Abstract

Cordycepin, an active ingredient of the insect fungus *Cordyceps militaris*, is a category of compounds that exhibit significant therapeutic potential. The aim of this work was to find out a method for cordycepin isolation from fermented broth of *C. militaris* 3936 followed by its optimization so as to extract maximum cordycepin. Further anti-proliferatory and anti-angiogenic effect of cordycepin was studied on A549 human lung cancer cell line and chorio-allantoic membrane (CAM) respectively. The suitable physical and nutritional conditions for cordycepin production were studied by individually varying one variable at a time. Solvent- solvent extraction method was used to extract cordycepin from liquid culture of *C. militaris* 3936. Crude concentrated extract of fermented broth was sequentially partitioned with hexane, chloroform and n-butanol. Effects of solvent-solvent ratio, extraction time and temperature on cordycepin extraction were investigated. The extracted cordycepin was further purified on silica gel column. Cytotoxic effect of cordycepin on A549 cell proliferation was evaluated by MTT assay. Apoptotic effect of cordycepin was observed using cell morphology, DAPI staining and DNA fragmentation studies. Flow cytometry (FCM) was used to analyze cell cycle status after cordycepin treatment. The optimum culture conditions for maximum cordycepin production (846 mg/L) were pH 5.5, temperature 25°C, inoculum size 8 % v/v, inoculum age 72 h, incubation time 24 d and optimum culture medium included 1.5 % dextrose, 0.8 % yeast extract, K₂HPO₄ 0.3 %, KH₂PO₄ 0.1 %, NaCl 0.05 %, MgSO₄ 0.05 % and NaCl 0.05 %. The bioactive metabolite, cordycepin was also isolated from *Cordyceps militaris* derived butanol fraction. Maximum yield of cordycepin was achieved at a solvent-solvent ratio of 1:2 (v/v) with extraction time of 90 minutes at 40°C. Final purity of the cordycepin was recorded up to 95 % based on high performance liquid chromatography, UV and NMR analysis. Results of MTT assay showed that cordycepin significantly inhibits cell proliferation with IC₅₀ value 64 µg/ml. The number of
dead cells increased with cordycepin treatment and showed changes in cellular morphology. In DNA fragmentation studies, a typical ladder pattern was observed on agarose gel and formation of apoptotic bodies were further confirmed using DAPI staining. The FCM analysis of cordycepin treated cells showed that apoptosis rate increased with increase in dose. Furthermore anti-angiogenic property of cordycepin was also investigated using chorioallantoic membrane (CAM) assay. Anti-angiogenic studies for extracted cordycepin showed that 40 µg/egg dosage of cordycepin was sufficient to inhibit the branching of blood vessels significantly (~50%) in a CAM assay. In conclusion, present work provides an excellent route for the extraction of cordycepin from fermented broth of C. militaris 3936. The extracted cordycepin was found to be biologically active and induced apoptosis in A549 human lung cancer cell line and could be a potential candidate for its therapy. Further cordycepin, potentially inhibited the angiogenesis further suggesting that the inhibition of angiogenesis is one of the mechanisms by which Cordyceps militaris can mediate an anti-cancer effect.