Original Research Article

ANTI-INFLAMMATORY EFFECT OF THREE NOVEL HYDROXY FLAVONES

Abstract

The aim of the present study was to evaluate the anti-inflammatory effect of three 7-hydroxy flavone derivatives; 7-HF, 7,3',4'-THF, and 7,8,3'-THF, **Materials and methods** The acute anti-inflammatory effect of 7-hydroxy flavones was the effect of 7-hydroxy flavones on certain mediators of pain and inflammation like cyclooxygenases (COX-1 and COX-2), and pro-inflammatory cytokines (IL-1b and TNF- were investigated by using in vitro tests. **Results** The investigated 7hydroxy flavones produced a significant, the test compounds inhibited both the isoforms of cyclooxygenase, a higher degree of inhibition on COX-2 was evident. Concentration-dependent inhibition of other inflammatory cytokines like tumour necrosis factor-a and interleukin-1b was identified in the present study **conclusion** of the present study reveal the pain and anti-inflammatory action of the investigated 7hydroxy flavones. The inhibitory effects of 7hydroxy flavone on various pro-inflammatory cytokines provide evidence for the mechanism of action of these compounds against pain and inflammation.

Keywords: Anti-inflammatory effect; Cyclooxygenases; Interleukin-1b .Flavone.

Introduction

Non-steroidal anti-inflammatory medicines (NSAIDs) are a class of pharmaceuticals that have anti-inflammatory, analgesic, and antipyretic properties. Glucocorticoids are also the cornerstone of

treatment for a number of chronic inflammatory and autoimmune illnesses. However, many people are unable to use them because of their intrinsic side effects. As a result, anti-inflammatory medications that are both safe and effective are being aggressively sought all over the world. Flavonoids appear to be promising as safe and effective anti-inflammatory medications among numerous types of experimental agents. Despite the fact that flavonoids have a number of important pharmacological properties, their therapeutic utility is limited due to their low bioavailability. However, Flavones have a higher bioavailability than other derivatives, according to a recent study[1], implying that flavonoids could be used to treat a wide spectrum of illnesses. Flavonoids are polyphenolic chemicals found in a wide range of angiosperms that have a wide range of health benefits and pharmacological activities. Many natural and synthetic flavone derivatives have been studied for their analgesic and anti-inflammatory properties in recent decades. In acute and chronic animal models of inflammation, flavonoids such as hesperidin, apigenin, luteolin, and, gossypin[3], titonine[4] and its derivatives, silymarin[5], and naringin[6]The anti-inflammatory effect of flavonoids was further validated by studies on a few monomethoxy flavones[7], monohydroxy flavones[8], and dihydroxy flavones[9,10]. A few structurally similar dimethoxy flavones were examined for their pharmacological activities in a recent study[11]. These dimethoxy flavones have been found to have potent antinociceptive action, especially in inflammatory pain models. As a result of this discovery, the anti-inflammatory potential of dimethoxy flavones was investigated, and the results were evaluated in this study. The effect of these compounds on important mediators of inflammation such as interleukin-1b (IL-1b), tumour necrosis factor-alpha (TNF-α), and the generation of prostaglandins were also investigated using appropriate in vitro techniques.

Material and methods

Carrageenan (Sigma Chemical Co., USA) was used as a phlogistic agent and diclofenac sodium (Novartis) was used as a standard anti-inflammatory agent. Diagnostic kits (Cayman., USA) were used for the in vitro assay of cyclooxygenases, IL-1b and TNF-α.

Effect of dimethoxy flavones on cyclooxygenase (COX) enzyme by immunoassay

Cyclooxygenase assay was carried out using COX (ovine) inhibitor screening assay (Cayman, USA). This assay directly measures prostaglandin-F_2 (PGF_{2a}) which is derived from stannous chloride reduction of PGH_2 produced in the cyclooxygenase reaction. 50µ of PGH_2 and various dimethoxy flavones dissolved and diluted in dimethyl sulfoxide (DMSO) to 5–50 lM concentration were incubated with reaction buffer and arachidonic acid. The reaction was allowed to proceed for 2 min and was quenched by addition of 50 µ of stannous chloride solution. The reaction was allowed to proceed for an additional 10 min. Further serial dilutions were made with the buffer and PGF_{2a} was mea- sured using enzyme immuno assay (EIA). The amount of PGF_{2a} was directly proportional to the amount of COX-1 and COX-2 present in the well. Ibuprofen and celecoxib were used as standard COX-1 and COX-2 inhibitors respectively.

Effect of dimethoxy flavones on cytokines— Interleukin-1b (IL-1b) and Tumor necrosis factor-a (TNF-a)

Freshly heparinised human whole blood was used for the immunometric assay, which is based on a double antibody ‘‘sandwich’’ technique. Microwell plates supplied with the commercial kit (Cayman, USA) have been coated with monoclonal antibody specific for TNF-a or IL-1b that can capture any TNF-α or IL-1b introduced into the well. Various dimethoxy flavones dissolved and diluted in DMSO to different concentrations (20–500 lM) were added to the well. Acetylcholinesterase (AChE) 50 **µ** that binds selectively to a different

epitope on the TNF-a or IL- 1b molecule, was also added to the well. When TNF-α or IL-1b (standard or sample) is introduced, the two anti- bodies form a sandwich by binding on opposite sides of the TNF-α or IL-1b molecule. The concentration of the analyte was then determined by measuring the enzymatic activity of the acetylcholinesterase by adding Ellman's reagent (which contains the substrate for AChE) to each well. The yellow color of the product of the AChE-catalyzed reaction was read spectrophotometrically at 412 nm. The intensity of this color was directly proportional to the amount of bound conjugate which in turn was proportional to the concentration of the TNF-α or IL-1b. Dexamethasone was used as a standard for both TNF-α and IL-1b assays

Results

Effect of trihydroxy flavones on cyclooxygenase – 1 (table-1)

The investigated hydroxy flavones inhibited cyclo oxygenase-1 activity in a concentration-dependent manner. Among the tested compounds, 7-hydroxy flavone produced a maximum inhibition of 86.23% in a 50 -M concentration. In a similar concentration 7,3'4'- THF produced inhibition of 78%, While 7,8,3', THF produced an inhibition of 67.24% (table-1). The IC_{50} values recorded for these compounds in increasing order are as follows: 7,3',4' – THF = 28.04 µM, 7,8,3' – THF = 35.81µM, 7 – HF = 22.72µM. The IC_{50} value of ibuprofen in this assay procedure was found to be14.68 **µ**M.

TABLE – 1
Effect of trihydroxy flavones on Cyclooxygenase – 1

Concentration (-M)	**Treatment mg/Kg, sc and** **% Inhibition**

	7-HF	7, 3',4'- THF	7,8,3' – THF
10	27.5	24.3	19.12
20	45.97	36.23	32.55
30	69.3	54.68	40.55
40	73.87	70.78	55.79
50	86.23	77.89	67.24
IC_{50} (-M)	**22.72565**	**28.04135**	**35.81687**

Each value represents the mean of 3 observations
* IC_{50} value calculated by linear regression analysis

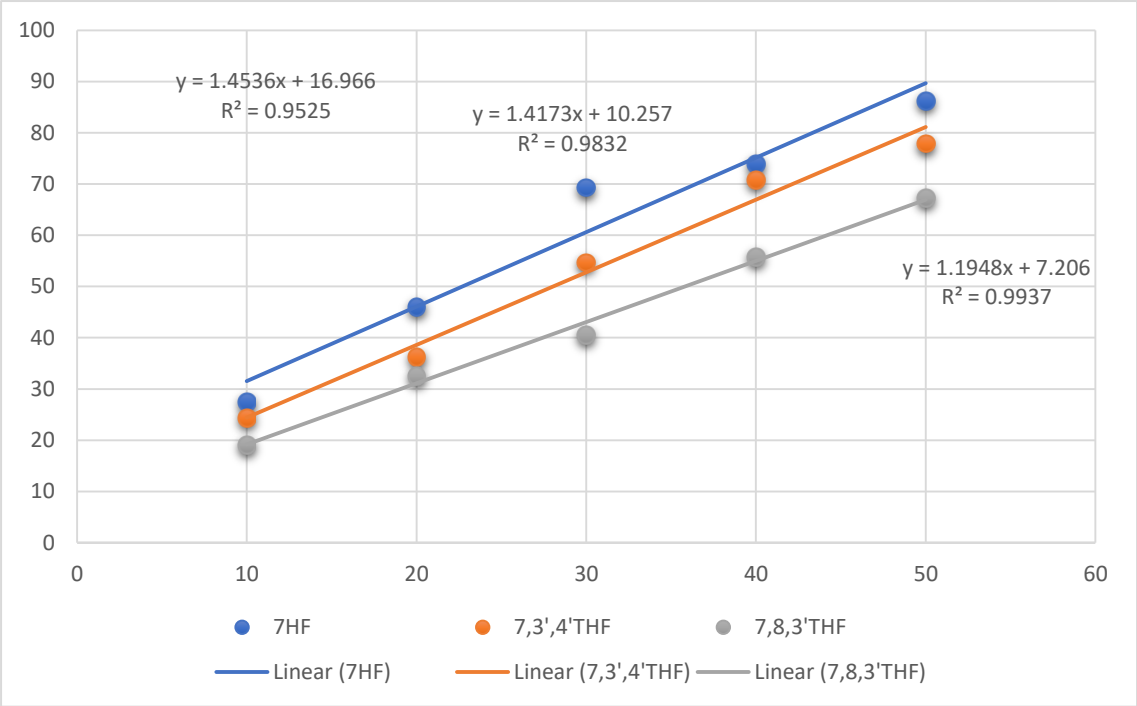

Fig 1: Effect of trihydroxy flavones on Cyclooxygenase – 1

5.5. Effect of trihydroxy flavones on cyclooxygenase 2(TABLE – 2)

Concentration-dependent inhibition of cyclooxygenase – 2 was clearly evident for all the tested trihydroxy flavones. A maximum of 71% inhibition was recorded for 7,3',4'-THF in a concentration of 50**µ**M. In the same concentration maximum inhibition of 59.18.89%, and 67.9% was recorded for 7 – HF, 7,8,3'- THF respectively (Table 2). The IC_{50} values recorded for the above compounds to inhibit cyclooxygenase – 2 are as follows: 7,8,3'–THF

= 32.32 µM, 7,3',4' – THF = 30.91µM , 7 – HF = 38.92 µM.The IC_{50} value for celecoxib in the same assay procedure was found to be 2.24 **µ**M.

It is pertinent to point out that all the investigated 7 hydroxy flavones almost equally inhibited both cyclooxygenase - 1 and cyclooxygenase – 2. The IC_{50} values for inhibition of cyclooxygenase 1 and 2 for these compounds are also almost identical.

TABLE – 2
Effect of trihydroxy flavones on Cyclooxygenase –2

Concentration (µM)	**Treatment mg/Kg, sc and % Inhibition**		
	7-HF	**7, 3',4' – THF**	**7,8,3'– THF**
10	17.48	24.52	25.36
20	30.97	39.91	39.54
30	45.97	49.76	46.43
40	50.48	59.48	58.68
50	59.18	71.18	67.9
IC_{50} (µM)	**38.9243**	**30.91239**	**32.32009**

Each value represents the mean of 3 observations
* IC_{50} value calculated by linear regression analysis.

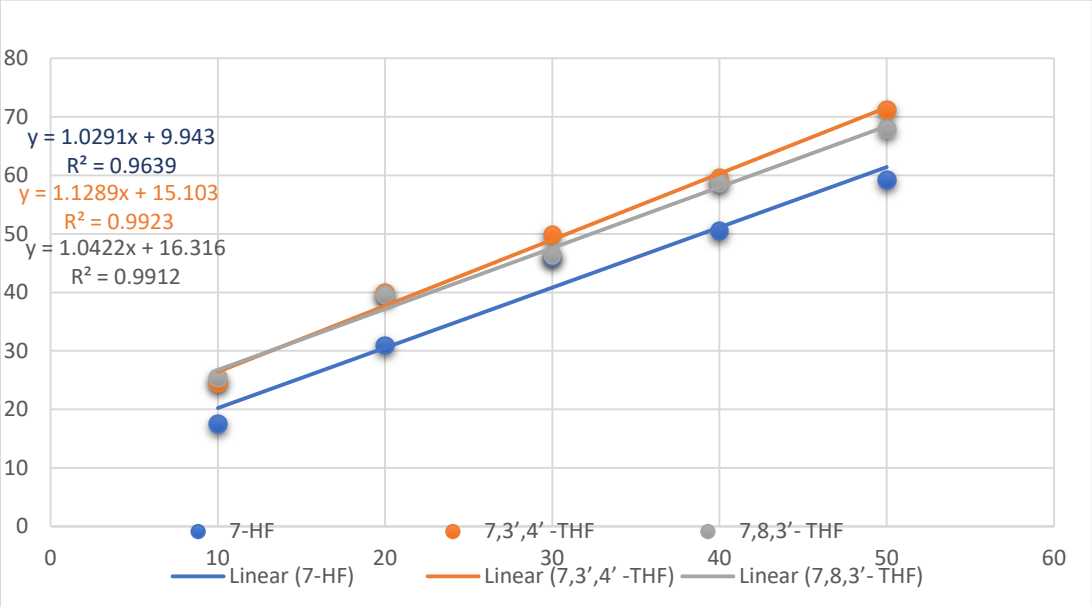

Fig 2: Effect of trihydroxy flavones on Cyclooxygenase –2

5.6.Effect of 7 hydroxy flavones on interleukin – 1β (TABLE 3)

Concentration-dependent inhibition of interleukin – 1β activity was recorded for all the tested trihydroxy flavones. Nearly 80.56% inhibition was recorded for 7- HF in a concentration of 50 □M. 7,8,3'- THF exhibited a maximum inhibition of 78.86% in the same concentration while that recorded for 7,3',4'- THF was 74% The IC_{50} values recorded for the above hydroxy flavones areas follows 7, 8, 3' – THF = 122.56 µM, 7, 3', 4' – THF = 128.89 µM, and 7 - HF = 138.01 µM

TABLE – 3
Effect of trihydroxy flavones on Interleukin – 1β

Concentration (-M)	**Treatment mg/Kg, sc and % Inhibition**		
	7-HF	**7,3',4'-THF**	**7, 8, 3'- THF**
10	26.23	21.24	20.46
20	35.89	29.82	29.21
30	40.12	38.76	39.09
40	64.98	59.45	64.98
50	**80.56**	73.96	**78.86**
IC_{50} (-M)	**138.01**	**128.89**	**122.56**

Each value represents the mean of 3 observations
* IC_{50} value calculated by linear regression analysis

The IC_{50} value of dexamethasone to inhibit interleukin 1β in the same experimental procedure was found to be 101.23 **µ**M. It can be appreciated that the IC_{50} value of all 7 HF is almost similar to that of dexamethasone, whereas the compounds possessed a slightly higher IC_{50} value (Table-3).

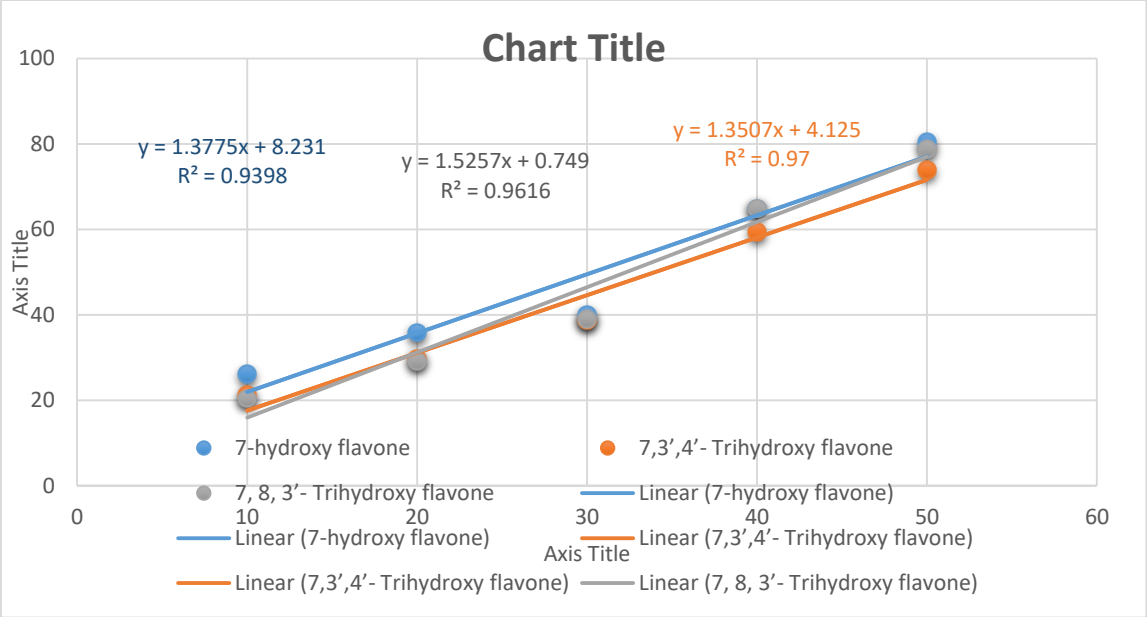

Fig 3: Effect of trihydroxy flavones on Interleukin – 1β

TABLE – 4: Effect of trihydroxy flavones on Tumor necrosis factor – α

The investigated trihydroxy flavones exerted a potent inhibition of the activity of the tumour necrosis factor α. The inhibition was concentration-dependent (Table 4).

A fairly high degree of inhibition was recorded for 7 hydroxy flavones in concentrations ranging from 10 – 50 **µ**M. A maximum of 72.16% inhibition was recorded with 7-HF. In a similar concentration, 7,8,3'- THF produced a maximum inhibition of 64.32%. The inhibitory action of 7,3'4'- THF on tumour necrosis factor α was revealed only in higher concentrations. A maximum of 63.54% inhibition was observed. The IC_{50} values calculated for the above compounds are as follows: 7– HF = 28.76 µM, 7,8,3' – THF = 35.13 µM and 7,3',4' – THF = 38.18 µM.

TABLE - 4

Concentration -M	**% Inhibition**		
	7-HF	**7,3',4' THF**	**7,8,3'THF**

10	23.13	12.97	21.46
20	38.16	22.68	30.23
30	57.15	38.77	46.66
40	67.28	50.01	58.14
50	**72.16**	63.54	64.32
IC-50*	28.76 (**µ**M)	38.18**µ**M)	35.13(**µ**M)

Each value represents the mean of three observations.

*IC – 50 value calculated by linear regression analysis. The IC_{50} value for dexamethasone to inhibit tumor necrosis factor-a was recorded to be 25.33 **µ**M. It can be appreciated that the IC_{50} values of 7- HF (27.08 **µ**M) and 7,8,3'- THF (34.54 **µ**M) are similar to dexamethasone value. The IC_{50} values recorded for 7,3',4'- THF (38.13**µ**M) are many-fold higher than the dexamethasone value.

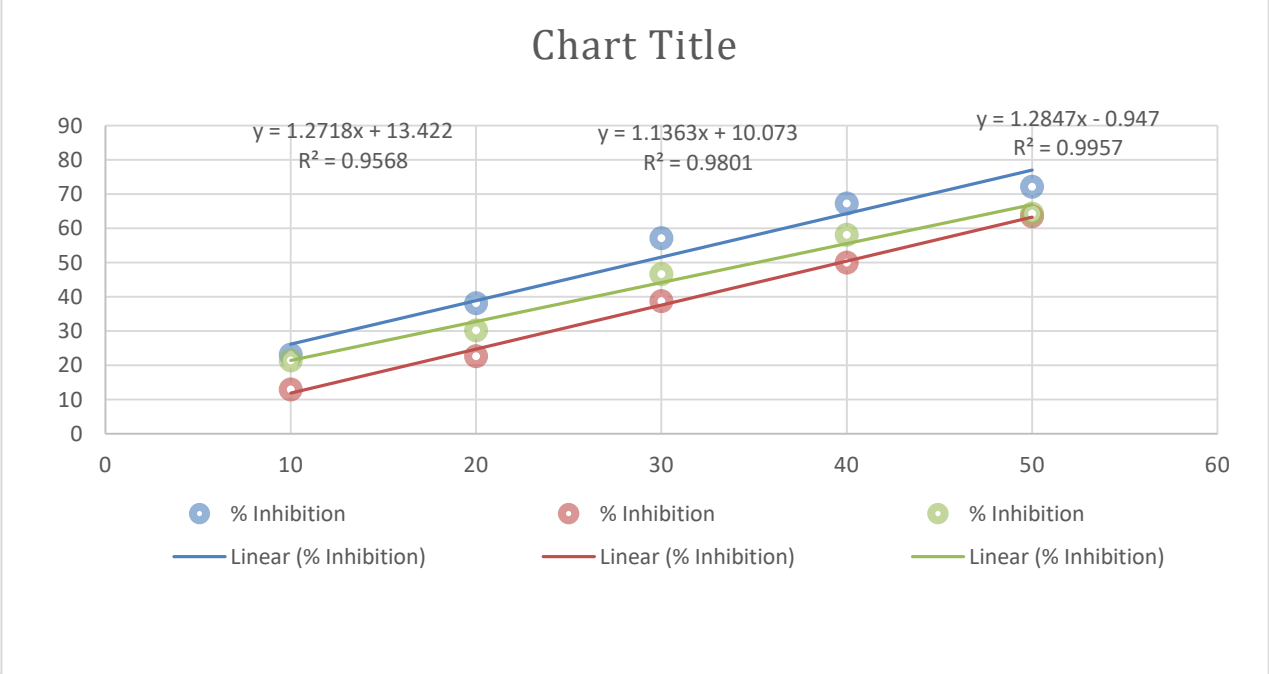

FIG – 4: Effect of trihydroxy flavones on Tumor necrosis factor – α

Discussion

The anti-inflammatory activity of flavonoids as reported by[10] revealed 50% inhibition of TNF-α with vitamin C, epicatechin, epigallocatechin, procyanidin B2, quercetin, and taxifolin. The same study has reported the inhibition of IL-1beta by epicatechin, epigallocatechin, procyanidin B2, quercetin was comparable to vitamin C [10]. In our study A concentration-dependent inhibition of interleukin–1β activity was recorded for all the tested trihydroxy flavones. Nearly 78% inhibition was recorded for 7,8,3'- THF in a concentration of 50 µM while that recorded for 7- HF was 80%. The IC50 values recorded for hydroxy flavones were 122.56 µM for 7, 8, 3'–THF, 128.89 µM for 7, 3', 4'–THF, and 138.01 µM for 7-HF. Our study also showed high degree of inhibition of TNF-alpha for 7-HF in concentrations ranging from 10 – 50 µM. A maximum of 72.16% inhibition was recorded. In a similar concentration, 7,8,3'- THF produced a maximum inhibition of 64.32%. The inhibitory action of 7,3'4'- THF on tumor necrosis factor-alpha was revealed only in higher concentrations. A maximum of 72.16% inhibition was observed. The IC50 values recorded for hydroxy flavones were 27.08 µM for 7–HF, 38.13 µM for 7,8,3'–THF and 34.54 µM for 7,3',4'–THF. The COX forms prostaglandins and thromboxanes which causes inflammation[12,13]. Many flavonoids exhibited inhibition of COX-1 and COX-2 which were reported in previous research studies[14.] also reported the COX-1 and COX-2 inhibitory property of flavonoids[15.] In our study, flavones inhibited COX-1 activity in a concentration dependent manner. Among the tested compounds, 7-hydroxy flavone produced a maximum inhibition of 86.23% in 50 µM concentration. In a similar concentration 7,3'4'- THF produced an inhibition of 76%, While 7- HF produced an inhibition of 58.8%. The IC50 values of COX-1 inhibition for hydroxy flavones were 27.61 µM for 7,3',4'–THF, 28.21µM for 7,8,3'–THF, and 39.40 µM for 7–HF. In contrary, revealed that flavones could not inhibit COX-1[16]. The COX-2 inhibition was maximum (71%) for 7,3',4'-THF in a concentration of 50 µM. In the same concentration maximum inhibition of 59.89%, and 67.9% with 7–HF, and 7,8,3'-THF respectively.

The IC50 values of COX-2 inhibition for hydroxy flavones were 31.98 µM for 7,3',4'–THF, 28.46 µM for 7,8,3'–THF, and 40.31 µM for 7–HF.

Conclusion

In conclusion, the present study has identified three 7hydroxy flavone derivatives with good anti-inflammatory activity. The inhibitory effects of 7hydroxy flavone on various pro-inflammatory cytokines provide evidence for the mechanism of action of these compounds against pain and inflammation.

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