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NEW BIOLOGICAL ANTICANCER ACTIVITIES OF ATROPINE ISOLATED FROM ALGERIAN HYOSCYAMUS ALBUS'S LEAVES

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Abstract

Background: Medicinal plants represent one of the most important source of drugs for several diseases treatment such cancer, due to its richness of alkaloids, polyphenols and terpenes. All these properties gives us the opportunity to come across new therapeutic agents extracted for plants. *Hyoscyamus albusL*. An old medicinal plant belong to Solonaceae family and which is rich source of alkaloids , tropane and nortropane which confers to this plant a number of very important and benefical therapeutic effet during time.

Purpose: Our purpose of the paper is to isolate the atropine as an alkaloid from hyoscyamus albus among its methanolic extract fractions and to evaluate the anticancer activity of atropine on different cancerous cell lines

Methodes: Atropine was isolated from *Hyoscyamus albus'* L. by using high performance chromatography (HPLC), analytic HPLC , mass spectromerty (MS) and proton NMR (NMR H^1) , as the effects of atropine were performed on, PC-3, U-87 MG , U-373 MG and DU-145 cells lines and were determined using MTT [3-(4,5-dimethylthiazolyl)-2,5-diphenyl-tetrazolium bromide] assay.

Results: Our results indicated that atropine represent an important component in hyoscyamus albus and have a strong cytotoxic activity against DU-145 with IC50=417 µg/ml and new activity on U-373 MG cells with IC50= 894 µg/ml.

Conclusion: The preliminary results confirm the interesting anticancer activity of *H.albus* and a support indicative for further investigations on new compounds characterization and molecular mechanisms study.

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

Introduction

Cancer is a disease which is charchtrized 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 S.B. and Park H. 2010).

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 JH, 2006). 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) (Murota, K.,2003), (Massinissa Y.,2018)

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 in the aim to test its cytotoxic activity on different cancer cell lines. The cytotoxic potential was studied by MTT assay. Moreover, this study characterized the compounds C2 by (HPLC), mass spectrometry (MS) and proton nuclear magnetic resonance (NMR H^1).

Methods

2.1 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 for further use.

2.2 Extraction

The vegetal 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.

2.3 Purification with Sephadex gel

The methanolic extract of *H. albus*'s leaves (HAMeOH) 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) pre-coated 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 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).

2.3 Cytotoxic activity of Fractions

The anticancer activity of compounds isolated from fraction of HAMeOH was evaluated with MTT [3-(4,5-dimethylthiazole-2-yl)-2,5diphenyltetrazolium bromide; Sisco, Italy] with method of mosman (Mosmann T, 1983)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 (100 µg/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 [10].

The cellular viability and mortality was calculated:
% Viability = (Abs test /Abs control) x 100
% Mortality = 100- % (Viability) (Mosmann T, 1983)
Experiment was conducted in triplicate.
IC50 values were calculated as the concentrations that show 50% inhibition of proliferation on any tested cell line.

2.4 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.

2.5 Analysis with mass spectrum (MS)

Electrospray ionization mass spectroscopic (ESI-MS) of compounds in fractions was performed using an applied 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 elucidation 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 (Olszewska M., 2008). 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.

2.6 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. The level of signification considered when $P \leq 0.05$

Results And Discussion

3.1 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}H NMR and mass spectrum) with literature values (Clarke's, 1986)

3.2 Cytotoxic activity

The graphs 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 which measure the enzymatic activity and depends to the reduction of MTT to formazan comparing to the standard agent which is DMSO for the preliminary results .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 µg/ml, 725 µg/ml , 425 µg/ml , 654 µg/ml, the compound 3 has an IC50 =445 µg/ml and 903µg/ml, 1000 µg/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 (Rahman AA, 2011).

In our study, we found that the fraction C contains the atropine which is strong alkaloid, and in previous

studies they proved that the quercetin has to prevent against prostate cancer especially (Rietjens IM, 2005). Also they mentioned that the quercetin has a capacity to inhibit the development of breast cancer (MCF-7 and MDA-MB231) (Scambia G, 1994) . 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 K, 2005) , and modulate the oxydoreductionprocess's (Garcia-Mediavilla, V, 2007).

Conclusion

H.albus have the anticancer activity against different cells line improved by it's fraction and atropine which represent the major product on fraction C as we have identified previously, for further research we need to proceed identifying the compounds including in this fraction and other fractions separated on hyoscyamus albus methanolic extract .

The compound atropine was characterized in the fraction C after HPLC preparative and analytic, MS spectrometry and NMR H^1 analysis so this compound will be tested on line cells directly to improve new biological activity.

Acknowledgments

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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

Figure 1. Profile of Preparative HPLC P2 of fraction C

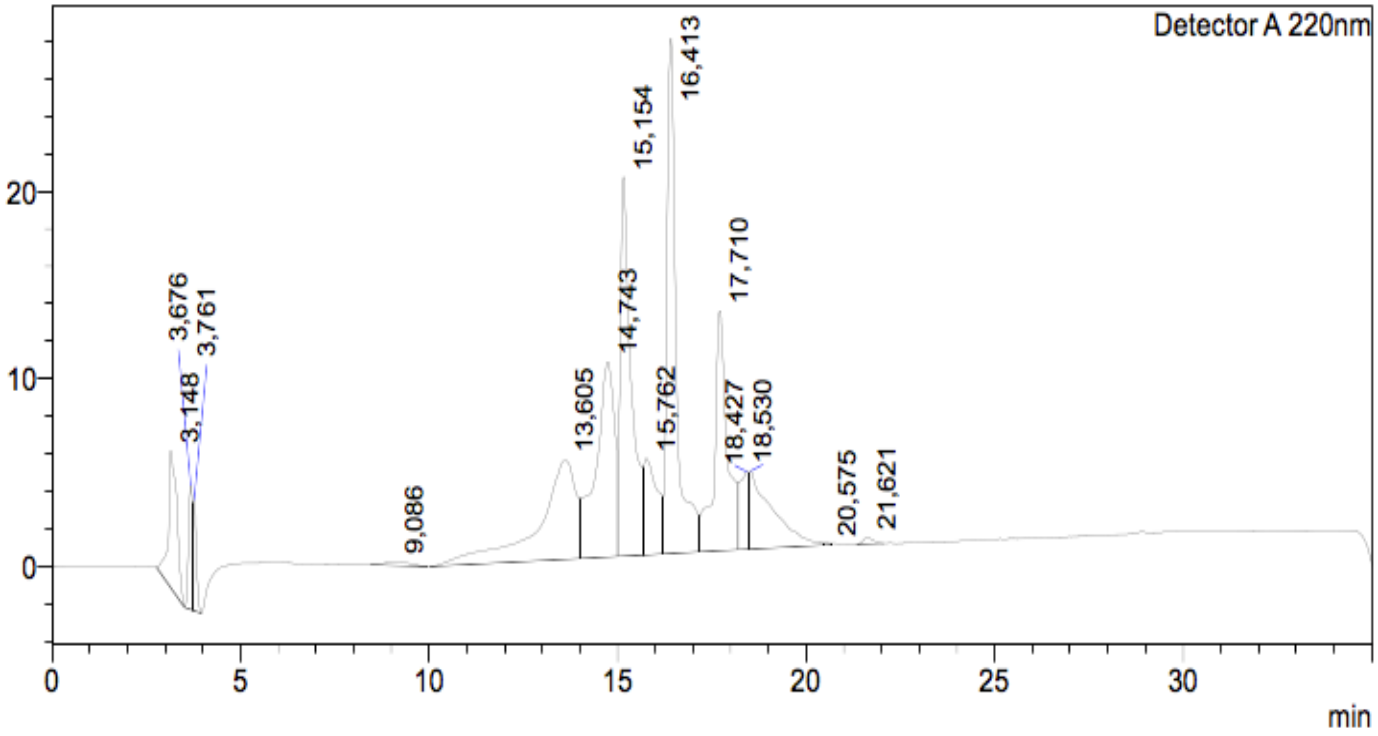


Figure 2. NMR H^1 Profile of P2 fraction C.

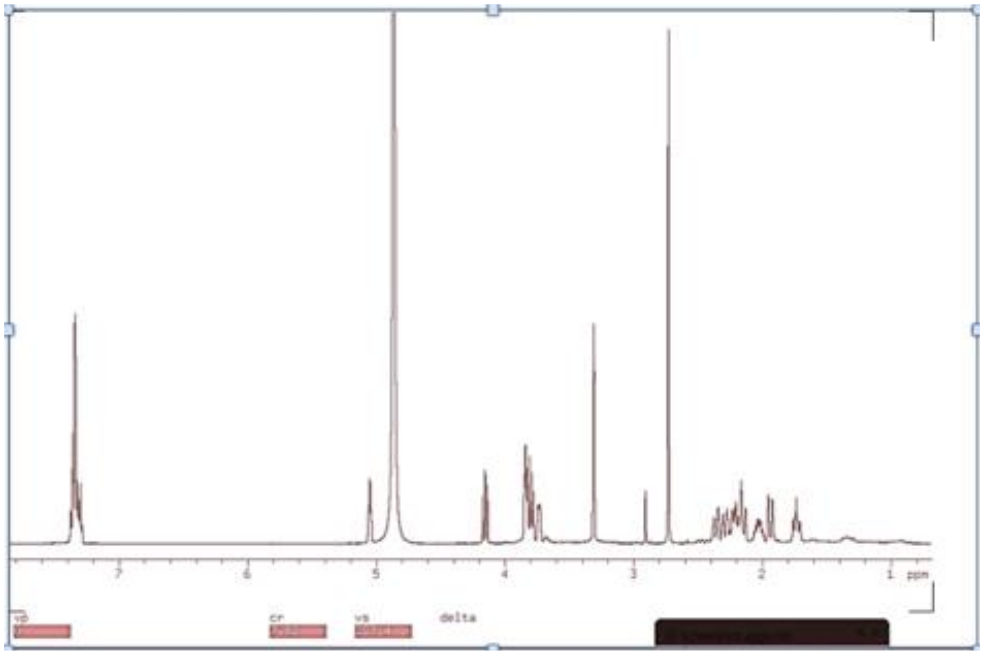


Figure 4. Atropine chemical form

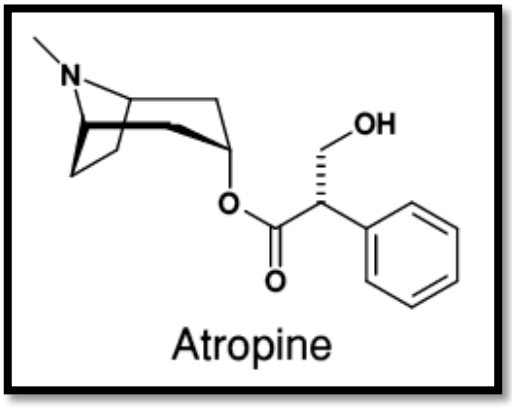
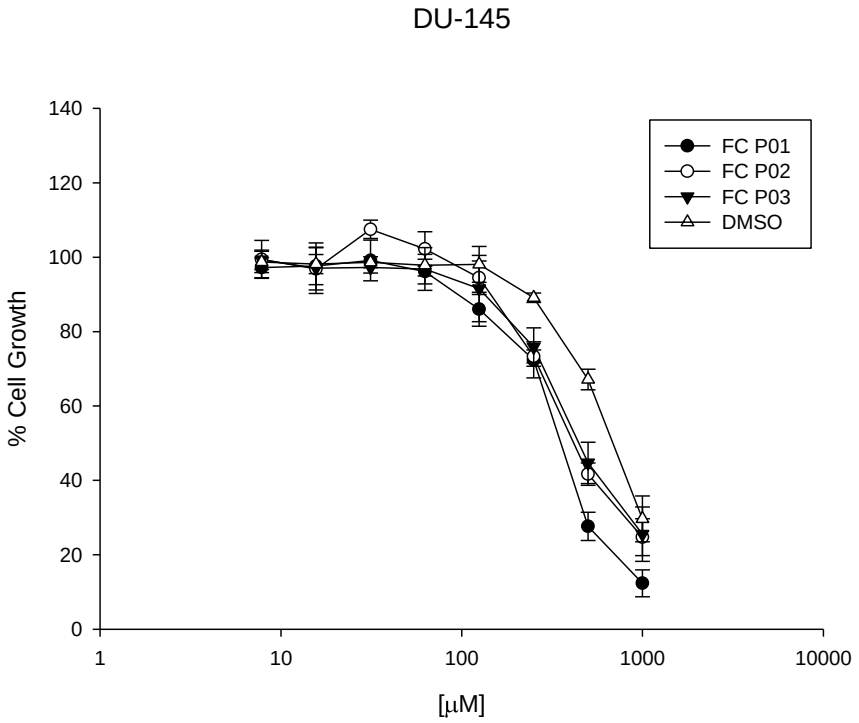
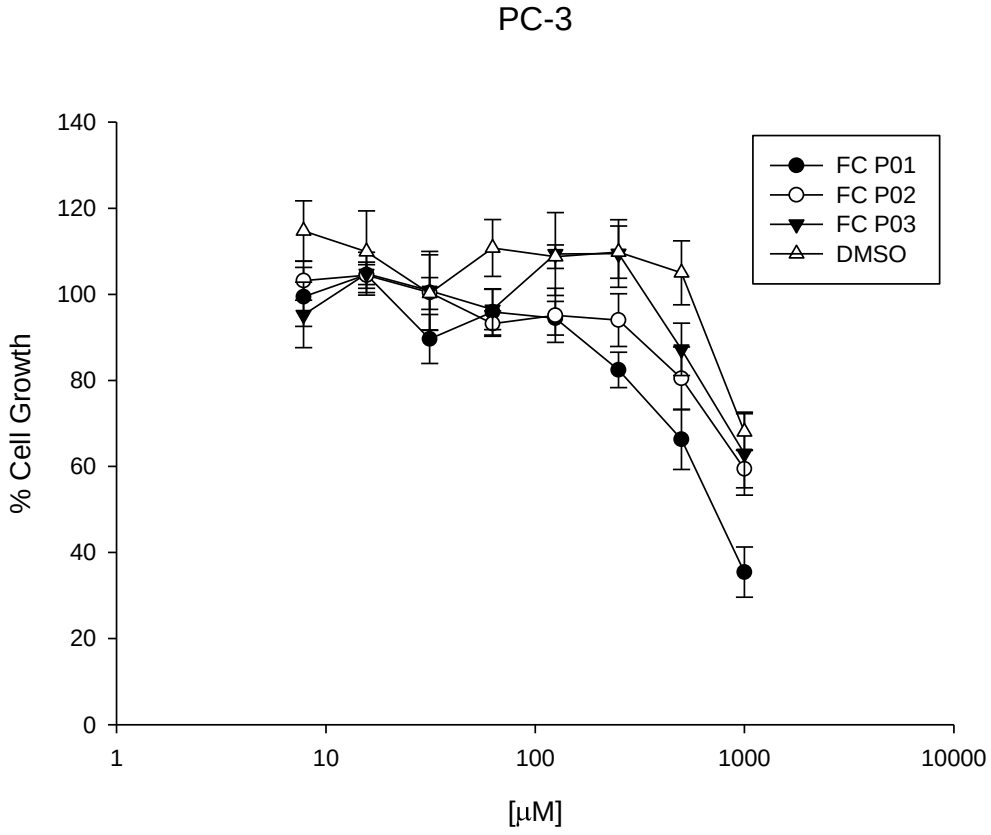
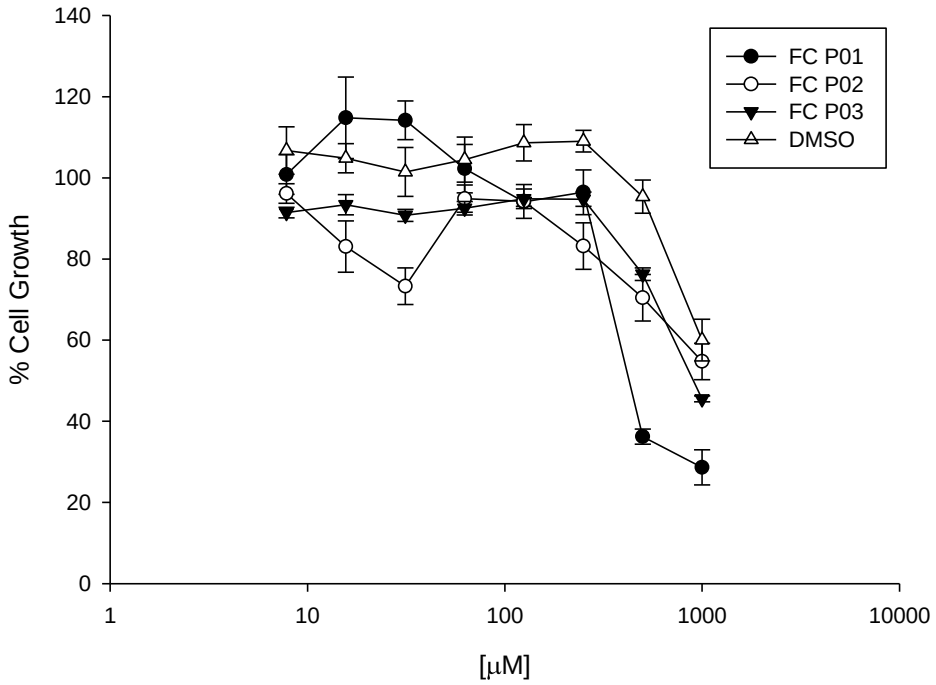


Figure 5. Percentage of viability of lines cells after treatment with compounds of HAMeOH 1, 2, 3.

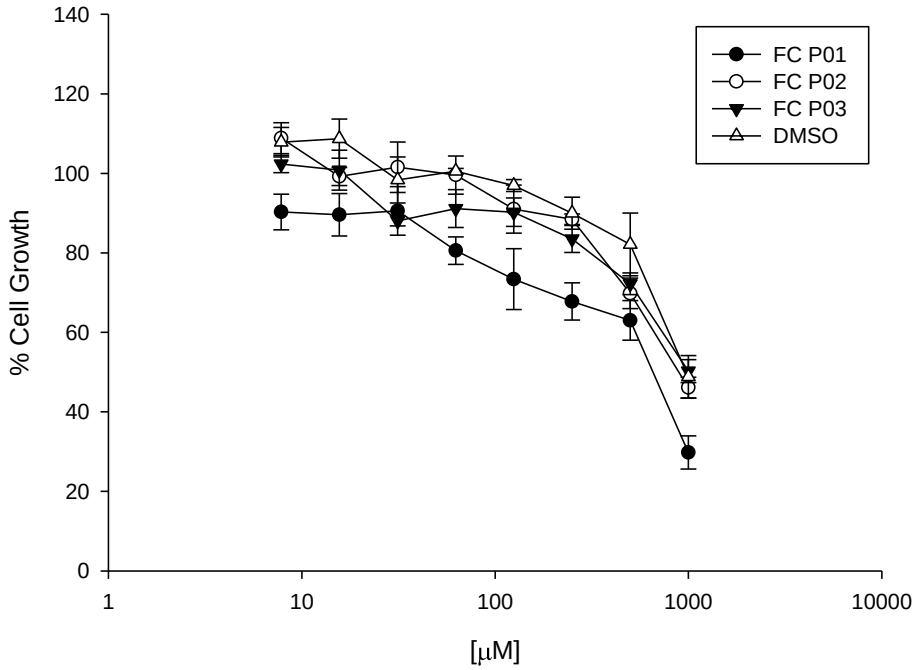




LN-229



U-373 MG



Graphical Abstract