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TOXICITY AND APOPTOSIS OF CRUDE ALKALOID EXTRACTS OF CONVOLULUES ARVENSIS ON TUMOR CELL LINES

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ABSTRACT

This study was to examine the impact of crude alkaloids isolate from Convolulues arvensis on the development of mouse liver cancer (HC cancer cell line) and human breast cancer (AMJ13 cell line). This study used the DPPH test to measure the antioxidant activity of C. arvensis, and the results revealed that this plant has antioxidant activity similar to ascorbic acid activity. In the concentration of 500µg/ml, the inhibition rate was 92.01%, while ascorbic acid was 92.23%. The impact of crude alkaloid on Tumor Cell Line Development AMJ13 and HC demonstrated that crud alkaloid is cytotoxic to a variety of cell lines, with the effect varying depending on concentration and cell type. Tumor cell line inhibition becomes more potent as extract concentration increases. in comparison, The highest inhibition rate in the HC cell line was 79.49.2% at 500µg/ml, and the lowest inhibition rate was 33.72% at the same concentration. At a con. of 500g/ml, the AMJ13 cell line showed a higher inhibition rate of 82.65 %. After 24 hours of exposure the effect of crude alkaloid extract on apoptosis-related genes (P53, BAX), Caspase8 and Caspase9 was investigated using the IC50 concentration determined from the effect of the crude alkaloid extract on the suppression of tumor cell growth). In the AMJ13 cancer cell line, P53, BAX, Caspase8, and Caspase9 fold gene expression were 0.05, 0.65, 0.15, and 2.98, respectively, whereas it was 1.36, 0.12, 0.19, and 3.45 in the HC cancer cell line. These findings revealed that caspase9 gene expression was significantly altered in cell lines compared to other genes. **Conclusion:** According to the result, C. arvensis' crude alkaloid, which is rich in antioxidants, may be helpful for treating tumors. This study also demonstrates that intrinsic mitochondrial pathway-mediated apoptosis was induced in the AMJ13 and HC cancer cell lines by the IC50 concentration of crude alkaloid extract.

KEYWORDS: *Antioxidant activity, the HC cell line, the AMJ13 cell line, and C. arvensis*.

INTRODUCTION

Over 60 of the available chemotherapeutic agents are derived from plants, which play a significant role in chemotherapy (Al-Hili, 2009). Studies are being conducted to determine whether or not these compounds offer defense against the mutag and carcinogenic effects of chemical compounds. Hoarse tails, also known as Convulules arvensis, is a perennial herb that grows to a height of 10 cm (Hojjat, et al., 2007). The phytochemistry of this plant revealed the presence of a number of substances, Examples include alkaloids, caffeic acid, and flavonoids (Such as isoquercetine and apeginin). Todd et al. (1995).

Traditional uses of this plant include the treatment of tuberculosis, kidney problems, stopping bleeding, and ulcers (Austin, 1997). The cytotoxic effects of this plant represent a novel method of use. Various extracts from C. arvensis can inhibit the growth of cells; This variation is affected by the cell line type, the kind and technique of extraction, and the con. used during incubation. The plant's ethyl acetate extract exhibits a notable inhibitor of human leukemia cell proliferation; this cytotoxic effect is thought to be dose dependent (Assem., 2000). The water extract of aerial parts of C. arvensis has an inhibitory effect on HIV-1-induced cytopathy. This was discovered after testing various plant extracts for their ability to inhibit HIV-1 and its vital enzymes. The vast majority of anticancer drugs currently in clinical use, according to Folkman et al. (2003), cause cell death via apoptosis. Apoptosis modification has emerged as an exciting target in cancer treatment due to its importance in tissue homeostasis and cancer development (Zhu and Zhang, 2013; AL-Tameme et al., 2023). Apoptosis is a tightly controlled and highly effective cell death program that is dependent on numerous factors working together. Components of the apoptotic signaling network are thought to be present in a nucleated cell and waiting to be activated by a stimulus that causes death.(AL-Tameme et al., 2023) Cell death can be brought on by a

variety of internal and external factors, including the absence of survival signals, cytotoxic medications or radiation therapy, erratic cell cycle signaling, or developmental death signals. Insufficient DNA repair mechanisms can lead to DNA damage and cell surface receptor ligation. A common cell death machinery is activated by death signals from various sources, which results in the visible signs of cell death.(Hojjat *et al*., 2007; Al-Tameemi et al., 2024).

METHODS

The plant used

Figure 1 illustrates where the plant in these experiments originated: the Diyala. Family of Convoluluceae, C. arvensis.

Figure 1: *Convolulus arvensis* plant.

Alkaloids extraction

Crude alkaloids were extracted from this plant in accordance with (Cannel, 1988)'s instructions. The alkaloids were identified using Dragendroff's reagent. After the extract was exposed to Dragendroff's reagent, a brown orange color emerged, indicating the presence of alkaloids. In this study, concentrations of (15.15, 31.25, 62.5, 125, 250, and 400) were used. and 500) µg\ml.

Antioxidant assay

Using the DPPH assay detailed in (Zhu, 2006), the antioxidant activity of C. arvensis was assessed. Every E. arvense concentration (15.15, 31.25, 62.5, 125, 250, 400, and 500) was combined in an equal volume (0.5 ml) with DPPH (60 M), and the mixture was left to sit for 30 minutes at 37. At a wavelength of 517 nm, the absorbance was measured. Ascorbic acid, a vitamin C component, served as a positive control. The percentage inhibition of DPPH, which was used to express the alkaloid extract's ability to scavenge free radicals, was calculated using the following equation.

DPPH inhibition (%)=(AC-AS/AC)X100

where AC and AS stand for the DPPH peak intensity and the test sample solvent, respectively.

Preparation of tumor cell line

The impact of the crude alkaloid from C. arvensis on the (AMJ13 and HC cell lines) was evaluated using this in vitro method. The solutions were made using the standard process employed by the Iraqi Center for Medical and Genetic Research. The RPMI-1640 medium used to maintain the viability of these cells contained 15 % serum, and they were incubation at 37°C in a humid environment with 5% CO2.

Toxicity assay

Freshney (2000) used the crystal violate stain to measure the cytotoxic assay. After seeding 96-well microplates with tumor cells, serum at concentrations of (15.25, 31.25, 62.5, 125, 250, 400, and 500) g/ml were diluted with tumor cells and incubaion for 24 h at 37 C. After the old media was replaced with new media, the plate was incubated for 24 hours. SFM with varying concentrations of each extract. Following the media removal and exposure times, 100µl wells of crystal violate dye were added to the wells to treat them. After 20 minutes at 37 degrees Celsius incubation, After cleaning the wells with phosphate-buffered saline (PBS), the plates were allowed to cool for 15 minutes before measuring the absorbency of the wells with an ELISA reader at 492 nanometers.

(qRT-PCR) Cell seeding

AMJ13 cell line was seeded into two falcons, and HC cell line was seeded into two falcons, each with 106 cells. The falcons were then incubated for 24 h. at 37°C with 6% CO2 to allow the cells to attach, proliferate, and form a confluent monolayer.

Exposure stage After monolayer formation, each cell line (AMJ13 and HC) was exposed for 24 hours. One falcon was treated with an alkaloid extract at its IC50 (33.90 gml for AMJ13 and 10.70 gml for HC), while the other was kept as a control (and only received SFM treatment).

Harvest cells Following the exposure period, the medium was removed from the falcons. 5 ml of PBS was used to wash the cells. Using the abm EXCellenCT Lysis Kit, each cell population was isolated by scraping the cell plate bottom in 50 l of cold PBS, followed by transferring the appropriate solution to a different 200 l eppendrof tube. To keep the biological material as fresh as possible, the tubes were immediately placed in an ice box. -80°C.

RNA Extraction

After frozen cells were thawed at room temperature according to the manufacturer's, The abm EXCellenCT Lysis Kit (Abm, Canada) was used to extract RNA from cultured cells. The First Chain cDNA Synthesis Kit (TonkBio, USA) was used as directed by the manufacturer to generate cDNA from pure extracted RNA. The concentration and purity of the cDNA were determined using a Nano-drop spectrophotometer.

Statistical Gene expression analysis using real-time PCR

In the cancer cell lines AMJ13 and HC, real-time PCR was used to assess the, BAX, P53, Caspase8, Casp. 9, and GAPDH' mRNA level. and they were lyophilized at -20°C in accordance with NCBI (National Center for

Biotechnology Information) recommendations. The discovered mRNA sequences were all carefully chosen. Using the NCBI's Primer-Blast tool, all of the genes in this study, including those in Table 1, were created.

The PCR amplification process made use of the USA-based Kappa Syber Green Master Mix Kit. The reaction took place in a 20µl container. Two liters of cDNA (100 ng), ten liters of KAPA SYBR Green Master Mix, and six liters of RNase-free water were used in each 20 l qRT-PCR reaction, and one liter of each primer (Forward and Reverse) at a concentration of one million times the concentration (100 µM). The thermal profile of gene expression in this research is shown in Table 2. By replacing the calibrator with sample 2ΔCt, the expression ratio was calculated:
ΔCT (test) = CT internal control - CT target gene of interest
Finally, the formula was used to calculate the expression ratio:
$2^{-\Delta Ct}$=ratio of normalized expression. The plates were allowed to cool for 15 minutes after the wells had been cleaned with phosphate-buffered saline (PBS), and then the absorbency (O.D.) of the wells was assessed with an ELISA reader at 492 nanometers., Caspase8 and Caspase9 were calculated. in addition to GAPDH (internal control gene):
ΔCT(test) = CT gene of interest (target, test) - CT internal control
ΔCT (calibrator)= CT internal control - CT The gene of interest (the calibrator, the target). Using the control samples, the calibrator was chosen ΔΔCT was computed using the following equation:ΔΔCT = ΔCT (test) - ΔCT (calibrator) Finally, The expression ratio was calculated using the formula:
$2^{-\Delta\Delta Ct}$= (Livak and Schmittegen, 2001) Normalized expression ratio).

Table 1: The primers used in experiment.

5´……...3´	F/R	Human/Mice	Primer
iCCG TCCi CAAi GCAi ATGi GATi Gi	F	H	*P53*
iGAA GATi GACi AGGi GGCi CAGi	R		
iGAT CTGi TAGi CTGi CCCi CAGi GATi	F	M	
iAGA TGAi CAGi GGGi CCAi TGGi AGTi	R		
iCCT CTCi CCCi ATCi TTC AGAi TCAi	F	H	*BAX*
iTCA AGTi CAAi GGTi CACi AGTi GAGi	R		
CGC AAGi AGAi GGCi CAGi AATi GAii	F	M	
iTGT GGAi GAGi AATi GTTi GGCi GTi	R		
iGAC CACi GACi CTTi TGAi AGAi GCTi	F	H	*Caspase 8*
iCAG CCTi CATi CCGi GGAi TATi ATCi	R		
iGCT CTGi AGTi AAGi TTTi AAGi Gi	F	M	
iGAT CTTi GGGi TTTi CCCi AGAi Cii	R		
iCTC TTGi AGCi AGTi GGCi TGGi TCi	F	H	*Caspase 9*
iGCT GATi CTAi TGAi GCGi ATAi CTi	R		
iGCT GTTi TCTi GCGi AAAi GGGi ACTii	F	M	
iAGG GCAi CAAi TCCi CTAi ACCi ACi	R		
iGGG TTCi TTTi GTGi CTGi AGCi GGi	F	H	*GAPDH*
iTGC AGAi TAGi GAAi GGGi CTTi TGi	R		
iTCT CCAi TGGi TGGi TGAi AGAi	F	M	
iTGG CCGi TATi TGGi GCGi CCTi	R		

Table 2: qRT-PCRi.

Step	Temperature	Duration	Cycles
Enzyme	95ºC	30 sec	Hold
Denature	95ºC	5sec	
Anneal/extend	62 ºC	20 sec	40
Dissociation	1min /95 ºC-30 sec /55 ºC-30sec/95 ºC		

RESULTS AND DISSCUSION

Crude alkaloid antioxidant activity extraction from plant

According to the results of the antioxidant activity, alkaloid extract is effective at scavenging free radicals. As shown in Table (3), seven different concentrations were tracked. A general con.-dependent inhibition was seen, with 500 µ g/ml performing better than the others. As shown in Figure 2, there were no differences in con. (15.15, 31.25) g/ml and con. (62.5, 125, 250, 400, and 500 g/ml and ascorbic acid).

Table (3): Alkaloid extract from C. arvensis has antioxidant properties.

SD ± Mean	(Concentrations) µg\ml
75.59 ± 6.29 a	15.15
87.21 ± 4.39 ab	31.25
86.60 ± 2.55 b	62.5
89.69 ± 2.30 b	125
90.10 ± 2.27 b	250
91.12 ± 2.54 b	400
92.01 ± 2.62 b	500
92.23 ± 1.46 b	Ascorbic Acid

The various letters signify statistical differences at the level of P 0.05.

Figure (2): Alkaloid extract of C. arvensis has antioxidant properties.

Copper and zinc concentrations were high in the C. arvensis plant, which also had high levels of vitamins C and E. Superoxide dismutase needed these to function against active oxygen species. (2005) Nagai and colleagues)

According to Huh and Han (2015), C. arvensis had a 94.7% DPPH scavenging activity for roots and rhizomatous stems at 4.0 mg/ml. DPPH and reactive hydroxyl radicals were used to assess the antioxidative activity of C. arvensis extracts. The results showed that the type and quantity of applied extracts had an impact on the free radical scavenging activity. The highest levels of hydroxyl radical scavenging (EC50 = 0.74 mg/ml) and DPPH (EC50 = 0.65 mg/ml) were produced by the n-butanol extract. Conadanovic-Brunet and coworkers, 2009 Onitin and luteolin, which were isolated from the methanolic extract of C. arvensis, were found to have DPPH free radical scavenging activity, with IC50 values of 35.8 0.4 microM and 22.7 2.8 microM, respectively.

Cytotoxicity of *C. arvensis* crude alkaloid extract on cell lines

Inhibition of the AMJ13 cancer cell line's growth by the alkaloid extract was shown in Table (4) to be 33.78% at concentrations of 15.15 gml, 48.92% at concentrations of 31.25 and 60.01%, 69.90%, 70.76%, and 78.62%, and 79.49% at concentrations of 31.25 gml, 62.5 gml, 125 gml, 250 gml, and 500µ gml, respectively. As shown in Figures 3 and 4, there were no differences between the concentrations (62.5, 125, 250) g/ml and (400 and 500 g/ml) at P 0.05. Table (5) demonstrated that the HC cell line had an inhibitory effect that began at concentrations of 15.1 gml and increased to levels of 31.2 gml, 62.5 gml, 125 gml, 250 gml, 400 gml, and 500 gml, with inhibitory effects of 60.01%, 59.60%, 75.20%, 76.91%, 79.40%, and 82.65%, respectively. Figures 3 and 4 illustrate that there were no significant differences between the concentrations of (15.1, 32.2, 62.5, 125, 250, 400, and 400 and 500 µg\ml) at the level P 0.05).

Table 4: Cytotoxicity of crude alkaloid extract on growth inhibition percentage in AMJ13 cell line after 24 hours of exposure.

Mean ± SD	(Concentrations in) µg\ml
33.78 ± 2.8 d	15.1.5
48.92 ± 7.3 c	31.25
60.01 ± 7.6 b	62.5
69.90 ± 8.6 b	125
70 .76± 8.1 b	250
78.62 ± 3.4 a	400
79.49 ± 11.4 a	500

The various letters signify statistical differences at the level of P 0.05.

Table 5: Alkaloid extract's cytotoxicity over 24 hours as measured by the percentage of HC cell line growth inhibition.

Mean ± SD	(Concentrations in) µg\ml
1.1±44.65 d	15.15
0.7 ± 56.50 c	31.25
3.8± 69.60 b	62.5
3.6± 75.2 b	125
3.4± 76.91 a	250
4.2± 79.40 a	400
3.5±82.56 a	500

The various letters signify statistical differences at the level of P 0.05.

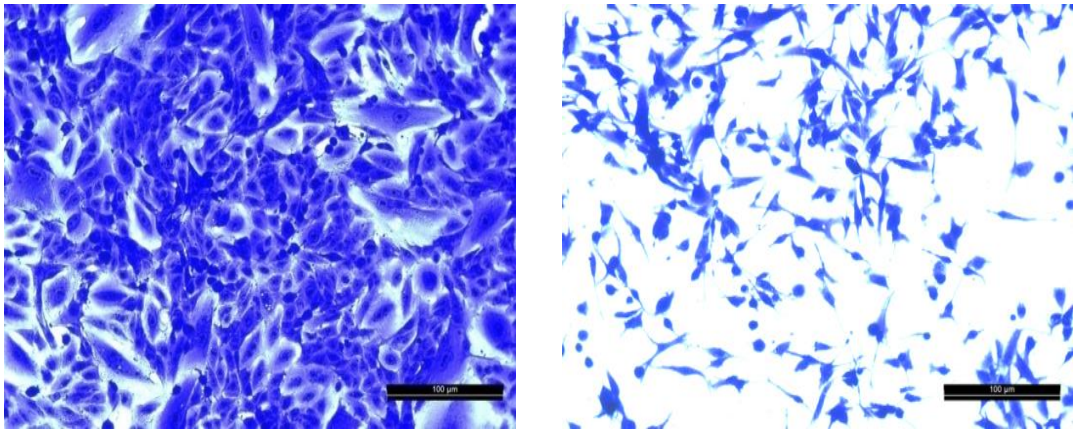

Figure 3: Effect crude alkaloid After 24 hours, in AMJ13 cell line was exposed of C. arvensis. A. AMJ13 as control B. crude alkaloid with treatment.

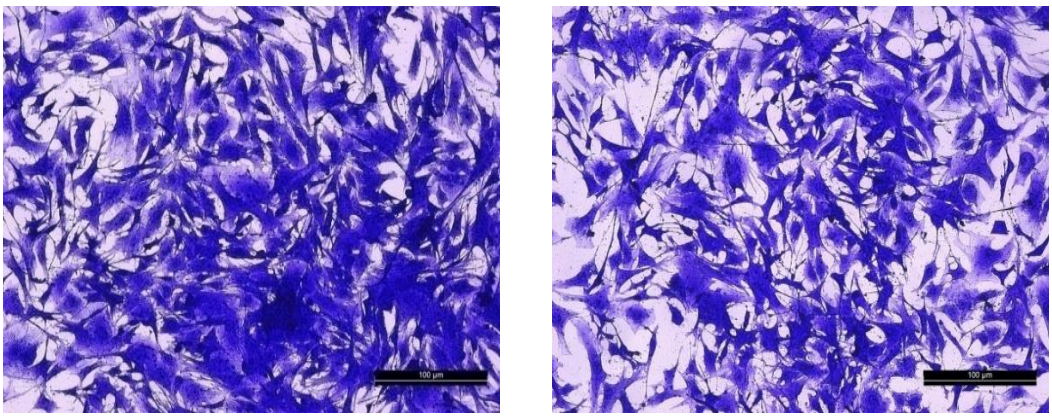

Figure 4: Effect crude alkaloids After being exposed for 24 hours, A. HC a control B. cell line treatment with alkaloids the HC cell line was affected by the alkaloid extract of C. arvensis.

On the (AMJ13 and HC cell lines), the toxicity activity of C. arvensis alkaloid extract was investigated. The cell line and extract concentration were factors in this cytotoxic activity. Human leukemic U937 cells were toxicity affected in a dose-dependent manner by the water extract from sterile stems of C. arvensis. Apoptosis was evident in cells cultured for 48 hours when DNA fragmentation, phosphatidilserine externalization, and the collapse of mitocondria transmembrane potential were all noted (Alexandru et al., 2007). Lung fibroblasts, breast adenocarcinoma, and human embryonic kidney cells were among the cancer cell lines tested for cytotoxicity with methanolic extract of the dried aerial part of C. arvensis. The relative percentages of live and dead cells in the cells were determined 72 hours after treatment. The extract killed all four test cell lines, but human embryonic kidney and breast adenocarcinoma cells were most negatively impacted. However, the degree of toxicity varied according to the type of cell and the extract concentration used. The plant extract had a significant cytotoxic impact on breast cancer cells compared to untreated cells.e (Aldass, 2011).

Quantitative Real Time PCR

GAPDH and other gene expression levels were measured using real-time PCR. Table (6) shows the Ct value for the housekeeping gene GAPDH, which was used in this study. P53, BAX, Caspase8, and Caspase9 each had a fold of gene expression of 2.98, 0.05, 0.65, and 0.15 in the AMJ13 cell line, respectively. The Ct values for GAPDH in the treatment and control groups were 25,09 and 26,90, respectively. According to Figure 7, the P53, BAX, Caspase8, and Caspase9 gene expression folds in the HC cell line were 3.45, 1.36, 0.12, and 0.19, respectively. In Table (7), the GAPDH Ct value is displayed. The treatment group's HC cell line range was (21.84) while the control group's range was (21.61).

Table 6: Gene expression fold in the AMJ13 cell line.

Fold of Gene expression	ΔΔCT	ΔCTC	ΔCTT	CT (GAPDH)	CT(Gene)	T/C	Gene
1.04	-4.18	-	-0.35	23.02	23.56	T	BAX
1.01	0.01	-4.50	-	25.94	21.32	C	
0.64	0.65	-	7.73	25.07	31.78	T	*P53*
1.01	0.01	7.08	-	26.92	32.97	C	

0.14	0.95	-	4.85	23.07	28.92	T	*Cas9*
1.01	0.01	2.15	-	26.91	29.03	C	
2.97	-1.56	-	-3.88	25.08	23.21	T	*Cas8*
1.01	0.00	-2.34	-	25.99	24.60	C	

Table 7: Gene expression patterns in the HC cell line.

Fold of Gene expression	ΔΔCT	ΔCTC	ΔCTT	CT (GAPDH)	CT(Gene)	T/C	Gene
1.65	-0.45	-	8.18	22.84	30.70	T	BAX
1.32	0.00	8.64	-	22.16	29.97	C	
0.87	2.96	-	10.12	22.84	30.06	T	*P53*
1.11	0.00	7.09	-	22.16	28.18	C	
0.10	2.37	-	11.24	21.84	33.86	T	*Cas9*
1.87	0.00	8.88	-	22.16	31.00	C	
3.76	-1.79	-	7.09	21.84	28.09	T	*Cas8*
1.98	0.00	9.34	-	21.16	31.10	C	

In contrast to necrosis, apoptosis is characterized by a specific sequence of events that leads to the disassembly of internal cell contents, including swelling and rupture of affected cells (Ge et al., 2003). The two main pathways by which apoptosis can take place are the mitochondria (intrinsic pathway) and the death receptor (extrinsic pathway). The same death program is reached by these two routes. The apoptosis-activating factor-1 (APaf-1) promotes mitochondrial leakiness by inducing specialized proteins. a protein that causes death attaches to cytochrome C via the mitochondrial pathway The common death pathway is started by the activation of TNF-receptor family cell surface receptors in the death receptor pathway (Abbas et al., 2008).

Plant extracts are toxic to cancer cells, according to several studies. Vetineria zizanioides root water extract was cytotoxic to MCF-7 cells by promoting nuclear material fragmentation and chromatin intensification (Anan et al. 1996). HepG2 cancer cells undergo morphological programmed apoptosis in response to Cinnamomum zeylanicum methanolic extract, as evidenced by cell shrinkage, nuclear material intensification, and fragmentation (Aberanthy, 2000). Berberine inhibits HL-60 leukemia cells by condensing chromatin and increasing the fraction of nuclear material, according to (Adams, et al. 2005). According to the alkaloid extract of Peganum harmala inhibits cancer cell growth by promoting nuclear fragmentation and the release of cytochrome C cells. Because of their effects on a number of cellular indicators, some of which were discovered in this study, plant extracts have been shown in all of these studies to be effective at inducing programmed cell death in cancer cells. (Adhami, 2003), These findings support the current study's discovery that alkaloid extracts have a significant effect on the induction of apoptosis in cancer cell lines.

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