

## EFFECT OF DIHYDROXY FLAVONES ON MORPHINE TOLERANCE

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### ABSTRACT

Flavonoids like gossypin and quercetin were earlier demonstrated to suppress the development of morphine tolerance. The present study investigated the effect of four dihydroxy flavone compounds (3,2'- dihydroxy flavone, 3,3'- dihydroxy flavone, 3,4'- dihydroxy flavone and 5,7,- dihydroxy flavone) on the development of tolerance to morphine antinociception in mice. Mice were treated with increasing doses of morphine (s.c.) for five days from 10 mg/kg/day with an increment of 5 mg/kg every alternate day. Different groups of mice were pretreated with the above dihydroxy flavones 30 minutes prior to morphine injection every day. On the sixth day, the antinociceptive effect of morphine (1 mg/kg, s.c.) was assessed in all the above treatment groups by employing acetic acid induced abdominal constriction assay and warm water tail immersion assay. Chronic treatment with morphine for five days resulted in decrement of analgesic response on the sixth day. The number of abdominal constrictions was significantly increased in morphine-tolerant animals compared to naïve mice. A similar attenuation of morphine-antinociceptive effect was recorded in tail immersion assay in mice exposed to morphine treatment for five days as revealed by a reduction in tail flick latency compared to morphine naïve mice. Pre-treatment with different dihydroxy flavone compounds did not alter the response recorded in morphine tolerant mice. This observation indicated that the investigated dihydroxy flavones failed to modulate the development of tolerance to morphine antinociception in mice.

**Key words:** Dihydroxy flavones, morphine tolerance, anti-nociception.

### INTRODUCTION

Morphine and related opioid analgesics remain the mainstay of management of pain due to myocardial infarction, traumatic, post operative and cancer pain. Repeated daily administration of opioid analgesics especially in chronic pain situations like cancer or terminal illness invariably decreases the analgesic effect due to development of tolerance. This remains a limiting factor and great impediment to the treatment of pain in many chronic painful situations.

Tolerance could result from alterations in receptor coupling, receptor number, the amount of effector protein or the capacity of an effector to be regulated by opioid receptors (Bailey CP and Connor M, 2005), leading to desensitization or down regulation of receptor-efficacy coupling mechanisms (Von-Zostrow et al. 2003). Many agents like N-methyl D-aspartate (NMDA) receptor antagonists such as ketamine (Sasnowski, 1993), dextromethorphan (Elliott et al. 1994),

calcium channel antagonist like nimodipine (Santillan et al. 1998) have been concurrently used along with opioid analgesics for preventing tolerance development. Thus, it is obvious that adjuvant use of other compounds can overcome opioid tolerance and ensure adequate relief in chronic painful situations.

Flavonoids are a group of naturally occurring polyphenolic compounds documented to possess antinociceptive effect in various experimental animal models (Viswanathan et al. 1984; Thirugnana sambantham et al. 1990 and 1993; Anjaneyulu and Chopra, 2003; Gadotti et al. 2005; Umamaheswari et al. 2006; Vidyalakshmi et al. 2010). Further, gossypin (flavonol glucoside) pre-treatment was found to significantly attenuate the acute tolerance development to morphine analgesia (Ramasamy and Viswanathan, 1997). In another study, quercetin (penta hydroxy flavone) was found to reverse morphine tolerance and dependence possibly by suppressing nitric oxide synthase activity (Naidu et al. 2003). The present study is designed to investigate the influence of a few dihydroxy flavone derivatives on the

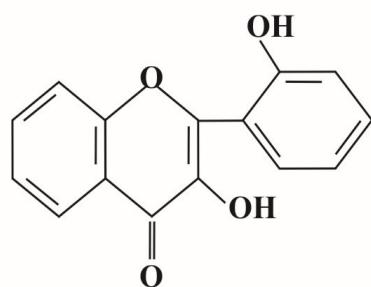
development of chronic tolerance to the antinociceptive effect of morphine in mice.

## MATERIALS AND METHODS

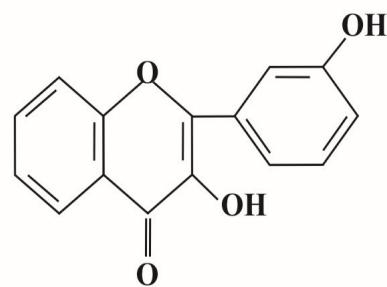
The following dihydroxy flavones (Research Organics, Chennai), with established antinociceptive activity were chosen for investigation; 3,2'- dihydroxy flavone (100 mg/kg), 3,3'- dihydroxy flavone (100 mg/kg), 3,4'- dihydroxy flavone (100 mg/kg) and 5,7,-dihydroxy flavone (50 mg/kg) (figure 1). A dose of dihydroxy flavone that produced almost 50% inhibition of nociception in the acetic acid induced nociceptive assay in earlier investigations (Thirugnana sambantham et al. 1990; Girija et al. 2002; Vidyalakshmi et al. 2010) was selected for the present work. Test compounds were prepared as a suspension in 1% carboxy methyl cellulose and injected subcutaneously to mice. Other chemicals used in the study are morphine sulphate (Vermor Pharmachemicals Labs, India) and acetic acid (SD Fine Chemicals).

**Figure – 1: Structure of investigated compounds:**

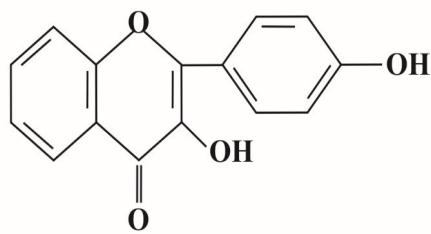
**3,2' – dihydroxy flavone**



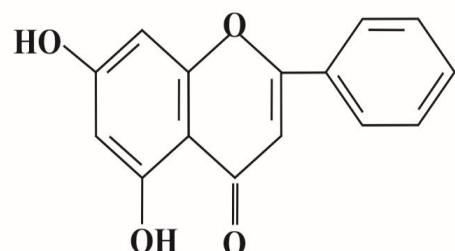
**3,3' – dihydroxy flavone**



**3,4' – dihydroxy flavone**



**5,7, - dihydroxy flavone**



**Animals:**

Male Swiss albino mice (20-25g) were used for the experiments. They were maintained in the institutional animal house under standard housing conditions. Food and water were available *ad libitum*. The experimental protocol was approved by the institutional animal ethical committee.

**Induction of morphine tolerance:**

In the present study, the modified method of Hamdy et al. (2004) was used to induce analgesic tolerance to morphine in mice. Mice were treated with increasing doses of morphine (10-20 mg/kg with an increment of 5 mg/kg every alternate day) for five days.

**Drug treatment:**

Different groups of mice were pre-treated with various dihydroxy flavones (in the doses described earlier) 30 minutes prior to morphine injection on all the five days. On the sixth day, the antinociceptive effect of morphine (1 mg/kg, s.c.) was assessed in these animals as detailed below.

**Assessment of anti-nociception:**

Acetic acid 0.6% was injected i.p. in a dose of 10 ml/kg and the number of abdominal constrictions was counted in the following 15 minutes period (Koster et al. 1959). Mice were treated with morphine (1 mg/kg, s.c.) 30 minutes prior to acetic acid challenge.

In tail immersion method (Sewell and Spencers, 1976) the mouse was restrained in a holder and the tail was immersed in a water bath maintained at  $55 \pm 0.5^{\circ}\text{C}$ . The reaction time to

flick the tail from the hot water was recorded before and 30 minutes after morphine (1 mg/kg) administration in all the test groups. A separate group of animals was treated with vehicle for five days (morphine naïve animals) and on the sixth day the antinociceptive effect of morphine (1 mg/kg, s.c.) was studied in these animals.

The results were analysed by Student's 't'-test. A *p* value of  $<0.05$  was considered statistically significant.

**RESULTS****Acetic acid induced abdominal constriction assay:**

The number of abdominal constrictions after i.p. injection of acetic acid in vehicle treated control animals was  $31.9 \pm 1.18$  (Table -1). In morphine naïve mice (group 2) treatment with morphine in a dose of 1mg/kg significantly reduced the number of abdominal constrictions to  $3.0 \pm 0.36$ . However, in animals that received increasing doses of morphine for five days (group 3, morphine tolerant mice) the reduction in the number of abdominal constrictions elicited by morphine (1 mg/kg) on the sixth day ( $11.6 \pm 0.37$ ) was significantly attenuated. Pre-treatment with various dihydroxy flavones has not significantly altered the response observed after repeated morphine injections. The number of abdominal constrictions in these animals (group 4 - 7) varied between  $10.5 \pm 0.56$  to  $12.8 \pm 0.95$  (table 1) which was not significantly different from that observed in morphine tolerant animals (group 3).

**Table – 1: Effect of dihydroxy flavones on tolerance to morphine antinociception <sup>a</sup> (Acetic acid induced abdominal constriction assay and warm water tail immersion assay)**

Group	Pre-treatment	Treatment	Number of abdominal constrictions (Acetic acid) (Mean $\pm$ SEM)	Increase in reaction time in seconds (Tail immersion) (Mean $\pm$ SEM)
1.	Vehicle	Vehicle	$31.9 \pm 1.18$	$0.11 \pm 0.08$
2.	Vehicle	Morphine 1mg/kg, sc	$3.0 \pm 0.36^*$	$2.0 \pm 0.23^*$
3.	Morphine (10-20mg/kg, s.c., 5 days)	Morphine 1mg/kg, sc	$11.6 \pm 0.37^{*\#}$	$1.3 \pm 0.13^{*\#}$

4.	3,2'-DHF 100mg/kg + Morphine (10-20mg/kg, s.c., 5 days) <sup>b</sup>	Morphine 1mg/kg, sc	11.8 ± 0.9* <sup>#</sup>	1.13 ± 0.23* <sup>#</sup>
5.	3,3'-DHF 100mg/kg + Morphine (10-20mg/kg, s.c., 5 days) <sup>b</sup>	Morphine 1mg/kg, sc	12.6 ± 0.8* <sup>#</sup>	1.25 ± 0.11* <sup>#</sup>
6.	3,4'-DHF 100mg/kg + Morphine (10-20mg/kg, s.c., 5 days) <sup>b</sup>	Morphine 1mg/kg, sc	10.5 ± 0.56* <sup>#</sup>	1.21 ± 0.08* <sup>#</sup>
7.	5,7,-DHF 50mg/kg + Morphine (10-20mg/kg, s.c., 5 days) <sup>b</sup>	Morphine 1mg/kg, sc	12.8 ± 0.95* <sup>#</sup>	1.31 ± 0.19* <sup>#</sup>

<sup>a</sup> Antinociceptive effect of morphine (1mg/kg, s.c.) was recorded on the sixth day in various treatment groups (2-7).

<sup>b</sup> Mice were pretreated with dihydroxy flavone s.c 30 min before morphine treatment every day for 5 days.

\* p< 0.01 compared to vehicle treatment.

# p< 0.01 compared to morphine (1mg/kg, s.c) treatment in naive mice (Group 2).

#### Warm water tail immersion assay:

Morphine 1 mg/kg treatment in naive mice (group 2) significantly increased the reaction time (2 ± 0.23 seconds) compared to vehicle treated control animals (0.11 ± 0.08 seconds, table-1). This was significantly attenuated in mice treated with high doses of morphine for five days (1.3 ± 0.13 seconds, group 3). Pre-treatment with different dihydroxy flavones did not alter the attenuation of the antinociceptive response of morphine. The increase in reaction time observed in animals exposed to various dihydroxy flavones and morphine for five days (group 4 – 7) ranged between 1.13 ± 0.23 and 1.31 ± 0.19 seconds and this was not significantly different from the value (1.3 ± 0.13 seconds) recorded in morphine tolerant animals (group 3).

## DISCUSSION

Opioid analgesics like morphine are the mainstay in the management of severe pain such as cancer. The compelling reasons to the use of opioid analgesics in pain or pain of terminal illness in the above settings are manifold. The excellent pain relief, tranquillity and even euphoria afforded by the use of opioids are very beneficial to the extremely suffering patient (Gutstein and Akil, 2006). However, the clinical usefulness of opioid analgesics is limited by significant side effects especially by the development of tolerance,

physical dependence and addiction potential. But, it is a concerted opinion that the possibility of development of tolerance and physical dependence should not in any way prevent the physician from fulfilling his primary obligation to provide adequate pain relief to the patient. Understanding the molecular basis of morphine tolerance has helped in the development of novel treatment options to overcome opioid tolerance.

Many agents like NMDA receptor antagonists such as ketamine (Sasnowski, 1993), dextromethorphan (Elliott et al. 1994), alpha-2 adrenergic agonist like clonidine (Ramasamy et al. 1981; Maldonado, 1997; Gowing et al. 2002), calcium channel antagonist like nimodipine (Santillan et al. 1998) have been successfully investigated and also employed therapeutically as adjuvants to overcome morphine tolerance. In addition, nitric oxide synthase inhibitors also have been demonstrated to attenuate the development of morphine tolerance (Meller et al. 1992; Kolesnikov et al. 1993; Mao et al. 1995). Such strategies to overcome morphine tolerance will offer definite advantage in the treatment of chronic pain as these adjuvant drugs will have an opioid-sparing effect.

In addition to the above compounds, a few reports indicate that some flavonoid compounds like gossypin (Viswanathan et al. 1990; Ramasamy and Viswanathan, 1997) and quercetin (Naidu et al. 2003) suppress the development of tolerance to opioid analgesia and also the expression of opioid

withdrawal symptoms. Hence, it was considered interesting to investigate a few structurally related dihydroxy flavone compounds for their possible role on morphine tolerance.

The results of the present study clearly indicated that repeated administration of morphine in increasing doses for five days resulted in tolerance development to its antinociceptive effect. This was evident in both acetic acid induced abdominal constriction assay and in tail immersion method. Pre-treatment with various dihydroxy flavones (3,2'- dihydroxy flavone, 3,3'- dihydroxy flavone, 3,4'- dihydroxy flavone or 5,7,- dihydroxy flavone) has not altered the development of tolerance to the antinociceptive effect of morphine in mice. This has been confirmed from the results of the two antinociceptive procedures carried out. Thus the investigated dihydroxy flavones failed to alter the development of tolerance to morphine antinociception in mice.

In earlier studies, quercetin (3,5,7,3',4'-pentahydroxy flavone) (Naidu et al. 2003) and gossypin (3,5,7,3',4'- pentahydroxyflavone-8-O-glucoside) (Ramasamy and Viswanathan, 1997), have been demonstrated to suppress the development of analgesic tolerance to morphine in experimental animals. However, the present results indicated that this property is not shared by the dihydroxy flavones investigated in this study. Though, it is not possible to offer a satisfactory explanation for the lack of efficacy of dihydroxy flavones on morphine tolerance, a correlation between the chemical structure and activity may be

considered. The compounds earlier demonstrated to suppress morphine tolerance (quercetin and gossypin) are both pentahydroxy flavones. Gossypin in addition also possesses a glycoside moiety in the 8<sup>th</sup> position of flavone nucleus. Polyhydroxylation of flavone nucleus perhaps may be an essential feature to overcome the development of tolerance to opioids. More detailed investigations with other flavone derivatives and in particular polyhydroxy flavones may help us to identify effective compounds that will be useful to overcome morphine tolerance.

## CONCLUSION

Different classes of drugs are employed to overcome the tolerance development to morphine analgesia. Based on previous reports, the present study screened four dihydroxy flavone compounds for their effect on morphine tolerance. Even though, the investigated dihydroxy flavones did not influence the morphine analgesic tolerance, it is suggested that a broader study with more polyhydroxy flavones may be rewarding.

## ACKNOWLEDGEMENT

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