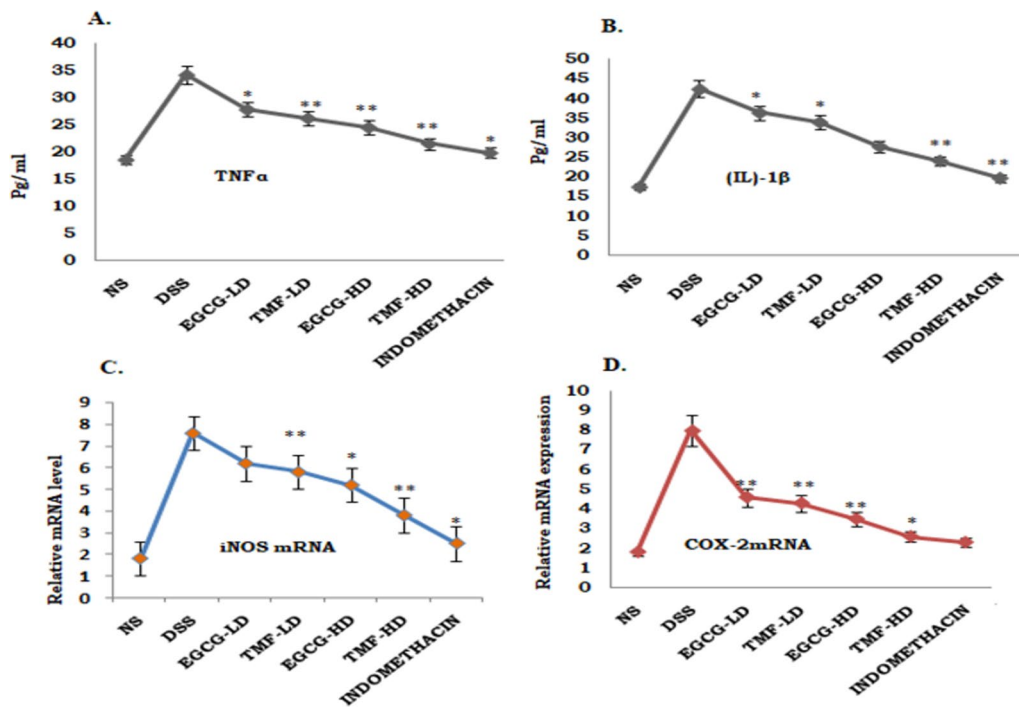
Panda *et al. Futur J Pharm Sci*   (2021) 7:207

Page 9 of 13

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
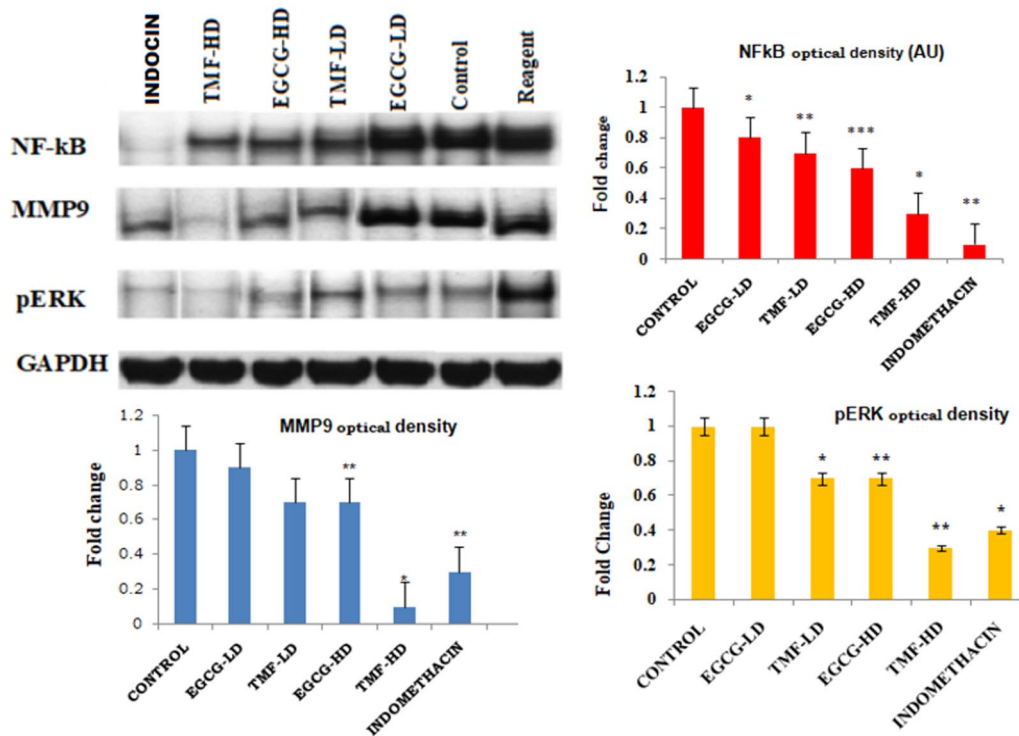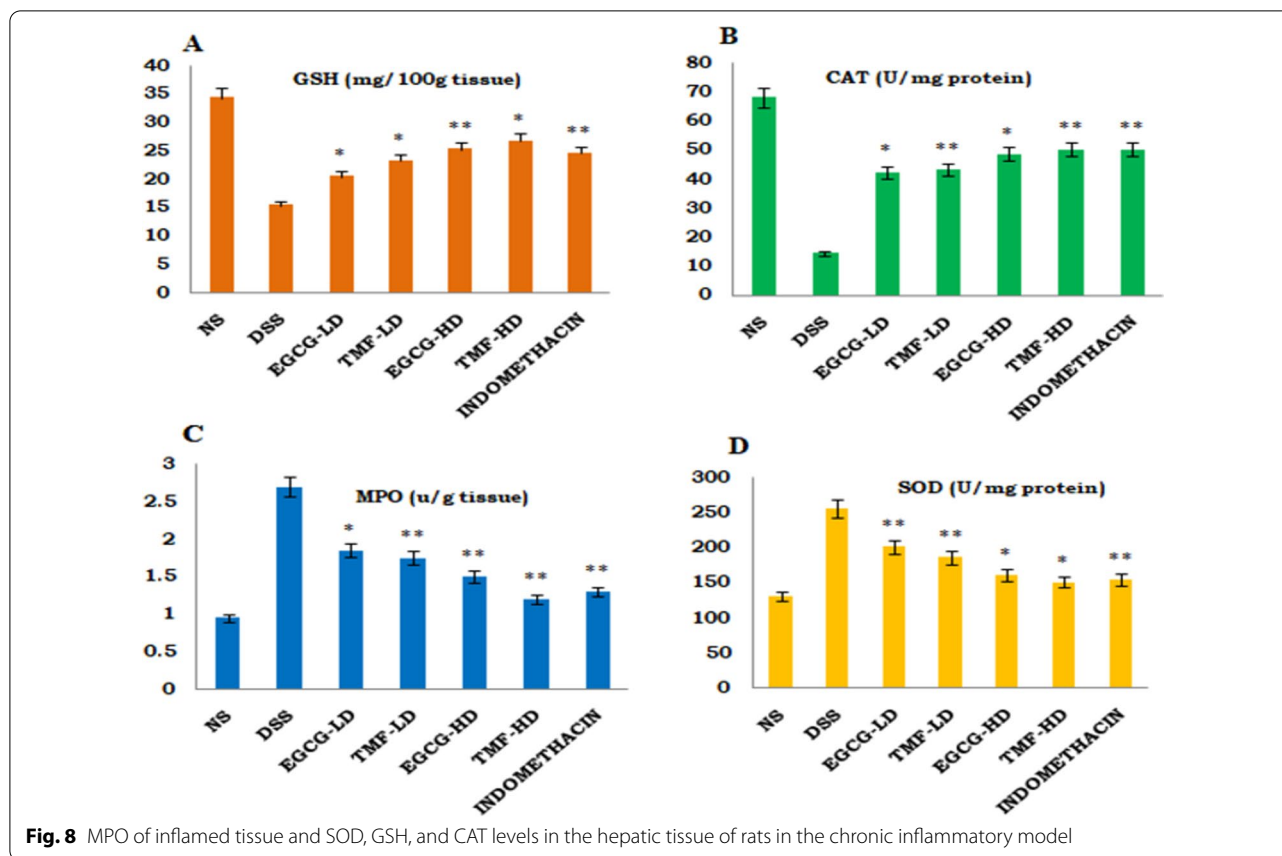

Fig. 7 Immunoblot images of NF-κB, ERK, and MMP9 signaling with their quantification

Panda *et al. Futur J Pharm Sci*   (2021) 7:207

Page 10 of 13

Fig. 8 MPO of inflamed tissue and SOD, GSH, and CAT levels in the hepatic tissue of rats in the chronic inflammatory model

(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 PGE_2 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

Panda *et al. Futur J Pharm Sci*   (2021) 7:207

Page 11 of 13

start a nuclear localization sequence (NLS) function. So, IK-Bα can be regarded as an NF-kB inhibitor. During the inflammatory reaction, the pro-inflammatory cytokine such as TNF, ILs, and free radicals ROS, RNS is involved in the phosphorylation of IK-Bα. So that the NF-kBp65 became active after detachment of p-IK-Bα from the binding site. The NFkB translocates to the nucleus and binds to the kappaB site in the promoter region or enhancer region of DNA. Further, the p65 protein of NF-kB will complex with the promoter of MMP9 to increase MMP9 protein expression [49–51]. The inflammation of the rectum and anal regions is mostly attributed to NFkB 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 IkBα phosphorylation [54]. That results in the activation of NF-kB 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 (PGG$_2$ and PGH$_2$) 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-Iβ 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-kB signaling, MMP9 gene expression, inflammatory cytokines, and inflammatory marker enzymes.

Panda *et al. Futur J Pharm Sci*   (2021) 7:207

Page 12 of 13

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References

1. Mukherjee PK, Nema NK, Venkatesh P, Debnath PK (2012) A changing scenario for the promotion and development of Ayurveda-way forward. J Ethnopharmacol 143:424–434
2. Mobaseri A (2012) Intersection of inflammation and herbal medicine in the treatment of osteoarthritis. Curr Rheumatol Rep 14:604–616
3. Choudhary M, Kumar V, Malhotra H, Singh S (2015) Medicinal plants with potential anti-arthritic activity. J Intercult Ethnopharmacol 4:147–179
4. Vogel H (2007) Analgesic, anti-inflammatory, and anti-pyretic activity. In: Vogel HG (ed) Drug discovery and evaluation. Springer, pp 983–1116p
5. Baildam E (2013) Juvenile idiopathic arthritis. In: Watts RA, Conaghan PG, Denton C, Foster J et al (eds) Oxford textbook of rheumatology, 4th edn. Oxford University Press, pp 910–918
6. Kashiwagi N, Nakano M, Saniabadi AR, Masakazu A, Toshikazu Y (2002) Anti-inflammatory effect of granulocyte and monocyte adsorption apheresis in a rabbit model of immune arthritis. Inflammation 26:199–205
7. Brand DD, Latham KA, Rosloniec EF (2007) Collagen-induced arthritis. Nat Protoc 2:1269–1275
8. Pietrosimone KM, Jin M, Poston B, Liu P (2015) Collagen-induced arthritis: a model for murine autoimmune arthritis. Bio Protoc 5:e1626
9. Zheng K, Zhang S, Wang C, Zhao W, Shen H, Green J (2015) Health-related quality of life in Chinese patients with mild and moderately active ulcerative colitis. PLoS ONE 10:e0124211
10. Shao J, Liu Z, Wang L, Song Z, Chang H, Han N, Yin J (2017) Screening of the optimized prescription form Suqingwan in terms of its therapeutic effect on DSS-induced ulcerative colitis by its regulation of inflammatory and oxidative mediators. J Ethnopharmacol 18:54–62
11. Panda SP, Panigraphy UP, Panda S, Jena BR (2019) Stem extract of *Tabebuia chrysantha* induces apoptosis by targeting sEGFR in Ehrlich ascites carcinoma. J Ethnopharmacol 235:219–226
12. Ospina GLF, Aragon DM, Vergel NE, Isaza G, Perez JE (2011) Anti-inflammatory and antioxidant activities of *Phenax rugosus* (poir.) wedd and *Tabebuia chrysantha*. VITAE COLUMBIA 18:49–55
13. Garzón-Castaño SC, Lopera-Castrillón IA, Jiménez-González FJ, Siller-López F, Veloza LA, Sepúlveda-Arias JC (2018) Nrf2-mediated antioxidant activity of the inner bark extracts obtained from *Tabebuia rosea* (Bertol) DC and *Tabebuia chrysantha* (JACQ) G. Nicholson. F1000Res 7:1937
14. Alberti C, Pinciroli P, Valeri B et al (2012) Ligand-dependent EGFR activation induces the co-expression of IL-6 and PAI-1 via the NFkB pathway in advanced-stage epithelial ovarian cancer. Oncogene 31:4139–4149
15. Fan Y, Mao R, Yang J (2013) NF-κB and STAT3 signaling pathways collaboratively link inflammation to cancer. Protein Cell 4:176–185
16. Du GJ, Zhang Z, Wen XD, Yu C, Calway T, Yuan CS, Wang CZ (2012) Epigallocatechin gallate (EGCG) is the most effective cancer chemopreventive polyphenol in green tea. Nutrients 4:1679–1691
17. Most J, van Can JGP, van Dijk JW, Goossens GH, Jocken J, Hospers JJ, Bendik I, Blaak EE (2015) A 3-day EGCG-supplementation reduces interstitial lactate concentration in skeletal muscle of overweight subjects. Sci Rep 5:17896
18. Jung YD, Ellis LM (2001) Inhibition of tumor invasion and angiogenesis by epigallocatechin gallate (EGCG), a major component of green tea. Int J Exp Pathol 82:309–316
19. Dulloo AG, Duret C, Rohrer D, Girardier L, Mensi N, Fathi M et al (1999) Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans. Am J Clin Nutr 70:1040–1045
20. Hou Z, Sang S, You H, Lee MJ, Hong J, Chin KV, Yang CS (2005) Mechanism of action of (-)-epigallocatechin-3-gallate auto-oxidation-dependent inactivation of epidermal growth factor receptor and direct effects on growth inhibition in human oesophageal cancer KYSE 150 cells. Cancer Res 65:8049–8056
21. Panda SP, Panigrahy UP et al (2020) A trimethoxy flavonoid isolated from stem extract of *Tabebuia chrysantha* suppresses angiogenesis in angiosarcoma. J Pharm Pharmacol 72:990–999
22. Jackson MT, Moradi B, Smith MM, Jackson CJ, Little CB (2014) Activation of matrix metalloproteinases 2, 9 and 13 by activated protein C in human osteoarthritic cartilage chondrocytes. Arthritis Rheumatol 66:1525–1536
23. Guo Q, Wang Y, Xu D, Nossent J, Paylos NJ, Xu J (2018) Rheumatoid arthritis: pathological mechanisms and modern pharmacologic therapies. Bone Res 6:15
24. Zaidi D, Wine E (2018) Regulation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κβ) in inflammatory bowel diseases. Front Pediatr 6:317
25. Srinu B, Parameshwar R, Kali Charan G et al (2019) Synthesis, antitubercular activity, and molecular docking studies of novel 2-(4-Chlorobenzylamino)-4-(cyclohexylmethylamino)-pyrimidine-5-carboxamides. Russ J Gen Chem 89:836–843
26. Kumar NB, Dickinson SI, Schell MJ, Manley BJ, Poch MA, Pow-sang J (2018) Green tea extract for prevention of prostate cancer progression in patients on active surveillance. Oncotarget 9:37798–37806
27. Sun K, Song X, Jia RY, Yin Z, Zou Y, Li L, Yin L, He C, Liang X, Yue G, Cui Q, Yang Y (2018) Evaluation of analgesic and anti-inflammatory activities of water extract of *Galla Chinensis* in vivo models. Evid Based Complementary Altern Med 2018:1–7
28. Kulkarni S (2012) Handbook of experimental pharmacology, 4th edn. Vallabh Prakashan
29. Brownlee G (1950) Effect of deoxycortone and ascorbic acid on formaldehyde induced arthritis in normal and adrenalectomized rats. Lancet 28:157–159
30. Alamgeer UH, Uttra AM, Rasool S (2015) Evaluation of in vitro and in vivo anti-arthritic potential of *Berberis calliobotrys*. Bangladesh J Pharmacol 10:807–819
31. Kanojia D, Morshed RA, Zhang L, Miska JM, Qiao J et al (2015) βIII-Tubulin regulates breast cancer metastases to the brain. Mol Cancer Ther 14:1152–1161
32. Bradley PP, Priebat DA, Christensen RD, Rothstein G (1982) Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. J Invest Dermatol 78:206–209
33. Misra H, Fridovich I (1972) The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 247:3170–3175
34. Ellman GL (1959) Tissue sulphydryl groups. Arch Biochem Biophys 82:70–77
35. Sinha KA (1972) Colorimetric assay of catalase. Ann Biochem 47:389–394
36. Ma W-T, Gao F, Gu K, Chen D-K (2019) The Role of monocytes and macrophages in autoimmune diseases: a comprehensive review. Front Immunol 10:1140
37. Menegatti S, Bianchi E, Rogge L (2019) Anti-TNF therapy in spondyloarthritis and related diseases, impact on the immune system and prediction of treatment responses. Front immunol 10:382
38. Moita E, Gil-Izquierdo A, Sousa C, Ferreres F, Silva LR, Valentano P, Dominguez-Perles R, Baenas N, Andrade PB (2013) Integrated analysis of COX-2 and iNOS derived inflammatory mediators in LPS-stimulated RAW macrophages pre-exposed to *Echium plantagineum* L. bee pollen extract. PLoS ONE 8:e59131
39. Yap HY, Tee SZ, Wong MM, Chow SK, Peh SC, Teow SY (2018) The pathogenic role of immune cells in rheumatoid arthritis: implications in clinical treatment and biomarker development. Cells 7:161
40. Castano R, Miedinger D, Maghani K, Ghezzo H, Trudeau C et al (2013) Matrix metalloproteinase-9 increases in the sputum from allergic occupational asthma patients after specific inhalation challenge. Int Arch Allergy Immunol 160:161–164

41. Wang T, Jin X, Liao Y, Sun Q, Luo C, Wang G, Zhao H, Jin Y (2018) Association of NF-κβ and AP-1 with MMP-9 overexpression in 2-chloroethanol exposed rat astrocytes. Cells 7:e96
42. Liu T, Zhang L, Joo D, Sun SC (2017) NF-κβ signaling in inflammation. Signal Transduct Target Ther 2:17023
43. Fana XZ, Zhu HJ, Wu X, Yan J, Xu J, Wang DG (2014) Effects of doxycycline on cx43 distribution and cardiac arrhythmia susceptibility of rats after myocardial infarction. Iranian J Pharm Res 13:613–621
44. Cui J, Chen S, Zhang C, Meng F, Wu W, Hu R et al (2012) Inhibition of MMP-9 by a selective gelatinase inhibitor protects neuro vasculature from embolic focal cerebral ischemia. Mol Neurodegener 7:21
45. Parks WC, Mecham RP (1998) Matrix metalloproteinases, 1st edn. Academic Press, pp 1–362
46. Manicone AM, McGuire JK (2008) Matrix metalloproteinases as modulators of inflammation. Semin Cell Dev Biol 19:34–41
47. Rodriguezac D, Morrison CJ, Overall CM (2010) Matrix metalloproteinases: What do they not do? New substrates and biological roles identified by murine models and proteomics. Biochim Biophys Acta 1803:39–54
48. Giridharan S, Srinivasan M (2018) Mechanisms of NF-κB p65 and strategies for therapeutic manipulation. J Inflamm Res 11:407–419
49. Chou YC, Sheu JR, Chung CL, Chen CY, Lin FL et al (2010) Nuclear factor-kappa β (NF-κβ), a key transcription factor for the production of MMP-9, can be activated by various proinflammatory cytokines and promotes inflammation. Chem Biol Interact 184:403–412
50. Yun-Feng L, Xiang-Bo X, Xi-Hua C, Gang W, Bin H, Jie-Dong W (2012) The nuclear factor-κβ pathway is involved in matrix metalloproteinase-9 expression in RU486-induced endometrium breakdown in mice. Hum Reprod 27:2096–2106
51. Bond AJC, Andrew HB, Andrew CN (2001) Inhibition of transcription factor NF-κB reduces matrix metalloproteinase-1, -3 and -9 production by vascular smooth muscle cells. Cardiovasc Res 50:556–565
52. Deenaz Z, Eytan W (2018) Regulation of nuclear factor Kappa-Light-Chain-Enhancer of activated B cells (NF-κβ) in inflammatory bowel diseases. Font Pediatr 6:317
53. Plotnikov A, Zehorai E, Procaccia S, Seger R (2011) The MAPK cascade signaling components, nuclear roles, and mechanisms of nuclear translocation. Biochim Biophys Acta 1813:1619–1633
54. Chen B, Liu J, Ho T, Ding X, Mo YY (2016) ERK-mediated NF-κβ activation through ASIC1 in response to acidosis. Oncogenesis 5:e279
55. Legeay S, Rodier M, Fillon L, Faure S, Clere N (2015) Epigallocatechin gallate: a review of its beneficial properties to prevent metabolic syndrome. Nutrients 7:5443–5468
56. Adhami VM, Siddiqui IA, Sarfaraz S, Khwaja SI, Hafeez BB, Ahmad N, Mukhtar H (2009) Effective prostate cancer chemopreventive intervention with green tea polyphenols in the TRAMP model depends on the stage of the disease. Clin Cancer Res 15:1947–1953
57. Amdekar S, Roy P, Singh V, Kumar A, Singh R, Sharma P (2012) Anti-inflammatory activity of Lactobacillus on carrageenan-induced paw edema in male Wistar rats. Int J Inflamm 2012:752015
58. Brown JN, Roberts J (2006) Histamine, bradykinin, and their antagonists. In: Gilman AG, Hardman JG, Limbird LE (eds) Goodman and Gilman's the pharmacological basis of therapeutics, 11th edn. Mc Graw Hill Co, New York, pp 645–667
59. Bischoff SC (2008) Quercetin: potentials in the prevention and therapy of disease. Curr Opin Clin Nutr Metab Care 11:733–740
60. Almeida R, Rao VS, Matos ME (1989) Inhibition of formaldehyde-induced arthritis by a purified fraction prepared form Wilbrandia verticillata which contains novel norcucurbitacin glucosides. Braz J Med Biol Res 22:1397–1399

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