International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2017): 7.296

Effect of Jatropha Extract on Cancer Cells as a New Source of Cancer Treatment

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Abstract: ***The experiments were carried out in the cell and tissue culture labs at the Central Laboratory - Cairo University. The aim of the study was to measure the effect of Jatropha Curcas L. extract in killing or inhibiting cancer using different concentrations. the study was conducted under different levels with the addition of 12 concentration of extract. It was noticed that at the concentration of (600um / L) the Cancer cells were eliminated by (83%) while the extract was removed by (200 um / L) by (39%) of the percentage of cancer cells, (60%) compared with the concentration of 600 um / L, the healthy living cell was approximately (12%).***

Keywords: Jatropha Curcas, Cancer, liver cancer

1. Introduction

In an age where the causes of disease and with the search for a persistent human for a successful alternative and available to reach the most appropriate and safe way of treatment it was important to find a new way to treat This disease, which has become very threatening to the Third World in particular and the world as a whole in general Where there was a link between progress, well-being of societies, and cancer

Due to the seriousness of the disease and its spread, this causes great financial burdens on the state and on patients. It was necessary to find a cure acceptable to the consumer with an acceptable cost The Jatropha plant is an important economic crop due to its low water and sand needs and its importance in the production of biofuels and the important medical benefits in the drug industry. It was therefore important to study the effect of Jatropha in inhibition of cancer. The goal of the study was achieved by reaching a new treatment for ovarian cancer

2. Materials

(Liver cancer) cell line.

- Phosphate buffer saline, pH 7.2 (1x), calcium and magnesium free (Gibco)
- 0.5% Trypsin–EDTA (10x) (Gibco) diluted to 1X by PBS.
- (DMEM) Dulbecco's Modified Eagle Medium ,high glucose (Lonza)
- Complete media : DMEM supplemented with 10% Fetal bovine serum (SeraLab) + 1%antibiotic (penicillin G potassium+ streptomycin (sigma))
- 0.4% (wt/vol) Trypan blue (Sigma)
- Neutral red stock solution 4 mg/ml: 40 mg neutral red dye was dissolved in 10 ml PBS protected from light by foil.
- Neutral red medium (40 µg/ml): the neutral red stock solution diluted with culture medium and Incubated overnight at the same temperature of cultured cells.
- Neutral red destain solution: 50% ethanol 96% (Riedel-de-Ha¨en), 49% deionized water,1% glacial acetic acid (Sigma)

3. Procedure

According to (R.Guillermo et al., 2008).

1) Cells were grown under aseptic conditions with complete medium in a 25 cm3 cell culture flask with humidified atmosphere and 5 % CO2 at 37°C.
2) Cultured monolayer at 80% confluence subjected to wash with PBS then trypsinized by 2 ml (0.25%) trypsin–EDTA solution, incubated for 2 min. then flask was lightly tapped to detach the cells, the reaction stopped by adding 5 ml complete culture medium.
3) The cell suspension counted using hemocytometer, and cell viability checked by trypan blue (100% viability).
4) The cells suspension was diluted with complete medium to have approximately 100,000 cell/ml, agitated gently and placed in a sterile reservoir. 200 µl of the cell suspension (containing ≈20,000 cell per well) was dispensed by multichannel pipette into the inner 60 wells of the 96 well plate, the peripherals wells were filled with PBS, then the plate incubated for 24 hours.
5) After cell seeding and attachment, the media discarded gently and different concentrations of the compounds prepared (1, 10, 20, 40, 60, 80, 100, 200, 300, 400, 500 and 600 µg/ml) by diluting with DMEM media (after filtered by 0.2 µm syringe filter).
6) Measuring the effect of 12 concentrations of Jatropha extract on healthy cells and ovarian cancer:
7) 200µl of treatment media was dispensed into 4 replicates for each concentration, other wells were filled with media only (as a negative control) and wells filled with media containing Doxorubicin HCL (4µg/ml) as a positive control. After that the 96 well plate covered by lid, incubated at 37° C for 24 h.
8) After the incubation period, the cultures examined under inverted microscope, recording changes in morphology of the cells due to cytotoxic effects of the test chemical and photos was taken then the medium was decanted from the wells gently without disturbing the materials.
9) 100µl of Neutral red medium (which was prepared and incubated at 37C for 24h. and centrifuged at 1800 rpm for 10 min. to remove any precipitated dye crystals) was

added into each well and incubated again for 3h at 37°C.

10) After incubation, the dye containing medium was decanted and each well was rinsed gently for two times with 150µl PBS solution to remove the unabsorbed neutral red dye contained in the wells.
11) 150µl of destain solution added and incubated for 10 min with shaking.
12) The absorbance of acidified ethanol solution containing extracted neutral red dye was measured using spectrophotometer (BioTek, ELX808).
13) The viability % and dose response curve were calculated for having the concentration of the test chemical reflecting the half maximum inhibitory concentration of the cell proliferation.

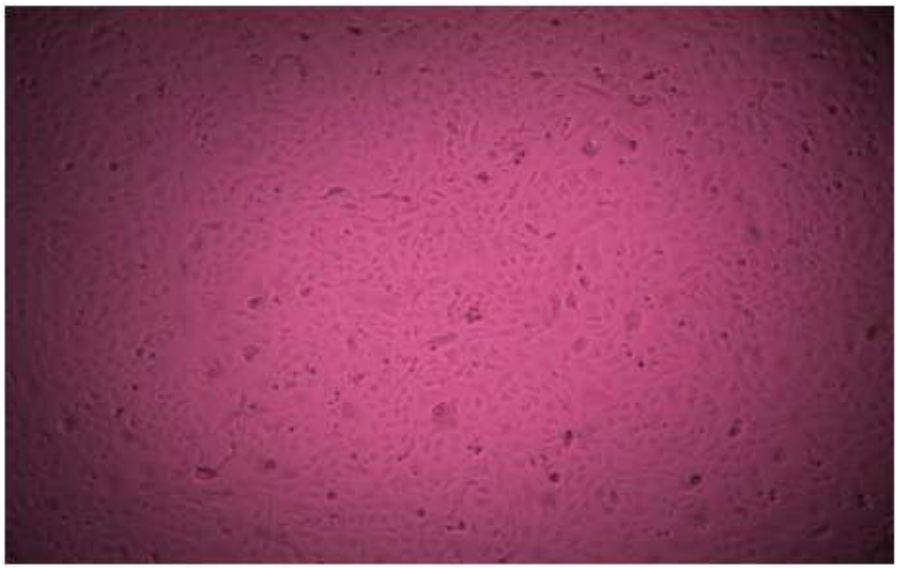
4. Results and Discussion

4.1 Effect of Jatropha extract on ovarian cancer in the laboratory

Sample Number	Optical density	%Viability
1	0,039	0
2	0.043	0
3	0.083	33
4	0.110	57.77
5	,129	75
6	,13	76
7	.13	76
8	.140	86
9	,154	98
10	.158	100
11	.16	100
12	.166	100

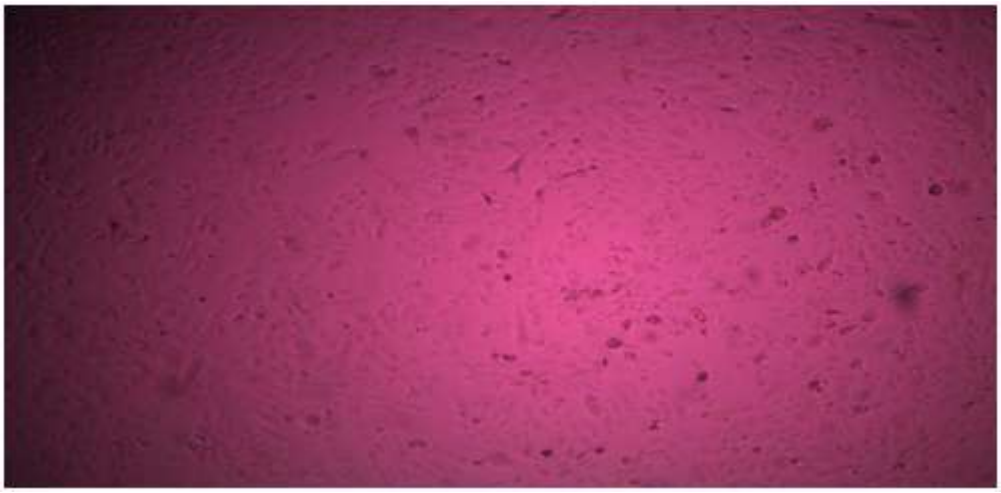
Neutral red uptake assay for the estimation of cell viability/ cytotoxicity.

Treated cells by 40X magnification power:
Negative control

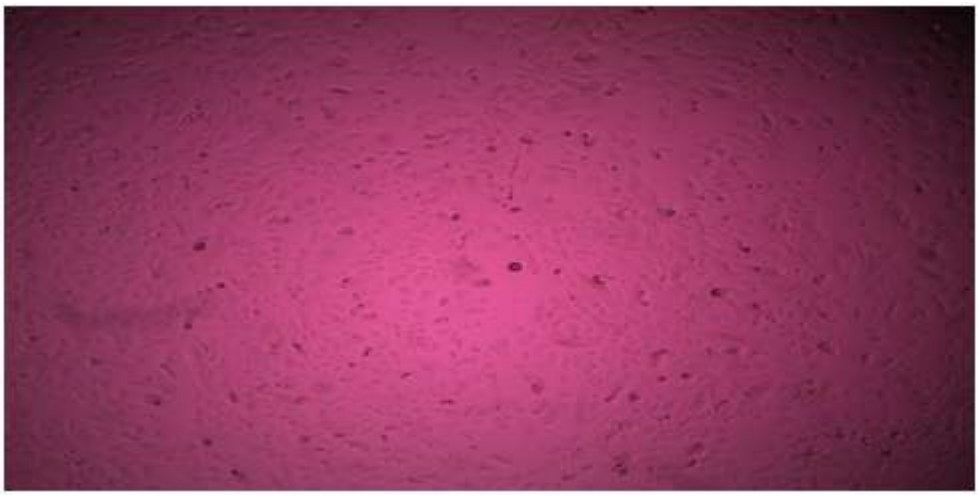


- Positive Control

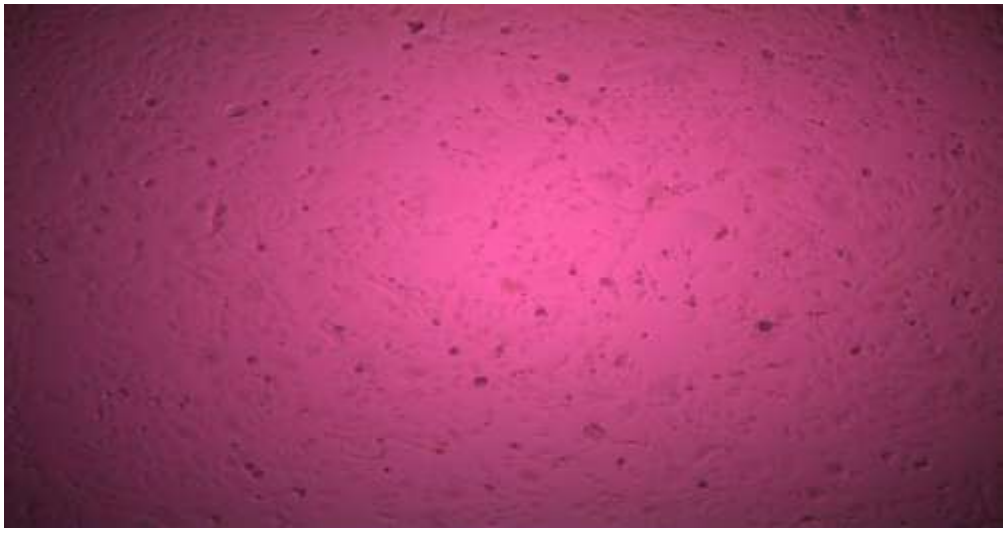
- 10µg/ml



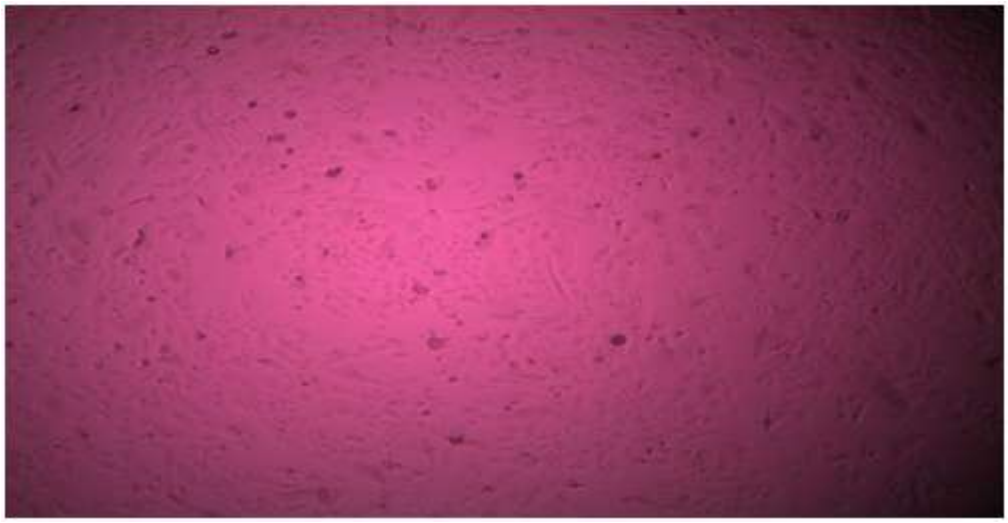
- 20µg/ml



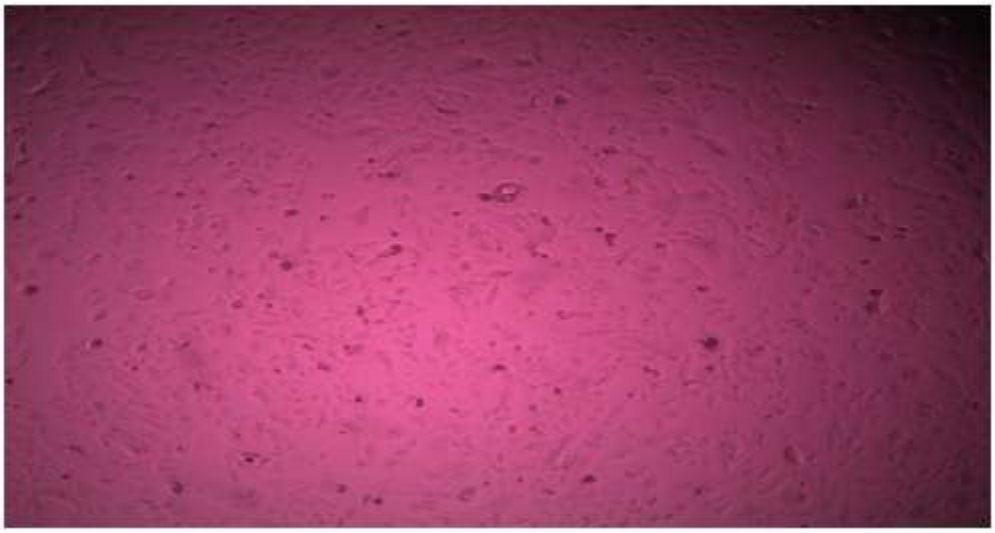
- 40µg/ml



- 60µg/ml



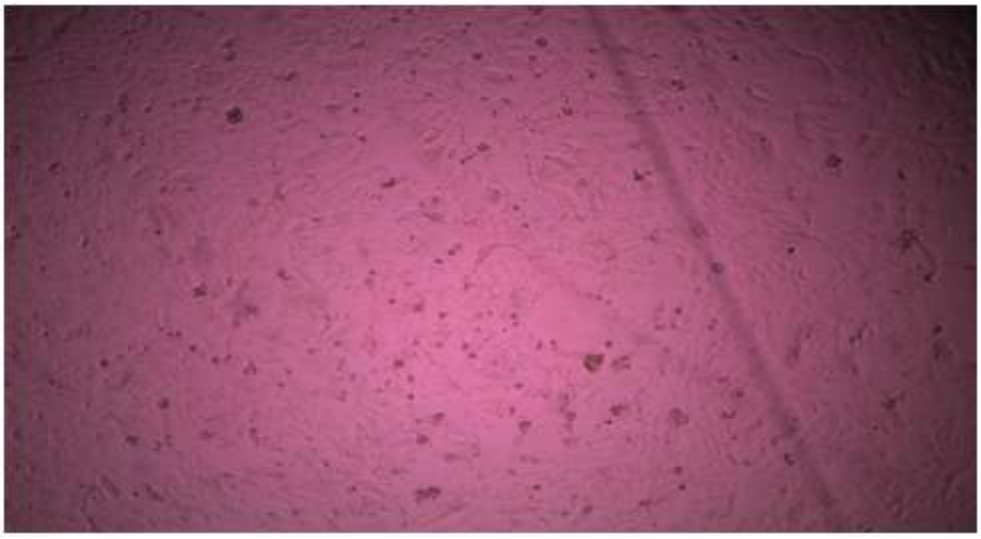
- 80µg/ml



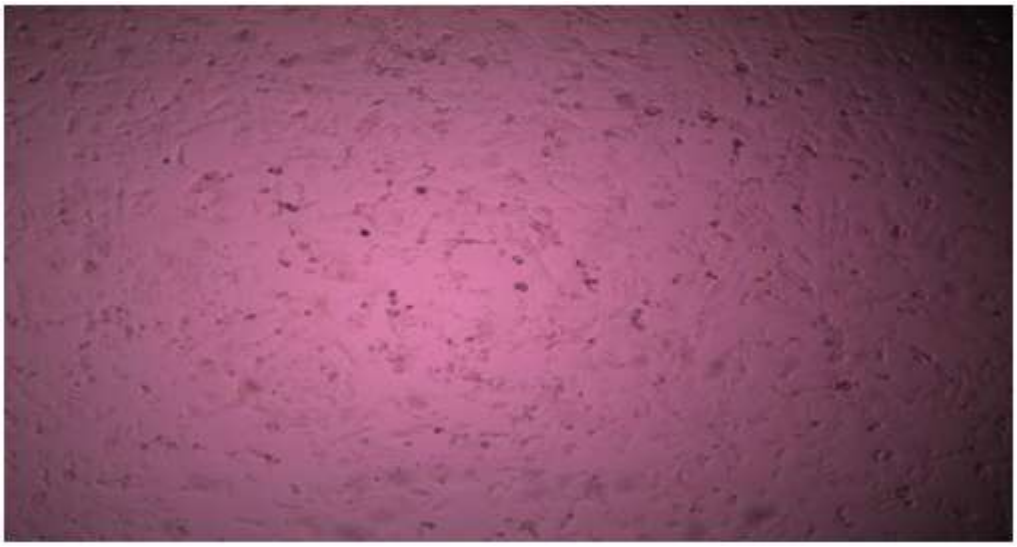
- 100µg/ml

- 200µg/ml

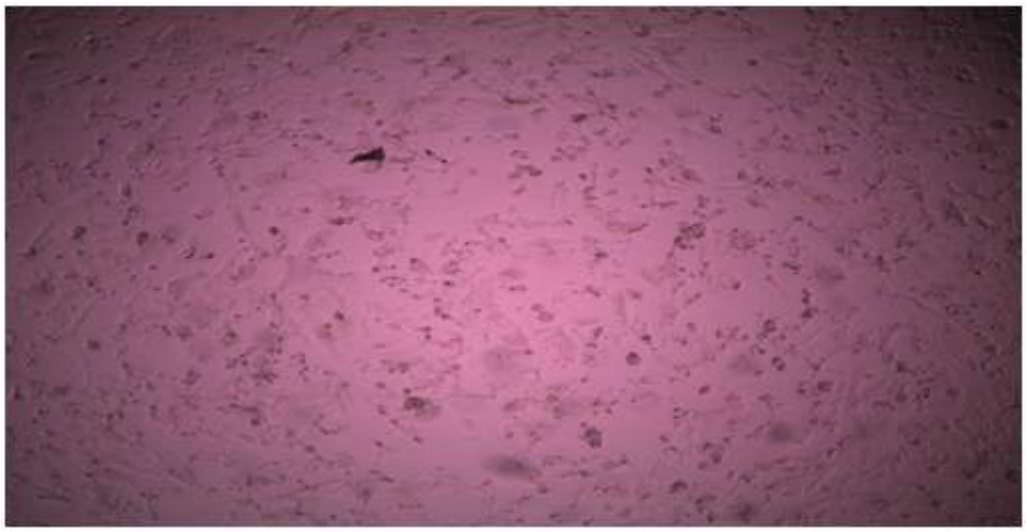
- 300µg/ml



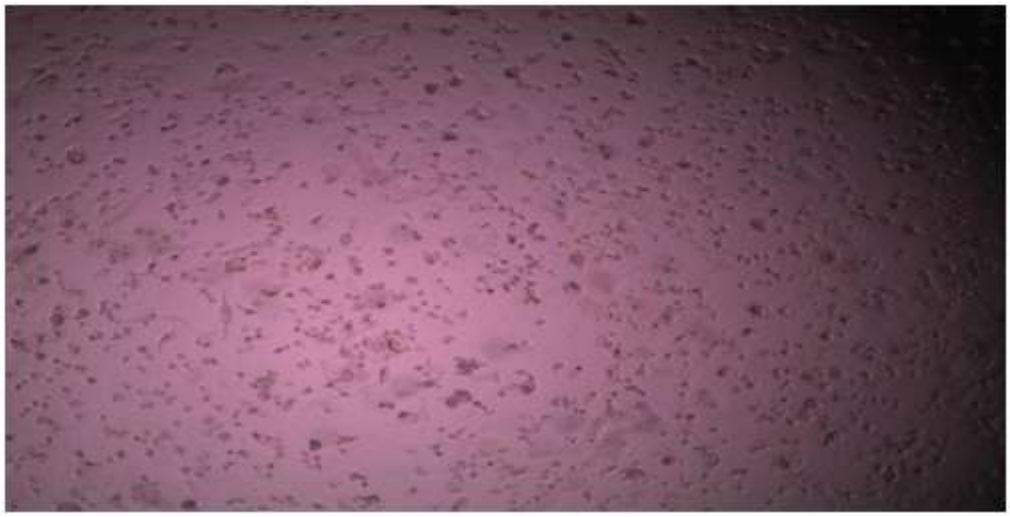
- 400µg/ml



- 500µg/ml



- 600µg/ml



Each sample has four replicates, the mean was calculated and the blank value (0.0477) subtracted then divided by the mean of negative control to calculate the viability %.

The half maximal inhibitory concentration (IC50) = 318.5 µg/ml

5. Comment

- The low doses have higher viability % than the negative control due to hormetic effect.

- Images of cells after 24hour of treatment taken by 40X magnification power with negative and positive control was sent by the Email.

6. Acknowledgments

I would like to extend my thanks and appreciation to Dr. Mohamed Nashat for his help in writing the research and his important observations. I also extend my gratitude and appreciation to Dr. Fatima Zahran of the plant to cultivate the cells and embryos for helping me.

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