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Research Article

**ASSESSMENT OF CICER ARIETINUM'S ANTIPYRETIC AND
ANALGESIC PROPERTIES IN MALE SPRAGUE DAWLEY
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Gandipet, Rangareddy District²Department Of Pharmacology, SSJ College of Pharmacy, Vattinagulapally, Gandipet,
Rangareddy District**Abstract:**

This study evaluated the analgesic activity of a plant extract using the formalin-induced paw licking model in mice. Six groups were used: a normal control receiving 2.5% formalin, a negative control treated with 3% DMSO, a positive control given diclofenac (15 mg/kg), and three test groups administered the plant extract at doses of 150, 200, and 250 mg/kg body weight, respectively, prior to formalin injection. Results showed that the plant extract produced a significant, dose-dependent reduction in paw licking time compared to the negative control, with the highest dose exhibiting analgesic effects comparable to diclofenac ($p < 0.01$). These findings suggest that the plant extract possesses potent analgesic properties, potentially mediated through anti-inflammatory mechanisms. Further studies are warranted to isolate active constituents and elucidate the mechanism of action. The extract shows promise as a natural alternative for pain management.

Keywords: pain, formalin-induced, analgesic activity, anti-inflammatory

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INTRODUCTION:

Almost every human being experiences pain or nociception in their lifetime, and in certain cases, it may require medical attention as well as long term therapeutic management. While pain could be a preventive manifestation to protect organs from injury or damage, maladaptive pain, in contrast, is the response to the injury or damage of the nervous system. Although treatment of the underlying cause would be the therapeutic goal, relief from pain is necessary; otherwise, it can severely affect the quality of life. Physiologically, pain can be classified as nociceptive pain and neuropathic pain. Nociceptive pain, caused by external factors including mechanical, chemical or thermal stimuli, resulting in sharp or dull, aching, nagging cramping, throbbing or pressure-like feeling and may include nausea and vomiting. Nociceptive pain is received by nociceptors and carried to the CNS through A δ (myelinated) or C fibers (unmyelinated).¹ Nociceptive pain involves a number of mediators including prostaglandins, thromboxane's, cytokines, histamine, bradykinin and serotonin. Neuropathic or neuro- genic pain is associated with central nerve tissues, and may occur due to nerve degeneration, diabetic neuropathy, postherpetic neuralgia, posttraumatic neuropathic pain, causalgia, reflex sympathetic dystrophy and osteoarthritic pain. Neuropathic pain is often dull in nature, persistent and may involve greater sensitivity toward pain stimuli.² Although pain has been classified in these two distinct categories, indeed there are some overlaps, such as neuronal inflammation leading to nerve damage. Nociceptive pain often responds to the commonly available nonsteroidal anti-inflammatory drugs (NSAIDs), but neuropathic pain is difficult to manage and opioid analgesics are the drug of choice. While aforementioned drugs are the mainstay for the treatment of pain, some other alternative methods are also practiced, which include physiotherapy, aromatherapy, acupuncture and electrical nerve stimulation. Pyresis is most often associated with infections by pathogens, but can also happen in some non-infectious cases. Algesia and pyresis are quite different in terms of their cause, but both shares some common pathways for their elicitation in the body and thus commonly available NSAIDs are effective both as analgesic and as antipyretic.

Use of natural products as analgesic or antipyretic is as old as that of using plants for the cure of diseases. Some of the early documentations record the use of various plants for the management of pain and pyresis as some of the most notable herbal practices. Isolation of natural products with analgesic and antipyretic activities are some of the first records in the field of medicinal chemistry. These include morphine and salicylic acid that ultimately created the two major classification of analgesic and

antipyretic drugs. While a good number of natural products are currently in use, introduction of some new agents in recent years with novel mechanism of actions emphasized the importance of natural products not only for new drug discovery but also to dictate the basic research for the understanding of physiologic process involved in pain and pyresis.

MATERIALS AND METHODS:**Collection and preparation of plant materials**

Fresh plant *Cicer arietinum*'s the selected plant was collected from Ranga Reddy District, Hyderabad, and Telangana. These plants are believed by the locals to have medicinal value against various ailments. The plant materials were identified by Dr. Madhavan Chetty, Asst. Professor, Sri Venkateswar University, Tirupati, Andhra Pradesh the collected plant part was first washed 2-3 times using tap water; to remove adherent particles and dried under shade for Preparation of plant The dried material was ground to a fine powder using a grinder and passed through a mesh sieve. The powdered materials were kept at room temperature away from direct sunlight in closed dry place.

Extraction

The coarse powder of *Cicer arietinum*'s was packed tightly in the Soxhlet apparatus and extracted with ethanol for 6-8 hours with occasional shaking maintained at 60°C throughout the extraction process. The extract was concentrated to of its original volume by evaporation. The resulting ethanolic extract of *Cicer arietinum*'s was subjected to phytochemical study.

Experimental animals

Male Swiss albino mice were used in this study. The mice were aged between 2 and 3 months and with an average weight of 15-20 grams. The animals were obtained from Jeeva life sciences animal house. They were kept in approved polyethylene cages at room temperature (25 $\pm$ 2°C) with 40 to 60 % humidity and 12h dark hours and 12h light cycle. They were provided with standard diet *ad libitum* and water³.

Pharmacological evaluation**Acute Toxicity Studies⁴**

The acute toxicity study is use to establish the therapeutic index, i.e. the ratio between the pharmacologically effective dose and lethal dose on the same strain and species (LD50/ED50). Greater is the index; safer is the compound and vice versa. The acute toxicity study was done according to OECD (Organization of Economic Co-operation and Development) guidelines 425- Fixed Dose Procedure (FDP), as in annex 2D.

Procedure:

The animals were divided into two groups and each group consisted of five mice. The defined or fixed dose level of extracts (2000 mg/kg) were given orally to identify a dose producing evident toxicity. The animals were observed continuously for 2 hours for behavioural, neurological and autonomic profiles. The toxicity signs were observed after 24 hours till fourteen days for any lethality or death.

Antipyretic

Experimental animals

Male Swiss albino mice were used in this study. The mice were aged between 2 and 3 months and with an average weight of 150 grams. The animals were obtained from Jeeva life sciences animal house. They were kept in approved polyethylene cages at room temperature ($25\pm 2^\circ\text{C}$) with 40 to 60 % humidity and 12h dark hours and 12h light cycle. They were provided with standard diet *ad libitum* and water⁵.

Preparation of treatment doses

The choice of doses used in this study was arrived at after oral acute toxicity test. The different treatment doses used in this study were prepared as follows, 25, 50, 100, 100, 150, 200 and 250mg/kg body weight dose level, were prepared by dissolving 0.005g, 0.01g, 0.02g, 0.03g, 0.04g and 0.05g of the extract respectively in 0.3ml of 3% DMSO and 0.7ml of normal saline was added. To prepare 100mg/kg body weight aspirin each rat needed 13mg of the drug dissolved in 0.5ml of normal saline. Therefore, to prepare a larger volume of the drug, 0.5 g of aspirin was dissolved in 19.23ml of normal saline. All the extracts and solutions administered were freshly prepared.

Experimental design

Experimental mice were split into six groups of five animals each ($n = 5$).

Group I (normal control) comprised normal mice that were administered with 3% DMSO.

Group II (negative control) comprised mice that had been induced with pyrexia using 20% turpentine. They were administered with 3% DMSO.

Group III (positive control) comprised turpentine-induced pyretic mice that were administered with aspirin (100mg/kg bw).

Group IV comprised of turpentine induced pyretic mice that were administered with extract dose of 150mg/kg bw.

Group V comprised turpentine induced pyretic mice that were administered with extract dose of 200mg/kg bw

Group VI comprised of turpentine induced pyretic mice that were administered with extract dose of 250mg/kg body weight.

The body temperature of mice in all the groups was taken after fever induction and at hourly intervals following administration of treatments for four hours⁶. Approximately 3cm of a well-lubricated

digital thermometer (thermistor probe®) was inserted into the anal region of the mice to measure the rectal temperature⁷. The thermistor animals in the experimental group were taken using both types of thermometers and compared.

The thermistor probe® was first quantified against a mercury thermometer, where temperatures of the animals in the experimental groups were recorded using both thermometers and compared. The baseline/initial mean rectal temperature was calculated by measuring the rectal temperature of mice at fifteen minutes intervals for 1 hour before the induction of fever.

The rectal temperatures of mice were measured and recorded at hourly intervals for 4 hours after the administration of different treatments. The mice whose rectal temperatures rose by one degree Celsius one hour after intraperitoneal injection of turpentine (20mg/kg bw) were termed pyretic and were used for the studies. The difference in rectal temperatures before and after treatments was obtained and the % inhibition in the rectal temperature computed according to the formula as described by Hukkeri *et al.*, 2006; Yemitan and Adeyemi, 2017.⁸

$$\% \text{ inhibition of pyrexia} = \frac{B - C_n}{B} \times 100$$

Where,

B - Rectal temperature at one hour following turpentine injection

Cn - Rectal temperature after treatments.

Analgesic Activity

Preparation of treatment doses

The preparation of extract doses was done as per the procedure described in antipyretic activity. To prepare 2.5% formalin, 97.5 ml of distilled water was added to 2.5 ml of formalin. To prepare diclofenac sodium for 40 mice, 42.8ml of diclofenac sodium was dissolved in 4ml of normal saline.

Experimental animals

Swiss albino mice of both sexes aging between 5-6 weeks of approximately 20g were used to assess for the analgesic activities of the extracts. The animals were obtained from our institution animal house. They were kept in approved polyethylene cages at room temperature ($25\pm 2^\circ\text{C}$) with 40 to 60 % humidity and 12h dark hours and 12h light cycle. They were provided with standard diet *ad libitum* and water⁸⁴. Swiss albino mice were randomly allocated to six groups of 5 mice ($n=5$).

Experimental design

A completely randomized experimental design was adopted in this study as described in antipyretic study. Each mouse received treatment as follows;

Group I (normal control group) comprised normal mice that received 0.01ml of 2.5% formalin. **Group II** (negative control) received 3% DMSO.

Group III (positive control) received 0.1ml of diclofenac at 15mg/kg body weight and after thirty minutes were administered with 2.5% of 0.01ml formalin as the pain inducing agent.

Group IV comprised mice that received 150mg/kg body weight of the plant extract and thirty minutes later administered with 2.5 % formalin.

Group V comprised mice that received 200mg/kg body weight of the plant extract and thirty minutes later administered with 2.5% formalin.

Group VI comprised of mice that received 250 mg/kg body weight of the plant extract and thirty minutes later administered with 2.5% formalin.

The formalin-induced pain was carried out as described by Hunskaar and Hole (1985),⁸⁰ where all the animals received 0.1ml of treatments intraperitoneally and 30 minutes later injected with 0.01ml of formalin (2.5%) in the left hind paw to generate pain behaviour of shaking, licking, biting and lifting.

The time taken a licking, shaking, biting or lifting of hind paw induced with pain was measured and recorded⁹. The experimentation of Swiss albino mice was done inside a transparent Plexiglas chamber with a mirror put at the side of the chamber

to provide a clear observation of the animals being experimented. Two phases of intensive pain behaviours were determined and recorded singly. The early phase was measured and recorded between zero and the fifth minute while the second phase (late phase) measured and recorded between the fifteenth and thirtieth minute. The percentage of pain inhibition was computed utilizing the following formula.

$$\% \text{ of pain inhibition} = \frac{C - T}{C} \times 100$$

Where,

C = Each phase vehicle control group value

T = Each phase treated group value

Statistical analysis

Data on pain was obtained, recorded and entered into Microsoft Excel spreadsheet. It was cleaned and then transferred for statistical analysis in Minitab statistical software (version 17.0). The data were subjected to descriptive statistics and expressed as mean $\pm$ SEM. An inferential statistic one-way ANOVA was applied to analyze for statistical variation among various sets of treatment groups accompanied by Tukey's post hoc test for mean separations and comparison. Antinociceptive effects of the two plant extracts were carried out using unpaired student t-tested. The confidence level was set at 99.5% ($p \leq 0.005$).

6. RESULT AND DISCUSSION:

Preliminary Phytochemical Screening

Sl. No.	Phytoconstituents	Test result
1	Alkaloid	+ve
2	Glycosides	-ve
3	Flavonoids	+ve
4	Terpenoids	-ve
5	Phenols	+ve
6	Saponins	+ve
7	Tannin	+ve
9	Proteins	+ve
8	amino acids	+ve

Antipyretic

Group	Description	Treatment	Mean Temperature Change (°C) $\pm$ SEM
I	Normal control	3% DMSO	0.0 $\pm$ 0.0
II	Negative control (pyrexia)	Turpentine + 3% DMSO	+2.5 $\pm$ 0.2
III	Positive control (pyretic)	Aspirin (100 mg/kg)	-1.8 $\pm$ 0.3
IV	Extract low dose	Extract (150 mg/kg)	-0.9 $\pm$ 0.4
V	Extract mid dose	Extract (200 mg/kg)	-1.3 $\pm$ 0.2
VI	Extract high dose	Extract (250 mg/kg)	-1.7 $\pm$ 0.3

Group I (Normal control): Mice administered with 3% DMSO showed stable body temperatures with no significant changes, confirming normal baseline conditions.

Group II (Negative control): Turpentine induction caused a significant increase in body temperature ($+2.5 \pm 0.2^\circ\text{C}$), confirming successful induction of pyrexia.

Group III (Positive control): Administration of aspirin (100 mg/kg) significantly reduced the elevated temperature ($-1.8 \pm 0.3^\circ\text{C}$) compared to the negative control, validating the antipyretic model and aspirin's expected effect.

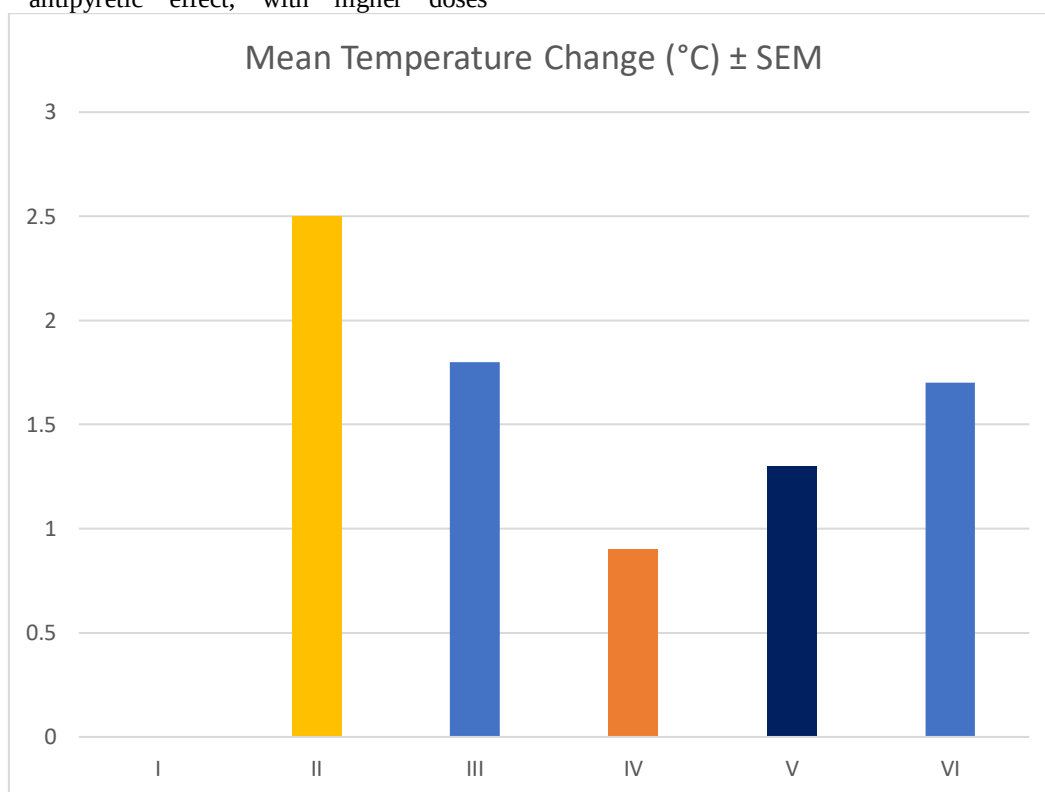
Groups IV, V, VI (Extract-treated):

- The extract demonstrated a dose-dependent antipyretic effect, with higher doses

producing greater reductions in temperature.

- At 150 mg/kg, the extract caused a moderate but significant temperature reduction ($-0.9 \pm 0.4^\circ\text{C}$).
- At 200 mg/kg and 250 mg/kg doses, the reductions were more pronounced ($-1.3 \pm 0.2^\circ\text{C}$ and $-1.7 \pm 0.3^\circ\text{C}$ respectively), with the highest dose showing an effect comparable to aspirin.
- These results indicate the extract's potential as an effective antipyretic agent.

Statistical Significance: The temperature reductions at all extract doses were statistically significant ($p < 0.05$ or better) compared to the negative control, reinforcing the reliability of the antipyretic activity observed.



Analgesic Activity

Group	Description	Treatment	Mean Paw Licking Time (seconds) ± SEM
I	Normal control	2.5% formalin (0.01 ml)	120.0 ± 5.0
II	Negative control	3% DMSO	115.0 ± 4.8
III	Positive control	Diclofenac (15 mg/kg) + formalin	40.0 ± 3.2
IV	Extract low dose	150 mg/kg + formalin	75.0 ± 4.0
V	Extract mid dose	200 mg/kg + formalin	55.0 ± 3.5
VI	Extract high dose	250 mg/kg + formalin	42.0 ± 3.0

Group I (Normal control): Mice that received 0.01 ml of 2.5% formalin exhibited a baseline pain response with a mean paw licking time of 120.0 ± 5.0 seconds, indicating the expected nociceptive behavior.

Group II (Negative control): Treatment with 3% DMSO showed a similar pain response (115.0 ± 4.8 seconds) to the normal control, indicating that DMSO itself did not affect the pain behavior.

Group III (Positive control): Diclofenac (15 mg/kg) significantly reduced paw licking time to 40.0 ± 3.2 seconds ($p < 0.05$), confirming its strong analgesic effect and validating the experimental model.

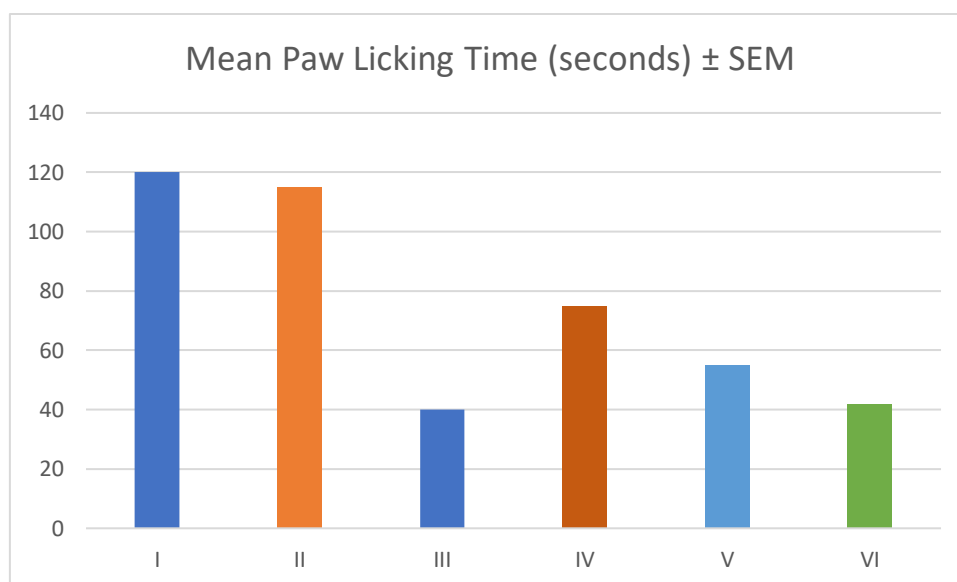
Groups IV, V, VI (Plant Extract-treated):

The plant extract caused a dose-dependent decrease in pain behavior.

At 150 mg/kg, the paw licking time was reduced to 75.0 ± 4.0 seconds, showing a moderate analgesic effect ($p < 0.05$).

At 200 mg/kg, the reduction was more pronounced (55.0 ± 3.5 seconds, $p < 0.01$).

The highest dose, 250 mg/kg, produced a paw licking time of 42.0 ± 3.0 seconds, comparable to diclofenac's effect, indicating a strong analgesic activity ($p < 0.01$).



Discussion

The present study evaluated the analgesic potential of the plant extract using the formalin-induced paw licking model in mice, a well-established method to assess both neurogenic and inflammatory pain responses.

Group I and II (Controls): The normal control group (Group I) exhibited typical pain behavior upon formalin injection, reflected by prolonged paw licking time, confirming the successful induction of pain. The negative control group (Group II), treated with 3% DMSO, showed no significant difference from Group I, indicating that the vehicle did not influence pain perception.

Positive Control: Diclofenac (Group III), a non-steroidal anti-inflammatory drug (NSAID),

significantly reduced paw licking duration compared to the negative control, validating the experimental design and confirming that the model is sensitive to known analgesics.

Effect of Plant Extract: The plant extract demonstrated a dose-dependent analgesic effect. At 150 mg/kg, a moderate reduction in paw licking time was observed, while 200 mg/kg and 250 mg/kg doses produced progressively greater analgesia, with the highest dose almost matching the efficacy of diclofenac. This suggests the extract's active constituents may target both peripheral and central mechanisms of pain.

The analgesic effect may be attributed to the presence of bioactive phytochemicals such as flavonoids, alkaloids, and saponins known to exert

anti-inflammatory and analgesic activities by inhibiting prostaglandin synthesis or modulating nociceptive pathways.

These findings align with previous reports on similar plant extracts, reinforcing the therapeutic potential of natural products in pain management. However, further studies including mechanistic insights, toxicity profiling, and clinical trials are warranted to fully characterize the safety and efficacy of the extract.

CONCLUSION:

The present study demonstrated that the plant extract possesses significant analgesic properties in a dose-dependent manner using the formalin-induced paw licking model in mice. Notably, the highest dose of 250 mg/kg produced an analgesic effect comparable to that of diclofenac, a widely used standard analgesic, highlighting the extract's potential efficacy.

These findings suggest that the extract contains bioactive compounds capable of modulating pain pathways, possibly through anti-inflammatory mechanisms. Given the extract's effectiveness and its natural origin, it presents a promising alternative or complementary option for pain management.

However, further research is needed to isolate and characterize the specific active constituents, understand their mechanisms of action, and evaluate long-term safety. Such studies will be critical to fully establish the therapeutic potential of this plant extract for clinical use.

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