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Antinociceptive effect of aqueous extract of *Emblica officinalis* in mice

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Abstract
Aim: To evaluate the anti-nociceptive activity of *Emblica officinalis* using acetic acid induced writhing test and eddy's hot plate method in mice and to investigate the role of opioid receptors in the central anti-nociceptive activity of *Emblica officinalis*.
Materials and Methods: Peripheral analgesic activity of *Emblica officinalis* was evaluated using acetic acid induced writhing test. Indomethacin (10 mg/kg, p.o) was used as the standard drug and *Emblica officinalis* was used at a dose of 300 mg/kg, p.o. Central anti-nociceptive activity of *Emblica officinalis* was evaluated using eddy's hot plate method. Morphine (5 mg/kg,i.p) was used as the standard drug and *Emblica officinalis* was used at a dose of 300 mg/kg, p.o. To assess the role of opioid system, the other groups of animals were pretreated with μ opioid receptor antagonist (CTAP-1 mg/kg, i.p), δ opioid receptor antagonist (Naltrindole-1 mg/kg, i.p) and κ opioid receptor antagonist(Nor-Binaltorphimine-1 mg/kg,i.p) and then treated with vehicle/*Emblica officinalis*. Results were analysed using one way ANOVA followed by Tukey's multiple comparison test. p value of <0.05 was considered significant.
Results: *Emblica officinalis* decreased the number of writhings significantly ($p<0.001$) in acetic acid test when compared to control group. Administration of *Emblica officinalis* increased the latency time significantly ($p<0.05$) when compared to control group in hot plate model. Further, administration of CTAP prior to *Emblica officinalis* reversed the anti-nociceptive effect significantly ($p<0.001$) when compared to *Emblica officinalis* group.
Conclusion: *Emblica officinalis* possesses analgesic effect in both central and peripheral models of nociception. The central anti-nociceptive activity probably mediated via μ opioid receptor.

Keywords: Analgesia, nociception, opioid, receptor

1. Introduction
Pain is an unpleasant sensory perception which may be associated with actual or potential tissue damage and is always subjective [1]. There are two components involved in sensation and perception of pain. One is the perception of actual or threatened tissue damage and other is an affective state of unpleasantness – giving pain its unique aversive quality [2]. Pain can be classified as acute or chronic. Conventional drugs such as opioids and NSAIDs are used to reduce the pain and inflammation are associated with risks and potential side effects. Therefore, the development of analgesics and anti-inflammatory drugs with fewer side effects are necessary. To evaluate the possible mechanisms involved in the ant nociceptive action of the aqueous extract of *Emblica officinalis* (EO) the hot plate and acetic acid-induced writhing assay procedures were employed.

2. Materials and Methods
2.1 Animals
Inbred adult Swiss albino mice (25-35 g) of either sex were sourced from the institutional animal house of Pondicherry Institute of Medical Sciences. Animals were housed in clean polypropylene cages and maintained at 24±2 ˚C temperature in a 12 hr/12 hr, day/night cycle with free access to food and water. The animals were acclimatized to laboratory conditions every time before testing. Experiments were conducted between 9.00 and 14.00 hours to avoid circadian variation and to maintain uniformity. All the procedures were reviewed and approved by the Institutional Ethical Committee (Reg. no. 1081/a/07/CPCSEA, Proposal no. 6). The care of animals was taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Each group consisted of six animals.

2.2 Drugs and chemicals

The investigational drug used in the study was aqueous extract of EO fruit and was procured from M/s. Natural Remedies Pvt. Ltd., Bangalore, India. The Quality Control Laboratory, M/s. Natural Remedies, Bangalore, India (lab reference no.FP1012062), did the estimation and purity of active principle of the powder. Phytochemical and HPLC analysis of the dried powder of EO showed the presence of tannic acid (>30%) and Gallic acid (>10%) as the main constituents. The other constituents comprised of alkaloids, flavonoids, carbohydrates, polyphenols and amino acids. The powder was reconstituted in distilled water and then fed to the animals according to the appropriate doses required.

Morphine (Verve Health Care Ltd), CTAP, Naltrindole, Nor-Binaltorphimine, Celecoxib, Chlorpheniramine (Sigma chemical company, St. Louis, MO, USA), Indomethacin (Jagsonpal Pharmaceuticals Pvt. Ltd), Misoprostol (Cipla Pharmaceuticals Pvt. Ltd) Carrageenan (Sigma chemical company, St. Louis, MO, USA), Acetic acid (Forbes Pharmaceutical Pvt. Ltd) and Distilled water as a vehicle.

2.3 Evaluation of analgesic activity

a) Eddy's hot plate test in mice

To detect the central analgesic activity of EO extract, Eddy's hot plate test was carried out using Eddy's hot plate apparatus. The test was carried out according to the method described by Woolfe *et al.* [3] the temperature was maintained at 55±1°. Mice were placed on hot plate and the reaction time was recorded when the animals start licking the paws and jumping. A cut off time of 15 s is maintained to prevent any injury to the animal. The basal reaction time was noted before and 30, 60, 90 and 120 minutes after the administration of the drugs. Mice were divided into nine groups of six in each. The control group received vehicle (distilled water-5 ml/kg p.o.) [4], Morphine (5 mg/kg i.p) [5] was used as the standard drug. EO extract was administered at a dose of 300 mg/kg p.o.[6]. A significant increase in mean reaction time (s) between these readings is an indication of possible analgesic response.

Further experiments were carried out to elucidate the possible mechanism of action by which the other three groups were pretreated with μ (CTAP-1mg/kg i.p.) [7], δ (Naltrindole-1 mg/kg i.p.), and κ (Nor-Binaltorphimine-1 mg/kg i.p.), receptor antagonist 15 min prior to the administration of vehicle and the same was repeated with test drug in the remaining three groups [8].

b) Acetic acid induced writhing test in mice

Acetic acid induced writhing test in mice was carried out to detect the peripheral analgesic activity of EO extract. The test was carried out according to the procedure described by Koti *et al.* [9] the mice were divided into three groups of six in each. Control group received distilled water (5 ml/kg p.o.).[4] Indomethacin (10 mg/kg p.o.) [10] was used as standard drug. EO extract was administered at a dose of 300 mg/kg p.o. Each mouse was injected 0.1 ml/10 g i.p. of 1% acetic acid, 30 min after administering the drugs. Five minutes after acetic acid induction, the number of writhings (abdominal constriction and stretching of the hind limbs) were counted for a period of 15 min. Significant reduction in the number of abdominal constriction in any treatment group compared with the vehicle treated group was considered as analgesic response. The percentage of inhibition was calculated, using the formula: (C-T/C) x100, where C is the number of writhings in control group and T is the number of writhings in the treatment groups [11].

2.4 Statistical Analyses

The data was presented as mean±SEM and analysed by one way ANOVA followed by Tukey's multiple comparison test for the possible significance identification between the various groups and $p<0.05$ was considered statistically significant. GraphPad In Stat software of version 3.06 was used for analysis of data.

3. Results

3.1 Peripheral analgesic activity of aqueous extract of EO assessed by writhing test in mice

Table 1 and Figure 1 shows the peripheral analgesic effect of EO extract and standard drug (Indomethacin) in acetic acid induced writhing test. EO extract showed significant analgesic activity at a dose of 300 mg/kg. The number of acetic acid induced writhes decreased significantly ($p<0.001$), when compared to control group with inhibitory rate of 52.4%. The standard drug, indomethacin (10 mg/kg), also showed significant analgesic activity ($p<0.001$), when compared to control group with the inhibitory rate of 58.4%. So the peripheral analgesic activity of EO extract is comparable to that of the standard drug indomethacin.

Table 1: Peripheral analgesic effect of EO extract in acetic acid induced writhing test in mice

S. No	Groups	Dose	No. of writhings	% Inhibition
1.	Control (DW)	5 ml/kg p.o	47.33 ± 2.51	-
2.	Indomethacin	10 mg/kg p.o	20.33 ±1.67***	58.4
3.	EO	300 mg/kg p.o	24.00 ± 1.15***	52.4

Values are presented as mean ± SEM (n = 6); *** $p< 0.001$ when compared with the control group. DW- Distilled water, EO - *Emblica officinalis*

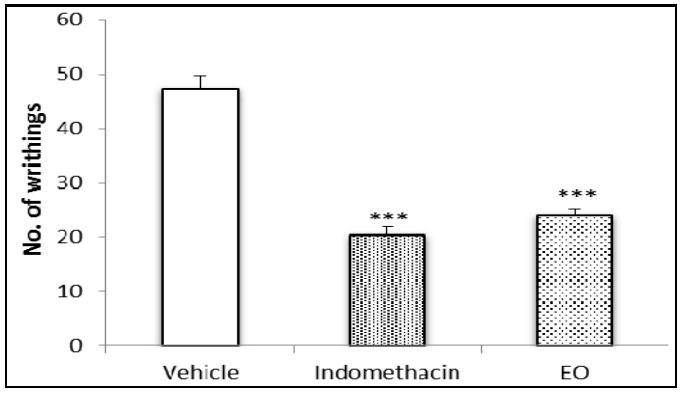


Fig 1: Peripheral analgesic effect of EO extract in acetic acid induced writhing test in mice

3.2 Central analgesic activity of aqueous extract of EO assessed by hot plate test in mice

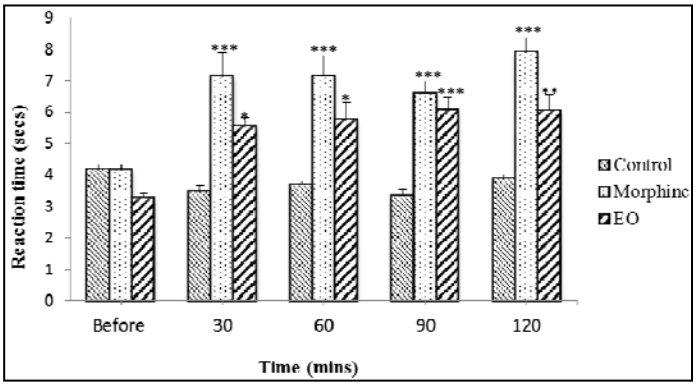
In this method, mean reaction time to hot plate at 30, 60, 90 and 120 min period, the standard drug (morphine 5 mg/kg) showed significant increase in the reaction time ($p<0.001$) when compared to control group. EO extract (300 mg/kg), the test drug, also showed significant increase in the reaction time at 30, 60 min ($p<0.05$), 90 min ($p<0.01$) and at 120 min ($p<0.001$) when compared to control group (Table 2 and Figure 2). There was no significant difference between

morphine and EO extract group at 30, 60 and 90 min. But at 120 min, significant difference in reaction time was observed ($p<0.05$) between morphine and EO extract group.

Table 2: Central analgesic effect of EO extract in hot plate test in mice

Reaction time in seconds							
S. No	**Groups**	**Dose**	**Before**	**30 min**	**60 min**	**90 min**	**120 min**
1.	Control (DW)	5 ml/kg p.o	4.18±0.15	3.46±0.2	3.69±0.11	3.34±0.21	3.86±0.15
2.	Morphine	5 mg/kg i.p	4.16±0.17	7.12±0.76***	7.14±0.66***	6.59±0.39***	7.89±0.47***
3.	EO	300 mg/kg p.o	3.28±0.14	5.54±0.29*	5.75±0.55*	6.05±0.42***	6.04±0.52**

Values are presented as mean ± SEM (n = 6); *p< 0.05, ** p<0.01 and ***p< 0.001 when compared with the control group. DW- Distilled water, EO – *Emblica officinalis*



* denotes p<0.05, **p<0.01, ***p< 0.001 when compared with the control group. EO – *Emblica officinalis*

Fig 2: Central analgesic effect of EO extract in hot plate test in mice

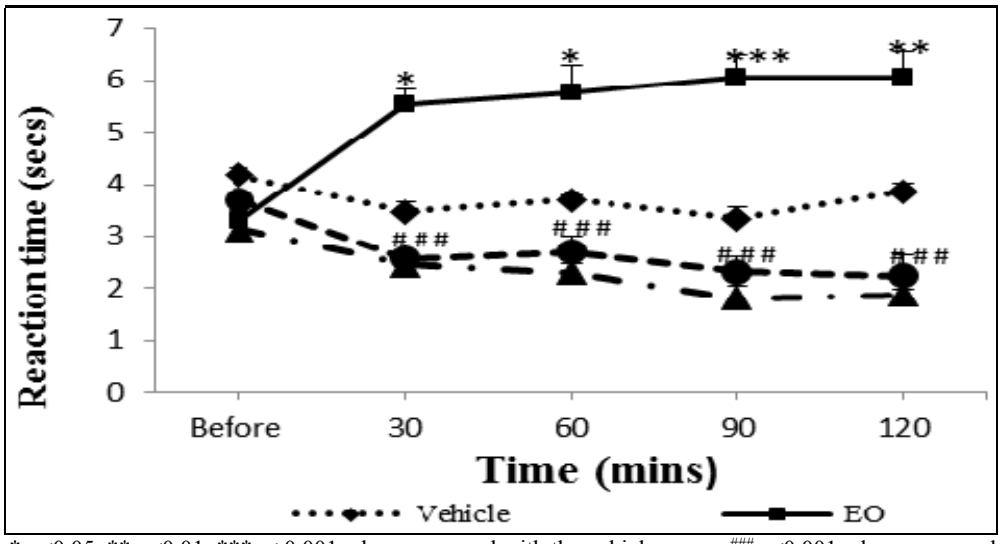
3.3 Mechanism of central analgesic activity of aqueous extract of EO assessed by hot plate test in mice

Table 3 and Figure 3 shows the pretreatment of μ receptor antagonist on the analgesic effect of EO extract. Mu receptor antagonist (CTAP) significantly reversed the analgesic effect of EO extract ($p<0.001$) at 30, 60, 90 and 120 min. table 4 and Figure 4 shows the pretreatment of δ receptor antagonist on the analgesic effect of EO extract. Delta receptor antagonist (Naltrindole) significantly reversed the analgesic effect of EO extract only at 120 min ($p<0.05$) whereas at 30,60 and 90 min there was no reversal of analgesic effect of EO. Table 5 and figure 5 shows the pretreatment of κ receptor antagonist (Nor-BNI) on the analgesic effect of EO extract. Kappa receptor antagonist did not reverse the analgesic effect of EO extract at all the time periods.

Table 3: Effect of pretreatment with μ receptor antagonist (CTAP) in the analgesic effect of EO extract in the hot plate test in mice.

S. No	**Groups**	**Dose**	**Reaction time in seconds**				
			Before	**30 min**	**60 min**	**90 min**	**120min**
1	Vehicle (DW)	5ml/kg p.o	4.18±0.15	3.46±0.2	3.69±0.11	3.34±0.21	3.86±0.15
2	EO	300 mg/kgp.o	3.28±0.14	5.54±0.29*	5.75±0.55*	6.05±0.42***	6.04±0.52**
3	CTAP + Vehicle (DW)	1mg/kg ip+ 5ml/kg p.o	3.14±0.34	2.44±0.14	2.30±0.18	1.80±0.24	1.87±0.12
4	CTAP + EO	1ml/kg i.p + 300 mg/kgp.o	3.70±0.10	2.56±0.26###	2.70±0.31###	2.32±0.30###	2.24±0.40###

Values are presented as mean±SEM (n=6); *p<0.05, **p<0.01 and ***p<0.001 when compared with the vehicle group. ###p<0.001 when compared with EO group. DW-distilled water, EO- *Emblica officinalis*



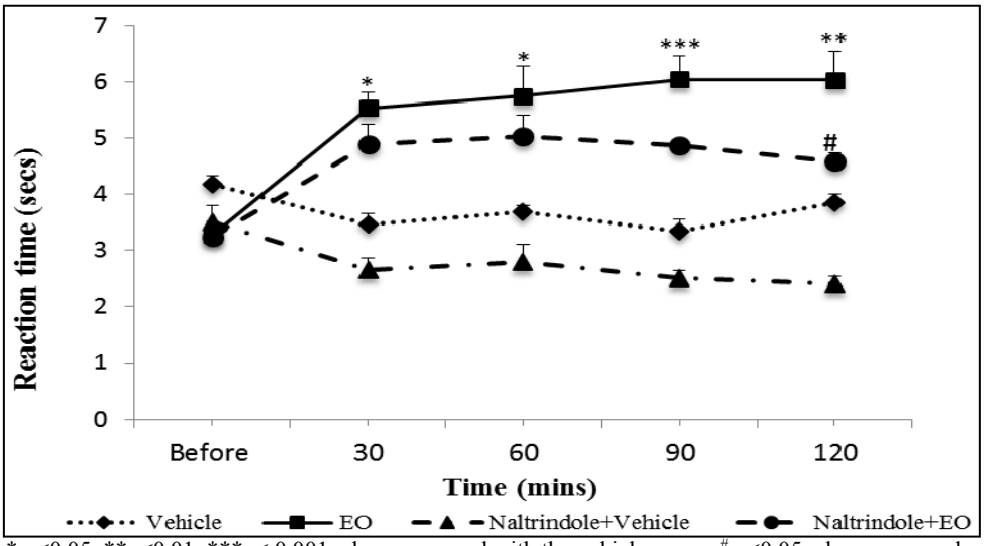
* p<0.05, ** p<0.01, ***p< 0.001 when compared with the vehicle group. ### p<0.001 when compared with the EO group. EO – *Emblica officinalis*

Fig 3: Effect of pretreatment with μ receptor antagonist (CTAP) in the analgesic effect of EO extract in the hot plate test in mice

Table 4: Effect of pretreatment with δ receptor antagonist (Naltrindole) in the analgesic effect of EO extract in the hot plate test in mice

S. No	Groups	Dose	Reaction time in seconds				
			Before	30 min	60 min	90 min	120min
1	Vehicle (DW)	5ml/kg p.o	4.18±0.15	3.46±0.2	3.69±0.11	3.34±0.21	3.86±0.15
2	EO	300 mg/kgp.o	3.28±0.14	5.54±0.29*	5.75±0.55*	6.05±0.42***	6.04±0.52**
3	Naltrindole + Vehicle (DW)	1mg/kg ip+ 5ml/kg p.o	3.50±0.30	2.65±0.21	2.80±0.30	2.50±0.15	2.41±0.13
4	Naltrindole + EO	1mg/kg i.p + 300 mg/kgp.o	3.25±0.27	4.90±0.36	5.04±0.37	4.87±0.10	4.58±0.16*

Values are presented as mean±SEM (n=6); *p<0.05, **p<0.01 and ***p<0.001 when compared with the vehicle group. ###p<0.001 when compared with EO group. DW-distilled water, EO- *Emblica officinalis*



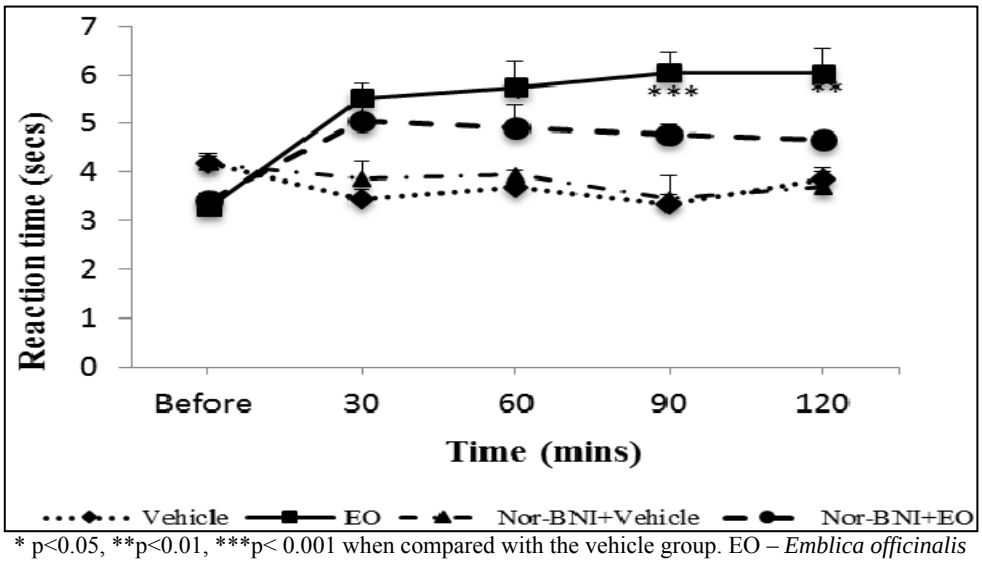
* p<0.05, **p<0.01, ***p< 0.001 when compared with the vehicle group. # p<0.05 when compared with the EO group. EO – *Emblica officinalis*

Fig 4: Effect of pretreatment with δ receptor antagonist (Naltrindole) in the analgesic effect of EO extract in the hot plate test in mice

Table 5: Effect of pretreatment with κ receptor antagonist (Nor-Binaltorphimine) in the analgesic effect of EO extract in the hot plate test in mice.

S. No	Groups	Dose	Reaction time in seconds				
			Before	30 min	60 min	90 min	120min
1	Vehicle (DW)	5ml/kg p.o	4.18±0.15	3.46±0.2	3.69±0.11	3.34±0.21	3.86±0.15
2	EO	300 mg/kgp.o	3.28±0.14	5.54±0.29*	5.75±0.55*	6.05±0.42***	6.04±0.52**
3	Nor-BNI + Vehicle (DW)	1mg/kg ip+ 5ml/kg p.o	4.18±0.22	3.86±0.37	3.94±0.09	3.46±0.46	3.70±0.38
4	Nor-BNI + EO	1mg/kg i.p + 300 mg/kgp.o	3.43±0.15	5.05±0.47	4.91±0.47	4.77±0.22	4.68±0.15

Values are presented as mean±SEM (n=6); *p<0.05, **p<0.01 and ***p<0.001 when compared with the vehicle group. ###p<0.001 when compared with EO group. DW-distilled water, EO- *Emblica officinalis*



* p<0.05, **p<0.01, ***p< 0.001 when compared with the vehicle group. EO – *Emblica officinalis*

Fig 5: Effect of pretreatment with κ receptor antagonist (Nor-Binaltorphimine) in the analgesic effect of EO extract in the hot plate test in mice

4. Discussion

Medicinal plants have been a major source of therapeutic agents since ancient times to cure human disease. Despite the major advances in the modern medicine, the development of new drugs from natural products is still considered important.[12] But, because of limited documented experimental evidences regarding their pharmacological effects, the use of traditional medicines remain restricted to a locality/region where they are being practiced traditionally, and not accepted globally. Current phytopharmacological research is focused on validating the therapeutic effects of traditional medicine in experimental studies. [13]

The fruits of EO are widely used in the Ayurveda and are believed to increase defense against diseases. It has its beneficial role in cancer, diabetes, liver disease, ulcer, anemia and various other diseases. Similarly, it has application as antioxidant, immunomodulatory, antipyretic, analgesic, cytoprotective, antitussive and gastroprotective effects [14].

Eventhough EO extract is found to have analgesic and anti-inflammatory activity in some studies, the mechanism of action was not done earlier.

In our study, central and peripheral analgesic effects of aqueous extract of *Emblica officinalis* was evaluated using hot plate test and acetic acid induced writhing test in mice. Possible mechanism of central analgesic effect of EO was also detected using hot plate test.

The hot-plate test which is a thermal model of nociception is a specific test carried out to verify involvement of central mechanism with compounds/drugs showing analgesic activity. In our study, aqueous extract of EO (300 mg/kg) showed significant increase in reaction time in the hot plate test at 30, 60, 90 and 120 min when compared to control group. So this shows that this extract possess central analgesic activity. Another study conducted by Ibrahem MS *et al.,* showed that methanolic extract of EO at a dose of 250 mg/kg possess central analgesic activity in hot plate test.[15] The exact mechanisms by which aqueous extract of EO produces central analgesic effect was not done previously. Since opioidergic system is commonly involved in central analgesic effect, our study was done by specifically blocking the opioid receptors. According to our results, the analgesic effect of the EO extract (300 mg/kg) was significantly reversed by pretreatment of animals with CTAP (μ opioid receptor antagonist) at 30, 60, 90 and 120 min and with naltrindole (δ opioid receptor antagonist) at 120 min. The reversal of analgesic effect of EO extract did not take place when pretreated with nor-Binaltorphimine (κ opioid receptor antagonist) at all time periods and with naltrindole at 30, 60 and 90 min. This suggested that the aqueous extract (300 mg/kg) might have produced analgesic effect by interaction with μ opioid receptors and partially with δ opioid receptors. However the results have to be confirmed using other central analgesic models. Further studies can also be done to detect whether other neurotransmitters like adenosine, serotonin, noradrenaline and acetylcholine are involved in the central analgesic effect of *Emblica officinalis*.

Writhing test is a chemical method used to induce pain of peripheral origin by injection of irritants like phenylquinone, acetic acid in mice. Analgesic activity of the test compound is inferred from decrease in the frequency of writhings. The manifestations of abdominal writhings in mice were first described by Sigmund *et al* as an arching of back, extension of hind limbs and contraction of abdominal musculature [16]. It has been postulated that acetic acid, which was used to induce writhing, acts indirectly by releasing endogenous mediators that stimulate pain nerve endings. Increased levels of prostaglandin E_2 and prostaglandin $F_2\alpha$ as well as lipoxygenase, liberation of sympathetic nervous system mediators in the peritoneal fluid and the release of cytokines, such as TNF-α, IL-1β and IL-δ, by resident peritoneal macrophages and mast cells have been reported to be responsible for pain sensation caused by i.p. administration of acetic acid [17].

Our results showed that EO at a dose of 300 mg/kg has peripheral analgesic properties similar to indomethacin (NSAIDs). Another study done by Goel *et al.*, also showed that the crude extract of EO had significant analgesic activity in acetic acid induced writhing test in mice at a dose of 600 mg/kg. [18] Acetic acid induced writhing is a highly sensitive and useful test for analgesic drug development, but not a selective pain test as it gives false positives with sedatives, muscle relaxants and other pharmacological activities as stated by Elisabetsky *et al.* [19] However further elaborate and explorative studies need to be conducted in order to detect its peripheral mechanism of analgesic action.

5. Conclusion

To conclude, the aqueous fruit extract of EO demonstrated significant central and peripheral analgesic activities in experimental animals in this study. Central analgesic activity could be mediated via μ opioid receptor and anti-inflammatory activity could be mediated by inhibiting the synthesis, release or action of prostaglandins. However future elaborate studies need to be conducted to detect the mechanism of peripheral analgesic effect in animal models. The analgesic activities may be attributed by the various phytoconstituents of EO extract like flavonoids, tannis, ellagic acid and other alkaloids. The unacceptable side effects associated with analgesics and anti-inflammatory agents are not seen with EO extract. Thus, the preliminary data of the present investigation provide some evidence for the effectiveness of *Emblica officinalis* in the treatment of pain, arthritis, rheumatism as claimed in the Ayurvedic system of medicine.

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