

SHORT COMMUNICATION

Evaluation of Cuban red propolis extract on liver sinusoidal endothelial cell cultures

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Key words: Cuban red propolis, liver sinusoidal endothelial cells, Transmission Electron Microscopy, Scanning Electron Microscopy, naphthoquinones, *in vitro* studies.

An ethanol extract of Cuban red propolis (CRP), mainly composed of naphthoquinones,¹ has been reported to possess scavenging action against oxygen radicals as well as hepatoprotective and anti-inflammatory effects on animal models.²⁻⁶ The 1:4-naphthoquinones-propolis derived fraction exerts scavenging action against oxygen radicals and inhibits tertbutyl/Fe²⁺-induced lipid peroxidation in rat microsomes.⁷

Five types of cells constitute the liver functional unit. They are divided into parenchymal cells or hepatocytes and non-parenchymal cells or sinusoidal cells. The sinusoidal cells are composed by fenestrated sinusoidal endothelial cells, Kupffer cells or liver macrophages, fat-storing cells or liver fibroblasts, and pit cells or liver NK cells. Each of them has a specific ultrastructure and function, directly related to their functions. In addition, they display different reactions against induced liver damage or disease.⁸

From the morphological point of view, CRP extract induces recovery of hepatocytes structure until nearly normal conditions in rodents after acute induced liver damage in centrolobular and periportal liver regions,^{3,6} but its effect on non-parenchymal cells has not been studied before.

Since *in vitro* studies allow to evaluate the effect of a product on a specific cell type without any systemic effect as occurs *in vivo*; the

effects of ethanol extracts of CRP and CRP-derived naphthoquinones on the morphology of cultured liver endothelial cells (LEC) were studied in this work by Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM).

Isolation and purification of LEC from male Wistar rats (250-300 g) was carried out by collagenase perfusion of the liver, isopycnic sedimentation in a two-step 25-50 % Percoll gradient and selective adherence on plastic and collagen type I, III. The cells were cultured in 24-multiwells on top of collagen-coated coverslips as described.⁹ Three hours after culture, LEC were washed with complete medium containing 2 % FCS and immediately treated with a 50 % ethanol extract of CRP. This propolis was collected from beehives that belong to the National Apicultural Station of Havana City, Cuba. Highly concentrated ethanol extracts of CRP were dissolved in 1 mL medium to yield final concentrations of 1.5 and 0.1 mg/mL of CRP. In addition, highly concentrated ethanol extracts of CRP-derived naphthoquinones were dissolved in 1 mL of medium to obtain a final concentration of 0.1 mg/mL of naphthoquinones. Fifteen hours after incubation, cells were washed in medium and processed for electron microscopical studies.

Controls consisted of cells incubated in medium, to which only 0.46 or 0.046 mg/mL of ethanol was added.

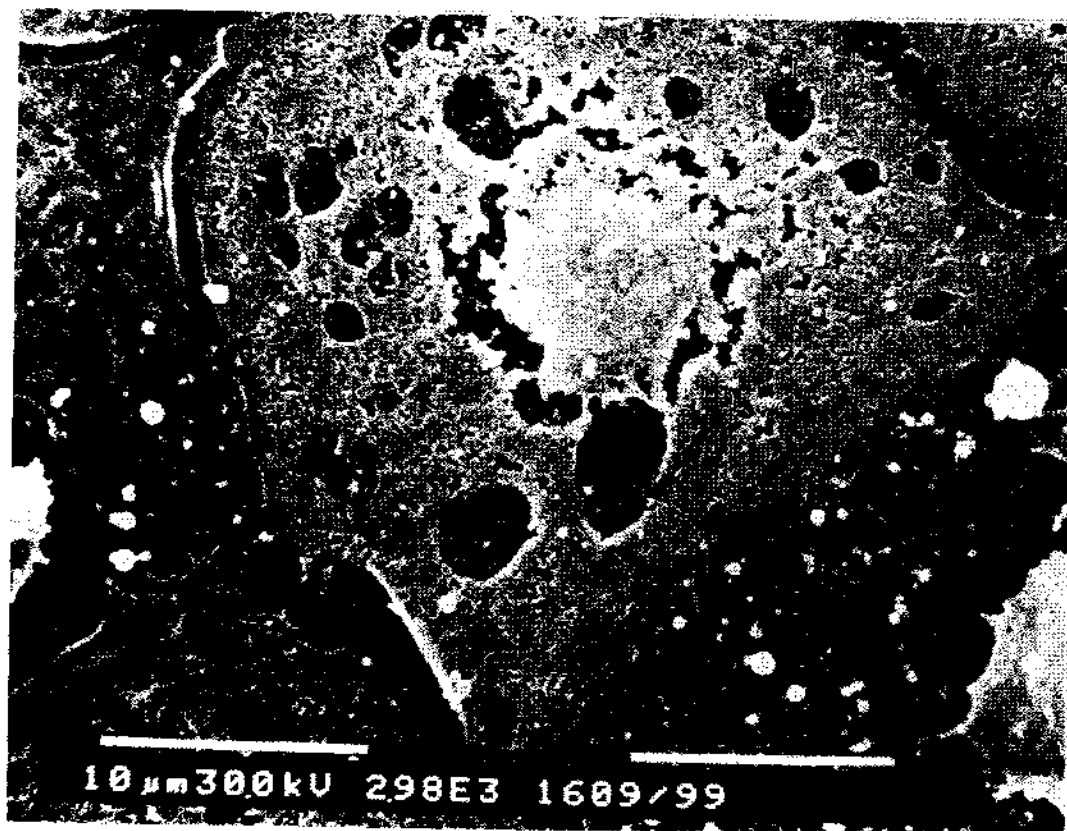
For SEM, treated LEC were fixed with 1.5 % glutaraldehyde in 0.1 mol/L sodium cacodylate buffer, pH 7.4 for 24-72 h and postfixed with 1 % osmium tetroxide in 0.1 mol/L sodium cacodylate buffer for 1 h. The samples were dehydrated in graded ethanol solutions; hexamethyldisilazane (HMDS) dried⁹ and sputter-coated with a thin layer of 10 nm gold. Cells were studied with a Philips SEM 505. For TEM, cultured cells were fixed as described above; subsequently they were stained *in block* with an aqueous solution of 3.5 % uranyl acetate, dehydrated in graded ethanol solutions and embedded in Epon. Ultrathin sections of 100 nm were stained with uranyl acetate and lead citrate subsequently and observed in a Philips EM 400.

Non-treated control LEC monolayers composed of vacuolized cells with normal cell ultrastructure were obtained (Fig. 1).

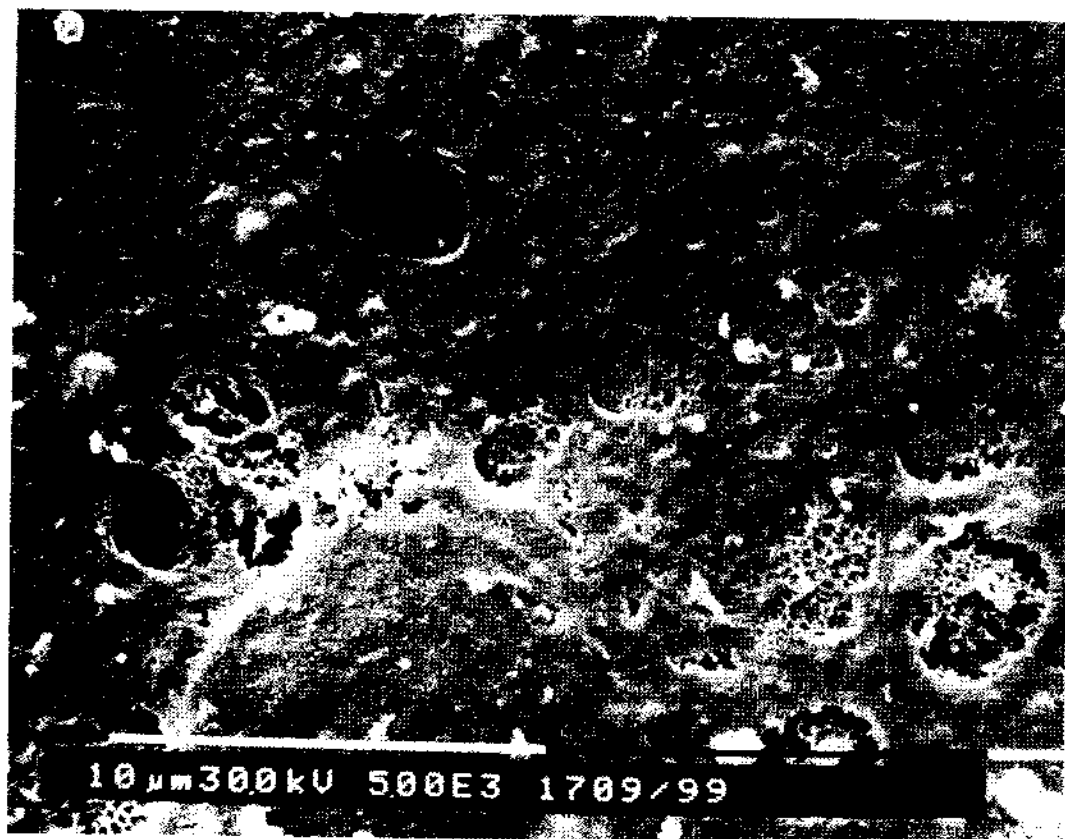
At concentration of 1.5 mg/mL of CRP a lot of gaps (fused fenestrae) were observed in LEC (Fig. 2a), it was due to the hyperosmoticity of the solution. Cultured LEC treated with 0.1 mg/mL of CRP showed vacuolization, loss of cytoplasmic integrity and damaged sieve plates with loss of fenestrated pattern (Fig. 2b). In some LEC, fenestrae were mainly found in the nuclear region.

CRP-derived naphthoquinones induced damage in cultured LEC, characterized by gaps and complete defenestration (Fig. 3).





A



B

Fig 2. Cultured LEC treated with CRP by SEM. A) Observe the effect of a hyperosmotic CRP solution (1.5 mg/mL). X 6556
B) Note LEC defenestration after exposure to 0.1 mg/mL of CRP. X 11000.

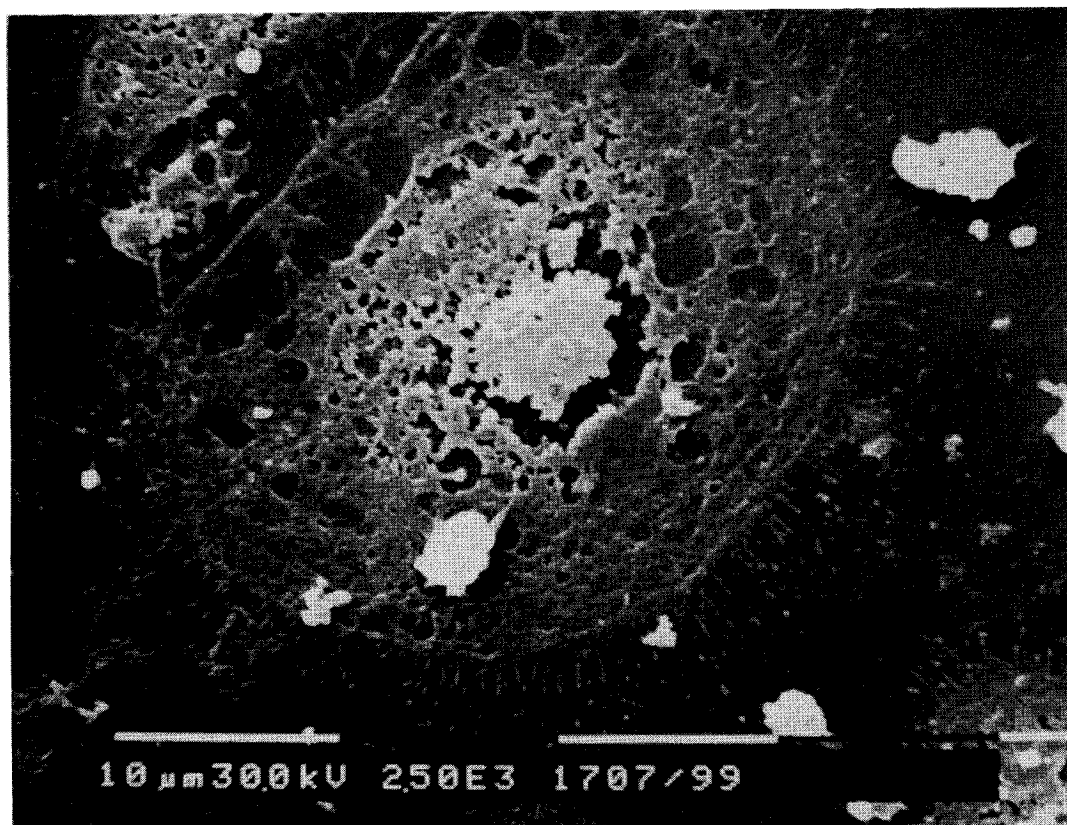


Fig. 3. Cultured LEC treated with 0.1 mg/mL of CPR-derived naphthoquinones. Gaps and defenestration are shown. X 5500.

