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ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF *VELLARUGU CHOORANAM (ENICOSTEMMA AXILLARE LINN.)* IN ALBINO RAT MODELS

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ABSTRACT

The Vellarugu Chooranam (VC) (*Enicostemma axillare* (Lam.) is one of the important drug in Siddha system, because it was used various formed in the system. The aim of the study was to evaluate the analgesic and anti-inflammatory activities of *Vellarugu chooranam (Enicostemma axillare Linn*) in male albino rat models (180 ± 5 g). The analgesic activity of *Vellarugu chooranam* was assessed by acetic-acid writhing test. The Anti-inflammatory effect was analyzed by carrageenan induced paw edema and pleurisy induced methods. The analysis of experimental data was performed by statistical process of ANOVA to determine the variability of sample, while Newman-Keul's multiple range was performed for evaluation of comparative analgesic and anti-inflammatory activity of *Vellarugu chooranam* with control and standard. The writhing test showed a significant increase in the mean reaction time to stimuli in both 100 mg/kg and 200 mg/kg BW doses throughout the observation period in 30 minutes after treatment, which was comparable to the standard ***Diclofenac sodium*** and control group. In analgesic and anti-inflammatory studies showed, the inhibition of pain percentage and inflammation was noted. This result was Comparing with control and treatment groups. The results was 1.22 ±0.14, 1.42±0.19 and percentage in paw values is 65.73%, 60.11% respectively. The carragenan induced pleurisy in rat models showed decrease in pleural exudates and Leukocytes count was 0.24±0.13, 0.20±0.11 and 0.008±0.12, 0.50%±0.09% respectively.

KEYWORDS: Anti-inflammatory, Analgesic, *Vellarugu chooranam*, carragenn induced pleurisy.

INTRODUCTION

Inflammation is a complex biological response of vascular tissues against aggressive agents such as pathogens, irritants or damaged cells. Acute inflammation is the initial response and is characterized by the increased movement of plasma and 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 blood flow, elevated cellular metabolism, vasodilatation, release of soluble mediators, extravasation of fluids and cellular influx [Ferrero-Miliani et al.2007]. Upon the presence of the inflammatory agent, cell membranes induce the activation of phospholipase A2 followed by release of arachidonic acid and inflammatory mediators such as cytokines, serotonin, histamine, prostaglandin and leukotrienes that increase vascular permeability, thus facilitating the migration of 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—oedema, hyperanalgesia, and erythema—develop immediately following cutaneous injection, resulting from action of pro-inflammatory agents—bradykinin, histamine, tachykinins, complement and reactive oxygen, and nitrogen species. Many saponins tested have displayed significant antinociceptive, anti-inflammatory and antipyretic activities possibly due to their nonglycosidic moiety, the sapogenin, but also many diverse activities have also been reported such as anti-allergic, antifungal,analgesic and others [Hostettmann et al .2005 ,Tomlinson et al 2004.and Francis et al 2002]. Moreover a variety of siddha formulation preparation have been proved to be useful in animal models of inflammation [De La Lastra et al.2005. Song et al.2012and, Kang et al 2005].

Paw swelling or footpad edema is a convenient method for assessing inflammatory responses to antigenic challenges and irritants. Typically, 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 Vellarugu chooranam regarding the anti-inflammatory activities of this Siddha preparation.

Inflammation and pain are common nonspecific manifestations of many diseases. Although non-steroidal anti-inflammatory drugs (NSAIDs) and opiates have been used classically in these conditions, but some adverse reactions occur with these drugs such as gastrointestinal disturbances, renal damage, respiratory depression, and possible dependence [Domaj et al.1999]. In comparateviely to other systems, the fewer side effects are observed in natural sources and siddha formulations.

MATERIALS AND METHODS

The study was performed in Vellarugu chooranam (VAC) has a reference from *Gunapadam Mooligai* Part - 1 was indicated for Erigunmam (peptic ulcer disease). The raw drug was collected from Tirunelveli district, Tamilnadu. The purified plant is authenticated by faculties of department of medicinal botany, Government Siddha Medical College, Palayamkottai.

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 ad Libitum. All animal experiments are approved by the Institutional Animal Ethical Committee, and were in accordance with the guidelines of the committee for the purpose of Control and Supervision of Experiments on Animal (CPCSEA), Government of India (321611005/KMCP /27/2018).

Acute inflammation

The Carrageenan-induced rat paw edema 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 Vellarugu chooranam was evaluated by carrageenan-induced rat paw edema method (Winter et al.1962). 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. Rats of Group II were treated with carrageenan (1%w/v) in saline in the subplantar region of the right hind paw. Rats in 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, prepared in normal saline. After 1 hr, 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 hr. The perimeter of paw was measured by using vernier callipers. Measurements were taken at 0–4 hr after the administration of the carrageenan.

The anti-inflammatory activity was calculated by using the relation

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

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 [Vinegar et al.1969] 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 ether 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.

Analgesic activity of Vellarugu chooranam

Acetic acid-induced writhing test

The acetic-acid writhing test was performed using the reported procedure,[2] Groups of rats (n=6), were administered with 100 and 200 mg/Kg of Siddha formulation *Vellarugu chooranam*, 10 mg/Kg Diclofenac as positive control group and 1 mL distilled water as negative control group. After 30 minutes the animals were administered with i.p. injection of 0.1 mL acetic acid (0.6%). Then the count of abdominal contractions of animals during 30 minutes after acetic acid injection was reported and the Percentage Analgesic Activity (PAA) was calculated by using the following formula:

$$PAA = ((C- CD)/CD) \times 100$$

C = Mean of contractions count in animals treated with different doses of siddha formulation *Vellarugu chooranam* and Diclofenac sodium

CD = Mean of contractions count in animals served as negative control

RESULT AND DISCUSSION

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

Table 1: Vellarugu chooranam on Carrageenan Induced Paw Edema in Rats.

Treatment	Dose (mg/kg, p.o.)	Mean increase in paw volume(ml)	% Decrease in paw volume
Normal control	10ml/kg saline	1.12 ± 0.11	
Toxic control	0.1 ml, 1% carrageenan	3.56 ± 0.30*a	
Standard control	10mg/kg Indomethacin	1.22 ± 0.14*b	65.73%
Treatment control	100mg/kg Vellaruguchooranam	1.42 ± 0.19*b	60.11%
Treatment control	200mg/kg Vellaruguchooranam	1.30 ± 0.16*b	63.48%

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: Effect of siddha formulation Vellarugu chooranam on Carrageenan Induced Pleurisy in Rats.

Treatment	Dose (mg/kg, p.o.)	Pleural exudates (ml)	Leukocytes (×103 cells/ml)
Normal control	10ml/kg saline	0.16±0.08	0.40±0.05
Toxic control	0.1 ml, 1% carrageenan	0.48±0.20*a	4.24±0.39*a
Standard control	10mg/kg Indomethacin	0.18±0.10*b	0.48±0.08*b
Treatment control	100mg/kg Vellaruguchooranam	0.24±0.13*b	0.58±0.12*b
Treatment control	200mg/kg Vellaruguchooranam	0.20±0.11*b	0.50±0.09*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.

Anti-inflammatory Activity of Siddha formulation Vellarugu chooranam

The effect of siddha formulation Vellarugu chooranam on carrageenan-induced edema in rats is shown in Table 1. The results obtained indicate that the Siddha formulation Vellarugu chooranam had significant anti-inflammatory activity in rats. The siddha formulation Vellarugu chooranam was reduced the edema induced by carrageenan by 60.11% and 63.48% on oral administration of 100 and 200 mg/kg, as compared to the untreated control group. Indomethacin at 10 mg/kg inhibited the oedema volume by 65.73%.

The administration of Vellarugu chooranam on carrageenan-induced pleurisy in rats was explained in Table 2. The volume of pleural exudates in the toxic control group was reduced in 0.48±0.20 ml. Animals treated with the siddha formulation Vellarugu chooranam (100 and 200 mg/kg, p.o.) decreased the pleural exudates to 0.24±0.13 ml and 0.20±0.11.Treatment with Indomethacin (10 mg/kg, p.o.) produced the exudates of 0.18±0.10 ml. The leukocyte count for the control group was found to be $4.24\pm0.39\times10^3$ cells/ml. Animals treated with the siddha formulation Vellarugu chooranam and standard produced a leukocyte migration of $0.58\pm0.12\times10^3$, $0.50\pm0.09X10^3$ and $0.48\pm0.05\times10^3$ cells/ml, respectively.

Acetic acid-induced writhing response

The second study showed that the application of different doses of Siddha formulation *Vellarugu chooranam* had significant analgesic effects in the animals under investigation. The results of doses 100 and 200 mg/Kg were significant and comparable with the effect of Diclofenac sodium in analgesic activity (Table 3).

Table 3: Effects of Siddha formulation *Vellarugu chooranam* on acetic acid–induced writhing response.

Groups	Treatment	(number of writhing movements) (Mean ± S.E)	Percentage %
Group I	Distilled water	29.00 ± 2.45	
Group II	Diclofenac sodium 10mg/kg	6.08 ± 0.90*b	79.03%
Group III	100mg/kg *Vellaruguchooranam*	14.25 ± 1.65*b	50.86%
Group IV	200mg/kg *Vellaruguchooranam*	13.05 ± 1.30*b	55.00%

Values are expressed as mean ± SEM.
* (b) Values are significantly different from Toxic control G2 at P<0.01.

Statistical analysis

The results are reported as mean ± S.E.M. The statistical analyses were performed using one way analysis of variance (ANOVA). Group differences were calculated by post hoc analysis using Tukey's test. For all tests,

differences with values of P<0.05 were considered significant.

DISCUSSION

Due to the increasing frequency of intake of NSAID's and their reported common side effects, there is 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 relief to inflammation. Carrageenan induced inflammation is a biphasic phenomenon [Vinegar et al. 1982]. The first phase of edema is attributed to release of histamine and 5-hydroxytryptamine. Plateau phase is maintained by kinin like substances and second accelerating phase of swelling is attributed to prostaglandin like substances. The knowledge of these mediators involved in different phases is important for interpreting mode of drug action. The tests performed with the siddha formulation Vellarugu chooranam in the pleurisy model showed that the siddha formulation Vellarugu chooranam behaves as an inhibitor of leukocyte migration and the formation of pleural exudates when given orally, as reported earlier [Milgate et al.1995]. Thus it can be concluded that the Siddha formulation Vellarugu chooranam possess significant anti-inflammatory activity in rats. Further studies involving the purification of the preparation and the investigations in the biochemical pathways may result in the development of a potent anti-inflammatory agent with a low toxicity and better therapeutic index.

The analgesic activity was assessed by writhing test which has been reported to be useful for investigation of peripheral anti nociceptive activity and performed as a chemical pain mode [Abdollahi et al.2003 and Golshani et al.2004]. The siddha formulation *Vellarugu chooranam* demon trated a dose-dependent, significant anti nociceptive activity in animal models of pain. Acetic acid believed to increase the PGE2 and PGF2α in peritoneal fluid [Krasteva et al.2008]. The analgesic activity shown in models of pain is indicative that siddha formulation *Vellarugu chooranam* might possess centrally and peripherally mediated anti nociceptive properties.

Chemical components of Siddha formulation *Vellarugu chooranam* such as flavonoids, saponins or phenolic compounds may be responsible for the anti nociceptive activities of this formulation. Since the findings of this study revealed a significant analgesic effect of the siddha formulation *Vellarugu chooranam*, it can be concluded that terpenoids and specially saponins of siddha formulation *Vellarugu chooranam* may be responsible for the observed analgesic effect which should be proved by further investigations.

CONCLUSION

It can be concluded that possesses anti-nociceptive properties which are probably mediated via inhibition of prostaglandin synthesis as well as central inhibitory mechanisms which may be of potential benefit for the management of pain and inflammatory disorders.

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