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POMEGRANATE EXTRACT REDUCES VIABILITY AND CELL GROWTH OF HUMAN PANCREATIC CANCER CELLS *IN VITRO*

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BY

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THESIS

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POMEGRANATE EXTRACT REDUCES VIABILITY

AND CELL GROWTH OF HUMAN PANCREATIC

CANCER CELLS *IN VITRO*

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THE UNIVERSITY OF TEXAS AT AUSTIN, 2010

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Pomegranate extract (PE) is a standardized whole fruit extract of pomegranate (*Punica granatum* L.), a fruit with known anti inflammatory and anti cancer properties. In the present study, the effects of PE on two different cell lines of human pancreatic cancer cells, AsPC-1 and PANC-1 were examined. Both cell lines are epithelial cancers but differ morphologically and in their response to treatment with PE. PE inhibited proliferation of both cancer cell lines in a dose dependent and time dependent manner. The reason for this was the potency of PE to cause significant cell cycle arrest in the pancreatic cancer cells, which was noted to be cell specific. AsPC-1 was arrested in G_2 phase while PANC-1 was arrested in G_0/G_1phase of the cell cycle. PE also checked the viability of confluent cancer cells in a concentration dependent manner. In case of AsPC-1 this was due to the action of PE to induce apoptosis, as demonstrated by the Annexin-V assay. In case of PANC-1, a highly invading cancer greatly affected by the presence of

surface stem cell markers $CD44^{+}/CD24^{+}$, PE was capable of modifying the stem cell markers and induce an increase in the non tumorigenic sub population of cells. A study was also conducted to look at the action of PE in causing inhibition of pancreatic cancer cell proliferation, in comparison to a clinically used chemotherapeutic agent, Paclitaxel. The results indicated that the effect of PE, at clinically feasible doses was superior to that of clinically used doses of Paclitaxel. This data suggests that PE, which is proven to be a safe dietary supplement, looks promising in the prevention and treatment of human pancreatic cancer cells without the side effects of standard drug therapy and could possibly have a role in controlling stem cancer cell population

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Introduction

In this past decade a lot of emphasis has been laid on pancreatic cancer research and this is in view of the case fatality rate of this particular cancer. Although not very prevalent, pancreatic cancer is one of the most lethal of all cancers. It is the fourth most leading cause of deaths due to cancer in both genders. The median survival rate after diagnosis is 3-6 months and the five year survival rate is less than 5% (WHO, 2009). The major reason for such high death rate is that pancreatic cancer usually presents with symptoms very late in the course of the disease and moreover these symptoms are vague like abdominal pain, back pain, fatigue, diarrhea and depression, making diagnosis really difficult (NCI, 2002). In addition to the problem with diagnosis, pancreatic cancer is unyielding to treatment by known chemotherapeutic agents. 5-Fluorouracil and recently, Gemcitabine are the only two chemotherapeutic agents, successfully used to treat pancreatic cancer, but like any other chemotherapeutic agent; these also present with a lot of side effects, leading to reduced patient compliance (El Kamar *et al*., 2003). This definitely calls for an urgent need to look for other means to control pancreatic cancer without affecting patient compliance. Phytochemicals, being natural products are safer and have lesser side effects than routine chemotherapeutic agents. Pomegranate (*Punica granatum*) is a fruit used in many cultures (the genus name, *Punica*, is derived from the Roman name for Carthage, where the best pomegranates were known to grow). The pomegranate tree is native to the region of Persia and is now cultivated over the entire Mediterranean area, Asia, and America. Pomegranate extract consists of a mixture of various phytochemicals, the most important being punicalagins, which are unique pomegranate tannins with free-radical scavenging properties in laboratory experiments. It has been used in various cultures for its culinary as well as therapeutic values, over several millennia (Bilderback L, 2007; Kulkarni *et*

al., 2007). Studies have demonstrated that pomegranate possesses anti-inflammatory, anti-hypertensive, and anti-diabetic properties (Larossa *et al.*, 2009; Xu *et al.*, 2009). Also some recent work has shown that pomegranate induces apoptosis in animal models of prostate cancer as well as inhibits invasion of human breast cancer cells (Khan *et al.*, 2009; Rettig *et a.l*, 2008). These qualities of pomegranate make it a good candidate for treatment of pancreatic cancer without the usual side effects of standard chemotherapeutics.

In the present study we analyzed the action of standardized extract of whole pomegranate fruit on the growth and viability of human pancreatic cancer cells *in vitro*. Two different human cancer cell lines PANC-1 and AsPC-1 were examined. Our lab is the first to study the effect of standardized, commercially available and certified safe pomegranate extract (PE) on these two human pancreatic cancer cell lines. We demonstrate that PE effectively inhibits growth and viability of human pancreatic cancer cells by inducing cell cycle arrest, apoptosis and modifying the cell surface glycoproteins CD44 and CD24. We also compared the effect of PE to that of Paclitaxel, a clinically tried chemotherapeutic agent, on the pancreatic cancer cell proliferation

Materials and Methods

Materials- POMELLA, an HPLC-standardized extract of pomegranate that retains the natural polyphenolic ratio of whole pomegranate fruit, was the received from Verdure Sciences (Noblesville, IN). Fetal bovine serum, glutamine, RPMI-1640 medium, trypsin/EDTA and phosphate-buffered saline (PBS) were obtained from Hyclone (Logan, UT). Dimethyl sulfoxide (DMSO), ribonuclease, TritinX-100, propidium iodide, sulforhodamineB (SRB), tricholoroacetic acid and Tris-base buffer were purchased from Sigma Chemical (St. Louis,MO). CD44 and CD24 antibodies were bought from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Annexin V kit used was from BD Biosciences.

Human Pancreatic cancer cell lines - The two cancer cell lines were obtained from ATCC (Manassas, VA). PANC-1 is a pancreatic ductal carcinoma obtained from a 56 yr old Male Caucasian patient. AsPC-1 is a pancreatic adenocarcinoma derived from a metastatic site (ascites) in a 62 yr old Female Caucasian patient. These two cell lines have different phenotypic and genotypic properties (Deer *et al*, 2010). PANC-1 has greater capacity to adhere and invade cells and is comparatively resistant to apoptosis, as seen from our experiments. AsPC-1 on the other hand grows faster and forms larger tumors. The PANC-1 and AsPC-1 cell lines were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum and 2mM glutamine and maintained in monolayer culture at 37°C and 5% CO_2 in a humidified incubator. Cells were passed twice weekly using 0.05% trypsin.

Proliferation and cytotoxicity assays- For the proliferation assays, PANC-1 cells were passed into 24- well plates at 5,000 cells per well and AsPC-1 cells were passed at 2,000 cells per well and allowed to grow for 24 hr. After this the control plate was treated with DMSO while the

other plates were treated with serial concentrations of PE and observed for changes every 24 hr for 96 hr. For the cytotoxicity assay, PANC-1 cells were passed 20,000 cells per well and AsPC-1 cells were passed 5,000 cells per well and grown to confluence of 80% over 2 days before adding treatment with DMSO or PE as indicated. Proliferation and cytotoxicity were measured using SRB assay, which has been validated to accurately reflect viable cell number (Skehan *et al*., 1990). Briefly following incubation, cells were fixed by adding 10% TCA for 60 minutes at 4°C. The plates were rinsed with dH_2O, dried overnight, and stained with 0.4% SRB for 30 minutes at room temperature. Plates were washed with acetic acid to remove unbound dye and blotted dry. The bound dye was solubilized by adding 1 ml 10 mM Tris base buffer. Optical density of the dye was measured on a microplate reader at a wavelength of 564 nm. DMSO at the concentrations used had no effect on cell viability.

Cell cycle analysis- To determine the cell cycle distribution, cells were grown to confluence on 6 well plates and treated with DMSO (control) or serial concentrations of PE for 24 hr. Each sample of 1 x 10^6 cells was trypsinized, washed with PBS, fixed in 1% methanol- free formaldehyde for 20 minutes in ice. The cell suspension was added to 70% ice-cold ethanol overnight. Cells were pelleted and stained with 50 µg/mL propidium iodide, 0.1mg/mL RNAse and 0.05% TritinX-100 for 45 minutes at 37°C. Cell cycle analysis of the stained cells was performed immediately with FACS-Calibur (BD, Franklin Lakes, NJ). A minimum of 10,000 cells per sample were used for the assay and analysis was conducted with Flowjo software.

Annexin-V assay- The cells were grown to confluence in 6 well plates and treated with DMSO (control) or serial concentrations of PE for 24 hr. Each sample of 1 x 10^6 cells was trypsinized and washed with PBS twice. Each sample was stained with Annexin V (FITC) (10 µL in 100 µL

of buffer) for 15 minutes and counterstained with 7-AAD (10 µL in 390 µL of buffer). The stained cells were analyzed immediately with FACS-Calibur to look for apoptosis.

CD24/CD44 Assay- The cells were grown to confluence in 6 well plates and treated with DMSO (control) or serial concentrations of PE for 24 hr. Each sample of 1 x 10^6 cells was trypsinized and washed with PBS twice. Each sample was stained with 5µL of CD44 and 20µL of CD24 in 100 µL of BSA and placed on ice for 60 minutes. The stained cells were washed and dissolved in PBS and analyzed with FACS-Calibur.

Statistics- Data is presented as the mean of triplicate or quadruplicate determinants with standard error. Statistical analysis was performed to assess the difference between the means of the untreated and treated samples using two tailed student's t-test with SPSS statistical software and GraphPad Prism software. A p value of less than 0.05 was considered statistically significant.

Results

Effect of PE on the proliferation and viability of PANC-1 and AsPC-1 cells

As shown in Figure 1A, PE causes a dose dependent decrease in the growth of both the cell lines of human pancreatic cancer cells with a half maximal inhibitory concentration (IC_{50}) of approximately 100 µg/mL. As shown in Figures 1B and 1C, PE is also potent in inhibiting proliferation in both cell lines in a time dependent manner reaching IC_{50} in approximately 48 hr. Likewise PE effectively suppresses viability in both cancer cell lines in a dose dependent manner as seen in Figure 2, with an IC_{50} of approximately 200 µg/mL.

Effect of PE on cell cycle arrest in PANC-1 and AsPC-1

PE causes a cell dependent arrest in the cancer cell lines. As seen in Figure 3A, in the case of AsPC-1 PE causes cell cycle arrest in G_2 phase while PANC-1 is arrested in G_0/G_1 phase of cell cycle (Figure 3A) indicating it involves different mechanisms in its action on the two cell lines. The action of PE is dose dependent with its potency increasing with dose and it does this at sub lethal concentrations. It is noted that PE causes more potent cell cycle arrest in PANC-1 cells than AsPC-1.

Role of PE in inducing apoptosis in pancreatic cancer cells

To look at the effect of PE on apoptosis in the pancreatic cancer cells, Annexin-V assay was used. As seen in Figure 4, PE significantly induces apoptosis in AsPC-1 cancer cells in a dose dependent manner and this dose in much higher than the dose required to cause cell cycle arrest. PE does not induce apoptosis in PANC-1 cancer cells.

Effect of PE on CD44/CD24 cell surface glycoproteins

To study how PE controls PANC-1 cancer cells without inducing apoptosis, we examined its effect on CD44/CD24 cell surface markers. As shown in Figure 5, PE modifies cell surface glycoproteins in dose dependent manner by increasing the $CD44^{-}/CD24^{-}$ population with increasing concentrations.

Effect of PE in comparison to Paclitaxel on cell proliferation

To compare the effects of PE with that of chemotherapeutic Paclitaxel, an SRB assay was performed. The concentration of Paclitaxel used is in conjunction with the dose used in clinical trials, 300nM being the highest concentration used. As seen in Figure 6 (A-F), compared to Paclitaxel, PE has a more potent effect on reducing cell growth both in a dose dependent and time dependent manner. In the dose response data, with AsPC-1 PE attains IC_{50} at 50µg/mL in 48 hr while Paclitaxel fails to attain IC_{50}in 48 hr. In case of PANC-1 cells, both PE and Paclitaxel reach IC_{50} at approximately 50µg/mL and 50 nM respectively, but Paclitaxel does not induce any further reduction in cell growth with increasing concentrations unlike PE, which reduces cell growth to less than 20% at 250 µg/mL in 48 hr. In the time response data, PE seems to have a greater effect on cell growth inhibition in both the cell lines when compared to the effect of Paclitaxel.

Discussion

Pancreatic adenocarcinoma remains to have a grim prognosis and life expectancy despite increased research in this area (Janes RH *et al*.,1996). There are no screening specifications because of vague presentations and unavailability of any specific screening techniques. Even resectable pancreatic tumors with comparatively better prognosis, have a 3 year survival rate of only 15 % (Calle EE *et al*., 2003). Post operative maintenance with chemotherapy and chemoradiation has debatable results (Kalser MH *et al*.,1985; Klinkenbijl JH *et al*., 1993). The best result obtained with post operative chemoradiation and chemotherapy with two drugs was a 5 year survival rate of 10% (Neoptolemos JP *et al*., 2004). There have been various attempts to look into the mechanisms involved in the unyielding nature of pancreatic cancer. Pancreatic cancer stem cell research has been able to identify a sub population of pancreatic cancer cells with $CD44^+CD24^+/ESA^+$ surface markers, which show stem cell properties of self renewal, ability to produce increased progeny and increased expression of the developmental signaling molecule sonic hedgehog (Li C *et al*, 2007), unlike the scenario observed in mammary cancer cells where the stem cancer sub population is $CD44^-/CD24^+$ (Wright MH *et al*, 2008). This sub population with $CD44^+/CD24^+/ESA^+$ surface markers showed 100 fold increased tumorigenic potential when compared to non tumorigenic cells. 75% of the mice injected with $CD44^+/CD24^+/ESA^+$ cancer cells showed tumor formation compared to none injected with $CD44^-/CD24^-/ESA^-$ cancer cells of equal number (Li C *et al*, 2007).

Phytochemicals, the natural chemicals obtained from fruits and vegetables could be an alternate source for chemoprevention and chemotherapeutics in human cancer treatment. Several of these phytochemicals have been established to be effective in inhibiting cancer cell growth *in vitro* and check formation of tumors *in vivo* (Amin R *et al*., 2009). Moreover phytochemicals

being natural compounds are better tolerated and are exempt of side effects of standard chemotherapeutic drugs. Very few studies have been conducted to look at the effects of phytochemicals in the treatment of human pancreatic cancer (Awale S *et al*., 2008; Li J *et al*., 2009). We hypothesized that whole pomegranate extract inhibits growth and viability of human pancreatic cancer cells *in vitro*. To test this hypothesis, in the present study we used two human pancreatic cancer cell lines PANC-1 and AsPC-1, which have different phenotypic and genotypic properties (Deer *et al*, 2010). AsPC-1 is a pancreatic adenocarcinoma derived from a metastatic site while PANC-1 is a pancreatic ductal carcinoma. Being epithelial cancers, both the cell lines are aggressive in nature and difficult to treat. AsPC-1 grows fast and forms large tumors while PANC-1 forms aggressively invading tumors (Deer *et al*, 2010). PANC-1 cells have been demonstrated to form spheres, which indicates increased invasion capacity in vitro and also have been shown to have a higher expression of stem cell markers $CD44^+/CD24^+/ESA^+$ (Gaviraghi M et al., 2010). In our studies we used PE, which is a standardized extract of pomegranate that is commercially available as a dietary supplement and is certified 'generally regarded as safe' by the Food and Drug Administration. The extract is standardized by HPLC to contain no less than 70% total polyphenolic compounds, and the final polyphenolic composition is similar to that of whole pomengranate fruit (Mertens- Talcott *et al*., 2006). Research in the past has shown pomegranate juice and seed oil to have a role in controlling proliferation and metastasis of various differentiated mouse and human breast cancer cell lines *in vitro* (Jeune *et al*., 2005; Kim *et al*., 2002; Toi *et al*., 2003; Dai *et al*., 2010). Previous studies have also demonstrated the action of pomegranate extracts in inhibiting proliferation of other cancer cell types *in vitro*, including lung, prostate, colon and oral cancer (Khan *et al*., 2007; Albrecht *et al*.,

224; Sreeram *et al*., 2004; Adams et al., 2006; Rettig *et al*., 2008). Our study is the first to examine the effect of PE on PANC-1 and AsPC-1 human pancreatic cancer cell lines.

As indicated in Figure 1A, PE potently inhibits cell growth in AsPC-1 cancer cell line in a dose dependent manner with an IC_{50} of 100µg/mL. PE also demonstrates a time dependent decrease in AsPC-1 cell proliferation reaching IC_{50} in less than 48 hr at a concentration of 150µg/mL (Figure 1B). This action of PE is further justified by the flow cytometry data showing that PE causes significant cell cycle arrest of AsPC-1 in G_2 phase and it does so in a dose dependent manner (Figure 3A). Figure 2 demonstrates PE to be significantly effective in controlling viability of AsPC-1 in a dose dependent manner. This is consistent with the Annexin-V data which elicits the action of PE to induce apoptosis of AsPC-1 cell population in a dose dependent manner (Figure 4). We further compared the action of PE to a known chemotherapeutic, Paclitaxel in controlling cell growth in AsPC-1. According to the results shown in Figure 6, the inhibitory action of PE at clinically feasible doses gives better results when compared to clinically used doses of Paclitaxel. Thus our data indicates that PE can induce cell death and control growth of human pancreatic adenocarcinoma *in vitro* and its comparability to clinically used chemotherapeutic drug indicates its likely benefits without the standard side effects of synthetic drugs.

Figure 1 A and 1C illustrate the role of PE in controlling the growth of PANC-1 cells in a time dependent and dose dependent manner (IC_{50} less than 100 µg/mL). This action is consistent with the cell cycle analysis data which shows that PE induces significant G_0/G_1 arrest in PANC-1 cells in a dose dependent manner (Figure 3B). As indicated in figure 2, PE also significantly stops cell viability in PANC-1 cells in a dose dependent manner, but this data could not be validated with Annexin-V data since PE failed to induce apoptosis in PANC-1 cells. This

brought us to conclude that the action of PE on viability of PANC-1 depends on an alternate mechanism. PANC-1 cells form extremely invading tumors as earlier demonstrated by their capacity to form spheres and also show increased expression of stem cell markers (Gaviraghi M et al., 2010). Hence we decided to examine the cell markers CD44 and CD24 in PANC-1 cancer cell population. As seen in Figure 5, PE modifies the cell markers to cause a significant increase in the non tumorigenic cell sub population with $CD44^{-}/CD24^{-}$ (Li C *et al*, 2007). Comparative study with Paclitaxel demonstrates PE to have a superior action in reducing cell growth in PANC-1 at clinically feasible doses (Figure 6). Our data thus indicates that PE can inhibit cell growth and curb tumorigenicity in highly invading ductal cancers like PANC-1 and could possibly have a role in controlling pancreatic cancer stem cells.

To summarize, our present study validates PE as a potent inhibitor of cell growth in human pancreatic epithelial cancer *in vitro*. Our data also indicates that PE significantly induces apoptosis in certain pancreatic cancers and controls potency of highly tumorogenic pancreatic cancers by modifying critical cell surface markers. This action of PE on the cell markers could likely affect the stemness in pancreatic cancer, a possibility which needs to be further studied. Comparative studies with clinically tried chemotherapeutics also indicate the likely clinical application of PE in the near future without the adverse effects of chemotherapeutics, thus increasing patient compliance and decreasing post chemotherapeutic morbidity. This is the first study recognizing the role of PE as a possible chemopreventive and chemotherapeutic agent. Further study will focus on the effects of PE on death receptor pathways which are highly expressed in human pancreatic cancer cells and its action on pancreatic tumor growth *in vivo*.

Figure Legends

Figure 1- Proliferation Assay- The cancer cells were grown on 24- well plates, PANC-1 cells at 5,000 cells per well and AsPC-1 cells at 2,000 cells per well for 24 hr. The cells were treated with serial concentrations of PE and observed for changes every 24 hr for 96 hr. Proliferation was measured using SRB assay. **A-**Shows the effect of PE on the growth of pancreatic cancer cells with serial concentrations of PE after 72 hr of treatment. **B-** Shows the time dependent effect of PE on the growth of pancreatic cancer cells over a period of 96 hr at a constant dose of 150µg/mL.

Figure 2- Cytotoxicity (Viability) Assay- The cancer cells were grown to confluence of 80% over a period of 2 days before being treated with serial concentrations of PE and observed for changes after 48 hr. Cytotoxicity was measured using SRB assay.

Figure 3- Cell Cycle Arrest- Cancer cells were grown to confluence on 6 well plates and treated with DMSO (control) or serial concentrations of PE for 24 hr. Trypsinized and washed cells, fixed with 1% methanol- free formaldehyde were pelleted and stained with 50 µg/mL propidium iodide, 0.1mg/mL RNAse and 0.05% TritinX-100 for 45 minutes at 37°C. Cell cycle analysis of the stained cells was performed immediately with Accuri. A minimum of 10,000 cells per sample were used for the assay and analysis was conducted with Flowjo software. **A-** Shows the effect of serial concentrations of PE on the cell cycle of AsPC-1 after 24 hr of treatment. **B-** Shows the effect of serial concentrations of PE on the cell cycle of PANC-1 after 24 hr of treatment.

Figure 4- Annexin- V Assay- The cells were grown to confluence in 6 well plates and treated serial concentrations of PE for 24 hr. Cells were trypsinized, washed and stained with Annexin V

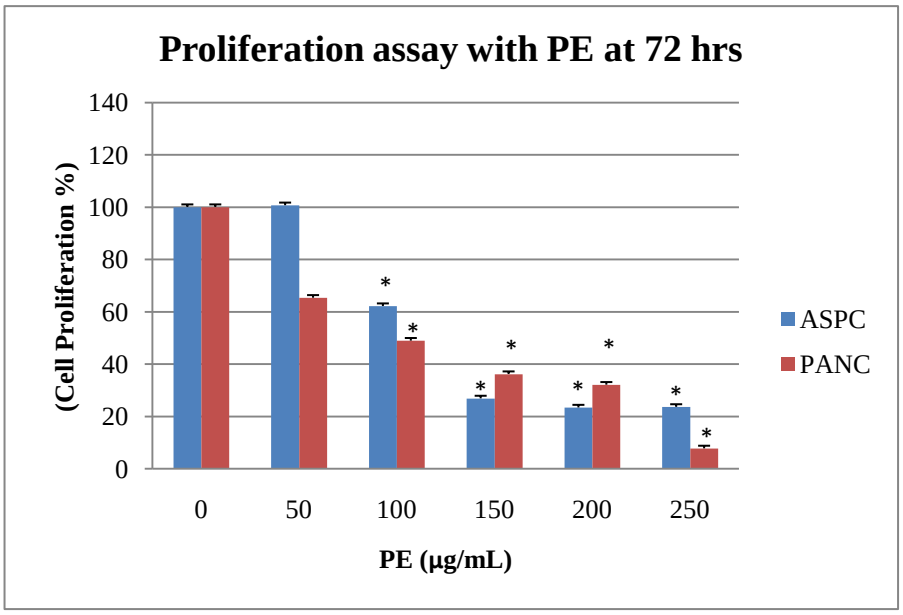
(FITC) (10 µL in 100 µL of buffer) for 15 minutes and counterstained with 7-AAD (10 µL in 390 µL of buffer). The stained cells were analyzed with Accuri.

Figure 5- CD44/CD24 Cell Surface Markers- The cells were grown to confluence in 6 well plates and treated serial concentrations of PE for 24 hr. Cells were trypsinized, washed and stained with 5µL of CD44 (labelled with APC) and 20µL of CD24 (labelled with FITC), in 100 µL of BSA. The stained cells were washed and dissolved in PBS and analyzed with Accuri.

Figure 6- Comparative studies with Paclitaxel- - The cancer cells were grown on 24- well plates, PANC-1 cells at 5,000 cells per well and AsPC-1 cells at 2,000 cells per well for 24 hr. The cells were treated with serial concentrations of PE or Paclitaxel and observed for changes every 24 hr for 72 hr. Proliferation was measured using SRB assay. **A**- Shows the effect of PE on the growth of AsPC-1 cancer cells with serial concentrations of PE after 48 hr of treatment. **B**- Shows the effect of PE on the growth of AsPC-1 cancer cells with serial concentrations of Paclitaxel after 48 hr of treatment. **C**- Shows the effect of PE on the growth of PANC-1 cancer cells with serial concentrations of PE after 48 hr of treatment. **D**- Shows the effect of PE on the growth of PANC-1 cancer cells with serial concentrations of Paclitaxel after 48 hr of treatment. **E**- Shows the time dependent effect of PE (100 µg/mL) and Paclitaxel (100 nM) on the growth of AsPC-1 cancer cells over a period of 72 hr. **F**- Shows the time dependent effect of PE (100 µg/mL) and Paclitaxel (100 nM) on the growth of PANC-1 cancer cells over a period of 72 hr

Figure 1.

A)


Proliferation assay with PE at 72 hrs
(Cell Proliferation %)
140
120
100
80
60
40
20
0
0
50
100
150
200
250
PE (µg/mL)
ASPC
PANC

B)

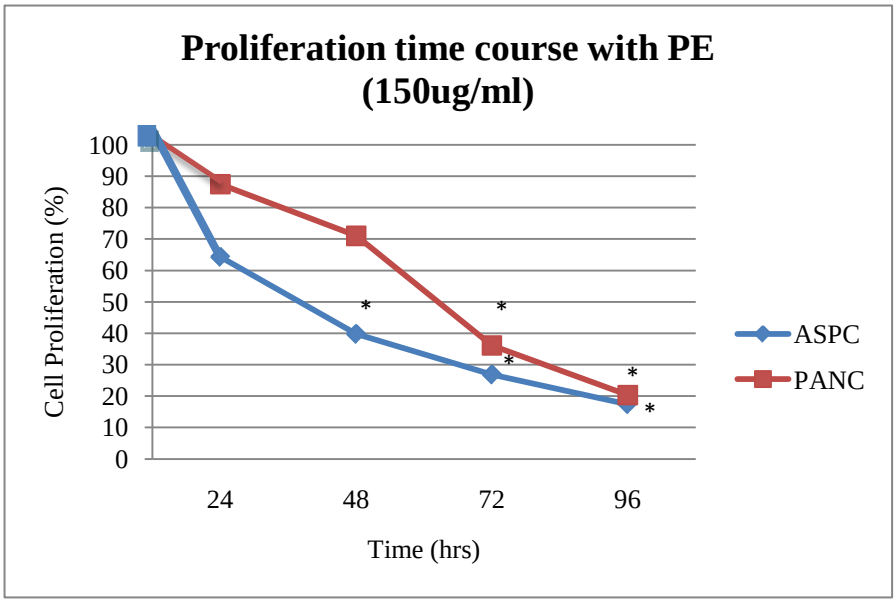

Proliferation time course with PE (150ug/ml)
Cell Proliferation (%)
100
90
80
70
60
50
40
30
20
10
0
24
48
72
96
Time (hrs)
ASPC
PANC

Figure 2.

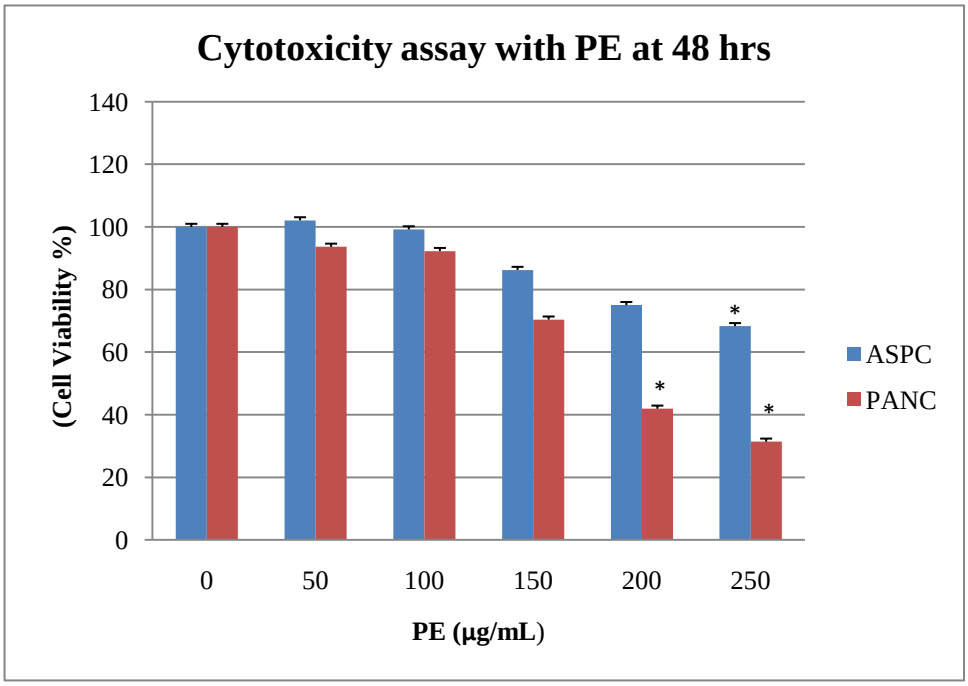
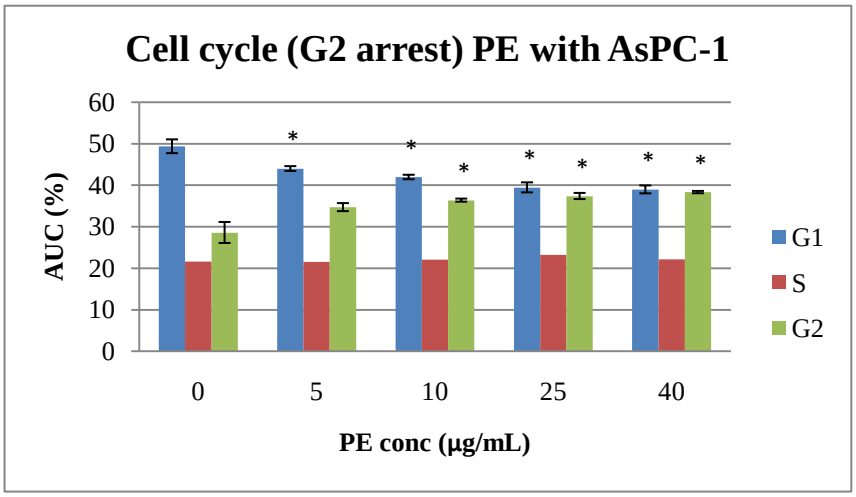
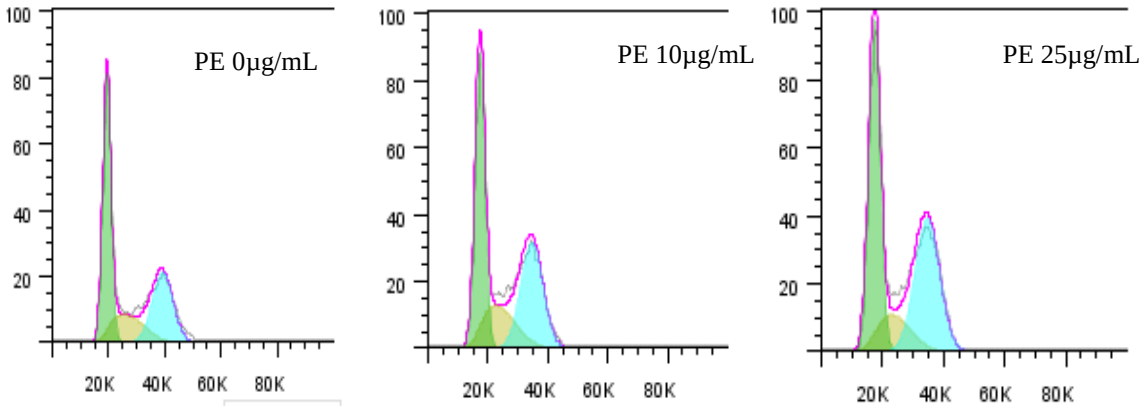

Cytotoxicity assay with PE at 48 hrs
(Cell Viability %)
140
120
100
80
60
40
20
0
0
50
100
150
200
250
PE (µg/mL)
ASPC
PANC
*
*
*

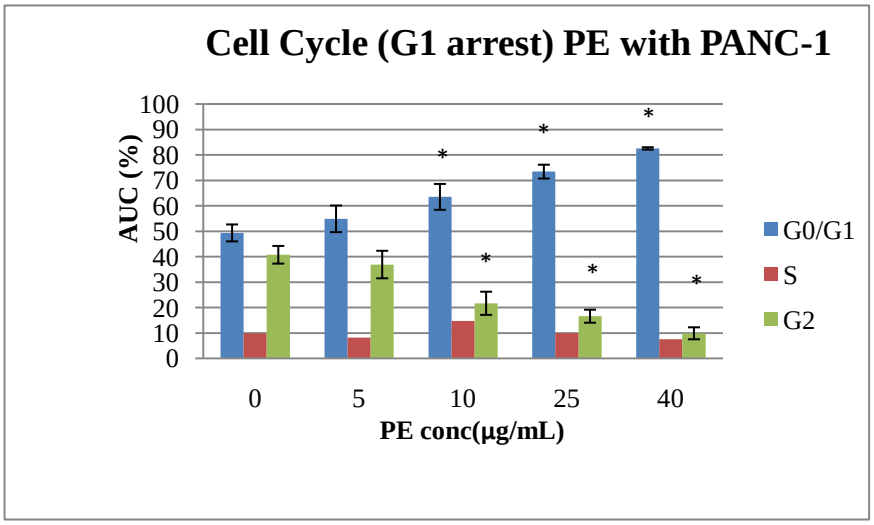
Figure 3.

3.1)


Cell cycle (G2 arrest) PE with AsPC-1
AUC (%)
60
50
40
30
20
10
0
0
5
10
25
40
PE conc (µg/mL)
G1
S
G2


PE 0µg/mL
PE 10µg/mL
PE 25µg/mL
100
80
60
40
20
20K
40K
60K
80K

3.2)


Cell Cycle (G1 arrest) PE with PANC-1
AUC (%)
100
90
80
70
60
50
40
30
20
10
0
0
5
10
25
40
PE conc(µg/mL)
G0/G1
S
G2

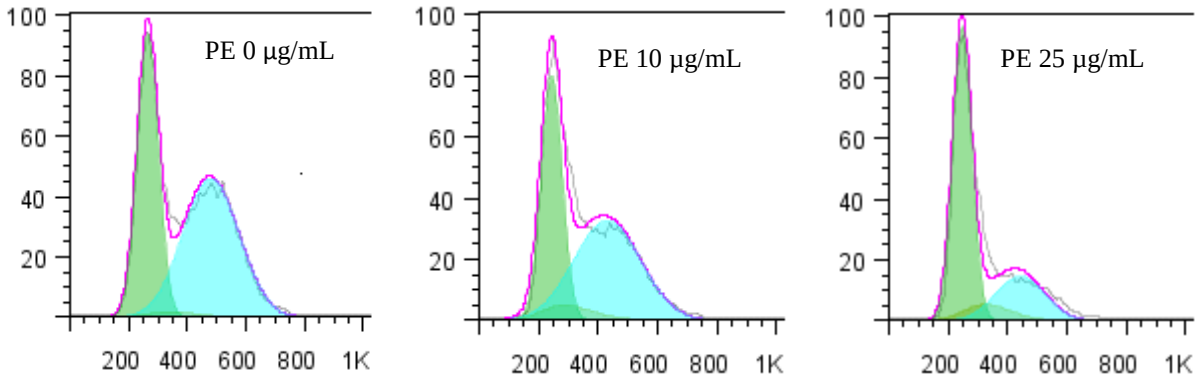

PE 0 µg/mL
PE 10 µg/mL
PE 25 µg/mL
100
80
60
40
20
200
400
600
800
1K

Figure 4

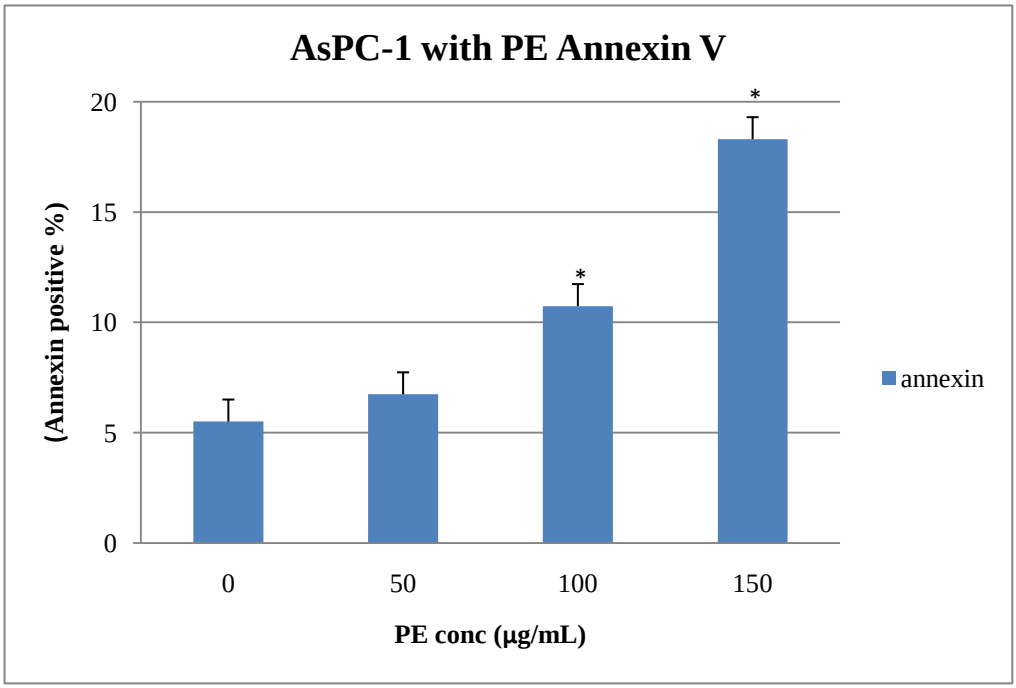

AsPC-1 with PE Annexin V
(Annexin positive %)
20
15
10
5
0
*
*
0
50
100
150
PE conc (µg/mL)
annexin

Figure 5

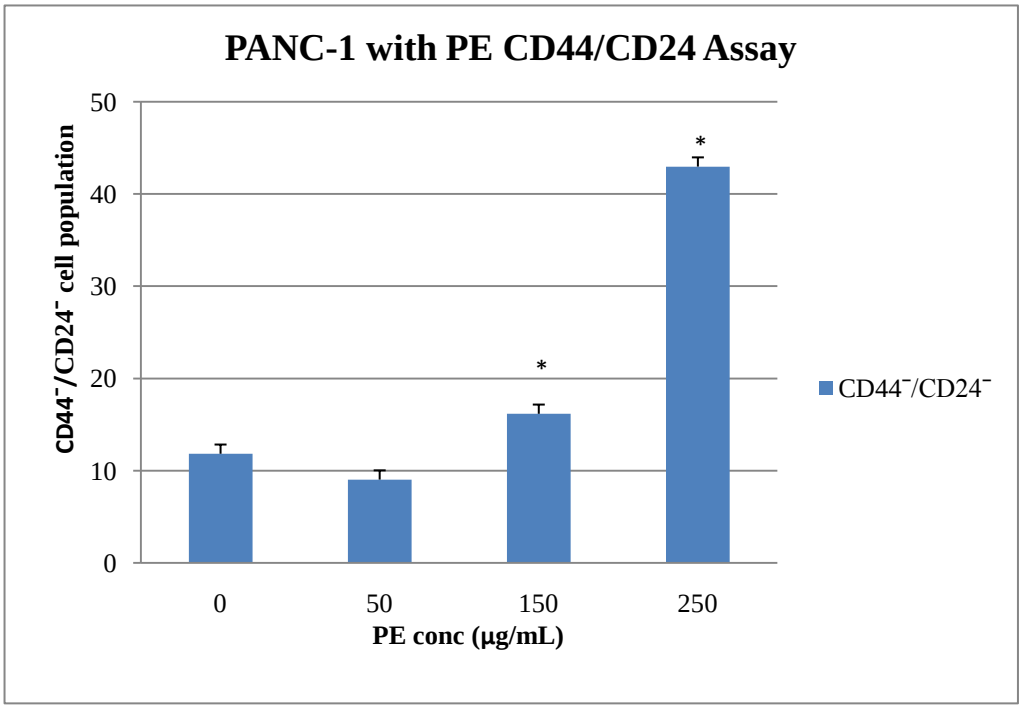
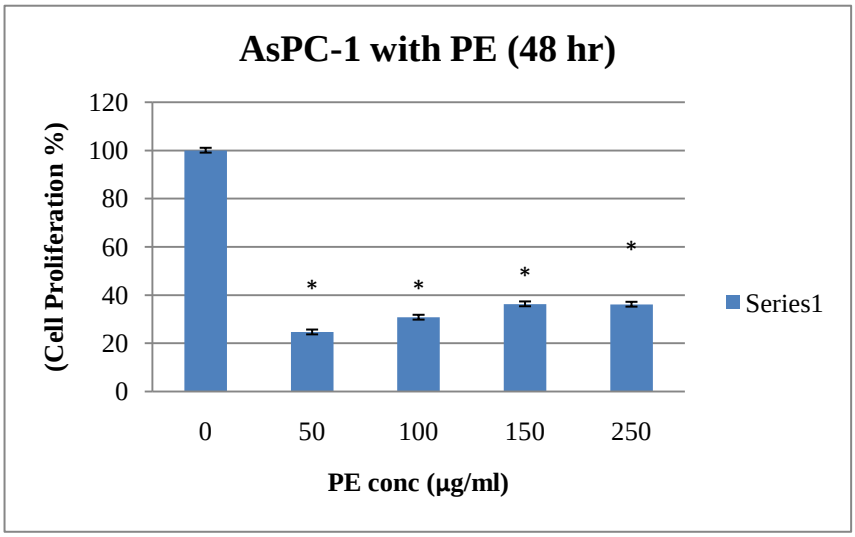
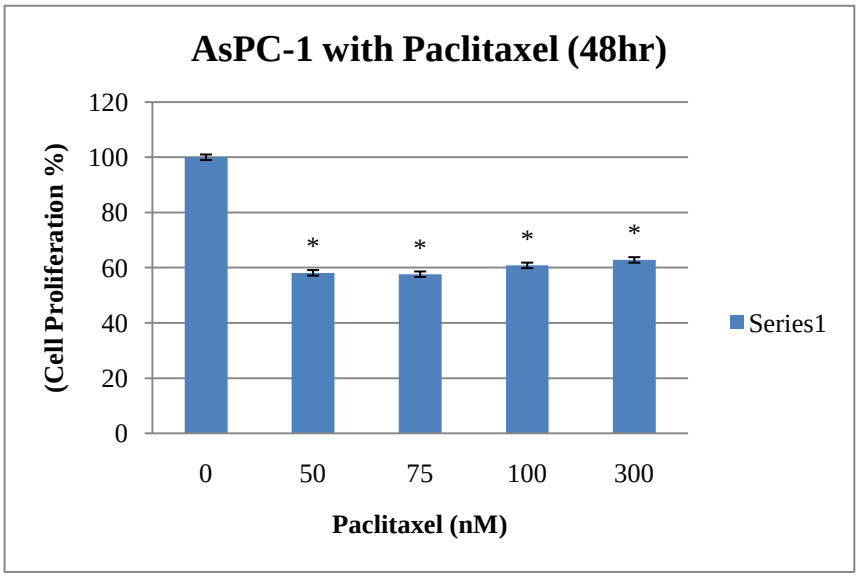

PANC-1 with PE CD44/CD24 Assay
CD44⁻/CD24⁻ cell population
50
40
30
20
10
0
*
*
0
50
150
250
PE conc (µg/mL)
CD44⁻/CD24⁻

Figure 6

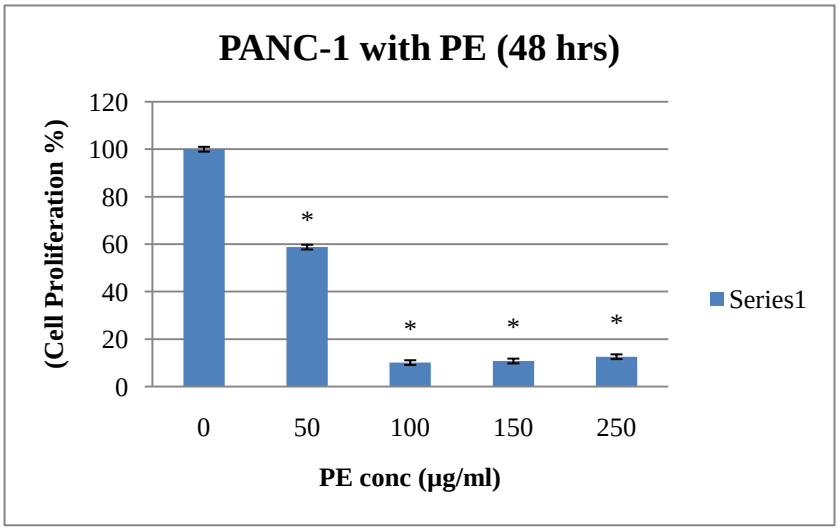
6.1)



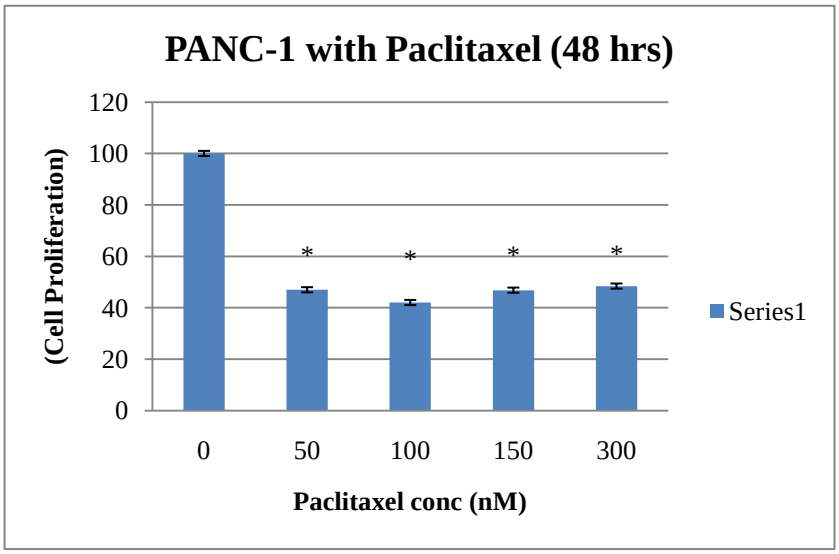
6.2)



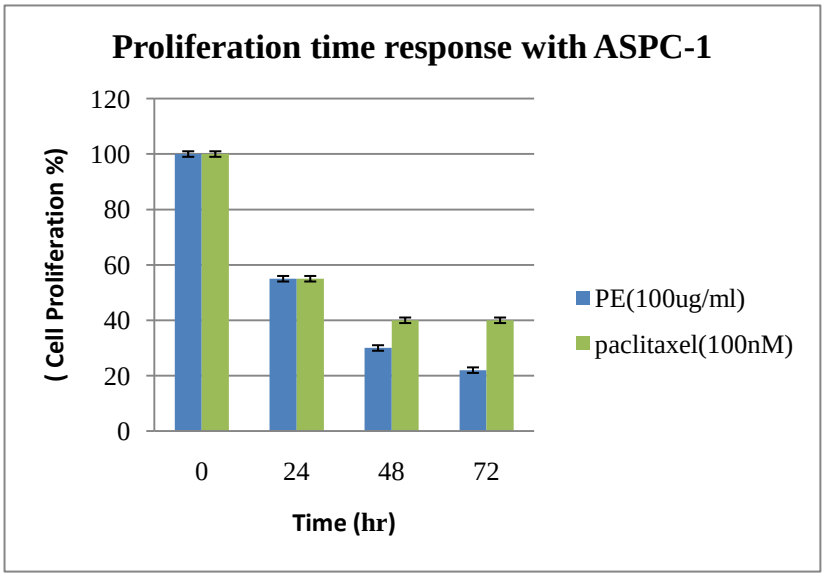
6.3)


PANC-1 with PE (48 hrs)
(Cell Proliferation %)
120
100
80
60
40
20
0
*
*
*
*
0
50
100
150
250
PE conc (µg/ml)
Series1

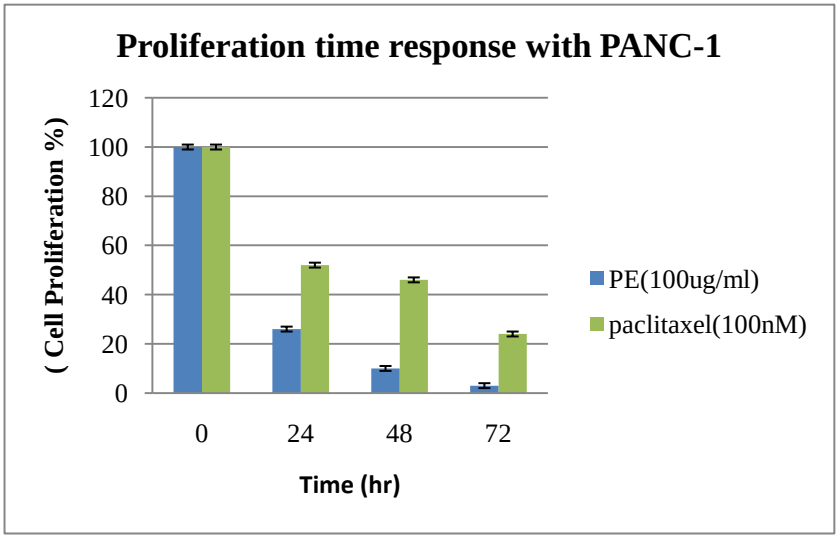
6.4)


PANC-1 with Paclitaxel (48 hrs)
(Cell Proliferation)
120
100
80
60
40
20
0
*
*
*
*
0
50
100
150
300
Paclitaxel conc (nM)
Series1

6.5)


Proliferation time response with ASPC-1
(Cell Proliferation %)
120
100
80
60
40
20
0
0
24
48
72
Time (hr)
PE(100ug/ml)
paclitaxel(100nM)

6.6)


Proliferation time response with PANC-1
(Cell Proliferation %)
120
100
80
60
40
20
0
0
24
48
72
Time (hr)
PE(100ug/ml)
paclitaxel(100nM)

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