Article

*In vitro* Assessment of Apoptosis Induction and Cell Cycle Arrest in Colon Cancer Cell Line of a Chemically Analyzed *Hypericum Triquetrifolium* Extract

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Received: date; Accepted: date; Published: date

**Abstract:** Naturally-derived drugs and plant-based products are attractive commodities that are being explored for cancer treatment. This *in vitro* study aimed to investigate the role of apoptosis, cell cycle modulation, and cell cycle arrest in the *Hypericum triquetrifolium* extract (HTE)-induced cytostatic effects in human colon cancer cell line (HCT-116). 50% ethanol (in water) HTE induced cell death via an apoptotic process, as assayed by Annexin V-Cy3 assay. Exposing HCT-116 to 0.064, 0.125, 0.25, and 0.5 mg/mL for 24 h led to 50%, 71.6%, 85%, and 96% apoptotic cells, respectively. HCT-116 cells treated with 0.25 and 0.5 mg/mL HTE, for 3 h resulted in 38.9% and 57.2% cleavage of caspase-3-specific substrate, respectively. RT-PCR analysis revealed that HTE extract had no effect on mRNA levels of Apaf-1 and NOXA. Moreover, 0.125 mg/mL and 0.25 mg/mL THE for 24 h was clearly shown to attenuate the cell cycle progression machinery in HCT-116 cells. GC/MS analysis of the extract identified 21 phytochemicals that are known as apoptosis inducers and cell cycle arrest agents. These results suggest that HTE-induced apoptosis of human colon cells is mediated primarily through the caspase-dependent pathway. Thus, HTE appears to be a potent therapeutic agent for colon cancer treatment.

**Keywords:** keyword 1; keyword 2; keyword 3 (List three to ten pertinent keywords specific to the article; yet reasonably common within the subject discipline.)

1. Introduction

Cancer is the second-leading cause of mortality in humans worldwide. It is estimated that one in three women and one in two men in the United States will develop cancer in their lifetime. An increase in the number of individuals diagnosed with cancer each year, due in large part to aging and growth of the population, as well as improving survival rates, has led to an ever-increasing number of cancer survivors {Mullan, 1985 #2;Wang, 2012 #1}. Colorectal cancer (CRC) is the second leading cause of cancer death. The development of CRC proceeds through a series of genetic alterations involving the activation of oncogenes and the loss of tumor suppressor genes. The first step in colon carcinogenesis involves the loss in functionality of the APC (Adenomatous Polyposis Coli) gene, which is a tumor suppressor gene. This is followed by the inactivation of the p53 gene (a sensor essential for the checkpoint control that arrests cells with damaged DNA in the G1 phase), which results in the formation of polyps on the inside of the colon wall. Although much has yet to be understood as to why some individuals develop CRC while others do not, certain genetic and environmental factors are known to increase a person’s chance of developing the disease {Mullan, 1985 #2} {Bedi, 1995 #3}.

Surgery, chemotherapy, and radiotherapy, either individually or combined, have been considered as conventional strategies for cancer treatment in the last century. With the rapid development of molecular medicine, novel therapeutic approaches such as immunotherapy, molecular targeted therapy, and hormonal therapy, have been proposed to improve clinical outcome for cancer patients. However, these therapeutic approaches are not always effective and survival rates are still poor {Mullan, 1985 #2; Wang, 2012 #1}.

There has been a substantial increase in the use of complementary and alternative medicines, including dietary supplements and medicinal plants, for cancer treatment. Several *in vitro*, cellular, and animal studies have examined the effects of herbal and other specialty products on the development and progression of CRC {Satia, 2009 #4}.

The use of agents targeting the cell cycle machinery has long been considered as an ideal strategy for cancer therapy. These drugs target the abnormal expression of cyclin-dependent kinases (CDKs), mitotic kinases/kinesins, or they affect cellular checkpoints, resulting in cell cycle arrest and the subsequent induction of apoptosis in cancer cells. Cell-cycle-based agents can be grouped into categories that reflect their molecular targets. For example, CDK inhibitors target inhibition of CDKs, which selectively blocks tumor growth without compromising normal cells; checkpoint inhibitors target the S and G2 checkpoints, and mitotic inhibitors affect mitosis {Chen, 1999 #6;Mahadevan, 2011 #7;Schoffski, 2009 #5}.

Apoptosis induction is a useful mechanism for modulating cancer progression, especially when there are mutations that alter the ability of the cell to undergo apoptosis and allow transformed cells to keep proliferating rather than dying. It would be therapeutically advantageous to tip the balance in favor of apoptosis over mitosis in tumors, if possible. The progressive accumulation of genetic alterations (APC, p53, and ras) governs the transition of the normal colorectal epithelium to adenocarcinomas {Fearon, 1990 #8}.

Despite the great progress in modern medicine, traditional medicine has always been practiced {Zaid, 2012 #9}. Herbal medicine such as garlic, onion, black seeds, olive oil, and leaf, as well as HTE, are prescribed for cancer treatment and prevention {Chandra, 2010 #11;Volanis, 2010 #10}. However, the safety and effectiveness of alternative medicine are not always scientifically proven. Based on the knowledge of traditional herbal medicine and on preliminary studies, this *in vitro* study aims to investigate the role of apoptosis, cell cycle modulation, and cell cycle arrest in the observed HTE extracts-induced cytostatic effects in colon cancer cell line HCT-116.

2. Materials and Methods

2.1. Materials

Cells of the human colorectal cell line HCT-116 were purchased from ATCC® CCL-247™, and all tissue culture reagents, including fetal bovine serum and standard culture medium RPMI-1640, were purchased from Biological Industries (Beit Haemek, Israel). LDH kit was purchased from Promega, WI, USA, cell cycle kit was purchased from Thermo Fisher, USA, and RNA isolation kit was purchased from QIAGEN. MTT reagent and all other materials were purchased from Sigma Aldrich. HTE (aerial parts) was purchased from Al Alim - Medicinal Herb Center, Zippori, Israel.

2.2 Preparation of Plant Extracts

100 g of air-dried HTE plant material was added to 1 L of 50% EtOH (in water) and boiled for 30 min, then stirred for 24 h at room temperature. The obtained extract was filtered through a filter paper, aliquoted and frozen at -80 °C until use {Kadan, 2018 #13;Saad, 2005 #12}.