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Evaluation of carrageenan induced anti-inflammatory activity of methanolic extract of *Mollugo cerviana* in albino wistar rats

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Abstract
Inflammation induced by carrageenan is acute, non-immune, well-researched, and highly reproducible. The anti-inflammatory activity of test compounds by examining their ability to reduce or prevent the development of carrageenan-induced paw swelling is one of the important studies. In the present study attempts are made to validate the anti-inflammatory activity of Methanolic extract of *Mollugo cerviana.* The anti-inflammatory activity of the Methanolic extract of *Mollugo cerviana* was evaluated by carrageenan-induced rat paw oedema method. The number of migrating leukocytes in the exudates was determined. The Methanolic extract of *Mollugo cerviana* reduced the edema induced by carrageenan by 55.48% and 57.62% on oral administration of 100 and 200 mg/kg, as compared to the untreated control group. Animals treated with the Methanolic extract of *Mollugo cerviana* and standard produced a leukocyte migration of $0.55\pm0.06\times10^3$, $0.51\pm0.05x10^3$ and $0.47\pm0.04\times10^3$ cells/ml, respectively. The pleurisy model showed that the Methanolic extract of *Mollugo cerviana* behaves as an inhibitor of leukocyte migration and the formation of pleural exudates.

Keywords: *Mollugo cerviana,* acute inflammation**,** Carrageenan Induced Pleurisy in Rats, anti-inflammatory activity.

1. Introduction
Inflammation is a complex biological response of vascular tissues against aggressive agents such as pathogens, irritants, or damaged cells. The acute inflammation causes the increased movement of plasma and innate immune system cells, such as neutrophils and macrophages, from the blood into the injured tissues. The inflammation causes increased blood flow, elevated cellular metabolism, vasodilatation, the release of soluble mediators, extravasation of fluids and cellular influx [1]. During inflammation cell membranes induce the activation of phospholipase A2 followed by the release of arachidonic acid and inflammatory mediators such as cytokines, serotonin, histamine, prostaglandin and leukotrienes that increase vascular permeability. When inflammation takes place leukocytes migrate to the site of inflammation [2]. Inflammation induced by carrageenan is acute, non-immune, well-researched, and highly reproducible. Proinflammatory agents like bradykinin, histamine, tachykinins, complement and reactive oxygen, and nitrogen species often became in the cutaneous injection. Subsequently there will be a development of signs of inflammation such as edema, hyperalgesia and erythema. Many saponins tested have displayed significant antinociceptive, anti-inflammatory and antipyretic activities possibly due to their nonglycosidic moiety, the sapogenin. There is evidence for the antiallergic, antifungal and analgesic activities [3–6]. Moreover, a variety of plant extracts have proved to be useful in animal models of inflammation [7–10].
Paw swelling, or footpad edema, is a convenient method for assessing inflammatory responses to antigenic challenges and irritants. The anti-inflammatory activity of test compounds by examining their ability to reduce or prevent the development of carrageenan-induced paw swelling is one of the essential studies. In the present study, attempts are made to validate the anti-inflammatory activity of Methanolic extract of *Mollugo cerviana.*

2. Materials and Methods
2.1. Animals
Male albino rats (180 ± 5 g) were obtained from animal house, K.M. College of pharmacy, Madurai maintained in standard laboratory conditions. They were given standard laboratory diet and water ad libitum. All animal experiments are approved by the Institutional Animal Ethics Committee, and were by the guidelines of the committee for Control and Supervision of Experiments on Animal (CPCSEA), Government of India.

2.2. Acute inflammation

Carrageenan-induced rat paw oedema is used widely as a working model of inflammation in the search for a new anti-inflammatory drug. The anti-inflammatory activity of the Methanolic extract of *Mollugo cerviana* was evaluated by carrageenan-induced rat paw oedema method [11]. Albino Wistar rats (180 ± 5 g) were used. Anti-inflammatory activity was measured using carrageenan induced rat paw oedema assay. The rats were divided into 5 groups of 5 animals each. Group I. were given normal saline and treated as the negative control. Rats of Group II was treated with carragenan (1%w/v) in saline in the sub planter 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 of Methanolic extract of *Mollugo cerviana* (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 one hour, 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 the hourly interval for 4 h. The perimeter of the paw was measured by using vernier calipers. Measurements were taken at 0–4 h after the administration of the carrageenan. The anti-inflammatory activity was calculated by using the relation

$$\% \text{ inhibition of edema} = \frac{T - T_0 \text{ X } 100}{T}$$

T, Thickness of paw in control group; T_0, Thickness of paw edema in the test compound treated group.

2.3. 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 [12,13] and each were pretreated with Methanolic extract of *Mollugo cerviana* (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 received 0.25 ml of an intra-pleural injection of 1 % carrageenan on the right side of the thorax. The animals were sacrificed 3 hours 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 hole 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.

2.4. Statistical analysis

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.

3. Results

The effect of Methanolic extract of *Mollugo cerviana* on carrageenan-induced edema in rats is shown in Table 1. The results obtained indicate that the Methanolic extract of *Mollugo cerviana* had significant anti-inflammatory activity in rats. The Methanolic extract of *Mollugo cerviana* reduced the edema induced by carrageenan by 55.48% and 57.62% on oral administration of 100 and 200 mg/kg, as compared to the untreated control group. Indomethacin at 10 mg/kg inhibited the edema volume by 60.06%.

The effect of Methanolic extract of *Mollugo cerviana* on carrageenan-induced pleurisy in rats is shown in Table 2. The volume of pleural exudates in the toxic control group was 0.38±0.10 ml. Animals treated with the Methanolic extract of *Mollugo cerviana* (100 and 200mg/kg, p.o.) decreased the pleural exudates to 0.20±0.07 ml and 0.17±0.06. Treatment with Indomethacin (10 mg/kg, p.o.) produced the exudates of 0.15±0.05 ml. The leukocyte count for the control group was found to be 4.16±0.37×103 cells/ml. Animals treated with the Methanolic extract of *Mollugo cerviana* and standard produced a leukocyte migration of $0.55\pm0.06\times10^3$,$0.51\pm0.05\text{x}10^3$ and $0.47\pm0.04\times10^3$ cells/ml, respectively.

4. Discussion

Due to the increasing frequency of intake of Non-Steroidal Anti-inflammatory Drugs (NSAID) common side effects are reported. The scientific exploration of plant extracts having fewer side effects become a significant work. The continuous search for indigenous drugs, to provide relief to inflammation can be a solution. Carrageenan-induced inflammation is a biphasic phenomenon [14]. The first phase of edema is attributed to the release of histamine and 5-hydroxytryptamine. Plateau phase is maintained by kinin like substances and second accelerating phase of swelling is assigned to prostaglandin like substances. The mode of action of drugs can be interpreted based on the knowledge of mediators involved in different aspects. The pleurisy model showed that the Methanolic extract of *Mollugo cerviana* behaves as an inhibitor of leukocyte migration and the formation of pleural exudates when given orally, as reported earlier [15].

5. Conclusion

Thus it can be concluded that the Methanolic extract of *Mollugo cerviana* posseses 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 low toxicity and better therapeutic index.

6. References

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