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# Anticarcinogenic and antimicrobial activity effects of the ellagic acid extract

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ABSTRACT

Recently, plant-derived compounds have been attracted increasable attention as alternative cancer remedies to enhance cancer prevention and healing, and as efficient antimicrobials, because of their low toxicity, low cost and fewer side effects. Ellagic acid (EA) is a natural phenolic constituent; previous studies have reported its antitumor properties when used in in vitro models. In this study, we have investigated the activity of a low concentration of EA against four different human cancer cell lines (SK-N-SH, Caov-3, SW-1088 and BxPC-3) which are very hard in the treatment and there is no available data about EA influence on them. Additionally, the effect of EA has assessed against (*H. Pylori (Helicobacter pylori), P.aeruginosa (Pseudomonas aeruginosa), A. tumefaciens: Agrobacterium tumefaciens (Rhizobium radiobacter)* and *E. herbicola: Enterobacter agglomerans (Erwiniaherbicola)* and two are Gram-positive bacterium *S. aureus (Staphylococcus aureus)* and *C. acnes (Cutibacterium acnes)*) strains which are resistant to the antibiotics. The results suggest that EA may have a potential role as an adjunct therapy for neuroblastoma, ovarian, pancreatic and astrocytoma cancers, in addition to its activity as an antimicrobial agent as it has been proved in this study against *H. pylori, P.aeruginosa, A. tumefaciens, E. herbicola, S. aureus* and *C. acnes strains*.

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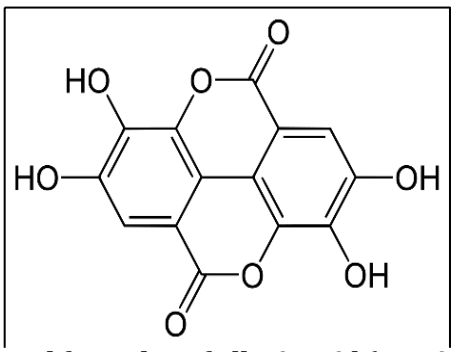
## INTRODUCTION

Understanding the challenge of how the tumour occurs and grows, many natural nutritional products have been used to treat cancer through different mechanisms (Amin *et al.,* 2009). Ellagic acid (EA) one of the natural products established as cancer therapy because this acid inhibits the carcinogenic properties of a variety of chemical compounds including benzo [a] pyrene-7,8-diol-9,10-epoxide, aflatoxin B, N-Methyl-N-nitrosourea, 3-methylcholanthrene, and 7,12-dimethylbenz [a] anthracene (Rossi *et al.,* 1991). EA structure as shown below looks like the dimeric derivative of benzoic acid having four OH groups along with a couple of lactone rings that represent the hydrophilic portion (Amakura *et al.,* 2000, Clifford and Scalbert, 2000).

It naturally exists in its bound form as glycoside derivatives or as Ellagitannins in the nuts (for example walnuts) and fruits (for example raspberries, blueberries, strawberries, grapes, and pomegranates) (kim *et al.,* 2009). Ellagitannins are a class of hydrolyzable tannins that have been considered as anti-oxidant, anti-inflammatory and anti-tumorigenic properties also prevent angiogenesis and genomic instability that cause cancer growth (Stroner *et al.,* 2007, Umesalma and Sudhandiran, 2011). Many studies reported that radiation-induced tumour cell killing but at the same time it elevates the normal tissue toxicity. To overcome this challenge, EA was used to exhibits cytotoxic effects on tumour cells by induced apoptosis within 48h

without or very negligible toxicity to normal cells (Girdhani *et al.,* 2005, Baliga and Triphala, 2010, Bhosle., 2010, Ahire, 2016). Researcher showed the therapeutic effect of EA in the regression of various types of tumours, involving lung cancer, colorectal carcinoma, esophageal cancer, metastatic melanoma, hepatocellular carcinoma, tongue cancer, breast cancer, bladder cancer, endometrial carcinoma, and prostate cancer (Barulmozhi *et al.,* 2010, Park *et al.,* 2005, Zhang *et al.,* 2014, Chen *et al.,* 2015, Farbood *et al.,* 2015. Ceci *et al.,* 2016, Abdelazeem *et al.,* 2017, Ahire *et al.,* 2017) the capability of EA to inhibit these types of cancer cell lines makes this compound a powerful and effective therapy for cancer prevention. The anticarcinogenic properties of EA have drawn increasing attention globally because the main problem of using Ellagic acid as anticancer agents is their poor bioavailability inside the human body. This study revealed the potential role of EA in Neuroblastoma, Ovarian, Astrocytoma and Pancreatic cancer chemotherapy and chemoprevention. However, there is no evidence regarding the effect of EA on these types of cancer. This study first provides cytotoxicity evidence for EA in terms of the inhibition of cell proliferation in SK-N-SH, Caov-3, and SW-1088 and BxPC-3 cancer cell lines. In addition to studies the anticarcinogenic effect of EA, this work included evaluating the antibacterial activity of Ellagic acid toward both gram-positive and gram-negative bacteria.




**Structural formulae of Ellagic acid (Rossi *et al.,* 1991)**

## MATERIALS AND METHODS

### Cell Culture

Human Neuroblastoma Cell Line (SK-N-SH) and Human Ovarian Cancer Cell Line (Caov-3), Human Pancreatic Cancer Cell Line (BxPC-3) were purchased from American Type Culture Collection ATCC (Middlesex, UK), Human Astrocytoma Cell Line (SW-1088) was originally obtained from Sigma Aldrich and stored in the Cell Bank of the Biomedical Research Centre at the University of Salford. SK-N-SH, Caov-3, SW-1088, and BxPC-3 cell lines were used as model cancer cells for this study.

### Cell Maintenance

SK-N-SH cells were maintained in Eagle's Minimum Essential Medium (EMEM) (Gibco, Merelbeke, Belgium) supplemented with 10% fetal bovine serum FBS (Fisher Scientific, USA) and 1% L-Glutamine (Lonza, UK) as well as to 1% Penicillin-Streptomycin-Amphotericin B 100X (Lonza, UK) as antiseptic. Caov-3 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) (Lonza, UK) supplemented with 10% fetal bovine serum FBS and 1% L-Glutamine (Lonza, UK) as well as to 1% Penicillin-Streptomycin-Amphotericin B 100X (Lonza, UK) as antiseptic. SW-1088 cells were maintained in Leibovitz's L-15 medium (Thermo Fisher, UK) supplemented with 10% fetal bovine serum FBS (Fisher Scientific, USA) and 1% L-Glutamine (Lonza, UK) as well as to 1% Penicillin-Streptomycin-Amphotericin B 100X (Lonza, UK) as antiseptic. BxPC-3 cells will be maintained in Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Thermo Fisher, UK) supplemented with 10% fetal bovine serum FBS (Fisher Scientific, USA) and 1% L-Glutamine (Lonza, UK) as well as to 1% Penicillin-Streptomycin-Amphotericin B 100X (Lonza, UK) as antiseptic. A 75 cm2 flask was used to make the cell cultures, and then they were incubated in 5% CO2/ 95% humidified air at 37° C. Once the cells reached 90% confluency, flasks containing SK-N-SH, Caov-3, SW-1088 and BxPC-3 cells were passaged under sterile conditions. 5 ml of phosphate buffered saline solution, or PBS was used to wash the cells and then incubated for 2 min in trypsin solution at a temperature of about 37° C to allow the cells to get detached from the base of the flask. An equal volume of complete growth media was added, and the cell suspension was transferred into a 50 ml conical tube. Centrifugation of cells was done at an rpm of 1200 and time allowed was 3 mins. After centrifugation, the supernatant layer was removed, and the cell pellet was resuspended in fresh supplemented growth media. Cells were then counted under the microscope on a haemocytometer and used as required.

### The Storage and resuscitation of the cell lines

Firstly, the cells in the 75 cm2 flasks were trypsinised followed by the centrifugation of the cell suspension. It was centrifuged for 3 min at about 1200 rpm. Then the resuspension of pellets was done in a 4ml medium which is freezing (Life Technologies, UK) and cryovials were filled with 1ml aliquots (Thermo Fisher Scientific, Loughborough, UK). Firstly, cells were stored for a short period of 24 hours at a temperature of -80° C and then it was stored in liquid nitrogen until required. When the cells were to be reused, they were removed from liquid nitrogen and immediately thawed to 37°C, followed by addition into 10 ml fresh media for

growth. In order to harvest the cells, they were centrifuged, and for the resuspension 25ml, fresh media was used. Finally, the contents were transferred to a 75 cm2 flask and grown.

**Concentration (IC50) by MTT Assay**

The MTT assay was used to assess the effects of ellagic acid on cancer cells viability. A 100 µl from all cells suspensions (SK-N-SH, Caov-3, SW-1088 and BxPC-3) were dispensed into 99 microliters well plates with flat bottoms (Falcon, USA). The concentration was 5 ×103 cells per well and incubated 24h under standard conditions; 4 × 103 cells/well for 48h incubation, and 3 × 103 cells/well for 72h incubation. After 24h, the cells were treated with (0.15, 0.312, 0.625, 1.25, 2.5, 5 and 10µM) of the Ellagic acid extract. The removal of culture medium was done after a recovery period 24h, 48h and 72h. This was followed by incubation with medium containing 30 µl of MTT solution (3 mg/ml MTT in PBS) (3-(4, 5-Dimethylthiazol-2-yl)-2, 5Diphenyltetrazolium Bromide) for about 4h at a temperature of 37° C. After 4h this medium was removed by gentle inversion and tapping onto paper. Control wells received only 100 µl growth media while dimethyl sulfoxide or (DMSO) was added into every single well, then the plates were kept in the dark for 15-20min. The absorbance of each well was measured by a multiscan reader at a wavelength of 540 nm and correcting for background absorbance using a wavelength of 650 nm. The cells' viability was determined according to the optical density (OD) of the wells which contained no Ellagic acid extract. The inhibitory concentration 50% (IC50) was defined as the minimum concentration of the Ellagic acid extract that reduced the viability of the incubated cells after 72h by 50%.

**In vitro antimicrobial susceptibility studies Preparation of microorganism**

In order to study the antimicrobial activity of Ellagic acid extract six different phytopathogenic microorganisms were used in this test, out of these six microorganisms, four are Gram-negative bacterium (H. Pylori (Helicobacter pylori), P.aeruginosa(Pseudomonas aeruginosa), A. tumefaciens: Agrobacterium tumefaciens (Rhizobium radiobacter)and E. herbicola: Enterobacter agglomerans (Erwiniaherbicola) and two are Gram-positive bacterium (S. aureus (Staphylococcus aureus) and C. acnes (Cutibacterium acnes)). Two of the bacteria microorganisms tested (H. pylori and C. acnes) were collected from microbiology laboratory in Al-Yarmouk Teaching Hospital, Iraq while the other four of these bacteria were collected from microorganism's bank in biological resource centre (IBRC), Iran. The pure cultures of this organism were in lyophilised or freeze-dried form; therefore, they were reconstituted by the addition of sterile water. This resulted in the required suspension of microbial cultures. Sterility was also maintained during inoculation, and for this purpose, sterile loops were used to transfer the cells to liquid broth medium. The next step involved the incubation of the liquid cultures to ensure the optimum replication and growth of bacterial cells. Finally, they were stored in a refrigerator for subsequent usage. Time given for incubation was 24 hours, and a thick spread of microbes was obtained for assays. The medium used for testing and maintaining the bacterial strains was Nutrient agar (NA).

**Minimum inhibitory concentration MIC**

Microliter plate assay containing about 96 microliter plate wells was used for the determination of MIC. The serial dilution used for the assay had a ratio of 1:2 (Depaiva *et al.,* 2003). In order to perform the test all, the 96 microliter plate wells were sterilised. A 1:2 serial dilution prepared at 20mg/ml was used in order to evaluate and study the active Ellagic acid extract. Therefore, the final concentrations were 2000, 1000, 500, 250, 125, 62.5, 31.3 and 15.6 µg/ml. The microdilution was performed in 96-well microtiter plates with U-shaped wells. Peptone water was used to make the dilutions of a specific density. The adjustment of density was made to a 0.5 McFarland turbidity. In this way, the final dilution made from bacterial colony was 5 ×105 CFU/ml. Only 0.5ml was taken from the culture medium to make the controls as a negative control, and antibiotic powder dilution of Chloramphenicol and Penicillin were utilized in the test. For filling up of the wells both ethanol 100% and Ellagic acid extract were added in the concentrations of 50 µl and 100µl respectively. Ellagic acid extract was taken from its stock solution and added in the serial two folds' dilution manner. Then the inoculum of 0.5 McFarland bacterial suspension was added in the concentration of 50 µl. In this way, every single well got 5 × 105 CFU/ml of inoculum. Addition of the inoculum was followed by covering of the plates, placing them in a suitable plastic bag and finally incubating them at about 37°C for 24 hrs. The study was performed to determine the lowest concentration of active Ellagic acid which can inhibit the growth of organisms within these wells by visual reading sensitivity towards the extracts of Ellagic acid.

**RESULTS**

**Percentage of cell death of SK-N-SH cell line**

To estimate the effect of EA on SK-N-SH cells viability, SK-N-SH cells were treated with (10, 5, 2.5, 1.25, 0.62, 0.31, 0.051 µM) EA at 24, 48 and 72h (Fig 1) $p < 0.0005$. EA significantly increased the cells death of SK-N-SH at 10 µM (90, 91 and 92.9

%) at 24, 48 and 72h respectively $p < 0.0001$ and 5 µM (82, 83 and 85 %) at 24, 48 and 72h respectively $p < 0.0005$ versus other concentrations as illustrated in figure 2 and 3.

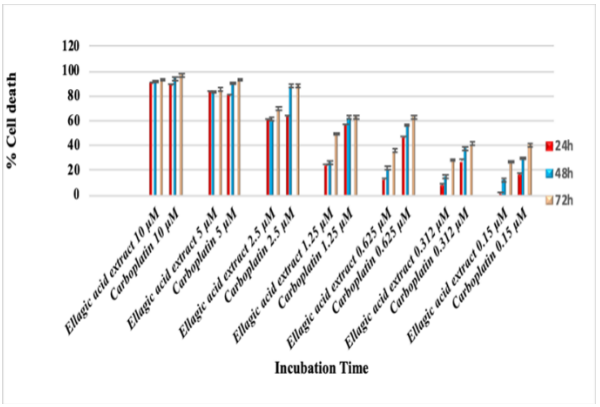



**Figure 1: *In vitro* cell death percentage of the Human Neuroblastoma Cell Line (SK-N-SH) was estimated by MTT assay in 96-well plates following 24, 48 and 72h exposure to 10, 5, 2.5, 1.25, 0.62, 0.31, 0.051 µM Ellagic acid.** Data are shown as % mean ± SEM of cell death for 3 separate experiments. Treatment significantly different from the untreated controls $p < 0.0005$

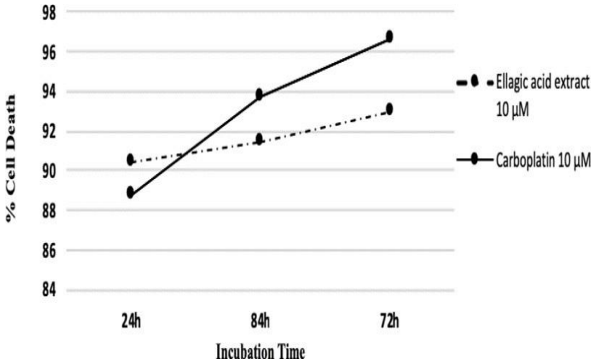



**Figure 2: *In vitro*, the comparison of cell death percentage of the Human Neuroblastoma Cell Line (SK-N-SH) cells were treated with 10 µM EA and 10 µM Carboplatin (control). The absorbance was measured at 540 nm (reference wavelength 650 nm) using a microplate reader.** The results represent the mean ± SEM of 3 independent experiments. EA has a strong inhibition ability for SK-N-SH cells. $p < 0.0001$ at 24, 48 and 72h respectively vs control

**Percentage of cell death of Caov-3 cell line by EA**

Caov-3 cell line showed highly cytotoxicity effect at concentration 10 µM (83 and 91 %) respectively at 48h and 72h as compared to other concentrations which showed low death percentage as illustrates in Figure 4 $p < 0.000001$. Caov-3 cell line treated with EA in concentration 10 and 5 µM compared to the carboplatin control (Fig 5, 6) showed a highly significant difference ($p < 0.00005$, $p < 0.0001$) respectively.

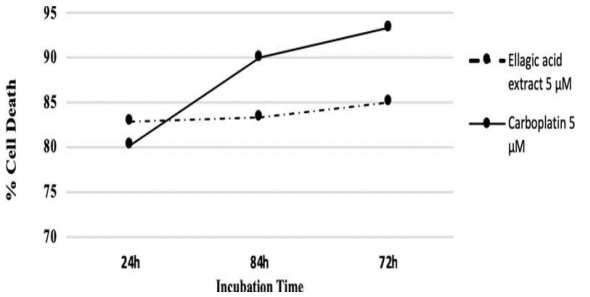



**Figure 3: *In vitro*, the comparison of cell death percentage of the Human Neuroblastoma Cell Line (SK-N-SH) cells were treated with 5 µM EA and 5 µM Carboplatin (control). The absorbance was measured at 540 nm (reference wavelength 650 nm) using a microplate reader.** The results represent the mean ± SEM of 3 independent experiments. EA has a strong inhibition ability for SK-N-SH cells. $p < 0.0005$ at 24, 48 and 72h respectively vs control

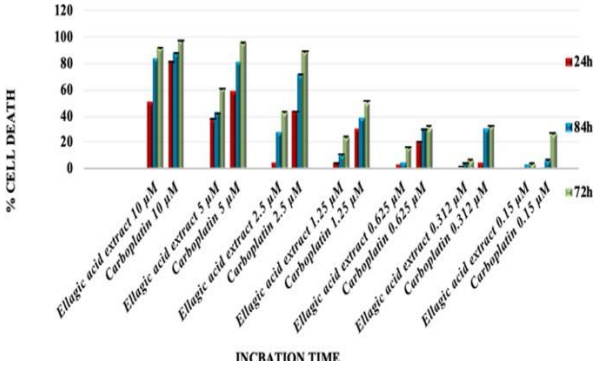



**Figure 4: *In vitro* cell death percentage of the Human Ovarian Cancer Cell Line (Caov-3), was estimated by MTT assay in 96-well plates following 24, 48 and 72h exposure to 10, 5, 2.5, 1.25, 0.62, 0.31, 0.051 µM Ellagic acid.** Data are shown as % mean ± SEM of cell death for 3 separate experiments. Treatment significantly different from the untreated controls $p < 0.00001$

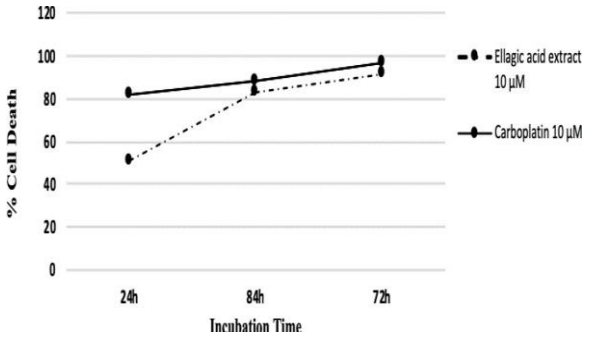



**Figure 5: *In vitro*, the comparison of cell death percentage of the Human Ovarian Cancer Cell Line (Caov-3) cells were treated with 10 µM EA and 10 µM Carboplatin (control). The absorbance was measured at 540 nm (reference wavelength 650 nm) using a microplate reader.** The results represent the mean ± SEM of 3 independent experiments. EA has a strong inhibition ability for Caov-3 cells. $p < 0.00005$ at 24, 48 and 72h respectively vs control

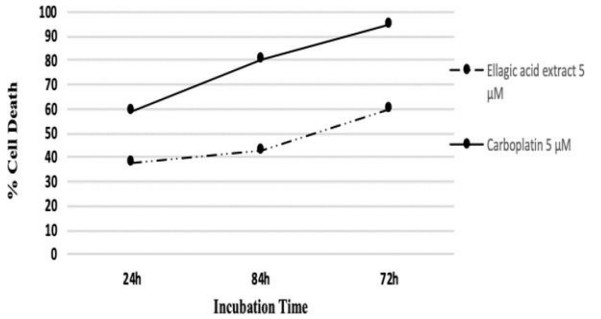



**Figure 6: In vitro the comparison of cell death percentage of the Human Ovarian Cancer Cell Line (Caov-3) cells were treated with 5 µM EA and 5 µM Carboplatin (control). The absorbance was measured at 540 nm (reference wavelength 650 nm) using a microplate reader.** The results represent the mean ± SEM of 3 independent experiments. EA has a strong inhibition ability for Caov-3 cells. $p < 0.0001$ at 24, 48 and 72h respectively vs. control

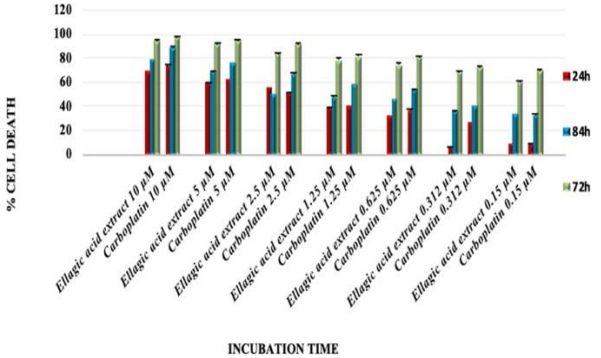



**Figure 7: *In vitro* cell death percentage of the Human Astrocytoma Cell Line (CW-1088) was estimated by MTT assay in 96-well plates following 24, 48 and 72h exposure to 10, 5, 2.5, 1.25, 0.62, 0.31, 0.051 µM Ellagic acid.** Data are shown as % mean ± SEM of cell death for 3 separate experiments. Treatment significantly different from the untreated controls $p < 0.0001$

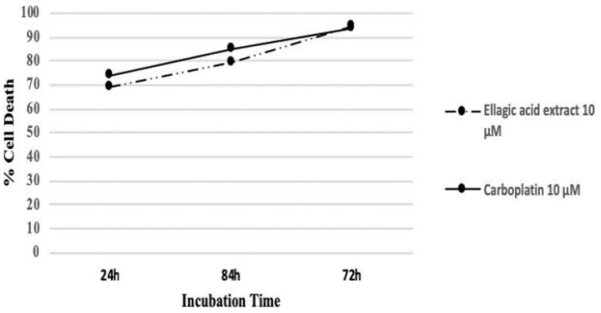



**Figure 8: In vitro the comparison of cell death percentage of the Human Astrocytoma Cell Line (CW-1088) cells were treated with 10 µM EA and 10 µM Carboplatin (control). The absorbance was measured at 540 nm (reference wavelength 650 nm) using a microplate reader.** The results represent the mean ± SEM of 3 independent experiments. EA has a strong inhibition ability for CW-1088 cells. $p < 0.0005$ at 24, 48 and 72h respectively vs control

**Percentage of cell death of CW-1088 cell line by EA**

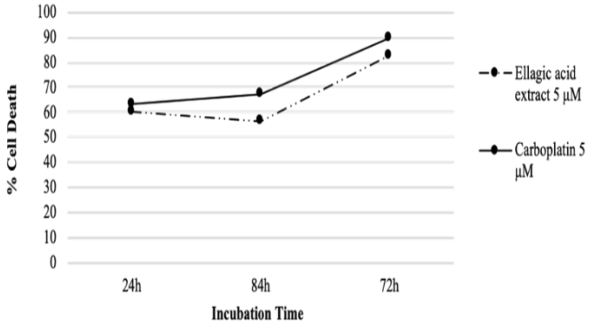



**Figure 9: In vitro the comparison of cell death percentage of the Human Astrocytoma Cell Line (CW-1088) cells were treated with 5 µM EA and 5 µM Carboplatin (control). The absorbance was measured at 540 nm (reference wavelength 650 nm) using a microplate reader.** The results represent the mean ± SEM of 3 independent experiments. EA has a strong inhibition ability for CW-1088 cells. $p < 0.0005$ at 24, 48 and 72h respectively vs. control

To determine the EA effect on CW-1088 cells viability, MTT assay was conducted. The results of the MTT assay showed that 10 µM EA was clearly capable of reducing cell viability after 72h, $p < 0.0001$ (Fig 7, 8, 9).

**Percentage of cell death of BxPC-3 cell line by EA**

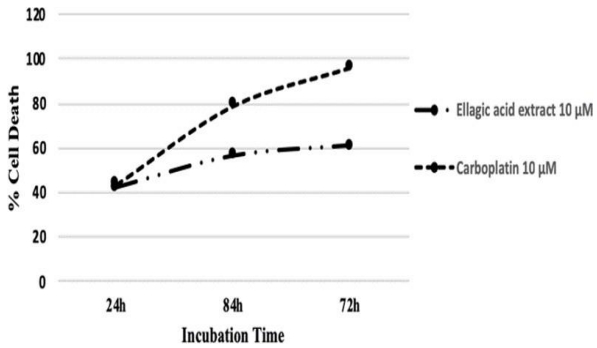



**Figure 10: *In vitro* the comparison of cell death percentage of the Human Pancreatic Cell Line (BxPC-3) cells were treatment with 10 µM EA and 10 µM Carboplatin (control). The absorbance was measured at 540 nm (reference wavelength 650 nm) using a microplate reader.** The results represent the mean ± SEM of 3 independent experiments. EA has a weak inhibition ability for BxPC-3 cells. $p < 0.005$ at 24, 48 and 72h respectively vs. control

The BxPC-3 pancreatic cancer cell line was treated with EA at concentration 10 µM (Fig 10), and the statistical analysis showed the low death percentage (42, 56 and 60.8%) respectively as compared with control at 24, 48 and 72h ($p < 0.005$).

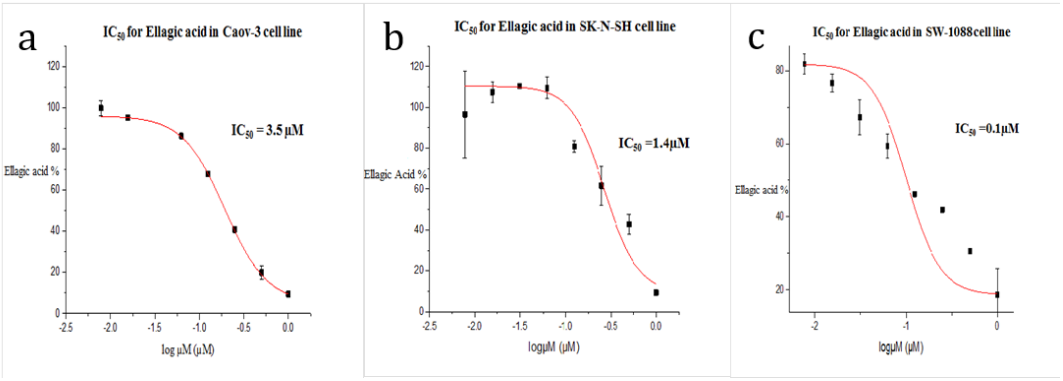



**Figure 11: Dose-response curves of $IC_{50}$ for EA:** a) Caov-3, b) SK-N-SH and c)SW-1088 cells were treated for 72h with 10, 5, 2.5, 1.25, 0.62, 0.31, 0.051 µM dose ranges of EA. The normalized dose response for EA was plotted over log transformed EA concentrations. $IC_{50}$ values were determined using nonlinear regression analysis (Origin 9.1). Error bars represent the standard error of the mean (SEM) for triplicate data

**Table 1: MIC of Ellagic acid against many bacterial isolates using the microtitre plate technique**

| Bacterial Isolates | Serial dilutions of EA (µg/ml) 2000 1000 500 250 125 62.5 31.3 15.6 | MIC (µg/ml) |
|---|---|---|
| *Helicobacter pylori* | 13 | 2000 |
| *Pseudomonas aeruginosa* | 10 9 8 | 500 |
| *Agrobacterium tumefaciens (Rhizobium radiobacter)* | 12 11 | 1000 |
| *Enterobacter agglomerans (Erwinia herbicola)* | 19 17 | 1000 |
| *Staphylococcus aureus* | 16 14 13 12 11 10 9 8 | 15.6 |

**Half Maximal Inhibitory Concentration (IC50) Value of Ellagic acid**

The dose-response curve generated by Origin 9.1 using nonlinear regression analysis for EA in SK-N-SH, Caov-3 and SW-1088 cells is shown in figure (11a, b, and c). The IC50 values were obtained to a range of concentrations of EA from 10, 5, 2.5, 1.25, 0.62, 0.31, 0.051 µM by MTT assay. The results of IC50 for EA were (3.5, 1.4, 0.1 µM) in SK-N-SH, Caov-3 and SW-1088 cells respectively, while IC50 of BxPC-3 was > 9 µM EA.

**Minimum inhibition concentration (MIC)**

In the current study, the MIC of EA against for many kinds of gram-negative and gram-positive bacteria by using microtitre plate assay was variable. The MIC was high against gram-negative bacteria which ranged from 2000µg/ml to 500 µg/ml versus gram-positive bacteria (15.6 µg/ml) as illustrated in Table 1.

**DISCUSSION**

This study has investigated the Ellagic acid as can use for the anticancer drug, so this research has given information in greater detail due to the use of MTT assay to investigate the inhibitory effect of EA on the viability of cancer cells (SK-N-SH, Caov-3, SW-1088, and BxPC-3). Most studies have established that EA may be used in the future as an anticancer drug (Zhang *et al.,* 2014) but the mechanism underlying the bioavailability inside the human body is poorly understood. Several studies reports have provided evidence for a tumour suppressive function of EA; they found that 4, 49-di-O-methyl ellagic acid derivative (4, 49-DiO THEMEA) was 13-fold more effective in the inhibition of colon cancer cell proliferation than another compound in the same family through the 4, 49-DiOMEA modulated Wnt signalling. Also, this derivative was very active against these cells which resistant to the chemotherapeutic agent called 5-fluoracil, while no action was observed in nonmalignant colon cells. Results suggest that structural-activity differences between Ellagic acid and it is derivative (4, 49-DiOMEA) might represent the source for a novel plan in anticancer drug innovation depend on these chemical compounds modifications (Ana *et al.,* 2015). The ant proliferative action of EA could be explained by its ability to suppress the DNA binding of certain carcinogens, including nitrosamines (Mandal *et al.,* 1988, Mandal and Stoner, 1990), also polycyclic aromatic hydrocarbons (Teel *et al.,* 1986). Girdhani *et al.,* 2005 investigated the mechanism of action of several anticancer and antioxidant agents, including EA, on both cancer cells and normal to build up efficient protocols in practical radioprotection and cancer

radiotherapy. They found that EA has cytotoxic effects involving oxidative damage, membrane change, and damage of DNA when combined with ionizing radiation on tumour cells (in vitro study). Further research indicated that EA could suppress the radiation-induced activation of tyrosine kinases receptor (TKR) plus NF-κB signalling, also can alter the cell survival, DNA repair efficacy and may possible ceramide signalling (Girdhani *et al.,* 2005). Rocha *et al.,* 2012 revealed that pomegranate juice or a mixture contain EA component increases breast cancer cell attachment and reduces cancer cell migration. Also, the reported that cytokines which consider pro-inflammatory molecules are significantly reduced by this natural product causing decrease inflammation and inhibit cancer development (Rocha *et al.,* 2012). EA exerts chemo-preventive effects on colon carcinogenesis, and it decreases TGF-β and IL-6 levels on the prostatic cancer cells line called LNCaP (Vanella *et al.,* 2013).

Several studies investigated the best IC50 and concentrations of Ellagic acid which can kill the cells in different lines and the action mode of cytotoxicity like Osteogenic Sarcoma Cell line called CRL1343 in concentration 4-100 µg/mL (IC50 =6.5 µg/mL). In this cell line, the apoptosis was Induced by up-regulating Bax and activating caspase-3 (Han *et al.,* 2006). Pancreatic cancer Cell line (MIA PaCa-2, and PANC-1) in concentration 10-50 mM, stimulates the mitochondrial pathway of apoptosis-related with mitochondrial depolarisation, cytochrome C release and downstream caspase activation (Edderkaoui *et al.,* 2008). That is mean in this study we need high concentration more than 10mM of Ellagic acid to kill a half number of cells. Therefore there is no IC50 curve will be produced.

Finally, ovarian carcinoma Cell line (ES-2 and PA-1) in concentration 10-100 µM, elevates p53 and Cip1/p21genes, decreases cyclin D1 and E levels and induces caspase-3-mediated apoptosis via rising the Bax/Bcl-2 ratio (Chung *et al.,* 2013).

According to our study, noticed that 10 µM at 72h EA has the ability to kill the many cancer cell lines including Human Neuroblastoma Cell Line (SK-N-SH), Human Ovarian Cancer Cell Line (Caov-3), Human Astrocytoma Cell Line (SW-1088) (Fig 1-9) with very low concentration in IC50 as show in figure 11. In contrast, EA has a low ability to inhibited BxPC-3 pancreatic cancer cell line at 10 µM by 60% after 72h (Fig10). Our results go in line with another study which found that EA at high concentrations between (10-50 mmol/L) be able to excite the cell death and completely inhibit the proliferation of the human pancreatic adenocarcinoma cells (MIA PaCa-2 and PANC) (Edderkaoui *et al.,* 2008). While our data did not agree with a new study which indicated that EA at low concentrations (0.5-3 µM) triggers the same effect (apoptosis and reduces the proliferation) of the human pancreatic cancer cell (MIA PaCa-2 and HPAF-II). In addition, in vivo study of EA reported that use it alone can reduce the size and cellularity of pancreatic cancer in a mouse model (Edderkaoui *et al.,* 2013).

Our data investigate the antimicrobial effect of Ellagic acid against gram negative and positive bacteria. According to the Table 1, the gram-positive was more sensitive to inhibition by low concentration of EA (15.6 µg/ml compared to gram-negative bacteria especially H. pylori (2000 µg/ml) , this agreement with recent paper which report that MIC of Ellagic acid ranged from 5 to 30 mg/L against fifty-five of H. pylori strains causing kill of this bacteria ( in vitro model), as well as repair the damage of gastric mucosal induced by H. pylori (De *et al.,* 2018). Other study found that Ellagic acid has biological activity against Staphylococcus epidermis, Bacillus cereus, Klebsiella pneumonia and Salmonella typhi) using the plate-agar method. The minimum concentration dose of EA was 0.15 mg/ml, whereas the minimum effective doses of streptomycin and gentamycin were 0.3 mg/ml and 2 mg/ml, respectively (Kadhem *et al.,* 2010).

This activity attributed to the presence of polyphenols which be effective against gram negative and positive bacteria (Nikitina and Kusmina, 2007) or in tannin, Ellagic acid may induce complexity with substrate or enzyme in microbe's cell. EA biological activity may be associated with its action on the bacterial cell membranes. In addition to the ability of EA to form complexes with the essential metals in bacteria cell leading to the death (Misanori *et al.,* 2001).

In summary, our study gives important information about the EA effect on cancer cells. The results presented here suggest that EA might be valuable in the treatment of these types of cancer because of its properties of a growth suppressor for the specific killing of the tumour cells. More studies are needed; for example, the cellular mechanism of action of EA mediated apoptosis of cells, in addition, the EA-induced apoptosis also needs to be investigated in suitable vivo models.

## CONCLUSION

These results suggest that EA may have a potential role as an adjunct therapy for neuroblastoma, ovarian, pancreatic and astrocytoma cancers, in addition to its activity as an antimicrobial agent as it has been proved in this study against H. pylori, P.aeruginosa, A. tumefaciens, E. herbicola, S. aureus and C. acnes strains.

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