

## Abstract

Mushroom metabolites are investigated worldwide in search of new therapeutic agents from natural source, as it has a historical use in human diet and medicine. In this present study, a bioactive active protein fraction was isolated by Cibacron blue affinity chromatography (designated as CBAEP) from five different locally edible mushrooms species (*T. clypeatus*, *C. indica*, *P. ostreatus*, *A. hygrometricus* and *V. volvacea*) and explored its anticancer and immunomodulatory activity. These CBAEPs showed *in vitro* antiproliferative activity (10-100 µg/ml) on several tumor cell lines as well as it stimulated mouse immune cells *in vitro* significantly. The highest immunomodulatory activity was found in *T. clypeatus* and *P. ostreatus* whereas most antiproliferative activity in *C. indica* and *P. ostreatus* were studied. CBAEP from *P. ostreatus* inhibited growth of HeLa cells through the induction of apoptosis with appearance of the sub G0/G1 peak in cell cycle analysis and nuclear and DNA fragmentation. Enhanced production of ROS in presences of CBAEP caused induction of apoptosis in HeLa cells. The Western blot analysis indicated that CBEAP induced activation of caspase 9 and release of cytochrome C from mitochondria, as well as increased in ratio of several proapoptotic to antiapoptotic proteins that favored apoptosis. It was observed that the phosphorylated forms of both Akt and MAPK (p38 and ERK) were down-modulated in presence of CBAEP without altering total levels of Akt and MAPK protein expression. Moreover, the CBAEP prolonged the survival of tumor bearing host without any visible toxicity. It inhibited *in vivo* tumor growth of DL cells through the induction of apoptosis. It increased Bax/Bcl<sub>2</sub>, which causes the release of Cytochrome C resulting activation of caspase-3 subsequently inducing apoptosis. On the other hand, CBAEP also enhances immune cell proliferation and up-regulates the Th1 immune response which might prevent tumor recurrence. Analysis of CBAEP by gel electrophoresis showed that the protein were in range of 5-80kDa without having lectin or glycoprotein in nature. Active protein component of this fraction was purified by ion exchange followed by gel permeation chromatography. Our findings thus revealed antitumor potential of a bioactive protein fraction from edible mushroom which can be used in development of new strategies for cancer therapies.

**Keywords:** Mushroom proteins, Anticancer activity, Immunomodulation, Apoptosis, MAPK/Akt.