

Anti-nociceptive effect of synthesized di-hydroxy flavones: Possible mechanism

K Girija*, M Kannappa Reddy & S Viswanathan**

*College of Pharmacy, Mother Theresa Institute of Health Sciences, Pondicherry, India

and

Medicinal Chemistry Research Centre, Institute of Pharmacology, Madras Medical College, Chennai 600 003, India

Received 4 March 2002; revised 30 May 2002

Renewed interest on the research on the flavonoids is gaining more importance. Earlier literature on flavonoids indicated a significant anti-nociceptive action for flavones and mono-substituted flavones. However, they exhibited a ceiling effect. The present study was undertaken by new synthesizing six disubstituted flavones (DHF) since poly substituted ones are expected to produce more potent effect. Their anti-nociceptive effect and the role of opioid involvement were studied using acetic acid induced abdominal constriction assay. All the six DHFs administered in elicited a dose related inhibition of abdominal constrictions indicating the presence of the anti-nociceptive response. However, these substances also showed a similar ceiling effect. Like other flavonoid substances, they also utilized opioid pathways. It is suggested that these newly synthesized DHFs can be included along with other flavonoids while attempting clinical trial for analgesic use.

Flavonoids have been extensively investigated for their anti-nociceptive, anti-inflammatory and anti-ulcer properties. A combination of these pharmacological properties in a single substance is unique since most of the available anti-inflammatory analgesics are ulcerogenics. With the report of Ramaswamy *et al.* on the anti-nociceptive effect on hydroxyethyl rutoside¹, further studies with gossypin²⁻⁴, epicatechin, flavone⁵ were carried out. It has been observed that these flavonoid substances produced significant opioid and ATP sensitive (K^+_{ATP}) channel mediated anti-nociceptive response like morphine. However, they exhibited a ceiling effect of 60-70% of the activity. A detailed structure activity relationship using mono-substituted flavone derivatives⁵ indicated only a marginal difference in the anti-nociceptive efficacy of different flavone substances.

In the present study, attempts have been made to explore more efficacious group of flavonoid substances by synthesizing di-hydroxyflavones (DHF) and investigate them for their anti-nociceptive activity and its mechanism.

Male Swiss albino mice weighing between 25-30 g, purchased from King Institute, Chennai, were housed under normal 12 hr: 12 hr light dark cycle at room temperature (28°-30°C) with free access to food (pellets obtained from Gold Mohar Ltd., Bangalore) and water. The experiments were conducted between 10.00 and 12.00 hrs.

The protocol was approved by Institute Ethical Committee, Madras Medical College, Chennai.

Measurement of anti-nociception—The acetic acid induced abdominal constriction assay⁶ was used. The number of abdominal constrictions for a period of 15 min following ip injection of 0.6% acetic acid (10 ml/kg) was counted. A significant reduction in number of constrictions when compared with vehicle treated animals was considered as anti-nociceptive response. Each animal was used only once for the study. Prior to this testing, the effect of DHFs on the motor activity was tested using rota rod.

Drug treatment—The newly synthesized six DHFs were suspended in 1% carboxymethyl cellulose (CMC) and were administered 60 min prior to acetic acid challenge in varying doses of 25-400 mg/kg, s.c. Vehicle treated animals served as control.

Role of opioid system—The role of opioid system was studied by administering naloxone (5 mg/kg ip) 45 min after DHF administration.

Role of K^+_{ATP} channels—This was studied by administering glibenclamide (10 mg/kg, ip) as known K^+_{ATP} sensitive blocker (dose related based on earlier studies)^{7,8}. 40 min after DHFs treatment and anti-nociception was measured.

Drugs and chemicals used—The following six DHFs were synthesized at Herboranics, Chennai following the standard procedure. Their physico-chemical authentication was also carried out.

**Correspondent author: E-mail: visu53@hotmail.com

- 3,6 - di-hydroxy flavone
 3,2' - di-hydroxy flavone
 6,7 - di-hydroxy flavone
 3,4' - di-hydroxy flavone
 3',4' - di-hydroxy flavone
 7,2' - di-hydroxy flavone

The authenticity of these substances was identified using physicochemical properties like Melting point, Elemental analysis, TLC, UV and IR.

Acetic acid, glacial (AR, IDPL), carboxymethyl cellulose sodium salt (Glaxo Laboratories, Bombay),

glibenclamide (Dr Reddy's Laboratory, Hyderabad), morphine sulphate (Govt. Opium and Alkaloid Works, Ghazipur) and naloxone hydrochloride (Endo Labs, NY, USA) were used.

Statistics—The data were subjected to ANOVA followed by Dunnett's 't' test. A level of $P < 0.05$ was considered as statistically significant.

Experiments conducted with rota rod employing all six DHFs failed to modify significantly the motor activity of the mice tested. Therefore, it can be presumed that the data obtained with acetic acid assay as

Table 1—Effect of naloxone/glibenclamide on different DHF induced inhibition of acetic acid writhing in mice

[Values, express number of abdominal constrictions, are mean $\pm$ SE]

Treatment mg/kg, sc	In absence of naloxone	In presence of naloxone	In presence of glibenclamide
Vehicle (10 ml/kg)	32.10 $\pm$ 0.70	31.50 $\pm$ 1.20	27.90 $\pm$ 2.10
3,6-DHF 100	16.50 $\pm$ 1.30	26.70 $\pm$ 0.95*	27.50 $\pm$ 0.40*
6,7-DHF 100	18.50 $\pm$ 0.30	26.80 $\pm$ 0.70*	26.40 $\pm$ 1.50*
3,2'-DHF 100	20.00 $\pm$ 0.60	27.70 $\pm$ 1.20*	25.40 $\pm$ 1.20*
3,4'-DHF 100	18.70 $\pm$ 0.30	27.20 $\pm$ 0.50*	27.50 $\pm$ 0.40*
3',4'-DHF 100	17.30 $\pm$ 0.20	24.70 $\pm$ 0.70*	26.10 $\pm$ 1.20*
7,2'-DHF 100	14.30 $\pm$ 1.40	25.00 $\pm$ 0.40*	25.70 $\pm$ 0.70*

Naloxone, 5 mg/kg, ip, was administered 15 min prior to acetic acid

Glibenclamide, 10 mg/kg, was administered 15 min prior to acetic acid

* $P < 0.01$ compared with the value observed without naloxone treatment

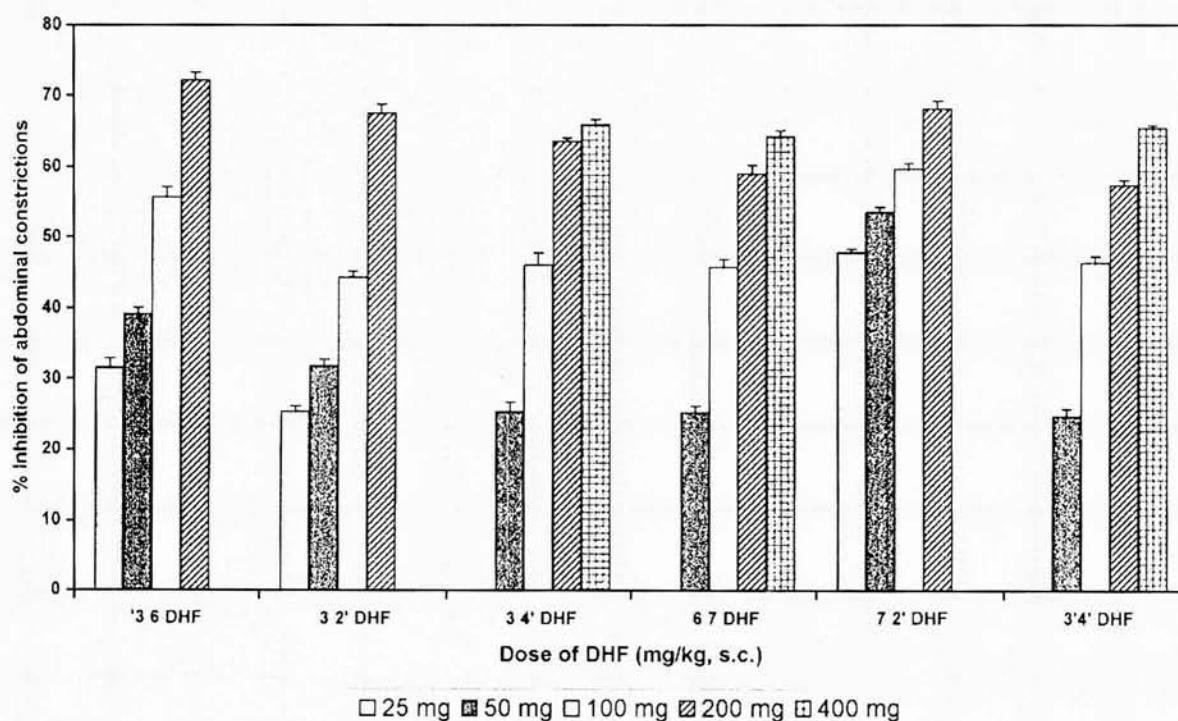


Fig. 1—Effect of di-hydroxy flavones on the acetic acid induced abdominal constrictions in mice (n=6). Values are mean $\pm$ SE.

true one and does not represent a false positive response.

Anti-nociception—All the six DHFs tested produced a dose related reduction in number of abdominal constrictions. A maximum reduction of 60-70% was recorded when compared with control (Fig. 1).

In the naloxone treated animals (5 mg/kg, ip) the reduction in the number of abdominal constrictions produced by all the six DHFs was significantly attenuated (Table 1).

Role of K^+_{ATP} channels—Glibenclamide, treatment *per se* induced hypoglycemia, hyper-insulinemia and no significant change in number of abdominal constrictions. However, its treatment in DHFs exposed animals antagonised the number of abdominal constrictions (Table 1).

The findings of the present study clearly indicate that the all newly synthesized DHFs, exhibited significant anti-nociceptive activity as tested by the standard and sensitive acetic acid induced abdominal constriction assay procedure. This effect was though dose-related, exhibited a ceiling effect. A maximum of 60-70% inhibition was only recorded like other flavone substances. Despite the earlier available literature indicated that the poly-substitution of flavones possibly enhance their effect⁵, we could not observe such an effect in the anti-nociceptive response. Additional substitution might prove beneficial. However, the limitation that these DHFs exhibited a ceiling effect, they are still advantages as they are free from side effects⁵. Amongst the six DHFs investigated, the efficacy appeared to be $7,2' > 3,6 > 3,2' > 3,4' = 6,7 > 3',4'$ based on their ED_{50} values. The anti-nociceptive effect recorded for DHFs was attenuated by naloxone pretreatment. In other words, in naloxone pretreated animals, the DHFs were unable to produce anti-nociceptive response. This finding was similar to other flavonoid substances as observed earlier⁹. Additionally, like morphine, there appears a role for K^+_{ATP} sensitive channels in the anti-nociceptive and hyper-insulinemic effect of DHFs.

Considering the modification of structure, i.e. di-hydroxy substitution attempted in this study to

improve the efficacy of the anti-nociceptive effect of flavones, the results revealed that still the ceiling effect persisted. It appears that the original notion that polysubstitution improves efficacy needs additional structural changes either with hydroxy or other radicals to improve the efficacy of flavonoid induced anti-nociception.

In summary, we have synthesized six DHFs, which can be included in the arena of flavonoids as potential candidates for relief of pain.

The authors acknowledge the assistance rendered by Dr.V.Krishnamurthy, Research Organics, Chennai in synthesizing DHFs and Dr.J.Mohanasundaram, Professor of Pharmacology, Chengleput Medical College, Chengleput.

References

- 1 Ramaswamy S, Padmanabha Pillai N, Gopalakrishnan V & Parmar N S, Analgesic activity of bioflavonoid in mice. *Indian J Pharmacol*, 12 (1980) 33.
- 2 Viswanathan S, Thirugnanasambantham P, Bapna J S & Kameswaran L, Flavonoid induced delay of small intestinal transit. Possible mechanism of action. *Arch Int Pharmacodyn*, 270 (1984) 151.
- 3 Viswanathan S, Thirugnanasambantham P, Ramaswamy S & Kameswaran L, Studies on the possible tolerance and mechanism of gossypin analgesia. *Indian J Exp Biol*, 23 (1985) 525.
- 4 Viswanathan S, Thirugnanasambantham P, Ramaswamy S & Bapna J S, A study on the role of cholinergic and gamma aminobutyric acid systems in the anti-nociceptive effect of gossypin. *Clin Exp Physiol Pharmacol*, 20 (1993) 193.
- 5 Thirugnanasambantham P, Viswanathan S, Krishnamurthy V, Mythirayee C, Ramachandran S & Kameswaran L, Analgesic activity of certain flavone derivatives: a structure-activity study. *J Ethnopharmacol*, 28 (1990) 207.
- 6 Koster R, Anderson M & De Beer E J, Acetic acid for analgesic screening. *Fed Proc*, 18 (1959) 412.
- 7 Shewade D G & Ramaswamy S, Prolactin induced analgesia is dependent on ATP sensitive potassium channels. *Clin Exp Pharmacol Physiol*, 22 (1995) 635.
- 8 Berridge T L, Doxey J C & Roach A G, Comparison of the effects of efaroxan and glibenclamide on plasma glucose and insulin levels in rats. *Eur J Pharmacol*, 213 (1992) 213.
- 9 Rajendran N N, Thirugnanasambantham P, Parvathavarthini S, Viswanathan S & Ramaswamy S, Modulation by insulin rather than blood glucose of the pain threshold and flavone induced anti-nociception in mice. *Indian J Exp Biol*, 39 (2001) 1009.