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Anti-inflammatory activity of ethanolic, hydroethanolic, aqueous and chloroform extracts of *Nyctanthes arbor-tristis* leaves

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Abstract
Background*: **Nyctanthes arbor-tristis** commonly known as Harsinghar, Night jasmine or Parijat. It is a shrub or small tree up to 10 m in height with gray to greenish rough bark distributed wild in Sub-Himalayan regions and Southwards to Godavari. The present study was an attempt to evaluate the anti-inflammatory activity of Nyctanthes arbor-tristis leaf extract.*
Method: *Melonex was used as standard drug for evaluation of anti-inflammatory activity by Carrageenan induced hind paw oedema. The anti-inflammatory activity of the ethanolic, hydroethanolic, aqueous and chloroform extracts of Nyctanthes arbor-tristis was evaluated using carrageenan induced hind paw edema method.*
Result : *NAEE at 250 mg/kg body weight showed significant (P<0.05) inhibition of paw volume in the later phase of inflammation i.e. in between 5-6 hours of observation period. Whereas NAEE at 500 and 1000 mg/kg body weight showed inhibition of paw volume at 2 hours, but at 3 hours it showed maximum increase in paw volume. From 4 hours onwards upto 6 hours of observation period there was significant (P<0.05) inhibition of paw volume. NAHE at 250, 500 and 1000 mg/kg body weight showed inhibition of paw volume at 2 hours, but at 3 hours it showed maximum increase in paw volume. From 4 hours onwards upto 6 hours of observation period there was significant (P<0.05) inhibition of paw volume. NAAE at 250,500 and 1000 mg/kg body weight showed inhibition of paw volume at 2 hours, but from 3 hours to upto 4 hours it showed increase in paw volume. From 5 hours there was significant (P<0.05) inhibition of paw volume upto 6 hours of observation period. NACE at 250, 500 and 1000 mg/kg body weight also showed inhibiton of paw volume at 2 hours, but at 3 hours it showed increase in paw volume. From 4 hour onwards there was significant (P<0.05) inhibition of paw volume upto 6 hours of observation period.*
Keywords*: Nyctanthes arbor-tristis, Anti-inflammatory, Carrageenan induced hind paw oedema method.*

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I. Introduction

Inflammation is a pathophysiological condition of mammalian tissue to a different type of hostile agents including infectious organisms, toxic chemical substances, physical injury, or tumor growth leading to local accumulation of plasma fluid and blood cells. Edema formation, leukocyte infiltration, and granuloma formation exemplify such components of inflammation. The primary objective of inflammation is to localize and eradicate the irritant and repair the surrounding tissue (Wikipedia). For the survival of the host, inflammation is a necessary and is a beneficial process. However, occasionally the inflammatory response fails to resolve itself once the injury or irritant has been removed. The consequence is that the resultant long term inflammation actually causes additional injury to the affected tissue. Some common condition caused by chronic inflammation are rheumatoid arthritis, eczema, and psoriasis. Mainly two types of anti-inflammatory agents are available such as glucocorticosteroids and nonsteroidal anti-inflammatory drugs (NSAIDs). Non-steroidal anti-inflammatory drugs (NSAIDS), which function through the inhibition of enzyme cyclooxygenase, are a more favorable option for the treatment of chronic inflammatory disorders. But due to having harmful side effects, like gastric lesions, caused by NSAIDs and tolerance and dependence produced by opiates, the use of these drugs as analgesic agents has not been successful in all the

cases. Therefore, analgesic drugs which do not contain any adverse side effects are being searched all over the world as alternatives to NSAIDs and opiates (Whelton., 1999).

Inflammatory disorders are a major problem of concern in the cattle industry, particularly in drought-stricken animals, racehorses, and hunting dogs, among other species. The complete etiology of the inflammatory diseases is still not clear to the scientific community and due to many inflammatory pathways proposed, suggest that probably only a complex molecule can effectively compete with different macromolecular substrates. Inflammation may lead to joint tissue destruction, cancer, cardiovascular events, diabetes and brain liver kidney degenerative diseases (Wikipedia). However these conventional anti-inflammatory agents produced various adverse effects (Tripathi.,2015).In order to avoid adverse effects, there is growing interest in the pharmacological evaluation of various plants used in Indian traditional system of medicine (Salunke *et al*.,2012). ***Nyctanthes arbor-tristis*** commonly known as Harsinghar, Night jasmine or Parijat.

It is a shrub or small tree up to 10 m in height with gray to greenish rough bark distributed wild in Sub-Himalayan regions and Southwards to Godavari. In Indian garden it is also planted for ornamental purpose due to its highly sweet-smelling flowers. The leaves of *N.arbortristis* shows antifungal activity against *Alrernaria alternate* (Chauhan.,1978). Aqueous extract of leaves is proved to be hepatoprotective (Chauhan., 1978). Kiew and Bass.(1984) isolated an alkaloid principle named nyctanthin form the leaves. Iridoid glucosides were isolated from the plant and have antileishmanial activity (Tandon *et al*., 1991).The present study was aimed to evaluate the anti-inflammatory activity of ethanolic, hydroethanolic, aqueous and chloroform extracts of *Nyctanthes arbor-tristis* leaves in laboratory animals.

II. Materials And Methods

Plant material

The fresh leaves of *N. arbor – tristis* will be collected from in an around Khanapara campus in the month of July to September for pharmacological experimental purpose. Leaves were identified and authenticated by Botanical Survey of India (BSI), Eastern Regional Centre, Shillong.

Processing of Plant Materials:

After identification and characterisation by BSI, leaves were further collected. The collected leaves were gently washed with fresh water to remove soil and dust particles. Leaves were then shade dried at room temperature for about 7-10 days.They were regularly turned over, to prevent fermentation and rot. Dried leaves were then grounded or pulverised to powder by Laboratory Willey Mill and kept at room temperature in air tight containers after proper labelling until preparation of extracts.

Preparation of Ethanolic, Hydroethanolic, Aqueous and Chloroform Extracts

Powdered plant materials were extracted with ethanol, hydroethanol (1:1) and distilled water respectively as per the procedure of Prasad (1965). Finely powdered plant powders were soaked with individually for 72 hours, three times, with intermittent agtitation. The extracts were then double filtered using muslin cloth and Whatman No.1 filter paper. The filtrate obtained was concentrated in rotary evaporator and completely dried over regulated water bath maintained at 50ºC. The extracts were refrigerated at 4ºC until the experiments for screening was done. Standard procedures (Lateef *et al*., (2003;2006); Sujon *et al*. (2008) were used with a few modifications.

Experimental Animals

Wistar albino mice weighing 30-40g were taken from the Dept. of Veterinary Pharmacology and Toxicology, Assam Agricultural University, Khanapara. All the mice were kept in polypropylene cages and they were divided into groups of 6 mice each. Paddy husk was used as litter material which was regularly changed every week. All the animals were provided with a balanced ration and clean drinking water *ad libitum* and were maintained in standard laboratory conditions (12:12 hour light/dark) cycle at an ambient temperature ranging between (22-27^0 C).

Acute Toxicity Study

The study was carried out according to OECD (Organization for Economic Co-operation and Development) 425 guidelines. For acute toxicity study, nulliparous and non-pregnant female albino mice,weighing 30-40g, were randomly selected. Ethanolic, hydroethanolic,aqueous and chloroform extracts of the leaves of *Nyctanthes arbor-tristis* was administered orally to the mice. Limit test was performed. Prior to administration of the test extracts animals were fasted overnight but given water *ad libitum.* Group-I served as vehicle control (20% Tween-80) and Group II-V kept ethanolic, hydroethanolic, aqueous and chloroform leaf extract of *Nyctanthes arbor-tristis* @ 2000 mg/kg orally, as single dose. The animals were closely observed for behavioral changes, toxicity and mortality upto 72 hours and animals were further observed for 14 days to

record mortality if any. Based on acute toxicity study three doses were selected and used for evaluation of anti-inflammatory activity in mice with six animals in each group for each of the following tests.

Anti-inflammatory activity

Carrageenan-induced hind paw oedema method

This test was performed according to the method of Winter *et al*. (1962). The paw volume was measured by using plethysmometer. The mice were divided into 14 groups of 6 animals each. Group I kept as vehicle control, Group II served as standard drug melonex @ 5mg/kg. Group III-XIV received ethanolic, hydroethanolic, aqueous and chloroform leaf extract of *Nyctanthes arbor-tristis* orally at the dose rate of 250, 500, 1000 mg/kg body weight respectively.The animals were pre treated with *Nyctanthes arbor-tristis* extract orally 30 min prior to carrageenan injection. 0.1 ml of 1% carrageenan was injected sub-cutaneously under the planter surface of the right hind paw. Paw oedema volume was measured by plethysmometer at different time intervals i.e. 30 min (prior to carrageenan injection), 0 hr (at the time of carrageenan injection) and at 1, 2, 3, 4, 5, 6 hours after carrageenan injection.

Statistical Analysis

Results were expressed as Mean ± S.E.M. Statistical analysis was performed by MS-excel to calculate mean, standard error of mean (SEM), analysis of variance (ANOVA), and co-efficient of correlation (r) values as per standard method Snedecor and Cochran (2004).

III. Result

The anti-inflammatory activity of the ethanolic, hydroethanolic, aqueous and chloroform extracts of *Nyctanthes arbor-tristis* was evaluated using carrageenan induced hind paw edema method. The paw volumes compared with "0" hour is displayed in Table: 1.1, 1.2, 1.3 and 1.4 Fig. 1.1,1.2,1.3 and 1.4 . In the control group, the animals showed a biphasic reaction.The swelling increased instantly within the first hour and then it subsided in the second hour, only to increase again in the third hour which continued upto fifth hour and then it subsided.The resulting paw volumes at time 0, 1, 2, 3, 4, 5 and 6 hours are 0.89 ±0.02, 1.05±0.02, 0.95±0.02, 1.15±0.02, 1.22±0.03, 1.38 ±0.031 and 1.12±0.03 respectively. Paw volume observed in Standard (Melonex) group are 0.26±0.02, 0.31±0.02 and 0.38±0.01, 0.54±0.02, 0.61±0.01, 0.36±0.01 and 0.27±0.01 at time 0, 1, 2, 3, 4, 5 and 6 hours respectively. Standard drug increased paw volume significantly upto 4 hours. From 5 hours there was significant ($P<0.05$) inhibition of paw oedema upto 6 hours of observation period. NAEE at 250 mg/kg body weight showed significant ($P<0.05$) inhibition of paw volume in the later phase of inflammation i.e. in between 5-6 hours of observation period. Whereas NAEE at 500 and 1000 mg/kg body weight showed inhibition of paw volume at 2 hours, but at 3 hours it showed maximum increase in paw volume. From 4 hours onwards upto 6 hours of observation period there was significant ($P<0.05$) inhibition of paw volume. NAHE at 250, 500 and 1000 mg/kg body weight showed inhibition of paw volume at 2 hours, but at 3 hours it showed maximum increase in paw volume. From 4 hours onwards upto 6 hours of observation period there was significant ($P<0.05$) inhibition of paw volume. NAAE at 250,500 and 1000 mg/kg body weight showed inhibition of paw volume at 2 hours, but from 3 hours to upto 4 hours it showed increase in paw volume. From 5 hours there was significant ($P<0.05$) inhibition of paw volume upto 6 hours of observation period. NACE at 250, 500 and 1000 mg/kg body weight also showed inhibiton of paw volume at 2 hours, but at 3 hours it showed increase in paw volume. From 4 hour onwards there was significant ($P<0.05$) inhibition of paw volume upto 6 hours of observation period.

TABLE 1.1 ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC LEAF EXTRACT OF *Nyctanthes arbor-tristis* ON CARRAGEENAN INDUCED HIND PAW OEDEMA METHOD

Group	Time (hour)						
	0	1	2	3	4	5	6
Control	0.89±0.02	1.05±0.02	0.95±0.02	1.15±0.02	1.22±0.03	1.38 ±0.03	1.12±0.03
Standard (Melonex)	0.26±0.02	0.31±0.02	0.38±0.01	0.54±0.02	0.61±0.01	0.36±0.01	0.27±0.01
NAEE (250 mg/kg)	0.31±0.02	0.42±0.02	0.60±0.02	0.89±0.03	0.95±0.01	0.80±0.03	0.71±0.03
NAEE (500 mg/kg)	0.31±0.02	0.38±0.03	0.36±0.02	0.78±0.02	0.61±0.02	0.42±0.02	0.36±0.02
NAEE (1000 mg/kg)	0.25±0.01	0.62±0.01	0.50±0.02	0.91±0.02	0.70±0.02	0.50±0.02	0.59±0.03

TABLE 1.2 ANTI-INFLAMMATORY ACTIVITY OF HYDROETHANOLIC LEAF EXTRACT OF *Nyctanthes arbor-tristis* ON CARRAGEENAN INDUCED HIND PAW OEDEMA METHOD

Group	Time (hour)						
	0	1	2	3	4	5	6
Control	0.89 ±0.02	1.05±0.02	0.95 ±0.02	1.15±0.02	1.22±0.03	1.38 ±0.03	1.12±0.03
Standard (Melonex)	0.26±0.02	0.31±0.02	0.38±0.01	0.54±0.02	0.61 ±0.01	0.36±0.01	0.27±0.01
NAHE (250 mg/kg)	0.35±0.01	0.54±0.02	0.49±0.01	0.62±0.02	0.59±0.02	0.56±0.02	0.45±0.01
NAHE (500 mg/kg)	0.32±0.01	0.54±0.01	0.45±0.01	0.77±0.01	0.64±0.01	0.60±0.02	0.45±0.03
NAHE (1000 mg/kg)	0.31±0.01	0.57±0.01	0.47±0.01	0.78±0.03	0.63±0.03	0.43±0.03	0.32±0.01

TABLE 1.3 ANTI-INFLAMMATORY ACTIVITY OF AQUEOUS LEAF EXTRACT OF *Nyctanthes arbor-tristis* ON CARRAGEENAN INDUCED HIND PAW OEDEMA METHOD

Group	Time (hour)						
	0	1	2	3	4	5	6
Control	0.89±0.02	1.05 ±0.02	0.95±0.02	1.15±0.02	1.22±0.03	1.38 ±0.03	1.12±0.03
Standard (Melonex)	0.26±0.02	0.31±0.02	0.38±0.01	0.54±0.02	0.61 ±0.01	0.36±0.01	0.27±0.01
NAAE (250 mg/kg)	0.25±0.01	0.44 ±0.02	0.31±0.02	0.54±0.01	0.61±0.01	0.52±0.02	0.44±0.01
NAAE (500 mg/kg)	0.25±0.01	0.46±0.01	0.35±0.01	0.53±0.01	0.63±0.01	0.54±0.01	0.44±0.02
NAAE (1000 mg/kg)	0.25±0.01	0.43±0.01	0.34±0.01	0.52±0.02	0.70±0.01	0.56±0.01	0.46±0.01

TABLE 1.4 ANTI-INFLAMMATORY ACTIVITY OF CHLOROFORM LEAF EXTRACT OF *Nyctanthes arbor-tristis* ON CARRAGEENAN INDUCED HIND PAW OEDEMA METHOD

Group	Time (hour)						
	0	1	2	3	4	5	6
Control	0.89 ±0.02	1.05±0.02	0.95±0.02	1.15±0.02	1.22±0.03	1.38 ±0.03	1.12±0.03
Standard (Melonex)	0.26±0.02	0.31±0.02	0.38±0.01	0.54±0.02	0.61±0.01	0.36±0.01	0.27±0.01
NACE (250 mg/kg)	0.24±0.01	0.65±0.02	0.55±0.04	0.79±0.02	0.69±0.02	0.51±0.03	0.40±0.02
NACE (500 mg/kg)	0.23±0.01	0.55±0.02	0.44±0.01	0.76±0.01	0.63±0.01	0.54±0.01	0.44±0.01
NACE (1000 mg/kg	0.26±0.01	0.42±0.01	0.33±0.01	0.58±0.03	0.55±0.01	0.44±0.01	0.36±0.01

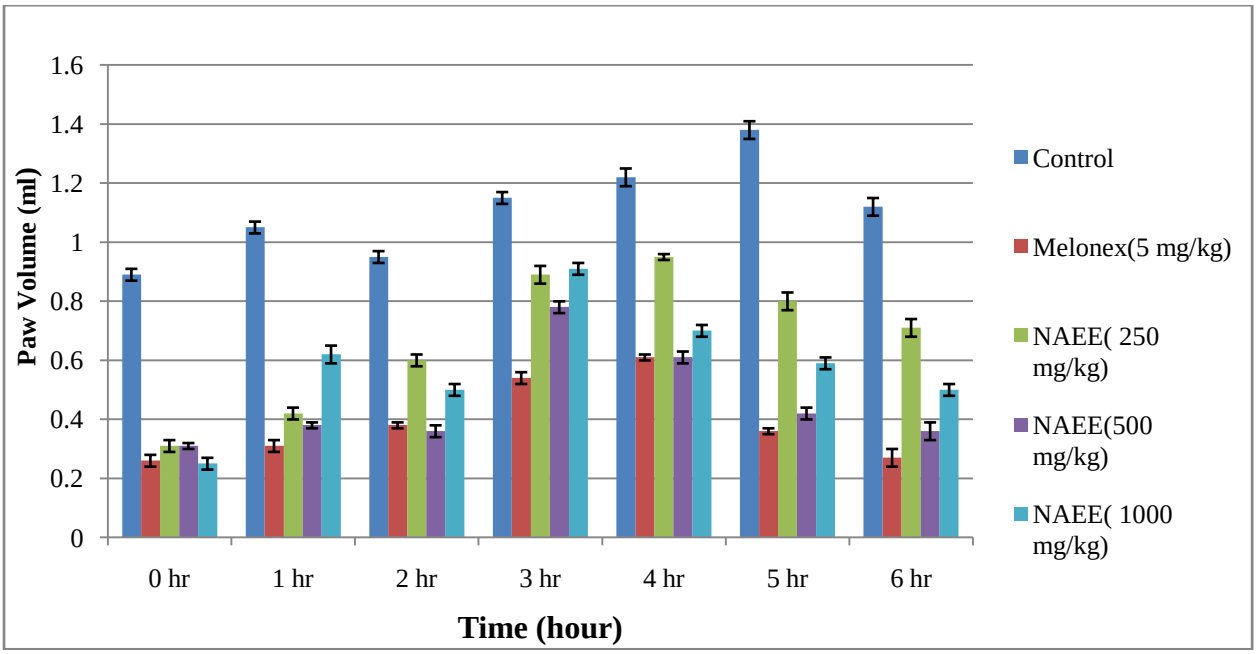

FIG.1.1GRAPH REPRESENTING MEAN CHANGE IN PAW VOLUME OF MICE TREATED WITH ETHANOLIC LEAF EXTRACT OF *Nyctanthes arbor-trists* AND MELONEX BY CARRAGEENAN INDUCED HIND PAW OEDEMA METHOD

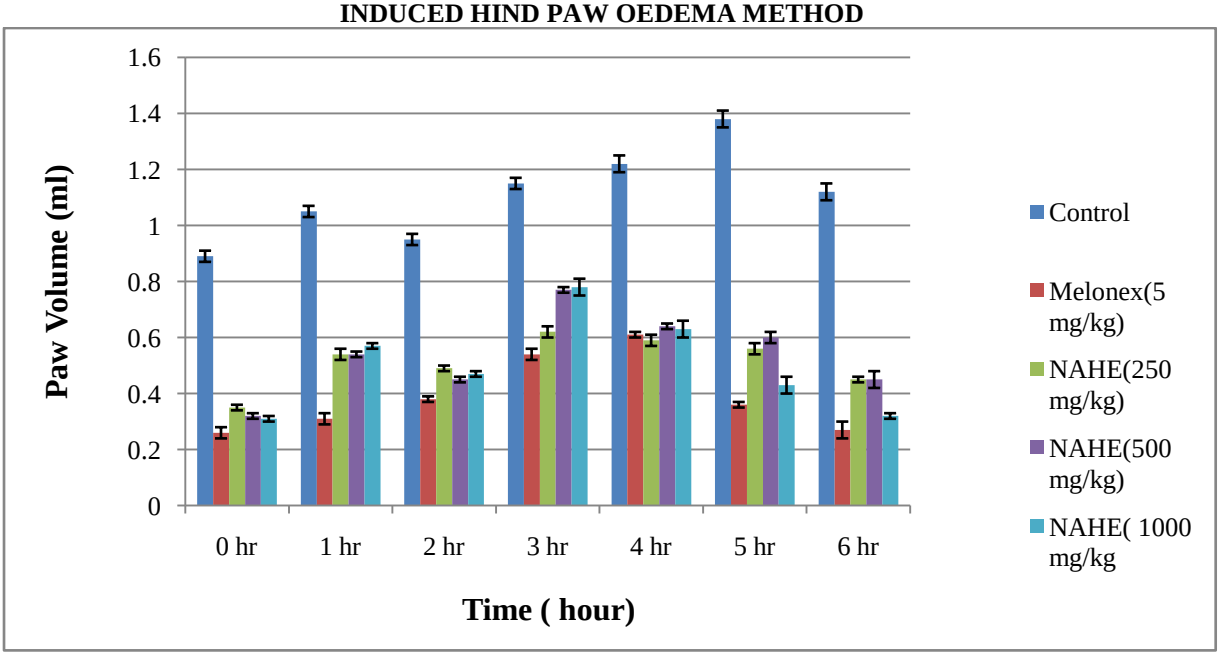

FIG.1.2 GRAPH REPRESENTING MEAN CHANGE IN PAW VOLUME OF MICE TREATED WITH HYDROETHANOLIC LEAF EXTRACT OF *Nyctanthes arbor-trists* AND MELONEX BY CARRAGEENAN INDUCED HIND PAW OEDEMA METHOD

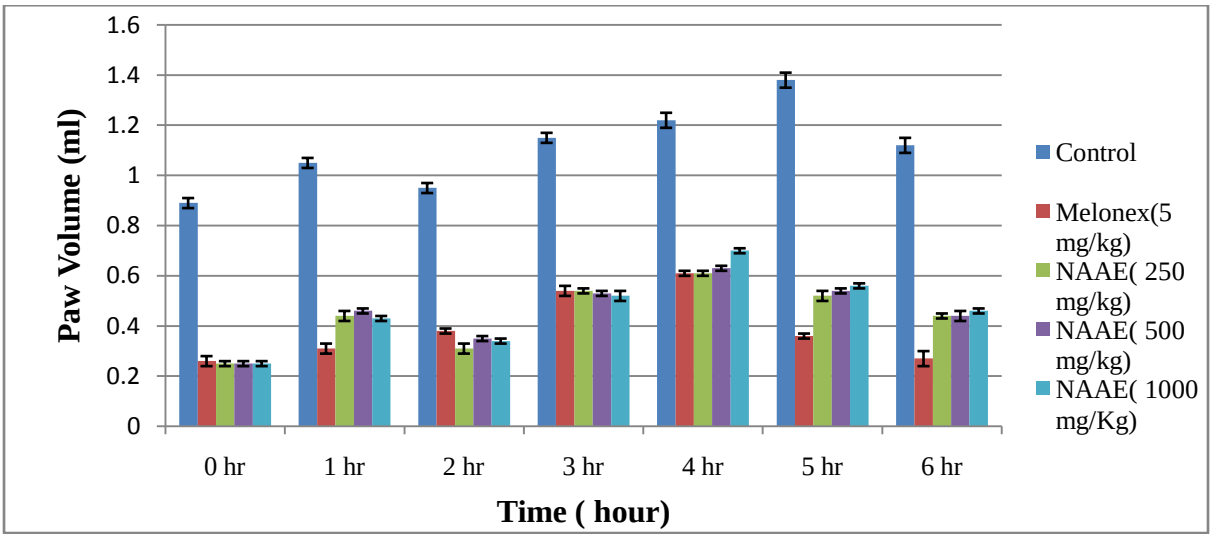

FIG.1.3 GRAPH REPRESENTING MEAN CHANGE IN PAW VOLUME OF MICE TREATED WITH AQUEOUS LEAF EXTRACT OF *Nyctanthes arbor-trists* AND MELONEX BY CARRAGEENAN INDUCED HIND PAW OEDEMA METHOD

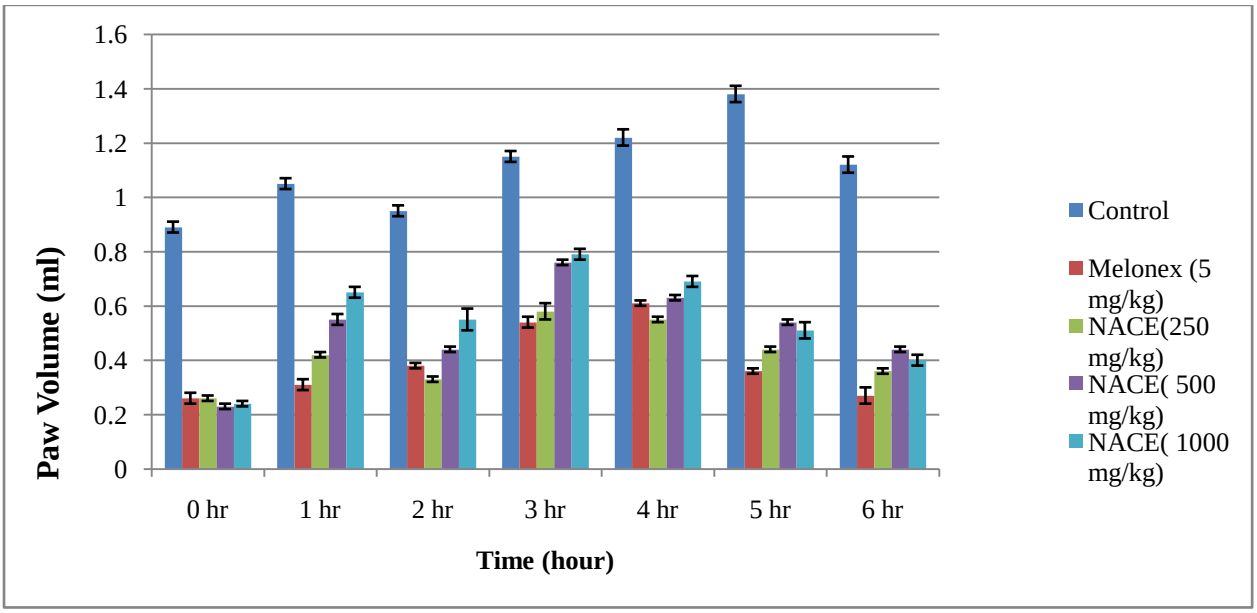

FIG.1.4 GRAPH REPRESENTING MEAN CHANGE IN PAW VOLUME OF MICE TREATED WITH CHLOROFORM LEAF EXTRACT OF *Nyctanthes arbor-trists* AND MELONEX BY CARRAGEENAN INDUCED HIND PAW OEDEMA METHOD

IV. Discussion

Inflammation is a common phenomenon and it is a reaction of living tissue towards injury. For the investigation of anti-inflammatory activity of *Nyctanthes arbor-tristis*, the commonly used *in vivo* model, the carrageenan-induced hind paw edema model by (Winter *et al.*, 1962). It has been reported that inflammation occurs in two phases. The first phase begins immediately after the injection of carrageenan and diminishes at 2 hour. This phase of inflammation is accompanied by the release of serotonin and histamine while the second phase begins at 3 hour and persisted for at least 4 hour. This phase is mediated by several agents e.g., bradykinin, prostaglandin and lysosome (Vijayalakshmi *et al.*, 2011). The later phase of inflammation is reportedly, sensitive to most of the currently available drugs (NSAIDs). Upto 4 hours post carrageenan injection no significant oedema inhibitory response was observed at any of the doses and/or extracts used, except NAEE@ 250 mg/kg body weight. Significant anti-inflammatory activity started from 4 hours onwards following carrageenan injection. It is well known that both the cyclooxygenase and lipoxygenase pathways are involved in the inflammatory process however, cyclooxygenase inhibitors are more effective in inhibiting carrageenan-induced inflammation (Nurcan *et al.*, 2012). Cyclooxygenase pathway is involved in the release of several

mediators particularly prostaglandins, bradykinin and lysosomes thereby, the edema inhibition by *Nyctanthes arbor-tristis* leaf extracts @ 1000 mg/kg doses may be due to the inhibition of these mediators. It has been reported that leaves of the *Nyctanthes arbor-tristis* contain biologically active compounds such as glycosides, steroids, phenolic compounds, flavonoids and alkaloids (Sathiya *et al*., 2008,; Ramachandran *et al.*,2014,; Chidi *et al*.,2015, and Hazarika.2019). In addition,Nurcan *et al*., 2012 also observed involvement of these compounds in anti-inflammatory activity.

V. Conclusion

NAEE at 250 mg/kg body weight showed significant ($P<0.05$) inhibition of paw volume in the later phase of inflammation i.e. in between 5-6 hours of observation period. Whereas NAEE at 500 and 1000 mg/kg body weight showed inhibition of paw volume at 2 hours, but at 3 hours it showed maximum increase in paw volume. From 4 hours onwards upto 6 hours of observation period there was significant ($P<0.05$) inhibition of paw volume. NAHE at 250, 500 and 1000 mg/kg body weight showed inhibition of paw volume at 2 hours, but at 3 hours it showed maximum increase in paw volume. From 4 hours onwards upto 6 hours of observation period there was significant ($P<0.05$) inhibition of paw volume. NAAE at 250,500 and 1000 mg/kg body weight showed inhibition of paw volume at 2 hours, but from 3 hours to upto 4 hours it showed increase in paw volume. From 5 hours there was significant ($P<0.05$) inhibition of paw volume upto 6 hours of observation period. NACE at 250, 500 and 1000 mg/kg body weight also showed inhibiton of paw volume at 2 hours, but at 3 hours it showed increase in paw volume. From 4 hour onwards there was significant ($P<0.05$) inhibition of paw volume upto 6 hours of observation period.

Future prospects

1. This study was the preliminary step towards screening of *Nyctanthes arbor-tristis* plant and it paves the way for further attention and research to identify the active compounds responsible for biological / pharmacological activities.
2. Further investigations are needed to find out the actual molecular mechanism of the active constituents present in the leaves of *Nyctanthes arbor-tristis.*

Acknowledgement

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Abbreviations

NAEE:*Nyctanthes arbor-tristis* ethanolic extract NAHE: *Nyctanthes arbor-tristis* hydroethanolic extract NAAE: *Nyctanthes arbor-tristis* aqueous extract NACE: *Nyctanthes arbor-tristis* chloroform extract NSAIDs: Non-steroidal Anti-inflammatory Drug OECD: Organization for Economic Co-operation and Development

Conflict of interest

The author declared no conflict of interest.

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Author's contributions

All the authors read and approved the final manuscript.

Animal welfare and Ethics statement

The animal experimentation was carried out according to the Committee for the Purpose of Control and Supervision of experimental animals (CPCSEA) guideline and Institutional Animal Ethical Committee Approved all the procedure for investing experimental pain in conscious animals.

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