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In vitro Cytotoxicity Activity of *Punica granatum* Rind Extract Against RD Cell Line

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Abstract:

Cancer is the major public difficulty and one of the top causes of death in the prosperous countries. Conventional plants are precious source of novel cytotoxic agents and are still in performance playing a better role in health concern. The study was intended to estimation of the anti cancer activity of aqueous extract of punica granatium on Rhabdomyo sarcoma (RD) cell line. The rind punica granatum aqueous extract was tested for its inhibitory effect in 96 micro plate formats against RD cell line. The cytotoxicity of Punica granatum on RD cell was evaluated. Dose response curve constructed the ranges between 100 µg/ml to 10 µg/ml for Punica granatum, while the inhibition values was on RD cell ranges were between 59-66% at different exposure periods.

Introduction:

Cancer is the major public difficulty and one of the top causes of death in the prosperous countries. Conventional plants are valuable source of novel cytotoxic agents and still in performance playing a better role in health concern. Throughout history and crosswise the world, the plant kingdom has provided a diversity of medicines for cancer treatment. In current period, plants have been a source of analgesics, anti inflammatories, antiasthematics, antiarrhythmic agents, antihypertensives, antimicrobial agents known to be frequent (1).

Pomegranate (Punica granatum L) belongs to family Punicaceae, is an edible fruit cultivated in many countries and consumed around the world. It is well documented that the edible part of pomegranate is rich in anthocyanins and hydrolysable tannins, a group of polyphenolic compounds that possess antioxidant and anti inflammatory activities (2). Recently, pomegranate juice was found to revert the potent down regulation of the expression of endothelial nitric oxide synthase induced by oxidized low density lipoprotein in human coronary endothelial cells (3). Dietary supplementation of polyphenolic rich pomegranate extract to atherosclerotic mice was shown to inhibit significantly the development of atherosclerotic lesions (4). It has also been shown that pomegranate extract can suppress NF-κB activation in vascular endothelial cells (5). Studies have also shown that of the popular antioxidant-containing beverages such as green tea, presumably due to the presence of hydrolysable tannins in the rind, along with anthocyanins and ellagic acid derivatives (6). In a comparative analysis, anthocyanins from pomegranate fruit were also shown to possess higher anti oxidant activity than vitamin–E (α -tocopherol), ascorbic acid and β – carotene (7). Pomegranate extract has also been shown to protect from NSAID and ethanol–induced gastric ulceration (8).

In view of this, the focus of the present study was to evaluate, the effects of the aqueous extract of Punica granatum peel on the growth of Rhabdomyo sarcoma (RD) cell line.

Material and Methods:

Preparation of pomegranate extraction:

An aqueous extraction of pomegranate was prepared using fresh seeds 100 g which soaked in 250 ml boiling distilled water for about 6 hours on a hot plate and homogenized. The mixture was then filtered through a piece of soft cloth and filter paper to remove all the

residual materials. Then, it dried at 45˚C by using hot air oven, with circulatory fan, and kept at 4˚C until the use. For the following experiments, 10g of powdered plant was dissolved into 100ml PBS (as solvent), the suspension then filtered and sterilize by using 0.2 µm sterile millipore filtering system, and the stock solutions have been kept in sterile containers at 4˚C until use.

**** Cell Growth Assay***

In vitro method was used to establish the effect of the aqueous extracts of pomegranate on RD cell lines. Solutions were prepared according to ICCMGR standard method.

In the preparation of any in vitro assay, the frozen cell line was withdrawn and maintained in RPMI-1640 containing 10% bovine calf serum. Where the in vitro cells culture forms a monolayer. These cells were treated with trypsin/ versine mixture in order to pursue subculture process. The percentage of inhibition was calculated according to the following equation (9):

Inhibition % = [(optical density of control wells -optical density of test wells)/optical density of control wells] x 100

Activity against cell lines was determined by the inhibition assay using an ELISA assay. Concisely the cells cultured in the micro titration plate were exposed to a range of plant extract concentrations during the log phase of growth and the effect was determined after recovery time. The following protocol as described in (10) was followed to the extracts of Pomegranate rind:

- After trypsinization, cell suspension seed in a micro titration plates at 50000 cells/ml RPMI-1640 growth medium supplemented with serum 5%, was used for seeding.
- Plates then incubated for 24 hours at 37º C.
- By using maintenance medium, two-fold serial dilution were prepared starting from (100, 75, 50, 25, 10 µg/ml).
- After incubation for 24 hrs, cells were exposed to different extract dilutions. Only 200 µl of each concentration has been added for each well (6-replicates for each tested concentration). 200 µl of maintenance medium was also added to each well of control group. The times of exposure were (24, 48 and 72 hrs). The plates have sealed with self adhesive film and returned to the incubator at 37º C.
- After the end of the exposure period, the medium and the cells decanted off and replaced by 200 µl of 0.01% crystal violet dye. After 20 min. the stain was washed gently with tap water for three times. The plate was left until become dry.

The optical density of each well was measured by using a micro-ELISA reader at 492 nm transmitting wave length.

Statistical Analysis:

The results represent the mean ± SEM and are the average of three values per assay and each assay was repeated three times.

Statistical evaluation of the untreated control cells along with the extract and solvent-treated cells was calculated using Student's t-test. A probability of 0.05 or less was statistically significant.

Results:

In vitro confirmation of Punica granatum toxicity on RD cell line could be determined. Percentage of cell inhibition was obtained by performing crystal violate stain exclusion technique.

The effect of extract of Punica granatum on the growth of RD cell line was examined and dose response curve constructed between the range 10µg/ml and 100 µg/ml for pomegranate, express decreasing number of viable cells with decreasing concentration of extract.

As shown in figure 1 the susceptibility of cells to the extract exposure was characterized during 72 hr period of exposure to the extract. Results indicated that the antiproliferative effect strengthens with decrease in the concentration of extract.

This cell line (RD) shows high sensitivity towards the extract. This explain that the concentration 10µg/ml at 24 hr and 48 hr of exposure period gives (57 and 44) % respectively, while at 72 hr the highest concentration 100µg/ml gives 66%.

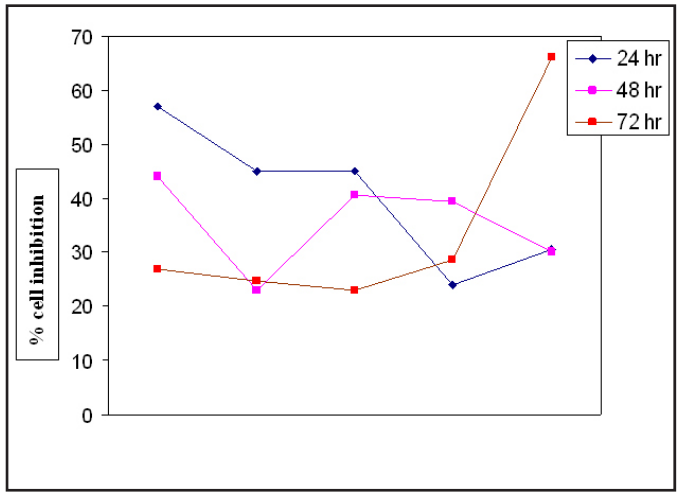

Figure 1: drug response curve of pomegranate extract for RD cell line.

Discussion:

Several phytochemicals have been proposed as potential chemopreventive agents based on animal and laboratory evidence of antitumor effects. Suggested mechanisms of anticancer effects of polyphenols include the inhibition of cancer cell growth by interfering with growth factor receptor signaling and cell progression, promotion of cellular differentiation, modulation of phosphodiesterase/ cyclooxygenase pathways, inhibition of kinases involved in cell signaling, and inhibition of inflammation (11,12,13).

This study has shown that, in vitro pomegranate extracts can inhibit the growth of RD cell lines. It has been found that the pomegranate extract at concentration of 10 µg/ml induced cell inhibition with 24 hr, 59% of cell inhibition, while at concentration 100 µg/ml of the extract induced cell inhibion 66% with 72 hr.

The effects of ellagic acid on cell cycle events and apoptosis were studied in cervical carcinoma, cells (CaSKi), showed that ellagic acid induced G1 arrest with 48 hr, inhibited overall cell growth and induced apoptosis in these cells after 72 hr of treatment (14). Other studies have showed that pomegranate juice and its purified polyphenols are potent antioxidants which may be a mechanism where by they inhibit cancer cell proliferation and induce cancer cell to undergo apoptosis. Although the purified polyphenols showed significant antiproliferative, antiapoptosis and antioxidant effects alone, the superior bioactivity of pomegranate juice suggested multifactorial effects and chemical synergy of the action of multiple compounds as compared with single purified active ingredients (15).

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[illegible] RD [illegible] *Punica granatum*

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[illegible] *punica granatium* [illegible] 96 [illegible] *Punica granatum* [illegible] (RD) [illegible] Rhabdomyo [illegible] /µg 10 [illegible] /µg 100 [illegible] RD [illegible] *Punica granatum* [illegible] RD [illegible] *Punica granatum*.

[illegible] RD [illegible] *Punica granatum* [illegible] 59-66 % [illegible].