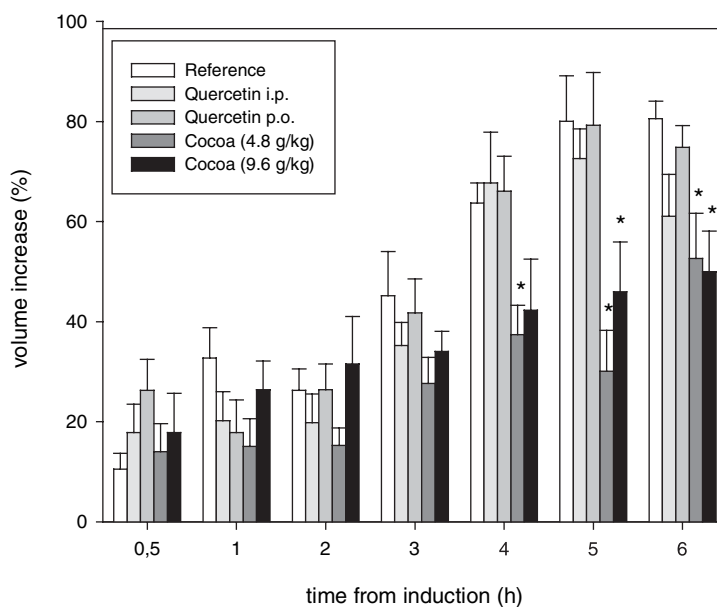


Anti-inflammatory effects of cocoa in rat carrageenin-induced paw oedema

S. Ramos-Romero, E. Ramiro-Puig, F. J. Pérez-Cano, C. Castellote, A. Franch and M. Castell
 Department of Physiology, Faculty of Pharmacy, University of Barcelona, Spain

Acute inflammation is the fastest immune system response to damage. Normally, short-lasting and limited inflammation develops beneficial effects. However, permanent inflammation becomes harmful and painful. Carrageenin-induced paw oedema is a reproducible acute inflammatory model used in the screening of anti-inflammatory drugs. Cocoa, a product from *Theobroma cocoa* seeds, contains flavonoids with potential anti-inflammatory properties. Previous studies have shown that *in vitro* cocoa extract inhibits some pro-inflammatory cytokines (TNF α , monocyte chemotactic protein 1 (MCP-1) and IL-6)⁽¹⁾. The aim of the present study was to establish the *in vivo* anti-inflammatory effect of cocoa. Female Wistar rats (Harlan Iberica SA, Barcelona, Spain) received water (reference animals; *n* 10), quercetin (3.1 g/kg body weight orally; *n* 10) or cocoa at doses of 4.6 (*n* 10) or 9.6 (*n* 10) g/kg body weight orally for 7 d. On day 8 some reference animals were injected with quercetin (10 mg/kg rat intraperitoneally (ip)). After 1 h all animals were injected with 0.1 ml carrageenin λ (1 %, w/v) into subplantar area of the right hind paw. Paw volume was measured by plethysmometry at 30 min post carrageenin injection and every hour for 6 h. Peritoneal macrophages (PM) and paw inflammatory exudates were obtained at the end of the study. PM were stimulated with lipopolysaccharide (LPS; 10 μ g/ml) and supernatant fractions were obtained after 24 h incubation. TNF α levels in exudates and supernatant fractions were determined by an ELISA technique. A significant reduction in paw oedema ($P < 0.05$) was observed in both groups treated with cocoa from 4 h after carrageenin injection (Figure). At 6 h paw volumes from cocoa-treated animals were about 35 % lower than that of the reference group. Quercetin treatment did not modify paw volume. TNF α levels in paw inflammatory exudates were lower in animals treated with the highest cocoa dose (Table). However, TNF α secreted by PM was reduced in the group treated with 4.6 g cocoa/kg and in animals injected with quercetin ip. In summary, a cocoa-rich diet could play a role as an anti-inflammatory adjuvant. This immunomodulatory action can be partially attributed to a reduction in cytokine release by pro-inflammatory cells such as macrophages.



	TNF α (pg/ml)					
	Inflammatory exudate		Non-stimulated PM		LPS-stimulated PM	
	Mean	SE	Mean	SE	Mean	SE
Reference	82.4	12.5	5991.3	685.2	11780.9	1444.6
Quercetin: ip	106.6	18.3	327.6**	87.7	1810.3**	410.6
Orally	63.4	15.1	4395.6	1147.3	7925.2	1954.4
Cocoa: 4.8 g/kg	65.4	10.1	1801.2*	1037.7	2444.0**	1217.7
9.6 g/kg	38.5*	12.2	6719.7	2896.3	12309.7	4489.7

Mean values were significantly different from those for the reference group: * $P < 0.05$, ** $P < 0.001$.

1. Ramiro E, Franch A, Castellote C, Pérez-Cano F, Permanyer J, Izquierdo-Pulido M & Castell M (2005) *J Agric Food Chem* 53, 8506–8511.