ORIGINAL RESEARCH PAPER

Immunology

EVALUATION OF ANTI-CANCER POTENTIAL OF PROTEINACIOUS EUPHORBIA TIRUCALLI AND OPUNTIAFICUS-INDICACACTUS EXTRACTS : IN VITRO STUDY

KEY WORDS: Cactus, Euphrbiatirucalli, opuntia, lung cancer, colon cancer, anti-cancer , cell cycle , apoptosis.

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ABSTRACT

Proteinacious materials of ***Euphorbia tirucalli*** and ***Opuntia ficus-indica*** have been utilized widely in society of medicine. This treatise aimed to evaluate the anti-cancer impact of cactus derived extracts on lung squamous cell carcinoma (A549) and colon cell carcinoma (Caco2). Data recorded revealed that a noteworthy inhibitory impact on cells proliferation went with some morphological changes that was cell type and concentration dependent. Likewise, up regulation of both casp-3 and Bax gene went with down regulation of Bcl-2 was identified 24 hrs post cell treatment. Information recorded uncovered that treatment with Euphorbia tirucalli and Opuntia ficus-indica extracts demonstrated clear anticancer potential accompanied with significantly elevated arrested cells during G2-M phase in both cell lines and significant apoptotic cell % induced under the effect of Opuntiaficus-indicaextract ($P<0.05$) than in case of Euphorbia extract treatment

INTRODUCTION

Worldwide cancer is a main public health issue, in 2018 the estimated new cases of colon cancer is 97,220 with its estimated death 50,630 being the third most common in both genders.Whereas, the estimated new cases of lung cancer are 234,030 with its estimated death 154,050 top in both genders **(American cancer society,2018)**. In addition, around50% patients with lung carcinoma do not survive within the first year of diagnosis and death rate about 88.2% within five years **(Zappa, et al.,2016)**. The likelihood of severe side effects from conventional cancer therapies has resulted in an ever-increase demand of using alternative approaches to tumor treatment **(Amin, et al.,2009).**Cactus plant and fruit (Opuntiaficus-indica) and (Euphorbia tirucalli) exhibit antioxidant and anti-inflammatory activities due to their numerous derivatives such as ascorbic acid, betalains, quercetina, quercetin**(Zou, et al,.2005)** alkaloids, indicaxanthin, neobetanin and its great content in polyphenols compounds **(El-Mostafa, et al,.2014)**. These properties help in the prevention of degenerative disorders for instance, gastric diseases, hyper-glycemia, -cholesterolemia and arteriosclerosis and more importantly cancer. In comparison to fruits such as tomatoes, white grapes or grapefruits, the cactus fruit has twice higher antioxidant activity than others **(Yeddes, et al,.2014).** The oil of cactus pear seed has been studied to contain high levels of unsaturated fatty acids with antioxidant and antimicrobial activity, as well as anti-thrombotic, cardio protective, hypolipidemic and anti-arrhythmic effect **(R.Rachdi, et al,.2017).**The flavonoids found in cactus fruits are more efficient antioxidants than vitamins due to their ability to produce stable radicals to prolong the effect of pro-oxidation lipids, proteins and DNA **(Yeddes, et al,.2014)**. Furthermore, extracts of polyphenolic compounds cactus have been shown to induce a hyperpolarization of the plasma membrane and to increase the intracellular pool of calcium in human Jurkat T-cell lines **1-(Aires, et al,.2004).** Experimental researches and epidemiological studies have emphasis the evidence of plant derivatives phytochemical compounds in decreasing cancer threats and the inhibition of tumor development and metastasis **(Thaipong K, et al,.2006).** In the past two decades 50 % of the medication used have been either derived from plants directly or natural products reformed by chemicals. The availability and non-toxic properties of the natural compounds makes the main advantage for treatment of tumor over conventional ones **(Amin, et al.,2009).**Therefore, our present study quest for safe and effective anticancer effect of cactus derivatives against [lung] and [colon] cell lines by examining the anti-proliferative potential, genetic and apoptotic profiles in addition to the cell cycle status.

Materials and Methods

Cell lines

Lung (A549) and colon (CaCo-2) cancer cells were provided from International Center for Advanced Researches (ICTAR-Egypt), cell culture department. Cells were kept up in RPMI 1640 medium (GIBCO - USA) in a humidified climate of 5% CO^2 at 37°C (Jouan - France) and maintained according the protocol of produced.

Cactus Extract Preparation

Cactus **(Euphorbia tirucalli and *Opuntia ficus-indica*)** ingredients arranged in **Helal cactus farm- Zagzig-Egypt,** and suspended in sterile (PBS) phosphate buffer saline (Sigma –Aldrich –USA) as 3.08 mg/ml. The blend was warmed in a water bath at 70°C for 30 mins and sterilized using 0.22 μm syringe filter (Millipore-USA).

Cytotoxicity

Toxicity of cactus derived proteinacious materials was tested against lung and colon cancer cell lines relative to concentration. Test products were 2 fold serially diluted in RPMI-1640 in A549 and Caco-2 cells 96 well pre-cultured plates. Twenty four hrs later the detached cells were washed-out using PBS. Viability of residual live cells was monitored using MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) obtained from (Sigma Aldrich-USA) ; added as 0.05 ml of 0.5 mg/ ml / well for 4 hrs at 37oC . Insoluble developed purple formazan crystals by cleavage of the tetrazolium ring by succinate dehydrogenase acknowledged from the mitochondria were dissolved using DMSO (ICI-UK). Optical densities of dissolved crystal were estimated at 570 nm utilizing ELISA plate reader (ELx-800-Biotek- USA). OD was plotted against concentration, and the IC50 of cactus was dictated by utilizing software of MASTER PLEX 2010.

Real Time PCR

RNA was separated from 24 hrs IC_{50} cactus models treated and untreated lung and colon cancer cell lines utilizing RNeasy smaller than expected Kit (Qiagen - USA) as indicated by producer's directions. Grouping of removed RNA was

assessed utilizing a Beckman double spectrophotometer (USA) **(Apak R, et al,.2008)**. The level of apoptosis related genes; p53 **(F: 5`-TCA GAT CCT AGC GTC GAG CCC-3` and R: 5`-GGG TGT GGA ATC AAC CCA CAG-3`), Bax (F: 5`-ATG GAC GGG TCC GGG GAG CA-3`and R: 5`-CCC AGT TGA AGT TGC CGT CA-3`) and Bcl-2 (F: 5`-GTG AAC TGG GGG AGG ATT GT-3`and R: 5`-GGAGAA ATC AAA CAG AGG CC-3`)** was resolved utilizing continuous PCR. Ten 10 ng of the removed aggregate RNA from each example were utilized for cDNA utilizing High Capacity cDNA Reverse Transcriptase pack (Applied Biosystems-USA). cDNA was accordingly intensified utilizing syber green PCR Master Kit(Fermentas-Lithuania) utilizing Step One instrument (Applied Biosystems-USA), as takes after: 10 min at 95°C for protein actuation took after by 40 cycles of 15 sec at a temperature of 95°C, 20 sec at 55°C and 30 sec at 72°C for the enhancement step. Changes in the expression of each target gene were normalized relative to the mean critical threshold (CT) values of β-actin as house hold gene Expression of Apoptosis linked Genes was performed using Real Time PCR.

Cell Cycle Analysis

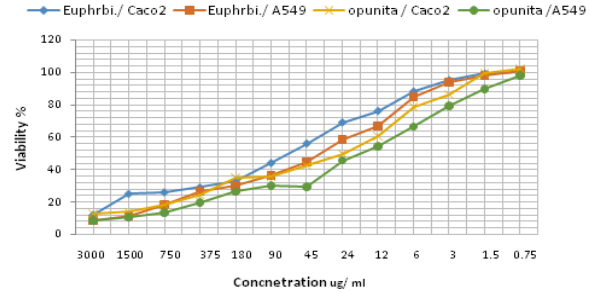
A549 and CaCo-2 cells were treated with the IC_{50} values of cactus extracts and incubated at 37°C for 24 h. For cell cycle investigation the cells were collected and settled delicately with 70% ethanol (in PBS), kept up at 4°C overnight and afterward re-suspended in PBS containing 40 µg/ml PI and 0.1 mg/ml RNase and 0.1% Triton X-100 in the dark. After 30 min at 37°C, the cells were investigated utilizing a stream cytometer (Becton Dickinson, San Jose, CA, USA) furnished with an argon particle laser at 488 nm wavelength.

Statistical Analysis

All tests were done three separate replicates. Results were measurably examined utilizing one-path investigation of fluctuation (ANOVA).The distinction was considered factually noteworthy at p 0.05.

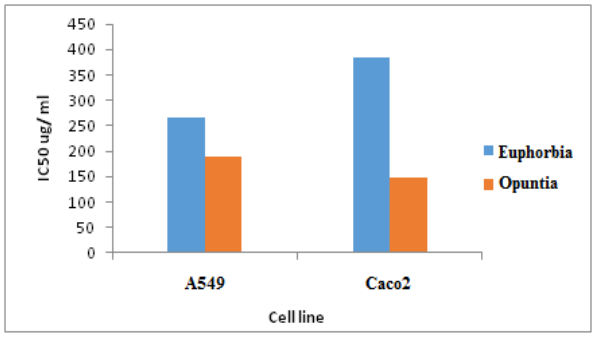
RESULTS

Assessment of cytotoxic impact of cactus extracts to human colon (Caco-2) and lung (A549) cells utilizing MTT test as cell reasonability tests showed a measurement subordinate decrease in cell viability alongside expanding the concentration of Opuntiaficus-indicacactus extract for 24 h post treatment recording IC50 estimates in the order of 148.5 µg/ml and 190.2 µg/ml for CaCo-2 and A549 respectively. Recorded outcomes showed a huge more noteworthy cytotoxic potential to CaCo-2 that A549 cells respectively (P>0.01). Also, it was noticed that Euphorbia was less toxic than Opuntiaficus-indicarecording IC50 values of 348 µg/ml and 265 µg/ml post A549 and Caco-2 cell treatment respectively. It was noticed that Caco-2 was significantly (P<0.05) sensitive to Opuntiaficus-indicathan A549 [Fig.1-2]



[Fig.1] Evaluation of cell viability using MTT stain relative to concentration

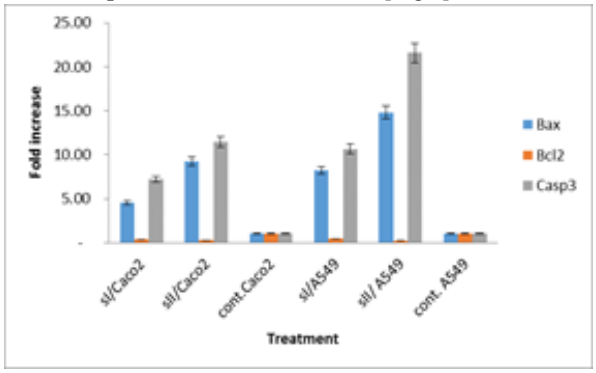
Regarding the cytotoxic effect of cactus extracts on A549 and Caco2 , it was noticed that Opuntia extract was significantly (P<0.05) toxic to both cell lines than Euphorbia Also, the IC50 of Euphorbia extract was significantly reduced in case of A549 treatment than in case of Caco2 (P<0.05) and Opuntia showed a significantly (P<0.05) reduced IC50 value in case of Caco2 than A549 [Fig.2]



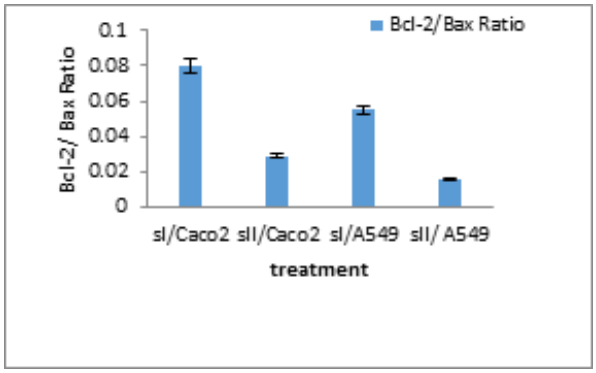
[Fig.2] Evaluation of Inhibitotry concentration (IC50) of cuctas extract using Master plex -2010 software

Real time PCR

Assessment of apoptotic empowering movement was distinguished in both cell lines post treatment, and was joined by a potential significant (P<0.05) up-regulation of star apoptotic genes (Casp3 and Bax). In the mean time, down regulation of the counter anti apoptotic gene (Bcl-2) was recorded [Fig.3]. Concurrently the Bcl2/Bax ration indicted a significant decreased Bcl2/ Bax ratio post treatment of ***Opuntia ficus-indica*** treated A549 cells than ***Euphorbia*** (P<0.05) . in the mean time ***Euphorbia*** showed a significant changed (P<0.05) of Bcl2 / Bax ratio in case of A549 than Caco-2 ***Euphorbia*** extract treated cells [Fig.4]



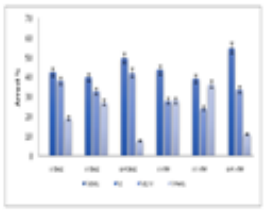
[Fig 3] Evaluation of genetic profile of A549 and Caco2 cactus treated cells using rt-PCR treated cells ; S1 Opuntiaficus-indica and S11 = Euphorbia

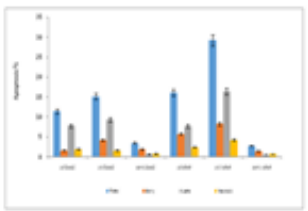


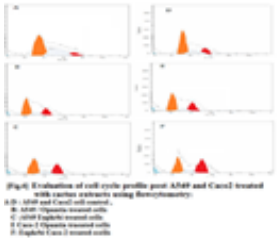
[Fig 4] Evaluation o BCl-2 / Bax gene s ratio in cactus treated A549 and Caco2 treated cells ; S1 Opuntiaficus-indica and S11 = Euphorbi

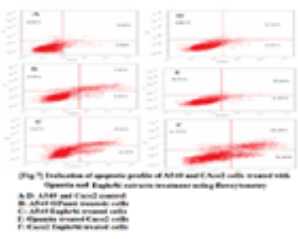
Cell Cycle Analysis

Examination of cell cycle utilizing flowcytometry indicated that cactus extracts indicated a measurably significant (p<0.05) capture of both A549 and Caco-2 cells at G2/M phase and insignificant arrest was detected during the G0-G1 and S phases (P>0.05). The level of arrested distributed cells was lifted by about two fold increase in cactus treated cells contrasted with untreated control cells [Fig.5].









DISCUSSION

Organic resources have dependably been a superior supplement than chemicals in drug making and ailment treatment particularly that chemicals frequently have risky short or long term side effects **(Dai J, et al,.2010)**. It is known that numerous polyphenolic mixes, for example, phenolic acids, flavonoids, anthocyanidins, and tannins, have noteworthy cancer prevention agent and anticancer activities, are rich in the materials of plant. A few examinations have demonstrated the positive relationship of the expanded dietary use of normal cell reinforcements with the diminished coronary illness and tumor mortality, and in addition with longer future. Numerous dietary polyphenolic constituents got from the plants or plant removes showed nearly high cell reinforcement properties than the standard cancer prevention agents, vitamins E or C by in vitro.

Nutrition containing cell reinforcement rich foods grown from the ground fundamentally lessens the danger of numerous malignancy disease proposing that cancer preventing agents could be compelling operators for the restraint of cancer expansion **(Dai J, et al,.2010)**.The anti-cancer capability of cactus was addressed for long time, in any case, observable outcomes were noted post treatment with cactus ingredient(**Wani B.A, et al,.2013).** Lung cancer and colon cancer are the most well-known dangerous cancer worldwide, and are rated with highly in people. The utilization of chemotherapy for the clinical management of lung and colon cancer causes eminent symptoms **(Delbridge AR,2015 and Huang J, et al,.2015)** in this way, new compelling medications to manage lung malignancy are required. Past examinations have demonstrated that polyoxometalates apply their antitumor properties by managing cell intrusion, multiplication and movement in an assortment of malignancies, for example, bosom, kidney, lung, ovary, pancreas and prostate canvcer **(Meng G, et al,.2015)**. Euphorbia and Opuntiaficus-indicamay have not been accounted for to incite apoptosis in human lung and colon malignancy cells. It creates the impression that the capacity of cactus plant to hinder cell expansion may rely on the cell compose; distinctive cell types have diverse prevalent apoptotic flagging pathways. Apoptosis ;programmed cell death, is an essential homeostatic system adjusting cell division and cell passing, while at the same time keeping up the fitting cell number in the body **(YangY, et al,.2014)** In this way, the ID of novel medications that trigger the apoptotic of tumor cells has turned into an appealing methodology in anticancer medication research **(ZouY, et al,.2015)**. The Bcl-2 relatives are basic intracellular players in the apoptotic apparatus **(Shen Y, et al,.2015)**. A few investigations have revealed that Bax, Bcl-2 and caspase-3 are key atoms that reason apoptosis in lung and colon diseased cells **(Zhivotovsky B,2003)**. Mix treatment with euphorbia and Opuntiaficus-indicaupgrades apoptosis by expanding the proportion of Bax/Bcl-2 in A549 and Caco-2 cells **(Nair R, et al,.2014)**. Formononetin-prompted apoptosis was joined by up-regulation of Bax, Casp3 and down regulation of Bcl-2, with the ensuing brokenness of caspase-3 in A549 cells (**Hartl M and Bister K,2013)** euphorbia and Opuntiaficus-indicacactus extracts expanded caspase3 and Bax protein levels, and diminished the Bcl-2 protein level in the lung and colon cancer cell line A549 and Caco-2 **(Zhang Y and Chen C,2014).**

CONCLUSION:

From the presented data it can be concluded that Cactus derived proteinacious extracts showed to have a promising medicinal important derivatives. Cactus extracts showed anti-proliferative potential relative to cell type and Cactus type. Also, the anticancer potential was proved through the expression of apoptotic genes and cell cycle arrest / DNA accumulation indicating cell division arrest and the total apoptotic % of treated cells compared with the profile of cell control

Recommendations

At long last, it can be prescribed that the utilization of cactus extracts can be utilized as a part of various formulae with various transporting frameworks to be focused on. Likewise, fractionation of cactus derivatives to consider the more potentially effective fractions to be evaluated considering different application; anti- analgesic, anti-inflammatory and anticancer as well. Finally in-vivo application must be considered.

REFERENCES:

1. Aires, V.; Adote, S.; Hichami, A.; Moutairou, K.; Boustani, E.S.E.; Khan, N.A. Modulation of intracellular calcium concentrations and T cell activation by prickly pear polyphenols. Mol. Cell. Biochem. 2004, 260, 103–110. [Google Scholar]
2. Apak R., Güçlü K., Özyürek M., Celik S.E. Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay. Microchim. Acta. 2008;160:413–419. doi: 10.1007/s00604-007-0777-0.
3. American cancer society 2018 facts and figures]
4. Amin, A., Gali-Muhtasib, H., Ocker, M., & Schneider-Stock, R. (2009). Overview of Major Classes of Plant-Derived Anticancer Drugs. International Journal of Biomedical Science :IJBS, 5(1), 1–11.]
5. Dai J., Mumper R.J. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Molecules. 2010;15:7313– 7352. doi: 10.3390/molecules15107313.
6. Delbridge AR, Strasser A. The BCL-2 protein family, BH3-mimetics
7. El-Mostafa, K., El Kharrassi, Y., Badreddine, A., Andreoletti, P., Vamecq, J., El Kebbaj, M., ...&Cherkaoui-Malki, M. (2014). Nopalcactus (Opuntiaficus-indica) as a source of bioactive compounds for anticancermedications, so as to clarify the neurotic changes recognized nutrition, health and disease. Molecules.
8. Hartl M, Bister K. Analyzing Mycincelltransformationand
9. evolution. Methods Mol Biol. 2013;1012:21–49. doi:10.1007/978-1-62703-429-6_3
10. Huang J, Sun C, Wang S, He Q, LiD. microRNA miR-10b inhibition reduces cell proliferation and promotes apoptosis in non-small cell lung cancer (NSCLC) cells. MolBiosyst. 2015;11:2051–2059. doi: 10.1039/C4MB00752B.
11. Lin M.-W., Lin A.-S., Wu D.-C., Wang S.S.W., Chang F.-R., Wu Y.-C., Huang Y.-B.Euphol from Euphorbia tirucalli selectively inhibits human gastric cancer cell growth.
12. Meng G, Wang W, Chai K, Yang S, Li F, Jiang K. Combination treatment with triptolide and hydroxycamptothecin synergistically enhances apoptosis in A549 lung adenocarcinoma cells through PP2A-regulated ERK, p38 MAPKs and Akt signaling pathways. Int J Oncol. 2015;46:1007–1017
13. Nair R, Roden DL, Teo WS, McFarland A, Junankar S, Ye S, Nguyen A, Yang J, Nikolic I, Hui M, et al,.2014. c-Myc and Her2 cooperate to drive a stem-like phenotype with poor prognosis in breast cancer. Oncogene. 2014;33:3992–4002. doi: 10.1038/onc.2013.368.
14. RaouenRachdi, FeydaSrarfi, and Najet Slim Shimi, "Cactus
15. Opuntiaficus-indicaas natural flocculant for urban wastewater treatment," Water
16. Science and Technology, vol. 76, no. 7, pp. 1875–1883, 2017]
17. Shen Y, Meng L, Sun H, Zhu Y, Liu H. Cochinchinamomordica seed suppresses proliferationand metastasis in human lung cancer cells by regulating multiple molecular targets.
18. Thaipong K., Boonprakob U., Crosby K., Cisneros-Zevallos L., Hawkins Byrne D. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J. Food Composit. Anal. 2006;19:669–675. doi: 10.1016/j.jfca.2006.01.003.
19. Wani B.A., Ramamoorthy D., Rather M.A., Arumugam N., Qazi A.K., Majeed R., Hamid A., Ganie S.A., Ganai B.A., Anand R., et al. Induction of apoptosis in human pancreatic MiaPaCa-2 cells through the loss of mitochondrial membrane potential (ΔΨm) by Gentianakurroo root extract and LC-ESI-MS analysis of its principal constituents. Phytomedicine. 2013;20:723–733. doi: 10.1016/j.phymed.2013.01.011.
20. Xu X, Wen H, Hu Y, Yu H, Zhang Y, Chen C, Pan X. STAT1-caspase 3 pathway in the apoptotic process associated with steroid-induced necrosis of the

femoral head. J MolHistol. 20149571-6.
21. Yang Y, Zhao Y, Ai X, Cheng B, Lu S. Formononetin suppresses the proliferation of human non-small cell lung cancer through induction of cell cycle arrest and apoptosis. Int J ClinExpPathol. 2014;7:8453–8461
22. Yeddes, N., Chérif, J. K., Guyot, S., Sotin, H., &Ayadi, M. T. (2013). Comparative Study of Antioxidant Power, Polyphenols, Flavonoids and Betacyanins of the Peel and Pulp of Three Tunisian Opuntiaficus-indicaForms. Antioxidants, 2(2), 37–51.
23. Zappa, C., &Mousa, S. A. (2016). Non-small cell lung cancer: current treatment and future advances. Translational Lung Cancer Research
24. Zhivotovsky B. Caspases: The enzymes of death. Essays
25. Biochem. 2003;39:25–40. doi: 10.1042/bse0390025.
26. Zou Y, Qin X, Xiong H, Zhu F, Chen T, Wu H. Apoptosis of human non-small-cell lung cancer A549 cells triggered by evodiamineT h r o u g h M T D H - dependentSignaling pathway. Tumor Biol. 2015;36:5187–5193. doi:
27. 10.1007/s13277-015-3174-z.
28. Zou, D. M., Brewer, M., Garcia, F., Feugang, J. M., Wang, J., Zang, R., ...&Zou, C. (2005). Cactus pear: a natural product in cancer chemoprevention. Nutrition journal