

Full Length Research Paper

Anticancer activity of n-hexane extract of *Cichorium intybus* on lymphoblastic leukemia cells (Jurkat cells)

Mohammad Saleem^{1,2*}, Khizar Abbas¹, Faiza Naseer¹, Mobasher Ahmad¹,
Nawazish-i-Husain Syed¹, Fatima Javed¹, Khalid Hussain¹ and Samia Asima³

¹University College of Pharmacy, University of the Punjab, Lahore, Punjab, Pakistan.

²College of Pharmacy, GC University, Faisalabad, Punjab, Pakistan.

³Pharmacy Department, The University of Lahore, Pakistan.

Received 18 February, 2013; Accepted 9 June, 2014

Cichorium intybus commonly known as chicory is a member of the family Compositae. It has been known for various pharmacological activities and anticancer effects against various cell lines including breast cancer MCF-7, prostate cancer LNCaP, amelanotic melanoma C32 and renal adenocarcinoma ACHN. In the present study, aerial parts of *C. intybus* were collected, identified, soaked in n-hexane non polar solvent, filtered and evaporated till the extract residue was isolated, dried and stored at 4°C and then checked for cytotoxic activities. Lymphoblastic leukemia cells (Jurkat cells) were used to evaluate the cytotoxic effects of n-hexane extract of *C. intybus*. Extracts were added to the cultured cells of selected cell line in various concentrations (10, 25, 50, 75 and 100 µg/ml) and incubated for 24 h. Trypan blue exclusion assay, MTS assay and FACS analysis were carried out to analyze cell viability, cell proliferation and apoptosis, respectively. The non polar n-hexane extract significantly reduced the number of viable cells and cell proliferation percentage but induced the apoptosis haphazardly. Results of this study demonstrate that n-hexane extract of *C. intybus* has potent anti-proliferative and cytotoxic activity against Jurkat cells, a human leukemia cell line.

Key words: *Cichorium intybus*, Jurkat cells, lymphoblastic leukemia cells, MTS, trypan blue assay, apoptotic analysis.

INTRODUCTION

Cichorium intybus is a small herb (Keshri et al., 1998) also known as chicory belonging to the family Compositae (Pushparaj et al., 2007) or Asteraceae (Shaikh et al., 2012). Its common name is Kasni. It widely exists in Mediterranean region, Iran, Europe and Northern Asia (Amirghofran et al., 2000) and also present

in Punjab, New Frontier and Hyderabad (Keshri et al., 1998). *C. intybus* is one of the plants cited by Hadith (Wani et al., 2011) and traditionally used in many indications. Its roots and leaves are used as bitter tonic cholagogue, cardio protective, diuretic, laxative, anti-hypertensive, anti-rheumatic and anti-diabetic agent

*Corresponding author. E-mail: saleem2978@hotmail.com. Tel: +92-321-4289022.

(Pushparaj et al., 2007). Seeds are used for the treatment of ulcer, skin and microbial diseases (Daniela et al., 2009). Its ethanolic extract has antitumor effects (Shaikh et al., 2012) and aqueous extracts act against mitogen induced lymphocytic proliferation (Amirghofran et al., 2000) and hepatic inflammation (Gadgoli et al., 1997). It has contraceptive activity in female rats (Keshri et al., 1998) and analgesic and sedative activity as well (Wesolowska et al., 2006).

Phytochemical studies shows that it contains polyphenols including flavonoids such as kaempferol, luteolin, epigenins and quercetin (Heimler et al., 2009), sesquiterpene lactones, coumarins and vitamins (Varotto et al., 2000). The phenolic compound trazin-3-p-coumaroylquinic acid contains hydroxycinnamic acid and coumaroyl groups isolated from this herb causes caspase 3 mediated apoptosis in gastric tumor cells (Hsieh et al., 2010). Other constituents are alkaloids, carbohydrates, triterpenoids, tannins, volatile oils and fatty acids in minute quantities. Aqueous extract of chicory contains reducing sugar including vanillic acid, syringic acid and butelic acid. Other water soluble contents are rutin and inulin which replace fat or sugar and reduces the food calories (Niness, 1999). Lactucin and lactopicrin, two isolated compounds show activity against the malarial parasite, bacteria (Bischoff et al., 2004; Shaikh et al., 2012) and fungi (Monde et al., 1990; Yusuf et al., 2002.). The objective of the present study was to evaluate the cytotoxic effect of n-hexane extracts of whole plant of *C. intybus* on lymphoblastic leukemia Jurkat cells.

MATERIALS AND METHODS

Plant material

Whole plant of *C. intybus* was collected from the fields of Punjab, Pakistan. The plant was identified by the Department of Botany, GC University Faisalabad Pakistan. A voucher specimen was kept in the herbarium as future reference. The plant was thoroughly washed with distilled water then shade dried, grinded into powder and stored at room temperature.

Extraction and isolation

The plant was put in macerating flask with ample non polar solvent n-hexane for 5 days with occasional shaking within mixture. The macerate was filtered with filter paper and the extract was obtained by evaporating the solvent in rotary evaporator at reduced pressure, until the semisolid mass was obtained. The extract was stored at 4°C until further use on selected cell line. To prepare the stock solution, 10 mg of solid residue was dissolved in 1 ml (500 µl ethanol + 500 µl water). Serial dilutions of various concentrations 10, 25, 50, 75 and 100 µg/ml were freshly prepared from stock solution before use.

Cell line and culture conditions

The Jurkat cell line was incubated after obtaining in the incubator provided with 5% CO₂ at 37°C. The cell culture was grown in RPMI-

1640 along with 10% (v/v) fetal bovine serum (Biowhitaker, Lonza, Belgium), 100 Units/ml of penicillin, 2 Mm L-glutamine and 100 µg/ml of streptomycin (Sigma St. Louis, MO).

Trypan blue exclusion assay

The effect of n-hexane extract of *C. intybus* on cell viability of the Jurkat cell line has been determined by the trypan blue exclusion assay. The Jurkat cell line was implanted in 6 well plates at the concentration of 10⁵ cells/well. The cells were incubated for 24 h before the addition of extract or vehicle. After 24 h, cells were collected, suspended in 0.4% trypan blue (Sigma-Aldrich, St-Quentin Fallavier, France) and number of cells were counted by haemocytometer.

MTS assay

The determination of the effect of n-hexane extract of *C. intybus* on cell proliferation of Jurkat cells was done by MTS assay. The Jurkat cell line was cultured in RPMI-1640 medium in the presence of vehicle and extract for 24 h in 96 well plates. After this, 20 µl/well of MTS reagent (Aqueous One® Reagent, Promega, United States of America) was applied and cells were incubated again for 2 h. The numbers of living cells were calculated by using multiwell ELISA plate reader by observing absorbance at 490 nm wavelengths.

Apoptosis analysis

For the analysis of late and early apoptosis, the Annexin V-FITC apoptosis kit (BD Pharmingen, USA) was used. The phosphatidyl serine is externalized in apoptotic cells for what the Annexin V has strong affinity. In short, the cells were exposed both to vehicle and n-Hexane extract for 24 h then washed with BPS. The cells were resuspended in the binding buffer, 5 µl of Annexin V-FITC and 10 µl propidium iodide (final concentration of 50 µg/ml) were added to each sample and cells were incubated for 20 min. Then 10,000 events for FACS analysis were considered and expressed as dots.

Statistical analysis

The data was expressed in terms of bar graph along with means ± SEM for at least three individual experimental trials, applying one way ANOVA. The data were subjected to statistical analysis. The level of significance was established according to standard notations.

RESULTS

n-Hexane extract of *C. intybus* decreased cell viability of Jurkat cell line

Trypan blue exclusion assay was used to evaluate the cell viability of Jurkat cells. n-hexane extract of *C. intybus* reduced the cell viability significantly, after 24 h of the addition of the extract of vehicle. The reduction happened in a concentration dependent manner and observed at a concentration greater than 10 µg/ml as shown in Figure 1. Cell viability determination indicated that *C. intybus* n-hexane extract at various concentrations: 10, 25, 50, 75 and 100 µg/ml reduced cell viability by 85, 75, 70, 58 and 50.3%, respectively, as compared to the control group

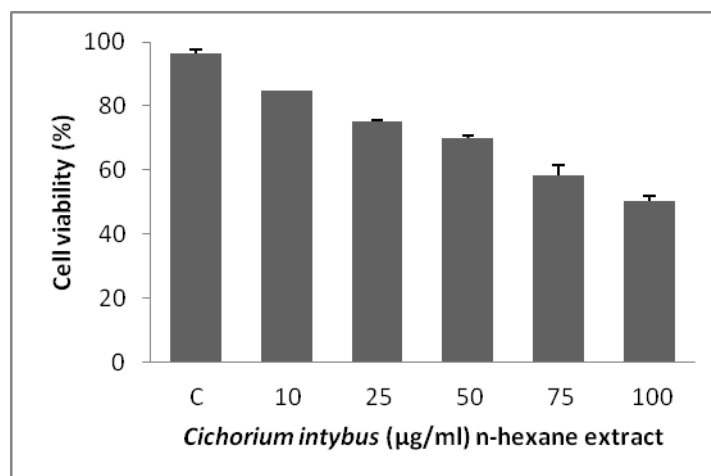


Figure 1. n-Hexane extract of *C. intybus* decreased cell viability of Jurkat cells in concentration dependent manner. The cells were exposed to either vehicle or different concentration of n-hexane extract of *C. intybus*. Trypan blue assay was used to determine the concentration of living cells. Values are shown as means \pm SEM; n = 3. P < 0.05 versus control.

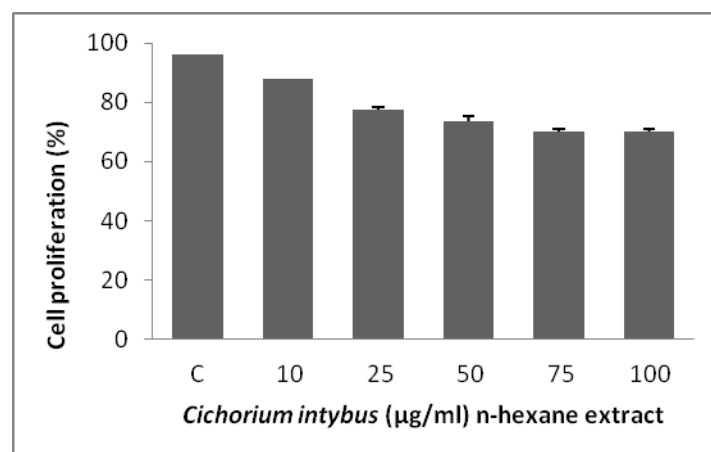


Figure 2. n-Hexane extract of *C. intybus* decreased cell proliferation of Jurkat cells in concentration dependent manner. The cells were exposed to either control or different concentration of n-hexane extract of *C. intybus*. MTS assay was used to determine the concentration of living cells. Values are shown as means \pm SEM; n = 3. P < 0.05 versus control.

having value of 96.3%.

n-Hexane extract of *C. intybus* decreased cell proliferation of Jurkat cell line

The cytotoxic effect of *C. intybus* n-hexane extract was further assessed by using the MTS assay for cell proliferation. This extract significantly decreased the percentage of living cells in a concentration-dependent manner.

For example in controlled group cell proliferation is 96.3% but at max concentration of extract 100 µg/ml, the cell proliferation declined at 70% as shown in Figure 2. This result had shown that as the concentration of extract increases, the percentage of living cells significantly decreases.

n-Hexane extract of *C. intybus* enhanced apoptosis

n-Hexane extract of *C. intybus* affect apoptosis in Jurkat

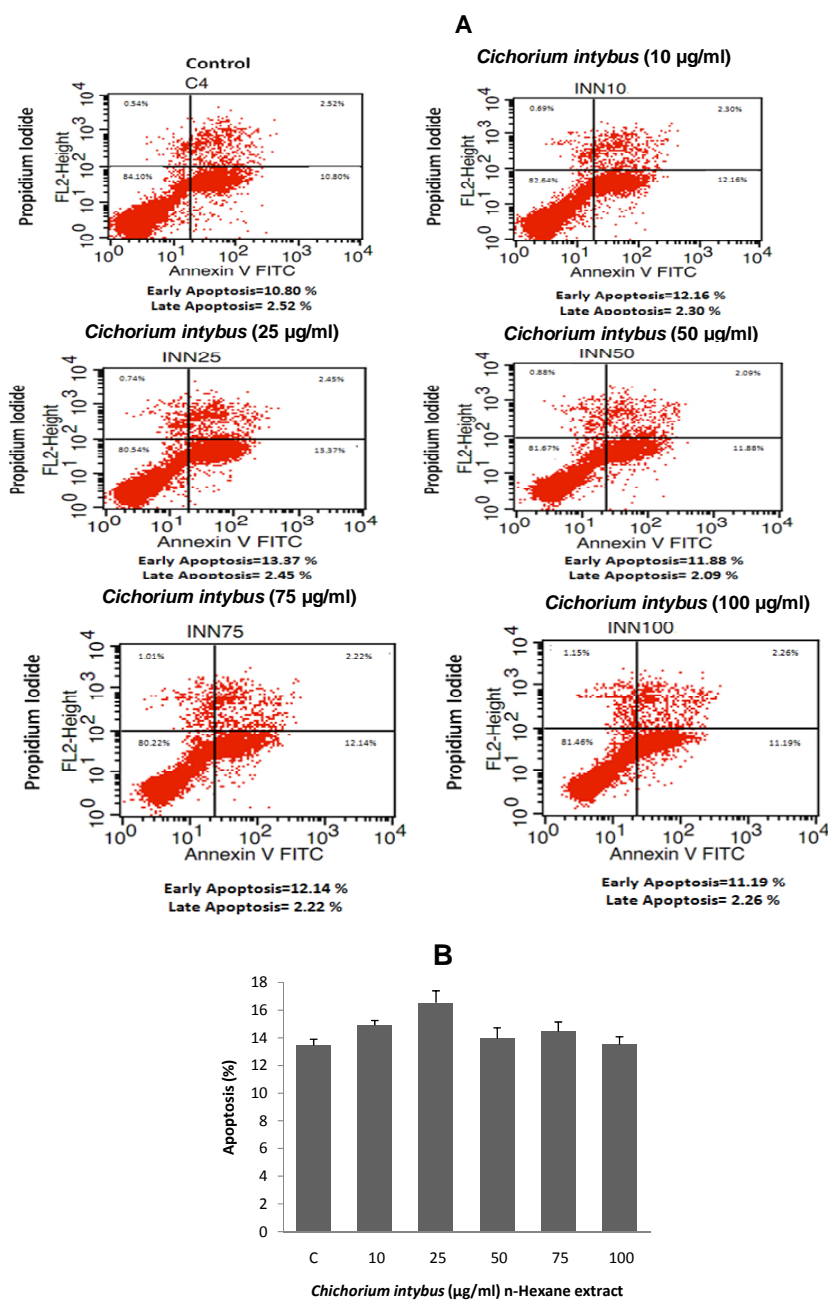


Figure 3. *C. intybus* n-hexane extract promotes apoptosis in lymphoblastic leukemia cell line, the Jurkat cells: Jurkat cells were seeded in six well plates, treated with either vehicle or different concentration of extract and incubated for 24 h. Thereafter 5 µl of Annexin-V and 10 µl of propidium iodide were added to each well, mixed well and wrapped in aluminum foil and then subjected to FACS analysis. (A) Representative flow cytometry analysis of cells. It indicated that lower left quadrant had cells which were negative for both Annexin V-FITC and propidium iodide, the lower right showed cells in early stage of apoptosis which were positive for Annexin-V, upper left showed dead cells which were positive for propidium iodide and the upper right showed cells in late stage of apoptosis and positive for Annexin V-FITC and propidium iodide-positive cells. (B) Represent corresponding cumulative data. Values are shown as means ± SEM; n = 3. P < 0.05 versus control.

cell line. Results have shown that increasing concentrations of extract were associated with increase in apoptosis up to 25 µg/ml. The number of apoptotic

cells decreased at 50 µg/ml and increased at 75 µg/ml concentration. Further increase in concentration again decreased the apoptosis as shown in Figure 3A and B.

DISCUSSION

Nowadays, many drugs are showing resistance, so it is direly needed to search for and develop new drugs. Natural products may be the good source of new therapeutic agents (Saleem et al., 2014). *C. intybus* has previously been shown to be cytotoxic against breast cancer MCF-7 (Dahab and Afifi, 2007), prostate cancer LNCaP, amelanotic melanoma C32 and renal adenocarcinoma ACHN (Conforti et al., 2008). Hsieh et al. (2010) had proven its *in vitro* anti-proliferative properties and caspase 3 mediated apoptosis induction in gastric tumor cell lines.

In the present study, we have observed the cytotoxic effect of *C. intybus* n-hexane extract against Jurkat cells, a human lymphoblastic leukemia cell line, by using trypan blue assay, MTS assay and FACS analysis. It was shown from the trypan blue exclusion assay that n-hexane extract of *C. intybus* decreased cell viability by increasing concentration of the extract. Antiproliferative activity of *C. intybus* was determined by performing MTS assay. It was shown that there is decrease in cell proliferation. Apoptosis induction was evaluated by FACS analysis. The rate of apoptosis induction by *C. intybus* n-hexane extract was found maximum at the dose of 25 µg/mL. As dose of the extract was increased, apoptotic rate decreased. Thus, 25 µg/ml may be considered the most effective dose for the induction of apoptosis in Jurkat cells.

Previous studies showed that *C. intybus* n-hexane extract consists of volatile oils, fatty acids and triterpenoids (Nandagopal and Kumari, 2007). The essential oils or volatile oil have great potential of cytotoxicity, antimicrobial, analgesic, anti-inflammatory and insect repellent activities and furthermore anticancer effects are via apoptotic pathway (Sharma et al., 2013). Similarly, triterpenoids are also considered as new promising anticancer drugs (Petronelli et al., 2009). Thus, n-hexane extract of *C. intybus* may contain volatile oils and triterpenoids and cytotoxic potential of this extract may be due to the presence of these compounds.

Conflict of Interests

The authors declare no conflict of interests.

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