Evaluation of genotoxic and cytotoxic doliprane on cultured lymphocytes

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Abstract
The main objective was to evaluate the genotoxicity and compare it with the use of the simplest painkiller prescribed to the patient and what are its effects on lymphocyte culture with different concentrations (0.05, 0.1, 0.5, 1, 1.5, 2, 2.5, and 3mg/ml), and the result was the discovery of cell damage, deformation of divisions, and thus chromosomal changes and the emergence of micronuclei in Cell divisions at high concentrations, where the highest concentration in which divisions appeared was 2.5 mg/ml. Knowing which regions of the chromosome are associated with the effect of this drug is an important first step.
Keywords: genotoxic, cytotoxic, chromosomal abnormalities, and doliprane.

1. Introduction

The use of the analgesic doliprane has spread over the past two years due to the global spread of the coronavirus, and this drug has also spread accordingly. Through research, it was found to be another aspect of paracetamol, an analgesic and antipyretic. excreted from However, relatively small amounts remain metabolized by the hepatic cytochrome B450 system to produce a peripheral metabolite (N- acetyl-para-benzo-quinone imine) responsible for the toxic effects of paracetamol [1, 2]. When taken at recommended doses, paracetamol does not irritate the stomach wall, affect blood clotting like other NSAIDs, or affect kidney function. It has been shown that high doses above 1 mg may increase the risk of upper gastrointestinal complications. Numerous studies on the toxicity of paracetamol and conflicting results on genotoxicity, the devastating effect of substances that weaken genetic material in cells [3,4]. Drugs are substances that can change the function of organs, including pain relievers. "Paracetamol, non-steroidal anti-inflammatory drugs (NSAIDs), and opioids are three basic pain relievers commonly used as over-the-counter analgesics" [5,6]. Its mechanism of action is known to be safe at therapeutic doses and to have few side effects [6]. All chemicals affect the cellular level, including DNA. Drugs have mechanisms, such as DNA repair, that affect cells and DNA to control drug-induced damage. Each drug affects genetic material and depends on dosage and dosing interval [7,8]. "Many physical, chemical and biological factors are known to damage chromosomes. Physical cholesterinogens include X-rays, ultraviolet light, cold shock, magnetic fields, and sound waves. Some genes, viruses, and protozoa are examples of biological" reproduction. Abnormal chromosomes often arise as a result of errors during cell division [9,10]. Chromosomal abnormalities often result from one or more errors during thedivision of sex cells (meiosis) and errors during thedivision of other cells (mitosis). "Lymphocytes are the main cells of the body's defense system against foreign pathogens" [11,12]. Doliprane is widely used without a prescription for many common ailments The dose of Doliprane is 500 mg every 5-6 hours for as long as symptoms persist (do not exceed 2000 mg per day). The maximum Driplan dose is 2000 mg daily. This medicine is usually used for 3 days for fever and 7 days for pain. It takes 20 minutes for this medicine to start working. This drug is not known to be habit-forming. An overdose of readily available tablets containing Doliprane usually causes unsatisfactory effects on the human body [13,14]. Although there are extensive reports of paracetamol toxicity related to liver defect and renal function, is known about its effects on cultured lymphocytes and human genetic materials [15,16]. In a study, the first study about doliprane routine cytotoxicity assays, bioassays, and cultured lymphocyte counts were used to determine the cytotoxic and genotoxic effects of a commonly used topical culture additive tablet containing doliprane on lymphocytes.

2. Material and Method

Cell viability assays were performed under sterile conditions. 200 microliters of live cell suspension were taken, after adding the same amount of trypan blue with continuous mixing well. The hemocytometer was moistened with water and stored on the counter. A chamber filled and 20 µL suspension cells and examined with a light-microscope at 10x magnification. Cells counted then live cells were colorless and dead cells stained blue. Calculate the property of live cells [17,18].
Cultivate lymphocytes from the surrounding blood, culture 0.3 ml in a flask containing 5 ml of a dedicated lymphocyte culture medium (lymphoprime: complete medium for peripheral blood lymphocyte culture), and place in a carbon dioxide gas incubator at 370°C. cultured in with 5% carbon dioxide. The addition of dolphin to lymphocytes is a pill used locally that has been crushed and dissolved in sterile and (D.D.W) to make a concentration of 75 mg/ml. Dolipraine additive for

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lymphocytes is a locally used tablet that is crushed and dissolved in sterile, distilled water to a concentration of 75 mg/mL. The method was done according to the method of Wang et.al.2006[17]. "MTT Assay for Cytotoxicity MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, tetrazole)" To check for chemical cytotoxicity. Cells convert MTT to an insoluble color. These may be crystals dissolved in dimethyl sulfoxide (DMSO) and measured by spectrophotometry. Prepare MTT by diluting 5 mg/ml MTT in phenol red-free Lymphoprime medium. MTT working solution was prepared by diluting 5 mg/ml MTT 1:10. The solution was filtered by a 0.2 µ filter and stored at 4-6 °C. Harvest cells by precipitate at 4,000 rpm for 15 minutes. Plated of cell in 96 flat bottom dishes containing 150 µl of themedium at 37 °C. The percentage of the examined cell borders outside the density was set at 0.1-0.5 x 10 5 cells/mL. Concentrations of doliprane (3.0,2.5, 2.0, 1.5, 1.0, 0.5, 0.1, and 0.05 mg/ml) were added to the pits. "Incubation continued for 44 hours. Four hours before the end, add 20 µL of MTT solution (5 mg/mL) to all holes, transfer cultures from the well to Eppendorf tubes, and centrifuge at 500 rpm for 15 minutes". The supernatant was removed and 100 µL of DMSO then add to all tubes. The tubes were placed at room temperature for 20 minutes, and the optical density was examined at 560 nm [18,19].

Blood samples were cultured in a medium (Lymphoprim) containing varying concentrations of doliprane and for each concentration cultured in two tubes in an inclined position at 37°C for 72 hours. Mitotic index (MI) and chromosomal aberrations in the structural index in cultures of human lymphocytes and micronuclei were quantified according to the standard protocol (20) and using solid staining techniques as a study method. Two hours before harvesting, metaphase cells were arrested by adding 0.1 µg/ml colcemid (colchicine) solution to the first culture tube. Cells and the second tube, add 0.5 µg/ml of cytochalasin at 48 hours of culture for the purpose of detecting micronuclei, then complete the culture for 72 hours in the 37C incubator, then all the tubes after completing the culture time, collect by centrifugation at 1000 rpm for 10 minutes and resuspend. in "hypotonic potassium chloride solution (0.075 M KCl, 0.45%)" for 15 min., fix three times with glacial acetic acid-methanol (1:3, vol/vol). A few drops were applied to wet, cooled, air-dried, and stained slides with 10% Giemsa for 15 minutes. Slides were examined using an oil-immersed lens. division index is1000 lymphocytes from each culture were examined and the proportion of dividing cells was determined. In addition, there are 50 well-seeded metaphases from each of the malformations recorded [18].

3. Result

Viability and cell number are Calculated for treated cells as concentration increases doliprane and the interpretation of results is in a table: 1. Relationship between increased doliprane concentration and cell Viability is shown in Figure 1. Percentage of doliprane in cell density Figure: 2. Cells exposed to Doliprane have a higher concentration of cytotoxicity. The preparation of cytotoxicity is high with increasing concentrations of doliprane. MTT test was performed to examine the cytotoxicity of doliprane. The results of the examination were the property of cytotoxicity with increasing concentrations of doliprane. All cells died at the high concentration of doliprane (3.0 mg/mL) the percentage of live cells at this concentration was 0 and the property of cytotoxicity was 100 (Fig: 2). And that cytotoxicity of cells with different concentrations of doliprane is calculated according to the formula,

$$\text{Percentage of dead cells} = 1 - [\text{O.D of Test} / \text{O.D of Control}] \times 100.$$

Table 1: Viable cell count and viability percentage after treatment with doliprane

Control &conce. Of doliprane	Doliprane (mg/ml)	The volume of cells (ml)	Number from the cells /20µl	The number from the viable cells/20µ	proportion of survival
control	0.0	5.0	250	240	96
1	0.5	5.0	230	200	86.9
2	1.0	5.0	220	170	77.3
3	1.5	5.0	215	100	46.5
4	2.0	5.0	200	50	25
5	2.5	5.0	150	20	13.3
6	3.0	5.0	50	0	0

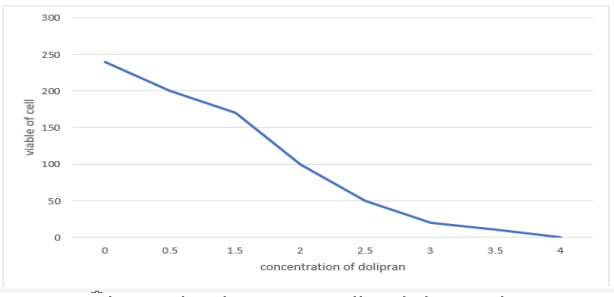

Fig: 2. Relationship between cell viability and increased doliprane concentration

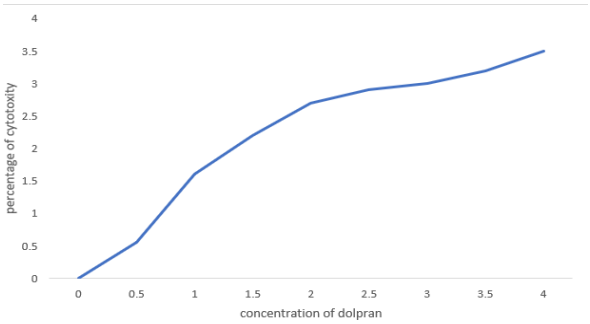

Fig: 5. MTT Assay for Cellular Cytotoxicity

The results of the cytogenetic study of all research samples showed unstable chromosomal changes that included (a gap, and a normal group) for lymphocyte culture drugs for different concentrations. Chromosomal aberrations (1.3% including gap) were recorded. The recorded deviations were in the form of gaps and breaks. The mitotic index for this group was (%). compared with the control. The recorded chromosomes had aberrations in the form of gaps, Moreover, there was a significant prevalence of inhibition with a statistically significant ($P < 0.05$) decrease in the mitotic index to become (6.8%) while in the control group. (Table). As in Table 2 and Figures (2 and 1), the mitotic index (MI), the frequency of metaphase nuclei, was measured against human mitotic lymphocytic cell lines using the mitotic index assay. Assess the genotoxicity of physical and chemical agents. The exponent is given by the formula

$$\%MI = \frac{No.of\ Dividing\ cell}{No.of\ dividing\ and\ nondividing\ cells} \times 100$$

As for the micronucleus test, it was found that the percentage of nuclei in the diploid cells was 1.17% in the control groups (Fig.4). In the group of samples after treatment with doliprane concentration, there was a significant ($P < 0.05$) increase in the average micronuclear frequencies in binucleated lymphocytes by 2.5% compared to the control group. (Fig.3،4 & Table 2)

Table (2): The percentage of the total chromosomal aberrations, micronuclei in the binucleated and mitotic index in human lymphocyte cultures

Groups	Mitotic index	% Total chromosomal aberrations	% Micronuclei in binucleated cells
Control	8.7	0.3	0.9
Conce.0.05*	8.7	0.4	1.0
Conce.0.1	8.7	0.6	1.2
Concen.0.5	8.5	0.9	1.6
Conce.1	7.9	1.2	1.9
Conce.1.5	7.5	1.5	2.2
Conce.2	7.0	1.9	2.6
Conce.2.5	6.8**	2.1**	2.8**

*The concentration of doliprane in culture ** high significance compare with control

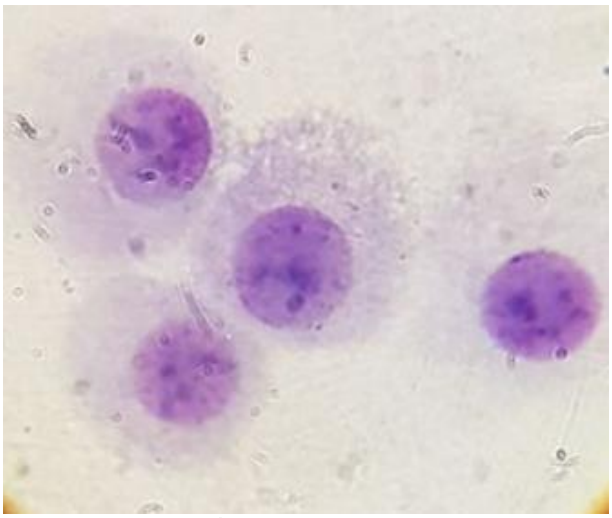

Figure (3) The figure shows cell division (400X)

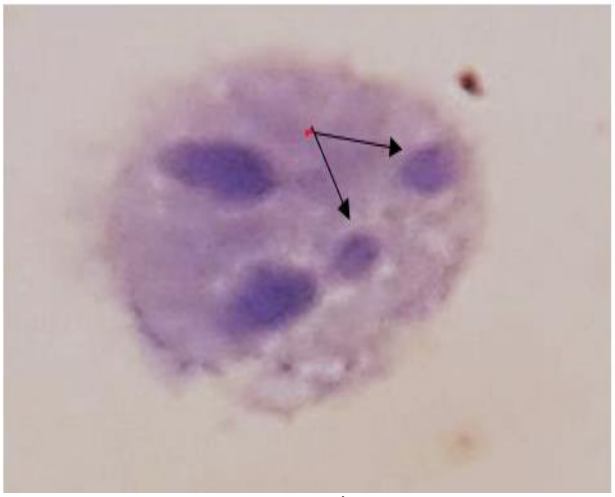

Figure (4) micronuclear at (1000X)

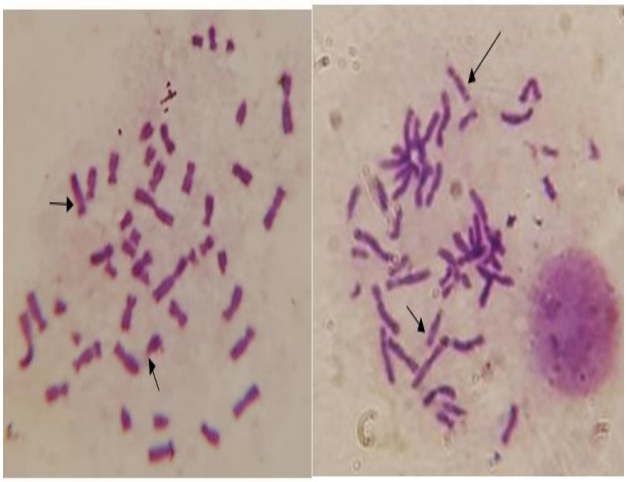

figure (5) chromosomal abnormalities

4. Discussion

Numerous surveys and studies have shown the cytogenetic effects of drugs on human cells of various ethnic groups. [21] The phenomenon of using painkillers and dependence on illegal drugs has become a major problem in our country, especially since the spread of corona, and this drug was widely used. Evidence from research on chromosomal abnormalities and research on the health status of drug addicts or illicit drugs from developing countries, especially after the recent national epidemic. [22,23] The results for this region show the following important points, the user results show a significantly increased frequency of cells with chromosomal damage compared to the healthy control group. A higher frequency of chromosomal

damage was seen when different concentrations of the drug were used. MTT selection is an important test for measuring the toxicity of a compound and its efficacy in cell division. Measure the activity of the purple enzyme that reduces MTT. It mainly occurs in the mitochondria and is activated by the intense activity of mitochondrial. this can be used to determine the cytotoxicity of potential drugs and other toxic substances [24,]. Another thing is the future impact. This is a serious problem when toxicity is linked to genes and cancer incidence [25]. Therefore, it is necessary to know the toxicity and duration of theuse of drug compounds as they interact with cellular molecules. The effects of substances on genetic material were studied by examination of the mitotic index (MI) and chromosome functioning when lymphocytes divide into human blood cells. doliprane significantly increases cell division rates at low concentrations (0.05 to 0.1) for approximately the same time (4, 12, and 72 hours). Doliprane also has an inhibitory effect on MI at high concentrations (1-2.5). This effect is dependent on time. The genotoxic effect of doliprane is expressed by a high incidence of chromosomal aberrations in treated cells and is time and concentration. Mutation rates were significantly in cells higher (and micronuclei examined by Karmakar et. al. [26] treated at low (0.05, 0.1) and long-term (72 h) concentrations, indicating acentric chromosomal fragments or whole chromosomes. are small chromatin-containing bodies arising from the nuclei that bind to daughter nuclei after cleavage. Therefore, micronucleus testing has been viewed as a biomarker of damage to the mitotic apparatus leading to chromosomal breakage or chromosomal loss [27,28]. A number of studies have concluded that this genotoxic effect of doliprane may be related to the production of micronuclei through the presence of micronuclei in some cells of this drug user. That agrees with [29,30,31,32]. CONCLUSIONS: Increasing doliprane concentration decreases cell viability. All cells were killed at a doliprane concentration of 3 mg/ml. at the highest concentration. When MT was screened to measure compound cytotoxicity, that cytotoxicity to cells increased and increased doliprane concentration. From these results, it can be assumed that concluded that this compound is doliprane to consume for mild illness, can affect cell division and cell vitality, and is genotoxic at high concentrations and frequent intake. doliprane exhibits the potential of genotoxic in Exo and in vivo, possibly through indirect cytotoxic effects or enzyme inhibition. they need to clarify if it doesn't happen.

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