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# Screening of Novel 4-chlorothiophene Compound for Anti-inflammatory Activity in Rats

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## ABSTRACT

**Background:** Every disease process involves inflammation. Pharmacologists have always been in quest for an ideal anti-inflammatory agent. In this study, 4-chlorothiophene was screened for both acute and chronic anti-inflammatory activity as an attempt in quest for a new ideal agent. **Methods:** Anti-inflammatory activity was assessed by carrageenan induced paw edema method and cotton pellet induced granuloma method using Wistar albino rats. **Results:** The test drug, 4-chloro thiophene showed significant dose and time dependent anti-inflammatory action. At 40 mg $kg^{-1}$ dose, it showed 84.61% reduction in edema volume in acute phase which was superior to indomethacin. In chronic model the test drug at 40 mg $kg^{-1}$ showed 39.53% reduction in cotton pellet induced granuloma weight. The 20 mg $kg^{-1}$ of 4-chlorothiophene dose action was also comparable to indomethacin. The results were statistically significant with $p<0.05$. **Conclusion:** The 4-chloro thiophene was found to possess significant anti-inflammatory properties in both acute and chronic inflammation and so 4-chlorothiophene can be a promising anti-inflammatory agent.

**Key words:** Thiophene, 4-chlorothiophene, anti-inflammatory, carrageenan, cotton pellet

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## INTRODUCTION

Every living being on this planet suffers from diseases and so also from undesirable effects of inflammation which is an integral part of disease and healing process. Inflammation has always contributed in evolution of species (Daniel and Ruslan, 2012). Inflammation is essential as it helps injuries or infections to heal. But it comes at the cost of a transient decline in tissue function which can in turn contribute to the pathogenesis of various diseases of altered homeostasis (Medzhitov, 2010). It has been a mystery as to how to solve the problem of inflammation when it also equally beneficial. Hence, anti-inflammatory agents are required to halt the process when inflammation is identified as being more harmful than beneficial.

The current common anti-inflammatory agents in use are NSAIDs, steroids, herbal formulations and others which have their own respective disadvantages (Adams *et al*., 2011). Some are also abused when they are available as over the counter drugs (Delaney *et al*., 2011). Hence, in the light of all the above said developments, search for an ideal agent is in process.

A novel fused thiophene derivative was synthesized and was found to have analgesic activity at 15-30 mg $kg^{-1}$ (Wardakhan *et al*., 2008). There are also reports from other studies that thiophene derivatives possess anti-inflammatory properties. (Ahmed *et al*., 2013; Pillai *et al*., 2004). Hence, the newly synthesized 4-chloro substituted derivative of thiophene compound was screened for anti-inflammatory properties. The study was conducted with following objectives: To evaluate acute anti-inflammatory activity by carrageenan induced paw edema model and chronic anti-inflammatory activity by cotton pellet induced granuloma model.

## MATERIALS AND METHODS

**Test compounds:** The study compound, 4-chlorothiophene was obtained from PES College of Pharmacy, Bangalore, India. The $LD_{50}$ values were estimated to be more than 2 g $kg^{-1}$ for rats which is far greater than the maximum testing dose in the current study. It was used at two doses namely, low dose being 20 mg $kg^{-1}$ and high dose being 40 mg $kg^{-1}$ b.wt. Indomethacin was used at 10 mg $kg^{-1}$ for acute model and at 3 mg $kg^{-1}$ for chronic model which served as standard. The vehicle, Tween 80 was used as control. Ketamine was used for anaesthesia.

**Animals used:** Forty eight male Wistar albino rats (*Rattus norvegicus*) weighing around 150-250 g were used. The rats were housed in the central animal house of the

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Department of Pharmacology, PES Institute of Medical Sciences, Kuppam, under suitable conditions of housing, temperature, ventilation and nutrition.

**Study approval:** Ethics clearance for experiments on animals was obtained from Institutional Animal Ethics Committee (IAEC CERTIFICATE: PESIMSR/Pharma/IAEC/07/2010-11).

**Methods:** The methods employed were carrageenan induced paw edema method for acute inflammation and cotton pellet induced granuloma method for chronic inflammation.

**Carrageenan induced rat paw edema method (Vogel, 2008a):** Four groups each consisting of six randomly allocated male Wistar rats with an average body weight between 150-250 g were used. The animals were starved overnight. To ensure uniform hydration, all the rats were given 5 mL of water by stomach tube 30 min prior to the test. The individual groups of rats were administered control (Tween 80)/standard (indomethacin 10 mg $kg^{-1}$)/test drug (4-chloro thiophene 20 mg $kg^{-1}$ and 4-chloro thiophene 40 mg $kg^{-1}$) per oral in a fixed volume of 1 mL per rat. Thirty minutes later, the rats were challenged by subcutaneous injection of 0.05 mL of 1% solution of carrageenan into the plantar region of right hind paw. The paw was marked at the level of the lateral malleolus and immersed in mercury up to this mark. The paw volume was measured plethysmographically immediately after injection i.e., at 0 h, again at 3 and 6 h and eventually 24 h after challenge. The increase of paw volume due to edema after 3, 6 and 24 h was calculated as percentage compared with the volume measured immediately after injection of the irritant for each animal. The difference of edema volumes at the various time intervals gives data for the anti-inflammatory effect and the percentage inhibition was compared between control, standard and test drug groups.

**Cotton pellet induced granuloma method (Vogel, 2008b):** Four groups consisting of six randomly allocated male Wistar rats in a similar manner as in acute model with an average weight between 150-250 g were taken and each rat was anaesthetized with ketamine at a dose of 100 mg $kg^{-1}$ (Kushikata *et al*., 2011). The groin skin was shaved and disinfected with 70% ethanol. Two incisions were made, one in each side of the groin. By a blunt forceps, subcutaneous tunnels were formed and one sterilized cotton pellet weighing 20 mg was placed in each groin region and sutured. The animals were treated for 7 days orally with Tween 80, standard drug indomethacin at 3 mg $kg^{-1}$ (Romay *et al*., 1998) and respective test drugs as per respective groups. Then on the 8th day, the animals were sacrificed with overdose of anaesthesia, the pellets along with surrounding granuloma was dissected and dried for 24 h at 65°C in hot air oven until the weight remained constant. The net dry weight i.e., after subtracting the weight of the cotton pellets was determined. The average weight of the pellets of the control group, standard group, as well as of the test group was calculated. The percentage reduction in granuloma weight relative to control group and standard group was determined and the results obtained were evaluated statistically.

**Statistical analysis:** Statistical test applied was one way ANOVA followed by Tukey's multiple comparison test for both models using graphpad prism version 5, computer software. Value of $p<0.05$ was considered to significant.

## RESULTS

**Acute model: Carrageenan induced rat paw edema method:** Table 1 shows the mean paw volume and edema volume of control, standard and test drug groups. Control group showed 0.2 mL of mean edema volume at 3 h after injection of irritant. The edema volume increased to 0.26 mL at 6 h and subsequently decreased to 0.13 mL at 24 h. Indomethacin group showed 0.16 mL of mean edema volume after 3 h of irritant injection. Later at 6 h, it showed a volume of 0.11 and 0.05 mL at 24 h. The test drug at 20 mg $kg^{-1}$ showed 0.13 mL of edema at 3 h, 0.08 mL at 6 h and 0.03 mL at 24 h. Test drug at 40 mg $kg^{-1}$ showed 0.1 mL of edema volume at 3 h. Subsequently the volume reduced to 0.05 mL at 6 h and further reduced to 0.02 mL at 24 h.

Figure 1 is a line diagram of the percentage reduction in edema volume of each drug groups. The standard drug indomethacin at 10 mg $kg^{-1}$ showed 20, 57.69 and 61.54% reduction in edema volumes at 3, 6 and 24 h, respectively. The test drug, 4-chlorothiophene showed a dose and time dependent reduction in edema. At 20 mg $kg^{-1}$, 4-chlorothiophene showed 35, 69.23 and 76.92% reduction in edema volume at 3, 6 and 24 h, respectively while at 40 mg $kg^{-1}$, it showed 50, 80.77 and 84.61% reduction in edema volume at 3, 6 and 24 h, respectively.

**Chronic model: Cotton pellet induced granuloma method:** As shown in Table 2, the weight of cotton pellet granuloma was highest in control group with 107±2.66 mg. Indomethacin treated group showed granuloma weight of 70±1.88 mg. The test drug, 4-chlorothiophene at 20 mg $kg^{-1}$ showed 66.8±1.42 mg and 4-chlorothiophene at 40 mg $kg^{-1}$ showed

Table 1: Mean paw volume (mL) and edema volume (mL) of control, standard, 4-chlorothiophene 20 mg $kg^{-1}$ and 4-chlorothiophene 40 mg $kg^{-1}$ groups at 0, 3, 6 and 24 h

| Groups | Hours | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | | 3 | | 6 | | 24 | |
| | Mean paw volume±SD (mL) | Mean edema volume (mL) | Mean paw volume±SD (mL) | Mean edema volume (mL) | Mean paw volume±SD (mL) | Mean edema volume (mL) | Mean paw volume±SD (mL) | Mean edema volume (mL) |
| Control Tween-80 | 0.55 ±0.02* | - | 0.75±0.03* | 0.20 | 0.81±0.03* | 0.26 | 0.68±0.01* | 0.13 |
| Standard indomethacin (10 mg $kg^{-1}$) | 0.76±0.04* | - | 0.92±0.06* | 0.16 | 0.87±0.04* | 0.11 | 0.81±0.05* | 0.05 |
| 4-chlorothiophene (20 mg $kg^{-1}$) | 0.77±0.02* | - | 0.9±0.03* | 0.13 | 0.85±0.05* | 0.08 | 0.8±0.03* | 0.03 |
| 4-chlorothiophene (40 mg $kg^{-1}$) | 0.84±0.04* | - | 0.94±0.03* | 0.10 | 0.89±0.03* | 0.05 | 0.86±0.04* | 0.02 |

*p<0.001

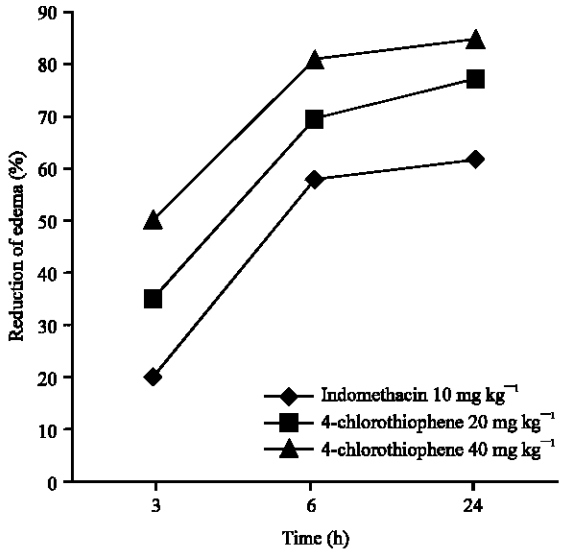


Fig. 1: Line graph showing percentage reduction of edema in control, standard, 4-chlorothiophene 20 mg $kg^{-1}$ and 4-chlorothiophene 40 mg $kg^{-1}$ groups of acute model

64.7±1.4 mg. The test drug, 4-chlorothiophene at 20 mg $kg^{-1}$ showed 37.57% reduction and 4-chlorothiophene at 40 mg $kg^{-1}$ showed 39.53% reduction whereas indomethacin at 3 mg $kg^{-1}$ showed only 34.58% reduction in cotton pellet granuloma weight which are presented in Table 2.

## DISCUSSION

The most widely used tests for screening anti-inflammatory compounds are carrageenan induced paw edema method (Posadas *et al*., 2004) and cotton pellet induced granuloma method. Previous studies have also shown that thiophene derivatives have anti-inflammatory action (Ahmed *et al*., 2013; Pillai *et al*., 2004). Hence, 4-chlorothiophene was evaluated for anti-inflammatory activity. In carrageenan induced edema model, anti-edematous effect of drugs is evaluated. Occurrence of edema secondary to carrageenan injection serves as an index of acute inflammatory changes and can be determined from differences in the paw volume measured immediately, at 3, 6 and 24 h. The test drug is expected to reduce edema volume. In cotton pellet induced granuloma model, the dry weight of cotton granuloma indicates the amount of granulomatous tissue developed due to chronic inflammation. A drug with anti-inflammatory properties is expected to reduce the granulomatous tissue.

Table 2: Mean dry weight and percentage reduction in cotton pellet granuloma in control, standard and test drug groups in all groups

| Groups | Mean dry weight±SD (mg) | Reduction in granuloma weight (%) |
|---|---|---|
| Control | 107.0±2.66* | - |
| Standard | 70.0±1.88* | 34.58 |
| 4 chlorothiophene (20 mg $kg^{-1}$) | 66.8±1.42* | 37.57 |
| 4 chlorothiophene (40 mg $kg^{-1}$) | 64.7±1.40* | 39.53 |

*p<0.001

In carrageenan induced edema model, 4-chloro thiophene at 40 mg $kg^{-1}$ has shown maximum percentage inhibition of 50% at 3 h, 80.7% at 6 h and 84.61% at 24 h compared to indomethacin at all three readings. The 4-chloro thiophene at 20 mg $kg^{-1}$ has also shown higher percentage inhibition than indomethacin at 3, 6 and 24 h. So, the observations indicated that 4-chloro thiophene at 20 and 40 mg $kg^{-1}$ showed a dose dependent action and that the action was superior and had started earlier compared to indomethacin.

In cotton pellet method, indomethacin at 3 mg $kg^{-1}$ dose has again shown a lesser response of 34.58% inhibition of granuloma weight when compared to 4-chlorothiophene at 20 mg $kg^{-1}$ which has shown 37.57% inhibition and 4-chlorothiophene at 40 mg $kg^{-1}$ which has shown 39.53% inhibition. Among the test drugs, 4-chlorothiophene at 40 mg $kg^{-1}$ has shown greater granuloma inhibitory response than 4-chlorothiophene at 20 mg $kg^{-1}$. The 4-chlorothiophene at 20 and 40 mg $kg^{-1}$ has shown granuloma inhibition which was comparable to indomethacin. However, the comparative study indicates that 4-chlorothiophene at 20 and 40 mg $kg^{-1}$ was more effective in chronic inflammation when compared to indomethacin.

## CONCLUSION AND LIMITATIONS

In carrageenan induced paw edema method, the test drug 4-chlorothiophene, has inhibited the edema induced by carrageenan in rats significantly at a dose of 20 and 40 mg $kg^{-1}$ and to a greater extent when compared to indomethacin at 10 mg $kg^{-1}$. In cotton pellet granuloma method, 4-chlorothiophene at 20 and 40 mg $kg^{-1}$ has produced good anti-inflammatory effect which was marginally greater when compared to the indomethacin at 3 mg $kg^{-1}$. At the end of the study it is concluded that the test drug, 4-chloro thiophene which was investigated possesses significant anti-inflammatory activity.

In acute model, the study could have been further conducted beyond 24 h to further follow the duration of anti-inflammatory responses of all drugs. In chronic model, the test drugs were given as a single daily dose. Perhaps, more frequent dosing would have further shown more effective results. The adverse effects of the drugs must have been included for the study. Further comprehensive studies must be carried out in different species of animals to evaluate pharmacokinetic and pharmacodynamic properties of the test drug.

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