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ORIGINAL ARTICLE

Cytotoxic and antioxidant properties of essential oil of *Centaurea behen* L. in vitro

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Abstract *Centaurea* species of Asteraceae family are widely use in traditional medicine. Despite wide medicinal use of *Centaurea* sp., there is limited knowledge concerning *Centaurea behen* toxicity. Therefore, in this study, it is aimed to determine cytotoxic and oxidative effects of essential oil of *C. behen* on human blood cell cultures. 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) and lactate dehydrogenase (LDH) release assays were performed to determine cytotoxic effects. In addition, total antioxidant capacity (TAC) and total oxidative status (TOS) were examined to determine oxidative potentials. The results indicated that all tested concentrations of essential oil of *C. behen* were cytotoxic and led to decreases of cell viability in both assays. Besides, *C. behen* led to significant increases of TOS levels and decreases of TAC levels. As a conclusion, the present study showed for the first time the cytotoxic and oxidant effects of essential oil of *C. behen* on cultured human whole blood cells.

Keywords *Centaurea behen* L. · Asteraceae · Cytotoxicity · Oxidative effect · Peripheral human blood

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Introduction

The family Asteraceae, which is the biggest family of the flowering plants with approximately 24,000–30,000 species and 1600–1700 genus, separated around the world especially in West Asia and Mediterranean region (Funk et al. 2005; Garcia-Jacas et al. 2000). The genus *Centaurea* is represented with 194 species that 118 are endemic in Turkey (Davis et al. 1988). Nevertheless, Turkey is the primary center of variety of *Centaurea* species (Uzunhisarcikli et al. 2005). Many members of this genus, are used in traditional medicine as bitter tonic, stomachic, diuretic, anti-malarial and as a mild astringent (Al-Easa and Rizk 1992) and to treat various ailments include rheumatism, diabetes, diarrhea and hypertension (Sarker et al. 1997). In addition, it has been stated that *Centaurea* species exerts a vide range of biological effects including anti-microbial, anti-fungal, anti-plasmodial (Karamenderes et al. 2006; Kaskoos

2013), anti-ulcerogenic, anti-oxidant, anti-viral, anti-protozoal and anti-cancer properties (Ugur et al. 2009a, b, 2010; Erol-Dayi et al. 2011; Kilic 2013; Pires Tania et al. 2018). Moreover, *Centaurea* was also a source of some phytochemical studies for its potentially active substance especially flavonoids (Flamini et al. 2004; Mishio et al. 2006) and sesquiterpene lactones (Gonzalez et al. 1984; Medjroubi et al. 2005; López-Rodriguez et al. 2009). In another study, Escher et al. (2018) identified chlorogenic, caffeic, ferulic, and p-coumaric acids, isoquercitrin, and coumarin as major compounds of *Centaurea* genus also. In the same study, authors showed that temperature effected content of flavonoids in a statistically important manner. In fact, it has been reported that medicinal effects of this species may related to bitter crystalline unsaturated lactones (Kurian and Sankar 2007).

Up to know *Centaurea behen* has been evaluated biological and biochemical properties in a few reports (Chougule et al. 2014; Esmaeili and Khodadadi 2012). Although the effects of this species growing in Turkey have not been investigated on human healthy tissues. Thus, it deemed interested to the researchers to carry out this study concerning determination of toxicity and oxidative features of this medical plant. The goal of this work is to determine the cytotoxic and antioxidant potentials of essential oil of *C. behen* on human whole blood cultures for the first time.

Materials and methods

Plant material and isolation of the essential oils

Centaurea behen L. samples were collected during to flowering stage from Harput (Elazığ-Turkey), on June, 2017 an altitude of 1200 m and identified by Dr. Şükrü HAYTA. Aerial parts of *C. behen* (100 g) were dried and subjected to hydrodistillation using a Clevenger-type apparatus for 3 h.

Cell cultures

The protocol that identified previously by Evans and O'Riordan (1975) was used to set up cultured human whole blood samples with a slight modification. The samples were taken from five healthy males (aged 26–28 years), without recent anamnesis of exposure to mutagens, not under drug therapy, non-alcoholic, non-smokings. The heparinized blood samples (0.6 mL) were cultured in 6.6 mL culture medium (Karyotyping Medium, Gibco, Barcelona, Spain) with 5.0 mg mL of phytohemagglutinin (Sigma Aldrich, Steinheim, Germany) (Geyikoğlu and Turkez 2006). Different concentrations of pure essential oil of *C. behen* (at 10, 25, 50,100, 200 and 400 μg mL^{-1} were added into the culture tubes before the incubation. The concentrations of *C. behen* were selected according to a previous report by Çelik et al. (2014).

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide) assay

The cell proliferation was tested via commercially available kit (MTT kit Cayman Chemical Company, USA). As the positive control, Triton-X (%1, Sigma-Aldrich) was used. Cells were incubated in a humidified 5% CO_2/95% air mixture at 37 °C and induced with pure essential oil of *C. behen* at varied concentrations for 48 h. In brief, MTT was put into the cultures for 3 h and formed formazan crystals were dissolved in dimethyl sulfoxide. (Sigma-Aldrich). Then, absorbances of samples were detected at 570 nm by Elisa plate reader (Sigma-Aldrich, USA) (Lewerenz et al. 2003; Wang et al. 2010).

LDH (lactate dehydrogenase) assay

LDH release activity was detected spectrophotometrically by using a LDH kit (*Cayman Chemical*, USA). Triton-X (%1) was also used as the positive control in this assay. Briefly, 10^4–10^5 μL cells/well were added in plates and treated with varied concentrations (10–400 μg mL^{-1}) of pure essential oil of *C. behen* for 48 h. Then, to settle down the pure essential oil of *C. behen*, and plate was centrifuged at 400 g for 5 min. After this process, supernatant (100 μL) was put into fresh wells that contained reaction mixture (100 μL) from the kit and incubated for 30 min at room temperature. And then, the absorbances of the samples were determined at 490 nm, by using a microplate reader (Elisa reader Bio-Tek(r), USA). The obtained results were calculated according to the kit procedure (Hussain et al. 2005).

TAC and TOS analysis

The TAC and TOS levels were determined by using commercial kits (Rel Assay Diagnostics, Gaziantep, Turkey). As the positive control hydrogen peroxide (25 µM, Sigma-Aldrich) and ascorbic acid (10 µM, Sigma-Aldrich) were used. Plasma samples were treated with pure essential oil of *C. behen* for 48 h.

Statistical analysis

Statistical analysis of the present work was performed by using SPSS Statistics packaged software program (version 22.0 Chicago, IL, USA). The homogeneity of variances (homoscedasticity) was checked using F-test. After confirmation of homoscedasticity and normality, Duncan's test was used to identify statistically important differences from control or each other (Granato et al. 2014). Finally, statistical variations were indicated at significance level of 0.05.

Results

The obtain results of the MTT and LDH release assays in cultured human lymphocytes exhibited that all tested concentrations of *C. behen* have cytotoxic effect, as shown in Figs. 1 and 2, respectively.

The TAC and the TOS levels were evaluated by using spectrophotometric methods. As shown in Table 1, TOS levels increased all tested concentrations of *C. behen.* On the other hand, TAC levels decreased in cultured whole human blood cells.

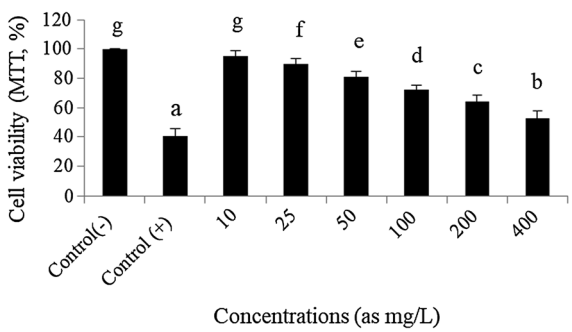

Fig. 1 The obtain MTT data in human whole blood cell cultures treated with *Centaurea behen* for 48 h. The results are demonstrated as mean ± SD for each group. The different letters demonstrate important variations from each other ($p < 0.05$)

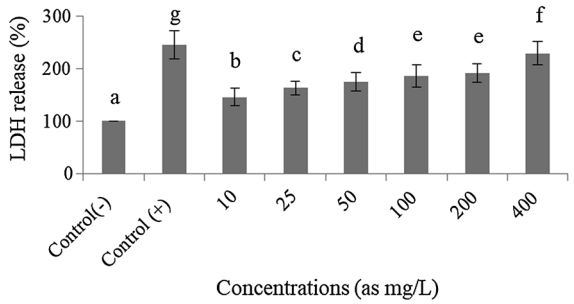

Fig. 2 The LDH release (%) values in human whole blood cell cultures induced with *Centaurea behen* for 48 h. The different letters indicate important differences from each other ($p < 0.05$)

Table 1 *In vitro* TAC and TOS status in cultured human whole blood cells after exposed to essential oil of *C. behen* for 48 h

Doses	TAC level	TOS level
Control (−)	6.2 ± 0.7^{d}	11.6 ± 2.3^{a}
Control (+)	12.5 ± 1.5^{e}	39.1 ± 3.2^{e}
10 mg/L	6.0 ± 0.8^{d}	12.2 ± 2.6^{a}
25 mg/L	5.1 ± 0.6^{c}	14.3 ± 2.8^{b}
50 mg/L	5.0 ± 0.8^{c}	15.5 ± 2.4^{b}
100 mg/L	4.3 ± 0.5^{b}	17.8 ± 2.1^{c}
200 mg/L	3.2 ± 0.7^{a}	23.9 ± 2.6^{d}
400 mg/L	2.9 ± 0.6^{a}	25.8 ± 2.9^{d}
400 mg/L	2.9 ± 0.6^{a}	25.8 ± 2.9^{d}

The results are indicated as mean ± SD for each group. The different superscript letters show statistically important discrepancy ($p < 0.05$)

Discussion

Medicinal plants are widely used in folk medicine and for improve new drugs (Newman and Cragg 2016). Currently, the *Centaurea* genus has a great of interest due to its chemical properties and wide distribution (Erel et al. 2014). Thus, in this paper, essential oil of *C. behen* was determined for its cytotoxic potential on cultured human peripheral blood samples. To determine cytotoxicity potential, MTT colorimetric test was performed. Cultured human peripheral blood samples were treated with different concentration of essential oil of *C. behen*. The obtain results showed that essential oil of *C. behen* exhibited cytotoxic action at all tested concentrations (from 10 mg L^{-1} to 400 mg L^{-1}) as shown in Fig. 1. Besides, LDH

release assay was also used to determine cytotoxic effects of essential oil of *C. behen*. The obtain data confirmed that essential oil of *C. behen* led to significant increases of the LDH release at all tested concentrations (Fig. 2).

The obtain results from cytotoxicity testing are in accordance with the previous studies in the literature. In fact, in a recent study, Escher et al. (2018) noted that *Centaurea cyanus* showed low cytotoxicity and pro-oxidant action without cause cell damage or death. In another study, it has been reported that 80% methanol extract of *C. alexanderina* exerted significant cytotoxic effect against A-495 lung cell line (Kubacey et al. 2012). Likewise, Csupor-Loffler et al. (2009) showed that some *Centaurea* species including *C. jacea*, *C. Spinulosa* and *C. biebersteinii* exhibited strong cytotoxic activities on the HeLa cervical, the A431 epidermal and MCF-7 breast cancer cell lines. Medjroubi et al. (2005) stated that cytotoxic activity of the *C. musimomum* against cells derived from human carcinoma of the nasopharynx with growth inhibition of 89% at 10 mg/L. In a different study, Seghiri et al. (2009) isolated algerianin (a new acylated flavonoid glucoside) from *C. africana* and tested its cytotoxic potential with MTT assay. At the end of the study, they reported that algerianin has important cytotoxic effect on human myeloid leukaemia HL-60 cells at Moreover, Bach et al. (2011) showed that onopordopicrin and cnicin have high cytotoxic effect on human-derived macrophages that were isolated from chloroform extracts of *C. tweediei* and *C. diffusa* weeds. Similarly, Erel et al. (2011) reported that cnicin, isolated from *C. calolepis,* has cytotoxic activity against human malignant melanoma (SK-MEL), human ductal carcinoma (BT-549) and pig kidney epithelial (LLC-PK) cells.

On the other hand, it has been reported that the imbalance between oxidants and antioxidants cause to formation of reactive oxygen species and this situation is addressed to oxidative stress (Yeum et al. 2004; Chen et al. 2011; Duan et al. 2016). The increases of TOS levels or decrease of TAC levels are leads to comprise of reactive oxygen species that may cause genotoxic and cytotoxic damages (Geyikoğlu et al. 2005; Lau et al. 2008; Türkez and Sisman 2007). For this reason, we evaluated cytotoxicity (MTT and LDH release) as well as and TAC and TOS levels as oxidative parameters for determining biological efficacy of *C. behen* in vitro.

There are limited studies associated to the antioxidant activities of *C. behen* in the literature. To our best knowledge, the antioxidant and oxidant potential of *C. behen* have not been evaluated on cultured human whole blood cells. To evaluate antioxidant/oxidant effects, TAC and TOS levels were detected by colorimetric methods. The obtained data demonstrated that *C. behen* decreased TAC levels and increased TOS levels at doses of 25 mg L^{-1}–400 mg L^{-1} according to the controls in cultured human peripheral blood cells as shown in Table 1. These results are disagreement with the literature. Chougule et al. (2012) reported that hydro-alcoholic extract of *C. behen* possesses strong free radical scavenging activity. In another study, Chougule et al. (2014) stated that *C. behen* showed a protective effect against CCl_4 induced hepatotoxicity. In the study, authors declared that protective effect of *C. behen* might be related with its antioxidant properties. In addition, there are some studies dealed with the antioxidant activities of other *Centaurea* species (Erol-Dayi et al. 2011; Conforti et al. 2008; Chougule et al. 2012). These differences may be explained with different contents of the plant due to local conditions.

Conclusions

As a conclusion, the obtain results of this work concluded that essential oil of *C. behen* exhibited cytotoxic effect and increased oxidative stress on cultured human whole blood cells for the first time. Finally, isolation, characterization and mechanism elucidation of active compounds of *C. behen* would be of interest.

Compliance withe ethical standards

Conflict of interest No potential conflict of interest was reported by the authors.

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