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THE EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF SIDDHA FORMULATION MUNNAIILAI KUDINEER *(PREMNA CORYMBOSA, BURM.F.)* CARRAGEENAN INDUCED ALBINO WISTAR RATS

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ABSRACT

The Munnaiilai kudineer (MIK) is*(Premna corymbosa, Burm.f.)*used in Siddha system for the management of arthritis, gastric ulcer, hypertension, giddiness and hemorrhoids. The classical siddha text Gunapadam mooligai part 1 **(Dr.K.S.Murugesa Muthaliyar).** The aim of study is to evaluate the anti-inflammatory activity of Munnaiilai kudineer. Test compounds are assessed for acute anti-inflammatory activity by examining their ability to reduced incarrageenan-induced hind paw edema and pleurisy induced rat models.The end of result showed MunnaiIlai Kudineer behaves as an inhibitor of leukocyte migration and the formation of pleural exudates,after oral administration of MIK had significantly reduced in swelling in Wister albino rat.

KEYWORDS: Anti inflammatory, MunnaiIlai Kudineer, Wisterrats, Siddha formulation.

INTRODUCTION

Siddha medicine is a traditional system, it was originated from South Indian based system of medicine. The Siddha system is an ancient medicinal practices and spiritual disciplines as well as alchemy and mysticism. In Siddha system of medicine are commonly used in plant, mineral, and animal resources, which is acquired from the natural surroundings.

Munnaiillai (Premnacorymbosa) is a potent medicinal plant in the Siddha system. Traditionally the leaves are used in the treatment of vatha diseases, giddiness, loss of appetite and in pain management.

Inflammation is a complex biological response of vascular tissues against aggressive agents such as pathogens, irritants,or damaged cells. Acute inflammation is the initial responseand is characterized by the increased movement of plasmaand innate immune system cells, such as neutrophils and macrophages, from the blood into the injured tissues. The standard signs of inflammation are expressed by increased bloodflow, elevated cellular metabolism, vasodilatation, release ofsoluble mediators, extravasation of fluids and cellular influx (Ferrero-Milianet al.2007). Upon the presence of the inflammatory agent, cell membranes induce the activation of phospholipase A2 followed byrelease of arachidonic acid and inflammatory mediators such ascytokines, serotonin, histamine, prostaglandin and leukotrienes that increase vascular permeability, thus facilitating the migra-tionof leukocytes to the site of inflammation (Dassoler et al. 2004).

Inflammation induced by carrageenan is acute, nonimmune, well-researched,and highly reproducible. Cardinal signs of inflammation edema,hyperalgesia, and erythema developed after immediately following subcutaneous injection, resulting from action of proinflammatory agents,bradykinin, histamine, tachykinins, complement and reactive oxygen, and nitrogen species. The saponins displayed significant antinocicep-tive, anti-inflammatory and antipyretic activities possibly due totheir nonglycosidic moiety. The sapogenin is diverse activities havebeen reported such as antiallergic, antifungal, analgesic (Hostettmann et al. 2007, Milgate et al.1995, and Francis et al.2007). Moreover a variety of siddha formulation preparation have proved to be useful in animal models of inflammation [De La Lastra.C.A et al.2007, Liu Yet al.2012and Kang 2010].

Paw swelling or foot pad edema is a formidable method for assessing inflammatory responses to antigenic challenges and irritants. The test compounds are assessed for acute anti-inflammatory activity by examining their ability to reduce or prevent the development of carrageenan-induced paw swelling. In the present study attempts are made to validate the claims of MunnaiIlai Kudineer (MIK)regarding the anti-inflammatory activities.

METHODS AND MATERIALS

Collection of Plant Material

The plant material was freshly collected in and around palayamkottai atTirunelveli district. It was identified and authenticated by the Medicinal Botanist and Gunapadam experts at GOVERNMENT SIDDHA MEDICAL COLLEGE AND HOSPITAL.

Prepation of Munnaiillai Kudineer

Fresh leaves of Munnaiillai (leaf) was cleaned and made drying in shade. Then it was made into crystal powder. The Kudineer was prepared by boiling 7.5gms of powderin 200ml of water and finally to made for decoction.

Animals

Male albino rats (180 ± 5 g) were obtained from animal house,K.M.College of pharmacy, Madurai and maintained in standard laboratory conditions. They were given standard laboratory diet and water and libitum. All the animals are approved by the Institutional Animal Ethics Committee, and were in accordance with the guidelines of the committee for the purpose of Control and Supervision of Experiment's on Animal (CPCSEA), Government of India.(KMCP/20/2018).

Acute inflammation

Carrageenan-induced rat paw oedema is used widely as a working model of inflammation in the search for new anti-inflammatory drug. The anti inflammatory activity of the siddha formulation MunnaiIlai Kudineer was evaluated by carrageenan-induced rat paw edema method [Winteret al.1962 and Vinegar et al.1994]. Albino Wistar rats (180 ± 5 g) were used. Anti inflammatory activity was measured using carrageenan induced rat paw edema assay. The rats were divided into 5 groups of 5 animals each. Group I. were given normal saline and treated as negative control. Group II was treated with carragenan (1%w/v) in saline in the subplanter region of the right hind paw rats. Group III were administered Indomethacin(10 mg/kg, bw) and considered as standard. Rats from Group IV and V were given two doses siddha formulation (100 and 200 mg/kg bw). Acute paw edema was induced by injecting 0.1 ml of 1% (w/v) carrageenan solution, pre-pared in normal saline. 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 will be measured at hourly interval for 4 h. The perimeter of paw was measured by using verniercallipers. Measurements were taken at 0–4 h after the administration of the carrageenan. The edema was calculated by using.

$$\%\text{inhibition of edema} = \frac{T - T0}{T} \times 100$$

T-Thickness of paw in control group; T0-Thickness of paw edema in the test compound treated group.

Carrageenan Induced Pleurisy In Rats

The animals were divided into five groups of five rats each as described in the carrageenan induced paw edema model [Tomlinson et.al.1994, Vinegar R et al.1982] and each were pretreated with siddha formulation(100 and 200 mg/kg, p.o.), Indomethacin (10 mg/kg, p.o.) or normal saline (0.1 ml).One hour later all the animals were received 0.25 ml of an intrapleural injection of 1 % carrageenan on the right side of the thorax. The animals were sacrificed 3 h after carrageenan injection by either inhalation. One 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 Neubauer chamber.

The values of each experimental group were expressed as mean ± SEM and compared with the control group.

Statistical analysis

Results of anti-inflammatory activity are expressed as Mean increase in paw diameter ± SD. Results was analyzed using one way ANOVA. Differences were considered as statistically significant at $P < 0.05$ are compared to control

Table 1: Result of MunnaiIlai Kudineer on Carrageenan Induced rat paw edema.

Treatment	Dose (mg/kg, p.o.)	Mean increase in paw volume (ml)	% Decrease in paw volume
Normal control	10ml/kg saline	1.05 ± 0.09	
Toxic control	0.1 ml, 1% carrageenan	3.45 ± 0.24*a	
Standard control	10mg/kg Indomethacin	1.20 ± 0.12*b	65.21%
Treatment control	100mg/kg MunnaiIlaiKudineer	1.40 ± 0.18*b	59.42%
Treatment control	200mg/kg MunnaiIlaiKudineer	1.32 ± 0.14*b	61.73%

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.

Table 2: Result ofMunnaiIlai Kudineer on Carrageenan Induced Pleurisy in rats.

Treatment	Dose (mg/kg, p.o.)	Pleural exudates (ml)	Leukocytes (×103 cells/ml)
Normal control	10ml/kg saline	0.14±0.05	0.37±0.03
Toxic control	0.1 ml, 1% carrageenan	0.42±0.14*a	4.20±0.36*a
Standard control	10mg/kg Indomethacin	0.15±0.06*b	0.46±0.05*b
Treatment control	100mg/kg MunnaiIlaiKudineer	0.21±0.09*b	0.54±0.08*b
Treatment control	200mg/kg MunnaiIlaiKudineer	0.16±0.07*b	0.51±0.06*b

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.

RESULT AND DISSCUSSION

The effect of MunnaiIlai Kudineeron carrageenan-induced edema in rats is shown in Table 1. Theresults obtained indicate that the siddha formulation MunnaiIlai Kudineer had significant anti-inflammatory activity in rats. The MunnaiIlai Kudineer reduced the edema induced carrageenan by 59.42% and 61.73% on oral administration of 100 and 200mg/kg, as compared to the untreated controlgroup.Indomethacinat 10 mg/kg inhibited the edema volume by 65.21%.

The result of MunnaiIlai Kudineer on carrageenan-induced pleurisy in rats is shown in Table 2. Thevolume of pleural exudates in the toxic control group was0.42±0.14ml.All the animals were treated with the siddha formulation MunnaiIlai Kudineer (100 and 200 mg/kg, p.o.) was decreased the pleural exudates to 0.21±0.09 ml and 0.16±0.07.Treatment with Indomethacin (10 mg/kg, p.o.) produced the exudates of0.15±0.06 ml. The leukocyte count for the controlgroup was found to be $4.20\pm0.36\times10^3$ cells/ml. Animal streated with the siddha formulation MunnaiIlai Kudineer and standard produced aleukocyte migration of $0.54\pm0.08\times10^3$, $0.51\pm0.06X10^3$ and $0.46\pm0.05\times10^3$ cells/ml, respectively.

DISCUSSION

The frequency of intake of NSAID's and their reported common side effects, thereis need to focus on the scientific exploration of Siddha formulation drugs having fewer side effects. So, there is a continuous search for indigenous drugs, which can provide reliefto inflammation.Carrageenan induced inflammation is a biphasic phenomenon [Vinegar et al.1969]. The first phase of edema is attributedto release of histamine and 5-hydroxytryptamine. Plateauphase is maintained by kinin like substances and second accelerating phase of swelling is attributed to prostaglandin like substances.

The tests was performed with the MunnaiIlai Kudineer in the pleurisy induced rat models showed that the MunnaiIlai Kudineer behaves as an inhibitor of leukocyte migration and theformation of pleural exudates when given orally, as reported earlier [Mikami et al.1983].

The concluded of the siddha formulation MunnaiIlai Kudineer possess significant anti-inflammatory activity in albino rats. Further studies involving the biochemical pathways may result in the developmentof a potent anti-inflammatory action and lowtoxicity and hightherapeutic value.

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