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

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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 (DHFs) 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 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 rutoside, further studies with gossypin epicatechin, flavone were carried out. It has been observed that these flavonoid substances produced significant opioid and ATP sensitive (K\textsubscript{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 derivatives 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 (DHFs) 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.

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

**Measurement of anti-nociception**—The acetic acid induced abdominal constriction assay\textsuperscript{g} 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\textsubscript{ATP} channels**—This was studied by administering glibenclamide (10 mg/kg, ip) as known K\textsubscript{ATP} sensitive blocker (dose related based on earlier studies)\textsuperscript{78}, 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.
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>
<thead>
<tr>
<th>Treatment</th>
<th>In absence of naloxone</th>
<th>In presence of naloxone</th>
<th>In presence of glibenclamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (10 ml/kg)</td>
<td>32.10 ± 0.70</td>
<td>31.50 ± 1.20</td>
<td>27.90 ± 2.10</td>
</tr>
<tr>
<td>3,6-DHF 100</td>
<td>16.50 ± 1.30</td>
<td>26.70 ± 0.95*</td>
<td>27.50 ± 0.40*</td>
</tr>
<tr>
<td>6,7-DHF 100</td>
<td>18.50 ± 0.30</td>
<td>26.80 ± 0.70*</td>
<td>26.40 ± 1.50*</td>
</tr>
<tr>
<td>3,2'-DHF 100</td>
<td>20.00 ± 0.60</td>
<td>27.70 ± 1.20*</td>
<td>25.40 ± 1.20*</td>
</tr>
<tr>
<td>3,4'-DHF 100</td>
<td>18.70 ± 0.30</td>
<td>27.20 ± 0.50*</td>
<td>27.50 ± 0.40*</td>
</tr>
<tr>
<td>3',4'-DHF 100</td>
<td>17.30 ± 0.20</td>
<td>24.70 ± 0.70*</td>
<td>26.10 ± 1.20*</td>
</tr>
<tr>
<td>7,2'-DHF 100</td>
<td>14.30 ± 1.40</td>
<td>25.00 ± 0.40*</td>
<td>25.70 ± 0.70*</td>
</tr>
</tbody>
</table>

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 ± SE.
flavone substances. Despite the earlier available evidence, they are still advantages as observed such an effect in the anti-nociceptive literature indicated that the poly-substitution of flavones possibly enhance their effects, we could not observe earlier. Additionally, like morphine, there is a ceiling effect. 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\textsuperscript{+}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 effect\(^5\), 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 effects. 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\textsubscript{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 earlier\(^9\). Additionally, like morphine, there appears a role for K\textsuperscript{+}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.

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**References**