

Research Article

# *Basil* and *Dracocephalum kotschyi* Alcoholic Extracts Affect *BCL2* expression and HepG2 Cell Proliferation

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## Abstract

**Background:** Liver cancer is the second most common reason for cancer-related death globally, and thus, a major public health challenge. Plant-based drugs are promising therapeutic agents against cancers. *Basil* and *Dracocephalum kotschyi* extracts also exhibit therapeutic impact on various cancers. To assess the effects of the extract of *Basil* and *Dracocephalum kotschyi* on cell proliferation and *BCL2* expression in the HepG2 cell line.

**Materials and Methods:** In this experimental study, the HepG2 cell line was selected for treatment with the alcoholic extracts of *Basil* and *Dracocephalum kotschyi*. Cell proliferation was examined in the presence of various concentrations of the *Basil* and *Dracocephalum kotschyi* extracts by MTT assay. Moreover, the effect of the alcoholic extracts of *Basil* and *Dracocephalum kotschyi* on *BCL2* expression was evaluated using quantitative real-time polymerase chain reaction (qRT-PCR).

**Results:** Cell proliferation analyses showed anticancer characteristics of the alcoholic extracts of *Basil* and *Dracocephalum kotschyi*. In addition, treatment with the extracts of *Basil* and *Dracocephalum kotschyi* led to the decreased expression of *BCL2* in HepG2 cell line.

**Conclusion:** These findings indicated that *Basil* and *Dracocephalum kotschyi* are promising candidates for future anticancer research.

**Keywords:** *Basil*, *BCL2*, *Dracocephalum kotschyi*, HepG2, Liver cancer.

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Editor-in-Chief:

Dr. Alireza Rafiei

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## 1. Introduction

Liver cancer remains fifth and eighth most common cancer in men and women, respectively. The incidence of this cancer is generally 2 to 4 times higher in men [1]. Some of the risk factors related to liver cancer include aflatoxin exposure through diet, smoking, alcohol consumption, and oral contraceptives [2]. This malignant neoplasm is the

second leading cause of cancer-related death worldwide. Approximately 70 to 85% of liver cancer patients constitute cases of hepatocellular carcinoma (HCC). More than 80% of HCC cases occur in East Asia and sub-Saharan Africa [3]. To overcome the limitations on HCC research, various cell line models have been proposed all of which use human hepatoma cells (HepG2) [4]. Although there have been major improvements in cancer therapeutics, substantial shortcomings still remain. Natural plants are excellent sources of bioactive components exerting their health beneficial effects, and very often, these sources are components of gourmet food preparations. Certain bioactive components from the plants have been reported for their anticancer activities [5, 6]. *Basil* (*Ocimum basilicum*) is an annual plant that belongs to the Lamiaceae family, which is native in central Africa to Southeast Asia. It exhibits substantial medicinal properties and contains various antioxidant compounds. Basil in traditional medicine has been employed for the treatment of coughs, headaches, constipation, and fever [6, 7]. Previous studies have also reported the potential of *Basil* extract as an anti-tumor factor [8]. On the other hand, *Dracocephalum kotschyi*, a member of the Lamiaceae family, is a medicinal plant and is endemic to various regions of Iran, such as Alborz Mountains and North of Khorasan. It has been used against various types of cancers, including leukemia and gastrointestinal malignant tumors [9, 10]. The antihyperlipidemic, antitumor, antioxidant, antihypoxic, and immunomodulatory activities of the *Dracocephalum* have already been reported. Moreover, this plant is used in Iranian medicine as an antispasmodic and analgesic, and it also demonstrates immunosuppressive, anticancer, anti-inflammatory, and antibacterial effects [11]. Xanthomycol, a flavonoid compound, has been reported to be present in *Dracocephalum kotschyi* leaves of the plant, and has been speculated to be the main factor in the anticancer activity of this plant [10]. Several chemotherapeutic drugs are botanical metabolites or semi-synthetic derivatives [12]. Members of the *BCL2* gene family play a major role in regulating programmed cell death by controlling pro-apoptotic and anti-apoptotic intracellular signals. In cancer, apoptosis via dysregulation of *BCL2* family genes is a recurring event. Thus, inhibition of anti-apoptotic *BCL2* family proteins represents a potential therapeutic strategy. Previous investigations have shown *BCL2* overexpression in various cancers, such as glioma, melanoma, breast, lung, and colorectal cancer cells [13]. HepG2 cells are non-tumorigenic and the functional specifications of HepG2 include several differentiated hepatic functions, including secretion of plasma proteins, triglyceride metabolism, lipoprotein metabolism, glycogen synthesis, or insulin signaling [14]. Therefore, human liver cancer cell line (HepG2) was selected for this experimental study. Since the anticancer activities of *Basil* and *Dracocephalum* have been reported, we hypothesized that alcoholic extracts of both plants have anticancer effects on HepG2 cell line. The main objective of this study is to investigate if alcoholic extracts of *Basil* and *Dracocephalum* inhibit HepG2 cell proliferation and modify *BCL2* gene expression.

## 2. Materials and Methods

### 2.1. Preparation of plan extracts

The leaves and aerial parts of *Dracocephalum kotschyi* were collected from central Iran (Semrom city in Isfahan province). The aerial parts of *Basil* were collected before flowering from Nazhvan farmlands at Isfahan, Southwest Iran. One hundred grams of dried and powdered aerial parts of *Basil* and *Dracocephalum kotschyi* was chopped separately and soaked in 250 mL ethanol. Then, the mixture was placed on the shaker for 24 h in a fully packed container at room temperature using maceration method. The mixture was filtered through millipore (0.22  $\mu\text{m}$  pore size) and concentrated by vaporizing with a rotary evaporator. Finally, specific concentrations of the alcoholic extracts of *Basil* and *Dracocephalum kotschyi* (0 (control), 10, 50, 100, and 500  $\mu\text{g/mL}$ ) were prepared.

### 2.2. Cell culture

HepG2 cell line was purchased from Pasteur Institute of Cellular Bank of Iran (Tehran, Iran) and cells were cultured at 37 °C, under 5% CO<sub>2</sub> in DMEM (Dulbecco's modified Eagle's medium, Gibco, USA) supplemented according to National Cell Bank of Iran (NCBI) recommendations. To compare the effects of the alcoholic extracts of *Basil* and *Dracocephalum kotschyi* on the HepG2 cell proliferation and *BCL2* expression, cell culture was treated with various concentrations of each extract (0, 10, 50, 100, and 500  $\mu\text{g/mL}$ ) for 24 h.

### 2.3. MTT assay

MTT assay was used to determine the effects of the extracts on cell proliferation [15]. The effects of both the alcoholic extract of *Basil* and *Dracocephalum kotschyi* on cell proliferation were measured by MTT-assay [16]. The HepG2 cell line was treated with various concentrations of each extract for 24 h. Then, MTT dye (Sigma-Aldrich, USA) (dissolved in DMSO) was added at final concentration of 100 $\mu\text{g/mL}$ . The mixture was incubated for 4 h at 37 °C in a CO<sub>2</sub> incubator. The medium was then removed, and the violet crystal was dissolved in DMSO for 30 min. The absorbance was determined using a spectrophotometer at 540 nm (BioTek, Winooski, VT, USA).

### 2.4. RNA isolation

Total cellular RNA was isolated from the cells using the RNeasy Mini, RNA isolation kit (Qiagen) based on manufacturer's instructions. The quality of the extracted RNA was determined at a 260/280 nm wavelength ratio measured by a NanoDrop spectrometer (Thermo Scientific, Waltham, MA, USA).

## 2.5. Complementary DNA synthesis

Complementary DNA (cDNA) was prepared using the QuantiTect Reverse Transcription Kit (Qiagen), according to the manufacturer's instructions.

## 2.6. Real time polymerase chain reaction

Primers were designed using the National Center for Biotechnology Information (NCBI) website and Gene Runner software (Table 1). Real-time quantitative PCR (RT-qPCR) reactions were performed in triplicates using an ABI PRISM 7500 instrument (Applied Biosystems, USA). Briefly, in a total volume of 10  $\mu$ L, 1  $\mu$ L cDNA was added to a master mix containing 5  $\mu$ L SYBR premix ExTaq II (TaKaRa, Kusatsu, Shiga Prefecture, Japan), 0.5  $\mu$ L forward primer, 0.5  $\mu$ L reverse primer, and 3  $\mu$ L DEPC-treated water. The run program was set at 95 °C for 10 min followed by 40 cycles of 95 °C for 15 s, 60 °C for 10 s, and 72 °C for 20 s. GAPDH was used as an internal reference gene to normalize the expression of the *BCL2* gene. The gene expression level was then calculated as described in a previous study [17]. The value was used to plot the expression of the gene using  $2^{-\Delta\Delta Ct}$  method.

TABLE 1: Primer sequences used for qRT-PCR (5'-3').

| Gene         | Forward Primer       | Reverse Primer          | Size (bp) |
|--------------|----------------------|-------------------------|-----------|
| <i>BCL2</i>  | CGGTGGGGTCATGTGTGTG  | CGGTTCAGGTAAGTCAGTCATCC | 90        |
| <i>GAPDH</i> | CCACTCCTCCACCTTTGACG | CCACCACCCTGTTGCTGTAG    | 107       |

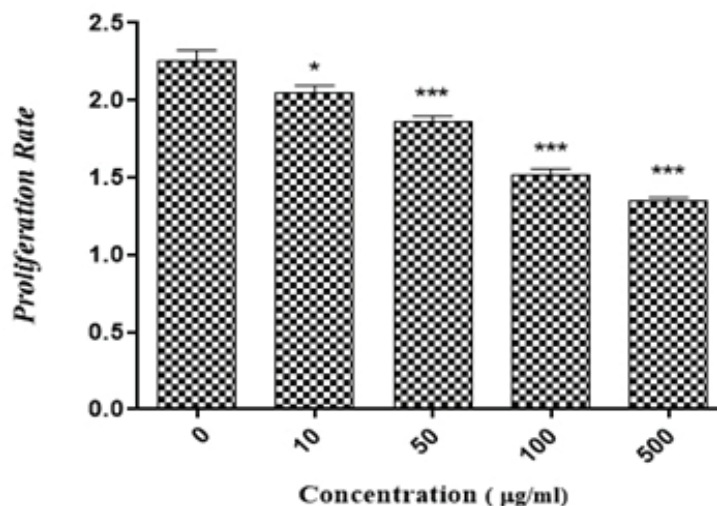
## 2.7. Statistical analysis

The statistical analyses were carried out using Graph Pad Prism, version 8.0.2 (Graph Pad, San Diego, CA, USA). Normality was evaluated by the Kolmogorov–Smirnov test. One-way ANOVA was used to compare the data. Data was expressed as the mean  $\pm$  SD.  $P < 0.05$  was considered to be statistically significant.

## 3. Results

### 3.1. Cell proliferation analyses after *Basil* extract treatment

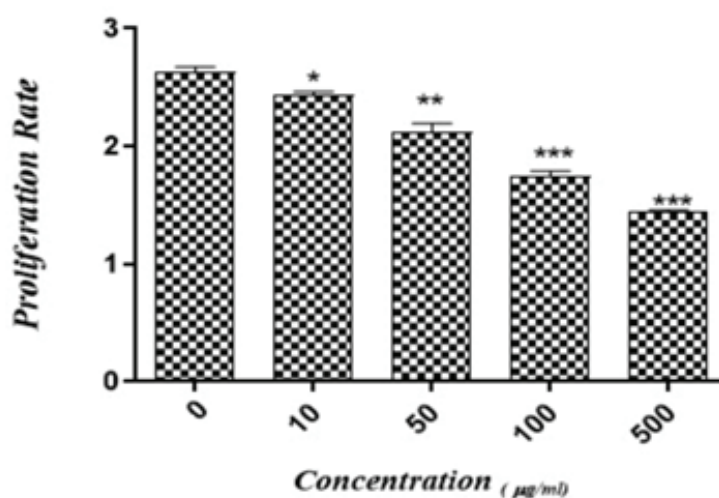
MTT assay was used to determine the effects of *Basil* extract on the proliferation of the HepG2 cell line after treatment for 24 h. The results of the MTT assay demonstrated that treatment with the alcoholic extract of *Basil* led to significant decrease in cell proliferation in a dose-dependent manner (Figure 1).



**Figure 1:** The rate of cell proliferation after treatment with various concentrations of *Basil* extract (0, 10, 50, 100, and 500 µg/ml) in HepG2 cell line after 24 h (\*:P < 0.05, \*\*\*: P <0.0005). Results are presented as mean ± SD.

### 3.2. Cell proliferation analyses after *Dracocephalum kotschy* extract treatment

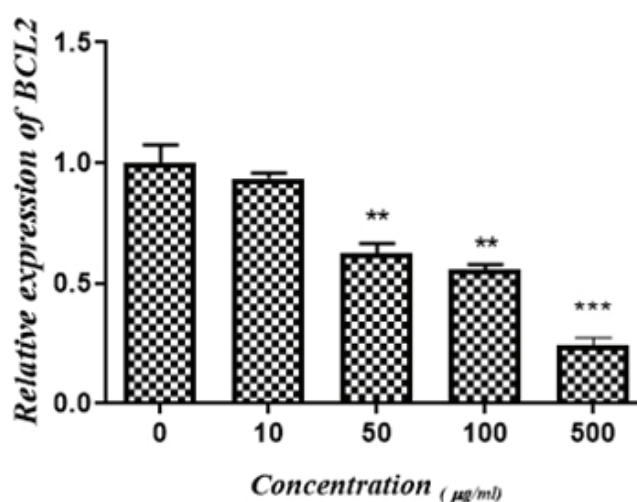
The MTT assay was also used to determine the effects of *Dracocephalum kotschy* extract on the proliferation of the HepG2 cell line after treatment for 24 h. Similar to the *Basil* extract, treatment with the alcoholic extract of *Dracocephalum kotschy* led to decrease in cell proliferation in a dose-dependent manner (Figure 2).



**Figure 2:** The rate of cell proliferation after treatment with various concentrations of *Dracocephalum kotschy* extract (0, 10, 50, 100, and 500 µg/ml) in HepG2 cell line after 24 h (\*: P < 0.05, \*\*: P < 0.004, \*\*\*: P < 0.0005). Results are presented as mean ± SD.

### 3.3. *Basil* extract downregulates *BCL2* expression

The results indicated that treatment with *Basil* extract led to a significant decrease in the expression of *BCL2* gene in a dose-dependent manner. We observed the highest decrease in *BCL2* expression after treatment with 500 µg/mL of the alcoholic extract of *Basil* (Figure 3).



**Figure 3:** Reduction in *BCL2* expression after treatment with various concentrations of the *Basil* extract (0, 10, 50, 100, and 500 µg/mL) in the HepG2 cell line in comparison to control (\*\*:  $P < 0.004$ , \*\*\*:  $P < 0.0005$ ). Results are presented as mean  $\pm$  SD.

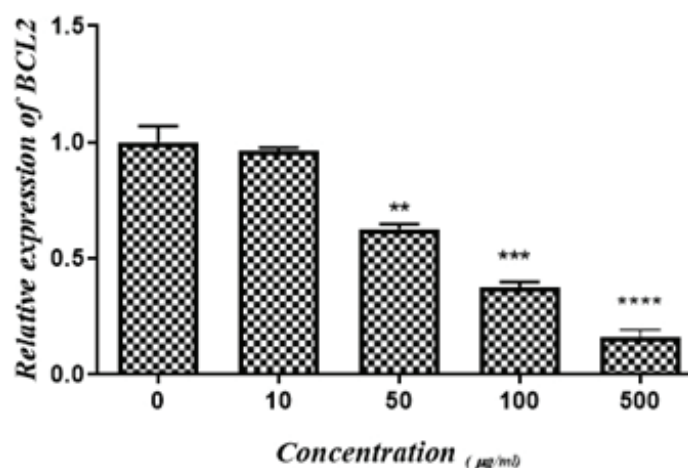
### 3.4. *Dracocephalum kotschy* downregulates *BCL2* expression

The expression of the *BCL2* gene was significantly decreased after treatment with the alcoholic extract of *Dracocephalum kotschy* in a dose-dependent manner (Figure 4). The highest decrease in *BCL2* expression was observed after treatment with 500 µg/mL of the alcoholic extract of *Dracocephalum kotschy*. Our data suggested that the alcoholic extract of *Dracocephalum kotschy* leads to a decrease in the mRNA levels of *BCL2* (Figure 4).

## 4. Discussion

Cancer is the second leading cause of deaths worldwide. Despite major improvements in cancer's therapeutic strategies, significant deficiencies still remain. Some undesired side effects of chemotherapy are seldom observed. Natural therapies, such as the usage of plant-derived products in cancer therapy, might decrease such side effects. Recently, several plant products have been reported to exhibit anti-cancer properties *in vitro* [18]. Certain bioactive compounds from the plants have been confirmed for their anticancer activities [19]. However, a myriad of plant products have shown promising anti-cancer





**Figure 4:** Reduction in *BCL2* expression after treatment with various concentrations of the *Dracocephalum kotschy* extract (0, 10, 50, 100, and 500 µg/mL) in the HepG2 cell line compared to control (\*\*:  $P < 0.004$ , \*\*\*:  $P < 0.0005$ , \*\*\*\*:  $P < 0.0001$ ). Results are presented as mean  $\pm$  SD.

properties *in vitro* but have yet to be evaluated in humans. Further study is required to determine the efficacy of these plant products in treating cancers in humans [18]. In the current study, we studied the effect of the alcoholic extracts of *Basil* and *Dracocephalum kotschy* as anti-cancer agents against the HepG2 cell line. MTT assay was used to explore the impact of the alcoholic extracts of *Basil* and *Dracocephalum kotschy* on cell proliferation in HepG2 cell line. The results demonstrated that cell proliferation decreased significantly after treatment with both alcoholic extracts of both extracts for 24 h in a dose dependent manner. Thus, the alcoholic extracts of *Basil* and *D. kotschy* exhibited anti-proliferative effects in HepG2 cell line. On the other hand, we investigated the effect of the alcoholic extracts of *Basil* and *Dracocephalum kotschy* on the *BCL2* expression. Our results indicated that treatment with the alcoholic extracts of *Basil* and *Dracocephalum kotschy* led to decrease in *BCL2* expression in a dose dependent manner. The most prominent reduction in *BCL2* expression reduce was observed after treatment with 500 µg/mL of the alcoholic extract of *Dracocephalum kotschy*. It has already been shown that the expression of *Bcl* and *Bax* could be differently regulated by quercetin in HepG2 cell line; thus, it has been suggested that the balance in the expression of these proteins might be involved in the regulation of the apoptotic process in this cell line. Quercetin treatment led to decreased *Bcl* expression [20]. Shimizu et al investigated the effect of *Basil* leaf extract on metastasis of aggressive human pancreatic cancer cells *in vitro* and *in vivo*. They suggested that leaves of *Basil* could be a potential source of novel anticancer compounds [21]. Sani et al studied the cytotoxic, anti-proliferative, and apoptotic effects of *Dracocephalum kotschy* extract against lung cancer cell lines (Calu-6 and Mehr-80). The different fractions and compounds of *Dracocephalum kotschy* extract exhibited significant cytotoxic activities against Calu-6 and Mehr-80 cells [11]. In a previous study, the main cytotoxic component of *Dracocephalum kotschy*, xanthomicrol, was investigated as a potential anti-cancer agent. It was observed that xanthomicrol in the leaf extract of *Dracocephalum kotschy* inhibited the proliferation of several types of malignant tumors [22]. The *Dracocephalum*

*kotschy* constituents include xanthomicrol, limonene, luteolin, geraniol, apigenin, and calycopterin. Previous studies have demonstrated that all *Dracocephalum kotschy* parts (roots, aerial parts, flowers, and leaves) harbored active constituents. Moreover, a number of *Dracocephalum kotschy* medicinal properties have been confirmed, such as antioxidant, antibacterial, anticancerous, antinociceptive, antihyperlipidemic, antispasmodic, and cytotoxic [23]. Based on the results of this study and previous studies, it can be concluded that *Basil* and *Dracocephalum kotschy* exhibit an anticancer effect. The identification of other factors constituting *Basil* and *Dracocephalum kotschy* extract could also be involved in the therapeutic potential of these plants. However, the effects of these extracts should be examined on several other cancer cell lines, including biochemical and molecular investigations carried out in animal models, to establish their therapeutic efficacy. In conclusion, we hypothesized that the alcoholic extracts of *Basil* and *Dracocephalum kotschy* might cause cancer cell apoptosis and downregulation of *BCL2* expression, thus making them potential therapeutic agents for cancer. Future research in this area could help in the exploration of novel effective anticancer drugs.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## Acknowledgements

This study was supported by Mansour Moghimi (Associate Professor of Clinical and Surgical Pathology) from Shahid Sadoughi University of Medical Sciences.

## Author Contributions

M.M; Contributed to conception and design. N.H, F.KH, and S.R; Contributed to all experimental work and molecular experiments. S.R and M.M Contributed to conception, design data, statistical analysis, and interpretation of data. N.H drafted the manuscript, which was revised by M.M, F.KH, and S.R.

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