Anti-Cancer and Acute Toxicity Activity of Benzo[e]Indol-2-Ylidene)-3 -(4-Methoxyphenyl) Imino Propanal

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Abstract

Background: The new indole Schiff base compound benzo[e]indol-2-ylidene)-3-(4-methoxyphenyl) iminopropanal](2P)was investigated for antioxidant, toxicity and anti-tumor ability for A547 cell, PC3 cell and WRL 68 cell.

Results: 2P has antioxidant ability for IC50 (75 µg/mL) compared with control ascorbic acid (27 µg/mL). Antitumor ability to 2P for A547 cell and PC3 cell were decreased significantly after treated with 2P in dose dependent manner at 24,48 and 72 hours, while no toxicity effect on the WRL 68 cell. The antitumor effect was through accelerated apoptosis, the alteration in nuclear morphology. Increasing in the cells membrane permeability, decreasing in the nucleus intensity with the increasing of concentration of 2P compound was observed.

MMP is disturbed in the apoptosis by forming of permeability and the result was showed that 2P activated release cytochrome c significant higher than control.2P break down the cell cycle progress in G1 phase ($P < 0.05$) compared with control. Conclusion: this result was conducted the new 2P compound has antioxidant and antitumor ability that may be used 2P a new anticancer drug.

***Keywords**: Acute Toxicity, Anti-tumor, Apoptosis, Synthetic*

Introduction

Cancer is the world's second most prevalent cause of death, killing more than 8 million people per year; the prevalence of cancer is predicted to rise by more than 50 per cent over the coming decades[1]. It is resulting from uncontrolled cell proliferation with an absence of cell death making abnormal cell mass or tumor, new vascularization with time, this primary tumor develops and spread to another body sites, causing metastasis then death. Cancer caused by damage or mutations in the genetic material of the cells due to environmental or inherited factors[2]. Between 2005 and 2015, cancer cases increased by 33%, for men, the most common cancer globally was prostate cancer (1.6 million cases). Prostate cancer is the serious problem among worldwide men society, in the UK, with a prevalence of 105 per 100 000 population, also common in northern Europe and the USA (mainly in the African American population) It is uncommon in India but the incidence is increasing, although the overall number of deaths from prostate cancer has declined in the past 10 years, due to the increasing use of screening methods focused on prostate specific antigen (PSA)[3]. In the second hand lung cancer (LCA) had become epidemic and come up as a leading cause of cancer related death in USA and Europe, killing three times much more than prostate cancer in men also twice as many women as breast cancer[4]. According to the recent statistics which illustrate the new cases in Iraq in 2018 for both sexes and all ages that 8% as lung cancer incidence and 14% of total deaths, according to the recent World Health Organization WHO announced report [5].

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Chemotherapy is still the best way to treat many type of cancers, considering the site of it , how it big, if it is spread to other body parts and if it effects on normal body function or health (side effect)[6]. Antioxidant can enhance the immune defenses and lower the risk of cancer and degenerative diseases by minimize cell oxygen toxicity or injury to prevent, intercept and repair damage resulting from (ROS). It is work at three levels: (a) prevention , by maintains formation of (ROS) to the minimum level, e.g., desferrioxamine; (b) interception, scavenges (ROS) by using catalytic and non catalytic molecules, e.g., ascorbic acid and tocopherol; and (c) repair, damaged target molecules, e.g., glutathione [7].

Schiff bases that prepared from the condensation reaction of aromatic primary amines with aromatic aldehydes or ketones are most stable then that prepared from the condensation reaction of aliphatic primary amines with aliphatic aldehydes oraliphatic ketones. They containing a carbon nitrogen double bond with the nitrogen atom connected to an aryl or alkyl group but not hydrogen[8]. Indole derivatives are an important class of organic heterocyclic which have played vital and significant role in curing so many ailments throughout the history of pharmaceuticals and medicines. Also they are one of the most attractive frameworks with a wide range of biological and pharmacological activities[9]. This study aim to synthesize a new Indole Schiff base to find anti -tumor for PC-3 cell.

Methods

Synthesizing a new Indole Schiff base

(2g, 0.01mol) of 1,1,2-Tri methyl-1H-benzo [e] indole was dissolved in10 ml of DMF and cooled in to ice bath. The solution of (4.6 ml, 0.03 mol) of phosphoryl chloride in 10 ml of dimethylformamide was cooled in an ice bath also and then was added dropwise to first solution with stirring for 1 h at below 5°C. After that, the reaction mixture was reflux for 3 hours. The mixture of reaction was poured on ice water, the pH was mend to 8.0 by added aqueous solution of (25%NaOH), the solid product was precipitated and the product was filtered, washed with water and then dried to afford solid yellow product. Yield (91%), m. p.199-200°C.

Synthesis of 2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene)-3-((4-methoxyphenyl) imino)propanal

A solution of (0.4 g, 1.5 mmol) of 2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene)malonaldehyde was dissolved in ethanol 20 ml and (0.19 g, 1.5 mmol) of 4-methoxy aniline was dissolved in ethanol 10ml and then added glacial acetic acid 2ml to the solution. The mixture was refluxed in a water bath at 78 ºC for 1h. A solvent was reduced to one quarter; yellow precipitate was formed direct, filtered off, washed with ethanol and dried in oven. The completion of the reaction was checked by using TLC (3:1) hexane: ethyl acetate with pre-coated silica gel, which gave one spot. Rf=0.7 cm. Yield (88%),IR data in (cm-1):3301 υ(N-H) 3120 υ (CH aromatic) 2923υ (CH aliphatic), 1665υ (CH=O), 1640 υ(C=C), 1607 υ(CH=N),1376 υ(CH3), 1254υ(C-N), 1076υ(C-O), and 829 υ(C-H bending).

1H NMR (400MHz, CDCl3) δ (ppm): δ = 14.37ppm (s, 1H, NH), 9.74ppm (s, 1H, CHO), 8.32 ppm (s, 1H, CH=N), 8.05-6.92ppm (d, 8H, Ar-H) and(t, 8H, Ar-H) 3.8ppm (s, 3H, OCH3), 1.87 (s, 6H, 2xCH3). APT13C NMR (100 MHz, CDCl3) δ (ppm): δ = 186.91 (CHO), 185.09(NH-C=C), 153.00 (CH=N), 157.75, 148.01, 133.80, 132.36, 129.75, 129.03, 128.17, 126.44, 124.14, 122.78, 119.70 and 118.41(Ar-H). 115.11 (C=C-CHO), 56 (OCH3), 55.61 (CH3-C-CH3) and 22.69 (2x CH3).

Antioxidant Activity

To investigate the antioxidant activity for new Indole Schiff base synthesis compound by applied FRAP and DPPH method as described by Walaa,et al [10].

Toxicity Effect of New Synthetic Compound

This in vitro technique was conducted to examine the potential cytotoxic effect on tumor cell lines (PC3 and A547) and standard cell line WRL 68 at various concentrations of 2P compounds.The cell sheet washed with PBS after growth medium was aspirated.Trypsin solution have been added to the cell by (2-3mL). To cover the monolayer full with gentle rocking, the flask have been flipped over. The flask was needed 1 to 2 minutes of incubation at 37 ° C before the cells were disconnected from the flask.Fresh complete medium RPMI (15-20 mL) was applied and cells were distributed by pipetting from the wedding surface into growth medium.At the required concentration, cells were redistributed to culture flasks, or plates whatever was desired and incubated in a 5 % CO2 incubator at 37 ° C. MTT assay was done as describe by Walaa,et al [10].

Apoptosis Activity

To investigate apoptosis activity for 2P used Cytotoxicity 3 Kit. Solutions of 2P were diluted at different concentration (5, 10, 15μg/ml) was prepared and 25 μl was added to the PC3 cells. The cells were incubated at 37 ° C for 24 hours. A 50 μl of live cell staining solution was added to each well.The cells were incubated at 37°C for 30 minutes.The medium and the staining solution and 100 μl/well of fixation solution and was plate incubated for 20 minutes at room temperature. The fixation solution was gently aspirated and 100 μl/ well of 1x wash buffer was added.The wash buffer was removed and 100 μl / well of 1X permeabilization buffer was added and incubated for 10 minutes at light free room temperature.The permeabilization buffer was aspirated and the plate cleaned three times with a 1x wash buffer of 100 μl / well.Wash buffer was Aspirated, and at room temperature 100 μl of 1X blocking buffer was added and incubated for 15 minutes.The Buffer of Blocking have been aspirated and (50 μl/well) of the primary antibody Solution was added, with 60 minutes of Incubation and protection from light at room temperature.The primary antibody solution was aspirated and plate washed three times with 100 μl/well 1x wash buffer.

Wash Buffer was aspirated and secondary Antibody / Staining Solution added 50 μl / well. with 60 minutes of room temperature and protection from light Incubation secondary antibody / staining solution was aspirated and plate washed three times with 1x wash buffer at 100 μl / well One hundred μl/well of 1x wash buffer was added. The plate was sealed and evaluated on the array scan HCS reader.Sealed plates were Stored in dark at 4°C. Within 24 hours after assay completion the plates has been evaluated. Sealed plates were stored in dark at 4°C. Within 24 hours after assay completion the plates has been evaluated.

PC3- cell cycle analysis

Using the commercial CycleTestTMPlus DNA Reagent Package (BD Bioscience system), for PC3- cell cycle analysis.Cells were seeded in twelve-well plates at 5 x 10, 5 cells per well. The plates were incubated at 37°C, 5% CO2 for 24 hrs.

A- After incubation, the medium was removed and the cells were treated with the compound at 100 and 200 μg/ml for 48 hrs for the cell cycle analysis. The next steps were followed in order to obtain a fine cell suspension:

1. The growth medium was aspirated and the cell sheet washed with PBS.

2. Two to three ml trypsin/EDTA solution was added to the cell. The flask was turned over to cover the monolayer completely with a gentle rocking. The flask allowed incubating at 37°C for 1 to 2 minutes, until the cells were detached from the flask.

3. Fresh complete RPMI medium (15-20 ml) was added and cells were dispersed from the wedding surface into growth medium by pipetting.

4. Cell suspension was placed into a labeled 17 x 100 mm tube.

5. Cells were centrifuged for 5 minutes at 300 g at room temperature.

6. The supernatant was aspirated and 1 ml of Buffer Solution was added. Cells were re-suspended by gently vortexing at low speed.

7. Cells were centrifuged for 5 minutes at 300 g at room temperature.

8. Steps f and g were repeated. After centrifugation, the supernatant was aspirated and the pellet was re-suspended in 1 mL of Buffer Solution.

9. The cells were counted using a hemacytometer and cells concentration was adjusted to 1.0 x 10 6 cells/ ml with Buffer Solution for immediate staining and flowcytometric analysis.

B- Staining Protocol:

1. Cell suspension was centrifuged at 400 g for 5 min at room temperature (20°C–25°C). All the supernatant was carefully decanted and 250 μl of Solution A (trypsin buffer) was added to each tube. The tube was gently mixed by tapping the tube by hand.

2. The mixture was incubated for 10 minutes at room temperature

3. Aliquot of 200 μl of Solution B was added to each tube with gentle mixing by tapping the tube by hand.

4. The mixture was incubated at room temperature for 10 minutes.

5. Aliquot of 200 μl of cold (2°C–8°C) Solution C (PI stain solution) was added to each tube with gentle mixing by tapping the tube by hand.

6. Mixture was incubated for 10 minutes in the dark on ice or in the refrigerator (2°C–8°C).

7. Finally the mixture was filtered using 50 µm nylon mesh into a new sterile tube for flow cytometer analysis.

C- Data Acquisition and Analysis:

With forward scatter (FSC) and side scatter (SSC) detection, BD FACS may provide linear fluorescence amplification capability for flowcytometers. In the blue-to-green range, the flowcytometer was fitted with an argon-ion laser emitting PI excitation at 488 nm. At an acquisition rate of at least 60 events per second, all samples were run and histograms were analyzed with suitable DNA analysis tools.

4. Solution C (8 ml), this solution have been used to binds to the DNA at a final concentration at least125µg/ml. It was kept away from light and stored at 2°C–8°C.

Statistical Analyses

A one-way ANOVA (Duncan) variance analysis was performed to evaluate whether or not group variance was significant, and statistical significance was defined as $p < 0.05$. Data were represented using Graph Pad Prism version 6 (Graph Pad Software Inc., La Jolla, CA) as mean± standard deviation and statistical significance.

Results

Synthesis of compound

The a new compound 2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene)-3-(4-methoxyphenyl) imino) propanal has been synthesized according to condensation reaction of 2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene) malonaldehyde with 4-methoxy aniline in equivalent ratio 1:1

Infra-Red Study (FT-IR)

The results of the IR Spectrum for the new synthesized compound 2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e]indol-2-ylidene)-3-(4-methoxyphenyl) imino) propanaldisplayed absorption bands in range between 400 - 4000 cm^{-1}.

IR Spectrum shows disappearance one absorption band of (NH_2) group which belonged to p-methoxy aniline and appearance a new absorption band of imino groups (CH=N) which is approved to formation new compound. The FT-IR results figure 2 showed absorption band at 3301 cm^{-1} which assigned to (N-H) of indole ring [11], absorption bands at 3120 and 2923 cm^{-1} were attributed to stretching vibration of aromatic (C-H) and aliphatic (C-H) respectively [12], strong absorption band at 1665 cm^{-1} was belonged to the carbonyl group (C=O) also strong absorption band at 1640cm^{-1} referred to (C=C) group as well as a new strong absorption band of azomethine group (CH=N) which appeared at 1607 cm^{-1} was approved the formation of a new compound, other absorption bands were appeared on spectrum such as, at 1376cm^{-1} which belonged to bending vibration of CH_3, at 1254cm^{-1} which attributed to (C-N) at 1076 attributed to (C-O) group and finally absorption band at 829 cm^{-1} was attributed to out -of-plane (C-H) group.

4.1.2 Nuclear Magnetic Resonance Study (NMR)

Nuclear Magnetic Resonance Study (NMR)

The ^1H and APT^{13}C NMR spectra of the a new synthesized Schiff base were recorded in deuterated chloroform $CDCl_3$ with chemical shifts expressed in ppm using tetramethylsilane TMS as an internal standard. The ^1H NMR and APT ^{13}C NMR spectrums were confirmed the suggested chemical structure for a new synthesized compound through disappearance signals and appearance of newsignals. Such as disappearance of one proton atom of the carbonyl group of aldehyde and a signal of NH_2 group of 4-methoxy aniline on spectrum as well as appearance new signal of one proton atom of imine group (Schiff base).This is approval to the formation of the new compound.

^1H-NMR spectrum of the new synthesized compound 2-(1,1-dimethyl-1,3-dihydro-2H-benzo[e] indol-2-ylidene)-3-(4-methoxyphenyl)imino) propanalshowedfigure 8 shows single singlet with chemical shift δ = 14.37 ppm which belonged to NH for indole ring . Also single singlet at δ = 9.74 ppm which attributed to one proton of carbonyl group .Important singale signal at δ = 8.32 ppm which assigned to new functional group of Schiff base CH=N) [13], single and doublet signals were observed in region between 6.92-8.05ppm which attributed to ten protons of aromatic rings .single singlet at δ = 3.8 ppm which attributed to three protons of methoxy group . Finally the spectrum shows strong single signal at 1.87ppm assigned to six protons of two methyl groups .

APT ^{13}C NMR was supported ^1H NMR results through appearance a number of signals of carbon atoms on APT ^{13}C NMR spectrum which were corresponding

to a number of signals of carbon atoms of the chemical structure of the a new proposed compound. Since a number of carbon atoms bearing protons of quaternary carbons were appeared at the positive side (above base line) such as some of carbon atoms of aromatic rings and indole ring, and a number of carbon atoms bearing protons of CH and CH_3 appeared at the negative side (under base line), like carbon atoms of carbonyl group, methoxy group, methyl groups and some of carbon atoms of aromatic rings.

Anti- oxidant activity

Activity of antioxidant of 2P compound was assessed via DPPH and FRAP test and compared with ascorbic acid in concentration gradient from (25, 50.100,200) µg/mL as shown in table 1. The result was revealed that the 2P compound has higher antioxidant activity.

Table 1 : Antioxidant activity value

	FRAP µmol Fe^{+2}/mg	DPPH inhibition %	IC 50 µg/mL
Ascorbic acid 25 µg/mL	45 ±1.414*	48±2.828*	27
Ascorbic acid 50µg/mL	52±1.414*	62±2.828*	
Ascorbic acid100µg/mL	63±2.828*	75.5±2.121*	
Ascorbic acid200µg/mL	73±2.828	83.5±2.121	
2P 25 µg/mL	26.5±2.121	20±2.828	75
2P 50µg/mL	38.5±2.121	37.5±3.536	
2P 100µg/mL	49±4.243	51±4.243	
2P 200µg/mL	69±1.414	77±2.828	

Data was presented as mean ±SD , *Significant at P< 0.001

Anti cancer ability

Concentration (12.5,25,50,100,200 and 400) ug/ml the viability for A547 cell and PC3 cell compared with viability of WRL 68 cell at 24h , 48h and 72 h were presented in the (Table 2) that showed effect of 2P in the viability of A547 cell and PC3 cell significantly (P< 0.05) compared with the viability of WRL 68 cell.

Table 2: Showed the Viability of the A547 cell,PC3 cell and WRL cell after treatment with Benzo[e]Indol-2-Ylidene)-3 -(4-Methoxyphenyl) IminoPropanal in different doses and different time.

Cells		Viability of cell in Concentration 2P/ ug/ml					
		12.5	25	50	100	200	400
A547 cell	24 h	95.2± 1.3	86.2±3.9	79.2±3.1	63.8±1.9	54.8±1.2	46.4±4.1
	48 h	83.2± 1.7	72.8±0.9	76.2±5.2	61.7±0.7	52.7±3.3	47.3±3.4
	72 h	65.1± 3.4	62.1±0.9	53.9±4.6	50.7±2.9	42.8±1.7	37.9±1.6
PC3 cell	24 h	96.6±1.4	96.2±0.7	90.0±2.4	63.8±2.7	37.8±1.7	25.1±1.8
	48 h	85.1± 1.2	73.2±0.7	63.3±3.0	56.1±1.2	47.4±4.1	44.9±2.4
	72 h	65.6± 4.5	60.1±1.3	50.8±1.3	44.8±1.3	40.7±1.7	33.7±2.4
WRL 68 cell	24 h	95.9± 1.0	95.2±0.8	95.3±1.2	93.6±2.1	84.8±1.2	69.3±2.6
	48 h	95.9± 1.0	95.2±0.8	95.3±1.2	93.6±2.1	88.3±1.5	79.3±0.7
	72 h	85.6± 2.0	85.4±1.1	76.9±5.8	78.4±2.3	69.6±5.2	65.7±3.7

Data was presented as mean ±SD , Significant at P< 0.05

2P induced apoptosis in PC3 cells

To prove of happened the apoptosis, the alteration in nuclear morphology of PC3 cells was examined by using Hoechest staining after treated the PC3 cells with different doses (5,10,15) µg/mL (Figure 1A).

The density of the nucleus has been found to be closely linked to apoptosis and chromatin modifications. in (Figure 1B). An rising in the permeability of the cell membrane was observed in (Figure 1C). The result was showed decreasing in the nucleus intensity and increasing in cell permeability with the increasing of concentration of 2P compound treated the cells.

2IP induced MMP disruption and release of cytochrome c.

MMP is distrusted in the apoptosis by forming of permeability, this ability of 2P on the MMP of PC3 cell was studied by using of mitochondria specific Dye, as seen in Figure 1D, the mitochondria membrane permeability in the PC3 cell processed with 2P showed significant ($P < 0.05$) diminution by decrease in the intensity of fluorescent compared with control (Figure 1A).

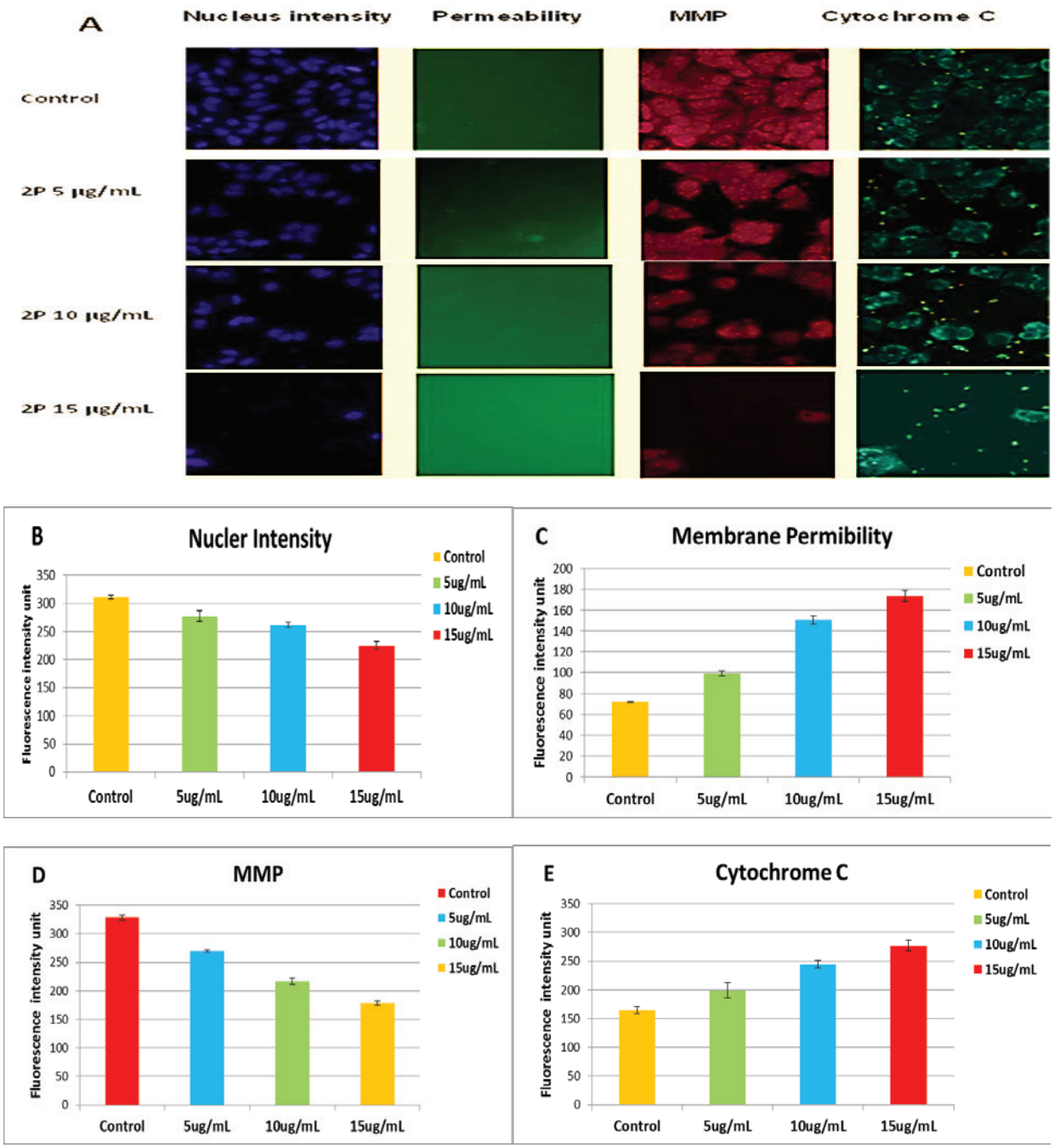

Figure 1:Effect of Benzo[e]Indol-2-Ylidene)-3 -(4-Methoxyphenyl) IminoPropanal (2P) on MMP, permeability and cytochrome c release. (A) Represented images of PC-3 cells treated with doses 5 µg/mL, 10 µg/mL, 20 µg/mL of 2P, and with Hoechst for nuclear, cell permeability dye, MMP and cytochrome c. The image from each row were obtained from the same field of each sample (magnification 20X0. (B□E) Average fluorescence intensities of Hoechst ,cell permeability dye, MMP, and cytochrome c in PC-3 cells treated with 2P. Data were presented as mean ±SD of florescence intensity readings measured from different photos taken. * Significant at P< 0.05

PC3 Cell cycle analysis

For detecting the effect of 2P on the DNA materials of PC3 cells applied the cell cycle phase divided into (G1) (G2) and (M) after therapy (Figure 2). The result was showed that 2P break down the cell cycle progress in G1 phase (P <0.05),(57.00 ± 0.283, 59.35 ± 0.354)% in 100 and 200 ug/mL respectively compared with control is (53.20 ± 0.283)% . The result display there is a significant G1 step arrest in PC3 in a concentration-dependent manner whereas, the cells in both G2 and M phase was reduced with an increased in the treatment.

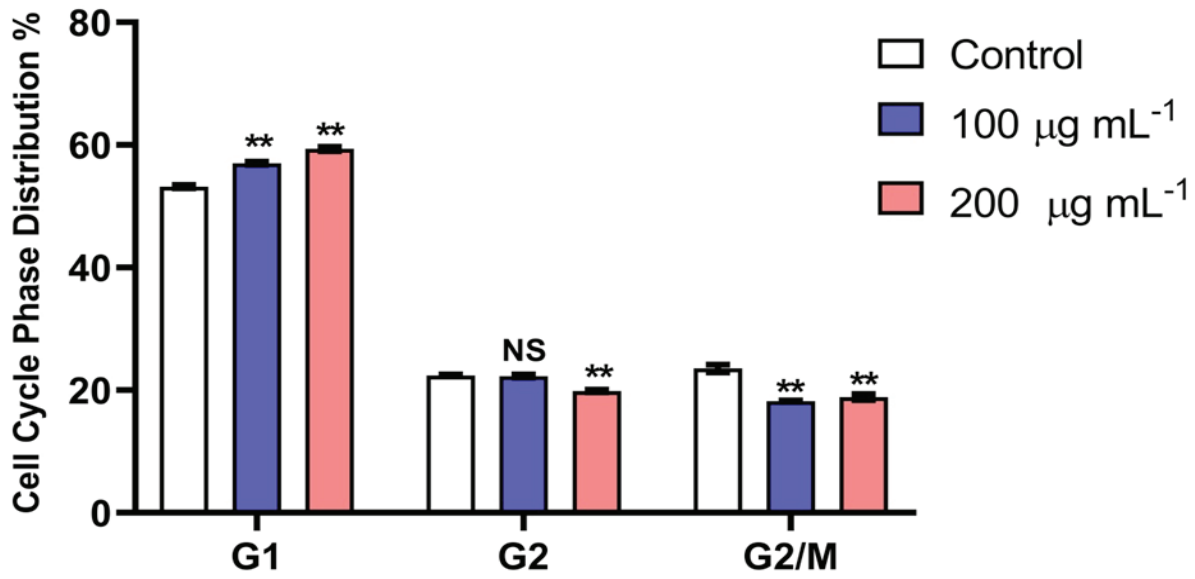

Figure 2: 2P induced G1 phase cell cycle arrest in PC-3 cell. Indication of G1 phase arrest in the cell cycle progression of PC-3 cell by 2P. **Indicate a significant difference (P < 0.05). Abbreviation: Benzo[e]Indol-2-Ylidene)-3 -(4-Methoxyphenyl)IminoPropanal (2P).

Discussion

Indole ring is an aromatic heterocyclic compound, and it is a white powder at room temperature. The indole chemical formula is C8H7N. It has an aromatic bicyclic structure consisting of a five-membered pyrrole ring fused together with a benzene ring to form two isomeric benzopyrrole. Indole derivatives are an important class of organic heterocyclic which have played vital and significant role in curing so many ailments throughout the history of pharmaceuticals and medicines. Also they are one of the most attractive frameworks with a wide range of biological and pharmacological activities [9]. Recent research has shown that the indole ring is the reactive center which handles oxidants because of its high resonance stability and very low energy activation barrier to free radical reactions[14].In this study the result of antioxidants activity was revealed that 2P compound has high level of antioxidant capacity upon the FRAP and DPPH assay as previous study reported phenols are involved in radical scavenging [15].

Many nutrient and synthetic drugs were followed to avoid or prevent oxidative damage which may lead to cell disruption to cancer cell depending on several mechanisms and means such as oxidative stress and apoptosis. This properties has been examined in this study and the result exhibited the ability of 2P compound significantly has anticancer activity for PC-3 cell.

Previous study appeared potent inhibitory effect of heterocyclic compounds which considered important class of molecules widely used for candidate as antitumor drug synthesis. The effect on proliferation in various types of aggressive prostate cancer cell lines (PC-3) dose dependent, the result significantly reducing to 80% of vehicle-treated controls [16].

A synergistic effect of new drug with effect of Titanocene−Gold Complexes Containing N-Heterocyclic Carbene Ligands Inhibit Growth of human Prostate cancer cell (PC-3) resulting heterometallic species which are highly apoptotic, exhibit strong antimigratory effects on the (PC-3) cell line [17]. A study gives a first prove in vivo that α-tomatine has antitumor affection of human androgen-independent prostate cancer(PC-3) cell line, potentially advantages was to prevention, thus acting as a therapy will need for more investigation [18]. A Natural dietary compound koenimbin reported as inhibitor of human prostate cancer, targeting PC-3-derived prostate cancer stem cells with koenimbin remarkably by induced nuclear condensation, forming of apoptotic bodies, and G0/G1 phase arrest of PC-3 cells [19].

The inspection of 2P compound anti-tumor activity effect on the PC-3 cell was achieved through Hoechst stain to tracing out nuclear morphology changing which following the treatment of the cell by using different dosage of 2P. The result was showed decreasing in the nucleus intensity and increasing in cell permeability with increasing of 2P compound concentration of treated cells. MMP changes or alteration after treating the PC-3 cells with 2P compound was studied by using mitochondria specific dye, the MMP in the PC3 cell treated with 2P showed significant ($P<0.05$) reduction by decrease in the intensity of fluorescent compared with control. The effect of cytochrome C was detected in the treated PC-3 cells with 2P compound, the result showed that 2P activated release cytochrome C significantly ($P<0.05$) higher than control. The data above reflect the ability of 2P compound to increase cell apoptosis by different ways using multiple strategies and scenarios, one of it is the alteration in membrane permeability.In this study the anti-proliferation activity of 2P compound was investigated on PC-3 cell which give a significant effecting on the cancer cells, in the other hand has less effecting on the normal cells WRL 68 cell where no effect on nuclear intensity, membrane permeability, MMP and cytochrome C. While there is a significant effect on the nuclear intensity, membrane permeability, MMP and cytochrome c in the PC-3 cell in different dose manner. Those effect because 2P improve the apoptosis processing by the antioxidant ability of 2P. Reactive oxygen species (ROS) Play a big part in the physiological process, involved in some important apoptotic signaling pathways, such as p38 and p53 pathways [20]. Programmed cell death is essential for animal development, homeostasis of the tissues and pathogenesis, apoptosis process has vital role in program cell death to maintain cell tissue homeostasis. The 2p compound has been found in this study inducing cell programmed death to PC-3 cell through apoptotic event including change in nuclear intensity, membranes permeability, release cytochrome C and DNA fragmentation in PC-3 cells. Activation of caspase-3, the primary executioner caspase in the majority of systems, irreversibly commits a cell to apoptosis. Therefore, caspase-3 activation serves as a reliable marker of apoptotic cells [21].

Appropriately to the cell cycle analysis 2P had induced PC3 cell cycle changing with increase in cell arrest in the G1phase. The result display that there is a significant G1 phase arrest in concentration dependent manner in PC3 cells. Whereas, the cells in both G2 and M phase was reduced with an increased in the treatment. The apoptosis process is highly related with cell cycle arrest. Controlling for cell cycle is essential for the maintenance of cellular division [22]. Many carcinogenicmaerial as natural and synthetic compounds were demonstrated in previous studies to enhance chemoresistance and tumorigenesis through cell cycle dysregulation and apoptosis inhibition in cancer cell [23],[24]. Like Koenimbin was induced PC3 cell cycle arrest with high percentage in the G0/G1 phase that revealed to inhibition of cellular proliferation through G0/G1 phase arrest [19].Generation of ROS interact with DNA caused alteration of the cell cycle phase [25]. DNA damage, and cell cycle perturbations induced by two representative gold(III) complexes in human leukemic cells with different cisplatin sensitivity [26].This made 2P effect on the disturb DNA lead to PC3 cell cycle arrest. The effect on the S phase arrest coupled with Bcl-2 down regulation cause anti- proliferation effect to PC3 cell [27]. Melatonin was shown that indole increases neuroendocrine markers and this cause increases cell sensitivity to apoptosis induced by cytokines [28].

proliferation properties for 2P compound have been related to effect on the transcription factors, protein kinases, Inflammatory pathways and growth signals pathways were activated by cell cycle proteins, cell adhesion molecules, and anti-proliferation proteins, but their function as antioxidants or pro-antioxidants was presumed [29].

Conclusion: 2P indole Schiff synthetic compound has anti-oxidant property and anti-proliferation effect could be promoted the role of bioactive compounds as

comparatively low toxicity, low cost, fast accessibility and potential role as adjuvant that potentiate the impact of chemotherapeutic drugs as chemo-preventive agents.

Ethical Clearance: Taken from College of Medicine, University of Diyala committee

Source of Funding: The source of funding self.

Conflict of Interest: Nil

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