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Anti-nociceptive, anti-inflammatory and anti-pyretic activities of latex and leaves methanol extract of *Euphorbia helioscopia*

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PEER REVIEW

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Comments

In this study, the authors quantitated the analgesic and antipyretic effects of the *E. helioscopia* by combining different methods of nociception measuring techniques. This study's merits qualify it to be published in APJTD.
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ABSTRACT

Objective: To evaluate anti-nociceptive, anti-inflammatory and anti-pyretic activities of latex and leaves methanol extract of *Euphorbia helioscopia* against chemical (acetic acid induced writhing and formalin tests) and thermal pain stimuli (hot plate test), carrageenan induced paw edema and brewer's yeast induced pyrexia in mice respectively.

Methods: Pain, inflammation and pyrexia were induced in mice. Leaves methanol extract and latex were administered to mice, orally at doses, 100, 200 and 300 mg/kg.

Results: Leaves methanol extract at all used doses had 100% pain protection against peripherally induced pain pathway (investigated by acetic acid induced writhing test and late phase of formalin test). While central anti-nociceptive effect (measured by hot plate test and early phase of formalin test) was found dose dependent with extract and latex. Similarly a dose dependent trend was observed in anti-inflammatory and anti-pyretic effects and leaves methanol extract showed maximal anti-inflammatory (81.25%) and anti-pyretic (45.36%) effects at 300 mg/kg dose.

Conclusions: From the data obtained, it can be concluded that *Euphorbia helioscopia* possesses marked anti-nociceptive, anti-inflammatory and anti-pyretic activities that can be attributed to the inhibition of synthesis of prostaglandins and other mediators responsible for pain, inflammation and pyrexia.

KEYWORDS

Anti-nociceptive, Anti-inflammatory, Anti-pyretic, *Euphorbia helioscopia*, Latex, Leaves methanol extract

1. Introduction

Inflammation is a natural defense mechanism of the body against cells or tissues injury either due to chemical, mechanical or thermal stimuli or infections[1]. Prostaglandins are key mediators of inflammatory response and end products of arachidonic acid metabolism[2]. Pain, redness, heat, swelling and edema are fundamental signs of inflammation. Hence, anti-

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nociceptive and anti-inflammatory drugs are needed to treat inflammatory diseases like asthma, cardiovascular diseases, and arthritis[1-3]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used to treat these diseases but long term use of these drugs causes GIT ulceration, renal dysfunction and bleeding. Prostaglandins synthesis is also increased in fever, thus, drugs that can inhibit prostaglandin synthesis may possess anti-pyretic action also. The search for new analgesic and anti-inflammatory drugs which do not present NSAIDs like side effects is going on globally[4].

Euphorbia helioscopia (*E. helioscopia*) (Family: Euphorbiaceae) grown in Nilgiri Hill, is also found throughout the province of Punjab in Pakistan[5]. Traditional uses of *E. helioscopia* are as follows: stem and leaves are used for febrifuge and vermifuge actions[6], seeds mixed with roosted pepper are used in cholera, seeds oil relieves constipation and roots possess anthelmintic activity[7]. Number of studies have unveiled multiple pharmacological activities, such as antibacterial, antiviral, antifungal[8-10], anticancer and/or antitumor[11], allelopathic[12], anti-allergic and anti-asthmatic[13], antioxidant[8,14,15], anti-nociceptive effect[16], insulin secretagogue[17], vasodepressor[18], phytotoxicity[19]*etc.*, of this plant.

Aerial parts of alcoholic extract of *E. helioscopia* have been investigated for anti-nociceptive activity using formalin test and acetic acid induced writhing test[16,20]. Number of studies have revealed anti-nociceptive activity of plants *e.g. Melanthera scandens*[21]. This study was designed to evaluate anti-nociceptive, anti-pyretic, and anti-inflammatory activities of latex and leaves methanol extract (L.MT) of *E. helioscopia*.

2. Materials and methods

2.1. Plant collection

E. helioscopia was collected from the suburbs of city of Lahore-Pakistan. It was authenticated by a taxonomist of Botany Department, Government College University, Lahore-Pakistan. After authentication, a voucher specimen (1501) was deposited at Government College University herbarium. It was dried under shade then leaves and stem were separated and ground to fine powder. Latex was collected by cutting the leafy parts of stem and collected in clean and sterile bottle.

2.2. Preparation of extracts and samples

Extracts of leaves and stem were prepared by two methods: 1) Cold extraction method (maceration) using water and ethanol as solvents separately; 2) Hot sequential extraction method (using Soxhlet apparatus) by employing petroleum ether, chloroform and methanol in increasing order of polarity. The solvents were removed by rotary evaporator at 40 ℃.

L.MT and latex were selected in the current study, keeping in view their excellent *in vitro* antioxidant property in our earlier work[22].

L.MT and latex were dissolved in distilled water to make solutions for administration to mice orally.

2.3. Animal ethical committee approval

The study was performed after getting approval from Animals Ethical Committee of University College of Pharmacy, University of the Punjab, Lahore-Pakistan (AEC/UCP 1009/4313 Ph).

2.4. Estimation of anti-nociceptive activity using three different assays

Albino mice of either sex (n=5) weighing 25-30 g were used in the study. Food was withdrawn 2 h prior to start of experiments.

2.4.1. Acetic acid induced writhing test

Mice were divided into nine groups as follows: Group I, control; Group II, standard (brufen, 100 mg/kg, orally); Group III, standard (tramadol, 10 mg/kg, orally); Groups IV-VI, treated with aqueous solutions of L.MT (100, 200 and 300 mg/kg, orally respectively); Groups VII-IX, treated with latex (100, 200 and 300 mg/kg, orally respectively). Acetic acid (1% or 100 mg/kg) was injected intraperitoneally, at the rate of 1 mL/100 g mouse, half an hour after the administration of standards/tested samples. Then, five minutes after the injection, the number of specific abdominal contractions (writhes) were counted till 20 min. Decrease in number of abdominal contractions is indicative of anti-nociceptive property of tested drug. The result was expressed as % pain protection that was calculated with following formula[23]:

$$\%\ \text{Pain protection} = \frac{\text{Total writhings in control} - \text{Total writhings in treated}}{\text{Total writhings in control}} \times 100$$

2.4.2. Formalin test

In this assay, anti-nociceptive property was assessed against formaldehyde induced pain. Thirty minutes after the oral dosing of standard drugs [paracetamol (PCM), 400 mg/kg and tramadol, 10 mg/kg] and tested samples (L.MT and latex, 100, 200 and 300 mg/kg each), 50 μL of formalin (2.5%) was injected into the plantar region of hind paw of each mouse of all groups. The observation of this experiment was scored, according to behavioral response, using the following scale:

0=walking or standing on injected paw, 1=partial elevation of paw, 2=total elevation of injected paw, 3=licking or biting of injected paw.

Observations were recorded for 45 min post injection. Pain was categorized into two phases: a) early phase that lasts up to 10 min; b) late phase that lasts till 45 min[24].

2.4.3. Hot plate test

Brufen (100 mg/kg), tramadol (10 mg/kg), aspirin (400 mg/kg) and PCM (400 mg/kg) were used as standard drugs. L.MT and latex (100, 200 and 300 mg/kg each) and standards were administered orally 20 min before experimentation. The temperature of hot plate was maintained at (55±0.5) ˚C and cut off time was selected at 60 seconds in order to prevent tissue injury at paw. The latency time of all the groups was noted at 30 min, 60 min, and 90 min. The percent pain protection (analgesia) was calculated with following formula[25]:

$$\%\ \text{Pain protection} = \frac{\text{Test latency-Control latency}}{\text{Cut off time-Control latency}} \times 100$$

2.5. Determination of anti-inflammatory activity by employing carrageenan induced paw edema

Mice of either sex were randomly divided into eight groups each containing 5 animals. Control group (Group I) was given distilled water (1 mL/100 g). Indomethacin (2 mg/kg) was used as standard (Group II). Groups III-V were administered L.MT and groups VI-VIII were administered latex at dose levels 100, 200 and 300 mg/kg. Carrageenan (1%, 0.05 mL) was injected subcutaneously in the plantar surface of right hind paw of individual mouse half an hour after administration of either vehicle, standard or tested sample solution. The inflammation was measured using vernier caliper immediately after carrageenan injection and then after 1, 2, 3 and 4 h. The average paw swelling in standard as well as tested samples was compared with that of carrageenan control and anti-inflammatory activity (percent inhibition of paw edema) was calculated using following formula[26]:

$$\%\ \text{Inhibition of paw edema} = \frac{\text{Edema of control-Edema of treated group}}{\text{Edema of control}} \times 100$$

2.6. Determination of anti-pyretic activity with Brewer's yeast induced pyrexia test

Albino mice of either sex (weight: 25-30 g) were randomly divided into eight groups (n=5). The normal body temperature of all the mice was recorded with digital thermometer and then 20% (2 g/kg) Brewer's yeast was injected subcutaneously (1 mL/100 g body weight of mouse) to all mice for inducing pyrexia. Animals were kept fasted overnight but water was available *ad libitum* and then rectal temperature was recorded. Pyrexia was confirmed with 0.5 ˚C increase in body temperature while animals showing fewer rises were excluded from the study. Group I was given distilled water orally, Group II received PCM (150 mg/kg orally), while remaining Groups III-V were administered L.MT and Groups VI-VIII were administered latex at 100, 200 and 300 mg/kg orally respectively. After completing the dosing, the rectal temperature was again noted at 1, 2, 3 and 4 h post dosing. The percent decrease in pyrexia was calculated with following formula[27]:

$$\%\ \text{Decrease in pyrexia} = \frac{\text{T. induced-Cn}}{\text{T. induced-T. normal}} \times 100$$

Where T. induced represented body temperature after induction of pyrexia, Cn was body temperature at 1, 2, 3 and 4 h intervals and T. normal indicated normal body temperature.

2.7. Statistical analysis

Results were presented as mean±SD. One way ANOVA followed by Tukey's *post hoc* test was applied using SPSS software version 12 to determine significance level. P<0.05 was considered statistically significant.

3. Results

3.1. Acetic acid induced writhing test

Number of writhings decreased significantly (P<0.05) in all the treated groups when compared with acetic acid control group (Table 1). % Pain protection was calculated from number of writhings. Brufen and tramadol, used as standards, showed 100% anti-nociceptive effect against chemically induced pain. Extract/latex exhibited dose dependent and time dependent effect. After 20 min, L.MT (300 mg/kg) showed 100% anti-nociceptive effect like standard drugs while latex exhibited 87.5% pain protection (Figure 1).

Table 1

Effect of latex and L.MT of *E. helioscopia* on acetic acid induced writhings in mice.

Groups	0-5 min	5-10 min	10-15 min	15-20 min
AA control	22.00±1.24	15.00±1.21	11.00±1.22	8.00±1.11
Brufen 100 mg/kg	0*	0*	0*	0*
Tramadol 10 mg/kg	0*	0*	0*	0*
L.MT 100 mg/kg	18.00±1.02*	10.00±1.05*	6.00±1.15*	3.00±1.05*
L.MT 200 mg/kg	15.00±0.98*	8.00 ±1.02*	5.00±1.11*	2.00±1.08*
L.MT 300 mg/kg	0*	0*	0*	0*
Latex 100 mg/kg	17.00±1.11*	8.00±0.78*	5.00±0.95	4.00±1.07*
Latex 200 mg/kg	15.00±1.08*	9.00±0.88*	6.00±0.96	3.00±1.02*
Latex 300 mg/kg	11.00±1.25*	7.00±0.99*	4.00±0.97*	1.00±0.98*

AA control=acetic acid control; each value is mean±SD (n=5); *P<0.05 with respect to control group.

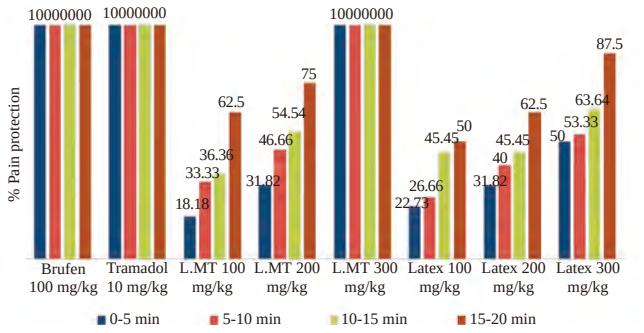

Figure 1. % Pain protection calculated from number of writhings produced in acetic acid induced writhing test. Each value is mean of five animals.

3.2. Formalin test for anti-nociceptive action

Paw lifting and paw licking were two parameters that were scored after injecting formalin (2.5%) into the plantar area of hind paw. Tramadol and PCM were used as standard drugs. In early phase and late phase, paw lifting and licking scores of control, standards (tramadol and PCM) and extract/latex treated groups were presented in Figure 2. L.MT (300 mg/kg) and latex (300 mg/kg) showed less score of paw lifting and licking in early phase as compared to standards. While in late phase, PCM exhibited 100% analgesia as no paw lifting and licking were observed. Hence, it was found that anti-nociceptive effect of L.MT (100, 200, and 300 mg/kg) was parallel to PCM (Figure 2).

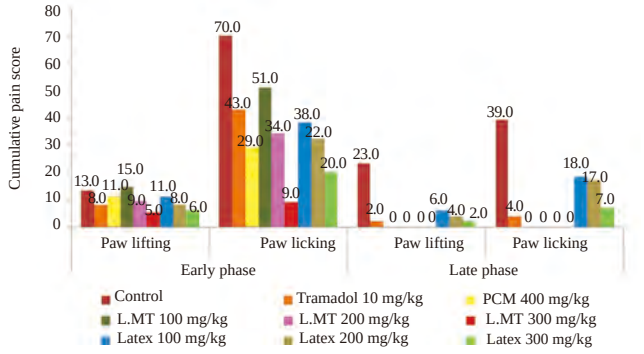

Figure 2. Effect of L.MT, latex, tramadol and PCM on formalin induced paw lifting and licking in mice. Each value is mean of five animals.

3.3. Hot plate test

Latency time (to feel pain) of control group was 12, 11, and 13 seconds at 30, 60 and 90 min interval of experiment. Brufen, tramadol, PCM and aspirin were used as standard drugs and showed maximum, 44%, 28%, 55% and 21% pain protection respectively after 90 min. Among all the treated groups, L.MT (300 mg/kg) exhibited maximum pain protection *i.e.* 42 % (Figure 3).

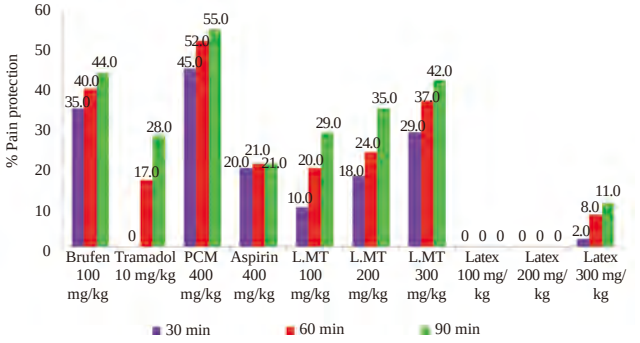

Figure 3. Effect of L.MT and latex, brufen, tramadol, aspirin and PCM on latency of pain onset in hot plate test. Each value is mean of five animals.

Hot plate test unveiled anti-nociceptive activity of those compounds that acted via central mechanism to inhibit the pain. The doses used for peripherally acting anti-nociceptive drugs did not show good activity in this assay. Central inhibition of pain needed high doses.

3.4. Carrageenan induced paw edema

Carrageenan induced paw edema was treated with standard anti-inflammatory drug (indomethacin, 2 mg/kg) and tested samples were used at three concentrations. Decrease in paw size (mm) with each therapy was described in Table 2. % Inhibition of edema was calculated from values given in Table 2 and graphically represented in Figure 4. Among all the treated groups, maximum anti-inflammatory activity (81.25%) was recorded with L.MT, 300 mg/kg.

Table 2

Anti-inflammatory evaluation of latex and L.MT of *E. helioscopia* on carrageenan induced paw edema.

Groups	Paw size (mm)					
	Before carrageenan injection	After carrageenan injection				
		0 h	1 h	2 h	3 h	4 h
Control	1.25±0.24	1.25±0.20	1.25±0.22	1.25±0.21	1.25±0.20	1.25±0.25
Carrageenan control	1.26±0.35	3.20±0.28	3.20±0.21	3.20±0.19	3.20±0.24	3.20±0.20
Indomethacin (2 mg/kg)	1.26±0.29	3.60±0.36	3.30±0.34*	2.60±0.31*	2.60±0.17*	2.60±0.20*
L.MT (100 mg/kg)	1.26±0.31	4.10±0.21	3.40±0.15*	3.30±0.24*	3.20±0.22*	2.80±0.14*
L.MT (200 mg/kg)	1.27±0.32	4.30±0.19	3.60±0.26*	3.50±0.19*	3.40±0.35*	3.10±0.17*
L.MT (300 mg/kg)	1.26±0.25	3.20±0.11	3.20±0.17*	3.10±0.19*	3.00±0.20*	2.90±0.17*
Latex (100 mg/kg)	1.24±0.45	4.10±0.15	3.20±0.18*	2.70±0.17*	2.70±0.19*	2.70±0.21*
Latex (200 mg/kg)	1.25±0.42	4.10±0.34	3.00±0.19*	2.80±0.23*	2.80±0.22*	2.80±0.25*
Latex (300 mg/kg)	1.25±0.34	4.00±0.22	3.90±0.17*	3.10±0.21*	3.10±0.20*	3.10±0.17*

Each value is mean±SD, n=5, *P<0.05 when compared with carrageenan control at 0 h.

Table 3

Anti-pyretic effect of L.MT and latex of *E. helioscopia* in mice.

Groups	Normal rectal temperature before drug administration	Rectal temperature (°C) after drug administration (orally)				
		Induced temperature 24 h after injection	1 h	2 h	3 h	4 h
Control	36.59±0.15	38.95±0.25	38.82±0.15	38.71±0.14	38.62±0.16	38.55±0.24
PCM (150 mg/kg)	37.10±0.11	39.49±0.13	39.18±0.06*	38.89±0.11*	38.60±0.20*	38.41±0.05*
L.MT (10 mg/kg)	37.08±0.10	39.62±0.11	39.42±0.09*	39.10±0.15*	38.91±0.14*	38.71±0.05*
L.MT (200 mg/kg)	37.00±0.21	39.22±0.09	39.00±0.14*	38.91±0.18*	38.72±0.16*	38.40±0.03*
L.MT (300 mg/kg)	37.06±0.23	39.00±0.21	38.71±0.12*	38.56±0.14*	38.32±0.23*	38.12±0.14*
Latex (100 mg/kg)	37.04±0.20	39.25±0.07	39.11±0.08*	38.99±0.19*	38.69±0.14*	38.49±0.21*
Latex (200 mg/kg)	37.06±0.09	39.74±0.09	39.52±0.07*	39.22±0.18*	38.95±0.10*	38.73±0.11*
Latex (300 mg/kg)	37.09±0.14	39.52±0.18	39.23±0.21*	38.91±0.21*	38.70±0.14*	38.55±0.12*

Each value is mean±SD, n=5, *P<0.05 when compared with induced temperature values at 24 h after yeast injection.

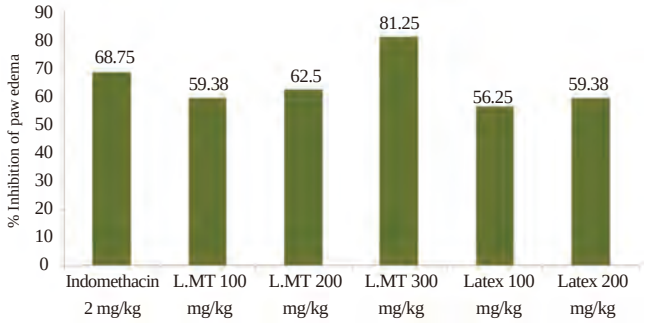

Figure 4. Anti-inflammatory effect of latex and L.MT of *E. helioscopia* in mice. Each value is mean of five animals.

3.5. Anti-pyretic test

PCM, 150 mg/kg, was used as standard anti-pyretic drug. There was significant decrease (P<0.05) in temperature of treated groups when compared with induced temperature value (Table 3). Anti-pyretic effect increased with time. PCM showed 45.19% decrease in pyrexia after 4 h. Similar pattern of activity was found in latex and L.MT treated groups. L.MT (300 mg/kg) displayed maximum anti-pyretic effect (45.36%) among all the treated groups (Figure 5).

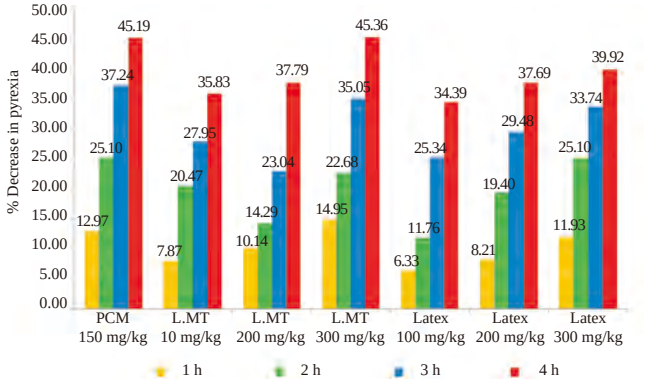

Figure 5. Percent decrease in pyrexia with latex and L.MT of *E. helioscopia* in mice. Each value is mean of five animals.

4. Discussion

L.MT and latex of *E. helioscopia* exhibited dose dependent anti-nociceptive effect in acetic acid induced writhing test, formalin test and hot plate test.

Acetic acid induced writhing test is well established and recommended to investigate anti-nociceptive property of medicinally rich herbs. In this test, pain was induced with chemical irritation of visceral organs with intraperitoneal injection of acetic acid that damages the phospholipids and causes the release of arachidonic acid, cyclooxygenase and prostaglandins which are mediators of pain and inflammation[23]. This method explores the peripheral anti-nociceptive activity of the compounds due to lowered nerves sensitivity and response of drugs at a dose which does not show anti-nociceptive activity in other anti-nociceptive activity testing methods[28]. Complete inhibition of abdominal constrictions was observed with L.MT (300 mg/kg) as compared to latex (maximum, 87.5% pain protection). These findings strongly suggest that latex and L.MT have peripheral anti-nociceptive activity via inhibition of peritoneal receptors that lead to blockage of cyclooxygenase actions.

Hot plate test is a thermal stimulus to induce pain. This method was adapted to measure central anti-nociceptive activity of the drugs. Tramadol, brufen, aspirin and PCM were used as standard anti-nociceptive drugs. Tramadol is an opioid agonist and acts through opioid receptors. Brufen and aspirin are NSAIDs that irreversibly inhibit cyclooxygenase enzyme; hence, brufen also has vasodilator action, while PCM is selective COX 2 inhibitor[29]. The results of this study showed maximum % pain protection (55%) with PCM at 90 min. Standard drugs showed anti-nociceptive activity in the following increasing order: aspirin<tramadol<brufen<PCM. PCM acts both centrally and peripherally. L.MT (300 mg/kg) exhibited maximum % pain protection *i.e.* 42% among all treated drugs. This effect was near to that of brufen. The recommended mechanism of compounds in this study seems to be the inhibition of cyclooxygenase enzymes that in turn inhibit the generation of pro-inflammatory mediators.

Formalin induced paw edema is biphasic (early and late phase) well known chemical test to evaluate anti-nociceptive activity of natural compounds as well as synthetic drugs. Early phase is under central control while in late phase, peripherally acting drugs control the pain. Bradykinin, serotonin and histamine are mediators to induce paw edema in early phase while late phase was due to prostaglandin overproductions[30]. In early phase, latex

and L.MT showed better inhibition on pro-inflammatory mediators as compared to standard drug. In late phase, L.MT and PCM showed zero pain score, indicating 100% pain protection against formalin induced algesia. Formalin test is indicative of central and peripheral effects of drugs/chemicals. The results of formalin test are supporting the results of hot plate test and acetic acid induced writhing test. Thus, it can be hypothesized that anti-nociceptive activity was due to inhibition of prostaglandins synthesis which results into blockage of sensation at the site of pain and inflammation.

The anti-nociceptive activity of *E. helioscopia* found in the present study is in agreement with the findings of Alibabaei *et al.* and Shirani *et al*[16,20].

Prostaglandins are mediators of inflammation. L.MT and latex of *E. helioscopia* possessed anti-inflammatory activity in carrageenan induced paw edema assay; the underlying mechanism of anti-inflammatory action can be attributed to inhibition of cyclooxygenase and prostaglandins synthesis.

Pyrexia occurs with increased production of prostaglandins and Brewer's yeast induced pyrexia by this mechanism. It is one of the best methods employed to test antipyretic activity of plant material as well as synthetic compounds[26]. The etiology of yeast induced pyrexia could be the prostaglandins production and this fever is called pathogenic fever[31]. There is a chain of reactions that mediate antipyretic effect of drugs, for example, prostaglandins synthesis inhibition is associated with antipyretic effect of NSAIDs and this inhibition requires blocking of cyclooxygenase activity. Additionally, there are several other pyrexia inducing mediators and their inhibition is required to get antipyretic action[32]. The oral administration of latex and L.MT of *E. helioscopia* significantly lowered the rectal temperature in yeast induced febrile mice. Thus, it supports the hypothesis that some pharmacologically active compounds in *E. helioscopia* block prostaglandin synthesis.

In the earlier study, quercetin, kaempferol and myricetin had been quantified in various extracts of *E. helioscopia* leaves[33]. Hence, it is proposed that antipyretic, anti-nociceptive and anti-inflammatory activities of *E. helioscopia* might be due to these flavonoids. Alibabaei *et al.*, concluded that flavonoids of *E. helioscopia* caused prostaglandins synthesis inhibition to produce anti-nociceptive effect; this is in consistent with our findings[20].

L.MT of *E. helioscopia* had pronounced anti-nociceptive, anti-inflammatory and anti-pyretic effects. The latex of *E. helioscopia* also showed these effects but these were less marked as compared to L.MT. Inhibition of prostaglandins synthesis and other mediators of cell or tissue damage seems to be the mechanism responsible for these activities of under studied plant.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

L.MT of *E. helioscopia* had been studied before for anti tumor activity, antioxidant effects, antibacterial and poisonous effects. This study analyzed the anti-nociceptive, anti-inflammatory and anti-pyretic effects of the latex of *E. helioscopia*.

Research frontiers

L.MT of *E. helioscopia* had pronounced anti-nociceptive, anti-inflammatory and anti-pyretic effects. The latex of *E. helioscopia* also showed these effects but these were less marked as compared to L.MT. Inhibition of prostaglandins synthesis and other mediators of cell or tissue damage seems to be the mechanism responsible for these activities of under studied plant.

Innovations & breakthroughs

This paper combines the different methods of nociception measuring techniques to quantitate the analgesic and antipyretic effects of the *E. helioscopia*. Scientific reasoning as well as the execution of the experiments are presented in a structured, easy-to-understand way.

Applications

Further purification of the active ingredients and characterizing the chemical reactive moieties could lead to specific compound selection for pharmaceutical development.

Peer review

In this study, the authors quantitated the analgesic and antipyretic effects of the *E. helioscopia* by combining different methods of nociception measuring techniques. This study's merits qualify it to be published in APJTD.

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