Effect of Tropan Alkaloids in Cell Line in Vivo and Vitro in Mice Laboratory

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Abstract

The purpose of this study was to see how the alkaloids tropan isolated from the leaves of Convolvulus arvensis affected the cancer cell line MCF 7 and the normal cell line MEF. The alkaloid Tropan has been reported to be found in plant leaves, however this study indicates that tropan is found in larger concentrations in Iraqi plants utilizing the TLC technique and solvent systems. On the cancer cell line MCF 7 and the normal cell line MEF, the cytotoxicity of Tropan alkaloid extract was examined. Tropan's toxic impact was determined by the rate of proliferation inhibition. At doses of 250 mg/ml, the Tropan extract inhibited MCF 7 cells at a higher proportion (36.67%). Tropan extract inhibits the pace of MEF cell line growth (11.3 %). This study, on the other hand, looked at the toxicity of Tropan in laboratory mice. The findings revealed that alkaloids extracts have a rather acute lethal impact in mice, with an LD50 of 79.52 mg/kg. After (I.P) administration of alkaloids extract at doses of (0.2, 0.4, 0.8 mg/kg) for 30 days, the therapeutic impact of both extracts was investigated in tumor-bearing mice. The results demonstrated a (35.12 %) reduction in tumor volume in the animals treated with alkaloids extract, particularly at a dose of 0.8 mg/kg.

Keyword: Tropan : alkaloids : cell line .mice

1. Introduction

Cancer is the common cause of death in human. It is a disease aracterized by uncontrolled growth and spread of cells in humans worldwide [1]. In general the term cancer is a form of malignant diseases which occurs in many parts of the body. This disease is reflected by a rapid and controlled cell proliferation leading abnormal growth. Convolvulus arvensis belong to family Convolvulaceae-Morning- glories, commonly named as Field bindweed, Creeping Jenny, etc., [2] Convolvulus arvensis was used as rejuvenation tonic headache, eye problems, therapy for various diseases affecting kidney, liver, scular, immune systems and also maintenance of Blood pressure,the rib pain effective immunomodulating activity and aphrodisiac properties [3]. Phytochemical compounds present in Convolvulus arvensis are saponins, alkaloids and Saponins present in Convolvulus arvensis .can be used as inhibitory effecton beast canser cell line 19 [4]. Natural compounds due to their chemical diversity and structural complexity are likely to possess enough efficacy to be considered as potential precursors for therapeutic drugs in the treatment of oxidative damage and related disorders [5]. Pharmacological investigation of this plant demonstrated the presence of alkaloids, flavonoids, glycosides and steroidal saponins like diosgenin and protodioscin [6]. The alkaloids group of secondary metabolites have pronounced physiological action on animals and therefore have therapeutic and biological importance. The alkaloid class of this plant fruits have not been tested for cytotoxic activity in perspective with antioxidant activity. In present study, we evaluated cytotoxicity of tertiary and quaternary alkaloids fractions isolated from Convolvulus arvensis leaf on leukemic cell line along with its effect on cell's antioxidant machinery [7].

2. Materials and Methods

The extracted of the plant Convolvulus arvensis were purchased from local traditional medicinal shop of Baghdad and were identified in the Department of biology, University Baghdad University.

Figure. (1) Convolvulus arvensis

Preparation of extraction

Powdered plant (50 g.) was macerated in 2N Hydrochloric acid (100 mL.)
and stirred gently by a magnetic stirrer.The extract was filtered by a
Buchner funnel, and the alkaloids salts were basify with ammonia
solution to free the basic alkaloid using pH paper. By Dragendroff's reagent, apply, by using a capillary tube, some spots of extract on a filter paper, spray it with the freshly prepared Dragndroff's reagent. Brown orange color after exposure of extract to Dragendroff's reagent indicates the presence of alkaloids [7].

Thin Layer Chromatography (TLC)It is a simple and accurate method of separation and identification of phytochemicals [8], it is performed for most steps in research in order to get an overview for the present

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substances in the plant.

Cell culture

MCF 7 cell lines and MCF was purchased from IRAQ Centre of canser research and cultured in Roswell Park Memorial Institute- 1640 (RPMI) medium with 10 % fetal bovine serum provided with streptomycin 100 U/ml and 50 µg/ml penicillin. [9].The cells were incubated at 37° in a humidified atmosphere of 5 % CO2.

Cell viability assay

The cell viability of MCF7 were carried out by measuring purple formazan formed after 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction by live cell mitochondrial reductase. The cells were platted at a density of 5×104/ml in a flat transparent 96-well plate and incubated at 37° with required atmospheric humidifier concentration of CO2 (5 %). Each well was then dispensed with different concentration of crude alkaloids, such as 15, 31, 62, 125, 250, 400, and 500 µg/ml of the medium. Staurosporine (5 mM) and dimethyl sulfoxide (DMSO; 0.1 %) were taken as positive and vehicle control, respectively. After the period of 24 h, 20 µl of MTT dye (5 µg/ml of phosphate buffer) was added in each well and the plate was again kept for 4 h. The formed formazan crystals were dissolved in 200 µl DMSO, after the centrifugation of plate at 650 g. Finally absorbance was read at 565 nm against blank sample, which contain an equal amount of solvent as test compounds [10].

3. Statistical Analysis

The data of all the experiments were expressed as mean±standard deviations of number of experiments (n=3). [11]. Statistical calculations and graphs for various experiments.

4. Result and Discussion

Detection of tropan alkaloids

Preliminary chemical detection of some components of Convolvulus arvensis leaf extract.The results of the chemical detection of the extract of the leaves of the plant using the previously mentioned reagents and solutions showed the presence of the compounds shown in the table 1.

Results	Detergenet	Compound
+	Dragendroff'	Alkaloid
(+) positive test		

The plant contains alkaloid compounds and this was indicated by (12) diagnosis by gas chromatography technique GC-SM. Alkaloids from the Tropane alkaloid group such as Tropine, Pseudotropine, Tropinone and Pyrolidine alkaloid group such as Cuscohygrine and Hygrine were identified from the aerial parts of the plant. Using TLC thin layer chromatography technique see in Figure 2 , which showed that the plant contains alkaloids and as well as tropane alkaloids compounds were identified using TLC technology.

Fig. (2) TLC for tropan in solvent system

Effect of alkaloid tropan in cell lines

For test alkaloid tropan in cell line used to two cell line MCF7 and normal cell line MEF in different concentration for the test to 24 hours incbutionin 37 c and the concentration15.5,31.2,62.5,125,250 µg/ml. ml.

Table (2) shows that the alkaloid extract had an inhibitory effect on the growth of the cancer cell line for a period of 24 hours exposure to mcf7 , starting with a concentration of 15.1 mcg/mL with a significant difference from the control as the percentage of inhibition reached 20.07%, and increased to 36.67% at a concentration of 250 mg/mL The difference was significant between the concentrations used and the control, but it was not a significant difference between the concentrations 125 and 250, but between all other concentrations it was significant .

Table (3) showed that the alkaloid extract on the natural line MEF had little effect on inhibition. At concentrations 15.1, 31.2, 62.5, 125 and 250 µg/ml, the percentage of inhibition was 0% and increased to 10.2% and 11.3% for concentrations 125and 250 µg/ml. ml, respectively. No significant difference was observed between the concentrations 15.1, 31.2, 62.5, 125 and 250 µg/ml as shown in Figure (3).

Table (1) extraction from leaves alkaloid by solvent compound

Table (2) Effect alkaloid tropan inhbation concentration of cell line MCF7 in different concentration in 24 hours incubation. .

Inhibition% ±standard deviations	Con. µg ml-1
2.6 ± 20.07 c	*15.1
5.1 ± 26.45 c	*31.2
.9 ± 29.41 c4	*62.5
8.9 ± 33.32 b	*125
6.1 ± 36.67 b	*250

Different letters means the presence of significant different at (P<0.05).

Table (3) Effect alkaloid tropan inhbation concentration of cell line MEF in different concentration in 24 hours incubation	
Inhibition% ±standard deviations	**Con. µg ml-1**
0 c	15.1
0 c	31.2
0 c	62.5
1.5 ± 10.2 b	125
11.3 a 1.6 ±	250
. Different letters means the presence of significant different at P<0.05	

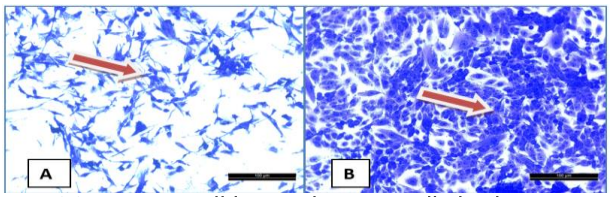

Fig (3)A- MCF7 cell line with tropan alkaloid in 250 µg ml-1 B-MCF7 cell line with control (100X)Crystal Violate

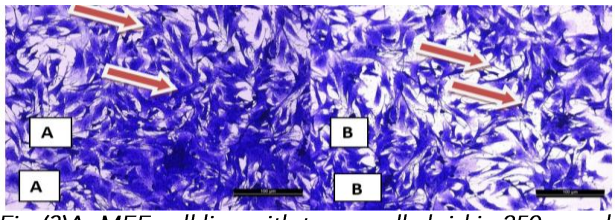

Fig (3)A- MEF cell line with tropan alkaloid in 250 µg ml-1B-MEF cell line with control (100X) Crystal Violate

The results of this study reinforce the findings of many researchers in local studies about the effectiveness of plant extracts against cancer cells, and this effectiveness depends mainly on the concentration of these compounds used, the type of extract and the sensitivity of cancer cells The results showed toxic effects on the growth of MCF7, cancer cell lines for all alkaloid extract concentrations.

The results showed an effect of the alkaloid extract that started with high concentrations on the normal MCF7 line, but at lower rates than in the cancer cells, as shown in Table (2). The inhibitory effect on cancer cells is attributed to the alkaloid extract containing many compounds. Chemical that is effective in inhibiting the growth of cancer cells or stopping their growth

An antioxidant should be a potential candidate in regulating oxidative stress according to the disease microenvironment and normal physiology, for example in neurodegenerative diseases an antioxidant must have to cross blood-brain barrier but most of the antioxidant cannot, as like carotenoids [12] Beyond involvement of ROS in disease and their development, interestingly increased level of free radicals can inhibit tumor cell growth. The therapeutic application of pro-oxidant, which induces oxidative stress to a cytotoxic level in cancer cell can selectively kill them. Fascinatingly, the antioxidant and pro-oxidant capabilities can be achieved by a single agent depending upon the concentration used, curcumin, a chemotherapeutic agent, for an instance. Hence, the screening for the new and potential natural product as an effective oxidative stress manager at various physiological circumstances continues to give the best application of resourceful natural compounds.

Alkaloids of tropan of Convolvulus arvensis leaves act as an antioxidant at lower concentrations and cytotoxic at higher doses. Alkaloids extract induced potent cell death at higher concentrations (250 µg ml-1), which explicit its cytotoxic effects to MCF7 cells. Contrarily. Future studies may be targeted on identifying the bioactive individual compounds in the alkaloid extracts along with their antioxidant/cytotoxic properties and mechanism of actions.

Acute toxic effect

LD50

Used Up & down method to test the drag for lethal dosge to tropan alkaloids for leaves the result 79.52 gm\kg for mic

Table (4) LD50 for tropan alkaloids Up & down method					
(LD50)	**Last dosage (xf)**	**K table**	**death anmail or survive in 24 hours**	**Increased for dosage (d)**	**extraction**
79.52 gm\kg	75	0.181	xoxx	25 gm\kg	Tropan

Survive rhe anmail in 24 hours in effect tropan)O(

Death the anmail in 24 hours)X(

Effect of alkaloid extract of extended-release on tumor size

Injection of the alkaloid extract at a dose of 0.8 mg/Kg showed a significant decrease in the tumor volume in mice throughout the treatment period, with a probability (P≤0.05) compared to the control. On days 24, 27 and 30, the tumor volume rates were 92.89), 51.87 and (35.12). Mm 3, respectively, compared to the control. As for the difference between the tumor volume rates of the groups treated with the three doses, the results showed that there were significant differences with probability (P≤0.05) for all days compared to the control, and the most effective dose in the tumor volume average for the last days in particular was 0.8 mg/ Kg, the volume on the last day was 35.12 mm 3 while the average for the second and third doses was 240.15) and 91.24 mm 3, respectively, compared to the control that gave a rate of 3855.80 mm 3.

Table (5): Effect of different doses of the alkaloid extract of extended release on the tumor volume mm 3 for mice carrying mammary gland carcinoma.				
0.8 mg/Kg	**0.4 mg/Kg**	**0.2 mg/Kg**	**control**	**Dose day**

standard error ± average	standard error ± average	standard error ± average	standard error ± average	
^{aA}2.37 ±221.12	^{aA}5.81± 220.38	^{bA}2.28± 220.06	^{gA}1.44± 220.88	0
^{aB}1.03± 222.33	^{aA}5.81± 220.16	^{bB}5.79± 220.12	^{gA}4.33± 237.65	9
^{bB}0.06± 211.34	^{aB}5.81± 210.02	^{bA}22.82± 210.11	^{fA}31.87±477.86	12
^{cC}0.01± 184.33	^{abB}5.84±191.13	^{aB}2.54± 295.23	^{eA}1.00± 682.15	15
^{dD} 2.30±144.15	^{bC}8.52± 186.22	^{aB}5.95±291.93	^{dA}0.65± 963.81	18
^{eD}3.18 ±114.45	^{cC}2.32± 174.82	^{bB}5.75± 250.96	^{dA}11.41±981.53	21
^{fC}1.86 ± 92.89	^{dcC}2.78± 156.23	^{Bb}28.81±250.25	^{cA}28.86± 1250.34	24
$^{f D}$0.92± 51.87	^{dC}0.03±125.27	^{Bb}0.05±243.21	^{bA}28.92± 2550.70	27
$^{f D}$0.92± 35.12	^{eC}17.35±91.24	^{Bb}0.05±240.15	^{bA}28.92± 3855.80	30

-The different lowercase letters within the column indicate the presence of significant differences at the probability level (P≤0.05).

- The different capital letters within the class indicate the presence of significant differences at the level of probability (P≤0.05).

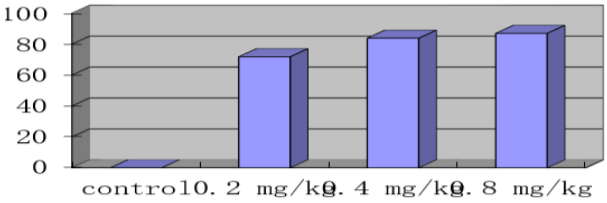

Figure (9-3): Effect of different doses of the crude alkaloid extract extended-release on the rate of tumor suppression (GI)% of mice bearing murine adenocarcinoma at the end of the experiment.

Figure 4: Anatomical character of mice carrying murine mammary adenocarcinoma, where the arrow indicates the tumor size after the end of the experiment.

This study suggests another direct effect of these extracts in their ability to reduce tumor size because they contain compounds capable of exhibiting anti-angiogenesis action (necessary for the maintenance and proliferation of tumor cells), which leads to hypoxia and cell death. neoplastic and necrotizing tumors [13].

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