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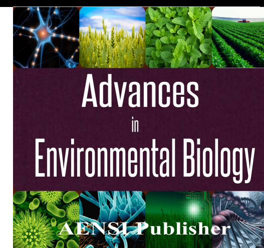

Cytotoxic and cell's death effect of Nettle extract on cancer cells of Hela cell line

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ABSTRACT
Chemotherapy is one of the effective ways of treating cancer. Having effective chemical compounds the plant Nettle is of utmost importance in examining the cellular toxicity. In this article the effects of cytotoxic in leaf and stem of the plant Nettle have been studied on cancer cells in cervix. During the research to study the effect of cellular toxicity of ethanol extract of the plant Nettle cancer cells of Hela, purchased from Pastor Institute cultivated and reproduced in Iran, have been placed next to different densities of extract (0.156, 0.312, 0.625, 1.25, 2.5, 5, 7.5 and 10) and MTT test has been done about time. Also painting Acridine orange - propidiom iyrodid (AO/PI) contributed to the changes in cells using apoptotic and necrotic through fluorescence microscope. In cancer cells of Hela ethanol extract of Nettle in densities of 5, 0.625, 10 mg/ml have significantly reduced the cells growth to controlling group. As it turned out the highest percentage of growth control has been calculated in densities of 10 mgml^{-1} and the quantity of its IC_{50}, in 3.96. Examination on painted cells through using fluorescence microscope resulted in morphological changes in four, and disintegrated and condensed chromatin in apoptotic cells in 24 hours. Accordingly Nettle extract restrains the growth of such cells and causes death to apoptosis cells. This examination along with the following ones may reap benefits in choosing an effective medicine for cancer.

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INTRODUCTION

Cancer today after natural occurrences, urban, machinery life and also heart diseases occupies the highest rank in death rate, and unfortunately ever-lasting. However in controlling cancer supplementary medicines can be of high use. In Iran traditional medicine as a comprehensive school having exclusive basics rooted in the history of people. It is declared that introducing medicines using in traditional medicine especially herbal ones open a window towards compiling research projects in acquiring new medicines in treating cancer [13]. Plants of different site especially in higher altitude are main sources of medicine and producing secondary metabolites which can serve as significant sources of medicines [2]. In comparison with chemical-based treatment, herbal-based seems to be worthwhile as it costs cheaper, is easier and available, and also in some cases is of fewer side effects [15]. One of these plants is Nettle with the scientific name *Urtica dioica* L. from the family of Urticaceae. According to its medical benefit and being superabundance in Iran, we intend to study the effect of cellular toxicity of hydro alcoholic extract of such a plant through using MTT on cancer cells of Hela. Genealogically gets back to prehistory, and its therapeutic benefits known to people, Nettle has been using as a food. Rarely are such plants arborous but are grassy and herbal in stroke with stipular leaves and single or mutual fairy-faced veins. Flowers usually are peculiar having five sepals and four mutual stamens to sepals. In a research methanol extract of the plant Nettle has shown a significantly anti- inflammatory activity in total segments of the plant including root, stem, leaf and flower with an average toxicity. Alternatively pole extract, water, and methanol as well as ethanol in four segment of the plant had a weak anti-inflammatory activity whereas methanol especially solution extract in water, chlorine extract and lipophilic methane in root, stem and leaf have shown respectively a considerable toxicity and a highly anti-inflammatory effect with the least toxicity. All in all lipophilic extract of the plant Nettle is of high effect in curing inflammatory disorders in clinical assessment especially arthritis [8]. Nettle extract is antibacterial on most Gram positive and some Gram negative bacteria, however they have had no effect on bacteria Pseudomonas aerogenosa. [12]. Moreover not only are they anti-inflammatory and anti-microbe but also are anti-diabetic in toxicology and medical

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assessment of the plant Nettle. The results provide the fact that the leaf of Nettle seems to be an interesting resource in active biological compounds so that using it in traditional medicine in treatment of different diseases give a justification to our hands [4]. Nettle has shown also to be anti-cholesterol in a research conducted on rats [3]. Nettle also has the property of non-carcinogenic, cytotoxic. In a series of research for its anti-proliferative effect on prostate cancer cells the root of Nettle has been used [10]. By stimulating non-Adrenalin and non-cholinergic paths, Nettle extract reduces heart activity and makes the heartbeat slower, moreover, plays a major role in reducing blood pressure in living tissues. Such an important thing is independent from its cholinergic property [1]. Nettle is antioxidant and prevents from liver tissues damages. A series of studies have been carried out to see the effect of Nettle seeds on preventing liver damages resulted in being effective against inculcated aflatoxin through carcinogenic liver cells in rats [9]. Phenol compounds in Nettle is a mostly available resource of natural antioxidant for pharmacy industries and food supplement. It is also anti-microbe, anti-injury and pain killer [5]. In this study, we determined anticancer activity in Nettle extract in order to understand the usefulness of this plant as a foodstuff as well as a medicine.

MATERIALS AND METHODS

Plant Material:

The plant *Urtica dioica* L. was collected from around Amol. Voucher specimens were deposited with the faculty of biology herbarium (as NO 720-654). Then, about 400 gram of its shoot were collected, washed with cold water, cut into small pieces, dried in the shade, and mechanically ground to produce a fine powder. Dry powder was extracted with ethanol 70% for about 2 days at room temperature. Dry ethanolic extracts were obtained after removing the solvent by evaporation. The dried ethanolic extract was used for evaluation of cytotoxicity activities.

Cell lines:

The HeLa cell line used for assay was purchased from the cell bank, Pasture Institute (Tehran, Iran). Cells were grown in RPMI-1640(PAA) [each 500 ml of RPMI-1640 was supplemented with 10% of fetal calf serum (FBS) (PAA), penicillin (100 IU/ml) streptomycin (100μgml^{-1}) (PAA)] in a humidified atmosphere of 5% CO_2 at 37ºC. The cells were dissociated with 0.2% trypsin (Gibco) in phosphate saline buffer solution. The stock cultures were grown in 25 cm^2 tissue culture flasks and all cytotoxicity experiments were carried out in 96 well plates.

Isolation of peripheral blood mononuclear cells:

Peripheral blood was immediately drawn into heparinized sterile 15 ml conical tube, layered on to an equal volume of Ficoll and centrifuged at 1500 g for 20 min. Cells were then harvested from the Ficoll-plasma interface and washed three times in RPMI 1640 medium. Cells were suspended at 1×10^4 cellml^{-1} in RPMI 1640 supplemented as above. Cell viability was always >95%, as estimated by trypan blue exclusion.

MTT Assay for cytotoxic activity:

Cytotoxic effect of the extracts against HeLa cells was measured by MTT assay (MTT, Sigma). 100 μl of cell suspension (1×10^4 cellml^{-1}) were cultured in 96 well microplate and were incubated for 24 h (37ºC, 5% CO_2 air humidified). Then 100 μl of different concentrations of extract including 0.156, 0.312, 0.625, 1.25, 2.5, 5, 7.5 and 10 mgml^{-1}, were added and the microplates were further incubated for 72 h at the same qualification. Dilution of stock solutions was made in culture medium yielding final extracts concentrations with a final DMSO concentration of 0.1%. This concentration of DMSO did not affect cell viability. Control cells were incubated in culture medium only. All concentrations of plants extracts were in triplicates on the same cell group. For examination of the effect of the extract on normal cells, we used peripheral blood mononuclear cells at 1×10^4 cellml^{-1} in RPMI1640. To consider the cell survival, each well was then incubated with 20 μl of MTT solution (5 mgml^{-1} in phosphate buffer solution) for 3-4 h. Then, the media in each well was gradually replaced with 100 μl DMSO and gently shaken to dissolve the formazan crystals. The absorbance of each well was determined at 492 nm using the ELISA reader (Awareness, USA). The percentage of growth inhibition was calculated using following formula,

%cell inhibition = 100-{(At-Ab)/ (Ac-Ab) × 100

Where,

At=Absorbance value of test compound

Ab=Absorbance value of blank

Ac=Absorbance value of control

The effects of extracts were expressed by IC_{50} values (the drug concentration reducing the absorbance of treated cells by 50% with respect to untreated cells).

Observation of morphological changes:

The HeLa cells were cultured in 96 well culture plates (1×10^4 cells per well) in RPMI-1640 supplemented with 10% FBS for 72 h. The cells were treated extract of *Urtica dioica* L. at various concentrations. After 72 h, cellular morphology was observed under the inverted microscope and pictures were taken.

Detection of apoptotic cells:

Morphological changes of apoptosis were assessed using acridine orange-propidium iodide (AO/PI) staining. Cell suspensions (1×10^4 cellml^{-1}) were seeded in 96-well plate and incubated for 24 h. In the next day, the Cells were treated with 1.25 mgml-1 concentration of extract for 4, 24, 48 and 72h. Cells were then fixed with 70% ethanol for 20 min and stained with a solution composed of Ao/PI (50 µl of 10 µgml^{-1} AO and 50 µl of 50 µgml^{-1} PI) for 30 min, after being washed with PBS, cells were examined using fluorescence microscopy Motic AE 31 (Australia) and photographed.

Statistical Analysis:

Results were presented as means ± SEM. Differences between control and test values were determined by Student's t test and were accepted as significant when $P<0.05$. The IC_{50} values were calculated from linear regression analysis.

Results:

According to fig.1, the percentage of controlling cancer cells of Hela can been seen in different densities of the plant extract; the highest percentage of growth control is 89.22% in the density of 10 mgml^{-1}, and the least one is 33.58% in the density of 0.312 mgml^{-1}. Also in the densities of 7.5, 5, 2.5, 1.5, 1.25, 0.625, 0.156 mgml^{-1} the extract has been able to control the cancer cells respectively 33.7 %, 62.64%, 53.67%, 47.28%, %62.4, 57.92%. This extract in densities of 0.156, 0.312, 0.625, 1.25, 2.5, 5, 7.5 and 10 mgml^{-1} could control the cancer cells of Hela respectively 33.7%, 33.58%, 62.64%, 53.57%, 47.28%, 62.04%, 57.92% and 89.22%. The highest effect was in the density of 10 mgml^{-1} in 89.22%. Morphology of untreated and treated cells was observed under inverted microscope, to evaluate apoptotic features of the cells.

Table 1: Cytotoxic activity of ethanolic extract of *Urtica dioica* L. on HeLa Cell Line at Different Concentrations by MTT Assay

concentration	absorbance	Inhibition %	IC_{50}
control	1.07 ± 0.09	-	
0.156	0.8 ± 0.11	33.7	
0.312	0.81 ± 0.13	33.58	
0.625	0.54 ± 0.12	62.64	
1.25	0.63 ± 0.21	53.67	
2.5	0.85 ± 0.37	47.28	3.96
5	0.55 ± 0.07	62.04	
7.5	0.73 ± 0.42	57.92	
10	0.4 ± 0.21	89.22	
DMSO	0.97 ± 0.08	18.01	
Lymph	0.63 ± 0.12	60.5	

From Fig. 1, it can be seen that untreated control cells showed normal appearance, which were elongated and flattened outgrowths, while the treated cells showed obvious morphological changes at concentration of 10 mgml-1 of extract.

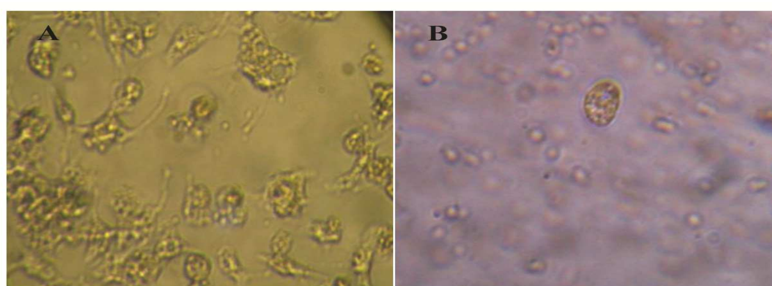

Fig. 1: Morphological changes of cancer cell line after being treated with extract under inverted microscope (A) Hela cell-Control, (B) Treated Hela cell with 10 mgml^{-1} of extract (Magnification 200×).

The transformed cells were shrunken, rounded, shiny, and condensed and showed surface blebs. In addition, morphological changes in nuclei were observed in all cell line. It indicated that the extract caused marked

apoptotic changes in a time-dependent manner, characterized by nuclear shrinkage and chromatin condensation (Fig. 2).

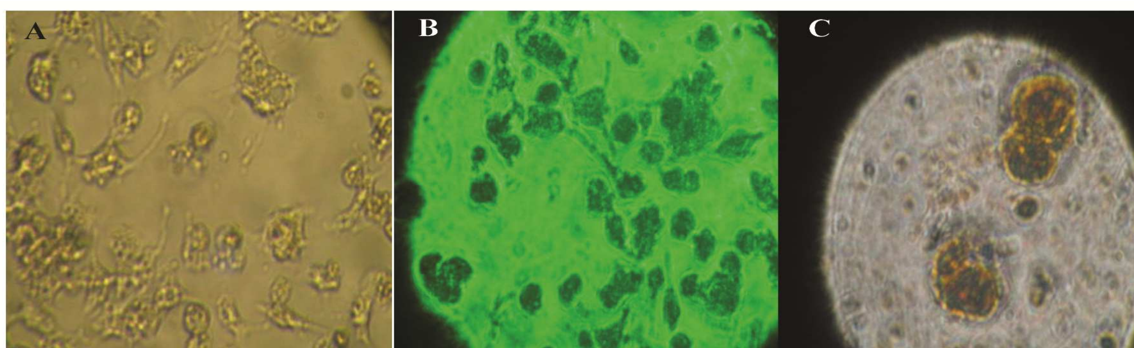

Fig. 2: Induction of morphological changes in shape and in nuclear (a) Control Hela cells (b) Hela cells treated with 10 mgml^{-1} concentration of *Urtica dioica* L. for 4 and 24 h (c) (Magnification 200×)

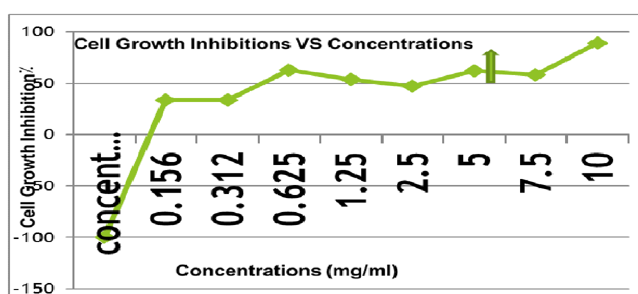

Fig. 3: Growth Inhibition of *Urtica dioica* L. Extract against Hela cell Line by MTT Assay

Discussion and Conclusion:

Spreading cancer throughout the world and also in Iran a need is felt for researchers to have medicines having fewer side effects, medical interferences and much better therapeutic benefits. Today over 60 percent of non-carcinogenic compounds derived from herbal, marine, microorganism resources for people suffering from cancer. Hirano et al. [6] showed the effect of Urticaceae being special anti-tumor on prostate cancer cells. In this research also densities of 0.625, 5 and 10 of Nettle extract significantly reduced cancer cells of Hela in a way that it comes in the line of other achievements. Hofseth et al. [7 considered the effect of open radicals one of causes in forming tumor through conducting studies on foliage of the plant Nettle so that it carries on a good antioxidant. Abdel-Kader et al. showed that Hydromethanol extract (20%) in shoot and leave of Nettle contains bioactive contain with biological effect such as Flavonoids that perform the role of eradicating tumor growth. In this study, extract of Nettle has the role of inhibitory on the growth of cancer cells with the highest (89.22% of inhibition). It is likely that some non-carcinogenic properties of the above-mentioned plant is for its Flavonoids properties in a way that along with other research comes across a similar conclusion. In a research conducted by Roa et al. [14] on Nettle, *U. pilulifera* they deducted that this species of such a plant contains Genisetin to restrain Tyrosine kinase and prevents cancer cells from producing in prostate cells through apoptosis. In the present study, the effect of the extract on cancer cells, morphological changes, disintegration and condensation of chromatin representing apoptotic changes have been seen in 4 and 24 hours. The obtained results similar to the ones conducted can be of Genisetin contributes increasing apoptosis in cancer cells of Hela. Discovering non-carcinogenic, herbal and powerful as well as harmless compounds, the effect of killing of ethanol extract, separated from the plant Nettle growing in various parts of the country, and has on cancer cells of Hela been assessed.

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