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Research Article

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Anti-inflammatory and analgesic activity of methanolic and chloroform extract of leaves of *Nyctanthus arbortristis* Linn.

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ABSTRACT

Nyctanthus Arbortristis Linn. is an medicinal plant having application in various disorders and have particular emphasis on potency specified in the screening assays. The present study was designed to evaluate the anti-inflammatory and analgesic activities of the methanolic and chloroform extract of leaves of Nyctanthus Arbortristis Linn. (Family-Oleacea).The aim of the study is to access the anti-inflammatory activity against carrageenen induced Rat Paw oedema. The analgesic activity was determined for its central and pharmacological actions using Eddy's Hot Plate, and Tail Immersion Method. Therefore, it concluded the methanolic and chloroform extract of the leaves of Nyctanthus Arbortristis possessing anti-inflammatory and analgesic activities.

Key words: *Nyctanthus arbortristis* Linn., Anti-inflammatory Activity, Analgesic activity, Rat paw oedema method, Cotton pellet granuloma method, Tail immersion method, Eddy's hot plate method.

INTRODUCTION

The practice of herbal medicine dates back to the very earliest period of known human history. There is evidence of herbs having been used in the treatment of diseases and for revitalising body system in almost all ancient civilisation. Ayurveda, the Science of Life,has provided the rational basis for treatment of various ailments, Pain, inflammation and fever are very common complications in human beings.Several plants and their products are claimed and proved to posses analgesic and anti-inflammatory property. *Nyctanthus Arbortritis* Linn.(family-Oleacea) is an aromatic herb which grows upto10m.The leaves are dessicately opposite, ovate, crenate to subcrudate at base.Over the last decades the leaves has been used in the treatment of inflammatory, analgesic, hepatoprotective and particular emphasis on potency specified in the screening assays. Hence in the present study, the methanolic and chloroform extract of the leaves of *Nyctanthus Arbortristis*Linn.was examined for its anti-inflammatory and analgesic properties.

EXPERIMENTAL SECTION

Plant Material

The leaves of *Nyctanthus Arbortrististis* Linn. were collected from the hills of Sathuragiri [viruthunagar District,T.N], India in the month of November-2012 and were identified by the department of botany, Kalasalingam University, Tamilnadu. The plant material were washed under running tap water to remove adhering dust, dried under shade and pulverised to get coarse powder.

Preparation of extract

The coarsely powdered parts were soxhlet extracted with methanol and chloroform for 8h. The filtrate was evaporated to dryness under reduced pressure using rotary vacuum evaporator. The extracts were lyophilised until further use. [Extracted value for methanol 12%, chlorolform 3-6% w/w].

Selection of animal, caring & handling

This was done as per the guidelines set by the Indian National Science Academy, New Delhi, India. Healthy Wistar rats of either sex and of approximately the same age, weight about 180-300gm were used in the study.They are fed with standard chow diet and ad libithium. They were housed in polypropylene cages and maintained under standard environmental conditions like temperature(27.0 $\pm$ 1.0^{o}c), relative humidity(55-65%) and 12hr light/12hr dark cycle. The experimental protocol was subjected to scrutinise of the Institutional Animal Ethical Committee and was cleared by same before starting.

Acute toxicity studies:

The acute toxicity studies of methanol and chloroform extract of *Nyctanthus Arbortritis* Linn. were carried out according to OECD guidelines. 200mg/kg dose of both the extracts were found to be non-toxic in rats and it was taken for further study.

Anti-inflammatory activity:

STUDY DESIGN

1) For acute inflammation: Carrageenan induced rat paw oedema.
2) For sub-acute inflammation: Cotton Pellet Granuloma method.

Carrageenan induced rat paw oedema inhibition method:

Wistar albino rats were divided into 4 groups each containing 6 rats.

Group I : Control treated with Normal Saline per orally(0.1ml).
Group II : Diclofenac(20mg/kg) per orally.
Group III : Test group A: Chloroform extract(200mg/kg) per orally.
Group IV : Test group B: Methanolic extract(200mg/kg) per orally.

Acute inflammation was produced by injecting 0.1ml of 1% Carrageenan suspension in normal saline into the subplantar region of the right hind paw after 30 minutes of drug administration. A mark was made on the leg at the malleous to facilitate uniform dipping at subsequent readings. The volume of paw oedema was measured with the help of Plethysmograph by mercury displace method immediately before & 3 hours after drug administration. The percentage inhibition of oedema in various treated groups was then calculated by using statistical analysis.

Cotton Pellet Granuloma Method

Rats were divided into four groups as earlier, each group consisting of 6 rats. Under light ether anaesthesia, the hair in the axillary and groin region were cut and sterile cotton pellets of 10mg each were implanted in the subcutaneous tissue on either sides of axilla and sterile grass pith (25x2mm) in the groin region. Wounds were then sutured and animals were then caged individually after recovery from anaesthetia. The rats then received treatments as described earlier. The scheduled drug administration was started on the day of implantation and repeated every twenty four hours, regularly for 7 days. Change in food intake, motor activity, and diarrhoea, if any were noted.

On the 8th day, the rats were sacrificed and cotton pellets and grass piths were removed. The pellets free from the tissue were dried overnight to their dry weight. Net granuloma formation was calculated by subtracting the initial weight noted (i.e.10mg). The grass piths were served in 10% formalin for histopathological studies.

Analgesic activity

Eddy's hot plate:

The hot plate test was used to measure response latency according to the method described by Eddy and Leimbach (1953). The hot plate maintained at constant temperature (55^{o}c).

Wistar Albino rats weighing 150-200gm were divided in four groups of 6 animals each:

Group-I : Normal saline(10ml/kg).
Group-II : Pentazocin(25mg/kg).
Group-III : Methonolic Extract(250mg/kg).
Group-IV : Chloroform Extract(500mg/kg).

Animals were placed in a pers pex cylinder on a heated surface, and the time placement of the animal on the hot plate and the occurance of discomfort, indicated by either licking the paws (or) jumping on the surface was recorded as response latency.

Mice with baseline latencies of more than 10sec were eliminated from the study, and the cut off time for hot plate latency was set at 15sec (Franzolti - et al; 2000). The latency of discomfort was measured at 0,30,60,90, & 120 min after oral administration of the test sample. The average basal reaction time and the % increase in basal reaction time were calculated using standard t-test.

Tail Immersion Method

The given samples of methonolic and chloroform extracts were evaluated using the Tail Immersion method (Chandrashekar KS. 2000).Wistar Albino rats weighing about 150-200gm were taken.They were divided in to 7 groups having 6 each, numbered and placed in to individual restraining cages leaving the tail hanging out freely. The animals are then allowed to adopt in the cages for 30min before testing. The lower 5cm portion of the tail was marked and immersed in to a cup of freely filled warm water of exactly 55^{o}c.

Within a few second the rat reacts by withdrawing the tail. The reaction time was recorded by the stop watch. After each determination the tail was carefully dried. The reaction was determined before oral feeding of the drug and methanol and chloroform extract (250mg/kg and 400mg/kg body weight p.o) which was recorded as 0min reading. The control standard, and test substances were given to the animals by gastric tube.

After the drug was administered the reaction time was recorded at an interval of 1/2hr, 1hr, 1 1/2hr, 2hr and 2 1/2hr. The cut off time of the immersion in 15seconds. The mean reaction time was recorded for each group and compared with the value of standard drug pentazocin.

Statistical analysis

Statistical analysis was carried out using repeated measures ANOVO followed by Dunnet's multiple comparison tests using Graph pad prism software version 4.03*P values<0.05 were considered significant.

RESULTS AND DISCUSSION

Effect on carrageenan induced paw oedema inhibition test (Table -1, Graph-1)

In carrageenan induced rat paw oedema test, the doses of 200mg/kg and 200 mg/kg methanolic and chloroform extract of the leaves of *Nyctanthus Arbortristis*Linn. plant showed statistically significant (P<0.05) inhibitory effect on"mean increase in paw volume". *Nyctanthus Arbortristis*Linn. showed acute anti-inflammatory activity higher than control group, but it did not show so strong effect as Diclofenac sodium, which produced significant inhibition (58.13%) (P<0.05) .It was found that reduction in the inflammation was 48.83 % (P<0.05) with 200mg/kg methanolic extract and 53.48 % (P<0.05) with 200 mg/kg of chloroform extract.

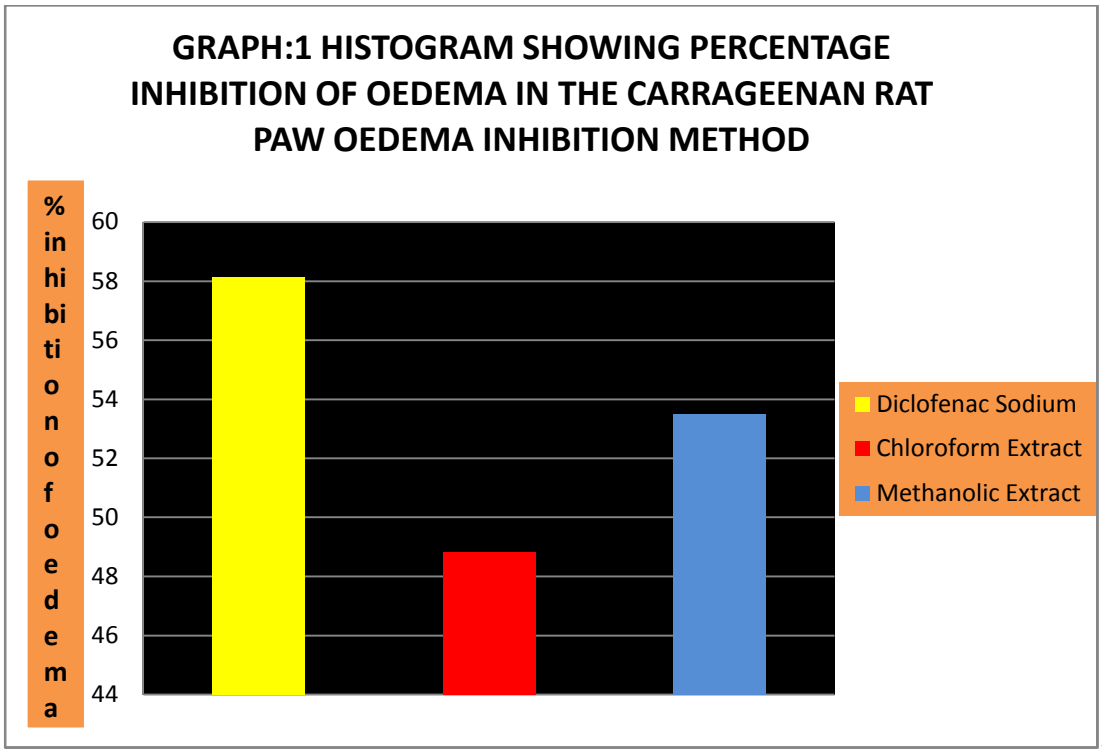


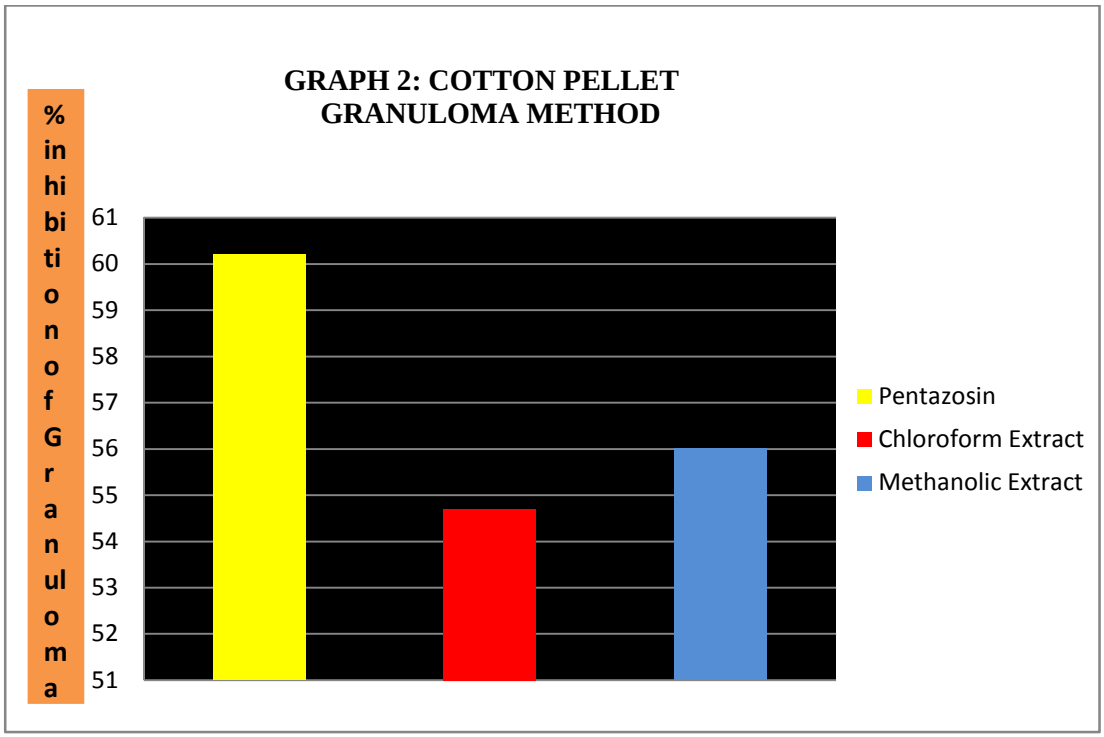
Table 1: Showing oedema volumes (ml) in carrageenan induced rat paw oedema inhibition test

Groups	Dose/route	Paw oedema(ml) (%)Mean ± S.E.M	% of Inhibition	P value
Group I (Control)	0.1 ml/kg(p.o)	0.43±0.30	-	-
Group II (Diclofenac Sodium)	20mg/kg (p.o)	0.18±0.33	58.13	0.05*
Group III Test - A	200 mg/kg(p.o)	0.22±0.30	48.83	0.05*
Group IV Test-B	200mg/kg (p.o)	0.20±0.36	53.48	0.05*

Table 2: Dry weight of cotton pellet in the cotton pellet granuloma method

Groups	Dose/route	Paw oedema ml (%) Mean ± S.E.M	% of Inhibition	P value
Group I (Control)	0.1 ml/kg(p.o)	18.27±6.32	-	-
Group II (Pentazocin)	20mg/kg (p.o)	7.27±0.18	60.20	0.05*
Group III Test - A	200 mg/kg (p.o)	8.27±0.34	54.70	0.05*
Group IV Test-B	200mg/kg (p.o)	8.03±0.27	56	0.05*

*p.o: per orally, S.E.M: Standard Error of Mean *P < 0.05: Significant with respect to control group*



Effect on tail flick response in rat (Table 3, Graph 3)

In the analgesic activity test using tail immersion method methanol and chloroform extract at a dose of 250mg/kg and 500mg/kg exhibited significant($P<0.0001$ and $P<0.001$ respectively) inhibition of pain. While the standard drug pentazocin inhibition was found after 180 minutes at a dose of 25mg/kg body weight(Table 3). So it can be claimed that the analgesic activity of *Nyctanthus Arbortristis* extract was Significant in comparison with negative control animals, as the extract at the doses of 250mg/kg and 500mg/kg body weight showed significant analgesic activity in mice.

Table 3: Effect of methanolic & chloroform extract of leaves of *Nyctanthus arbortritis* Linn. on tail flick response in rat

Group	Treatment (mg/kg)	Mean Pain ± S.E.M (Seconds)
Control-Normal Saline	10 ml/kg	2.09±0.47
Standard-Pentazocin	25mg/kg	4.91±1.20
Methanolic Extract	200mg/kg	3.50±0.17
Chloroform Extract	200mg/kg	3.82±0.48

Effect on hot plate induced pain in rat (Table-4, Graph- 4)

Hot plate test is commonly used to assess narcotic analgesics, other centrally acting drugs, including sedatives and muscle relaxants (or) Psychotomimetics have shown activity in this test. However, in contrast

to the effect for morphine, indomethacin and other NSAIDS have no effect according to the hot plate test (Yamamoto and Nozoki -Taguchi, 1996)- Table 4 shows the result of the hot plate test.

Table 4:-Effect of methanolic & chloroform extract of leaves of *Nyctanthus arbortristis* Linn. on hot plate induced pain in rat

Group	Treatment (mg/kg)	Mean Prodrug Reaction time ±S.E.M	Mean Post drug Reaction time ±S.E.M
Control-Normal Saline	10mg/kg	2.31±0.19	2.11±0.58
Standard-Pentazocin	25mg/kg	2.57±0.54	4.07±0.74
Methonolic Extract	200mg/kg	2.31±0.60	3.07±0.38
Chloroform Extract	200mg/kg	1.66±0.12	3.21±0.51

CONCLUSION

The result of the current investigation reveals that the chloroform and methanolic extracts of the leaves of *Nyctanthus arbortristis* Linn. possess protective effect against inflammation and pain sensation. The methanolic and chloroform extract significantly showed analgesic and anti-inflammatory activity compared to standard drug. Hence the methanolic extract has more analgesic and anti-inflammatory in a dose dependent manner when compared to standard drug Pentazocin and Diclofenac Sodium.

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