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Research Article

Accumulation of Cynarin, the Hepatoprotective Compound, in Ethephon Treated Callus Cultures of Globe Artichoke (*Cynara scolymus* L.)

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Abstract

Background and Objective: Cynarin, found in globe artichoke, is one of the substances reported to have very strong effect on hepatoprotective properties. The aim of this study is to develop an alternative system for production of hepatoprotective compound, cynarin, from cell cultures of globe artichoke using ethephon as an elicitor. **Materials and Methods:** Effects of different concentrations (45, 90 and 180 $\mu\text{L L}^{-1}$) of ethephon used for different periods (6, 9, 12, 15, 18 and 21 days) on callus growth, total phenol, antioxidant activity, cynarin accumulation and antiviral activity were evaluated. **Results:** The different elicitation treatments of ethephon generally increased cell growth. An inverse relationship was observed between fresh weight and ethephon concentrations. The highest value of total phenol was recorded with 90 $\mu\text{L L}^{-1}$ ethephon at day 12. However, the highest antioxidant activity was obtained with 180 $\mu\text{L L}^{-1}$ ethephon at day 15. Cynarin was detected in the untreated callus at 6, 9 and 12 days only, while it was detected in ethephon treated callus of all days of growth periods. HPLC analysis revealed that application of 90 $\mu\text{L L}^{-1}$ ethephon at 9 and 12 days caused the highest accumulation of cynarin in callus cultures. Untreated and treated callus cultures at day 12 were assayed for their antiviral activity against Bovine Viral Diarrhea Virus (BVDV), using the plaque reduction assay. Treated callus with 45 and 90 $\mu\text{L L}^{-1}$ ethephon registered high antiviral activity while untreated callus gave moderate activity. However, callus treated with 180 $\mu\text{L L}^{-1}$ ethephon had the lowest antiviral activity. **Conclusion:** Cynarin was detected in the untreated cell during growth period till 12 days, while it was detected in ethephon treated cell till 21 days of growth period. The current study suggests that ethephon treated callus extracts with highest cynarin content possess higher antiviral activity and could be an effective agent against viral infections.

Key words: Globe artichoke, callus culture, ethephon, cynarin, elicitor

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

INTRODUCTION

Plants are a valuable source of a wide range of secondary metabolites, which are used as pharmaceuticals, agrochemicals, flavors, fragrances, colors, bio pesticides and food additives. Cynarin (1,3-dicaffeoylquinic acid), found in globe artichoke is one of the substances reported to have very strong effect on hepatoprotective properties^{1,2}. The mode of action of cynarin is similar to one of the most effective liver-protectants (dicaffeoylquinic acid)³⁻⁶. Cynarin is the widely known substance possessing also anti-HIV⁷ choloretic, anticholestatic and diuretic properties^{8,9}. Cynarin is found in both leaves and heads of artichoke and in methanolic extracts at about 1.5% level^{10,2,11}. However, it is important to consider that phenolic content of globe artichoke is strongly related to physiological stages, genotype, plant parts, environmental conditions, agricultural management and post-harvest conditions^{12,2,13}.

The biotechnological production of secondary metabolites in plant cell and organ cultures is an attractive alternative to the extraction of the whole plant material¹⁴. The faster proliferation rates and shorter biosynthetic cycle of cell and organ cultures lead to a higher rate of metabolism when compared to field grown plants¹⁵. Further, plant cell/organ cultures that are under controlled conditions proliferate at their optimum growth rates when compared to the cultivated plants, which are facing environmental, ecological and climatic variations. Various strategies have been developed for use in biomass accumulation and the synthesis of secondary compounds, such as strain improvement, optimization of medium and culture environments, elicitation, precursor feeding, metabolic engineering, permeabilization, immobilization and bio transformation methods, bio reactor cultures and micropropagation¹⁶. In this respect, elicitation has been shown to be the most efficient strategy that leads to the highest enhancement in production of many secondary metabolites, among the strategies for the improvement of the biosynthesis in plant cell cultures¹⁷. Elicitors in the plant cell cultures include inorganic ions such as chemical abiotic elicitors and culture filtrates or cell extracts of fungal and bacterial origin as biotic elicitors as well as methyl jasmonate or jasmonic acid, salicylic acid and ethephon which are stress mediators¹⁸. In this context, a relatively limited number of studies have been carried out to assess the possibility of using plant biotechnology techniques for studying the production of cynarin from *in vitro* cultures of globe artichoke^{19,20,21}. The plaque assay is an accurate technique for the direct quantification of infectious virions and screening for antiviral substances through counting of discrete plaques (infectious

units and cellular dead zones) using Mad in Darby bovine kidney cells (MDBK), infected by Bovine Viral Diarrhea Virus (BVDV)²². The aim of this study was to develop an alternative system for production of hepatoprotective compound, cynarin, from cell cultures of globe artichoke using ethephon as an elicitor.

MATERIALS AND METHODS

Establishment of *in vitro* cultures: Callus cultures were established as described by Bekheet *et al.*²³ and were maintained on MS medium supplemented with 0.1 mg L⁻¹ naphthaleneacetic acid (NAA), 0.5 mg L⁻¹ 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.5 mg L⁻¹ kinetin by transferring to a newly prepared same medium every 3 weeks. Cultures were incubated at 25±2°C under dark condition. Start callus was 5±0.2 g per replicate and nine replicates were used in each treatment.

Elicitation: In this experiment, ethephon was added to callus cultures medium as an elicitor. Ethephon solution was sterilized using a 0.22 µm filter and added to callus cultures in three concentrations (45, 90 and 180 µL L⁻¹).

Determination of cell growth: Cell growth was determined as fresh cell weight (g FW).

Sample preparation: Gradually, after 6, 9, 12, 15, 18 and 21 days of ethephon elicitation, samples were harvested, weighed and immersed in liquid nitrogen to avoid any possible enzymatic degradation; then the samples were freeze-dried. The lyophilized samples were grounded to a fine powder and then kept in -80°C for further analysis.

Extraction of the phenolic compounds: Ground elicitor samples (100 mg) were extracted with 80% ethanol (1.0 mL) overnight in a shaker at room temperature, then put in ultrasonic water bath for 20 min, vortex for 10 sec and then centrifuged at 10000 rpm for 10 min. The supernatant was collected and the same procedure was repeated twice.

Determination of total phenolic content: Total phenolic content was determined by the Folin-Ciocalteu micro method²⁴. A 20 µL aliquot of extract solution was mixed with 1.16 mL of distilled water and 100 µL of Folin-Ciocalteu reagent followed by 300 µL of 200 g L⁻¹ Na₂CO₃ solution. The mixture was incubated in a shaking incubator at 40°C for 30 min and its absorbance was measured at 760 nm. Gallic

acid was used as standard for the calibration curve. Total phenolic content was expressed as gallic acid (mg gallic acid g⁻¹ dry weight).

Determination of antioxidant activity

DPPH radical-scavenging activity: The antioxidant capacity of extracts from the different extractions was measured using the stable free radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assay according to Gabr *et al.*²⁵. Briefly, the stock reagent solution (1 mM) was prepared by dissolving 22 mg of DPPH in 50 mL of methanol and stored at -20°C until use. The working solution (0.06 mM) was prepared by mixing 6 mL of stock solution with 100 mL of methanol to obtain an absorbance value of 0.8±0.02 at 515 nm. Extracts (0.1 mL) were vortexed for 30 sec with 3.9 mL of DPPH working solution. After a 30 min incubation period at room temperature in the dark, absorbance was recorded at 515 nm. The DPPH solution without extract served as control. Scavenging activity was calculated as follows:

$$\text{DPPH radical-scavenging activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where:

A_{control} = Absorbance of the control reaction at 515 nm (containing all reagents except the test compound)

A_{sample} = Absorbance of the test compound

ABTS radical-scavenging activity: The 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay was performed according to Gabr *et al.*²⁵. Briefly, ABTS⁺ solution was produced by the reaction of ABTS solution (7 mM) and potassium persulfate solution (2.4 mM) at a ratio of 1:1 (v/v). After incubation for 12-16 h at room temperature in the dark, 1.0 mL of the resulting ABTS⁺ solution was diluted with 60 mL of methanol resulting in an absorbance of 0.706±0.001 at 734 nm. Forty microliters of diluted samples was added to 1.96 mL of the resulting blue-green ABTS⁺ and incubated in the dark for 10 min at 37°C. Blank was run in parallel in an identical manner. Scavenging activity was calculated as follows:

$$\text{ABTS radical-scavenging activity (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where:

A = Absorbance at 734 nm

Determination of cynarin: Cynarin content was determined in the different callus treatments using High Performance Liquid Chromatography (HPLC) according to the method described by Menin *et al.*²⁶. Briefly, 50 mg of grounded samples was extracted for 20 min using 1 mL 80% aqueous ethanol (v/v) in an ultrasonic bath. Samples were then centrifuged for 10 min at 6000 rpm. The supernatants were collected and the pellets were re-extracted twice with 500 µL 80% ethanol. After centrifugation (10 min at 10,000 rpm), the supernatant was filtered through a 0.45 µm Anotop 10 filter (Whatman, Maidstone, UK) into a 2 mL glass vial and 10 µL of the filtrate was injected into an HPLC instrument (Dionex Summit P680A HPLC-System), equipped with P680 pump, ASI-100 automated sample injector, a Narrow-Bore Acclaim PA C16-column (3 µm, 2.1×150 mm, Dionex) and PSA-100 photodiode array detector (Dionex) and software Chromeleon 6.8 (Dionex, USA). The mobile phases consisted of a 1:1,000 (v/v) mix of degassed glacial acetic acid: ultrapure water (Eluant A) and a 1:1,000 (v/v) mix of glacial acetic acid: acetonitrile (Eluant B). The elution gradient started at 5% (v/v) B: 95% (v/v) A and increased linearly to 35% (v/v) B: 65% (v/v) A over 28 min. The column was equilibrated with 100% (v/v) A between injections. The flow rate was 0.5 mL min⁻¹ and the absorbance of the output was monitored at 300 nm and at 330 nm. Cynarin reference standard was purchased from Sigma-Aldrich.

BVDV plaque reduction assay: HPLC analysis revealed that the highest cynarin content was recorded with callus treated with 45 and 90 µL L⁻¹ ethephon as well as un-treated callus at 12 days. Therefore, all treatments on day 12 were assayed.

Madin-Darby Bovine Kidney (MDBK) cells were seeded into six-well culture plates (Nunc™, Nunc, Denmark) till reaching 90% confluence. The cells were washed in phosphate buffered saline (PBS) and then infected with bovine viral diarrhea virus (BVDV) in PBS containing 1% horse serum and 1 mM MgCl₂. After 2 h, the virus inoculum was removed and the cells were washed with PBS. Different concentrations of the test samples were added to 1.5 mL of DMEM containing 0.5% Sea plaque™ agarose and supplemented with 5% horse serum, 2 mM L-glutamine, 50 U mL⁻¹ penicillin and 50 µg mL⁻¹ streptomycin (BioWhittaker, Lonza Group, GmbH, Germany). Each sample concentration was analyzed in duplicate. Positive control with virus only without compounds and negative control without virus and samples were prepared. The cells were incubated at 37°C and 5% CO₂ for 3 days. Then the cells were fixed with 1.5 mL of 10% formaldehyde solution for at least 2 h at room temperature. The agarose layer was gently

removed by using warm water and the fixed mono layers were stained with 0.3% methylene blue. Plaques were identified by microscopic examination and the percentage inhibition of plaque formation was estimated by comparing mean plaque numbers in test wells with the mean plaque number in the control wells. Plaques are counted in terms of plaque forming units (PFU) per milliliter.

Statistical analysis: The data presented in the study were statistically evaluated as Mean $\pm$ SD for each group. Data were analyzed by Student's t-test and one-way ANOVA followed by the Tukey multiple comparison test using the SPSS statistical program. Differences were considered significant at $p \leq 0.05$.

RESULTS

Influence of ethephon on callus fresh weight: Data presented in Fig. 1 demonstrate the cell growth, in terms of fresh cell weight of globe artichoke callus cultures as a result of ethephon treatments. During elicitation periods, the fresh weight of calli treated with the different concentrations of ethephon was gradually increased from day 6 and reached the maximum of cells growth at day 21. However, callus fresh weights of untreated cultures were increased dynamically with increasing ethephon compare to untreated cultures.

Influence of ethephon on total phenols content: The influence of the different concentrations of ethephon on total phenol content in the different calli cultures during the 21 days of culture is presented in Table 1. The total phenol content in the treated callus cultures with 45 or 90 μL ethephon was significantly higher ($p \leq 0.001$) than those untreated callus cultures. Treatment of callus with 180 $\mu\text{L L}^{-1}$ ethephon decreased the total phenol content at days 9 and 12 compared with those untreated callus cultures. It was noticed that the value of total phenol differed depending upon ethephon concentration and period of time. The highest value of total phenol was reached with callus cultures treated with 90 $\mu\text{L L}^{-1}$ ethephon (15.198 mg g^{-1} DW) at day 12. The

lowest total phenol content was observed at day 21 with all ethephon concentrations (3.175, 4.433 and 1.629 mg g^{-1} DW), respectively.

Influence of ethephon on antioxidant activity: In the current study, DPPH and ABTS methods based on the different mechanisms to evaluate antioxidant capacity of the different cultures was used. The results of the DPPH $^{\cdot}$ and ABTS $^{\cdot+}$ free radical scavenging assays showed that antioxidant capacities of ethephon treated callus cultures were significantly higher than untreated callus cultures (Table 2 and 3). The DPPH $^{\cdot}$ free radical scavenging assay showed the same trend as total phenol, antioxidant capacity in the callus cultures treated with 45 or 90 μL ethephon was significantly higher ($p \leq 0.001$) than in the untreated callus cultures. Treatment of callus cultures with 180 $\mu\text{L L}^{-1}$ ethephon decreased the total phenol content at day 9 and 12 compared with the un-treated callus culture. The highest antioxidant activities either with DPPH or ABTS $^{\cdot+}$ were recoded with callus cultures treated by 180 μL ethephon (93.362 and 98.186%, respectively) at day 15. It was noticed that callus cultures treated with 45 $\mu\text{L L}^{-1}$ ethephon reached the highest antioxidant activity at day 12.

Fig. 1: Dynamic profiles of cell growth, in terms of fresh weight, of callus treated with different ethephon concentrations. Eth: Etethephon. Values are Mean $\pm$ SD

Table 1: Effect of ethephon concentrations on total phenol (mg g^{-1} DW) in callus cultures at different periods of culture (day)

Period (day)	Ethephon concentrations (μL)			
	Control	45	90	180
6	1.937 $\pm$ 0.033 ^a	8.356 $\pm$ 0.089 ^b	6.348 $\pm$ 0.016 ^c	8.125 $\pm$ 0.033 ^d
9	2.968 $\pm$ 0.016 ^a	9.187 $\pm$ 0.033 ^b	9.441 $\pm$ 0.033 ^c	2.460 $\pm$ 0.016 ^d
12	3.862 $\pm$ 0.022 ^a	13.756 $\pm$ 0.016 ^b	15.198 $\pm$ 0.016	3.533 $\pm$ 0.016 ^d
15	2.983 $\pm$ 0.016 ^a	5.541 $\pm$ 0.033 ^b	8.806 $\pm$ 0.016 ^c	14.760 $\pm$ 0.049 ^d
18	2.537 $\pm$ 0.033 ^a	4.756 $\pm$ 0.033 ^b	5.206 $\pm$ 0.016 ^c	3.210 $\pm$ 0.033 ^d
21	1.537 $\pm$ 0.029 ^a	3.175 $\pm$ 0.016 ^b	4.433 $\pm$ 0.016 ^c	1.629 $\pm$ 0.022 ^d

Values are Mean $\pm$ SD, In each row same letters means non-significant difference, different letter means significance difference at 0.05 probabilities

Table 2: Effect of ethephon concentrations on scavenging of DPPH free radical (%) in callus cultures at different periods of culture (day)

Period (day)	Control	Ethephon concentrations (μL)		
		45	90	180
6	7.851 $\pm$ 0.195 ^a	69.045 $\pm$ 0.263 ^b	60.484 $\pm$ 0.263 ^c	60.670 $\pm$ 0.175 ^{cd}
9	16.315 $\pm$ 0.088 ^a	79.280 $\pm$ 0.175 ^b	91.625 $\pm$ 0.088 ^c	16.005 $\pm$ 0.877 ^a
12	17.851 $\pm$ 0.292 ^a	85.174 $\pm$ 0.088 ^b	82.444 $\pm$ 0.614 ^c	17.370 $\pm$ 0.526 ^a
15	18.681 $\pm$ 0.195 ^a	42.928 $\pm$ 0.702 ^b	67.494 $\pm$ 0.175 ^c	93.362 $\pm$ 0.614 ^d
18	7.987 $\pm$ 0.390 ^a	36.849 $\pm$ 0.351 ^b	35.298 $\pm$ 0.439 ^c	32.568 $\pm$ 0.088 ^d
21	4.257 $\pm$ 0.097 ^a	13.834 $\pm$ 0.088 ^b	28.598 $\pm$ 0.088 ^c	19.851 $\pm$ 0.175 ^d

Values are Mean $\pm$ SD. In each row same letters means non-significant difference, different letter means significance difference at 0.05 probabilities

Table 3: Effect of ethephon concentrations on scavenging of ABTS⁺ free radical % in callus cultures at different periods of culture (day)

Period (day)	Control	Ethephon concentrations (μL)		
		45	90	180
6	20.984 $\pm$ 0.366 ^a	82.211 $\pm$ 0.489 ^b	68.221 $\pm$ 1.221 ^c	76.857 $\pm$ 5.862 ^d
9	23.661 $\pm$ 0.733 ^a	84.974 $\pm$ 0.489 ^b	90.760 $\pm$ 0.611 ^c	49.050 $\pm$ 4.152 ^d
12	28.929 $\pm$ 0.122 ^a	87.047 $\pm$ 0.733 ^b	88.428 $\pm$ 0.244 ^{bc}	44.819 $\pm$ 0.366 ^d
15	32.556 $\pm$ 0.122 ^a	78.670 $\pm$ 0.122 ^b	81.693 $\pm$ 0.977 ^{bc}	98.187 $\pm$ 0.122 ^d
18	28.929 $\pm$ 0.366 ^a	61.658 $\pm$ 0.244 ^b	46.287 $\pm$ 0.244 ^c	62.522 $\pm$ 0.489 ^{bd}
21	25.561 $\pm$ 0.489 ^a	46.546 $\pm$ 0.366 ^b	46.891 $\pm$ 1.099 ^{bc}	45.596 $\pm$ 1.221 ^{bcd}

Values are Mean $\pm$ SD. In each row same letters means non-significant difference, different letter means significance difference at 0.05 probabilities

Table 4: Plaque reduction assay using treated and untreated callus cultures

	Concentration (ng)	Number of plaques (PFU)	Inhibition (%)
Untreated callus	48.6	72 $\times$ 10 ^{-4.3}	60
Ethephon 45 $\mu\text{L L}^{-1}$	48.8	18 $\times$ 10 ^{-4.3}	85
Ethephon 90 $\mu\text{L L}^{-1}$	48.9	24 $\times$ 10 ^{-4.3}	80
Ethephon 180 $\mu\text{L L}^{-1}$	49.3	96 $\times$ 10 ^{-4.3}	20

Plaques are counted in terms of plaque forming units (PFU) per milliliter

Fig. 2: Effect of ethephon concentrations on cynarin contents during different period of callus elicitation
Eth: Ethephon

Influence of ethephon on cynarin content: Cynarin content extracted from the ethephon treated and untreated callus cultures were chromatographically separated by HPLC. Cynarin accumulation in ethephon treated compared to untreated calluses presented in Fig. 2. Interestingly, the level

of cynarin in callus cultures treated with 90 $\mu\text{L L}^{-1}$ ethephon at days 9 and 12 was higher than those callus cultures treated with the other two ethephon concentrations (45 or 180 $\mu\text{L L}^{-1}$) and the untreated callus (control). It was observed that the untreated callus cultures were not able to accumulate cynarin at days 15, 18 and 21. Callus treated with 45 $\mu\text{L L}^{-1}$ ethephon showed increase in cynarin content from day 6-12; then it decreased from day 15-21. In callus treated with 180 $\mu\text{L L}^{-1}$ ethephon, cynarin content was decreased till day 12 followed by increase at day 15 and then again decrease at days 18 and 21.

Assessment of antiviral activity of samples based on plaque assay: Evaluating the antiviral activities of samples was carried out using MDBK cells infected with BVDV. The number of plaque forming units (PFUs) after exposure to untreated and treated cultures at day 12 was examined and compared to PFUs in the positive control, cells with BVDV only that had 120 $\times$ 10^{-4.3} PFU. The assessment of antiviral activity after ethephon elicitation addition to callus cultures is presented in Table 4. The data pointed out that treated callus with 45 and 90 $\mu\text{L L}^{-1}$ ethephon have high antiviral activity, while un-treated callus has moderate activity and treated callus with 180 $\mu\text{L L}^{-1}$ ethephon has low antiviral activity.

DISCUSSION

The present study is a pioneer in production of cynarin compound from cell culture of globe artichoke treated by ethephon as an elicitor. Subsequently, this study was carried

out to investigate the effects of ethephon added to culture medium at three concentrations (45, 90 and 180 $\mu\text{L L}^{-1}$) on callus growth, total phenol, antioxidant capacity and cynarin production from callus cultures. According to our results, cell growth of treated calli was increased from days 6-18 compared with untreated callus, while, at day 21, untreated callus showed increasing in cell growth compared to those treated.

In the regard of alternatives for production of desirable medicinal compounds from plants, biotechnological approaches are found to have potential as a supplement to traditional agriculture in the industrial production of bioactive plant metabolites²⁷. Elicitation is one of the most effective techniques currently used for improving the biotechnological production of secondary metabolites. Elicitation procedures are often used to increase the production of phenolics, archiving in most cases higher yields than in non elicited cultures. The cultivation period in particular can be reduced by the application of elicitors, although maintaining high concentrations of product¹⁵.

These results agree with those of Dong and Zhong²⁸. They reported that cells are able to adapt themselves to the new stress and application of elicitation in the lag phase severally inhibited cell growth. However, this may vary among different cell lines. Furthermore, Ye *et al.*²⁹ found that elicitation in the early exponential phase was more effective than that in later phases. On the contrary, Saw *et al.*³⁰ reported that ethephon did not affect cell growth of *Vitis vinifera* cell suspension. In this respect, it was found that elicitor concentration plays a very important role in elicitation process. High dosage of elicitor has been reported to induce hypersensitive response leading to cell death³¹.

Regarding influence of ethephon on total phenolic accumulation, the results obtained revealed that ethephon was significantly effective with the treated callus compared to those untreated cultures. Data clearly showed that the lowest value of total phenolics was recorded with untreated callus culture. In this context, Cai *et al.*^{32,33} reported that cell suspension cultures of *Vitis vinifera* produced high level of phenolic acids with ethephon treatments compared to un-treated culture. Furthermore, a similar result was reported in *V. vinifera*, common grape vine, in which ethephon has been shown to increase anthocyanin content over 2-fold³⁰. To the best of our knowledge, cynarin accumulation in callus under chemical elicitor has not been previously reported. Cynarin contents were affected by ethephon concentrations and the period of time. Interestingly, cynarin was not detected in untreated callus cultures beginning from day 15. In this respect, few studies have been carried out to assess the possibility of using plant biotechnology techniques for

studying as well as producing different polyphenolic compounds from *in vitro* cultures of globe artichoke^{19,34,20,25,23}. The main reason may be attributed to difficulty in establishment of cell cultures and callus induction from globe artichoke^{35,36}. However, Trajtemberg *et al.*³⁷ reported that the content of cynarin is higher in callus than in leaves of *C. cardunculus* var. *cardunculus* L. and they mentioned that *in vitro* tissue culture can be employed as a source to obtain cynarine for pharmaceutical purposes. In this regard, Menin *et al.*²⁶ established an effective protocol for callus induction from globe artichoke (*C. cardunculus* var. *scolymus*), which accumulates three to five fold-higher level of dicaffeoylquinic acids than the leaves under UV-C stress. They reported that their protocol may be useful in future, not only as a production system for dicaffeoylquinic acids, but also as a tool to understand the metabolic pathway leading to caffeoylquinic acid. Recently, Pandino *et al.*²¹ studied for the first time the phytochemical accumulation in cell suspension culture of globe artichoke. They did not detect cynarin during the growth period of 25 days of cell suspension culture.

Many experiments showed that artichokes exhibit hepatoprotective and hypocholesterolemic properties^{38,2}. In this study we aimed to examine which ethephon treated callus culture can exhibit more *in vitro* antiviral activity using plaque reduction assay. Results revealed that callus treated with 45 and 90 $\mu\text{L L}^{-1}$ ethephon had higher antiviral activity than the un-treated callus followed by callus treated with 180 $\mu\text{L L}^{-1}$ ethephon. This maybe attributed to the cynarin content in samples, as its concentration is directly proportional with the antiviral activity. This comes in agreement with Elsebai *et al.*³⁹, who recently reported that 2 lactones derived from leaves of artichoke inhibited HCV infection *in vitro*. Also Elsebai *et al.*⁴⁰, assessed the effect of artichoke leaf water extract as a potential anti-HCV agent *in vivo* and observed significant therapeutic potential.

CONCLUSION

In the present study, it is the first time to report the accumulation of cynarin in elicited callus culture of globe artichoke. Cynarin was detected in the untreated cell during growth period till 12 days, while it was detected in ethephon treated cell till 21 days of growth period. The current study suggests that ethephon treated callus extracts with highest cynarin content possess higher antiviral activity and could be an effective agent against viral infections. Further assays to assess biological effects of ethephon treated callus extracts will be carried out especially against HCV due to its high prevalence in Egypt.

SIGNIFICANCE STATEMENTS

This is the first time to report the accumulation of cynarin in elicited callus culture of globe artichoke. The current study suggests that ethephon treated callus extracts with highest cynarin content possess higher antiviral activity and could be an effective agent against viral infections. Further assays to assess biological effects of ethephon treated callus extracts will be carried out especially against HCV due to its high prevalence in Egypt.

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