



ANTIPROLIFERATIVE ACTIVITY OF THE PHYTOCHEMICAL DIVICINE FROM *VICIA FABA L.*

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ABSTRACT

The objective of the present study was to investigate the effect of divicine obtained from fava bean to work as an anti cancer against 60 human cell lines. A498 Renal cancer cell line gave the best result as growth percent was 23.85. Leukemia, breast and prostate cancer cell lines were not sensitive to Divicine. The sensitivity of the rest of the cell lines varied against divicine. It can be concluded that Divicine has an Antiproliferative activity.

KEY WORDS: *Vicia faba*, Antiproliferative, cell lines.

INTRODUCTION

Favism is a life threatening hemolytic anemias that can result from the ingestion of fava beans (*vicia faba*) by individuals with low activity variants of erythrocytic glucose 6- phosphate dehydrogenase (G6PD). Fava beans pyrimidines, divicine and isouramil, have been implicated in the onset of favism primarily on the basis of their ability to deplete reduced glutathion (GSH) in suspensions of human G6PD-deficient red cells (Mager *et al.*, 1965) (Beutler, 1978). Divicine and isouramil, the aglycons of vicine and convicine respectively are probably related chemically to those nucleic acid bases which are substituted pyrimidines. Increased levels of glutathione have been linked with resistance of cytotoxic drugs. (Mickisch *et al.*, 1990). One approach for some anticancer drugs such as 6- mercaptopurine is depending on the changes in glutathione content and the activities of glutathione-s-transferase, glutathione peroxidase and glutathione reductase which have been detected in tumors (Dillio *et al.*, 1995 & Blair *et al.*, 1997). The aim of this study was to investigate whether vicine has cytotoxic effect against 60 different human cell lines depending on the background of link between favism and vicine in absence of G6PD as an oxidant agent.

MATERIALS & METHODS

Vicia faba

Fava bean seeds were collected from the local market in Baghdad city. They were identified at the department of Pharmacognosy and medicinal plants of the college of pharmacy- University of Baghdad. Afterward, seeds were weighted then dried at 40°C for 24 h. Then, the dried seeds were weighted again and ground into powder prior to extraction.

Preparation of *Vicia faba* the extract

Vicine pure crystalline was extracted from mature seeds of fava beans (*Vicia faba*) according to the procedure described by Arbid and Marquardt.

Preparation of Divicine

Divicine pure crystalline sample was obtained by acid hydrolysis from Vicine. By refluxing 1.0g in 5% aqueous acetic acid (10 ml) for 5 minutes, the solid dissolving rapidly, followed by the precipitation of divicine. After cooling 30 minutes at 0°C the divicine (Yield 0.42g, 68%) was filtered, washed with water and dried. (Marquard *et al.*, 1983 & Jamalian *et al.*, 1976).

Antiproliferative activity of divicine

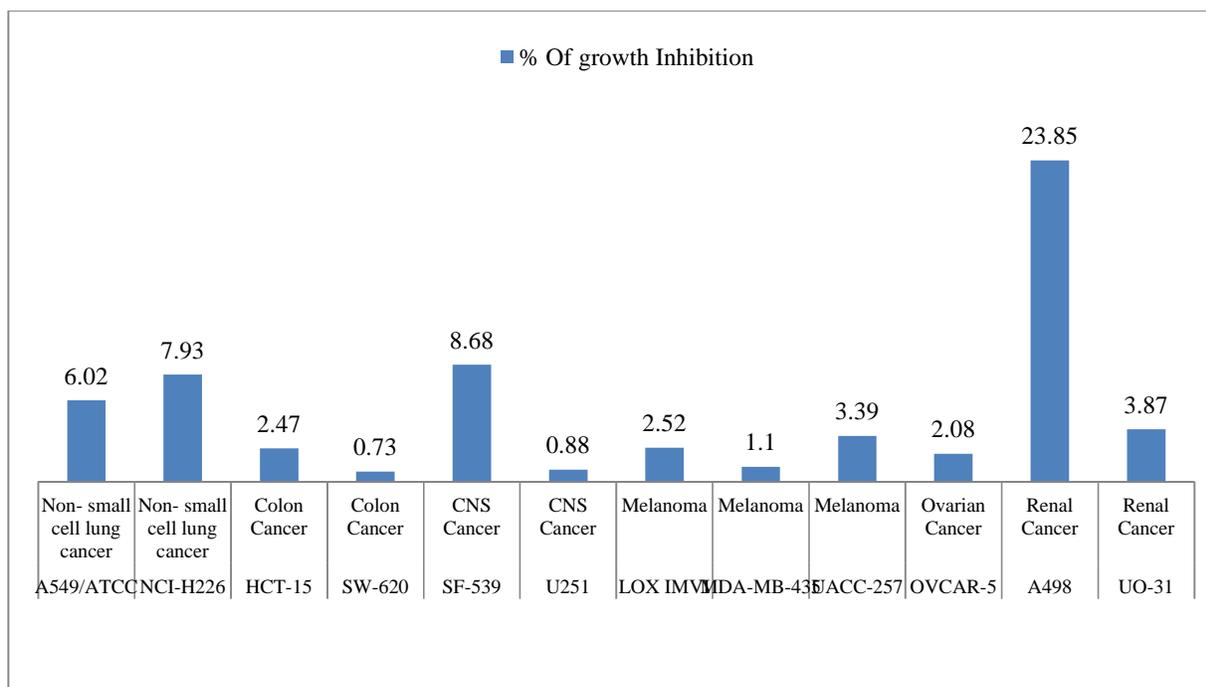
The phytochemical divicine which extracted from the seeds of fava beans was submitted to the National Cancer Institute (NCI) to investigate its anticancer activity in a dose of (10^{-5}) against 60 human tumor cell lines derived from Leukemia (CCRF-CEM, HL-60(TB), K-562, MOLT-4, RPMI-8226 and SR), Non- small cell lung cancer (A549/ATCC, EKVX, HOP-62, HOP-92, NCI-H226, NCI-H23, NCI-H322M, NCI-H460 and NCI-H522). Colon Cancer (COLO 205, HCC-2998, HCT-116, HCT-15, HT29, KM12 and SW-620), CNS Cancer (SF-268, SF-295, SF-539, SNB-19, SNB-75 and U251), Melanoma (LOX IMVI, MALME-3M, M14, MDA-MB-435, SK-MEL-2, SK-MEL-28, SK-MEL-5, UACC-257 and UACC-62), Ovarian Cancer (IGROV1, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, NCI/ADR-RES, SK-OV-3), Renal Cancer (786-0, A498, ACHN, CAKI-1, RXF 393, SN12C, UO-31), Prostate Cancer (PC-3 and DU-145) and finally Breast cancer (MCF7, MDA-MB-231/ATCC, BT-549, T-47D and MDA-MB-468). These cell lines were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mL- glutamine cells (5000-40000 cells/ well) were plated into 96 well micro titer plates and allowed to grow for 24 hr at 37°C in a humidified atmosphere supplemented with 5% CO₂ divicine dissolved in dimethyl sulfoxide, then added to the cells at a final concentration of 0.01 µM. Afterwards cells were incubated for another 48 hrs. At the end of the incubation period cells were fixed in situ and stained with the protein – staining dye sulforehodamine B. After solubilization of the dye, the optical density of the stain was

measured at 515nm. Finally, one dose response parameters were calculated for the drug growth inhibition and lethality. The protocol used by the NCI for the anticancer assessment has been described in detail. (Monks *et al.*, 1991).

RESULTS

In the vitro cytotoxicity parameters (growth percent and lethality) were obtained for divicine in NCI's anticancer screening program are listed in (Figure 1). The one dose data is reported as a mean graph of the percent growth of treated cells. The number reported for this study was growth relative to the no-drug control and relative to the time 0 number of cells. This allows detection of both growth inhibition (Values between 0-100) and lethality

(values less than 0). The percentage of growth inhibition is listed in (Figure 1 and 2). Treatment with divicine yielded a highest response in percent of growth inhibition for A498 cell line was 23.85 in renal cancer. Leukemia, breast and prostate cancer cell lines were not sensitive to Divicine. For non - small cell lung cancer only A549/ATCC and NCI-H226 cell lines gave us a response (6.02, 7.93 respectively). While in colon cancer only HCT-15 and SW-620 cell lines gave us a very weak response (2.47 and 0.73 respectively). In the other hand SF-539 and U251 cell lines for CNS cancer gave us (8.68 and 0.88 respectively). Moreover, three melanoma cell lines gave us a good response LOX IMVI (2.52), MDA-MB-435 (1.1) and UACC-257 (3.39). Finally, OVCAR-5 cell lines for ovarian cancer gave us (2.08).



Graph 1: The percent of growth inhibition

DISCUSSION

At this study Divicine has showed a great Antiproliferative effect against numeral cell lines. Two cell lines of non-small cell lung cancer (A549/ATCC and NCI-H226) are sensitive to divicine (Figure-1). Divicine deplete reduced glutathione to established favism and this may be attributed to anticancer activity of divicine. It has been suggested that distribution of GSH status can affect the extra cellular redox state and initiate apoptosis (Coffee *et al.*, 2000; Young *et al.*, 2000a; Davis *et al.*, 2001). As well, CNS cancer cell lines are also sensitive to divicine but less for lung cell cancer. Melanoma and ovarian cancer cells appear to be sensitive towards divicine. Moreover, the sensitivity of ovarian cancer towards divicine has an immense important since there is a frantic need for chemo effective drugs against ovarian cancer (Picard *et al.*, 2001). Furthermore, renal cancer cell lines A408 and Uo-31 were most sensitive to divicine than other cell lines. At this point, the mechanism responsible for the sensitivity for

melanoma and renal cancer cell lines are unclear. However, it has previously been shown that incubation of isolated rat renal cortical cells with 6-MP at 0.5 and 1mM concentration for 2 Hr. Significantly increased cell death as measured by the release of lactate dehydrogenase compared with buffer/ only incubation. This increase in lactate dehydrogenase release could be partially blocked by alloprenelol, uninhibitor of xanthine oxidase that catalyses the oxidation of 6 MP to thiouric acid and superoxide unaion (Lash *et al.*, 1997). The activity of 6-MP which is related to GSH intracellular contained may be similar to Divicine activity in favasim depending on the depletion of cellular GSH. Finally, these finding suggest that the toxicity of 6-MP and Divicine could be impart due to generation of reactive oxygen specious and oxidative stress. It can be concluded that divicine has an Antiproliferative effect against some human cell lines mainly A498 Renal cancer cells.

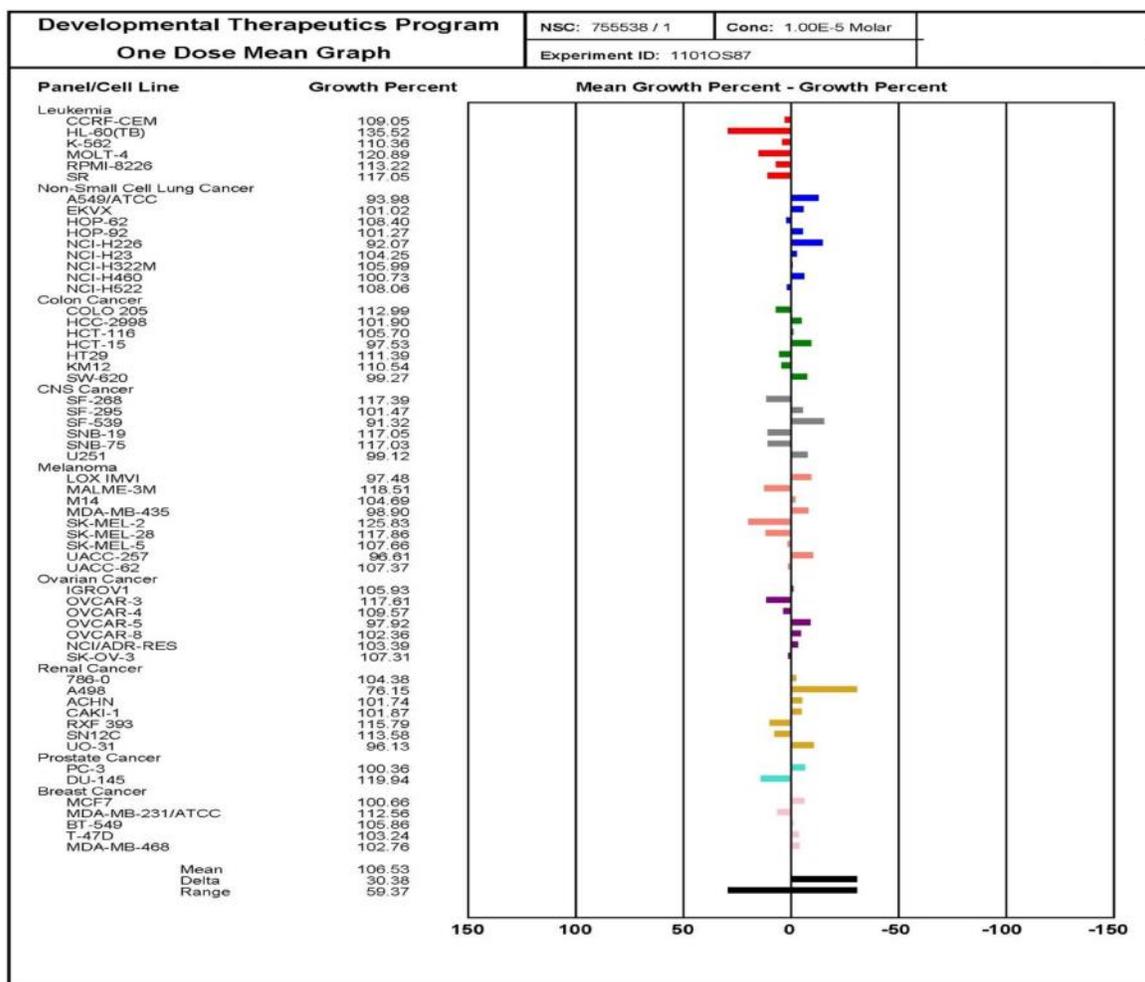


FIGURE 1: NCI's anticancer screening for 60 human cell lines against Divicine

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