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# ARTICHOKE (*CYNARA SCOLYMUS* L.) EXTRACTS ARE SHOWING CONCENTRATION-DEPENDENT HORMETIC AND CYTOTOXIC EFFECTS ON BREAST CANCER CELL LINES

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**ABSTRACT:** Chemomapping of artichoke (*Cynara scolymus* L.) leaf extracts revealed novel bioactive compounds with putative anti-tumor effects, and therefore, we set to analyze the cytotoxic effects of aqueous and alcoholic extracts on T-47D and ZR-75-1 human breast cancer cell lines. A concentration dependent hormetic response was detected in the case of aqueous extract, while the alcoholic extract featured a pronounced cytotoxic effect as inferred by the MTT proliferation assays. Immunocytochemical analyses of treated cells were suggesting that the observed cytotoxicity could be associated with cellular senescence and/or apoptosis. Our experimental data are suggesting that the artichoke could be a good candidate for the complementary treatment of certain human cancers but further studies are needed to shed light on all aspects related to its hormetic dose response and cytotoxic effect not talking about the putative hepatoprotective effect in relation to cancer therapy efficiency.

**Keywords:** artichoke, *Cynara scolymus L.*, human breast cancer, hormesis, cytotoxicity

## INTRODUCTION:

The artichoke - *Cynara scolymus* L. - is emerging like a promising medicinal plant as its extracts are featuring a high degree of consistency regarding the generated effects across experimental and clinical trials (for review see Ben Salem *et al.*, 2015). Among health-promoting properties there were identified and thoroughly characterized the antioxidant, cardiovascular-and hepato-protective, choleretic, postprandial hypoglycemic, hypocholesterolemic, hypotriglyceridaemic and prebiotic effects.

Several antioxidant bioactive compounds had been identified in globe artichoke, and their antioxidant activity were confirmed in multiple pre-clinical studies (Betancor-Fernandez *et al.*, 2003; Wang *et al.*, 2003; Llorach, 2002; Fintelmann, 1996a; Adzet *et al.*, 1987). Further studies were indicating an antioxidant-based hepatoprotective effect based on rat hepatocytes cultured endothelial cells (Gebhardt and Fausel, 1997; Gebhardt, 1995; Adzet *et al.*, 1987), monocytes (Zapolska-Downar *et al.*, 2002), and an *in vivo* rat model (Magielse *et al.*, 2014; Jimenez-Escrig *et al.*, 2003). Hepatoprotective effect of artichoke was associated with its polyphenolic content that could counteract lipid peroxidation, occurring at the membraneous structures of rat hepatocytes (Gebhardt, 1995), and also by facilitating bile production assisted fat digestion (Kraft, 1997) together with toxin removal (Fintelmann, 1996b).The antioxidants of artichoke were also suggested to inhibit the oxidation of LDL (Brown and Rice-Evans, 1998).

The cardiovascular protective effect of artichoke was proposed to be associated partly with the up regulation of endothelial nitric oxide synthase (eNOS) gene expression leading to an increased nitric oxide production and vasodilator activity as inferred from cultured human umbilical vein endothelial cells (Li *et al.*, 2004; Lupattelli *et al.*, 2004). Moreover, the hypocholesterolemic effect of artichoke might bring a substantial contribution to cardiovascular protection. Initially, the cynarin, a polyphenol component of artichoke extracts, was proposed to reduce serum cholesterol and triglyceride levels based on i*n vitro* and animal studies (Lietti, 1997; Wojcicki, 1978). However, the luteolin, a flavonoid from artichoke, was found to be responsible for inhibition of cholesterol synthesis, while cynarin had no effect on cholesterol synthesis (Gebhardt, 1996). Other white rat based research was showing that artichoke extracts may prevent the development of atherosclerotic plaques (Samochowiec, 1962). The antiatherosclerotic action was suggested to be the result of an antioxidant effect that reduces LDL oxidation (Brown and Rice-Evans, 1998), and the inhibition of cholesterol synthesis (Wegener, 1995). More recently, both caffeoylquinic acids and luteolin glucosides were proposed to be responsible for the antiatherogenic effects of artichoke (Wang *et al.*, 2003). Other observations showed that the artichoke exerted positive effects on serum lipid profile together with blood glucose levels. Moreover, artichoke leaf extract had been proven to increase the anti-inflammatory response in non-alcoholic

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steatohepatitis induced rats (Safaa *et al*., 2013) and non-alcoholic fatty liver patients (Rangboo *et al*., 2016).

The anti-tumoral effects of artichoke extracts have also been analyzed, and it was observed that antioxidants such us rutin, quercetin and gallic acid were able to reduce viability of leukemia cells by inducing apoptosis (Nadova *et al*., 2008). Experimental conditions when MDA-MB231 human breast cancer cells were incubated with the edible part extract of artichoke at high concentration and for shorter time interval, made the apoptotic program activated and tumor progression arrested (Mileo *et al*., 2012). However, at lower concentration of extract exposure and longer incubation intervals, the brest cancer cell growth had been inhibited via senescence through epigenetic and ROS-mediated mechanisms (Mileo *et al*., 2015). Polyphenolic extracts made from the edible part of artichoke reduced cellular viability in the case of human liver cancer HepG2 inducing apoptosis in a dose-dependent manner (Miccadei *et al*., 2008). Wild and cultivated artichoke species vegetative parts were used to make extracts, and subsequently test them, using DLD1 colorectal cancer cells (Simsek and Uysal, 2013). Such treated cells entered apoptosis having their DNA fragmented, while the induction of pro-apoptotic BAX gene and inhibition of anti-apoptotic BCL-2 gene expressions were observable. Cultured malignant pleural mesothelioma cells showed pronounced cytotoxicity when treated with artichoke leaf extract, and the identified misregulated pathways were related to cancer initiation and progression (Sharma *et al*., 2016). In order to assign the observed effects to bioactive compounds, an *in silico* target prediction has revealed 47 compounds in relation to 93 genes that are involved in cancer progression, survival and resistance, including activation of apoptosis and restraining invasion (Sharma *et al*., 2016; Pulito *et al*., 2015).

In a previous study using UHPLC-ESI-MS technique, we have carried out the qualitative chemomapping of aqueous and alcoholic artichoke leaf extracts, and could identify 48 and 50 bioactive compounds, respectively (Vígh *et al*., unpublished results). Individual assessment of the newly found bioactive compounds in artichoke extracts were indicating anti-tumoral properties in the case of 7-methoxy-4-methylcoumarin, diosmetin, salvigenin, naringin and ursolic acid. Moreover, our studies have confirmed the presence of rutin, quercetin and vitamin B6 in artichoke leaf extracts, and these are bioactive compounds whose anti-tumoral effects have been reported in earlier studies (Mocellin *et al*., 2016; Orfali *et al*., 2016; de Araújo *et al*., 2013). Some published papers associated the artichoke specific anti-tumoral effect with its antioxidant properties, and the later was considered to be related to the polyphenol and flavonoid content of the obtained extracts (Miccadei *et al*., 2008; Nadova *et al*., 2008). It is also interesting to notice that there are significant differences between the bioactive compound profiles of our artichoke leaf extracts, so that most strikingly, very few polyphenols were detected in alcoholic extracts as compared to the aqueous, while in the case of flavonoids did exist only few differences between the two artichoke leaf extracts. The above mentioned differences prompted us to assess the putative cytotoxicity of aqueous and alcoholic artichoke leaf extracts on T-47D and ZR-75-1 human breast cancer cell lines. We were able to detect a concentration-dependent hormetic response for the aqueous extract, while the alcoholic extract showed marked cytotoxicity as inferred by the MTT proliferation assays performed at different time intervals on cancerous cell lines. Immunocytochemical analyses of treated cells were suggesting that the observed cytotoxicity could be associated with cellular senescence and/or apoptosis.

## MATERIALS AND METHODS:

*Preparation of plant extract*

Dried artichoke leaves were obtained from TTDR 2000 Ltd., Hungary. 10 g of dried artichoke leaves were extracted within 200 ml boiling distilled water for 5 min. The obtained primary extract was allowed to cool down at room temperature, then filtered and stored in refrigerator. Such an extract was named „aqueous artichoke extract" (AqA). To obtain the alcoholic extract 50 g of dried artichoke leaves were extracted twice with 500 ml 50% ethanol solution by stirring for 4 hours on a magnetic stirrer at room temperature. As a result, primary alcoholic extracts were obtained that later were mixed together, filtered then evaporated under vacuum and stored in refrigerator. Such an extract was named „alcoholic artichoke extract" (AlA).

*Cell culture*

Human breast cancer cell lines T-47D and ZR-75-1 were obtained from ATCC (American Type Culture Colection, USA). These ductal carcinoma cell lines were grown in RPMI-1640 (Lonza) culture medium supplemented with 1% Antibiotic Antimycotic Solution (Invitrogen-Gibco), 10% heat-inactivated FBS (Fetal Bovine Serum; Sigma) and 0,1% 1 mM Na-pyruvate (Biochromag). All cell culture experiments were carried out at 37°C in 5% $CO_2$ incubator.

*MTT (cytotoxicity) assay*

Cells were used in this studies when 80% confluence was reached in T75 flasks (Greiner Bio-One GmbH). Cells were washed twice with sterile PBS, and harvested with Trypsin in the incubator. For counting the cells we used Trypan blue dye (Sigma) exclusion method and counted with Bürker chamber. After it the cells were seeded into 96-well plates at a density of $10^4$ cells/well and left to attach to the plates. After 24 hours cells were incubated for two and three days with various concentrations (10-5-2.5-1.25-0.62-0.31-0.16-0.08-0.04 v/v%) of extracts. After the exposure time removed the extracts and the cells were incubated with ten microliter of MTT (5 mg/ml) (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) (Sigma) at 37°C for three hours. After dissolving the formazan crystals in MTT solubilization solution, plates were read in a microplate reader (BioTek EL808) at 570 nm. This experiment was performed in sextuplicates and repeated three times.


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*Immunostaining and microscopy*

The treatments with both artichoke extracts were carried out in six-well plates and at the examined concentration range. At the bottom of each well, a coverslip was laid and cells were let to grow on the surface of coverslip. After 2- and 3–day treatments the cells were fixed with formaldehyde and stained as described by Mathe *et al*. (2004) and Lemos *et al*. (2000). The microtubules were detected with YL1/2 rat monoclonal anti-α-tubulin antibody (Sera Lab, Inc.), while the DNA was counterstained with the DAPI dye (Molecular Probes). Digital images of optical sections were collected with an Olympus CellR fluorescent microscope.

*Statistical analysis*

Statistical analysis were performed by the SPSS 16.0 software. Statistical differences among treated and untreated cells were determined by one-way ANOVA (Analysis of Variance). To compare several groups, Tukey post-hoc test was applied and mean differences with $p< 0.05$ were considered statistically significant.

## RESULTS AND DISCUSSION:

In order to gain more information about the possible effects exerted by the artichoke extracts on human cancerous breast cells, we set to analyze T-47D and ZR-75-1 cell lines. The T-47D is classified like a luminal A subtype of breast cancer cell line that is endocrine (oestrogen receptor expression) and often chemotherapy responsive (Holliday and Speirs, 2011). The ZR-75-1 is luminal B subtype of breast cancer cell line being usually endocrine responsive and variable to chemotherapy. The luminal A and B subtypes are amenable to hormone therapy. Interestingly, genomic differences were detected between certain breast cancerous cell lines, like the T-47D cells are having the mutant allele of p53 gene, while ZR-75-1 cells are bearing the normal p53 allele (Huovinen *et al*., 2011). It had been also demonstrated that ZR-75-1 cells are containing the normal BRCA1/2 allele (Gilardini Montani *et al*., 2013). Surprisingly, the hMAT gene expression shows a 10-fold increase in T-47D cells as compared to the normal expression levels detected for the ZR-75-1 cells (Bandyopadhyay *et al*., 1996). All the above mentioned differences are suggesting that the T-47D and ZR-75-1 cell lines representing different subtypes of breast cancer could have different prognosis and treatment response.

**Aqueous artichoke leaf extract induces hormetic response associated cytotoxicity on T-47D cultured cells**

To investigate whether the aqueous artichoke extract could influence the viability of T-47D breast cancer cells, 2 to 3 days long treatments were carried out in culture media containing the dilution series of the original extract and MTT assays were carried out (see Fig. 1 and 2). Interestingly, in the case of the 2-day treatment, we could observe in the case of 10 – 0.63% concentration range of aqueous artichoke extract that the treated cancerous cell viability was approximately 4 times reduced as compared to the control untreated cells. It seems likely that the 0.31% concentration could be considered the LD50 value in this experiment. Throughout the 0.16 – 0.04% extract-specific concentration interval, the viability of the treated cells steadily increased, so that at 0.08% and 0.04% concentrations their viability exceeded approximately 1.3 times those of the untreated control cells.

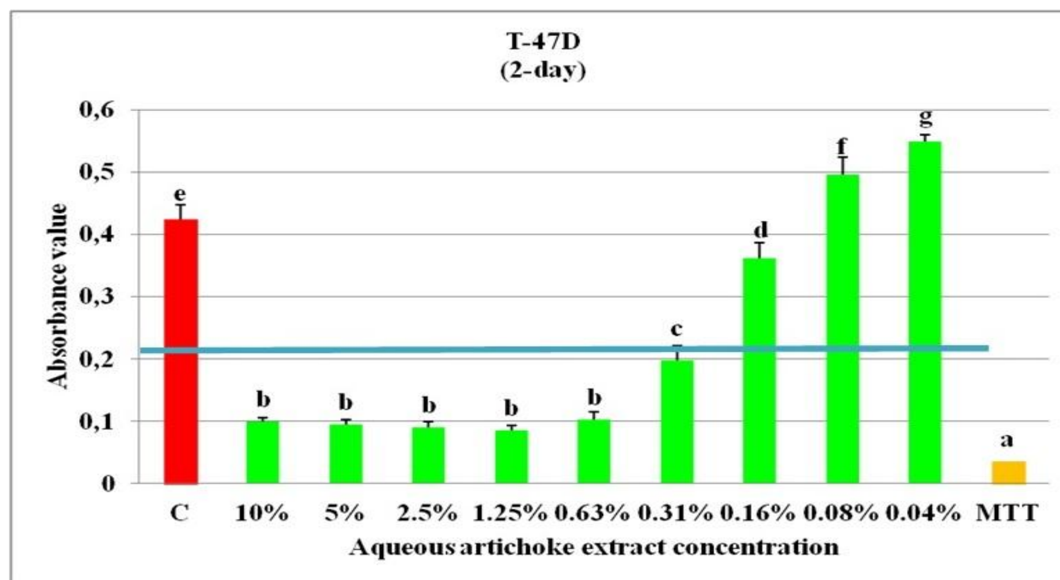


Fig. 1 Cell viability after 2-day treatment using aqueous artichoke extract. The T-47D cell viability was measured by MTT assay. The applied concentrations are indicated in %. **C**-stands for untreated or positive control cells. Blue line indicates the presumptive LD50. **MTT**- represents the negative control. One-Way ANOVA Tukey test was used for statistical analysis. Different letters on columns indicate statistical significance at $p<0.05$.


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Looking at the 3-day treatment experiments, in the 10 – 0.63% concentration range of aqueous artichoke extract, the viability of treated cancerous cells was 5 times reduced, yet constant, throughout the assessed concentration values as compared to control untreated cells (see Fig.2). It seems likely that the 0.31% concentration could be close to the LD50 value in this experiment. Started from the 0.16% concentration point, and ended with the 0.04% concentration of artichoke leaf extract, the viability of treated cells was increasing progressively. At 0.08% and 0.04% extract concentrations, the viability of treated cells surpassed approximately 1.5 times the values of untreated control cells. In this respect, the 3-day treatment recapitulates the 2-day experiment, though the increased number of viable cells was evident.

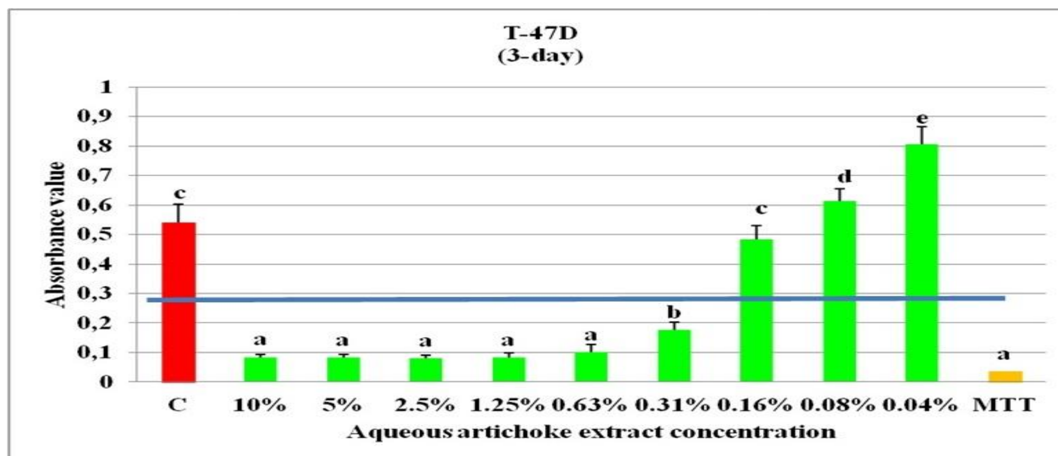


Fig. 2 Cell viability after 3-day treatment using aqueous artichoke extract. The T-47D cell viability was measured by MTT assay. The applied concentrations are indicated in %. **C**-stands for untreated or positive control cells. Blue line indicates the presumptive LD50. **MTT**- represents the negative control. One-Way ANOVA Tukey test was used for statistical analysis. Different letters on columns indicate statistical significance at $p<0.05$.

**Alcoholic artichoke leaf extract induces marked cytotoxicity on T-47D cultured cells**

We have assessed the alcoholic artichoke extract effect regarding the viability of T-47D breast cancer cells in 2 to 3 days long treatments in culture media containing the dilution series of the original extract (see Fig. 1 and 2). In 2-day treatment experiments, we could observe in the 10 – 0.08% concentration range that the treated cancerous cell viability was greatly reduced. Among the observed low viability values some fluctuations did occur. Therefore, in the 10-1.25% concentration range the viability of treated cells was approximately 8 times lower compared to control cells. Similarly in the 0.63-0.08% concentration rage, the viability of treated cells increased, yet compared to controls was approximately 4 times lower. Nevertheless, in the case of the lowest applied 0.04% concentration, the viability of treated cells increased approximately to 50% compared to control, meaning that this concentration might correspond to LD50 value. Taken together, we can conclude that the applied alcoholic artichoke extract concentrations are featuring toxicity.

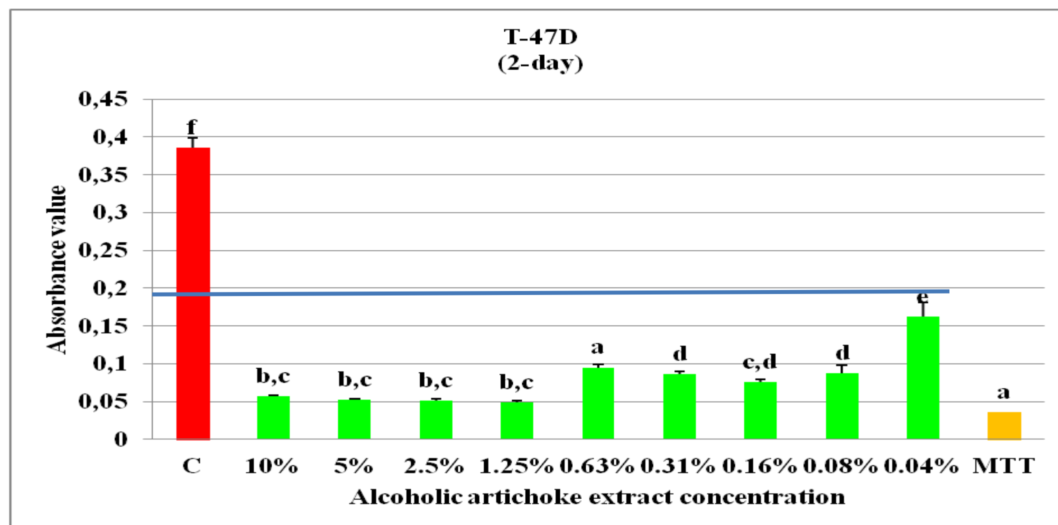


Fig. 3 Cell viability after 2-day treatment using alcoholic artichoke extract. The T-47D cell viability was measured by MTT assay. The applied concentrations are indicated in %. **C**-stands for untreated or positive control cells. Blue line indicates the presumptive LD50. **MTT**- represents the negative control. One-Way ANOVA Tukey test was used for statistical analysis. Different letters on columns indicate statistical significance at $p<0.05$.


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At the 3-day treatment experiments, we could observe in the 10 – 0.08% concentration range that the viability was greatly reduced for treated cancerous cells. Throughout the above mentioned concentration range the low viability values show some degree of variation. Accordingly, in the 10-0.63% concentration range the viability of treated cells was approximately 9 times lower as compared to control cells. In the 0.31-0.08% concentration rage, the viability of treated cells increased, yet compared to controls was approximately 5.5 times lower. Nevertheless, in the case of the applied lowest 0.04% concentration, the viability of treated cells increased but, did not reach 50%, suggesting that this working concentration does not corresponds to a LD50 value. We can conclude that the 3-day long applied alcoholic artichoke extract concentrations are featuring a greater level of toxicity than those observed in the 2-day treatment experiments.

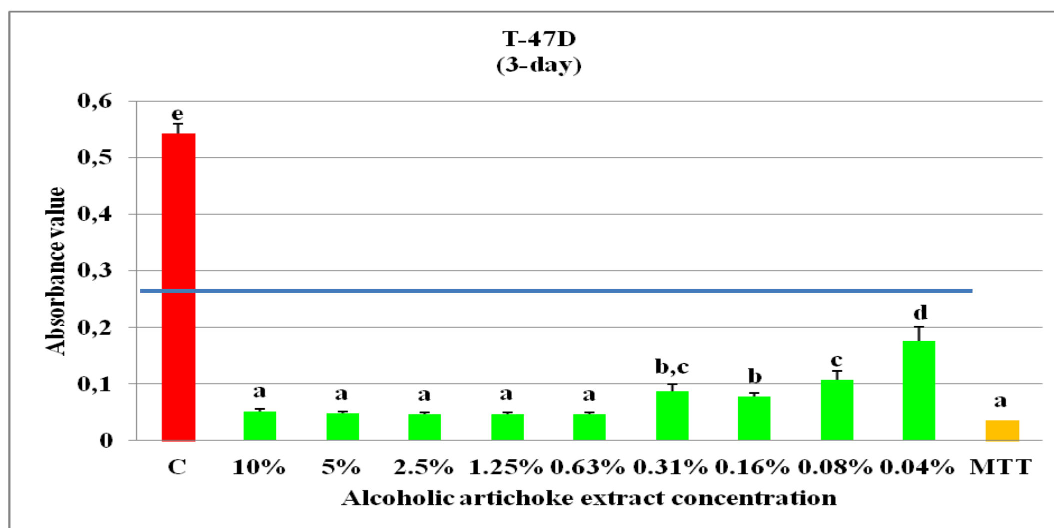


Fig. 4 Cell viability after 3-day treatment using alcoholic artichoke extract. The T-47D cell viability was measured by MTT assay. The applied concentrations are indicated in %. **C**-stands for untreated or positive control cells. Blue line indicates the presumptive LD50. **MTT**- represents the negative control. One-Way ANOVA Tukey test was used for statistical analysis. Different letters on columns indicate statistical significance at $p<0.05$.

**Aqueous artichoke leaf extract induces hormetic response associated cytotoxicity on ZR-75-1 cultured cells**

We also assessed the aqueous artichoke effect on the viability of ZR-75-1 breast cancer cells by carrying out 2 and 3 days long treatments in culture media containing dilution series of the original extract (see Fig. 5 and 6). In the 2-day treatment, we observed for the 10 – 0.63% concentration range of aqueous artichoke extract that the treated cancerous cell viability was approximately 6 times lower than in control untreated cells. It is also interesting that the 0.16% concentration would be the equivalent of the LD50 value in this experiment. Throughout the 0.16 – 0.04% extract-specific concentration interval, the viability of the treated cells increased steadily, so that at 0.04% concentration the cell viability exceeded 1.2 times those of the untreated control cells.

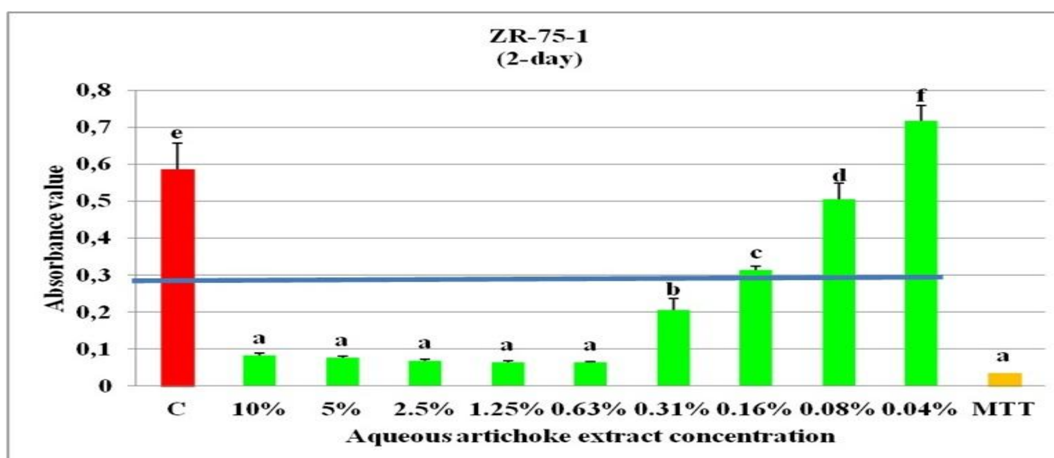


Fig. 5 Cell viability after 2-day treatment using aqueous artichoke extract. The ZR-75-1 cell viability was measured by MTT assay. The applied concentrations are indicated in %.**C**-stands for untreated or positive control cells. Blue line indicates the presumptive LD50. **MTT**- represents the negative control. One-Way ANOVA Tukey test was used for statistical analysis. Different letters on columns indicate statistical significance at $p<0.05$.


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Looking at the 3-day treatment experiments, in the 10 – 0.31% concentration range of aqueous artichoke extract, the viability of treated cancerous cells was markedly reduced, yet constant, throughout the assessed concentrations at about 8.5 times lower viability was detected as compared to control untreated cells (see Fig.5). The 0.16% concentration could be considered the LD50 value in this experiment. In the 0.08 - 0.04% concentration range of artichoke extract, the viability of treated cells was increasing progressively. At 0.04% extract concentration, the viability of treated cells surpassed 1.2 times the untreated control cells specific values. Therefore, the 3-day treatment seems to recapitulate the 2-day experiment with respect to cell viability.

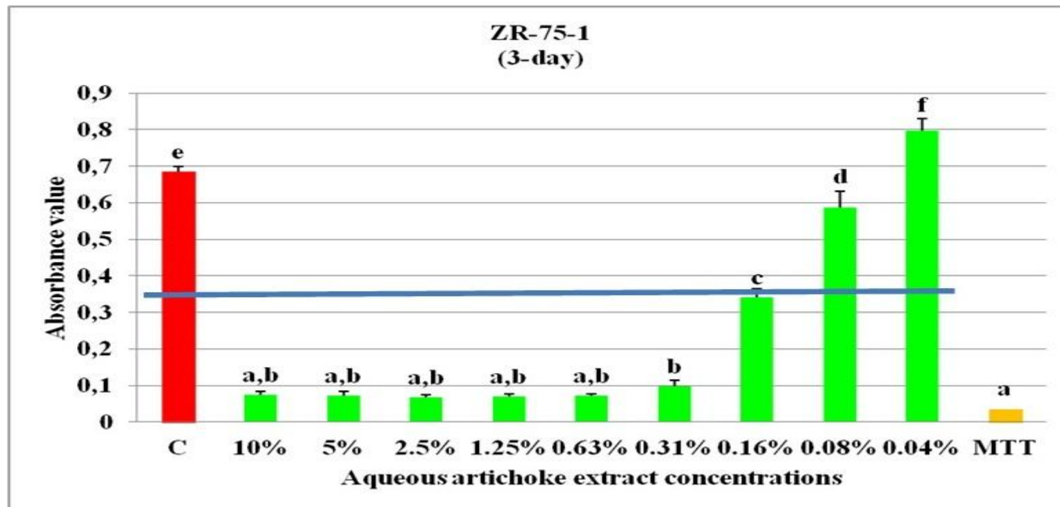


Fig. 6 Cell viability after 3-day treatment using aqueous artichoke extract. The ZR-75-1 cell viability was measured by MTT assay. The applied concentrations are indicated in %. **C**-stands for untreated or positive control cells. Blue line indicates the presumptive LD50. **MTT**- represents the negative control. One-Way ANOVA Tukey test was used for statistical analysis. Different letters on columns indicate statistical significance at p<0.05.

**Alcoholic artichoke leaf extract features a marked cytotoxic effect in case of ZR-75-1 cultured cells**

We analyzed the alcoholic artichoke extract effect on the viability of ZR-75-1 breast cancer cells in 2 to 3 days treatments in culture media containing the dilution series of the original extract (see Fig. 7 and 8). The 2-day treatment experiment showed that in the 10 – 1.25% concentration range that the treated cancerous cell viability was critically reduced, so that the viability of treated cells was more than 10 times lower as compared to control cells. Next, in the 0.63-0.08% concentration rage, the viability of treated cells increased slightly but remained, being 7 times lower than the control value. In the case of the applied lowest 0.04% concentration, the viability of treated cells increased but not substantially, and did not reach any value that would correspond to LD50. All these data are indicating that the applied alcoholic artichoke leaf extract featured pronounced toxicity in 2-day experiments.

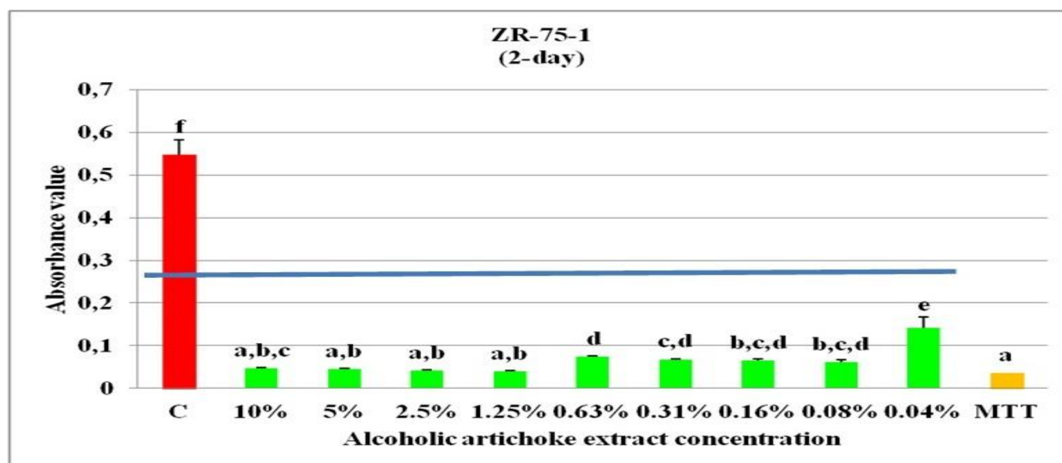


Fig. 7 Cell viability after 2-day treatment using alcoholic artichoke extract. The ZR-75-1 cell viability was measured by MTT assay. The applied concentrations are indicated in %.**C**-stands for untreated or positive control cells. Blue line indicates the presumptive LD50. **MTT**- represents the negative control. One-Way ANOVA Tukey test was used for statistical analysis. Different letters on columns indicate statistical significance at p<0.05.


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At the 3-day treatment experiments, we could observe throughout the analyzed concentration range that the viability was greatly reduced for treated cancerous cells. The observed viability values showed some minor variation throughout the monitored concentrations but did not differ significantly from each other (see Fig.8). The viability of treated cells was approximately 13 times lower as compared to control cells. All the observed viability values were far from reaching the expected LD50 value. It is obvious that the 3-day treatment with alcoholic artichoke extract shows the most pronounced toxic effect on the ZR-75-1 cancerous cell line.

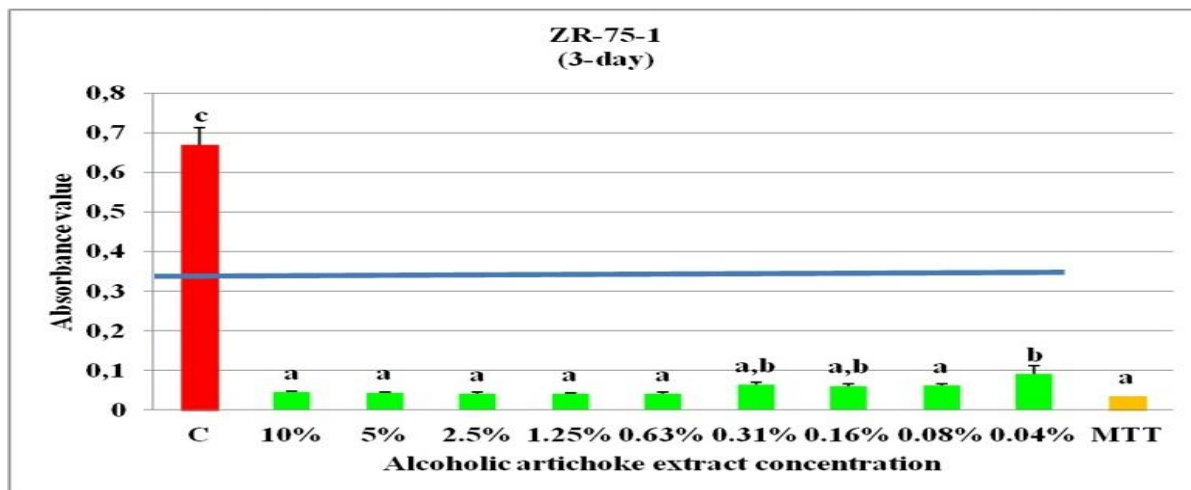


Fig. 8 Cell viability after 3-day treatment using alcoholic artichoke extract. The ZR-75-1 cell viability was measured by MTT assay. The applied concentrations are indicated in %. **C**-stands for untreated or positive control cells. Blue line indicates the presumptive LD50. **MTT**- represents the negative control. One-Way ANOVA Tukey test was used for statistical analysis. Different letters on columns indicate statistical significance at $p<0.05$.

**Immunostaining reveals senescence and apoptosis like effects in artichoke leaf extract treated breast cancer cells**

Having seen the above described viability reducing effects in the case of our artichoke leaf extracts treated cells we set to analyze the cells phenotype by assessing their chromosomal structure. Among the artichoke leaf extract treated cells we were able to detect some normal interphase and mitotic cells, but there were also polyploidy cells, binuclear cells, and cells showing minispindle and sometime multipolar spindle (Fig.9). The polyploidy cells quite often were showing overcondensed and/or fragmented chromosomes features that are specific to cells entering senescence and/or apoptosis.

However, such an immunostaining does not allow us to quantify the observed phenotypes, but it indicates that further studies are needed based primarily on FACS to assess all the existed cells at a given time point and applied concentration. In this way a more precise picture will unfold regarding the cellular aspects of the cytotoxic effect generated by artichoke leaf extracts.

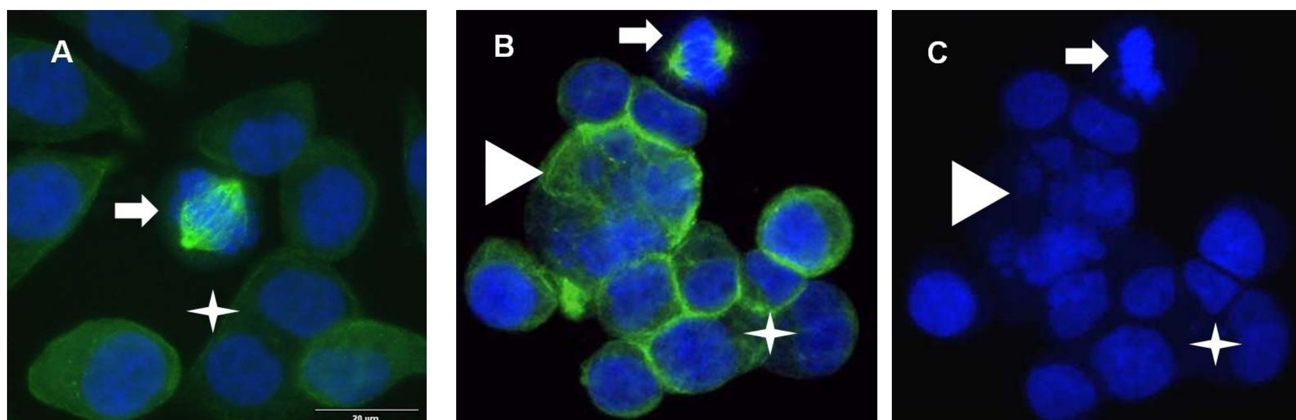


Fig. 9 Immunostainigs of artichoke extract treated breast cancer cells.

**Note. A.** Normal looking cells in interphase, at mitotic metaphase (arrow) and binucleated cell (star); green indicates microtubules, blue stands for DNA. **B-C.** Polyploidy cell with fragmented chromosomes and/or micronuclei (arrowhead). Minipindle with overcondensed chromosomes (arrow). Binucleated cell (star); green indicates microtubules, blue stands for DNA. **C.**


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## CONCLUSIONS:

Our studies are indicating a pronounced cytotoxic effect for both aqueous and alcoholic artichoke leaf extracts on the T-47D, ZR-75-1 human breast cancer cell lines. The observed cytotoxic effect was more pronounced in the case of alcoholic artichoke leaf extract for both cell lines. Indirect evidences are indicating that the alcoholic artichoke extract is inducing apoptosis on the treated cells even at very low concentrations. In previous study we have analyzed the chemical composition of alcoholic and aqueous artichoke extracts, and we were able to detect some bioactive compounds only in the alcoholic extract (Vígh *et al*., accepted for publication). In the alcoholic artichoke leaf extract we could identify specific compounds like scopoletin, apigenin, diosmetin, isohoifolin, salvigenin, naringin dihydrochalcone, ursolic acid, stearidonic acid methyl ester and stearidonic ethyl ester, whose individual anti-tumoral properties have been already confirmed. It seems likely that these bioactive compounds by acting on different cellular mechanisms do produce a strong inhibitory effect on the viability of T-47D and ZR-75-1 human breast cancer cell lines.

Interestingly, when we assessed the effects of aqueous artichoke leaf extracts on the T-47D and ZR-75-1 human breast cancer cell lines the observed viability varied in function of concentration and time (see Table 1).

**Table 1.**
Concentration dependent variation of T47-D and ZR-75-1 cells' viability**.**

| Aqueous artichoke treated cells | 2-day treatment | | 3-day treatment | |
|---|---|---|---|---|
| | conc. range (%) | viability | conc. range (%) | viability |
| T-47D | 10-0.63 | 4x↓ | 10-0.63 | 5x↓ |
| | 0.31 | LD50 | 0.31 | LD50 |
| | 0.08 | 1.2x↑ | 0.08 | 1.2x↑ |
| | 0.04 | 1.3x↑ | 0.04 | 1.5x↑ |
| ZR-75-1 | 10-0.63 | 6x↓ | 10-0.63 | 8.5x↓ |
| | 0.16 | LD50 | 0.16 | LD50 |
| | 0.04 | 1.2x↑ | 0.04 | 1.2x↑ |

Note:↑indicates increased , while ↓ symbolizes decreased values.

At higher artichoke leaf extract concentrations the breast cancer cell lines viability was reduced, while from the DL50 values on by further decreasing the extract concentration, the viability of cells started to increase and ultimately at the lowest concentration exceeded at about 1.2 times the viability of control cells. In other words, at higher concentrations, our aqueous artichoke leaf extract markedly reduced breast cancerous cell viability, while as the concentrations got lower, the inhibitory effect disappeared, and even an increased viability so a stimulatory effect could be observed.

The overall shape the aqueous artichoke leaf extract induced concentration-dependent response is biphasic (see Fig. 1, 2, 5, 6). This kind of data are indicating a hormesis dose response that is characterized by a low concentration stimulation and a high concentration inhibition. Such a hormetic dose response was observed in case of chemicals and radiation, and it was suggested that biological stress responses should be integrated within a hormetic context (Mitchell, 2007; Calabrese and Baldwin, 2000; Calabrese, 1999; Calabrese and Baldwin, 1999).

Hormesis implies different interpretations for the low and high dose responses. At high doses, within the toxicological concentration range, the measured values indicate cellular damage. The stimulation seen at low concentrations, more likely it represents the manifestation of an adaptive response that leads to modest increases related to some kind of biological performance.

Our results put in the context of hormesis are suggesting that the elevated levels of toxicity seen in case of aqueous and alcoholic artichoke leaf extracts are presumably due to senescence and/or apoptosis, while the increased viability seen at low aqueous artichoke concentrations is probably related to some adaptive stress response that would facilitate the division of the examined cancerous cell lines. In this respect further studies are needed to establish what molecular and cellular mechanisms are contributing to this adaptive stress response, and it would be equally important to shed light on the mechanisms behind the high concentration toxicity of our artichoke leaf extracts.

There are several considerations that we should take into account regarding future studies since the T-47D and ZR-75-1 human breast cancer cell lines are extensively used in biomedical research, and are classified as estrogen receptor positive (Ford *et al.*, 2011). Estrogen receptor has been reported to play a critical role in promoting the growth of breast tumor cells. In the future, it would be of a great relevance to assess the estrogen receptor modulator effect of our extracts since for other plant extract (Huaier extract) significantly reduced the mRNA and protein levels of estrogen receptor α (ERα) in T-47D and ZR-75-1 ERα-positive cell lines. The downregulation of ERα protein levels was correlated with activation of the proteasomes, and inhibition of the estrogen-stimulated proliferation and reversed the estrogen-induced activation of the nuclear factor κB (NFκB) pathway (Wang *et al.*, 2013). Recently, it has been demonstrated that the malignant pleural mesothelioma cell lines were showing pronounced cytotoxicity when treated with artichoke leaf extract, and the identified misregulated pathways were related to cancer initiation and progression (Sharma *et al.*, 2016). Similarly we intend


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to carry out the transcriptome analysis of the T-47D and ZR-75-1cells treated with our artichoke leaf extracts in order to assess the high concentration specific reduced viability, and low concentration induced increased viability as it would be very interesting to find out the artichoke induced gene expression associated with the hormetic response. Besides the applied extract concentrations we will have to pay attention to the duration of the treatments since it was demonstrated that at lower concentration exposure and longer incubation, the cancer cell growth had been inhibited via an apoptosis independent mechanism. Experimental data obtained on long term exposed MDA-MB231 human breast cancer cells to polyphenols extracted from the head of fresh artichoke did induce senescence driven growth arrest through epigenetic and ROS-mediated mechanisms (Mileo *et al*., 2015).

All together our data are suggesting that the aqueous and alcoholic artichoke extracts might be applicable for the supplementations of certain anti-tumor therapies. However, further studies are needed to carefully assess their potential effects before any step is taken regarding their use as complementary remedies. In this respect it would be important to take into account and assess the possible hepatoprotective effect of our artichoke extracts since we were able to identify several hepatoprotective bioactive compounds in them (Vígh *et al.*, 2017, accepted for publication). It is known that the hepatotoxicity related to chemotherapy causes delays of treatment in cancer patients and often requires supplementation of anti-tumor therapy with hepatoprotective agents (Angelini *et al*., 2012).

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