Summary
SUMMARY

Parallel to the advancements in curative medicine, significant progress has also been made in identifying preventive medicines. Many natural products especially phytochemicals have been identified as nutraceuticals with proven beneficial effects in the prevention of many chronic ailments. Carotenoids, phytosterols, saponins, terpenoids and polyphenolic compounds like flavonoids are some of the nutraceuticals employed all over the world for their health benefits.

The flavonoid polyphenolics are an important group of nutraceuticals due to their ubiquitous presence in nature and multiple health benefits they offer. Apart from the role as nutraceuticals, many recent investigations have also explored the potential usefulness of flavonoids in certain specific disease conditions. An important finding in this regard is a combination of antinociceptive and anti inflammatory effect for many flavone derivatives in different experimental models.

Some other reports have also revealed that flavonoid compounds are devoid of gastric mucosal irritation and in fact some of them are effective in healing experimentally induced ulcers. The above observations aroused curiosity among many workers, because the drugs that are currently employed to treat pain and inflammation invariably provoke gastric ulceration. Potent narcotic analgesics like morphine also have many significant limitations for their use.
Hence, to identify safer analgesic and antiinflammatory agents, a few investigations have been carried out earlier to delineate the pharmacological profile of flavone compounds with particular reference to their antinociceptive and anti-inflammatory effects. Flavone, its monohydroxy, monomethoxy, a few dihydroxy and a few flavonol glucosides have been documented to possess anti nociceptive and anti inflammatory effects. Possible structure activity correlations have also been attempted in the above said effects. However, the maximum anti nociceptive efficacy of these compounds in an established antinociceptive assay was found to be around 70 percent similar to previously reported compounds.

**Selection of dihydroxy flavones**

In order to identify more effective flavone compounds, the present study was designed with four new dihydroxy flavones. A few flavone compounds with one hydroxyl group in 3’ position and another hydroxyl substitution in a carefully chosen second position of the flavone nucleus were selected for present investigation. In addition, another compound 2’, 4’ dihydroxy flavone which has not been investigated so far was also included in the present study. These compounds were subjected to a battery of tests to investigate their safety, antinociceptive, anti inflammatory and also their possibility of their ulcerogenic potential. Additionally the mechanisms involved in the antinociceptive and anti inflammatory effects were analysed.
Safety profile

The acute toxicity testing carried out in mice revealed that the tested dihydroxy flavones did not produce any mortality even in a dose of 2g/kg indicating the safe nature these compounds. This observation confirms an earlier report stating that “The margin of safety for the therapeutic dose of flavonoid in humans can be considered to be very large, probably much superior to any other drug in current use” (Havsteen. 2002).

Antinociceptive study

The assessment of antinociceptive potential of the chosen dihydroxy flavones was carried out by employing three different well established assay models. A consistent and dose related antinociceptive effect of dihydroxy flavones was clearly evident from the results of acetic acid induced nociception, formalin induced nociception and hot water tail immersion assay in mice. In a dose of 200 mg/kg, all the tested dihydroxy flavones exhibited nearly 100% inhibition of abdominal constriction response. Similarly 100% inhibition of the late phase (inflammatory phase) of formalin nociception was produced by these compounds. The early phase of formalin (neurogenic phase) was inhibited to an extent of 80 %. In the thermal model of nociception, where the pain intensity is maximum, different dihydroxy flavones produced an inhibition ranging between 68-78 percent.

The results of the three different antinociceptive assays provide concrete evidences for the analgesic potential of different dihydroxy flavones.
The inflammatory pain in acetic acid induced nociception and formalin induced nociception were completely (100%) inhibited by the tested compounds. Moreover the neurogenic pain in the early phase of formalin nociception and thermal pain was also maximally inhibited by dihydroxy flavones. The effectiveness of dihydroxy flavones in amelioration of various types of pain is revealed by the present study.

Many mono and disubstituted flavone derivatives and flavonol glucosides investigated earlier were reported to produce only a maximum of 70 percent inhibition of nociception in acetic acid induced abdominal constriction assay. The present investigations have identified more effective antinociceptive flavones than those previously reported.

**Structure activity relationship**

A possible correlation between chemical structure and antinociceptive efficacy has been attempted from the results of the present study in comparison with previous reports. The antinociceptive efficacy of dihydroxy flavones tested in the present study was found to be much superior to that of respective mono hydroxy flavones reported earlier. It can also be stated that introduction of a hydroxyl group at 3’ position of the flavone nucleus enhanced antinociceptive efficacy of the respective monohydroxy flavones.
Mechanism of antinociceptive action

Opioid

Earlier reports have indicated a role for opioid system in the antinociceptive action of many flavone derivatives. Such a possibility was also examined in the present study. Naloxone, a non selective opioid antagonist was able to reverse the antinociceptive activity of all the presently investigated dihydroxy flavones. This observation confirms the earlier finding that opioid mechanism plays an important role in the antinociceptive action of dihydroxy flavones. The identification of opioid receptor involvement in the antinociceptive action of dihydroxy flavones, prompted for designing suitable experiments to investigate the acute and chronic tolerance to their antinociceptive effect.

The effectiveness of these compounds to inhibit nociception remained unchanged even after repeated administration, either in acute or chronic fashion. This observation indicates the absence of either acute or chronic tolerance to the antinociceptive action of dihydroxy flavones. Similar observation has been reported for many flavone derivatives by earlier workers and as such with the available data it is difficult to offer an explanation for the lack of tolerance to the antinociceptive effect of dihydroxy flavones through the involvement of opioid mechanism in the above action. Participation of various pathways, other than opioid system has been identified in the antinociceptive action of flavones by several investigators cannot be excluded. Involvement of peripheral opioid receptors like those present on
sensory nerve endings and inflammatory cells has also been proposed as a possible mechanism of antinociceptive action of dihydroxy flavones. Perhaps the above possibilities may be responsible for the absence of tolerance noted for many flavone derivatives.

**Anti inflammatory effect**

Earlier studies indicated significant anti-inflammatory activity for many monohydroxy flavones. Hence it was considered interesting to screen the dihydroxy flavones for this activity. Moreover in the present study all the four dihydroxy flavones were found to exert potent antinociceptive action, especially in inflammatory models of pain. Therefore these compounds were screened for their effect on acute inflammation employing carageenan induced paw edema in rats. This is a well established animal model and substances that are found to significantly reduce the paw edema have been therapeutically correlated to posses a good anti inflammatory activity.

The results of the present study indicated a dose and time dependent anti inflammatory activity of all the tested dihydroxy flavones. Nearly 81-88 \% of inhibition of inflammation was evident for the tested compounds in a dose of 50mg /kg and hence may be considered to be equi effective in their action. In general, the anti inflammatory efficacy of dihydroxy flavones appear to be greater than monohydroxy flavones reported earlier. Thus the present study has identified that the dihydroxy flavones too posses potent antinociceptive and anti inflammatory actions.
Effect of dihydroxy flavones on the mediators of pain and inflammation

Pain and inflammation are complex processes and many endogenous mediators/chemicals have been identified to initiate and perpetuate these processes. The effect of dihydroxy flavones on some of these mediators has been investigated in the present study. Some of them are implicated in both in pain and inflammation, while a few have a predominant role in inflammation and hence a causative factor for pain generation. Currently used analgesic and anti-inflammatory drugs have been shown to interact at these sites in mediating their effects. Previous reports also indicate the action of a few flavonoids on some of these mediators. In the present study, the dihydroxy flavones were investigated for their effect on cyclooxygenase enzyme system, TNF-α, interleukin-6, nitric oxide and reactive oxygen species / free radicals by selecting appropriate in vitro and in vivo tests.

The results indicated a significant inhibition of the activity of the afore mentioned mediators by the tested dihydroxy flavones. The potent antinociceptive and anti-inflammatory effect exhibited by the dihydroxy flavones may be attributed to the inhibition of several mediators of pain and inflammation.

Synergism with other drugs

It is conventional to combine drugs to achieve enhanced therapeutic effect and minimise the side effects. In chronic pain situations, NSAIDs are usually combined with opioids to reduce the tolerance development. Any
agent that can potentiate the anti inflammatory effect of NSAID with simultaneous reduction in their side effects will also be highly desirable. Previous studies indicated a role of flavonoids in exerting such a potentiation with various analgesics and anti inflammatory drugs. Hence in the present study, a combination of dihydroxy flavones with either morphine or diclofenac in minimally effective doses was investigated for their combined antinociceptive effect. Similarly, a combination of dihydroxy flavones and diclofenac in minimally effective doses was investigated for its effect on acute inflammation induced by carageenan. The results of the protocol indicated that dihydroxy flavones potentiated the antinociceptive effect of either morphine or diclofenac. At the same time, a potentiated anti inflammatory effect was also observed while combining minimally effective doses of dihydroxy flavones and diclofenac. These observations open up a new avenue in the management of pain and inflammation. Dihydroxy flavones may be considered as suitable candidates to enhance the therapeutic efficacy of conventional analgesics and anti inflammatory effects with minimal side effects and more therapeutic benefits.

Ulcerogenic potential of Dihydroxyflavones

In general, compounds possessing a combination of antinociceptive and antiinflammatory actions like NSAID have a propensity to induce gastric mucosal damage and ulceration. The test compounds employed in the present study exhibited a significant antinociceptive and antiinflammatory effect. Hence, the dihydroxyflavones were studied for their effect on gastric
mucosa and also against aspirin induced ulceration in rats. The test compounds per se did not induce any gastric mucosal damage in rats. Moreover aspirin induced ulceration was significantly attenuated by dihydroxyflavones pretreatment. These observations are similar to many previous investigations on flavonoids.

Dihydroxy flavones have been shown in the present study to influence several mediators of inflammation and pain apart from COX-I. This might be the reason for the absence of gastric mucosal damage by dihydroxyflavones. Moreover the dihydroxyflavones may promote the generation of defensive factors and oppose the formation of aggressive factors on gastric mucosa. This has been revealed by their scavenging effect on reactive oxygen species/ free radicals.