

Ultrastructural Changes of Aescin on 5RP7 Cell Line by Using a Transmission Electron Microscope

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Keywords: Aescin, 5RP7, TEM (Transmission Electron Microscope)

Cancer takes the first place around the world of deadly illness. The known cancer therapies aim to destroy the proliferating cancer cells using some cytotoxic agents. Saponins are a vast group of glycosides. Cytotoxic plant compounds can potentially be used as antitumor agents.

Extracts of *Aesculus hippocastanum* (horse chestnut) seed have been used in the treatment of chronic venous insufficiency, edema, and hemorrhoids. Most of the beneficial effects of horse chestnut are attributed to its principal component B-escin or aescin. Recent studies suggest that B-escin may possess anti-inflammatory, antihyaluronidase, and antihistamine properties. Natural products isolated from medicinal herbs have been the potential sources of novel anticancer drugs over the last few decades. The medicinal use of plant extracts seems to be a more natural, less expensive approach and in general involves minimal unwanted side effects.

The rat embryo fibroblast cells, 5RP7 were cultured in sterile plastic tissue culture flasks in DMEM medium supplemented with 10 % (v/v) fetal calf serum (GIBCO BRL Paisley, Scotland), L-glutamine (1mM final concentration) and penicillin/streptomycin at 100 units/mL as adherent monolayers. Fibroblast cells were incubated at 37 °C in 5 % CO₂ / 95 % air in the humidified atmosphere provided by an incubator.

They were deposited on Formvar-coated 200-300 mesh copper grids and dried. Cells were fixed with 2.5 % glutaraldehyde in 0.1M phosphate buffer (pH:7.4) and left in phosphate buffered saline (PBS) overnight at +4°C. After being embedded in Agar and stained in 2 % osmium tetroxide. Cells were dehydrated in graded ethanol: 70, 90, 96 and 100 %. Then cells were embedded in EPON 812 epoxy. They were thin sectioned using a diamond knife to a maximum thickness of 100 nm. The sections were stained with lead citrate and uranyl acetate. Cancer cells with aescin as a saponin of interaction was displayed on TEM. Furthermore, Transmission Electron Microscope was used to determine ultrastructure of the cells[1].

We postulated that effects of the various concentrations of aescin on the morphology of 5RP7 cells were also examined by Transmission Electron Microscope (TEM). Sample morphology and size were determined using TEM.

1. Bozzola J.J, Russell D.L., Electron Microscopy (1999) p1-670.

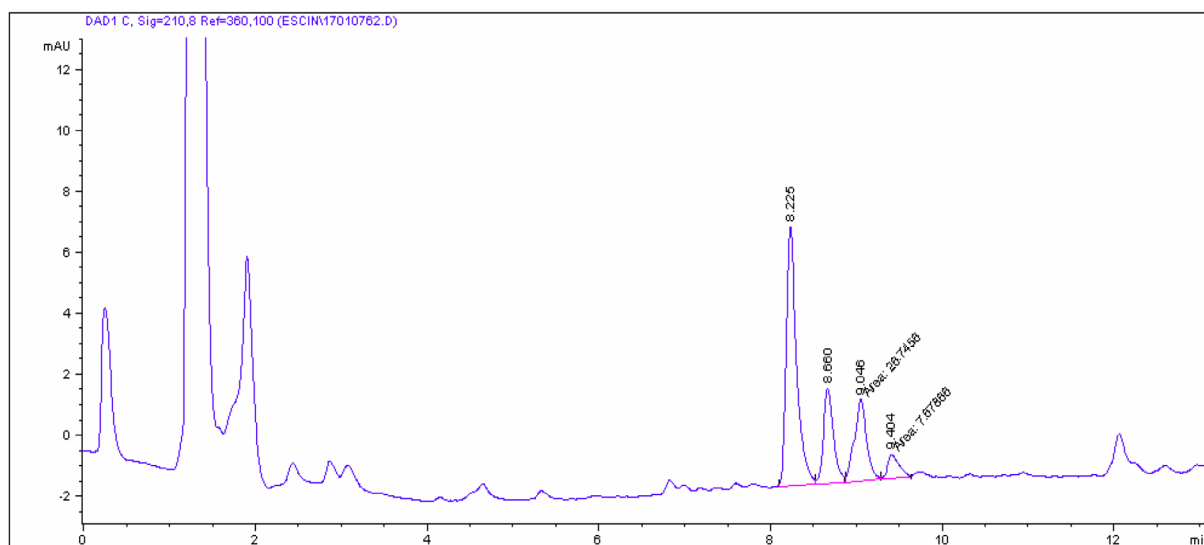


Figure 1. Chromatography of horse chesnut extract (25 µg/ml)