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# Research Article
# Crude Artichoke Extract's Antioxidant, Anti-Inflammatory and Autophagy-Promoting Effects on the Cancer Cell Line SH-SY5Y

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## Abstract

**Background and Objective:** In this study, the impact of various concentrations of *Cynara scolymus* ethanol extract was explored on mitigating drug resistance in the SH-SY5Y neuroblastoma cell line, despite aggressive multimodal therapy yielding a poor prognosis for patients. The primary objective was to investigate the effects of *C. scolymus* ethanol extract on cell survival and to examine autophagy as a potential mechanism influencing tumor and cell resilience to chemotherapy. **Materials and Methods:** The *C. scolymus* extract was diluted with the cultivation medium for testing against SH-SY5Y cell lines. Cell survival was evaluated using the MTT cell proliferation assay, with extract concentration ranging from 50 to 200 µg/mL. The GC-Mass analysis identified 28 components which were phenolic while 13 flavonoids and its several sub-classes were also identified. Antioxidants, proinflammatory markers and the autophagy marker LC3 were measured using a flow cytometer. **Results:** A significant increase in TNF-α, IL-10, IL-6 and IL-1B in SH-SY5Y NB cell line compared to normal cells. *Cynara scolymus* extract showed dose-independent anti-inflammatory and antioxidant properties. The levels of TNF-α, IL-10, IL-6 and IL-1B in treated cells are notably lower than those in untreated cells. *Cynara scolymus* extract has anti-oxidant properties in a manner that is independent of time and dose. This increase in the LC3 autophagy marker suggests a distinct process, indicating a partial departure from dependence on mTOR inhibition. **Conclusion:** *Cynara scolymus* extract has the potential to reduce drug resistance in SH-SY5Y cells by leveraging its antioxidant and anti-inflammatory properties, coupled with an impact on autophagy presenting it as a safe anticarcinogenic agent.

Key words: *Cynara scolymus* L., neuroblastoma, autophagy, antioxidants, neuroinflammation, cytokines

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Noncommunicable Diseases (NCDs) are diseases that are not transmitted directly from one person to another and account for more than 70% of all fatalities worldwide. Cancer is the second greatest cause of death among NCDs, trailing only cardiovascular disease[1]. Cancer incidence and mortality are quickly growing over the world as the world's population ages. Environmental pollution, chemical toxins pollution, ionising radiation, free radical toxin, microorganisms (bacteria, fungi, viruses, etc.) and their metabolic toxins, genetic features, endocrine imbalance and immunological dysfunction, among other things, are among the causes[2].

Globally, neuroblastoma (NB) is the most prevalent extracranial solid tumour in children[3]. It is formed by nerve sympathetic cells. Neuroblastoma affects 11 to 13 children under the age of 15, with rates ranging from 1 per million in children aged 10-14 years to 65 per million in children aged 1 year[3-5]. Approximately 50% of high-risk patients do not respond to first-line treatment alternatives and relapse within two years. Despite great breakthroughs in NB treatment, therapeutic resistance remains a serious obstacle to access to curative cancer medicines. As a result, uncovering the mechanisms of therapeutic resistance in NB is critical. The NB cell resistance is typically induced by a combination of genetic (mutation, amplification) and epigenetic changes (DNA hypermethylation, histone modifications), overexpression of drug efflux transporters and autophagy.

The majority of malignancies are still diagnosed and treated at very advanced stages, with poor overall survival and significant mortality rates despite progresses in early cancer detection and its therapeutic interventions. Usually radiation is being utilized to boost the efficacy of chemotherapy. However, it appears that combining chemotherapy with other anti-tumor drugs would be the most successful approach to cancer treatment. Because cancer cells can develop resistance to both radiotherapy and chemotherapy, combined chemotherapy aids in disrupting the molecular pathways that contribute to cancer resistance. As a result, chemotherapy's effectiveness is improved and the outcomes of its clinical studies are more promising. Finding the right anti-tumor medication to use in conjunction with chemotherapy is critical for poly-chemotherapy. High anti-tumor activity, multi-targeting and low toxicity are some of the most important qualities of a good anti-tumor agent. As a result, novel therapy strategies have been studied, with naturally occurring substances having established anti-carcinogenic properties showing the greatest promise[6-10].

Chemoprevention is a promising approach that blocks, inhibits, reverses or delays the carcinogenesis process using natural food ingredients and/or synthetic drugs[11-13]. Dietary benefits are attributed, at least in part, to polyphenols, which have antitumor properties in both animal models and humans[14,15].

Because of its high polyphenolic content, artichoke has received renewed attention due to the present expanding interest in dietary plants. Preclinical studies show that artichoke has active components with antiproliferative and chemopreventive actions *in vitro* and *in vivo*[16,17] and it has been postulated that artichoke active ingredients such as luteolin, apigenin, myricetin and quercetin could be used as an adjuvant drug during chemo and/or radiation[18-20].

This information initiates our curiosity to study the possible anti-inflammatory, antioxidant, cytotoxic and autophagy-promoting effects of crude extracts of *Cynara scolymus* L. (artichokes) on the neuroblastoma cancer cell line SH-SY5Y as a well-known drug resistant cancer cell.

## MATERIALS AND METHODS

**Study area:** The study was conducted at the National Research Center, Egypt from June, 2022 to August, 2022.

**Preparation of *Cynara scolymus* ethanol extract:** *Cynara scolymus* L. (CS, lot number-715) were procured in March, 2019, from the supermarket of Riyadh, Saudi Arabia. Inedible plant parts (leaves, bracts and floral stems) of globe artichoke were washed thoroughly, oven dried at 37°C and crushed to fine powder (0.260 mesh). The extraction processes and analytical techniques employed to obtain and standardize CS has been described in this study. Briefly, 1 kg of dried plant material was subjected to ultrasonic extraction with 80% (3×1L) of ethanol for 1 hr. After filtration, ethanol was evaporated using a vacuum rotary evaporator (Buchi, Rotavapor at R-100, BUCHI Labortechnik, Flawil, Switzerland) and water was removed from the frozen extract by freeze-drying to obtain a freeze-dried ethanol extract (237.49 g). The residue obtained was transferred to capped glass bottle and stored in refrigerator at 4°C prior to use.

**Total phenolic and flavonoid contents in the CS extract:** *Cynara scolymus* extract (CSE) total phenolic content was calculated as mg/g gallic acid equivalent (GAE) using sodium carbonate solution and the Folin-Ciocalteu phenol reagent[21]. This was done in accordance with the gallic acid standard curve ($y = 0.5752x-0.0059$, $R^2 = 0.9951$). A quercetin standard curve ($y = 3.2461x+0.0138$, $R^2 = 0.9941$) was used to calculate the total flavonoid concentration of CSE[22].

**Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS) analysis of constituents from the CS extract:** The phenolic components present in the ethanolic extract of CS were identified and quantified by LC-MS/MS analysis, which was earlier validated for 23 components on High Performance Liquid Chromatography (HPLC) with a diode array detector (DAD) coupled to Time-of-Flight Mass Spectrometry with an Electrospray Interface (ESI-TOF-MS) instrument. The CS extract (10.14 mg) was solubilized in methanol (10 mL) under an ultrasound-assisted sonicator. The sample solution was poured into a sample vial for testing after being filtered with a 0.22 µm membrane. Luteolin (5 mg/L), rutin (1 mg/L), apigenin (5 mg/mL) and quercetin (5 mg/mL) were applied as internal standards (IS). The LC-MS/MS analysis was performed using LC-MS 2020 system (Shimadzu) equipped with an online DGU-20A3R degasser, two LC-20ADXR pumps, an SIL-20AXR auto-sampler and a column heater (CTO-20AC) which is coupled to a SPD333 M20A diode array detector and a LCMS-2020 quadrupolar mass analyzer (Shimadzu, Japan). The AQUASIL $C_{18}$ column (150 mm×3 mm×3 µm) was used for LC separations. The temperature of the column was maintained at 40°C and 5 µL of injection volume with a 0.2 mL/min flow rate was used. A 0.1% formic acid in water (v:v) was applied as mobile phase A and 0.1% formic acid in methanol (v:v) as mobile phase B. The gradient elution conditions were established as follows: 0-10 min 20% B, 10-20 min 25% B, 20-40 min 50% B, 40-42 min 100% B, 42-47 min 20% B and 49-55 min 20% B. The UV chromatograms were recorded at 325 nm. Electrospray ionization (ESI) was applied in both positive ion ($ESI^+$) and negative ion (ESI) mode. The mass spectrometric data were recorded from 100 to 1,000 in the positive and negative ion modes under the centroid mode. The identification of peaks was conducted by comparing retention time ($t_R$), UV and mass spectra (MS) of the phenolic components with those of pure available standards as well as reported in literature.

**MTT assay:** The MTT assay was conducted using SH-SY5Y cells, a human neuroblastoma cell strain obtained from the National Cancer Institute in Cairo, Egypt. The cells were cultured, counted and seeded in a 96-well plate at a concentration of $1\times10^4$ cells per well in DMEM medium supplemented with 10% FBS. After one day of inoculation, adherent cells were cultured in serum-free DMEM and exposed to varying concentrations (50, 100, 150 and 200 µM) of rosemary essential oil for 24 and 48 hrs. The culture media were collected and held at -20°C, while the cells were treated with a 5 mg/mL solution of 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) for 4 hrs. The reaction was stopped with 100 µM L DMSO and absorbance at 570 nm was measured with an enzyme-linked immunosorbent assay reader.

**Cell viability:** In a 96-well culture plate, cells were plated at a density of $5\times10^3$ cells per well for an overnight assessment of cell viability. Proliferation was assessed using the MTT cell proliferation assay (Cell Titer 96 Non-Radioactive Cell Proliferation Assay, Promega) in accordance with the manufacturer's instructions after treatment under varied settings for 24 hrs. The Spectra Max 190 microplate spectrophotometer (Molecular Devices, Sunnyvale, California) was used to detect absorbance at 570 nm. The experiments were conducted in triplicate and the formula utilized to compute relative cell viability (represented as a percentage of control) was:

$$\text{Control (\%)} = \frac{\text{Mean OD of treated cells}}{\text{Mean OD of control cells}} \times 100$$

At each time point, treated cells were compared with control cells that had been treated with the vehicle only.

**Biochemical analysis:** Biochemical analysis included the assessment of various parameters using kits from Randox Company by the manufacturer's instructions.

**Measurement of Glutathione (GSH) level:** The GSH level was determined utilizing a kit from Randox Company, following the protocol outlined by Moron *et al.*[23].

**Measurement of malondialdehyde (MDA) level:** The MDA, serving as an indicator of lipid peroxidation, was quantified using a kit from Randox Company, as per the instructions provided by Ohkawa *et al.*[24].

**Measurement of superoxide dismutase (SOD) level:** The SOD level was assessed using a kit from Randox Company, following the manufacturer's guidelines.

**Measurement of IL-1β and IL-10 levels:** To determine the levels of IL-1β and IL-10, supernatants from the cells were assessed using ELISA kits from BD Bioscience Pharmingen in accordance with the manufacturer's protocol. The procedure involved the use of a sandwich ELISA for detecting the ability of cells to secrete IL-1β in response to cytokines.

A flat-bottom 96-well microtiter plate (Greiner Bio-One, Kempten, Germany) was coated with 100 µL/well of anti-human IL-1β monoclonal antibody (2 mg/mL in a combination of sodium carbonate and sodium bicarbonate, pH 9.5) overnight at 4°C. The plate was then rinsed with phosphate-buffered saline (PBS; pH 7.0) and 0.05% Tween 20 and then blocked with 10% foetal calf serum. The IL-1β standards (recombinant human IL-1β) were produced in a solution of PBS (pH 7.0) and 10% FCS through serial dilution. Standards or supernatants (100 µL/well) were plated in triplicate and incubated at room temperature for 2 hrs. Following three washes, 100 µL of biotinylated anti-human IL-1β monoclonal antibody (100 ng/mL in PBS, pH 7.0 and 10% FCS) was applied to each well. Next, add 100 µL of streptavidin-peroxidase conjugate to each well. The chromogen substrate was administered at 100 µL per well and after 30 min, 10% $H_2SO_4$ was added to terminate the reaction. The absorbance was measured at 450 nm with an automated microplate reader (Bio-Tek Instruments, Richmond, California, USA).

Additionally, the levels of IL-10 were determined using a specific ELISA kit, following a similar procedure.

**Measurement of TNF-α levels:** To determine the TNF-α levels, supernatants from each treatment were incubated at room temperature and analyzed using specific Enzyme-Linked Immunosorbent Assay (ELISA) kits, following the manufacturer's instructions. The results were presented as the Mean±Standard Deviation, measured in picograms of each cytokine per milliliter.

**Flow cytometry analysis for autophagy detection:** For the detection of the autophagy marker LC3, flow cytometry was performed by manufacturer's instructions using the Accuri C6 flow cytometer from Becton Dickinson. To sum up, 200 µL of protein block solution (2% BSA; Cat. No. 810652; Merck KGaA) were added to 100 µL of cell suspension and left for 20 min at room temperature. The samples were then fixed for 20 min at room temperature using 200 µL of 1% paraformaldehyde, as directed by the IntraPrep permeabilization reagent (Cat. No. GAS003; Invitrogen; Thermo Fisher Scientific, Inc.).

Following fixation, the samples were treated for 15 min at room temperature in the dark with 10 µL of primary antibody (rabbit anti-LC3A/II, 1:100; Cat. No. 4108; Cell Signalling Technology, Inc.). After two PBS/BSA washes, the cells were centrifuged for 5 min at 2000 rpm. After discarding the supernatant, the cells were exposed to 10 µL of secondary polyclonal antibody (IgG) labelled with Fluorescein Isothiocyanate (FITC) for 15 min at room temperature in the dark. Following two further PBS/BSA washes, the labelled cells were ready for flow cytometric analysis by fixing them with 200 µL = K of 0.5% paraformaldehyde for an overnight period at 37°C.

After 15 min of room temperature incubation with 10 µL of the secondary antibody alone, control cells were promptly examined using the Accuri C6 flow cytometer (Becton Dickinson, Sunnyvale, California, USA). The AccuriC6 programme was used to create flow cytometry histograms and staining values were computed as a proportion of the total number of cells counted. Three replications of each experiment was conducted.

**Statistical analysis:** The data exhibited normal distribution and for single mean comparisons, equality of variances was assessed using Levine's test, followed by a Student's t-test for independent samples to determine significance. A significance level of 0.05 was employed for all analyses. The statistical package for the social sciences release 19 (SPSS, SN:5087722) was utilized for all data analyses.

## RESULTS

The yield of *C. scolymus* extract (CSE) was found to be 23.79%. The total contents of phenolic and flavonoid were determined to be 62.43±2.56 mg GAE/g and 47.51±2.10 mg RE/g, respectively. The phytoconstituents were identified by the data provided by the LC-MS/MS instrument. The response values in the negative and positive ion mode were observed different for different phyto-constituents. Based on LC-MS/MS chromatogram of CS ethanol extract, significant segregation was achieved in 30 min. Thus, for all the detected peaks in the chromatogram, a probable list of molecular formulas was obtained from inbuilt data analysis software. The identification was accomplished by comparing the retention time (tR), accurate quality and fragmentation patterns. A total 28 chemical components were confirmed from the ethanol extract of CS and their results were summarized in Table 1. The symbol "▲" in Table 1 designates the compositions among them that have been confirmed by comparison with chemical reference standards and the results were presented in Fig. 1. As 28 components including 1-O-caffeoylquinic acid, 3-caffeoylquinic acid, 4-O-caffeoylquinic acid, chlorogenic acid, caffeic acid, rosmarinic acid, cynarin isomer 1, cynarin isomer 2 and 1,5-dicaffeoylquinic acid were phenolic compounds identified in CS extract. Whereas, 13 flavonoids and its several sub-classes (flavones and their derivatives) were identified in the extract of CS.

Table 1: Characterization and quantitative screening of phenolic, flavonoid and other polar chemical components in ethanol extract of *Cynara scolymus* by LC-MS/MS

| Peak No | tR (min) | Molecular formula | Fragment ion (mz) | ppm | Identity | Class |
|---|---|---|---|---|---|---|
| 1 | 4.23 | $C_{16}H_{18}O_9$ | 353, 191, 179 | -0.9 | 3-caffeoylquinic acid | Phenolic acid |
| 2 | 6.21 | $C_{16}H_{18}O_9$ | 353, 191, 179, 135 | -0.7 | 1-O-caffeoylquinic acid | Phenolic acid |
| 3 | 6.18 | $C_{16}H_{18}O_9$ | 191, 179, 135 | -1.2 | Chlorogenic acid | Polyphenol |
| 4 | 6.28 | $C_9H_8O_4$ | 135, 163, 89 | 1.7 | Caffeic acid | Phenol |
| 5 | 6.47 | $C_{25}H_{24}O_{12}$ | 353, 335, 191, 179, 135 | -2.5 | Dicaffeoylquinic acids | Phenolic acid |
| 6 | 6.59 | $C_{25}H_{12}O_4$ | - | -4.3 | unknown | - |
| 7 | 6.89 | $C_{18}H_{16}O_8$ | 359, 179, 197 | 7.4 | Rosmarinic acid | Polyphenol |
| 8 | 7.41 | $C_{25}H_{24}O_{12}$ | 515, 497, 335, 317, 229 | -3.4 | Cynarin isomer 1 | Polyphenol |
| 9 | 7.69 | $C_{27}H_{30}O_{15}$ | 593, 473, 431, 383, 311 | -0.1 | Luteolin 7-O-rutinoside | Flavonoid glycoside |
| 10 | 7.75 | $C_{21}H_{18}O_{12}$ | 447, 327, 285, 241, 201 | -2.0 | Luteolin 7-O-glucuronide | Flavonoid glycoside |
| 11 | 7.89 | $C_{16}H_{18}O$ | 353, 191, 173, 135 | -1.1 | 4-O-caffeoylquinic acid | Phenolic acid |
| 12 | 7.97 | $C_{21}H_{20}O_{11}$ | 447, 327, 285, 241 | -2.1 | Cynaroside | Flavonoid glycoside |
| 13 | 8.14 | $C_{25}H_{24}O_{12}$ | 515, 497, 317, 229, 225 | -2.0 | Cynarin isomer 2 | Polyphenol |
| 14 | 8.36 | $C_{25}H_{24}O_{12}$ | 353, 335, 191, 179, 135 | -3.5 | 1, 5-dicaffeoylquinic acid | Polyphenol |
| 15 | 8.62 | $C_{27}H_{30}O_{14}$ | 577, 308, 269, 225 | 2.4 | Isorhoifolin | Flavonoid glycoside |
| 16 | 9.17 | $C_{21}H_{18}O_{11}$ | 431, 341, 311, 283 | -1.3 | Apigenin 7-O-glucuronide | Flavonoid glycoside |
| 17 | 9.93 | $C_{21}H_{20}O_{10}$ | 431, 413, 341, 311 | -2.1 | Apigenin-glucoside | Flavonoid glycoside |
| 18 | 10.12 | $C_{20}H_{10}O_6$ | 345.0263 | 7.0 | Unknown | - |
| 19 | 11.74 | $C_{11}H_{12}O_4$ | 207.0546 | 3.2 | Unknown | - |
| 20▲ | 14.21 | $C_{15}H_{10}O_6$ | 285, 241, 217, 199, 175 | -1.4 | Luteolin | Flavonoid |
| 21▲ | 15.84 | $C_{15}H_{10}O_8$ | 319, 291, 219, 74 | 1.6 | Myricetin | Flavonoid |
| 22▲ | 16.12 | $C_{15}H_{10}O_7$ | 302, 275 245, 203, 175 | 3.38 | Quercetin | Flavonoid |
| 23▲ | 16.56 | $C_{15}H_{10}O_5$ | 269, 225, 197, 181 | -5.7 | Apigenin | Flavonoid |
| 24 | 21.32 | $C_{18}H_{34}O_5$ | 329, 293, 229, 211, 171 | -1.7 | Trihydroxy-octadecenoic acid | Fatty acid |
| 25 | 22.68 | $C_{16}H_{32}O_4$ | 287, 269, 85 | 1.0 | 10, 16-Dihydroxyhexadecanoic acid | Fatty acid |
| 26 | 24.15 | $C_{16}H_{12}O_5$ | 285 | 1.4 | Methylapigenin | Flavonoid |
| 27 | 26.57 | $C_{15}H_{10}O_6$ | 287, 259, 245, 165, 127 | 2.1 | Kaempferol | Flavonoid |
| 28▲ | 27.09 | $C_{27}H_{30}O_{16}$ | 300, 271, 255, 243 | -3.5 | Rutin | Flavonoid glycoside |

Table 2 indicated the significant increase of the TNF-α, IL-10, IL-6 and IL-1B in SH-SY5Y NB cell line compared to normal cells. Moreover, demonstrate the anti-inflammatory and antioxidant properties of crude extracts of artichoke on the SH-SY5Y NB cell line in comparison to normal cells. When tested on the SH-SY5Y NB cell line, artichoke extract showed dose-independent anti-inflammatory and antioxidant properties. The levels of TNF-α, IL-10, IL-6 and IL-1B in treated cells are notably lower than those in untreated cells. Artichoke extract has anti-oxidant properties in a manner that is independent of time and dose. Figure 2 depicted the cell viability of SH-SY5Y neuroblastoma cell line when exposed to *Cynara scolymus* L., extract, contrasted with normal cells under treatment. Figure 3 illustrated the variance in autophagic activity levels between control cells and those treated with *C. scolymus* L. extract.

## DISCUSSION

For the components obtained from the extraction processes, researchers working with artichoke extracts for cancer treatment usually use gas chromatography/mass spectrometry, GC/MS as accurate analytical tool. This analytical process, without a doubt, will provide information on the components present in the extract and their associated yields, allowing for simpler interpretation of the anticancer results and correct identification of the mechanisms underlying their effects on cancer cell lines. The chemical components in ethanol extract of *C. scolymus* by LC-MS/MS (Table 1) revealed that ethanol extract has significant chemical compounds belonging to the classes of phenolic and flavonoids comparable to those of other studies by Palermo *et al.*[25]. These active constituents' phytoconstituents have shown promising biological activities and holds considerable potential to explain the diverse biological properties of the various extracts of *C. scolymus*. The potency of their therapeutic effects was correlated with the concentration of phenolic and flavonoids in the plant extract (Table 1, Fig. 1).

It is important to thoroughly evaluate the inflammatory mediators linked to cancer that might be used in anticancer therapy. It's worth noting that inflammation has both pro and anti-tumor properties. As part of the immune response, inflammation can activate immune cells and cause the release of inflammatory substances such as cytokines and enzymes that slow tumour growth. Several biotherapies have been developed to enhance these antitumor effects with the goal of curing cancer. In contrast, inflammation may play a role in cancer genesis, progression and metastasis[26].

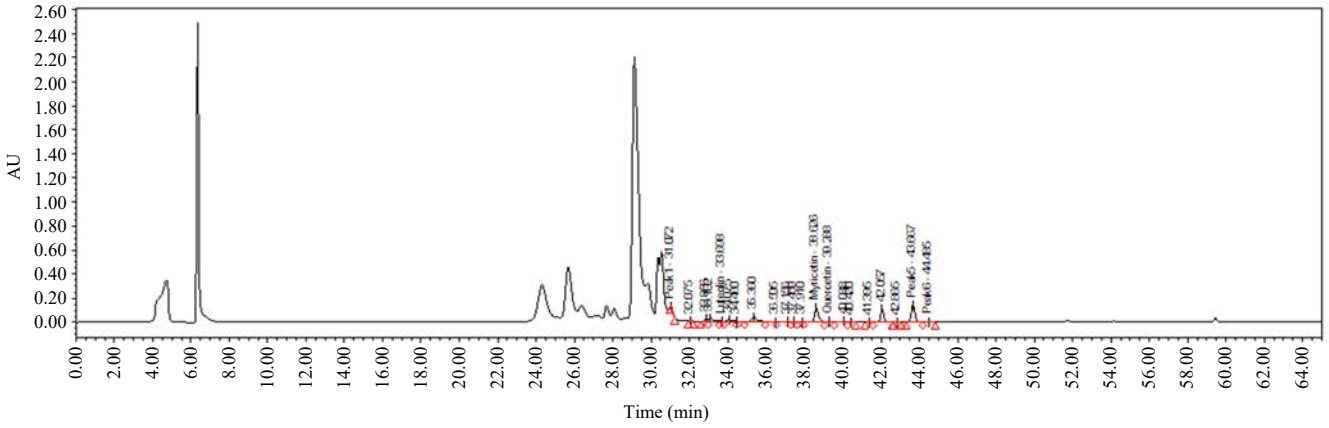



Fig. 1: Quantitative screening chromatogram of phenolic and flavonoids in the ethanol extract of the CS by LC-MS/MS

Table 2: Cells treated with different concentration of artichoke choke extract for 24 and 48 hrs

| | | Concentration of plant extract | | | | |
|---|---|---|---|---|---|---|
| Parameter | Groups | 200 mM | 150 mM | 100 mM | 50 mM | 0 mM |
| IL-1-b | Normal 24 hrs | 1.26±0.16 | 1.17±0.62 | 1.34±0.46 | 1.52±0.16$^{e}$ | 0.81±0.001$^{d}$ |
| | Cancer 24 hrs | 66.82±49.95$^{e}$ | 96.16±2.64$^{e}$ | 96.19±0.06$^{e}$ | 98.29±2.20$^{e}$ | 277.61±1.27$^{abcd}$ |
| | Normal 48 hrs | 1.17±0.16 | 1.26±0.41 | 1.17±0.41 | 1.45±0.17$^{e}$ | 0.81±0.001$^{d}$ |
| | Cancer 48 hrs | 97.16±1.73$^{e}$ | 96.82±1.14$^{e}$ | 97.53±1.19$^{e}$ | 96.58±1.51$^{e}$ | 277.61±1.27$^{abcd}$ |
| IL-10 | Normal 24 hrs | 54.48±1.52$^{e}$ | 57.746±0.341$^{e}$ | 57.52±1.17$^{e}$ | 54.47±3.87$^{e}$ | 28.77±0.0001$^{abcd}$ |
| | Cancer 24 hrs | 253.16±0.001$^{de}$ | 252.93±0.54$^{cde}$ | 253.83±0.53$^{bde}$ | 246.16±0.002$^{abce}$ | 312.13±0.48$^{abcd}$ |
| | Normal 48 hrs | 48.37±0.16$^{bcde}$ | 56.21±3.50$^{ae}$ | 55.63±3.10$^{ae}$ | 54.83±2.89$^{ae}$ | 28.77±0.0001$^{abcd}$ |
| | Cancer 48 hrs | 250.64±0.53$^{bcde}$ | 253.17±1.01$^{acde}$ | 251.80±0.55$^{abde}$ | 247.33±0.28$^{abce}$ | 312.13±0.48$^{abcd}$ |
| IL-6 | Normal 24 hrs | 42.45±0.34$^{e}$ | 42.179±0.972$^{e}$ | 42.53±0.59$^{e}$ | 42.58±0.64$^{e}$ | 39.19±0.04$^{abcd}$ |
| | Cancer 24 hrs | 242.26±0.22$^{de}$ | 242.57±0.43$^{de}$ | 242.29±0.08$^{de}$ | 243.10±0.06$^{abce}$ | 315.13±0.06$^{abcd}$ |
| | Normal 48 hrs | 42.71±0.57$^{e}$ | 42.63±0.50$^{e}$ | 43.28±0.90$^{de}$ | 42.02±0.95$^{ce}$ | 39.19±0.04$^{abcd}$ |
| | Cancer 48 hrs | 243.09±0.70$^{e}$ | 242.65±0.58$^{e}$ | 243.37±0.95$^{e}$ | 242.316±0.00$^{e}$ | 315.13±0.06$^{abcd}$ |
| TNF-α | Normal 24 hrs | 23.49±0.58 | 22.88±1.60 | 22.90±0.64 | 23.11±0.08 | 24.16±0.01 |
| | Cancer 24 hrs | 142.13±0.50$^{e}$ | 142.95±0.58$^{e}$ | 142.16±0.50$^{e}$ | 142.13±0.50$^{e}$ | 242.28±0.24$^{abcd}$ |
| | Normal 48 hrs | 23.88±0.62$^{bcd}$ | 23.17±0.001$^{acde}$ | 21.23±0.08$^{abde}$ | 22.28±0.10$^{abce}$ | 24.16±0.01$^{bcd}$ |
| | Cancer 48 hrs | 142.47±1.03$^{e}$ | 143.47±0.78$^{ce}$ | 141.66±0.83$^{be}$ | 142.44±1.07$^{e}$ | 242.28±0.24$^{abcd}$ |
| NO | Normal 24 hrs | 61.31±0.26$^{bd}$ | 62.31±0.57$^{a}$ | 61.61±0.51 | 62.46±0.30$^{a}$ | 61.98±0.74 |
| | Cancer 24 hrs | 82.49±0.58$^{e}$ | 82.53±1.18$^{e}$ | 82.48±0.27$^{e}$ | 82.18±0.04$^{e}$ | 100.79±0.77$^{abcd}$ |
| | Normal 48 hrs | 62.44±0.33 | 61.77±0.26 | 61.81±0.31 | 62.13±0.50 | 61.98±0.74 |
| | Cancer 48 hrs | 83.16±0.05$^{de}$ | 82.67±0.53$^{e}$ | 82.10±0.81$^{e}$ | 81.84±0.56$^{ae}$ | 100.79±0.77$^{abcd}$ |
| MDA | Normal 24 hrs | 61.81±0.76$^{e}$ | 61.95±0.58$^{e}$ | 61.53±0.18$^{e}$ | 61.96±0.60$^{e}$ | 63.15±0.50$^{abcd}$ |
| | Cancer 24 hrs | 82.16±0.86$^{e}$ | 81.97±0.56$^{e}$ | 81.24±0.08$^{e}$ | 81.54±0.19$^{e}$ | 100.10±0.92$^{abcd}$ |
| | Normal 48 hrs | 61.96±0.60$^{e}$ | 62.13±0.88 | 61.99±0.58$^{e}$ | 62.13±0.48 | 63.15±0.50$^{ac}$ |
| | Cancer 48 hrs | 82.13±0.84$^{e}$ | 82.63±0.02$^{e}$ | 81.97±0.59$^{e}$ | 81.54±0.20$^{e}$ | 100.10±0.92$^{abcd}$ |
| $H_2O_2$ | Normal 24 hrs | 1.44±0.16$^{e}$ | 1.61±0.06$^{de}$ | 1.53±0.11$^{e}$ | 1.26±0.16$^{b}$ | 0.99±0.31$^{abc}$ |
| | Cancer 24 hrs | 3.49±0.28$^{e}$ | 3.49±0.28$^{e}$ | 3.44±0.26$^{e}$ | 3.65±0.002$^{e}$ | 5.32±0.29$^{abcd}$ |
| | Normal 48 hrs | 1.36±0.47 | 1.37±0.16 | 0.99±0.31 | 1.41±0.29 | 0.99±0.31 |
| | Cancer 48 hrs | 3.65±0.002$^{e}$ | 3.33±0.28$^{e}$ | 3.55±0.09$^{e}$ | 3.65±0.002$^{e}$ | 5.32±0.29$^{abcd}$ |
| ROS | Normal 24 hrs | 55.77±0.36$^{cde}$ | 51.79±5.36$^{cd}$ | 46.82±1.02$^{ab}$ | 46.83±0.76$^{ab}$ | 47.48±0.27$^{a}$ |
| | Cancer 24 hrs | 141.83±0.57$^{e}$ | 142.55±0.67$^{ce}$ | 141.55±0.54$^{be}$ | 142.23±0.11$^{e}$ | 190.49±0.58$^{abd}$ |
| | Normal 48 hrs | 57.54±1.19 $^{bcde}$ | 54.78±0.78$^{acde}$ | 45.32±0.28$^{abe}$ | 45.83±0.58$^{abe}$ | 47.48±0.27$^{abcd}$ |
| | Cancer 48 hrs | 141.68±0.61$^{e}$ | 142.18±0.96$^{e}$ | 141.94±0.70$^{e}$ | 142.28±0.10$^{e}$ | 190.49±0.58$^{abcd}$ |

p-value between the different groups for each parameter using Kruskal-Wallis test with Mann-Whitney test as multiple comparisons to compare between each two groups., $^{a}$There is Sig. difference with (200 mM) group for each group type (Normal 24 hrs, Cancer...) for each parameter, $^{b}$There is Sig. difference with (150 mM) group for each group type (Normal 24 hrs, Cancer...) for each parameter, $^{c}$There is Sig. difference with (100 mM) group for each group type (Normal 24 hrs, Cancer...) for each parameter, $^{d}$There is Sig. difference with (50 mM) group for each group type (Normal 24 hrs, Cancer...) for each parameter and $^{e}$There is Sig. difference with (0 mM) group for each group type (Normal 24 hrs, Cancer...) for each parameter

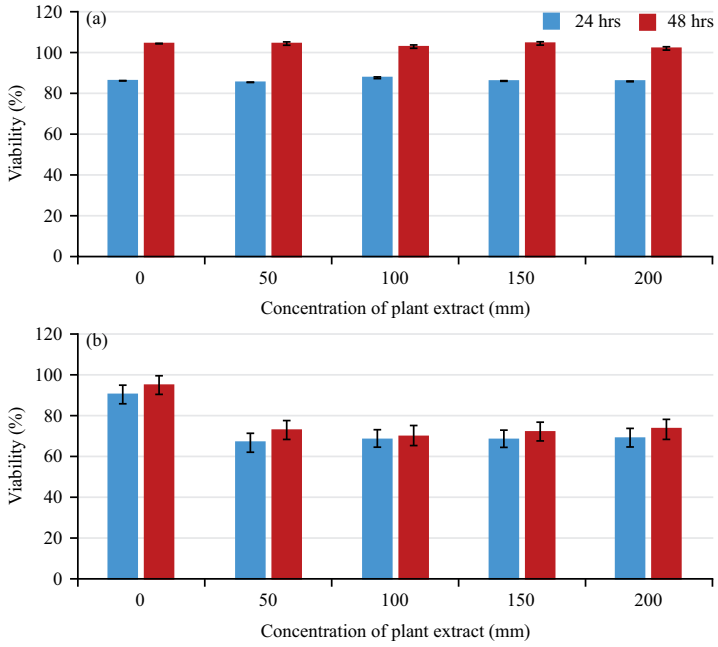
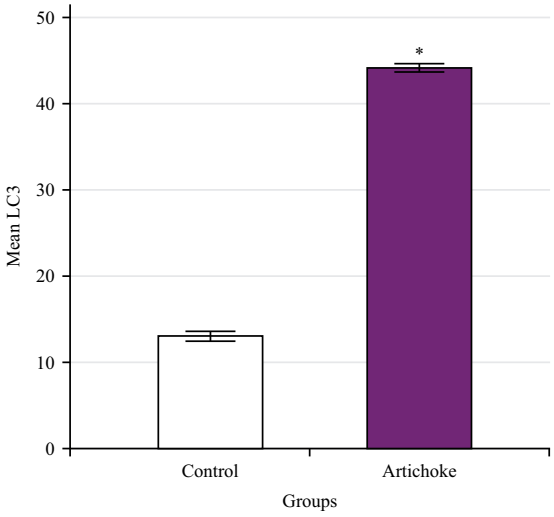



Fig. 2(a-b): Cell viability of *Cynara scolymus* L. extract against SH-SY5Y neuroblastoma cell line compared to treated normal cells, (a) Normal cells and (b) Cancer cells

| Groups | LC3 Mean±SD |
|---|---|
| Control | 12.96±0.55 |
| Artichoke-treated | 44.13±0.45* |

*Significantly different from control (p<0.0001)



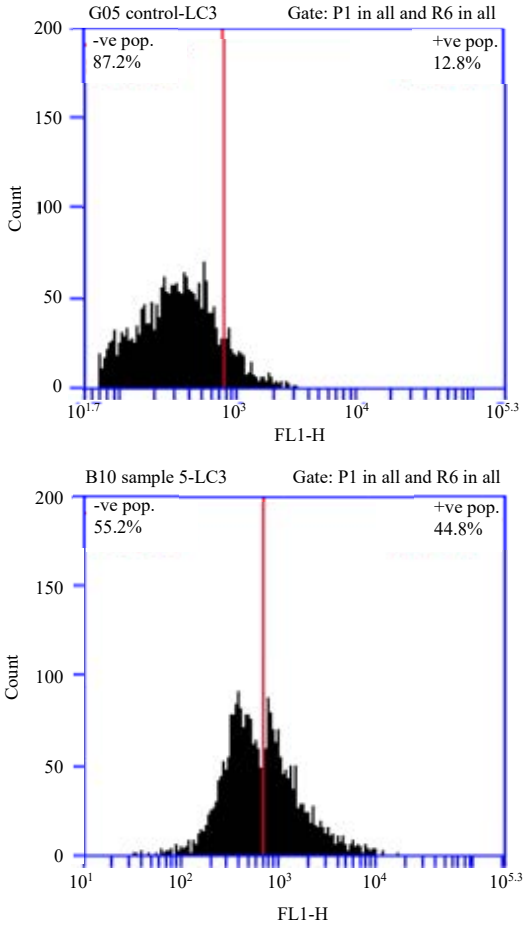




Fig. 3: Level of autophagic activity between control and *C. scolymus* L. extract treated cells

Cytokines released by cancer cells or cells in the tumour microenvironment stimulate angiogenesis, tumour cell migration and metastasis and the establishment of an immunocompromised environment[27]. Cytokines' tumor-promoting actions also apply to NB cell line. It is interesting to know that cytokines among which is IL-6, TNF-α and IL-1B are involved in the pathogenesis of NB cell lines and that elevated levels of IL-6 correlated with features of high-risk NB and poor prognosis. Because of its pleiotropic NB-promoting activities, IL-6 may be a promising therapeutic target[28-30]. Table 2 showed that artichoke crude extracts induce a significant but time and dose independent lowering effects on the four measured cytokines.

Oxidative stress is one of the most important factors in cancer development and a well-studied event that contributes to tumour growth and progression[31]. Continuous inflammation has been found to cause a preneoplastic state. Chronically inflamed cells secrete more reactive oxygen/nitrogen species, which recruit more activated immune cells to overcome the endogenous antioxidant response. This results in the amplification of dysregulated processes and irreversible oxidative damage to nucleic acids, proteins and lipids, which can cause genetic and epigenetic changes. This causes oncogene and tumour suppressor gene deregulation, which drives the beginning of carcinogenesis[32].

Tables 2 demonstrated the significant increase of NO, MDA, $H_2O_2$ and ROS as biomarkers of oxidative stress in untreated NB SH-SY5Y cell line compared to normal cells. This can find support in multiple studies which proved that cancer cells have higher levels of ROS than normal cells, which is attributable to factors such as oncogene mutations and mitochondrial damage as well as their metabolism[33]. Table 2 also demonstrated the significant but dose and time independent anti-oxidant effects of artichoke extract. This was clearly seen as significantly lower NO, MDA, $H_2O_2$ and ROS compared to normal un-treated NB SH-SY5Y cells. This can find support in the previous work of Yuan *et al.*[34], which proved the free radical scavenging and antioxidant effect of ethanolic extract of artichoke leaves.

It is noteworthy to correlate the remarkable decrease of NO to the presence of the flavonoids in the artichoke ethanolic extract (cynarin-isomers 1 and 2, cynaroside and luteolin) (Table 1). These compounds with cynarin being the most potent led to the downregulation of Inducible Nitric Oxide Synthase (iNOS) mRNA and protein expression which might explain the remarkable decrease of NO in artichoke- treated NB SH-SY5Y cell line[35].

The anti-proliferative effect of artichoke-ethanolic extract against the NB SH-SY5Y cell line, as reported (Fig. 2), was consistent with several studies that show dose-dependent reductions in cell viability effects of the MSTO-211H, HL-60 and L1210 leukaemia cell lines[36], T-47D breast cancer cell line[37] and MPP-89 and NCI-H28 mesothelioma cell lines. Both caffeoylquinic and chlorogenic acids, when applied at concentrations between 400 and 1200 μM for 24, 48 and 72 hrs, have a strong cytotoxic effect on HepG2 cell lines, which explains the extract's observed anti-proliferative activity. The reduction in cell viability was dose and time-dependent. In an attempt to explain the dose and time independent response of NB SH-SY5Y cell line to artichoke ethanolic extract, we can suggest that medication responses vary throughout cancer cell types originating from the Central Nervous System (CNS). Multidrug resistance (MDR) is an inherent feature of many brain tumors and may have a role in disease progression or relapses[38]. Drug resistance is frequently the cause of therapeutic failure for extracranial solid tumours in children, as is widely known[39]. Multiple biological processes contribute to neuroblastoma cell line resistance, including enhanced drug efflux via ATP-binding cassette transporter expression and cancer cells' incapacity to initiate an apoptotic response. Furthermore, the production of sphingolipid species like ceramide in a neuroblastoma cell line plays a significant part in drug resistance[40].

Autophagy is essential for the destruction of damaged organelles and old proteins, as well as the preservation of cellular homeostasis[41,42]. In the field of cancer biology, autophagy is involved in both the promotion and repression of tumors as well as the formation and proliferation of cancer cells[43]. As a result, autophagy-regulated drugs may have a role in cancer cell survival or death[44,45]. Moreover, autophagy is essential for a number of cancer processes, such as treatment resistance, metastasis, carcinogenesis and interactions with the microenvironment[41,45]. According to certain research, autophagy inhibits the growth of tumours and animals lacking certain autophagy effectors exhibit an increase in spontaneous tumour growth. Another consequence of prolonged autophagy is "autophagic cell death", also known as "type II programmed cell death"[42].

Given that several medications that target autophagy has demonstrated significant promise in decreasing the viability and proliferation of cancer cells, it would seem that autophagy is a viable target in cancer therapy. The considerable increase of autophagy in the artichoke-treated NB SH-SY5Y cell line seen in Table 3 and Fig. 3, as well as the remarkable decrease in NB cell viability (Fig. 2), could demonstrate the autophagy promoting anticancer effects of artichoke crude extract. This is supported by Yang *et al.*[46], who claimed that cynaropicrin (CYN), a natural chemical derived from

artichoke, is increasingly being investigated as a possible cancer treatment drug. The CYN dramatically increased autophagy by increasing autophagy initiation and reducing autophagosome-lysosome fusion. They reported that CYN, a component of artichoke, decreased NB cell growth *in vitro* and *in vivo* and that the process included endoplasmic reticulum stress/autophagy/Nrf2 signaling/apoptosis. The significant increase of autophagy in artichoke-treated NB SH-SY5Y cell line (Fig. 3) could be also attributed to Luteolin as an important flavonoid in artichoke. It was proposed that inhibiting autophagy slowed down luteolin's anti-tumor activity[47]. As a result, autophagy may operate as an anti-tumor mechanism and artichoke through autophagy-promoting effect, reduces the viability and proliferation of cancer cells.

## CONCLUSION

Artichoke is a medicinal plant that may be a safe anti-carcinogenic agent. It contains active compounds (such as polyphenols and flavonoids) with anticancer properties. Ethanolic extract exerts its effect through promoting autophagy, cytokines' down-regulating action, antioxidants, cytotoxic effects and suppressive effects on the growth of NB SH-SY5Y, as drug-resistant cells. Finally, since the field of cancer treatment is still relatively innovative and expanding quickly, more research is probably going to be done soon, especially with regard to methods for dissecting, evaluating and administering the components of artichoke extract to treat different kinds of cancer.

## SIGNIFICANCE STATEMENT

This work reveals neuroinflammation and oxidative stress as key targets for drug resistance in neuroblastoma cell lines, paving the way for better treatment options. In this work, artichoke crude extract showed dose-independent anti-inflammatory and antioxidant effects on the SH-SY5Y neuroblastoma cell line. Artichoke use may benefit survivors of high-risk neuroblastoma who require ongoing multidisciplinary follow-up and reduce the long-term morbidity that often accompany cure with current therapy.

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