IJPSR (2023), Volume 14, Issue 11 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 25 March 2023; received in revised form, 14 June 2023; accepted 25 August 2023; published 01 November 2023

EVALUATION OF *IN-VIVO* ANTI-INFLAMMATORY ACTIVITY OF MONO HERBAL SIDDHA FORMULATION KANDUPARANGI CHOORANAM (*PYGMAEOPREMNA HERBACEA* - ROOT POWDER) IN CARRAGEENAN-INDUCED PAW EDEMA & PLEURISY IN WISTAR ALBINO RATS

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Keywords:

Kanduparangi chooranam, Pygmaeopremna herbacea, Carrageenan, Edema, Pleursy

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ABSTRACT: Introduction: Monoherbal or polyherbal formulation, which is used in Siddha medication, have a wide variety of phytochemical substances as primary and secondary metabolites, which show many pharmacological activities, including the anti-inflammatory effect. **Materials and Methods:** The anti-inflammatory activity of *Kanduparangi chooranam* (KC) was evaluated by using carrageenan-induced paw edema and carrageenan-induced pleurisy Wistar albino rat models. KCwas administered orally at doses such as 100 and 200 mg/kg BW, and it was compared with the standard drug indomethacin (10 mg/kg, body weight). The changes in % of inhibition of paw volume, level of pleural exudates and migratory leukocyte count were noted. **Results:** In the carrageenan-induced rat paw oedema model, the test drug KC was found to be the paw oedema volume at the dose of 100mg and 200 mg/kg, body weight was 61.94% and 63.05 %, respectively ($p<0.001$) whereas Indomethacin which had 65.83% paw volume inhibition. In the carrageenan-induced pleurisy rat models, the test drug KC was found to be values of the pleural exudates level at the dose of 100mg and 200 mg/kg, body weight were0.27±0.12ml and 0.23±0.1ml respectively ($p<0.001$) whereas Indomethacin which showed 0.21±0.08 ml and as well as the migratory leukocyte count of KC was found to be0.55 ± 0.11 x 10^3cells / ml and 0.50 ± 0.10 x 10^3cells / ml, respectively ($p<0.001$) whereas Indomethacin had 0.45±0.08x10^3 cells/ml. These results suggest that *Kanduparangi chooranam* has significant anti-inflammatory activity, which is useful in the management of painful inflammatory conditions.

INTRODUCTION: Inflammation is the main biological response of vascular tissues to harmful stimuli from an injury, infection, environmental agents, malignancy, and changes in cell structure. By initiating the healing process of tissues, inflammation acts as a protective mechanism for the body.

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DOI: 10.13040/IJPSR.0975-8232.14(11).5399-03

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.14(11).5399-03

Inflammation is a complex process that results in the activation of leucocytes, the release of the chemicals of the immune system like cytokines, complement factors *etc.*, and then the production and discharge of inflammatory mediators and prostaglandins [1].

Inflammation is divided into two major types *i.e.*, acute and chronic inflammation. Acute inflammation represents the four main features: pain, redness, heat, and swelling, which finally may leads to loss of function. Whereas chronic inflammation represents persistent pain, redness, and swelling due to various persistent biological,

physical, and chemical irritants or by long-term deficiency of blood (ischemia), oxygen (hypoxia) and nutrients [2]. So, many anti-inflammatory synthetic drugs (NSAID's) are now available in the market to treat painful musculoskeletal conditions and inflammation but often result in adverse effects such as gastric irritation, nephrotoxicity [3] *etc*., So, herbal drugs are of the utmost importance because of their safety, efficacy, affordability and devoid of drastic side effects [4]. Hence, there is a need for the production of novel herbal drugs as mentioned in the Siddha classical textbooks. *Kanduparangi* (*Pygmaeopremna herbacea* (Roxb.) Moldenke) which is documented in the Siddha classical literature *Gunapadam Part- I, Mooligai Vagupu* [5], and it belongs to the family, Verbenaceae [6]. This plant has been used as an ingredient in many of the Siddha preparations, which are prescribed for a variety of musculoskeletal disorders (*e.g.* arthritis, rheumatism), generalized body pain and burning pain. The present study was therefore undertaken to evaluate the anti-inflammatory effect of the *Kanduparangi chooranam* (root powder of *Pygmaeopremna herbacea*) by using the carrageenan-induced paw edema model and carrageenan-induced pleurisy model of Wistar albinorats.

MATERIAL AND METHODS:

Source of the Raw Material for the Preparation of the Drug: The root part of *Pygmaeopremna herbacea* (Roxb.) Moldenke (*Kanduparangi ver)* was purchased from a well-reputed country shop named *M.Gopala Asan Naatu Marunthu Kadai* in Nagercoil, Tamilnadu, India.

Authentication of the Raw Drug: The root sample was found and authenticated by Dr. S. Sutha, Ph.D., Head of the Department of Medicinal Botany, Government Siddha Medical College, Palayamkottai.

Purification of the Raw Drug: The root of the *Pygmaeopremna herbacea* (Roxb.) Moldenke was purified according to the proper procedure as per classical Siddha literature by removing the adulterants and cut into small pieces, dried in the shade and then collected.

Preparation of the Drug: The purified raw drug was dried well in shadow and made into micronized powder based on the Siddha literature, *Gunapadam I -Mooligai vaguppu* [6]. Finally, the powder was sieved using pure white cloth which is mentioned as *Vasthirakayam* in Siddha.

TABLE 1: INFORMATION ABOUT KANDUPARANGI (*PYGMAEOPREMNA HERBACEA*)

Sl. no.	Comman name Tamil/ English	Botanical name/ Family	Phyto-chemistry	Pharmacological actions	Usage in Siddha
1	Kanduparangi/ Blue glory or Beetle killer	*Pygmaeopremna herbacea* (Roxb.) Moldenke / Verbenaceae	Acidresin Icetexane Sirutekkone (Bharangin) Scutellarein	Antipyretic, Antimicrobial, Analgesic, Anticonvulsant, Antiasthmatic, Antioxidant, Anti-carcinogenic, Antitumor, Antiulcer, Hepatoprotective, Stimulant, Sedative, Laxative	Vatha, Pitha and Kaba disorders, Bronchial asthma, Fever, Mental disorders, Sinusitis, Burning pain, *Chronic rheumatism*, General body pain, Fever with chills & Hallucinations

Acute Toxicity Study: For toxicity studies, two different groups of six albino rats of both sexes were administrated orally with the test substances in the range of doses 100- 2000 mg/ kg/body weight and the mortality rates were observed after 72 hours. The *Kanduparangi chooranam* showed no mortality at 2000mg/ kg/body weight. Therefore 3000 mg/ kg/body weight was considered an LD_{50} cut-off dose (safe dose) and hence $1/10^{th}$ (300 mg/ kg/body weight), 1/5 (600 mg/ kg/body weight) of LD_{50} doses were selected as safe doses (2019).

Animals used for the Evaluation of Anti-inflammatory Activity (2020): Male Wistar albino rats with 175- 185 g body weight were used in this study, which was obtained from the animal house, K. M. College of Pharmacy, Madurai. The animals were maintained in standard laboratory conditions by being given a standard laboratory diet and water *ad libitum* and fasted for 16 hours before starting the experiment. The Institutional Animal Ethics Committee approved this animal experiment and was by the guidelines of the Committee for Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India [IAEC/C.B.S.BHARATHCHRISTIAN/M.D(S)/TN MGRMU/84/2020].

Drugs (Synthetic Anti-Inflammatory Agents): The commercial name of the reference anti-inflammatory drug used in this study is Indomethacin. It is chemically called 1-(4-Chlorobenzoyl)- (- methoxy-2methylinode -3-yl) acetic acid and was obtained from Pharmco Pharmaceuticals Company.

Chemical Used for the Induction of Inflammation: Carrageenan, type IV (Sigma, USA): Carrageenan is a polysaccharide isolated from two species, the marine alga, *Gigartina acicularis* and *G. Pistillata*, which grow together in the sea.

Statistical Analysis of the Data: The results of various studies were expressed as mean ± SEM and analyzed statistically using one-way ANOVA, followed by Newmann Keul's multiple range tests. $P<0.05$ was considered statistically significant. The analysis was performed using the GraphPad Prism software package (Version 4.0).

Anti-Inflammatory Activity Assessment- Method 1:

Carrageenan-Induced Paw Edema Assay in Wistar Albino rats:

TABLE 2: GROUP OF ANIMALS IN THE CARRAGEENAN-INDUCED PAW EDEMA ASSAY

Groups	Control	Administration
I	Negative	Normal saline 0.5 ml/kg, body weight
II	Toxic	Carrageenan (1%w/v) in saline in the sub-plantar region of the right hind paw
III	Standard	Administered Indomethacin at the dose of 10 mg/kg, body weight
IV	Test control 1	Root powder of *Pygmaeopremna herbacea* at the dose of 100 mg/kg body weight.
V	Test control 2	Root powder of *Pygmaeopremna herbacea* at the dose of 200 mg/kg body weight

Method- Carrageenan-induced Paw Edema Assay [8, 9]: Paw swelling or footpad edema, is a convenient method for assessing inflammatory responses to antigenic challenges and irritants. The animals were divided into five groups of five rats each as described in **Table 1** and each was pretreated with *Kanduparangi chooranam*, Siddha formulation (100 and 200 mg/kg. p.o.), Indomethacin (10 mg/kg, p.o.) or normal saline (0.1 ml). Acute paw edema was induced in 2 ways, one was injecting 0.1 ml of 1% w/v Carrageenan in physiological saline into the sub-plantar tissues of the right hind paw of each rat and the other way, after 1 h, 0.1 ml, 1 % Carrageenan suspension in 0.9% NaCl solution was injected into the sub-plantar tissue of the right hind paw. The linear paw circumference was measured after the administration of carrageenan at intervals of 60 minutes, 120 minutes, 180 minutes and 240 minutes. The perimeter of the paw was measured by using vernier calipers. The inhibition of the percentage of paw edema volume in drug-treated groups was compared with the carrageenan toxic control group.

The Percentage of Inhibition was calculated by using the formula:

Percentage of inhibition of paw edema volume = $(T-T_0) / T \times 100$

Where, T_0 represents the percentage difference in increased paw volume after the administration of test drugs to the rats and T represents the percentage difference in increased paw volume in the control group.

TABLE 3: OBSERVATIONS OF THE EFFECT OF KANDUPARANGI CHOORANAM ON THE PERCENTAGE OF INHIBITION IN CARRAGEENAN-INDUCED RAT PAW EDEMA

Groups	Control	Dose (mg or ml /kg, body weight)	Mean increase in paw volume (ml)	Decrease in paw volume (%)
I	Normal	10ml, 1% saline	0.91 ± 0.06	-
II	Toxic	0.1 ml, 1% Carrageenan	3.60 ± 0.28*a	-
III	Standard	10mgIndomethacin	1.23 ± 0.12*b	65.83%
IV	Treatment	100mg KC	1.37 ± 0.17*b	61.94%
V	Treatment	200mg KC	1.33 ± 0.14*b	63.05%

Values are expressed as mean ± SEM.Values were compared by using analysis of variance (ANOVA) followed by Newman-Keul's multiple range tests. * (a) Values are significantly different from normal control G1 at $P<0.01$. * (b) Values are significantly different from Toxic control G2 at $P<0.01$.

Results and Inference of the Effect of Kanduparangi Chooranam on the Percentage of Inhibition of Paw Volume in Carrageenan-Induced Rat Paw Edema: The root powder of the *Pygmaeopremna herbacea* did not show any sign of toxicity up to 2000mg/kg/body weight, and hence it was considered to be safe. As indicated in **Table 3**, the *Kanduparangi chooranam* reduced carrageenan-induced paw edema volume in Wister albino rats by 61.94% and 63.05 % on oral administration of 100 and 200 mg/kg, as compared to the untreated control group. Indomethacin at 10 mg/kg inhibited the paw edema volume by 65.83%. The results obtained indicate that the *Kanduparangi chooranam* had significant anti-inflammatory activity in rats.

Anti-Inflammatory Activity Assessment- Method 2:

Carrageenan-Induced Pleurisy Assay in Wistar Albino Rats:

TABLE 4: GROUP OF ANIMALS FOR THE CARRAGEENAN-INDUCED PAW EDEMA ASSAY

Groups	Control	Administration
I	Negative	Normal saline 0.5 ml/kg, body weight
II	Toxic	Carrageenan (1%w/v) in saline in the sub-plantar region of the right hind paw
III	Standard	Administered Indomethacin at the dose of 10 mg/kg, body weight
IV	Test control 1	Root powder of *Pygmaeopremna herbacea* (KC) at the dose of 100 mg/kg body weight
V	Test control 2	Root powder of *Pygmaeopremna herbacea*(KC) at the dose of 200 mg/kg body weight

Method - Carrageenan-induced Pleurisy Assay [10]: Carrageenan-induced pleurisy is another convenient method for assessing inflammatory responses. The animals were divided into five groups of five rats each as described in **Table 4** and each was previously treated with Siddha formulation (100 and 200 mg/kg. p.o.), Indomethacin (10 mg/kg, p.o.) or normal saline (0.1 ml). Pleurisy was induced one hour later in all the animals by receiving 0.25 ml of an intra-pleural injection of carrageenan on the right side of the thorax. The animals were sacrificed 3 h after carrageenan injection by ether inhalation. 1 ml of heparinized Hank's solution was injected into the pleural cavity and gently massaged to mix its contents. The fluid was aspirated out of the cavity and the exudates were collected. The number of migrating leukocytes in the exudates was determined with the Neubauer chamber. The values of each experimental group were expressed as mean SEM and compared with the control group.

TABLE 5: OBSERVATIONS OF THE EFFECT OF KANDUPARANGI CHOORANAM ON THE PLEURAL EXUDATES LEVEL AND LEUKOCYTE MIGRATION IN CARRAGEENAN-INDUCED PLEURISY

Groups	Control	Dose (mg or ml /kg, body weight)	Pleural Exudates (ml)	Leukocytes ($\times 10^3$ cells/ml)
I	Normal	10ml, 1% saline	0.19±0.06	0.39±0.06
II	Toxic	0.1 ml, 1% Carrageenan	0.44±0.18*a	4.34±0.38*a
III	Standard	10mgIndomethacin	0.21±0.08*b	0.45±0.08*b
IV	Treatment	100mg KC	0.27±0.12*b	0.55±0.11*b
V	Treatment	200mg KC	0.23±0.10*b	0.50±0.10*b

Values are expressed as mean ± SEM.Values were compared by using analysis of variance (ANOVA) followed byNewman-Keul's multiple range tests. * (a) Values are significantly different from normal control G1 at P<0.01. * (b) Values are significantly different from Toxic control G2 at P<0.01.

Results and Inference of the Effect of Kanduparangi Chooranam on the Pleural Exudates Level and Leukocytes Migration Count in Carrageenan-Induced Pleurisy in Rats: As indicated in **Table 3**, the volume of pleural exudates in the toxic control group was 0.44±0.18ml.Treatment with Indomethacin (10 mg/kg, body weight) produced exudates of 0.21±0.08 ml. Animals treated with the *Kanduparangi chooranam* at the dose of 100 and 200 mg/kg showed decreased pleural exudates to 0.27±0.12ml and 0.23±0.1ml, respectively. The leukocyte migration was increased in the toxic control group at around $4.34\pm0.38\times10^3$cells/ml. The leukocyte count for the control group, which was treated with Indomethacin (10mg/kg, body weight), was found to be $0.45\pm0.08\times10^3$ cells/ml. Animals treated with the *Kanduparangi chooranam* at the

dose of 100 and 200 mg/kg, body weight showed that producing leukocyte migration of 0.55 ± 0.11 x 10^3cells / ml, 0.50 ± 0.10 x 10^3cells / ml, respectively. Anti-inflammatory effect of the *Kanduparangi Chooranam* at the dose of 200 mg/kg, body weight showed a significant reduction ($P < 0.01$)of inflammation by the reduction in the level of pleural exudates and leucocyte migration count in Wistar Albino rats as compared to the standard control Indomethacin (10mg/kg, body weight).

CONCLUSION: The results of this study showed that the monoherbal Siddha formulation *Kanduparangi chooranam*, which has been widely used by traditional healers and medical practitioners of Siddha Medicine to treat various conditions present with rheumatism for decades, exhibits significant paw edema volume inhibition, reduction in pleural exudates level and decreased leucocyte migration count in Wistar Albino rats. The results clearly show the anti-inflammatory properties of the test drug. However, this study needs further research to include studies at the cellular level to determine the mechanisms of reduction of the triggers that induce the inflammation more specifically.

ACKNOWLEDGEMENT: Sincere thanks are due to all those who helped in preparing this paper.

CONFLICTS OF INTEREST: The author hereby declares that he has no conflicts of interest to disclose.

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How to cite this article:
Christian CBSB: Evaluation of *in-vivo* anti-inflammatory activity of mono herbal Siddha formulation Kanduparangi chooranam (*Pygmaeopremna herbacea* - root powder) in carrageenan-induced paw edema & pleurisy in wistar albino rats. Int J Pharm Sci & Res 2023; 14(11): 5399-03. doi: 10.13040/IJPSR.0975-8232.14(11).5399-03.