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Downregulated BCL-2 and Caused Apoptosis in the (MDA-MB-231) Breast Cancer Cell Lines via Natural Compound (PCAE) That Majority Founds in the Clausena Excavata

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Abstract

Propyl–cannabinol allyldimethylsilyl ether (PCAE) is one of the natural compound that majority founds in the Clausena excavata plant that belong to the Rutaceae family has used in the medical treated especially in the Asia. The compound under study is presumed to have anticancer effect against cancer cell lines. The mechanism action of compound is still exactly unknown. This study designed to evaluate the effect of (PCAE) on (MDA-MB-231) breast cancer cell lines through induce caspase 9, 3 and depressioin BCL-2. Western blot and mRNA expression assays were studied. The results showed clearly increasing in level of caspase 9 with decreasing in upregulated of BCL-2 and slightly increasing in the caspase 3. In conclusion, PCAE is a potent anti-cancer agent that can used to fight cancer.

Keywords: program cell death (apoptosis), caspases9, caspase3, BCL-2. Phytochemical compounds.

Introduction:

A program cell death or apoptosis has been critical role in immune system, development and homeostasis of organisms [1]. The serious disease such as cancer, stoke, viral infection or autoimmune diseases can happen as a result of this process frailer [2]. Generally, the understanding of apoptosis mechanism is considered critical matter in successful therapy because the proteins that inducing apoptosis could be targeted therapeutically [3, 4].

Caspase 9 and subsequent activation of other proteins have been considered as a sign of intrinsic apoptosis that mediated via mitochondrial outer membrane permeablization (MOMP) [5]. Furthermore, the extrinsic apoptosis can launch through transmembrane death receptor [6]. Hence, all these process can regulate via the caspase families of proteins. Generally, caspase 9 can induce caspase 3 and 7 once it has activated [7]. In addition, all the hallmarks of intrinsic apoptosis which include DNA fragmentation, cell shrinkage and membrane blebbing are happened through cleavage caspase activity [8]. As mention by (Hsiao., *et all*), caspase 9 and others caspase have sequential and distinct effects on mitochondria that have represented by prevent cytochrome c from accessibility to complex III, this in itself could be stimulated ROS production [9, 10]. As mention in the previous studies, caspase 8 has cleaved Bid into tBid that do some changes in the model of

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mitochondria but actually the role of tBid in intrinsic apoptosis has not elucidated yet [11]. In general, the role of both caspase 3 and 8 that take place in intrinsic apoptosis is no understood [12, 13]. Therefore, more studies are needed to look into the distinct functions of each caspase. Hence, we used (MDA-MB-231) breast cancer cell lines to study the effect of Propyl–cannabinol allyldimethylsilyl ether (PCAE) in inducing apoptosis.

Materials and methods

Materials

Deionized water was used in current study. Cancer cells line, Dulbecco's modified eagle's medium (DMEM), dimethyl sulfoxide (DMSO), trypsin, phosphate–buffered saline (PBS), bovine serum and 3-(4, 5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide (MTT) were purchased from Sigma chemical company (St. Louis, MO, USA).

Cell culture

After enhanced (DMEM) media with 1% amphotericin B, 1% penicillin-streptomycin and 10%fetal bovine serum, the (MDA-MB-231) breast cancer cell lines were cultured in (DMEM) media under temperature 37°C and 5% CO2.

Western blotting assay

After cultured cells, the cell pellet was gated through centrifuging (1,000× g for 10min). Then, the pellet was lysed via treated with "fifty Mm lysis buffer". After that, 40µg of protein was added into ten percent of gel followed with electrophoresis process. Then, all the proteins were sent to membrane. After blocked membranes, Primary mouse antibodies (1:2,000 caspase 9, 1:2,000 caspase 3 and 1:2,000 Bcl-2) were used to incubate membranes against β-actin (overnight at 4°C). Then after one hour secondary antibody "1:1000 goat anti-mouse" was used. Chemilumiescence has used to detect protein antibody complex then ChemiDoc MP imaging system was used.

Extraction of RNA

The Aurum total RNA (Bio-Rad) kit has used to extraction RNA. After centrifuge treated cells (20,000×g) for 2 min, 350µL of lysis solution was added to cells then followed with 350µL ethanol. After that the column of RNA binding has inserted into "2ml wash tube" followed by added 700 µL of lysate to RNA column. Then after centrifuging process, wash solution (700) µL has been added to the RNA column with centrifuging in the end. The Ribonucleases-free deoxyribonucleases 1 was added followed with wash solution (700) µL. after centrifuging for 1 minute, the RNA was got and Bio spectrometer Nanodrop was used.

Synthesis of cDNA

The cDNA kit has used to create first–strand complementary DNA from 600 ng RNA. Master cycler Gradient (Germany) was used. Twenty microliter of cDNA reaction was used. Finally, when created cDNA has got, it kept at -30°C.

Amplification of cDNA

The amplification process has done via thermal cycler. All the components of the PCR reaction was listed in (table 1).

Table (1) the PCR reaction components

components	Volume (µL)

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Taq	25
Primer (F)	1
Primer (R)	1
Template	4
Water	19
Volume (T)	50

PCR condition

Caspase 9 and 3

The conditions of PCR cycling have listed as mention in (table 2):

Table (2) PCR cycling conditions

proteins	cycles	time	Temperatures
Caspase 9 and 3	Amplification	1 minute	95C°
	Denaturation	15 seconds	95C°
	Annealing	15 seconds	56.6C°
	Primer extension	10 seconds	72C°
	Final extension	7 minutes	72C°
Bcl-2	Amplification	1 minute	95C°
	Denaturation	15 seconds	95C°
	Annealing	15 seconds	60C°
	Primer extension	10 seconds	72C°
	Final extension	7 minutes	72C°

Table (3) primer sequences

primers	Forward primer	Reverse primer
Caspase 9	CCA GAG ATT CGC AAA CCA GAG G	GAG CAC CGA CAT CAC CAA ATC C
Caspase 3	TCA CAG CAA AAG GAG CAG TTT	CGT CAA AGG AAA AGG ACT CAA
Bcl-2	GAT GTG ATG CCT CTG GGA AG	CAT GCT GAT GTC TCT GGA ATC T
β- actin	AGG TGA CAC TAT AGA ATA GAT CAT TGC TCC TCC TGA GC	GTA CGA CTC ACT ATA GGG AAA AGC CAT GCC AAT CTC ATC

Preparation of agarose gel electrophoresis

RNA was separated through using agarose gel (1.5%) electrophoresis. Agarose gel (1.5%) is composed of 1.5g of agarose powder mixed with 100 mL running buffer in 200mL flask. Running buffer was prepared by dissolved 2mL of stock TAE (50x) with (98) water. After melting preparing gel, 1µL 10mg/mL of dye has added. Finally, the gel was molding to the tray and left to solidity. Finally, TAE buffer has used.

Preparation samples

One µL loading dye and buffer have added to each 5µL of samples. Then, after inserted the samples in the wells the electrophorese process was run under sixteen minutes at 110 volts.

Results

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Western blot analysis

Caspase 9:

After treated cells (MDA-MB-231) breast cancer with Propyl –cannabinol allyldimethylsilyl ether (PCAE), caspase 9 level expressions have been examined through using western bot assay.

Caspase 9 was examined for 24h and 72h. The MDA-MB-231 breast cancer cells were treated with IC50 (9.83 ± 0.040) of PCAE. As shown in (figure 1(A)) there are markedly increasing in the caspase 9 levels after treated with PCAE for 24h and 72h. In addition, the highest elevation has seen after 72h of treated compare with untreated cells. These results have indicated that PCAE has a huge role in inducing caspase 9 in treated cells in time dependent manner.

Caspase 3:

The caspase 3 protein expression was also measured by western blot assay. As depicted in (figure 1(B)), Caspase 3 levels were slightly increased compare with untreated cells after 24h. At the same time, the increasing in the protein level was very clearly after 72h of treatment.

Bcl-2

As shown in (figure 1(c)), there are gradually decreasing in Bcl-2 expression compare with untreated cells. The lowest level was after treated (MDA-MB-231) breast cancer cells with IC50 of PCAE for 72h. (Figure 1(D) represented β actin).

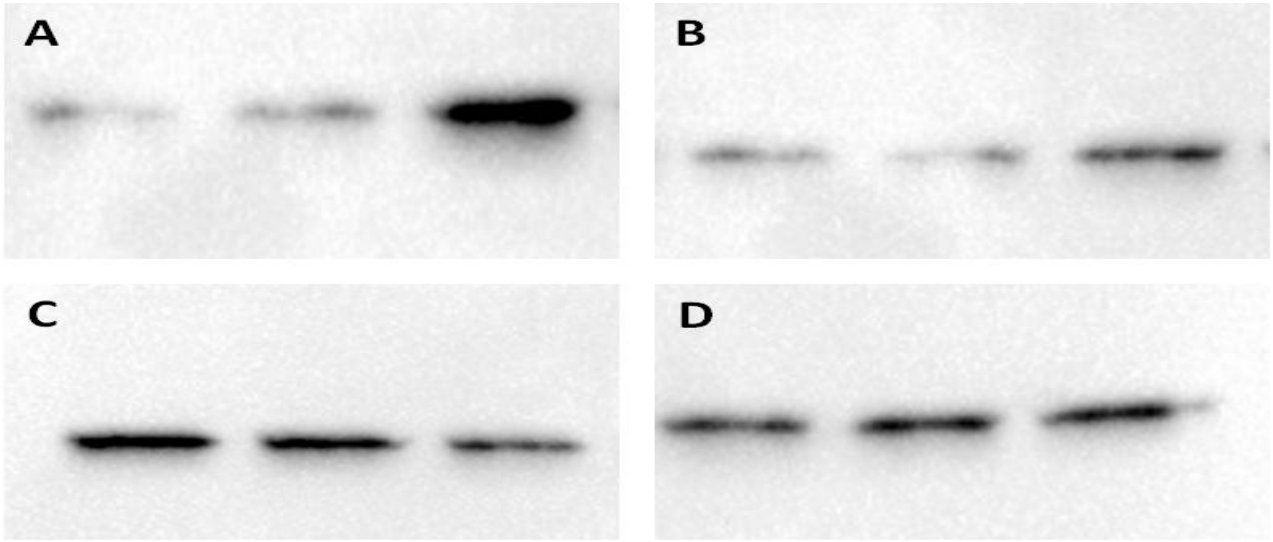

Figure1: western blot analysis showed the expression of proteins under study.

Caspase 9 mRNA:

The mRNA expression levels of caspase 9 in (MDA-MB-231) breast cancer have examined. The highest expression of caspase 9 has seen after 72h of treated with IC50 of (PCAE) compare with untreated cells. In addition, there was slightly increasing in protein expression after 24h compare with control as depicted in (figure 2(A)).

Caspase 3 mRNA:

Caspase 3 mRNA gene expression was slightly increased after 24h of exposure to IC50 of (PCAE) as compared to the negative control (figure 2(B)). The expression level in the caspase 3 gene has gradually continued to increase after 72hour of treatment compare with control.

Bcl-2 mRNA:

According to the results, the level of Bcl-2 mRNA expression in the treated cells has been decline especially after 72h of treatment compare with control as has seen in (figure 2(c)). Furthermore, the

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level of Bcl-2 mRNA expression was slightly decreased after 24h of treated cell with (IC50) of (PCAE) compare with untreated cells.

β actin

The result of β actin expression has shown in the (figure 2(d)). IC50 of (PCAE) did not effect on the concentration of β actin expression gene. The expression gene was equal at all study conditions.

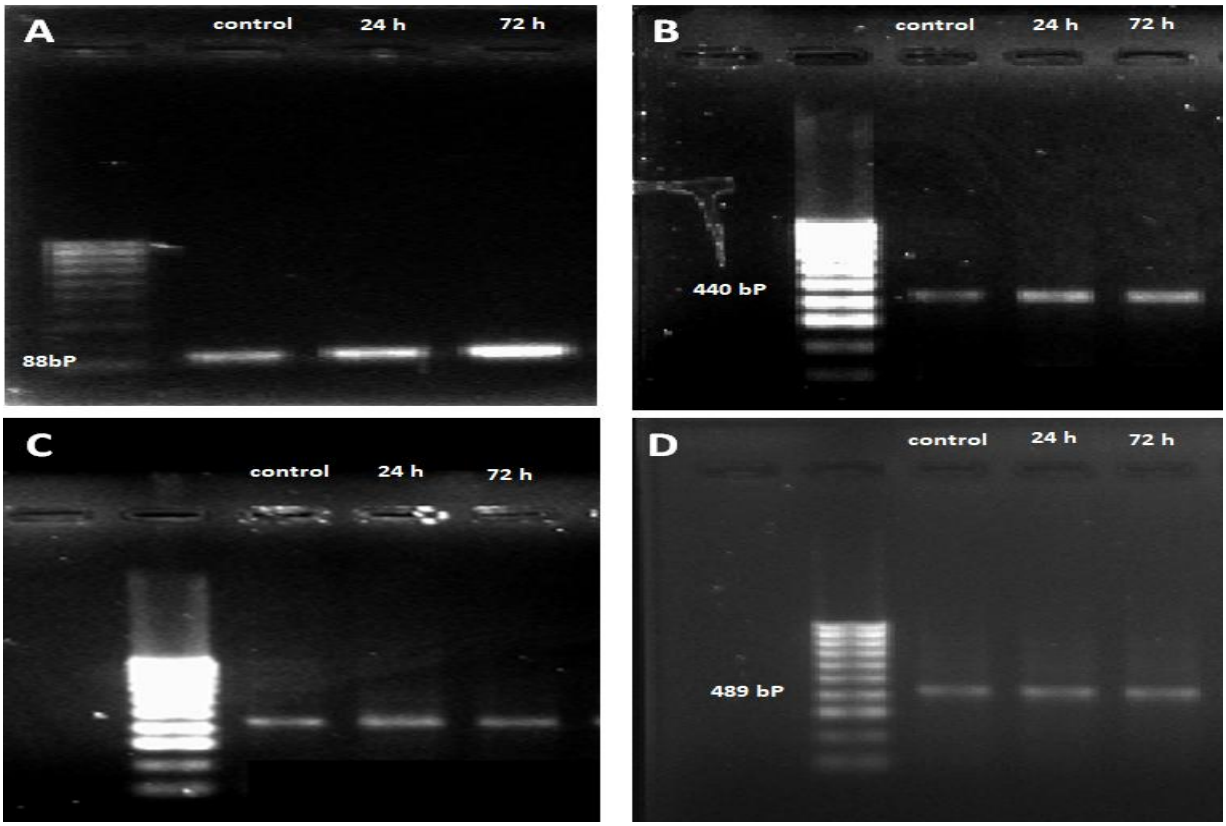

Figure 2: mRNA expression in treated (MDA-MB-231) breast cancer cells with IC50 of (PCAE)

Discussion

The apoptosis processes have been two pathways: intrinsic and extrinsic pathways [14]. Also, caspases have been two groups: initiator caspases such as caspase 2, 8, 9 and 10, and effector caspases such as caspases 3 and 7 [15, 16]. Furthermore, caspase 9 is activated by phosphorylation at Tyr53 [17]. In addition, the sequential activation of caspase 8 and 3 could be inducing when binding the death receptors with their respective ligands [18].

The distinct activity of caspase in executes the all hallmarks of intrinsic apoptosis and cytochrome c is less understood. According to previous studies and current results, they has been appeared that these proteins have a clearly effects on the mitochondria and inducing cytochrome c release [19]. In addition, caspase 9 was induced ROS production but the effect of caspase 3 and 8 was less in intrinsic apoptosis [20]. Recently, the studies suggested that the mitochondria disruption can happen by caspase activation [21]. Moreover, the cells with less activation of caspases due to cytochrome c release show decrease release of other effector proteins [22].

The current study suggests that PCAE can induce the activation of mitochondria apoptotic initiator and effectors proteins such as caspase 9 and 3 to ensure cell death happiness in (MDA-MB-231) breast cancer cell lines through suppression of anti–apoptotic BCL-2 family proteins. The ability of PCAE to promote apoptosis via inhibitor BCL-2 family proteins effect is important implication in cancer treatment. Typically, chemotherapeutic drugs have induced mitochondria–dependent apoptosis [23]. The increasing of anti–apoptotic BCL-2 family proteins and decreasing the expression of caspases in cancer cells will decline the mitochondria disruption and defect apoptosis. PCAE may have the ability to overcome of the mitochondria disruption and promote caspase activations.

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