Background: Hepatocellular carcinoma (HCC) is one of the most frequent tumors representing the fifth commonest malignancy worldwide. Unfortunately, the overall response rate of liver cancer treatment is unsatisfactory mainly due to late diagnosis and poor treatment efficacy, especially resistance to chemotherapeutic drugs and metastasis to other organs. (+)-Catechin (CAT) is the flavonoid found to inhibit lipid peroxidation in cell culture and in-vivo models. CAT is claimed to be effective in treating carbon tetrachloride induced liver damage and also reported to inhibit angiogenesis in-vivo. However, there is no report on the effect of CAT on HCC.

Methods: The antiproliferative and antitumor effect of CAT was tested in-vitro on the human hepatic carcinoma cell line (HepG2), and in-vivo on Balb/c mice bearing hepatic tumors. Cell line was treated with CAT at various concentrations and the proliferation of cells was measured by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT), sulforhodamine B (SRB) and lactate dehydrogenase (LDH) assays. Cell apoptosis was detected by Hoechst 33258 (HO) and Acridine orange/ethylene dibromide (AO/EB) staining and DNA fragmentation analysis. In addition, we examined the combined effect of CAT and cisplatin over time. The effect of (+)-catechin on tumor growth in-vivo was studied. The HCC tumor model was established in mice by injecting N-nitrosodimethylamine/carbon tetrachloride (NDEA/CCl4) and the effect of CAT on tumor growth in-vivo was studied. The levels of liver injury markers, tumor markers, and oxidative stress were measured. The expression levels of apoptosis-related genes in in-vitro and in-vivo models were determined by RT-PCR and ELISA.

Results: The (+)-catechin induced cell death was considered to be apoptotic by observing the typical apoptotic morphological changes under fluorescent microscopy and DNA fragmentation analysis. We found that the combination of CAT and cisplatin produced synergistic effects on the of HepG2 cell line in a dose dependent manner. Typical morphological changes including chromatin condensation and blebbing and characteristic DNA ladder formation in agarose gel electrophoresis were observed in CAT-treated HepG2 cells. The CAT treatment significantly reduced the tumor incidence and multiplicity. Morphology of liver tissue and levels of marker enzymes indicated that CAT offered protection against hepatocarcinoma. The CAT was also found to significantly upregulate different antioxidative enzymes. The levels of lipid peroxidation and nitrite were significantly decreased by CAT. The induction of apoptosis is correlated with the increased mRNA expressions of p53, bax, cytochrome-c and caspase-3, -7, -8 and -9 and decreased mRNA expressions of bcl-2. Subsequently, CAT decreased the mRNA expressions of mdm2, p65, c-jun, c-fos in both in-vitro and in-vivo models. The protein expression levels of p53, bax, and caspase-3 were increased, whereas, that of p65, c-jun and bcl-2 were decreased on CAT treatment. Immunohistochemistry data revealed less localization of p53, p65 and c-jun in CAT treated tumors as compared to localization in control tumors.

Conclusion: Our data demonstrated that CAT could significantly inhibit the proliferation of different cancer cells in-vitro and suppress tumor growth in-vivo. Therefore, the results from this study provided critically important experimental facts to suggest that CAT may be a potential therapeutic agent for treating cancer.