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Inonotus obliquus mushroom suppresses proliferation of colorectal carcinoma: Translational pharmacological approach in cancer prevention

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Background: Chaga mushroom (*Inonotus obliquus*) has been used as a folk remedy to treat several illnesses including gastrointestinal disorders. However, its effects on intestinal inflammation and colorectal cancer (CRC) have not been clearly elucidated.

Objectives: We investigated the effects of an aqueous extract of *Inonotus obliquus* (IOAE) on HCT116 and DLD1 cells and in three mice models, DSS-induce experimental colitis, AOM/DSS-induced colitis associated colon cancer (CACC) and adenoma in $APC^{Min/+}$ mice.

Methods: Cell cytotoxicity was assessed by MTT assay. Apoptosis induction and cell cycle arrest were analyzed by flow cytometry. Immunohistochemical analysis of intestinal tissues was performed for inflammation scoring and expression of proteins. Cytoplasmic and nuclear protein lysates were isolated for western blotting. Total RNA was isolated and reverse-transcribed to cDNA for PCR amplification of inflammation related genes.

Results: HCT116 and DLD1 cell lines: IOAE suppressed cell proliferation by inducing mitochondrial intrinsic apoptosis, autophagy, and S phase cell cycle arrest. IOAE suppressed β-catenin and its downstream targets cyclin D1 and c-Myc along with CRC oncogene CDK8. IOAE also inhibited the nuclear and cytoplasmic levels of NF-κB.

DSS-induced colitis mice: IOAE ameliorates colonic inflammation by suppressing iNOS and Cox-2 and myeloperoxidase accumulation. IOAE inhibited the mRNA expression of inflammation mediators (TNF-α, IL-1β, IL-6, IFN-γ and iNOS) in colon.

AOM/DSS-induced CACC mice: IOAE suppressed the number of colorectal tumor. IOAE diminished the expressions of iNOS, Cox-2, cyclin D1 and c-Myc, and dramatically inhibited the mRNA expression of pro-inflammatory cytokines in colon. These results indicate potent anti-inflammatory and anti-proliferative effects of IOAE in CACC model of mice.

$APC^{Min/+}$ mice: IOAE suppressed polyp formation in small intestine. IOAE inhibited the levels of β-catenin along with cyclin D1, c-Myc and CDK8. IOAE triggered caspase-3 activation and PARP cleavage in intestinal tissues. IOAE inhibited the mRNA expression of inflammation mediators also.

Conclusions: IOAE suppressed colorectal carcinoma *in vitro* and *in vivo* through anti-inflammation and downregulation of β-catenin/NF-κB pathway. Considering recent anticancer approaches involving natural products with least side effects, we advocate that Chaga could be a beneficial supplement in prevention of colorectal cancer.

Role of natural product in treatment of cancer

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Traditional medicine has a long history of serving peoples all over the world. India is without doubt an herbal hub. Medicinal plants that are native to India and their use in various traditional systems of medicine are indeed inspiring. The ethnobotany and ubiquitous plants provide a rich resource for natural drug research and development. In recent years, the use of traditional medicine information on plant research received considerable interest. In the present study, eighteen plant extracts of different species used by Indian traditional healers for the treatment of ulcers, cancers, tumors, warts, and other diseases, were tested *in vitro* for their potential anticancer (antiproliferative and cytotoxic) activity. The chloroform, ethanolic, and water extracts were tested against five human cancer cell lines like MCF-7, HOP-62, MOLT-4, HCT-15 and PRO cell lines using SRB assay. Seven out of the eighteen extracts showed remarkable cytotoxic potential. The highest activity was found in the leaf/stem ethanol extracts from *Rivea hypocrateriformis* and *Trichosanthes tricuspidata* against all the five human cancer cell lines screened. The present finding may pave the way for the bioactivity guided fractionation and isolation of important moieties for the anticancer chemotherapy and in part help to minimize the serious lacuna in scientific validation of these herbs.