Int J Pharma Res Health Sci. 2017; 5 (2): 1654-1658
DOI:10.21276/ijprhs.2017.02.10
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CODEN (USA)-IJPRUR, e-ISSN: 2348-6465

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International Journal of Pharma Research and Health Sciences

Available online at www.pharmahealthsciences.net

Original Article

New Biological Anticancer Activities of Atropine Isolated from Hyoscyamus Albus's Leaves

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ARTICLE INFO

Received: 16 Mar 2017
Accepted: 28 Mar 2017

ABSTRACT

The flora of medicinal plants represent one of the most important source of drugs in different kind of dieses treatment such cancer, regarding to their antioxidant and anti-cytotoxic activities due to its richness to polyphenolic compounds ,. For these causes we are looking for new anti-cancer agents and compounds extracted from plants which represent a very important portal for cancer therapy and oncology research further.*Hyoscyamus albus* is a plant which is part of the Solanaceae family, generally it used in traditional medicine as a nervous sedative and para sympatholytic agents. They have isolated from this plant some tropane alkaloids such as scopolamine, hyoscyamine. The present work has for objective to purify compound P2 of Fraction C from methanolic extract of H.albus and evaluate it's cytotoxic activity on different cell lines.

Atropine (P2 of Fraction C) was isolated from leaves of *Hyoscyamus albus'* L. byusing high performance chromatography (HPLC), mass spectromerty (MS) and proton NMR (NMR H^1).

The effects of different compounds extracted from methanolic extract of *H.albus* on DU-145, PC-3, U-87 MG and U-373 MG cells lines were determined using MTT [3-(4,5-dimethylthiazolyl)-2,5-diphenyl-tetrazolium bromide] assay.

Our results indicated that the compound 2 fraction C (atropine) has a strong cytotoxic activity against DU-145 with IC50=417 µg/ml and on U-373 MG cells with IC50= 894µg/ml.

Keywords: *Hyoscyamus albus* L., Solanaceae, HPLC, cytotoxic activity, HAMeOH, NMR H^1.

1. INTRODUCTION

Currently many person killed every year by cancer which is a disease which occurs when changes in a group of normal cells within the body lead to uncontrolled growth causing a lump called a tumor. The cancer is caused by endogenous and exogenous factors which drive to the accumulation of genetic alterations [Lee and Park, 2010]. [1]

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H.albus is a plant which belongs to Solanaceae family; it's used in traditional medicine as a nervous sedative and para sympatholytic. They were isolated some tropane alkaloids such as scopolamine, hyoscyamine and with spectral techniques they isolated 2, 3 – dimethyl nonacosane. Some tests showed the antitumor proprieties of quercetin like inhibition of proliferation and migration of cancer cells [Lim *etal*., 2006]. [2] They found that some compounds like kaempferol, quercetine, anthocyanes, coumaric acid and ellagic acid could inhibit the growth of human cancer cells like breast line cells (MCF-7), oral (KB,CAL-27), colon (HT-29, HCT-116) and prostate (LNCaP, DU-145) [Zhang *etal*., 2007] [3] .The aim of our study is to separate different fractions of H.albus and characterize an important compound on the plant which represent the major pic on one of these fractions and test its cytotoxic activity on different cancer cell lines . The cytotoxic potential was studied by MTT assay. Moreover, the present study characterized the compoundsC2 by (HPLC), mass spectrometry (MS) and proton nuclear magnetic resonance (NMR H^1).

2. MATERIAL AND METHODS

Plant material

The leaves of *H. albus* was collected from Batna city, Algeria in Mai 2015. It was identified by Dr. OUDJHIH, Laboratory of Botanic, Department of Agronomy, university of Batna Algeria. Plant leaves were dried for 40 days at an ambient temperature under shade, after; the leaves were crushed to obtain a fine and homogeneous powder and conserved in dry place.

Extraction

The plants materials were powdered (500g) and extracted by using 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.

Purification with Sephadex gel

The methanolic extract of *H. albus*'s leaves was submitted to column chromatography over Sephadex LH-20 (Pharmacy Department,Italy), using methanol as eluent (mobile phase).The obtained fractions (FA,B,C,D,E,F) (Merck). were analyzed by Thin layer chromatography (TLC) on Selica gel 60 F_{257} plate (Merck) precoated aluminum plates (thickness = 200µm) using butanol - glacial acetic acid-water system and anisaldehyde sulfuric and $FeCl_3$ reagents as a spray reagent, finally the similar profiles were combined.

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

We obtained four fractions FA (0.73 g), FB (0,75 g) ,FC (2.04 g) ,FD (2.11 g) ,FE (0.36g) ,FF (0.32 g) ,FG (1.37 g).

Cytotoxic activity of Fractions

The anticancer activity of compounds from fraction of HAMeOH was evaluated with MTT [3-(4,5-dimethylthiazole-2-yl)-2,5diphenyltetrazolium bromide; Sisco, Italy] with method of [Mossmann, 1983; Skehan, 1990] [4, 5] with some modifications.

DU-145 (human prostate cancer cell lines), PC-3(human prostate cancer cell lines), LN-229(cells are androgen-sensitive human prostate adenocarcinoma)and U-373 MG (human glioblastoma line cells)were kindly provided by the United States National Cancer Institute (NCI).

The cells were grown in The Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal bovine serum (FBS), Penicillin G (100 U/mL) and streptomycin sulfate (100µg/mL) at 38°C and 4.7 % of CO_2 for one week.

one week later the cells were washed with saline phosphate buffer (PBS) and treated with « Trypsine EDTA » and incubated 4 min at 38 °C and 4,7% of CO2 .

After incubation time , we introduced the culture medium in conic tubes and we centrifuged min at 1040 to min to separate the cells for the medium. After agitation, 100 µl of each line were mixed with 100 µl of Trypan to calculate the number of cells using the microscope using hemocytometer.

In the 96-well plates wells, we putted in each well 100 µl of each cell and incubated 72 h at 38 °C and 4,7% of CO_2.

Our compounds p1,p2(atropine),p3 were solubilized in 10 % of Dimethyl sulfoxide DMSO (1µg/ml, 10 µg/ml, 100 µg/ml and 1000 µg/ml) with is prepared in DMEM with concentrations (10, 20, 30, 40 and 50 µg /ml) and incubated 72 hours. DMEM and the DMSO were used as controls and final DMSO concentration did not affect cell viability.

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 [Skehan ,1990]. [6]

The cellular viability and mortality was calculated:

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

% Mortality = 100- % Viability [Mossmann, 1983]. [5]

Experiment was conducted in triplicate.

IC50 values were calculated as the concentrations that show 50% inhibition of proliferation on any tested cell line.

Preparative HPLC

We have used the reverse phase high performance liquid chromatography (HPLC) to analyze the present compounds in the fractions of *H.albus* This HPLC equipped with a C18 column (kintex 5UXB- C18) and UV-photodiode array detection (DAD) was performed at 220 nm and method file 10-60 in 20 minutes .pump. with gradient system consisting of solvent A (Acetonitrile) and solvent B (Methanol) with a flow rate 500µl/min and the volume of injection was 20 µl and the temperature of 25°C.

Analysis with mass spectrum (MS)

(ESI-MS) Electrosprayionization mass spectroscopic of compounds in fractions was performed using an applied

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Biosystems (LC/MSD TRAP x CT) Agilent 6110. Mass spectra were achieved by electrospray ionization in positive mode. We adjusted the prob-flow to 1 ml/min .the continuous mass spectra were obtained by scanning from 100 to 1000 m/z.

The structure elucidated of P2 of fraction C (Atropine) was determined by comparison of its physicochemical and spectroscopic data (^{1}H NMR and mass spectrum) with literature values [Moffat, A. C.,al, 1986]. NMR was recorded on a Bruker Advance DPX400 equipment (Germany) operation 400 MHz for NMR H^1 and using methanol deuterium (MeOH-d4) as a solvent to solubilize the compounds.

Statistical analysis

The results of activity were represented the mean S.E.M. Statistical differences between the and the control were evaluated by one ANOVA followed by Tukey test.

3. RESULTS AND DISCUSSION

Identification of P2 of fraction C Atropine:

The structure elucidation of P2 of fraction C (Atropine)was determined by comparison of its physicochemical and spectroscopic data (^{1}HNMR and mass spectrum) with literature values[Moffat, A. C.,al,1986.

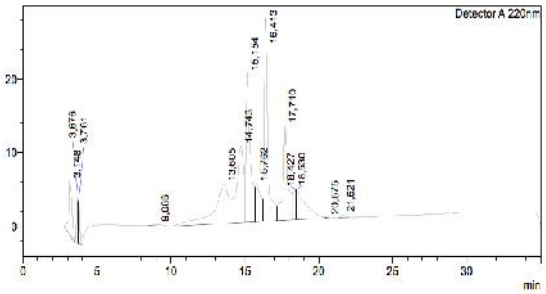

Fig 1: Profile of Preparative HPLC P4 of fraction C

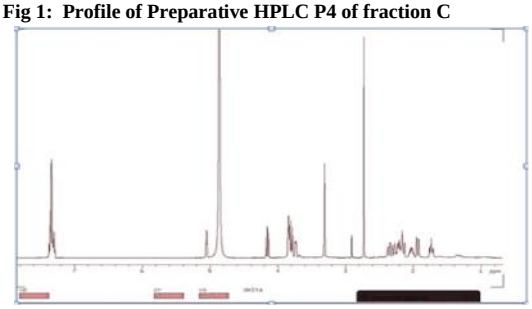

Fig 2: NMR H^1 Profileof P2 fraction C.

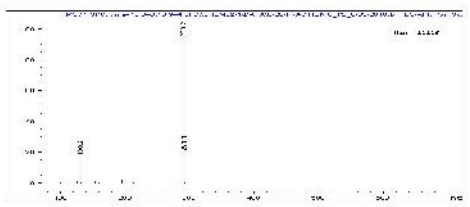

Fig 3: Mass spectrum Profile of P2 fraction C.

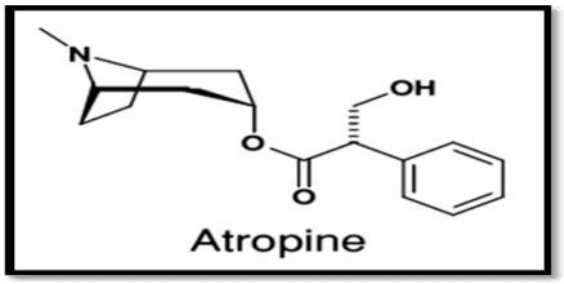

Fig 4: Atropine chemical form

Cytotoxic activity

Thegraphs in figure 5 and table 1 present the percentage of viability of different cells DU-145, PC-3, LN-229 and U-373 MG after treatment with different concentrations of compounds extracted from fraction C of HAMAoH (figure5). The MTT is a colorimetric assay whichmeasure the enzymatic activity and depends to the reduction of MTTtoformazan comparing to the standard agent which is DMSO , for the preliminary results

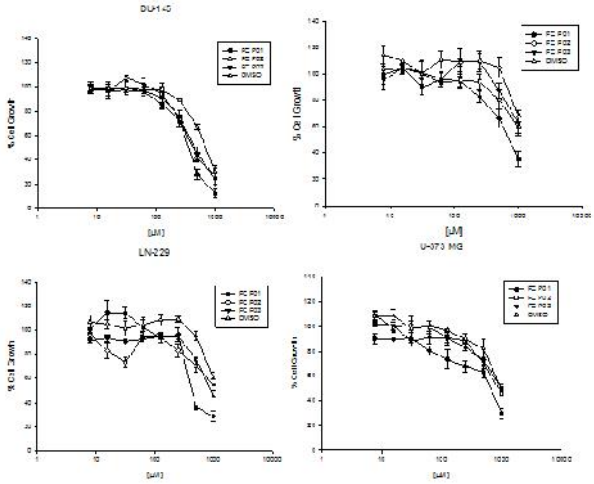

Fig 5: Percentage of viability of lines cells after treatment with compounds of HAMeOH 1, 2, 3

Table 1: IC 50 values of compounds of methanolic extract of *H.albus* on anti-cancer activity

Cell lines	Fraction C P01 µg/mL IC50	Fraction C P02 Atropine IC50	Fraction C P03 µg/mL IC50	DMSO µg/mL IC50
DU-145	353 (78%)	417 (73%)	445 (80%)	689
PC-3	725 (85%)	……	……..	……
LN-229	425 (99%)	…….	……...	……..
U-373 MG	654 (69%)	894 (54%)	1000 (49%)	956

The results indicated that the compound 2 fraction C of HAMeOH have a strong activity against line cells showed marked anti-cancer activity with IC50 = 417µg/ml, and 894 µg/ml for the DU-145U-373 MG respectively.

Therefore, the compound 1 of fraction C have an activity against DU-145 , PC-3, LN-229, U-373 MG with IC50=353

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µg/ml, 725µg/ml , 425µg/ml , 654µg/ml, the compound 3 has an IC50 =445 µg/ml and 903µg/ml, 1000µg/ml against DU-145 and LN-229 and U-373 MG. They have found that the flavonoids are the best candidates with protective effects against the different kinds of cancer [Rahmane *et al*., 2011]**.** [6]

In our study, we found that the fraction C contains the atropine , and in previous studies they proved that the quercetin has to prevent against prostate cancer especially [Rietjens *et al*., 2005] [7.] Also they mentioned that the quercetin has a capacity to inhibit the development of breast cancer (MCF-7 and MDA-MB231) [Scambia et *et al.,* 1994] [8]**.** The quercetin is known by its antioxidant activity against oxidative stress. Also the quercetin protects the cells against the damages caused by free radicals by antioxidant effect.

The previous studies showed that the quercetin induce the apoptosis of cancer cells and inhibit the protein kinase C [Murota et Terao, 2003] **[9]**, and modulate the oxydoreduction process's [Garcia-Mediavilla *et al*., 2007] **[10].**

4. CONCLUSION

the anticancer activity has been proved by h.albus against different cells line used specially the fraction C which contain majorly the atropine as we have identified previously for further reasearch we need to proceed identifying the compounds including in this fraction and other fractions separated on hyoscyamus albus methanolic extract .

The compound atropinewas characterized in the fraction Cafter HPLC preparative and analytic, MSspectrometry and NMR H^1analysis so this compound will be tested on line cells directly.

5. ACKNOWLEDGMENTS

The department of pharmacy University Federico II- Naples Italy supported the present work; aswe shall also thank the Department of Biochemistry, Biophysics and General Pathology, Second University of Naples,80138 Naples, Italy.

6. REFERENCES

1. Lee S.B. and Park H. Anticancer activity of guava (Psidium guajava L.) branch extracts against HT-29 human colon cancer cells. Journal of Medicinal Plants Research. 2010; 4: 891-896.
2. Lim JH, Park JW, Min DS, Chang JS, Lee YH, Park YB, Choi KS, Kwon TK . NAG-1 up-regulation mediated by EGR- 1 and p53 is critical for quercetin-induced apoptosis in HCT116 colon carcinoma cells. Apoptosis, 2006; 12:411-421.
3. Zhang J, Li Q, Di X, Liu ZH, Xu G. Layer-by-layer assembly of multicoloured semiconductor quantum dots towards efficient blue, green, red and full color optical films. Nanotechnology 2008; 19:435-606.
4. Mosmann T: Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983; 65:55-63.
5. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR. New colorimetric cytotoxicity assay for anticancer-drug screening.J Natl Cancer Inst. 4; 1990; 82(13):1107-12.
6. Rahman AA, Lophatananon A, Stewart-Brown S, Harriss D, Anderson J, Parker T,Easton D, Kote-Jarai Z, Pocock R, Dearnaley D, Guy M, O'Brien L, Wilkinson RA,Hall AL, Sawyer E, Page E, Liu JF. UK Genetic Prostate Cancer Study Collaborators; British Association of Urological Surgeons' Section of Oncology,Eeles RA, Muir K. Hand pattern indicates prostate cancer risk. Br J Cancer. 4; 2011; 104(1):175-177.
7. Rietjens IM, Boersma MG, Van DWH, Lim JH, Park JW, Min DS, Chang JS, Lee YH, Park YB, Choi KS, Kwon TK. NAG-1 up-regulation mediated by EGR- 1 and p53 is critical for quercetin-induced apoptosis in HCT116 colon carcinoma cells. Apoptosis, 2006; 12: 411-421.
8. Scambia G, Ranelletti FO, Panici PB. Quercetin potentiates the effect of adriamycin in a multidrug-resistant MCF-7 human breast cancer cell line: P-glycoprotein as a possible target. Cancer Chemother Pharmacol 1994; 34:459-464.
9. Murota, K., Terao, J. Antioxidative flavonoid Q: implication of its intestinal absorption and metabolism. Arch. Biochem. Biophys. 2003; 417: 12–17.
10. Garcia-Mediavilla, V., Crespo, I., Collado, P.S., Esteller, A., Sanchez- Campos, S., Tunon, M.J., et al, The anti-inflammatory flavones quercetin and kaempferol cause inhibition of inducible nitric oxide synthase, cyclooxygenase-2 and reactive C-protein, anddown-regulation of the nuclear factor kappaB pathway in chang liver cells. Eur. J. Pharmacol. 2007: 557: 221–229.
11. Arts IC, Jacobs DRJ, Gross M, Harnack LJ, Folsom AR Dietary catechins and cancer incidence among postmenopausal women: the Iowa Women's Health Study (United States). Cancer Causes Control 2002; 13:373-382.
12. Jung HG, Kim HH, Paul S, Jang JY, Cho YH, Kim HJ, Yu JM, Lee ES, An BJ, Kang SC, Bang BH. Quercetin-3-O-b-D-glucopyranosyl-(1 fi 6)-b-D-glucopyranoside suppresses melanin synthesis by augmenting p38 MAPK and CREB signaling pathways and subsequent cAMP down-regulation in murine melanoma cells, Saudi Journal of Biological Sciences 2015; 22: 706–713.
13. Olszewska M. Separation of quercetin, sexangularetin, kaempferol and isorhamnetin for simultaneous HPLC determination of flavonoid aglycones in inflorescences,

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leaves and fruits ofthree Sorbus species. J. Pharm. Biomed. Anal. 2008; 48: 629–635.
14. Clarke's Isolation and Identification of Drugs, 2nd ed., Moffat, A. C., et al., eds., The Pharmaceutical Press (London, GB: 1986), p. 363-364

Conflict of Interest: None

Source of Funding: Nil

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