STUDY OF THE ANTI-INFLAMMATORY AND ANALGESIC EFFECTS OF NOVEL RIGID BENZOFURAN-3, 4-DIHYDROXY CHALCONE BY FORMALIN, HOT-PLATE AND CARRAGEENAN TESTS IN MICE

MAHMOUD REZA HEIDARI[a,b]*, ALIREZA FOROUMADI[b,c] , HOJAT NOROOZI[a], ALI SAMZADEH-KERMANI[d] , AND BEHZAD SARVAR AZIMZADEH[e]

[a]*Department of Toxicology and Pharmacology, Faculty of Pharmacy, Pharmaceutics and Physiology Research Centers, Kerman University of Medical Sciences, Kerman, Iran*

[b]*Kerman Neuroscience Research Center, Kerman University of Medical Sciences, Kerman, Iran*

[c]*Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran*

[d]*Faculty of Sciences, Tarbiat Modarress University, Tehran, Iran*

[e]*Department of Cardiology, Faculty of Medicine, Kerman University of Medical Sciences, Kerman, Iran*

ABSTRACT

It is reported that dihydroxy chalcones have analgesic and anti-inflammatory effects. Study of the structure activity relationship (SAR) shows that benzofuran-3-one derivatives may be more effective in this respect. In this study, a new (Z)-2-(3,4-dihydroxybenzylidene)-5-methoxybenzofuran-3(2*H*)-one (compound **5**) was synthesized and its analgesic and anti-inflammatory effects were evaluated by formalin, carrageenan and hot-Plate methods in mice.

The results showed that, compound **5** induced significant antinociceptive and anti–inflammatory effect ($P< 0.01$). Maximum analgesia (42.6 %) was obtained at dose of 25 mg/kg in the first phase of formalin test. The effect of compound **5** was higher (87.7%) in chronic phase of inflammation induced by formalin ($P< 0.01$). Administration of 25 mg/kg of compound **5** inhibited the inflammation induced by carrageenan, 32.8% and 41.7%, 1 and 3 hour after carrageenan injection, respectively. In addition, this dose of compound **5**, induces significant analgesia (20.2%) in hot plate test 45 minutes after injection ($P<0.01$). Therefore it seems that compound **5** has potential for discovery of a compound with potent anti-inflammatory and analgesic effects and its scaffold could be use for further structural modifications.

Keywords: Benzylidene benzofuran-3-one, analgesic activity; anti-inflammatory effect; formalin; carrageenan; hot-plate.

INTRODUCTION

Analgesic and anti-inflammatory drugs are one of the most products that used in many of disease for relief of pain and inflammation. Most analgesic and anti-inflammatory drugs available in the market, still present a wide range of many problems such as efficacy and undesired effects including GIT disorders and other unwanted effects, (Heidari *et al*., 2007; Girard *et al*., 2008), that limit their clinical usefulness and remain to be solved and leaving an open door for new and better compounds (Heidari *et al*., 2006; Tao *et al*., 2008). This situation highlights the need for advent of safe, novel and effective analgesic and anti-inflammatory compounds ((Kolesnikov and Sõritsa, 2007; Tao *et al*., 2008). It is found that chalcones with 3,4-dihydroxycinnamoyl structure strongly inhibited lipid peroxidation in rat liver microsomes (Sogawa *et al*., 1994). Some chalcone derivatives have been reported as anti-inflammatory or antiallergic agents (Sogawa *et al*., 1993). Potential anti-inflammatory and cancer chemo preventive activity of synthetic chalcones have been reported (Won *et al*., 2005). Nakadate *et al*., have reported that known hydroxy chalcones inhibit 12-lipoxygenase and cycloxygenase in the mouse epidermis (Nakadate *et al*., 1985). A series of benzofuran-3-one derivative have been reported to have potent anti-inflammatory activity (Hsieh *et al*., 1998) and inhibition of ocular inflammation (Chiou *et al*., 1992)]. The benzofuran-3-one derivatives are rapidly and extensively metabolized after systemic administration (Sogawa *et al*., 1993). These facts suggest that chalcone may be nontoxic anti-inflammatory agents. Therefore, in an attempt to continually developed potent anti-inflammatory agents, in the present study a new rigid benzofuran-3-one derivative were synthesized and invest-tigated the analgesic and anti-inflammatory activity by chemical, formalin (Hunskaar and Hole, 1987; Shibata *et al*., 1989; Heidari *et al*., 2007), Carrageenan (Gilligan and Lovato, 1994; Winter *et al*., 1962; Amann and Schuligoi, 2000; Girard *et al*., 2008) and thermal, hot-plate (Heidari *et al*., 2006; Tomaszewski *et al*., 1992; Tao *et al*., 2008) test in experimental animals respectively.

**Corresponding author*: Tel.: +98-341-3205001-2, Fax: +98-341-3205003, e-mail: heidarimr@kmu.ac.ir

MATERIAL AND METHODS

Synthesis

The synthesis of (Z)-2-(3,4-dihydroxybenzylidene)-5-methoxybenzofuran-3(2*H*)-one (compound **5**) was achieved through the route outlined in Scheme **1**.

5-Methoxybenzofuran-3-one **4** was prepared by firstly conversion of 2,5-dimethoxybenzoic acid **1** to 2,5-dimethoxybenzoyl chloride **2** via chlorination by oxalyl chloride and then the reaction between compound 2 and diazomethane at room temperature for 45 min. gave compound **3** as a yellow oil. The resulting yellow oil **3** was dissolved in glacial acetic acid at room temperature for 30min. to give **4.** Compound 4 was crystallized from ether to give slightly yellow crystals in 64% yield (Tomaszewski *et al*., 1992). Acid catalyzed condensation of 5-Methoxybenzofuran-3-one **4** with 3,4-dihydroxy-benzaldehyde afforded (Z)-2-(3,4-dihydroxybenzylidene)-5-methoxybenzofuran-3(2*H*)-one (compound **5**). (Siddaiah *et al*., 2006; Nakib *et al*., 1990; Lawrence *et al*., 2003; Foroumadi *et al*., 2007)

Dry hydrogen chloride gas was passed through an ice-cold solution of 5-Methoxybenzofuran-3-one **4** (0.02 mol) and substituted benzaldehyde (0.025 mol) in acetic acid (5 mL) for 3 min. The reaction mixture was allowed to stand at room temperature for 24 h. The precipitate was filtered, dried, and crystallized from ethanol (Lawrence *et al*., 2003).

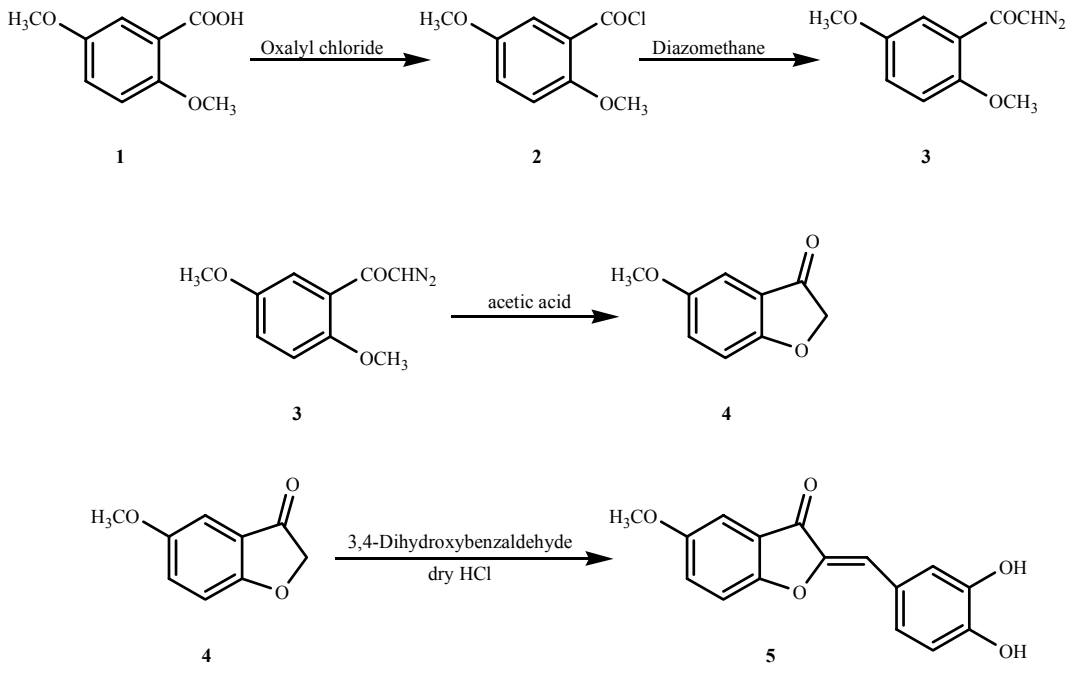
Pharmacology

Animals

In this experiment male Swiss albino mice weighing 22-28g were used. The animals were obtained from neuroscience research center of Kerman University of Medical Sciences. The animals were housed in a room temperature 21±2 at 12/12h light/dark cycle. They had free access to food and water except during the time of experiments (Zarrindast and Heidari, 1994). Animals were acclimatized to the laboratory for at least one hour before testing and were used for once experiment only. The experiments were carried out between 8.00 and 18.00 hours. The animals were placed into groups of 7 as controls and test groups (Zarrindast *et al*., 1996; Heidari *et al*., 2007). According to international rules considering animal experiments, all efforts were made to minimize animal suffering and to reduce the number of animal used [Zimmermann, 1983).

Formalin test

In this experiment the formalin test was carried out according to standard methods. Briefly in this method, 25 µl of 0.5% formalin were injected subcutaneously to the right hind paw of mice. The pain response was recorded for a period of 40 min. The summation of time (in second) spent in licking ad biting response of paw during first 5 min and 10-40 min block was measured as an indicator of acute and chronic phase (inflammation phase) of pain response respectively (Hunskaar and Hole, 1987; Heidari *et al*., 2007; Heidari *et al*., 1996; Girard *et al*., 2008).



Scheme 1: Procedure for synthesis of (Z)-2-(3,4-dihydroxybenzy-lidene)-5-methoxybenzofuran-3(2*H*)-one (compound **5**).

The animals received different experimental doses of Compound **5**, including 12.5, 25 and 50 mg/kg, Ibuprofen 200 mg/kg, morphine 2.5 mg/kg, i.p. as reference drug (Daru-pakhsh, I.R. Iran) (Heidari *et al*., 2007).

Hot-plate test

The temperature of a metal surface in the hot-plate test, was set at 55±1.0ºC. The time taken by the animals to lick the fore or hind paw or jump out of the place was taken as the reaction time. Latency to the licking paws or jumping from plate was determined before and after treatment. The latency was recorded at the time of 0 (just before any treatment) and 15, 30, 45 and 60 min after intraperitoneal administration of compound **5**. A latency period of 30s was defined as complete analgesia as cut off time to prevent damage to mice (Heidari *et al*., 2004; Heidari *et al*., 2006; Tao *et al*., 2008). Maximum possible effect was calculated as:

$$\%MPE = \frac{\text{Test latency- Control latency}}{\text{Cut off – Control latency}}$$

Compound 5, with doses of 12.5, 25 and 50 mg/kg were injected IP in treated groups. Ibuprofen 200 mg/kg, Morphine 2.5 mg/kg, were injected IP as reference drug. Volume of solutions for intraperitoneal injection was 10ml/kg in this experiment (Heidari *et al*., 2007; Heidari *et al*., 2006).

Carrageenan induced paw edema

Anti-inflammatory activity was evaluated using the Carrageenan induced mouse paw edema according to standard methods (Gilligan and Lovato, 1994; Winter *et al*., 1962; Amann and Schuligoi, 2000; Girard *et al*., 2008). In this method, 25 µl of 0.5% Carrageenan suspension was injected subcutaneously in to the plantar surface of the right hind paw and normal saline in to left paw. The volume of each paw was measured using a plethysmometer immediately, 1 and 3 hour after each treatment.

Group I served as control group received vehicle (DMSO in normal saline) 10 ml/kg IP. Group II, III and IV animals received Compound 5, with dose of 12.5, 25 and 50 mg/kg as a fine suspension in DMSO and normal saline. Group V, VI were administered Ibuprofen and Morphine with dose of 200 and 2.5 mg/kg respectively as reference drugs.

The difference volume of two paws was considered as the inflammation induced by carrageenan. Percent inhibition of the edema was calculated as:

$$\%\ \text{Inhibition of edema} = \frac{V1-V2}{V2} * 100$$

V2: The difference volume of two paws in control group in 1 and 3 hour after carrageenan injection

V1: The difference volume of two paws in treated groups in 1 and 3 hour after carrageenan injection

Statistic analysis

The mean of Antinociception% in formalin test or the mean of Maximum Possible Effect (MPE%) in Hot-plate test and the mean of Percent inhibition of edema in carrageenan test in seven mice were calculated according to the method section and statistical significance between groups were analyzed by ANOVA followed by Newman - Keuls test. P values less than 0.05 were considered significant (Zarrindast and Heidari, 1994; Zarrindast *et al*. 1997; Heidari *et al*., 2007).

RESULTS

Formalin test

Different doses of Compound 5, 12.5, 25, and 50 mg/kg, induced analgesia in the formalin test. Maximum analgesia, 42.6% and 87.7% were observed with the dose of 25 mg/kg, in acute and chronic phase of formalin test respectively P<0.01 (fig. 1).

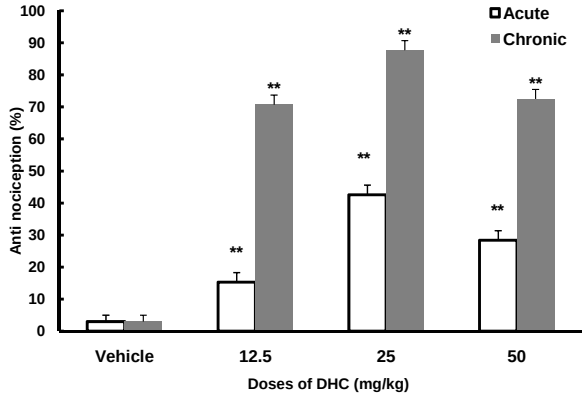


Fig. 1: Analgesic effect of Compound **5**, on acute and chronic phase of formalin test.

Vehicle or different doses of Compound 5, 12.5, 25 and 50 mg/kg were injected intraperitoneally, 20 minutes before injection, of 25µl of 0.5% formalin SC in sub plantar mouse paw. Each point indicates the Mean ± SEM of pain response to formalin in seven animals.

**, P<0.01 significant difference from respective control group.

The analgesic effect of Compound 5, was lower than morphine 2.5 mg/kg but higher than Ibuprofen 200mg/kg as reference drug in acute phase of formalin test P<0.05 (fig. 2), and was equal to them in chronic phase (Data not shown).

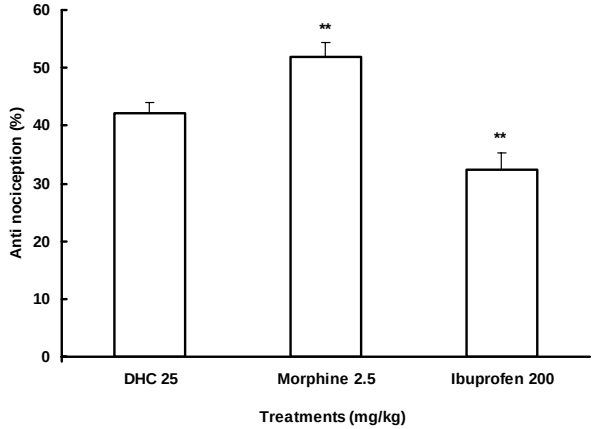


Fig. 2: Comparison the analgesic effect of Compound **5**, with Morphine and Ibuprofen on acute phase of formalin test.

Compound 5, with dose of 25 mg/kg or Morphine 2.5 mg/kg or Ibuprofen 200 mg/kg were injected intraperitoneally, 20 minutes before injection, of 25µl of 0.5% formalin SC in sub plantar mouse paw. Each point indicates the Mean ± SEM of pain response to formalin in seven animals.

**, P<0.01 significant difference from control group.

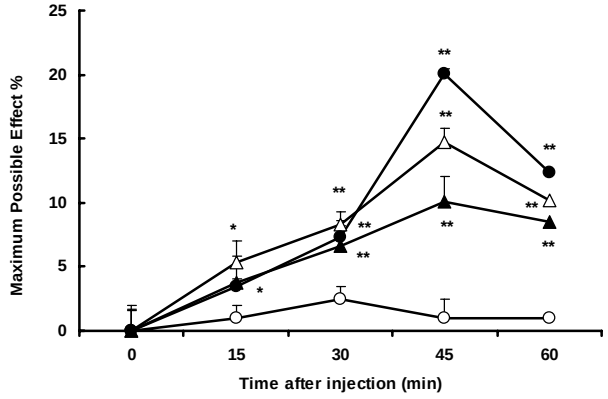


Fig. 3: Maximum possible effect of Compound **5** in Hot-plate test.

Normal saline 10 ml/kg (○), or Compound 5, with dose of 12.5 (▲), 25 mg/kg (●), or 50 mg/kg(Δ) were injected intraperitoneally to mice. Each point indicates the Mean ± SEM of pain response (MPE) in seven animals.

*, P<0.05; **,P<0.01 significant difference from control group.

Hot-Plate test

Doses of 12.5, 25 and 50 mg/kg of Compound 5, induced analgesia in the hot plate test. The highest antinociception induced by dose of 25 mg/kg 20.2%, 45 min. after injection, P<0.01 (fig. 3). The effect of morphine 2.5 mg/kg was not different from Compound 5, 25 mg/kg, in 45 min. after injection but was higher in other times P<0.01 (fig. 4). The effect of Ibuprofen 200mg/kg was higher than Compound 5 at 25 mg/kg only in 30 min. after injection P<0.01. The analgesic effect of Compound 5, was equal to Ibuprofen afterward.

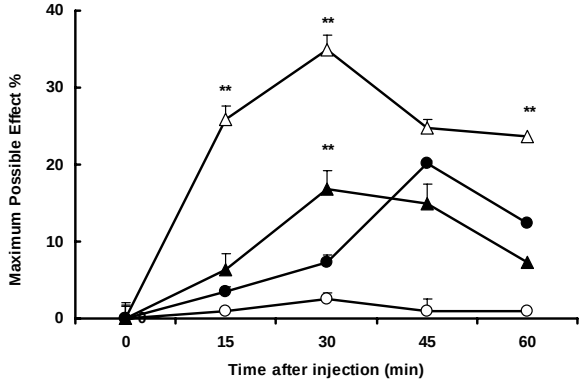


Fig. 4: Comparison of analgesic effects of Compound **5** with Morphine and Ibuprofen in mouse Hot-plate test.

Normal saline 10 ml/kg (○), or Compound **5**, with dose of 25 mg/kg (●), or Ibuprofen 200 mg/kg (▲) or morphine 2.5 mg/kg (Δ) were injected intraperitoneally to mice. Each point indicates the Mean ± SEM of pain response (MPE) in seven animals.

*, P<0.01 significant difference from control group.

Carrageenan test

Interplanetary injection of carrageenan in the hind paw induced gradual increase in the edema paw volume in the control group. Compound 5, with most effective dose in formalin test, 25 mg/kg, inhibited inflammation induced by carrageenan 32.8%, 1 hour after carrageenan injection that was higher than morphine 2.5 mg/kg and equal to Ibuprofen 200 mg/kg. Compound **5**, inhibited inflammation induced by carrageenan 41.7%, 3 hour after carrageenan injection that was higher than morphine 2.5 mg/kg and lower than Ibuprofen 200 mg/kg (fig. 5).

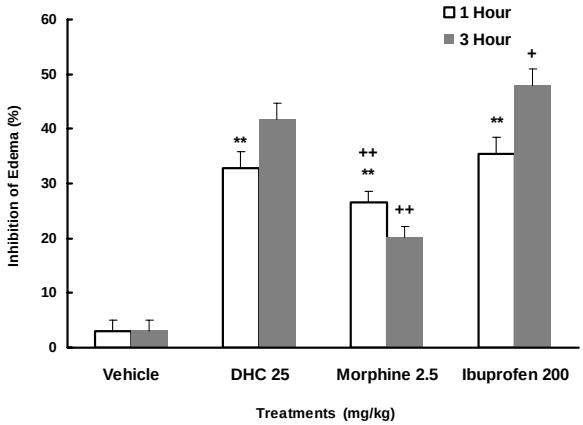


Fig. 5: Effect of Compound **5** on inflammation, 1 hour and 3 hour after injection of carrageenan

Compound 5, with dose of 25 mg/kg or Morphine 2.5 mg/kg or Ibuprofen 200 mg/kg were injected intraperitoneally, after injection, of 25µl of 0.5% carrageenan SC in sub plantar mouse paw. Each point indicates the Mean ± SEM of inhibition edema % in seven animals.

**, P<0.01 significant difference from respective control group

+, P<0.05, + +, P<0.01 significant difference from respective DHC group.

DISCUSSION

There are some reports indicating that chalcone derivatives have anti-inflammatory properties (Nakadate *et al*., 1985; Hsieh *et al*., 2000). In this investigation a new rigid benzofurane, 3,4 Dihydroxy chalcone, (Compound **5**) was synthesized and some animal models, thermal, Hot-Plate (Heidari *et al*., 2004; Heidari *et al*., 2006; Tao *et al*., 2008), chemical, formalin and carrageenan test (Gilligan and Lovato, 1994; Amann and Schuligoi, 2000; Girard *et al*., 2008), were used to evaluate the antinociceptive and anti-inflammatory effects of this newly synthesized Compound 5. The results of this study showed the significant analgesic effects of different doses of Compound 5, in both phase of formalin test. In formalin test the initial phase is a direct stimulation of nociceptors and in late phase is thought to be secondary to inflammatory reaction (Shibata *et al*., 1989; Heidari *et al*., 1996; Tjolsen *et al*., 1992; Girard *et al*., 2008), and therefore it seems that the analgesic effect of Compound 5, may be mediated centrally and peripherally. Some chalcone derivatives have been reported as anti-inflammatory or antiallergic agents (Sogawa *et al*., 1993). It is found that chalcones with 3,4-dihydroxycinnamoyl structure strongly inhibited lipid peroxidation in rat liver microsomes (Sogawa *et al*., 1994). Nakadate *et al*., have reported that known hydroxylchalcones inhibit 12-lipoxygenase and cycloxygenase in the mouse epidermis (Nakadate *et al*., 1985). Dihydroxy chalcones are a potent chemical mediator and cyclooxygenase Inhibitor (Lin *et al*., 1997). Therefore the analgesic and anti-inflammatory effect of new rigid 3,4 Compound **5**, may be partly mediated through lipoxygenase and cyclooxygenase pathwys. There is a report indicating that 4–dimethylamino-3́,4 ́-dimethoxy chalcones exerts their anti-inflammatory effects via down regulation of inducible nitric oxide synthase (iNOS) protein expression and nitrite production (Herencia *et al*., 2001).

The most effective dose of Compound **5** was 25 mg/kg. However doses of higher than this dose did not increased the analgesic effect of it. It seems that this dose produce enough concentration for induction of maximum analgesic effect (Heidari *et al*., 2007; Shargel and Yu, 1999). Higher doses induced some adverse effect in animals probably due to high levels of concentration and produced toxic effect (Heidari *et al*., 2007; Shargel and Yu, 1999).

Compound **5** showed antinociceptive activity in hot-plate test. Since Hot-Plate test is a central antinociceptive test (Gilligan and Lovato, 1994; Heidari *et al*., 2006; Tao *et al*., 2008), thus Compound **5**, may act via central mechanisms. The analgesic effect of Compound **5**, in the hot plate test might again be due to the inhibitory action on prostaglandin synthesis (Gilligan and Lovato, 1994; Heidari, *et al*., 2006; Hsieh *et al*., 2000). The validity of this test has been shown even in the presence of substantial impairment of motor performance, and the activity is supraspinally mediated (Heidari *et al*., 2006) therefore Compound **5**, may be exhibiting its analgesic effect by involving both peripheral and central nervous mechanisms.

The peak of analgesic effect of Compound **5**, was seen in 45 minutes after injection, it seems that the concentration of compound 5, reached to maximum in this time or due to some active analgesic metabolite (Heidari *et al*., 2007; Shargel and Yu, 1999).

The analgesic effect of Compound **5**, 25 mg/kg was higher than Ibuprofen 200mg/kg in acute phase of formalin test and equal to ibuprofen at some time interval in Hot-Plate test. Morphine, a centrally active analgesic drug (Heidari *et al*., 2007; Kolesnikove and Soritsa, 2008; Tao *et al*., 2008), with dose of 2.5mg/kg produced analgesic effect more than Compound 5, in Hot-Plate and formalin test in this investigation. Therefore the potency of Compound **5** was lower than morphine. However the effect of Compound **5**, 25mg/kg was equal to Ibuprofen 200 mg/kg in the second phase of formalin test. Since the late phase is thought to be secondary to inflammatory reaction (Shibata *et al*., 1989; Heidari *et al*., 1996; Tjolsen *et al*., 1992; Girard *et al*., 2008), therefore it seems that Compound 5, has more anti-inflammatory effect rather than analgesic effect.

However other mechanisms responsible for the effect of Compound **5**, remain to be investigated (Ahurqoub *et al*., 2006).

Compound **5**, significantly suppressed the carrageenan induced rat paw edema 1 and 3 h after carrageenan injection. Carrageenan induced rat paw edema is commonly used as an experimental animal model for evaluation of the anti-inflammatory potential of compounds (Gilligan *et al*., 1994; Winter *et al*., 1962; Amann and Schuligoi, 2000; Girard *et al*., 2008) and is believed to be biphasic. The initial phase is due to the release of histamine, serotonin and kinin in the first hour after the administration of carrageenan, a more pronounced second phase is attributed to release of bradykinin, prostaglandin and lysosime. The later phase is reported to be sensitive to most of the clinically effective anti-inflammatory agents (Gilligan *et al*., 1994; Winter *et al*., 1962; Amann and Schuligoi, 2000).

The result of present study indicates that Compound **5**; possess significant analgesic and anti-inflammatory activity in animal tests. Further detailed investigation is underway to determine the exact mechanisms, which are responsible for the anti-inflammatory activity. In conclusion, the present results provide a new way for further study in the light of developing new analgesic and anti-inflammatory compounds.

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