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Cytotoxic Effect of Vincristine and Paclitaxel on the Normal Human Lymphocytes.

Inssaf IH Al –Shemmary*.

College of Medicine, University of Anbar, Al-Ramadi, Iraq.

ABSTRACT

Vincristine and paclitaxel are microtubule inhibitors anti-cancer drugs. The aim of this study was to determine the cytotoxic effect of vincristine and paclitaxel on the primary human lymphocytes culture at 72 hours. Human peripheral blood was obtained from 20 volunteers in Al – Yarmouk Teaching Hospital / Ministry of Health. Ficoll was used for isolation of normal lymphocytes. Serial dilutions (250, 125, 62.5, 31.25µg/ml) of two drugs were added to the lymphocytes culture, the viable cells count was 3×10^6cells.Using methyl thiazolyltetrazolium (MTT) assay. The results showed that the cytotoxic effect or inhibition growth rate of vincristine and paclitaxel on the human lymphocytes was depended on the dose or concentration. The inhibition rate of lymphocytes in the high concentration (250 µg/ml) were 38.77%, 27.07% for vincristine and paclitaxel respectively and in the low concentration (31.25 µg/ml) were 15.27%, 6.34% for vincristine and paclitaxel respectively and there were significant differences (*P<0.01*) between inhibition rates of concentrations in both drugs. The study concludes that vincristine was higher inhibition rate than paclitaxel on the primary normal human lymphocytes culture at 72 hours.

Keywords: vincristine, paclitaxel, human lymphocytes culture, MTT assay.

Corresponding author

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INTRODUCTION

Microtubule inhibitors anti-cancer drugs included vinca alkaloids as vincristine and taxanes as paclitaxel [1]. Vincristine is an alkaloid antineoplastic drug which derived from *Vincarosea* plant [2,3].The mechanism of action may involve inhibition of tubulin polymerization, which may disrupts assembly of microtubules, an important part of the cytoskeleton and the mitotic spindle, results in mitotic arrest in metaphase and stop cell division that may lead to cell death [4].Vincristine is used for treatment acute lymphoblastic leukemia in children, Hodgkin's and non-Hodgkin's lymphomas [5], multiple myeloma and in several pediatric tumors such as neuroblastoma, rhabdomyosarcoma, Ewing's sarcoma, and Wilms' tumor [6].

Paclitaxel is an alkaloid ester derived from the Pacific yew *(Taxusbrevifolia)* and the European yew *(Taxusbaccata).*The mechanism of action through poisoning of mitotic spindle by high binding to microtubules with enhancement of tubulin polymerization and stabilization of the polymer rather than disassembly. Thus, they shift the depolymerization-polymerization process to accumulation of microtubules. The overly stable microtubules formed are nonfunctional, and chromosome desegregation does not occur, results in cell death.[7,8,9].Paclitaxel is used for treatment ovarian cancer, metastatic breast cancer, small and non small cell lung cancers, head and neck cancers, esophageal cancer, prostate cancer, bladder cancer and AIDS-related Kaposi's sarcoma[10,11].

Lymphocyte is a type of white blood cells (leukocytes) ina vertebrate that derived from hematopoietic stem cells. Lymphocytes represent a key of immune system. The types of lymphocytes are: B cells, T cells and natural killer cells [12,13]. B cells produce plasma cells which in turn produce antibodies and T cells produce different active cells after recognition of antigen or foreign bodies such as bacteria, viruses, and toxins on antigen- presenting cells[14].Natural killer cells (NK) are lymphocytes to be cytotoxic by the ability to kill other cells[15,16]. The normal count of lymphocytes in human adult is 20 - 40 % of the total white bloodcells. They are found in the circulation and concentrated in the central lymphoid organs and tissues such as tonsils, spleen and lymph nodes where the initial immune response occurs [17,18].

MATERIALS AND METHODS

This study was carried out in the laboratories of Al – Yarmouk Teaching Hospital / Ministry of Health, Baghdad governorate, during the period extended from August 2016 to December 2017. A total of 20 blood sample were obtained from 20 volunteers (10 males and 10 females)after obtaining the approvals, their ages ranged from 18 - 30 years. All study participants were carrying Iraqi nationality.

Peripheral Blood Lymphocytes Preparation: Human lymphocytes (normal) were isolated from peripheral blood by using Ficoll(Pharmacia, Piscataway, NJ) [19]. Blood was diluted 1:1 with phosphate buffered saline(PBS) and layered onto Ficoll with ratio of blood and PBS to Ficoll was 4:3. The blood was centrifuged at 1340 rpm for 10 minutes at room temperature. The lymphocyte layer was removed and washed twice in PBS by using centrifugation at 1200 rpm for 10 minutes, then washed with Roswell Park Memorial Institute medium (RPMI-1640)[20].

Cell Culture:Lymphocytes were placed into 25 cm falcon after adding 10 ml of RPMI -1640.This medium was prepared by dissolving 16.35g powder of RPMI -1640 with Hepes buffer and L-glutamine, 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. Incubation at 37°C [21,22].

Drugs preparation: Vincristine (Pfizer, Italy)and paclitaxel(Ebewe) were prepared in the different concentrations (250, 125, 62.50, 31.25µg/ml). 200µl of each drug was added to the cells culture (200 µl of cell suspension in the each well of micro titration plate of 96 wells flat bottom), the viable cell count was3 × 10^6cells. Four replicates for each concentration. Incubation at 37 º C for 72hr [23].

Cell viability assay: Methyl thiazolyltetrazolium (2mg /ml) was added to the cell culture. ELISA reader used to count viable cells at 550 nm. The percentage of inhibition growth rate was calculated by (A – B) / A x 100. A : is the mean of the optical density for untreated cells (control). B: is the mean of the optical density for treated cells [24].

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Statistical analysis: The descriptive data of the results was demonstrated as ranges, percentages, means, standard errors and LSD (*P≤0.01*) for comparison [25].

Ethical approval

Verbal and written agreement were obtained from each subjects involved in this study

RESULTS

This study showed that the inhibition growth rate or cytotoxic effect of two drugs on the normal human lymphocytes cultures was depended on the dose or concentration for each drug. The inhibition rate of lymphocytes in the high concentration of drugs(250µg/ml) were 38.77%, 27.07% for vincristine and paclitaxel respectively and in the low concentration of drugs (31.25µg/ml) were 15.27%, 6.34% for vincristine and paclitaxel respectively. There were significant differences (*P<0.01*) between inhibition rates for concentrations in both drugs as in the table 1,2 for vincristine and paclitaxel respectively and figures1,2for vincristine and paclitaxel respectively. In comparison between cytotoxic effect of vincristine and paclitaxel, this study recorded that vincristine was higher cytotoxic effect than paclitaxel on the normal human lymphocytes as in table 3 and figure3.

Table 1: Inhibition rate of vincristine on the normal human lymphocytes at72hr

Concentration (µg/ml)	Mean ± SEM of IR %
31.25	15.27 ± 0.84 d
62.50	23.04 ± 1.83 c
125	30.83 ± 2.07 b
250	38.77 ± 2.16 a
LSD value	4.923 **
P-value	0.0036
** (*P<0.01*).	

IR=inhibition rate, SEM=standard error of mean, different letter=differ significantly,**=significant differences

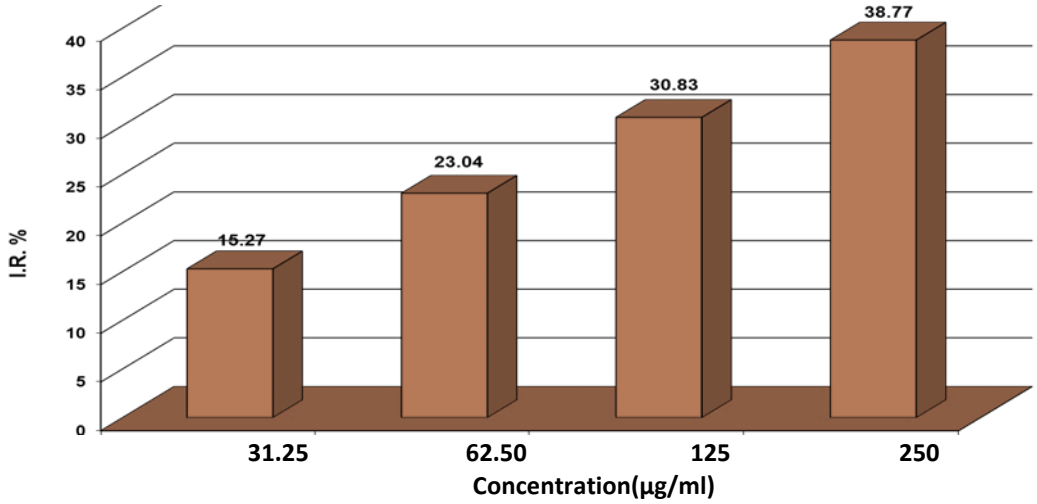

Figure 1: Inhibition rate of vincristine on the normal human lymphocytes at 72hr.

Table 2: Inhibition rate of paclitaxel on the normal human lymphocytes at 72hr

Concentration (µg/ml)	Mean ± SEM of IR %
31.25	6.34 ± 0.42 d

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62.50	12.40 ± 0.61 c
125	20.70 ± 1.54 b
250	27.07 ± 1.76 a
LSD value	4.198 **
P-value	0.0029
** (*P<0.01*).	

IR=inhibition rate, SEM=standard error of mean, different letter=differ significantly,**=significant differences.

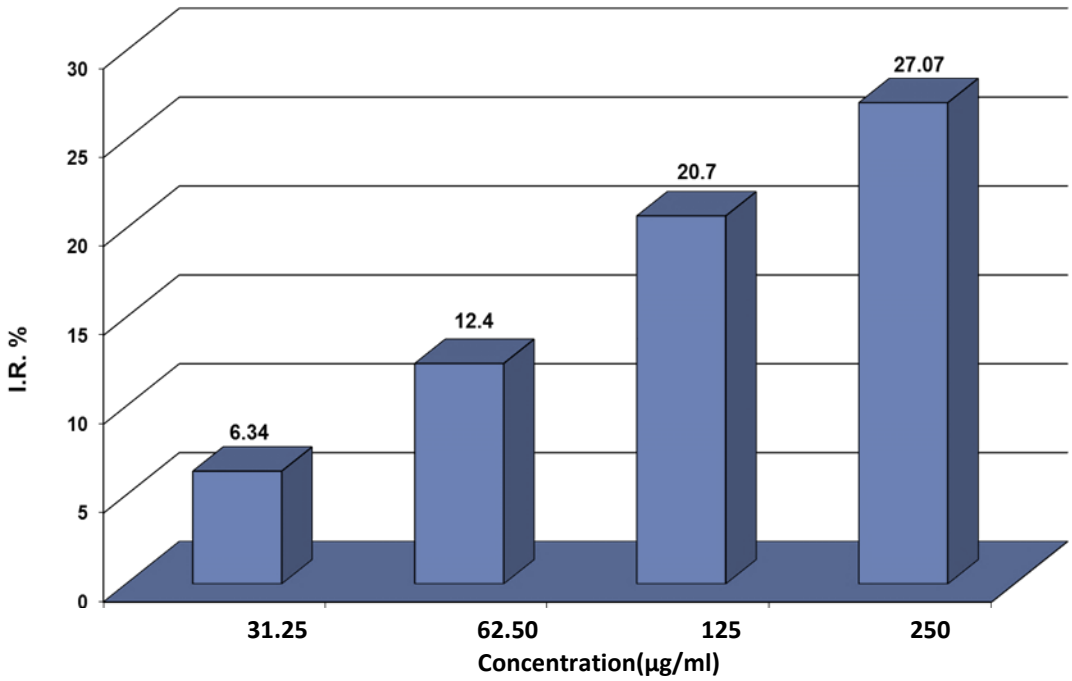

Figure 2: Inhibition rate of paclitaxel on the normal human lymphocytes at 72hr.

Table 3: Comparison of inhibition rates between vincristine and paclitaxel on the normal human lymphocytes at 72 hr.

Concentration (µg/ml)	Mean ± SEM of IR %		LSD value
	V	P	
31.25	15.27 ± 0.84 d	6.34 ± 0.42 d	5.032 **
62.50	23.04 ± 1.83 c	12.40 ± 0.61 c	4.956 **
125	30.83 ± 2.07 b	20.70 ± 1.54 b	4.738 **
250	38.77 ± 2.16 a	27.07 ± 1.76 a	5.973 **
LSD value	4.923 **	4.198 **	---
P-value	0.0036	0.0029	---
** (*P<0.01*).			

IR=inhibition rate, SEM=standard error of mean, different letter=differ significantly, **=significant differences, V= vincristine, P= paclitaxel.

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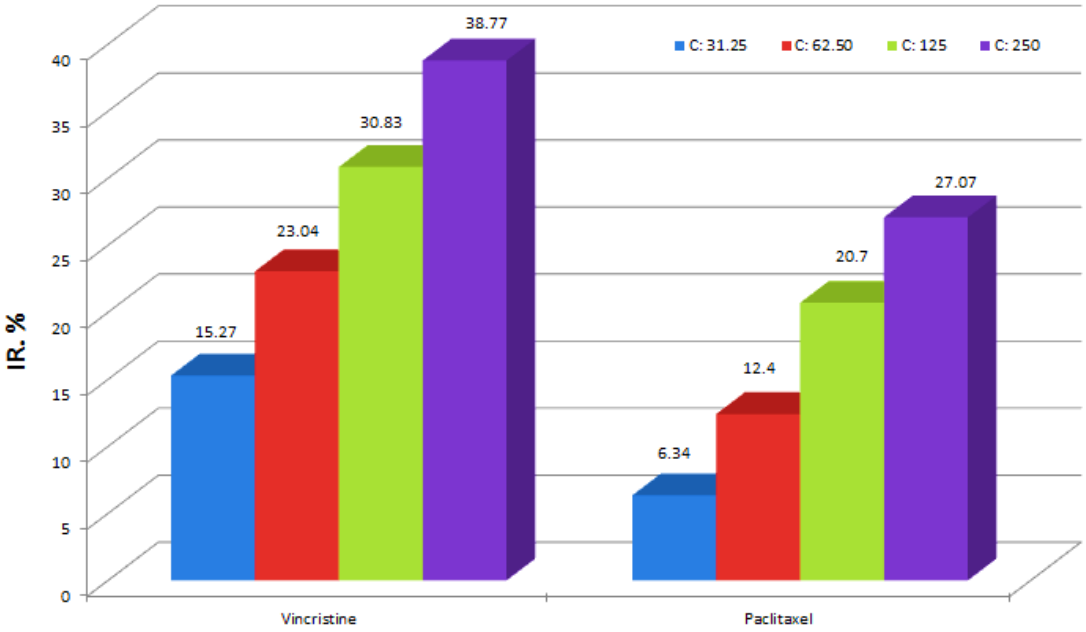

Figure 3: Comparison of inhibition rates between vincristine and paclitaxel on the normal human lymphocytes at 72 hr.

DISCUSSION

The present study showed that the cytotoxic effect of vincristine and paclitaxel on the normal human lymphocytes at 72hr were depended on the concentration, therefore the inhibition growth rate was increased with the Increase concentrations in both drugs. Also this study showed that the inhibition rate of vincristine was higher than inhibition rate of paclitaxel on the normal human lymphocytes at 72hr.

The cytotoxic effect of vincristine and paclitaxel as a microtubular inhibitor anti-cancer drugs due to mitotic spindle is part of larger intracellular skeleton (cytoskeleton) that is essential for the structures movements in the cytoplasm of all cells [26].The mitotic spindle consists of chromatin plus a system of microtubules composed of the protein tubulin. The mitotic spindle is essential for the equal partitioning of DNA into the two daughter cells when cell divides. Anticancer drugs inhibit this process by affecting the equilibrium between the polymerized and depolymerized forms of the microtubules, therefore causing cytotoxicity[27,28].Previous study reported that all anti-cancer drugs proved a cytotoxic effect to the normal human cells[29,30], but the comparison between two drugs, this study recorded that paclitaxel was less cytotoxic effect than vincristine on the primary lymphocytes culture in spite of paclitaxel has a broad range of anticancer activity in many organs.MTT assay was used to determine the cytotoxic effect of two drugs because this method is a rapid spectrophotometric for determining cell viability inculture as well as widely use in researches [31,32].

Lymphocytes are play an important role in the immune system because lymphocytes are the cells that determine the specificity of the immune response against different microorganisms and other foreign bodies. The immune system provide the body by protection against infections and has an important role in the control of malignant disease. The immune response is represented by innate and adaptive response[33,34,35]. The innate immune system is responsible for the initial response to infection and inflammation also is mediated by cells such as macrophages, neutrophils and dendritic cells as well as natural killer cells (NK). Previous several studies showed that the effect of natural killer (NK) cells in malignant tumors were obtained a better clinical outcome [36,37,38].Therefore the cytotoxic effect of anti- cancer drugs on the lymphocytes which affected immune system activity.

CONCLUSION

The study was included two drugs of microtubule inhibitors anti-cancer drugs as vincristine and paclitaxel. The cytotoxic effect of two drugs was depended on the dose or concentration. In spite of paclitaxel

has broad spectrum anticancer activity but this study recorded that the cytotoxic effect of vincristine was higher than cytotoxic effect of paclitaxel on the primary human lymphocytes culture at 72 hr.

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