

# Rana Zaini Publications

## Bioactive Chemicals from Carrot (*Daucus carota*) Juice Extracts for the Treatment of Leukemia

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**ABSTRACT** Overwhelming evidence indicates that consumption of fruits and vegetables with antioxidant properties correlates with reduced risk for cancers, including leukemia. Carrots contain beneficial agents, such as  $\beta$ -carotene and polyacetylenes, which could be effective in the treatment of leukemia. This study investigated the effect of carrot juice extracts on myeloid and lymphoid leukemia cell lines together with normal hematopoietic stem cells. Leukemia cell lines and nontumor control cells were treated with carrot juice extracts for up to 72 hours *in vitro*. Induction of apoptosis was investigated by using annexin V/propidium iodide staining followed by flow cytometric analysis, and results were confirmed by using 4'-6-diamidino-2-phenylindole morphology. Effects on cellular proliferation were investigated via cell cycle analysis and cell counts. Treatment of leukemia cell lines with carrot juice extract induced apoptosis and inhibited progression through the cell cycle. Lymphoid cell lines were affected to a greater extent than were myeloid cell lines, and normal hematopoietic stem cells were less sensitive than most cell lines. This study has shown that extracts from carrots can induce apoptosis and cause cell cycle arrest in leukemia cell lines. The findings suggest that carrots may be an excellent source of bioactive chemicals for the treatment of leukemia.

**KEY WORDS:** • apoptosis •  $\beta$ -carotene • cell proliferation • *Daucus carota* • leukemia • polyacetylenes

### INTRODUCTION

**L**EUKEMIA IS A COMPLEX PROGRESSIVE malignant disorder, characterized by distorted differentiation, proliferation, and development of white blood cells and their precursors in the bone marrow and blood.<sup>1</sup> Leukemia is generally termed acute or chronic according to cellular maturity and classified by the lineage that it affects. There are 4 major types of leukemia: acute myeloid leukemia, acute lymphoblastic leukemia, chronic myeloid leukemia,

nosis. The rates of survival in children are higher; 8 in 10 children with leukemia survive for at least 5 years.<sup>3</sup>

The current treatments for leukemia involve chemotherapy, interferon therapy, hormone therapy, radiation therapy, and stem cell transplantation.<sup>4</sup> However, the specific treatment depends on the type and stage of the disease and the age and sex of the patient. Current leukemia treatments have several disadvantages, such as decreased levels of blood cells (*e.g.*, erythrocytopenia and neutropenia), leading to

## Effects of Bioactive Compounds from Carrots (*Daucus carota* L.), Polyacetylenes, Beta-Carotene and Lutein on Human Lymphoid Leukaemia Cells

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**Abstract:** New therapies for leukaemia are urgently needed. Carrots have been suggested as a potential treatment for leukaemia in traditional medicine and have previously been studied in other contexts as potential sources of anticancer agents. Indicating that carrots may contain bioactive compounds, which may show potential in leukaemia therapies. This study investigated the effects of five fractions from carrot juice extract (CJE) on human lymphoid leukaemia cell lines, together with five purified bioactive compounds found in *Daucus carota* L, including: three polyacetylenes (falcarinol, falcarindiol and falcarindiol-3-acetate) and two carotenoids (beta-carotene and lutein). Their effects on induction of apoptosis using Annexin V/PI and Caspase 3 activity assays analysed via flow cytometry and inhibition of cellular proliferation using Cell Titer Glo assay and cell cycle analysis were investigated. Treatment of all three lymphoid leukaemia cell lines with the fraction from carrot extracts which contained polyacetylenes and carotenoids was significantly more cytotoxic than the 4 other fractions. Treatments with purified polyacetylenes also induced apoptosis in a dose and time responsive manner. Moreover, falcarinol and falcarindiol-3-acetate isolated from *Daucus carota* L were more cytotoxic than falcarindiol. In contrast, the carotenoids showed no significant effect on either apoptosis or cell proliferation in any of the cells investigated. This suggests that polyacetylenes rather than beta-carotene or lutein are the bioactive components found in *Daucus carota* L and could be useful in the development of new leukemic therapies. Here, for the first time, the cytotoxic effects of polyacetylenes have been shown to be exerted via induction of apoptosis and arrest of cell cycle.

**Keywords:** Leukaemia, Polyacetylenes, Carotenoids, *Daucus carota* L, Apoptosis, Cell proliferation.

### 1. INTRODUCTION

Leukaemia is a major source of morbidity and mortality worldwide and is the most common childhood cancer [1], however the causes of childhood leukaemia remain mostly unclear [2]. According to the Leukaemia & Lymphoma Society about 28,500 patients are diagnosed annually in the UK with a blood cancer [3]. Approximately 40% of patients diagnosed with leukaemia survive for at least five years after diagnosis [4]. The main treatments for leukaemia are chemotherapy, radiotherapy, growth factors, and bone marrow or stem cell transplants. Treatment for leukaemia varies depending on age, level of fitness and type of leukaemia [5]. The current therapies however, show a wide range of side effects: e.g. drop in blood cell count, complete hair loss, diarrhoea, tiredness, nausea and reduced fertility [6]. Thus, new treatments with potentially different mechanisms than those presently used as chemotherapy agents could support existing treatments of leukaemia, if their use could reduce side effects without compromising efficacy. Promising sources of such new agents could be found in common foods, which contain bioactive compounds with potential anti-cancer properties.

We have previously demonstrated that carrot (*Daucus carota* L) juice extract contains bioactive agents which prevent leukaemia cell proliferation and induce cell death in human leukaemia cell lines preferentially to non-tumour control cells [13]. Carrot juice extract (CJE) treatment of four human myeloid and four human lymphoid leukaemia cell lines showed an inhibition in progression of cell cycle and induction of programmed cell death (apoptosis) [13]. In addition, lymphoid leukaemia cell lines were more sensitive to CJE treatment than myeloid cell lines [13].

*Daucus carota* L is a rich source of a number of compounds thought to have bioactive properties, including polyacetylenes and carotenoids. Polyacetylenes found within *Daucus carota* L include falcarinol (FaOH), falcarindiol (FaDOH) and falcarindiol-3-acetate (FaDOAc) and have demonstrated bioactive actions in a number of cell lines [14-16]. Falcarinol from Apiaceae vegetables including carrots has been shown to be cytotoxic against an acute lymphoblastic leukaemia cell line (CEM-C7H2), with an IC<sub>50</sub> of 3.5 μM [17]. Moreover, Purup and colleagues (2009), investigated the inhibitory effect of polyacetylenes from carrots (falcarinol and falcarindiol) on the proliferation of human lymphoid leukaemia cell lines.