Synthesis, antinociceptive activity and structure activity relationship of flavone derivatives

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Abstract: Flavonoids are phenolic compounds that have always attracted pharmaceutical researchers and food manufacturers. Nature has indirectly provided us flavones in our daily diet i.e. tea, fruits, juices and vegetables. Flavones have got special position in research field of natural and synthetic organic chemistry due to their biological capabilities. Flavone derivative has been synthesized in good yield from ketone and corresponding aldehydes. The structures have been established by different spectroscopic techniques like ^{1}H NMR, ^{13}C NMR, IR and elemental analysis. The compounds were then screened for its acute toxicity and antinociceptive response in mice models with writhings induced by acetic acid, tail immersion and formalin-induced nociception assay procedures and structure activity relationship was established. The compounds were safe up to a maximum dose of 1200 mg/kg body weight in mice. The effects following pretreatment with naloxone were also studied to reveal the involvement of opioid receptors in the antinociceptive action. The flavone derivatives showed significant reduction in number of abdominal constrictions, increase in paw licking response time in both phases and a significant raise in latency time in nociception models. Moreover, the antinociceptive response was significantly attenuated by pretreatment with naloxone suggesting the involvement of opioid system in the antinociceptive action. The promising effects were shown by halogenated flavone. The flavone derivatives showed analgesic response in all models of nociception suggesting the involvement of opioid system in the antinociceptive action.

Keywords: Flavone derivatives, antinociceptive, naloxone, opioid, structure activity relationship.

INTRODUCTION

Flavones as a panacea have always attracted pharmaceutical researchers and food manufacturers (Cushnie and Lamb 2005, Harborne and Baxter 1999). Nature has indirectly provided us flavones in our daily diet, i.e. tea, fruits, juices and vegetables. Flavones has got special position in the research field of natural and synthetic organic chemistry due to their biological capabilities such as, antimicrobial (Proestos *et al.,* 2005), anti-oxidant (Mellou *et al.,* 2005, Rusak and Gutzeit 2005, Zhao *et al.,* 2003), anti cancer (Yenjai *et al.,* 2004) and antinociceptive effect (Vishwanathan *et al.,* 1984, Thirugnana *et al.,* 1990). They are synthesized from intermediate product of chalcone, where a three carbon atom heterocyclic ring interconnects two aryl rings. Its derivatives are derived from modifications in its polyphenolic structure (Havasteen 1983, Middleton and Chithan 1993).

Drugs having analgesic and antiinflammatory capabilities also have gastric mucosal injury and ulceration as an adverse effect (Carlos *et al.,* 2013). However, the adverse effects of flavone derivatives in humans appear to be rare (Elliott *et al.,* 2000). Generally the safety margin of flavones derivatives is better than the currently used drugs (Havasteen 2002). The above research data put emphasis on further research work on flavones derivatives. Here in the first objective was to report synthesis of flavones derivatives and its evaluation for its analgesic activity in mice. The second objective was to explore the possible mechanism of action that may be involved and also to establish the structure activity relationship (SAR) of the flavone derivatives.

MATERIALS AND METHODS

Chemicals and instruments

Chemicals/ reagents used in the synthesis of the flavone derivatives were of Sigma Chemical Company (Aldrich, Germany). Solvents like ethanol, n-hexane, ethyl acetate, etc. were synthesis grade purchased from E. Merck and used as such without prior distillation.

^{1}H-NMR and ^{13}C NMR spectra were recorded in deutrated chloroform (CDCl3) on Bruker SF spectrometers operating at 300 and 75 megahertz (MHz) frequencies respectively. Chemical shifts values are

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expressed in δ (ppm) downfield relative to TMS, which was used as an internal standard. Infrared spectra was recorded on Thermoscientific USA (Nickolet 6700), Infrared spectrometer on KBr disk method. All melting points are uncorrected and were taken in open capillary tubes using an Electrothermal 9100 apparatus (Barnstead UK). Reaction extents and final products purities were checked on TLC plates (Merck 60 F254, Darmstadt Germany) and spots were visualized under UV Lamp (180-365 nm) and with subsequent staining with iodine vapours.

General procedure for the synthesis of flavone derivatives (F1, F2, F3, F4 and F7)

To an ethanolic solution of 2-hydroxy acetophenone (15 mili mol), sodium hydroxide (10 ml, 40% ethanolic) was added dropwise at room temperature. Then corresponding benzaldehyde derivatives (15mili mol) were added dropwise to this mixture and stirred for 24 hours at room temperature (25±2ºC). The reaction was monitored by TLC and upon completion of the reaction, it was poured onto crushed ice and neutralized with 1N HCl solution resulting in yellow precipitates of corresponding chalcones. The chalcones were filtered and washed with water to remove the impurities.

In next step, the respective chalcones were cyclized to flavone derivatives in 15ml DMSO in the presence of iodine (375mg) at 140ºC for 1 hour separately. Upon completion of reactions, the mixtures were cooled to room temperature and poured into water followed by extraction with ethyl acetate (25ml×3), treated with sodium thiosulphate solution (20%), brine solution and dried over sodium sulphate. The final products (mixture of flavone and chalcone) were subjected to column chromatography using n-hexane: ethyl acetate (9:1).

Pharmacological activities

Drugs and Animals

Tween-80 was purchased from Daejung Chemicals, Korea. Diclofenac sodium, morphine sulphate naloxone and indomethacine were purchased from the local market. Swiss Albino mice of either sex weighing 30-35gm were purchased from National Institute of Health (NIH) Islamabad. The animals were housed in individual cages at the animal house of University of Malakand with free access to water and standard diet and starved for 12-18 hours before experimentation. Ethical Committe of the Department of Pharmacy, University of Malakand approved the experimental protocols and ensured its compliance with provisions of the "Animal Bye-Laws 2008, Scientific Procedures Issue-I of the University of Malakand".

Acute toxicity

The synthesized compounds (F1, F2, F3, F4 and F7) suspended in 2% tween-80 were subjected to acute toxicity study on mice. Briefly, mice (n=6) were given different doses of flavones by i.p route to six groups each in two phases and were kept under observation for 30 minutes followed by six hourly observation for 24 hours. The animals were kept for the next 7 days for any manifestation of toxicity (Lorke 1983, Niaz *et al.,* 2013).

Writhing test

Antinociceptive effects of the synthesized compounds were assessed by using mice as an animal model with slight modifications. The synthesized compounds at different dose concentrations in 2% tween-80 were dispensed to respective groups of animals (n=6) by s.c. route and diclofenac sodium was administered intraperitoneally. The control group only received 2% tween-80 in normal saline. After 30 minutes of time interval, 0.1ml of acetic acid at a concentration of 1% (v/v) was injected intraperitoneally. Data was recorded in the form of number of writhes (abdominal constriction, extension of hind legs and turning of the trunk) during 30 minutes of test period, starting 3 minutes after the administration of acetic acid (Koster and Anderson 1959, Ali *et al.,* 2013).

Formalin test

This test was performed by the method of assessing the licking response of formalin-induced in paw of mice. 20 µl of 1% formalin prepared in 0.9% saline was administered by s.c. route into the dorsal hind paw and immediately placed in the transparent box for observing the licking response. The duration of reaction time (paw licking or biting) was determined between 0-5 min (first phase) and 15-30min (second phase). Animals in different groups were treated (s.c. route) with synthesized compounds (50mg/kg), indomethacin (10mg/kg) and morphine (5mg/kg), 30min prior to administration of formalin. Naloxone (2mg/kg) was administered 20 min prior to treatment of animals with test compound and standard. Control animals received the vehicle (0.1ml/10 gm). The reaction time of the animals in respective groups was compared to control group and expressed as percent inhibition (Pandurangan *et al.,* 2013).

Tail immersion test

Albino mice were used to assess the central antinociceptive response of the synthesized compounds. Briefly, animals in respective groups were treated with synthesized compounds (50mg/kg) and vehicle (2% tween-80) by s.c. route 30 minutes and morphine and naloxone by s.c. route 15 minutes before the screening. The latency period (time taken by the mice to deflect the tail) was determined by immersing 1-2 cm of mice tail in water that was kept warm at 53±1ºC (Mohammad and Chandra 2014, Moniruzzaman and Imam 2014).

STATISTICAL ANALYSIS

Data are presented as mean ± SEM. Analysis of variance and Dunnett's test is statistically manipulated with GraphPad prism 5 version 5.01 software.

RESULTS

2-phenyl-4H-chromen-4-one (F1)
^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.22 (dd, *J*=8.0, 1.7 Hz, 1H), 7.75-7.53 (m, 5H), 7.50-7.37 (m, 4H), 6.85 (s, 1H).^{13}C NMR (75 MHz, $CDCl_3$) δ 178.48, 163.42, 156.26, 133.79, 131.77, 131.61, 129.04, 126.66, 125.71, 125.24, 123.95, 118.09, 107.58 IR (KBr), *υ, cm^{-1}*, 1635.4, 1463.3, 1372.4, 766.0. Found, %: C 81.07; H 4.54. $C_{15}H_{10}O_2$. Calculated, %,: C 81.19; H 4.60 (Donghee *et al.*, 2012, Jie *et al.*, 2011).

2-(4-(dimethylamino)phenyl)-4H-chromen-4-one (F2)
^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.24 (dd, *J*=7.9, 1.7 Hz, 1H), 7.89-7.81 (m, 2H), 7.68 (ddd, *J*=8.7, 7.1, 1.7 Hz, 1H), 7.55 (dd, *J* = 8.4, 1.3 Hz, 1H), 7.40 (ddd, *J*=8.1, 7.1, 1.1 Hz, 1H), 6.80 – 6.76 (m, 2H), 6.73 (s, 1H), 3.10 (s, 6H). ^{13}C NMR (75 MHz, Chloroform-*d*) δ ppm=178.20, 163.7, 156.50, 152.60, 133.22, 127.75, 125.58, 124.81, 124.03, 117.84, 111.66, 104.39, 40.10. IR (KBr) *υ, cm^{-1}*, 2919.4 (CH) 1730.3(C=O), 1197.8 and 1363.2 (C-N), 1558.1(C=C), 3311.5 (=C-H) 1127.2 (C-0). Found, %: C 76.96; H 5.70; N 5.28. $C_{17}H_{15}NO_2$. Calculated, %,: C 76.59; H 5.60; N 5.60.

2-(4-Chlorophenyl)-4H-chromen-4-one (F3)
^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.24 (dd, J=8.0, 1.6 Hz, 1H), 7.83 (d, 2H), 7.72 (td, J=8.7, 7.1, 1.7 Hz, 1H), 7.53 (d, J=8.4 Hz, 1H), 7.47 (d, 2H), 7.40 (t, J=7.6 Hz, 1H), 6.75 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.2, 162.1, 156.1, 137.8, 133.9, 130.2, 129.3, 127.5, 125.7, 125.3, 123.9, 118.0, 107.6. IR (KBr) *υ, cm^{-1}*, 1662, 1374, 1092, 827, 754. (Donghee *et al.*, 2012, Jie *et al.*, 2011).

2-(2,6-dichlorophenyl)-4H-chromen-4-one (F4)
^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.30 (dd, *J*=8.0, 1.7 Hz, 1H), 7.83-7.68 (m, 1H), 7.58-7.36 (m, 5H), 6.47 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.06, 162.03, 156.96,, 133.65, 133.39, 128.48, 126.58, 125.75, 125.50, 124.89, 123.43, 120.38, 117.58. IR (KBr) *υ, cm^{-1}*, 2918.5, 1714.7, 1659.3, 1191.3, 747.8. Found, %: C 61.94; H 2.49. $C_{15}H_8Cl_2O_2$. Calculated, %,: C 61.88; H 2.77.

2-(3,4-dichlorophenyl)-4H-chromen-4-one (F7)
^{1}H NMR (300 MHz, Chloroform-*d*) δ 8.24 (dd, *J*=.0, 1.7 Hz, 1H), 8.04 (d, *J*=2.1 Hz, 1H), 7.74 (tq, *J*=7.0, 2.2 Hz, 2H), 7.65-7.56 (m, 2H), 7.46 (ddd, *J*=8.2, 7.1, 1.1 Hz, 1H), 6.80 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.03, 160.82, 156.08, 135.96, 134.09, 133.70, 131.69, 131.11, 128.06, 125.78, 125.55, 125.25, 123.87, 118.06, 108.19. IR (KBr) *υ, cm^{-1}*, 1659.3, 1413.7, 1378.8, 750.04, 747.8. Found, %: C 61.92; H 2.53. $C_{15}H_8Cl_2O_2$. Calculated, %,: C 61.88; H 2.77.

Acute toxicity
In the *in vivo* acute toxicity studies on the flavone derivatives, there were no gross physical and behavioral changes; including, rigidity, sleep, diarrhea, depression, abnormal secretion and hair erection for 24 hours and no mortality occurred within the observation period of two weeks.

Since the compounds even at a dose of 1200 mg/kg didn't show any mortality rate in mice so it is considered to be comparatively safe, during the study it was observed that the systemic administration of the compounds didn't produce any sedation, alteration in locomotor activity or motor dysfunction in animals.

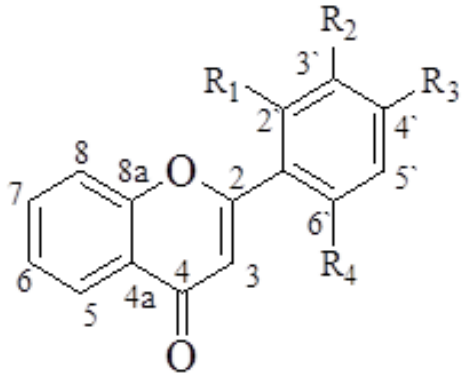

Fig. 1: Structure of flavones derivative

Writhing test
The synthesized compounds (F1, F2, F3, F4 and F7) caused significant inhibition of the nociception response induced by acetic acid with maximum effect of 71.09% (P<0.001, n=6), 64.45% (P<0.01, n=6), 78.19% (P<0.001, n=6), 75.59% (P<0.01, n=6) and 82.07% (P<0.001, n=6) respectively at a dose of 50 mg/ kg as shown in table 2. The results were comparable to that of the standard drug diclofenac sodium that displayed 86.42% (P<0.001, n=6) at a dose of 50 mg/kg.

Formalin test
The administration of the compound F1 at a dose of 50 mg/kg significantly inhibited both phases to 58.20% (P<0.001, n=6) and 76.43% (P<0.001, n=6) of formalin-induced paw licking response respectively. Similarly, the inhibition response of F2 was observed as 52.44% (P<0.001, n=6) and 67.34% (P<0.001, n=6). The halogenated flavones F3 and F4 also showed an inhibitory response on both phases, but the maximum response was observed in F7 that was appeared to be 69.36% (P<0.001, n=6) and 88.61% (P<0.001, n=6). Results are shown in table 3, fig. 2a and 2b.

Table 1: Physical parameters of compounds

Flavone	R_1	R_2	R_3	R_4	Yeild	Appearance	R_f	M.P (ºC)
F1	-H	-H	-H	-H	68.7%	Creamy white solid	0.58	96-98
F2	-H	-H	$-N(CH_3)_2$	-H	73.6%	Brick red solid	0.67	107-109
F3	-H	-H	-Cl	-H	84.2%	White crystals	0.69	178-181
F4	-Cl	-H	-H	-Cl	81.6%	White solid	0.63	182-185
F7	-H	-Cl	-Cl	-H	79.3%	White solid	0.61	195-197

Table 2: Acetic acid induced analgesic activity data of the compounds.

Treatment/Dose	Number of writhing	% inhibition
Control (2% Tween 80)	74.83±1.01	---
F1 (50mg)	21.63±0.80***	71.09
F2 (50mg)	26.26±0.93**	64.45
F3 (50mg)	16.32±0.97***	78.19
F4 (50mg)	18.26±1.06**	75.59
F7 (50mg)	13.41±0.76***	82.07
Diclofenac sodium (50 mg)	10.16±0.70***	86.42

All the values were expressed as mean ±SEM (n=6). **P<0.01, ***P<0.001 when compared to control group.

Table 3: Formalin-induced paw-licking response

Treatment/Dose	Licking time (Sec)		Inhibition (%)	
	1st Phase	2nd Phase	1st Phase	2nd Phase
Control (2% Tween 80)	48.83±1.627	72.83±1.287	----	----
F1 (50mg)	20.41±1.191***	17.16±1.619***	58.20	76.43
F2 (50mg)	23.22±1.132***	23.78±1.668***	52.44	67.34
F3 (50mg)	18.26±1.067***	11.52±1.425***	62.60	84.18
F4 (50mg)	19.34±1.411***	15.21±1.039***	60.39	79.11
F7 (50mg)	14.96±1.536***	8.29±1.441***	69.36	88.61
Indomethacin (10mg)	39.83±1.541	18.66±1.542***	18.43	74.37
Morphine (5mg)	6.416±1.165***	2.83±1.260***	86.86	96.11
F1 (50mg) + Naloxone (2mg)	49.21±1.579	76.26±1.542	-0.77	-4.70
F2 (50mg) + Naloxone (2mg)	47.41±1.515	73.62±1.428	2.90	-1.08
F3 (50mg) + Naloxone (2mg)	46.12±1.239	71.65±1.396	5.54	1.62
F4 (50mg) + Naloxone (2mg)	49.02±1.245	74.33±1.416	-0.38	-2.05
F7 (50mg) + Naloxone (2mg)	45.47±1.618	68.16±1.364	6.88	6.41
Indomethacin (10mg) + Naloxone (2mg)	40.50±1.784**	19.16±1.429***	17.05	73.69
Morphine (5mg) + Naloxone (2mg)	47.66±1.520	71.83±1.142	2.39	1.37

All the values were expressed as mean ±SEM. *P<0.05, **P<0.01 and ***P<0.001 when compared to control group (one way ANOVA followed by Dunnetts: compare all vs control test).

Animals pretreated with morphine at a dose of 5 mg/kg significantly inhibited both phases to 86.86% (P<0.001, n=6) and 96.11% (P<0.001, n=6) of formalin-induced paw licking response respectively. Pre-treated animals with naloxone reversed the inhibitory effects of synthesized compounds.

It is also evident from the results that naloxone caused an obvious reversal of the analgesic response of morphine in both early and late phases of formalin test. Indomethacin at 10 mg/kg caused marked reduction in the paw-licking time to 74.37% (P<0.001, n=6) in the second phase while mild reduction in paw-licking time (18.43%) in the first phase was observed.

Tail immersion

Table 4 shows that the analgesic response of flavone derivatives (F1, F2 and F7) at a dose of 50 mg/kg was significant in tail immersion test. The maximum analgesic effect of F1 was observed at 75 min (latency increased 72.87%, P<0.001). Similarly, the F2 showed a maximum response of 63.09%, P<0.01 at 75 min and it was observed to be 76.94%, P<0.001, 74.48%, P<0.001 and 83.10%, P<0.001 for halogenated flavones F3, F4 and F7 respectively. Whereas, morphine, a centrally acting opioid analgesic agent, exhibited powerful activity recorded at 60 min after treatment (84.80%, P<0.001), as shown in table 3. Animals treated with naloxone produced significant reduction in the analgesic activity of morphine and flavone derivatives (table 4).

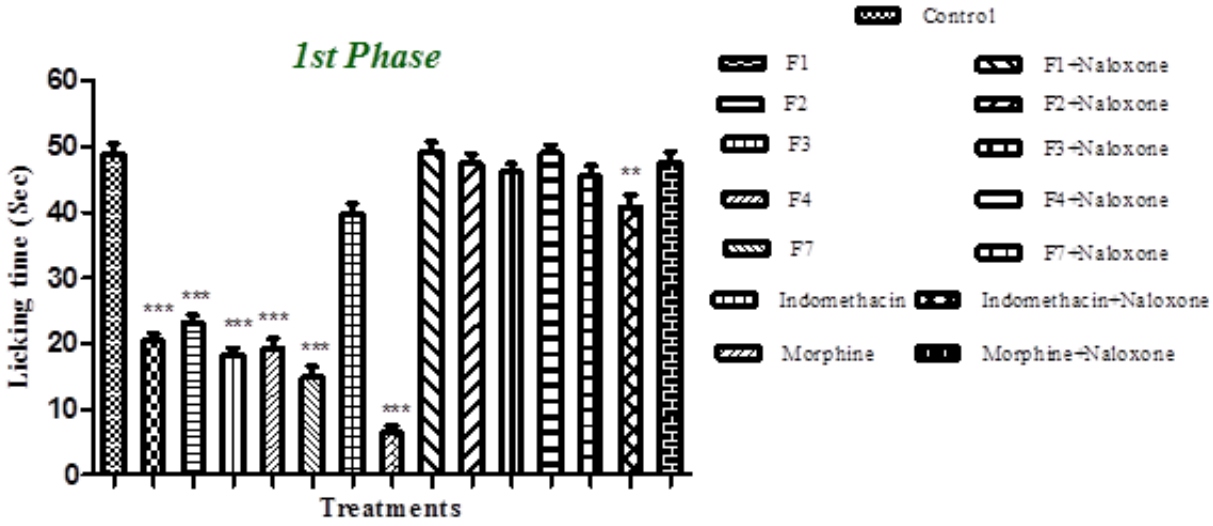

Fig. 2a: Analgesic effect of compounds on formalin induced licking response in first phase of the test. Values are mean ± SEM, n=6, $^{**}P<0.01$, $^{***}P<0.001$ significantly different compared with control.

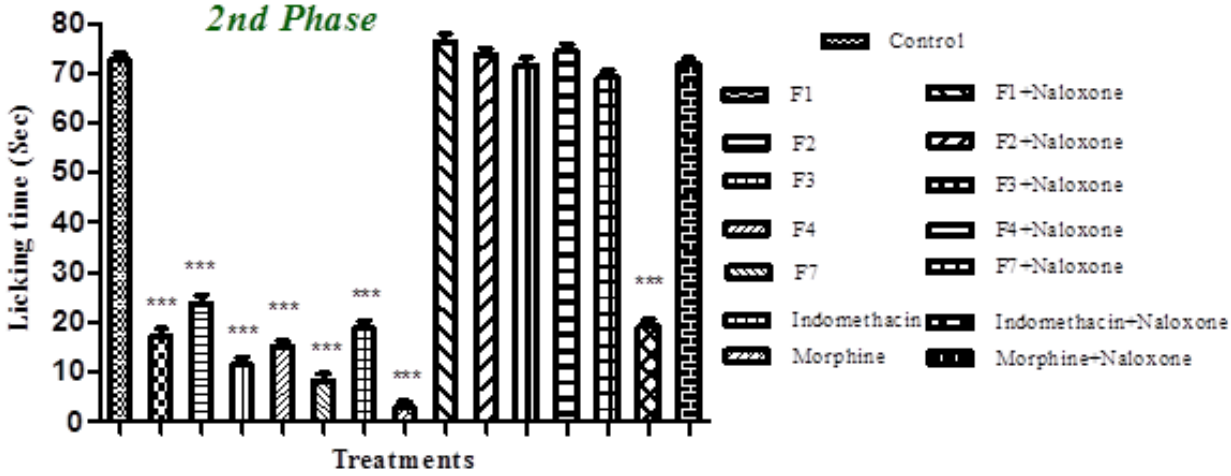

Fig. 2b: Analgesic effect of compounds on formalin induced licking response in second phase of the test. Values are mean ± SEM, n=6,$^{***}P<0.001$ significantly different compared with control.

DISCUSSION

The analytical data of F1 is compared with standard publications and is in accordance with the reported results. The H3 of all flavones gave singlet at 6.85, 6.73 and 6.80 ppm. For N,N- dimethyl group of F2, singlet of 6H is observed at 3.10. ^{13}C NMR show signals for the C=O group at 178.48, 178.20 and 178.03 cm^{-1} respectively. The IR absorption peaks for this group is at1635.4, 1730.3 and 1659.3in F1, F2 and F7 respectively. The (C-Cl) peaks are observed at 750.04 and 747.8 cm^{-1} in F7. The analytical data of halogenated flavones F3 and F4 are also given in results. The elemental analysis data of the compounds is within the range.

The writhing method is a minimal noxious stimulus and very sensitive method even with which weak analgesic agents can be detected (Ali *et al.,* 2013). In writhing induced by acetic acid, the peritoneal fluids in animals are found to contain high levels of histamine, prostaglandins and serotonin which is most frequently used for peripheral analgesic activities (Collier *et al.,* 1968, Deraedt *et al.,* 1980).

The acetic acid induced constrictions is a non-selective screening method because of the release prostaglandins which is an endogenous mediator and capable of accelerating the neurons sensitivity and peripheral nociceptors combine to opioids, non-steroidal antiinflammatory (NSAIDs) and other central acting drugs (Vaz *et al.,* 1996). All the compounds showed marked inhibition in the acetic acid induced constrictions, which clearly show the potent analgesic effect of the compounds but the maximum responses were observed in

Table 4: Analgesic activity (Tail flick mathod) data of the compounds

	Time in Sec (Tail Flick)/ Response (%)					
Treatment/Dose	15 min	30 min	45 min	60 min	75 min	90 min
Control (2% Tween 80)	0.78±0.030	0.88±0.021	0.98±0.021	0.93±0.038	0.86±0.025	0.92±0.041
F1 (50mg)	1.09±0.123* (27.10%)	1.38±0.112* (36.23%)	1.93±0.131** (49.22%)	2.78±0.124** (66.54%)	3.17±0.163*** (72.87%)	3.02±0.138*** (69.53%)
F2 (50mg)	0.97±0.112 (19.58%)	1.23±0.135* (28.45%)	1.70±0.141** (42.35%)	2.08±0.111** (55.28%)	2.33±0.133** (63.09%)	2.04±0.123** (54.90%)
F3 (50mg)	1.05±0.207* (25.71%)	1.34±0.411* (34.32%)	1.96±0.161** (50.00%)	3.36±0.201** (72.32%)	3.73±0.206*** (76.94%)	3.25±0.218*** (71.69%)
F4 (50mg)	1.02±0.214* (23.52%)	1.31±0.241* (32.82%)	1.86±0.361** (47.31%)	3.08±0.421** (69.80%)	3.37±0.302*** (74.48%)	2.75±0.280*** (66.54%)
F7 (50mg)	1.09±0.029* (28.44%)	1.36±0.021* (35.29%)	2.15±0.042** (54.41%)	4.32±0.039*** (78.47%)	5.09±0.063*** (83.10%)	4.46±0.048*** (79.37%)
Standard (Morphine 5mg)	1.54±0.024** (49.35%)	2.11±0.066** (58.29%)	4.52±0.038*** (78.31%)	6.12±0.054*** (84.80%)	4.98±0.050*** (82.70%)	4.74±0.074*** (80.59%)
F1 (50mg) + Naloxone (2mg)	0.86±0.037	0.81±0.065	0.93±0.049	0.99±0.038	0.87±0.029	0.83±0.044
F2 (50mg) + Naloxone (2mg)	0.92±0.048	0.86±0.036	0.88±0.031	0.94±0.047	0.89±0.038	0.84±0.061
F3 (50mg) + Naloxone (2mg)	0.82±0.108	0.91±0.044	0.84±0.045	0.89±0.064	0.94±0.026	0.78±0.042
F4 (50mg) + Naloxone (2mg)	0.91±0.033	0.88±0.051	0.81±0.051	0.83±0.034	0.86±0.061	0.87±0.056
F7 (50mg) + Naloxone (2mg)	0.82±0.046	0.96±0.031	1.05±0.047	0.97±0.035	0.91±0.051	0.93±0.034
Morphine (5mg) + Naloxone (2mg)	0.75±0.022	0.87±0.036	0.96±0.040	0.95±0.034	0.89±0.030	0.97±0.042

All the values were expressed as mean ±SEM. $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ when compared to control group (one way ANOVA followed by Dunnetts: compare all vs control test).

F7, F3 and F4 showed the introduction of the halogen group to the flavones increase the response of halogenated flavones (F3, F4 and F7) in comparison with simple flavone F1. These results also suggest that change in the position of halogen among the halogenated flavones also increase or decrease the activity response. Base upon these findings, the order of activity among the halogenated flavones is F7 ℘ F3 ℘ F4. Further the incorporation of *N*,*N*-Dimethyl as in F2 decreased the response and confirm that the addition of groups to flavone may lead to either increase or decrease in the activity of compounds.

For confirmation of the analgesic mechanism, the compounds were further screened for formalin induced paw licking response. As the formalin pain model is very useful for explaining the mechanism of analgesia and pain (Tjolsen *et al.*, 1992). This model involves two phases of pain, the first phase and the late or second phase (Hunskaar and Hole 1987, Murray *et al.*, 1988).

In the current study, the mice were pretreated with the flavones derivatives which caused an evident decrease in paw-licking/ biting response of mice during observations in both phases like morphine. It is clear that the NSAIDs, peripherally acting drugs inhibit the second phase and opioids, centrally acting drugs like morphine acts on both phases to reduce the paw-licking response (Santos *et al.*, 1994, Shibata *et al.*, 1989) in formalin-stimulated nociception test. The ability to suppress both phases of the formalin-stimulated pain response by compounds showed that the analgesic effect is due to central mechanism of pain inhibition. Similar results were observed like writhing test and the maximum response was achieved by halogenated flavones in comparison with F1. An opioid antagonist like naloxone distinctly inhibits the effects of morphine and the compounds suggest that the analgesic effect is mediated by opioid receptors.

The writhing model and late phase formalin model represents the pain response of inflammatory origin (Tjolsen *et al.*, 1992, Tjolsen and Hole 1997). These findings indicated that the compounds can be potent anti-inflammatory agents.

The central analgesic effects of the compounds were further confirmed by tail immersion screening method which is consider more specific for such studies (Turner 1965).

Previous studies also showed potent antinociceptive activities flavone derivatives that supports our findings (Thirungnana *et al.*, 1993, Girija *et al.*, 2002, Umamaheswari *et al.*, 2006). In the literature, there are evident reports of major contribution of flavone derivatives in opioid mechanism involvement. Flavonoid derivatives like gossypin, monohydroxy and monomethoxy, dihydroxy flavone derivatives and quercetin is found to use opioid routes in their analgesic effects (Thirungnana *et al.*, 1993, Girija *et al.*, 2002, Umamaheswari *et al.*, 2006, Viswanathan *et al.*, 1985, Thirungnana *et al.*, 1990, Naidu *et al.*, 2003). The current study also confirmed the marked reduction in antinociceptive action of compounds by naloxone. These findings support the previous reports and convincingly indicated the involvement of opioid receptors in antinociceptive response of compounds.

CONCLUSION

In conclusion, the present study confirms the antinociceptive action of flavones derivatives. Antinociceptive action of this compound has been augmented and established in three different nociception mice models. In addition, opioid receptors involvement in its antinociceptive action was demonstrated from the results. These findings will open a new channel to synthesize halogenated flavones and explore the potent antinociceptive compounds.

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