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Antipyretic, anti-inflammatory and analgesic properties of nilavembu kudineer choornam: a classical preparation used in the treatment of chikungunya fever

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ABSTRACT

Objective: To investigate the efficacy of ethanolic extract of nilavembu kudineer choornam (EENKC) in inflammation, pain and fever using animal models to support its actions. **Methods:** Acute toxicity study of EENKC was performed in mice to fix the effective dose. The antipyretic, anti-inflammatory and analgesic activity of EENKC was evaluated in brewer's yeast induced pyrexia in rats, carrageenan-induced inflammation in rats and acetic-acid induced writhing in mice model. **Results:** Acute toxicity revealed that EENKC didn't show death and toxic signs up to 2 000 mg/kg. In brewer's yeast induced pyrexia and carrageenan-induced inflammation EENKC at the doses of 200 and 400 mg/kg inhibited fever and inflammation significantly ($P<0.01$ and <0.05) compared to control animals. In mice, the number of writhing induced by acetic-acid was significantly ($P<0.01$) reduced after treatment with both the dose of EENKC than control animals. EENKC 200 mg/kg inhibits inflammation higher level in carrageenan-induced paw edema, but there is no significant difference when compared to indomethacin 10 mg/kg. **Conclusions:** The present findings revealed that EENKC possesses antipyretic, anti-inflammatory and analgesic activity which supports nilavembu kudineer choornam efficacy in chikungunya fever.

1. Introduction

Chikungunya fever is a viral disease caused by an alpha virus that is spread by bite of *Aedes aegypti* (*Ae. aegypti*) mosquito. The name is derived from the "Makonde" word meaning "that which bends up" in reference to the stooped posture developed as a result of the arthritic symptoms of the disease. The disease causes crippling arthralgia and frequent arthritis that accompany fever, chills, headache, nausea, vomiting, low back pain and rash are clinically distinctive[1]. Chikungunya is an emerging vector-borne disease and has been reported from countries of South and East Africa, South Asia, South-East Asia and, in 2007, from Italy. In the South-East Asia region, outbreaks were documented from India, Indonesia, Maldives, Myanmar, Sri Lanka and Thailand. Although it is not a killer disease, high morbidity rates and prolonged polyarthritis lead to considerable disability in a proportion of the affected population and can cause substantial socioeconomic impact in affected countries. Chikungunya is a new, emerging disease and it has not received sufficient coverage yet in the medical curricula of member states[2]. Several indigenous drugs have been described in Siddha and Ayurveda for the management of inflammation, fever and other diseases. In the long term treatment of chronic disorders, like rheumatic diseases, combinations of different plants families and species active principles often exhibit remarkable potency and tolerance[3]. It is well documented that the plant possessing anti-inflammatory property also having antipyretic and analgesic activities or vice versa. The nilavembu kudineer choornam (NKC) is a classical preparation used in the treatment of chikungunya fever as Siddha medicine in Tamil Nadu, India. It is a combination of commonly used food materials along with medicinal plants. NKC composition is equal proportion of heartwood of *Santalum album* Linn. (Santalaceae), rhizomes

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of *Zingiber officinale* Rosc. (Zingiberaceae), fruits of *Piper nigrum* Linn (Piperaceae), whole plant of *Andrographis paniculata* (Burm) Wall. ex Nees (Acanthaceae), tubers of *Cyperus rotundus* Linn. (Cyperaceae), roots of *Vetiveria zizanioides* (Linn) Nash. (Poaceae), whole plant of *Hedyotis corymbosa* (Linn.) Lam. (Rubiaceae), root of *Plectranthus vettiveroides* (Linn.) Nash. (Lamiaceae) and whole plant of *Trichosanthes cucumerina* Linn. (Curcurbitaceae). All these plants are used traditionally in the treatment of fever, inflammation, arthralgia, arthritis, gastric ulcer, jaundice and general debility conditions[4-6]. In chikungunya, only symptomatically treatment has recommended with paracetamol and non-steroidal anti-inflammatory drugs (NSAIDs). Till-date, there is no specific treatment and vaccine for the prevention of chikungunya fever[2]. NKC is prescribed in the government hospitals, Tamil Nadu, India at the time of chikungunya fever season. But, up-to-date, its efficacy is not scientifically documented and hence, the present study was undertaken to investigate anti-pyretic, anti-inflammatroy and analgesic potential of EENKC in animal models to support its actions.

2. Materials and methods

2.1. Extraction of NKC

The NKC was procured from Dr.Anbarasu, MD (Siddha)., Government Primary Health Center, Pappanadu, Tanjore, Tamil Nadu. The extraction of NKC (240 g) was carried out by soxhlet apparatus using 95% of ethanol as a solvent for 72 h. The extract was concentrated under vacuum using a rotary evaporator and dried. The total yield of the extract was 29.43 g. The EENKC was stored at 2-8 ℃ until the completion of pharmacological studies.

2.2. Animals

Female Wistar albino rats (150-200 g) were used to assess the antipyretic activity. In the case of anti-inflammatory and analgesic activity, Wistar albino rats either sex (150-200 g) and male Swiss albino mice (25-30 g) were used respectively. Female mice (25-30 g) were used to assess the acute toxicity. All animals were kept and maintained under standard laboratory conditions of temperature (22±2) ℃, humidity (45±5) ℃ and 12 h light: 12 h dark cycle. The animals were fed with normal laboratory diet and allowed to drink tap water *ad libitum*. The studies were carried out in accordance with institutional ethical guidelines for the care of laboratory animals of KMCH College of Pharmacy, Coimbatore, India. The study was initiated after the approval of institutional animal ethical committee (KMCRET/PH/14/2008).

2.3. Chemicals

Carrageenan and brewer's yeast was procured from Himedia laboratories, Mumbai, India. Other solvents and drugs used were of analytical grade and obtained commercially.

2.4. Acute toxicity study

Acute oral toxicity study was performed as per Organization for Economic Cooperation and Development guidelines[7]. The EENKC was administered to 3 female mice in a single dose by gavage using oral needle. If 2 mice died or 2-3 mice are alive out of 3 mice after 24 h, another 3 mice were received EENKC as per guidelines to find the acute toxicity. Animals are fasted 3 h prior to dosing (food was withheld for 3 h but not water). During the period of fasting, mice were weighed and EENKC was administered. After the EENKC administration, food was withheld 2 h. Animals are observed individually after dosing at least once during the first 30 min, periodically during the first 24 h, with special attention given during the first 4 h, and daily thereafter, for a total of 14 d. Animals are removed, if any humanely killed for animal's welfare reasons or are found dead. The postmortem examination was carried out in dead animals to determine cause of death.

2.5. Brewer's yeast induced pyrexia

Animals were selected for the experiment after confirmation of approximate constant rectal temperature for 7 d. The antipyretic activity of the EENKC was evaluated based on brewer's yeast-induced pyrexia in rats. Pyrexia was induced by subcutaneous injection of 10 mL/kg of 15% w/v brewer's yeast suspension in 0.09% of saline below the nape of the neck[8,9]. The rectal temperature of each rat was measured using a digital thermometer (BD™ rapid flex) before injection of the yeast. 18 h post challenge, induction of fever was confirmed by measuring rectal temperature of the rats.

2.6. Experimental design for antipyretic activity

The rats showed rectal temperature of at least 38.0 ℃ were selected for to assess the antipyretic activity[9]. Fever induced rats were divided in to four groups of six animals in each group. Group 1 served as negative control which received propylene glycol (10 mL/kg), group 2 served as positive control which received paracetamol (150 mg/kg), and group 3 and 4 served as extract treatment which received EENKC 200 and 400 mg/kg respectively. After dosing the respective group, the rectal temperature was recorded over a period of 1 to 5 h[8]. Paracetamol and EENKC were suspended in propylene glycol before prior to oral administration.

2.7. Carrageenan induced rat paw oedema

Acute inflammation was produced by subplantar injection of 0.1 mL of 1% suspension of carrageenan in normal saline, in the right hind paw of the rats, 1 h after the oral administration of doses of the EENKC, positive and negative control substances to the respective groups[8].

2.8. Experimental design for anti-inflammatory activity

The rats were divided into four groups of six animals in each group. Group 1 served as negative control which received propylene glycol (10 mL/kg), group 2 served as positive control which received indomethacin (10 mg/kg), group 3 and 4 served as extract treatment which received

EENKC 200 and 400 mg/kg. The paw volume was measured plethysmographically (Inco-Niviqure, Version 60.1, India) at 0, 1, 3 and 5 h after the carrageenan injection. Indomethacin and EENKC was suspended in propylene glycol before oral administration[8].

2.9. Acetic acid induced writhing test

Acetic acid 0.6% v/v (10 mL/kg) was injected by intraperitoneal route one hour after administration of the EENKC, positive and negative control substance to the respective groups[10].

2.10. Experimental design for analgesic activity

Male Swiss mice were divided four groups of six animals in each group. Group 1 served as negative control which received propylene glycol (10 mL/kg), group 2 served as positive control which received aspirin (100 mg/kg), group 3 and 4 served as extract treatment which received EENKC 200 and 400 mg/kg. After administration of acetic acid, number of writhes (*i.e.* index of pain reaction against chemical stimuli characterized by abdominal muscle contraction together with turning of trunk and extension of hind limbs) was counted over a period of 20 min[10]. The standard drug and EENKC were suspended in propylene glycol prior to oral administration.

2.11. Statistical analysis

All the data expressed as mean ± SEM were evaluated by one-way analysis of variance (ANOVA), followed by Dunnett's test for multiple comparisons and values of $P < 0.05$ were considered as statistically significant.

3. Results

3.1. Acute toxicity study

The oral administration of EENKC at the dose 2 000 mg/kg did not exhibit any signs of toxicity up to 14 d and no animals were died. This indicates that EENKC was nontoxic in mice up to an oral dose of 2 000 mg/kg of body weight. Therefore, the biological evaluation was carried out using 200 and 400 mg/kg dose levels.

3.2. Antipyretic activity

Administration of EENKC 200 mg/kg and paracetamol (150 mg/kg) was significantly ($P<0.01$) reduced pyrexia at 1 to 5 h compared to 0 h of the same group animals. At a dose of 400 mg/kg, EENKC did not show significant reduction in pyrexia at 1 and 2 h compared 0 h. But, it reduced pyrexia significantly ($P<0.01$) at 3, 4 and 5 h compared to 0 h of the same group animals. The EENKC 200 mg/kg has equal and consistent efficacy to reduce pyrexia compared to paracetamol 150 mg/kg at 1 to 5 h (Table 1).

3.3. Anti-inflammatory activity

The efficacy of the EENKC 400 mg/kg was not significant at 1 h, but it showed significant activity at 3 h ($P<0.01$) and 5 h ($P<0.05$). The EENKC 200 mg/kg showed more significant ($P<0.01$) anti-inflammatory activity at 1 h compared to control group than standard drug indomethacin (10 mg/kg, $P<0.05$). The efficacy of the EENKC 200 mg/kg was more consistent and significant ($P<0.01$) than EENKC 400 mg/kg. The higher level of inhibition of inflammation with EENKC 200 mg/kg revealed the greater efficacy than indomethacin, but there is no statistically significant efficacy (Table 2).

3.4. Analgesic activity

Both the dose (200 and 400 mg/kg) of EENKC and aspirin (100 mg/kg) pretreatment animals significantly ($P<0.01$) reduced the painful response produced by acetic acid, manifested as writhing compared to the control group animals (Table 3).

Table 1
Effect of ethanolic extract of nilavembu kudineer choornam on brewer's yeast induced pyrexia.

Treatment	Dose (mg/kg)	Rectal temperature in ℃ at various times intervals						
		-18 h	0 h	1 h	2 h	3 h	4 h	5 h
Control	Vehicle (5 mL/kg)	37.41 ± 0.04	38.48 ± 0.04	38.70 ± 0.06	38.61 ± 0.07	38.71 ± 0.04	38.80 ± 0.03	38.85 ± 0.03
Paracetamol	150	37.13 ± 0.05	38.66 ± 0.06	37.66 ± 0.10*	37.45 ± 0.11*	37.30 ± 0.04*	37.25 ± 0.02*	37.21 ± 0.06*
EENKC	200	37.33 ± 0.13	38.56 ± 0.12	37.68 ± 0.10*	37.58 ± 0.14*	37.51 ± 0.03*	37.43 ± 0.09*	37.20 ± 0.05*
EENKC	400	37.16 ± 0.07	38.21 ± 0.16	38.01 ± 0.23ns	37.63 ± 0.15ns	37.46 ± 0.07*	37.26 ± 0.11*	37.23 ± 0.14*

All data were expressed in mean ± SEM (n=6). *$P<0.01$ compared with 0 h of the same group. nsNo significance with 0 h of the same group.

Table 2
Effect of ethanolic extract of nilavembu kudineer choornam on carrageenan-induced rat paw edema.

Treatment	Dose (mg/kg)	Rat paw edema volume at different time interval (mL)			
		0 h	1 h	3 h	5 h
Control	Vehicle (5 mL/kg)	1.438 ± 0.03	1.706 ± 0.06	1.872 ± 0.05	2.011 ± 0.02
Indomethacin	10	1.429 ± 0.01	1.524 ± 0.03*	1.626 ± 0.03**	1.665 ± 0.07**
EENKC	200	1.416 ± 0.04	1.406 ± 0.05**	1.487 ± 0.04**	1.635 ± 0.05**
EENKC	400	1.423 ± 0.03	1.630 ± 0.04ns	1.562 ± 0.07**	1.775 ± 0.05*

All data were expressed in mean ± SEM (n=6). *$P<0.05$ compared with control group. **$P<0.01$ compared with control group. nsNo significance compared with control group.

Table 3
Effect of ethanolic extract of nilavembu kudineer choornam on acetic acid induced writhing in mice.

Treatment	Dose (mg/kg)	Number of writhes (per 20 min)
Control	Vehicle (5 mL/kg)	90.33 ± 3.38
Aspirin	100	44.50 ± 2.30*
EENKC	200	46.16 ± 1.49*
EENKC	400	49.33 ± 1.70*

All data were expressed in mean ± SEM (n=6). *P<0.01 compared with control group.

4. Discussion

In chikungunya, fever is one of the commonest clinical features and it was reported in 92% of the cases and varies from low grade to high grade, lasting for 24 to 48 h. Fever rises abruptly in some, reaching 39 to 40 , with shaking chills and rigor and usually subsides with use of antipyretics[2]. Fever is caused as a secondary impact of infection, malignancy or other diseased states. It is the body′s natural defence to create an environment where infectious agent or damaged tissue cannot survive. It leads to enhanced formation of pro-inflammatory mediator′s (cytokines like interleukin 1 β , α , β , and tumor necrosis factor-alpha (TNF- α)), which increase the synthesis of prostaglandin E2 (PGE2) near peptic hypothalamus area, triggering the hypothalamus to elevate the body temperature. The inhibition of cyclooxygenase-2 (COX-2) expression leads to reduction in the elevated body temperature by inhibits PGE2 synthesis has reported as common mode of action to antipyretic agents[11]. The significant reduction of fever by EENKC on the brewer′s yeast induced pyrexia in rat supporting the antipyretic potentials of NKC in chikungunya fever. This property may be due to inhibiting the enzyme cyclooxygenase and reducing the level of PGE2 within the hypothalamus.

In chikungunya, arthralgia (pain in a joint) will be associated with fever and it was reported in 87% of the cases. Recently, many patients presented with arthralgia without fever. The joint pain tends to be worse in the morning, relieved by mild exercise and exacerbated by aggressive movements. Migratory polyarthritis (inflammation of several joints) with effusions may be seen in around 70% cases, but resolves in the majority. Ankles, wrists and small joints of the hand were the worst affected. Larger joints like knee and shoulder and spine were also involved. There is a tendency for early and more significant involvement of joints with some trauma or degeneration[2]. Carrageenan-induced hind paw edema is the standard experimental model of acute inflammation. Carrageenan is commonly used for testing anti-inflammatory drugs due to absence of apparent systemic effects and an antigenic nature. Moreover, reproducibility of this model is high. Carrageenan-induced edema is a biphasic response. The first phase is mediated through the release of histamine, serotonin and kinins whereas the second phase is related to prostaglandin release and slow reacting substances which peak at 3 h[12]. The EENKC treated animals showed good anti-inflammatory activity at 1, 3 and 5 h in carrageenan induced inflammation compared of control animals. This result supports the anti-inflammatory activity of NKC in chikungunya fever. It may be due to inhibition of the release of first and second phase inflammatory mediators[13]. Moreover, backache (67 %) and headache (62 %) associated with chikungunya fever is common clinical feature[2]. Pain is a complex process mediated *via* physiological mediators such as prostaglandins, bradykinins, substance-P. The constrictions induced in mice by acetic acid results from an acute inflammatory reaction with production of PGE2 and prostaglandin F2 in the peritoneal fluid[10]. Therefore, NKC may be suppress the formation of above substances or prevent their action to produce analgesic activity.

At present, to manage chikungunya, no vaccine is available and symptomatic treatment given for mitigating pain and fever using anti-inflammatory drugs along with rest usually recommended. While recovery from chikungunya is the expected outcome, convalescence can be prolonged (up to a year or more), and persistent joint pain may require analgesic (pain medication) and long-term anti-inflammatory therapy[14]. It was well documented that 25% of chronic NSAID users will develop ulcer disease and 2 to 4 % will bleed or perforate. Concerns rose due to potential cardiovascular risk of COX-2 inhibitors and other NSAIDs have complicated clinical decision to prescribe further[15]. Therefore, preparation which possess less or no toxicity, multiple therapeutic effects with cost effective have greater need than NSAIDs and analgesics to treat fever, inflammation and pain associated with chikungunya fever. Pharmacological actions of few medicinal plants present in NKC were studied extensively. Piperine, isolated from *Piper nigrum* has anti-inflammatory, antinociceptive, and anti-arthritic property[16,17]. Neoandrographolide, one of the principal diterpene lactones, isolated from a medicinal herb *Andrographis paniculata* possesses significant anti-inflammatory effects[18]. Hepatoprotective role of the whole plant of *Hedyotis corymbosa* against paracetamol overdose-induced and *Trichosanthes cucumerina* in carbon tetrachloride induced liver damage in rats was well documented[19-21]. Rhizomes of *Zingiber officinale* has anti-inflammatory, cytoprotective, anti-ulcer action in NSIADs induced ulcer model, hepatoprotective and antioxidant property in paracetamol induced animal model[22-24]. Hence, antipyretic, anti-inflammatory and analgesic action of NKC

may be due to the presence of above phytoconstituents.

The present study finding, first time, supports the efficacy of nilavembu kudineer choornam and we conclude that it might be a safer, better alternative medicine in short and long-term treatment of chikungunya fever due to its multiple therapeutic actions compared to NSAIDs and analgesics.

Conflict of interest statement

The authors declare that there is no any conflict of interest to disclose.

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