ANTIOXIDANT, ANTICANCER AND IMMUNOCYTOCHEMISTRY ACTIVITIES OF THREE SYNTHETIC COUMARIN DERIVATIVES

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ABSTRACT

Many biological studies have indicated that coumarin and its derivatives can act as chemoperventive agents. In this study, three synthetic coumarin derivatives were investigated for their free radical scavenging, anticancer and cell signaling activities. Compound A showed antioxidant effects with 100% decoloration of DPPH at concentration of more than 150 µg/ml with IC_{50} of 102.12 ± 0.15 µg/ml. Compound B and C induced antioxidant effects with 100% decoloration at 100 and 50 µg/ml, respectively. Also, the IC_{50} of compounds B and C was 28.35 ± 0.04 and 6.12 ± 0.05 µg/ml, respectively. Moreover, these compounds suppressed PC-3 prostate and MDA-MB-453 breast cancer cells proliferation with IC_{50} of 20 µM, 70 µM and 50 µM, respectively for PC-3 cells. However, it needs higher dosage to inhibit MDA-MB-453 breast cancer cell growth with IC_{50} at 25 µM, 137 µM and 100 µM, respectively. All of these compounds activated the apoptotic death signals downstream by activating caspase pathway. And showed many apoptotic characterizations including, DNA fragmentation and apoptotic bodies formation.

Key words: Antioxidant, Anticancer, Free radical scavenging activity, Synthetic coumarin derivatives, Apoptosis, Prostate cancer, Breast cancer.

INTRODUCTION

Natural and synthetic coumarin and their derivatives showed a wide range of biological and pharmacological applications and activities. These activities and applications as anti-inflammatory (Witaicenis *et al.*, 2014), antimicrobial (Ojala *et al.*, 2000), anticoagulant (Manolov *et al.*, 2006) and Antiviral (Shokoohinia *et al.*, 2014). One important biological activity of coumarin derivatives is to prevent cancer as antioxidants. This preventive action includes scavenging free radicals which have the capability to damage many important cellular components, such as lipids, protein and DNA (Witaicenis *et al.*, 2014). The most important pharmacological activity of coumarin and its derivatives is as anticancer against different types of cancer, such as melanoma, ovarian, renal, breast, prostate,

colon, leukemia, pancreatic, lung and liver (Garazd *et al.,* 2017; Saidu *et al.,* 2012; Kimura *et al.,* 2005; Devji *et al.,* 2011; Musa *et al.,* 2001 and Klenkar and Molnar, 2015).

Globally, the second leading cause of death after heart diseases in high-income countries is cancer, but it is the third after both heart and parasitic diseases in middle- and low-income countries. Cancer types are variable according to geographic area. In women, breast cancer is the most common type worldwide. And the most regular type of cancer among males in Africa is prostate cancer which is one of the major cancer types among males worldwide (American Cancer Society, Global cancer Facts & Figures, 3rd and Siegel *et al.,* 2017).

The main challenges of any new anticancer agents are to induce cytotoxicity and mediate apoptosis. Apoptosis is a programmed cell death, which is characterized with nuclear DNA fragmentation, formation of apoptotic bodies, chromatin condensation, and cell shrinkage (Chen *et al.,* 2009; Kalinichenko and Matveeva, 2008). Many anticancer agents stimulate immune response, by targeting specific molecules to interfere cancer cell growth (Padma, 2015). The most biochemical mediator in apoptosis cell death signaling that trigger cellular substrates cleavage is Caspase-3 (Kumar, 2007 and Yedjou *et al.,* 2010).

Therefore, the main objective of this paper is to investigate the antioxidant and anticancer activities of three coumarin derivatives which were synthesized previously by the second author (figure 1) (Ababe, 2012). Also, their anti-breast-cancer activities against four breast cancer cell lines MDA-MB-231, MCF-7, BT-474, and SK-BR-3 were determined by first author (Mahmoud, 2012). Moreover, is to explore their pro-apoptotic activities such as nuclear DNA fragmentation and formation of apoptotic bodies. Beside, the detection of the immuno- signaling mediator, caspaase-3/7.

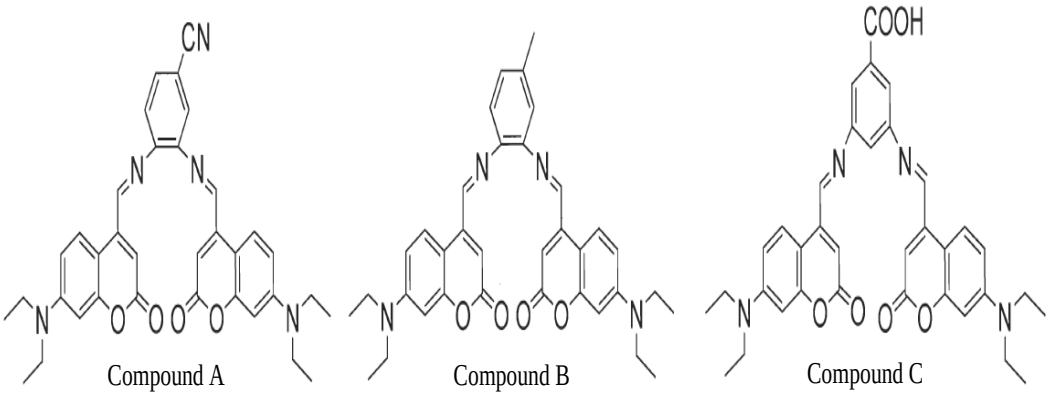


Figure 1. **Chemical structure of the three coumarin derivatives**

MATERIALS AND METHODS

Chemicals, reagents and Cells

All chemicals and solvents were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). Tested compounds, compound A (3E, 4E) - 3 - ((7 - (diethylamino) - 2 - oxo - 2H - chromen - 4 - yl) methyleneamino) - 4 - ((7 - (diethylamino) - 2 - oxochroman - 4 - yl) methyleneamino) benzonitrile), compound B (3E, 4E) - 3 - ((7 - (diethylamino) - 2 - oxo - 2H - chromen - 4 - yl) methyleneamino) - 4 - ((7 - (diethylamino) - 2 - oxochroman - 4 - yl) methyleneamino) toluene), and compound C (3E, 4E) - 3 - ((7 - (diethylamino) - 2 - oxo - 2H - chromen - 4 - yl) methyleneamino) - 4 - ((7 - (diethylamino) - 2 - oxochroman - 4 - yl) methyleneamino) benzoic acid) were synthesized previously by the second author (Ababe, 2012). All *in vitro* reagents were purchased from Roche Applied Science (Indianapolis, IN, USA), Invitrogen Life Technologies (Grand Island, NY, USA), Gibco, (Grand Island, NY, USA) and Thermo Fisher Scientific (Waltham, MA, USA).

Antioxidant activity

The antioxidant activity was measured by testing the (2,2-diphenyl-1-picrylhydrazyl) DPPH free radical scavenging activities of the tested compounds according to the method described by (Brand-Williams *et al.,* 1995). The dimethyl sulfoxide (DMSO) solution of DPPH was used as blank. 100 µl of the tested compounds at concentration of 10, 50, 100 and 150 µg/mL was added to 1000 µl of (20mg/L DPPH). The solution was mixed and incubated for 20 min at dark. The absorbance (A) of DPPH was determined at 517 nm using the UV-VIS spectrophotometer and the free radical scavenging activity of each test material was expressed as inhibition percentage of free radical DPPH according to this equation

$$\text{Inhibition \%} = \frac{A_b - A_t}{A_b} \times 100 =$$

A_b = Absorbance of blank

A_t = absorbance of tested compounds

Also, IC_{50} (sample concentration required for 50% inhibition of the cells), was calculated by the linear regression analysis.

Cell culture conditions

PC-3 and MDA-Mb-453 cells were cultured in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12) supplemented with 1% antibiotic penicillin-streptomycin solution, 10% fetal bovine serum, 1% L-glutamine, and 0.37% sodium bicarbonate . The cells were seeded in a 96-well microplate at the density of 1×10^4 for two days at 37 °C in a 5% $C0_2$ humidified incubator.

MTT cytotoxicity assay:

The anticancer activity of compounds A-C was tested against the PC-3 and MDA-Mb-453 cell lines using the 3-(4, 5 dimethylthiazol-2yl)-2, 5-diphenyl tetrazolium bromide assay (MTT) according to Chen *et al.,* 2009. The whole experiment was repeated 3 times in triplicate for each concentration. Cells were seeded in a 96-well microplate at the density of 1 x 10^4 for two days at 37 °C in a 5% $C0_2$ humidified incubator. Cells were treated for 24 h and 48 h using various concentrations of compounds A, B and C at 25-300 µM of each compound. Control wells received DMSO and media only. The medium replaced with 200 µL of MTT solution (5 mg/mL) in new medium and incubated for 5 h. The medium was removed and the resulting formazan crystals is solubilized in 200 µL of DMSO. The optical density was read at 560 nm and reference wavelength at 650 nm on a Tecan infinity F500 microplate reader (Tecan System Inc., San Jose, CA, USA).

$$\text{The \% of cell viability} = \frac{\text{OD of treated cells}}{\text{OD of controlcells}} \text{ X } 100$$

Morphological examination of apoptotic changes

The apoptotic morphological changes were examined using procedure described by Chen *et al.* 2009. PC-3 and MDA-MB-453 cells were seeded in a chamber slide, Thermo Scientific (Rochester, NY, USA). Cells were exposed to the 3 compounds at 50 µM concentration for 48 h. Treated cells were stained with Hoechst 33342 (chromatin dye) 2 µg/ml at 37 °C for 30 min. The cells' morphological changes were examined using fluorescence microscopy (Leica DM5500 Bmicroscope, Leica Microsystems) equipped with a blue filter.

DNA isolation and fragmentation assay

The DNA fragmentation assay was determined according to the protocol provided with apoptotic DNA ladder detection kit (Roche). Cells were grown in 25 cm^3 tissue cultural treated flasks. When PC-3 and MDA-MB-453 cells turned to 50% confluent , new media containing the compounds (1%) was replaced the old media. Control flasks received DMSO (1%) instead of compounds (1%). And then cells were incubated for 2 more days. Treated cells were washed with versene then detached using trypsin-EDTA. Followed by centrifugation at 5000 RPM at 4 °C for 10 min. The supernatant was removed and cells were resuspended with 200 µl phosphate buffer saline (PBS) and 200 µl of lysis buffer, which incubated for 20 min at RT. DNA precipitated with 400 µl isopropanol. The DNA was collected using the kit filter tubes. Then the DNA was obtained using 200 µl elution buffer (70 °C). The collected DNA was stored for the next analysis at -20 °C. The extracted DNA was quantified by a spectrophotometer. Then, 25 µg of the extracted DNA was mixed with 2µl of the kit loading buffer which was loaded in 1% agarose gel. A 5 µl of DNA marker was loaded as well. The gel electrophoresis ran at 124 volts for 60 min

using 1X Tris-borate-EDTA soltuion (TBE). The apoptotic DNA fragmentations were visualized under UV light and Ethidium Bromide dye.

Immunocytochemistry determination of Caspase-3/7 activity

Caspase-3/7 activity was determined as described by the manufacturer protocol (Invitrogen Life Technologies, Grand Island, NY, USA). PC-3 and MDA-MB-453 cells were grown for 2 days at 37 ℃ in a 5% $C0_2$ humidified incubator. Treated cells with 50 µM of compounds concentrations were washed with versene then detached using trypsin-EDTA. Only 100 µl of the treated cells was mixed with100 µl of the detection reagent at final concentration of 5 µM. Cells were incubated for 90 min at 37 °C in a 5% $C0_2$ incubator. After that cells were mixed with 150 µl of PBS, the caspase-3/7 activity was detected using BD FACSCalibur flow cytometer at fluorescence excitation and emission maxima: 502/530 nm.

Statistical analysis

Statistical analysis and graphical data are expressed as mean ± SD for three replicate, The statistical difference was determined by using the Enova test, *P*-values of less than 0.05 were considered statistically significant.

RESULTS

Free radical scavenging activity

The antioxidant activity of compounds A, B and C was determined using the DPPH assay, the results were expressed as both IC_{50} µg/ml and the decoloration %. The free radical scavenging effects of compounds A, B and C were variable with IC_{50} of 102.12 ± 0.15, 28.35 ± 0.04 and 6.12 ± 0.05 µg/ml, respectively. Compound A showed slight antioxidant activity with decoloration percentage of 7.8, 21.2, 56.1 and 92.8 % at four different concentrations of 10, 50, 100 and 200 µg/ml respectively. In contrast, compound C showed a strong free radical scavenging activity with decoloration percentage of 81.6, and 100 at concentration of 10 and 50 µg/ml respectively. While, compound B was moderate antioxidant, with decoloration percentage of 29.4, 78.3 and 100 % at concentrations 10, 50 and 100 µg/ml respectively.

Anti-proliferation activity of the three coumarin derivatives on prostate and breast cancer cell lines

The anti-cancer activity of compounds A, B and C was determined using MTT assay against both PC-3 prostate adenocarcinoma cells and MDA-MB-453 breast carcinoma cells. This cytotoxicity test indicated that, these compounds showed both dose-dependent and time-dependent after 2 days of treatment. Compound A showed no to slight activity against both cell lines after 24 h treatment with 25, 50, 100, 200, and 300 µM of concentrations (figure 2). However, after 2 days and with lower concentrations, it suppressed almost 100 % of the cells (figure 3). So, after 48 h treatment compound A showed significant loss of cells' viability for PC-3 and MDA-MB-453 lines with IC_{50} of 20 and 25 µM respectively. Both of compounds B and C showed very slight anti-proliferation activity against the two cell lines (figure4 and

6). But, after 48 h of treatment, compound B showed moderate toxicity with IC_{50} of 70 µM against PC-3 cells and IC_{50} of 135 µM against MDA-MB-453 cells (figure 5). However, compound C showed significant stronger activity against both PC-3 and MDA-MB-453 lines with IC_{50} of 50 and 100 µM respectively (figure 7).

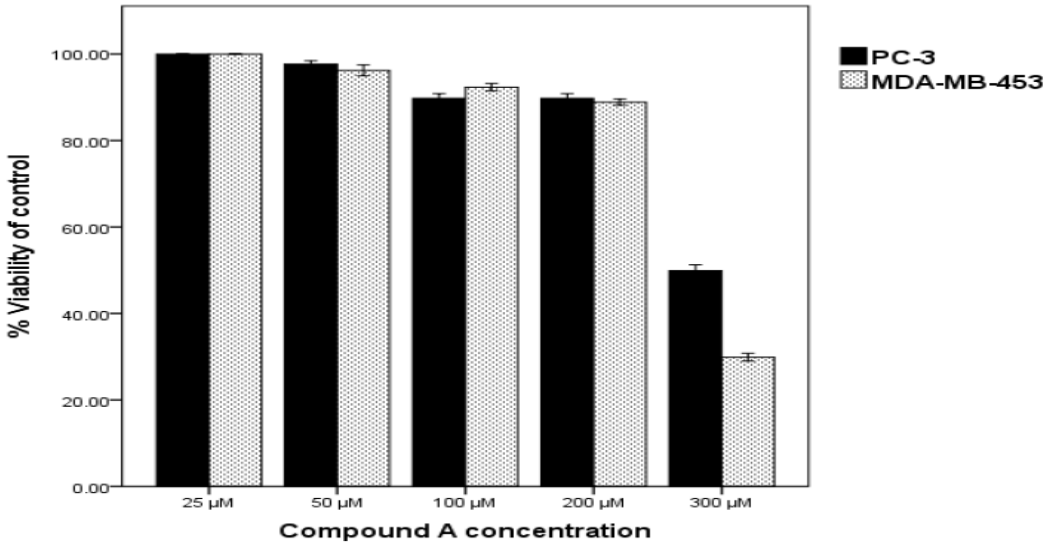


Figure 2. **The effect of compound A on PC-3 and MDA-MB-453 cells' growth. The cells were treated with 25, 50, 100, 200 and 300 µM of compound A for 24 h.**

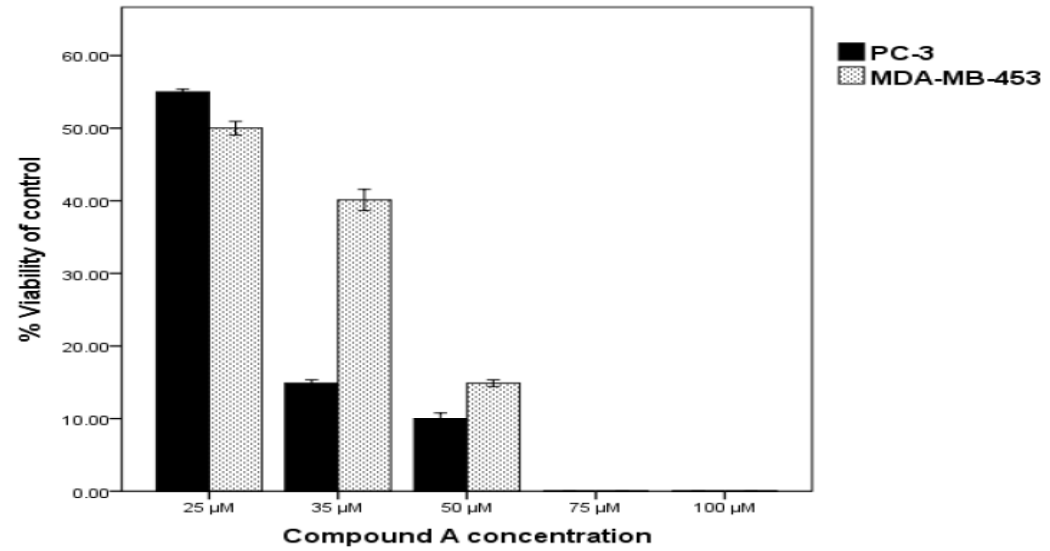


Figure 3. **The effect of compound A on PC-3 and MDA-MB-453 cells' growth. The cells were treated with 25, 35, 50, 75 and 100 µM of compound A for 48 h.**

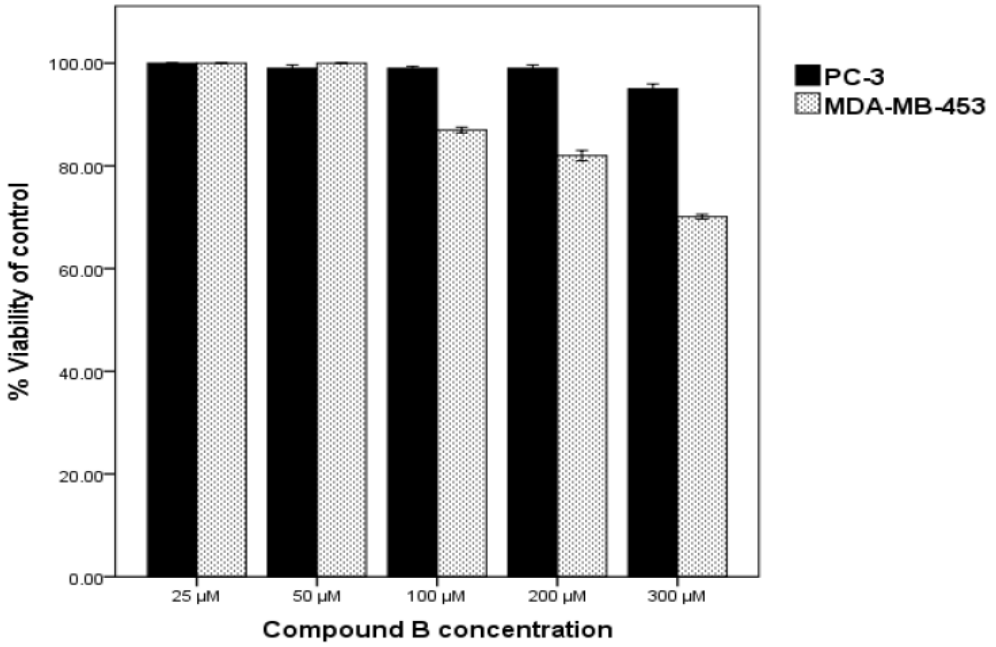


Figure 4. **The effect of compound B on PC-3 and MDA-MB-453 cells' growth. The cells were treated with 25, 50, 100, 200 and 300 µM of compound B for 24 h.**

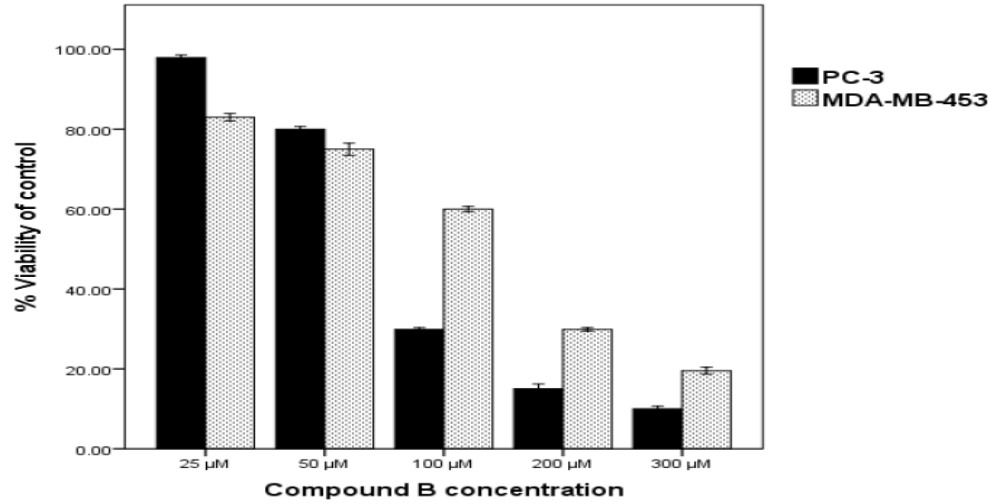


Figure 5. **The effect of compound B on PC-3 and MDA-MB-453 cells' growth. The cells were treated with 25, 50, 100, 200 and 300 µM of compound B for 48 h.**

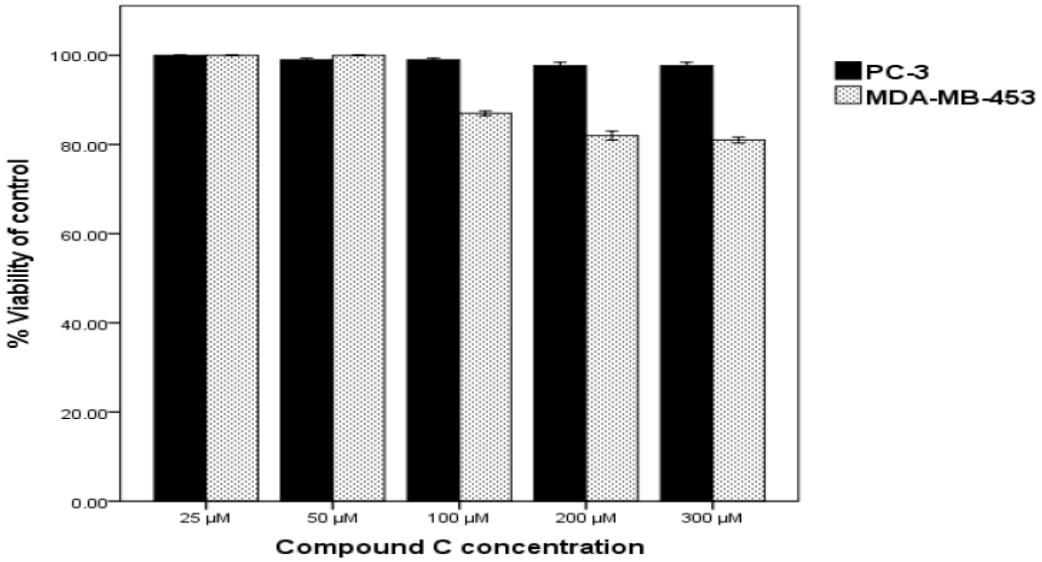


Figure 6. **The effect of compound C on PC-3 and MDA-MB-453 cells' growth. The cells were treated with 25, 50, 100, 200 and 300 µM of compound C for 24 h.**

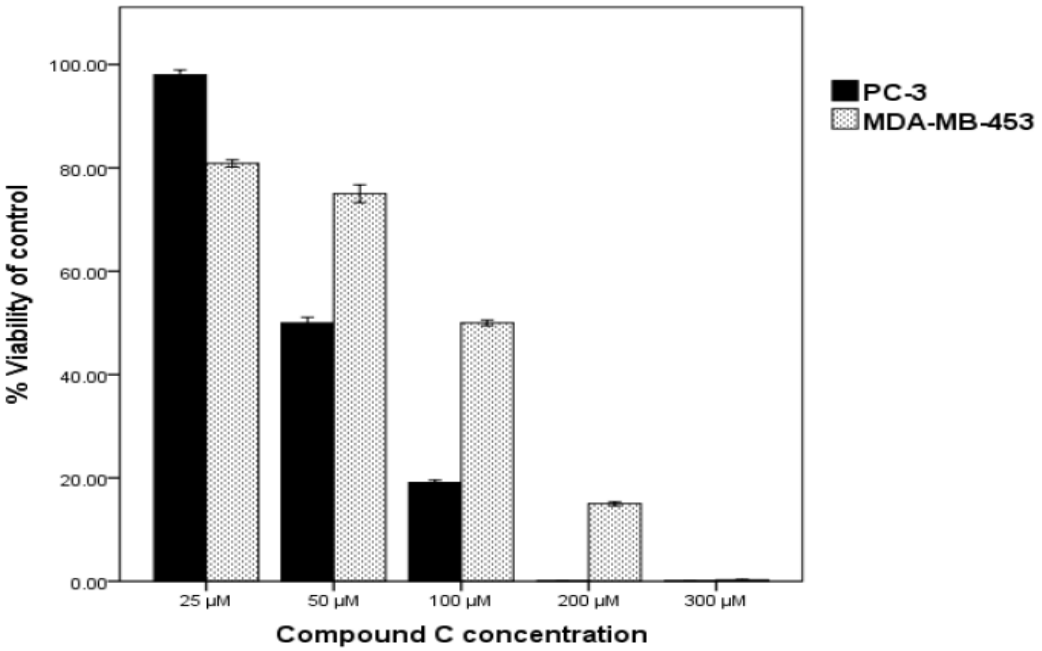


Figure 7. **The effect of compound C on PC-3 and MDA-MB-453 cells' growth. The cells were treated with 25, 50, 100, 200 and 300 µM of compound C for 48 h.**

Morphological examination of apoptotic changes

According to the cytotoxicity results for compounds AB and C, these compounds were examined their ability to produce pro-apoptotic activity. Chen *et al.*, 2009, and Kalinichenko and Matveeva, 2008, indicated that programmed cell death has many characteristics including chromatin condensation, cell shrinkage, nuclear DNA fragmentation and formation of apoptotic bodies. The apoptotic morphological changes were examined for these 3 compounds using the fluorescent chromatin Hoechst 33342 dye. PC-3 prostate cells were treated with compounds A, B and C at 50 µM for 48 h then stained with Hoechst 33342 dye, these cells had clear apoptotic bodies (figure 7, top 2 panels). Also, MDA-MB-453 breast cells were treated with compounds A, B and C at 50 µM for 48 h then stained with Hoechst 33342 dye, these cells had clear apoptotic bodies (figure 8, bottom 2 panels).

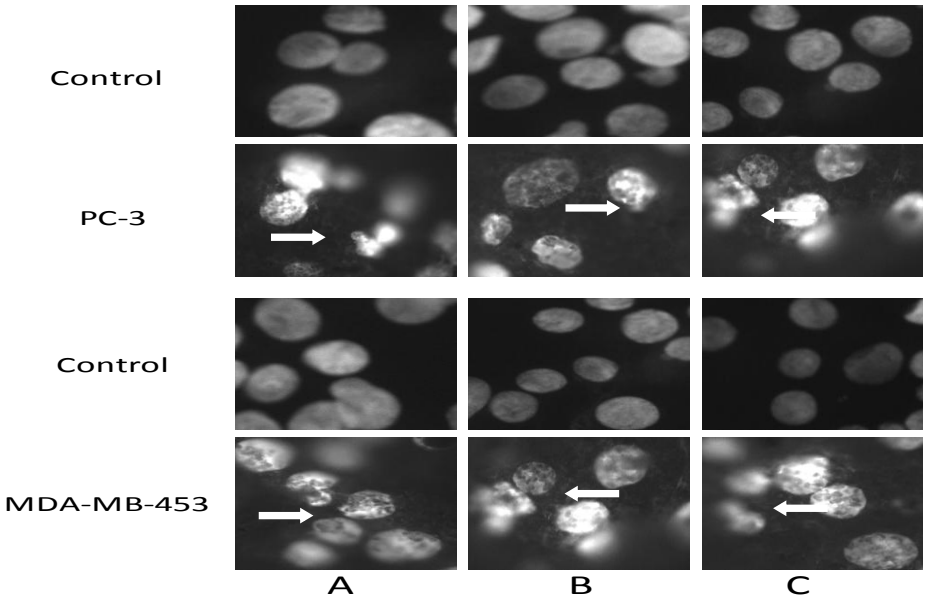


Figure 8. **Morphological and Cellular changes were induced by coumarin derivatives.**

The apoptotic morphological changes were examined for compounds A, B and C using the fluorescent chromatin Hoechst 33342 dye. Both PC-3 prostate cells and MDA-MB-453 cells were treated with compounds A, B and C at 50 µM for 48 h then stained with Hoechst 33342 dye. These cells had clear apoptotic bodies for PC-3, top 2 panels and MDA-MB-453 cells, bottom 2 panels.

DNA isolation and fragmentation assay

The DNA fragmentation assay was determined according to the protocol provided with apoptotic DNA ladder detection kit (Roche). Since compound A was promising with IC_{50} 20-25 µM. It went through further apoptotic investigation such DNA fragmentation.

Both PC-3 cells and MDA-MB-453 cells were treated with and without compound A for 48 h using 1% agarose gel. Control cells showed no DNA formation, however, both PC-3 and MDA-MB-453 cells formed DNA fragmentations as a hallmark of apoptotic cells (figure 9).

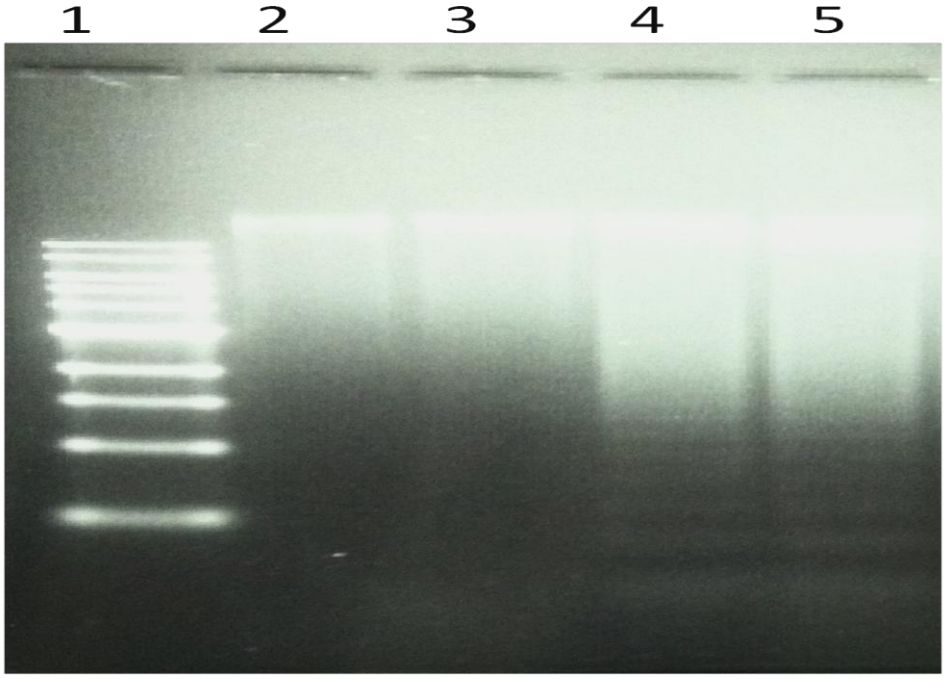


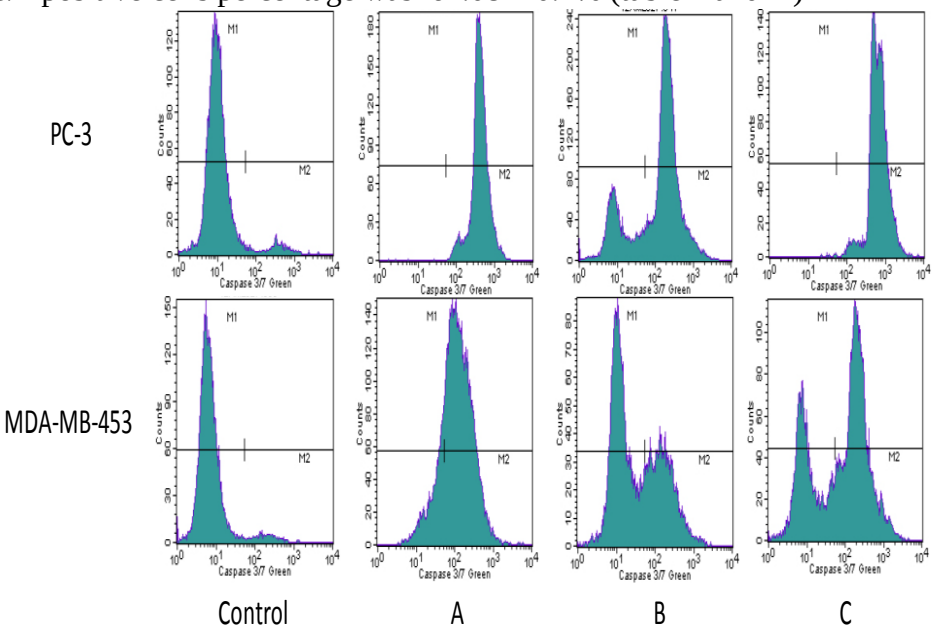
Figure 9. **The DNA fragmentation assay was determined according to the procedure provided with apoptotic DNA ladder detection kit (Roche).**

Both PC-3 and MDA-MB-453 cells were treated with the compound A for 48 h using 1% agarose gel. DNA marker (lane 1), PC-3 untreated cells (lane 2), MDA-MB-453 untreated cells (lane3), PC-3 treated cells (lane 4), MDA-MB-453 treated cells (lane5).

Caspas-3/7 activity

Caspas-3/7 activity was assayed against both PC-3 and MDA-MB-453 cells (Histogram 1). Cells were grown and treated with the compounds A, B and C at 50 µM for 2 days at 37 °C in a 5% $C0_2$ humidified incubator. Compound A showed significant very strong Caspas-3/7 activity for PC-3 and MDA-MB-453

lines with 99.98 ± 0.6 % and 80.90 ± 0.6 % respectively. However, compound B showed moderate to light Caspas-3/7 activity for PC-3 and MDA-MB-453 lines with 76.35 ± 2.3% and 37.47 ± 0.3 % respectively. But, compound C showed significant very strong Caspas-3/7 activity for PC-3 with 99.93 ± 0.1% while the caspase-3/7 positive cells percentage was 64.08 ± 0.1% (table 1 and 2)



Histogram 1. **Shows Caspase-3/7 negative cells (M1) and Caspase-3/7 positive cells after 48 h treatment for compounds A-C for both PC-3 top panel and MDA-MB-453 bottom panel.**

Table(1): Shows caspase-3/7 negative cells (viable cells) % and Caspase-3/7 positive cells (apoptotic cells) % after 48 h treatment for compounds A-C for PC-3 prostate cell line. The values expressed as mean ± SD% of 3 replicates for each experiment.

PC-3 Prostate cell line		
Compound's Concentration	**Viable cells Caspase-3/7 negative cells (Mean ± SD) %**	**Apoptotic cells Caspase-3/7 positive cells (Mean ± SD) %**
Control	96.23 ± 3.2	03.77±3.2
A 50 µM	00.02 ± 0.6	99.98 ± 0.6
B 50 µM	23.65 ± 2.3	76.35 ± 2.3
C 50 µM	00.07 ± 0.1	99.93 ± 0.1

Table (2): Shows caspase-3/7 negative cells (viable cells) % and Caspase-3/7 positive cells (apoptotic cells) % after 48 h treatment for compounds A-C for MDA-MB-453 breast cell line. The values expressed as mean ± SD% of 3 replicates for each experiment.

MDA-MB-453 cell line		
Compound's Concentration	Viable cells Caspase-3/7 negative cells (Mean ± SD) %	Apoptotic cells Caspase-3/7 positive cells (Mean ± SD) %
Control	99.96 ± 0.4	00.04 ± 0.4
A 50 µM	19.10 ± 0.6	80.90 ± 0.6
B 50 µM	62.53 ±0.3	37.47 ± 0.3
C 50 µM	35.93 ± 0.1	64.08 ± 0.1

DISCUSSION

The three coumarin derivatives were evaluated for their antioxidant and anticancer activities against PC-3 prostate adenocarcinoma cells and MDA-MB-453 breast carcinoma cells. Compound A showed antioxidant effects with 100% decoloration at concentration of more than 150 µg/ml with IC_{50} of 102.12 ± 0.15 µg/ml. Compound B and C induced antioxidant effects with 100% decoloration at 100 and 50 µg/ml and with IC_{50} of 28.35 ± 0.04 and 6.12 ± 0.05 µg/ml, respectively. The antioxidant activity was variable from strong to very slight due to the differences in compounds' chemical structures, in agreement with Mladenović *et al.,* 2011, and Salem *et al.,* 2016. Where the presence of cyano group reduces the free radical scavenging effect. However, the presence of carboxylic group increases the antioxidant activity.

The *in vitro* anticancer activities of these three coumarin derivatives results indicated that all of these compounds exhibit no to slight activity against both PC-3 prostate adenocarcinoma cells and MDA-MB-453 breast carcinoma cells after one day of treatment. However, by increasing the treatment time to 48 h, these 3 compound showed both time- and dose-dependent effects. The IC_{50} results indicated that compound A was very toxic for both cell lines, may be due to the presence of cyano group in its structure. Salem *et al.* 2016, indicated that some coumarin derivatives have cytotoxicity activity related to the coumarin moiety and cyano group in their structure. Also, cyanide compounds show acute toxicity and cause DNA damages when evaluated on mammalian cells (Bhattacharya and Rao, 1997). Furthermore, all the IC_{50} results indicated that MDA-MB-453 cell line always required higher dosage than PC-3 cell line. These exact compounds were previously investigated for their anti-breast-cancer activity by the first author (Mahmoud, 2012) and showed selectivity toward estrogen receptor (ER) over-expressing cancer types. PC-3 cells were found to be both

HER-2 and ER over-expressing prostate cell line (Andersson *et al.,* 2014; Malmberg *et al.,* 2011 and Pisolato *et al.*, 2016). However, MDA-MB-453 cells is HER-2 over-expressing breast cancer cell line and not ER over-expressing breast cancer cell line (Asada *et al.* 2002 and Al-Bader *et al.* 2011). So, MDA-MB-453 cell line was more resistant than PC-3 cell line, which may be because its growth depends only on HER-2 protein, and compounds A, B and C are selective against ER over-expressing cancer cells.

Evidently, any deficiency in apoptotic process will turn normal cells to cancer cells. Thus, the most important expected feature of any anti-cancer agent is to induce apoptosis (Phonnok *et al.*, 2010). In view of the fact that caspase-3/7 has a major role in apoptotic death signals downstream by cleaving the cellular substrates (Kumar, 2007). These three tested compounds were cytotoxic for both PC-3 and MDA-MB-453 cells, these cytotoxic effects were clear at the concentration of 50 μM of all compounds. Thus, compounds A, B and C were tested at the previous concentration and showed many apoptotic characterizations including, DNA fragmentation and apoptotic bodies formation. Also, these compounds were investigated for their caspase-3/7 activities, and found to activate the apoptotic death signals downstream after 48 h of treatment comparing with controls.

In conclusion, the evaluation of the biological activities of compounds A, B and C, showed that they produced antioxidant effects and were cytotoxic for both PC-3 and MDA-MB-453 cells. MDA-MB-453 cell line was more resistant than PC-3 cell line, which may be because its growth depends only on HER-2 protein, and these compounds are selective against ER over-expressing cancer cells. In general, compound A induced the highest cytotoxicity and compound B was the lowest. Also, these compound activated the apoptotic death signals downstream by activating caspase pathway. And showed many apoptotic characterizations including, DNA fragmentation and apoptotic bodies formation.

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النشاط الكيميائي الخلوي المناعي كمضاد للسرطان وكمضاد للاكسده لثلاث مشتقات مخلقه من الكومارين

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اكدت العديد من الدراسات البيولوجيه ان ماده الكومارين و مشتقاتها يمكن استخدامها كمواد وقائيه كيميايه ضد الامراض السرطانيه. في هذه الدراسه تم دراسه النشاط البيولوجي لثلاثه مشتقات كيومارينه مخلقه فتم فحص نشاطهم كمستضدات للشقوق الحره و كمضادات سرطانيه كذلك مدي تأثيرها علي ارسال الاشارات الخلويه. اظهر المشتق (أ) فاعليه كمضاد اكسده من خلال قدرته علي ازاله اللون لمركب ثنائي فينيل بكريل هيدرازيل بنسبه ١٠٠% عند اكثر من ١٥٠ ميكروجرام /مل و كان له القدره علي التثبيط النصفي (IC_{50}) بواسطه ٠,١٥ ± ١٠٢,١٢ ميكروجرام /مل. بينما المركب (ب) ازال اللون بنسبه ١٠٠% عند ١٠٠ ميكروجرام /مل و كان له القدره علي التثبيط النصفي (IC_{50}) بواسطه ٠,٠٤ ± ٢٨,٣٥ ميكروجرام /مل. بينما مركب (ج) ازال اللون بنسبه ١٠٠% عند ٥٠ ميكروجرام /مل و كان له القدره علي التثبيط النصفي (IC_{50}) بواسطه ٠,٠٥ ± ٦,١٢ ميكروجرام /مل. ايضا قد تم دراسه مدي تأثير هذه المشتقات (أ) و (ب) و (ج) كمثبطات لنمو الخلايا السرطانيه علي خلايا سرطان البروستاتا PC-3 فكان التركيز المثبط النصفي للخلايا هو ٢٠ و ٧٠ و ٥٠ ميكرومولرعلي التوالي .بينما تأثيرها المثبط ضد خلايا سرطان الثدي MDA-MB-453 فكان ٢٥ و ١٣٧ و ١٠٠ ميكرومولر علي التوالي. اوضحت هذه الدراسه علي قدره هذه المشتقات علي تنشيط ارسال الاشارات الخلويه المنبهه لقتل الخلايا السرطانيه من خلال تنشيط بروتينات Caspase . كما اظهرت هذه المشتقات بعض الخصائص الخاصه بموت الخلايا المبرمج مثل تكسير(تجزؤ) الحمض النووي ال DNA للخلايا السرطانيه و كذلك تكوين الجسيمات الصغيره.