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Original Research Article

Preclinical screening of a novel compound, 2-chlorothiophene for analgesic activity in swiss albino mice

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ABSTRACT

Background: Pain is often the first indication of disease or injury. Analgesics are the drugs used clinically for controlling pain. They relieve pain as a symptom, without affecting its cause. Currently available options are nonsteroidal anti-inflammatory drugs (NSAIDs) and opioid analgesics for the management of pain. Long term use of existing analgesics causes significant disturbances in the body system. A search for new, safe and cost effective analgesic compound is in progress. Hence a study on 2-chlorothiophene, a novel compound has been carried out in different experimental animal models.
Methods: The central analgesic activity of 2-chlorothiophene was evaluated by eddy's hot plate method and compared to standard central analgesic, morphine. Both central and peripheral analgesic activities of 2-chlorothiophene were evaluated by formalin induced paw licking in mice and compared to a standard drug, aspirin.
Results: There were 40mg/kg dose of 2-chlorothiophene has shown maximum Pain Inhibition Percentage (PIP) of 46.15% at 60 min compared to 128% by morphine in eddy's hot plate method. Under Formalin test, 20mg/kg dose of 2-chlorothiophene has shown maximum PIP of 22.91% in early phase and 52.63% in late phase compared to 12.5% and 47.37% by aspirin. The results were statistically significant with $p<0.05$.
Conclusions: 2-chlorothiophene found to have minimal central analgesic activity and significant peripheral analgesic activity as evident in eddy's hot plate and formalin tests.

Keywords: Analgesic, Eddy's hot plate, Formalin test, Thiophene, 2-chlorothiophene

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[1,2.] It may be physiological or pathological.

It is the most frequent reason for seeking medical attention.[3] It is a major symptom in many medical conditions and can significantly interferes with a person's quality of life and general functioning.[4] Accessibility of the adequate pain treatment is a human right.[5]

This discomfort of pain can be removed by a group of compounds called analgesics**.** Long term use of existing analgesics causes adverse effects. Opioids cause adverse effects such as sedation, mental clouding, blurring of vision, respiratory depression, constipation and urinary retention. NSAIDs cause the adverse effects involving several systems of the body such as gastrointestinal tract, kidneys, blood, skin, nervous and cardio vascular systems.

Test compound

There were 2-chlorothiophene was obtained from PES College of Pharmacy, Bangalore, India. Over the past few

years, research groups have conducted a comprehensive programme towards the synthesis of thiophene and their fused derivatives which have reported to possess wide range of activity.

It has been reported that the parent compound, thiophene and its derivatives possess analgesic,[6] anti-inflammatory activities.[6,7,8] The LD_{50} values were estimated to be at more than 2g/kg which is far greater than the maximum testing dose in the current study. During this current study, 2-chlorothiophene was tested for analgesic activity at 10, 20 and 40mg/kg doses as the previous studies have proved that the parent compound has predominant analgesic activity at 15 to 30mg/kg.[9]

METHODS

Both central and peripheral analgesic activities of 2-chlorothiophene were evaluated using experimental animal models such as eddy's hot plate method and formalin induced paw licking in mice. The study was conducted in the Animal house affiliated to the People's Education Society Institute of Medical Sciences and Research, Andhra Pradesh after obtaining approval from the Institutional Animal Ethics Committee. All the animals were handled with gentle care as per the CPCSEA guidelines.

Experimental animals

The study was carried out in healthy male Swiss albino mice (Mus musculus) weighing between 20 to 25 g as they are the most widely used strain for assay of analgesics.[10] Female mice were excluded to avoid the effect of oestrous cycle. They were kept in propylene cages in 12:12 hours light: dark cycle, under standard laboratory conditions and had water ad libitum. Food was withdrawn 12 hours before and during the experiment.

Eddy's hot plate method[11,12]

The paws of mice are very sensitive to heat at temperatures which are not damaging the skin. The time for nociceptive response such as jumping or licking of the paw will be prolonged after the administration of centrally active analgesics. This test has been done to determine the central analgesic activity of 2-chlorothiophene.

Animals

A total of 30 male Swiss albino mice were taken for this study. Mice which have responded with in 5 seconds were included in the study.[13]

Drugs and reagents

There were 10% Tween-80 or Polysorbate-80 (Merck Specialties private limited, Mumbai), was used as a drug suspending agent, as 2-chlorothiophene is water insoluble. Morphine was used as a standard drug and injected intraperitoneally using tuberculin syringe. The test compound, 2-chlorothiophene was administered orally using gavage.

Mice were randomly made into 5 groups each containing 6 in number. Group I was considered as control and received 0.5 ml of Tween-80 per oral. Group II was considered as standard and received morphine-5 mg/kg/ip.[14,15] Groups III, IV and V were considered as test groups and received 2-chlorothiophene per orally at 10mg/kg, 20mg/kg, 40mg/kg respectively. Mice grouped 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)	0.5ml PO
II	morphine	5mg/kg/ip
III	2-chlorothiophene	10mg/kg/PO
IV	2-chlorothiophene	20mg/kg/PO
V	2-chlorothiophene	40mg/kg/PO

Mice were placed on the Eddy's hotplate, a commercially available equipment which consists of an electrically heated copper or glass plate surface, the temperature of which can be maintained between 55-56°C to evoke thermal stimulus. Time for response such as jumping or licking of the paw was recorded using a stop watch. The cut-off time, 15 seconds was considered to avoid paw damage.[12,16]

Statistical analysis

It was done by paired student-t test for comparison between mean value 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 study was found to have statistically significant with a p value <0.05.

Formalin induced paw licking in mice

Animals

A total of 30 male Swiss albino mice were taken for this study.

Drugs and reagents

There were 10% Tween-80 or Polysorbate-80 was used as a drug suspending agent. Aspirin was used as a standard and 2-chlorothiophene as test compound. All drugs were administered orally using gavage.

Mice were randomly made into 5 groups each containing 6 in number. Group I was considered as control and received 0.5 ml of Tween-80 per oral. Group II was

considered as standard and received aspirin 100 mg/kg per oral.[17,18] Groups III, IV and V were considered as test groups and received 2-chlorothiophene per orally at 10mg/kg, 20mg/kg, 40mg/kg respectively. 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)	0.5ml PO
II	aspirin	100mg/kg/PO
III	2-chlorothiophene	10mg/kg/PO
IV	2-chlorothiophene	20mg/kg/PO
V	2-chlorothiophene	40mg/kg/PO

The procedure was done as per the standard method suggested by Murray et al and Hunskaar and Hole.[11] After 1 hour of respective drug administration, 0.02 ml of 5% formalin was injected into the sub plantar region of hind paw of mice.[19] (Figure 1).

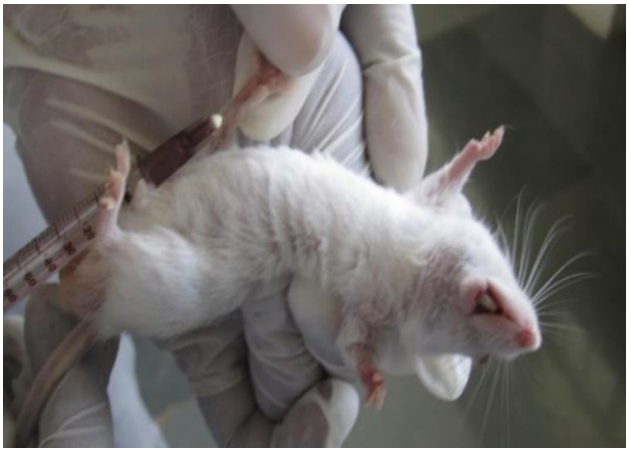

Figure 1: Sub plantar injection of formalin into hind paw of mice.

Pain response was indicated by the paw licking or biting of the paw. Analgesic response was indicated if both paws were resting on the floor with no obvious favouring of the injected paw.

Statistical analysis

It was done by unpaired student-t test for comparison between control and each drug group whereas ANOVA was applied for the entire model followed by Dunnett's Multiple Comparison Test. The study was found to be statistically significant with significant p value <0.05.

RESULTS

Eddy's hot plate method

Latency time was recorded before and after 20, 60 and 90min following administration of the drug.

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

Recording time	Mean latency time ±SD	PIP (%)	Paired t test (p value)
'0' min	3.67±0.03	-	-
'20' min	3.37 ±0.03	(-8.17)	< 0.0001
'60' min	4.03 ±0.04	9.81	< 0.0001
'90' min	3.57±0.03	(-2.72)	< 0.0001

ANOVA p <0.0001

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

Recording time	Mean latency time ±SD	PIP (%)	Paired t test (p value)
'0' min	3.78±0.08	-	-
'20' min	6.93±0.82	83.33	< 0.001
'60' min	8.62±0.46	128.04	< 0.0001
'90' min	7.48±0.47	97.88	< 0.0001

ANOVA p <0.0001

Table 5: Mean latency time and PIP produced by 2-chlorothiophene-10 mg/kg in eddy's hot plate method in mice.

Recording time	Mean latency time ±SD	PIP (%)	Paired t test (p value)
'0' min	3.52±0.08	-	-
'20' min	3.83±0.34	8.81	< 0.0001
'60' min	4.50±0.40	27.84	< 0.0001
'90' min	3.82±0.04	8.52	< 0.0001

ANOVA p <0.0001

Table 6: Mean latency time and PIP produced by 2-chlorothiophene-20 mg/kg in eddy's hot plate method in mice.

Recording time	Mean latency time ± SD	PIP (%)	Paired t test (p value)
'0' min	3.32 ± 0.18	-	-
'20' min	3.62 ± 0.15	9.04	< 0.001
'60' min	4.37 ± 0.24	31.63	< 0.001
'90' min	4.07 ± 0.14	22.59	< 0.001

ANOVA p <0.0001

Table 7: Mean latency time and PIP produced by 2-chlorothiophene-40mg/kg in eddy's hot plate method in mice.

Recording time	Mean latency time ± SD	PIP (%)	Paired t test (p value)
'0' min	3.25±0.24	-	-
'20' min	4.27±0.58	31.38	< 0.0001
'60' min	4.75±0.47	46.15	< 0.0001
'90' min	4.38±0.53	34.77	< 0.0001

ANOVA p <0.0001

Prolongation of latency time proves the analgesic activity of the test compound. Prolongation of latency time for 50 to 100 percent was considered positive.[11]

Pain inhibition percentage (PIP) was calculated by comparing the values before and after drug administration. Mean latency time and PIP were determined for control, morphine and test groups separately and shown in Tables 3 to 7 and Figure 2.

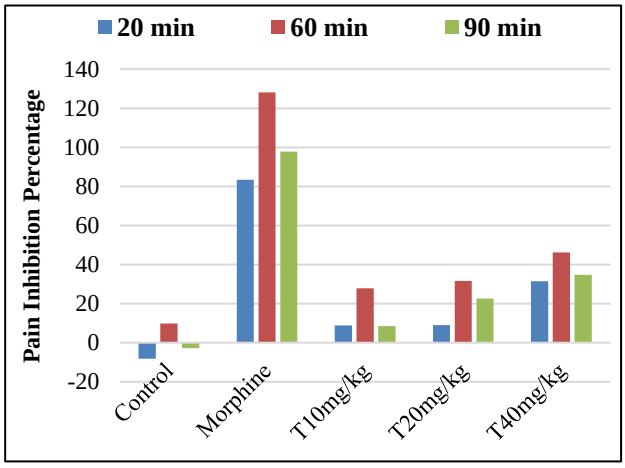

Figure 2: PIP produced by control, morphine and test drug (T) - 10, 20 and 40mg/kg groups at 20, 60 and 90 min in eddy's hotplate method in mice.

Formalin test

Central analgesic activity of test compound was determined by the early phase whereas the peripheral analgesic action was determined by the late phase of formalin test. Number of paw lickings were recorded in early phase of 0-5 min and in late phase of 20-30 min.[20] Pain inhibition percentage was calculated by comparing the drug treated values to that of control group.

Pain inhibition percentage was calculated 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 shown in Tables 8, 9 and Figure 3.

DISCUSSION

Eddy's hot plate method

The PIP produced by the control group of mice at 20, 60 and 90 min indicates that no much significant change in latency time. Morphine group of mice has shown maximum PIP at 60 min and can be considered as standard for comparison.[21] 40mg/kg dose of 2-chlorothiophene has shown maximum action (46.15%) at 60 min which is far less than that of morphine group (128.04%).

Formalin induced paw licking test

Early phase

The maximum PIP produced by 20mg/kg dose of 2-chlorothiophene (22.91%) is better than that of 100mg/kg dose of aspirin (12.5%).

Late phase

The maximum PIP produced by 20mg/kg dose of 2-chlorothiophene (52.63%) is better than that of 100mg/kg dose of aspirin (47.37%) as similar to parent thiophene compound.[9]

The study infers that 2-chlorothiophene compound has maximum central analgesic activity at 40mg/kg at 60 min which is lesser than that of 5mg/kg dose of morphine and maximum peripheral analgesic activity at 20mg/kg which is better than that of 100mg/kg dose of aspirin. This study is a simple screening test for the presence or absence of analgesic activity for 2-chlorothiophene compound. The purpose of the study is served by demonstrating the analgesic activity in the doses employed.

It can be considered to have good peripheral analgesic action with maximum PIP of 52.63% as evident in late phase of formalin test. If the test drug provides substantial positive results under future clinical trials, it's contribution to the community may become very significant as the access to pain management is still very much a human right.[22]

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

Group	Mean No. of licks ±SD	PIP (%)	Unpaired t test (p)
control (10% tween-80)	32.00±1.90	-	-
aspirin (100 mg/kg/PO)	28.00±1.67	12.50	<0.01
2-chlorothiophene (10 mg/kg/PO)	28.50±1.38	10.94	<0.01
2-chlorothiophene (20 mg/kg/PO)	24.67±3.33	22.91	<0.001
2-chlorothiophene (40 mg/kg/PO)	25.50±2.59	20.31	<0.001

ANOVA p <0.0001

The drug can't be claimed for central analgesic activity as the maximum PIP is <50% as evident in eddy's hot plate method and early phase of formalin test.[11]

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

Group (n=6)	Mean No. of licks ±SD	PIP (%)	Unpaired t test (p)
Control (10% tween-80)	38.00±2.28	-	-
Aspirin (100 mg/kg/PO)	20.00±2.00	47.37	< 0.0001
2-chlorothiophene (10 mg/kg/PO)	22.00±2.53	42.11	< 0.0001
2-chlorothiophene (20 mg/kg/PO)	18.00±2.97	52.63	< 0.0001
2-chlorothiophene (40 mg/kg/PO)	19.83±1.47	47.82	< 0.0001

ANOVA p <0.0001

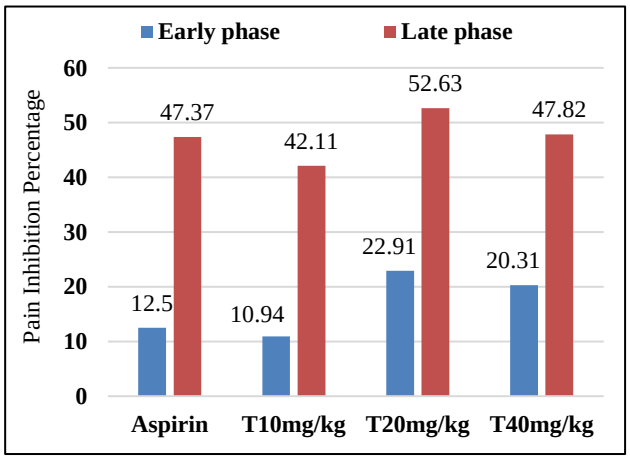

Figure 3: PIP shown by aspirin (100mg/kg) and Test drug(T) at 10, 20 and 40mg/kg in formalin test.

Under eddy's hot plate method, all drugs have shown peak activity at 60 min and declined activity at 90 min. If the observations might have been continued, then the complete time course of action for all the drugs could have been recorded. Further studies may be conducted in more number of animal models to evaluate and confirm the findings. These results can't be interpolated for human. Further investigation can throw light on the minimum effective dose and ceiling doses of the drug.

CONCLUSION

From this study, it can be safely concluded that the test drug, 2-chlorothiophene has good peripheral analgesic action at 20mg/kg dose as evident in late phase of formalin test. It has shown maximum central analgesic activity (but <50%) at 40mg/kg dose as evident in eddy's hotplate method.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Animal Ethics Committee

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