Eng. Sci., 2022, **18**, 217–223

Engineered Science

DOI: https://dx.doi.org/10.30919/es8e676

Evaluation of Anti-inflammatory Potentials of Novel Flavonols and Their Metal Complexes

Venkatachalam H,[1] Jayashree B S[2,*] and Akhila H Shrungeswara[2]

Abstract

Synthesis of novel flavonols and their complexation with Zn, Fe, Cu, and Co was carried out. The synthesized compounds were characterized by spectral methods. The characterized compounds were evaluated for their anti-oxidant activity and the test compounds exhibiting promising anti-oxidant activity were screened for their *in-vitro* anti-inflammatory activity by Cyclooxygenase (COX) and Lipoxygenase (LOX) inhibition assay. Among the synthesized compounds, three potential test compounds such as 3-(4-Benzyloxy-phenyl)-2-hydroxy-4H-naphthalen-1-one (F2), the zinc complex of F2, (F2M1) and 2-Hydroxy-3-(4-methylsulfanyl-phenyl)-4H-naphthalen-1-one (F4) were screened for their acute oral toxicity as per the Organization for Economic Co-operation and Development (OECD) guidelines and were found to be non-toxic to the animals at a dose of 2000mg/kg body weight. They were further evaluated for *in-vivo* anti-inflammatory activity both by carrageenan-induced acute paw edema method, carrageenan-induced air-pouch model, and Complete Freund's Adjuvant (CFA)-induced arthritis model.

Keywords: Anti-inflammatory activity; Anti-oxidant activity; Flavonols; Metal complexes.
Received: 04 October 2021; Revised: 11 March 2022; Accepted: 15 March 2022.
Article type: Research article.

1. Introduction

Flavonoids are naturally occurring phenolic compounds and they possess a wide variety of pharmacological activities. They easily chelate with metal ions and form complexes due to their specific chemical structure. The biological activity of an organic ligand can be enhanced when co-coordinated with a suitable metal ion because of its increased ability to act as a free radical acceptor. In a study carried out by Borissova *et al.*,[1] the Mg^{2+} complex of rutin was found to show a comparable activity concerning rutin. However, the same did not show significant activity against histamine-induced paw edema.

Li *et al.*[2] reported xylene-induced ear edema in a mouse model, where, the complexes of La^{3+}, Ho^{3+}, Yb^{3+}, Lu^{3+}, and Y^{3+} with luteolin showed comparable inhibitory activity to that of luteolin and standard dexamethasone. An increase in the anti-inflammatory activity was reported by Lippai and Speier[3] when rutin and dihydroquercetin were chelated with transition metal ions such as Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+} in an *in vitro* model of asbestos-induced cell injury. Pereira *et al.*[4] reported that, the naringin Cu(II) complex exhibited superior anti-inflammatory activity than that of naringin and was comparable to that of the control drug indomethacin. The antioxidant and anti-inflammatory potentials of the copper naringenin complex were much superior to free naringenin as reported by Dowling *et al.*[5]

In summary, the anti-inflammatory activity of flavonoid–metal complexes is governed by both flavonoids as well as the metal ion used for chelation. The anti-inflammatory activity could be associated with the antioxidant capacity of the complex. In this context, an attempt was made to synthesize the metal complexes of novel flavonols, and was purified and characterized. These compounds were further evaluated for their *in vitro* antioxidant and anti-inflammatory activity. The compounds with promising anti-inflammatory activity were further tested for their oral toxicity and *in vivo* anti-inflammatory activity.

2. Materials and methods

2.1 Synthesis of chalcones and flavonols

The chalcones intermediates used for the synthesis of flavonols were synthesized by Algar - Flynn - Oyamada (AFO) method[6] which comprises the initial formation of chalcone

[1] *Department of Chemistry, Manipal Institute of Technology, Manipal Academy of Higher Education, Manipal, Karnataka 576104, India.*
[2] *Department of Pharmaceutical Chemistry, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal, Karnataka 576104, India.*
**Email:* jayashree.sy@gmail.com (Jayashree B S)

(1,3-diaryl-2-propen-1-one) by condensing 2-hydroxyacetophenone with an aryl aldehyde in the basic medium (Claisen-Schmidt condensation) followed by oxidative cyclisation to obtain flavone. The general scheme for the synthesis of chalcones and flavonols is given in Figs. 1 and 2. The substituents R_1, R_2, R_3, R_4, and Ar in the general scheme of synthesis of chalcones and Flavonols are given in Table 1. The structure, chemical name, and experimental data of flavonols are reported in Table S1 of the supporting document.

The methanolic solution of these flavonols was treated with salts of zinc, iron, copper, and cobalt in equimolar proportion and stirred constantly to yield the complex as a precipitate and was kept aside for the evaporation of the solvent, washed with water, dried, and stored in a desiccator.

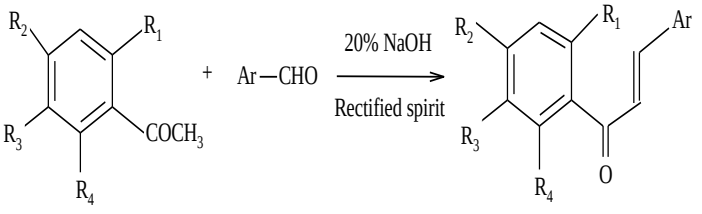


Fig. 1 General scheme for the synthesis of chalcones.

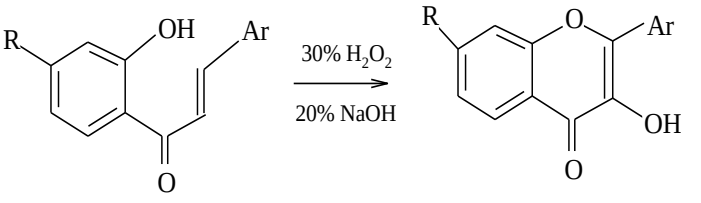


Fig. 2 General reaction for the synthesis of flavonols.

2.2 General Method of synthesis of complexes

0.001 mol of the flavonoids were dissolved in 10 mL of methanol by warming and 0.001 mol of the corresponding salts of Zn, Fe, Cu, and Co were added to the hot solution with constant stirring. During the preparation of Zn, Fe, and Co complexes, an inert atmosphere was maintained by passing nitrogen gas to prevent the interaction of atmospheric air and moisture. Stirring was continued till the complex got precipitated and the precipitated complex was kept aside for the evaporation of the solvent, washed with distilled water, dried, and stored in a desiccator. Proposed reactions for the formation of complexes are given in Figs. S1 and S2 of the supporting document.

Various metal salts used for complexation were $ZnCl_2$, $FeSO_4 \cdot 7H_2O$, $Cu(CH_3COO)_2 \cdot H_2O$ and $CoCl_2 \cdot 6H_2O$. Experimental data of complexes are given in Table 2.

Table 1. Substitutions in the chalcones and flavonols.

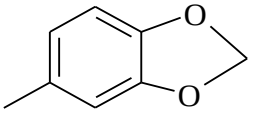
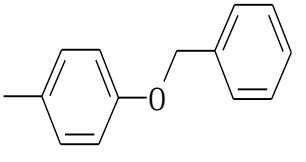
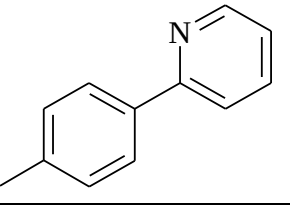
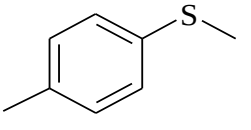
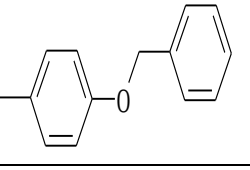
Compound Code	R_1	R_2	R_3	R_4	Ar
C1	OH	H	H	H	
C2	OH	H	H	H	
C3	OH	H	H	H	
C4	OH	H	H	H	
C5	OH	OH		H	

Table 2. Experimental data of complexes.

Compound code	Proposed Mol. Formula	Mol. Weight	% Yield	M. P (˚C)	R_f Value*
F1M1	$C_{16}H_9O_5ZnCl_2$	416	88	222	0.51
F1M2	$C_{16}H_9O_5FeSO_4$	432	76	230	0.48
F1M3	$(C_{16}H_9O_5)_2Cu(CH_3COO)_2$	743.6	60	314	0.56
F1M4	$C_{16}H_9O_5CoCl_2.2H_2O$	445.9	63	218	0.60
F2M1	$C_{23}H_{17}O_3ZnCl_2$	477.3	78	168	0.62
F2M2	$C_{23}H_{17}O_3FeSO_4$	492.9	76	178	0.54
F2M3	$(C_{23}H_{17}O_3)_2Cu(CH_3COO)_2$	863.6	68	290	0.70
F2M4	$C_{23}H_{17}O_3\ CoCl_2$	470.8	58	180	0.59
F3M1	$C_{20}H_{12}NO_3ZnCl_2$	450.3	72	232	0.63
F3M2	$C_{20}H_{12}NO_3FeSO_4$	465.9	64	272	0.55
F3M3	$(C_{20}H_{12}NO_3)_2Cu(CH_3COO)_2$	876.7	69	338	0.45
F3M4	$C_{20}H_{12}NO_3\ CoCl_2$	443.8	68	212	0.51
F4M1	$C_{17}H_{13}O_2SZnCl_2.H_2O$	437	71	218	0.54
F4M2	$C_{17}H_{13}O_2SFeSO_4$	432.9	82	226	0.58
F4M3	$(C_{17}H_{13}O_2S)_2Cu(CH_3COO)_2$	743.6	85	290	0.62
F4M4	$C_{17}H_{13}O_2S\ CoCl_2$	410.8	56	226	0.74
F5M1	$C_{22}H_{15}O_5ZnCl_2$	495.3	68	214	0.68
F5M2	$C_{22}H_{15}O_5FeSO_4$	510.9	72	208	0.72
F5M3	$(C_{22}H_{15}O_5)_2Cu(CH_3COO)_2$	899.6	69	238	0.66
F5M4	$C_{22}H_{15}O_5\ CoCl_2$	488.8	76	224	0.64

*Ethyl acetate:n-hexane – 1:3

Characterization and purity assessment

The complexes were recorded for UV-vis peaks from 190 – 800 nm on a Shimadzu UV-Vis spectrophotometer (model - UV 1650 Japan), IR in the range of 400 – 4000 cm^{-1} on Shimadzu GC-MS QP5050 Japan, NMR in the range of 0 – 12 ppm TMS Scale on Bruker Ascend 400 MHz spectrometer and mass spectra using Shimadzu GC-MS QP5050 Japan.[7]

The synthesized compounds were tested for their antioxidant activity by DPPH• and ABTS radical scavenging and nitric oxide scavenging activity.[8] Among these compounds, 8 test compounds exhibited promising anti-oxidant activity and they were taken further for the evaluation of anti-inflammatory activity by *in vitro* methods such as Cyclooxygenase (COX) and Lipoxygenase (LOX) inhibition assay. The interaction of our compounds with LOX and COX enzymes was studied using an assay kit (Cayman chemical - product code 760700 and 701050 respectively).

2.3 Evaluation of Anti-inflammatory activity

2.3.1 Lipoxygenase (LOX) inhibition assay

The test compound solutions were prepared with a concentration of 250 µM and serially diluted to get 25 and 2.5 µM concentrations. Curcumin was used as a standard with a concentration of 75µM. The Lipoxygenase assay was performed as per the manufacturer's instructions. The percent inhibition or percent IA for each inhibitor was determined using one of the following equations,

$$\%\ \text{inhibition} = \left[\frac{\text{I A-Inhibitor}}{\text{I A}}\right] \times 100$$

$$\%\ \text{I A} = \frac{\text{Inhibitor}}{\text{I A}} \times 100$$

The IC_{50} value (concentration at which there was 50% inhibition) was determined by plotting a graph of Percent Inhibition or Percent Initial Activity as a function of the inhibitor concentration.

2.3.2 Cyclooxygenase COX inhibition assay

The Cayman's COX colorimetric inhibitor screening assay involved the measurement of the peroxidase component of COXs. The peroxidase activity was estimated colorimetrically by monitoring the appearance of oxidized N, N, N', N'-tetramethyl-p-phenylenediamine (TMPD) at 590 nm.

The test compound solutions were prepared with a concentration of 250 µM and serially diluted to get 25 and 2.5 µM concentrations. Curcumin was used as standard with a concentration of 75 µM. The COX enzyme assay was performed as per the manufacturer's instructions. Calculations were performed similarly to that of LOX assay.

2.3.3 Acute oral toxicity studies

The acute oral toxicity studies for the selected compound were performed as per the OECD 425 guideline in female Wistar rats. Synthetic flavonoids are congeners of natural flavonoids which are consumed in food. Hence, as per the guidelines, a limit dose test was performed initially before doing the main test. A dose of 2000 mg/kg body weight was administered orally to one rat and observed for 48 h for mortality. After 48 h, another rat was dosed with the same dose and was observed in a total of five rats. The animals were observed at the cage side for each hour for the first 24 h and then every 6 h for 48 h. Further, these rats were kept for long-term observation for 14 days. The lethality was noted for 72 h.

Carrageenan-induced paw-edema model was carried out on Wistar rats weighing between 120-150 g and the Carrageenan-induced air pouch model as well as Complete Freund's Adjuvant (CFA)-induced arthritis model was studied on Wistar rats weighing 180-230 g, procured from Central Animal Research Facility, Manipal University. The rats were maintained at 12 h light and dark cycle at a temperature and humidity of 26 ± 1 °C and 50 ± 5 % respectively in plastic cages. The standard food pellet and water *ad libitum* were provided to all the rats. The experiments were performed after obtaining the approval of the experimental protocol from the Institutional Animal Ethical Committee (No. IAEC/KMC/55/2013).

2.3.4 Carrageenan-induced paw-edema model in rats

Test compounds F2, namely 3-(4-Benzyloxy-phenyl)-2-hydroxy-4H-naphthalen-1-one, and its complex with zinc, F2M1, and F4 (2-Hydroxy-3-(4-methylsulfanyl-phenyl)-4H-naphthalen-1-one) were tested and taken for acute paw inflammation as per the method described by Mudgal *et al*.[9] Diclofenac 10 mg/kg was used as a positive control. The test compounds were dosed by oral route, after 30 min of the treatment, 0.1 mL of freshly prepared carrageenan in 1% w/ v normal saline was injected into the sub-plantar region of the left hind paw of male Sprague Dawley (SD) rats to induce acute inflammation (naive control group was administered with normal saline in place of carrageenan), using a digital plethysmometer, the paw volume was measured (in milliliters) just before carrageenan injection, and successively at 1, 2, 3 and 5th hour after injecting carrageenan. The difference in the paw volumes was calculated by subtracting the basal paw volume from the inflamed paw volume at each time point.

2.3.5 Carrageenan-induced air-pouch model in rats

Inflammation of the Air-pouch was attained as per the literature[10] with required modifications. The air-pouch was developed by injecting 20 mL of sterile air using a 0.22 mm syringe filter unit (Millipore, USA) to the shaved dorsal part of Wistar rats on day 1, day 3, and day 5. Further 10 mL of air was injected into the same air pouch. Test compounds namely, F2 and F2M1 were selected for the study at doses 50, 100, and 200 mg/kg. Diclofenac (10 mg/kg) was used as a positive control. Rats were administered with test compounds/standard. 2 mL of 1% w/v carrageenan in saline was later injected into the air-pouch after 30 minutes to trigger the inflammation.

Blood was collected from the retro-orbital plexus of all the rats 6 h after the carrageen challenge following which, the rats were sacrificed, and air-pouch-lavage was collected and tested for inflammatory markers. The protection observed by the test compounds and the reference standard, diclofenac was assessed based on comparing the air pouch lavage volume, spleen weight, and level of inflammatory markers such as nitrite and myeloperoxidase (MPO) with that of the carrageenan control.

2.3.6 Estimation of inflammatory markers in plasma

After 6 h of carrageenan challenge, blood was withdrawn from the retro-orbital plexus, and collected in centrifuge tubes holding 10% potassium-EDTA. The plasma was collected and stored at -80 °C after centrifuging (8000 rpm, 10 min at 4 °C) the blood using a cooling micro-centrifuge.

2.3.7 Estimation of inflammatory markers in air pouch lavage

Rats were sacrificed after 6 h of administering carrageenan and the air pouch was washed thrice with 5 mL of cooled sterile saline. The lavage was aspirated by using the syringe after a gentle massage. The volume of the lavage was measured and the total leucocyte count was quantified using a Veterinary Blood Cell Counter. MPO and nitrite levels in air-pouch lavage were measured as per the procedures described below.

2.3.8 Estimation of MPO and nitrite in air pouch lavage

MPO activity in lavages was determined by previously reported methods with minor amendments.[11]

Nitrite levels were estimated in air pouch lavage by using Griess Reagent, adopting the established procedure.[12] In a clear 96-well plate, 100 µl of Griess reagent was added to a 100 µl of the supernatant medium, incubated at 37 °C for 20 min, and the absorbance was measured at λ value of 540 nm.

2.4 Complete Freund's Adjuvant (CFA)-induced arthritis model in rats

Wistar rats were randomized into different treatment groups

(six rats per group, a total of six groups) based on their body weight. The control group was administered with an intra-plantar injection of 0.1 mL of 5 mg/mL of Complete Freund's Adjuvant (CFA), to the left hind paw whereas the naive control group received 0.1 mL paraffin oil. Treatment groups received CFA along with the test compound (F2) at three different doses such as 50 mg, 100 mg, and 200 mg/kg. All the groups were orally dosed with the test compound and standard drug diclofenac (10 mg/kg) from day 8 to day 21 (once daily, a total of 14 days).

The CFA was freshly prepared as a suspension in paraffin oil with *Mycobacterium butyricum* (Product# 264010, DIFCO). On day 1, male Wistar rats were administered with an intraplantar injection of 0.1 mL of 5 mg/mL of Complete Freund's Adjuvant (CFA), to the left hind paw (ipsilateral paw) whereas naive control rats were administered with 0.1 mL paraffin oil. Attributes such as body weight, clinical scoring, and ipsilateral and contralateral paw (non-induction paw) volume were measured on days 1, 7, 14, and 21 respectively and the difference in the paw volume was calculated by subtracting the basal paw volume on day 1 (before the CFA injection). Further, on day 21, the whole blood was collected by a retro-orbital puncture for hematological analysis using the Veterinary Blood Cell Counter (PCE-210VET, Erma Inc., Tokyo, Japan). Further, the radiographic changes were determined by X-ray of the hind and ipsilateral paws of the animals along with their hematological parameters on day 21.

The radiological alterations were recorded for the severity of arthritis on day 21 at Oral Medicine and Radiology, Kasturba Medical College, Manipal University. The rats were anesthetized and radiographs of both the ipsilateral and contralateral paws were recorded using an x-ray (Model 2100, Kodak Co., Japan). The machine was operated at a 60 kV peak, at 8 mA, the time of exposure was 0.08 sec, and the focus distance was 40-50 inches.[10]

3. Results and discussion

3.1 Characterization of the compounds

The molecular ion peak IR, ^{1}H NMR, and Mass spectral data obtained for the synthesized flavones, flavonols, and their complexes were in support of the proposed structures as shown in the spectral characterization data given in the supporting document.

The complexes of flavonols were analyzed volumetrically for the estimation of metals. Zinc and cobalt were estimated by titration against EDTA, whereas, iron was estimated using potassium dichromate solution and copper was estimated using the iodometric method. The results obtained by volumetric methods were validated by atomic absorption spectrometry (AAS).

The results of the volumetric analysis and AAS are given in Table S2 of the supporting document.

The IR spectra for flavones exhibited a prominent absorption band in the region of 1606 – 1704 cm^{-1}, characteristic of the presence of α, β-unsaturated carbonyl group, and other band observed in the region of 3257 – 3625 cm^{-1} may be due to the presence of phenolic -OH group on ring A.

The IR spectra for the synthesized complexes exhibited prominent absorption in the region of 1600 – 1613 cm^{-1} corresponding to the carbonyl group and a band in the region of 3216 – 3504 cm^{-1} corresponding to the phenolic – OH group of the ring B. There was a slight shift in both these peaks, which could be attributed to the involvement of the carbonyl group and 3-OH group in forming the coordinate bond with the metal ion.

Various peaks observed in ^{1}H NMR spectra for all the chalcones, flavones, and flavonols were concordant with the proposed structures. The molecular ion peaks obtained from the mass spectra for all the flavonols were also in concordance with their structures.

To confirm this, ^{1}H NMR studies were carried out. However, because of the solubility issues and interference of the metal ions, a ^{1}H NMR study for all the complexes was not possible. ^{1}H NMR spectra for a few flavonol-zinc complexes were recorded with good resolution.

The absence of a signal at δ, in the range of 9.15 – 9.67 ppm corresponding to the proton of 3-OH group in ^{1}H NMR spectra for the zinc complexes flavonols such as F1, F2, and F4 and cobalt complex of F4 indicated for the interaction of the 3-OH group in the formation of the complex.

The stoichiometry of the complexes was ascertained by Job's method.[13]

3.2 Anti-inflammatory activity

The compounds exhibiting promising antioxidant activity were taken for their *in vitro* anti-inflammatory activity by LOX and COX inhibition assay. The results of the LOX inhibition assay are given in Table 3. The standard used for this study was curcumin at a concentration of 75 µM. However, it did not show any inhibition at this concentration.

Results of *in vivo* Anti-inflammatory activity are given in Figs. S3-S18 in the supporting document.

Table 3. LOX inhibition assay.

Compound Code	Percentage Inhibition at a concentration (Mean ± SEM)		
	250 µM	25 µM	2.5 µM
F1	12.9 ± 0.24	10.5 ± 0.19	12.9 ± 0.14
F1M1	14.3 ± 0.12	17.1 ± 0.09	15.7 ± 0.2
F1M2	16.2 ± 0.09	14.3 ± 0.08	17.1 ± 0.11
F1M4	15.7 ± 0.20	21.4 ± 0.3	18.6 ± 0.25
F2	7.1 ± 0.09	15.2 ± 0.11	19 ± 0.13
F2M1	------	18.6 ± 0.26	19 ± 0.3
F2M4	2.9	17.6 ± 0.28	18.1± 0.25
F4	9.5	20 ± 0.21	19.5 ± 0.16

3.2.1 COX – LOX inhibition by test compounds

The compounds such as F1, F1M1, F1M2, F2, F2M1, F2M4, and F4 exhibiting promising antioxidant activity following the

DPPH, ABTS radical scavenging, and lipid peroxidation methods were taken further for in vitro anti-inflammatory activity both by LOX and COX inhibition assay. It was found that the tested compounds exhibited low enzyme inhibition for LOX assay and did not exhibit a concentration-dependent inhibition of the enzyme.

Further, test compounds such as F1M4 exhibited maximum inhibition of 21% at a concentration of 25 μM, whereas compounds F2 and F2M1 exhibited inhibition of 19% at 2.5 μM concentration. However, the standard, Curcumin did not show any inhibition at 75 μM concentration.

The test compounds which exhibited anti-inflammatory activity by the LOX enzyme inhibition assay did not show any activity for the COX inhibition assay.

3.2.2 Acute oral toxicity

After careful consideration of the results obtained based on antioxidant and enzyme inhibition assays, three potential candidates namely, F2, F2M1, and F4 were evaluated for their evaluation by in vivo anti-inflammatory activities such as Carrageenan-induced acute paw edema, Carrageenan-induced air pouch, and CFA-induced arthritis models.

These candidates were initially screened for their acute oral toxicity, determined according to OECD 425 guidelines. During this acute toxicity study, it was found that, even after the administration of a maximum of 2000 mg/kg of the test compounds, mortality was not observed. Thus, they were found non-lethal and non-toxic to the animals and were taken further for in vivo studies.

3.2.3 Effect of selected test compounds on Carrageenan-induced acute paw edema

Three test compounds such as F2, F2M1, and F4 were evaluated for their potency against carrageenan-induced acute paw edema. It was observed that the treatment with the test compound F2 did not show any significant decrease in the paw edema in rats at 1hr duration after the treatment. However, at the dose level of 200 mg/kg, it showed a significant reduction in the paw edema after 2 h of exposure to carrageenan treatment. Further, at a 3 h duration after the treatment, it showed a reduction in the paw edema at 200 mg/kg. Again with a dose of 50 mg/kg, the test compound showed a substantial reduction in the paw edema 5 h after the treatment.

However, the compound F2M1 did not show any significant decrease in the paw edema at 1hr and 2 h duration after the treatment. However, it showed a significant reduction in the paw edema 3 h after the treatment at a dose of 400mg/kg.

Compound F4 did not elicit any considerable reduction in the paw edema caused by carrageenan administration at 1h, 2h, and 3h duration after the treatment. The result of carrageenan-induced acute paw edema is given in Figs. S3-S10 of the supporting document.

3.2.4 Effect of the selected test compounds on carrageenan-induced air-pouch

Two of the promising test compounds F2 and its zinc complex, F2M1 were selected based on the results obtained from carrageenan-induced acute paw edema on Wistar rats, for further evaluation by carrageenan-induced air-pouch model. It was found that both F2 and F2M1 treatments did not show any difference in spleen weights, air pouch lavage volume, and nitrite levels in air pouch lavage. Whereas, both the treatments did show a significant difference in myeloperoxidase levels (MPO) in air-pouch lavage compared to positive control. The results are shown in Figs. S11-S13 of the supporting document.

3.2.5 Effect of the selected test compound on CFA-induced arthritis

Test compound F2 was further evaluated against chronic inflammation and it was observed that the increase in the paw volume was effectively reduced by the treatment with F2 (100 mg/kg) and it protected the increase in the WBC count in arthritis-induced rats when compared to the positive control. Further, compound F2 could also protect the increased monocytes and lymphocytes compared to the disease control, and the reduction in the body weight was less compared to that of the standard drug group as well as the disease control group. Further, the total WBC count in CFA-induced arthritis drastically decreased when compared to that of the normal control. The decrease in the total WBC and the absolute count could be due to the induction of arthritis as well as the distress induced by the disease. Further, the test compound F2 protected in lowering both the monocytes and lymphocytes at a dose of 200 mg/kg. The percentage protection obtained in terms of reduction in WBC was far more than that of the standard diclofenac.

To further support this, the radiographs obtained by X-ray showed protection against inflammation both in contralateral and ipsilateral paws of CFA-induced rats and was comparable to that of the standard diclofenac as shown in Table S2 of the supporting document. The anti-inflammatory activity of the flavonols could be attributed to their modulation of multiple inflammatory pathways involving inhibition of the enzyme, antioxidant activity, and inhibition of NF-κB.[14] Similarly, the compounds F2, F2M1, and F4 exhibited anti-inflammatory action and it could be partly by the anti-oxidant property, inhibition of prostaglandins synthesis, and immune modulation.

4. Conclusion

Flavonols and their complexes were synthesized, characterized, and evaluated for their antioxidant activity. Out of the eight test compounds evaluated for their *in-vitro* anti-inflammatory activity, three potential test compounds such as F2, F2M1, and F4 were selected and screened for their acute oral toxicity. Further, these three test compounds were evaluated for their *in vivo* anti-inflammatory activity by the Carrageenan-induced acute paw edema method. Test compound, F2 was also evaluated against chronic inflammation and exhibited considerable results. Therefore,

these compounds can be further studied to elucidate the exact mechanism of action.

Acknowledgment
The authors thank Manipal Academy of Higher Education, Manipal, India for providing the facilities to perform the studies.

Conflict of Interest
The authors declare no conflict of interest.

Supporting information
Applicable.

References
[1] P. Borissova, S. Valcheva, A. Belcheva, *Acta Physiologica et Pharmacologica Bulgarica*, 1994, **20**, 25-30.
[2] J. Li, L. Wang, H. Bai, B. Yang, H. Yang, *Medicinal Chemistry Research*, 2011, **20**, 88-92, doi: 10.1007/s00044-009-9289-2.
[3] I. Lippai, G. Speier, *Journal of Molecular Catalysis A: Chemical*, 1998, **130**, 139-148, doi: 10.1016/s1381-1169(97)00208-2.
[4] R. Pereira, N. Andrades, N. Paulino, A. Sawaya, M. Eberlin, M. Marcucci, G. Favero, E. Novak, S. Bydlowski, *Molecules*, 2007, **12**, 1352-1366, doi: 10.3390/12071352.
[5] S. Dowling, F. Regan, H. Hughes, *Journal of Inorganic Biochemistry*, 2010, **104**, 1091-1098, doi: 10.1016/j.jinorgbio.2010.06.007.
[6] T. Oyamada, *Bulletin of the Chemical Society of Japan*, 1935, 10, 182-186.
[7] L. G. Naso, V. R. Martínez, E. G. Ferrer, P. A. M. Williams, *Journal of Trace Elements in Medicine and Biology*, 2021, **64**, 126690, doi: 10.1016/j.jtemb.2020.126690.
[8] H. Venkatachalam, Y. Nayak, B. S. Jayashree, *International Journal of Chemical Engineering and Applications*, 2012, 216-219, doi: 10.7763/ijcea.2012.v3.189.
[9] J. Mudgal, V. S. Gowdra, G. Mathew, P. G. Nayak, N. D. Reddy, N. Namdeo, R. R. Kumar, C. Kantamaneni, M. R. Chamallamudi, G. K. Nampurath, *Chemico-Biological Interactions*, 2014, **210**, 34-42, doi: 10.1016/j.cbi.2013.12.012.
[10] S. Talwar, K. Nandakumar, P. G. Nayak, P. Bansal, J. Mudgal, V. Mor, C. M. Rao, R. Lobo, *Journal of Ethnopharmacology*, 2011, **134**, 323-328, doi: 10.1016/j.jep.2010.12.015.
[11] P. P. Bradley, R. D. Christensen, G. Rothstein, *Blood*, 1982, **60**, 618-622, doi: 10.1182/blood.v60.3.618.bloodjournal603618.
[12] G. Mathew, A. Jacob, E. Durgashivaprasad, N. D. Reddy, M. K. Unnikrishnan, *International Immunopharmacology*, 2013, **15**, 182-189, doi: 10.1016/j.intimp.2012.10.028.
[13] D. C. Harris, *Nature*, 1968, **100**, 304, doi: 10.1038/100304b0.
[14] L. Wang, Y.-C. Tu, T.-W. Lian, J.-T. Hung, J.-H. Yen, M.-J. Wu, *Journal of Agricultural and Food Chemistry*, 2006, **54**, 9798-9804, doi: 10.1021/jf0620719.

Author information

***Venkatachalam H**, Teaching experience over 18 years. The area of research interest is pharmaceutical chemistry and involved in the synthesis and activity evaluation of molecules of biological importance.*

***Jayashree B S**, presently working as a professor at Manipal college of Pharmaceutical Sciences, Manipal and has a teaching experiences of 33 years. Guided 50 PG students both in Pharmaceutical chemistry and Regulatory affairs and 11 Ph D students. Received various research grants from government funding agencies such as AICTE, INSA DST. Published around 100 research papers both in National and international Journals. Granted one patent and the second one is being filed. A board member for many universities and widely travelled to many countries for presenting research work and have been invited as a speaker both at national and International level. Reviewer and editorial board member for many j reputed journals.*

***Dr Akhila H Shrungeswara**, Completed post graduate degree in biochemistry from University of Mysore and qualified GATE-2013 with an All India Rank 166 out of 12920 also qualified K-SET (Karnataka State Eligibility Test for Lectureship). Area of research includes inflammation, metabolic disorders, cell signaling and molecular biology.*

Publisher's Note: Engineered Science Publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.