Complex Synthesis of Cis-[Pt(Asc) $(NH_3)_2$] and its Effect on Human Breast Cancer MCF-7 Cell *in vitro*

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Abstract: *Bosom malignancy is the most regularly analyzed disease and the imperative reason for growth-related passing among ladies, accounting for 23% of all new tumor cases and 14% of tumor passing's. L-Ascorbic acid, commonly known as vitamin C is well-known in chemistry since long back. It has tremendous medical applications in several diseases. Therefore, in this paper five concentrations of complex cis-[Pt(Asc)$(NH_3)_2$] where Asc=L-ascorbic acid derivative on MCF-7 cell line to detect the changes in five cellular parameters (nuclear intensity, mitochondrial membrane potential, valid cell count, cytochrome C, and membrane permeability) after exposure with 24 h are investigated. The results showed that 400 µg/mL has the highest significant effect on the five parameters (nuclear intensity, mitochondrial membrane potential, valid cell count, cytochrome C, and membrane permeability) when compared with Doxorubicin 20 µM (substance used as anti-cancer) which represent the positive control. Also, the 200 µg/mL showed results close to those of the untreated cells which represent the negative control (-ve) with a very few significant differences.*

Keywords: *breast cancer; vitamin C; cis-platinum; high-content screening*

INTRODUCTION

The bosom disease is the most generally analyzed malignancy and the imperative reason for tumor-related demise among ladies, accounting for 23% of all new growth cases and 14% of growth passing's. Bone is a standout amongst the most extraordinary target destinations of metastasis for bosom malignancy, and up to 70% of ladies with cutting-edge infection create bone metastases [1]. Such sores have irritating impacts, including torment, spinal pressure, pathologic cracks, and hypercalcemia, all of which enormously trade off the personal satisfaction and result [2]. Platinum-based medications, for example, oxaliplatin, cisplatin and carboplatin, are utilized as a part of the treatment of a significant number of the more forceful and difficult to treat tumors, including those of the lung (non-little and little cell diseases), inside, bosom, throat, cervix testicles and ovaries, and in addition non-Hodgkin's lymphoma [3]. Carboplatin is the minimum dangerous of the platinum-based medications, yet like all coordinators of DNA harm, its viability diminishes on a patient-by-quiet premise over numerous chemotherapy cycles because of the rise of protection. In this foundation, understanding the atomic premise of medication protection could prompt the clinical capacity to beat gained protection in tumors utilizing a resensitizing particle, bringing about an enhanced treatment outcome [4].

Since the disclosure of the antitumor action of cis-plating by Rosenberg et al. [5], more than 2000 cis-Platin Analogues have been arranged'. Hollis et al. [6] combined the Vitamin C. analogs, the cis-[Pt(diamine)(A)] and cis-[Pt$(RNH_2)_2(HA)_2$] where

diamine=$(NH_3)_2$. These complexes represent the first progress metal ascorbates to respect finish basic portrayal. The Cl2orbate ligand in these mixed ligand complex is bound to Pt in a one of a kind mold. There are numerous examinations investigating the potential restorative and synergist [7] utilization of metal ascorbates a large portion of which are in patent details. Cisdiamineplatinum(II) ascorbate edifices have demonstrated guarantee as antitumor operators [8]. These platinum buildings might be viewed as second era mixed ligand complex in view of cis-platin, which is most likely the best-known case of a little, metal-containing drug [9]. Synthesis and characterization of ascorbic acid derivative and cis-$[Pt(Asc)(NH_3)_2]$. The ascorbic acid derivative was prepared of the interaction of Ascorbic acid with acetone with gas Hydrochloric acid. cis-$[Pt(Asc)(NH_3)_2]$ was prepared of the interaction of Ascorbic acid derivative with cis-$[Pt(Cl_2)(NH_3)_2]$. Cytotoxicity effect of complex cis-$[Pt(Asc)(NH_3)_2]$ on MCF-7 cell line by using (HCS) High-Content Screening. Five independent parameters including effective cell count, cytochrome C, membrane of mitochondrial, total nuclear intensity cell membrane permeability and potential, which refers to the cell health. Different concentrations of the complex cis-$[Pt(Asc)(NH_3)_2]$ leaves (25, 50, 100, 200, and 400 µg/mL) were used to treat the MCF-7 cell line. The study aimed to: 1- The new platinum (II) complex cis-$[Pt(Asc)(NH_3)_2$ have been prepared and, 2- Study the effect of the new platinum(II) complex cis-$[Pt (Asc) (NH_3)_2]$ on human breast cancer MCF-7 Cell.

■ EXPERIMENTAL SECTION

Materials

Calf thymus (CT) or the plasmid DNA was protected with platinum complexes in 35 mM $Pt(Cl_2)(NH_3)_2$ at 39 °C in the dark medium. After 38 h, the models were comprehensively dialyzed beside the medium essential for subsequent the biochemical or the biophysical analysis. An Aliquot of these tasters was applied to define the value of r_s defined as the number the molecules of platinum complex inevitable per nucleotide remainder by flameless atomic fascination spectrophoto metry or by differential pulse polarography.

Solutions of 2-helical CT DNA at an absorption of 0.042 mg mL^{-1} were incubated with pronuclear complex one at an r_d of 0.05 or 0.1 and binuclear complex two at an r_b of 0.1 in $Pt(Cl_2)(NH_3)_2$ at 39 °C (r_b is as the molar ratio of platinum complex to the nucleotide phosphates at the 1st of incubation with the DNA). At numerous time intermissions, and the aliquot of reaction mixture was quiet and examined by the DPP for the platinum not destined to the DNA. The number of molecules of complexes one and two destined to the DNA (r_s) was obtained by deducting the amount of free (unbound) molecules of the complexes one and two from the total quantity of complexes present in the response. No fluctuations in the pH of a reaction mixture comprising DNA and the platinum mixtures were dignified within 58 h after the mixing DNA with platinum complex. The amount of platinum mixtures was bound to the DNA amplified with the time.

Instrumentation

The basic liquid chromatograph comprised of a Model 1lOA Altex pump (Beckman Instruments, Berkeley, CA, U.S.A.) a fixed-wavelength UV detector (280 nm, Altex Model 153) and an Altex injector (Model 210) fitted. A 1260 Infinity II LC system (the Agilent Technologies, Waldbronn, Germany) was applied to inject 40 mL aliquots of managed samples on the Poroshell 180 EC-C_{20} column (5×40 mm, 2.6 µm, Agilent Technologies, Waldbronn, Germany) maintained at the area temperature. Quantitation was attained by the MS–MS discovery in the positive ion modes for materials, palladium, and the internal standard, using a 6420A Triple Quadrupole LC/MS quad mass spectrometer (the Agilent Technologies, Waldbronn, Germany), prepared with a Jet-stream IonSpray™ interface at 400 °C. The capillary voltage was set at 4500 V.

Procedure

Preparation of 5,6-O-isopropylidene-L-ascorbic acid

A saturated solution of L-ascorbic acid (10.00 g, 57.00 mmol) in 100 mL of freshly distilled acetone, with HCl gas, was stirred at room temperature for 20 min, and to this solution, 80 mL of n-hexane was added, stirred, and decanted. The residue was washed with

acetone-hexane (4:7) four times, then the solution was removed under reduced pressure to give L-ascorbic acid derivative (10.96 g, 91.33% yield) as white crystalline residue, m.p. 218-221 °C, Rf (0.61).

Preparation of complex cis-[Pt(Asc)(NH$_3$)$_2$]

A solution of L-ascorbic acid derivative (0.2 g, 0.92 mol) in hot water (10 mL) was added to a solution of cis-[Pt(Asc)(NH$_3$)$_2$] (0.277 g, 0.92 mmol) in hot water (25 mL). A few drops of trimethylamine were added, and the resulting mixture was heated under reflux for 2 h. This produced a dark-green solution was filtered off and reduced to half volume. Ethanol (2 mL) was added, and the mixture was set aside to evaporate slowly at room temperature. The dark-green solid thus formed was filtered off and dried in a vacuum oven. Yield (0.36 g, 87.8%). m.p. (dec.) 294 °C, Rf (0.54).

The high content screening assay (HCS)

Five autonomous parameters counting substantial cell count, total nuclear intensity cell membrane permeability, mitochondrial membrane potential, and cytochrome C which refers to the cell health. Different concentrations 25, 50, 100, 200, and 400 µg/mL of the complex cis-[Pt(Asc)(NH$_3$)$_2$] leaves were used to treat MCF-7 cell line. The assay was carried on at the Centre for Natural Product Research and Drug Discovery, Department of Pharmacology, Faculty of Medicine, University of Malaya Kuala Lumpur. This assay was carried out according to Diana et al. [10].

Statistical analysis

The Statistical Analysis System-SAS (2012) program was utilized to impact of contrast factors in study parameters. The least significant difference–LSD test (ANOVA) was utilized to significantly compare Chi-square test and percentage. A gauge of the correlation coefficient between factors was used in this investigation [11].

■ RESULTS AND DISCUSSION

Synthesis of L-Ascorbic Acid Derivative

The L-ascorbic acid molecule has four hydroxyl groups, and all these groups are active for classical esterification and other reactions. Synthesis of L-ascorbic acid derivatives at 2 and 3- position needs the first conversion of L-ascorbic acid into its 5,6-isopropylidene derivative because carbon-6 hydroxyl group (a primary hydroxyl group) is the most reactive group [12]. The acetal is stable across alkaline conditions, but it is readily hydrolyzed in dilute acid [13], consequently it is very useful as blocking agent, and was used in this work to protect the hydroxyl group at C-5 and C-6 leaving the hydroxyl group at C–2 and C-3 free for the required chemical modification. Accordingly, L-ascorbic acid derivative was prepared from the reaction of acetone with L-ascorbic acid in acidic media, according to the literature [9] (Fig. 1). The IR spectrum of L-ascorbic acid derivative show stretching band at 3244 cm^{-1} for O-H, 2995 cm^{-1} for C-H aliphatic, 1755 cm^{-1} for C=O lactone, 1664 cm^{-1} for C=C, 1141 cm^{-1} for C-O. The ^{1}HNMR spectrum (400 MHZ, DMSO) of L-ascorbic acid derivative show stretching band a single signal at 11.27 ppm which belong to the proton of the (OH) hydroxyl group at C–2, show stretching band a single signal at 8.46 ppm which belong to the proton of the (OH) hydroxyl group at C–3, show stretching band a doublet signal at 4.71 ppm which belong to the proton of the H-C4, show stretching band a quartet signal at 4.26 ppm which belong to the proton of the H-C5, show stretching band a doublet signal at 4.11 ppm which belong to the proton of the H-C6, show stretching band a doublet signal at 3.89 ppm which belong to the proton of the H-C6, show stretching band a single signal at 1.26 ppm which belong to the protons of the two CH$_3$ aliphatic groups.

Synthesis of Complex Cis-[Pt(Asc)(NH$_3$)$_2$]

The complex was prepared from the reaction of the ligands with cis-[Pt(Cl)$_2$(NH$_3$)$_2$] solution which is shown in Fig. 2.

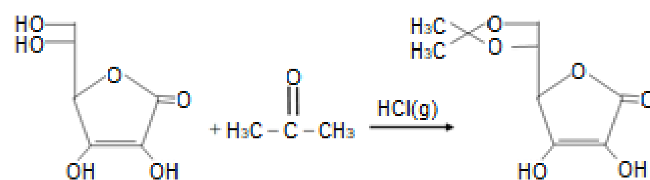

Fig 1. The reaction of acetone with L-ascorbic acid in acidic media

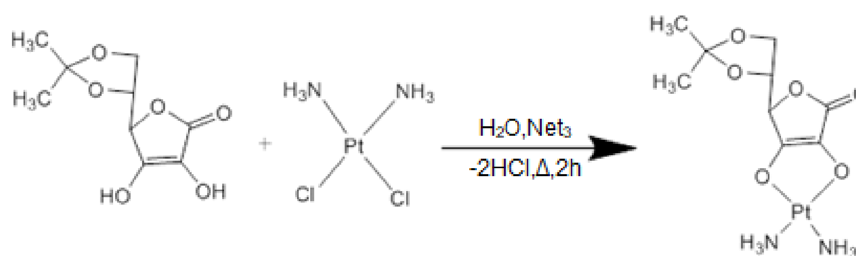

Fig 2. The reaction of the ligands with cis-[Pt $(Cl)_2(NH_3)_2$] solution

Table 1. Cytotoxicity effect of different concentrations of complex cis-[Pt(Asc)$(NH_3)_2$] on valid cell count after 24 h of incubation at 37 °C

	Mean 1 ± SE	Mean 2 ± SE	Mean Diff ± SE of diff	Summary
Control (Untreated) vs. Doxo 20 µM	3655±185.5	2040±161.5	1615±171.5	***
Control (Untreated) vs. 25 µg/mL	3655±185.5	3353±42	301.5±171.5	ns
Control (Untreated) vs. 50 µg/mL	3655±185.5	3315±43	339.5±171.5	n.s
Control (Untreated) vs. 100 µg/mL	3655±185.5	±43.53189	466±171.5	n.s
Control (Untreated) vs. 200 µg/mL	3655±185.5	2635±154.5	1020±171.5	**
Control (Untreated) vs. 400 µg/mL	3655±185.5	2099±114.5	1556±171.5	***

*** : There a high significant height ($p \leq 0.001$)
** : There a significant height ($p \leq 0.01$)
n.s : Non-significant

Table 2. Cytotoxicity effect of different concentrations of complex cis-[Pt(Asc)$(NH_3)_2$] on membrane permeability after 24 h of incubation at 37 °C

	Mean 1 ± SE	Mean 2 ± SE	Mean Diff ± SE of diff	Summary
Control (Untreated) vs. Doxo 20 µM	60.48±4.570	37.61±5.25	22.87±5.116	*
Control (Untreated) vs. 25 µg/mL	60.48±4.570	59.32±1.22	1.165±5.116	n.s
Control (Untreated) vs. 50 µg/mL	60.48±4.570	54.92±3.68	5.56±5.116	n.s
Control (Untreated) vs. 100 µg/mL	60.48±4.570	56.34±3.23	4.145±5.116	n.s
Control (Untreated) vs. 200 µg/mL	60.48±4.570	42.54±3.64	17.94±5.116	*
Control (Untreated) vs. 400 µg/mL	60.48±4.570	42.20±2.1	18.28±5.116	*

* : There a significant height ($p \leq 0.05$)
n.s : Non-significant

The IR spectrum of complex cis-[Pt(Asc)$(NH_3)_2$] show stretching band at 3213, 3253 cm^{-1} for NH_3, 2981 asymmetric, 2894 symmetric cm^{-1} for C-H aliphatic, 1743 cm^{-1} for C=O lactone, 1612 cm^{-1} for C=C, 1139 cm^{-1} for C-O. The ^{1}HNMR spectrum (400 MHZ, DMSO) of complex cis-[Pt(Asc)$(NH_3)_2$] shows stretching band a single signal at 4.35 ppm which belongs to the protons of the NH_3, stretching band a doublet signal at 4.21 ppm which belongs to the proton of the H-C4, stretching band a quartet signal at 3.9 ppm which belongs to the proton of the H-C5, stretching band a doublet signal at 3.78, 3.7 ppm from the proton of the 2H-C6, and stretching band a single signal at 1.25 ppm from the protons of the two CH_3 aliphatic groups.

Cytotoxicity Effect of Complex Cis-[Pt (Asc)$(NH_3)_2$] on MCF-7 Cell Line by Utilizing High-Content Screening (HCS)

High-Content Screening (HCS) is a cell imaging-based approach that assumed a key part in the location of danger and order of mixed ligand complex in view of watched examples of reversible and irreversible cell damage. HCS gives multipara metric investigation of reversible and irreversible cellular injury. Five

concentrations 25, 50, 100, 200, and 400 µg/mL of complex cis-[Pt(Asc)$(NH_3)_2$] on MCF-7 cell line to detect the changes in five cellular parameters (valid cell count, cytochrome C, membrane of mitochondrial, total nuclear intensity cell membrane permeability and potential) after 24 h of contact. Table (1-4) showed that the expose at 400 µg/mL has the highest significant effect on the five parameters (valid cell count, membrane permeability, nuclear intensity, mitochondrial membrane potential and cytochrome C) including when it was compared to Doxorubicin 20 µM (a substance used as anti-cancer) which represents the positive control (+ve) when ($p \leq 0.01$), ($p \leq 0.05$). At 200 µg/mL can be showed that the result is closed to those of the untreated cells which represent the negative control (-ve) with a very few significant differences (Fig. 3).

The results of valid cell count are listed in Table 2, and Fig. 4 showed that the complex cis-[Pt(Asc)$(NH_3)_2$] significantly affected on MCF-7 cell line viability as it reduces 42.57% of cells at 400 µg/mL when compared with (–ve) while the viability for the other concentrations was 8.26, 9.3, 12.74 and 27.9% (for 25, 50,100 and 200 µg/mL respectively) did not show significant differences from –ve. The reduction in the viability of MCF-7 cell line correlated to the toxic effect of complex cis-[Pt(Asc)$(NH_3)_2$]. Membrane permeability (Table 2-3 and Fig. 2-3) significantly increased and the percentages of increasing were 1.91, 9.19, 6.84, 29.66 and 30.22% for 25, 50, 100, 200 and 400 µg/mL respectively when ($p \leq 0.05$).

From Table 3 and Fig. 5, complex cis-[Pt(Asc)$(NH_3)_2$] significantly increased the nuclear intensity of MCF-7 cell line The highest percentage of increasing showed at 400 µg/mL (30.22%) when compared with –ve. It was found that complex cis-[Pt (Asc)$(NH_3)_2$] at this concentration has a moral effect ($P < 0.05$) upon permeability cancer cells due to damages occur in the cellular membrane which leads to increase in the cellular membrane. Other concentrations did not seem as this effect it has been reported that changes in cell membrane permeability are often related with a toxic or apoptotic response, and the loss of cell membrane integrity is a common phenotypic feature of marked cytotoxicity.

Table 3. Cytotoxicity effect of different concentrations of complex cis-[Pt(Asc)$(NH_3)_2$] on Nuclear Intensity after 24 h of incubation at 37 °C

	Mean 1 ± SE	Mean 2 ± SE	Mean Diff ± SE of diff	Summary
Control (Untreated) vs. Doxo 20 µM	337±6.825	447.6±31.44	-110.7±19.97	**
Control (Untreated) vs. 25 µg/mL	337±6.825	361.9±6.485	-24.90±19.97	n.s
Control (Untreated) vs. 50 µg/mL	337±6.825	368.8±6.22	-31.84±19.97	n.s
Control (Untreated) vs. 100 µg/mL	337±6.825	360.6±0.565	-23.64±19.97	n.s
Control (Untreated) vs. 200 µg/mL	337±6.825	341.2±9.905	-4.290±19.97	n.s
Control (Untreated) vs. 400 µg/mL	337±6.825	408.5±13.50	-71.55±19.97	*

**: There a high significant height ($p \leq 0.01$); *: There a significant height ($p \leq 0.05$); n.s : Non-significant

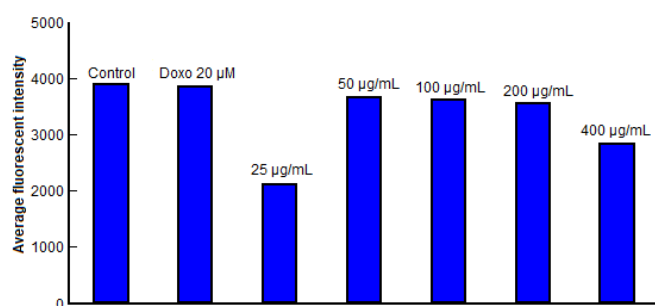

Fig 3. Effect of different concentrations of complex cis-[Pt(Asc)$(NH_3)_2$] on valid cell count after 24 h of incubation at 37 °C

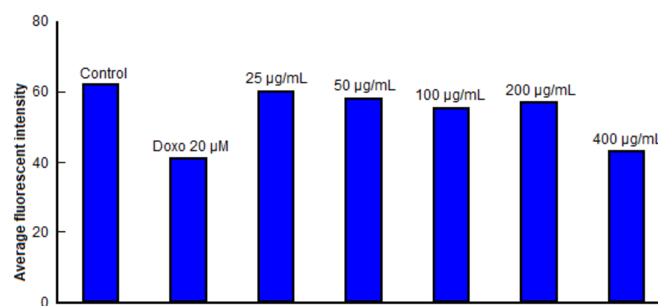

Fig 4. Effect of different concentrations of complex cis-[Pt(Asc)$(NH_3)_2$] on Membrane Permeability after 24 h of incubation at 37 °C

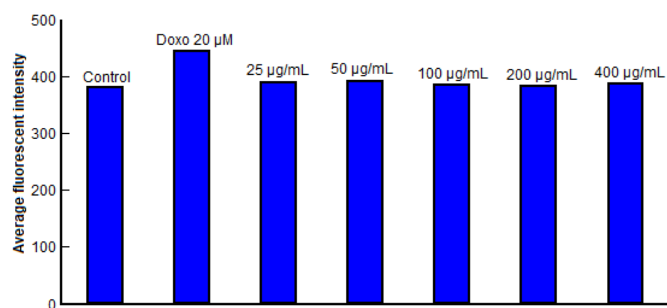

Fig 5. Effect of different concentrations of complex cis-[Pt(Asc)$(NH_3)_2$] on nuclear intensity after 24 h of incubation at 37 °C

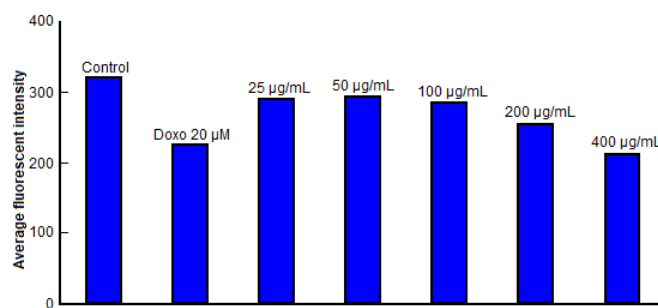

Fig 6. Effect of different concentrations of complex cis-[Pt(Asc)$(NH_3)_2$] on mitochondrial membrane potential (MMP) after 24 h of incubation at 37 °C

Table 4. Cytotoxicity effect of different concentrations of complex cis-[Pt(Asc)$(NH_3)_2$] on mitochondrial membrane potential (MMP) after 24 h of incubation at 37 °C

	Mean 1 ± SE	Mean 2 ± SE	Mean Diff ± SE of diff	Summary
Control (Untreated) vs. Doxo 20 µM	317.3±11.63	218±7.865	99.27±13.33	***
Control (Untreated) vs. 25 µg/mL	317.3±11.63	284.8±8.37	32.45±13.33	n.s
Control (Untreated) vs. 50 µg/mL	317.3±11.63	279±1.965	38.30±13.33	n.s
Control (Untreated) vs. 100 µg/mL	317.3±11.63	275.5±12.45	41.78±13.33	n.s
Control (Untreated) vs. 200 µg/mL	317.3±11.63	258.8±13.19	58.47±13.33	*
Control (Untreated) vs. 400 µg/mL	317.3±11.63	205.7±4.7	111.6±13.33	***

***: There a high significant height ($p \leq 0.001$); *: There a significant height ($p \leq 0.05$); n.s : Non-significant

From Table 4 and Fig. 6, complex cis-[Pt(Asc)$(NH_3)_2$] significantly increased the nuclear intensity of MCF-7 cell line. This increasing was dose-dependent (7.38, 9.43, 7.00%, 1.25 and 21.21 for 25, 50, 100, 200, and 400 µg/mL, respectively. The highest percentage of increase was 21.21% at 400 µg/mL when compared with –ve. The same results also show that all the values were less significantly from +ve control when ($p \leq 0.01$). A study on human MCF-7 cell line reported that treatment with cis-platinum and carboplatin induces DNA damage in cancer cells which is an indicator of apoptosis [14]. Parameters mitochondrial layer potential (MMP) and cytochrome C discharge. (MMP). A list in Table 2 and Fig. 4 indicate that all concentrations significantly decreased the MMP (10.24, 12.07, 13.17, 18.43, and 35.17 for 25, 50, 100, 200, and 400 µg/mL, respectively). The expose at 200 and 400 µg/mL affects significantly more than other concentrations as it reduces 18.43 and 35.17% of MMP when was compared with –ve. Other concentrations (25, 50, and 100 µg/mL) did not show any significant differences from each other when ($p \leq 0.01$), ($p \leq 0.05$), and cytochrome C releasing listed in Table 4 and Fig. 6 rise significantly with the increasing of concentration when compared with –ve and the percentages of increasing were 1.26, 2.33, 3.68, 16.56, and 24.02% for 25, 50, 100, 200 and 400 µg/mL, respectively, when $p \leq 0.05$. The data indicates that there was no significant difference between all concentrations and all the values were less significantly from +ve control when $p \leq 0.01$.

The diminished and splendid intensity of Hoechst blue stain was added to the nuclear concentration, and such perceptions are the characteristic highlights of apoptotic cell morphology: atomic buildup, atomic discontinuity, cell reduction, arrangement and gathering of apoptotic bodies [14]. In the expansion, the utilization of layer penetrability color and the expanding power of this color particularly at the most noteworthy introduction focus supporting the way that the concentrate could initiate apoptosis of MCF-7 cells since this can just stain the cells when plasma film porousness increment because of the loss of plasma film

uprightness. The MMP dye was accustomed sight the practicality of active mitochondria; it's the power to accumulate in mitochondria that maintain their inner membrane potential [15]. It was prompt that changes within the membrane potential square measure because of the gap of the mitochondrial permeableness transition pore, permitting the transition of ions and tiny molecules like atomic number 20 ions and in consequence, this ends up in the decoupling of the metabolism chain and so the discharge of cytochrome into the cytoplasm. Finally, the discharge of cytochrome activates a sequence of caspases, amino acid proteases, that square measure in the main liable for the degradation and digestion of the cell from within. Accordingly, the high concentration from complex cis-[Pt(Asc)$(NH_3)_2$] effect on cell indications of cancer cell line (MCF-7) when it exposed to the complex for 24 h at temperature 37 °C, which leads to stimulate the cells to enter programmed cell death (apoptosis). Distinctive features for early stages of programmed cell death include disturbance of activity of mitochondria and changes in membrane permeability, oxidation system and shorthand inside which leads to opening membrane pores and allow ions and micro-molecular through the membrane cellular membrane leading to process of pores opening in the cellular membrane to ionic balance, then separation of respiratory chain and free cytochrome C inside the cell.

Using a cytotoxicity test for drugs regarded an essential part for discovering new drugs. It is a very complicated process that affects metabolism paths (multiple) after exposing the cells to toxic cells, eventually leads to simulate the cells to die. Death happens as apoptosis or necrosis accompanied with many changes in the form of nucleus, cell permeability and mitochondria functions leading to the loss of mitochondria membrane and cytochrome C freedom from mitochondria. There are two main paths of programmed cell death: - internal and external paths. The internal path exists around mitochondria as starting point of programmed cell death after getting several signals such as damage the DNA, lack of oxygen and oxidation stress leading to cytochrome (C) secretion from mitochondria membrane, besides of the proteins helping in programmed cell death within cytoplasm [17-18]. When secretion is made of these proteins in the cytoplasm, cytochrome (C) correlated with stimulation factor to carry out programmed cell death (Apaf-1) which leads to polymerize (Apaf-1) into complicated composite septuplet parts, then this composite linked with (caspase-9). Thus multi-protein formation emerged which is called (apoptosome), the last leads to break and activate several active enzymes in protein analysis. External path of programmed cell death depends on receptors of Tumor Factor Necrosis to stimulate programmed cell death [16]. When these receptors linked with their counterparts inside the cell activating caspase – 9, 10 and the formation of a complex compound to begin signaling the occurrence of cellular death and activation of destructive enzymes. All platinum showed cytotoxic activity and antitumor cells subjected eukaryotic cells response for programmed cell death which could be stimulated by catalysts inside or outside the cell. There must be balanced between cell duplication and cell distinction from another side. Any disruption in this balance will lead to activation of diseases such as cancer. Many researches and reviews

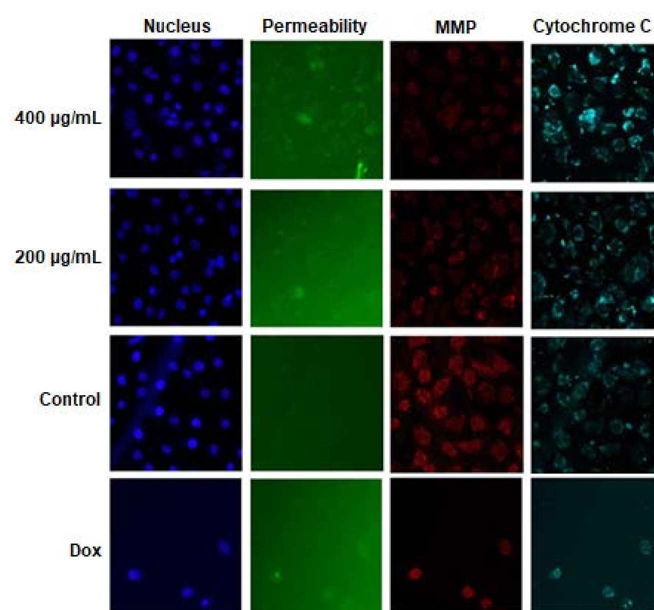

Fig 7. Effect of different concentration of complex cis-[Pt(Asc)$(NH_3)_2$] on cancer line cell (MCF-7) and their comparison with a treated cell with the drug (Doxorubicin) and untreated cells for 24 h at 37 °C

indicated that complex of cis- through multiple mechanisms and pathways including: cell cycle arrest, anti-angiogenic, anti-metastatic and apoptosis, the results of these studies agree with the findings of the present study that the platinum complex has a clear effect on the cell's induction of the cancer cells of the cancer line MCF-7 to enter the apoptosis process. Fig. 7 shows the effect of different concentration of complex cis-$[Pt(Asc)(NH_3)_2]$ on cancer line cell (MCF-7) and their comparison with treated cell with drug (Doxorubicin) and untreated cells for 24 h at 37 °C.

CONCLUSION

The complex of cis-$[Pt(Asc)(NH_3)_2]$ has been prepared of the interaction of ascorbic acid derivative with cis-$[Pt(Cl_2)(NH_3)_2]$. The ascorbic acid derivative was prepared by the interaction of ascorbic acid with acetone with gas hydrochloric acid. Result showed that the expose of complex cis-$[Pt(Asc)(NH_3)_2]$ at different concentrations (25, 50, 100, 200, and 400 µg/mL) of complex cis-$[Pt(Asc)(NH_3)_2]$ on MCF-7 cell line leaded to changes in five cellular parameters (nuclear intensity, mitochondrial membrane potential, valid cell count, cytochrome C, and membrane permeability) after expose for 24 h. The effect of treatment at 400 µg/mL was more significant on the five parameters in comparison to that with Doxorubicin 20 µM (a substance used as anti-cancer) which represents the positive control. The treatment at 200 µg/mL showed a result close to those of the untreated cells which represent the negative control (-ve) with a very few significant differences. The high concentration from cis-$[Pt(Asc)(NH_3)_2]$ affected on cellular indicators of cancer cell line (MCF-7) when it exposed to the complex for (24 h) at a temperature (37 °C) which leads to stimulate the cells to enter programmed cell death (apoptosis).

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