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INVESTIGATION FOR POTENTIAL EFFECT OF *NYCTANTHES ARBORTRISTIS* IN EXPERIMENTALLY INDUCED ASTHMA

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ABSTRACT

Aim of study: In this study, we investigate the potential effect of Nyctanthes arbortristis leaf extract in experimentally induced asthma in rats. The leaf has shown the prominent effect against the inflammation.

Materials and methods: Asthma was induced experimentally in rats using Sephadex and in another model croton oil was applied on ear of rats; this model also mimics the biological event similar to asthma.

Results: The results demonstrated that the Sephadex induced lung inflammation and croton oil induced ear edema both were palliated by the *Nyctanthes arbortristis* leaf extract given to the rats. The prophylactic effect of the leaf extract was evaluated by the lung weight and histological examination and investigation of oxidative and anti-oxidant markers in lung tissues.

Conclusions: This study demonstrated for the first time that ethanolic leaf extract of *Nyctanthes arbortristis* (ENAL), was effective in prevention of experimentally induced asthma in rats. In vivo data indicated that ENAL were as much effective as Fluticasone propionate i.e. standard drug used to treat asthma which might be due to regulation of inflammation and anti-oxidant property. This study gave us good scientific evidence that the ENAL is prophylactic agent for the asthma.

INTRODUCTION: Natural products from plant, animal and minerals have been the basis of the treatment of human disease. It is estimated that about 80% of people in developing countries still rely on traditional medicine based exclusively on species of plants for their primary health care. Herbal medicines are currently sought after and their popularity is increasing progressively. About 500 plants with medicinal use are mentioned in ancient literature and around 800 plants have been used in indigenous systems of medicine. India is a vast repository of medicinal plants that are used in traditional medical treatments (Chopra et al., 1956). The use of herbal medicines becoming popular and the toxicity, side effects of allopathic medicines are likely to be averse. This led to sudden increase in the number of herbal drug manufactures (Agarwal,

2005). *Nyctanthes arbortristis* is indigenous to India, disseminated wild in its northern parts and southwards to Godavari. It is also disseminated in Bangladesh, Indo-Pak subcontinent and South-East Asia (Khatune et al., 2003), tropical and sub-tropical South East Asia (Wallander and Albert, 2000). The plant is used in Ayurveda for various pharmacological actions and this plant has also studied for its potential in various pharmacological activities such as antibacterial (Mahida and Mohan, 2007), anti-inflammatory (Das et al., 2008; Rathee and Hassarajani, 2007; Saxena et al., 1984), antidiabetic (Husain et al., 2010; Rathod et al., 2010; Suresh et al., 2010), hepatoprotective (Hukkeri et al., 2006; Lucas and Sekhar, 2000; Langhate et al., 2003; Vishwanathan and Juvekar, 2010), antiarthritic (Rathore et al., 2007), antioxidant (Narendhirakannan and Smeera, 2010; Rathee and Hassarajani, 2007), antimicrobial (Vats et al., 2009), anthelmintic (Das et al., 2010), antileishmanial (Khatune et al., 2001; Poddar et al., 2008; Shukla et al., 2011; Tandon et al., 1991), antiviral (Gupta et al., 2005), CNS depressant (Das et al., 2008).

Generally some anti-inflammatory drugs are equally effective in asthma, therefore the ethanolic leaf extract of *Nyctanthes arbortristis* gives the scope to investigate its potential effect in asthma. In order to perform such study, models were selected to induce asthma experimentally via Sephadex (intratracheally) and croton oil. Sephadex is generally used as acute inflammatory model by provoking lung inflammation and croton oil is administered epicutaneous to induce the inflammatory events similarly as that of clinical asthma. Sephadex model has been used in parallel with allergen-induced lung inflammation models by many groups in the last decades in order to simulate different aspects of inflammatory lung disorders in animal settings.

Although the Sephadex model can be classified as an acute inflammation model, it is widely reported to show similarity in inflammatory profile to clinical asthma in several respects such as, inflammatory cell infiltrates dominated by eosinophils, oedema formation, tumour necrosis factor (TNF), eosinophil peroxidase (EPO), and cysteinyl leukotrienes (cysLT), strain-related airway hyper-reactivity (Belvisi et al., 2000; Birrell et al., 2000; Guo et al., 1999; Hammerbeck et al., 2000). The proinflammatory component in croton oil is tumor promoter phorbol 12-myristate 13-acetate (PMA).

It is known that epicutaneous application of PMA results in histological and biochemical changes including vascular leakage, leukocyte infiltration, epidermal hyperplasia, activation of protein kinase C, increased release of arachidonic acid and its metabolites, enzyme induction and increased protein, RNA and DNA synthesis (Towbin et al., 1995). The above mentioned biochemical changes occur clinically in to the lung in case of asthma, so this model was used to study the asthma.

MATERIALS AND METHODS:

1. **Collection and identification of plant material:** The leaves of *Nyctanthes arbor-tristis* were collected from the local area of Pune. The plant herbarium was prepared and submitted for authentication at Botanical Survey of India, Pune and voucher specimen (Auth 08-111) was deposited.

2. **Preparation of extract:** The leaves were shed dried and powdered. The powdered material was macerated with ethanol (95%) and in order to achieve complete maceration continuous stirring with the help of mechanical stirrer was applied for 4 hours and then filtered. Filtrate was collected and resultant macerate was again subjected for maceration process using same solvent. The above mention process was repeated four times. The final filtrate was concentrated under reduced pressure. The crude extract was dried, weighed and percentage yield was calculated. The final extract was refrigerated in airtight, amber colored bottle at 4-5°C. (Reibling and Walker, 1975).

3. **Phytochemical screening:** The extract was screened for the presence of major phytoconstituents using standard protocol (Khandelwal, 2008; Rangari, 2002).

4. **Experimental animals** Sprague-Dawley rats of either sex, weighing 100-150 g were procured from National Institute of Bioscience, Pune. Animals were placed separately in polypropylene cages (five per cage) randomly with paddy husk as bedding. The animals were maintained under

standard laboratory conditions temperature 23±2^{o}C, relative humidity 55 ± 10 % and the same condition was maintained throughout all the experiments. Animals had free access of water and standard laboratory feed, ad libitum. The animals were shifted from animal house to the laboratory one hour prior to the start of the experiment. All the experimental procedures and protocols used in this study were reviewed and approved (SCOP/IAEC/Approval/2008-09/06) by the Institutional Animal Ethics Committee (IAEC) of Sinhgad College of Pharmacy, Pune, constituted under Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

5. **Selection of dose:** The literature survey showed that a study was conducted using rat for anti-inflammatory study of ENAL and the doses of (2, 4 and 8g/kg, p.o) were given to the rats for five days to investigate its anti-inflammatory effect using various models such as, carrageenin induced granuloma pouch, cotton pellet granuloma, and the doses of (2 and 4g/kg, p.o) were significant relative to the saline treated group respectively (Saxena et al., 1984). So it has been considered that the dose of (2, 4 and 8g/kg, p.o) for five days might be useful for the study.

6. **Croton oil induced ear edema:** Before experiment onset day one, rats were weighed. Test drug treated animals were given ENAL for five days (2, 4 and 8g/kg, p.o) and other groups were given normal saline solution for the same days. On the sixth day, animals were anaesthetized and 25µl of inflammation inducing irritant was applied to the outer surface of the right ear by micropipette. The irritant consisted of croton oil (Sigma-Aldrich, India) 2.5% in acetone (Kim et al., 1998; Kim et al., 2008). After 6 hours (Towbin et al., 1995), animals were anaesthetized, uniform ear pieces were removed using curved scissor and weighed. Fluticasone propionate as standard (Sigma-Aldrich, India) (0.05%, topically) was applied on ear of the rats 6 hours prior to application of irritant. Control rats received acetone as a vehicle only (25µl).

7. **Sephadex induced lung inflammation:** A Sephadex (G-200 superfine, Sigma-Aldrich, India) suspension was prepared with sterile 0.9% NaCl, 4-5 days before instillation. Before experiment onset day one, rats were weighed. Test drug treated animals were given ENAL for five days (2, 4, 8g/kg, p.o) and other groups were given normal saline solution for the same days. On sixth day, rats were weighed, anesthetized by Ketamine (50mg/kg, i.m), and intratracheally instilled with 1ml/kg of normal saline or Sephadex (5mg/ml) (Belvisi et al., 2000; Birrell et al.,2000; Guo et al., 1999), Fluticasone propionate (Sigma-Aldrich, India) (0.3mg/ml) (Hammerbeck et al., 2000) followed by Sephadex (5mg/ml) two hours later. Animals were supervised until fully awake and active. 24 hours after instillation, rats were weighed again and sacrificed. Lungs and thymes were excised, rinsed with normal saline and weighed separately and one lobe was preserved in formalin for the histopathological examination while, rest of the lobes were used for anti-oxidant study.

a. **Anti-oxidant study**

i. **Tissue preparation:** Lung tissue was washed in ice-cold 1.15% KCl and homogenized in a homogenizing buffer (50 mM Tris-buffer, pH 7.4) using a Teflon homogenizer. The homogenate was centrifuged at 9000 rpm for 20 minutes at 4°C to remove debris. The supernatant was further centrifuged at 15 000 rpm for 20 minutes at 4°C in cold centrifuge to get post mitochondrial supernatant (PMS) which was used for various biochemical assays.

ii. **Malondialdehyde (MDA) level in lung tissue:** MDA production was determined by the method of (Uchiyama and Mihara, 1978). MDA, an end product of lipid peroxidation reacts with Thiobarbituric acid to form a red coloured complex. The measurement of MDA levels by Thiobarbituric acid reactivity is the most widely used method for assessing lipid peroxidation. To 0.5 ml of supernatant, 0.2 ml of 8.1% Dodecyl sodium sulphate salt (SDS), 1.5 ml of 1% phosphoric acid, 0.2ml of distilled water and 1.0 ml of 0.6% 2-thiobarbituric acid were added. The mixture was heated in a boiling water bath for 45 minutes. Subsequently, the heated mixture was cooled in an ice bath, followed by an addition of 4.0 ml of n-butanol to extract the cold Thiobarbituric acid reactants. The optical density of the n-butanol layer was determined at 532 nm after centrifugation

at 1,000 rpm for five minutes and expressed as ng MDA/g of wet tissue.

iii. **Reduced glutathione (GSH) levels:** Reduced glutathione levels were estimated based on the ability of the SH group to reduce 5,5'-dithiobis- (2-nitrobenzoic acid) to form 1 mole of 2-nitro-5-mercaptobenzoic acid per mole of SH. The method of (Sedlak and Lindsay, 1968) was employed in the determination of GSH levels. To 0.5 ml of supernatant, 1.5 ml of 0.2 mol/L Tris HCl buffer (20 mmol/L EDTA, pH 8.2), 0.1 ml of 0.01 mol/L of 5, 5'-dithiobis-(2-nitrobenzoic acid) and 7.9 ml of methanol were added. The mixture was incubated at 37ºC with occasional shaking for 30 minutes. The mixture was then centrifuged at 3000 rpm 15 minutes and the absorbance of the supernatant was determined at 412 nm. The GSH concentrations of the samples were derived from the standard curve prepared using known amounts of GSH. GSH levels are expressed as µg/g of wet tissue.

iv. **Super Oxide Dismutase (SOD) levels:** To 0.l ml supernatant, 1ml of sodium carbonate (1.06 gm in 100 ml water), 0.4 ml of 24 mM NBT and 0.2 ml of EDTA (37 mg in 100 ml water) was added and zero minute reading was taken at 560 nm. Reaction was initiated by addition of 0.4 ml of 1mM Hydroxylamine hydrochloride, incubated at 25°C for 5 minutes and the reduction of NBT was measured at 560nm and expressed as Units/g of wet tissue. (Kono, 1978).

v. **Catalase (CAT) levels:** CAT activity was measured based on the ability of the enzyme to break down H_2O_2. This method was employed in the assay of CAT activity (Aebi, 1984). 0.4 ml of homogenate added to 980 µl of the assay mixture containing 900 µl of 10 mmol/L of H_2O_2, 50 µl of Tris buffer (pH 8.0) and 30 µl of distilled water. The rate of decomposition of H_2O_2 was monitored spectrophotometrically at 240 nm and expressed as Units/g of wet tissue.

b. **Histopathology of lung tissue:** Paraffin wax embedded lung tissue was cut into about 2 µm sections and mounted on microscope slides. Sections were stained for eosinophils, by use of conventional haematoxylin and eosin. Giemsa stain for detection of micro-organisms.

8. **Statistical analysis:** Values reported were expressed as mean ± SEM. Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Tukey's test. The values were considered to be significantly different when $p< 0.05$.

RESULTS:

Phytochemical screening: Phytochemical analysis of the ENAL revealed the presence of alkaloids, glycosides, sterol, tannins, flavonoids, phenols and quantitative estimation was done for total flavonoid and phenolic contents and found to be 44.03 and 174mg/g of dried extract respectively.

Effect of ENAL on ear weight using croton oil induced ear edema in Sprague-Dawley rats: In croton oil treated group, ear weight increased significantly compared to control group (**Fig. 1**). Five days treatment with ENAL (4, 8g/kg) decreased the ear weight significantly compared to croton oil treated group.

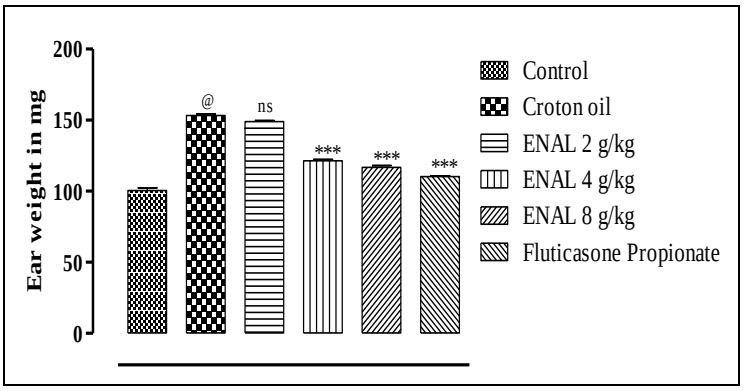

FIG. 1: EFFECTS OF ENAL ON EAR WEIGHT USING CROTON OIL INDUCED EAR EDEMA IN SPRAGUE-DAWLEY RATS. (One way ANOVA followed by Tukey's test). (n=6). @p< 0.001 compared with control group. ***p< 0.001 and ns= non-significant compared with croton oil treated group.

Effects of ENAL on Sephadex induced lung inflammation in Sprague-Dawley rats: Sephadex was used as an allergen, intratracheal administration of Sephadex invokes various inflammatory changes into lung tissue such as eosinoplillia, edema formation, local release of TNF, EPO, eotaxin and cysLT (Evaldsson et al., 2011) which eventually increases the lung weight. In Sephadex treated group, lung weight increased significantly compared to control group (**Fig. 2**). Five

days treatment with ENAL (4,8g/kg) decreased the lung weight significantly compared to sephadex treated group.

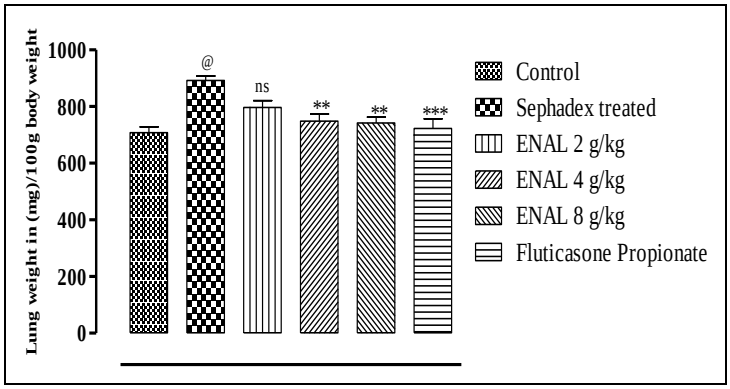

FIG. 2: EFFECTS OF ENAL ON LUNG WEIGHT USING SEPHADEX INDUCED LUNG INFLAMMATION IN SPRAGUE-DAWLEY RATS. (One way ANOVA followed by Tukey's test). (n=6). @p< 0.001 compared with control. **p< 0.01, ***p< 0.001and ns= non-significant compared with Sephadex treated group.

Effect of ENAL on thyme weight using Sephadex induced lung inflammation in Sprague-Dawley rats: It has been observed that entry of an antigen into the systemic circulation leads to increased antibodies formation and lymphatic cells thus eventually causing thymus hyperplasia. Thymus gland is important marker used in immunological events. In Sephadex treated group, thyme weight increased significantly compared to control group (Fig. 3). Five days treatment with ENAL (8g/kg) decreased the thyme weight significantly compared to control group.

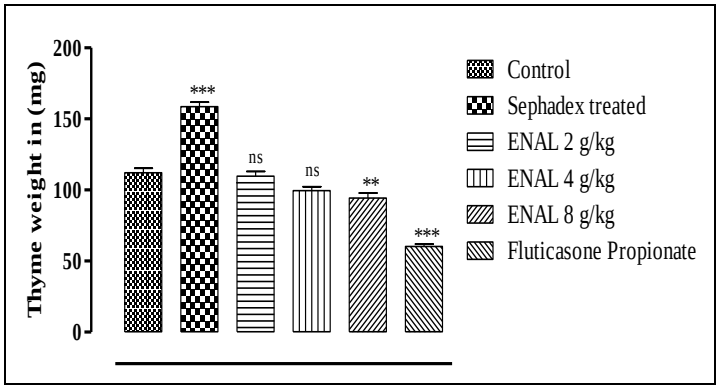

FIG. 3: EFFECTS OF ENAL ON THYME WEIGHT USING SEPHADEX INDUCED LUNG INFLAMMATION IN SPRAGUE-DAWLEY RATS. (One way ANOVA followed by Tukey's test). (n=6). **p< 0.01, ***p< 0.001and ns= non-significant compared with control group.

Effect of ENAL on MDA level in lung tissue using Sephadex induced lung inflammation: The increased level of MDA, which is a major degradation product of lipid peroxidation, leads to cell death. In Sephadex treated group, MDA level increased significantly compared to control group (**Fig. 4**). Five days treatment with ENAL (4,8g/kg) decreased the MDA levels significantly compared to sephadex treated group.

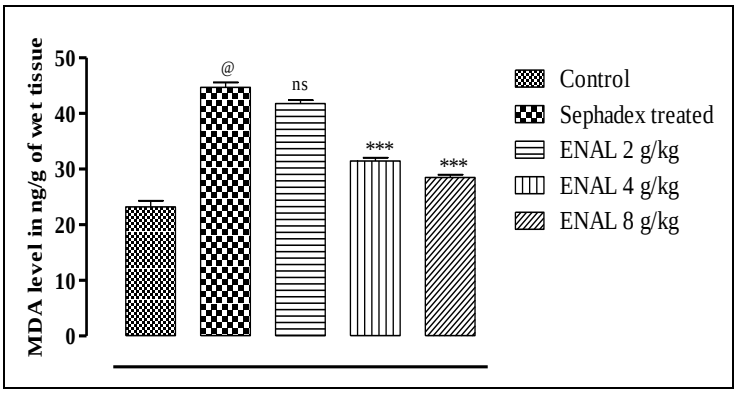

FIG. 4: EFFECTS OF ENAL ON MDA LEVEL IN LUNG TISSUE USING SEPHADEX INDUCED LUNG INFLAMMATION. (One way ANOVA followed by Tukey's test). (n=6). @p< 0.001 compared with control group. ***p< 0.001 and ns= non-significant compared with Sephadex treated group.

Effect of ENAL on GSH level in lung tissue using Sephadex induced lung inflammation: In Sephadex treated group, GSH level decreased significantly compared to control group (Fig. 5). Five days treatment with ENAL (2, 4, 8g/kg) increased the GSH levels significantly compared to sephadex treated group.

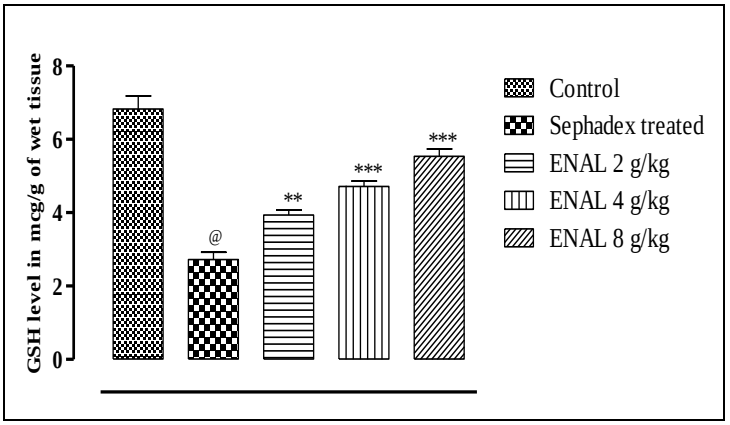

FIG. 5: EFFECTS OF ENAL ON GSH LEVEL IN LUNG TISSUE USING SEPHADEX INDUCED LUNG INFLAMMATION. (One way ANOVA followed by Tukey's test). (n=6). @p< 0.001 compared with control group.

Effect of ENAL on CAT level in lung tissue using Sephadex induced lung inflammation: In Sephadex treated group, CAT level decreased significantly compared to control group (**Fig. 6**). Five days treatment with ENAL (4, 8g/kg) increased the CAT

levels significantly compared to sephadex treated group.

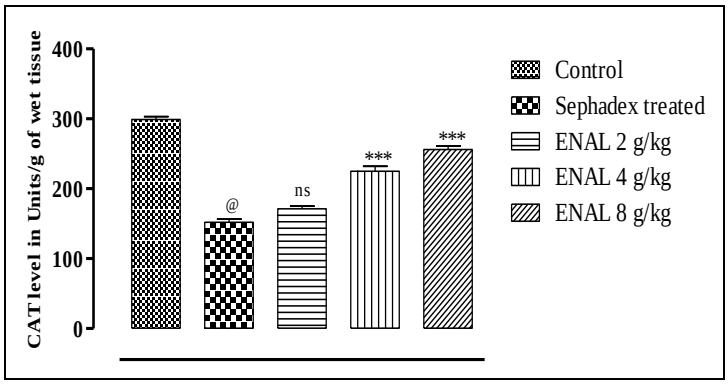

FIG. 6: EFFECTS OF ENAL ON CAT LEVEL IN LUNG TISSUE USING SEPHADEX INDUCED LUNG INFLAMMATION. (n=6). (One way ANOVA followed by Tukey's test). @p< 0.001 compared with control group. ***p< 0.001 and ns= non-significant compared with Sephadex treated group.

Effect of ENAL on SOD level in lung tissue using Sephadex induced lung inflammation: In Sephadex treated group, SOD levels decreased significantly compared to control group (**Fig. 7**). Five days treatment with ENAL (4, 8g/kg) increased the SOD levels significantly compared to sephadex treated group.

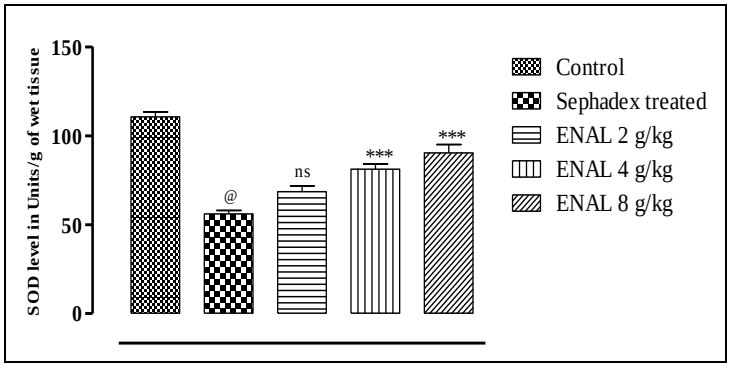

FIG. 7: EFFECTS OF ENAL ON SOD LEVEL IN LUNG TISSUE USING SEPHADEX INDUCED LUNG INFLAMMATION. (One way ANOVA followed by Tukey's test). @p< 0.001 compared with control group. ***p< 0.001 and ns= non-significant compared with Sephadex treated group.

Histopathology of lung tissue: Microscopy was performed to identify the cells of the inflammatory plaques and examine tissue distribution cell types.

No inflammatory cells and inflammation was seen (**Fig. 8, A**), but small congestions in alveoli were observed in some sections from vehicle control group. These increases most likely reflect minor damages from instillation procedure. But overall the lung section was seen normal.

In section from the sephadex treated group (**Fig. 8, F**), beads were seen placed in interstice throughout the lungs, many congestion of RBC's within the alveoli and adjacent tissue, and showing plenty of necrosis, monocytes, polymorphs, monocytes with interstitial inflammation, peribronchial inflammation. Lung section of ENAL (2g/kg) treated group revealed that minimum inflammatory changes (slightly less inflammatory cells and monocytes infiltration) were observed.

However, necrosis was still present. In Fluticasone propionate (0.3g/kg) and ENAL (4 and 8g/kg) treated groups inflammatory changes observed were comparatively less than ENAL (2g/kg). Less congestion of RBC's, reduced monocytes cell infiltration, macrophages and anthracotic pigments were observed. No necrosis was seen in these groups.

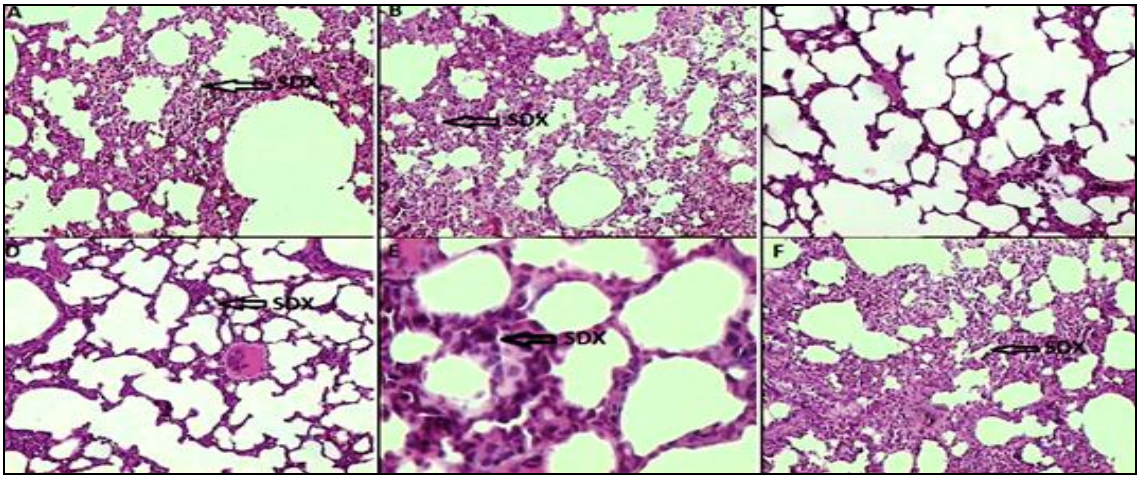

FIG. 8. HAEMATOXYLIN-STAINED LUNG SECTIONS FROM SPARGUE-DAWLEY RAYS. Animals had been intratracheally instilled with **A** Sephadex (SDX, 5 mg/kg) and ENAL (4g/kg, p.o.), **B** SDX (5 mg/kg) and ENAL (2g/kg, p.o.), **C** NaCl (Vehicle control), **D** SDX (5 mg/kg) and Fluticasone propionate (0.3mg/kg) **E** SDX (5 mg/kg) and ENAL (8 g/kg, p.o.) and **F** SDX (5 mg/kg). There was no bacterial infection observed.

DISCUSSION: The present study for the first time ascertains the prophylactic action of ENAL against asthma. In earlier research works, it has been demonstrated that leaves of *Nyctanthes arbortristis* have an anti-inflammatory action. This activity has been verified by using various in vivo animal models such as hyaluronidase induced paw edema (Saxena et al., 1984). Hyaluronidase is known to produce an increase in vascular permeablitiy. Leaves of *Nyctanthes arbortristis was* found to prevent significantly the hyaluronidase induced oedema indicating anti-inflammatory potential of *Nyctanthes arbor-tristis*.

Leaves of *Nyctanthes arbortristis* have also shown anti-inflammatory activity against other models of subacute inflammation such as granuloma pouch technique. In this model, the assessment is done on the basis of weight, thickness of the cavity wall and volume of the exudates to study inflammatory reaction connected with cellular hypertrophy and its sequelae. Inflammatory response is the major pathological event in asthma. Hence, the present study was designed to evaluate antiasthmatic potential of *Nyctanthes arbortristis* using *in vivo* anti-asthmatic models such as Sephadex induced lung inflammation and croton oil induced ear oedema.

In Sephadex induced lung inflammation model, Sephadex was used as an allergen and this allergen upon intratracheal administration invokes various inflammatory changes into lung tissue such as eosinoplillia, edema formation, local release of TNF, EPO, eotaxin and cysLT (Evaldsson et al., 2011) which eventually increases the lung weight. The Sephadex model of lung oedema in the rat is a model of acute alveolitis and bronchiolitis leading to inflammatory cell infiltration and interstitial oedema which appears to parallel many of the pathophysiological features associated with human interstitial lung diseases (Cotgreave, 1988).

In this model, we observed that ENAL significantly prevented the lung inflammation and was as much effective as Fluticasone propionate as indicated by significant reduction in lung weight. Furthermore, ENAL reduced the thymus weight relatively to normal control. This might be due to either of steroidal moiety or flavonoid present in ENAL.

Reactive oxygen species, including superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($OH^{\bullet}$) are generated as byproducts of normal metabolism (Rice-Evans and Miller, 1996). Cumulative oxidative damage leads to numerous diseases and disorders (Halliwel et al., 1991). The enhanced production of free radicals and oxidative stress can also be induced by a variety of factors such as radiation or exposure to heavy metals and xenobiotics (Kim et al., 1990).

Sephadex administration intratracheally caused free radical generation in lung tissue. These free radicals initiate the peroxidation of membrane poly unsaturated fatty acids (PUFA), cell necrosis, GSH depletion, membrane damage and loss of SOD, CAT enzyme activity. The previous study has revealed in

vitro antioxidant activity of *Nyctanthes arbortristis* leaves. Hence , in present investigation in vitro antioxidant activity of different concentrations of ethanol extracts of *Nyctanthes arbortristis* leaves and stem were determined by DPPH radical scavenging assay, Reducing power ability, Hydrogen peroxide scavenging assay and Total antioxidant assay (Narendhirakannan et al., 2010).

The present *in vivo* study demonstrated that ENAL significantly reduced the MDA level in lung tissue and preserved antioxidant enzymes e.g. SOD, CAT and GSH. Thus, it indicates that anti-inflammatory- antiasthmatic action of ENAL is through its antioxidant mechanism.

Moreover, the histopathological study showed microscopically distinguishing changes in lung tissue. Normal lung tissue does not show monocytes infiltration polymorphs and macrophages but it was seen that after 24 hours, intratracheal injection of Sephadex produced the remarkable changes in lung tissue, plenty of eosinophils, monocytes, macrophages and necrosis. However, it was reduced by the ENAL and no anthracotic pigments and necrosis were seen after the treatment of ENAL. These changes strongly suggested that the absence of anthracotic pigments and necrosis might be due to antioxidant activity of ENAL.

Another model used for exploring antiasthmatic potential was croton oil induced ear oedema. The proinflammatory component in croton oil is tumor promoter phorbol 12-myristate 13-acetate (PMA). It is known that epicutaneous application of PMA results in histological and biochemical changes including vascular leakage, leukocyte infiltration, epidermal hyperplasia, activation of protein kinase C, increased release of arachidonic acid and its metabolites, enzyme induction and increased protein, RNA and DNA synthesis (Towbin et al., 1995). The above mentioned biochemical changes occur clinically into the lung in case of asthma. The results of present study revealed that ENAL significantly prevented edema formation in treated ears which confirms that ENAL is capable of inhibiting above mentioned histological and biochemical changes in lung.

Thus, results of present investigation confirm the antiasthmatic potential of leaves of *Nyctanthes arbortristis* which is shown to be mediated through its anti-inflammatory and antioxidant mechanism.

CONCLUSION: This study demonstrated for the first time effectiveness of *Nyctanthes arbortristis* leaves, in prevention of experimentally induced asthma in rats using Sephadex induced lung inflammation and croton oil induced ear edema model. The results of present study indicated that ENAL was as much effective as Fluticasone propionate i.e. standard drug used to treat asthma which might be due to regulation of inflammation and anti-oxidant property. Furthermore, phytochemical screening of ENAL revealed presence of alkaloids, glycosides, sterol, tannins, flavonoids and phenols. Flavonoids are known to have anti-inflammatory activity.

Thus, the results of present research work confirm the antiasthmatic potential of leaves of *Nyctanthes arbortristis,* which is shown to be mediated through its anti-inflammatory*,* and antioxidant mechanism, flavonoids and other phytoconstituents in combination may be responsible for it. Thus, *Nyctanthes arbortristis* could be an important prophylactic agent for the asthma.

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