

**ANTIPROLIFERATIVE AND ANTIOXIDANT EFFECTS OF *ERUCA SATIVA* (JARJEER) LEAVES EXTRACT ON CARCINOMA OF WOMEN'S BREAST**SAFAA A DERBALA<sup>1</sup>, MANAR E ELKADY<sup>1</sup>, REHAB A ELBANHAWY<sup>1</sup>, ABDEL-AZIZ AF<sup>2</sup><sup>1</sup>Urology and Nephrology Center, Mansoura University, Egypt. <sup>2</sup>Department of Biochemistry, Faculty of Science, Mansoura University, Mansoura, Egypt. Email: manarbio2005@yahoo.com

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**ABSTRACT****Objective:** This work aims to investigate the influence of *Eruca sativa* leaves extract on the cell viability of the breast carcinoma cell line (MCF-7).**Methods:** *In vitro*, breast cancer cell line (MCF-7) treated by *E. sativa* leaves extract for 48 h. The cell viability, proliferation, and apoptosis were assessed using colorimetric (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, flow cytometric technique, and antioxidant enzymes (superoxide dismutase [SOD] and catalase [CAT]) measurement.**Results:** This study demonstrated that the incubation of MCF-7 cells with *E. sativa* for 48 h caused a significant reduction in cell viability and proliferation of MCF-7 cell line. In parallel, *E. sativa* treatment induces a significant increase in apoptosis of MCF-7 cells compared to control. Moreover, flow cytometry analysis demonstrated that the inhibition of MCF-7 cell proliferation existed at the G2 and M phase in the cell-division cycle. Finally, the intracellular antioxidant enzymes SOD and CAT activities were significantly increased in the administered cells compared with unadministered MCF-7 cells.**Conclusions:** Taken together, *E. sativa* treatment reduces cell viability and proliferation concomitant with enhanced antioxidant enzymes expression and apoptosis of breast cancer cell line MCF-7. This may help in protection from breast cancer or preclinical recommendation.**Keywords:** *Eruca sativa*, Cell line of breast cancer, Apoptosis, DNA fragmentation, Antioxidants.© 2021 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2021v14i2.39937>. Journal homepage: <https://innovareacademics.in/journals/index.php/ajpcr>**INTRODUCTION**

Carcinoma of the breast is the second overriding reason for mortality in womenfolk. Breast cancer incidence rates were 32.0% followed by hepatocellular carcinoma (13.5%) among females in Egypt [1]. Medicinal herbs are regarded as the key sources of bioactive compounds that can be utilized for the medical management of varied diseases such as cancer. However, among the 250,000–500,000 herb sorts on land, only 1–10% has been examined by chemical and pharmaceutical experts for their potentiality and medicative worth, particularly for chemotherapy impact [2]. *Eruca sativa* or rocket is an “endemic” sort of the Cruciferae and productized chiefly in lands that surround the Mediterranean Sea [3]. The rocket contains great quantities of ascorbic acid and antioxidant compounds [4]. The previous studies reported that rocket species exhibit many medicinal possessions. Consistently, arugula salad types have now been proven a valuable resource for antioxidant complexes, as a rich source of three essential vitamins such as vitamin C, tetraterpenoids, and polyphenols [5]. Importantly, several studies suggested a relationship between the potential to reduce the chance of some specific types of carcinoma and augmented consumption of Cruciferae vegetables, composing their rich sources [6]. The present study aims to evaluate the *in vitro* antitumor activity of alcoholic extract of *E. sativa* leaves on the cell viability of the breast carcinoma cell line (MCF-7). As well as antioxidant, activities of *E. sativa* leaf extracts were evaluated.

**METHODS****Preparation of the alcoholic extract of *E. sativa* leaves**

One hundred and ten gram powder of shade dried leaves of *E. sativa* was extracted with 75 ml of 95% ethanol. After that, the mixture was shaken, using a magnetic stirrer for 3 h/day and allowed to stand for 21 h for 3 days. Then, the mixture was filtered on Whatman filter paper

0.45 µm, and the extract was dissolved in 450 ml 95% ethanol and filtered again. The dissoluble ethanol extract was condensed to dryness at 60°C under low pressure. Dissolvent eradication of the resultant ethanol extract was weighed 13.5 g. The percent yield was computed utilizing the following form: (weight of extract/original weight×100 giving 12.27% yields) of green fatty crude *E. sativa* leaf ethanol extract. The herbal extract was kept in the fridge and was suspended in distilled water before administration [7].

**Cell line**

MCF-7 mammary bust carcinoma cell-division line (ER+) got acquired from initial malignant tumor of a case, but cell line was purchased from the Research and Development of Medical Experimental Research Center, Kafrelsheikh University. Cells were expanded and raised in an adherent culture in minimum essential medium supplemented with 10% (v/v) fetal bovine serum, 1 mM Pyruvic Acid Sodium Salt, 10 µg/ml beef insulin, and 1% (v/v) antibiotic–antimycotic agent. Cells were raised until 37°C confluence in a moistened climate of 5% CO<sub>2</sub> in the ambient ether and were passed out once a week with 0.25% trypsin.

**Cell lysate preparation**

MCF-7 cells got placed into 12-well dishes at 5×10<sup>6</sup> cells/well and permitted attachment for 48 h. MCF-7 human breast carcinoma cells were incubated for 48 h with the 50% inhibitory concentration (IC<sub>50</sub>) level of treated cells while comparison cells were incubated with vehicle only. After incubation, trypsin was utilized to assemble cells, flushed 3 times with phosphate-buffered saline (PBS) (pH 7.4) and the pieces were transmitted into extract solution that comprised 20 mM potassium phosphate buffer (pH 7) and a protease inhibitor cocktail. The cells were homogenized and centrifuged at 10,000 rpm at 4°C for 10 min. The resulting supernatant will be used for the measurement of antioxidant enzymes activity.

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

The inhibition of MCF-7 cell proliferation on medical treatment with test sample was set by the MTT assay according to the method of Oka *et al.* [8] using (Sigma Aldrich Chemical Company, UK) MTT assay kit. The rationale of this way is built upon cellular reduction of MTT to an azure formazan result by dehydrogenases of mitochondria of living cells. The blue color intensity made by this process is proportionate to the number of living cells. Trypan Blue (0.4%) melted in PBS was utilized to count cells. 0.2 ml trypsin-suspended cell was placed into 0.3 ml medium and 0.5 ml 0.4% Trypan Blue. Cells were reckoned with a hemocytometer after 5 min. Cells were seeded down into a 96-well dish with density  $5 \times 10^3$  cells/well and incubated ( $37^\circ\text{C}$  and 5%  $\text{CO}_2$ ). After 24 h, media was substituted by new media encompassing variant concentrations of test samples and incubated for another 24 h comparison cells administered with intermediary only were placed to the same dish. Ten microliters of 5 mg/ml MTT solution were added for each one and these dishes were wrapped with the tin sheet and incubated for 3 h. Then, the intermediary was taken away and the remnant cells were melted in dimethyl sulfoxide. The absorbency was gauged at 595 nm. The mean values for the absorbance of the ones incubated with test specimens were compared to the mean worth for the absorbance of the comparison cells to compute the per cent of living cells (% of comparison cells) and when subtracted from 100 give the % of cell death (inhibition). The outcomes were given as the average $\pm$ SE of three independent experiments; each repeated 3 times.

### Assay for apoptosis and DNA damage

Programmed cell death (apoptosis) and damaged DNA were observed by utilizing an Annexin V-Fluorescein isothiocyanate (V-FITC) apoptosis detection kit (BD Pharmingen™, USA). Administered and comparison cells were flushed two occasions with PBS and re-suspended in a 1-fold binding buffer at a concentration determined to be  $1 \times 10^6$  cells/ml. Five microliters of Annexin V-FITC Conjugate and 10  $\mu\text{L}$  of propidium iodide (PI) solution were added to 500  $\mu\text{L}$  of each cell suspension, incubated for 10 min at ambient temperature and then analyzed by flow cytometry instrument (FAC Star caliber, Becton Dickinson, USA) utilizing excitation at 488 nm and a 515 nm bandpass filter for FITC detection and a filter over 600 nm for PI detection. The percentage of cells in each stage was computed with the software Cell Quest [9].

### Assays of superoxide dismutase (SOD) and catalase (CAT)

Determination of SOD activity was done using kit commercially available (Bio diagnostic, Cairo, Egypt). The SOD activity depends on its ability to inhibit the phenazine methosulfate-mediated reduction of nitro blue tetrazolium dye [10]. CAT activity was also determined by a ready-for-use colorimetric kit (Bio diagnostic, Cairo, Egypt). The CAT activity relies on the ability of the enzyme to react with a known quantity of  $\text{H}_2\text{O}_2$ . The reaction is stopped after exactly 1 min with CAT inhibitor. In the presence of peroxidase (horseradish peroxidase), remaining  $\text{H}_2\text{O}_2$  reacts with 3, 5-Dichloro -2-hydroxybenzenesulfonic

acid and 4-aminophenazone to form a chromophore with color intensity inversely proportional to the amount of CAT in the original sample [11]. These assays were done agreeable to the instructions provided by the manufacturer. SOD and CAT activities in each specimen were expressed as U/ml.

### Statistical analysis

Data were analyzed utilizing IBM SPSS statistics 21 (SPSS Inc., Chicago, Illinois, USA). They were shown as mean $\pm$ standard division. Independent sample t-test was utilized to measure variations between groups. Level of significance for probability value was  $<0.05$  as well as  $<0.01$  and  $p < 0.001$  signified a highly significant result.

### RESULTS

Our study assessed the cytotoxicity of *E. sativa* affected MGF-7 cells by MTT assay. Forty-eight hours after treatment with *E. sativa*, the incubation of MGF-7 cells with *E. sativa* for 48 h brought about a reduction in the viable cell, with approximate  $\text{IC}_{50}$  of 41.13  $\mu\text{g}/\text{mL}$ . The  $\text{IC}_{50}$  was  $41.13 \pm 0.03$   $\mu\text{g}/\text{mL}$ . In parallel, *E. sativa* dose-dependently impeded cell line proliferation MGF-7 (Fig. 1).

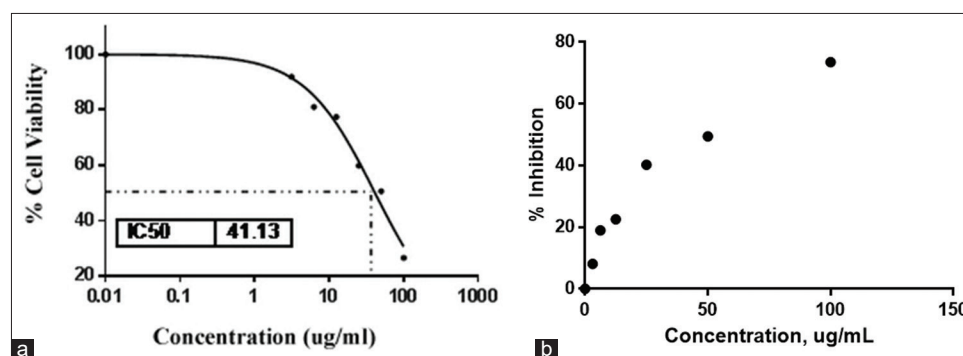
Herein, the ability of *E. sativa* to induce apoptosis was examined using Annexin V-FITC apoptosis kit. Our analysis of flow cytometry of Annexin V-FITC showed that showed a significant increase in apoptosis of MGF-7 cells (55.5 $\pm$ 3.6)% treated by the *E. sativa* alcoholic extract for 48 h in compared with untreated MGF-7 cells as control (8.5 $\pm$ 0.5)% (Table 1 and Fig. 2).

To find out extra info on the machinery behind the growth inhibition effects of *E. sativa* cell on MGF-7 cell-cycle distribution, we used flow cytometry techniques. Forty-eight hours after treated with *E. sativa*, our analysis of flow cytometry demonstrated that the inhibition of MGF-7 cell proliferation existed at the G2 and M phase cell-division cycle, as reflected with a larger number of accumulated cells (Table 1 and Fig. 3). Specifically,

**Table 1: Assessment of cell-division cycle and induction of apoptosis in MCF-7 cells administered with *Eruca sativa* after 48 h**

Parameters	MCF-7 cell line	MCF-7 cell line treated	p value
Sub G1	62.5 $\pm$ 2.9	16.6 $\pm$ 2.2	0.000***
G0	24.5 $\pm$ 1.9	35.9 $\pm$ 3.4	0.014*
S phase	3 $\pm$ 0.5	12.5 $\pm$ 1.4	0.003**
G2M	7.8 $\pm$ 1.5	34.2 $\pm$ 0.7	0.000***
Necrotic cell	8.5 $\pm$ 1.2	21.1 $\pm$ 5.6	0.019*
Apoptotic cell	8.5 $\pm$ 0.5	55.5 $\pm$ 3.6	0.000***
Viable cell	69.9 $\pm$ 1.4	14.0 $\pm$ 0.7	0.000***
Early apoptotic cell	13.0 $\pm$ 0.6	9.7 $\pm$ 1.8	0.07

Data are presented as mean $\pm$ standard division. Comparison of MCF-7 cell line treated with MCF-7 cell line non-treated as control group. \* $p < 0.05$  and \*\* $p < 0.01$  were considered significant differences, while \*\*\* $p < 0.001$  was highly significant



**Fig. 1: Effect of *Eruca sativa* to the cell viability of MGF-7 cells. (a)  $\text{IC}_{50} = 41.13 \pm 0.02$   $\mu\text{g}/\text{mL}$  derived from at least  $n = 4$  replicates. Note that the incubation of MGF-7 cells with *E. sativa* for 48 h caused a diminish in living cell, with approximative  $\text{IC}_{50}$  of 41.13  $\mu\text{g}/\text{mL}$ . (b) shows that *E. sativa* dose dependently inhibited the proliferation of MGF-7 cell line**

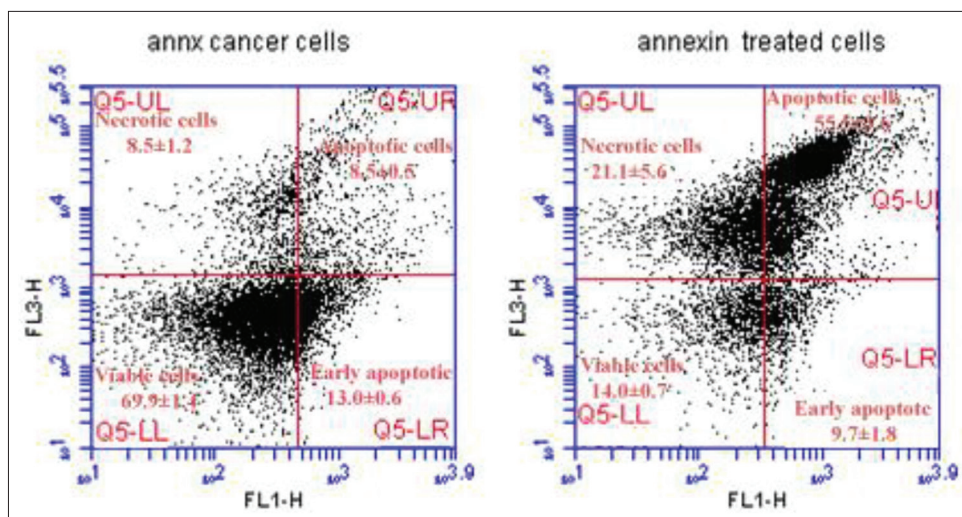


Fig. 2: Quadrant statistical analysis of flow cytometry of Annexin V-FITC. Note, a significant increase in apoptosis of MGF-7 cells (55.5±3.6) % treated by the *Eruca sativa* alcoholic extract for 48 h in compared with untreated MGF-7 cells as control (8.5±0.5)%

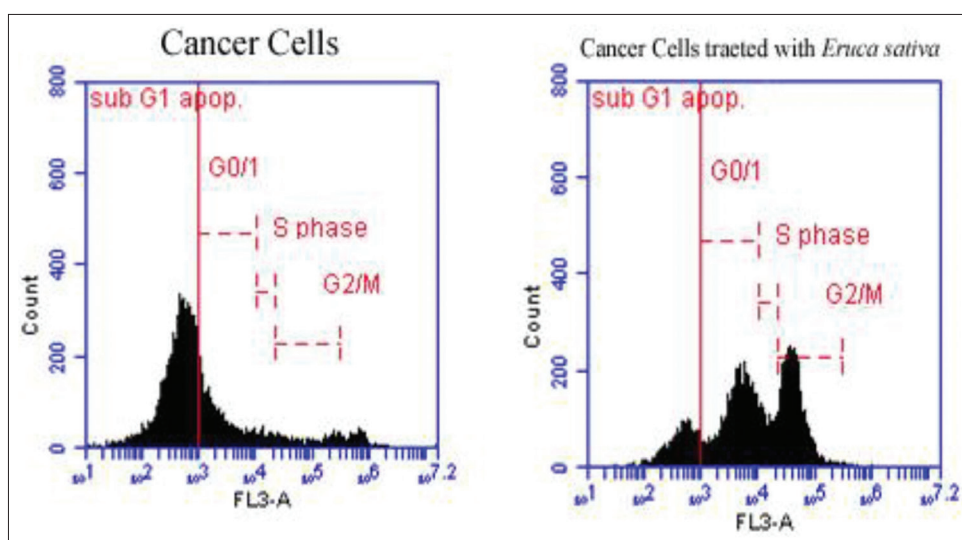


Fig. 3: Assessment of cell-division cycle and induction of apoptosis in MCF-7 cells administered with *Eruca sativa* after 48 h. Cell-division cycle assay, cell-cycle distribution of MCF-7 cells was analyzed by flow cytometry. Note that the inhibition of MGF-7 cell proliferation happened at the G2 and M stage of the cell cycle, as reflected with a larger number of accumulated cells

7.8±1.5% of the comparison ones were cumulated in the G2 and M phases, while 34.2±0.7% of the cells were discovered in the G2 and M phases in the treated cells with *E. sativa*. The mean histogram plot of cell-division cycle analysis also signified that *E. sativa* treatment induced an accumulation of MGF-7 cells in G2 and M phases with the accompaniment of losses from Sub G1 stage. These outcomes implied that *E. sativa* brought about a stopping point in the cell cycle in G2/M phases.

The antioxidant defense system abilities and oxidative degradation were assessed of MCF-7 cells by measuring SOD and CAT which play a key part in the cellular antioxidant defense machinery. These enzymes actions in MCF-7 cell-division line were mining augmented 48 h following *E. sativa* treatment compared with controls (p<0.001) (Table 2).

**DISCUSSION**

Carcinoma of Women’s Breast is still the dominant mode of death worldwide. The carcinoma therapy main objective is to reach maximal remedial destruction of tumor cells utilizing the least concentration of the dose. A significant portion of the work on a plant-based diet and carcinoma prevention gives ground for expecting the virtually helpfully

**Table 2: Effect of *Eruca sativa* on the actions of intracellular enzymatic antioxidant SOD and CAT within MCF-7 cells**

Parameters	MCF-7 cell line	MCF-7 cell line treated	p value
CAT (U/ml)	1.26±0.11	2.14±0.15	<0.001***
SOD (U/ml)	1.64±0.12	2.06±0.11	<0.001***

Data are presented as mean±standard division. Comparison of MCF-7 cell line treated with MCF-7 cell line non-treated as control group. \*\*\*p<0.001 was highly significant

impact of high cruciferous veggies recipes [12]. Here, the incubation of MGF-7 cells with *E. sativa* for 48 h significantly showed reduced cell viability and proliferation. Furthermore, *E. sativa* treatment of MGF-7 cells increased G2/M accumulation increased the actions of intracellular enzymatic antioxidant (SOD and CAT). In general, our result simply that *E. sativa* induces apoptosis and G2/M arrest concomitant with reduced cell viability and proliferation in breast cancer MGF-7 cells line.

The cytotoxic action on mammary tumor cell lines defined agreeable to the dosage values of medications exposure required to diminish survivor



cell lines to 50% (IC<sub>50</sub>). We found that MCF7 cell line treatment with *E. sativa* increased cytotoxic activities as reflected with inhibition of cell proliferation in a time-dependent manner with an IC<sub>50</sub> of 41.13 µg/ml. This may suggest its anticancer potential. These findings confirm with the past study by Michael *et al.* [12] showed that IC<sub>50</sub> (µg/ml) of 70% aqueous ethanolic extract of *E. sativa* on MCF7 cell line was 21.7. While, Azarenko *et al.* [13] indicated that the main isothiocyanate Erucin existed in arugula species (cabbage, cauliflower, kale, arugula, *E. sativa*) were inhibited MCF7 cell proliferation in a time-dependent manner with 50% inhibition the IC<sub>50</sub> that happens at a concentration of 28 mM. Pawlik *et al.* [14] found that MCF7 cells after 96 h of treatment with *E. sativa* IC50 were 9.7 µM. This may suggest the anticancer potential of *E. sativa*.

The arrest of cell-division cycle progression is recognized as a stopping point in the cell-division cycle transition such as G0, G1, S, and G2/M stages for cell reduplication and subdivision. In the current study, *E. sativa* effectively induced G2/M arrest. Hence, cell cycle growth-inhibitory at G2M by *E. sativa* was accompanied by the arrest in mitosis. Since both non-cancerous cell and cancerous cell proliferation is contingent on the cell-division cycle in well-balanced, many composites that act against cancer, brought about an extended mitotic arrest through induced cell G2/M arrest [15].

It illustrated that *E. sativa* might be a suitable candidate as described previously for breast cancer therapy as long as it induced conditions of oxidative stress in MCF-7 cells. There was an enhancement in the actions of SOD and CAT. Our investigation exhibited that *E. sativa* augmented the action of cellular SOD and CAT in 48 h. This may be due to an increase in free radicals contribute to augment in action and plane of enzymatic antioxidant. The antioxidants activity of *E. sativa* is due to the presence of polyphenols contents [5]. The present findings agree with the past study by Alam *et al.* [16] demonstrated that *E. sativa* seeds have antioxidant action and do a preventive impact on Mercury (II) chloride-induced nephrotoxicity. Consistently, Hussein *et al.* [17] reported that the extracts of *E. sativa* in ethanol increasing/preserving the plane of antioxidant molecules and enzymatic antioxidant in rats with an ethanol-induced liver injury.

## CONCLUSION

In summary, the incubation of MGF-7 cells with *E. sativa* for 48 h significantly showed reduced cell viability and proliferation. Furthermore, *E. sativa* treatment of MGF-7 cells increased G2/M accumulation increased the actions of intracellular enzymatic antioxidant SOD and CAT. In general, our results imply that *E. sativa* brings about apoptosis and G2/M arrest concomitant with reduced cell viability and proliferation in breast cancer MGF-7 cells line this may help in clinical pretreatment and protection for breast cancer.

## AUTHORS' CONTRIBUTIONS

All authors participated equally in this research work.

## AUTHORS' FUNDING

This study was self-funded.

## CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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