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Immunomodulatory and radioprotective property of glucans isolated from lactobacillus species

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ABSTRACT

Bacterial exopolysaccharides (EPS) have been reported to protect the radiation induced damage during radiotherapy. Recent studies suggested that, lactic acid bacteria (LAB) and their secreted products have unique radioprotective properties. In the present study, we evaluated the ability of EPS isolated from two LAB species i.e., *Lactobacillus acidophilus* and *Lactobacillus plantarum* as a radioprotective agent against radiation induced mice small intestinal damage. EPS was isolated, identified as glucan and named as glucan A (GA) derived from *Lactobacillus acidophilus* and glucan P (GP) derived from *Lactobacillus plantarum*. GA and GP were subjected to *in vitro* immunomodulatory and *in vivo* radioprotective activity. Both GA and GP exhibited significant mitogenic activity and increased interleukin-2 (IL-2) and interleukin-10 (IL-10) secretion in a dose dependent manner. Similarly, after 9Gy whole body irradiation, glucan pre-treated mice showed improved spleen weight, reduced radiation induced hematopoietic disorder and radiation enteritis when compared to the irradiation control mice. Both glucans were also protected mice from early death and increased survival rate when compared to irradiation control. As a result, it is possible that glucan from *Lactobacillus acidophilus* and *Lactobacillus plantarum* could be served as a novel radioprotective agent to inhibit radiation-induced intestinal damage.

1. Introduction

Radiotherapy is a valuable medical tool, particularly for killing cancer cells (Bing et al., 2014; Lee et al., 2015). Gamma ray irradiation is among the most common radiotherapeutic approach used to treat brain tumors, malformations, and other abnormalities. Radiation when used for colon cancer treatment, although is known to kill or slow the growth of cancer cells, it is observed that the healthy tissue surrounding the tumor site is also affected. Rectal irritation, which can cause diarrhoea, painful bowel movements, or blood in the stool, is a common side effect in radiotherapy mediated colon cancer treatment. During radiotherapy, intestinal stem cells respond immediately to irradiation by losing function via apoptosis, mitotic inhibition, and reproductive sterilization (Potten et al., 1994). Radiation induced cell death causes attenuation and blunting of the height of villi in the small intestine, resulting in malabsorption, gastrointestinal bleeding, and fatal destruction (Potten & Grant, 1998; Somosy et al., 2002). Gamma radiation exposure is also responsible for bone marrow damage and depletion of white blood cells (WBC). Hence, there is a need to investigate potential radioprotective agents to reduce the radiation induced side effect during cancer treatment.

Lactic acid bacteria (LAB) have been used as probiotics to treat a variety of intestinal disorders such as lactose intolerance, irritable bowel syndrome, and inflammatory bowel disease – with lactobacillus species shown to have positive impact on the health of the human gastrointestinal system. Considering that a wide range of LAB were found to be capable of producing exopolysaccharides (EPS). EPS produced by these organisms were shown to have multifarious biological and pharmaceutical activities such as antioxidant, anticancer, antiviral, anti-inflammatory and immunomodulatory properties (Liu et al., 2010; Pan & Mei, 2010). β-glucan, also known as a biological response modifier (BRM), is a well-studied macromolecule that exhibited

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Interaction studies of flavonoids with Bcl-2 protein to re-activate apoptosis in JurkatT-cells by induced TRAIL

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Abstract. Immune cell malignancy such as Acute T- cell Lymphoblastic Leukaemia is generally associated with high rate of relapse and often does not respond to salvage therapy. Thus, identification of novel treatment regimens or cell apoptosis pathways and therapeutic agents without major side effects is necessary. TRAIL-induced apoptotic pathway is one such pathway that is usually blocked by anti-apoptotic proteins like Bcl-2. This research estimated and compared the ability of few common flavonoids to re-activate TRAIL-induced apoptosis by blocking Bcl-2 protein. Studies were carried out to understand the interaction between binding energy of the Flavonoids with Bcl-2 protein in cancer cells. The pharmacokinetic and toxicity profiling was performed to study the potency of the flavonoids as a lead candidate. Baicalein was selected as lead molecule because of its lower binding energy and its ability to increase Mitochondrial Membrane Potential as studied from its ADME properties. For validation of apoptosis of Baicalein by TRAIL-induced owing to Bcl-2 analysis of cell cycle and Gene expression studies were carried out on Jurkat T cells.

Keywords: flavonoids, acute lymphoblastic leukaemia, Jurkat cells, Bcl-2 protein, TRAIL, docking, pharmacokinetic analysis, Mitochondrial Membrane Potential, baicalein, ADME properties

INTRODUCTION

T-cell acute-lymphoblastic leukaemia (T-ALL) a variety of malignancy of T-cells, where the bone marrow produces defective T-cells which are immature and which subsequently accumulate in liver, spleen and lymph nodes. B-lymphoblastic counterpart is naturally distinct from T-ALL and shows dissimilar response to treatment systems (Elizabeth *et al.*, 2016). Indian Incidence rate varies with region, gender and age. Incidence rate according to study in 2018 was 101/100000 in boys and 62/100000 in girls. T-ALL is slightly more common in boys than girls with a peak incidence of 2-5 years. T-ALL constitutes about 15%-20% of total ALL though in India higher proportion up to 50% is reported (Agarwal & Sahi, 2020). Symptoms of T-ALL include anaemia, weakness, fever, purpura, nosebleeds, bleeding gums and sweats. T-ALL frequently causes swollen lymph nodes in the central part of chest (mediastinum) which may possibly distress breathing or the circulation (Jordan *et al.*, 2017).

Causes of T-ALL are certain genetic conditions and mutations in genes like NOTCH1, WT1, EZH2 and so on, radiation exposure, exposure to chemicals like benzene, viruses like HTLV-1. Diagnostic tests for T-ALL include certain lymph node biopsy, lumbar puncture, blood tests, karyotyping, bone marrow tests and

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Biotechnology Section

Original Article

In-vitro Study of Macelignan as a Potential Anticancer Drug against Colorectal Cancer using HCT116 Cell Line

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ABSTRACT

Introduction: Many recent studies have shown that lignans from many plant sources have an effective impact on cancer treatment and it is evident that many medicinal plants are rich in lignans. Genus *Leucas* is known for its medicinal use and is rich in lignans. Macelignan is a polyphenolic derivative might play significant roles as clinically useful anticancer agents in treating Colorectal Cancer (CRC).

Aim: Isolation, characterisation and pharmacological profiling of bioactive compound lignan from *Leucas aspera* and *Leucas cephalotes* and to assess the anticancer potential using in-vitro methods using Human Colorectal Cancer (HCT116) cell lines.

Materials and Methods: This in-vitro study was conducted from August 2018 to January 2020 at The Oxford College of Engineering in Bengaluru, Karnataka, India. Anticancer potential of Macelignan was evaluated through 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, Reactive Oxygen Species (ROS) measurement, cell cycle study, apoptosis analysis, and gene expression studies. One-way Analysis of Variance (ANOVA) was performed for the total phenolic content estimation and the results were expressed as mean±SD with n=3 trials.

Results: The MTT assay result indicated that macelignan has an IC50 value of 22.8 µM with 73% of cells showing inhibition, ROS production was enhanced 2.5-fold at a maximum concentration at 100 µM. Macelignan (12.5 µM and 25 µM) significantly prevented cell growth in G0/G1 and G2 phases of the cell cycle, while the apoptotic study showed that 12.5 µM and 25 µM macelignan induced early and late apoptosis in HCT116 cells with 21.28% and 19.17%, 21.54 % and 29.02% apoptosis at cellular level, respectively. This set of tests sought to examine the effect of macelignan on the *Caspase 3* gene expression in HCT116 cells by semi-quantitative Polymerase Chain Reaction (PCR). The study showed that *Caspase 3* expression was upregulated up to 1.98 and 2.87 folds when treated with macelignan.

Conclusion: The macelignan could serve as a potent drug derivative for the treatment of colon cancer with further study on the mechanism of action, structure-activity relation, toxicity profiling, bioavailability.

Keywords: Apoptosis, *Caspase 3*, Cell growth, Cytotoxicity, Human colorectal cancer, ROS production

INTRODUCTION

Cancer is one of the most serious health issues affecting the length and quality of human life and has been classified as one of the deadliest diseases affecting mankind worldwide. Limited success has been witnessed even with enormous effort put to cope with the disease. Since conventional therapeutic strategies do not meet the essential requirements for successful cancer therapy, the use of natural bioactive compounds isolated from medicinal plants such as *Leucas aspera* as anticancer agents has emerged as an alternative safe, inexpensive, and convenient method. Four of the most common cancers are lung, breast, prostate, and colon cancer [1].

Colorectal Cancer (CRC) is predominantly the 2nd most fatal cancer and the 3rd most widespread malignant tumour globally. A 2018 survey reported 1.8 million new CRC cases and 881,000 deaths, accounting for nearly 10% of new cancer cases and deaths on the global death scale [2]. Generally, CRC is characterised as an uncharacteristic growth on the internal lining of colon epithelial cells that are surgically removed upon early diagnosis [3]. The existing treatment for CRC consists of chemotherapy with solo drug fluoropyrimidine and numerous agent regimes including capecitabine, oxaliplatin, and irinotecan [4].

Leucas aspera Linn. (*L.aspera*) is a widely distributed herbaceous plant across the Indian subcontinent, which belongs to the family *Lamiaceae* and is an annual, branched plant. The taxonomic classification and anatomy of this plant are well documented through many research studies [5-8]. The plant is known to contain many potent metabolites like triterpenoids, oleanolic acid, urosolic acid, β-sitosterol, nicotine, sterols, glucoside, diterpenes, and phenolic compounds [8]. Indian traditional medicines Ayurveda and Siddha use this plant. The plant is reported to have pharmacologic activities like carminative, antihistaminic, antipyretic, and antiseptic. It is used to treat diseases like jaundice, anorexia, dyspepsia, fever, helminthic infestation, respiratory and skin diseases [9].

From the study on green synthesis of silver nanoparticles of *L.aspera*, it has been reported that these plants serve as potent herbs [10], and have been reported to contain many phytotoxic components [11].

Leucas cephalotes another species commonly called "Dronapushpi" in Sanskrit belonging to the family *Lamiaceae* as *L.aspera*, is a weed and grows in monsoon. Reportedly, two protostane-type triterpenoids named leucastrins A and B and oleanolic acid were isolated from *L.cephalotes* [12]. Triterpenoids β-sitosterol [13], stigmasterol [14], lupeol [15,16], labellinic acid isolated were also reported from *L.cephalotes* [17]. Aliphatic esters [18], essential oils [19,20], flavones [20] were other metabolites that have been successfully isolated from *L.cephalotes*.

Macelignan, a class of secondary metabolite classified as phytolignan, a polyphenolic derivative might offer new anticancer therapeutic ability and play significant roles as clinically useful anticancer agents in treating CRC [21]. In this context, the present study was undertaken to evaluate the anticancer efficacy of the macelignan isolated from *L.aspera* and *L.cephalotes* on CRC cell line HCT116, and a sincere attempt was made to provide scientific validation for the role of macelignan as a therapeutic lead molecule in treating CRC.