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Acute Anti-inflammatory activity of Eraippu Noi Chooranam on carrageenan induced paw edema in wistar albino rats

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Abstract

The aim of the present study was to explore the probable anti-inflammatory activity of aqueous extract of Eraippu Noi Chooranamusing Carrageenan induced inflammation in wistar rats. The medicinal value of the ingredients of ENC has been mentioned in ancient literature as useful in disorders of inflammation. The anti-inflammatory effect was done by carrageenan induced hind paw edema method using digital plethysmometer. Wistar rats were treated orally with distilled water (as negative control group), 10mg/kg of Diclofenac sodium in normal saline intra peritonially (as standard group) and aqueous extract of ENC (135, 270 and 400 mg/kg) as test group 30 min before 0.1 mL 1% carrageenan injection. Paw volume was measured before and at 0,30 min, 1, 2, 3, 4, 5 and 6th hr after the injection of carrageenan. The results were expressed as the Mean ±SEM and the statistical significance of differences between groups was analyzed by One Way Analysis of Variance (ANOVA) followed by Dunnett's test. The sub plantar injection of carrageenan caused a time-dependent paw edema in the rats. Oral administration of aqueous extract of ENC(135, 270 and 400 mg/kg) inhibited paw swelling. The maximum inhibition of paw volume was observed at 2 and 3 hr after treatment with ENC at each does but the activity was almost static after 4 hrs. We can conclude from the outcome of the present work that the low dose of ENC exhibits similar effect as comparable to the high dose which shows low dose of ENC itself possess significant anti inflammatory activity.

Keywords: Eraippu Noi Chooranam, Anti-inflammatory, Carrageenan, Diclofenac sodium, Aqueous extract

Introduction

The World Health Organization (WHO) defined health as "a complete state of physical, mental, and social well-being and not merely the absence of disease or infirmity." So during the past decade, traditional systems of medicine have become a topic of global importance. Current estimate suggest that, in many developing countries a large proportion of the population relies heavily on traditional practitioners and traditional medicines to meet primary health care needs. Although modern medicine may be available in these countries, traditional medicines have often maintained popularity for historical and cultural reasons. Concurrently, many people in developed countries have begun to turn to alternative or complementary therapies, including medicinal herbs.

Traditional medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about their mode of action is available. Many traditional medicines are being prescribed widely for the treatment of inflammatory conditions. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional systems of medicine.

Thus, the present investigation was carried out to evaluate the antiinflammatory potential of Eraippu Noi Chooranamin experimental animal models.

Materials and Methods

Procurement and Authentication of Raw Drugs

The ingredients of ENC was purchased from reputed country shop. The raw drugs were identified and authenticated by medicinal botanist of National Institute of Siddha. All the ingredients were powdered and bottled up at the ratio mentioned.

Preparation of Eraippu Noi Chooranam.

The raw drugs were purified as mentioned in Siddha literature. ENC was prepared using the procedure described in Siddha literature with slight modifications.

Animal Care and Husbandry

The study protocol involving animals was reviewed and approved by Institutional Animal Ethical Committee (IAEC), KMCH College of pharmacy, Kovai estate, with the experimental protocol number IAEC NO: KMCRET/MD(S)/10/2014-15. Experiments were performed as per the instructions prescribed by the Committee for the Purpose of conduct and Supervisions of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India. Male and female Wistar albino rats, (150-200 g) obtained from Sree Venkateshwara Enterprises Pvt. Ltd, Bangalore, were housed in the animal house KMCH College of pharmacy, Kovai estate. Each group of rats was separately housed in polypropylene cages in a well ventilated room under an ambient temperature of 22±3°C and 30-70% relative humidity, with a 12-h light/dark artificial light cycle. They were provided with food (SaiDurga Animal Feed, Bangalore) and purified water ad libitum.

Acclimatization

The rats were acclimatized for 3 days to the laboratory conditions and were identified by a unique tail marking using permanent red marker pen. During the acclimatization, individual animal was subjected to daily general observation and prior to final assignment to the study the animals were subjected to a detailed clinical examination to ensure the selected rats were in a good state of health.

Randomization & Grouping

Following completion of acclimatization, the rats were randomized into 5 groups, each group consisting of 6 animals

Acute Oral Toxicity Study

The lethal dose (LD 50) OF ENC was determined by OECD guideline (423 guideline). The LD50 of the test drug ENC was found to be more than 2000 mg/kg .

Evaluation of Anti-Inflammatory Activity

Carrageenan-induced Paw Edema in Rats:[1](Acute Study)

Experimental design

Group-I: Negative control (0.1ml of 1% carrageenan)
Group-II: Standard Diclofenac sodium (10mg/kg, i.p) + (0.1ml of 1% carrageenan)
Group-III: Eraippu Noi Chooranam (135mg /kg) + (0.1ml of 1% carrageenan)
Group IV: Eraippu Noi Chooranam (270 mg/kg) + (0.1ml of 1% carrageenan)
Group V: Eraippu Noi Chooranam (400 mg/kg) + (0.1ml of 1% carrageenan)

Preparation and administration of dose

The doses of - Eraippu Noi Chooranam were prepared in distilled water, whereas Diclofenac sodium was dissolved in normal saline.

Procedure

One day before the experiment, three basal readings of hind paw in each rat were recorded. Group I received distilled water, Group II animals received Diclofenac sodium (10 mg/kg i.p). Group – III, IV &V animals received the Eraippu Noi Chooranam 135 mg/kg, 270mg/kg, and 400mg/kg respectively. Prior to the above administrations, food alone was withdrawn from all the groups overnight (water was provided ad libitum). After 30 minutes of respective treatment, the rats were challenged with subcutaneous carrageenan injection (0.1 ml of 1% carrageenan) into the sub plantar region of left paw to produce the acute inflammation. At the level of lateral aspect of malleolus, the paw was marked with ink and immersed in mercury up to the mark. Digital Plethysmometer was used to measure the paw volume at 0,30 min, 1, 2, 3, 4, 5 and 6th hr after carrageenan injection. The difference between initial reading and subsequent reading gave the actual edema volume. The average paw edema in the group of ENC treated rat was compared with control. Percent inhibition in inflammation was calculated by the formula

% of inhibition = [1-(Vt/Vc)] × 100 Where,

Vt is Increase in paw volume in test animal and
Vc is Increase in paw volume in control group.
The results were obtained as mean increase in paw volume (ml) and percentage inhibition in paw oedema.

Statistical analysis

The statistical analysis of the evaluation of the anti-inflammatory activity of aqueous extract of ENC against the carrageenan induced paw oedema in Wistar rats were analyzed using Anova followed by Dunnett's t test and expressed as mean ± SEM. Differences between the mean of treated animals and control groups were considered significant at $P < 0.05$

Results

The results of carrageenan induced rat paw edema are illustrated in Table 1. The result obtained indicates that the test drug exhibit significant ($P < 0.05$) anti-inflammatory activity in wistar rats. The ENC at the test doses 135,270 and 400 mg/kg bw reduced the edema caused by carrageenan induced method to 34.12% & 34.26% and 34.56% respectively at 6hrs whereas the standard drug Diclofenac sodium showed 39.6% of inhibition at 6hrs when compared to the control group. The pre-treatment with ENC resulted in a significant reduction ($P < 0.05$) in carrageenan induced paw edema in rat (Table1 and fig1). The percent inhibition at 1 hr after treatment with ENC was comparatively less at all the doses when compared to the effect of Diclofenac sodium. The maximum inhibition of paw volume was observed at 2 and 3 hr after treatment with ENC at each does but the activity was almost static after 4 hrs (Table2and fig2). The low dose of ENC exhibits similar effect as comparable to the high dose which shows low dose of ENC itself possess significant anti inflammatory activity.

Table 1 Effect of Eraippu Noi Choranam on carrageenan-induced paw oedema

Groups	Mean increase in paw volume (CU.MM)							
	0 min	30 min	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
Control	3.735 ± 0.15	6.575 ± 0.25	7.163 ± 0.16	7.277 ± 0.22	7.243 ± 0.16	7.087 ± 0.27	6.923 ± 0.13	6.802 ± 0.11
Standard	3.8±0.23	6.067± 0.28	4.913 ±0.18**	4.69 ±0.20**	4.51 ±0.19**	4.327 ±0.15**	4.183 ±0.15**	4.057 ±0.17**
ENC LD	4.067± 0.19	6.177±0.11	6.78±0.17	5.825±0.15**	5.125±0.08**	4.885±0.14**	4.57±0.16**	4.477±0.18**
ENC MD	4.088± 0.15	6.278±0.19	6.59±0.13	5.918±0.16**	5.352±0.13**	4.925±0.12**	4.58±0.13**	4.468±0.13**
ENC HD	4.01±0.18	7.005±0.21	6.377±0.18**	5.718±0.12**	5.295±0.9**	4.865±0.14**	4.617±0.15**	4.447±0.15**

Results are expressed as Mean± S.E.M. where n=6, Statistical analysis done by using ANOVA followed by Dunnett's testwhere **p<0.01 Group II, III, IV, V compared with Group I.

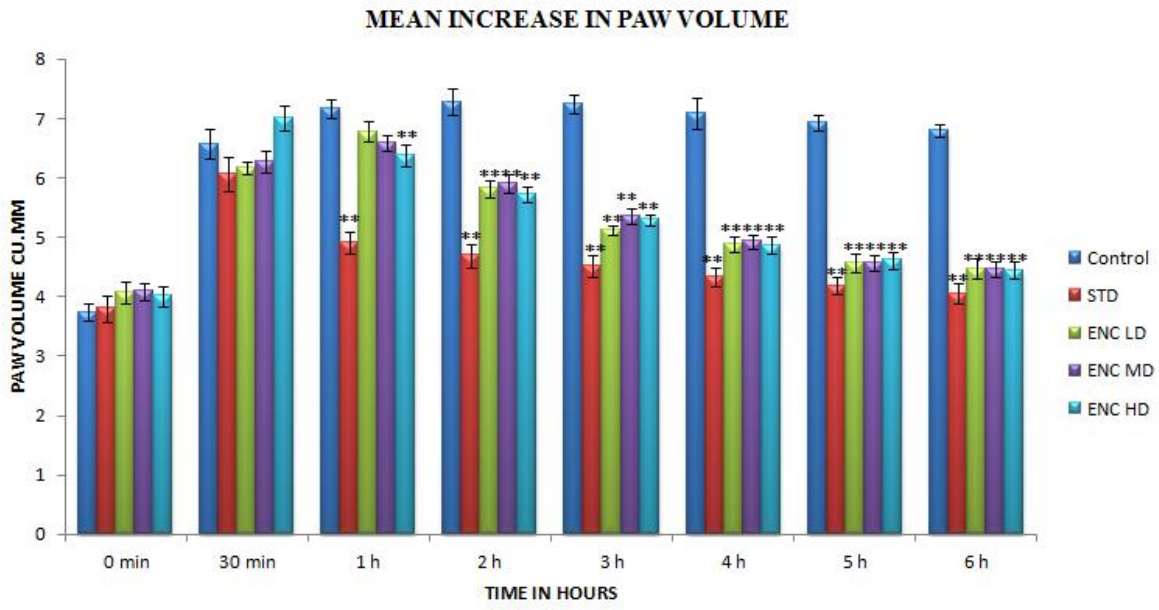

Fig.1 Effect of ENC on Mean increase in paw volume of carageenan induced paw edema in rat models

Results are expressed as Mean± S.E.M. where n=6, Statistical analysis done by using ANOVA followed by Dunnett's testwhere **p<0.01 Group II, III, IV, V compared with Group I.

Table 2 Effect of Eraippu Noi Choranam on % of inhibition of edema

GROUPS	% of inhibition					
	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
STANDARD	31.42	35.58	37.71	38.79	40.08	39.6
ENC LD	5.31	19.92	29.14	31.03	33.96	34.12
ENC MD	7.96	18.68	26.1	30.47	33.82	34.26
ENC HD	10.89	21.29	26.8	31.31	33.24	34.56

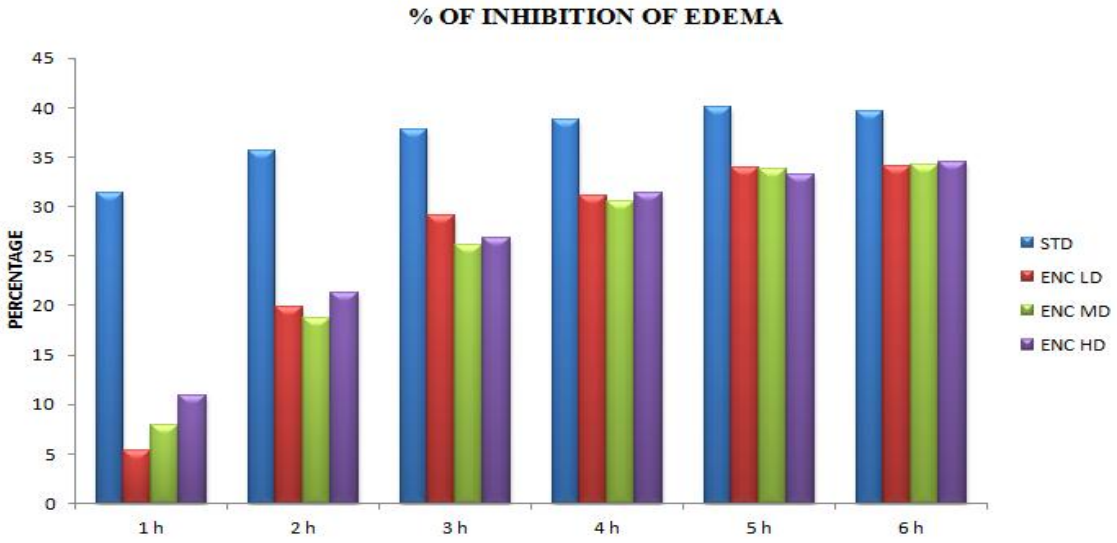

Fig.2Effect of Eraippu Noi Choranam on % of inhibition of edema

Discussion

Carrageenan is the known agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and devoid of apparent systemic effect. It induces oedema is a biphasic response which is shown to be mediated by histamine and serotonin during first 1hr. After which increased vascular permeability is maintained by the release of kinins upto 2.5 hr, followed by the release of kinins and finally through the release of bradykinin, prostaglandin and lysosomes from 2.5 to 6 h. The later phase is reported to be sensitive to most of the clinically effective antiinflammatory agents[2]. The mediators of this phase appear to be prostaglandins, the release of which is closely associated with migration of leucocytes into the inflamed site[3].The paw edema induced by Carrageenan model in rats is known to be sensitive to cyclo-oxygenase (COX) inhibitors and was used to evaluate the effect of non-steroidal anti-inflammatory drugs (NSAID)[4,5.] This method was selected for this study since it is the most prominent experimental model in search for new antiinflammatory drugs and evaluation of anti-inflammatory effect of natural products[6,7]. It was found that the administration of the test drug ENC at all the three doses of 135,270 and 400 mg/kg, p.o.) and Diclofenac sodium (10mg/kg, i.p) play a crucial role as protective factors against the acute inflammation induced by carrageenan.

The injection of carrageenan produces a typical biphasic oedema which was associated with the production of several inflammatory mediators such as histamine, bradykinin, prostaglandins, serotonin, nitric oxide, and cytokines[8,3,6]. The results revealed that administration of ENC inhibited theedema starting from the first hour andduring all phases of inflammation, whichis probably due tothe inhibition of differentaspects of chemical mediators ofinflammation.

Conclusion

From the above study it can be suggested that Eraippu Noi Chooranam possess promising anti-inflammatory activity.

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