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Antagonistic effect between *Citrullus colocynthis* extract and TiO2 nanoparticles in anticancer combination therapy

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Abstract:

The objective of this study is to determine the level of some element and chemical components of aquatic extract of fruit of Citrullus colocynthis and study the cytotoxic effect of crude extract of plant alone and TiO2 nanoparticles (NPs) alone on two cancer cell lines: Glioblastoma-Multiforme cell culture (AMGM), mice mammary carcinoma cell line 2003 (AMN3) and Recombinant mouse epithelial cell line expressing human poliovirus receptor (CD155) on the cell surface (L20B) compared with combination of them in vitro.

The levels of Cu, Cd, Mn, K, Fe, Co, Zn, N and Ti were very low and phosphor was absent. The level of total proteins was 0.138 %. Twenty nine compounds were identified by GC-MS. The major components were 2,3-Dihydroxypropyl elaidate (30.33 %), followed by 9,12-Octadecadienoic acid (Z,Z)- (29.36 %).

All concentrations of plant extract reduced cellular viability of both cancer cell lines, (AMN3 and AMGM), compared with control. The IC50 value was 8.6 mg/ml and 0.013 mg/ml respectively. While there were no effect on L20B cell line. In the treatment of TiO2 NPs, there were reductions in AMN3 cell proliferation in the concentrations (0.2 mg/ml- 0.02 µg/ml). IC50 value was (0.42 mg/ml). While the reduction in AMGM cell line was observed at 2 mg/ml- 0.2 µg/ml concentrations. IC50 value was 0.05 mg/ml.

In combination treatment against AMN3 cell line, most concentrations of combination's treatment, except the high concentrations, reduced cell viability. IC50 value was 1.25 mg/ml of plant with 0.47 mg/ml of NPs. The same were found with AMGM cell line. IC50 value was 6.35 mg/ml of plant combined by 0.635 mg/ml of NPs. All combination points located in the antagonism area in AMN3 and AMGM. Although there were good anticancer activities of each of plant extract and nanoparticles alone but there were Antagonistic effect between them in combination therapy.

Keyword: *GC-MS; cell line; combination; Citrullus colocynthis; nanoparticles; TiO2.*

Introduction:

Citrullus colocynthis is a desert plant with a rich history as an important medicinal plant, it belong to family of Cucurbitaceae and known as Handhal, Colocynth Bitter Apple. Its origin is native of Turkey, Africa and Asia, (Khare, 2007).

The Parts used is pulp and seeds. Its medical Uses is: treatment of hemorrhoids, rheumatism, reducing blood sugar, purgative. Medical uses in Iraq is used for the treatment of rheumatism; constipation and hemorrhoids, (Albayaty, 2011). The pulp is used for varicose veins and piles. A paste of root is applied to various inflammations and swellings. The cataplasm of leaves is applied in migraine and neuralgia. In addition, the pharmacopoeia of India indicated the use of the fruit in jaundice; the root in diseases of the liver and spleen and the leaf in cutaneous affections and alopecia, (Khare, 2007). Recently, some study found that C. colocynthis is safe at its antidiabetic dose and is safe for use as an antidiabetic remedy, (Atole et al., 2009) with broad spectrum antimicrobial, (Marzouk et al., 2011). There is a few study found about anticancer activity of this plants.

The pulp contains caffeic acid derivatives (chlorogenic acid), colocynthium, gum, pectic acid, calcium, magnesium, phosphates, lignin and water in pulp contains, fatty acids; oleic acid and linoleic acid, Roots contain aliphatic compounds. Ethanolic extract shows significant anti-inflammatory activity in albino rats, (Khare, 2007).

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Leaves and flowers contain quercetin and kaempferol. The ethanolic extract of leaves and flowers exhibits antibacterial activity against a number of Gram-positive and Gram-negative bacteria. The powder is toxic at (0.6–1) g, (Khare, 2007).

The fruit contain flavones glucosides (isosaponarin , isovitexin and isoorientin 3'-O-methyl ether) and cucurbitacin glucosides (2-O-β-D-glucopyranosyl cucurbitacin I and 2-O-β-D-glucopyranosyl cucurbitacin L), (Delazar et al., 2006) which have some pharmacological and traditional medicinal uses such as important contributors to tissue injury, inflammation, cancer and many other ailments, the antioxidant properties, (Tannin-Spitz et al., 2007), (Duangmanoet al., 2010), (Sun et al., 2010), (Liu et al., 2008)

Kernels contain 52.0% oil, 28.4% protein (60% in defatted flour), 2.7% fiber, 3.6% ash, and 8.2% carbohydrate. They are good sources of essential amino acids, especially arginine, tryptophan and methionine, vitamins B1, B2, and niacin. The oil contains mostly oleic (15.9%) and linoleic (62.8%) acids, (Akobundu, 1982).

The mineral composition is Calcium ranged from 569 mg/100 g. Copper is 5.1 mg/100g, Iron 11.6 mg/100 g, Magnesium is 210 mg/100 g, Phosphorus is 30.0 mg/100 g, Potassium is 465 mg/100 g, Sodium is 11.9 mg/100 g, Zinc is 1.1 mg/100g, (Sadou et al., 2007).

Titanium dioxide (TiO2) nanoparticles is a metal oxides with crystal size between 1 and 100 nm. It possess different physicochemical properties compared to their balk particles.

TiO2 NPs can be produced by a variety of techniques ranging from simple chemical to mechanical to vacuum methods, including many variants of physical and chemical vapor deposition techniques (Ramelan et al ., 2012a), (Ramelan et al ., 2012b) or even biological synthesis (Kirthi et al., 2011) in shaped of powders, crystals, thin films, nanotubes and nanorods. Each shape has different applications which including: photocatalysis, sensors, solar cells, hydrogen storage, electrochromics and memory devices, degradation of pesticides, photoinduced hydrophilic coatings and self-cleaning devices (Mital and Manoj 2011) and might alter their bioactivity, (Mohan et al., 2013).

TiO2 NPs shows inhibitory effect on the growth of E. coli strain. Hence TiO2 NPs can be considered as potent antibacterial compound, but not as much as the established compound such as ampicillin (Ahmad et al., 2013). Some study showed that it has toxicity in BRL 3A rat liver cells, (Hussain and Sardar 2013) and Rat Embryo Fibroblast REF-3 Cell Line, (Suker and Albadran 2013), Phototoxic to Marine Phytoplankton, (Miller et al., 2013). It induced cytotoxicity, oxidative stress and DNA damage in human amnion epithelial (WISH) cells, (Saquib et al., 2012) and induce a mouse epidermal (JB6) cell apoptosis through activation of the caspase-8/Bid and mitochondrial pathways (Zhao et al 2009). Its anticancer effect is poorly studied and understood.

The observations of (Hamilton et al., 2009) suggest that any modification of a nanomaterial, resulting in a wire, fiber, belt or tube, be tested for pathogenic potential. The toxicity and pathogenic potential change dramatically as the shape of the material is altered into one that a phagocytic cell has difficulty processing, resulting in lysosomal disruption. In addition, the cytotoxicity of TiO2 NPs on two different breast cancer epithelial cell lines: (MDA-MB-468) (human breast adenocarcinoma, highly invasive) and Michigan Cancer Foundation (MCF)-7) depends on the crystal phase of the same TiO2 NPs. So, pure anatase structure induced apoptosis specifically in MDA-MB-468 cells including increased proapoptotic Bax expression, caspase-mediated PARP cleavage, and DNA fragmentation, thus resulting in cell apoptosis, (Lagopati et al ., 2014).

TiO2 alone presented a minor cytotoxicity for C6 (glioma line cells) and B16 cells (mouse melanoma cell lines); however, it did not cause any toxic effect on the RG2 (rat glioma cell lines) and U373 (human glioma cell line), which indicates its high biocompatibility with these cells, (Lopez et al., 2013). Exposing human hepatocarcinoma cell lines (HepG2) to TiO2 NPs cause DNA damage, this damage was double strand breaks, as well as chromatin condensation, nuclear fragmentation, and apoptosis, (El-Said et al., 2014).

The aim of the present study was to develop an efficient anticancer drug using C. colocynthis aquatic seed' extract alone and Titanium dioxide nanoparticles alone and if there are any avntagonistic effect between them in combination therapy.

Materials and methods:

Plants extraction

The dry fruit of C. colocynthis were get from Iraq Medical Herbarium / Ministry of Health/ / Baghdad / Iraq. Fifty gram of dried prouder of fruit was extracted in a Soxhlet extractor with D.W at 40 °C for 4 -5 h. The extract was filtered using Whatman No.1 filter paper and sterilized through 0.22 µ micro filters, (Harborn, 1984).

Chemical analysis

Element and total proteins: The levels of Cd, Mn, K, Fe, Cu, Co, Zn, N, P and Ti were analyzed using a flame atomic absorption spectrophotometer, (Nove AA-350, analytik Jena), while the level of Nitrogen were recorded by Macro Kjeldahl method, (Egli, 2008). The percent of total proteins were also calculate according to percentage of nitrogen.

GC-MS: To analysis the compounds of fruit of C. colocynthis Gas Chromatography – Mass Spectrometry GC-MS (Shimadzu GC-2010 Plus, Shimadzu GCMS-Q2010 Ultra) was used. The properties of Capillary column were: Inert Cap 1MS, 0.25mm, 30m, 0.25µ4m, Gl Sciences, Japan. Auto Injector: AOC-20i, Shimadzu. Injection volume was 5 µl. Carrier gas was helium and constant flow rate was 1 ml/min. Program's oven temperature illustrated by the following sequence: the temperature of column oven was started at one hundred Celsius for tree minute, it increased to 240 °C for nine minute, then 280 °C for five minute and finally it increased to 300 °C for two minute. The rate was fifteen.

To identify the components of C. colocynthis extract, direct comparison of the retention times and mass spectral data with standard compounds by computer matching with the (NIST

mass spectral search program for the NIST/EPA/NIH mass spectral library version 2.0 f / 2008 and NIST08.LIB).

Cytotoxicity activity

Cell culture

Two cancer cell line and one cell line were obtained from the Iraqi center for cancer and medical genetic research (ICC-MGR), experimental therapy department, cell bank unite. The cell line were: Glioblastoma-Multiforme cell culture (AMGM) (passage 68), (Al-Shammari et al., 2014), mice mammary carcinoma cell line 2003 (AMN3) (passage 200), (Al-Shamery, 2003), and Recombinant mouse epithelial cell line expressing human poliovirus receptor (CD155) on the cell surface (L20B) (passage CD155), (Nadkarni and Deshpande 2003). They maintained using RPMI 1640 (USbiological - USA) supplemented with 15% calf bovine serum (Gibco, USA), 100 units/ml penicillin, and 100 µg/ml streptomycin as recommended by cell bank unite at ICCMGR. The cells were seeded in Flat bottomed 96-well polystyrene and incubated for 24 hours at 37°C under a humidified atmosphere containing 5% CO2. A population of cells per well was 1.5*104 cells.

Cytotoxicity assay:

• TiO2 NPs alone: confluent monolayer cells was exposed to different concentrations of TiO2 NPs (2 mg/ml-0.02µl).

• Cytotoxicity assay of C. colocynthis extract alone: Series of dilution (20 mg/ml-0.2µl) of crude extracts, dissolved in phosphate buffer saline and diluted with serum free medium, were added to another confluent monolayer cells.

• Combination therapy: various concentration of TiO2 NPs were add to another confluent monolayer cells. Series of dilution of C. colocynthis extracts were added to the same cells. There was negative control (PBS) for each experiment and four replicates for each tested concentration. The cells were incubated for 24. at 37°C under a humidified atmosphere containing 5% CO2. At the end of exposure time, the media of the plate were removed and stained by 5 Methyl thiazolyl tetrazolium (MTT) solution according to (Freshney, 2005). The absorbency was determined on an ELISA micro well system microplate reader at 492 nm.

Parameters

1. Percentage of cell growth or percentage cell viability (C.V) = (mean absorbance in test wells/ mean absorbance in control wells) * 100, (Kamuhabwa et al., 2000).

2. The inhibiting rate of cell growth was calculated as (G.I) = (A-B)/Ax100. Where A is the mean of optical density of untreated wells and B is the optical density of treated wells, (Gao et al., 2003).

3. Compusyn Computer software, (version 2011), was used for calculate IC50.

4. Determine the synergistic effect of TiO2 NPs and C. colocynthis fruit extracts in combination treatment has done using the method of (Bijnsdorp, et al., 2011). A combination index (CI) was calculated from drug cytotoxicity curves. In order to calculate a CI, the Compusyn Computer software, (version 2011), Calcusyn was used, taking the entire shape of the growth inhibition curve into account for calculating whether a combination is antagonistic, synergistic or additive.

5. Statistical analysis of the results has done using SPSS, version 10. Analysis of variance (ANOVA) and the least significant difference (LSD) were used. P-values at levels ($P \leq 0.05$, $P \leq 0.01$).

Results and Dissections:

Chemical analysis

The level of some elements (determined by Atomic Absorption Spectrometry) and total proteins in C. colocynthis were showed in table (1). This result were comfortable with the study of (Akobundu, 1982) and (Sadou et al., 2007).

GS-MS chromatogram of C. colocynthis extracts showed twenty nine peaks, (Fig. 1). The percentage of identified compounds were presented in (table 2). The major components was 2,3-Dihydroxypropyl elaidate (30.33 %), followed by 9,12-Octadecadienoic acid (Z,Z)- (29.36 %).

Table (1): The level of some elements and total proteins in C. colocynthis.

Elements	Cu	Zn	Cd	MN	K	Fe	Co	Ti	P	N	protein
Amount %	0.00023	0.00008	0.00006	0.00046	0.0173	0.00677	0.00013	0.00023	N	0.0221	0.13813

N: Nil

Table (2): Compositions of aquatic extract of C. colocynthis.

P.	R. Time	Area %	Compounds
1	7.11	0.21	Stannane, phenyltrivinyl-
2	11.49	0.15	10-Methyl-10-nonadecanol
3	12.52	0.15	Phenol, 2,4-bis(1,1-dimethylethyl)-

4	14.44	0.53	1,2,4-Oxadiazol-5(4H)-one, 4-(2-chlorophenyl)-3-(2,6-difluorophenyl)-
5	14.68	0.51	1-Eicosyne
6	16.39	6.73	l-(+)-Ascorbic acid 2,6-dihexadecanoate
7	18.35	29.36	9,12-Octadecadienoic acid (Z,Z)-
8	18.45	30.33	2,3-Dihydroxypropyl elaidate
9	18.76	3.95	Octadecanoic acid
10	19.52	0.61	9,12-Octadecadienoic acid (Z,Z)-
11	20.83	0.88	Glycidol stearate
12	22.68	0.67	4H-Pyran-3-carbonitrile, 5-aminocarbonyl-2-ethoxy-4-(4-fluorophenyl)-6-methyl-
13	22.93	0.74	4-[4-Chlorophenyl]benzoic acid
14	23.24	0.7	3,10-Dichloro-9-anthraldehyde
15	23.46	0.6	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester
16	24.23	0.51	D:A-Friedooleanan-28-oic acid, 3.beta.-hydroxy-
17	24.5	3.96	Methyl 5,11,14-eicosatrienoate
18	24.63	3.47	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-
19	24.78	0.96	Glycidol stearate
20	25.72	5.6	Phthalic acid, 6-ethyloct-3-yl 2-ethylhexyl ester
21	25.9	1.44	2-[(2,4-Dihydroxybenzylidene)hydrazino]-4-morpholino-6-(1-pyrrolidinyl)-1,3,5-triazine
22	25.95	1.3	3-Octyl-[1,2]dithiolane
23	26.03	1.59	1,2-Cyclopentanediol, bis(4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptane-1-carboxylate
24	26.16	0.5	Phosphine, cyclohexylbis[5-methyl-2-(1-methylethyl)cyclohexyl]-
25	26.23	0.88	Propanedinitrile, 2-[5-(2-pyrimidylthio)-2-furfurylidene]-
26	26.27	1.24	1,4-Benzodiazepin-2(1H,3H)-one, 7-chloro-5-phenyl-3-propyl-
27	26.38	0.57	1H-[1,2,4]Triazole-3-carboxylic acid (2-cyclohex-1-enyl-ethyl)-amide
28	26.47	0.67	Pentane-1,5-diamide, N,N›-bis(2-nitrophenyl)-
29	26.67	1.17	1-Phenanthrenol, tetradecahydro-4b,8,8-trimethyl-, [1R-(1.alpha.,4a.beta.,4b.alpha
		100	

P: Peaks; R. Time: Retention time.

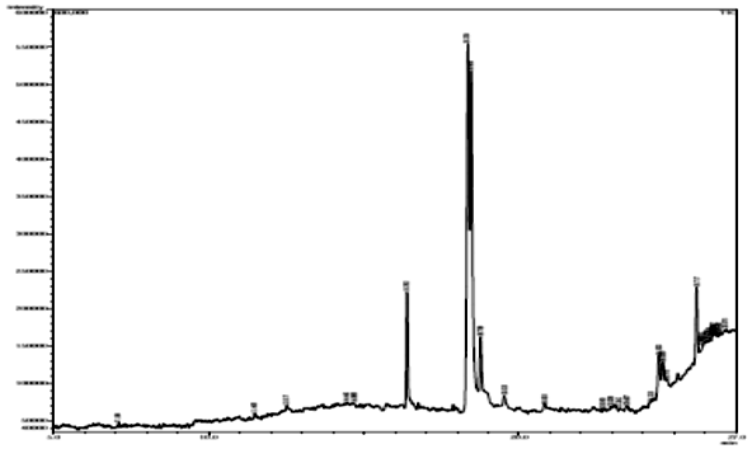

Fig. (1). Chromatogram of aquatic extract of C. colocynthis using GC-MS.

Cytotoxicity activity

• AMN3 cancer cell line

Plant extract alone: all concentrations of plant extract alone showed a significant reduction ($P \leq 0.01$) of the cellular viability compared with control. The highest inhibitory rate was showed in concentration 0.2 µg/ml mg/ml. It was 94.855 %. The IC50 values was 8.6 mg/ml, (Fig. 2).

TiO2 NPs alone: a significant reduction in cell proliferation was observed in the presence of the concentrations (0.2 mg/ml- 0.02 µg/ml) of TiO2 NPs alone. The highest inhibitory rate were showing at concentration between (20 µg/ml- 0.02 µg/ml), (93.6 -95.8) %. The IC50 value was (0.42 mg/ml), (Fig. 2). The higher concentration (2mg/ml) was induct cellular viability.

Combination: results demonstrated statistically significant ($P \leq 0.01$) differences of cell viability between most concentrations of combination's treatment, except the high concentrations, compared with treatment alone and with control, (Fig. 2). IC50 value was 1.25 mg/ml of plant with 0.47 mg/ml of NPs in combination.

All combination points located in the antagonism area. Table (3) showed Combination Index (CI) data for non-constant combination. Figure (4) showed normalized Isobologram for combination.

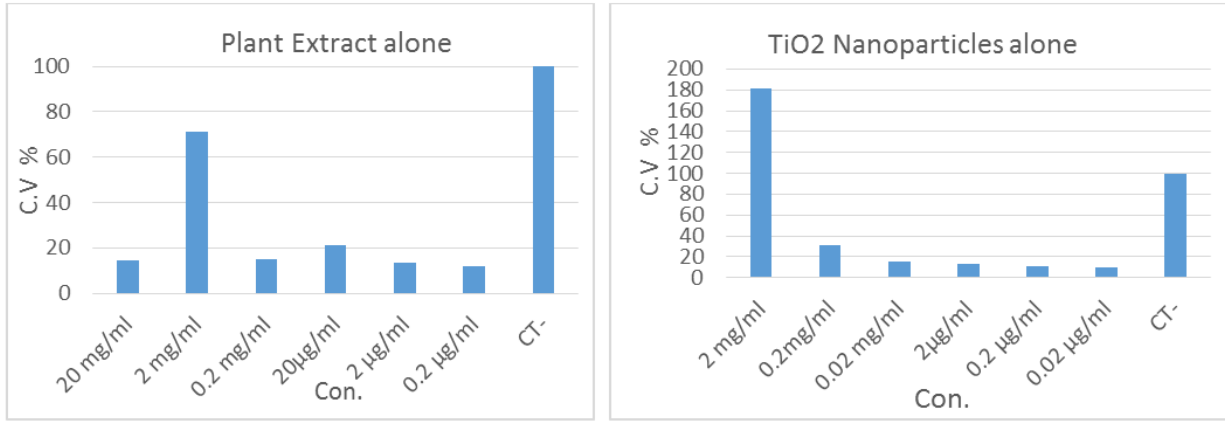

Fig. (2): Cytotoxicity assay of: (A) plant extract alone, (B) TiO2 NPs alone on AMN3 cancer cell line. CT- negative control, % C.V.: percentage of cell viability.

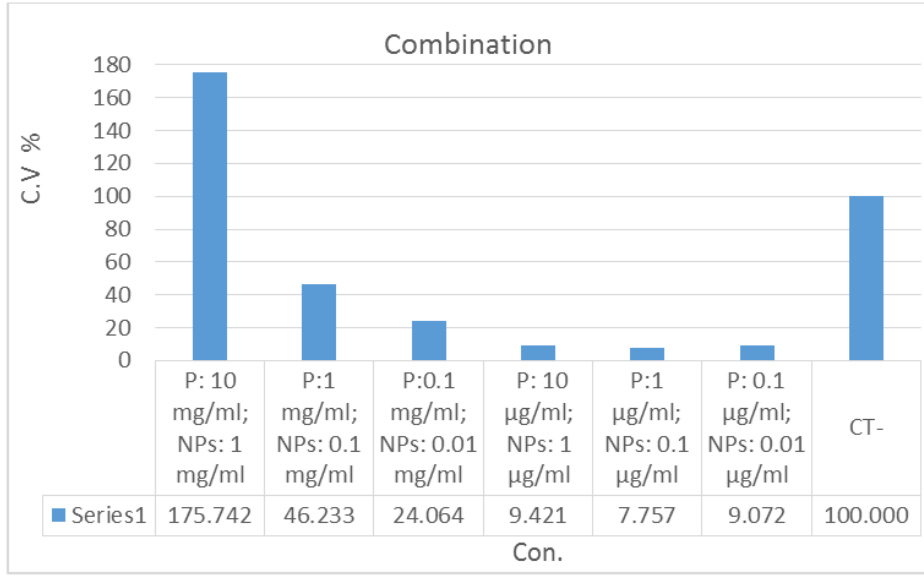

Fig. (3): Cytotoxicity assay of combinations of aquatic extract of C. colocynthis (P) with TiO2 NPs on AMN3 cancer cell line. * The mean difference is significant in comparison with control (CT-) at levels ($P \leq 0.01$); % C.V.: percentage of cell viability.

Table (3): Combination Index (CI) Data for Non-Constant Combination (plant with NPs) on (AMN3) cell Line.

Point	Dose of NPs (mg/ml)	Dose of Ex. Plant (mg/ml)	Effect %	CI
1	1.0	10.0	0.17574	7448.97
2	0.1	1.0	0.46233	116.336
3	0.01	0.1	0.24064	1.19111
4	0.001	0.01	0.9421	42557.6
5	1.0E-4	0.001	0.7757	16.8121
6	1.0E-5	1.0E-4	0.9072	68.9125

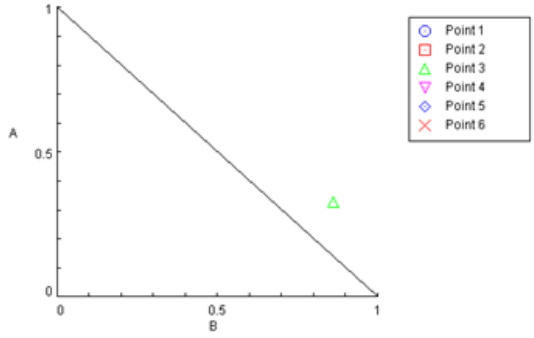

Fig. (5): Normalized Isobologram for Combination (plant with nanoparticles) on AMN3 cell line.

• (AMGM) cancer cell line

Plant extract alone: all concentrations of plant extract alone showed a significant reduction ($P \leq 0.01$) of the cellular viability compared with control. The highest inhibitory rate was showed in concentration 0.2 mg/ml. It was 79 %. The IC50 values was 0.013 mg/ml, (Fig. 6).

TiO2 NPs alone: a significant reduction in cell proliferation was observed in the presence of the concentrations (2 mg/ml- 0.2 µg/ml) of TiO2 NPs alone. The highest inhibitory rate were found at 2 mg/ml concentration, (81 %). The IC50 value was (0.05 mg/ml), (Fig. 7).

Combination: results demonstrated statistically significant ($P \leq 0.01$) differences of cell viability between the lower concentrations of combination's treatment, (Fig. 8) compared with control. IC50 value was found when combined 6.35 mg/ml of plant with 0.635 mg/ml of NPs. All concentrations of combination showed antagonisms effect, (figure 9). Table (4) shows combination Index (CI) data for non-constant combination of two points.

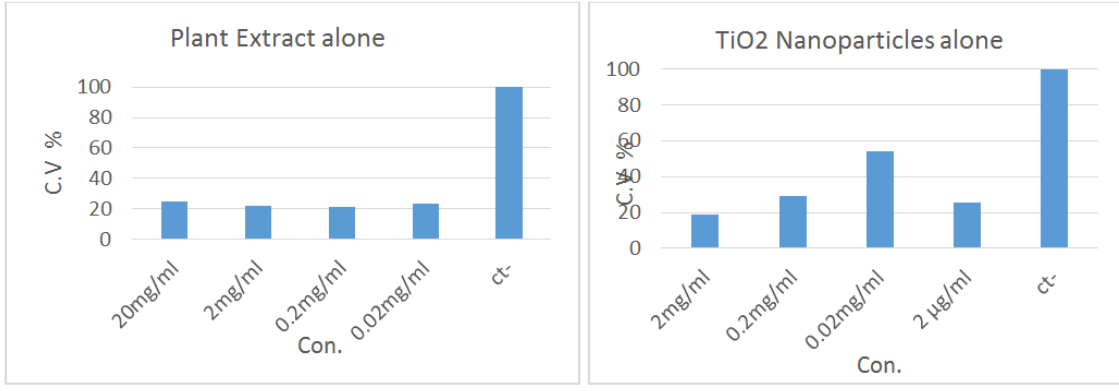

Fig. (6): Cytotoxicity assay of: (A) plant extract alone, (B) TiO2 NPs alone on (AMGM) cancer cell line. CT-negative control, % C.V.: percentage of cell viability.

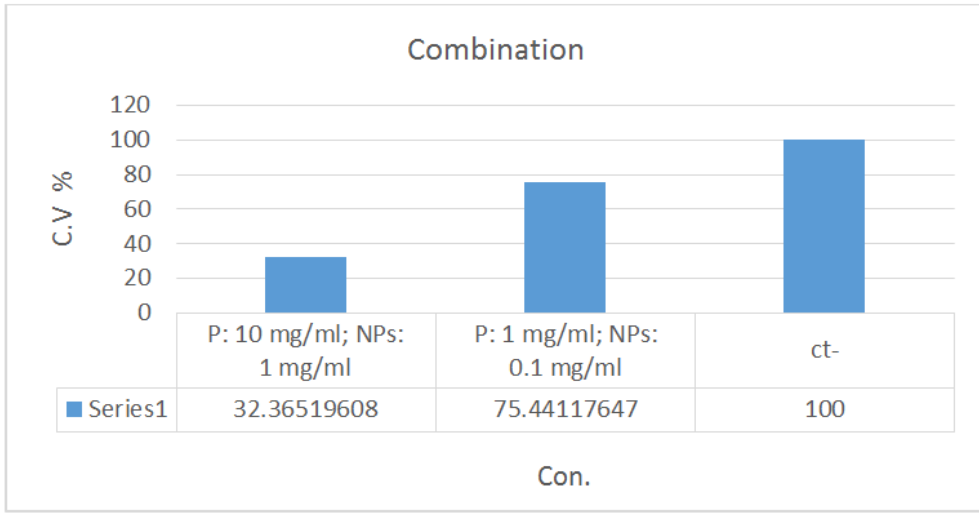

Fig. (8): Cytotoxicity assay of combinations of aquatic extract of C. colocynthis (P) with TiO2 NPs on AMGM cancer cell line. * The mean difference is significant in comparison with control (CT-) at levels (P ≤ 0.01); % C.V.: percentage of cell viability.

Table (4): Combination Index (CI) Data for Non-Constant Combination (plant with nanoparticles) on (AMGM) cell Line.

Point	Dose of NPs (mg/ml)	Dose of Ex. Plant (mg/ml)	Effect %	CI
1	1.0	10.0	0.32365	36.5801
2	0.1	1.0	0.75441	1.435E9

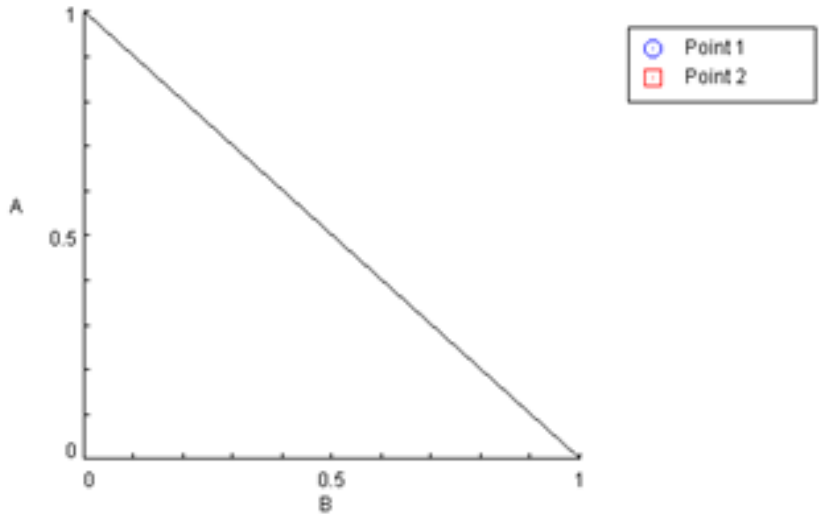

Fig. (9): Normalized Isobologram for Combination (plant with nanoparticles) on AMGM cell line.

L20B cell line: no significant decreases were obtained in cellular viability of this cell line treated with (2 mg/ml – 20 nanog/ml) concentrations of C. colocynthis alone, (P ≤ 0.01), compared with control, The IC50 values was 25 mg/ml, (Fig.10).

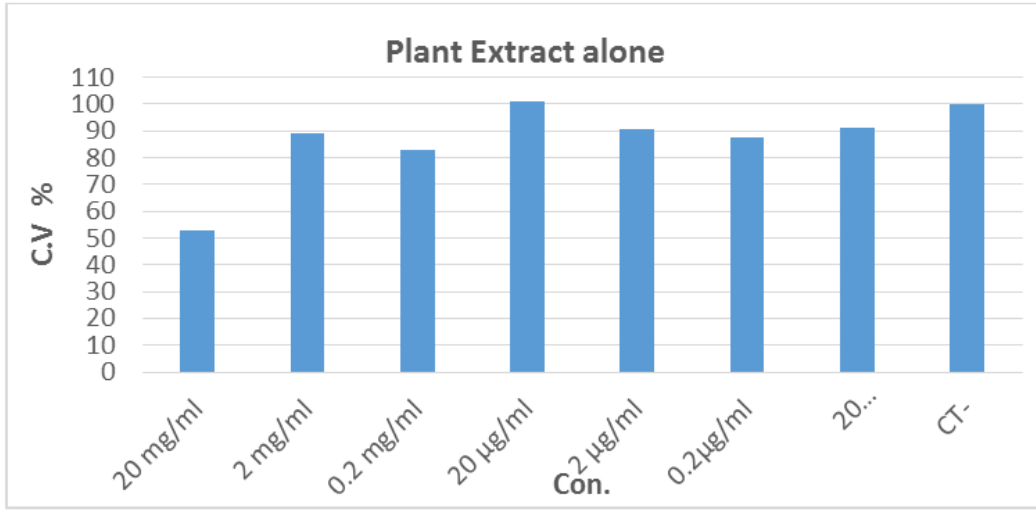

Fig. (10): Cytotoxicity assay of plant extract alone on L20B cell line. CT- negative control, % C.V.: percentage of cell viability.

Current result found that the second major components in C. colocynthis extract was lenolic acid (9,12-Octadecadienoic acid (Z,Z)-) it appeared (29.36 %) in plant. Some study found that this compound serve as a chemo-preventive and chemo-therapeutic agent in different cancers, (Field and Schley 20040), especially human breast cancers by up-regulating the estrogen-regulated tumor suppressor gene, PTP gamma expression, (Wang et al., 2006). Linoleic acid has an inhibitory effect on human breast cancer cell lines which can be due to its two double-bandings molecular structure, (Hasanzadeh et al., 2011). The anticancer effect of lenolic acid is associated with upregulation of GJIC mediated by enhanced Cx43 expression through inactivation of NF-κB and generation of ROS in MCF-7 cells, (Rakib et al., 2013).

The percentage of Phthalic acid, 6-ethyloct-3-yl 2-ethylhexyl ester that appear in C. colocynthis aquatic extract of current study was 5.6%. It found as major compound in other anticancer plant such as Calendula officinalis, (Abdul Jalill, 2014).

The size of anatase crystals of titanium dioxide NPs that used in this study was 50 nm. It was a good anticancer agent when it used alone. The result found that its anticancer effect was more efficient on AMN3 compared with its effect on AMGM cell line. Other study showed that it has toxicity in BRL 3A rat liver cells, (Hussain and Sardar 2013) and Rat Embryo Fibroblast REF-3 Cell Line, (Suker and Albadran 2013), human breast adenocarcinoma, highly invasive (MDA-MB-468), Michigan Cancer Foundation (MCF-7), (Lagopati et al ., 2014), depends on: the crystal phase of the same TiO2 NPs, size nanoparticles, charge nanoparticles and also on the cell type treated, (Thurn et al., 2007).

Although there were good anticancer activity of each of plant extract and nanoparticles alone, in this study, but there were antagonistic effect between them in combination therapy. Antagonism is a phenomenon where in two or more agents in combination have an overall effect that is less than the sum of their individual effects. This is might due to competition between the plant extract and nanoparticles on receptors of cancer cell's membrane. The observations of Thevenot and others in 2008 found that the surface functional groups of TiO2 NPs (21nm) may provide a degree of toxicity of different cancer cell death, especially prostate tumor line (JHU) and Lewis Lung Carcinoma (LLC), based on the interactions between the specific surface chemical group and the properties of a particular cancer cell's membrane.

Conclusion:

The aquatic extract of C. colocynthis contain Twenty nine different components with different levels of more than nine elements. The extract of C. colocynthis alone has anticancer activity on (AMN3 and AMGM) cell lines but it did not have cytotoxic effect on L20B cell line. The same features were seen in the treatment of each of TiO2 NPs alone and combinations between C. colocynthis aquatic extract and TiO2 NPs. But there was antagonism effect between Citrullus colocynthis aquatic extract and TiO2 NPs in combination treatment on cytotoxic effect cell line (AMN3 and AMGM) cell lines. More study about the effect of another size and shape of the same nanoparticles would be benefit.

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التأثير التضادري بين مستخلص نبات الحنظل ودقائق ثاني أوكسيد التيتانيوم النانوية في العلاج التجميعي للسرطان

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الخلاصه:

هدفت هذه الدراسة لتحديد مستويات بعض العناصر والمرطبات الكيميائية الموجودة في المستخلص المائي لثمار نبات الحنظل مع دراسة تأثير مستخلصه الخام لوحده وكذلك تأثير دقائق ثاني اوكسيد التيتانيوم النانوية لوحدها في خطين من خطوط الخلاليا السرطانية وخط طبيعي واحد بالمقارنة مع مزيج العلاج التجميعي لهما.

أظهرت النتائج ان مستويات كل من: النحاس، الكادميوم، المنغنيز، البوتاسيوم، الحديد، الكوبلت، الزنك، النيتروجين والتيتانيوم كانت قليلة جدا في المستخلص الخام بينما أنعدم وجود الفسفور. شخصت تسعة وعشرون مركب مختلف بواسطة جهاز كروموتوغرافيا الغاز المدمج بمكياف الكتلة GC/MS وكان المركب الرئيسي هو (30.33) 2,3-Dihydroxypropyl elaidate (%), تلاه مركب (29.36) -(Z,Z)-9,12-Octadecadienoic acid (%) .

خفضت جميع تراكيز النبات من حيوية خلايا الخطين AMGM و AMN3 عند استخدامها لوحدها بالمقارنة مع السيطرة . كانت الجرعة القاتلة لنصف عدد الخلايا تساوي 8.6 ملغم /مل و 0.013 ملغم /مل على التوالي.بينما لم تتأثر حيوية خلايا الخط الطبيعيL20B . عند استخدام دقائق ثاني اوكسيد التيتانوم لوحدها، لم ينخفض تكاثر خلايا الخط AMN3 بأستخدام التراكيز (0.02-0.2) مايكروغرام/ مل. كانت الجرعة القاتلة لنصف عدد الخلايا تساوي 0.42 ملغم /مل بينما خفضت التراكيز (2 غم/مل 0.2- مايكروغرام/ مل) من حيوية خلايا الخط AMGM ، كانت الجرعة القاتلة لنصف عدد الخلايا تساوي 0.05 ملغم /مل.

اظهر العلاج التجميعي بين المستخلص النباتي والدقائق النانوية انخفاض في حيوية خلايا الخط AMN3 عند معظم التراكيز المستخدمة عدا العالية منها، كانت الجرعة القاتلة لنصف عدد الخلايا بأستخدام العلاج التجميعي المكون من 1.25 ملغم/مل من مستخلص النبات مع 0.47 ملغم/مل من الدقائق النانوية. ولوحظت الصورة ذاتها عند استخدام الخط AMGM حيث كانت الجرعة القاتلة لنصف عدد الخلايا عند أستخدام علاج تجميعي مكون من6.35 ملغم/مل من مستخلص النبات مع 0.635 ملغم/مل من الدقائق النانوية. لوحظ ان جميع التراكيز المستخدمة من العلاج التجميعي قد ظهرت في منطقة التضادية للخطين AMN3 و AMGM. ومن هذا يستنتج انه على الرغم التأثير الواضح للفعالية المضادة للسرطان لكل من المستخلص الخام والدقائق النانوية عند استخدامهما لوحدهما لكن وجد تأثير تضادي بينهما في العلاج التجميعي.

كلمات مفتاحية: جي سي/ ماس، خطوط خلوية، نبات الحنظل، دقائق نانوية، تيتانيوم، علاج تجميعي