Original Article

Antitumor Activity of Methanolic Fractions Extracted From the Aerial Part of Algerian *Hyoscyamus albus* and apoptotic cell aspect screening

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ABSTRACT

Hyoscyamus albus is a plant which belongs to the Solanaceae family, used generally in traditional medicine as a nervous sedative and para sympatholytic which is a rich source of flavonoid, alkaloids and tropane. The present work is for an object to test the methanolic fractions extracted from the aerial parts of *H.albus* collected among the Aures region in Algeria, and evaluate their cytotoxic activity on different cancer cells lines with the characterization of microscopically morphology of apoptosis cell. The effects of the four different selected methanolic fractions of *H.albus* extract *(D, E, F, G)* obtained by column chromatography over Sephadex LH-20 on DU-145, PC-3, U-87 MG and U-373 MG cancer cells lines were determined using MTT [3-(4,5-dimethylthiazolyl)-2,5-diphenyl-tetrazolium bromide] assay and the cell were marked by acridine orange to visualize the apoptotic cells aspect. Our results indicate that the fraction C has a cytotoxic activity against three different cells with an IC_{50} = 73 μg/ml, 187 μg/ml, 96 μg/ml and 114 μg/ml for the DU-145, PC-3, U-87 MG and U-373 MG respectively, the morphological aspect of the apoptotic cell have revealed several physiologic change which attempt an apoptotic cell marked by a fluorescent agent. Additionally, fraction D showed an activity against DU-145 with IC_{50} = 186 μg/ml, the fraction F has an IC_{50} = 165 μg/ml and 198 μg/ml against DU-145 and PC-3 respectively. The fraction G showed a notable activity against DU-145, LNCaP and U-373 MG. The results demonstrated the anticancer properties of *H.albus fractions* and its therapeutic benefits, so they are indicative for several alternative investigations on molecular mechanisms underlying the activity and components identification.

Key words: *Hyoscyamus albus* L., Solanaceae, Acridine, Cytotoxic Activity, Fraction.

INTRODUCTION

During the last years, the discovery of new anticancer drugs remains the mains concerns in oncology, the natural environment has always been a very important source of active biological molecules, 60% of the cancer drugs currently used are originally from a natural products.

The cancer is known medically as a malignant tumor characterized by uncontrolled growth of abnormal cells. it is caused by endogenous and exogenous factors which lead to the accumulation of genetic alterations.[1] *H.albus* is a Solanaceae family plant; which has been used in traditional medicine from a long time ago as a nervous sedative, para-sympatholytic, mydriatic, anticholinergic, antispasmodique and analgesic.[2] In previous study researchers have isolated some tropane alkaloids (scopolamine, hyoscyamine) and with spectral techniques they isolated 2,3–dimethyl nonacosane.[3] Recently, a new groupe of polyhydroxylated nortropane alkaloid named calystegines have been isolated from different species of solanaceae like Hyoscyamus and Datura,[4,5] where the antidiabetic activity has been demonstrated.[6]

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The aim of this study is to screen the different fractions of *H. albus* to test their cytotoxic activity on different cells lines and to stain the morphological aspect of the apoptotic cell. The cytotoxic potential was studied by MTT assay. Moreover, the characterizations of apoptosis cell were used by biological colorant and fluorescent microscope.

MATERIALS AND METHODS

Plant Material

The aerial parts of *H.albus* were collected from Ighzer Naith Abdi, Batna, Algeria in Mai 2015, the plant was identified by Doctor OUDJHIH, Laboratory of Botanic, Department of Agronomy, University Batna 1, Algeria, then were dried for forty days at room temperature under shade, and the plant were crushed, pulverized and stored in dry place.

Extraction

The vegetal materials were powdered (1Kg) and extracted with ether of petrol, chloroform and methanol at room temperature. The solvents were removed in a rotary evaporator at 30°C for ether of petrol and chloroform and 40°C for methanol, the extracts were conserved and covered in refrigerator at 4°C until use in experiments.[7]

Purification on Sephadex gel

The methanolic extract of the aerial part of *H.albus* (HAMeOH) was subjected to column chromatography over Sephadex LH-20 (D.Farmacia. Italy), using methanol as eluent (mobile phase).

The first obtained preliminary fractions (A,B,C,D,E,F,G) were analyzed by Thin layer chromatography (TLC) on Silica gel 60 F_{257} plate (Merck) recoated aluminum plates (thickness = 200 µm) using butanol - glacial acetic acid-water system and anisaldehyde sulfuric and $FeCl_3$ reagents as a spray reagent, finally the similar profiles were regrouped.

All reagents and solvents used in experiment were of analytical grade and purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and Merck KGaA (Darmstadt, Germany).

The final fractions grouped were: FC (5,577 g), FD (4.22 g), FF (1,36 g), FG (1.76 g).[8]

Cytotoxic Activity of Fractions

The anti-tumoral activity of four selected fractions F , C , D ,G of HAMeOH was evaluated with MTT [3-(4,5-dimethylthiazole-2-yl)-2,5diphenyltetrazolium bromide; Sisco, Italy] with method of Mosmann.[9,10]

DU-145 (human prostate cancer cell lines), PC-3(human prostate cancer cell lines), LNCaP (cells are androgen-sensitive human prostate adenocarcinoma), U-87 MG (human primary glioblastoma) and U-373 MG (human glioblastoma line cells) were provided by the United States National Cancer Institute (NCI).

Cells were cultured in the Dulbecc modified Eagle medium (DMEM) supplemented with 5% fetal bovine serum (FBS), Penicillin G (100 U/mL) and streptomycin sulfate (100 µg/mL) at 38°C and 4.7% of CO_2 for one week and washed by saline phosphate buffer (PBS) and treated with « Trypsin EDTA » and incubated 4 min at 38 °C and 4,7% of CO_2.

100 µl of each line were mixed with 100 µl of Trypan to calculate the number of cells by hem cytometer. In 96 plates wells 100 µl of each cell were add and incubated for 72 h at 38 °C and 4,7% of CO_2.

The four fractions were solubilized in 10 % of DMSO (1µg/ml, 10 µg/ml, 100 µg/ml and 1000 µg/ml) which have been prepared in different DMEM concentrations (10, 20, 30, 40 and 50 µg /ml) and incubated 72 hours. DMEM and the DMSO were used as controls.

After 72 h of incubation, 25 µl de MTT were added in each well and after 3 hours of incubation we added also 100 µl of Lysis buffer of MTT and the absorbance was measured in spectrophotometric quantification (Mutiskan Ex) at 620 nm. Experiment was conducted in triplicate [10].

The cellular viability and mortality was calculated as described by:

% Viability = (Abs test /Abs control) × 100

% Mortality = 100 - % Viability.[9]

IC_{50} values were calculated as the concentrations that show 50% inhibition of proliferation on any tested cell line.

Apoptosis Cells Marking

Acridine orange ($5C_{34}H_{40}Cl_4N_6Zn$) is a vital biological colorant which land on nucleic acid, we have incubated 25 µl of cell suspension mixture with 1 µl of acridine solution (ThermoFisher A1301) for 10 to 20 minutes, the samples were mixed and visualized just after the incubation.[11]

We have placed 10 µl of cell suspension of microscope flake covered by a glass and to be cheeked with fluorescent microscope with fluorescein filtered lens X60.

STATISTICAL ANALYSIS

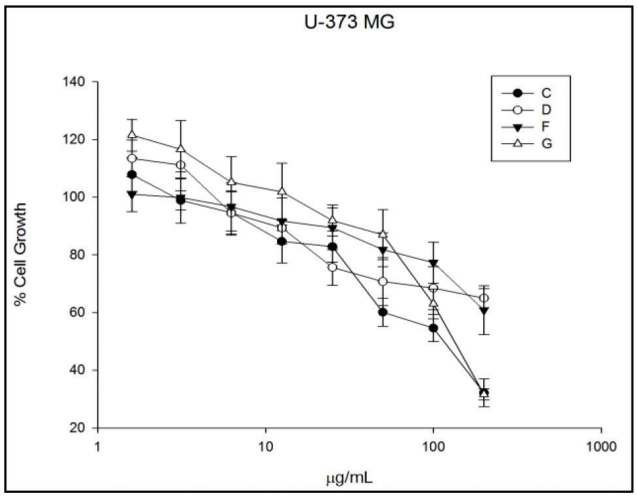
Results were depicted as mean ±sem. A graph pad prism 6 (ver. 5.02, GraphPad Software, Inc., CA, USA), were used to analyzed All data. The data were then analyzed

using one-way analysis of variance, followed by Tukey post hoc test for multiple comparison. The level of signification considered when P ≤0.05.

RESULTS AND DISCUSSION

Cytotoxic activity of Fraction C, D, F, G

The graphs on Figure 1 represent the percentage of viability of different cells DU-145, PC-3, LNCaP, U-87 MG and U-373 MG after treatment with different concentrations of fractions C, D, F, G (Figure 1). The MTT is a colorimetric assay which measures the enzymatic activity and depends to the reduction of MTT to formazan. The results IC 50 values of the four fractions of methanolic extract of *H.albus* are enumerated in **Table 1.**



Apoptosis Cell coloring

We reorganize that for the presence of many yellow dot's on the base of nuclei which show a chromatin condensation on the nuclei and also it fragmentation we also reorganize the budding of cytoplasm which define and confirm the biological mechanism of apoptosis the yellow dots have been shown on (Figure 2) by arrows.

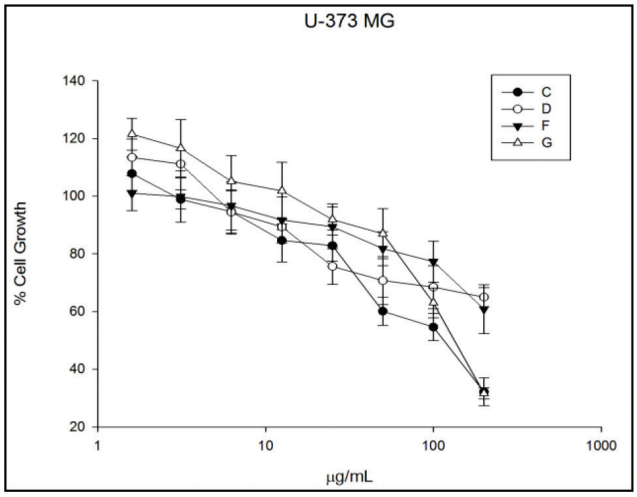
The results indicated that the fraction C of HAMeOH possessed a strong activity against cells lines showed marked anti-cancer activity, with IC_{50} = 73µg/ml, 187 µg/ml, 96 µg/ml and 114 µg/ml for the DU-145, PC-3, U-87 MG and U-373 MG respectively.

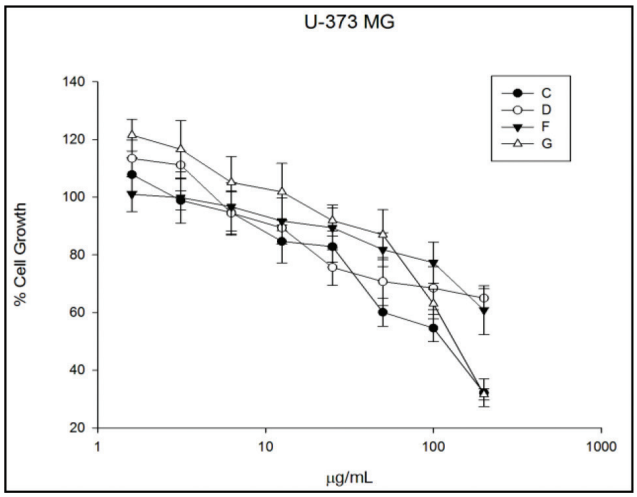
Therefore, the fraction D had just an activity against DU-145 with IC_{50}=186 µg/ml, the fraction F has an IC_{50} =165 µg/ml and 198 µg/ml against DU-145 and PC-3respectively. The fraction G showed the activity against DU-145, LNCaP and U-373 MG.

About the Fraction C we estimate the growth inhibition of the human prostate cancer cell lines, DU-145 and PC-3, and the human glioblastoma cell lines, LN-229 and U-373 MG, after 72 hours of treatment.

They found that the flavonoids are the best candidates for a protective effect against different kinds of cancer.[12] In a previous study which evaluate the cytotoxicity of more than 100 low molecular weight polyphenols used on normal and tumor cell lines, it shows that the compounds are more active on cancer strains rather than healthy ones.[13,14]

The flavonoids act at different levels of the carcinogenesis process: reducing the activation of procarcinogens to carcinogens by interacting with cytochromes P450, or by inducing the synthesis of certain cytochromes (CYP1A1 and CYP1A2, CYP1B1), Either by being metabolized by certain cytochromes, or by modulating the enzymatic activities of certain (stimulation or inhibition).[15] Cyto-





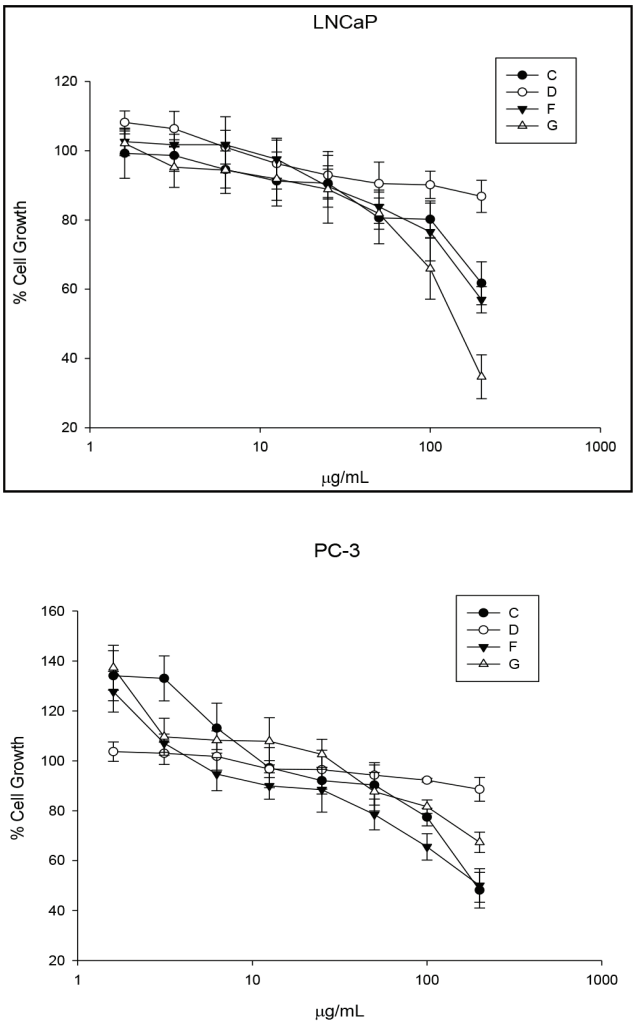


Figure 1: Percentage of viability of lines cells after treatment with four fractions of HAMeOH : C, D, F and G.

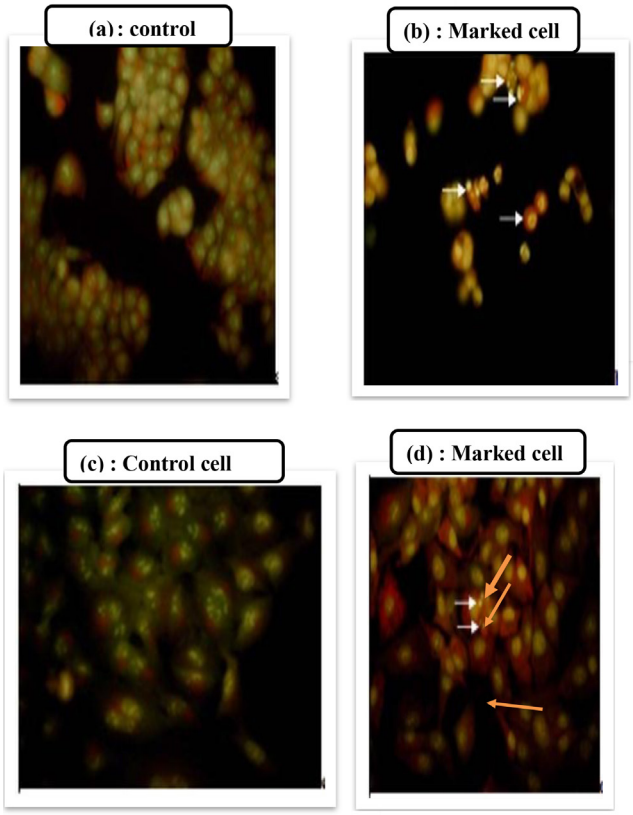


Figure 2: Visualization of apoptosis cell marked by acradine orange. Comparison between control cells treated by Fraction C on cell line PC-3 (a, b) and DU-145 cells (c, d)

Table 1: IC_{50} values of four selected fractions of methanolic extract of *H.albus*

Cell lines	Fraction C µg/mL IC_{50}	Fraction D µg/mL IC_{50}	Fraction F µg/mL IC_{50}	Fraction G µg/mL IC_{50}
DU-145	73	186	165	110
PC-3	187	---	198	---
LNCaP	---	---	---	142
U-87 MG	96	---	---	---
U-373 MG	114	---	---	133

chromes CYP1A1 and CYP1B1 are overexpressed in tumor tissues and metabolize procarcinogens to carcinogens.[16]

The anticancer activity of our extracts can be attributed also at abundance of terpenic compounds, Indeed, studies have demonstrated the anti-cancer activity of terpenes.[17] According to the same author, monoterpenes prevent the process of carcinogenesis during initiation and the stages of promotion / progression. Monoterpene pirillyl alcohol has been described to have anti-proliferative activity against glioblastoma cell by the inhibition of the Na / K-ATPase pump[17]. Other monoterpenes, such limonene, have been showed to prevent mammary, liver, lung, and other cancers. The activity of these constituents is related to the activation of cell death (apoptosis) induced by the caspases proteins in cancer cells.[18]

Some alkaloid plants have been already cited to inhibit proliferation of breast cancer cells by inhibiting anoïkis resistance or detachment-induced apoptosis, may prevent cancer progression and metastasis by blocking signals necessary for survival of localized cancer cells.[19, 20, 21]

In our study, we found that fraction C induce apoptosis while cell proliferation inhibition and we reorganize the presence of many dot's on the base of nuclei which show the chromatin condensation on the nuclei and also its fragmentation, we also reorganize the budding of cytoplasm which define and confirm the biological mechanism of apoptosis.[22]

CONCLUSION

In conclusion, the present investigation demonstrated that *H.albus* have an anticancer activity against different cells line which gives more importance to medicinal plants and improve their benefits and therapeutic effect, the fraction C and fraction F and induce apoptosis while the inhibition of the proliferation , for this reason we open the gate for future research to identify the unknown molecules on fractions which could be the first responsible of the obtained activity and investigate the molecular pathway induced while the anticancer activity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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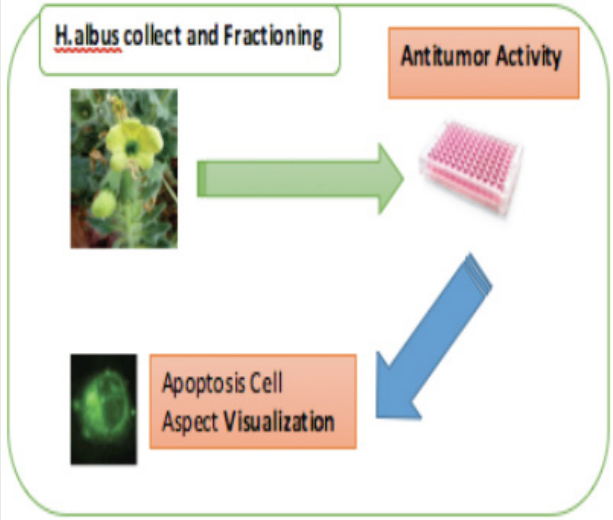
PICTORIAL ABSTRACT



SUMMARY

- Plant was collected from Aures Region in Algeria .
- Plant extracting and fractioning was done in
- LBPMC , University of Batna 2 .
- Antitumoral activity done to show the importance of medcinal plant
- Apoptosis screening was done to mark the pathway action of plant fractions
- The results show the importance of *H.albus* and open e new research project on compounds identification.

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