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Research Article

Therapeutic Kit Identify the Type and Dosage of the Drug for Chemotherapy

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ABSTRACT

Purpose: Broader drugs are used to treat cancer by mechanisms such as cell cycle and metastasis inhibition, interleaved signaling, and so on. The phenolic compounds of olivine, olivoropin, have multiple drug properties, including anti-inflammatory anti-inflammatory, anti-atherogenic, anti- cancer and anti-microbial properties, which inhibit the onset of cancer progression. This important function by inhibiting the oxidative stress infused into DNA has anti-cyclic effects.

Methods: After purification of the oleoropine, we cultured the MCF7, PC3, and LNCAP cancer cells in a RPMI medium. For culture of treated cells, Cyclophosphamide, Cell cept, Cyclosporine, Carboplatin, Syclophosphamide, Gemcitabine, Carboplatin and Oleoropine were excreted in 4 different doses in 3 replicates for 24 hours. We applied a 37-cab vacuum cleaner. After 24 hours treatment, the effect, survival and cytotoxicity of the cells were measured by MTT method. According to the obtained results, the mean dose of oleoropine inhibition was 97.5% in the PC3 cell line, 99% in the LNcap cell line and 97.7% in the MCF7 cell line, which is more efficient than other drugs.

Results: The oleoropine inhibits the proliferation of cancer cells and induces apoptosis, also by blocking the G1 to S phase in the front of the cell division, which is approximately the same effect of the chemotherapy drugs used with much lesser harm and a strong antioxidant is named Is. In comparison, the amount of oleoropine inhibition was significantly higher in these treated cell lines than in cyclophosphamide and carboplatin.

Conclusion: The most important advantages of this invention is the reduction of chemotherapy prescription error; choosing the appropriate and effective drug; -Prescribing appropriate drugs for the next stages (continued treatment) - Avoiding experimentation and trial and error -Paying attention to the clinical results beside the laboratory results.

Keywords: Diagnostic and therapeutic kit; Oleoropine; Chemotherapy drugs; Cancer

ABBREVIATIONS:

ALT: Alanine Aminotransferase; AST: Aspartate amino-Transferase; ALP: Alkaline Phosphatase

BACKGROUND

Cancer is known today as a cause of mortality in advanced and underdeveloped countries. To combat cancer, medical science has relied on toxic compounds (Blagosklonny, 2005). Many treatments do not differentiate between healthy cells and cancer cells, leading to unintended toxicity and side effects. In recent years, many efforts have focused on the identification and testing of antiangiogenic compounds such as cancer treatment. In this regard, broad-spectrum drugs are discovered and used with mechanisms such as cell cycle inhibition, inhibition of metastasis, inhibition of the formation of enzymes, inhibition of intercellular signaling, and ... in order to control the treatment. Several epidemiological studies have indicated that there is a small prevalence of common cancers such as prostate and breast and ... in the Mitterane region, which, due to the genetics of people, has the lowest saturated fat content along with food habits, which is one of the most effective compounds. Being in the Mediterranean diet is olive [1]. Many studies suggest that the olive as an unsaturated lipid is known to have a positive effect on

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cancer. It is also part of the Mediterranean diet. The phenolic compounds of olivorin have several medicinal properties including anti-inflammatory anti-oxidant, anti-atherogenic anti-cancer, anti-ischemic, fat-reducing, antimicrobial and anti-viral properties [2]. In studies of laboratory animals, the oleoropine properties of cancer have shown that the work of phenolic compounds of olive oil inhibits the stages of cancer progression, which accomplish this important function by controlling the oxidative stress involved in the DNA of antimicrobial, pro-apoptotic, and ... effects [3]. According to studies by Kolahdozan et al. [4], on cancer epidemiology in Iran, there are five common cancers that include prostate cancer and ... worldwide. In the present study, in order to compare the anti-cancer effect of drugs Valerupine chemotherapy has been used in prostate and breast cancer from LNCAP cell lines, MCF7 and PC3. The Lncap cell line is the metastatic activity of prostate adenocarcinomas, which grows readily in a semi-solid medium under laboratory conditions. This cancerous tuberculosis is highly resistant to human INF gamma and human fibroblasts, and develops atopic inflammatory mice at the site of tumor injection, and doubles the amount of 86 hours to produce cell and tumor acid phosphatase. *In vitro* 5, α-dehydrogenase stimulates cell growth and induces acid phosphatase production. In the vital environment of the body, the average growth and development of the tumor and its onset time vary significantly, however, regardless of the individual's gender, the rate of development of the tumor is related to the serum level of androgen [5]. The TRAMP-c3 tuberculosis line is a cancer of adenocarcinoma of the prostate that is common in young people. TRAMP1.2.3 Cytokine E Cadherin in the androgens receptor identified by immunohistochemical studies suggests no mutation in the P53, cell line (TRAM) (transgenic prostate cancer of the mouse). The basis for making these multi-cellular environments is the different deformities of the cell [5]. The MCF-7 cell line derived from metastatic breast cancer is an acronym for the term "lung cancer", which has been widely studied. Many studies have already been conducted on the MCF7 cell line for cancer screening, which is due to its stability and similarity to many aspects of breast cancer in the clinical environment, especially in women or men with prostate cancer [6-40]. In order to treat this malignancy, broad-based chemotherapy drugs are used, and two of these drugs were studied in this study. Cyclophosphamide and carboplatin were used, which are widely used in chemotherapy and even before and after. Cyclophosphamide prevents cell division through cross-linking of DNA strands and acts as an anti-neoplasmic agent. In addition, the drug has significant immunosuppressive activity. The drug is absorbed through the digestive system and spreads throughout the body, but it is mainly excreted in the urine in saliva, sweat, articular fluid, CSF, a small amount of the drug and metabolized in the liver to a metabolite.

MATERIALS AND METHODS

Extraction of Oleoropine

Oleoropin consists of hydroxytyrosol (3,4′-dihydroxyphenylethanol), phenolic acid, and glucose. The Memeli variety of olive tree leaves was picked randomly from the same tree grown in Zanjan (Iran). The optimal conditions were obtained as follows: ethanol concentration 75% (v/v), extraction temperature 50°C, ultrasonic power 600 W, extraction time 3 min, liquid to solid ratio 30:1 (ml/g), and extraction pressure 25 kPa. Under these optimal conditions, the oleoropin extraction was 7.08 ± 0.05%. A total yield of oleoropine of 7.67 ± 0.02% was obtained after three extractions using URPE. The extraction efficiency reached 92.3% after one extraction.oleoropine was extracted from dried leaves of olive with 70% alcohol (100 ml) for two weeks. The extracted solution (10% w/v) was refined using a 22 μm filter. The results indicated that URPE was an extremely useful and important extraction method for natural products. The developed method was tested on two herbal formulations. The oleoropine was successfully identified using HPLC to characterize elution time in comparison to an authentic standard. HPLC was then used in purification and fractions were lyophilized. Isolated oleoropine was greater than 94% in comparison to the authentic standard.

PBS buffer

This buffer, which acts as an extracellular medium, was used to wash cells. PBS buffer was autoclaved at 120°C for 45 minutes (Table 1).

Table 1: Buffer used to wash cells.

Compounds	Values in volume 1000 cc
NaCl	Mm791/39
KCl	Mm2/6
Na2HPO1-4H2	Mm8/66
KH2PO4	Mm7/41

Cell culture Cancer Cells MCF7, PC3, LNCAP were purchased from the Tehran Pasteur Institute. The medium used for cell culture is RPMI. The cells were cultured after being frozen in a flask containing RPMI, FBS10%, and streptomycin and penicillin antibiotics in a 90% humidity incubator and 37°C in presence of 5% CO_2 for 24 hours. In order to treat the cultured cells, chemotherapy drugs and oleoropine extract were applied on a culture medium in 3 replicates for 24 hours at 37°C in a 37-cabinet. After applying the 24-hour treatment, the effect,

survival and cytotoxicity of the cells were measured using MTT method.

DMEM culture medium

Table 2: Compounds of DMEM medium.

Compounds	Values needed to make 200 ml of DMEM
DMEM powder	7/2 gr
NaHCO9	368/0 gr
Pen/strep	2 ml penicillin/ml,10 mg streptomycin/ml10000

The DMEM powder and the $NaHCO_3$ powder were first weighed with the scales as shown in Table 2 and poured into a sterile gradient cylinder and the distilled water was added to 150 cc and completely dissolved with a magnet and pH adjusted to 7.1. Then the solution was brought to 200 cc volume and under the hood was added Pen/strep antibiotic and the final solution was filtered/2. The micron was passed under the hood and 10% volumetric FBS was added to it. If the DMEM environment is purchased ready to use, FBS should be added at 10%.

Cell passage

To pass on the cancer cells after preparing the hood and all the equipment and materials required for cell passage, transfer the petri-containing cell from the incubator to the hood and gently discard the cell medium and wash the cells using PBS solution. Dead cells and antitrypsin compounds are isolated from the cell environment. Then pour 600 µL of trypsin slowly and slowly transfer to cells to transfer trypsin to all parts of the cell. then incubated the flask for 3 minutes until trypsin decomposed the adherent proteins by dissolving the cells in the bottom of the flask, then removed the flask from the incubator, and by gently inflating the flask, the cells were separated and To make them unicellular. The cells were then examined under a microscope. After this step, cells were rapidly added to the medium containing 10% FBS to neutralize trypsin and not damage the cells themselves. After adding the medium using the Peptor, several times the cells were pipetted and the flask wall was thoroughly washed until all cells were collected, then centrifuged at 1000 rpm for 5 min and then the cell supernatant was removed. Shed and divided by a ratio of 1/3 to other flasks (or we can freeze 3-4 million with 1/3 flask cells approximately two cryotubes) and the medium containing serum added and cultured. The task is to count cells and transfer the appropriate number or proportion of cells to each flask.

Cell count

After trypsinizing the cells and removing the cells from the flask, neutralize by adding trypsin medium, then thoroughly rinsing the petri bottom with the petri medium to collect all the cells, then transferring the entire cell containing medium to Falcon 15 and incubating for a period of time. 5 minutes at rpm, precipitated 1000 cells, then removed the supernatant, added fresh cells to the cells, and completely separated the cells using a pipette. To count the cells, first pipette them thoroughly to make the cells completely uniform, then pour 30 microliters of cell-containing medium and 30 microliters of trypan blue into a vial and thoroughly mix with the sampler to homogenize and remove 10 microliters from the solution for viewing below. The microscope (magnification 10) was transferred to a homocytometer slide. This slide consists of 4 squares of 16 cells that were averaged to count cells in 4 squares. Multiply the mean number by the inverse of the dilution factor of the cell used and then multiply the number by 104 to obtain the cell value in cc (Figure 1).

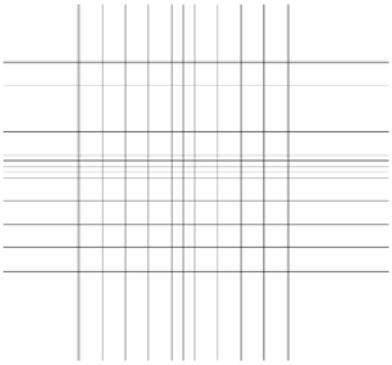

Figure 1: Homocytometer slice for cell counting.

MTT method

A method for determining the number of living cells in many different biological compounds. The MTT method is a colorimetric method to reduce the cell's vital capacity and its cytotoxic effects. The basis of this method is based on a change in the thickness of the insulin which is insoluble in water and is converted into crystalline form. The tetrazolium oxide is degraded by the suction disodium mitochondrial enzyme and produces an insoluble insoluble moiety in order to readily absorb the DMSO solution to form a color solution. This solution is obtained at a concentration of 5 mg/ml (W/V) by dissolving in PBS and then sterilized with a 0.2 micrometer filter and stored at 4°C. In this method, cells from 2 to 11 cells of 96 cell culture cells were grown and incubated for 24 hours.

MTT cell life assay

In MTT method, which is soluble in water and yellow in color, was converted to the formazan purple water-soluble formazan by the mitochondrial dehydrogenase of living cells. The amount of purple produced is proportional to the number of living cells (Liu et al. 1997).

1) In a 96-well plate, 8,000 cells per well were cultured for MDA-MB-231 and 20,000 cells for Mcf-7 with 200 µl of complete culture medium (DMEM medium plus 10%).

2) After 24 h, change the cell medium and each well with 200 µl of DMEM medium with 10% FBS with different

concentrations of alkaloid extract (1, 10, 20, 30, 40, 50, 60, 80, 100 g/ml). The plate was then wrapped in foil and incubated for 24 h in 37°C, 5% CO_2, and 95% humidity conditions.

3) After 24 hours, vacuum the medium in each well and reconstitute 180 µl of FBS-free medium with 20 µl of MTT solution (5 mg MTT in 1 ml PBS with pH=7.2) for 60 minutes. Stylish but of course the plate should be completely covered with foil, after 60 minutes transfer the plate to the incubator and leave for 5 hours under these conditions.

4) At this stage, after 5 h of incubation, cells were emptied into the well and added to each well of 200 µl DMSO and incubated for 10 min until the MTT formulation in DMSO was completely dissolved.

5) The sample OD was read at 540 nm.

1) In a 24-well plate, 100,000 cells were seeded per well with 750 µl of complete culture medium (DMEM medium with 10% FBS).

2) After 24 h, change the cell medium and add 200 µl of DMEM medium with 10% FBS plus 30 µg/ml concentration (LD50) of the alkaloid extract for 24 h in incubation under 37°C, CO_2 was placed at 5% and 95% humidity.

3) After 24 hours, vacuum the well inside each well and wash with PBS buffer and 100 µl of solution containing propidium iodide and annexin V bound to FITC (1 ml of Annexin-V-Flous 20 µl and 20 µl). Propidium iodide was added and placed at room temperature for 10-15 minutes.

4) Cells were observed for apoptosis under fluorescence microscopy at 450-500 nm.

The results of the MTT and cell survival were readed by the Eliza reader apparatus were studied at two wavelengths of 490 and 530 nm.

RESULTS AND DISCUSSION

In order to evaluate and compare the anti-cancer effects of the studied drugs and oleoropine on MCF7, LNcap and PC3 cell lines with MTT technique, and their inhibitory comparison based on different doses. Based on a chart plotted after comparing the efficacy of drugs with oleoropine (Figures 2-4).

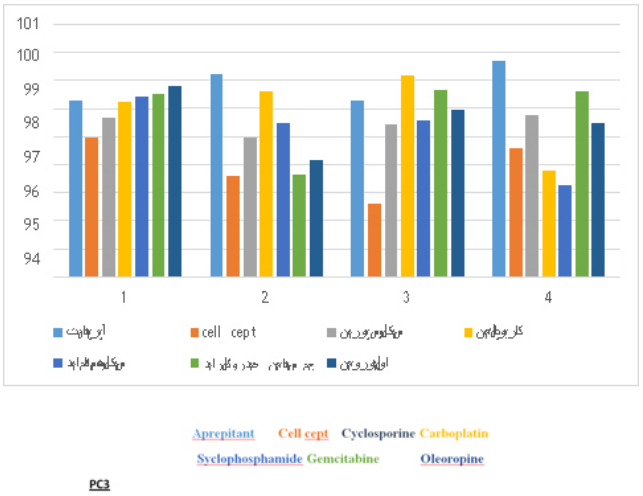

Figure 2: PC3 cell line.

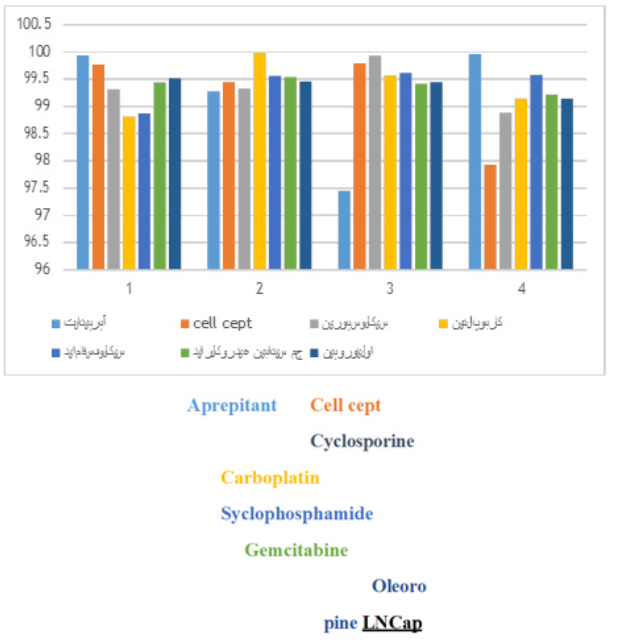

Figure 3: LNcap cell line.

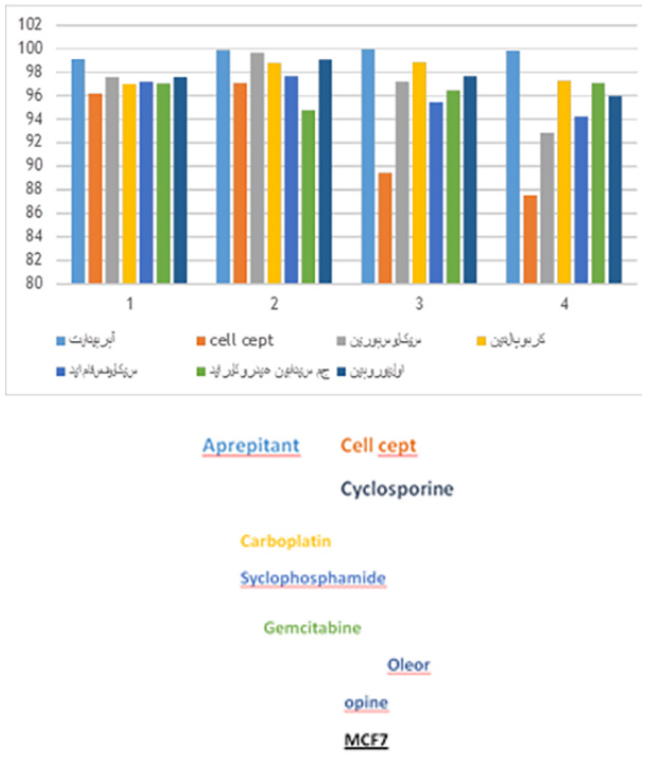

Figure 4: MCF7 cell line.

in PC3 cell line has shown a carboplatin and cyclofosfamide inhibitory effect of over 98% with inhibitory effect, but the amount of preventative action of oleoropine has been most prominent. In the second dose, the tuberculosis showed the greatest effect on carboplatin activity, and then cyclophosphamide exhibited an inhibitory effect of about 98%, and then oleoropine was inhibited by approximately 96%. In the third dose, carboplatin has the most inhibitory effect and oleoropine has a 99% inhibitory effect. For the fourth time, the amount of oleoropine inhibition has remained constant over the previous dose, and it can be stated that, with the exception of the second dose, the amount of inhibition of oleoropine was almost constant and was 98%. In LNCap cell line had an inhibitory effect on the drugs used, and their performance was found to be close to 99% in the first dose of carboplatin and cyclophosphamide and about 97.5% in oloropine. In the second half of the year, the drug was very close to each other. It can be said that carboplatin is the most inhibitor, which is about 100%, and subsequently oleoropine inhibits 99.5%. At 3 doses of cyclophosphamide and carboplatin, 99.5% prevents cell growth and subsequently oleoropine exhibits its function. In the application of the final dose of cyclophosphamide, the most inhibitory growth of the cells is shown, and then oleoropin has a deterrent effect of 99%.

CONCLUSION

A study conducted in this study suggests that there is a positive impact on the safety and low side effects of oleoropine in MCF7, LNCap, and PC3 cell lines, which are widely used in the study of common cancers in the world. In comparison, the inhibitory effect was significantly higher in these cell lines treated with oleoropine than other chemotherapy drugs.

CONFLICT OF INTEREST

Amirhossein Alimohammadian declares that he has no conflict of interest. NeginMaleki declares that he has no conflict of interest. Dr Mohammad zaefizadehBablan declares that he has no conflict of interest. NayyerLatifinavid declares that he has no conflict of interest.

ETHICAL APPROVALS

This article does not contain any studies with human participants or animals performed by any of the authors.

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