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ANTI-INFLAMMATORY ACTIVITY OF THE ETHANOLIC LEAVES EXTRACT OF *NYCTANTHES ARBORTRISTIS* ON WISTAR RATS

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ABSTRACT

Leaves of *Nyctanthes arbortristis* was extracted in ethanol to evaluate their anti-inflammatory activities on wistar rats. The anti-inflammatory activity was determined on Wistar rats by formalin induced paw edema method using Diclofenac sodium as standard drug at a dose of 10 mg/kg of body weight. Leaves extract was given at a dose of 200 mg/kg of body weight. Both standard drugs and extract were administered orally to the animals. Control received water orally. Results showed that *Nyctanthes arbortristis* had potent anti-inflammatory activities.

Keywords: *Nyctanthes arbortristis,* Ethanolic extract, Wistar rats, Diclofenac sodium.

I. INTRODUCTION

Ethnobotanical research occurred in the previous couple of decades has revealed the medication and anti-inflammatory properties of plants cited within the ancient literature. Inflammatory diseases embody an enormous array of disorders and conditions that are characterised by inflammation. Examples hypersensitivity reaction, asthma, hepatitis, inflammation of internal organ, preperfusion injury and transplant rejection etc. Those synthetic and semisynthetic drugs have advers effect.As per reduce the advers effect of anti-inflammatory drugs we use herbal drug which is effective but advers effect is very little.The herbal anti-inflammatory drug is Nyctanthes**. [1]**

Nyctanthes arbortristis is a valuable medicinal plant, belongs to the family Oleaceae. The plant generally grows in tropical and subtropical region of India. *N. Arbortristis* is called "Tree of sadness" (arbor-tristis). It also known as Harsinghar, Coral Jasmine, queen of the night and night flowering jasmine. It distributed in sub-Himalayan region and also found in Indian garden as ornamental plant, commonly known as Night jasmine & Parijat. The flowers start falling down after midnight and by the day break, the plant appears dull.The generic name 'Nyctanthes' has beencoined from two greek words 'Nykhta'(Night) and 'anthos' (Flower). It is usually a shrub or a small tree having beautiful, highly fragrant flowers, which bloom at night and fall down on the ground before sunrise, giving the ground underneath a pleasing blend of white and red. After the morning time the plant loses all its brightness and hence.[2]

Different parts of this plant are used in Indian systems of medicine for various pharmacological actions like as anti-leishmaniasis, anti-viral, anti-fungal, anti-pyretic, antihistaminic, anti-malarial, anti-oxidant, anti-inflammatory and many more activities. Herbs have been always the main principle form of medicine since traditions in India and nowa day it becomes most popular throughout the world. Adibasi human of tropical and subtropical regions of the world that has been traditionally used to provoke menstruation, [3, 4] for treatment of scabies and other skin infections as hair tonic, chalogogue and Herbal medicines are not only providing traditional and ethnic medicine but also promising for highly efficient novel bioactive molecules. Since old era, man has been dependent on *N. arbortristis* for Prevent and curing various body diseases. From ancient time various parts of different plants were used to treatment of pain, control suffering and counteract disease. Most of the drugs used in primitive medicine were obtained from plants and are the earliest and principle *N. arbortristis* source of medicines.[5, 6]

II. MATERIALS AND METHODS

A. Collection of Plant Material

The leaves of *Nyctanthes arbortristis* were collected in the month of August 2020 from rural area of Singur, Hooghly, West Bengal, India. The plant was identified by Dr. Sanjit Das, Pharmacognosist, Department of Pharmacognosy, Bharat technology, Uluberia, Howrah. The leaves were cleaned by distill water and dried under shade for 10 days. Coarsely powdered, and stored under air tight container for further study.

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B. Preparation of Ethanolic Extract [7, 8]

The coars powder is collected and weighted, 20gm of coarse powder is added to 60ml petroleum ether and left for 24hrs with occational shaking. The mixture is filter and the filter cake is collected and dried under the fan. The coarse powder is taken and mixed with 70ml of ethanol. The mixture is left for 72hrs. The mixture is filter and the filtrate is collected with carefully. The filtrate is concentrated by simple distillation process. Then the drug is collected in Eppendorf tube and storage in the refrigerator until the use.

A B C D

Figure 1: *Nyctanthes arbortristis* (A), Coarse powder of *Nyctanthes arbortristis* (B), Filtration (C), Extracted Drug (D)

C. Phytochemical Screening [9]

The ethanolic extract of leaves of *Nyctanthes arbortristis* was subjected to preliminary phytochemical screening for the detection of major active compound. The result of different chemical tests on the ethanolic leaves extract of *Nyctanthes arbortristis* showed the presence of alkaloids, amino acids, carbohydrate, flavonoids, glycosides, protein, tannins, tarpenoids, saponins, amino acid and phenolic compounds.

D. Selection and Maintenance of Animals

Healthy adult albino rats (Wistar rats) of either sex, weighing between 150 gm and 190 gm were obtained from M/S Sannyal Enterprises, Kolkata, India. The animals were acclimatized under laboratory condition in a polypropylene cage for 2 weeks before the starting of experiments. They were provided with standard diet and water and maintained under standard conditions of temperature (24 ± 1 ◦ C) and humidity (49%) with an alternating 12 h light/dark cycles. All the studies were conducted in conformity with the proper guidance for care and standard experimental animals study ethical protocols.

E. Acute Toxicity Test [10]

N. arbortristis shown toxic effect of ethanolic extract of leaves in rats in high dose. The median lethal dose (LD) 15 gm/kg was observed in rats. No mortality was at 2.0 gm/kg while 75% mortality was seen at a 30 gm/kg dose. An administration of ethanol extract of the leaves (1, 2 and 5 gm/kg/day) orally for 6 extract also showed irritant effects as it, dose-dependently, the formation of unformed semi-fluid collagenous pasty stools in albino mice because of a purgative effect. When extract instilled into the rabbit's eye produced conjunctival congestion with oedema, while the person who grounded the dried leaves developed vesicles on both palms.

G. Induction of inflammation

Inflammation will be induced in Wistar rat (males) by formalin 0.1ml (2.5%) is inject in right hind paw.

F. Experimental Design

After 14 days accilmatization, male wistar rats will be randomly divided into four groups (n=6). The wistar will be pre-treated daily with leave extract of *Nyctanthes arbortristis* (200mg/kg) 3 days, then wistar rats will be treated with Ds (diclofenac Sodium) 10mg/kg,p.o. for 1 week. Each group will receive the following treatment.

Group I-(Normal control):

Will receive clean water and normal food. No drug given.

Group II-(Formalin control):

Will receive formalin (0.01ml/2.5%) after 7days for one time.

Group III-(Test):

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Will receive extracted drug from leaves of *Nyctanthes arbortristis* (200mg/kg; p.o.) for 3days and formalin (0.01ml/2.5%;right hind paw) for one time after 30 mins of given the test drug.

Group IV-(Standard):
Will receive diclofenac sodium (10mg/kg; ip) for 3 days and formalin (0.0 1ml/2.5%; right hind paw) for one time after 30mins of given Diclofenac sodium.
At first the animal's right hind paw will measure by screw gauge and fed them for 6days.After 6days Normal controls are no drugs given, then Formalin controls are given formalin in right hind paw for one time. Then test group are given the Nyctanthes 200mg/kg; p.o. after 30min given formalin 0.01ml/2.5%;right hind paw, Then the standard group are given diclofenac sodium 10mg/kg;IP. After 30min will give formalin 0.01ml/2.5%;right hind paw.
After given the all dose measure the right hind paw inflamed of all groups animals and take the reading after induced (1hr, 3hrs, 6hrs, 24hrs, 72hrs).

G. Statistical Evaluation
Result will be expressed Mean ± SEM from 6 animal in each group comparison the groups made by using one way analysis of variance (ANOVA) followed by dunnett's multiple comparison test using graph pad prism version 9. $P<0.05$ will be considered as stastically significant.

III. RESULTS AND DISCUSSION

A. Effect of administration of formalin (0.01ml S.c) in paw inflamed wistar rats:
In formalin (0.01ml/2.5% s.c) treated paw oedema of '0' days (before induced), 1hrs, 3hrs, 6hrs, 24hrs and 72hrs were found to be 3.40±0.08, 6.50±0.1, 7.28±0.05, 7.61±0.127 and 7.21±0.12 respectively. Thus, formalin significantly increase of the paw oedema against normal group (I).

B. Effect of administration of diclofenac sodium (10mg/kg, I.P) in formalin induced paw inflamed wistar rats:
In Diclofenac sodium (10mg/kg,I.P) treated rats paw oedema of '0'days (before induced), 1hrs, 3hrs, 6hrs, 24hrs and 72hrs,were found to be 3.46±0.08, 06.31±0.1, 5.06±0.03, 4.67±0.02, 4.34±0.082 and 4.14±0.03 respectively. The above value have significantly decrease the paw oedema when compared to formalin group (II).

C. Effect of administration of ethanolic extract of leaves of Nyctanthes (200mg/kg,p.o.) in formalin induced paw inflamed wistar rats:
In extracted drug from leaves of *Nyctanthes arbortristis* (200mg/kg,p.o.) treated rats paw oedema of '0'days (before induced), 1hrs, 3hrs, 6hrs, 24hrs and 72hrs,were found to be 3.42±0.09, 6.11±0.15, 5.71±0.07, 5.71±0.01, 5.06±0.024 and 4.65±0.04 respectively. The above values have significant decrease the paw oedema when compared to formalin group (II).

Table 1. Effect of ethanolic extract of leave of Nyctanthes in formalin induced paw inflamed rats.

Group	Treatment	'0'Days	1 hrs	3 hrs	6 hrs	24 hrs	72 hrs
I	Normal control	3.35±0.10	3.35±0.1	3.37±0.1	3.34±0.01	3.37±0.01	3.34±0.1
II	Formalin control (0.1ML IP 2.5%)	3.40±0.08	6.50±0.1	7.28±0.05	7.61±0.06	7.67±0.127	7.21±0.12
III	Standard (0.1 ml Formalin+ Diclofenac sodium 10mg/kg)	3.46±0.08	6.31±0.06	5.06±0.03	4.67±0.02	4.34±0.082	4.14±0.03
IV	TEST (0.1ML Formalin + Extracted Drug 200mg/kg)	3.42±0.09	6.11±0.15	5.71±0.07	5.71±0.01	5.06±0.024	4.65±0.04

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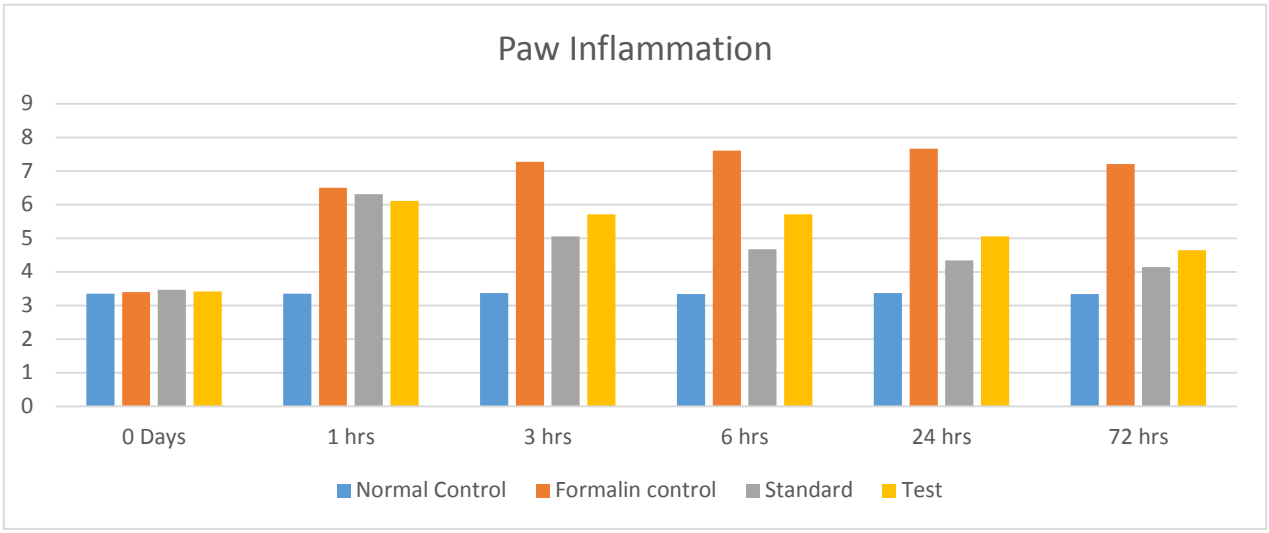
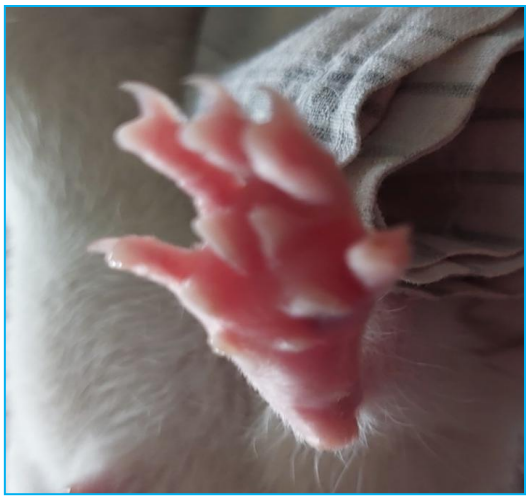


Figure 2: Graphical representation of activity of extracted drug on Paw inflamed rats.

A

B



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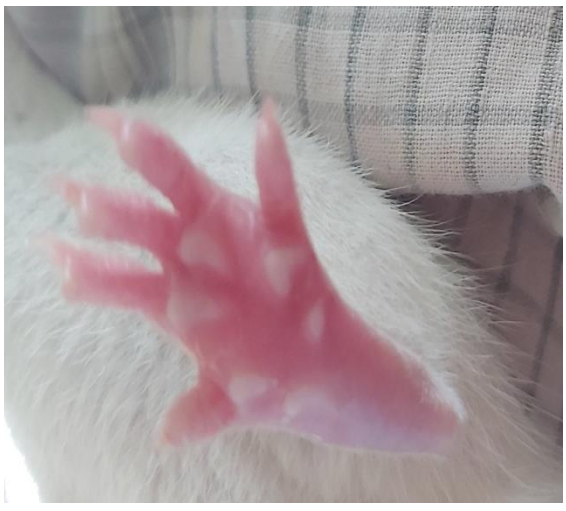

D

Figure 3: Normal control (A), Formalin control (B), Standard (C), Test (D)

Formalin administered at an occasional dose induces an edema that in the main results from an animal tissue inflammation mediate by neuropeptides like substance P. At higher doses, solution induces an edema that in

the main depends on the discharge of substance P, prostanoids, serotonin and amine. Bradykinin plays no important role within the vascular changes wherever as this amide has been rumored to participate within the stimulation of sensitive sensory neurons. This discrepancy can be explained by a distinction within the threshold of stimulation of the sensitive neurons which of the cells of the vascular walls, or by a formation of kinins in shut contact of the neurons. [11, 12] Ethanolic extract of phytochemicals like lupeol improves the paw oedema by various pathway. It is improves at low dosage inflammation by neurogenic inflammation mediated by neuropeptides such as substance p way. It also improve the higher dose way such as substance p, prostanoids, 5-hydroxytryptamine and histamine. [11, 13]

IV. CONCLUSION

The present study reveals that the ethanolic extract of *Nyctanthes arbortistis* significantly improved the paw oedema in formalin induced paw inflamed rats. Thus it can be concluded that the ethanolic extract of leaves of is potential against to formalin induced paw inflamed.

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