J. Pharm. Res. 2025;24(1):49–53

Print ISSN: 0973-7200
Online ISSN: 2454-8405

Journal of Pharmaceutical Research

RESEARCH ARTICLE

Pharmacological Investigation of Anti-Nociceptive, Anti-Inflammatory and Anti-Pyretic Potential of Ethanolic Flower Extract of *Carthamus tinctorius*

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ARTICLE INFO

Article history:
Received 14.02.2025
Accepted 20.03.2025
Published 31.03.2025

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https://doi.org/10.18579/jopcr/v24.i1.29

ABSTRACT

Carthamus tinctorius commonly known as Safflower, has been used as a folkloric medicine in the Ayurvedic system for its various therapeutic properties. The researchers have made a sincere attempt to evaluate the biological effects of the medicinal plant in the treatment of pain, inflammation and pyrexia indicating it as a promising candidate. The ethanolic flower extract of *Carthamus tinctorius* was subjected to Fourier Transform Infrared Spectroscopy (FTIR) studies to establish the role of possible phytoconstituents responsible for these activities. For the evaluation of therapeutic properties different methods like Anti-nociceptive activity using the standard Acetic acid induced writing response test in mice, anti-inflammatory activity in rats adapting Carrageenan induced paw edema and anti-pyretic activity by inducing suspension of Brewer's yeast were analyzed. The medicinal plant has acquired lot of prominence for its effective usefulness for various ailments used by local natives as a folkloric medicine. As no scientific studies or research has been published on pyrexia and inflammation by *Carthamus tinctorius* hence, a sincere attempt is made to explore the possible therapeutic activity of this medicinal plant.

Keywords: *Carthamus tinctorius;* Anti-nociceptive; Anti-inflammatory; Anti-pyretic; Fourier Transform Infrared Spectroscopy (FTIR)

INTRODUCTION

Inflammation is an immunological defense mechanism elicited in response to various mechanical injuries like burns, microbial allergens and other noxious stimuli which develops to redness, swelling etc.[1–3]. Pain is a common symptom of inflammatory disease associated with tissue damage leading to activation of nociceptors through the release of various chemical mediators like amino acids, peptides and cytokines leading to induction of pain and inflammation.[4]

Safflower, *Carthamus tinctorius* belonging to the family Asteraceae[3,5] is a folkloric traditional medicinal herb used for pain, inflammation and pyrexia treatment[3,6]. In China, Safflower injection has been used clinically for treating cerebrovascular disease, coronary heart disease and angiitis.[7]

MATERIALS AND METHODS

Plant material

The flowers of *Carthamus tinctorius* were collected from different regions of Rampachodavaram, Maredumalli and Jaddanki forest of East Godavari region of Andhra Pradesh. The plant has been identified and approved by taxonomist Dr. T. Raghuram, Maharani College, Peddapuram.

Extraction and isolation

The flowers were dried under shade for 3 weeks and pulverized to course crude powder and stored in a tight container. The required amount of coarse powder was subjected to Soxhlet Extractor for continuous hot extraction with ethanol for 8 to 10 hours. Then the extract was filtered, and the filtrate was evaporated to dryness.

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Chemicals and Apparatus

Chemicals used are of Analytical grade with standard Glassware.

FTIR Analysis

The FTIR Spectra of the ethanolic flower extract of *Carthamus tinctorius* was taken with "Bruker FTIR Spectrophotometer" and the spectra was recorded in the region of 3000 cm^{-1} to 720 cm^{-1}. The spectral data of the flower extract was presented in the Table 1.

These peaks suggest the presence of unsaturated fatty acids (from the C=C and C-H bonds) and ester groups (from triglycerides). The strong carbonyl (C=O) absorption around 1744 cm^{-1} confirms the ester linkage in the triglyceride structure.

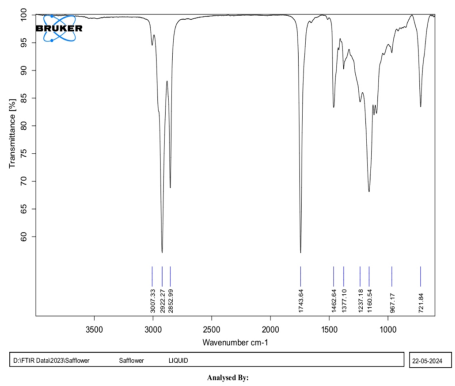

Fig. 1: FTIR Analysis of Ethanolic flowers extract of *Carthamus tinctorius*

Animals

Albino mice of either sex weighing 20-25 grams and Albino rats weighing 120-150 grams of either sex was used for carrying different activities. The animals were housed in standard polypropylene cages and kept under room temperature at 28°C relative humidity of 70 % in light/dark cycle and accessed to food and water ad libitum. The animal experimental procedure was approved by Institutional Animal Ethical Committee.

(i) In-vivo Anti-nociceptive studies

Acetic acid induced writhing responses: The mice were treated with standard method of Acetic acid induced writhing responses[8–10]. 4 groups of 3 mice each were used for the study.

- Group-I: Animals were treated with normal saline (5 ml/kg body weight I.P).
- Group-II: Mice were treated with standard drug Diclofenac sodium (10 mg/kg).
- Group-III: Animals were treated with ethanolic flower extract of *Carthamus tinctorius* 200 mg/kg body weight.
- Group-IV: Mice were treated with ethanolic flower extract of *Carthamus tinctorius* 400 mg/kg body weight.

About 30 minutes after administration 0.5 % Acetic acid (0.1ml) was injected intraperitoneally to the mice. The number of abdominal constrictions and stretching were counted in a period of 5-30 minutes.

The following formula was used to determine the percentage inhibition of *Carthamus tinctorius* flower extract at 200 and 400 mg/kg.

$$\text{Percentage inhibition} = \left(\frac{wC - wT}{wC}\right) \times 100$$

where, wc = Writhings in control group
wt = Writhings in treated group.

(ii) In-vivo Anti-inflammatory studies

Carrageenan Induced Paw Edema in Rats: Carrageenan induced paw edema is the most reliable and sensitive method and is most comparable to the previous articles[11–15]. This method is used to evaluate the anti-inflammatory activity of ethanolic flowers extract of *Carthamus tinctorius* at a dose of 200 and 400 mg/kg which is comparable to the standard reference drug Diclofenac sodium (10 mg/kg) p.o. 1 hour after the administration, edema was induced to the sub-plantar injection of 0.1mL of 1 % freshly prepared suspension of carrageenan. After the injection the inhibition was determined by measuring the thickness at intervals of 0, 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} hour. Paw sizes were measured immediately before and after carrageenan injection. Inhibitory activity of edema was calculated using the formula:

$$\text{Percentage inhibition} = \left(1 - \frac{VT}{VC}\right) \times 100$$

Where, VT= Edema volume of treated group
VC= Edema volume of control group.

The in-vivo activity in Carrageenan induced paw edema was evaluated and compared with the standard drug Diclofenac Sodium.

(iii) In-vivo Anti-pyretic studies

By employing an established method[15,16] to inject 15% suspension of Brewer's yeast (*Saccharomyces cerevisiae*) into the rectum which caused a temperature rise phenomenon. The rectal temperature was recorded by inserting a thermostat probe 3-4 cm deep. The animals received a subcutaneous injection of 10 ml/kg of 15 % w/v Brewer's yeast suspended in 0.5% w/v methylcellulose solution. The rat's rectal temperature was recorded 15 hours after

Table 1: FTIR Analysis of *Carthamus tinctorius* flowers extract

Wave number (cm^{-1})	Possible vibration	Functional Group
3007	C-H stretching in alkenes	C=C-H, indicative of unsaturated bonds
2922	C-H stretching in alkanes	Sp^3 hybridized C-H, from CH_2 and CH_3 groups
2853	C-H stretching in alkanes	Another Sp^3 C-H bond
1744	C=O stretching	Carbonyl group, likely from esters or triglycerides
1463	C-H bending	Methylene and Methyl groups
1377	C-H bending	Methyl groups, -CH_3
1237	C-O stretching	Typically found in Esters
1160	C-O stretching	Likely from Ester functional group in Fatty acids
967	C-H out of plane	Possibly from Trans alkenes
721	CH2 rocking	Indicative of long chain Hydrocarbons, often seen in oils.

the injection. Immediately the ethanolic flower extract of *Carthamus tinctorius* with different doses of 200 and 400 mg/kg were administered, and paracetamol was used as standard reference drug.

RESULTS AND DISCUSSION

The ethanolic flower extract of *Carthamus tinctorius* was tested for its suspected anti-nociceptive, anti-inflammatory and anti-pyretic characteristics in order to show that it had nociceptive, inflammation-reducing and body temperature lowering properties.

(i) Anti-nociceptive activity by Acetic acid induced writhing responses in Mice

Acetic acid triggered the analgesic mechanism of abdominal writhing in the visceral pain model, which primarily involves the release of arachidonic acid through cyclooxygenase and prostaglandin production.[17] At doses of 200 and 400 mg/kg, the ethanolic floral extract of *Carthamus tinctorius* markedly reduced the mice's acetic acid- induced writhing response. The standard drug Diclofenac sodium (10 mg/kg) demonstrated 88% protection against acetic acid-induced writhing in mice, the flower extract has shown notable analgesic effect by 72% reduction of nociception at a dose of 200 mg/kg and 83% inhibition at 400 mg/kg. The findings were tabulated in Figure 2 and Table 2.

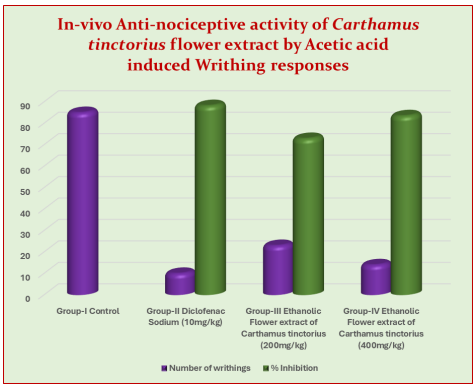

Fig. 2: No. of writhings and Percentage Inhibition of writhing by Ethanolic flowers extract of *Carthamus tinctorius*

(ii) Anti-inflammatory activity by Carrageenan Induced Paw Edema in Rats

Inflammation is a complex pathophysiological response that involves the activity of inflammatory mediators such as neutrophils, reactive oxygen species (ROS), nitric oxide (NO), Prostaglandins (PG's) and Cytokines[18]. The standard methodology carrageenan induced rat paw edema is adopted for screening of anti-inflammatory activity[19]. Inflammatory responses induce innate immune response by activating T-cells and Macrophages and stimulating the secretion of inflammatory cytokines, TNF-α is the major mediator[20]. In the pathogenesis of inflammatory disorders significant correlations exert between cytokine production, cyclooxygenase-2 (COX-2) protein expression and Prostaglandin synthesis in the rat paw tissues where edema was involved by carrageenan intra-plantar injection.[21] Ethanolic flower extract of *Carthamus tinctorius* at doses of 200 and 400 mg/kg significantly inhibited the protein expression of COX-2 levels in the rat edema paw tissues by 48 % and 55 % compared with the standard drug Diclofenac sodium 10 mg/kg produced 69 % of inhibition of edema. The results were tabulated in Table 3 and Figure 3.

(iii) In-vivo Anti-pyretic studies

Fever could potentially be caused by an infection or one of the consequences of tissue damage[22]. The mindful balance between heat production and loss is necessary for controlling body temperature. The hypothalamus is in charge of controlling body temperature. The ethanolic flower extract of *Carthamus tinctorius* showed notable antipyretic properties, as it significantly decreased the animals increased body temperature caused by Brewer's yeast. The reduction of body temperature at a dose of 200 mg/kg exhibited 50% reduction and 70% reduction of elevated body temperature

Table 2: Effect of *Carthamus tinctorius* flowers extract in mice using Acetic acid induced writhing response

Treatment Group	No. of writhings	% Inhibition
Group-I Control	85±0.15	-
Group-II Std Drug Diclofenac sodium [10 mg/kg]	10±0.36	88.23 %
Group-III *Carthamus tinctorius* [200 mg/kg]	23±0.46	72.9 %
Group-IV *Carthamus tinctorius* [400 mg/kg]	14±0.19	83.5 %

All the values were expressed in Mean ± SEM, n=3.

Table 3: Anti-inflammatory effect of *Carthamus tinctorius* flower extract by Carrageenan induced paw edema model

S.No	Treated Group	Mean inhibition in Paw Diameter (in mm)				
		0 Hour	1st Hour	2nd Hour	3rd Hour	4th Hour
1	Control	0.36±0.01	0.42±0.05	0.68±0.07	0.80±0.03	0.74±0.02
2	Std- Diclofenac Sodium (10 mg/kg)	0.21±0.04	0.33±0.01 22	0.45±0.02 34	0.47±0.06 42	0.23±0.09 69
3	Extract of *Carthamus tinctorius* (200 mg/kg)	0.32±0.03	0.38±0.06 10	0.51±0.03 25	0.52±0.01 34	0.42±0.04 48
4	Extract of *Carthamus tinctorius* (400 mg/kg)	0.32±0.07	0.35±0.02 17	0.48±0.09 30	0.51±0.08 40	0.40±0.01 55

All values are expressed in Mean ± SEM, n=3, p<0.001 when compared with standard values.

Table 4: Anti-pyretic activity of *Carthamus tinctorius* flower extract by Brewer's suspension

S.No	Treated Group	Rectal temperature (°C) after Yeast induction					
		0 Hour	1st Hour	2nd Hour	3rd Hour	4th Hour	5th Hour
1	Control	38.25±0.04	39.33±0.20	39.40±0.13	39.35±0.20	39.28±0.30	39.31±0.05
2	Std- Paracetamol (100 mg/kg)	38.50±0.05	38.45±0.80 30	37.85±0.50 40	37.43±0.30 50	3.681±0.2 70	3.650±0.01 80
3	Extract of *Carthamus tinctorius* (200 mg/kg)	38.38±0.30	39.15±0.05 10	38.83±0.20 20	38.50±0.40 30	37.79±0.20 40	37.55±0.06 50
4	Extract of *Carthamus tinctorius* (400 mg/kg)	38.45±0.15	39.05±0.02 20	38.25±0.04 30	37.95±0.04 40	37.15±0.02 60	36.6±0.02 70

All values are expressed in Mean ± SEM, n=3.

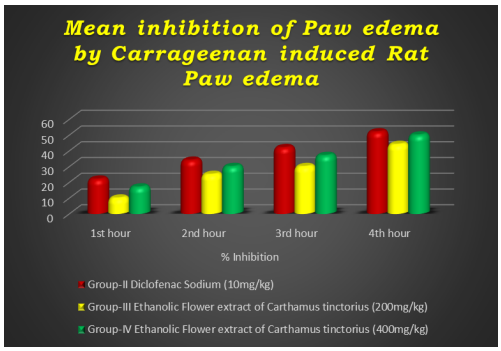

Fig. 3: Mean Percentage inhibition of edema by Ethanolic flowers extract of *Carthamus tinctorius*

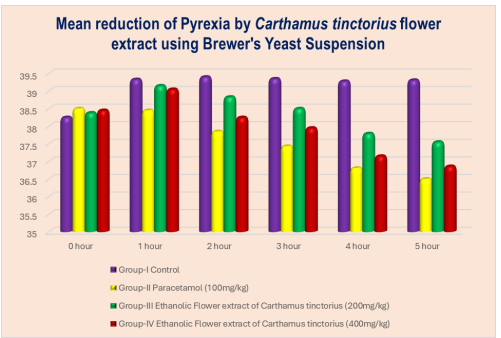

Fig. 4: Mean Reduction of Pyrexia by Ethanolic flowers extract of *Carthamus tinctorius*

at 400 mg/kg compared with the standard reference drug Paracetamol (100 mg/kg) which produced 80% of reduction. The results were tabulated in Table 4 and Figures 4 and 5.

CONCLUSION

In conclusion, our experimental results revealed that the ethanolic flower extract of *Carthamus tinctorius* exhibited significant anti-nociceptive, anti-inflammatory and anti-pyretic activities. The traditional usage of this medicinal herb to treat nociception, inflammation and temperature related conditions is supported by these genuine experimental results. The active phytoconstituents from this plant extract exhibited the biological activities needs to be distinguished by further investigation.

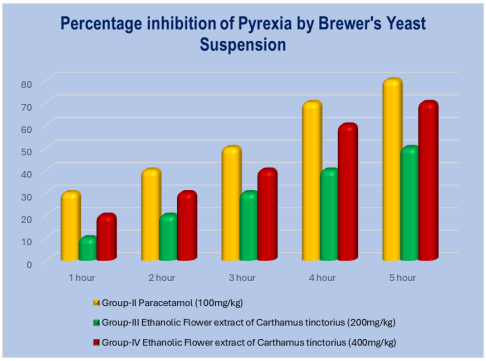

Fig. 5: Percentage inhibition Pyrexia by Brewer's suspension using Ethanolic flowers extract of *Carthamus tinctorius*

ACKNOWLEDGEMENT

The researchers are grateful to the administration of Aditya College of Pharmacy for providing all the required research facilities in conducting the experiment.

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