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Preclinical evaluation of a novel compound, 4-chlorothiophene for analgesic activity in swiss albino mice

Kummara Muni Kumar[1], Gondi Surendrakumar[2*]
[1](*Department of Pharmacology, PESIMSR, Dr. NTRUHS, Andhra Pradesh, India)*
[2](*Department of Pharmacology, S. V. Medical College, Tirupati, Dr. NTRUHS, Andhra Pradesh, India)*
*****[2]*Corresponding Author: Gondi Surendra Kumar*

Abstract:***Background:*** *Pain is one of the most common sensory and emotional experiences which may lead to physiological and psychological stress. Analgesic drugs are commonly used to alleviate pain sensation in several health illnesses. Chronic use of currently available analgesics such as opioids and nonsteroidal anti-inflammatory drugs (NSAIDs) may cause several adverse effects in the body.Still there exists a need for search of an ideal analgesic****. Aim:*** *To evaluate the analgesic activity of a novel compound, 4-chlorothiophene in Swiss albino mice.****Material and methods:****The central analgesic activity of 4-chlorothiophene was evaluated by eddy's hot plate method and early phase of formalin test whereas peripheral activity was evaluated by the late phase of formalin test.* ***Results:****40 mg/kg dose of 4-chlorothiophene has shown maximum Pain Inhibition Percentage (PIP) of 35.41% when compared to 128% by morphine in eddy's hot plate method. Under formalin test, it has shown 19.28% in early phase and 42.55% in late phase compared to 17.72% and 47.37% by aspirin. The results were statistically significant with p<0.05.*

Key words:*Analgesic, Eddy's hot plate, Formalin test, Thiophene,4-chlorothiophene*

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I. Introduction

Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage[1].It is one of the most common reasons for seeking health care. It affects the quality of life and general functioning of the individual[2].It potentially impairs the cognitive function[3].It may cause psychological disturbances such as depression, anxiety, fear and anger[4].Commonly used analgesics may cause significant adverse effects[5]. Opioids cause adverse effects such as respiratory depression, nausea, vomiting, mental clouding, dysphoria, pruritus, constipation, increased pressure in the biliary tract, urinary retention, hypotension and rarely delirium[6].NSAIDs may cause disturbances in the several body systems such as nervous, cardiovascular, gastrointestinal systems, blood, liver and kidneys[7].

Still there exists a need for search of an ideal analgesic compound with least toxic effects even with long term usage in the management of pain associated with chronic painful conditions. A novel fused thiophene derivative was synthesized and found to have good analgesic activity[8].Thusits derivative 4-chlorothiophene was selected as a test compound for evaluating analgesic activity during this current study.

II. Material and Methods

The analgesic activity of 4-chlorothiophene was evaluated by different experimental animal models. The central analgesic activity was evaluated by eddy's hot plate method whereas both central and peripheral analgesic activities were evaluated by formalin test.

Test compound: 4-chlorothiophene.

It is a synthetic compound supplied by PES College of Pharmacy, Bangalore, India. The parent compound, thiophene and its derivatives found to have analgesic and anti-inflammatory activities[9,10,11]. The LD_{50} values wereobserved to be more than 2 g/kg which is far greater than the maximum dose tested in this study. During this study, test doses were fixed at 10, 20 and 40 mg/kg as the past studies have proved that the parent compound has shown good analgesic activity at 15-30 mg/kg[8].

Study Location: The study was carried out in the central animal house located in the Department of Pharmacology, People's Education Society Institute of Medical Sciences and Research, Andhra Pradesh, India.

Experimental animals: The study was carried out in Swiss albino mice (Mus musculus) as they were widely used for evaluation of analgesic activity[12]. The study was approved by the Institutional Animal Ethics Committee. All the mice were handled as per the standard guidelines of CPCSEA. They were kept in 12:12 light: dark cycle, had water ad libitum and food was withdrawn 12 hours before the experimentation.

Control: Vehicle, 10% Tween-80 (Merck Specialties Private Limited, Mumbai) was used as control as the test compound, 4-chlorothiophene is water insoluble.
Standard: The central analgesic, morphine was used as a standard for comparison in eddy's hot plate method[13,14]. The peripheral analgesic, aspirin was used as a standard for comparison in formalin test[15,16].
Sample size: Six mice were included in each group as minimum of six is needed to have statistical significance. Total of 30 mice were included in each model and grouped as control, standard and three test groups.

Inclusion criteria:
1. Healthy mice
2. Male mice
3. Weight: 20-25 g
4. Mice which have shown latency time less than 5 secs (Eddy's hot plate method)[17].

Exclusion criteria:
1. Female mice to avoid the effect of estrous cycle
2. Pregnant mice
3. Diseased mice

Procedure methodology
Eddy's hot plate method:

This test has been done to evaluate the central analgesic activity of 4-chlorothiophene. Mice were divided into 5 groups each with 6 in number. Tween80-10% (0.5 ml) was given per oral to Group I. Morphine (5mg/kg) was given intraperitoneal to Group II. Test drug was given at 10, 20 and 40 mg/kg per oral to groups III, IV and V respectively. Group I considered as control, Group II as standard and Groups III, IV and V as test groups. Grouping of mice in eddy's hot plate method was shown in Table 1.

Table 1: Group classification of mice in eddy's hot plate method

Group	Drug	Dose
I	Control (10% Tween-80)	0.5 ml PO
II	morphine	5 mg/kg/ip
III	4-chlorothiophene	10 mg/kg/PO
IV	4-chlorothiophene	20 mg/kg/PO
V	4-chlorothiophene	40 mg/kg/PO

Eddy's hot plate contains electrically heated glass plate surface and the temperature of which was maintained at 55-56°C to evoke heat stimulus. Each mousewas placed on the eddy's hot plate separately and time with in which mice responded by jumping or licking of the paw was recorded[18]. The time of 15 seconds was kept as cut-off time to avoid damage to the paw of mice[19,20].

Statistical analysis

Data was analyzed using graph pad prism statistical software of version 5. Paired Student's *t*-test was used to ascertain the significance of differences between mean values at 0 min and 20, 60 or 90 min values in each group of mice whereas Analysis of Variance (ANOVA) was applied for the entire model in each group followed by Dunnett's Multiple Comparison Test. The level $P < 0.05$ was considered as the cutoff value or significance.

Formalin induced paw licking in mice:

This test has been done to evaluate both the central and peripheral analgesic activities of 4-chlorothiophene. Mice were divided into 5 groups each with 6 in number. Tween80-10% (0.5 ml) was given per oral to Group I. Aspirin (100 mg/kg) was given per oral to Group II. Test drug was given at 10, 20 and 40 mg/kg per oral to groups III, IV and V respectively. Group I considered as control, Group II as standard and Groups III, IV and V as test groups. Grouping of mice in formalin test was shown in Table 2.

Table 2: Group classification of mice in Formalin test

Group	Drug	Dose
I	control (10% Tween-80)	0.5 ml PO
II	aspirin	100 mg/kg/PO
III	4-chlorothiophene	10 mg/kg/PO
IV	4-chlorothiophene	20 mg/kg/PO
V	4-chlorothiophene	40 mg/kg/PO

The procedure was followed as per the suggestion of Murray et al and Hunskaar and Hole[18]. Formalin of 5% concentration (0.02 ml) was injected into sub plantar region of hind paw of mice[21].Pain response was identified by licking or biting of the paw. Resting of both paws on the floor with no favoring of the injected paw indicates analgesic response.

Statistical analysis

Data was analyzed using graph pad prism statistical software of version 5. Unpaired Student's *t*-test was used to ascertain the significance of differences between control and each drug group whereasANOVA was applied for the entire model followed by Dunnett's Multiple Comparison Test. The level $P < 0.05$ was considered as the cutoff value or significance.

III. Results

Eddy's hot plate method

Latency time was recorded at 0, 20, 60 and 90 min following drug administration. Analgesic activity of the test drug was proved by the prolongation of latency time. Pain Inhibition Percentage (PIP) was determined by comparing values before and after administration of the drug. Mean values of latency time and PIP for the respective groups of mice were presented in Tables 3 to 7 and Figure1.

Table 3: Mean latency time & PIP produced by the control in eddy's hot plate method

Sl. No.	Recording time	Mean latency time ± SD	PIP (%)	Paired t test (p value)
1	'0' min	3.67 ± 0.08	-----	----
2	'20' min	3.37 ± 0.19	(-8.17)	< 0.0138
3	'60' min	4.03 ± 0.22	9.81	< 0.0019
4	'90' min	3.57 ± 0.07	(-2.72)	< 0.0021

(ANOVA p < 0.0001)

Table 4: Mean latency time & PIP produced by morphine-5mg/kg in eddy's hot plate method

Sl. No.	Recording time	Mean latency time ± SD	PIP (%)	Paired t test (p value)
1	'0' min	3.78 ± 0.08	-----	----
2	'20' min	6.93 ± 0.08	83.33	< 0.0002
3	'60' min	8.62 ± 0.46	128.04	< 0.0001
4	'90' min	7.48 ± 0.47	97.88	< 0.0001

(ANOVA p < 0.0001)

Table 5: Mean latency time & PIP produced by 4-chlorothiophene-10 mg/kg ineddy's hot plate method

Sl. No.	Recording time	Mean latency time ± SD	PIP (%)	Paired t test (p value)
1	'0' min	3.91± 0.04	-----	----
2	'20' min	4.02± 0.03	2.81	< 0.0001
3	'60' min	4.69± 0.08	19.95	< 0.0001
4	'90' min	4.60± 0.04	17.65	< 0.0001

(ANOVA p < 0.0001)

Table 6: Mean latency time & PIP produced by 4-chlorothiophene-20 mg/kg in eddy's hot plate method

Sl. No.	Recording time	Mean latency time ± SD	PIP (%)	Paired t test (p value)
1	'0' min	3.36± 0.14	-----	----
2	'20' min	3.86± 0.11	14.88	< 0.0001
3	'60' min	4.34± 0.48	29.17	< 0.0014
4	'90' min	4.20± 0.39	25.00	< 0.0027

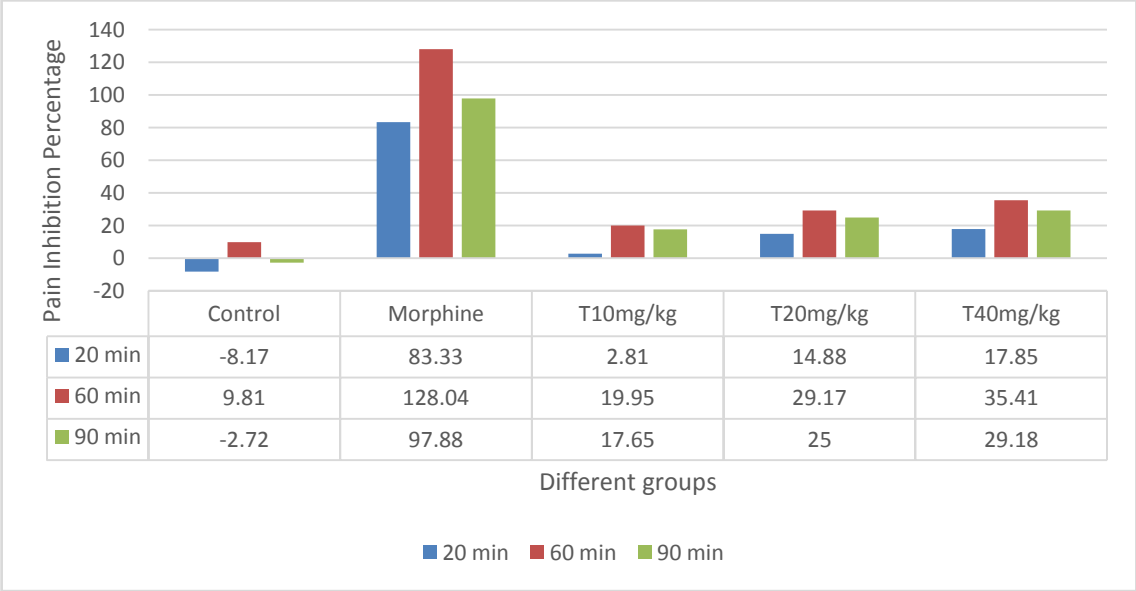
(ANOVA p < 0.0002)

Table 7: Mean latency time & PIP produced by 4-chlorothiophene-40 mg/kg in eddy's hot plate method

Sl. No.	Recording time	Mean latency time ± SD	PIP (%)	Paired t test (p value)
1	'0' min	3.53± 0.04	-----	----
2	'20' min	4.16± 0.03	17.85	< 0.0001
3	'60' min	4.78± 0.03	35.41	< 0.0001
4	'90' min	4.56± 0.03	29.18	< 0.0001

(ANOVA p < 0.0001)

Figure 1: PIP produced by control, morphine and test drug (T) – 10, 20 and 40 mg/kg groups at 20, 60 and 90 min in eddy's hot plate method



Formalin induced paw licking in mice:

Number of paw lickings were recorded in two phases. Early phase (0-5 min) values determine central analgesic activity whereas the late phase (20-30 min) values determine peripheral analgesic activity of 4-chlorothiophene[22].PIP was calculated by comparing drug treated values to that of control group using the formula:

PIP = {No. of Licks (control-treated group) / No. of Licks in control} X 100

Mean number of paw licks and PIP produced in early and late phases of formalin test were shown in Tables 8, 9 and Figure 2.

Table 8: Mean No. of paw licks & PIP produced in the early phase of formalin test

Sl. No.	Group	Mean No. of licks ± SD	PIP (%)	Unpaired t test (p)
1	control (10% tween-80)	32.00 ± 1.90	-----	----
2	aspirin (100 mg/kg/PO)	26.33±3.27	17.72	< 0.0043
3	4-chlorothiophene (10 mg/kg/PO)	28.33±2.58	11.47	< 0.0187
4	4-chlorothiophene (20 mg/kg/PO)	27.33±1.97	14.59	< 0.0019
5	4-chlorothiophene (40 mg/kg/PO)	25.83± 1.72	19.28	< 0.0002

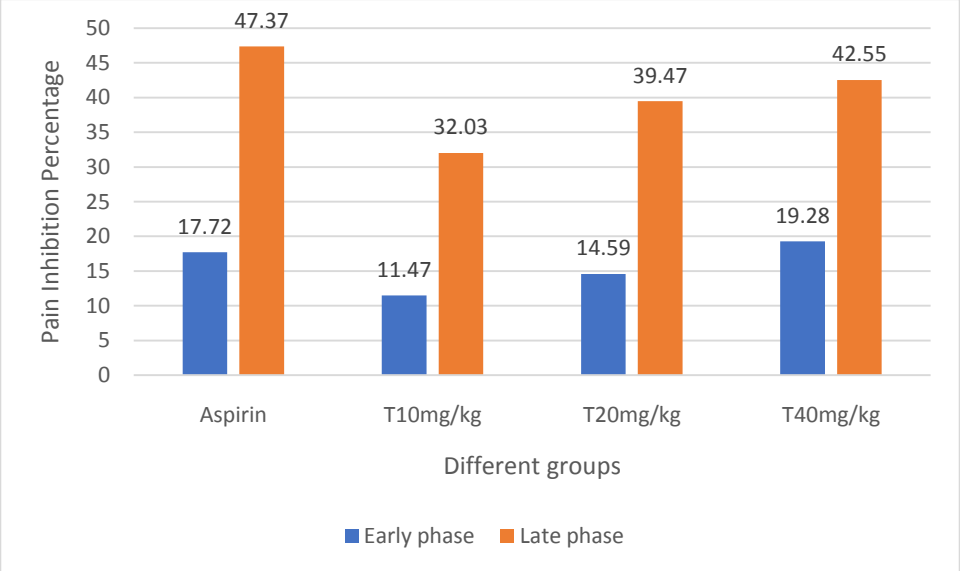
(ANOVA p < 0.0001)

Table 9: Mean No. of paw licks & PIP produced in the late phase of formalin test

Sl. No.	Group (n=6)	Mean No. of licks ± SD	PIP (%)	Unpaired t test (p)
1	control (10% tween-80)	38.00 ± 2.28	-----	----
2	aspirin (100 mg/kg/PO)	20.00 ± 2.00	47.37	< 0.0001
3	4-chlorothiophene (10 mg/kg/PO)	25.83±1.94	32.03	< 0.0001
4	4-chlorothiophene (20 mg/kg/PO)	23.00± 2.37	39.47	< 0.0001
5	4-chlorothiophene (40 mg/kg/PO)	21.83 ±2.64	42.55	< 0.0001

(ANOVA $p < 0.0001$)

Figure 2:PIP shown by aspirin (100mg/kg) and Test drug (T) at 10, 20 and 40 mg/kg in formalin test



IV. Discussion

Eddy's hot plate method

The control group of mice treated with 10% Tween80 have shown no much significant changewith respect to PIP at 20, 60 and 90 min[23]. The standard group of mice treated with morphine has shown maximum PIP of 128.04% at 60 min[23]. 4-chlorothiophene has shown maximum PIP at 60 min at all doses. The PIP found to be increased with increase in dose of 4-chlorothiophene from 10 to 40 mg/kg. 40 mg/kg dose of 4-chlorothiophene has shown maximum PIP of 35.41% at 60 min. Further increase in the dose of 4-chlorothiophene might have produced much increase in the PIP. Thus, maximum PIP that can be produced by 4-chlorothiophene might have been identified if higher doses(i.e.,>40 mg/kg) were tested.

Formalin induced paw licking in mice:

Early phase: The PIP produced by aspirin is negligible as it is only a good peripheral analgesic drug. The PIP was found to be increased with increase in dose of 4-chlorothiophene from 10 to 40 mg/kg. The maximum PIP of 19.28% was produced by 40 mg/kg dose of 4-chlorothiophene as similar to that of aspirin (17.72%).

Late phase: Aspirin has shown PIP of 47.37%. 4-chlorothiophene has shown gradual increase in PIP from 10 to 40 mg/kg doses and has shown approximate similar response to that of aspirin at 40 mg/kg dose (42.55%).

The study has shown that maximum central and peripheral analgesic activities of 4-chlorothiophene might have been recorded if further dose increments were tested. As per the current study observations, though 40 mg/kg dose of 4-chlorothiophene produced maximal response, it can't be claimed for central analgesic activity as it has shown very minimal response when compared to standard drugs as is evident in both eddy's hot

plate method and early phase of formalin test. It can be considered as a good peripheral analgesic drug due to its similar response to that of standard drug, aspirin as evident in late phase of formalin test. The results can't be interpolated to human. If the test drug provides promising results in future clinical trials, it may provide significant contribution for adequate pain management which is a human right[24].

V. Conclusion

It can be concluded that the test compound, 4-chlorothiophene has minimal central analgesic activity and good peripheral analgesic action at 40 mg/kg dose.

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