

# Cytotoxic effect of imatinib on the normal myeloid and cancer cells of patients with chronic myelogenous leukemia

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## Abstract:

**Background:** Chronic myelogenous leukemia( CML) is a type of leukemia in about 20 % of all types of leukemia. It is a malignant clonal, myeloproliferative disorders of the pluripotent hematopoietic stem cells. Aim of study:The present study was aimed to determine in vitro the cytotoxic effect of imatinib on normal myeloid stem cells and leukemic myeloid cells of patients with chronic myelogenous leukemia.

**Materials and methods:** Imatinib was prepared by serial dilution in concentrations (250, 125, 62.5, 31.25, 15.625,7.8125 µg/ml ), it was added to the cells culture. Using MTT assay.

**Results:** The results show the cytotoxic effect (inhibition rate of growth) of imatinib was in dose and time dependent. There were significant differences between concentrations and time exposure (24,48,72 hr). The highest inhibition rate on the leukemic cells was 89.907% in concentration 250µg/ml and the lowest inhibition was 43.918 % in concentration 7.8125 µg/ml for exposure time 72hr While the highest inhibition rate on the normal myeloid cells was 34.621 % in concentration 250µg/ml and the lowest inhibition was 14.588 % in concentration 7.8125 µg/ml for exposure time 72hr.

**Conclusions:** imatinib has a highly inhibited growth for the leukemic myeloid stem cells and less inhibited for the normal cells. This is a guide for choosing any drug with high cytotoxic effect on the leukemic cells and less cytotoxic effect on the normal cells.

**Keywords:** Pure imatinib, normal and leukemic myeloid cells culture.

## Introduction:

Chronic myelogenous leukemia( CML) is a type of leukemia in about 20 % of all types of leukemia (1). It is a malignant clonal, myeloproliferative disorders of the pluripotent hematopoietic stem cells (2,3,4). Characterized by formation of specific abnormal chromosome such as Philadelphia chromosome in 95 % of patients (5,6). It occurs mostly in adults and rarely in children (7).

Imatinib mesylate (Gleevec or Glivec )R is the first drug in protien kinase inhibitors group (8). In 2001 this drug is approved by the U.S. Food and Drug Administration (FDA) asa selective anticancer drug that develop as a guide to know the specific oncogene (9). It has anticancer effect through

inhibits tumor tyrosine kinase activity such as in CML and gastro-intestinal stromal tumor. It inhibits other tyrosin kinase receptors such as platelet- derived growth factor receptor (PDGFR), stem cell factor as well as c-Kit (10). Imatinib acts by inhibiting tyrosine kinase domain of the BCR – ABL oncoprotein which is commonly expressed in CML (CML result in mutation of the BCR – ABL gene) and prevents phosphorylation of the kinase substrate by ATP (11).

## Materials and Methods:

Fifteen patients with CML (newly diagnosed) and 15 persons with normal hematopoietic stem cells (myeloid cells). Both of normal and leukemic were: 6males and 9 females and their ages ranged between 35-70 years who attended to the National Center of Hematology/ Al – Mustansiriya University and Al – Kadhimyia Teaching Hospital in the period from April 2014 to April 2015 and this work was

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Human bone marrow was obtained from the posterior iliac crest by an aspiration needle under local anesthesia (10 ml xylocaine). (12). Ficoll- opaque was added for the isolation of myeloid cells (13).

Later, the cells were placed into 25 cm falcon after adding 10 ml of RPMI – 1640 (20 % FCS ), this medium was prepared by dissolving 16.35g powder of RPMI – 1640 with HEPES buffer and L – glutamine. Added 2 g of sodium bicarbonate powder, 1ml of ampicillin, 0.5 ml of streptomycin and 200 ml of fetal calf serum (20 % FCS) were added to one liter of medium. Incubated at 37° C .(14).The same procedure was used for normal and leukemic myeloid cells culture and the viable cells count was  $52 \times 10^5$  Cells.

Pure powder of Imatinib (SantaCruz Biotechnology, USA. SC-267106, Lot. RK1813) was prepared in the concentrations (250, 125, 62.5, 31.25, 15.625, 7.8125  $\mu\text{g/ml}$ ). This drug was dissolved in the dimethylsulfoxide (DMSO) (15).

Added 200 $\mu\text{l}$  of drug with concentrations 250, 125, 62.5, 31.25, 15.625, 7.8125  $\mu\text{g/ml}$  to the cells culture (200  $\mu\text{l}$  of cell suspension in the each well of micro titration plate of 96 wells flat bottom ). Four replicates were used for each concentration of drug. Incubated at 37 ° C for a selected time 24, 48, 72 hr for leukemic cells and 72hr for normal

cells (16).

MTT(Methyl thiazolyltetrazolium) solution 28  $\mu\text{l}$  (2 mg /ml) was added to calculate cells viability. Read at 550 nm by ELISA reader (17). The percentage of inhibition rate for cells growth was calculated as:  $(A - B) / A \times 100$ . A : is the mean of optical density for untreated wells (control). B: is the mean of optical density for treated wells (18). Statistical analysis: The descriptive data of the results was demonstrated as ranges, percentages, means, standard errors and LSD ( $P \leq 0.05$ ) for comparison (19).

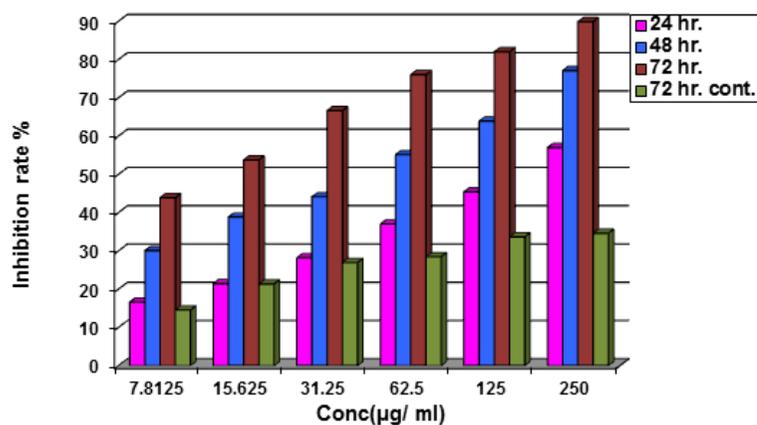
## Results:

This study showed no resistant to imatinib. The inhibition of growth rate (cytotoxic effect) of imatinib on the leukemic myeloid stem cells was dose and time dependant. There were significant differences ( $P \leq 0.05$ ) between concentrations and time (24, 48, 72hr) as in the table (1). While on the normal myeloid cells depending on the concentration, thus increased the effect with the increased concentration at 72hr and the inhibition rates were 14.588%, 21.361%, 26.991%, 28.504%, 33.697%, 34.621% for 7.8125, 15.625, 31.25, 62.5, 125, 250  $\mu\text{g/ml}$ , respectively in comparison to inhibition rate on leukemic cells as in figure (1).

**Table (1): Inhibition rate of imatinib on the leukemic myeloid cells culture**

Conc. ( $\mu\text{g/ml}$ )	Mean $\pm$ SEM*			LSD value
	24 hr.	48 hr.	72 hr.	
7.8125	16.64 $\pm$ 0.90	30.08 $\pm$ 1.90	43.91 $\pm$ 1.65	8.734 *
15.625	21.46 $\pm$ 0.64	38.87 $\pm$ 0.64	53.80 $\pm$ 1.21	7.095 *
31.25	28.20 $\pm$ 1.89	44.15 $\pm$ 1.51	66.68 $\pm$ 1.56	7.633 *
62.50	37.03 $\pm$ 1.07	55.15 $\pm$ 0.95	76.06 $\pm$ 0.83	7.018 *
125	45.45 $\pm$ 2.18	63.94 $\pm$ 1.49	82.05 $\pm$ 0.96	9.513 *
250	57.00 $\pm$ 3.82	77.12 $\pm$ 1.38	89.90 $\pm$ 0.19	8.644 *
LSD value	6.334 *	4.248	3.980 *	---
* ( $P \leq 0.05$ ).				

SEM\* = standard error of mean.  
\* = significant differences



**Figure (1):** Inhibition rate of imatinib on the leukemic myeloid cells of patients at 24,48,72 hr and normal myeloid cells at72hr.

## Discussion:

In this study showed highly effect of imatinib on the leukemic cells and less effect on the normal myeloid cells. The classical method for evaluating the effect of herb on cells is based on proportion of inhibition that indicates the rate of inhibition of the cell growth or percentage of toxicity.

Imatinib has a revolution in the treatment of BCR-ABL in CML (20). It inhibits tyrosine kinase domain of BCR-ABL oncoprotein and prevents phosphorylation of the kinase substrate. The disorder of the pluripotent hematopoietic stem cell is characterized by the t(9:22) Philadelphia chromosomal translocation leading to the production of BCR-ABL fusion protein. This is the important cause for CML and it is present in up to 95 % of patients with CML (21,22).

The major functions of BCR-ABL are: first; an increased proliferation, many cell lines were derived from blast crisis of patients with CML due to proliferation in the absence of growth factors or by low concentrations of cytokine or they have interleukin-3-driven autocrine loops. Second; reducing apoptosis, the transducer survival signals stimulated by BCR-ABL, result in decreasing the apoptosis rate. It is more easily demonstrable in cell lines than primary cells in culture in chronic phase because they need cytokines for survival (23,24). BCR-ABL may cause a prolonged arrest in G2 phase of the cell cycle leading to extensive DNA repair after DNA damage, while the normal cells undergo apoptosis in the same conditions. Third; disturbing interaction with the extracellular matrix, the tyrosin- phosphorylated in BCR-ABL positive cells included in the organization of the cytoskeleton (25).

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## التأثير السمي لدواء الايماتنيب على الخلايا النقوية الطبيعية والخلايا السرطانية مرضى ابيضاض الدم النقوي المزمن

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### الخلاصة:

تم تعريض الخلايا السرطانية النقوية لنقي العظم والمعزوله من المرضى المصابين بابيضاض الدم النقوي المزمن والخلايا النقوية الطبيعية الى دواء الايماتنيب وبتركيز مختلفه ولمدة 24,48,72 ساعه وقد اظهرت النتائج ان معدل تثبيط النمو يعتمد على التركيز ومدة التعرض وكانت اعلى نسبة تثبيط للنمو 89.907% و 43.918% للخلايا السرطانية والطبيعية على التوالي وبتركيز 250 مايكو كرام \ مل و اقل تثبيط 34.621% و 14.588% للخلايا السرطانية والطبيعية على التوالي وبتركيز 7.8125 مايكو كرام \ مل ولمدة تعريض 72 ساعه.