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# ANALGESIC ACTIVITY OF CERTAIN FLAVONE DERIVATIVES: A STRUCTURE-ACTIVITY STUDY

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## Summary

Flavone and 10 hydroxy and glucoside flavone derivatives were synthesised. They were tested for their analgesic effect in mice employing acetic acid-induced writhing and tail immersion methods. Subcutaneously all the tested compounds exhibited significant analgesic activity with varying potencies in both assay models. The activity of flavone and its 5-; 7-; 2′-; 5,7- and 7,8-hydroxy derivatives apparently involves an opiate-like mechanism, since their activity was reversed by naloxone pretreatment. It is suggested that flavonoid substances may utilise more than one mechanism in eliciting analgesia.

## Introduction

Renewed interest has been observed in recent years on the novel, multiple activities of flavonoids. Originally, flavonoids were documented to exert beneficial effects against hepato-toxicity, inflammation and cataract formation (Parmar and Ghosh, 1980). Recent experiments have revealed significant analgesic activity for certain flavonoids such as hydroxyethylrutoside(HR) (Ramaswamy et al., 1980) and gossypin (Viswanathan et al., 1984). On analysis of these reports, it is evident that the tested flavonoids, however, may not utilize a common mechanism to elicit analgesic activity. Further, only a few flavonoids have been tested for this activity. At this time, it appears that a detailed and systematic study on the possible analgesic capacity of the various flavonoid substances and the likely mechanism involved is

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warranted. An attempt has been made to synthesise flavone and its various hydroxy and glucoside derivatives and to relate their chemical structure with the nature of analgesic activity.

## Materials and methods

*Drugs and chemicals*

Flavone (Wheeler, 1952), flavonol (Oyamada, 1934), 5-hydroxy and 6-hydroxy (Looker et al., 1964), 7-hydroxy and 7,8-dihydroxy (Baker, 1933), 5,7-dihydroxy (Rama Rao et al., 1964), 2′-hydroxy (Shah et al., 1938; Baker and Besley, 1940), 4′-hydroxy (Jain et al., 1965) flavones; 6-hydroxy and 7-hydroxy flavone glucosides (Zemplen and Farkas, 1943; Barczai-Martos and Körösy, 1950) were synthesised in our laboratory. The purity of all the compounds was checked by melting point, chromatography with an authentic sample, elemental analysis and also by UV spectra. 6-Hydroxy flavone glucoside has not been reported in the literature. The purity and identity of this compound was confirmed by elemental analysis, UV, N.M.R. and M.S. studies, as follows. Analysis, calculated: $C_{21}H_{20}O_8$; C, 63; H, 5.0; found: C, 62.9; H, 5.10. $\lambda_{max}$ MeOH (nm) 268, 298: proton magnetic spectrum showed a signal at 4.8 ppm, corresponding to sugar C-1 proton and another multiplet signal at 3.2 ppm confirming the presence of a sugar moiety in the compound (Mabry et al., 1970). Mass spectrum of the compound showed a glycone (6-hydroxy flavone) base peak ($M^+$: 238) which is in accordance with an earlier report (Prox, 1968). Acid hydrolysis of the glucoside yielded 6-hydroxy flavone and glucose.

Other chemicals used were: morphine hydrochloride (Government Opium Alkaloid Works, Ghazipur), naloxone hydrochloride (Endo Labs.), acetic acid (BDH) and carboxymethyl cellulose (Loba).

*Assessment of analgesia*

Swiss male albino mice were used throughout the study. Food and water were available ad libitum. Two assay models, viz: acetic acid-induced writhing and the tail immersion method, were employed to detect analgesic activity.

In the manner of Koster et al. (1959), acetic acid (0.6%, 10 ml/kg i.p.) was injected and the number of writhings in the following 15-min period was observed. A significant reduction in the number of writhings by any treatment as compared to vehicle-treated animals was considered as a positive analgesic response. The percentage inhibition of writhings was calculated and plotted against the log dose of the drug and the dose producing a 50% inhibition was obtained from the graph ($ED_{50}$).

In the manner of Sewell and Spencer (1976), the tail of the mouse up to a constant level was immersed in a water bath at 55 $\pm$ 0.5°C. The reaction time to flick the tail from the liquid was recorded at +1, +15, +30, +60 and +120 min after flavonoid or morphine administration. A maximum immersion time of 30 sec was maintained to prevent thermal injury to the animals. A graph was plotted with the reaction time against the time after drug administration and the area under the

time-response curve (AUC, $cm^2$) was calculated. A significant increase in AUC as compared to control animals was considered to be a positive analgesic response.

Morphine was included in all the studies as a reference drug for comparison purposes. A dose of 0.25 mg/kg s.c. was used 30 min prior to acetic acid in the writhing assay and 10 mg/kg s.c. was employed in the thermal assay. Flavone and its derivatives were injected s.c. in the dose range of 6.25—400 mg/kg. All flavonoids were administered as a suspension in 1% carboxymethyl cellulose. The control animals received only the vehicle.

*Mechanism of analgesia studies*

The mechanism of analgesic action of the test compounds was investigated employing an antagonism assay using naloxone (5 mg/kg s.c.), a specific opioid antagonist. Naloxone was administered 10 min prior to flavonoid or morphine treatment. The analgesic activity was then documented either 60 min after flavonoid or 30 min after morphine, as described earlier (acetic acid writhing).

*Statistical analysis*

The results were analysed employing Dunnett's *t*-test (Dunnett, 1964).

## Results

*Analgesia assessment*

Using the writhing assay, the administration of morphine, flavone and its hydroxy or glucoside derivatives inhibited the number of writhings in a dose-related manner (Table 1). All the tested flavone compounds inhibited the writhing response but varied as to potency, depending on the position of hydroxylation or glucosylation. The $ED_{50}$ values of the flavonoids are summarised in Table 1.

Using the tail immersion assay, AUC was increased significantly by morphine. Flavone and its derivatives also increased the AUC (Table 2).

*Naloxone pretreatment studies*

Naloxone per se at the dose used did not modify the writhing response. In naloxone-pretreated animals, morphine or flavone and its 5-; 7-; 2′-; 5,7- and 7,8-substituted derivatives failed to produce any inhibition of writhing. However, the 3-; 6- and 4′-substituted flavones and the 6-hydroxy flavone glucoside continued to produce a significant inhibition of the writhing response (Table 3).

## Discussion

The present investigation of the analgesic activity of several structurally related flavonoid compounds confirmed their analgesic activity in two assay models. However, the potency of the different compounds varied widely as evidenced by the $ED_{50}$ values. One of the main aims of this study was, if possible, to relate changes in the structure of the flavonoids with their analgesic potencies. The modifications attempted in the present study can be grouped as (*i*) hydroxylation, (*ii*) dihydroxylation and (*iii*) glucosylation.

TABLE 1

EFFECTS OF FLAVONOIDS ON ACETIC ACID-INDUCED WRITHING IN MICE

| Treatment (s.c.) | Mean ± S.E.M. writhings observed/15 min (*N*) | | | | | | | $ED_{50}$ (mg/kg) |
|---|---|---|---|---|---|---|---|---|
| | 6.25 | 12.5 | 25 | 50 | 100 | 200 | 400 mg/kg | |
| Flavone | — | 26.2 ± 1.0 | 20.3 ± 1.8** | 18.0 ± 1.3** | 7.6 ± 0.8** | 1.3 ± 0.8** | — | 57.5 |
| 3-Hydroxy flavone | — | — | 25.0 ± 1.7* | 18.6 ± 1.9** | 8.5 ± 0.7** | 3.8 ± 1.7** | — | 55.0 |
| 5-Hydroxy flavone | — | 22.8 ± 2.7* | 21.8 ± 1.3** | 11.6 ± 1.5** | 7.5 ± 1.8** | — | — | 31.0 |
| 6-Hydroxy flavone | — | 22.8 ± 2.0* | 18.1 ± 2.1** | 11.5 ± 3.4** | 8.2 ± 2.7** | — | — | 31.6 |
| 7-Hydroxy flavone | — | — | 23.6 ± 1.7* | 20.8 ± 2.5** | 16.3 ± 2.6** | 12.0 ± 1.7** | — | 125.0 |
| 2'-Hydroxy flavone | — | — | — | 21.8 ± 1.8* | 13.6 ± 0.4** | 10.5 ± 1.5** | — | 87.0 |
| 4'-Hydroxy flavone | — | 25.3 ± 1.5 | 20.0 ± 1.8** | 14.4 ± 1.4** | 10.8 ± 2.0** | 8.6 ± 1.6** | — | 34.6 |
| 5,7-Dihydroxy flavone | — | 21.5 ± 1.4** | 17.3 ± 1.2** | 11.2 ± 1.5** | 9.0 ± 1.1** | — | — | 40.0 |
| 7,8-Dihydroxy flavone | — | — | — | — | 26.1 ± 1.5 | 17.3 ± 0.7** | 8.7 ± 1.1** | 200.0 |
| 6-Hydroxy flavone glucoside | 22.0 ± 1.7* | 13.8 ± 1.8** | 10.1 ± 0.9** | 1.7 ± 0.9** | — | — | — | 9.8 |
| 7-Hydroxy flavone glucoside | — | 23.2 ± 2.7 | 13.6 ± 2.9** | 11.0 ± 2.9** | 9.8 ± 3.7** | — | — | 20.9 |

Number of writhings for vehicle-treated animals was 30.5 ± 2.5 and in morphine (0.25 mg/kg; s.c.) treated animals 15.5 ± 0.3.
Significance compared to vehicle-treated animals: *$P < 0.05$; **$P < 0.01$. $N = 6$—9; dashes indicate doses not tested.

TABLE 2

ANALGESIC EFFECT OF FLAVONOIDS AS TESTED BY THE TAIL IMMERSION METHOD IN MICE

| Treatment | Mean ± S.E.M. AUC ($cm^2$) | | | | | |
|---|---|---|---|---|---|---|
| | 12.5 | 25.0 | 50 | 100 | 200 | 400 mg/kg |
| Flavone | 3.55 ± 0.8 | 9.75 ± 0.5** | 11.0 ± 1.5** | 11.2 ± 0.7** | 12.5 ± 1.0** | — |
| 3-Hydroxy flavone | 4.90 ± 0.7* | 7.9 ± 0.5** | 9.5 ± 0.9** | 10.9 ± 0.9** | 12.1 ± 1.0** | — |
| 5-Hydroxy flavone | — | 4.66 ± 0.6* | 6.45 ± 0.7** | 12.9 ± 0.9** | 11.8 ± 0.75** | — |
| 6-Hydroxy flavone | 7.46 ± 0.5** | 10.0 ± 0.8** | 13.25 ± 1.3** | 13.3 ± 1.20** | 13.4 ± 1.0** | — |
| 7-Hydroxy flavone | 5.6 ± 0.5** | 8.8 ± 0.7** | 11.3 ± 0.7** | 14.0 ± 0.85** | 15.0 ± 1.0** | — |
| 2′-Hydroxy flavone | — | 3.9 ± 0.6 | 5.8 ± 0.72** | 8.0 ± 0.9** | 9.8 ± 0.58** | — |
| 4′-Hydroxy flavone | — | 4.2 ± 0.7 | 8.5 ± 0.50** | 9.5 ± 0.6** | — | — |
| 5,7-Dihydroxy flavone | — | — | 8.6 ± 0.65** | 15.9 ± 1.7** | 19.0 ± 0.74** | — |
| 7,8-Dihydroxy flavone | — | — | — | 7.6 ± 0.75** | 9.6 ± 0.93** | 16.9 ± 2.6** |
| 6-Hydroxy flavone glucoside | — | 6.45 ± 0.7** | 7.3 ± 0.4** | 11.2 ± 0.5** | 15.8 ± 1.2** | — |
| 7-Hydroxy flavone glucoside | 4.0 ± 0.9 | 10.0 ± 1.1** | 10.8 ± 1.2** | 13.8 ± 1.6** | 16.3 ± 1.7** | — |

For vehicle-treated animals an AUC of 2.5 ± 0.5 $cm^2$ was recorded and in morphine-treated (10 mg/kg, s.c.) animals 18.1 ± 0.8 $cm^2$.
Significance compared to vehicle-treated animals: *$P < 0.05$, **$P < 0.01$. $N = 6$—9; dashes indicate doses not tested.

TABLE 3

EFFECT OF NALOXONE OR SALINE PRETREATMENT ON FLAVONOID-INDUCED ANALGESIC EFFECT IN ACETIC ACID-INDUCED WRITHING TEST

| Treatment (s.c.) | Dosage (mg/kg) | Mean ± S.E.M. writhings observed in 15 min (*N*) | |
|---|---|---|---|
| | | Saline | Naloxone |
| Vehicle | — | 30.8 ± 1.5 | 32.1 ± 2.2 |
| Morphine | 0.25 | 15.5 ± 0.3 | 29.2 ± 1.0* |
| Flavone | 60.0 | 16.4 ± 0.9 | 24.9 ± 2.0* |
| 3-Hydroxy flavone | 60.0 | 15.0 ± 0.8 | 12.0 ± 0.4 |
| 5-Hydroxy flavone | 40.0 | 14.2 ± 1.7 | 23.3 ± 2.0* |
| 6-Hydroxy flavone | 30.0 | 14.6 ± 2.0 | 11.5 ± 3.1 |
| 7-Hydroxy flavone | 125.0 | 14.3 ± 2.6 | 32.2 ± 2.3* |
| 2′-Hydroxy flavone | 100.0 | 13.6 ± 0.4 | 24.8 ± 1.7* |
| 4′-Hydroxy flavone | 40.0 | 14.1 ± 1.4 | 14.4 ± 1.2 |
| 5,7-Dihydroxy flavone | 40.0 | 14.8 ± 1.5 | 25.7 ± 1.9* |
| 7,8-Dihydroxy flavone | 200.0 | 17.8 ± 0.8 | 27.3 ± 1.8* |
| 6-Hydroxy flavone glucoside | 10.0 | 16.0 ± 0.9 | 13.4 ± 1.2 |
| 7-Hydroxy flavone glucoside | 20.0 | 16.8 ± 1.2 | 30.1 ± 2.5* |

Naloxone (5 mg/kg s.c.) was administered 10 min prior to flavonoid or morphine treatment. The dosage used for each flavonoid approximated the $ED_{50}$ value (Table 1).
Significance compared to saline-pretreated animals: $^*P < 0.01$; $N$ per group = 6—9.

*Effect of hydroxylation*

Substitution of a hydroxyl group at the 3rd position in the flavone skeleton did not significantly alter the analgesic effect of flavone ($ED_{50} = 57$ mg/kg), while substitution at the 5th or 6th position almost doubled the potency. In a contrary fashion, substitution at the 7th position reduced its potency. Hydroxylation in the B-ring had varied effects. Substitution at the 2′-position decreased the analgesic potency, whereas a hydroxyl group at the 4′-position accentuated the analgesic effect compared to flavone.

*Effect of dihydroxylation*

Substitution of two hydroxyl groups at the 5 and 7 positions showed a slightly enhanced analgesic potency as compared to flavone or 7-hydroxy flavone. A 7,8-substitution reduced the analgesic potency to almost one fourth as compared to flavone and about one half that of 7-hydroxy flavone.

*Effect of glucosylation*

Introduction of a glucose moiety in 6-hydroxy flavone potentiated the analgesic activity to almost six times that of flavone, while in 7-hydroxy flavone a three-fold increase was observed. Thus the substitution of a glucose moiety in the flavone skeleton uniformly had an enhanced analgesic effect.

Based on the results of the present study, the following generalizations can be made. The flavone skeleton has inherent analgesic capacity. 5-Substitution in-

creases the analgesic potential as evidenced by greater potency for 5-hydroxy flavone. Moreover, the decrease in analgesic potency resulting from hydroxylation at the 7 position can be offset by introduction of a hydroxyl group at the 5 position. Glucosides have greater analgesic potency as compared to their hydroxy flavones.

*Naloxone pretreatment*

When their mechanism of action was analysed, the activity of flavone and its 5-; 7-; 2′-; 5,7- or 7,8-substituted compounds clearly indicated the involvement of an opioid-like mechanism, since their analgesic activity was attenuated by naloxone. However, naloxone pretreatment did not alter the activity of compounds with 3-, 6- or 4′- substitution. These data favour the suggestion that these flavonoid compounds may utilise different mechanisms in eliciting analgesia (Ramaswamy et al., 1985; Thirugnanasambantham et al., 1985). The reported inhibitory effect of flavonoids on prostaglandin synthesis (Baumann et al., 1979) suggests an alternative mechanism. The present observations indicate that the position of substitution may play an important role in the opioid-like effect of flavonoids.

In conclusion, the results of the present study suggest that structural modification of the flavone skeleton can result in significant changes in analgesic potency. This finding warrants further investigation involving other groups as substituents.

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