

Antiinflammatory Activity of the Aqueous Extract of *Calea zacatechichi*

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The inflammatory process involves many biochemical pathways and a variety of mediators [1-5]. Anti-inflammatory drugs presently available for the treatment of various inflammatory disorders, have one or more adverse and undesirable side-effects [5,6]. In recent years, active principles of varied chemical structures have been isolated from plants possessing anti-inflammatory activity. *Calea zacatechichi* Schlecht (Compositae/Asteraceae) grows in fields in southern Mexican and several other countries. It is used in the folk medicine of Mexico for many medical purposes, especially as treatment of various inflammatory diseases: rheumatism, edema and respiratory pathway disorders. Different parts of the plant have been administered when the extremities are cold during fever and for pains in the legs and arms [7]. We only found a single reference related to anti-inflammatory effects for this plant [8]. Therefore, the aim of this study was to evaluate whether the aqueous extract of *Calea zacatechichi* showed anti-inflammatory activity.

METHODS: *Calea zacatechichi* was identified in the herbarium of the Department of Botany of the Facultad de Estudios Superiores Iztacala, UNAM and a specimen was deposited under accession number 26901.

Preparation of the aqueous extract. Powdered leaves (1 g) was added in 20 ml of distilled water, heated for 10 min at 95°C and centrifuged at 100 x g. The supernatant was adjusted to pH 7.4. It contained 60 mg/ml of extract material.

Experimental animals. Male Wistar rats (200-250 g) and Swiss albino mice (20-25 g), maintained under uniform laboratory conditions, were used.

Carrageenan-induced edema. Edema was induced in rats by injection of 0.1 ml of 1% carrageenan (Type II Sigma, St Louis, MO) in sterile saline into the subplantar tissue of the right hind paw. The left hind paw was injected with 0.1 ml of saline (control). Aqueous extract (10 and 100 mg/kg), indomethacin (Sigma, St Louis, MO) (10 mg/kg) and vehicle (0.1 ml /100 g) were administered po 30 min prior to carrageenan injection. The paw volume was measured before and 1,2,3, and 4 h after carrageenan injection by the mercury displacement method [9].

Neutrophil migration into peritoneal cavity. The rats groups were injected with 3 ml of carrageenan (100 µg/ml) prepared in sterile saline solution into the peritoneal cavity, and 4 h later the abdominal cavity was washed with phosphate buffered saline containing 5 U/ml of heparin (Sigma, St Louis, MO) and 5% of bovine serum albumin. The total cell counts were done in a Neubauer chamber and differential cell counts were performed by the Souza and Ferreira technique [10]. One hour before the carrageenan injection, the rats groups were treated with 10 and 100 mg/kg po of the aqueous extract, saline (control), indo-

methacin or dexamethasone (Merck-Sharp and Dohme) (10 and 1 mg/kg, po), respectively.

Statistical analysis. Significance of the differences was assessed by Student's t-test for unpaired samples and values of $p < 0.05$ were considered significant.

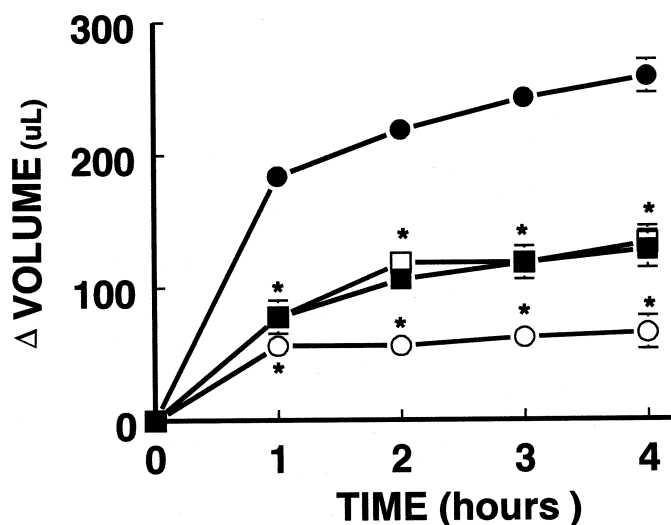


Figure 1. Effect of aqueous extract of the *Calea zacatechichi* on the edema induced by carrageenan (100 µg in 0.1 ml, intraplantarly) ●-● control group 0.1 ml of saline solution; ○-○ 10 mg/Kg of indomethacin; - - 10 mg/Kg and ■-■ 100 mg/kg, aqueous extract of *Calea zacatechichi* (orally). Values are the mean ± SEM for the number animals used (n=10), * $p < 0.05$ when compared vs treated groups.

RESULTS: The aqueous extract of *Calea zacatechichi* showed significantly anti-inflammatory activity preventing the formation of edema after administration of carrageenan. Carrageenan increased paw volumes by $257 \pm 6 \mu\text{L}$, whereas carrageenan concomitant with aqueous extract (100 and 10 mg/kg) increased paw volume to 133 ± 6 and $129 \pm 6 \mu\text{L}$, respectively (Fig. 1). Indomethacin also prevented the formation of edema (Fig. 1). The aqueous extract (100 and 10 mg/kg) displayed significantly decreased the number of neutrophils migrating into the peritoneal cavity (Fig. 2); by $44 \pm 7\%$ and $61 \pm 9\%$, respectively, compared with the control group. When we used dexamethasone, we observed that it inhibited migration by $50 \pm 8\%$.

CONCLUSIONS: This study showed that aqueous extract of *Calea zacatechichi* contain compounds with po-

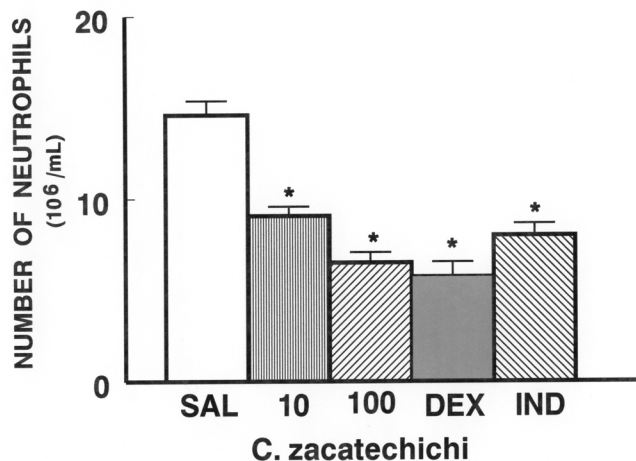


Figure 2. Effect of 10 and 100 mg/kg aqueous extracts of *Calea zacatechichi*, orally administered, on neutrophil migration into the cavity induced by carrageenan (300 µg). Saline (SAL) was used as vehicle control whereas dexamethasone (DEX) (1 mg/kg orally) and indomethacin (INDO) (10 mg/kg po) were used as standard drugs. Values are the mean ± SEM for the number of animals used (n=8), *p<0.05 when compared vs saline treated groups.

tential anti-inflammatory activity. We found that, at doses used, *Calea zacatechichi* inhibited edema formation by a percentage close to the inhibition produced by indomethacin, which, like most of the non-steroidal anti-inflammatory agents, inhibits the biosynthesis of prostaglandins [11]. This effect might explain the anti-inflammatory activity in carrageenan-induced rat paw edema by the aqueous extract of *Calea zacatechichi*. The antiedema effect showed by the extract aqueous correlated with their ability to decrease the number of neutrophils migrating into the peritoneal cavity, similar to that seen with dexamethasone [12]. We observed that the dose of dexamethasone used produced a higher inhibition of neutrophil migration than the effect produced by indometha-

cin, supporting the possible role of leukotrienes in the inflammation model [13,14]. These results suggested that anti-inflammatory activity showed by *Calea zacatechichi* could be related to the biosynthesis of prostaglandins and lipoxygenase products. In conclusion, *Calea zacatechichi* appears to be a promising source of anti-inflammatory agents.

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