SUBPLANTAR injection of Pilocarpus spicatus essential oil (PSEO), induced rat hindpaw oedema in a dose-dependent manner. The time course study revealed that when compared to carrageenan-induced oedema, the oedema response to PSEO was greater at 1 h post-injection, and thereafter remained relatively constant until 5 h post-injection. By 24 h, it was still at almost the 50% level. This effect of PSEO was characterized using several inhibitors of oedema formation. Pretreatment with the H1-receptor antagonist chlorpheniramine did not affect this response, while a significant reduction of paw oedema was achieved with the serotonin antagonist methysergide, but only 1 h and 2 h after injection of PSEO. The oedemagenic activity of PSEO was also suppressed by pretreating the rats with the eicosanoid synthesis inhibitors, phenylbutazone, EP 10161 and dexamethasone. This last drug showed the greatest potency. These findings suggested a probable injury to dermal mast cells and liberation of arachidonate metabolites and eicosanoids at the late phase of oedema induced by PSEO.

Key words: Eicosanoids, essential oil, Pilocarpus spicatus, rat hindpaw oedema, serotonin.

Introduction

Experimental acute inflammation can be produced by subplantar injection of a number of irritant substances in the hindpaw of rat. Among those of special interest is carrageenan, a sulphated saccharide derived from the red seaweed (Buchemna spinosum) that induces hindpaw oedema. Carrageenan-induced oedema involves predominantly the mediation of cyclooxygenase products of acid arachidonate1 and this oedema is suppressed by all nonsteroidal anti-inflammatory drugs (NSAIDS).2 Although this model system bears little relation to human disease conditions, it has significant predictive value for clinically useful anti-inflammatory agents.3,5 However, there is controversy about the effects of these drugs on the response to carrageenan. For example, compounds of gold, the most efficacious drugs for rheumatoid arthritis, have absolutely no effect on carrageenan-induced oedema.6 Therefore, the search for novel model systems continues in the development of new anti-inflammatory drugs.

The present paper describes the oedemagenic potential of Pilocarpus spicatus essential oil (PSEO) in the hindpaw of rat and the mediator substances involved in its action.

Materials and Methods

Animals: Male Wistar rats (120–180 g), housed at 25–27°C and maintained on standard pellet diet (Purina Chow) and water ad libitum, were randomly assigned to various treatment groups. Six animals were included in each group.

Drugs and chemicals: The essential oil of P. spicatus was supplied by the Natural Products Laboratory of the Federal University of Ceará, Fortaleza, Brazil and lambda carrageenan was purchased from Sigma Chemical Company, St Louis, USA. The other drugs were chlorpheniramine (Schering, São Paulo, Brazil), dexamethasone (Upjohn, São Paulo, Brazil), EP 10161 (Institut Henri Beaufour, Le Plessis, France), phenylbutazone (Geigy, São Paulo, Brazil) and methysergide (Sandoz, São Paulo, Brazil). PSEO was emulsified in Tween 80 (9:1 w/w) and diluted with 154 mM saline. EP 10161 was suspended in 5% DMSO and all other drugs were dissolved in 154 mM saline.

Paw oedema assay: Using unanaesthetized rats, PSEO (2.5% and 5%) or carrageenan (1%) was injected into the subplantar surface of the right hindpaw in a volume of 0.1 ml/rat. An equal volume of vehicle was injected into the contralateral paw. The volumes of both hindpaws up to the ankle joint were measured with a plethysmometer prior to and at different periods (1 h, 2 h, 3 h, 4 h, 5 h and 24 h) after induction of oedema. The difference in the volume between the hindpaws was a measure of the oedema present (ml).

Inhibition of oedema by drugs: Unanaesthetized rats that had been fasted overnight were treated with a single
dose of chlorpheniramine (10 mg/kg, i.p.), methysergide (1 mg/kg, p.o.), phenylbutazone (100 mg/kg, p.o.), EP 10161 (20 mg/kg, i.p.), dexamethasone (0.5 mg/kg, i.p.) or 154 mM saline (2 ml/kg, i.p.). One hour after drug administration, animals were given 0.1 ml of 5% PSEO into the subplantar region of the right hindpaw. An equal volume of vehicle was injected into the contralateral paw. The paw volume measurements were carried out as described earlier by a plethysmometer at different periods following PSEO injection. The mean paw oedema values for each drug treatment were established and compared with vehicle-treated control. The data were evaluated by use of ANOVA and \( t \)-independent test. Statistical significance was assigned for \( p < 0.05 \).

**Results**

The effect of graded doses of PSEO (2.5 and 5 mg) and carrageenan (1 mg) on the magnitude of hindpaw oedema is shown in Figure 1. Subplantar injection of PSEO induced a dose-related elevation of paw oedema at all time points. The oedemagenic effect of 5 mg PSEO was greater than that of carrageenan at 1 h post-injection, attained the same peak level at 3 h, remained relatively constant up to 5 h and was still pronounced (almost 50% of peak level) at 24 h.

Figure 2 shows the ability of some of the test drugs to inhibit the oedema induced by 5 mg of PSEO. The PSEO-induced rat hindpaw oedema was not affected by chlorpheniramine, while paw swelling that occurred at 1 h and 2 h was significantly reduced by methysergide. Methysergide was not effective at all other time points. The oedema caused by PSEO was also suppressed by phenylbutazone, EP 10161 and dexamethasone. At the peak effect of PSEO, the extent of oedema inhibition was 25%, 41% and 65% for phenylbutazone, EP 10161 and dexamethasone, respectively.

At 24 h, dexamethasone almost abolished the oedema (98% inhibition), while EP 10161 showed 23% inhibition. Phenylbutazone had no significant effect at this time.

**Discussion**

The results of the present study show that PSEO is highly potent in promoting a local oedematous reaction. This reaction is dose-dependent and has a time course of action very similar to the one we obtained with carrageenan. The action of PSEO, however, seems more potent because it demonstrated a greater magnitude of oedema than carrageenan at 1 h and 2 h post-injection.

In order to characterize the oedema response to PSEO, a number of specific pharmacological antagonists were utilized. Pretreatment with the \( H_1 \)-receptor antagonist chlorpheniramine was without any effect on PSEO response, while methysergide, a serotonin antagonist significantly inhibited the response at an earlier time. This observation suggested a possible injury to dermal mast cells and release of serotonin in the early phase of oedematous reaction. In agreement with the results are the reports by others that serotonin but not histamine is largely responsible for hindpaw oedema induced by compound 48/80 and carrageenan.\(^7\) Cyclooxygenase inhibition does not seem to affect serotonin-induced paw oedema in rats\(^8\) and probably for this reason phenylbutazone did not show any influence on early phase oedema reaction to PSEO. Nevertheless, the finding that phenylbutazone, EP 10161 and dexamethasone (cyclooxygenase, lypoxygenase and phospholipase A\(_2\) inhibitors, respectively) were potent in suppressing late phase oedema response to PSEO established that eicosanoids are the major inflammatory...
mediators. The rank order potency of these inhibitor drugs is dexamethasone > EP 10161 > phenylbutazone. The fact that EP 10161 produces a greater inhibitory effect than phenylbutazone indicates that leukotrienes are more important mediators than prostaglandins in this model. In contrast, carrageenan-induced oedema involves predominantly the mediation of prostaglandins.\(^1\)

In the present study, dexamethasone produced a more potent block in the oedemagenic effect of PSEO. Dexamethasone has been reported to inhibit increased vascular permeability independent from inhibition of phospholipase A\(_2\).\(^9\) It is possible that similar mechanisms might underlie the inhibitory action of dexamethasone against PSEO-induced inflammatory response.

The two major constituents of PSEO were methylnonylketone (\(~80\%) and methylundecylketone (\(~10\%). The oil also contains some minor constituents such as terpenes and phenols. It remains to be verified whether one or more of these constituents are responsible for the induction of PSEO-induced inflammatory reaction. It is probable that PSEO, besides promoting mast cells degranulation, may activate phospholipase A\(_2\) enzyme in vascular endothelial cells and may release arachidonic acid from membrane phospholipids. Arachidonate metabolism through cyclooxygenase and lipoxygenase pathways is known to produce highly potent inflammatory mediators, namely prostaglandins, thromboxanes and leukotrienes.\(^10-12\) Therefore it is reasonable to assume that PSEO-induced hindpaw oedema in rat involves serotonin at the early phase and eicosanoids, particularly leukotrienes, at the late phase.

**References**


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