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Ameliorating Effect Of *Capparis Aphylla Roth* In Carrageenan Induced Inflammation In Rats.

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Abstract

The purpose of this study was to evaluate the capability of Capparis aphylla Roth to lessen inflammation brought by carrageenan in rats. Rats chosen for this experiment were six females of 200–250 g, and each group had six animals, so six groups were formed (n=6). Group 1 did not receive any external treatments, so they drank water and ate a standard diet. The group called Model Control received carrageenan to promote acute inflammation. Group 3 was subjected to carrageenan and also given Indomethacin at a dose of 100 mg/kg. Animals in groups 4, 5, and 6 got carrageenan along with equal amounts of the MECA extract at 190 mg/kg, 240 mg/kg, and 300 mg/kg, respectively. All the required treatments were provided, and staff carefully observed for the whole day. The paws of rats treated with carrageenan became swollen, and this was strongly decreased by the use of Indomethacin as well as MECA. To sustain continuous damage, inflammation goes through processes such as matrix breakdown and formation of new blood vessels. It is one of the most common autoimmune diseases that is known to cause chronic and lasting inflammation, but scientists are unaware of the reason. In RA, the first damage to joints is in the synovial lining, which spreads to the cartilage and bone and causes the formation of a pathological growth called pannus. The process includes evaluating joint scoring, ESR, and CRP, whereas outcome criteria mean paying attention to mortality or x-ray vision of destroyed joints. Reducing the action of COX-II in the body could be the reason for MECA's anti-inflammatory effects.

Key words: *Anti-inflammatory, Carrageenan, Indomethacin, MECA, COX-II Inhibitor, Capparis aphylla*

INTRODUCTION

RA is a disease that progresses through the years, when the body's immune system attacks itself, causing inflammation in joints that leads to permanent joint damage. We do not know exactly what events cause the disease to start and develop further, but both immune and non-immune forces lead to joint damage. Several processes such as intracellular signaling, cell division, inflammation, breaking down of tissues, and creating more blood vessels encourage joint deterioration. The disease causes disabilities in nearly 1% of people around the world. Early diagnosis and prompt treatment are the main focuses of using advanced technology in rheumatology now. The main function of pro-inflammatory cytokines in

RA is to increase the growth of synovial tissue and cause damage to the cartilage, and this occurs with the help of other markers such as C-reactive protein and elevated rheumatoid factors. RF is still a main immunological signal found in more than 80 percent of RA patients. Radiographic images that show erosion and reduced bone density around the joints are still used as the main way to check joint damage. Experiments with Complete Freund's Adjuvant-induced arthritis in animals produce swelling in the joints, growth of pannus, and bone reduction, very much like these changes seen in human RA. Capparis aphylla Roth, a plant used to handle inflammation traditionally, is said to be helpful in giving pain relief, promoting sweating, killing bacteria, resisting fungi, and lowering inflammation. Traditionally, asthma, gout, and inflammation in the joints have been treated using the antigenic spermidine alkaloids found in its bark. The purpose of this investigation was to observe how Capparis aphylla Roth can help to treat inflammatory changes caused by carrageenan in rats, by manipulating pro-inflammatory cytokines.

2. Materials & Methods

2.1 Collection and Authentication of the plant material

The roots of *Capparis aphylla Roth.* were procured from a certified herbal farm located in Amreli, Gujarat. The plant specimen was taxonomically identified and authenticated by Dr. Geetha K.A., Senior Scientist at the Directorate of Medicinal and Aromatic Plants Research (DMAPR), Boriavi, Anand, Gujarat, India.

2.2 Preparation of extract of *Capparis aphylla* Roth. [8]

Take 3.2 kg of root bark from Capparis decidua and keep it in three cycles of percolation with ethyl alcohol at room temperature for 2–3 weeks. Triangular shaped were formed, and the percolates were then reduced under pressure to minimize the amount of solvent. In the end, 38 g of a dark busy residue was obtained. Then, the residue was dissolved in 1N hydrochloric acid and repeatedly extracted with ethyl acetate to remove everything that was neutral. To continue, we added ammonia and this led to the formation of yellow precipitates in the reaction. Next, chloroform was used to gather the precipitates gathered earlier, after which the solvent was evaporated to get a more concentrated alkaloid fraction. The chloroform extract that held the crude alkaloids was purified further by column chromatography on neutral alumina oxide. The elution was done by using a solvent mixture of ethyl acetate and methanol, where the former made up 4/5 of the mixture. The procedure allowed the researchers to obtain a yellow-colored compound. Minor impurities were discovered in the isolated compound, so the next step was to clean it further to fully learn about it.

2.3 Chemical and reagents

Astron Chemicals, Ahmedabad, Gujarat, India was where we purchased all the Dragendorff reagent, methanol, alumina oxide, ethyl acetate, sodium azide, sodium citrate, succinic acid, bromocresol, Brij-95, formalin, formic acid, Freund's adjuvant, and carrageenan. Also, the Total Protein Kit, RF Latex Kit, and CRP Kit were obtained from Coral Clinical Systems, Gujarat, India.

Animals

Female Wistar albino rats with weights ranging from 200 to 250 g were obtained from the Zydus Research center in Ahmedabad. Essential laboratory conditions were ensured for the animals and they were free to get food and water whenever they wanted. In the laboratory, the animals' housing had a temperature of 22 ± 2°C, a relative humidity of 50% ± 10%, and was lighted for 12 hours each day. Before the study started, all animals were adjusted to the laboratory environment and handled regularly for 3–4 days so they would feel comfortable. Working hours for experiments were from 08:00 AM to 4:00 PM to keep the observations uniform. Handling and experimental procedures done on animals were performed under the OECD Guidelines for the Care and Use of Laboratory Animals and were also approved by the Institutional Animal Ethics Committee (IAEC).

2.4 Preparation of the herbal formulation, reference drug solution:
The herbal formulation and standard formulation were administered orally at various doses, as a solution in water. Indomethacin was used as a standard drug.

2.5 Preparation of drug solution:
Distilled water was used to blend a sample of the dried extract in order to make stock solutions with 190 mg/kg, 240 mg/kg, and 300 mg/kg dosages. Likewise, Indomethacin was dissolved in distilled water at a ratio of 100 mg per kg. The amount of each stock solution given to the animals was determined based on their weight for correct and accurate dosing.

Animal Grouping
The rats were evenly put into six groups with six rats in each group. Groups 1 (Normal Control) was just given drinking water and the standard diet used by the laboratory. The subjects in Group 2 were given carrageenan to bring about inflammation. Besides carrageenan, Group 3 received Indomethacin in a dose of 100 mg/kg. Groups 4, 5, and 6 (Test Groups) received injections of carrageenan together with MECA at the doses of 190 mg/kg, 240 mg/kg, and 300 mg/kg respectively. After the treatment drugs were given, the animals were observed and data were taken for a full 24 hours.

Dose Fixation Study:
Capparis aphylla Roth. shows significant Antiasthmatic , Sedative , Anticonvulsant, Antidiabetic , Antistress. Methanolic plant extract have been shown its activity in 100mg/kg, 200mg/kg, 300mg/kg. Dependent on this data 5 doses were been decided.
Methanolic Extract of *Capparis aphylla* Roth.
Dose 1 (MECA) - 120 mg/kg,
Dose 2 (MECA) - 150 mg/kg,
Dose 3 (MECA) - 190 mg/kg
Dose 4 (MECA) - 240 mg/kg
Dose 5 (MECA) - 300 mg/kg

Carrageenan-Induced Rat Paw Edema
Experimenters use a method whereby test materials are checked for their ability to decrease edema in rats' hind paw after an inflammatory substance is injected. Male or female Wistar albino rats (with a weight of 200–250 g and at 12–14 weeks of age) took part in this study. Both the control group and groups receiving the drug received 5 ml of distilled water every day through their stomach tube. After half an hour, the inflammation was caused by putting a tiny amount of a 1% carrageenan solution under the skin in the plantar region of the left hind paw. The point where the skin was injected was marked with ink near the lateral malleolus to keep all measurements the same. Following that, mercury was poured into the paw up to the mark, and the size of the paw was captured by a plethysmograph. Immediately after the carrageenan task, the measurements were taken, then again after 3 and 6 hours and finally at the end of the 24-hour timeframe to study how edema became affected by the substance.

3. RESULTS

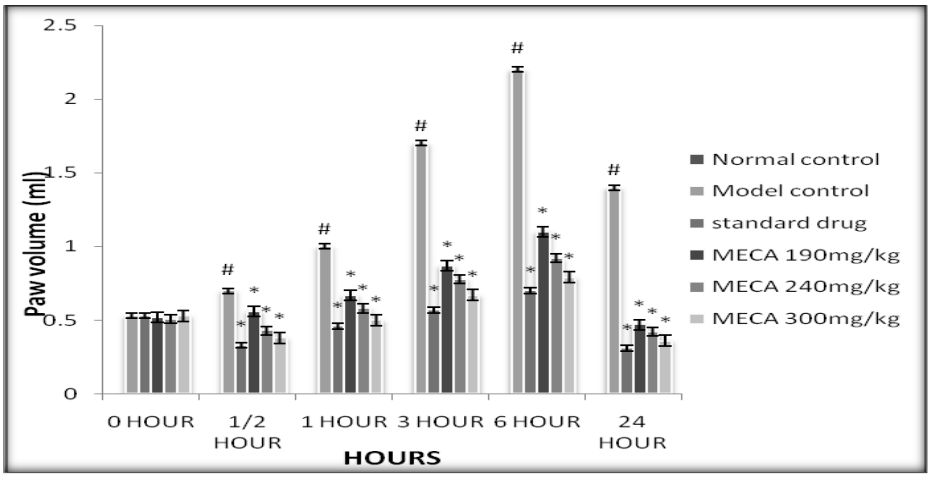
CARRAGEENAN-INDUCED RAT PAW EDEMA
A greater amount of swelling and redness occurred in the sub plantar injection model control group rather than in the normal control group. Three hours after treatment, the edema in the model control animals was higher than it was in normal controls. All the findings are listed in Table 1.

Table 1: Values of Paw Volume at Different Time

Groups	0 hour	½ hour	1 hour	3 hours	6 hours	24 hours
Normal control	0	0	0	0	0	0
Model control	0.53±0.08$^{\#}$	0.7±0.14$^{\#}$	1±0.13$^{\#}$	1.7±0.1$^{\#}$	2.2±0.06$^{\#}$	1.4±0.07$^{\#}$
Standard control	0.53±0.1*	0.33±0.1*	0.46±0.13*	0.57±0.15*	0.7±0.17*	0.31±0.17

MECA 190 mg/kg	0.52±0.12*	0.56±013*	0.67±0.23*	0.87±0.08*	1.1±0.13*	0.47±0.1*
MECA 240 mg/kg	0.51±0.2*	0.43±0.07*	0.58±0.15*	0.78±0.19*	0.92±0.2*	0.42±0.2*
MECA 300 mg/kg	0.53±0.16*	0.38±0.14*	0.5±0.15*	0.67±0.1*	0.79±0.1*	0.36±0.2*

At 0 hours, no signs of inflammation were observed in any group. From 3 to 6 hours and continuing through 6 to 24 hours, the model control animals exhibited a progressive increase in inflammatory response as paw edema developed. Treatment with MECA at doses of 190 mg/kg, 240 mg/kg, and 300 mg/kg resulted in a significant reduction in paw edema volume compared to the model control group from baseline up to 6 hours. Notably, the suppression of edema was more pronounced during the 3 to



6-hour interval and was further enhanced between 6 and 24 hours post-carrageenan injection.
Figure 1: Effect of methanol extract of *Capparis aphylla* Roth. on paw edema.

The data for values are expressed as mean ± SEM, using a set of six animals in every group (N=6). A one-way ANOVA was used first, and after that, Dunnett's post hoc test was carried out. Comparisons of normal controls with the model control have a significant difference shown as #P < 0.05, while that of the model controls with MECA treatment have a significant difference shown as *P < 0.05. Figure 1 shows the chart of these results. After carrageenan was given, there was no major increase in paw volume during the first phase. However, at all these times, the paw volume kept growing steadily over time. Using MECA in doses of 190 mg/kg, 240 mg/kg, and 300 mg/kg led to notable decrease in paw swelling in contrast to the control group.

4. DISCUSSION

When tissues such as blood vessels are exposed to hazardous substances such as pathogens or cell damage, inflammation results.[1]The immune system acts as a guard to get rid of dangerous agents and kick-start the healing process. Many animal models are used to study inflammation. For instance, acute inflammation is measured using carrageenan-induced paw edema, and chronic inflammation is studied by means of the Complete Freund's Adjuvant (CFA) model. Using steroids, nonsteroidal anti-inflammatory drugs (NSAIDs), and drugs that target the tumor necrosis factor -alpha (TNF-α) or interleukin-1 beta (IL-1β) has had only poor results in most cases.[14] Not to mention, using NSAIDs and analgesics provides relief from symptoms but does not halt the course of rheumatoid arthritis (RA) or keep joints from being damaged. Over time, using glucocorticoids through the mouth leads to serious results that must be handled with care. A lot of people are choosing herbal medicines as CAM because they are affordable, available everywhere, and have few minor side effects. It is common to consider them as the right choice for some issues, and they normally contain natural ingredients unless

mixed with man-made chemicals. It was found in the study that Capparis aphylla Roth. reduces inflammation when used as a herbal remedy. Rats are commonly used in exploration of different methods of inflammation using experimental models. Boosting the immune system because of inflammation leads to higher levels of proinflammatory cytokines that encourage the disease to worsen. This study involved these two models of inflammation: carrageenan induced paw edema in rats, as well as CFA induced arthritis. Strong inflammation was visible in the left hind paw of animals from the model group compared to the normal group after subplantar carrageenan injection. Throughout the experiment, the edema volume in the paws was recorded at 0, 0.5, 1, 3, 6, and 24 hours after giving the injection. As a result of carrageenan, antigen-presenting cells become active, and there is more bradykinin to encourage neutrophils to migrate into the peritoneal cavity. As a result of this, cells break down and leak toxic cationic proteins that raise the permeability of the nearby tissues. Treatment with MECA reduced the size of the swollen paw at all the tested doses and this continued during the whole recording period. Rheumatoid arthritis and CFA-induced arthritis present the same symptoms, such as joint swelling, pannus formation, destruction of joints, and bone resorption, so it is a valuable model to test therapies that help fight RA. Disease starts with an acute phase of inflammation at the joint, noted by the presence of mononuclear cells in the synovial lining, and ends with chronic inflammation, which includes the careful study of a single clinical case and leads to damage and loss of the bone and cartilage around the joint. Prolonged and untreated arthritis in dogs can end in bone stiffness and changes in their paws. A review of the literature showed that injection of 0.2 ml CFA into the left hind paw caused primary inflammation within 3 to 5 days in this trial. Edema in the paws that did not receive injection appeared in rats about 11 to 14 days after the test started. The score based on symptoms and arthritis while assessing the animal's sense of smell and other symptoms was increasing over time. These lead to the conclusion that CFA injection causes arthritis in rats. MECA reduced both prime and secondary lesions as well as the sufferance index, which means that Capparis aphylla Roth protected against adjuvant-induced arthritis.

5. CONCLUSION

Based on the results presented, it can be concluded that MECA demonstrated a strong anti-inflammatory activity by alleviating pathological damage through the suppression of proinflammatory cytokine levels, which in turn decreased acute phase protein concentrations. Additionally, its notable immunomodulatory effects likely contributed to this beneficial outcome. The enhanced efficacy of MECA may be due to the synergistic actions of spermidine alkaloids such as caparicine.

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