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RESEARCH ARTICLE

Anti-inflammatory effect of aqueous extract of *Emblica officinalis* in animal models

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Abstract

Background: The importance of inflammatory diseases and side effects of conventional drugs necessitate the discovery of new anti-inflammatory agents from natural sources. In this study,the mechanism of action of anti-inflammatory action of the aqueous extract of *Emblica officinalis* was evaluated in animal models.

Methods: Forty-eight male Wistar albino rats were divided into six groups and three groups were pretreated with indomethacin,celecoxib and chlorphineramine maleate. The two groups were pretreated with misoprostol and different doses of *Emblica offinalis* extract. The aqueous extract of *Emblica officinalis* was administeredbefore the injection of carrageenan. Paw edema was measured by a plethysmometer at 1st, 3rd and 5th hour and compared to standard drugs.

Results: The aqueous extract of *Emblica officinalis* decreased the rat paw edema in a dose-dependent manner. The effect on inflammation of the highest dose of extract was comparable to indomethacin. *Emblica officinalis* extract at dose of 600mg/kg showed decrease in paw edema when combined with misoprostol.

Conclusions: The aqueous extract of *Emblica officinalis* reduced the inflammation in a dose-dependent manner mediated by inhibiting the action of prostaglandins and is a good candidate for further studies of safety and efficacy. The clarification of active components of the plant is necessary.

Introduction

Inflammation is the response of the injured cells and body tissues through different factors such as microbes, chemicals, thermal and mechanical injuries. [1] Inflammation is a part of non-specific immune response that occurs in reaction to any type of bodily injury and the cardinal signs of inflammation as a result of vasodilation, increased permeability and release of inflammatory mediators leading to exudation of fluid and plasma proteins. Pain and the inflammation are the common ailments to which remedies are being sought on every day basis.[2]

Inflammation has two main components, cellular and exudative. The cellular components involve the movement of leucocytes. The exudative component involves the movement of important proteins such as fibrin and immunoglobulins. Although it is a defensive mechanism, the complex events and mediators involved in inflammation can induce and aggravate many diseases.[3] Inflammation occurs in three distinct phases namely acute, subacute and chronic (or proliferative). The acute response to tissue injury occurs in the microcirculation at the site of injury. Initially there is a transient constriction of arterioles, however within several minutes, chemical mediators released at the site cause relaxation of arteriolar smooth muscle and increase in vascular permeability. Protein rich

fluid seeps out of capillaries and accumulates in the interstitial space. This fluid contains many of the components of plasma including fibrinogen, kinins, complements and immunoglobulins that mediate inflammatory response. The subacute phase is characterized movement of phagocytic cell to the site of injury. In response to adhesion molecules released from activated endothelial cells, leukocytes, platelets and RBCs in injured vessels become sticky and adheres to the endothelial cell surfaces. As the inflammatory process continues, macrophages infiltrate the site of injury, actively removing the damaged cells of the tissue followed by a period of tissue repair. Blood clots are removed by fibrinolysis and their damaged tissue are regenerated or replaced with fibroblast, collagen or endothelial cells. However, inflammation can become chronic, leading to further tissue destruction and fibrosis.[4]

Anti-inflammatory drugs are used to reduce the inflammation. Steroidal anti-inflammatory drugs specifically glucocorticoids reduce the inflammation by binding to glucocorticoid receptor. These drugs are often referred to as corticosteroids, though that is a larger category. Non-steroidal anti-inflammatory drugs (NSAIDs) alleviate the pain by counteracting cyclooxygenase enzyme (COX).[5] However, long term administration of NSAIDs induce gastrointestinal irritation, kidney damage and respiratory depression.

Conventional analgesics namely opioids and NSAIDS which are used to reduce 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.

Purified natural compounds from plants can serve as template for the synthesis of new generation of analgesics and anti-inflammatory drugs with low toxicity and higher therapeutic value. Hence, the drug derived from natural products particularly from medicinal plants like *Emblica officinalis* (EO)are believed to be a vital source of chemical substance that have good potential therapeutic efficacy with fewer side effects.Various parts of this plant are used to treat several diseases, but the most important is the fruit. The fruit is used either alone or in combination with other plants to treat many ailments. *Emblica officinalis* is found to have hepatoprotective, cardioprotective, cytoprotective, antioxidant, anti-ulcer, antidiabetic, antimicrobial, analgesic, anti-inflammatory, antitussive properties etc. on animal models but the mechanism of action is not studied.[6]Hence the present study was carried out to evaluate the anti-inflammatory effects and the mechanism of action of aqueous extract of EO in experimental animals models.

Materials and methods

Animals

Inbred adult Wistar albino rats (140-200g) of either sex were sourced from the central 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 Animal 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).

Test drug

The test 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 HPLCanalysis of the dried powder of *Emblicaofficinalis*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.

Drugs and chemicals

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 was used as a vehicle.

Evaluation of anti-inflammatory activity

a) Carageeenan induced hind paw edema in rats

Carrageenan induced hind paw edema in rats was carried out to detect the anti-inflammatory activity of EO extract, using Mercury Plethysmometer. The test was carried out according to the procedure described by Winter *et al.*[7]Acute inflammation was produced by sub plantar injection of 0.1ml of freshly prepared 1% (w/v) suspension of carrageenan in normal saline in the right hind paw of all the rats. The left hind paw was injected with 0.1 mL of saline and used as a control. The drugs were administered orally one hour and intraperitoneally half an hour before

carrageenan injection.[8]The paw volumes were measured at 0, 1, 3 and 5 hrs after carrageenan injection using Mercury Plethysmometer by noting the displacement of mercury when the paw was dipped in mercury column up to a predetermined mark on the paw.[9] A mark was made on both the legs at lateral malleolus to facilitate uniform dipping and recording of paw volumes. The difference in paw volume indicates the degree of inflammation.The rats were divided into nine groups of six in each group. The control group received distilled water at a volume of 5 ml/kg p.o.Indomethacin (10 mg/kg, p.o.), Celecoxib (10 mg/kg, p.o.)and Chlorpheniramine (10 mg/kg i.p.) were used as the standard drugs. EO extract was administered at a dose of 150, 300 and 600 mg/kg p.o.This experiment was carried out with misoprostol to detect the mechanism of action where one group was administered with misoprostol (11.43µg/kg p.o.)and was compared with the other groupwhere misoprostol was administered one hr after the administration of EO extract . Average increase in paw edema at different time interval was determined and the percentage inhibition of edema was calculated according to the formula,$(Vc - Vt/V_C)$ x100, where,Vc is the mean edema volume in the control group and Vt is the mean edema volume in the treated group.

Statistical analysis

The data was presented as mean±SEM and analyzed 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.GraphPadInStat software of version 3.06was used for analysis of data.

Results

Anti-inflammatory activity of aqueous extract of *Emblicaofficinalis*assessed by carrageenan induced hind paw edema model in rats

The paw volume at different time intervals in different groups are shown in the Table 1 and Figure 1. In Indomethacin group there was a significant decrease in paw volumeat 1st hour ($p<0.01$) and a very significant decrease in paw volume at 3rd and 5th hour ($p<0.001$) when compared to control group. With celecoxib, there was a significant decrease in paw volume at all the time periods ($p<0.05$ and $p<0.001$)when compared to control group. With chlorpheniramine, there was no significant decrease in paw volume when compared to control group. EO extract at a dose of 150 mg/kg showed significant decrease in paw volume only at 5th hour ($p<0.05$). At a dose of 300 mg/kg, there was a significant decrease in paw volume at 3rd hour ($p<0.01$) and 5th hour ($p<0.001$). EO extract at a dose of 600 mg/kg showed significant decrease in paw volume at 1st ($p<0.05$), 2nd ($p<0.01$) and 5th ($p<0.001$) hour when compared to control group. However the reduction in paw volume with EO extract is less when compared to indomethacin and celecoxib group

The percentage of inhibition of paw edema with indomethacin at 1st, 3rd and 5th hour were 34.21%, 50.75% and 61.11%. With celecoxib, the percentage inhibition were28.94%,47.76% and 53.33% respectively. At 1st, 3rd and 5th hour, the percentage inhibition with EO extract 300 mg/kg were 7.9%, 28.35% and 40%. With EO extract 600 mg/kg the percentage inhibition were 28.94%, 35.82% and 45.56% respectively. So the maximum percentage of inhibition with EO extract is seen at 5th hour at a dose of 600 mg/kg (Figure 2).

Mechanism of action of anti-inflammatory activity of aqueous extract of *Emblicaofficinalis*assessed by carrageenan induced hind paw edema in rats

To detect the possible mechanism of action of anti-inflammatory effect of EO extract, one group of animals were treated with misoprostol and another group with EO extract and misoprostol. There was a significant decrease in paw volume in EO extract and misoprostol group at 1st ($p<0.05$), 3rd and 5th hour ($p<0.001$) when compared to misoprostol alone treated group (Table 2).

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.[10] 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.[11]

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

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, anti-inflammatory effects and its mechanism of action of *Emblicaofficinalis*was evaluated using carrageenan induced hind paw edema model in rats.

Morrisstated that Carrageenan-induced paw inflammation in rats is considered to be one of the best methods for screening anti-inflammatory drugs. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover, the experimental model exhibits a high degree of reproducibility.[12]The edema and inflammation induced by carrageenan are a biphasic event. In the initial 1 h after carrageenan administration, the edema and inflammation are mediated by histamine and serotonin. Later, the increased vascular permeability is maintained by the release of kinins up to about 2.30 h. Thereafter from 2.30 h to 5-6 hours, inflammation is mediated by prostaglandins and is also associated with migration of leucocytes into the inflamed site.[13]

In our study, oral administration of aqueous extract of EO showed significant decrease in paw edemaat doses of 300 mg/kg and 600 mg/kg at 1st, 3rd and 5th hour when compared to control group. But at a dose of 150 mg/kg there was no significant decrease in paw edema when compared to control group. Standard drugs, indomethacin (10 mg/kg) and celecoxib (10 mg/kg) also showed significant decrease in paw edema at all the times when compared to control group. But chlorpheniramine maleate did not show significant decrease in paw edema when compared to control group.

However, in a study conducted by Jaijoy K et al., water extract of fruit of Phyllanthusemblica Linn showed significant decrease in paw edemaat all three doses (150, 300 and 600 mg/kg) when compared to control group.[14] In another study conducted by Arunachalam et al., Phenolic fractions of EO (20 and 40 mg/kg p.o) significantly reduced paw volume in carrageenan induced paw edema model.[15] In earlier study on the anti-inflammatory activity of leaf extracts of EO in carrageenanand dextran-induced rat paw edema models, it was reported that the extracts did not inhibit the synthesis of the lipid mediators LTB4, TXB2, or PAF.[16] In a recent study by Golechha M et al., it was showed that the hydroalcoholic extract of EO at the dose of 700 mg/kg, exhibited maximum anti-inflammatory activity in carrageenan, PGE_2, histamine and serotonin induced paw edema. Additionally, in paw tissue the antioxidant activity of hydro alcoholic extract of *Emblicaoffinalis* (HAEEO) was also measured and it was found that HAEEO significantly increased glutathione, superoxide dismutase, and catalase activity and subsequently reduced lipid peroxidation evidenced by reduced malondialdehyde.[17] In our study, administration of EO extract (600 mg/kg p.o) and misoprostol (11.43 µg/kg p.o) shows significant decrease in paw volume when compared to misoprostol alone treated group. So this results indicates that EO extract possess anti- inflammatory activity which might be, by inhibiting the synthesis, release or the action of prostaglandin. But further in vitro studies are required to find the exact molecular mechanism of anti-inflammatory action of *Emblicaofficinalis*.

The previous scientific studies have been reported that alkaloids, flavonoids and tanins are responsible for analgesic and anti-inflammatory effects.[18] Therefore anti-inflammatory effect of the extract may be due to the presence of flavonoids, tanins, gallic acid and alkaloids either singly or in combination.

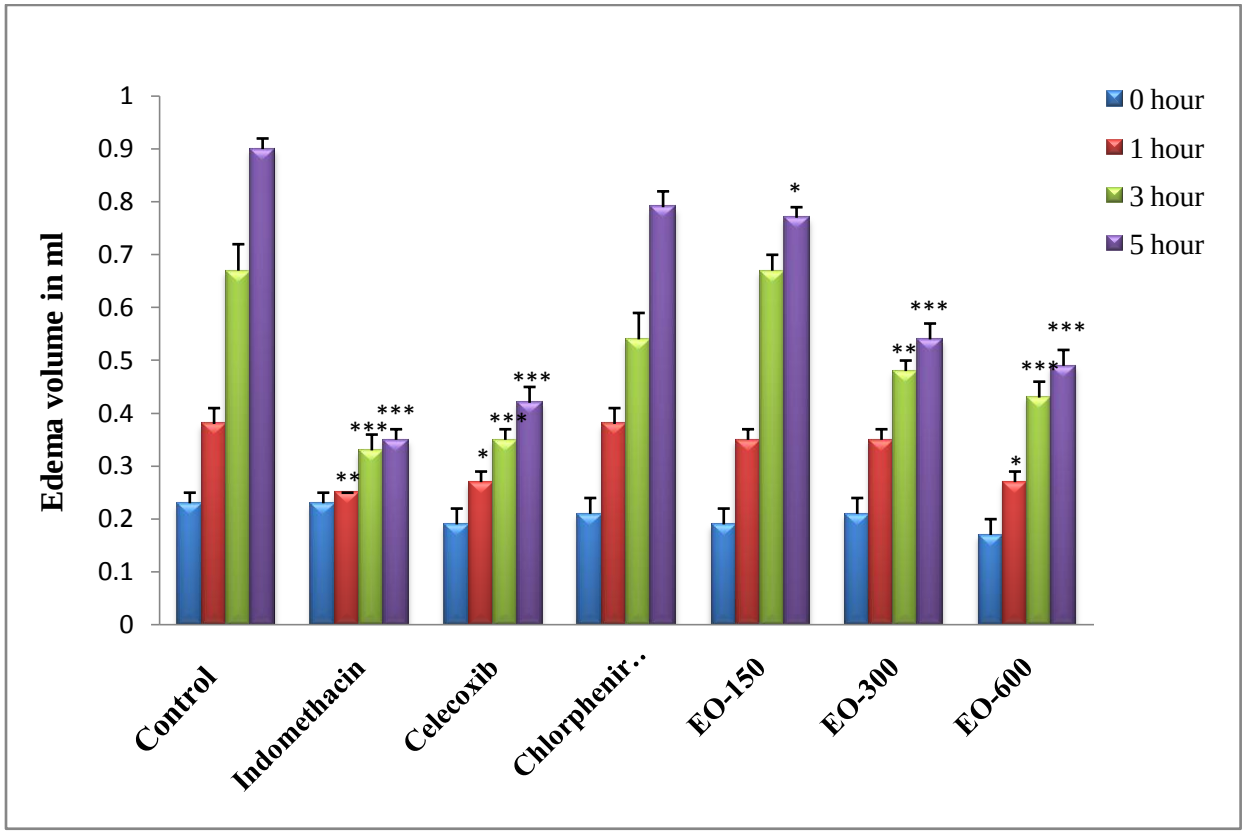
Table 1. Anti-inflammatory effect of different drugs in carrageenan induced paw edema in rat

S.No.	Groups	Dose	Time after 1% carrageenan injection			
			0 hr	1 hr	3 hr	5 hr
			EV (ml)	EV (ml)	EV (ml)	EV (ml)
1	**Control (DW)**	**5 ml/kg p.o**	**0.23±0.02**	**0.38±0.03**	**0.67±0.05**	**0.90±0.02**
2	**Indomethacin**	**10 mg/kg p.o**	**0.23±0.02**	**0.25±0.00****	**0.33±0.03*****	**0.35±0.02*****
3	**Celecoxib**	**10 mg/kg p.o**	**0.19±0.03**	**0.27±0.02***	**0.35±0.02*****	**0.42±0.03*****
4	**chlorpheniramine**	**10 mg/kg i.p**	**0.21±0.03**	**0.38±0.03**	**0.54±0.05**	**0.79±0.03**
5	**EO**	**150 mg/kg p.o**	**0.19±0.03**	**0.35±0.02**	**0.67±0.03**	**0.77±0.02***
6	**EO**	**300 mg/kg p.o**	**0.21±0.03**	**0.35±0.02**	**0.48±0.02****	**0.54±0.03*****
7	**EO**	**600 mg/kg p.o**	**0.19±0.03**	**0.27±0.02***	**0.43±0.03****	**0.49±0.03*****

Values are presented as mean ± SEM (n = 6); *p<0.05, ** p<0.01, *** p<0.001 when compared with the control group.

EV- Edema volume, DW- Distilled water, EO – *Emblica officinalis.*

Figure 1. Anti-inflammatory effect of EO extract incarrageenan induced hind paw edema in rat



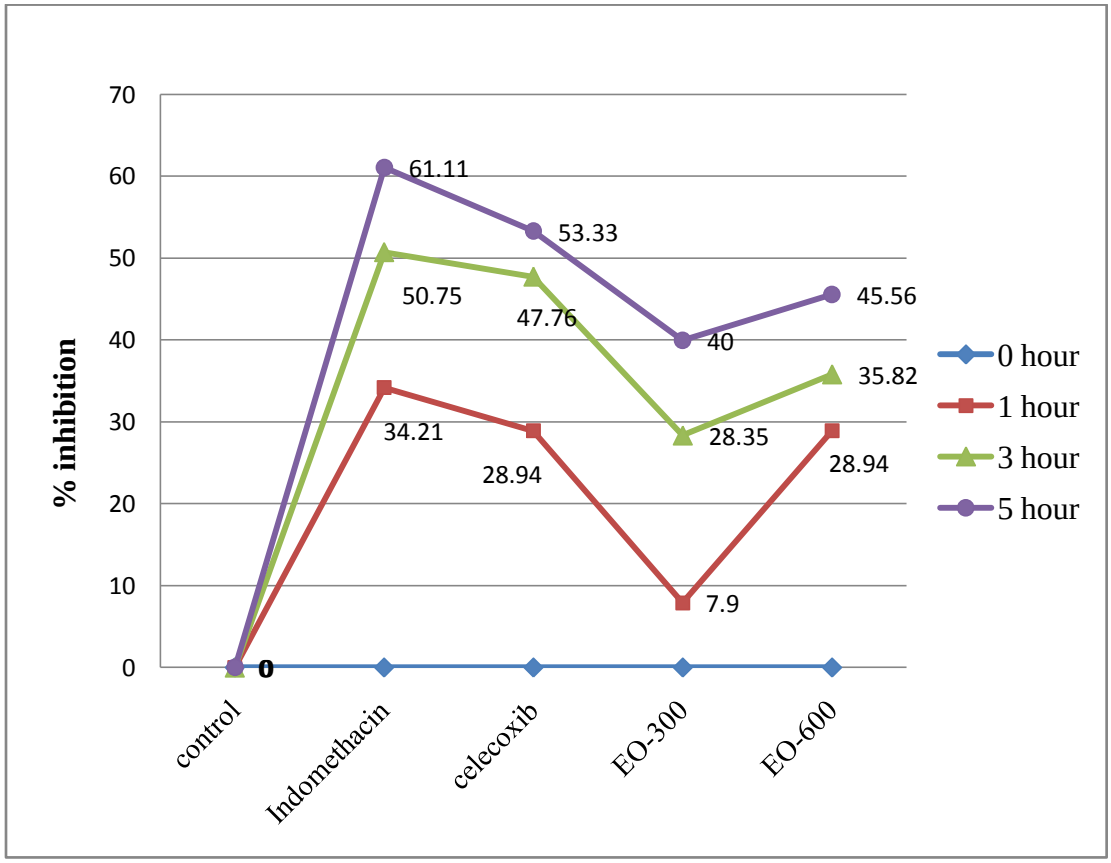
***p<0.05, ** p<0.01, ***p<0.001 when compared with the control group.**
EO – *Emblica officinalis*

Table 2: Anti-inflammatory effect of EO extract and misoprostol in carrageenan induced paw edema in rat

S.No.	Groups	Dose	Time after 1% carrageenan injection			
			0 hr EV (ml)	1 hr EV (ml)	3 hr EV (ml)	5 hr EV (ml)
1	Control (DW)	5 ml/kg p.o	0.23±0.02	0.38±0.03	0.67±0.05	0.90±0.02
2	Misoprostol	11.43 µg/kg p.o	0.27±0.02	0.38±0.03	0.71±0.03	0.99±0.03*
3	EO+ Misoprostol	600 mg/kg p.o+ 11.43 µg/kg p.o	0.23±0.02	0.27±0.02#	0.42±0.03###	0.60±0.02###

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

Figure 2. Percentage of inhibition of paw edema by various drugs in carrageenan induced hind paw edema in rats



EO – *Emblica officinalis*

Conclusion

To conclude, the aqueous fruit extract of EO demonstrated significant anti-inflammatory activities in experimental animals in this study. However future studies need to be conducted to find the effect on various inflammatory disorders in specific animal models. The anti-inflammatory activities may be attributed by the various phytoconstituents of EO extract like flavonoids, tannis, ellagic acid, vitamic c and other alkaloids. The unacceptable side effects associated with 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.

References

1. Dorababu Neerugatti D, Battu GR, Jangiti R. Anti-inflammatory effect of methanolic extract of *Tamilnadia uliginoza* (Retz.) roots. *International Journal of Phytopharmacology*. 2014; 5(1): 34-37
2. Ferrero MilianiL, NielsenOH, Andersen PS, Girardin SE. Chronic inflammation : importance of NOD2 and NALP3 in interleukin - 1β generation. *Clin Exp Immunol*.2007; 147(2): 227–235.
3. Pinheiro RM, Calixto JB. Effect of the selective COX-2 inhibitors, Celecoxib and Rofecoxib in rat acute models of inflammation.*Inflamm res*. 2002; 51:603–10.
4. Silva JR, Rao VN.Involvement of serotonin and eicosanoids in the rat paw edema response to theessential oil of *Pilocarpusspicatus*. *Mediators of Inflammation*.1992; 1: 167-9.

5. Medeiros MB, Amaral B, Morimoto MSS, Cardoso Vieira LG, Ferreira Perazzo F, Tavares Carvalho JC. Anti-inflammatory and Analgesic actions of etoricoxib (an NSAID) combined with misoprostol.*Inflammopharmacology*. 2007; 15:175–8.
6. Ismail TS, Gopalakrishnan S, Hazeena Begum V. Biochemical modes of action of *GmelinaAsiatica* in inflammation.*Indian Journal of Pharmacology*.1997; 29:306-9.
7. Winter CA, Risley EA, Nuss GW. Carrageenan-induced edema in hind paw of the rat as an assay for anti-inflammatory drug. *ProcSocExpBiol Med.* 1962; 111: 544-7.
8. Saivasanthi V, Gowthamigoud, Swathi k, Aakruthi, Sowmya rani, Gupta A*et al*. Evaluation of *carallumafimbrita* for analgesic, anti-inflammatory and anxiolytic activities.*Int J Pharma*. 2011; 1(1): 40-5.
9. Seewaboon S,Arunporn I,Nusiri L,Pritsana P,Parirat K,Supot B *et al*. Anti-inflammatory, Analgesic, and Antipyretic Activities of the Ethanol Extract of *Piper interruptumOpiz*. and *Piper chaba Linn.*ISRN Pharmacology. Volume 2012, Article ID 480265.
10. Venkateswaralu K. *Vitex negundo*: Medicinal values, Boiological activities, Toxicity studies and Phytopharmacological actions. *Int J Pharm Phytopharmacol Res*. 2012; 2(2):126-133.
11. Singh D, Singh B, Goel RK. Hydroethanolic leaf extract of *Ficus religiosa* lacks anticonvulsant activity in acute electro and chemo convulsion mice models. *Journal of Pharmaceutical Negative Results*. 2011; 2(2): 58-61.
12. Morris CJ. Carrageenan-induced paw edema in the rat and mouse. *Methods Mol Biol*. 2003; 225:115-21.
13. Querioz RF, Jordao AK, Cunah AC. Nitroxides attenuate carrageenan-induced inflammation in rat paws by reducing neutrophil infiltration and the resulting myeloperoxidase mediated damage. *Free Radic Biol Med*. 2012; 53(10): 1942-53.
14. Jaijoy K, Soonthornchareonnon N, Panthong A, Sireeratawong S. Anti inflammatory and analgesic activities of the water extract from the fruit of *Phyllanthus emblica* Linn. *International Journal of Applied Research in Natural Products*. 2010; 3(2): 28-35.
15. Muthuraman A, Sood S, Singla SK. The Antiinflammatory potential of phenolic compounds from *Emblica officinalis* in rat. *Inflammopharmacology*. 2011; 19(6): 327-34.
16. Asmawi MZ, Kankaanranta H, Moilanen E, Vapaatalo H. Anti-inflammatory activities of *Emblica offinalis* Gaertn leaf extracts. *J Pharm Pharmacol*. 1993; 45(6): 581-4.
17. Mahaveer Golechha M, Sarangal V, Ojha S, Bhatia J, Arya DS. Anti-inflammatory effect of *Emblica officinalis* in Rodent models of Acute and Chronic Inflammation: Involvement Possible Mechanisms. *Int J Inflam*. Volume 2014; Article ID 178408.
18. Moazem Hosen SM, Sarkar R, Mahmud S, Abdul Aziz NM. Medicinal potential of Phyllanthus emblica (Linn.) fruits extracts: Biological and Pharmacological activities. *Br J Pharm Res.*2014; 4(12): 1486-92.